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

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Abstract

Stream bed metal deposits affect the taxon richness, density and taxonomic diversity of primary and secondary producers by a variety of direct or indirect abiotic and biotic processes but little is known about the relative importance of these processes over a deposit metal concentration gradient. Inorganic matter (IM), algal, and non-photosynthetic detrital (NPD) dry biomasses were estimated for 10 monthly samples, between 2007 and 2008, from eight sites differing in deposit density. Invertebrate abundance, taxon richness and composition were also determined. Relations between these variables were investigated by canonical correspondence analysis (CCA), generalized estimating equation models and path analysis. The first CCA axis correlates with deposit density and invertebrate abundance, with lumbriculids and chironomids increasing in abundance with deposit density and all other taxa declining. Community structure changes significantly above a deposit density of approximately 8 mg cm^{-2} , when algal biomass, invertebrate richness and diversity decline. Invertebrate richness and diversity were determined by direct effects of NPD biomass and indirect effects of IM. Algal biomass only had an effect on invertebrate abundance. Possible pH, oxygen, food and ecotoxicological effects of NPD biomass on the biota are discussed.

Keywords

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

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

biological processes has been less studied. Metal-oxidising bacteria are significant biogenic agents (Crerar et al. 1979; Konhauser 1998; Tebo et al. 2004; Emerson et al. 2010). Stream bed organic matter can be partitioned into phototrophic (algal) and non-photosynthetic detrital (NPD) components: NPD consists of bacteria, fungi, extracellular biofilms and detritus of terrestrial or aquatic origin (Ledger & Hildrew 1998; Carr et al. 2005). Macintosh & Griffiths (2014) showed that deposit concentrations were influenced by NPD biomass and tentatively concluded that microbial lithotrophic activity was a likely agent of metal deposition in the streams studied.

While there are exceptions (e.g. Bott et al. 2012), previous studies have tended to focus on deposit effects on a single trophic level: in this study a more holistic approach is taken. We partition the stream bed deposit into inorganic matter (IM), algal and NPD components and examine corresponding differences in invertebrate richness, abundance and composition over spatial and temporal gradients. Specifically we investigate: (1) If deposit density affects algal abundance and invertebrate composition over a deposit metal concentration gradient. (2) Whether food availability determines invertebrate abundance and composition. Algae are an important food source for many aquatic invertebrates (Layer et al. 2013). Hence increasing deposit metal concentrations should have a negative impact on algal biomass which, in turn, will have implications for invertebrate assemblages. (3) The relative importance of direct and indirect effects of the deposit variables (IM, NPD and algal biomasses) in determining invertebrate abundance, diversity and biotic scores.

Materials and Methods

Study area

The analyses presented here are based on eight stream sites located within the Sperrin Mountains, Northern Ireland: sampled monthly from November 2007 to September 2008, high flows in January prevented adequate sample collection. Spatial survey data from 32 Sperrin Mountains sites, collected on one sampling occasion in April 2007, were used to confirm the algal and NPD deposit correlations (Macintosh & Griffiths, 2014).

Study sites were small, 1-2m wide, first order upland streams and tributaries of the Glenelly and Glenlark rivers, which are part of the Owenkilnew catchment. All but two of these sites drained separate areas. Streamflow tended to be 'flashy', with rapid fluctuations between high and low flow discharge (see Macintosh & Griffiths 2013; 2014 for site locations and general environmental information).

The sites were chosen to represent a range of metal deposit concentrations. All streams were located on open moorland, had well-oxygenated water and stony substrata: no aquatic macrophytes were observed. Sites

lack anthropogenic interference and are not impacted by mining activities. The benthic chlorophyll *a* (Chla) and phosphorus (P) concentrations indicate that these streams are oligotrophic (Dodds et al. 1998).

Sampling and laboratory analysis

Each of the eight sites was visited monthly to collect water, deposit and invertebrates samples. On each sampling occasion, seven stream bed stones were randomly removed from each site and bagged individually for the analysis of deposit composition (Fe, Mn, Al and P concentration, organic matter (OM) and IM content) and Chla concentration. Deposit material on the upper stone surface was removed by spatula, brush and rinsing with Millipore Milli-Q grade water. Depending upon density, the material from two to three stones was amalgamated and dried at 105 °C until there was no further weight loss.

Inorganic matter was determined as the material remaining after ashing deposit samples for 1 hour in a muffle furnace at 550 °C and OM as the loss-on-ignition (Lamberti & Resh 1985). Inorganic matter and deposit metal concentrations were strongly correlated with total deposit density ($r = 0.68 - 0.98$, $n = 80$, $P < 0.001$): inorganic matter comprised on average 67% of deposit (range 38-93%). Deposit material consisted of epilithic algae and ‘ochre rich sludge’ made up of detritus, fungi/bacteria and metal hydroxides: silt levels were low as a result of the flashy nature of the upland stream systems and the preponderance of peat in the catchment (Macintosh KA unpublished observation). Deposit density was calculated as the dry mass of material per unit surface area, the latter determined by covering the exposed stone surface with aluminium foil which was then weighed.

Metal concentration in the deposit material was determined by sequential acid digestion: hydrofluoric acid was used to break down silicates, and nitric and perchloric acids to oxidise organics. After acid treatment, deposit Fe, Mn, Al and P concentrations were measured by spectrometry (Macintosh & Griffiths (2013) using 2, 4, 6-tripyridyl-1, 3, 5-triazine, formaldoxime, pyrocatechol violet and molybdate-antimony methods respectively (HMSO 1978a; 1978b; 1980; Murphy & Riley 1958; 1962). Blanks (Millipore Milli-Q) and standards were included, in triplicate, for each chemical determinand.

Epilithic algal Chla concentrations were determined following the procedure of Marker et al. (1980), after cold extraction in the dark at 4°C. Published data on ash free dry weight (AFDW) and Chla concentrations of periphyton from streams without Fe deposits (Clark et al. 1979; Weitzel et al. 1979; Biggs & Close 1989; Biggs 1996; Hill et al. 2000; Pizarro & Vinocur 2000; Carpenter 2003) were compiled and the autotrophic index, an indicator of change in the relative importance of heterotrophic and autotrophic biomasses (Rice et al.

2012), calculated as AFDW/Chla. Indices below 200 were taken as indicative of sites where production was dominated by photosynthetic rather than by lithotrophic activity. From these low autotrophic index sites the AFDW attributable to photosynthetic organisms (algal biomass), was estimated from measured deposit Chla concentrations ($\log \text{AFDW} = 2.016 + 1.043 \pm 0.026 \log \text{Chla}$, $r^2 = 0.98$, $n = 37$). Deposit density was partitioned into IM, algal and NPD components. In the absence of direct measures, the difference between algal biomass and the corresponding OM values was used as an estimate of NPD biomass (Macintosh & Griffiths, 2014).

On each sample date, invertebrates were collected in non-pool habitat by a single, area-standardised kick sample, covering 100 cm length of stream bed x net width (25 cm), to give a semi-quantitative estimate of density (Rice et al. 2012). The animals were identified to family level (Croft 1986) and numbers counted. Published diet data (e.g. Mellanby 1963; Merritt & Cummins 1996; Mihuc 1997; http://water.epa.gov/scitech/monitoring/rsl/bioassessment/app_b-1.cfm) were used to allocate taxa to shredder, collector-gatherer, collector-filterer, herbivore and predator functional feeding groups.

Statistical analyses

The relation between in-stream deposit composition variables and invertebrate abundances was investigated by canonical correspondence analysis (CCA), as implemented in PC-Ord v5 (McCune & Mefford 2006). Six families, represented by fewer than 16 individuals (over 80 samples) and therefore judged as rare taxa, were omitted from the analysis. Potentially important environmental variables that might affect invertebrates (% dissolved oxygen (DO), temperature, pH, IM, deposit metal and biomass component concentrations) were used.

To test whether food availability influenced invertebrate abundance, correlations between algal and NPD biomasses and invertebrate family abundances over the sampling period were calculated. To test for a herbivore effect, the three ephemeropteran families were combined into a mayfly group.

The monthly data are temporally pseudoreplicated and therefore potentially correlated within sites. These data were analysed by generalized estimating equation (GEE) models, which are designed to deal with correlated data (Garson 2013), using SPSSv21. The models, fitted assuming a normal distribution and identity link function, were estimated using the model-based estimator because the number of sites was less than 10. The within-site correlation structure needs to be specified in GEE models. As indicated by quasi-likelihood under independence criterion (QIC) values, the independent option, that is assuming that successive measurements are uncorrelated, gave consistently better fits than first-order autoregressive or unstructured models. If the deposit increasingly influences biotic structure one would expect a non-linear relationship, the simplest of which to

model is the quadratic. The fits of quadratic and linear deposit density predictors were compared by the corrected version of QIC (QICC), to test if the responses were non-linear: the greater the difference in QICC statistics between models the stronger the evidence for a particular model. QICC statistics are interpreted in the same way as the more familiar AIC statistics (Burnham & Anderson 1998).

Path analysis (Quinn & Keogh 2002) was used to test all direct and indirect paths from IM, NPD and algal biomasses to the target invertebrate variable. We are unaware of any path analysis procedure that allows for temporally pseudoreplicated data and so these results need to be treated with some caution. However, the finding that the best fit GEE models were obtained with uncorrelated (independent) within site values suggests that the path analysis results are realistic. Various invertebrate measures were calculated. Taxon richness was estimated as the number of families while Shannon diversity, evenness and Berger-Parker indices, which take abundances into account, were calculated as measures of invertebrate diversity, evenness and dominance respectively (Magurran 1988). ASPT (average score per taxon), a measure of the sensitivity of the invertebrate fauna to organic pollution (oxygen stress), was calculated from faunal composition at each site.

All variables, except temperature, DO and pH, were $\log_{10}$ transformed to normalise the data. Non-linear lines were fitted to the physical, chemical and biological variable data using locally weighted scatterplot smoothers (LOWESS) because these do not impose a functional form on the relationship.

Results

Medians and ranges of deposit and biotic variables are summarised in Table 1: the variables vary by 2-4 orders of magnitude across sites and season. Seasonal trends in physical, chemical and biological variables are also shown in Online Resource1. Some variables, for example algal biomass and invertebrate abundance show marked seasonal changes while others, such as pH, metal concentrations, and NPD biomass, are less variable.

The first three axes of the CCA had significantly greater taxon-environment correlations than expected from 999 randomizations ($P < 0.001$) and explained 14, 11 and 7% respectively of the variance. Important chemical determinants of deposition, pH and DO, were weakly or non-significantly correlated with all axes (Table 2). Episodic low pH can potentially affect the biota but of nine invertebrate taxon abundance and diversity measures only trichopteran abundance showed a significant correlation with minimum site pH. There were strong positive correlations of deposit IM and metal concentrations with the first axis while invertebrate richness and diversity measures were negatively correlated with this axis. Lumbriculids and chironomids showed positive correlations with the first axis whereas significant negative relationships were found for all

stonefly families and sericostomatids (Fig. 1, Table 2). Mayflies were negatively correlated with the second axis, which was correlated with DO (+), P (+) and temperature (-). Gammarids (+), perlids (+) and chironomids (-) were correlated with the third axis, which was negatively correlated with algal biomass.

There were only three significant correlations between algal biomass and the abundance of particular families. All were positive, but none of the three families (leuctrids, nemourids and chironomids) would normally be classified as herbivores. In contrast, 9/15 correlations with NPD biomass were significant, eight of which were negative: only lumbriculid abundance was positively correlated with NPD biomass ($r = 0.54$, $P < 0.001$). The number of Plecoptera declined with increasing deposit density, IM content and NPD biomass, but increased with algal biomass ($r = -0.38, -0.38, -0.36, 0.34$ respectively, all $P < 0.01$): there were no correlations for Ephemeroptera and Trichoptera.

While NPD biomass rose log-linearly with deposit density, algal biomass and hence the autotrophic index, increased to a deposit density of approximately 8 mg cm^{-2} before declining (Fig. 2a, Table 3). The spatial survey sites, which were not pseudoreplicated, also showed a non-linear (quadratic) relation for algae and a linear rise for NPD biomass with deposit density ($r = 0.40, 0.68$ respectively). While there was no overall relation between algal and NPD biomasses ($r = 0.07$, $n = 75$, $P > 0.5$), above 8 mg cm^{-2} there was a negative relationship ($r = -0.67$, $n = 34$, $P = 0.05$), consistent with a potential competitive effect. As expected from the deposit density - deposit P relationship ($r = 0.69$), NPD biomass increased linearly with deposit P concentration but algal biomass showed a significant, dome-shaped, relationship (Table 3) i.e. algal biomass was lower in the most P-rich deposits.

ASPT and taxon richness showed no trends until deposit densities reached 8 mg cm^{-2} and declined at higher concentrations (Fig. 2b, c), but invertebrate density declined over the whole range (Table 3). Neither Berger-Parker dominance nor dominance measured as equitability (not shown) varied with deposit density.

Path analyses explained only 3-19% of the variation but invertebrate richness, abundance (Fig. 3), and composition measures showed similar responses to direct and indirect effects (Table 4), with NPD effects strongest and algal effects weakest. IM indirect effects were stronger than direct effects, whereas the NPD direct effects were stronger than indirect ones. Biotic scores, abundance, taxon richness and diversity declined and dominance increased with IM and NPD. These effects are consistent with the predominantly negative correlations between NPD biomass and taxon abundance noted above. Most of the indirect effects of IM were through NPD rather than algal biomass, e.g. for number of individuals, path coefficients via NPD and algal

biomass are -0.44 (0.871*-0.506) and 0.04 (0.116 * 0.366) respectively. Invertebrate abundance was correlated with algal biomass: as expected for a food chain effect this correlation was direct and positive.

Discussion

Results from our study support the findings of previous research that stream bed metal deposits reduce invertebrate taxon richness, abundance and diversity: mayfly and stonefly families were most affected, and oligochaetes and chironomids least. Top-down processes seem unlikely to account for these patterns since none of the study sites supported fish populations (Griffiths, D. unpublished observations) and there was no correlation between invertebrate predator and other invertebrate abundances.

The changes in NPD, algal and invertebrate relations at a deposit density of approximately 8 mg cm⁻² are consistent with negative food supply, chemical, and/or ecotoxicological effects of the deposit material. Aluminium is generally regarded as more toxic than Fe and Mn (Hirst et al. 2002), both of which can have toxic effects (Maltby & Crane 1994). Fe affects the survival and feeding activity of some invertebrates (Gerhardt 1992; Maltby & Crane 1994; Wellnitz et al. 1994). Fe uptake can occur from ingestion of metals whilst feeding, thereby reducing energy intake (Smock 1983; Gerhardt 1993; Maltby & Crane 1994; Wellnitz et al. 1994), and varies with feeding method, with indiscriminate feeders and filterers tending to have higher body concentrations than predators (Gerhardt 1993; Wellnitz et al. 1994; Hünken & Mutz 2007). Some invertebrates are affected by metal deposition on respiratory surfaces: the generally high sensitivity of mayfly larvae to Fe-rich deposits is consistent with an Fe precipitation effect on respiratory surfaces (Gerhardt 1992). The path analyses showed that NPD exerted direct negative effects on invertebrate richness, composition and diversity, while the negative effects of IM were indirect and driven by NPD. However, the absence of direct effects for IM suggests that the metals did not have direct ecotoxicological effects.

There was no evidence for an indirect negative effect of NPD biomass on invertebrate abundance via algal abundance (path coefficient $-0.51 \times 0.07 = 0.04$), that is for NPD to determine invertebrate abundance and composition by reducing algal populations, or by being less nutritious than algae. However, the significant positive path coefficient (0.37) of algae on invertebrate abundance does suggest a food supply effect, although there was no correlation between mayfly numbers, the most likely group of herbivores, and algal biomass. Layer et al. (2013) have shown changes in the importance of detritus and algae with increasing pH and corresponding changes in invertebrate richness, abundance and trophic composition. Identification of diet from literature sources is potentially misleading given the considerable spatial and temporal variation observed (Lamberti &

Moore 1984; Mihuc 1997). For example, while most sources identify nemourid stoneflies as collector-gatherers, Ledger & Hildrew (2000) showed that in at least some acid streams they feed, in part, on algae. From functional feeding groups identified from published data, collector-gatherers had positive and significantly different scores on CCA axis 1, a deposit-density axis, from the other feeding groups.

The EPT index (number of mayfly, stonefly and caddis species or individuals), an index of water quality, declines with pH and increasing metal concentrations (Hickey & Clements 1998; Malmqvist & Hoffsten 1999; Clements et al. 2000; Ledger & Hildrew 2005) but, as we found, not all orders always contribute to this relation (not stoneflies, Rosemond et al. 1992; not caddis, Malmqvist & Hoffsten 1999; not mayflies, Dsa et al. 2008).

Acidity generally has a marked effect on species richness and the composition of stream bacteria, algae, invertebrates and fish (e.g. Townsend et al. 1983; Mulholland et al. 1992; Rosemond et al. 1992; Ledger & Hildrew 2005; Layer et al. 2013). Despite our sites covering a similar pH range, we found no effects on deposit density, algal or NPD biofilm biomasses (Macintosh & Griffiths, 2014), or invertebrate community indices, consistent with these variables being determined by other in-stream factors.

The stream bed was blanketed by a bright orange mat at the most deposit-rich sites in our study. Sheath/stalk production by chemolithoautotrophs, frequently associated with metal deposits (Ghiorse 1984), can stabilise the deposit matrix and decrease oxygen concentration within the deposit (Emerson et al. 2010; Roden 2012). The deposits potentially reduce light levels for benthic primary producers, and consequently oxygen production by photosynthesis, and can also bind phosphorus (Sheldon & Wellnitz 1998; Withers & Jarvie 2008; Rentz et al. 2009). The observed decline in algal biomass at high phosphorus concentrations supports the latter possibility while the decline in ASPT scores above a threshold deposit density is consistent with a negative effect on oxygen concentration.

Conclusion

In our study, community structure changes above a deposit density of about 8 mg cm⁻², when algal biomass, invertebrate richness and diversity decline. The changes noted in invertebrate richness and composition are consistent with known responses to environmental stress (Rosenberg & Resh 1993), with lumbriculids and chironomids increasing in abundance and all other taxa declining. Previously reported responses in invertebrate species richness and composition tend only to be found when pH drops below 6 (Sutcliffe & Hildrew 1989;

Mason 1996). However, our study streams were circumneutral (median pH 6.7) and there was little evidence that episodes of low pH affected invertebrate abundance and composition.

Metal deposits blanket the stream bed, reduce oxygen concentrations therein and favour invertebrates with low biotic scores, thereby negatively influencing species composition. Deposit accumulations are also known to affect light penetration, reducing algal biomass above a threshold density, and thus influence invertebrate abundance. This essentially correlative study has addressed important questions regarding the direct and indirect effects of metal deposition on upland headwater stream community structure, particularly with regard to changes in algal biomass, invertebrate richness and diversity. Confirmation of its conclusions will require further analysis and more detailed measurements of oxygen concentrations and the distributions of algae, bacteria/fungi and invertebrates within the deposits.

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References

- Biggs, B. J. F., 1996. Patterns in benthic algae of streams Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, 31-56.
- Biggs, B. J. F. & M. E. Close, 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology* 22: 209-231.
- Bott, T. L., J. K. Jackson, M. E. McTammany, J. D. Newbold, S. T. Rier, B. W. Sweeney & J. M. Battle, 2012. Abandoned coal mine drainage and its remediation: impacts on stream ecosystem structure and function. *Ecological Applications* 22: 2144-2163.
- Burnham, K. P. & D. R. Anderson, 1998. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.
- Carpenter, K. D., 2003. Water-quality and algal conditions in the Clackamas River Basin, Oregon, and their relations to land and water management US Geological Survey Water-Resources Investigations Report 02-4189. 114
- Carr, G. M., A. Morin & P. A. Chambers, 2005. Bacteria and algae in stream periphyton along a nutrient gradient. *Freshwater Biology* 50: 1337-1350 doi:10.1111/j.1365-2427.2005.01401.x.
- Clark, J. R., K. L. Dickson & J. Cairns, 1979. Estimating aufwuchs biomass. In Weitzel, R. L. (ed) Methods and measurements of periphyton communities: a review. American Society for Testing and Materials, Philadelphia, 116-141.
- Clements, W. H., D. M. Carlisle, J. M. Lazorchak & P. C. Johnson, 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications* 10: 626-638.
- Crerar, D. A., G. W. Knox & J. L. Means, 1979. Biogeochemistry of bog iron in the New Jersey Pine Barrens. *Chemical Geology* 24: 111-135.
- Croft, P. S., 1986. British freshwater invertebrates. Field Studies Council, Shrewsbury.
- Dalzell, D. J. B. & N. A. A. Macfarlane, 1999. The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. *Journal of Fish Biology* 55: 301-315.
- Dills, G. & D. T. Rogers, 1974. Macroinvertebrate community structure as an indicator of acid mine pollution. *Environmental Pollution* 6: 239-262.
- Dodds, W. K., J. R. Jones & E. B. Welch, 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. *Water Research* 32: 1455-1462.

312 Dsa, J. V., K. S. Johnson, D. Lopez, C. Kanuckel & J. Tumlinson, 2008. Residual toxicity of acid mine
313 drainage-contaminated sediment to stream macroinvertebrates: relative contribution of acidity vs.
314 metals. *Water, Air & Soil Pollution* 194: 185-197 doi:10.1007/s11270-008-9707-y.

315 Emerson, D., E. J. Fleming & J. M. McBeth, 2010. Iron-oxidizing bacteria: an environmental and genomic
316 perspective. *Annual Review of Microbiology* 64: 561-83 doi:10.1146/annurev.micro.112408.134208.

317 Garson, G. D., 2013. Generalized linear models/generalized estimating equations. Statistical Associates
318 Publishing, Asheboro, NC.

319 Gerhardt, A., 1992. Effects of subacute doses of iron (Fe) on *Leptophlebia marginata* (Insecta: Ephemeroptera).
320 *Freshwater Biology* 27: 79-84.

321 Gerhardt, A., 1993. Review of impact of heavy metals on stream invertebrates with special emphasis on acid
322 conditions. *Water, Air & Soil Pollution* 66: 289-314.

323 Ghiorse, W. C., 1984. Biology of iron- and manganese-depositing bacteria. *Annual Review of Microbiology* 38:
324 515-550.

325 Greenfield, J. P. & M. P. Ireland, 1978. A survey of the macrofauna of a coal-waste polluted Lancashire fluvial
326 system. *Environmental Pollution* 16: 105-122.

327 Hickey, C. W. & W. H. Clements, 1998. Effects of heavy metals on benthic macroinvertebrate communities in
328 New Zealand streams. *Environmental Toxicology and Chemistry* 17: 2338-2346.

329 Hill, B. H., W. T. Willingham, L. P. Parrish & B. H. McFarland, 2000. Periphyton community responses to
330 elevated metal concentrations in a Rocky Mountain stream. *Hydrobiologia* 428: 161-169.

331 Hirst, H., I. Jüttner & S. J. Ormerod, 2002. Comparing the responses of diatoms and macroinvertebrates to
332 metals in upland streams of Wales and Cornwall. *Freshwater Biology* 47: 1752-1765.

333 HMSO, 1978a. Iron in raw and potable waters by spectrophotometry (using 2, 4, 6-tripyridyl-1, 3, 5-triazine).
334 Her Majesty's Stationery Office, London.

335 HMSO, 1978b. Manganese in raw and potable waters by spectrophotometry (using formaldoxime). Her
336 Majesty's Stationery Office, London.

337 HMSO, 1980. Aluminium in raw and potable waters by spectrophotometry (using pyrocatechol violet). Her
338 Majesty's Stationery Office, London.

339 Hünken, A. & M. Mutz, 2007. On the ecology of the filter-feeding *Neureclipsis bimaculata* (Trichoptera,
340 Polycentropodidae) in an acid and iron rich post-mining stream. *Hydrobiologia* 592: 135-150
341 doi:10.1007/s10750-007-0735-1.

342 Jarvis, A. P. & P. L. Younger, 1997. Dominating chemical factors in mine water induced impoverishment of the
 343 invertebrate fauna of two streams in the Durham Coalfield, UK. *Chemistry and Ecology* 13: 249-270.
 344 Kimball, B. A., R. L. Runkel, K. Walton-Day & K. E. Bencala, 2002. Assessment of metal loads in watersheds
 345 affected by acid mine drainage using tracer injection and synoptic sampling: Cement Creek, Colorado,
 346 USA. *Applied Geochemistry* 17: 1183-1207.
 347 Konhauser, K. O., 1998. Diversity of bacterial iron mineralization. *Earth-Science Reviews* 43: 91-121.
 348 Lamberti, G. A. & W. Moore, 1984. Aquatic insects as primary consumers. In Resh, V. H. & D. M. Rosenberg
 349 (eds) *The ecology of aquatic insects*. Praeger, New York, 164-195.
 350 Lamberti, G. A. & V. H. Resh, 1985. Comparability of introducing tiles and natural substrates for sampling lotic
 351 bacteria, algae and macroinvertebrates. *Freshwater Biology* 15: 21-30.
 352 Layer, K., A. G. Hildrew & G. Woodward, 2013. Grazing and detritivory in 20 stream food webs across a broad
 353 pH gradient. *Oecologia* 171: 459-471 doi:10.1007/s00442-012-2421-x.
 354 Ledger, M. E. & A. G. Hildrew, 1998. Temporal and spatial variation in the epilithic biofilm of an acid stream.
 355 *Freshwater Biology* 40: 655-670.
 356 Ledger, M. E. & A. G. Hildrew, 2000. Herbivory in an acid stream. *Freshwater Biology* 43: 545-556.
 357 Ledger, M. E. & A. G. Hildrew, 2005. The ecology of acidification and recovery: changes in herbivore-algal
 358 food web linkages across a stream pH gradient. *Environmental Pollution* 137: 103-11
 359 doi:10.1016/j.envpol.2004.12.024.
 360 Letterman, R. D. & W. J. Mitsch, 1978. Impact of mine drainage on a mountain stream in Pennsylvania.
 361 *Environmental Pollution* 17: 53-73.
 362 Macintosh, K. A. & D. Griffiths, 2013. Catchment and in-stream influences on metal concentration and ochre
 363 deposit density in upland streams, Northern Ireland. *Environmental Earth Sciences* 70: 3023-3030
 364 doi:10.1007/s12665-013-2363-6.
 365 Macintosh, K. A. & D. Griffiths, 2014. Spatial and temporal influences of in-stream factors on the chemistry
 366 and epilithic biomasses of upland stream metal deposits. *Aquatic Sciences* 76: 331-338
 367 doi:10.1007/s00027-014-0338-7.
 368 Magurran, A. E., 1988. *Ecological diversity and its measurement*. Chapman & Hall, London.
 369 Malmqvist, B. & P. Hoffsten, 1999. Influence of drainage from old mine deposits on benthic macroinvertebrate
 370 communities in Central Swedish streams. *Water Research* 33: 2415-2423.

371 Maltby, L. & M. Crane, 1994. Responses of *Gammarus pulex* (Amphipoda, Crustacea) to metalliferous
 372 effluents: identification of toxic components and the importance of interpopulation variation.
 373 Environmental Pollution 84: 44-52.

374 Marker, A. F. H., C. A. Crowther & R. J. M. Gunn, 1980. Methanol and acetone as solvents for estimating
 375 chlorophyll *a* and phaeopigments by spectrophotometry. Archiv für Hydrobiologie Beiheft Ergebnisse
 376 der Limnologie 14: 52-69.

377 Mason, C. F., 1996. Biology of freshwater pollution, 3rd edn. Longman, Harlow.

378 Mayes, W. M., E. Gozzard, H. A. B. Potter & A. P. Jarvis, 2008. Quantifying the importance of diffuse
 379 minewater pollution in a historically heavily coal mined catchment. Environmental Pollution 151: 165-
 380 175 doi:10.1016/j.envpol.2007.02.008.

381 McCune, B. & M. J. Mefford, 2006. PC-ORD. Multivariate Analysis of Ecological Data. MjM Software,
 382 Gleneden Beach, Oregon, U.S.A.

383 McKnight, D. M. & G. L. Feder, 1984. The ecological effect of acid conditions and precipitation of hydrous
 384 metal oxides in a Rocky Mountain stream. Hydrobiologia 119: 129-138.

385 Mellanby, H., 1963. Animal life in fresh water, 6th edn. Chapman & Hall, London.

386 Merritt, R. W. & K. W. Cummins, 1996. An introduction to the aquatic insects of North America, 3rd edn.
 387 Kendall/Hunt Publishing Co., , Dubuque, Iowa.

388 Mihuc, T. B., 1997. The functional trophic role of lotic primary consumers: generalist var. specialist strategies.
 389 Freshwater Biology 37: 455-462.

390 Mulholland, P. J., C. T. Driscoll, J. W. Elwood, M. P. Osgood, A. V. Palumbo, A. D. Rosemond, M. E. Smith &
 391 C. Schofield, 1992. Relationships between stream acidity and bacteria, macroinvertebrates, and fish: a
 392 comparison of north temperate and south temperate mountain streams, USA. Hydrobiologia 239: 7-24
 393 doi:10.1007/BF00027525.

394 Murphy, J. & J. P. Riley, 1958. A single-solution method for the determination of soluble phosphorus in sea
 395 water. Journal of the Marine Biological Association UK 37: 9-14.

396 Murphy, J. & J. P. Riley, 1962. A modified single solution method for the determination of phosphate in natural
 397 waters. Analytica Chimica Acta 27: 31-36.

398 Neal, C., S. Lofts, C. D. Evans, B. Reynolds, E. Tipping & M. Neal, 2008. Increasing iron concentrations in UK
 399 upland waters. Aquatic Geochemistry 14: 263-288 doi:10.1007/s10498-008-9036-1.

400 Niyogi, D. K., D. M. McKnight & W. M. Lewis, 1999. Influences of water and substrate quality for periphyton
 401 in a montane stream affected by acid mine drainage. *Limnology and Oceanography* 44: 804-809.
 402 Nyberg, P., P. Andersson, E. Degerman, H. Borg & E. Olofsson, 1995. Labile inorganic manganese - an
 403 overlooked reason for fish mortality in acidified streams? *Water, Air and Soil Pollution* 85: 333-340.
 404 Peuranen, S., P. J. Vuorinen, M. Vuorinen & A. Hollender, 1994. The effects of iron, humic acids and low pH
 405 on the gills and physiology of brown trout (*Salmo trutta*). *Annales Zoologici Fennici* 31: 389-396.
 406 Pizarro, H. & A. Vinocur, 2000. Epilithic biomass in an outflow stream at Potter Peninsula, King George Island,
 407 Antarctica. *Polar Biology* 23: 851-857.
 408 Prange, H., 2007. Ochre pollution as an ecological problem in the aquatic environment: solution attempts from
 409 Denmark. Edmund Siemers-Stiftung, Hamburg.
 410 Quinn, G. P. & M. J. Keogh, 2002. Experimental design and data analysis for biologists. Cambridge University
 411 Press, Cambridge.
 412 Rasmussen, K. & C. Lindegaard, 1988. Effects of iron compounds on macroinvertebrate communities in a
 413 Danish lowland river system. *Water Research* 22: 1101-1108.
 414 Rentz, J. A., I. P. Turner & J. L. Ullman, 2009. Removal of phosphorus from solution using biogenic iron
 415 oxides. *Water Research* 43: 2029-2035 doi:10.1016/j.watres.2009.02.021.
 416 Rice, E. W., R. B. Baird, A. E. Eaton & L. S. Clesceri, 2012. Standard methods for the examination of water and
 417 wastewater, 22nd edn. American Public Health Association, Washington, D.C.
 418 Roden, E. E., 2012. Microbial iron-redox cycling in subsurface environments. *Biochemical Society Transactions*
 419 40: 1249-1256 doi:10.1042/BST20120202.
 420 Rosemond, A. D., S. R. Reice, J. W. Elwood & P. J. Mulholland, 1992. The effects of stream acidity on benthic
 421 invertebrate communities in the south-eastern United States. *Freshwater Biology* 27: 193-209.
 422 Rosenberg, D. M. & V. H. Resh (eds), 1993. Freshwater biomonitoring and benthic macroinvertebrates.
 423 Chapman & Hall, New York.
 424 Scullion, J. & R. W. Edwards, 1980. The effects of coal industry pollutants on the macroinvertebrate fauna of a
 425 small river in the South Wales coalfield. *Freshwater Biology* 10: 141-162.
 426 Sheldon, S. P. & D. K. Skelly, 1990. Differential colonization and growth of algae and ferromanganese-
 427 depositing bacteria in a mountain stream. *Journal of Freshwater Ecology* 5: 475-485.
 428 Sheldon, S. P. & T. A. Wellnitz, 1998. Do bacteria mediate algal colonization in iron-enriched streams? *Oikos*
 429 83: 85-92.

430 Smock, L. A., 1983. The influence of feeding habits on whole-body metal concentrations in aquatic insects.
 431 Freshwater Biology 13: 301-311.

432 Stubblefield, W. A., S. F. Brinkman, P. H. Davies, T. D. Garrison, J. R. Hockett & M. W. McIntyre, 1997.
 433 Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*).
 434 Environmental Toxicology and Chemistry 16: 2082-2089.

435 Stumm, W. & J. J. Morgan, 1996. Aquatic chemistry, 3rd edn. J Wiley & Sons, New York.

436 Sutcliffe, D. W. & A. G. Hildrew, 1989. Invertebrate communities in acid streams. In Morris, R., E. W. Taylor,
 437 D. J. A. Brown & J. A. Brown (eds) Acid toxicity and aquatic animals. Society for Experimental
 438 Biology Seminar Series. Cambridge University Press, Cambridge, 13-29.

439 Tebo, B. M., J. R. Bargar, B. G. Clement, G. J. Dick, K. J. Murray, D. Parker, R. Verity & S. M. Webb, 2004.
 440 Biogenic manganese oxides: properties and mechanisms of formation. Annual Review of Earth and
 441 Planetary Science 32: 287-328 doi:10.1146/annurev.earth.32.101802.120213.

442 Townsend, C. R., A. G. Hildrew & J. Francis, 1983. Community structure in some southern English streams: the
 443 influence of physicochemical factors. Freshwater Biology 13: 521-544.

444 Verb, R. G. & M. L. Vis, 2000. Comparison of benthic diatom assemblages from streams draining abandoned
 445 and reclaimed coal mines and nonimpacted sites. Journal of the North American Benthological Society
 446 19: 274-288.

447 Verberk, W. C. E. P., P. J. J. van den Munckhof & B. J. A. Pollux, 2012. Niche segregation in two closely
 448 related species of stickleback along a physiological axis: explaining multidecadal changes in fish
 449 distribution from iron-induced respiratory impairment. Aquatic Ecology 46: 241-248
 450 doi:10.1007/s10452-012-9395-y.

451 Vuori, K.-M., 1995. Direct and indirect effects of iron on river ecosystems. Annales Zoologici Fennici 32: 317-
 452 329.

453 Weitzel, R. L., S. L. Sanocki & H. Holecek, 1979. Sample replication of periphyton collected from artificial
 454 substrates. In Weitzel, R. L. (ed) Methods and measurements of periphyton communities. American
 455 Society for Testing and Materials, Philadelphia, 90-115.

456 Wellnitz, T. A., K. A. Grief & S. P. Sheldon, 1994. Response of macroinvertebrates to blooms of iron-
 457 depositing bacteria. Hydrobiologia 281: 1-17.

458 Wellnitz, T. A. & S. P. Sheldon, 1995. The effects of iron and manganese on diatom colonization in a Vermont
 459 stream. Freshwater Biology 34: 465-470.

460 Withers, P. J. A. & H. P. Jarvie, 2008. Delivery and cycling of phosphorus in rivers: A review. Science of the
461 Total Environment 400: 379-395 doi:10.1016/j.scitotenv.2008.08.002.
462 Woodcock, T. S. & A. D. Huryn, 2005. Leaf litter processing and invertebrate assemblages along a pollution
463 gradient on a Maine (USA) headwater stream. Environmental Pollution 134: 363-375.
464 Younger, P. L., 2001. Mine water pollution in Scotland: nature, extent and preventative strategies. Science of
465 the Total Environment 265: 309-326.

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468 **Tables**

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

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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

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Table 2 Pearson correlations between the first three axes of the CCA and deposit (_d) variables (interset) and the abundances of the main invertebrate taxa. Significant values ($P < 0.01$) are shown in bold. $n = 80$.

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

Table 3 Slope coefficients of generalized estimating equation outputs with (a) log. deposit density and (b) log. deposit phosphorus concentration as the predictor variable for quadratic (x and x²) and linear models. ΔQICC is the absolute difference in QICC values for quadratic and linear models: slope coefficients are shown only for the better model i.e. the one with the smaller QICC value.

	Quadratic		Linear	ΔQICC
Variable	x	x ²	x	
(a) x = Deposit density				
Autotrophic index (log ₁₀)	-0.401±0.609	0.759±0.313*		1.22
Algal biomass (log ₁₀)	1.660±0.629**	-0.856±0.324**		2.09
NPD biomass (log ₁₀)			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 ₁₀)			-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 ₁₀)	-3.475±1.203**	-1.107±0.361**		3.31
NPD biomass (log ₁₀)			1.043±0.094***	1.78

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4 Path analysis summaries of the effects of inorganic matter, NPD and algal biomass densities on invertebrate richness, abundance, diversity and biotic scores. The values are path coefficients. U are unexplained path effects ($= \sqrt{(1 - r^2)}$). $n = 75$.

		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		

* $P < 0.05$

Figure legends

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

Fig. 2 (a) Algal (*circles*) and NPD biomasses (*triangles*) (b) ASPT (*circles*) and the number of taxa (*triangles*) and (c) the Shannon (*circles*) and Berger-Parker (*triangles*) indices as functions of deposit density. Lowess smoothed lines (tension 0.7) are shown.

Fig. 3 Diagram showing path coefficients between the deposit components and total invertebrate abundance. Heavy lines indicate significant coefficients. The coefficients for the predictor variables are the Pearson correlation coefficients between these variables.

Fig. 1

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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**

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544 Hydrobiologia

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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.