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1 **Changes in epilithic biomasses and invertebrate community structure over a deposit metal concentration**

2 **gradient in upland headwater streams**

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11 **Abstract**

12 Stream bed metal deposits affect the taxon richness, density and taxonomic diversity of primary and secondary

13 producers by a variety of direct or indirect abiotic and biotic processes but little is known about the relative

14 importance of these processes over a deposit metal concentration gradient. Inorganic matter (IM), algal, and

15 non-photosynthetic detrital (NPD) dry biomasses were estimated for 10 monthly samples, between 2007 and

16 2008, from eight sites differing in deposit density. Invertebrate abundance, taxon richness and composition were

17 also determined. Relations between these variables were investigated by canonical correspondence analysis

18 (CCA), generalized estimating equation models and path analysis. The first CCA axis correlates with deposit

19 density and invertebrate abundance, with lumbriculids and chironomids increasing in abundance with deposit

20 density and all other taxa declining. Community structure changes significantly above a deposit density of

21 approximately 8 mg cm^{-2}, when algal biomass, invertebrate richness and diversity decline. Invertebrate richness

22 and diversity were determined by direct effects of NPD biomass and indirect effects of IM. Algal biomass only

23 had an effect on invertebrate abundance. Possible pH, oxygen, food and ecotoxicological effects of NPD

24 biomass on the biota are discussed.

25

29

Introduction

Deposits of iron hydroxide in surface waters have been documented globally (Niyogi et al. 1999; Prange 2007; Neal et al. 2008) and are frequently reported in post-industrial landscapes impacted by acid mine drainage (Younger 2001; Kimball et al. 2002; Mayes et al. 2008). Stream metal deposits are also found in non-industrial, often upland, catchments with limited anthropogenic activity (Macintosh & Griffiths, 2013; 2014). In impacted areas metal rich precipitates are ubiquitous and envelop benthic habitats, with direct and indirect ecosystem effects. The deposits potentially have harmful effects on algae, invertebrates and fish (Vuori 1995; Jarvis & Younger 1997).

Stream bed deposits, which are rich in iron (Fe), manganese (Mn) and aluminium (Al), are associated with reductions in the species richness and density of periphyton (McKnight & Feder 1984; Sheldon & Skelly 1990; Wellnitz et al. 1994; Wellnitz & Sheldon 1995; Hill et al. 2000; Verb & Vis 2000) and macroinvertebrates (Dills & Rogers 1974; Greenfield & Ireland 1978; Letterman & Mitsch 1978; Scullion & Edwards 1980; McKnight & Feder 1984; Rasmussen & Lindegaard 1988; Wellnitz et al. 1994; Clements et al. 2000; Hirst et al. 2002).

Community composition also changes with increasing metal concentration and deposit density, with the reduction/loss of Ephemeroptera, Plecoptera and Trichoptera (EPT) species and dominance by chironomids and oligochaetes (Letterman & Mitsch 1978; Scullion & Edwards 1980; Woodcock & Huryn 2005; Bott et al. 2012). Fish abundance and diversity are also reduced in metal-enriched streams (Mulholland et al. 1992; Vuori 1995). Many of these studies have been conducted in waters affected by acid mine drainage but the faunal effects are similar in circumneutral streams (Greenfield & Ireland 1978; Rasmussen & Lindegaard 1988; Clements et al. 2000; Hirst et al. 2002). Hence metal deposits can affect all trophic levels in streams: some authors (Mulholland et al. 1992; Clements et al. 2000) have associated these changes with alterations in ecosystem function, with fewer grazers and filterers in Fe-rich streams. The (physiological) effects of Al on fish survival are well documented (Mason 1996); toxic effects have also been recorded for Fe and Mn (Peuranen et al. 1994; Nyberg et al. 1995; Stubblefield et al. 1997; Dalzell & Macfarlane 1999; Verberk et al. 2012).

Metal deposition in stream ecosystems is driven by a variety of physical, chemical and biological processes but little is known about their relative importance. The redox processes involved in Fe mobilisation and deposition are well understood (Stumm & Morgan 1996), as is the effect of pH on the richness and composition of stream organisms (Townsend et al. 1983; Mulholland et al. 1992; Layer et al. 2013). The role of

60   biological processes has been less studied. Metal-oxidising bacteria are significant biogenic agents (Crerar et al.

61   1979; Konhauser 1998; Tebo et al. 2004; Emerson et al. 2010). Stream bed organic matter can be partitioned

62   into phototrophic (algal) and non-photosynthetic detrital (NPD) components: NPD consists of bacteria, fungi,

63   extracellular biofilms and detritus of terrestrial or aquatic origin (Ledger & Hildrew 1998; Carr et al. 2005).

64   Macintosh & Griffiths (2014) showed that deposit concentrations were influenced by NPD biomass and

65   tentatively concluded that microbial lithotrophic activity was a likely agent of metal deposition in the streams

66   studied.

67   While there are exceptions (e.g. Bott et al. 2012), previous studies have tended to focus on deposit

68   effects on a single trophic level: in this study a more holistic approach is taken. We partition the stream bed

69   deposit into inorganic matter (IM), algal and NPD components and examine corresponding differences in

70   invertebrate richness, abundance and composition over spatial and temporal gradients. Specifically we

71   investigate: (1) If deposit density affects algal abundance and invertebrate composition over a deposit metal

72   concentration gradient. (2) Whether food availability determines invertebrate abundance and composition.

73   Algae are an important food source for many aquatic invertebrates (Layer et al. 2013). Hence increasing deposit

74   metal concentrations should have a negative impact on algal biomass which, in turn, will have implications for

75   invertebrate assemblages. (3) The relative importance of direct and indirect effects of the deposit variables (IM,

76   NPD and algal biomasses) in determining invertebrate abundance, diversity and biotic scores.

77

78   **Materials and Methods**

79   Study area

80   The analyses presented here are based on eight stream sites located within the Sperrin Mountains, Northern

81   Ireland: sampled monthly from November 2007 to September 2008, high flows in January prevented adequate

82   sample collection. Spatial survey data from 32 Sperrin Mountains sites, collected on one sampling occasion in

83   April 2007, were used to confirm the algal and NPD deposit correlations (Macintosh & Griffiths, 2014).

84   Study sites were small, 1-2m wide, first order upland streams and tributaries of the Glenelly and

85   Glenlark rivers, which are part of the Owenkillew catchment. All but two of these sites drained separate areas.

86   Streamflow tended to be 'flashy', with rapid fluctuations between high and low flow discharge (see Macintosh

87   & Griffiths 2013; 2014 for site locations and general environmental information).

88   The sites were chosen to represent a range of metal deposit concentrations. All streams were located on

89   open moorland, had well-oxygenated water and stony substrata: no aquatic macrophytes were observed. Sites

90 lack anthropogenic interference and are not impacted by mining activities. The benthic chlorophyll *a* (Chla) and

91 phosphorus (P) concentrations indicate that these streams are oligotrophic (Dodds et al. 1998).

92

93 Sampling and laboratory analysis

94 Each of the eight sites was visited monthly to collect water, deposit and invertebrates samples. On each

95 sampling occasion, seven stream bed stones were randomly removed from each site and bagged individually for

96 the analysis of deposit composition (Fe, Mn, Al and P concentration, organic matter (OM) and IM content) and

97 Chla concentration. Deposit material on the upper stone surface was removed by spatula, brush and rinsing with

98 Millipore Milli-Q grade water. Depending upon density, the material from two to three stones was amalgamated

99 and dried at 105 °C until there was no further weight loss.

100   Inorganic matter was determined as the material remaining after ashing deposit samples for 1 hour in a

101 muffle furnace at 550 °C and OM as the loss-on-ignition (Lamberti & Resh 1985). Inorganic matter and deposit

102 metal concentrations were strongly correlated with total deposit density ($r = 0.68 – 0.98$, $n = 80$, $P<0.001$):

103 inorganic matter comprised on average 67% of deposit (range 38-93%). Deposit material consisted of epilithic

104 algae and 'ochre rich sludge' made up of detritus, fungi/bacteria and metal hydroxides: silt levels were low as a

105 result of the flashy nature of the upland stream systems and the preponderance of peat in the catchment

106 (Macintosh KA unpublished observation). Deposit density was calculated as the dry mass of material per unit

107 surface area, the latter determined by covering the exposed stone surface with aluminium foil which was then

108 weighed.

109   Metal concentration in the deposit material was determined by sequential acid digestion: hydrofluoric

110 acid was used to break down silicates, and nitric and perchloric acids to oxidise organics. After acid treatment,

111 deposit Fe, Mn, Al and P concentrations were measured by spectrometry (Macintosh & Griffiths (2013) using 2,

112 4, 6-tripyridyl-1, 3, 5-triazine, formaldoxime, pyrocatechol violet and molybdate-antimony methods respectively

113 (HMSO 1978a; 1978b; 1980; Murphy & Riley 1958; 1962). Blanks (Millipore Milli-Q) and standards were

114 included, in triplicate, for each chemical determinand.

115   Epilithic algal Chla concentrations were determined following the procedure of Marker et al. (1980),

116 after cold extraction in the dark at 4°C. Published data on ash free dry weight (AFDW) and Chla concentrations

117 of periphyton from streams without Fe deposits (Clark et al. 1979; Weitzel et al. 1979; Biggs & Close 1989;

118 Biggs 1996; Hill et al. 2000; Pizarro & Vinocur 2000; Carpenter 2003) were compiled and the autotrophic

119 index, an indicator of change in the relative importance of heterotrophic and autotrophic biomasses (Rice et al.

120	2012), calculated as AFDW/Chla. Indices below 200 were taken as indicative of sites where production was
121	dominated by photosynthetic rather than by lithotrophic activity. From these low autotrophic index sites the
122	AFDW attributable to photosynthetic organisms (algal biomass), was estimated from measured deposit Chla
123	concentrations (logAFDW = 2.016 + 1.043±0.026 logChla, $r^2 = 0.98$, $n = 37$). Deposit density was partitioned
124	into IM, algal and NPD components. In the absence of direct measures, the difference between algal biomass
125	and the corresponding OM values was used as an estimate of NPD biomass (Macintosh & Griffiths, 2014).
126	On each sample date, invertebrates were collected in non-pool habitat by a single, area-standardised
127	kick sample, covering 100 cm length of stream bed x net width (25 cm), to give a semi-quantitative estimate of
128	density (Rice et al. 2012). The animals were identified to family level (Croft 1986) and numbers counted.
129	Published diet data (e.g. Mellanby 1963; Merritt & Cummins 1996; Mihuc 1997;
130	http://water.epa.gov/scitech/monitoring/rsl/bioassessment/app_b-1.cfm) were used to allocate taxa to shredder,
131	collector-gatherer, collector-filterer, herbivore and predator functional feeding groups.
132	
133	Statistical analyses
134	The relation between in-stream deposit composition variables and invertebrate abundances was investigated by
135	canonical correspondence analysis (CCA), as implemented in PC-Ord v5 (McCune & Mefford 2006). Six
136	families, represented by fewer than 16 individuals (over 80 samples) and therefore judged as rare taxa, were
137	omitted from the analysis. Potentially important environmental variables that might affect invertebrates (%
138	dissolved oxygen (DO), temperature, pH, IM, deposit metal and biomass component concentrations) were used.
139	To test whether food availability influenced invertebrate abundance, correlations between algal and
140	NPD biomasses and invertebrate family abundances over the sampling period were calculated. To test for a
141	herbivore effect, the three ephemeropteran families were combined into a mayfly group.
142	The monthly data are temporally pseudoreplicated and therefore potentially correlated within sites.
143	These data were analysed by generalized estimating equation (GEE) models, which are designed to deal with
144	correlated data (Garson 2013), using SPSSv21. The models, fitted assuming a normal distribution and identity
145	link function, were estimated using the model-based estimator because the number of sites was less than 10. The
146	within-site correlation structure needs to be specified in GEE models. As indicated by quasi-likelihood under
147	independence criterion (QIC) values, the independent option, that is assuming that successive measurements are
148	uncorrelated, gave consistently better fits than first-order autoregressive or unstructured models. If the deposit
149	increasingly influences biotic structure one would expect a non-linear relationship, the simplest of which to

150   model is the quadratic. The fits of quadratic and linear deposit density predictors were compared by the

151   corrected version of QIC (QICC), to test if the responses were non-linear: the greater the difference in QICC

152   statistics between models the stronger the evidence for a particular model. QICC statistics are interpreted in the

153   same way as the more familiar AIC statistics (Burnham & Anderson 1998).

154   Path analysis (Quinn & Keogh 2002) was used to test all direct and indirect paths from IM, NPD and

155   algal biomasses to the target invertebrate variable. We are unaware of any path analysis procedure that allows

156   for temporally pseudoreplicated data and so these results need to be treated with some caution. However, the

157   finding that the best fit GEE models were obtained with uncorrelated (independent) within site values suggests

158   that the path analysis results are realistic. Various invertebrate measures were calculated. Taxon richness was

159   estimated as the number of families while Shannon diversity, evenness and Berger-Parker indices, which take

160   abundances into account, were calculated as measures of invertebrate diversity, evenness and dominance

161   respectively (Magurran 1988). ASPT (average score per taxon), a measure of the sensitivity of the invertebrate

162   fauna to organic pollution (oxygen stress), was calculated from faunal composition at each site.

163   All variables, except temperature, DO and pH, were $\log_{10}$ transformed to normalise the data. Non-linear

164   lines were fitted to the physical, chemical and biological variable data using locally weighted scatterplot

165   smoothers (LOWESS) because these do not impose a functional form on the relationship.

166

167 **Results**

168 Medians and ranges of deposit and biotic variables are summarised in Table 1: the variables vary by 2-4 orders

169 of magnitude across sites and season. Seasonal trends in physical, chemical and biological variables are also

170 shown in Online Resource1. Some variables, for example algal biomass and invertebrate abundance show

171 marked seasonal changes while others, such as pH, metal concentrations, and NPD biomass, are less variable.

172   The first three axes of the CCA had significantly greater taxon-environment correlations than expected

173 from 999 randomizations ($P<0.001$) and explained 14, 11 and 7% respectively of the variance. Important

174 chemical determinants of deposition, pH and DO, were weakly or non-significantly correlated with all axes

175 (Table 2). Episodic low pH can potentially affect the biota but of nine invertebrate taxon abundance and

176 diversity measures only trichopteran abundance showed a significant correlation with minimum site pH. There

177 were strong positive correlations of deposit IM and metal concentrations with the first axis while invertebrate

178 richness and diversity measures were negatively correlated with this axis. Lumbriculids and chironomids

179 showed positive correlations with the first axis whereas significant negative relationships were found for all

180 stonefly families and sericostomatids (Fig. 1, Table 2). Mayflies were negatively correlated with the second

181 axis, which was correlated with DO (+), P (+) and temperature (-). Gammarids (+), perlids (+) and chironomids

182 (-) were correlated with the third axis, which was negatively correlated with algal biomass.

183   There were only three significant correlations between algal biomass and the abundance of particular

184 families. All were positive, but none of the three families (leuctrids, nemourids and chironomids) would

185 normally be classified as herbivores. In contrast, 9/15 correlations with NPD biomass were significant, eight of

186 which were negative: only lumbriculid abundance was positively correlated with NPD biomass ($r = 0.54$,

187 $P<0.001$). The number of Plecoptera declined with increasing deposit density, IM content and NPD biomass,

188 but increased with algal biomass ($r = $ -0.38, -0.38, -0.36, 0.34 respectively, all $P<0.01$): there were no

189 correlations for Ephemeroptera and Trichoptera.

190   While NPD biomass rose log-linearly with deposit density, algal biomass and hence the autotrophic

191 index, increased to a deposit density of approximately 8 mg cm^{-2} before declining (Fig. 2a, Table 3). The spatial

192 survey sites, which were not pseudoreplicated, also showed a non-linear (quadratic) relation for algae and a

193 linear rise for NPD biomass with deposit density ($r = 0.40, 0.68$ respectively). While there was no overall

194 relation between algal and NPD biomasses ($r = 0.07$, $n = 75$, $P>0.5$), above 8 mg cm^{-2} there was a negative

195 relationship ($r = $ -0.67, $n = 34$, $P=0.05$), consistent with a potential competitive effect. As expected from the

196 deposit density - deposit P relationship ($r = 0.69$), NPD biomass increased linearly with deposit P concentration

197 but algal biomass showed a significant, dome-shaped, relationship (Table 3) i.e. algal biomass was lower in the

198 most P-rich deposits.

199   ASPT and taxon richness showed no trends until deposit densities reached 8 mg cm^{-2} and declined at

200 higher concentrations (Fig. 2b, c), but invertebrate density declined over the whole range (Table 3). Neither

201 Berger-Parker dominance nor dominance measured as equitability (not shown) varied with deposit density.

202   Path analyses explained only 3-19% of the variation but invertebrate richness, abundance (Fig. 3), and

203 composition measures showed similar responses to direct and indirect effects (Table 4), with NPD effects

204 strongest and algal effects weakest. IM indirect effects were stronger than direct effects, whereas the NPD direct

205 effects were stronger than indirect ones. Biotic scores, abundance, taxon richness and diversity declined and

206 dominance increased with IM and NPD. These effects are consistent with the predominantly negative

207 correlations between NPD biomass and taxon abundance noted above. Most of the indirect effects of IM were

208 through NPD rather than algal biomass, e.g. for number of individuals, path coefficients via NPD and algal

209 biomass are -0.44 (0.871*-0.506) and 0.04 (0.116 * 0.366) respectively. Invertebrate abundance was correlated

210 with algal biomass: as expected for a food chain effect this correlation was direct and positive.

211

212 **Discussion**

213 Results from our study support the findings of previous research that stream bed metal deposits reduce

214 invertebrate taxon richness, abundance and diversity: mayfly and stonefly families were most affected, and

215 oligochaetes and chironomids least. Top-down processes seem unlikely to account for these patterns since none

216 of the study sites supported fish populations (Griffiths, D. unpublished observations) and there was no

217 correlation between invertebrate predator and other invertebrate abundances.

218 The changes in NPD, algal and invertebrate relations at a deposit density of approximately 8 mg cm^{-2}

219 are consistent with negative food supply, chemical, and/or ecotoxicological effects of the deposit material.

220 Aluminium is generally regarded as more toxic than Fe and Mn (Hirst et al. 2002), both of which can have toxic

221 effects (Maltby & Crane 1994). Fe affects the survival and feeding activity of some invertebrates (Gerhardt

222 1992; Maltby & Crane 1994; Wellnitz et al. 1994). Fe uptake can occur from ingestion of metals whilst feeding,

223 thereby reducing energy intake (Smock 1983; Gerhardt 1993; Maltby & Crane 1994; Wellnitz et al. 1994), and

224 varies with feeding method, with indiscriminate feeders and filterers tending to have higher body concentrations

225 than predators (Gerhardt 1993; Wellnitz et al. 1994; Hünken & Mutz 2007). Some invertebrates are affected by

226 metal deposition on respiratory surfaces: the generally high sensitivity of mayfly larvae to Fe-rich deposits is

227 consistent with an Fe precipitation effect on respiratory surfaces (Gerhardt 1992). The path analyses showed that

228 NPD exerted direct negative effects on invertebrate richness, composition and diversity, while the negative

229 effects of IM were indirect and driven by NPD. However, the absence of direct effects for IM suggests that the

230 metals did not have direct ecotoxicological effects.

231 There was no evidence for an indirect negative effect of NPD biomass on invertebrate abundance via

232 algal abundance (path coefficient -0.51*0.07 = 0.04), that is for NPD to determine invertebrate abundance and

233 composition by reducing algal populations, or by being less nutritious than algae. However, the significant

234 positive path coefficient (0.37) of algae on invertebrate abundance does suggest a food supply effect, although

235 there was no correlation between mayfly numbers, the most likely group of herbivores, and algal biomass. Layer

236 et al. (2013) have shown changes in the importance of detritus and algae with increasing pH and corresponding

237 changes in invertebrate richness, abundance and trophic composition. Identification of diet from literature

238 sources is potentially misleading given the considerable spatial and temporal variation observed (Lamberti &

239   Moore 1984; Mihuc 1997). For example, while most sources identify nemourid stoneflies as collector-gatherers,

240   Ledger & Hildrew (2000) showed that in at least some acid streams they feed, in part, on algae. From functional

241   feeding groups identified from published data, collector-gatherers had positive and significantly different scores

242   on CCA axis 1, a deposit-density axis, from the other feeding groups.

243   The EPT index (number of mayfly, stonefly and caddis species or individuals), an index of water

244   quality, declines with pH and increasing metal concentrations (Hickey & Clements 1998; Malmqvist & Hoffsten

245   1999; Clements et al. 2000; Ledger & Hildrew 2005) but, as we found, not all orders always contribute to this

246   relation (not stoneflies, Rosemond et al. 1992; not caddis, Malmqvist & Hoffsten 1999; not mayflies, Dsa et al.

247   2008).

248   Acidity generally has a marked effect on species richness and the composition of stream bacteria,

249   algae, invertebrates and fish (e.g. Townsend et al. 1983; Mulholland et al. 1992; Rosemond et al. 1992; Ledger

250   & Hildrew 2005; Layer et al. 2013). Despite our sites covering a similar pH range, we found no effects on

251   deposit density, algal or NPD biofilm biomasses (Macintosh & Griffiths, 2014), or invertebrate community

252   indices, consistent with these variables being determined by other in-stream factors.

253   The stream bed was blanketed by a bright orange mat at the most deposit-rich sites in our study.

254   Sheath/stalk production by chemolithoautotrophs, frequently associated with metal deposits (Ghiorse 1984), can

255   stabilise the deposit matrix and decrease oxygen concentration within the deposit (Emerson et al. 2010; Roden

256   2012). The deposits potentially reduce light levels for benthic primary producers, and consequently oxygen

257   production by photosynthesis, and can also bind phosphorus (Sheldon & Wellnitz 1998; Withers & Jarvie 2008;

258   Rentz et al. 2009). The observed decline in algal biomass at high phosphorus concentrations supports the latter

259   possibility while the decline in ASPT scores above a threshold deposit density is consistent with a negative

260   effect on oxygen concentration.

261

262   **Conclusion**

263   In our study, community structure changes above a deposit density of about 8 mg cm^{-2}, when algal biomass,

264   invertebrate richness and diversity decline. The changes noted in invertebrate richness and composition are

265   consistent with known responses to environmental stress (Rosenberg & Resh 1993), with lumbriculids and

266   chironomids increasing in abundance and all other taxa declining. Previously reported responses in invertebrate

267   species richness and composition tend only to be found when pH drops below 6 (Sutcliffe & Hildrew 1989;

268 Mason 1996). However, our study streams were circumneutral (median pH 6.7) and there was little evidence

269 that episodes of low pH affected invertebrate abundance and composition.

270   Metal deposits blanket the stream bed, reduce oxygen concentrations therein and favour invertebrates

271 with low biotic scores, thereby negatively influencing species composition. Deposit accumulations are also

272 known to affect light penetration, reducing algal biomass above a threshold density, and thus influence

273 invertebrate abundance. This essentially correlative study has addressed important questions regarding the

274 direct and indirect effects of metal deposition on upland headwater stream community structure, particularly

275 with regard to changes in algal biomass, invertebrate richness and diversity. Confirmation of its conclusions will

276 require further analysis and more detailed measurements of oxygen concentrations and the distributions of algae,

277 bacteria/fungi and invertebrates within the deposits.

278

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467

468 **Tables**

469 **Table 1** Annual medians and ranges of deposit and biotic variables across the eight study sites.

470

Variable	Median	Range
pH	6.7	4.6-8.7
DO (%)	105.2	93.6-116.5
Deposit density (g m^{-2})	58.5	10.3-883.6
IM (g m^{-2})	40.6	6.5-618.6
OM (g m^{-2})	18.4	1.5-265.0
Chla (g m^{-2})	0.0056	0.0002-0.2691
Autotrophic index	3101	70-389513
Ephemeroptera (m^{-2})	68	4-784
Plecoptera (m^{-2})	52	4-384
Trichoptera (m^{-2})	16	4-80
Chironomidae (m^{-2})	12	4-256
Lumbriculidae (m^{-2})	16	4-88
Other invertebrates (m^{-2})	16	0-168

471

472   **Table 2** Pearson correlations between the first three axes of the CCA and deposit ($_d$) variables (interset) and the

473   abundances of the main invertebrate taxa. Significant values ($P<0.01$) are shown in bold. $n = 80$.

474

Variable	1	2	3	Taxon	1	2	3
% DO	-0.23	**0.34**	-0.10	Lumbriculid	**0.72**	-0.14	0.09
Temperature	0.13	**-0.37**	-0.25	Gammarid	-0.06	**-0.33**	**0.35**
pH	-0.11	-0.16	0.06	Leuctrid	**-0.38**	0.19	**-0.44**
Fe$_d$ (log$_{10}$)	**0.73**	0.11	0.21	Nemourid	**-0.43**	0.22	-0.28
Mn$_d$ (log$_{10}$)	**0.56**	-0.05	**0.37**	Perlid	**-0.34**	**0.48**	**0.42**
Al$_d$ (log$_{10}$)	**0.30**	0.02	-0.09	Perlodid	**-0.37**	0.17	0.22
P$_d$ (log$_{10}$)	**0.72**	**0.37**	-0.03	Baetid	-0.22	**-0.67**	-0.03
IM$_d$ (log$_{10}$)	**0.53**	0.00	0.06	Ephemerellid	-0.21	**-0.38**	-0.12
Algal biomass (log$_{10}$)	-0.10	0.08	**-0.44**	Heptageniid	-0.27	**-0.38**	-0.21
NPD biomass (log$_{10}$)	**0.64**	0.16	0.15	Hydropsychid	-0.13	**-0.37**	-0.04
Number of taxa	**-0.40**	**-0.55**	0.09	Polycentropid	-0.27	-0.19	0.16
Number of individuals (log$_{10}$)	-0.24	**-0.46**	-0.24	Sericostomatid	**-0.43**	-0.27	0.06
Shannon index	**-0.33**	0.00	0.20	Chironomid	**0.30**	-0.15	**-0.38**
Berger-Parker index	0.25	-0.20	-0.14	Simuliid	0.06	-0.04	**-0.34**
ASPT	**-0.88**	0.22	0.02	Tipulid	-0.01	**-0.46**	0.06

475

476 **Table 3** Slope coefficients of generalized estimating equation outputs with (a) log. deposit density and (b) log.

477 deposit phosphorus concentration as the predictor variable for quadratic (x and x2) and linear models. ΔQICC is

478 the absolute difference in QICC values for quadratic and linear models: slope coefficients are shown only for the

479 better model i.e. the one with the smaller QICC value.

480

Variable	Quadratic		Linear	ΔQICC
	x	x^2	x	
(a) x = Deposit density				
Autotrophic index ($\log_{10}$)	-0.401 ± 0.609	$0.759\pm0.313^*$		1.22
Algal biomass ($\log_{10}$)	$1.660\pm0.629^{**}$	$-0.856\pm0.324^{**}$		2.09
NPD biomass ($\log_{10}$)			$1.126\pm0.049^{***}$	1.99
ASPT	$2.307\pm1.005^*$	$-2.079\pm0.517^{***}$		22.13
Number of taxa	$3.713\pm1.608^*$	$-2.862\pm0.827^{***}$		43.74
Number of individuals ($\log_{10}$)			$-0.208\pm0.083^*$	1.60
Shannon (richness)			$-0.194\pm0.083^*$	0.96
Berger-Parker (dominance)			0.039 ± 0.033	1.95
(b) x = Deposit phosphorus				
Algal biomass ($\log_{10}$)	$-3.475\pm1.203^{**}$	$-1.107\pm0.361^{**}$		3.31
NPD biomass ($\log_{10}$)			$1.043\pm0.094^{***}$	1.78

481 *P<0.05, **P<0.01, ***P<0.001*

482

483   **Table 4** Path analysis summaries of the effects of inorganic matter, NPD and algal biomass densities on

484   invertebrate richness, abundance, diversity and biotic scores. The values are path coefficients. U are unexplained

485   path effects (= $\sqrt{(1 - r^2)}$). $n = 75$.

486

		Direct	Indirect	Total
Number of taxa	IM	0.100	-0.461*	-0.361*
	NPD	-0.555*	0.011	-0.454*
	Algae	0.199	0.370*	-0.171
	U	0.866		
Number of individuals ($\log_{10}$)	IM	0.149	-0.398*	-0.249*
	NPD	-0.506*	0.157	-0.349*
	Algae	0.366*	-0.019	0.347*
	U	0.856		
Shannon	IM	0.149	-0.422*	-0.273*
	NPD	-0.496*	0.135	-0.361*
	Algae	0.111	-0.019	0.092
	U	0.922		
Berger-Parker ($\log_{10}$)	IM	-0.213	0.362*	0.149
	NPD	0.418	-0.187	0.231*
	Algae	-0.026	0.005	-0.021
	U	0.967		
ASPT	IM	-0.125	-0.377*	-0.502*
	NPD	-0.460*	-0.094	-0.554*
	Algae	0.209*	-0.047	0.162
	U	0.805		

487   *$P<0.05$

Figure legends

489

490 **Fig. 1** Biplot of CCA ordination of taxon (LC scores) and deposit variables (bold) across the first two axes.

491

492 **Fig. 2** (a) Algal (*circles*) and NPD biomasses (*triangles*) (b) ASPT (*circles*) and the number of taxa (*triangles*)

493 and (c) the Shannon (*circles*) and Berger-Parker (*triangles*) indices as functions of deposit density. Lowess

494 smoothed lines (tension 0.7) are shown.

495

496 **Fig. 3** Diagram showing path coefficients between the deposit components and total invertebrate abundance.

497 Heavy lines indicate significant coefficients. The coefficients for the predictor variables are the Pearson

498 correlation coefficients between these variables.

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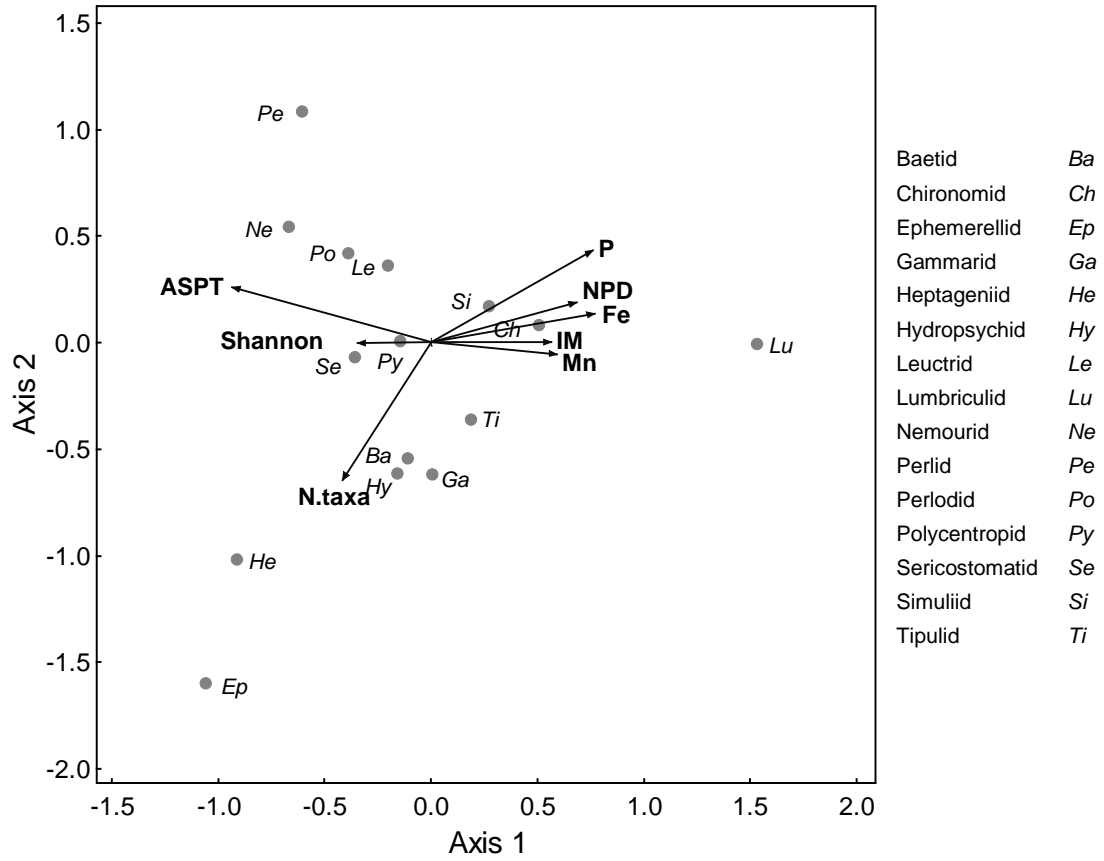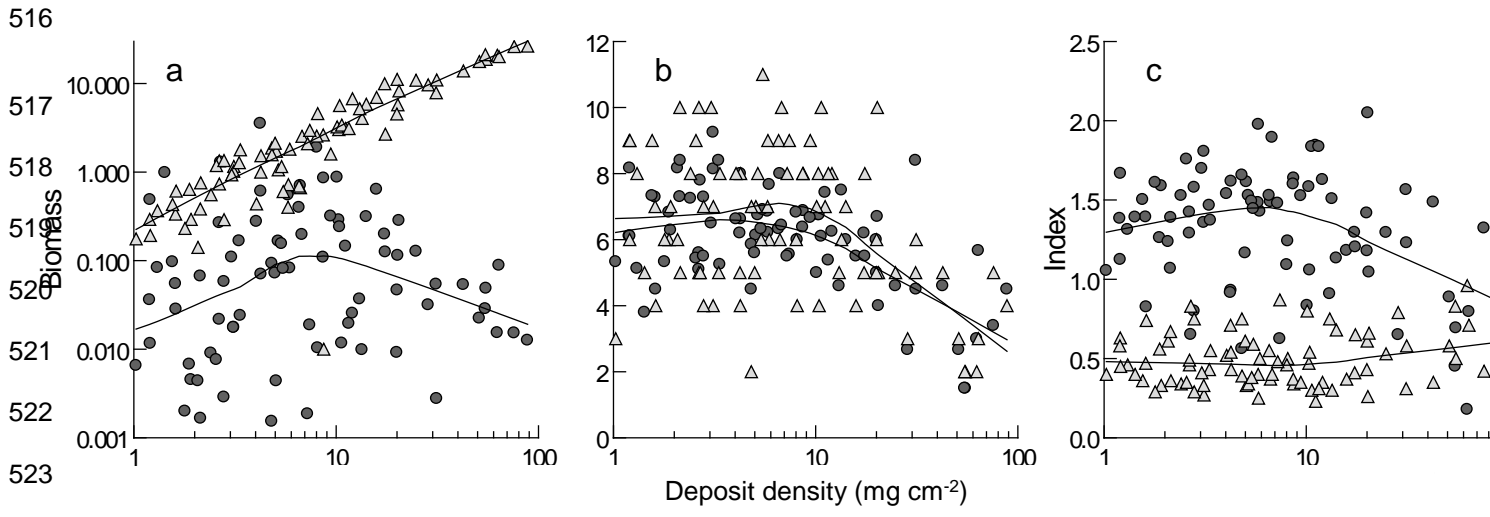508

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515 **Fig. 1**

Baetid	*Ba*
Chironomid	*Ch*
Ephemerellid	*Ep*
Gammarid	*Ga*
Heptageniid	*He*
Hydropsychid	*Hy*
Leuctrid	*Le*
Lumbriculid	*Lu*
Nemourid	*Ne*
Perlid	*Pe*
Perlodid	*Po*
Polycentropid	*Py*
Sericostomatid	*Se*
Simuliid	*Si*
Tipulid	*Ti*

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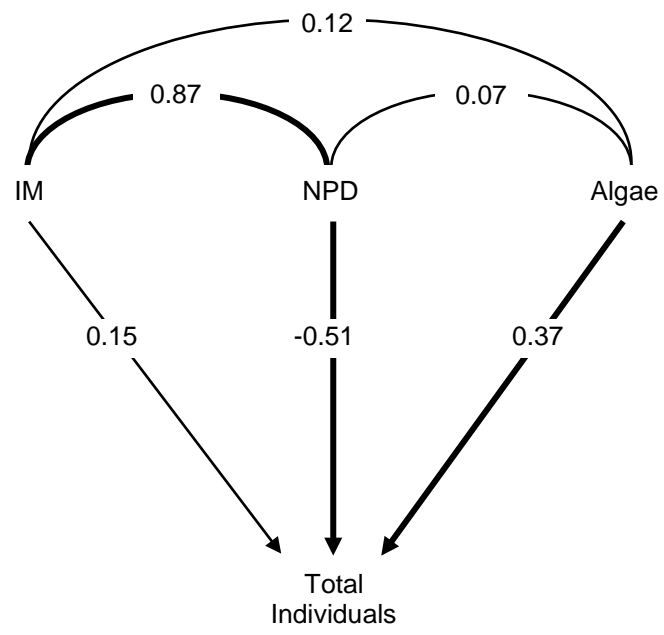
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524 **Fig. 2**

25

Fig. 3

540   Online Resource 1

541   **Changes in epilithic biomasses and invertebrate community structure over a deposit metal concentration**

542   **gradient in upland headwater streams**

543

544   Hydrobiologia

545

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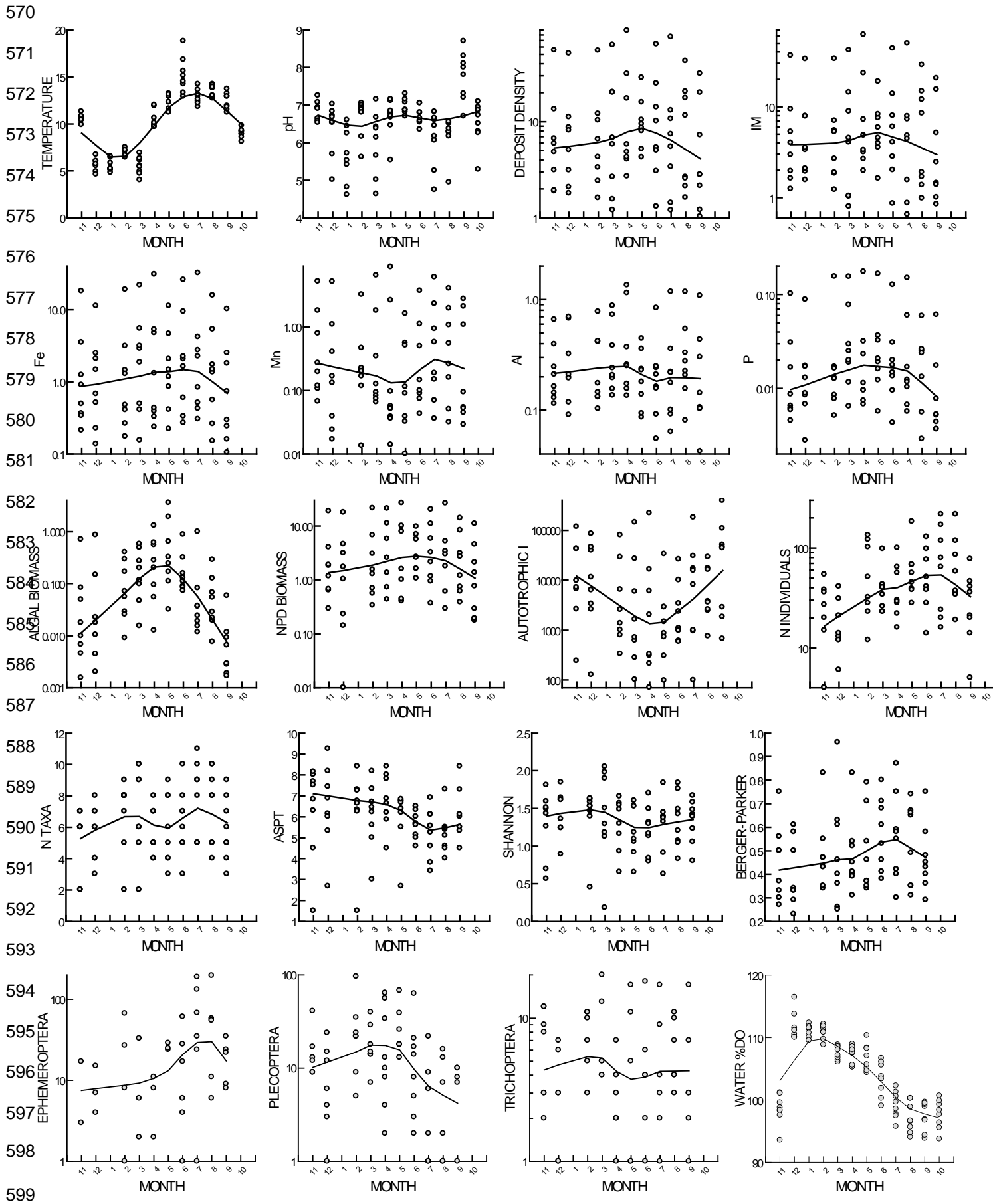
600   Fig. 1. Seasonal variation in physical, deposit chemical and biological variables, across all sites. The x-axis is

601   ordered by calendar month, from November 2007 to October 2008. The lines are Lowess smoothers (tension

602   0.5). Note that some variables are log transformed.