CENTRE OF EXPERTISE FOR WATERS

Factoring Ecological Significance of Sources into Phosphorus Source Apportionment Phase 2

CRE W CENTRE OF EXPERTISE FOR WATERS

Factoring Ecological Significance of Sources into Phosphorus Source Apportionment Phase 2

Full Report

M. Glendell, M. Stutter, I. Pohle, J. Palarea-Albaladejo, J. Potts, L. May

Hydro Nation International Centre

The James Hutton Institute

Scottish Government
Riaghaltas na h-Alba
gov.scot

CENTRE OF EXPERTISE FOR WATERS

Published by CREW – Scotland's Centre of Expertise for Waters. CREW connects research and policy, delivering objective and robust research and expert opinion to support the development and implementation of water policy in Scotland. CREW is a partnership between the James Hutton Institute and all Scottish Higher Education Institutes supported by MASTS. The Centre is funded by the Scottish Government.

This document was produced by:

M. Glendell[1], M. Stutter[1], I. Pohle[1], J. Palarea-Albaladejo[2], J. Potts[2], L. May[3]

1 The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH.

2 Biomathematics and Statistics Scotland, Craigiebuckler, Aberdeen AB15 8QH

3 UK Centre for Ecology & Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB

Please reference this report as follows: M. Glendell[1], M. Stutter[1], I. Pohle[1], J. Palarea-Albaladejo[2], J. Potts[2], L. May[3] (2020) Factoring Ecological Significance of Sources into Phosphorus Source Apportionment - Phase 2. CRW2017_09. Available online at: crew.ac.uk/publication/Eco-significance

ISBN number: 978-0-902701-71-7

Dissemination status: Unrestricted

Cover photographs are courtesy of Miriam Glendell[1]

Acknowledgements

We would like to thank Brian McCreadie, Graeme Cameron, Alison Bell, Mark Hammonds and Fiona Napier from the Scottish Environment Protection Agency for helpful comments and water quality monitoring data. Laura Poggio, Richard Hewitt and Malcolm Coull from the James Hutton Institute helped with catchment delineation in ArcGIS. J. Palarea-Albaladejo[2] was supported by the Scottish Government's Rural and Environment Science and Analytical Services Division (RESAS).

Contents

Tables & Figures

Executive Summary

Factoring Ecological Significance of Sources into Phosphorus Source Apportionment: Phase 2 Understanding the link between phosphorus and ecological impact in Scottish streams

BACKGROUND

Phosphorus (P) source apportionment is an important tool for prioritising mitigation strategies and assessing compliance as part of River Basin Management Planning[1] process within the EU Water Framework Directive. However, the methodology for P source apportionment in rivers is subject to significant errors and uncertainty as annual total P loads are assumed to correlate with ecological impact, despite a wealth of evidence to demonstrate other factors such as seasonality and P bioavailability that affect the processes and mechanisms responsible for the transport of P from source to river systems (Stutter *et al.*, 2014).

In 2014 CREW delivered a descriptive methodology[2] of how modelled Total Phosphorus (TP) loads could be modified to take account of their impact on ecology (Phase 1). In the absence of measured bioavailable P concentrations (by the Scottish Environment Protection Agency), the study examined indirect evidence that Soluble Reactive Phosphorus (SRP) loads from different sources had a different impact on the ecological response due to differences in bioavailability of P fractions and timing of inputs. These relationships were examined and tested (Appendix 1) within the context of other pollutants and in catchments with different characteristics.

This project (Phase 2), therefore aims to: (i) evaluate the relevance of the method developed in Phase 1 for the SAGIS tool to derive 'ecologically significant source apportionment' and (ii) examine potential factors affecting ecological status based on the regulatory data.

RESEARCH QUESTIONS (PHASE 2)

- What is the relative importance of different phosphorus (P) fractions and sources on diatom response?
- How important is P in affecting diatom status in the context of multiple stressors (nutrients, land cover and catchment hydrological characteristics) in running waters?
- How do factors influencing the phosphorus-diatom relationship vary between different catchments?

In this study, we use the term 'stressor' to mean an environmental factor that has an adverse impact on the ecological community.

MAIN FINDINGS

Data from 45 Scottish streams were examined to identify a relationship between diatom response (a key ecological indicator for water body status) and other factors including: nutrients, SRP loads from different sources, land cover proportions and hydrological catchment characteristics.

- The Trophic Diatom Index (TDI) was used to represent the ecological response. TDI allows a comparison of the observed state of a water body against that expected in the absence of anthropogenic disturbance by deriving the Ecological Quality Ratio (EQR TDI) between the observed and expected diatom status. Higher values indicate higher ecological status.
- In agreement with previous work, diatom response was negatively associated with P species (SRP and TP), nitrate (NO_3-N) and urban land cover and positively associated with seminatural land cover type. The relationship varied with season, with higher ecological status associated with spring.

1) What is the relative importance of different P fractions and sources on the diatom response?

- Total phosphorus concentration was more strongly associated with the diatom response EQR TDI than SRP, although the differences between them were small.
- A negative association was observed between P concentrations (mg L^{-1}) and diatoms, but this study did not find evidence of a relationship between P loads and diatoms in running waters

2) How important is P in affecting diatom status in the context of multiple stressors (nutrients, land cover and catchment hydrological characteristics) in running waters?

- Semi-natural land cover had the strongest positive association with EQR TDI, while urban land cover had a significant negative association with the ecological response.
- After excluding the overarching impact of land cover, the ratio of NH_3-N to NO_3-N as well as NO_3^- N concentrations also had a negative effect on the EQR TDI.

3) How do factors influencing the phosphorus-diatom relationship vary between different catchments?

- The analysis highlighted catchment-specific responses, whereby catchments could be grouped according to most strongly associated stressors.
- The relationship between TP, SRP and EQR TDI was not spatially consistent and varied more between catchments for TP than for SRP.

1 *https://www.sepa.org.uk/environment/water/river-basin-management-planning/*
2 *https://www.crew.ac.uk/publication/ecological-significance-phosphorus*

RECOMMENDATIONS

- The complex relationship between multiple stressors (P and N species, land cover proportions) and ecological response between catchments, supports the need for further research into factors that may affect the spatial variability in the stressor-response relationship. This could be informed by further collection of data with high temporal resolution in representative catchment types.

- Effective mitigation measures should target all stressors in concert, taking into account catchment-specific responses in different catchment types.

- In future research, the bio-available P (BAP) fraction should be measured and monitored alongside SRP and TP at a number of representative locations across Scotland to provide data for model development and validation.

- Reflecting the overriding importance of land cover highlighted in this study, it has been suggested that current mitigation policies may not be sufficient to achieve good ecological status and that targeted land cover change may need to be considered.

1 Introduction

Phosphorus (P) pollution remains an important cause of eutrophication of freshwaters worldwide (Withers *et al.*, 2014). In the UK, significant effort has been made to control the anthropogenic input of excess nutrients to freshwater bodies from a variety of sources. However, despite observable reductions in nutrient concentrations leading to an improvement in the chemical status of inland waters, in many cases a corresponding improvement in the ecological status has not yet been observed (Bowes *et al.*, 2012; Harris & Heathwaite, 2012). This may be due to incomplete understanding of these systems (Harris & Heathwaite, 2012), the difficulties in measuring and quantifying interactions between multiple stressors in river catchments (Friberg, 2010) as well as potential lag effects (Hamilton, 2012).

Phosphorus source apportionment is an important tool for prioritising mitigation strategies under River Basin Management Planning within the EU Water Framework Directive (Council of the European Communities, 2000). However, not all P sources are considered 'equal' in terms of their bioavailability and ecological impact (Stutter *et al.*, 2014). While prioritising mitigation actions according to the load of P from different sources may be more relevant to standing water bodies where P accumulates in sediments over time, mean P concentrations are likely to be more relevant in running waters (Stamm *et al.*, 2014). In both cases however, it is important to consider the likely differing characteristics of contributing sources in terms of their actual P bioavailability and therefore their ability to affect ecological outcomes.

Analytically, TP can be split into different fractions (Haygarth & Sharpley, 2000), which include particulate P (PP) represented by the solid fraction (> 0.45µm). The soluble (< 0.45µm) fraction (Total Dissolved Phosphorus, TDP) includes both inorganic forms of P (SRP), and soluble organic P usually considered as unreactive (Soluble Unreactive Phosphorus, SUP). Traditionally, SRP has been taken as largely composed of orthophosphate (PO_4^-) and is considered completely bio-available (Prestigiacomo *et al.*, 2016). While SRP is typically used in the setting of water quality standards, it may not be a perfect proxy for bio-available P (BAP) as the bioavailability of individual compounds included in SRP varies with their precise molecular form (Li & Brett, 2013). Therefore, there is a risk that equating SRP with bioavailable P will under-estimate the total bioavailable P as other P fractions, such as PP attached to sediment particles and SUP, can both contribute to the pool of bioavailable P (Baker *et al.*, 2014) (Fig. 1). Thus, both forms of P (TP and SRP), typically quantified in regulatory water quality monitoring schemes, may either over- or under-estimate the actual amount of bioavailable P from different sources (Ellison & Brett, 2006; Ekholm *et al.*, 2009).

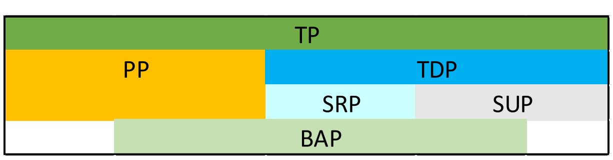

Fig 1. Pools of phosphorus (P): TP = Total P, PP = Particulate P, TDP = total dissolved P, SRP = Soluble reactive P, SUP = soluble unreactive P, BAP = biologically available P.

The bioavailability of different P fractions is usually measured through algal bioassays in laboratory settings (Ellison & Brett, 2006; McDowell *et al.*, 2016; Prestigiacomo *et al.*, 2016). As such determinations are not straightforward in field settings. To our knowledge, studies of the relative bioavailability of P forms from different contributing sources are limited. For example, Ekholm & Krogerus (2003) found that depending on the source, between 16 and 89 % of TP and 4.5 and 46 % of PP can be bioavailable. Furthermore, P removal technologies alter the bioavailability of P fractions from sewage treatment works (STWs) (Li & Brett, 2015). While P stripping in sewage treatment works can reduce the bioavailable fraction to 1% of particulate phosphorus (PP), the bioavailable fractions of soluble unreactive (SUP) and soluble reactive (SRP) phosphorus remain high at 72 and 93% respectively (Prestigiacomo *et al.*, 2016).

Moreover, the bioavailability of P forms from catchments dominated by different land uses has been shown to vary. This is between 12-73 % for TP (Ellison and Brett, 2006) and 6-81% for PP (Ellison & Brett, 2006; Egemose & Jensen, 2009; Poirier *et al.*, 2012; Baker *et al.*, 2014), with evidence that some land cover, such as agriculture and urban areas, make TP more bioavailable (Ellison & Brett, 2006; Prestigiacomo *et al.*, 2016). In addition, the proportion of bioavailable P in different P fractions varies temporally, both between seasons (Stutter *et al.*, 2007; Abell & Hamilton, 2013) and during storm events (Ellison & Brett, 2006). This makes it difficult to estimate bioavailable P from other 'proxy' P fractions without location-specific high-resolution measurements.

The timing of P inputs from different sources is relevant for their relative ecological impact. This is particularly apparent in running waters where P inputs from near-continuous sources such as STWs may be more important during the ecologically active summer season than intermittent inputs linked to hydrologically responsive diffuse agricultural sources (Stamm *et al.*, 2014; Shore *et al.*, 2017). Consequently, septic tanks are likely to have an intermediate effect on the scale of likely impacts as they act as semi-continuous P sources that are also responsive to high flow events throughout the year (Stutter *et al.*, 2014). In addition, ST effects may be greater in the summer, when the flows are low and river temperatures are higher, thus having a greater potential impact on river ecology. Thus, temporal variability in P input from different sources, as well as the spatial and temporal variability in their bioavailable P composition, need to be considered when prioritising effective pollution mitigation actions (Withers et al., 2014).

Diatoms are used in environmental assessments of water bodies around the world (Kelly *et al.*, 2012; Kelly, 2013; Stevenson, 2014; Poikane, Kelly & Cantonati, 2016) as they are strongly correlated to eutrophication gradients (Hering *et al.*, 2006). In the UK, diatoms are one of the ecological indicators used to assess ecological status under the EU Water Framework Directive (Kelly *et al.*, 2008; UKTAG, 2014). The Trophic Diatom Index (TDI) allows comparison of the observed state of a water body against that expected in the absence of anthropogenic disturbance by deriving the Ecological Quality Ratio (EQR TDI) between the observed and expected diatom status (Kelly *et al.*, 2008), with higher values indicating higher ecological status. TDI was shown to be correlated with both SRP and NO_3--N (Kelly *et al.*, 2008), however the relationship between TDI and bioavailable P has not yet been tested due to the absence of measured bioavailable P concentrations in UK rivers. In this study, we examine the link between diatoms as indicators of ecological status in Scottish rivers and concentrations of routinely measured P fractions (TP and SRP) as proxies for bioavailable P. Specifically, we address the following questions: 1) What is the relative importance of different P fractions and sources on the diatom response? 2) How important is P in affecting diatom status in the context of multiple stressors in running waters? 3) How do factors influencing the phosphorus-diatom relationship vary between different catchments?

2 Sites and Statistical Analysis

2.1 Sites

Ecological monitoring data was provided by the Scottish Environment Protection Agency (SEPA) for 88 locations across Scotland where continuous diatom sampling has taken place in spring and autumn between January 2007 and September 2017 (Appendix 2). The data comprised observed and calculated Trophic Diatom Index (TDI) and their Environmental Quality Ratio TDI (EQR TDI) (Kelly *et al.*, 2008; UKTAG, 2014) for spring and autumn sampling.

The ecology data was matched with monthly water quality data from the Scottish Environment Agency (SEPA) operational Harmonised Monitoring Scheme (HMS) at the nearest sampling location. From this data, diatom and chemistry monitoring locations within 200 m distance were selected for analysis, resulting in 625 complete observations from 45 study catchments with diverse characteristics (Fig. 2, see Appendix 2 for full list of data used in the analysis). Chemical parameters of interest included TP, SRP, NO_3-N, nitrite (NO_2-N), ammonia (NH_3-N), suspended solids (SS) and chloride (Cl). TP was derived as a reduced phosphomolybdenum blue complex from a manual sulphuric acid - persulphate digest of unfiltered samples, while SRP represented the molybdate-reactive P determined from a <0.45 µm filtered sample. Alkalinity was not included in the analysis as it is accounted for in the calculation of the TDI index (UKTAG, 2014).

Observed mean daily river discharge data was obtained from SEPA for the selected 45 locations. For each location, the upstream contributing area was delineated using the ArcHydro tools in ArcGIS 10.2.1 and a 10m or 50m resolution DEM. Delineated catchment outlines were visually sense-checked to eliminate any misalignments due to errors in DEM and the best-fitting outline was selected. Proportion of arable, improved grassland, urban, woodland and semi-natural land cover types (the latter including all types of unimproved grassland and dwarf shrub heath) were calculated for each catchment area in ArcGIS 10.2.1 based on the CEH Land Cover Map 2007 (Morton *et al.*, 2011).

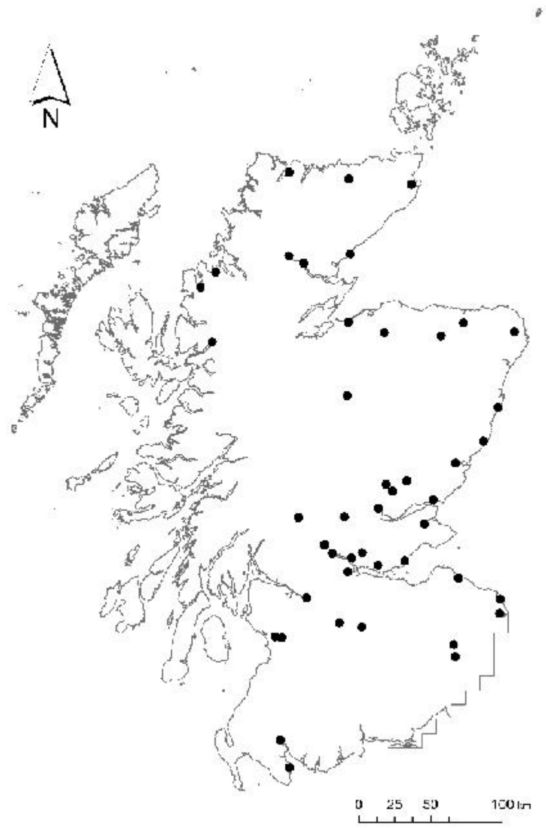

Fig. 2. Location of 45 catchments included in the study.

Hydrological characteristics of each study catchment were derived to describe hydrological flashiness (ratio of high to low flows Q5:Q95), seasonality (range of Parde coefficients) and oscillation (Richards-Baker Index (RBI)), using dimensionless indices describing variability of streamflow (see Appendix 1). All hydrological indices were calculated based on daily observed or simulated streamflow (based on the CEH Low Flows model) for the time period 2007-2016.

Modelled phosphorus source apportionment load estimates (kg yr^{-1}) from sewage treatment works (SWLOAD), septic tanks (STLOAD), combined storm overflows (CSOLOAD), urban (URLOAD), livestock (LSLOAD) and arable (ARLOAD) land cover types were obtained from SEPA as the output from the SAGIS source apportionment tool (Daldorph, 2017).

2.2 Statistical analysis

A stepwise 'weight of evidence' based approach, based on a range of different statistical modelling approaches and model structures, was chosen to maximise the insights that can be gained from regulatory water quality monitoring data. Each class of statistical models was used to get a different insight into the data structure and functional relationships as outlined below.

Principal Component Analysis was used to investigate the presence of main environmental gradients and correlations in the multivariate dataset. PCA simplifies the data structure by converting correlated variables (potential stressors) to a smaller number of uncorrelated axes or 'components' (Fig.3).

Further **formal statistical modelling** was undertaken using three different modelling approaches – regression trees for clustered data (RT), compositional linear mixed models (CLMM) and ordinary linear mixed models (LMM). For the LMM, either compositional or ordinary, we considered two alternative scenarios: (A) one with all variables, including land cover type distribution, and (B) another with all variables excluding land cover type distribution.

Regression trees (RT) were used to classify catchments into groups according to stressor-response relationships. This allowed to understand potential differences in relevant stressors of diatom response between different catchment types. The target variable (EQR TDI) was predicted from observations of catchment characteristics (water quality data, hydrology and land cover type). The predicted EQR TDI values (represented by the 'leaves') appear as the endpoints of the RT, while the 'tree branches' are split along breakpoints in the influential input variables. If a condition is satisfied, the classification path follows to the left and if a condition is not satisfied, the path follows to the right (Fig. 4).

Compositional Linear Mixed Models (CLMM) **were used to understand the influence of the relative proportions of water chemistry components and land cover types on the observed diatom response.** These relative amounts represent parts of a whole (as demonstrated by their units (mg/L and percentage land cover respectively) and imply that each part (i.e. a chemical) is not free to vary independently of the other measured parts (i.e. other chemicals). Thus, instead of taking the original absolute values of individual variables separately, the compositional data analysis method (CoDA, see e.g. Aitchison, 1986; Pawlowsky-Glahn, Egozcue, & Tolosana-Delgado, 2015) examined the relative influence of individual chemical and land cover variables in relation to the other chemical variables and land cover proportions. (See Fig. 5 for example output and Appendix 3 for further details).

Linear Mixed Models (LMM) (Zuur *et al.*, 2009) were used to statistically investigate the relationship between the absolute values of the predictor variables and the ecological response and to understand the relationships both at the level of individual catchments as well as deriving an overall response that could be generalised to unmeasured locations (Fig. 6).

3 Results

3.1 Principal Component Analysis

To address questions 1 and 2, **Principal Component Analysis (PCA) was used as a first step in the process of disentangling the association between nutrient concentrations, SRP loads, hydrological variables, land cover types, and the ecological response.** It allowed the identification of **environmental gradients across the study catchments and to simplify the multivariate data set.** In this analysis, a number of correlated variables are transformed into a smaller number of uncorrelated variables called principal components, which can then be interpreted as representing different environmental gradients (e.g. ecological quality, pollution stressor gradient). The first five principal components (eigenvalues > 1) accounted for 79.71% of the total variance in the data (Table 1). All chemical parameters were associated with PC1, which accounted for 33.89% of variation. Arable, improved grassland and urban land cover types as well as P loads from all sources were positively associated with PC1, while semi-natural land cover was negatively associated with PC1. Thus, **PC1 represented a land cover and hydro-chemical gradient, with sites in good ecological status related more closely (but not exclusively) to a higher proportion of semi-natural land cover, while sites in poor ecological status related more closely (but not exclusively) to higher chemical concentrations and P loads from all sources** (Fig 3). **Concentrations were more strongly positively associated with PC1 than nutrient loads,** especially TP which was more strongly associated with this environmental gradient than SRP or N. Conversely, semi-natural land cover type and Richards Baker Index (RBI, representing hydrological flashiness) were strongly negatively associated with PC1.

P loads from all sources other than arable were strongly positively associated with PC2. PC3 represented a gradient of differentiated hydrological response, with RBI and Q5:Q95 associated positively and baseflow index (BFI) associated negatively with this axis. (Table 1).

Thus, **PCA analysis indicated that pollutant concentrations, pollutant loads, and hydrological characteristics are likely to have different associations with the ecological response.** However, PCA does not allow to conclusively understand which of these predictors (e.g. which nutrient concentrations), or their ratios, are more influential. To this end, we conducted further formal statistical modelling as outlined below.

Table 1 Loadings (indicating the strength of relationship) and total variance explained by PCA axes 1 to 3. For clarity, only loadings >0.3 are shown. The most important variables with loadings >0.7 used in the interpretation of axes are marked in bold.

	PC1	PC2	PC3
z.logTP	**0.834**		
z.logSRP	**0.771**		
z.logAmmonia	**0.714**		
z.logCl	0.670	-0.369	
z.logNitrate	**0.743**	-0.414	
z.logNitrite	**0.763**		
z.logSS	0.581		
z.arable	0.561	-0.515	
z.improved.grassland	0.656	-0.326	
z.urban	0.536		
z.seminatural	**-0.775**	0.488	
z.woodland			
z.SWLOAD	0.550	**0.805**	
z.CSOLOAD	0.554	**0.807**	
z.LSLOAD	0.467	**0.850**	
z.ARLOAD	0.538	0.318	-0.338
z.URLOAD	0.557	**0.801**	
z.STLOAD	0.606	**0.737**	
z.RBI	-0.300	0.314	**0.776**
z.Parde_Range			0.452
z.BFI	0.320	-0.481	**-0.768**
z.q5.q95.ratio		0.539	**0.660**
Cumulative variance	33.89%	56.58%	67.70%

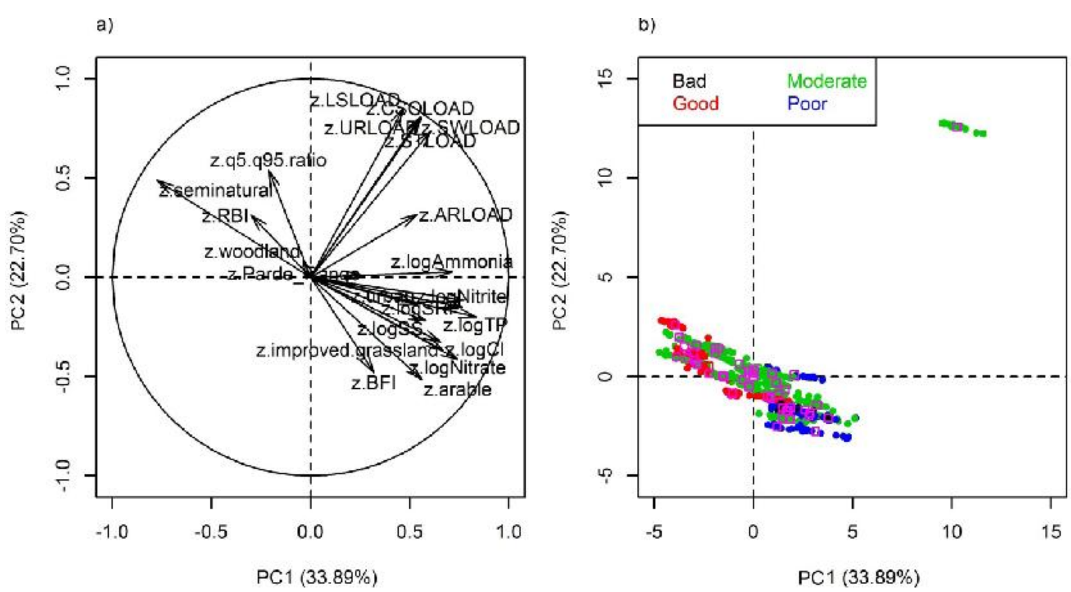

Fig. 3 PCA plots showing a) variable distribution according to PCA loadings and b) sampling-site PCA scores (the outliers representing extreme SRP loads from Clyde estuary). Site points were coloured according to ecological status.

3.2 Regression Tree Analysis

Regression tree analysis identified the presence and the hierarchy of importance of multiple stressors in the dataset and was relevant to all three research questions. This indicated a strong negative relationship between TP and diatom response (Fig. 4). Low EQR TDI ratios (average EQR TDI equal to 0.58) were associated with TP concentrations > 0.035 mg L^{-1} and urban land cover > 5% (called group A). EQR TDI values of about 0.67 on average can be expected in catchments where TP concentrations are > 0.035 mg L^{-1} and arable land cover exceeds 27% (group B) whereas EQR TDI values of around 0.76 can be expected where arable land cover is <27% (group C). In catchments with TP concentrations below 0.035 mg L^{-1} and more than 12% woodland cover, the average expected EQR TDI value was 0.74 (group

D). The highest EQR TDI (average value = 0.82) can be expected in catchments with TP concentrations < 0.035 mg L^{-1} and woodland cover < 12% (Group E).

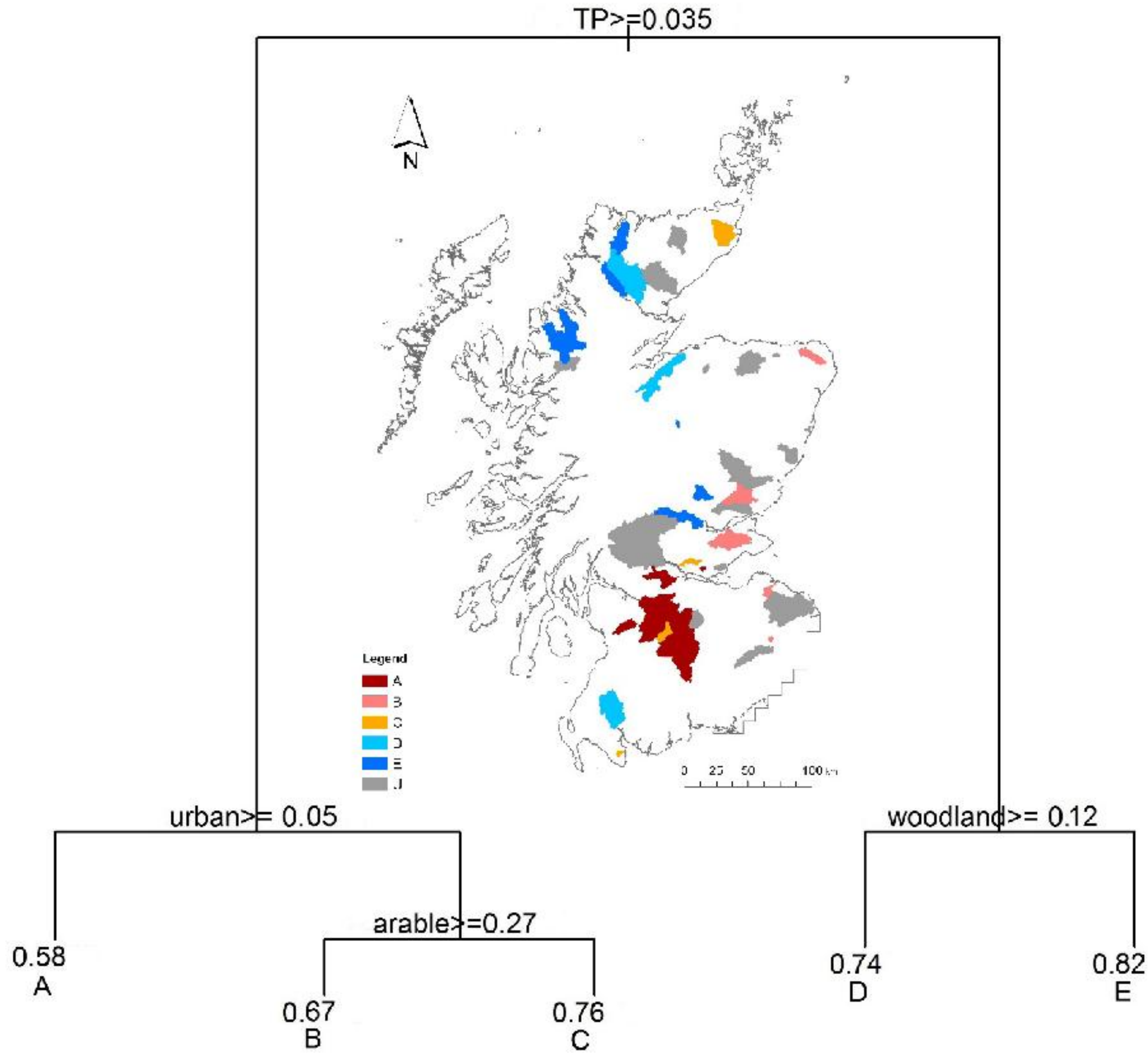

Fig. 4 Regression tree from observations of water quality, discharge, land cover and P apportionment loads from 45 catchments. The endpoints of the tree (leaves) show average EQR TDI according to splits (tree branches) of the variables at optimal threshold values. The map shows the spatial distribution of the predicted groups. Note that a subgroup of observations (U) had two possible endpoints.

This analysis indicated that TP concentration was most strongly associated with the diatom response, along with urban, arable and woodland land cover types and that these relationships varied between catchments.

3.3 Compositional Linear Mixed Model Analysis

Compositional analysis was undertaken to understand which pollutant and land cover ratios were most influential on the diatom response. These data were firstly explored using compositional PCA biplots (Aitchison & Greenacre, 2002) which represent their relative variation structure (Fig. 5). Whilst NO_3-N concentrations were the least associated to the other chemical parameters; NH_3-N and NO_2^--N concentrations, as well as TP and SRP concentrations, were strongly associated with each other (indicated by the proximity of the corresponding vectors) (see Fig 5. and Appendix 3 for related groupings). CLMM considering all predictors (scenario A) showed that the diatom response was negatively related to the balance TP+SRP *vs.* NH_3+NO_2^-+Cl^-+SS (represented by term $b_{2;}$ Table 2; Appendix 3) as well as to b_6 – the balance of NH_3-N to NO_2^--N (term b_6; Table 2; Appendix 3) and positively associated to the land cover balance l_1 related to the log-ratio of semi-natural and woodland land cover types to arable, grassland and urban land cover types (Table 2; Appendix 3). When land cover was excluded from the analysis (scenario B), balances b_2 and b_6 were negatively associated with the diatom response (Table 2; Appendix 3).

These results suggest that **the combined ratio of TP and SRP to other nutrients (balance b_2) and the trade-off between NH_3-N to NO_2–N (balance b_6) were the main chemical stressors negatively influencing EQR TDI.** While P species are significantly negatively linked to ecological status**, the anoxic conditions indicated by the NH_3-N to NO_2–N balance,** likely indicative of point-source pollution, including domestic discharges, **have an equally**

important negative relationship with EQR TDI that is ameliorated by the presence of seminatural habitats (balance l_1). Therefore, these combined relationships should be considered when setting mitigation targets.

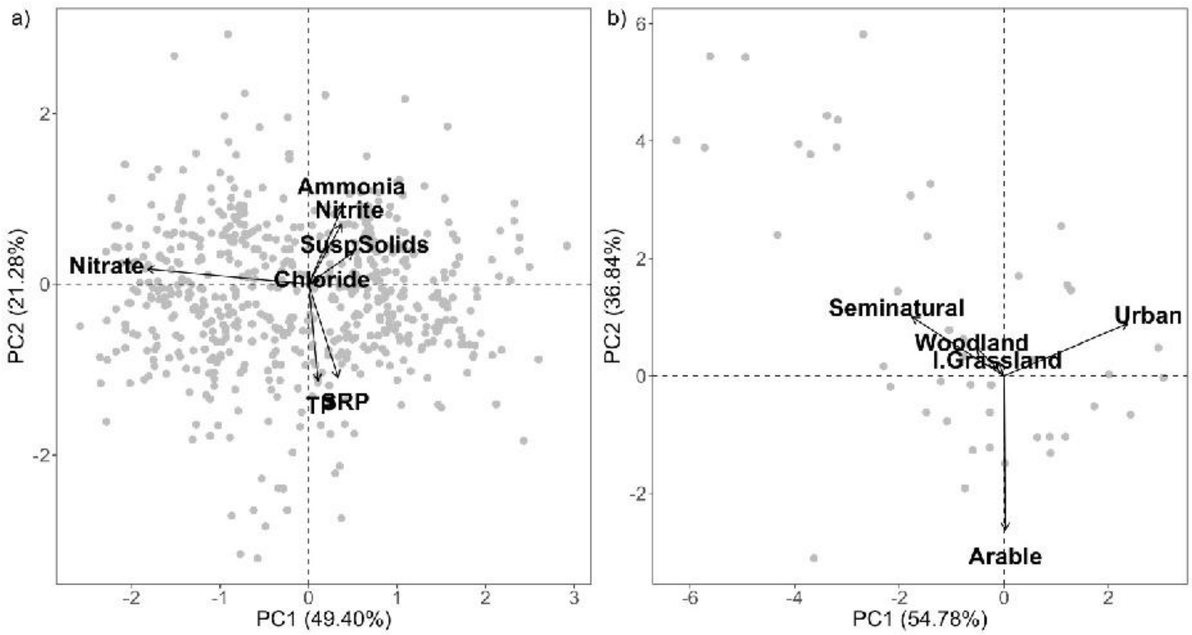

Fig. 5 Compositional biplots of chemistry concentrations (a) and land cover distributions at sampling-site level (b). Closeness of variable rays is directly related to proportionality relationships between variables.

Table 2 Estimates from averaged top compositional linear mixed models in two scenarios: (A) chemistry concentrations, SRP loads, land cover proportions, hydrological indices and season as predictors (R^2 = 0.61); (B) chemistry concentrations, SRP loads, hydrological indices and season as predictors (R^2 = 0.62).

(A)	Estimate	Std. Error	P
Intercept	0.65	0.04	< 0.001
balance b_2 (TP & SRP *vs*. Cl, SS, ammonia and nitrite)	-0.024	0.01	<0.05
balance b_6 (ammonia *vs*. nitrite)	-0.040	0.02	<0.05
balance l_1 (semi-natural & woodland *vs*. other)	0.042	0.01	<0.001
(B)			
Intercept	0.90	0.47	n.s.
balance b_2 (TP & SRP *vs*. Cl, SS, ammonia and nitrite)	-0.032	0.01	<0.01
b_6 (ammonia *vs*. nitrite)	-0.039	0.017	<0.05

3.4 Linear Mixed Model Analysis

Finally, **ordinary LMM were used to characterise the relationship between EQR TDI and absolute chemical concentrations, SRP loads, land cover and hydrological indices in individual catchments, as well as an overall relationship across all catchments.**

Averaged top five LMM in Scenario A showed that SRP, TP and urban land cover were negatively related to the diatom response (Table 3). However, as SRP and TP were highly correlated, the top five models were also examined separately. These included (nature of relationship between the predictor variable and EQR TDI in brackets):

a) SRP (-), semi-natural (+) and urban land cover (-) (R^2 = 0.62)
b) SRP (-), season (spring +) and semi-natural land cover (+) (R^2 = 0.61)
c) TP (-), semi-natural (+) and urban land cover (-) (R^2 = 0.65)
d) TP (-), season (spring +) and semi-natural land cover (+) (R^2 = 0.64)
e) Season (spring +), seminatural (+) and urban land cover (-) (R^2 = 0.64)

The models accounted for a similar amount of variability in EQR TDI (R^2 = 0.61-0.65) with model c) accounting for the highest amount of variability. **SRP, TP and urban land cover had negative association with EQR TDI while semi-natural land cover had a positive association. In all models, semi-natural land cover had the greatest effect-size (i.e. influence) on the diatom response,** as indicated by the standardised model coefficients. The relationship between TP and EQR TDI was more variable between catchments and thus influenced by site-specific factors than the relationship between SRP and EQR TDI (see graph of variable slopes in Fig. 6).

The combined evidence from all modelling approaches showed that TP was potentially more closely negatively associated with EQR TDI than SRP. However, the negative association of NO_3-N alongside P was also apparent, while semi-natural habitat had an overriding strong positive association.

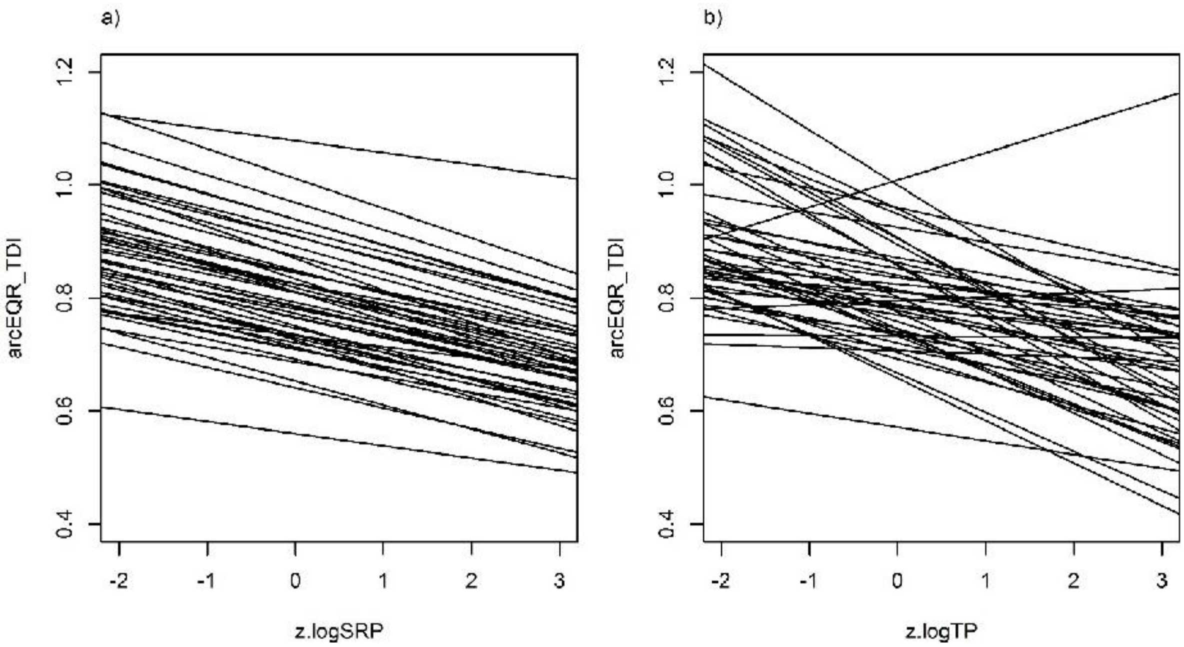

Fig. 6 LMM random slopes for a) SRP and b) TP showing greater variability in the stressor-response relationship between TP and EQR TDI than between SRP and EQR TDI in studied catchments. The slopes for TP indicate potentially stronger but variable control of TP on the ecological status, affected by site-specific catchment characteristics, whereas the response to SRP was more homogenous between locations and less affected by site-specific effects.

Table 3 Averaged top linear mixed models in two scenarios (A) chemistry concentrations, SRP loads, land cover proportions, hydrological indices and season as predictors (R^2 = 0.61); (B) chemistry concentrations, SRP loads, hydrological indices and season as predictors (R^2 = 0.60).

(A)	Estimate	Std. Error	P
Intercept	0.79	0.02	< 0.001
z.logSRP	-0.021	0.02	n.s.
z.logTP	-0.021	0.02	n.s.
z.seminatural	0.091	0.02	<0.001
z.urban	-0.049	0.02	<0.05
SeasonSpring	0.027	0.01	<0.05
(B)			
Intercept	0.79	0.02	<0.001
z.logTP	-0.05	0.02	<0.01
z.logNitrate	-0.055	0.02	<0.001
SeasonSpring	0.029	0.01	<0.05

4 Discussion

This study examined a) the relationship between diatoms as indicators of ecological status in Scottish rivers and routinely measured P fractions (TP and SRP) and b) evidence on whether P loads from different sources have a differentiated impact on the ecological response, due to potential differences in bio-availability of P fractions and timing of inputs. Specifically, we addressed the following research questions:

4.1 What is the relative importance of different P fractions and sources on the diatom response?

In this study, **the combined evidence from different modelling approaches has shown that TP is more strongly associated with EQR TDI than SRP.** This may be because TP accounts for additional P fractions such as particulate P attached to sediment particles as well as soluble P and unreactive P (see Glendell *et al.*, 2019). In addition, TP may be associated with other fine sediment-bound contaminants. However, the combined role of SRP and TP apparent from compositional analysis (Section 3.3) indicates that **neither fractions fully accounts for bioavailable P and is therefore likely to be associated with either an over- or under- estimation of the biological impacts** (Li and Brett, 2015). **To provide a more definitive conclusion with regards to BAP, we recommend that in future research, the BAP fraction should be measured and monitored alongside SRP and TP at a number of representative locations across Scotland to provide data**

for model development and validation.

Formal modelling in **the present study did not find a statistically significant link between modelled loads of SRP from different sources and the ecological response** in running waters. While this study supports the finding that in running waters, loads are indeed less relevant to ecological status than nutrient concentrations (Stamm *et al.*, 2014), the modelled loads used in this study are subject to poorly quantified uncertainties in both the model and the data. **Therefore, where possible, future work should focus on examining these relationships in catchments with high-resolution monitoring data where uncertainties associated with load estimation can be quantified** (Johnes, 2007; Defew, May, & Heal, 2013; Cassidy *et al.*, 2018)daily paired instantaneous P and flow data for 17 UK research catchments covering a total of 39 water years (WY.

4.2 How important is P in affecting diatom status in the context of multiple stressors in running waters?

Compositional analysis was undertaken to understand which trade-offs between pollutants and other stressors were most significantly associated with EQR TDI. We **concluded that the combined ratio of TP and SRP to other chemicals, rather than the two P species separately, was the main chemical predictor of EQR TDI in models that included land cover** (Section 3.3). In models that did not include land cover, the ratio of NH_3-N to NO_2^--N was found to be statistically more significant (Table 2). **NH_3-N has been shown to be indicative of domestic effluent from septic tanks (Richards *et al.*, 2016), suggesting that these P sources may be particularly important for ecological status. However, this study suggests that land cover has an overriding association with the stressor-response relationship and that the weaker signal from the NH_3 to NO_2 ratio can only be detected when this overriding association is removed from the model.**

Linear mixed models with chemistry, source apportionment and land cover type identified TP and SRP as the only statistically significant chemical variables associated with the diatom status, alongside semi-natural and urban land cover. These models accounted for up to 65% of the variability in EQR TDI. However, semi-natural land cover type had a stronger (positive) association with EQR TDI than either TP or SRP, based on the comparison of standardized regression coefficients (Table 3). **Thus, combined evidence from different modelling approaches shows that land cover type had an overriding association with EQR TDI, probably acting as a proxy for a number of possible mechanisms and processes, such as different bioavailability of P fractions, river morphology, riparian and/or aquatic habitat structure and toxic contaminants** (see also Glendell *et al.*, 2019). Significantly for the objectives of this study, land cover can act as a proxy for varying bioavailability of P fractions, which have been shown to differ between catchments dominated by different land cover types (Ellison and Brett, 2006; Stutter & Lumsdon, 2008; Egemose & Jensen, 2009).

In models without land cover, nitrate was the most strongly associated with EQR TDI, alongside TP (Table 3). **The importance of nitrate in this model indicates an additive stressor response relationship between TP and nitrate.** Both N and P can enhance the rates of primary production and lead to impairment of water quality (Stevenson, 2014; Wagenhoff, *et al.*, 2017; Wagenhoff *et al.* 2017; Paerl *et al.*, 2016; Crnkovic, *et al.*, 2018; Jarvie *et al.*, 2018). EQR TDI was also found to be related to both SRP and NO_3-N when the index was designed (Kelly *et al.*, 2008). The twin stressor from both N and P may be particularly important in upland low alkalinity rivers that are naturally both N and P limited and where targeting of both nutrients may be necessary to achieve good ecological status (Jarvie *et al.*, 2018). In contrast, in lowland high alkalinity headwater streams, which are also likely to be more densely populated and receive greater P loads from more bioavailable point sources such as sewage treatment works and septic tanks (Ekholm and Krogerus, 2003; Ekholm *et al.*, 2009; Richards *et al.*, 2016; Stutter, Graeber, Evans, Wade & Withers, 2018), reducing P concentrations may be the most effective mitigation strategy (Jarvie *et al.*, 2018). **Thus, we suggest, that targeting of all stressors in a concerted way would be the most meaningful mitigation strategy.**

4.3 How do factors influencing the phosphorus-diatom relationship vary between different catchments?

Catchment-specific regression coefficients derived from the mixed models show a greater variability in the relationship between EQR TDI and TP than between EQR TDI and SRP (Fig. 6). **These findings point towards a differentiated relationship between TP, SRP and diatom status in different catchment types** and may be related to complex interactions and factors such as river morphology and habitat condition as well as different bioavailability of P fractions in catchments with different characteristics.

Regression tree analysis also pointed towards the importance of both chemistry and catchment characteristics in predicting ecological status, with relevant breakpoints for both P concentrations and urban/arable land cover. This approach identified the total P concentration of 0.035 mg L^{-1} as the primary breakpoint in the data set, distinguishing between catchments in good and mixed ecological status (Fig. 3). This breakpoint appears plausible, as it lies within the range of previously reported limiting P concentrations in British streams between 0.01-0.05 mg L^{-1} (Jarvie *et al.*, 2018) and coincides with a threshold of 0.03 mg L^{-1} at which significant change in diatom assemblage was observed (Bowes *et al.*, 2012). This analysis also indicated that urban land cover (>5%) and arable land cover (> 27%) had a detrimental effect on the ecological response, with the lowest EQR TDI scores likely to be expected in catchments with a higher proportion of urban land cover. This may be linked to higher P loads from point sources such as STWs and septic tanks, and their likely higher bio-availability (Jarvie *et al.*, 2010). Particulate P from urban catchments during base-flow conditions has been found to be more bio-available than in catchments dominated by other land cover types (Ellison and Brett, 2006) and is therefore likely to have significant impact on river ecology (Shore *et al.*, 2017). Conversely, PCA, compositional and ordinary mixed models all showed the positive associations of semi-natural land cover with river ecology.

Interestingly, hydrological catchment characteristics likely to be linked to the timing of nutrient inputs such

as seasonality and flashiness were only marginally associated with EQR TDI. Only the compositional linear mixed models (Section 3.3) that did not include land cover as suggested a weak association between the hydrological variability indices BFI, RBI and EQR TDI. **However, season was a significantly associated with EQR TDI in both compositional and ordinary linear mixed models with a higher EQR TDI score expected in spring**, which is consistent with the findings of the index authors (Kelly *et al.*, 2008).

These findings suggest that mitigation strategies should target P impacts alongside multiple chemical and land use stressors, tailored to catchment-specific responses (Glendell *et al.*, 2019).

5 Conclusions and recommendations

This study applied a suite of statistical modelling approaches to regulatory water quality monitoring data to examine the association between routinely measured P fractions (TP and SRP) as proxies for bioavailable P and the ecological response. The stepwise 'weight of evidence' approach has shown that TP may potentially be more strongly associated with EQR TDI, although the differences with SRP were small and both fractions were negatively associated with the ecological response. This study did not find evidence of a relationship between P loads and diatoms in running waters. Semi-natural land cover type had the strongest positive association with EQR TDI, while urban land cover type had a strong negative association with the ecological response. The ratio of NH_3-N to NO_3-N as well as NO_3-N concentrations also had a negative association with the EQR TDI, when the overarching impact of land cover was not included among modelled variables.

The relationship between TP, SRP and EQR TDI, varied spatially, pointing towards different bioavailability of these two BAP proxies in catchments with contrasting characteristics. **Therefore, future research should test this hypothesis against empirical measurements of bioavailable P in different catchment types within the region of interest, as it can be expected that a relationship between bioavailable P and the ecological response would result in a statistically closer relationship than between either of the routinely measured P fractions. Furthermore, the results of this study support the need for further research into factors that affect the spatial variability in this stressor-response relationship in different catchment types. Furthermore, collecting data at a high temporal resolution in representative research catchments would also help to inform future modelling efforts.**

Reflecting the overriding importance of land cover highlighted in this study, "it has been suggested that current mitigation policies may not be sufficient to achieve good ecological status and that targeted land cover change may need to be considered. Such landscape redesign could include a shift to heterogenous landscape mosaics and mixed farming, perhaps as part of climate change adaptation. Thus, future regulatory efforts should target P impacts alongside multiple chemical and land use stressors, tailored to catchment-specific responses (Glendell *et al.*, 2019)**."** Advanced statistical modelling approaches, such as those used in this study, based on reanalysis of the growing body of regulatory data, will help to inform catchment-specific targeting of mitigation measures.

6 References

Aitchison, J., Greenacre, M., 2002. Biplots of compositional data. R. Stat. Soc. Ser. C 51 375-392.

Aitchison, J., 1986. The Statistical Analysis of Compositional Data., Journal of the Royal Statistical Society. Series B (Methodological). Chapman and Hall, London. https://doi.org/10.2307/2345821

Bond, N.R., 2015. hydrostats: Hydrologic indices for daily time series data. R package version 0.2.4.

Bowes, M.J., Ings, N.L., McCall, S.J., Warwick, A., Barrett, C., Wickham, H.D., Harman, S.A., Armstrong, L.K., Scarlett, P.M., Roberts, C., Lehmann, K., Singer, A.C., 2012. Nutrient and light limitation of periphyton in the River Thames: Implications for catchment management. Sci. Total Environ. 434, 201–212. https://doi.org/10.1016/j.scitotenv.2011.09.082

Buccianti, A., Pawlowsky-Glahn, V., 2005. New Perspectives on Water Chemistry and Compositional Data Analysis. Math. Geol. 37, 703–727. https://doi.org/10.1007/s11004-005-7376-6

Cassidy, R., Jordan, P., Bechmann, M., Kronvang, B., Kyllmar, K., Shore, M., 2018. Assessments of Composite and Discrete Sampling Approaches for Water Quality Monitoring. Water Resour. Manag. 1–16. https://doi.org/10.1007/s11269-018-1978-5

Crnkovic, C.M., May, D.S., Orjala, J., 2018. The impact of culture conditions on growth and metabolomic profiles of freshwater cyanobacteria. J. Appl. Phycol. 30, 375–384. https://doi.org/10.1007/s10811-017-1275-3

Daldorph, P., 2017. Source apportionment of nutrient contributions to rivers in England and Wales modelled with SAGIS. [WWW Document]. NERC Environ. Inf. Data Cent. URL https://catalogue.ceh.ac.uk/documents/8c5d9e38-0244-4a39-8600-a85513a6fecf

Defew, L.H., May, L., Heal, K. V., 2013. Uncertainties in estimated phosphorus loads as a function of different sampling frequencies and common calculation methods. Mar. Freshw. Res. 64, 373–386. https://doi.org/10.1071/MF12097

Egemose, S., Jensen, H.S., 2009. Phosphorus forms in urban and agricultural runoff: Implications for management of Danish Lake Nordborg. Lake Reserv. Manag. 25, 410–418. https://doi.org/10.1080/07438140903413228

Ekholm, P., Krogerus, K., 2003. Determining algal-available phosphorus of differing origin: Routine phosphorus analyses versus algal assays. Hydrobiologia 492, 29–42. https://doi.org/10.1023/A:1024857626784

Ekholm, P., Rita, H., Pitkänen, H., Rantanen, P., Pekkarinen, J., Münster, U., 2009. Algal-available phosphorus entering the Gulf of Finland as estimated by algal assays and chemical analyses. J. Environ. Qual. 38, 2322–33. https://doi.org/10.2134/jeq2008.0356

Ellison, M.E., Brett, M.T., 2006. Particulate phosphorus bioavailability as a function of stream flow and land cover. Water Res. 40, 1258–1268. https://doi.org/10.1016/j.watres.2006.01.016

Filzmoser, P., Hron, K., Reimann, C., 2009. Univariate statistical analysis of environmental (compositional) data: Problems and possibilities. Sci. Total Environ. 407, 6100–6108. https://doi.org/10.1016/j.scitotenv.2009.08.008

Glendell, M., Palarea-Albaladejo, J., Pohle, I., Marrero, S., McCreadie, B., Cameron, G., Stutter, M., 2019. Modeling the ecological impact of phosphorus in catchments with multiple environmental stressors. J. Environ. Qual. 48, 1336–1346. https://doi.org/10.2134/jeq2019.05.0195

Jarvie, H.P., Smith, D.R., Norton, L.R., Edwards, F.K., Bowes, M.J., King, S.M., Scarlett, P., Davies, S., Dils, R.M., Bachiller-Jareno, N., 2018. Phosphorus and nitrogen limitation and impairment of headwater streams relative to rivers in Great Britain: A national perspective on eutrophication. Sci. Total Environ. 621, 849–862. https://doi.org/10.1016/j.scitotenv.2017.11.128

Jarvie, H.P., Withers, P.J.A., Bowes, M.J., Palmer-Felgate, E.J., Harper, D.M., Wasiak, K., Wasiak, P., Hodgkinson, R.A., Bates, A., Stoate, C., Neal, M., Wickham, H.D., Harman, S.A., Armstrong, L.K., 2010. Streamwater phosphorus and nitrogen across a gradient in rural-agricultural land use intensity. Agric. Ecosyst. Environ. 135, 238–252. https://doi.org/10.1016/j.agee.2009.10.002

Johnes, P.J., 2007. Uncertainties in annual riverine phosphorus load estimation: Impact of load estimation methodology, sampling frequency, baseflow index and catchment population density. J. Hydrol. 332, 241–258. https://doi.org/10.1016/j.jhydrol.2006.07.006

Jordan, P., Menary, W., Daly, K., Kiely, G., Morgan, G., Byrne, P., Moles, R., 2005. Patterns and processes of phosphorus transfer from Irish grassland soils to rivers - Integration of laboratory and catchment studies. J. Hydrol. 304, 20–34. https://doi.org/10.1016/j.jhydrol.2004.07.021

Kelly, M., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, J., Rippey, B., Hirst, H., Yallop, M., 2008. Assessment of ecological status in U.K. rivers using diatoms. Freshw. Biol. 53, 403–422. https://doi.org/10.1111/j.1365-2427.2007.01903.x

Li, B., Brett, M.T., 2015. The relationship between operational and bioavailable phosphorus fractions in effluents from advanced nutrient removal systems. Int. J. Environ. Sci. Technol. 12, 3317–3328. https://doi.org/10.1007/s13762-015-0760-y

Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Simpson, I.C., 2011. Final report for LCM2007 – the new UK land cover map, CS Technical Report No 11/07 NERC/Centre for Ecology & Hydrology 108pp. (CEH project number: C03259).

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Nisi, B., Buccianti, A., Vaselli, O., Perini, G., Tassi, F., Minissale, A., Montegrossi, G., 2008. Hydrogeochemistry and strontium isotopes in the Arno River Basin (Tuscany, Italy): Constraints on natural controls by statistical modeling. J. Hydrol. 360, 166–183. https://doi.org/10.1016/j.jhydrol.2008.07.030

Otero, N., Tolosana-Delgado, R., Soler, A., Pawlowsky-Glahn, V., Canals, A., 2005. Relative vs. absolute statistical analysis of compositions: A comparative study of surface waters of a Mediterranean river. Water Res. 39, 1404–1414. https://doi.org/10.1016/j.watres.2005.01.012

Paerl, H.W., Scott, J.T., McCarthy, M.J., Newell, S.E., Gardner, W.S., Havens, K.E., Hoffman, D.K., Wilhelm, S.W., Wurtsbaugh, W.A., 2016. It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. Environ. Sci. Technol. 50, 10805–10813. https://doi.org/10.1021/acs.est.6b02575

Palarea-Albaladejo J., Rooke J. A., Nevison I. M., Dewhurst R. J., 2017. Compositional mixed modeling of methane emissions and ruminal volatile fatty acids from individual cattle and multiple experiments. J. Anim. Sci. 95, 2467–2480.

Parde, M., 1947. Fleuves et Rivieres. Colin, Paris.

Pawlowsky-Glahn, V., Egozcue, J.J., Tolosana-Delgado, R., 2015. Modeling and analysis of compositional data. John Wiley & Sons, Chichester, United Kingdom. https://doi.org/10.1017/CBO9781107415324.004

Reimann, C., Filzmoser, P., Hron, K., Kynčlová, P., Garrett, R.G., 2017. A new method for correlation analysis of compositional (environmental) data – a worked example. Sci. Total Environ. 607–608, 965–971. https://doi.org/10.1016/j.scitotenv.2017.06.063

Richards, S., Paterson, E., Withers, P.J.A., Stutter, M., 2016. Septic tank discharges as multi-pollutant hotspots in catchments. Sci. Total Environ. 542, 854–863. https://doi.org/10.1016/j.scitotenv.2015.10.160

Sela, R.J., Simonoff, J.S., 2012. RE-EM trees: A data mining approach for longitudinal and clustered data. Mach. Learn. 86, 169–207. https://doi.org/10.1007/s10994-011-5258-3

Shore, M., Murphy, S., Mellander, P.E., Shortle, G., Melland, A.R., Crockford, L., O'Flaherty, V., Williams, L., Morgan, G., Jordan, P., 2017. Influence of stormflow and baseflow phosphorus pressures on stream ecology in agricultural catchments. Sci. Total Environ. 590–591, 469–483. https://doi.org/10.1016/j.scitotenv.2017.02.100

Stamm, C., Jarvie, H.P., Scott, T., 2014. What's more important for managing phosphorus: Loads, concentrations or both? Environ. Sci. Technol. 48, 23–24. https://doi.org/10.1021/es405148c

Stevenson, J., 2014. Ecological assessments with algae: a review and synthesis. J. Phycol. 50, 437–461. https://doi.org/10.1111/jpy.12189

Stutter M.I., Jackson-Blake L., May L., Richards S., V.A., 2014. Factoring Ecological Significance of Sources into Phosphorus Source Apportionment [WWW Document]. URL https://www.crew.ac.uk/sites/www.crew.ac.uk/files/sites/default/files/publication/Factoring Ecological Significance of Sources into Phosphorus Source Apportionment.pdf

Stutter, M.I., Graeber, D., Evans, C.D., Wade, A.J., Withers, P.J.A., 2018. Balancing macronutrient stoichiometry to alleviate eutrophication. Sci. Total Environ. 634, 439–447. https://doi.org/10.1016/j.scitotenv.2018.03.298

Stutter, M.I., Lumsdon, D.G., 2008. Interactions of land use and dynamic river conditions on sorption equilibria between benthic sediments and river soluble reactive phosphorus concentrations. Water Res. 42, 4249–4260. https://doi.org/10.1016/j.watres.2008.06.017

UKTAG, 2014. UKTAG River Assessment Method Macrophytes and Phytobenthos Phytobenthos – Diatoms for Assessing River and Lake Ecological Quality (River DARLEQ2), UKTAG Guide to River.

Viglione, A., Parajka, J., Rogger, M., Salinas, J. L., Sivapalan, M., Blöschl, G., 2013. Comparative assessment of predictions in ungauged basins; Part 1: Runoff hydrograph studies. Hydrol. Earth Syst. Sci. Discuss. 10, 375–409. https://doi.org/10.5194/hessd-10-375-2013

Wagenhoff, A., Clapcott, J.E., Lau, K.E.M., Lewis, G.D., Young, R.G., 2017a. Identifying congruence in stream assemblage thresholds in response to nutrient and sediment gradients for limit setting: Ecol. Appl. 27, 469–484. https://doi.org/10.1002/eap.1457

Wagenhoff, A., Liess, A., Pastor, A., Clapcott, J.E., Goodwin, E.O., Young, R.G., 2017b. Thresholds in ecosystem structural and functional responses to agricultural stressors can inform limit setting in streams. Freshw. Sci. 36, 178–194. https://doi.org/10.1086/690233

Zambrano-Bigiarini, M., 2015. hydroTSM: Time series management, analysis and interpolation for hydrological modelling. R Packag. version 0.4-2-1. https://doi.org/doi: https://doi.org/10.5281/zenodo.83964

Zuur A. F., Ieno E. N., Walker N. J., Saveliev A. A., Smith G. M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer-Verlag, New York.

7 Appendices

Appendix 1 Statistical Modelling

Data pre-processing and exploration

Data was visually screened for outliers and checked for normality. Thereafter, all chemistry data was log transformed and the EQR TDI data (originally defined in the [0, 1] interval) was arcsin transformed to better accommodate linear mixed model (LMM) assumptions. Note that cases with EQR TDI = 1 (equating to 29 observations) were excluded to achieve satisfactory distribution of model residuals. For consistency and comparability of results between different statistical analyses, the same number of complete observations (596 cases) was used in all analyses. Principal Component Analysis (PCA) was used to investigate the presence of environmental gradients in the multivariate data set. Principal components with eigenvalues exceeding 1 were retained. All continuous variables were z-transformed to homogenise the scales and to facilitate comparison of effect sizes between different variables.

The following hydrological indices were calculated to characterise the catchment hydrological response:

- **Distribution**: The ratio of high to low flows (Q5:Q95 ratio) (Jordan *et al.* 2005) relates the streamflow which is exceeded 5 % of the days to streamflow with an exceedance frequency of 95 %. Streamflow quantiles were calculated using the function fdc in the R-package hydroTSM (Zambrano-Bigiarini, 2015).

- **Seasonality**: The Pardé coefficient (Parde, 1947) relates long term mean monthly streamflow to long term mean annual streamflow. To convert the Pardé coefficient into one single value expressing seasonality (rather than twelve values, one for each month), Viglione *et al.* (2013) introduced the range of the Pardé coefficients which is the difference between the maximum Pardé coefficient and the minimum Pardé coefficient.

- **Oscillation**: The Richards Baker Flashiness Index (RBI) (Baker, Richards, Loftus, & Kramer, 2004) relates the difference between the streamflow of the current to the previous day as:

$$RBI = \frac{\sum_{i=1}^{n}|Q_i - Q_{i-1}|}{\sum_{i=1}^{n} Q_i}$$

with Q_i being the streamflow of the current day, Q_{i-1} being the streamflow of the previous day, and n representing the number of observations.

The base flow index (BFI) was derived from streamflow records using the function baseflows implemented in the R-package hydrostats (Bond, 2015).

Statistical modelling

Formal statistical modelling was undertaken using three different modelling approaches – regression trees for clustered data (RE-EM algorithm), compositional linear mixed models (CLMM) and ordinary linear mixed models (LMM). For the LMM, either compositional or ordinary, we considered two scenarios of alternative model structures to account for model structural uncertainty: (A) one with all water chemistry variables, phosphorus source apportionment, land cover type distribution and hydrological variables, and (B) another with just chemistry, phosphorus source apportionment and hydrological variables.

A RT was fitted using the RE-EM algorithm for clustered data (Sela and Simonoff, 2012) as a non-parametric machine learning approach to understand potential differences in significant predictors of diatom response between different catchment types, with the data clusters defined by the different sites as a random effect (in our case repeated sampling occasions nested within 475 sampling sites).

CLMM were used to understand the influence of the relative distribution of water chemistry components and land cover types on the observed diatom response. These variables consist of positive and relative amounts representing parts of a whole, so-called composition, as demonstrated by their units (mg/L and percentage land cover respectively). This implies, amongst others, that the analytical results for each part are not fully free to vary independently of the other measured parts. Thus, instead of treating the original individual variables separately, compositional data analysis methods (CoDA, see e.g. Aitchison, 1986; Pawlowsky-Glahn *et al.*, 2015) focus on the relative information by working with log-ratios between them. In this manner, the compositions are mapped onto the real space and the modelling can then be conducted using ordinary methods on real log-ratio coordinates (for illustration in the environmental and water science context see e.g. Buccianti & Pawlowsky-Glahn, 2005; Otero *et al.*, 2005; Nisi *et al.*, 2008; Reimann *et al.*, 2017). This type of data transformation additionally guarantees that results do not change with changes in the relative units of measurement used (e.g. if data were rescaled from mg/L to proportions) or depending on whether we are working with either the full composition or only a subset of its parts of interest (a sub-composition).

A particular type of log-ratio coordinates, also called balances (Pawlowsky-Glahn *et al.*, 2015), which account for the relative importance of one subset of parts (in the numerator of the log-ratio) against another (in the denominator of the log-ratio) was built. The elements in each subset were chosen according to the strength of pairwise proportionality relationships. This was used as input to perform R-mode Ward's clustering to produce the subsets of most closely linked components going into numerator and denominator for each balance (see Appendix 5 for further details). The CLMMs resulted from using these log-ratio balances as predictors in ordinary LMMs along with the other covariates (Palarea-Albaladejo *et al.*, 2017), instead of using the original chemical and land cover variables.

LMM (see Zuur *et al.*, 2009) were used to statistically investigate the relationship between predictor variables as fixed effect and the ecological response as outcome variable, with site specified as a random effect. LMMs allowed to investigate the functional relationship between predictor variables and the ecological response for clustered data, enabling predictions both at the level of individual catchments as well as an overall response.

Selection between alternative mixed models (CLMMs and LMMs) to explain EQR TDI with nested fixed effect structures and the same random effect structure was based on the Akaike information criterion (AIC) (maximum likelihood estimation was used for this). The AIC measure ranked predictors according to their relative importance by the sum of Akaike weights over all possible models derived from the full model in which a predictor was included (Burnham and Anderson, 2002). Moreover, these models were screened for collinearity and only models with correlations between predictors lower than 0.6 were retained. Top models with delta AIC (AIC difference) <2 and r<0.6 were selected and then re-fitted using restricted maximum likelihood (REML). Model goodness-of-fit was evaluated using the pseudo-R^2 coefficient proposed in Nakagawa and Schielzeth (2013).

Appendix 2 summarises the data included in the analyses. Statistical test significance was concluded for p-values below the usual 5% significance level in all cases. Statistical analyses and modelling were undertaken in the R system for statistical computing v3.4 (R Core Team, 2018) using the packages FactorMineR, RE-EMtree, lme4, MuMln and compositions.

Appendix 2 Overview of data included in the analysis

Table A2.1a - Summary statistics for EQR TDI and chemical parameters for the 45 study locations

	SRP			TP			Ammonia				Cl		Nitrate			Nitrite			SS			EQR TDI		
Location	N	mean	median	N	mean	Median	N	mean	Median	N	mean	median	N	mean	median	N	mean	median	N	mean	median	N	mean	median
529	18	0.136	0.104	19	0.198	0.175	18	0.141	0.024	18	75.99	68.65	18	1.72	1.53	18	0.023	0.010	17	9.445	6.200	19	0.452	0.464
1466	14	0.015	0.013	14	0.050	0.050	14	0.094	0.034	14	46.54	47.25	14	3.43	3.28	14	0.033	0.022	13	5.548	4.440	14	0.637	0.643
1515	16	0.065	0.054	15	0.111	0.083	16	0.072	0.059	16	15.88	17.25	16	1.18	1.21	16	0.008	0.007	12	8.110	4.960	20	0.553	0.524
1518	6	0.050	0.050	6	0.091	0.096	5	0.041	0.038	6	11.48	11.70	6	0.80	0.82	6	0.007	0.007	6	3.937	3.900	21	0.598	0.598
1574	19	0.008	0.008	18	0.025	0.017	19	0.023	0.024	19	6.05	6.09	19	0.21	0.18	19	0.006	0.007	16	2.661	2.300	20	0.721	0.727
1615	16	0.086	0.057	16	0.124	0.099	16	0.169	0.073	16	27.90	27.25	16	1.34	1.43	16	0.022	0.014	14	5.199	4.515	16	0.602	0.600
3453	3	0.003	0.003	3	0.009	0.010	3	0.030	0.040	3	3.22	3.17	3	0.12	0.10	3	0.008	0.010	2	3.775	3.775	7	0.854	0.843
4638	18	0.015	0.014	18	0.045	0.046	18	0.033	0.031	18	8.97	8.44	18	0.38	0.35	18	0.006	0.007	15	6.806	5.750	18	0.550	0.528
5982	14	0.025	0.018	14	0.056	0.049	14	0.047	0.046	14	80.85	65.63	14	5.42	5.70	14	0.014	0.013	13	3.827	3.600	14	0.679	0.644
7762	16	0.014	0.010	16	0.031	0.032	17	0.038	0.036	17	9.38	9.36	17	1.46	1.41	17	0.006	0.005	16	2.537	2.138	20	0.822	0.844
7989	19	0.037	0.031	19	0.080	0.066	19	0.027	0.021	19	34.68	34.20	19	6.32	6.11	19	0.018	0.016	18	10.305	2.625	20	0.715	0.679
8175	19	0.124	0.118	18	0.174	0.165	19	0.050	0.045	19	30.09	30.00	19	6.03	5.95	19	0.022	0.021	18	11.789	6.025	19	0.553	0.522
8455	18	0.008	0.009	19	0.020	0.020	18	0.019	0.017	18	7.94	7.58	18	0.86	0.90	18	0.005	0.005	18	2.692	2.000	20	0.837	0.854
8517	18	0.005	0.005	18	0.018	0.017	18	0.023	0.017	18	10.72	10.60	18	1.03	1.05	18	0.007	0.005	16	2.928	2.550	18	0.861	0.903
8549	16	0.067	0.054	16	0.097	0.081	17	0.279	0.116	17	27.95	27.35	17	7.46	7.65	17	0.030	0.022	16	6.201	3.700	17	0.656	0.657
8801	16	0.007	0.009	16	0.018	0.012	16	0.014	0.017	16	5.63	5.19	16	0.33	0.33	16	0.005	0.005	15	2.598	2.000	18	0.824	0.837
9422	20	0.037	0.017	20	0.063	0.040	20	0.037	0.024	20	39.67	39.60	20	6.25	6.48	20	0.010	0.007	17	4.513	2.700	20	0.616	0.590
9457	20	0.026	0.020	19	0.051	0.044	20	0.023	0.024	20	26.19	25.00	20	2.83	2.87	20	0.008	0.007	16	4.375	2.825	20	0.602	0.599
9750	19	0.013	0.010	19	0.032	0.031	19	0.029	0.024	19	15.20	14.60	19	1.12	1.13	19	0.007	0.007	16	3.321	2.450	20	0.852	0.885
10476	13	0.035	0.033	14	0.050	0.050	13	0.029	0.021	13	25.78	25.20	13	6.50	6.42	13	0.016	0.016	14	3.439	2.625	16	0.546	0.545
17012	16	0.155	0.123	16	0.205	0.145	16	0.044	0.024	16	29.96	30.15	16	3.05	3.33	16	0.018	0.016	13	24.004	4.400	20	0.652	0.678
122480	15	0.214	0.220	14	0.240	0.254	15	0.359	0.286	15	35.60	32.60	15	2.14	2.25	15	0.049	0.039	12	6.762	5.500	16	0.547	0.524
122482	18	0.017	0.008	16	0.028	0.019	18	0.031	0.033	18	19.30	18.90	18	1.70	1.61	18	0.009	0.007	16	4.135	3.300	19	0.881	0.956

Appendix 2 Overview of data included in the analysis

Table A2.1b - Summary statistics for EQR TDI and chemical parameters for the 45 study locations

	SRP			TP			Ammonia				Cl		Nitrate			Nitrite			SS			EQR TDI		
122496	16	0.077	0.067	17	0.110	0.102	16	0.040	0.040	16	25.16	23.30	16	1.27	1.24	16	0.014	0.010	15	3.781	3.700	19	0.581	0.577
122706	16	0.035	0.038	15	0.072	0.068	16	0.033	0.038	16	21.45	15.15	16	0.26	0.25	16	0.009	0.007	13	6.011	3.600	19	0.573	0.587
122709	18	0.062	0.047	18	0.111	0.081	18	0.076	0.040	18	34.90	31.85	18	1.04	0.84	18	0.020	0.012	16	10.756	4.540	20	0.527	0.530
123153	13	0.008	0.008	12	0.014	0.014	13	0.033	0.040	13	12.11	11.70	13	0.28	0.20	13	0.009	0.010	13	2.423	2.000	23	0.781	0.785
200211	12	0.010	0.009	14	0.024	0.022	12	0.017	0.017	13	19.92	20.80	12	0.16	0.20	12	0.005	0.005	14	2.091	2.000	18	0.782	0.804
204343	14	0.033	0.033	15	0.044	0.045	14	0.015	0.017	14	25.59	25.38	14	5.05	4.99	14	0.013	0.011	14	3.848	3.750	20	0.606	0.616
206718	19	0.009	0.009	19	0.010	0.007	19	0.016	0.017	19	15.15	13.60	19	0.14	0.20	19	0.004	0.005	19	2.003	2.000	20	0.885	0.885
206732	17	0.008	0.008	18	0.012	0.012	17	0.011	0.005	17	13.78	14.10	17	0.13	0.11	17	0.004	0.004	17	1.615	2.000	18	0.731	0.749
206807	19	0.009	0.009	19	0.015	0.014	19	0.013	0.017	19	12.57	12.10	19	0.13	0.12	19	0.005	0.005	19	1.918	2.000	20	0.806	0.785
206811	16	0.009	0.009	16	0.010	0.010	16	0.012	0.012	16	9.77	10.09	16	0.13	0.13	16	0.004	0.004	16	1.869	1.600	18	0.805	0.817
206837	17	0.010	0.009	17	0.009	0.007	17	0.012	0.017	17	10.15	9.75	17	0.14	0.20	17	0.004	0.005	16	1.712	2.000	19	0.829	0.838
206845	17	0.009	0.009	18	0.012	0.009	17	0.013	0.017	17	8.37	7.98	17	0.14	0.18	17	0.004	0.005	17	2.959	2.000	19	0.844	0.827
207120	15	0.032	0.033	16	0.057	0.056	15	0.031	0.022	15	27.82	28.10	15	1.40	1.36	15	0.012	0.010	16	5.934	3.625	20	0.700	0.681
207127	14	0.020	0.016	14	0.032	0.035	14	0.014	0.017	14	10.85	10.85	14	0.13	0.14	14	0.005	0.005	13	2.396	2.000	20	0.740	0.759
207269	15	0.046	0.047	14	0.073	0.080	15	0.029	0.026	15	39.09	39.00	15	3.70	3.64	15	0.009	0.006	14	5.486	4.500	20	0.555	0.544
207277	14	0.050	0.035	15	0.080	0.060	14	0.202	0.069	14	137.49	131.50	14	1.03	0.97	14	0.019	0.009	15	8.228	5.000	19	0.508	0.493
207298	16	0.009	0.009	16	0.009	0.010	16	0.011	0.013	16	9.37	9.36	16	0.12	0.09	16	0.004	0.004	16	1.753	2.000	19	0.878	0.881
207309	13	0.009	0.009	12	0.005	0.004	13	0.014	0.017	13	3.55	3.32	13	0.10	0.08	13	0.004	0.005	11	3.044	1.897	19	0.812	0.825
207636	19	0.010	0.009	19	0.014	0.013	19	0.014	0.017	19	19.94	19.95	18	1.78	1.97	19	0.004	0.005	18	1.847	2.000	20	0.862	0.863
232930	10	0.019	0.019	9	0.042	0.044	10	0.024	0.018	10	27.85	27.70	10	4.90	5.03	10	0.007	0.007	9	8.237	6.750	11	0.556	0.543
300008	14	0.018	0.011	15	0.051	0.044	14	0.033	0.028	14	33.71	34.78	14	0.39	0.25	14	0.006	0.005	14	6.045	4.713	16	0.938	1.000
300379	12	0.090	0.084	13	0.154	0.137	12	0.090	0.064	12	29.39	28.80	12	3.78	3.49	12	0.032	0.025	10	11.670	8.646	16	0.946	1.000

Table A2.2 - Phosphorus load apportionment (kg/year) and hydrological indices for the 45 study locations

Phosphorus source apportionment (kg yr^{-1})									
Location	STW	Septic tanks	CSOs	arable	urban	RBI	Parde Range	BFI	q5:q95 ratio
529	0	0.1	0.19	0.11	0.24	0.404	1.361	0.475	17.321
1466	0.46	0.29	0.14	0.27	0.62	0.244	1.147	0.588	10.174
1515	0.61	0.16	0.41	0.5	0.11	0.407	1.625	0.455	16.319
1518	0.12	0.06	0	0.13	0	0.385	1.465	0.487	15.074
1574	2.34	1.2	0.98	1.95	0.24	0.231	1.506	0.459	19.477
1615	28.62	1.05	10.43	0.95	2.24	0.323	1.46	0.445	21.653
3453	0	0.08	0	0.03	0	0.411	1.182	0.416	26.508
4638	1.18	1.19	0.27	4.35	0	0.271	1.441	0.444	22.401
5982	0	0.41	0	0.76	0.02	0.315	1.379	0.505	18.194
7762	0.48	2.2	2.67	3.73	1.41	0.33	1.293	0.515	13.019
7989	23.08	3.17	1.88	5.45	0.29	0.168	1.735	0.581	14.297
8175	2.73	1.52	1.02	6.16	0.12	0.151	1.485	0.624	10.917
8455	0	0.51	0.1	0.62	0.03	0.353	1.5	0.493	13.451
8517	0	0.51	0.1	0.62	0.03	0.201	1.28	0.623	6.013
8549	0.61	0.56	0.32	0.58	0	0.247	2.025	0.494	13.963
8801	2.81	1.04	3.17	2.93	0.94	0.323	1.398	0.47	19.263
9422	0.24	0.5	0	1.06	0.01	0.379	1.645	0.402	24.607
9457	2.64	2.66	0.33	7.75	0.03	0.335	1.389	0.479	18.693
9750	1.03	0.48	0.19	2.13	0	0.345	1.56	0.465	18.557
10476	0.59	1.36	0.19	1.73	0.02	0.294	1.701	0.521	10.585
17012	0	0.11	0	0.27	0	0.366	1.74	0.454	22.412
122480	950.91	15.67	119.63	9.08	19.13	0.355	1.465	0.433	32.25
122482	0.17	0.87	0.18	0.42	0	0.323	1.481	0.504	14.215
122496	6.03	0.9	2.26	0.33	0.02	0.431	1.572	0.424	22.988
122706	0	0.23	0.45	0.3	0.3	0.541	1.592	0.39	23.906
122709	0	0.21	0.29	0.42	0.09	0.572	1.558	0.376	25.698
123153	0.06	0.53	0.05	0.03	0.05	0.312	1.135	0.427	36.625
200211	0	0.13	0	0.01	0	0.533	1.362	0.374	36.867
204343	0.02	0.7	0	4.45	0.01	0.231	1.303	0.577	12.356
206718	0	0.03	0	0.02	0	0.362	1.251	0.471	17.607
206732	0.27	0.35	0	0.03	0	0.394	1.218	0.497	12.965
206807	0	0.27	0	0.02	0	0.435	1.449	0.425	21.607
206811	0	0.04	0	0.01	0	0.557	1.419	0.372	35.264
206837	0	0.07	0	0.04	0	0.139	1.325	0.494	15.453
206845	0	0.11	0	0.04	0	0.61	1.311	0.33	53.937
207120	1.64	1.09	0.43	2.15	0.04	0.285	0.917	0.557	9.913
207127	0	0.05	0	0.01	0	0.369	0.58	0.514	10.469
207269	1.94	0.73	0.28	0.9	0.03	0.189	1.032	0.615	7.915
207277	0	0.23	0	0.02	0.17	0.16	1.026	0.633	7.222
207298	0	0.02	0	0.01	0	0.306	1.349	0.463	19.245
207309	0	0.03	0	0	0	0.374	1.152	0.51	12.507
207636	1.03	1.54	0.18	0.41	0.05	0.403	0.996	0.48	14.833
232930	0	0.06	0	0.06	0	0.224	0.793	0.608	8.311
300008	0	0.58	0	2	0.21	0.344	1.47	0.452	23.108
300379	0	0.05	0	0.16	0	0.492	1.202	0.452	17.571

Table A2.3 - Land cover proportions for the 45 study catchments

		Land cover proportion						
Location	**Catchment area (km²)**	**Arable**	**freshwater**	**improved grassland**	**Semi-natural**	**urban**	**woodland**	**other**
529	10.37	0.27	0.02	0.28	0.07	0.21	0.11	0.05
1466	32.48	0.36	0.01	0.32	0.10	0.06	0.15	0.01
1515	58.51	0.14	0.00	0.48	0.18	0.02	0.17	0.00
1518	8.76	0.10	0.00	0.56	0.18	0.00	0.15	0.00
1574	514.21	0.00	0.05	0.05	0.66	0.00	0.23	0.00
1615	191.63	0.07	0.03	0.29	0.30	0.08	0.23	0.00
3453	30.73	0.00	0.00	0.01	0.84	0.00	0.15	0.00
4638	1028.64	0.08	0.03	0.10	0.52	0.01	0.26	0.00
5982	54.93	0.48	0.00	0.15	0.23	0.01	0.13	0.01
7762	505.17	0.22	0.00	0.13	0.54	0.01	0.11	0.00
7989	125.87	0.45	0.00	0.27	0.11	0.11	0.05	0.00
8175	318.16	0.43	0.01	0.34	0.10	0.02	0.11	0.00
8455	222.11	0.12	0.00	0.17	0.59	0.01	0.10	0.00
8517	104.85	0.08	0.04	0.20	0.57	0.00	0.11	0.00
8549	247.01	0.52	0.00	0.23	0.13	0.03	0.09	0.00
8801	463.28	0.01	0.03	0.12	0.71	0.00	0.12	0.00
9422	120.01	0.56	0.00	0.18	0.12	0.01	0.13	0.01
9457	530.47	0.31	0.00	0.12	0.44	0.00	0.11	0.01
9750	175.14	0.24	0.01	0.16	0.36	0.00	0.23	0.00
10476	60.01	0.57	0.00	0.20	0.10	0.02	0.11	0.00
17012	13.4	0.63	0.01	0.13	0.17	0.00	0.06	0.00
122480	1965.92	0.07	0.01	0.28	0.39	0.08	0.17	0.01
122482	105.72	0.12	0.00	0.25	0.50	0.00	0.12	0.01
122496	88.8	0.03	0.00	0.47	0.26	0.02	0.20	0.01
122706	77.83	0.02	0.01	0.33	0.31	0.07	0.25	0.00
122709	26.99	0.14	0.00	0.68	0.09	0.05	0.03	0.00
123153	369.7	0.00	0.01	0.05	0.38	0.00	0.55	0.00
200211	192.09	0.00	0.01	0.01	0.79	0.00	0.19	0.00
204343	126.54	0.44	0.00	0.29	0.11	0.02	0.14	0.00
206718	213.8	0.00	0.05	0.00	0.92	0.00	0.02	0.01
206732	583	0.00	0.07	0.03	0.70	0.00	0.20	0.00
206807	423.24	0.00	0.01	0.01	0.92	0.00	0.05	0.00
206811	187.57	0.00	0.01	0.01	0.92	0.00	0.05	0.01
206837	441.48	0.00	0.09	0.00	0.84	0.00	0.05	0.02
206845	140.27	0.00	0.02	0.01	0.85	0.00	0.10	0.02
207120	276.08	0.20	0.00	0.28	0.22	0.01	0.28	0.00
207127	20.59	0.00	0.01	0.00	0.42	0.00	0.57	0.00
207269	119.55	0.28	0.00	0.36	0.28	0.01	0.07	0.00
207277	8.99	0.16	0.00	0.42	0.07	0.21	0.15	0.00
207298	153.72	0.00	0.04	0.00	0.92	0.00	0.01	0.03
207309	10.17	0.00	0.00	0.00	0.95	0.00	0.05	0.00
207636	329.21	0.10	0.02	0.15	0.44	0.01	0.28	0.00
232930	11.19	0.56	0.00	0.27	0.10	0.00	0.07	0.00
300008	264.45	0.02	0.02	0.25	0.55	0.00	0.15	0.00
300379	17.72	0.27	0.00	0.54	0.07	0.00	0.12	0.00

Appendix 3. Construction of real-valued log-ratio balances for compositional data and linear mixed modelling.

Ordinary statistical methods are generally designed for real-valued variables and use the absolute magnitude of the measurements as basic input for distinction and comparison between different observations. However, compositions consist of a number of variables which provide information about the relative levels of measurement between them using units like percentages, parts per million and similar. Compositional statistical methods exploit this information by focusing on the analysis of log-ratios between parts of the composition. Formally, given a D-part composition $x = [x_1, \dots, x_D]$, a balance b_i represents a contrast between two subsets of parts as

$$b_i = \sqrt{\frac{r_i s_i}{r_i + s_i}} \log \frac{\left(\prod_{k=1}^{r_i} x_{ik}^{+}\right)^{1/r_i}}{\left(\prod_{k=1}^{s_i} x_{ik}^{-}\right)^{1/s_i}}, \; i = 1, \dots, D-1, \quad (1)$$

where x_{ik}^{+} and x_{ik}^{-} refer to the subsets of r_i and s_i parts of $\mathbf{x}$ going, respectively, into the + (numerator) and – (denominator) groups. In accordance with the relative scale of the data, instead of using the ordinary Pearson's correlation measure, these subsets were determined according to proportionality between pairs of parts. Following Aitchison (1986), proportionality was measured by computing the matrix of log-ratio variances $\mathbf{T} = [\tau_{ij}]_{D\times D}$, where

$$\tau_{ij} = \mathrm{var}\left(\log\left(x_i / x_j\right)\right), \; i, j = 1, \dots, D,$$

with var referring to the ordinary variance measure. A log-ratio variance (τ_{ij}) that is close to 0 indicates that the two components x_i and *ix* are nearly proportional (highly co-dependent); that is, their log-ratio is nearly constant. The information in $\mathbf{T}$ was used as input to perform hierarchical clustering of variables (R-mode) so that clusters of homogeneous parts according to proportionality were identified. In particular, the well-known Ward's clustering method was used. Figure A3.1 shows for instance the resulting dendrogram for the water chemistry composition considered in this study.

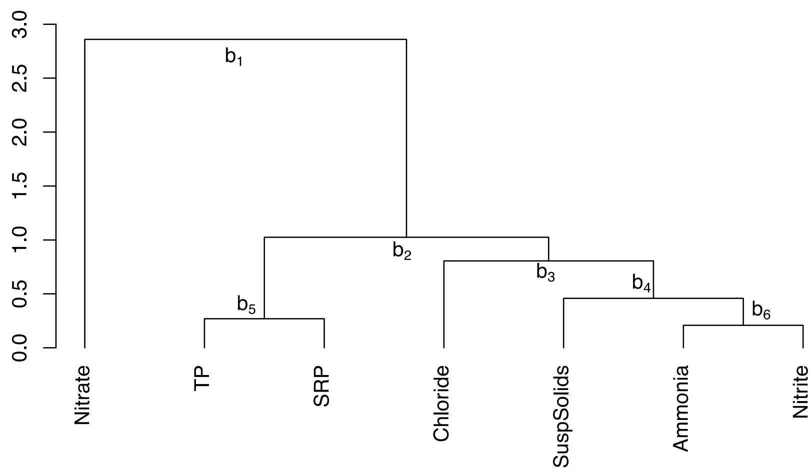

Fig A3.1 Groupings of water chemistry components (mg/L) according to proportionality relationships from pairwise log-ratio variances and associated compositional balances (b_i, i = 1,...,6).

The obtained hierarchical structure of proportionality relationships can be meaningfully used to inform the construction of orthogonal balances according to the successive splits into two mutually exclusive groups of parts until only groups of one part are left. This procedure is known as sequential binary partition (SBP; Egozcue and Pawlowsky-Glahn, 2005). To facilitate interpretation, the successive balances b_i, for $i = 1, \dots, D-1$, are represented in Fig. A3.1 at each node of the dendrogram defining a binary split. Parts on the left and right branches go into the + and – subsets respectively in Eq. (1). Note that compositions of D parts give rise to $D-1$ balances, which is in agreement with the actual number of degrees of freedom of the composition.

For the water chemistry composition, this meant six balances from a 7-part composition, with expressions given by:

$$b_1 = \sqrt{\frac{6}{7}} \log \frac{\text{Nitrate}}{(\text{TP} \cdot \text{SRP} \cdot \text{Chloride} \cdot \text{SuspSolids} \cdot \text{Ammonia} \cdot \text{Nitrite})^{1/6}},$$

$$b_2 = \sqrt{\frac{8}{6}} \log \frac{(\text{TP} \cdot \text{SRP})^{1/2}}{(\text{Chloride} \cdot \text{SuspSolids} \cdot \text{Ammonia} \cdot \text{Nitrite})^{1/4}},$$

$$b_3 = \sqrt{\frac{3}{4}} \log \frac{\text{Chloride}}{(\text{SuspSolids} \cdot \text{Ammonia} \cdot \text{Nitrite})^{1/3}},$$

$$b_4 = \sqrt{\frac{2}{3}} \log \frac{\text{SuspSolids}}{(\text{Ammonia} \cdot \text{Nitrite})^{1/2}},$$

$$b_5 = \sqrt{\frac{1}{2}} \log \frac{\text{TP}}{\text{SRP}} \quad \text{and}$$

$$b_6 = \sqrt{\frac{1}{2}} \log \frac{\text{Ammonia}}{\text{Nitrite}}.$$

and it accounts for the relative importance of TP and SRP in relation the other parts in the composition.

An analogous procedure was used to obtain meaningful log-ratio coordinates for the percentage 5-part land cover distribution according to proportionality between land cover categories, leading to balances denoted by l_i , with as $i = 1, \ldots, 4$, represented in Figure A3.2.

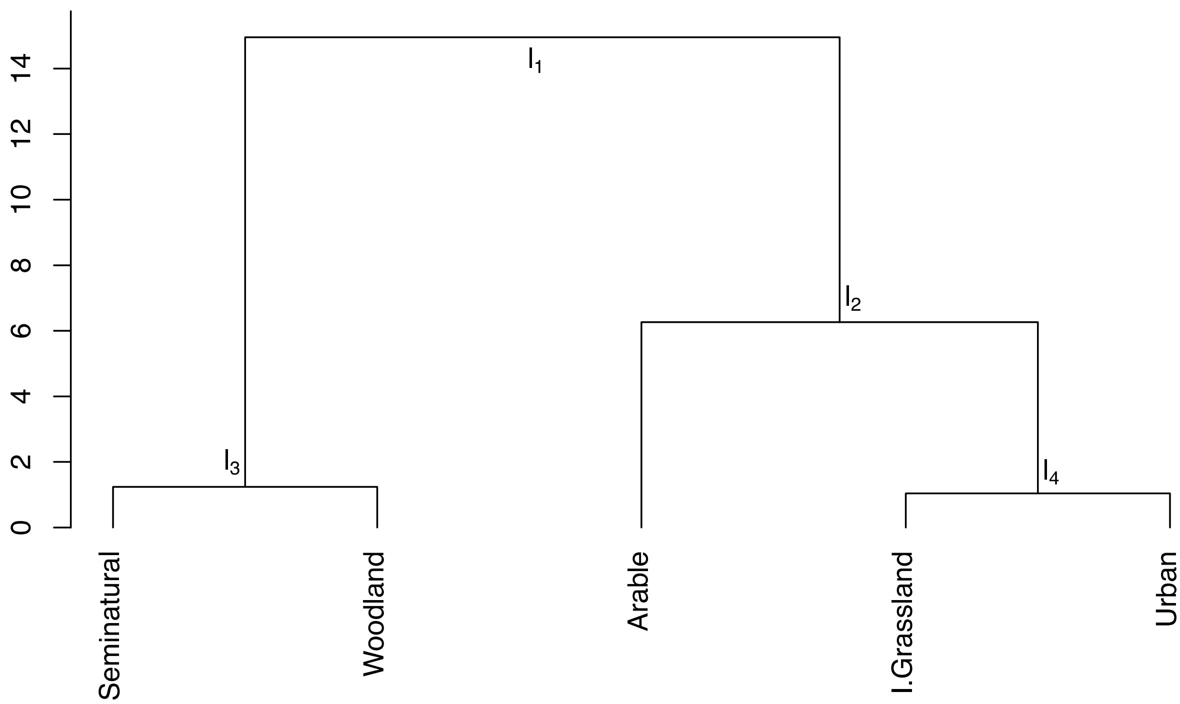

Fig A3.2 Groupings of percentage land cover types according to proportionality relationships from pairwise log-ratio variances and associated compositional balances (l_i , i = 1,...,4).

The expressions of the land cover types balances follow:

$$l_1 = \sqrt{\frac{6}{5}} \log \frac{(\text{Seminatural} \cdot \text{Woodland})^{1/2}}{(\text{Arable} \cdot \text{I. Grassland} \cdot \text{Urban})^{1/3}},$$

$$l_2 = \sqrt{\frac{2}{3}} \log \frac{\text{Arable}}{(\text{I. Grassland} \cdot \text{Urban})^{1/2}},$$

$$l_3 = \sqrt{\frac{1}{2}} \log \frac{\text{Seminatural}}{\text{Woodland}} \quad \text{and}$$

$$l_4 = \sqrt{\frac{1}{2}} \log \frac{\text{I. Grassland}}{\text{Urban}}.$$

The obtained sets of balances b_i and l_i convey all the information about the original compositions. Balances decompose that information in terms of ratios of parts, which is coherent with the relative scale of compositions. Moreover, balances are real-values variables which can be plugged into ordinary statistical modelling. In particular, we use them as explanatory variables into a linear mixed model (LMM) along with the other covariates. Formally, the vector of arsine-transformed observed EQR TDIs $\mathbf{y}_i$ from the ith catchment was modelled as

$$\mathbf{y}_i = \mathbf{B}_i \cdot \beta_1 + \mathbf{L}_i \cdot \beta_2 + \mathbf{C}_i \cdot \beta_3 + \mathbf{Z}_i \cdot f_i + \varepsilon_i, \quad i = 1, \ldots, 47,$$

$$f_i \sim N(\mathbf{0}, \sigma_f^2),$$

$$\varepsilon_i \sim N(\mathbf{0}, \sigma^2 \mathbf{I}),$$

where β_1 and β_2 were the vectors of coefficients of the fixed effects associated with, respectively, the water chemistry and land cover balances ($\mathbf{B}_i$ and $\mathbf{L}_i$ matrices) obtained as described above; and β_3 was the vector of coefficients for the other explanatory covariates ($\mathbf{C}_i$ matrix). The catchment random effects were represented by the $\mathbf{Z}_i \cdot f_i$ term, and ε_i was the within-group random error term.

Note that alternative sets of balances can be actually constructed in infinitely many ways following a SBP not necessarily based on the $\mathbf{T}$ matrix. However, they all correspond with mutual orthogonal rotations of the coordinate system where the log-ratio-transformed data points are represented. As a consequence, as long as the particular statistical method used is invariant under such rotations, the results will be comparable. This is the case of linear mixed models (Palarea-Albaladejo *et al.* 2017), where overall results like goodness of fit measures, predictions, etc. are the same regardless of the balance representation. Only the regression coefficients for each balance will be obviously different, although compatible, depending on the particular balance representation chosen.

CREW CENTRE OF EXPERTISE FOR WATERS

CREW Facilitation Team

Hydro Nation International Centre

James Hutton Institute

Craigiebuckler

Aberdeen AB15 8QH

Scotland UK

Email: enquiries@crew.ac.uk

www.crew.ac.uk

Hydro Nation International Centre

The James Hutton Institute

Scottish Government
Riaghaltas na h-Alba
gov.scot

CREW is a Scottish Government funded partnership between the James Hutton Institute and Scottish Universities.