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1 **Changes in epilithic biomasses and invertebrate community structure over a deposit metal concentration**  
2 **gradient in upland headwater streams**

3

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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<sup>-2</sup>, 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

26 **Keywords**

27 Metal deposits · Invertebrate richness and composition · Algal biomass · Non-photosynthetic detrital biomass ·  
28 Direct and indirect pathways

29

## 30 **Introduction**

31

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

39 Stream bed deposits, which are rich in iron (Fe), manganese (Mn) and aluminium (Al), are associated  
40 with reductions in the species richness and density of periphyton (McKnight & Feder 1984; Sheldon & Skelly  
41 1990; Wellnitz et al. 1994; Wellnitz & Sheldon 1995; Hill et al. 2000; Verb & Vis 2000) and macroinvertebrates  
42 (Dills & Rogers 1974; Greenfield & Ireland 1978; Letterman & Mitsch 1978; Scullion & Edwards 1980;  
43 McKnight & Feder 1984; Rasmussen & Lindegaard 1988; Wellnitz et al. 1994; Clements et al. 2000; Hirst et al.  
44 2002).

45 Community composition also changes with increasing metal concentration and deposit density, with the  
46 reduction/loss of Ephemeroptera, Plecoptera and Trichoptera (EPT) species and dominance by chironomids and  
47 oligochaetes (Letterman & Mitsch 1978; Scullion & Edwards 1980; Woodcock & Huryn 2005; Bott et al. 2012).  
48 Fish abundance and diversity are also reduced in metal-enriched streams (Mulholland et al. 1992; Vuori 1995).  
49 Many of these studies have been conducted in waters affected by acid mine drainage but the faunal effects are  
50 similar in circumneutral streams (Greenfield & Ireland 1978; Rasmussen & Lindegaard 1988; Clements et al.  
51 2000; Hirst et al. 2002). Hence metal deposits can affect all trophic levels in streams: some authors (Mulholland  
52 et al. 1992; Clements et al. 2000) have associated these changes with alterations in ecosystem function, with  
53 fewer grazers and filterers in Fe-rich streams. The (physiological) effects of Al on fish survival are well  
54 documented (Mason 1996); toxic effects have also been recorded for Fe and Mn (Peuranen et al. 1994; Nyberg  
55 et al. 1995; Stubblefield et al. 1997; Dalzell & Macfarlane 1999; Verberk et al. 2012).

56 Metal deposition in stream ecosystems is driven by a variety of physical, chemical and biological  
57 processes but little is known about their relative importance. The redox processes involved in Fe mobilisation  
58 and deposition are well understood (Stumm & Morgan 1996), as is the effect of pH on the richness and  
59 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 ( $\log\text{AFDW} = 2.016 + 1.043 \pm 0.026 \log\text{Chla}$ ,  $r^2 = 0.98$ ,  $n = 37$ ). 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/rs1/bioassessment/app\\_b-1.cfm](http://water.epa.gov/scitech/monitoring/rs1/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  $8 \text{ mg cm}^{-2}$  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  $8 \text{ mg cm}^{-2}$  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  $8 \text{ mg cm}^{-2}$  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<sup>-2</sup>  
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<sup>-2</sup>, 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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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 <sup>-2</sup> )	58.5	10.3-883.6
IM (g m <sup>-2</sup> )	40.6	6.5-618.6
OM (g m <sup>-2</sup> )	18.4	1.5-265.0
Chla (g m <sup>-2</sup> )	0.0056	0.0002-0.2691
Autotrophic index	3101	70-389513
Ephemeroptera (m <sup>-2</sup> )	68	4-784
Plecoptera (m <sup>-2</sup> )	52	4-384
Trichoptera (m <sup>-2</sup> )	16	4-80
Chironomidae (m <sup>-2</sup> )	12	4-256
Lumbriculidae (m <sup>-2</sup> )	16	4-88
Other invertebrates (m <sup>-2</sup> )	16	0-168

471

472 **Table 2** Pearson correlations between the first three axes of the CCA and deposit (<sub>d</sub>) variables (intersept) 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	<b>0.34</b>	-0.10	Lumbriculid	<b>0.72</b>	-0.14	0.09
Temperature	0.13	<b>-0.37</b>	-0.25	Gammarid	-0.06	<b>-0.33</b>	<b>0.35</b>
pH	-0.11	-0.16	0.06	Leuctrid	<b>-0.38</b>	0.19	<b>-0.44</b>
Fe <sub>d</sub> (log <sub>10</sub> )	<b>0.73</b>	0.11	0.21	Nemourid	<b>-0.43</b>	0.22	-0.28
Mn <sub>d</sub> (log <sub>10</sub> )	<b>0.56</b>	-0.05	<b>0.37</b>	Perlid	<b>-0.34</b>	<b>0.48</b>	<b>0.42</b>
Al <sub>d</sub> (log <sub>10</sub> )	<b>0.30</b>	0.02	-0.09	Perلودid	<b>-0.37</b>	0.17	0.22
P <sub>d</sub> (log <sub>10</sub> )	<b>0.72</b>	<b>0.37</b>	-0.03	Baetid	-0.22	<b>-0.67</b>	-0.03
IM <sub>d</sub> (log <sub>10</sub> )	<b>0.53</b>	0.00	0.06	Ephemerellid	-0.21	<b>-0.38</b>	-0.12
Algal biomass (log <sub>10</sub> )	-0.10	0.08	<b>-0.44</b>	Heptageniid	-0.27	<b>-0.38</b>	-0.21
NPD biomass (log <sub>10</sub> )	<b>0.64</b>	0.16	0.15	Hydropsychid	-0.13	<b>-0.37</b>	-0.04
Number of taxa	<b>-0.40</b>	<b>-0.55</b>	0.09	Polycentropid	-0.27	-0.19	0.16
Number of individuals (log <sub>10</sub> )	-0.24	<b>-0.46</b>	-0.24	Sericostomatid	<b>-0.43</b>	-0.27	0.06
Shannon index	<b>-0.33</b>	0.00	0.20	Chironomid	<b>0.30</b>	-0.15	<b>-0.38</b>
Berger-Parker index	0.25	-0.20	-0.14	Simuliid	0.06	-0.04	<b>-0.34</b>
ASPT	<b>-0.88</b>	0.22	0.02	Tipulid	-0.01	<b>-0.46</b>	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 x<sup>2</sup>) 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 <sup>2</sup>	x	
(a) x = Deposit density				
Autotrophic index (log <sub>10</sub> )	-0.401±0.609	0.759±0.313*		1.22
Algal biomass (log <sub>10</sub> )	1.660±0.629**	-0.856±0.324**		2.09
NPD biomass (log <sub>10</sub> )			1.126±0.049***	1.99
ASPT	2.307±1.005*	-2.079±0.517***		22.13
Number of taxa	3.713±1.608*	-2.862±0.827***		43.74
Number of individuals (log <sub>10</sub> )			-0.208±0.083*	1.60
Shannon (richness)			-0.194±0.083*	0.96
Berger-Parker (dominance)			0.039±0.033	1.95
(b) x = Deposit phosphorus				
Algal biomass (log <sub>10</sub> )	-3.475±1.203**	-1.107±0.361**		3.31
NPD biomass (log <sub>10</sub> )			1.043±0.094***	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 \* $P < 0.05$

488 **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.

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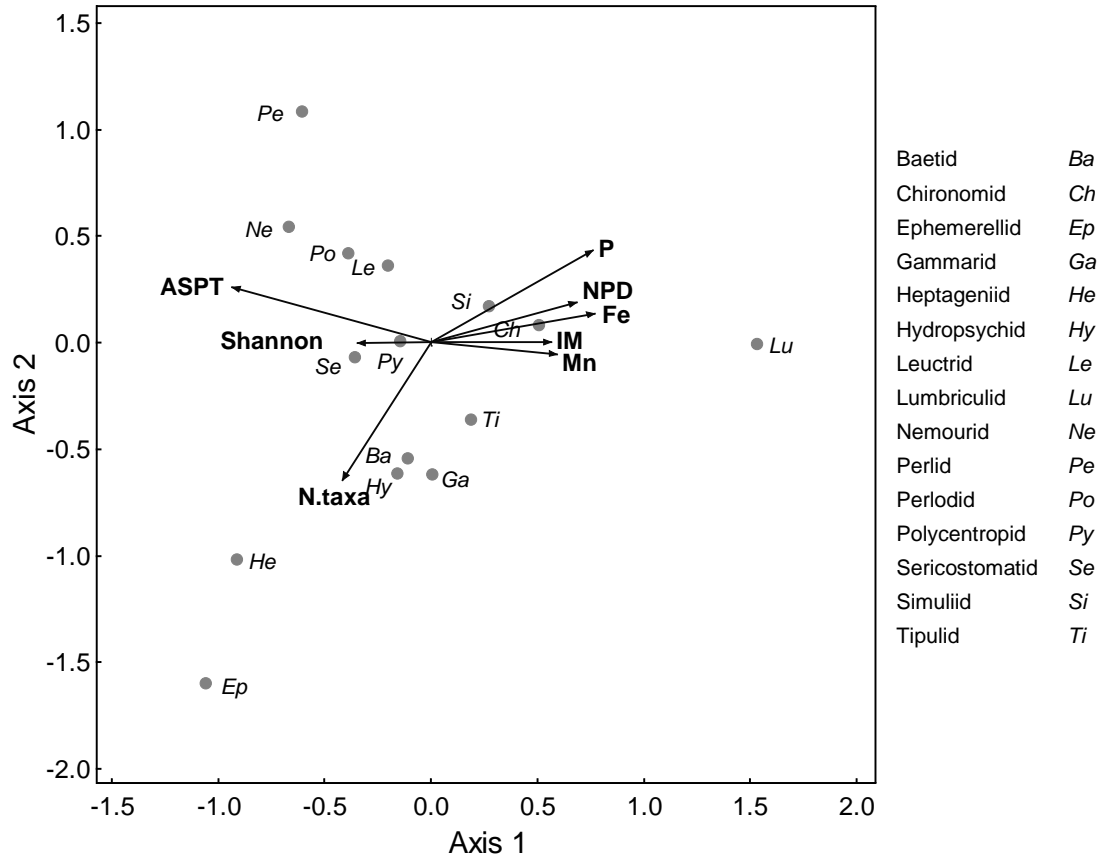
496 **Fig. 3** Diagram showing path coefficients between the deposit components and total invertebrate abundance.

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

498 correlation coefficients between these variables.

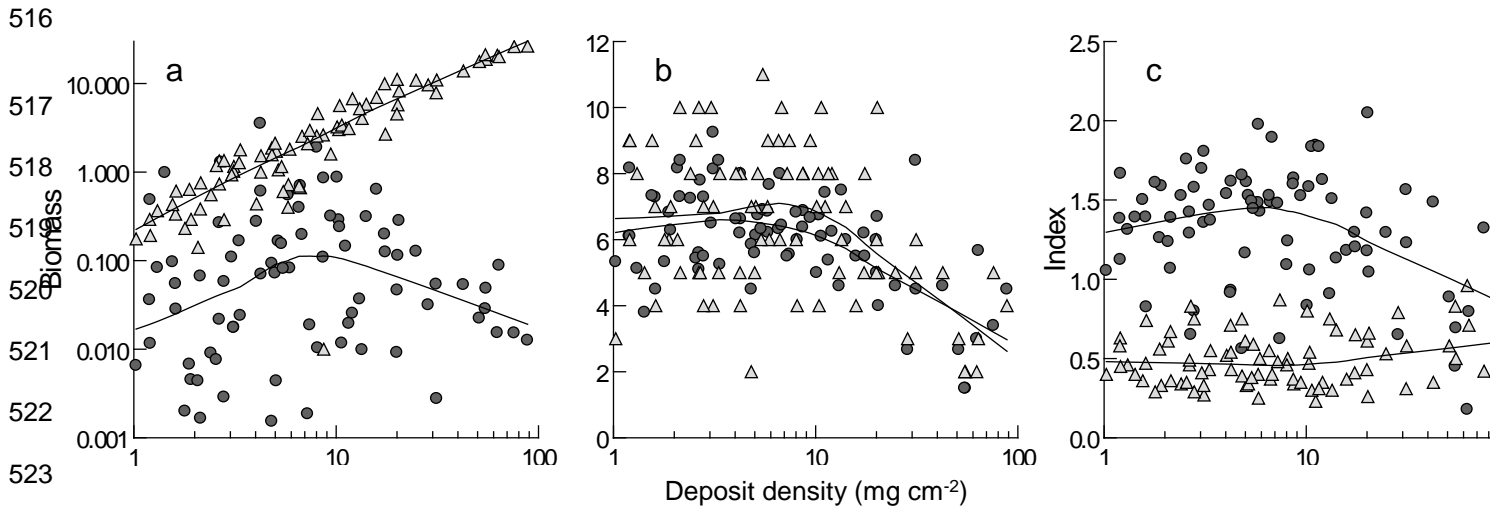


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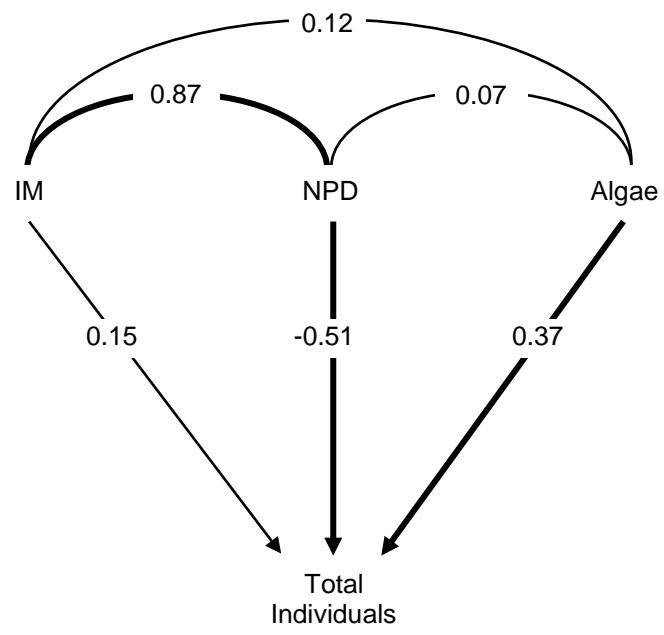
**Fig. 1**

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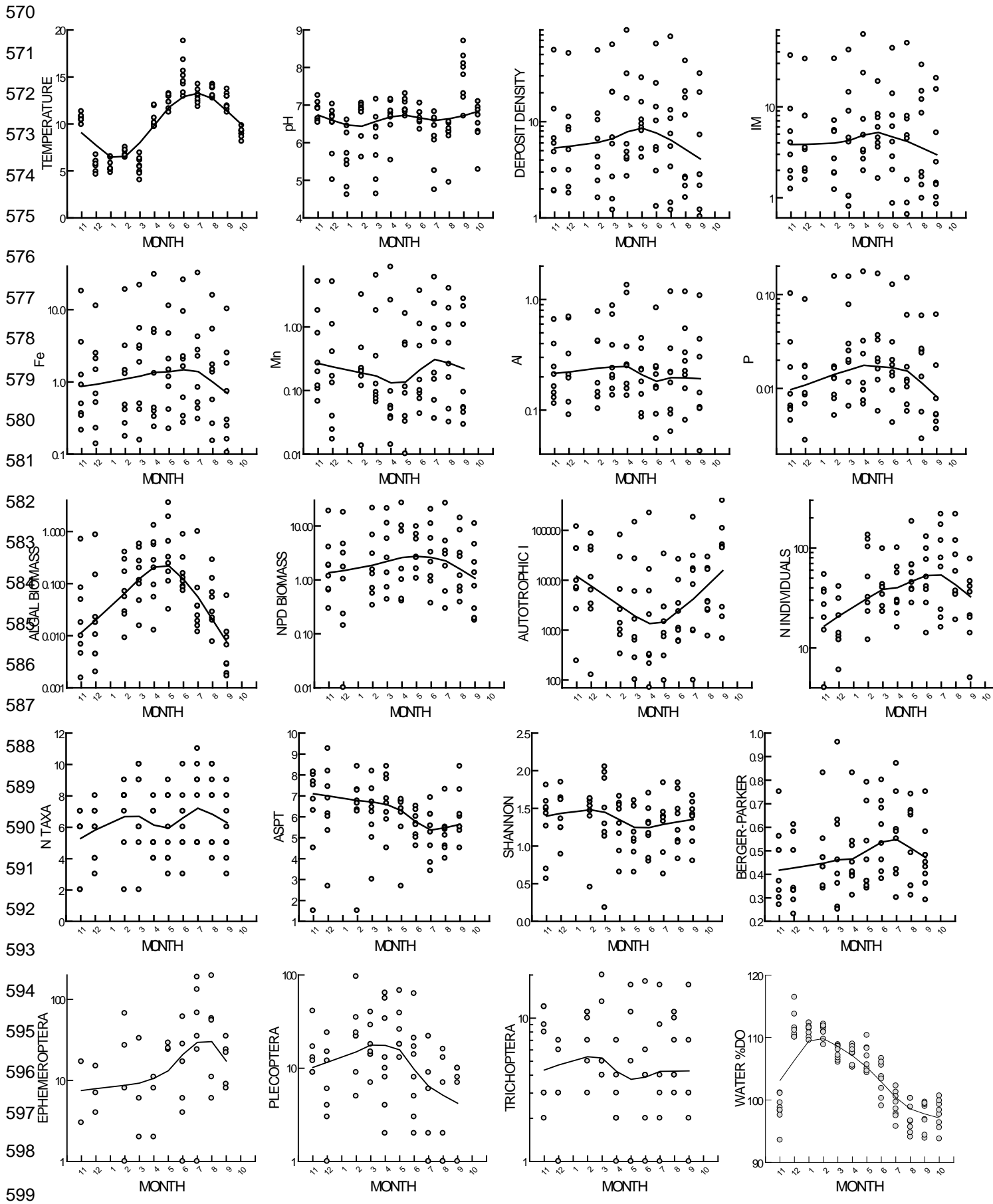
524 **Fig. 2**

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**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  
546 Katrina Ann Macintosh <sup>a,\*</sup> · David Griffiths <sup>a</sup>  
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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.