Structure and function of food webs in acid mine drainage streams

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Ecology at the University of Canterbury by Kristy L. Hogsden

University of Canterbury
Christchurch, New Zealand
2013
Acid mine drainage (AMD) is a significant environmental issue worldwide, which often causes severe contamination and marked species losses in receiving streams. However, little is known about how this stress alters food webs and ecosystem function. I conducted a literature review, which revealed that AMD-impacted streams generally had depauperate benthic communities dominated by a few tolerant species and impaired ecosystem processes. Next, using survey and experimental-based approaches, I investigated food web structure and energy flow in these highly stressed streams, which typically have low pH (< 3), high concentrations of dissolved metals (Al, Fe), and substrata coated with metal hydroxide precipitates, on the South Island, New Zealand. Inputs of AMD caused substantial loss of consumers and reduced the overall number of links between species generating small and simplified food webs, with few invertebrates and no fish. Comparative analysis of food webs from a survey of 20 streams with either anthropogenic or natural sources of acidity and metals, indicated that anthropogenic sources had a stronger negative effect on food web properties (size, food chain length, number of links); an effect driven primarily by differences in consumer diversity and diet. However, the presence of fewer trophic levels and reduced trophic diversity (detected using isotopic metrics), were common structural attributes in AMD-impacted webs along a pH gradient, regardless of impact level. Furthermore, complementary dietary analyses of consumer gut contents and stable isotope signatures (δ\(^{13}\)C and δ\(^{15}\)N) confirmed that primary consumers fed generally on basal resources and that there were few predatory interactions, which reflected low densities of small-bodied chironomids. This suggests that food quantity was unlikely to limit primary consumers but that reduced prey
availability may be an additional stressor for predators. In these radically re-structured food webs, trophic bottlenecks were generated at the primary consumer level and energy flow to higher consumers was disrupted. However, streams still retained some limited function, including slow leaf litter breakdown, which provided detrital resources and supported the small food webs. Overall, my findings have furthered our understanding of these highly stressed stream ecosystems by providing new insights into interactions among species and trophic levels that structure food webs and enable function.
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Preface

Acid mine drainage (AMD) generated from mining activities is a prominent and complex environmental issue worldwide. The consequences of this highly acidic, metal-rich drainage for freshwater ecosystems are substantial (Gray 1997; Palmer et al., 2010). A considerable amount of research has documented the negative effects of AMD on benthic stream communities over the past 40 years; particularly, notable declines in the abundance, richness, and diversity of microbes, algae, invertebrates, and fish taxa (e.g., Koryak et al., 1972; Scullion and Edwards, 1980; McKnight and Feder, 1984; Schulthesis et al., 1997; Lear et al., 2009). Surprisingly, few studies have attempted to examine the links among species and trophic levels in streams receiving inputs of AMD, despite the importance of these interactions for energy flow and ecosystem function (Woodward, 2009).

Food webs are increasingly being used to expand our understanding of the wider effects of stress on stream ecosystems by focusing on the network of interactions among species (Layman et al., 2007a; Layer et al., 2010; Woodward et al., 2010). A food web perspective is useful because populations of species are critically linked to how their resources, prey and predators respond to stress. Stream food webs are usually comprised of a complex network of energy flow pathways highly connected by species interactions among multiple trophic levels. However, owing to the loss of sensitive species, removal of entire trophic levels, or reduced numbers of interactions among species, stressed food webs are expected to be less complex (Odum, 1985; McCann, 2000). New tools and approaches for detecting and assessing human impacts at the food web level are just
being proposed, developed, or tested in streams (Layman et al., 2007b; Crane et al., 2011; Thompson et al. 2012).

My research focuses on food web structure and function in streams affected by AMD in order to improve our understanding of these highly stressed ecosystems. Specifically, my objectives were to assemble complete food webs, relate changes in food web structure to ecosystem function, and test a recently proposed tool for detecting mine pollution. I carried out this work in the West Coast region of the South Island, New Zealand; where historic and current mine drainage inputs have impacted extensive river systems since the late 1800s (Harding and Boothroyd, 2004). Nearby naturally acidic and naturally high metal streams provided a unique opportunity for comparative analysis of food web attributes and ecosystem processes with AMD-affected streams.

Thesis structure and chapter outlines

This thesis was written as a series of stand-alone papers intended for publication. As such, there is some overlap in material presented in the introduction and method sections of some chapters. References and supplementary material are found at the end of each chapter, however additional appendices with unpublished maps and more detailed methods are found at the end of the thesis. The formatting style is consistent throughout the thesis, but the content of each chapter is the same as in the published or submitted papers. All chapters were co-authored, but the fieldwork, statistical analysis, and writing were primarily my own.

Chapter one is a review of the current state of knowledge concerning the impact of AMD on benthic stream communities. The complex and interactive chemical and physical stressors associated with AMD are known to influence the structure and function
of biological communities in different ways. However, what these substantial changes in each community mean for energy flow through the stream food web remains a significant gap in our knowledge. In this chapter, I brought together published information on the effects of AMD on the structure and function of algal, microbial, benthic invertebrate, and fish communities. Using this information, I assembled generalised food webs and encourage a more holistic approach to studying these stressed aquatic ecosystems.

Chapter two provided novel insights into the ecological effects of AMD on stream ecosystems by taking a food web approach and described, for the first time, the structure of food webs in these highly stressed environments. This approach built on our existing knowledge of community change in AMD-impacted streams by focusing on interactions between species and among trophic levels. An additional objective of this chapter was to compare food web structure in mining-impacted streams with those in streams receiving natural sources of acidity and metals.

Chapter three examined the relationship between food webs and leaf litter breakdown, a key ecosystem process that provides detrital food resources for primary consumers. Links between food web structure and ecosystem function are not often made despite the fact that modified food webs in human-impacted ecosystems disrupt energy flow pathways. In this chapter, I took a comparative approach and determined leaf breakdown rates, detrital resource biomass, and food web structure in mining-impacted, naturally acidic, and circumneutral streams. Streams affected by mining were of two types: those with highly acidic water (pH < 3) or those with metal precipitates on substrata.
Chapter four focused on the quantity and quality of basal resources and prey available for invertebrate consumers in AMD-affected streams. Factors that affect food quantity and quality can translate directly into effects on energy flow and can limit production of higher trophic levels in stream food webs. I used a gradient approach to evaluate potential changes in the availability and quality of food resources in twelve streams as pH declined with increasing AMD stress. I also examined whether consumer diets reflected these changes using a combination of complementary stable isotope and gut content analyses techniques.

Chapter five examined the potential use of recently proposed isotopic metrics (Layman et al., 2007b) as a tool for detecting and investigating the effects of mine pollution on streams. These quantitative metrics depict food webs based on the relative spacing of consumers in isotopic ($\delta^{13}C - \delta^{15}N$) space and describe different aspects of food web structure (e.g., trophic diversity). To do this, I compared the isotopic metrics with traditional structural and functional metrics used for stream biomonitoring and assessed changes in all metrics (15 in total: 5 isotopic, 5 structural, 5 functional) along an AMD pollution gradient that included twelve streams.

Chapter six is a general discussion, in which I summarise my overall findings, highlight key results from my thesis and suggest how they could provide new insights and inform management and restoration efforts of mining-affected streams.
References


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For my family
Chapter 1

Consequences of acid mine drainage for the structure and function of benthic stream communities: a review

Filamentous algae downstream of an abandoned adit, Sullivan’s Mine, Denniston.
Abstract

Streams affected by acid mine drainage (AMD) are highly stressed ecosystems that occur worldwide. These streams typically have low pH, high concentrations of dissolved metals, and substrata coated with metal hydroxide precipitates. This combination of chemical and physical stressors creates a challenging environment for aquatic biota. We provide a synthesis of the effects of AMD on stream food webs to provide a holistic perspective of these highly stressed ecosystems. First, we reviewed the effects of AMD on the structure and function of algal, microbial, invertebrate, and fish communities. We then used this published information to propose generalized food webs and identify areas for future research. In general, AMD-affected streams have depauperate communities that are dominated by a few tolerant species, and ecosystem processes (e.g., decomposition) are often impaired. Biota respond differently to the individual stressors (e.g., pH compared to precipitates), which may complicate remediation efforts that focus primarily on neutralizing acidity and removing metals from mine discharges. Food webs in these streams are substantially altered because basal resources are less productive or inaccessible, microbial processing of organic matter is slow, many grazers and shredders are absent, and fish are replaced by invertebrates as top predators. Structurally, declines in species diversity and the loss of fish shorten and simplify food webs by decreasing the number of interactions among species. Functionally, most energy pathways are weakened by disrupted trophic links, and this problem should be a key target of restoration efforts. We think research that focuses on species interactions in a foodweb context is needed to provide a better understanding of community organization and functioning in these highly stressed ecosystems.
**Key words:** AMD, stress, periphyton, microbial, invertebrate, fish, primary production, decomposition, ecosystem process, food web.

**Introduction**

The global increase in mining activities raises increasing concern about effects on stream ecosystems, our lack of understanding of mining’s complex ecological effects, and the need for effective mitigation and restoration techniques (Palmer et al. 2010). Acid mine drainage (AMD) is one of the most widely documented consequences of mining that affects freshwaters worldwide. AMD is often associated with coal, pyritic sulfur, Cu, Zn, Ag, and Pb mining operations. AMD runs off active and abandoned mine sites into surface and groundwater systems and causes widespread contamination that is exported downstream (Gray 1997). Streams receiving mine drainage are highly acidic (often pH < 3) with elevated concentrations of dissolved metals (e.g., Al, Cu, Fe, Zn) and, in many cases, substrata are coated with metal hydroxide precipitates.

AMD exerts chemical and physical stresses on stream biota, but separating the effects of acidity, metal toxicity, and habitat degradation on biota in streams receiving AMD inputs is a significant challenge for freshwater scientists. Authors of a number of studies focused on patterns in community composition and relative abundance have demonstrated the negative effects of AMD on benthic communities. However, surprisingly few investigators have examined ecosystem processes in these streams. Focusing on the network of interactions among species by adopting a foodweb approach may be an effective way to understand stressed ecosystems and to complement existing research methods (Culp et al. 2005, Woodward 2009). This approach is useful because it
focuses on the relationships between consumers and resources and may provide insights into direct and indirect effects of AMD on stream ecosystems. To our knowledge, a complete food web in an AMD-affected stream has not been published. To develop a perspective on food webs, we reviewed the published literature on the effects of AMD on the structure and function of microbial, algal, invertebrate, and fish communities in affected streams, and we present potential mechanisms responsible for these patterns. We then used this published information to assemble generalized food webs. In some cases, we also drew on insights provided in studies in similarly stressed (i.e., acidified or metal-contaminated) streams. We did not include macrophytes, bryophytes, and meiofauna in this review even though they are important components of stream food webs.

**AMD: General Chemistry**

AMD is formed when sulfide minerals and associated heavy metals are exposed to weathering processes during excavation of mineral deposits (Kelly 1988). Sulfuric acid is generated by the chemical oxidation of sulfides exposed to air (atmosphere) and water during excavation, reactions that are catalyzed by the activity of sulfur-oxidizing bacteria. Metals are leached from the disturbed substrata under these highly oxidized and acidic conditions. The composition and concentration of metals found in AMD are related to the mineral deposits from which the drainage originated (Lottermoser 2003). The chemical and physical characteristics of streams affected by AMD can vary widely (Table 1), and the degree of impact depends primarily the extent of dilution and buffering capacity of the receiving stream (Gray 1997). Thus, a large stream underlain by limestone would be less susceptible than a small headwater stream flowing over granitic bedrock or
sandstone. The availability and speciation of metals is linked to pH. For example, when AMD enters a stream with a higher pH, the solubility of some metals may be exceeded (Table 2). Under these conditions of reduced acidity, metal hydroxides can precipitate out of solution and coat the streambed in the form of loose flocs or tight plaques. Precipitation of ferric iron as ferric hydroxide, frequently termed yellow boy is a visible sign of AMD that occurs when pH is >~3.5 (Harding and Boothroyd 2004).

**Table 1** Chemical and physical characteristics of streams affected by acid mine drainage (AMD). These streams were selected from the literature to demonstrate the wide variation in characteristics of AMD in streams from different sources and do not necessarily reflect the condition of all AMD-affected streams from a particular region.

<table>
<thead>
<tr>
<th>Stream</th>
<th>Source</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Metal concentration (mg/L)</th>
<th>Metal hydroxides (present/absent)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peña de Hierro, Spain</td>
<td>Ag, Cu, various</td>
<td>1.5</td>
<td>20,500</td>
<td>3325.0 Fe(^a), 82.1 Zn(^b), 34.9 Cu(^a), 1.8 Pb(^b)</td>
<td>n/a</td>
<td>Sabater et al. 2003</td>
</tr>
<tr>
<td>St. Kevin’s Gulch, USA</td>
<td>Ag</td>
<td>2.7</td>
<td>n/a</td>
<td>80.0 Zn(^b)</td>
<td>Present</td>
<td>Niyogi et al. 2001</td>
</tr>
<tr>
<td>Miller Creek, New Zealand</td>
<td>Coal</td>
<td>2.9</td>
<td>944</td>
<td>16.5 Al(^b), 5.2 Fe(^b)</td>
<td>Absent</td>
<td>Winterbourn et al. 2000</td>
</tr>
<tr>
<td>Mosteirão, Portugal</td>
<td>Cupriferous pyrite</td>
<td>3.0</td>
<td>n/a</td>
<td>1.4 Cu(^a), 2.3 Fe(^a), 0.2 Pb(^a)</td>
<td>n/a</td>
<td>Gerhardt et al. 2004</td>
</tr>
<tr>
<td>Gamble Gulch, USA</td>
<td>Ag</td>
<td>3.8</td>
<td>831</td>
<td>1.1 Zn(^b)</td>
<td>Present</td>
<td>Niyogi et al. 2009</td>
</tr>
<tr>
<td>Neubecks Creek, Australia</td>
<td>Coal</td>
<td>5.1</td>
<td>787</td>
<td>~0.2 Ni(^b), ~0.3 Zn(^b)</td>
<td>Present</td>
<td>Battaglia et al. 2005</td>
</tr>
<tr>
<td>Burnetts Stream, New Zealand</td>
<td>Coal</td>
<td>4.1</td>
<td>77</td>
<td>0.4 Al(^b), 0.7 Fe(^b)</td>
<td>Absent</td>
<td>Winterbourn et al. 2000</td>
</tr>
<tr>
<td>Slippery Rock Creek, USA</td>
<td>Coal</td>
<td>6.3</td>
<td>690</td>
<td>0.4 Fe(^b), 0.1 Al(^b), 0.07 Zn(^b)</td>
<td>Present</td>
<td>DeNicola and Stapleton 2002</td>
</tr>
<tr>
<td>Zerbe Run, USA</td>
<td>Coal</td>
<td>6.3</td>
<td>524</td>
<td>7.1 Fe(^b), 0.5 Al(^b)</td>
<td>Present</td>
<td>MacCausland and McTammany 2007</td>
</tr>
</tbody>
</table>
In addition, streams associated with active mine sites may be turbid because of suspended metal precipitates or inorganic material (e.g., coal fines). On the other hand, streams downstream of abandoned mines or adits (underground mine entrances) often appear clear because metals remain dissolved under highly acidic conditions. Metals can cycle between particulate and dissolved forms many times as they move downstream. Defining criteria that identify the level of AMD impact (e.g., mild, moderate, severe) on streams may be difficult because of the complex nature of chemical and physical stress associated with different dissolved metals and metal hydroxide precipitates and variation in natural pH ranges between regions.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Minimum pH for precipitate formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sn</td>
<td>4.2</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>3.5–4.3</td>
</tr>
<tr>
<td>Al</td>
<td>4.9–5.4</td>
</tr>
<tr>
<td>Pb&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>6.3</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>7.2</td>
</tr>
<tr>
<td>Zn</td>
<td>8.4</td>
</tr>
<tr>
<td>Ni</td>
<td>9.3</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>9.5</td>
</tr>
<tr>
<td>Cd</td>
<td>9.7</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>10.6</td>
</tr>
</tbody>
</table>
Effects of AMD on Benthic Communities

Periphyton

Periphyton is highly responsive to increased acidity and metal hydroxides that coat the streambed in AMD-affected streams (Douglas et al. 1998, Niyogi et al. 1999, Verb and Vis 2001, 2005, DeNicola and Stapleton 2002). Several investigators have shown that algal species richness and diversity are consistently low in the presence of AMD (Fig. 1) because acid-sensitive species are replaced by a few species of tolerant diatoms (e.g., *Eunotia* spp.) or filamentous green algae (e.g., *Klebsormidium* spp.) (Douglas et al. 1998, Verb and Vis 2000, Bray et al. 2008). Verb and Vis (2005) modelled species data from 56 streams and showed that pH was a strong predictor of periphyton community composition. However, this predictability tends to decline with decreasing stress along AMD impact gradients as other abiotic and biotic factors increasingly structure the community (Verb and Vis 2001, 2005, Bray et al. 2008). Species loss often is attributed to physiological sensitivity to low pH and high concentrations of dissolved metals. Tolerant species survive by reducing proton influx ($H^+$ ions), increasing proton pump efficiency, and forming metal complexes that prevent entry of metals into cells (Gross 2000, Novis and Harding 2007). Several authors have suggested that indirect effects of pH, which changes habitat quality through precipitation of metal hydroxides, may explain low species richness because deposits can coat streambed surfaces, smother algae, reduce light penetration, and decrease sites for colonization (McKnight and Feder 1984, Niyogi et al. 1999).

Algal biomass can vary substantially in AMD-affected streams. High biomass usually indicates dominance of large-celled filamentous green algae instead of diatoms.
Chapter 1 – Benthic communities in AMD streams

(Verb and Vis 2000, Bray et al. 2008). Tolerant filamentous green algae, such as *Ulothrix*, can proliferate when metals remain dissolved in highly acidic waters (pH < 3) and adequate light creates ideal conditions for growth downstream of abandoned mine adits (Winterbourn et al. 2000) or under conditions of improved water and substrate

quality after experimental diversion of AMD (Niyogi et al. 1999). On occasion, tolerant algal species may be abundant, possibly reflecting a preference for low pH or reduced interspecific competition for nutrients, light, or space (Sabater et al. 2003, Novis and Harding 2007). Some authors have speculated that reduced grazing pressure because of low density (or absence) of key grazers or the inability of tolerant grazers to consume dominant algae may lead to high algal biomass, but these interactions have not been tested in AMD-affected streams (Niyogi et al. 2002a, Bray et al. 2008). Low algal biomass has been reported commonly in the presence of metal hydroxides (McKnight and Feder 1984, Verb and Vis 2000, Bray et al. 2008) even when rates of deposition are low (Niyogi et al. 1999). Biomass was almost 50% lower at sites with ferric hydroxide deposition than at sites where it was absent (Sode 1983). Metal hydroxide deposits can suppress biomass by direct toxicity or by smothering the algae. Some metal deposits are more detrimental to algae than others (e.g., Al > Fe; Niyogi et al. 1999), but the mechanism for this difference is not clear. In field studies, periphyton biomass was more negatively affected by physical stress from metal hydroxides than by pH or metals (McKnight and Feder 1984, Niyogi et al. 2002a). High variability in algal biomass that could not be traced to AMD has been observed in some studies. These findings highlight the importance of considering other factors that might influence algal communities (e.g., nutrients, grazing, interspecific competition; Verb and Vis 2000, Sabater et al. 2003) when evaluating the effects of AMD.

Metal hydroxide deposition has been implicated as a cause of reduced primary production, but this key ecosystem process has been investigated in only a limited number of studies. Sode (1983) and Niyogi et al. (2002a) observed marked declines in
primary production at sites with high ferric iron and aluminium hydroxide precipitates, respectively. In 58 streams distributed along an AMD impact gradient, the rate of hydroxide deposition explained 50% of the variation in net primary productivity (<0.1–54 mg C m\(^{-2}\) h\(^{-1}\)) (Niyogi et al. 2002a). Moreover, along this AMD gradient, tolerant species were able to compensate to maintain function (and biomass) under chemical (pH, dissolved metals) but not physical (metal hydroxide deposits) stress. Production was low even at moderate levels of physical stress (Niyogi et al. 2002a). Primary production also may be limited by low bicarbonate concentrations and by diffusion of carbon dioxide into the atmosphere that increases as acidity rises in the water column of streams receiving AMD (Allan and Castillo 2007). Breakdown of the bicarbonate buffering system in acidified waters effectively reduces the amount of dissolved inorganic C (DIC) available for photosynthetic activity and benthic algal growth. For example, Fonyuy and Atekwana (2008) demonstrated the transformation of DIC to CO\(_2\) gas in AMD streams and reported seasonal losses of 50 to 98% of DIC. However, species able to use DIC at low concentrations can thrive despite this loss, as shown by metaphytic blooms of filamentous green algae in an acidified lake with low DIC (82 µmol/ L; Turner et al. 1987). Algal production also may be limited by other factors in AMD streams, including nutrient availability, light, flow, and grazing pressure. Some evidence suggests that AMD streams are nutrient poor (Hamsher et al. 2002, Novis and Harding 2007) because of low concentrations of inorganic N (<40 µg/L; P. M. Novis, Landcare Research, unpublished data) or precipitation of orthophosphates in acidic waters with high concentrations of Al (Gross 2000). However, high concentrations of dissolved inorganic N (>200 µg/L) and dissolved reactive P (>40 µg/L) have been recorded in some highly acidic AMD streams.
(D. K. Niyogi, University of Missouri, unpublished data). Clearly, significant gaps exist in our understanding of how primary production is affected by AMD and how nutrient availability and other controlling factors may influence the process in affected streams.

Bacteria and fungi

Microbial community structure and activity respond strongly to AMD. For the most part, microbial diversity, richness, and biomass are lower in streams receiving AMD relative to unaffected streams (Fig. 1). However, diverse and morphologically complex assemblages of bacteria, including Proteobacteria, Nitrospira, Firmicutes, and Acidobacteria, have been described on substrata in acidic, metal-rich AMD in both surface (López-Archilla et al. 2001, 2004) and subsurface waters (Baker and Banfield 2003). A diverse array of fungi, from yeasts to filamentous forms, also have been observed in AMD surface waters (Bridge Cooke 1976, López-Archilla et al. 2001, Amaral Zettler et al. 2002). However, fungal communities in AMD-affected and unaffected reaches or streams have been compared in only a few studies. In these experimental studies, fungal diversity estimated from conidia identification or production was lower on leaf litter conditioned in affected than in unaffected streams (Bermingham et al. 1996a, Niyogi et al. 2002b, 2009). Which factors drive community structure in these systems is unclear because information on bacterial and fungal communities is so scarce. However, some evidence suggests that pH may play a key role in determining microbial community composition. In a recent survey of 17 streams across an AMD gradient, bacterial communities in epilithic biofilm were less diverse in very acidic streams (pH < 3.5) and, based on analysis of deoxyribonucleic acid (DNA) sequences,
differences in community structure were driven by low pH (Lear et al. 2009). Low pH can limit the growth of some microbes, but it stimulates the growth of certain iron-oxidizing bacteria (e.g., *Gallionella*), which use available ferric iron for metabolism (Baker and Banfield 2003). Results of several studies suggest that high abundances of tolerant microbes in AMD streams may be caused by a physiological tolerance of acidic conditions; the absence of shredding invertebrates, which reduce leaf-litter surfaces for colonization or directly consume fungi; or the presence of filamentous green algae, which provides increased surface area for attachment and labile dissolved organic C (DOC) for bacterial growth (Niyogi et al. 2001, Sabater et al. 2003, Lear et al. 2009).

Microbial activity in AMD streams appears to be controlled primarily by the deposition of metal hydroxides, which accumulate as loose flocs or tight plaques on streambed substrata and limit colonization and respiration. Metal hydroxide deposits can reduce substrate availability and limit microbial colonization by coating leaf litter (Gray and Ward 1983, Siefert and Mutz 2001) or reducing periphyton biomass (Niyogi et. al 2002a). Thus, high microbial activity is restricted to periods before hydroxide deposits cover organic matter (Siefert and Mutz 2001, Schrief 2004, Schrief and Mutz 2005). In experimental studies of microbial respiration on leaf litter, breakdown rates consistently decline over time in the presence of metal hydroxide deposits (e.g., 20% in 7 wk, Schrief 2004; 60% in 8 mo, Schrief and Mutz 2005). Similar respiration rates have been reported for leaves coated with loose flocs (Niyogi et al. 2001, 2002b) and tight plaques (Schrief 2004), but the highest rates were always recorded at sites with low deposition. However, Niyogi et al. (2002b) found that respiration did not correspond to fungal biomass. This result was consistent with results obtained by Schrief and Mutz (2005), who observed that
leaves from sites with high metal hydroxide deposits had low respiration and moderate fungal biomass. Schleif and Mutz (2005) suggested that hydroxide deposits negatively affect respiration, but also might serve as substrate for colonization by certain species of bacteria or fungi. In the absence of metal hydroxides, microbial activity varies in response to high concentrations of different dissolved metals. Respiration rates were high in an affected stream with high concentration of dissolved Zn (Niyogi et al. 2001). However, respiration rates were lower in the presence of elevated levels of Fe and Ni than at unaffected sites (Bermingham et al. 1996a). The role of reduced microbial activity in litter decomposition has been studied (see Synthesis – A Food Web Perspective below), but consequences for organic matter retention and export to downstream communities have not yet been addressed.

**Invertebrates**

Invertebrate species richness, diversity, and abundance are frequently reduced in streams receiving AMD (Fig. 1). Communities often are numerically dominated by acid-tolerant species of chironomids, beetles, and true bugs, while sensitive species of mayflies, caddisflies, and molluscs are excluded (e.g., Koryak et al. 1972, Gray 1998, Winterbourn 1998). In many severely affected streams, either only a few species remain (Winterbourn 1998, Cherry et al. 2001, Battaglia et al. 2005) or invertebrates are completely absent (Soucek et al. 2003). When AMD enters an unaffected waterway, changes in community structure usually are attributed to rapid and substantial shifts in pH and metal concentrations that exceed the physiological tolerance limits of many species. Stress and mortality are caused by impaired regulation of ions and metabolically active
metals (Herrmann et al. 1993, Rainbow 2002). These toxic effects can occur in streams receiving short-term episodic inputs (Soucek et al. 2000a, MacCausland and McTammany 2007) or in chronically affected streams (Letterman and Mitsch 1978, Schultheis et al. 1997, Battaglia et al. 2005). In streams with pH > 3.5 where some metals (e.g., Fe) are insoluble, low invertebrate richness and abundance may be caused, in part, by loss of habitat and refugia as substrata are covered by metal hydroxides (Warner 1971, Koryak et al. 1972, Gray 1998, McKnight and Feder 1984). Some evidence indicates that species richness may be limited more by loose, unstable flocs than by tight plaques (DeNicola and Stapleton 2002, MacCausland and McTammany 2007). Furthermore, metal hydroxides can be directly toxic to invertebrates by clogging gill surfaces (e.g., Soucek et al. 2000b). Results from experimental field studies suggest that water chemistry is a stronger predictor than metal precipitates on stream substrata (DeNicola and Stapleton 2002) or contaminated sediments (Cherry et al. 2001, Battaglia et al. 2005) of the distribution and colonization of invertebrates. However, invertebrate responses to metals in water and in substrate are complex and species-specific (Courtney and Clements 2002).

If the regional species pool contains acid- or metal-tolerant species, then total invertebrate species diversity can remain comparable to that in unaffected streams. For example, Gerhardt et al. (2004) reported compensatory replacement of crustaceans, molluscs, and mayflies by beetles and true bugs and increased species diversity downstream of an abandoned cupriferous pyrite mine relative to a nearby reference site. Results of other studies suggest that life-cycle stage, feeding strategy, or reduced competition or predation may be important in understanding the dynamics of tolerant
species in AMD-affected streams. For example, acid-tolerant crayfish (*Cambarus* spp.) present in AMD-affected streams where pH was above established lethal limits had increased sensitivity to low pH and intermediate to high levels of dissolved metals during molting (Gallaway and Hummon 1991). Therefore, levels of AMD contamination that are otherwise tolerable can restrict molting success or be lethal during that period. Hünken and Mutz (2007) suggested that feeding on small particles retained in nets coated by metal hydroxides and reduced competition may have contributed to high abundances of a passive filter-feeding caddisfly (*Neureclipsis bimaculata*) in a mining-affected stream.

Results of several studies suggest that predators become numerically dominant in AMD-affected streams, whereas the abundances of other functional groups (filter feeders, collector-gathers, scrapers, and shredders) tend to decrease (Schultheis et al. 1997, Gerhardt et al. 2004, Barnden and Harding 2005; but see Hünken and Mutz 2007). Similar shifts in functional feeding groups have been observed in acidic (Townsend et al. 1983, Mulholland et al. 1992) and metal-contaminated streams (Clements et al. 2000), except that the decline in shredders varies from the widely observed pattern of replacement of herbivores by detritivores in low-pH streams (e.g., Guerold et al. 1995). Tolerant invertebrate predators can dominate highly acidic (pH ≈ 3) stream communities (Gerhardt et al. 2004) or those with abundant Fe floc and precipitates (Barnden and Harding 2005). The absence of fish from AMD-affected streams may promote the dominance of tolerant invertebrate predators and suppress invertebrate prey, as occurs in acidic streams (Hildrew et al. 1984). Kiffney (1996) demonstrated increased predation intensity by stoneflies and increased prey vulnerability in experimental AMD stream channels. Alternatively, reduced interspecific competition subsequent to losses of
sensitive predator species or an ability to feed on available prey may explain dominance of tolerant predators. The low abundance of most primary consumers may indicate that the quantity and quality of food resources available for invertebrates is altered in AMD-affected streams. Several investigators have suggested that low periphyton biomass associated with metal hydroxide deposits might reduce food availability or accessibility for grazers, collector-browsers, and scrapers (McKnight and Feder 1984, Niyogi et al. 2002a). Food quality may be affected by high concentrations of metals, which accumulate in periphyton, plant material, bacteria, and fungi (Bermingham et al. 1996b, Winterbourn et al. 2000). Uptake and accumulation of metals in invertebrates varies among metals and among taxa and can reflect differences in feeding habits (Beltman et al. 1999, Besser et al. 2001, Winterbourn et al. 2000). For example, Besser et al. (2001) found elevated concentrations of Zn, Cu, and Cd in a mayfly (Rhitrogena) that consumes periphyton, but the highest concentrations of Pb in a detritivorous stonefly (Zapada). Diet is clearly an important pathway for metal exposure in invertebrates, even when concentrations of dissolved metals are low in the water column (e.g., Fe; Winterbourn et al. 2000). However, whether metal concentration affects the palatability of these basal resources is not clear. A shredder (Gammarus pulex) consumed leaf litter coated with iron hydroxides at the same rate as leaves conditioned in neutral stream waters in a laboratory feeding experiment, a result suggesting that palatability was not affected by the presence of metal hydroxides (Schlief and Mutz 2006). Fe adsorbed to organic material may have increased the palatability of these leaves (Schlief and Mutz 2006) or Fe-loving bacteria present on the hydroxide surface might be a suitable food resource for some consumers. The relative importance of resource quantity and quality in
structuring invertebrate communities in AMD-affected streams requires further investigation.

Fish

Fish typically are absent from AMD-affected streams, particularly when pH < 5. However, if fish are present, their richness and abundance are always reduced (e.g., Sullivan and Gray 1992, Rutherford and Mellow 1994, Besser et al. 2001; Fig. 1). Eels (Gray 1998, Greig et al. 2010), centrarchids (Schorr and Backer 2006), and brown trout (Scullion and Edwards 1980, McCormick et al. 1994) have been recorded from AMD-affected streams. Schorr and Baker (2006) found that pH accounted for >70% of the variation in species richness and abundance, a result suggesting that acidity was the primary driver of fish communities in AMD-affected streams. However, if fish are locally adapted to tolerate low-pH waters, then the influence of metals might increase. Greig et al. (2010) showed that dissolved metals (including Fe, Al, Zn, Mn, and Ni) had the strongest negative effect on fish and that pH was a poor predictor of community structure in a survey of fish communities in AMD-affected (pH > 3.1), naturally acidic (pH = 4.3–6.0), and circumneutral (pH > 6.2) streams.

Direct toxicity of low pH and metals, accumulation of metal hydroxides on gill surfaces, streambed degradation, reduced egg viability, decreased food availability, or a metal-contaminated diet have been suggested as potential mechanisms for reduced or absent fish populations in AMD-affected streams (Letterman and Mitsch 1978, Scullion and Edwards 1980, Besser et al. 2001, Greig et al. 2010). Acidity and high concentrations of dissolved metals directly impair ion regulation and interfere with respiration in fish
and lead to decreased fitness or death (Baker and Schofield 1982, Wendelaar Bonga 1997, Pane et al. 2004). Metal hydroxides, particularly Al, negatively affect respiration by adhering to and clogging gills (Rosseland et al. 1992). Rapid accumulation of Al precipitates on gills and increased mortality of bluegills (*Lepomis macrochirus*) was linked to freshly mixed AMD-contaminated and circumneutral stream water in experimental stream channels (Henry et al. 2001). These results highlight the potential for heightened toxicity to fish downstream of the confluence of AMD-affected with unaffected streams. Deposition of metal hydroxides and sediments associated with mine drainage also can reduce availability and quality of fish habitat by coating the stream bed, which can negatively affect spawning and reproductive success. For example, accumulation of coal wastes and sediments accounted for a 30% reduction in spawning area and high mortality (>97%) of rainbow trout eggs (Scullion and Edwards 1980). Reduced fish density also has been attributed to low food availability and diversity rather than direct metal toxicity (Letterman and Mitsch 1978). Scullion and Edwards (1980) suggested that the change in fish density upstream (0.18/m$^2$) and downstream (0.03/m$^2$) of coal-mine discharge was linked to reduced availability of food because burrowing oligochaetes and chironomids buried in thick metal deposits dominated the downstream benthic invertebrate community. Fish in these polluted reaches fed almost exclusively on terrestrial insects (97% of stomach contents by volume), whereas fish in unpolluted reaches fed predominantly on aquatic invertebrates (86%). Even if food is not limited, accumulation of metals in invertebrates might be toxic to fish (Woodward 1995, Besser et al. 2001). Last, fish may actively avoid waters with high metal concentrations (e.g., Åtland 1998) resulting in low densities or absence from contaminated waters.
Synthesis: A Foodweb Perspective

A foodweb analysis has the potential to provide novel insights into community dynamics and ecosystem function in stressed ecosystems by focusing attention on interactions among species and among trophic levels. This perspective is useful because populations are dependent on how their resources, prey, and predators respond to stress. To our knowledge, no studies have been published that describe a complete food web in an AMD-affected stream, although a highly resolved stream food web has been described for an acidic stream in the UK (Schmid-Araya et al. 2002). Therefore, we assembled 3 generalized food webs that represent likely scenarios in AMD-affected streams (Fig. 2A–C).

Food webs are expected to become shorter and simpler under stress because of loss of sensitive species, declines in community diversity, or removal of entire trophic levels (e.g., top predators) (Odum 1985, McCann 2000). Therefore, we truncated and simplified our proposed food webs in accordance with observed AMD-related changes in the abundance and composition of the algae, invertebrate, and fish communities. Short food chains are maintained by intense and frequent stress and reduced resource availability (Post 2002). The severe and usually chronic nature of AMD reduces algal and benthic invertebrate diversity and eliminates fish (Fig. 2A, B). Furthermore, filamentous green algae, which can dominate the periphyton (Fig. 2A), or detritus coated by metal hydroxide deposits (Fig. 2B) may be physically inaccessible for grazing invertebrate consumers able to tolerate AMD conditions. The reduced availability of these basal resources and low numbers of invertebrate prey in AMD-affected streams places further restrictions on food-chain length by limiting the quantity and efficiency of energy transfer.
to higher trophic levels. In these food webs, fewer links connect species because fewer species are present (Fig. 2A, B). Reduced species diversity and a decreased number of connections among species results in simplified foodweb structure (Pimm 1982, Townsend et al. 1998, McCann 2000). In mildly affected streams (e.g., less acidic, low metal concentrations), food-chain length, foodweb size, and complexity should be higher relative to in strongly affected streams because of the presence of fish and increased invertebrate diversity (Fig. 2C). As a result, some energy pathways will be maintained (e.g., fish predation) but they might be inefficient. If invertebrate communities are impoverished or consist of species that can evade fish predation (e.g., burrowing chironomids or oligochaetes) then fish may rely increasingly on terrestrial arthropods as a food resource (Fig. 2C). We would expect energy-flow pathways between many consumers and their basal resources or prey to be weakened or disrupted in AMD-affected streams because of these substantial changes in foodweb structure.

Interpretation of these proposed food webs is limited by the lack of information on biotic interactions and ecosystem processes in these systems. Patterns observed in foodweb studies from other stressed ecosystems show different trends. For example, evidence from acid-stressed streams has shown that generalism and flexibility in feeding strategies can produce food webs with few species but many links (Hildrew 2009). Ledger and Hildrew (2001) reported that a predominantly detritivorous stonefly (*Nemurella pictetii*) grazed algae in an acidic stream lacking specialist grazers. In addition to shifts in feeding strategies, the presence of tolerant species and compensatory species replacements can maintain ecosystem processes despite the loss of certain species and truncation or simplification of the stream food web (Ledger and Hildrew 2005,
Hildrew 2009). In contrast, if sensitive species are dominant contributors then ecosystem processes may be substantially altered. For example, leaf-litter breakdown rates were significantly reduced in Zn-contaminated streams because of low production (or absence) of a metal-sensitive shredder (*Taenionema pallidum*), which plays a key role in this process (Carlisle and Clements 2005). Furthermore, under conditions of extreme stress so many species are lost that compensation by tolerant species is not possible and processes are completely disrupted.

![Food webs in streams affected by acid mine drainage (AMD). A.—Severely affected stream with pH < 3 and high concentrations of dissolved metals. B.—Severely affected stream with metal hydroxide deposits present. C.—Mildly affected stream. Broken lines signify disrupted interactions. Basal resources have been simplified into functional groups. Boxes around basal resources in panel B indicate resources are covered by metal hydroxide deposits. FISH1 = fish species 1; IPx = invertebrate predator species x; GRx = grazer species x; SHx = shredder species x; TAx= terrestrial arthropod species x; FGA = filamentous green algae.](image_url)
By taking a foodweb perspective, we can recognize more clearly the importance of modified interactions among communities that affect ecosystem processes. For example, reduced rates of leaf-litter breakdown (up to 50%) have been observed in experimental studies in AMD-affected streams (Gray and Ward 1983, Schultheis et al. 1997, Niyogi et al. 2001, Siefert and Mutz 2001). Deposits of metal hydroxides inhibit microbial access to litter, but decreased microbial activity in these streams usually occurs in combination with a decline in shredding invertebrates (Schultheis et al. 1997, Niyogi et al. 2001). The interplay between microbes and invertebrates is critical in this process, and if significant amounts of litter remain undecomposed and accumulate in the stream, then energy transfer and nutrient cycling processes will be limited.

Conclusions

Mine drainage generated from current and historic mining activities radically alters receiving streams by introducing highly acidic waters with high concentrations of dissolved metals and metal hydroxides that are deposited and coat substrata. This complex combination of stressors causes substantial changes in the structure and function of benthic communities and food webs. Diversity, richness, and abundance of algal, microbial, invertebrate, and fish communities decrease consistently in response to AMD. Exceptions occur when abundances of tolerant taxa increase (e.g., filamentous chlorophytes). Primary production, microbial respiration, and decomposition also decrease consistently, but few studies of these ecosystem processes have been published for AMD-affected streams. Differential responses of biota to the individual stressors imposed by AMD (acidity, dissolved metals, and metal hydroxide deposits) are evident in
measures of community structure (diversity, richness, abundance), whereas metal hydroxide deposits are always linked to reduced rates of ecosystem processes.

Few investigators have considered the mechanisms leading to the observed negative effects of AMD in stream ecosystems. In particular, altered biotic interactions have been suggested frequently but tested rarely. For example, under some circumstances, reductions in fish populations could be mediated by low resource availability rather than direct toxicity, or the accrual of filamentous green algae may be a result of release from grazing pressure instead of a direct response to high acidity (Scullion and Edwards 1980, Niyogi et al. 2002a). Tests of the strength, frequency and relative importance of interactions between consumers and resources are needed (e.g., grazing and predation). Tests could be extended to include multiple trophic levels to reveal more information about interactions in the system as a whole (Woodward et al. 2009).

Tolerant species in every community in AMD-affected streams could be limited by the quantity, and in some cases the quality, of food resources. Little is known about the quality (e.g., C:N:P ratios, lipid content) of algal and detrital resources and assimilation of these resources by tolerant primary consumers. Invertebrates certainly are exposed to metals via consumption of metal-contaminated basal resources, but whether or how sequestration affects food quality and palatability is not known. The importance of resource subsidies to and from AMD-affected streams and adjacent habitats has not been investigated adequately. If in-stream food resources are limited in AMD-affected streams, then terrestrial subsidies (e.g., arthropods) may help support aquatic communities (Scullion and Edwards 1980). Conversely, loss of the reciprocal subsidy of adult insects
from AMD-affected streams could negatively affect food webs in the surrounding terrestrial habitat (Burdon and Harding 2008). Similarly, the importance of connections with the hyporheic zone in AMD-affected streams has rarely been studied, despite the fact that this interface may provide a valuable source of organic C and nutrients. Limited evidence indicates that AMD contaminates the hyporheic zone in the same way it does surface waters (Nelson et al. 1993, Gray1996, Anthony 1999). Further work is needed to understand the importance of the flow of nutrients, detritus, prey, and consumers within AMD-affected stream food webs and between food webs in AMD-affected streams and adjacent ecosystems and habitats. Factors that affect food quantity and quality translate directly into effects on energy flow and consumer production.

The strong negative effects of AMD on stream communities are well documented for some communities (e.g., invertebrates) but not for others. The paucity of studies of AMD effects on microbes, macrophytes, meiofauna, and tertiary consumers (small mammals, amphibians, birds) is a significant gap in our understanding of these systems, particularly from a foodweb perspective. Potentially toxic water chemistry and reduced food resources are likely to affect other top consumers severely. Such an effect has been manifested as poor breeding performance of a songbird (*Seiurus motacilla*) along acidic streams in the Appalachians (Mulvihill et al. 2008). Drastic community changes can occur in a stream food web when just one species is lost because of stress, and these effects propagate throughout the food web. In AMD-affected streams, many species are lost concurrently, and this simultaneous impact might be the cause of the rapid and almost complete collapse of foodweb structure in some streams (Gray 1997).

Our review brings together the effects of AMD on the structure and function of
benthic communities, offers a foodweb perspective, and identifies biotic interactions and ecosystem processes as key areas for future research in AMD-affected streams. Our review also highlights the importance of considering the effects of metal hydroxides, which negatively affect each community but often are not considered in remediation efforts. We suggest that future investigators place a greater emphasis on a foodweb approach that incorporates both structural and functional attributes to assess the effects of AMD on stream ecosystems. This strategy should provide new and better insights into these stressed environments and how to restore them as functioning ecosystems, particularly as mining activities continue to increase worldwide.

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Chapter 2

Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs

Acid mine drainage from abandoned mine adits flows into Canel Creek, north of Greymouth.
Abstract

We compared food web structure in 20 streams with either anthropogenic or natural sources of acidity and metals or circumneutral water chemistry in New Zealand. Community and diet analysis indicated that mining streams receiving anthropogenic inputs of acidic and metal-rich drainage had much simpler food webs (fewer species, shorter food chains, less links) than those in naturally acidic, naturally high metal, and circumneutral streams. Food webs of naturally high metal streams were structurally similar to those in mining streams, lacking fish predators and having few species, whereas, webs in naturally acidic streams differed very little from those in circumneutral streams due to strong similarities in community composition and diets of secondary and top consumers. The combined negative effects of acidity and metals on stream food webs are clear. However, elevated metal concentrations, regardless of source, appear to play a more important role than acidity in driving food web structure.

Key words: acid mine drainage (AMD); food web; acidity; heavy metals; conductivity

Introduction

Human activities, such as the combustion of fossil fuels and mining activities, have caused widespread acidification and metal contamination of freshwater ecosystems in many parts of the world. These anthropogenic activities accelerate the release of acidifying substances and increase leaching and mobility of metals that have resulted in substantial species loss and marked changes in ecosystem processes (Driscoll et al., 2001; Palmer et al., 2010). However, freshwater ecosystems with natural sources of acidity and metals also occur globally. Where acidity arises naturally in streams from the leaching of
organic acids in soils or in poorly buffered catchments, the extent of species loss and changes in function are far less pronounced and in some places, biodiversity and ecosystem processes can be maintained (Collier et al., 1990; Petrin et al., 2008; Greig et al., 2010). Similarly, background metal levels can also be naturally elevated in freshwaters as metals enter surface waters through natural weathering of bedrock or soils (Garrett, 2000; Kelley and Hudson, 2007). The dissolution of metals by weathering tends to occur at a slow rate and concentrations of dissolved metals in freshwaters vary depending on the underlying geology. Relatively few studies have examined the influence of naturally high metal concentrations on the structure and function of stream communities and it is not known if or how they differ from communities in streams receiving anthropogenic inputs of metals. However, one study has shown that prior to mining, streams adjacent to a large lead-zinc ore deposit had few invertebrate taxa due to naturally elevated concentrations of metals in the water (see Scannell, 1996).

The greater negative effects of anthropogenic acidity on species diversity and some ecological processes is often attributed to the extent and source of acidity, increased mobility and bioavailability of metals (e.g. aluminum), and inability of organisms to adapt to rapid changes in pH, which extend beyond natural or historical ranges (Petrin et al., 2008). In contrast, strong similarities in community composition and ecosystem function are often reported between naturally acidic and circumneutral streams (Collier et al., 1990; Dangles et al., 2004). In regions with naturally low pH waters, many freshwater invertebrates and fish exhibit tolerance to a wide range of pH suggesting the species are pre-adapted to low pH (Collier et al., 1990; O’Halloran et al., 2008; Petrin et al., 2008).
Damage to freshwater ecosystems caused by anthropogenic acidification is most often associated with acidic precipitation caused by industrial emissions of sulphur dioxide and nitric oxides. However, acid mine drainage (AMD) is another major source of anthropogenic acidity worldwide. AMD is generated when sulphide minerals are exposed to weathering processes during mining activities (Kelly, 1998). Exposure to air and water generates sulphuric acid that is exported from mine sites and into nearby waterways. AMD impacted streams are characterized by extremely low pH (often < 3), high concentrations of metals (e.g. Al, Cu, Fe, Zn) and insoluble metal hydroxide deposits that coat stream substrata. Significant reductions in species richness and abundance of algae (Niyogi et al., 1999; Bray et al., 2008), invertebrates (Cherry et al., 2001; Battaglia et al., 2005), and fish (Sullivan and Gray, 1992; Greig et al., 2010) are typical of streams receiving mine drainage. These losses are often attributed to the substantial changes in pH and metal concentrations, which exceed the physiological tolerance limits of many species. For example, concentrations of dissolved metals typical of mining-impacted streams are known to be directly toxic to fish via inhibition of ion exchange and respiratory stress (e.g. Baker and Schofield, 1982 and references therein). Species loss may also be due, in part, to the loss of habitat and refugia as substrata are covered by metal hydroxide precipitates in streams where pH > 3.5 and some metals become insoluble (e.g. Fe; McKnight and Feder, 1984).

To date, most studies of ecological change in streams associated with anthropogenic stress have focused on shifts in community composition and abundance rather than functional organization and interactions among species. But more recently, in order to further understand the wider effects of stress on streams, a food web approach
has been taken (e.g. Culp et al., 2005; Woodward et al., 2010). Stressed food webs are expected to shorten and simplify owing to loss of sensitive species, removal of entire trophic levels (e.g. top predators), and decreases in interactions between species (Odum, 1985; McCann, 2000). Empirical evidence of modified food web structure has been reported in studies on freshwaters affected by acidification (Layer et al., 2010), warming (Woodward et al., 2010), and land use change (Thompson and Townsend, 2004). Changes in food web structure can be expected to have important implications for energy processing in these systems (Woodward, 2009).

Our objectives were to: 1) describe food web structure in streams receiving anthropogenic inputs of AMD; and 2) compare webs in mining streams to those in streams with natural sources of acidity and metals. We predicted that the extreme acidity and elevated metal concentrations of mining streams would generate a highly simplified food web structure compared to webs with natural sources. We expected that streams with natural sources of acidity and metals would have similar, intermediate-sized food webs including a more diverse consumer community comprised of acid and/or metal adapted species, and to be structurally more similar to webs in circumneutral streams.

**Methods**

**Study sites**

Food webs were sampled in 20 streams with either anthropogenic or natural sources of acidity and metals or circumneutral water chemistry on the South Island, New Zealand (see Supplementary Material; Appendix A). We selected five streams from each of the following four types: mining (low pH, high metal); naturally acidic (low pH, low metal); naturally high metal (alkaline pH, high metal); and circumneutral
(circumneutral pH, low metal). Mining, naturally acidic, and circumneutral streams were all located in close proximity to each other in three areas (90 – 130 km apart). All naturally high metal streams were concentrated in the Red Hills range, further to the northeast (~80 km from nearest mining stream). Despite the physical distance, stream faunas in these areas are comparable given the cosmopolitan nature of New Zealand’s freshwater biota (Harding et al., 1997). Mining streams received inputs of AMD from active or abandoned coal mines and were located in areas associated with the Brunner Coal Measures. Metal hydroxide precipitates were present on substrata in two mining streams. Natural acidity in streams in this area arises from the leaching of organic acids from soil and decomposing vegetation in surrounding forests (Collier et al., 1990). Streams with naturally high metal concentrations in our study contain nickel that is released from underlying ultramafic rocks and soils. All streams were selected to be as similar as possible with respect to other physicochemical factors and were in relatively isolated catchments dominated by native vegetation. Within each type, we selected streams across a gradient of elevation (20 - 550 m ASL) and vegetation cover (0 - 95 %), except naturally high metal streams (see Results). Samples were collected on a single occasion in January - February 2009 (austral summer) at sites within a 20 m reach that included both pools and riffles.

Water chemistry & physical characteristics of streams

Stream water pH, specific conductivity, temperature, and dissolved oxygen were measured using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA). Water samples (125 mL) collected for analysis of dissolved
metals were filtered in the field through a 0.45 µm mixed cellulose ester filter, acidified with nitric acid, and kept cool until analysis by ICP-MS (Hill Laboratories, Hamilton, New Zealand). Wetted channel width, depth, and velocity were recorded along three randomly selected transects in each 20 m reach. Substrate size was estimated by measuring 30 randomly selected particles from the streambed. Vegetation cover in the middle of the reach was estimated visually using a densiometer (Robert E. Lemmon Forest Densiometer Model-A, Oklahoma, USA).

*Basal resources – Algae & detritus*

Samples of algae and organic matter were collected to quantify basal food resources. Attached algae were removed from the surface of 15 randomly selected stones using a hard-bristled brush, rinsed with filtered stream water, and pooled into three 200 mL representative samples. The surface area of each stone was estimated from length measurements of the two main axes. In the field, a 50 mL subsample was filtered (0.7 µm-GF/F) and frozen for chlorophyll _a_ analysis to determine algal biomass. In the laboratory, algal pigments were extracted in buffered ethanol (90%) and quantified using fluorometry (Trilogy Fluorometer, Turner Designs, California, USA). Benthic organic matter was collected in five Surber samples (0.06 m²; 250 µm mesh) and divided into two size fractions: FPOM (250 – 1000 µm) and CPOM (> 1000 µm). Suspended organic matter (seston) was collected from 2 L of filtered (0.7 µm-GF/F) stream water. All FPOM, CPOM, and seston samples were dried, weighed, ashed (550°C for 4 h) and re-weighed to determine ash-free dry mass (AFDM).
**Consumer samples - Benthic invertebrates and fish**

Benthic invertebrates were collected in five Surber samples (0.06 m²; 250 µm mesh) from riffle and run habitats. They were fixed in ethanol (70%) and returned to the laboratory for processing. Taxa were identified and counted under a dissecting microscope to the lowest possible resolution according to Winterbourn et al. (2000a) and an unpublished key for hydrobiosid caddisflies (Brian Smith, NIWA, Hamilton, New Zealand). Following identification, molluscs and cased caddisflies were removed from their shells and cases, respectively. All invertebrates were classified into functional feeding groups, dried, weighed, ashed (550°C for 4 hr), and re-weighed together by group to determine AFDM. Kick net samples were collected at each stream to gain a more complete taxonomic list and collect individuals for gut contents analysis. Specimens for gut analysis were fixed in ethanol (70%) in the field. Fish and crayfish were sampled using quantitative electrofishing techniques in the 20 m reach. Stop nets were placed at the top and bottom of the reach and fish were removed during three successive passes. All fish were processed following Greig et al. (2010). Fish were returned to the stream except for a subset (up to 10 per species) that was retained for gut analysis. These fish were euthanized using 2-phenoxyethanol and preserved in ethanol (100%). Crayfish were collected for gut content analysis but were not included in invertebrate biomass estimates.

**Food web structure & properties**

Gut contents analysis was used to identify feeding links and construct food webs for each stream. Invertebrate guts were removed, mounted on slides in lactophenol-PVA stained with lignin pink, and examined under a microscope (400x magnification).
Invertebrates found in gut contents were identified to the lowest possible taxon and counted. Otherwise, food was itemised as animal prey, filamentous algae, diatoms, other algae, plant detritus (CPOM), and amorphous detritus (FPOM). The relative abundance of food items was visually estimated by scanning the whole slide and categorised as: rare (< 5%), common (5 – 20%), or abundant (> 20%). Stomach contents of fish were examined under a dissecting microscope. Prey items were identified to the lowest taxonomic resolution possible and counted or estimated as described above. All terrestrial insects found in guts were grouped into a single basal resource category (i.e. terrestrial insect) and counted.

Because many species were present at low densities in our streams, particularly the mining streams, we were only able to collect a limited number of individuals of each species for gut analysis. Frequently, this was less than the minimum of 10 individuals per species recommended by Thompson et al. (2001). In order to standardise our effort and generate comparable food webs across the four stream groups, we identified gut contents from several different species in each functional feeding group in all streams to get dietary information on a wide range of species. The number of guts analysed for each species ranged from 2 – 47. We then compiled a cumulative binary matrix (Cohen et al., 1990) for predators and prey and included links found in all 20 streams. Based on this cumulative matrix, we constructed individual food webs for each stream and were able to infer links for species where gut content data was missing but the species was present in samples for a particular stream. This approach assumes the presence of a feeding interaction between two species in all streams in which they are found but does not allow for differences in prey selection or the strength of interactions between streams (Havens,
1992). However, the procedure limited bias due to inadequate or unachievable sample size. We assessed invertebrates and fish in each stream for evidence of all six basal resources (algae, filamentous green algae, diatoms, FPOM, CPOM, terrestrial insects). If one or more was absent, all links with this resource were excluded for a particular stream. Links between predators and algal basal resources were also excluded to avoid links that were due to prey within prey (Thompson et al., 2001). However, detrital resource links were retained due to evidence of detritus in predator guts lacking prey. The constructed food webs did not incorporate meiofauna, bacteria, or fungi, and therefore provide a conservative estimate of overall food web structure.

The following food web properties were calculated for each stream: number of species, including basal resources (or web size, S), number of links (L); fraction of basal species (species with predators but no prey); fraction of intermediate species (species with predators and prey); fraction of top species (species with prey but no predators); maximum food chain length (number of links from a basal species to a top species, excluding feeding loops); predator – prey ratio (Cohen, 1977); linkage density (L/S; Pimm et al., 1991); and connectance (fraction of all possible links that are realised within a food web; L/S^2). This commonly used measure of connectance is less likely than other measures to be affected by differences in web size.

**Statistical analysis**

Principal components analysis (PCA) was conducted on chemical and physical variables. Conductivity and the concentrations of four metals were log_{10} -transformed to meet assumptions of normality. Separation of streams along PCA axes was determined
using multivariate analysis of variance (MANOVA) followed by univariate analysis of variance (ANOVA) on specific water chemistry variables. Differences in biomass of basal resources and consumers between stream groups were determined using ANOVA followed by Tukey’s post-hoc tests where significant differences were found. CPOM, FPOM, algal, invertebrate and fish biomass data were square-root transformed prior to analysis.

Non-metric multidimensional scaling (NMDS), conducted on a Bray-Curtis similarity matrix, was used to describe differences in food web properties between streams. Food web property data were square root transformed prior to analysis. Differences in food web properties between stream groups (i.e. mining, naturally acidic, naturally high metal, and circumneutral) were tested using analysis of similarities (ANOSIM). Next, similarity percentages (SIMPER) of food web properties were calculated to determine properties that contribute most to dissimilarity between stream groups. Finally, the BEST procedure was used to relate patterns in food web structure with physicochemical variables (Clarke and Warwick, 2001). In this procedure, the best matches are measured by Spearman rank correlation between the similarity matrices for food web properties (Bray-Curtis) and physicochemical variables (Euclidean distance).

ANOVA and MANOVA analyses were performed using SYSTAT 10 (SPSS 2000). All other multivariate analyses described above were conducted using Primer 6.1.12 (Clarke and Gorley, 2006).
Chapter 2 – Acidity, metals & food web structure

Results

Physicochemical variables

An ordination of water chemistry variables emphasized the separation of mining streams from all other streams (Fig. 1a). Separation occurred along a gradient of conductivity, concentrations of dissolved Al, Fe, Ni, Zn, and pH (Axis 1), but not temperature or dissolved oxygen (Axis 2) (MANOVA: Wilks $\lambda_{6,30} = 0.12$, $P < 0.001$. Univariate ANOVAs: Axis 1 $F_{3,16} = 29.03$ $P < 0.001$; Axis 2 $F_{3,16} = 1.78$ $P = 0.19$; Fig. 1a). pH differed significantly between all stream types, increasing from mining to naturally acidic, circumneutral, and naturally high metal streams (Table 1).

Mining streams were twice as acidic as most naturally acidic streams and up to six times more acidic than all other streams in the study based on the concentration of $H^+$ ions. Conductivity and the concentrations of four dissolved metals (Al, Fe, Ni, Zn) also differed significantly between stream types and were one to three orders of magnitude higher in mining streams. In addition, the concentration of Ni and conductivity were one order of magnitude higher in naturally high metal than in naturally acidic and circumneutral streams and were in the range of those observed in mining streams (Table 1).

The streams had similar physical characteristics, including velocity (0.05 - 0.31 m s$^{-1}$), width (1.3 - 7.2 m), depth (0.07 - 0.23 m), and substrate size (7.5 - 13.7 cm). Streams in all groups were represented across the elevation and vegetation cover ranges, except for naturally high metal streams, which were only at higher elevations (791 - 1232 m ASL) and had 0 % vegetation cover. Vegetation at these sites was predominately grasses and short shrubs, which did not provide any cover. Naturally high metal streams
differed from all other streams based on elevation and vegetation cover but not other physical variables (MANOVA: Wilks $\lambda_{6,30} = 0.20$, $P < 0.001$. Univariate ANOVAs: Axis 1 $F_{3,16} = 1.08$, $P = 0.39$; Axis 2 $F_{3,16} = 15.32$, $P < 0.001$; Fig. 1b).

Fig. 1 Principal components analysis (PCA) of (a) water chemistry, and (b) physical habitat variables for mining, naturally acidic, naturally high metal, and circumneutral streams. Lines indicate correlation of variables with axes.
Table 1 Physicochemical variables of the four stream types identified in Figure 1. Values shown are group means (± SE) with ranges below. Results of one-way ANOVA tests for differences between stream groups for each variable are also shown. N = 5 streams per type.

<table>
<thead>
<tr>
<th>Stream type</th>
<th>pH</th>
<th>Specific conductivity (µS₂₅ cm⁻¹)</th>
<th>Al (mg L⁻¹)</th>
<th>Fe (mg L⁻¹)</th>
<th>Ni (mg L⁻¹)</th>
<th>Zn (mg L⁻¹)</th>
<th>Elevation (m ASL)</th>
<th>Vegetation cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mining</td>
<td>mean ± se</td>
<td>3.5 ± 0.4</td>
<td>689.2 ± 137.1</td>
<td>12.4 ± 5.2</td>
<td>10.21 ± 5.5</td>
<td>0.06 ± 0.02</td>
<td>0.3 ± 0.1</td>
<td>318.6 ± 90.6</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>2.8 - 4.4</td>
<td>295.4 - 994.7</td>
<td>0.7 - 30.0</td>
<td>0.5 - 31.0</td>
<td>0.006 - 0.1</td>
<td>0.03 - 0.5</td>
<td>46.0 - 225.0</td>
</tr>
<tr>
<td>Naturally acidic</td>
<td>mean ± se</td>
<td>5.0 ± 0.3</td>
<td>34.4 ± 4.4</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.0006 ± 0.00007</td>
<td>0.007 ± 0.001</td>
<td>237.6 ± 96.5</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>4.1 - 5.9</td>
<td>21.7 - 43.2</td>
<td>0.09 - 0.65</td>
<td>0.1 - 0.6</td>
<td>0.0005 - 0.0009</td>
<td>0.003 - 0.013</td>
<td>20.0 - 544.0</td>
</tr>
<tr>
<td>Naturally high metal</td>
<td>mean ± se</td>
<td>8.1 ± 0.1</td>
<td>112.9 ± 9.7</td>
<td>0.01 ±0.01</td>
<td>0.03 ± 0.005</td>
<td>0.006 ± 0.003</td>
<td>0.003 ± 0.001</td>
<td>968.6 ± 100.1</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>7.7 - 8.4</td>
<td>87.4 - 138.4</td>
<td>0.003 - 0.06</td>
<td>0.02 - 0.05</td>
<td>0.002 - 0.02</td>
<td>0.001 - 0.007</td>
<td>791.0 - 1232.0</td>
</tr>
<tr>
<td>Circumneutral</td>
<td>mean ± se</td>
<td>6.6 ± 0.1</td>
<td>79.4 ± 8.7</td>
<td>0.07 ±0.02</td>
<td>0.09 ± 0.01</td>
<td>0.0005 ± 0.00001</td>
<td>0.007 ± 0.003</td>
<td>177.2 ± 36.2</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>6.3 - 6.9</td>
<td>58.1 - 105.7</td>
<td>0.05 - 0.13</td>
<td>0.06 - 0.11</td>
<td>0.0005 - 0.00055</td>
<td>0.003 - 0.019</td>
<td>46.0 - 255.0</td>
</tr>
<tr>
<td>ANOVA</td>
<td>$F_{3,16}$</td>
<td>62.0</td>
<td>63.9</td>
<td>15.8</td>
<td>10.8</td>
<td>12.5</td>
<td>11.0</td>
<td>18.7</td>
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<tr>
<td></td>
<td>$P$</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</tbody>
</table>
**Food web components - Basal resources & consumers**

Basal resource biomass varied both within and between mining, naturally acidic, and circumneutral streams but was consistently lower in naturally high metal streams (Fig. 2a-c). Algal biomass was relatively low in all streams and differed among stream groups \( F_{3,56} = 5.24, P = 0.003 \). This difference was driven by significantly lower algal biomass observed in naturally high metal streams (Fig. 2a). Across all streams, algal biomass was not related to vegetation cover \( R^2 = 0.15, P = 0.09 \). Both CPOM and FPOM biomass differed significantly between stream groups \( F_{3,96} = 5.73, P = 0.001 \) and \( F_{3,96} = 4.51, P = 0.005 \), respectively. Again, these detrital resources were significantly lower in naturally high metal streams (Figure 2b-c) but biomass increased in streams with greater vegetation cover (CPOM: \( R^2 = 0.34, P < 0.01 \); FPOM: \( R^2 = 0.28, P = 0.02 \)). Biomass of suspended seston was comparable across stream types \( F_{3,96} = 2.92, P = 0.07 \); Fig. 2d).

Invertebrate consumer biomass was significantly lower in mining streams but similar among other stream groups, except biomass in naturally high metal streams which was significantly lower than in circumneutral streams \( F_{3,96} = 28.42, P < 0.001 \); Fig. 3a). Species-poor communities in mining streams were comprised of only a few collector-browsers and predators including oligochaetes, orthoclad chironomids, and tanypod chironomids (Fig. 3c). Predatory dobsonflies (Archichauliodes diversus) and stoneflies (Stenoperla macellani) as well as two collector-browsers (mayfly Deleatidium spp. and stonefly Zelandoperla spp.) were common in naturally high metal streams (Fig. 3c). Species-rich communities in naturally acidic and circumneutral streams were very similar and contained a core group of filter-feeders (e.g. Hydropsyche spp., Coloburiscus humeralis) and collector-browsers (Deleatidium spp.; Fig. 3c). Fish were absent from all
mining and naturally high metal streams. In contrast, fish biomass was comparable in naturally acidic and circumneutral streams (Fig. 3b). Communities in both these stream groups included longfin eels (*Anguilla dieffenbachii*), shortfin eels (*Anguilla australis*), brown trout (*Salmo trutta*), bullies (*Gobiomorphus* spp.), and galaxiids (*Galaxias* spp.). All fish were predators (Fig. 3d). Fish and invertebrate consumer species used to construct food webs are listed in Table 2, as gut contents analysis was not conducted on some taxa (e.g. oligochaetes).

**Fig. 2** Mean biomass of basal resources in mining, naturally acidic, naturally high metal, and circumneutral streams (± SE). a) algae (chlorophyll *a*), b) coarse particulate organic matter (CPOM), c) fine particulate organic matter (benthic FPOM), and d) seston. Letters above bars indicate statistically significant differences as determined by Tukey’s posthoc test (*P* < 0.05) following significant one-way ANOVAs. *N* = 5 streams per type.
**Fig. 3** Mean biomass of (a) invertebrate and (b) fish consumers in mining (low pH, high metal), naturally acidic (low pH, low metal), naturally high metal (circumneutral pH, high metal), and circumneutral (circumneutral pH, low metal) streams. (± SE). Letters above bars indicate statistically significant differences as determined by Tukey’s posthoc test ($P < 0.05$) following significant one-way ANOVAs. $N = 5$ streams per type. Mean biomass of (c) invertebrate functional feeding groups and (d) fish (all predatory).
### Table 2
Consumer taxa present in food webs and the number of streams in which they were found (- = absent).

<table>
<thead>
<tr>
<th>Functional Feeding Group</th>
<th>Taxon</th>
<th>Mining</th>
<th>Naturally acidic</th>
<th>Naturally high metal</th>
<th>Circumneutral</th>
</tr>
</thead>
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<td>Vertebrate predator</td>
<td>Anguilla australis</td>
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<td>-</td>
<td>1</td>
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<tr>
<td></td>
<td>Anguilla dieffenbachii</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Galaxias brevipinnis</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Galaxias fasciatus</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gobiomorphus hubbci</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gobiomorphus huttoni</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Salmo trutta</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>3</td>
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<tr>
<td>Invertebrate predator</td>
<td>Ameletopsis perscitus</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Aphrophila spp.</td>
<td>-</td>
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<td>-</td>
<td>1</td>
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<tr>
<td></td>
<td>Archichauliodes diversus</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>3</td>
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<tr>
<td></td>
<td>Chironomidae (predatory)</td>
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<td>Costachorema spp.</td>
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<td>3</td>
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<td>-</td>
<td>3</td>
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<td></td>
<td>Hydrobiosella spp.</td>
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<td>3</td>
<td>-</td>
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<tr>
<td></td>
<td>Hydrobiosis frater</td>
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<tr>
<td></td>
<td>Hydrobiosis gollanis</td>
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<td>-</td>
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<td>Hydrobiosis spp.</td>
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<td>5</td>
<td>-</td>
<td>5</td>
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<td></td>
<td>Hydrochorema spp.</td>
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<td>Neurochorema spp.</td>
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<td>Plectrocnemia maclachlani</td>
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<td>Stenoperla macellani</td>
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<td>Stenoperla prasina</td>
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<td>Chironomidae (non-predatory)</td>
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<td>Homalaena sp.</td>
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<td>Hydora sp.</td>
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<td>Oniscigaster distans</td>
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<td>Pycnocentria spp.</td>
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<td>Zelandobius spp.</td>
<td>-</td>
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<tr>
<td></td>
<td>Zelandoperla sp.</td>
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<td>Zellessica meizon</td>
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<td>Shredder</td>
<td>Olinga feredayi</td>
<td>-</td>
<td>-</td>
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<td>Filter</td>
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<td>-</td>
<td>3</td>
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</table>
Chapter 2 – Acidity, metals & food web structure

Amorphous detritus (FPOM) was the main basal resource used by dominant primary consumers in mining and naturally acidic streams, while both detritus and algae were more commonly found in diets of primary consumers in circumneutral streams (Table 3). In contrast, green algae and diatoms formed the basis of diets for primary consumers in naturally high metal streams, although FPOM was also abundant for two consumers.

**Table 3** Basal resource use by numerically dominant primary consumers in each stream type (R = rare, < 5%; C = common, 5 – 20%; A = abundant, > 20%). * = non-predatory chironomids

<table>
<thead>
<tr>
<th>Stream type</th>
<th>Consumer taxon</th>
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<th>CPOM</th>
<th>FPOM</th>
<th>Algae</th>
<th>Filamentous algae</th>
<th>Diatom</th>
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<td></td>
<td></td>
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<td>C</td>
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<td>A</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>R</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>A</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tr>
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<td>-</td>
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<tr>
<td>Chironomidae*</td>
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<td>-</td>
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<td>-</td>
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<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
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<td>A</td>
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<tr>
<td><strong>Hydropsyche</strong></td>
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<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>C</td>
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</tr>
</tbody>
</table>

_food web structure_

Food webs in mining streams were smaller, had fewer links per species and overall, and higher connectance than webs in all other streams types (Table 4). The structure of naturally high metal stream food webs was more similar to that observed in
mining streams than naturally acidic or circumneutral streams, in terms of size (S), number of links, and maximum food chain length. Naturally acidic and circumneutral stream food webs were larger, had more links overall, and higher linkage density (Table 4). Connectance declined as web size increased from small mining webs to larger, more species-rich circumneutral and naturally acidic stream webs ($F_{1,18} = 37.83$, $P < 0.001$; Fig. 4).

![Graph showing Connectance (C) decreases as food web size (S) increases in all streams.](image)

**Fig. 4** Connectance (C) decreases as food web size (S) increases in all streams (open inverted triangles = mining, closed squares = naturally acidic, closed diamonds = naturally high metal, and closed triangles = circumneutral streams).

Streams were separated into three clusters in the NMDS ordination of food web properties (Fig. 5) and significant differences were observed between food web properties of the stream groups (ANOSIM: Global $R = 0.70$, $P < 0.001$). Specifically, pairwise comparisons of mining and naturally high metal streams indicated that these groups differed significantly from each other and from the other two streams types ($P < 0.02$). In
contrast, naturally acidic and circumneutral streams were very similar in terms of food web properties ($P = 0.52$). Average similarities (SIMPER) of food web properties within groups were high in mining, naturally acidic, naturally high metal, and circumneutral streams (average Bray-Curtis similarity = 92.3%, 89.3%, 90.5%, and 92.6%, respectively). Differences in numbers of links and web size made the greatest contributions to dissimilarity between food webs (Table 4). BEST analysis indicated that specific conductivity, dissolved nickel concentration, and elevation together provided the best explanation (62%) of the pattern in food web properties among stream groups (Global test $P < 0.001$). However, specific conductivity alone explained 49% of this pattern.

Fig. 5 NMDS ordination of streams using food-web properties. Statistically different groups are identified by broken lines based on ANOSIM ($P < 0.01$).
Table 4 Food web properties for each stream group. S = web size, L = links, max FCL = maximum food chain length, basal sp., intermediate sp., and top species = proportions in web, predator: prey ratio (Cohen 1977), C = connectance (L/S^2), L/S = links per species. Values shown are group means (± SE) and ranges. N = 5 streams per type.

<table>
<thead>
<tr>
<th>Stream type</th>
<th>Mining</th>
<th>Naturally acidic</th>
<th>Naturally high metal</th>
<th>Circumneutral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± se</td>
<td>range</td>
<td>mean ± se</td>
<td>range</td>
</tr>
<tr>
<td>S</td>
<td>6.4 ± 0.5</td>
<td>5 - 8</td>
<td>30.0 ± 3.5</td>
<td>17 - 37</td>
</tr>
<tr>
<td>L</td>
<td>8.8 ± 1.4</td>
<td>6 - 12</td>
<td>116.8 ± 21.4</td>
<td>37 - 165</td>
</tr>
<tr>
<td>max FCL</td>
<td>2.0 ± 0</td>
<td>2 - 2</td>
<td>2.8 ± 0.2</td>
<td>2 - 3</td>
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<tr>
<td>basal sp</td>
<td>0.53 ± 0.06</td>
<td>0.50 - 0.80</td>
<td>0.18 ± 0.02</td>
<td>0.11 - 0.24</td>
</tr>
<tr>
<td>intermediate sp</td>
<td>0.07 ± 0.07</td>
<td>0 - 0.33</td>
<td>0.71 ± 0.04</td>
<td>0.53 - 0.84</td>
</tr>
<tr>
<td>top sp</td>
<td>0.40 ± 0.03</td>
<td>0.33 - 0.50</td>
<td>0.11 ± 0.04</td>
<td>0.03 - 0.24</td>
</tr>
<tr>
<td>predator : prey</td>
<td>1.37 ± 0.19</td>
<td>1.00 - 2.00</td>
<td>1.07 ± 0.03</td>
<td>1.0 - 1.19</td>
</tr>
<tr>
<td>C</td>
<td>0.21 ± 0.02</td>
<td>0.17 - 0.24</td>
<td>0.13 ± 0.01</td>
<td>0.10 - 0.14</td>
</tr>
<tr>
<td>L/S</td>
<td>1.35 ± 0.12</td>
<td>1.00 - 1.71</td>
<td>3.73 ± 0.44</td>
<td>2.18 - 4.85</td>
</tr>
</tbody>
</table>
Discussion

Our findings confirm that anthropogenic acidity and metal contamination have a stronger negative effect on food web structure than natural sources. This effect is driven primarily by substantial changes in consumer communities and diet that resulted in differences in web size and number of links. Small, fishless mining stream food webs differed most from large, species-rich webs in naturally acidic and circumneutral streams that had many links overall (Fig. 6). However, we observed very little difference between the structures of naturally acidic and circumneutral webs due to comparable community composition and diet of consumers. Surprisingly, naturally high metal stream webs were more similar to mining than naturally acidic webs in terms of size, number of links, and the absence of fish as a top predator. Specific conductivity was identified as the most important water chemistry predictor distinguishing all streams based on food web properties. Together, these results highlight the potential importance of metals, regardless of source, as a driver of food web structure.

Short and simple mining food webs

Mining stream food webs were small, simple structures that were sparsely populated by low densities of tolerant invertebrates and lacked fish, which is typical of streams receiving AMD (e.g. Cherry et al., 2001; Greig et al., 2010). The combination of very few consumer species and the number of basal resource categories meant that only a small number of links were possible in our mining stream webs, because the proportion and position of species in different trophic levels limits the number of links (Schmid-Araya et al., 2002). Therefore, as a result of all consumers feeding on almost all available
basal resources, mining webs had a high proportion of realised links and high connectance. Basal resource quantity was sufficient to support a small consumer community in mining streams. However, accessibility and food quality may limit invertebrate population sizes. For example, filamentous green algae can proliferate in mining impacted streams but may be inaccessible to many invertebrates as a food resource (Niyogi et al., 1999; Bray et al., 2008). However, we found that chironomids commonly consumed filamentous green algae, where present in mining streams in addition to amorphous detritus, which was their primary basal resource. Furthermore, metal hydroxide deposits, which can coat leaf litter and restrict decomposition (Schlief and Mutz, 2005) may negatively affect consumer feeding on detrital resources.

**Comparison of food webs with anthropogenic vs. natural sources of acidity and metals**

Our analysis revealed distinct differences in web size and number of links between mining and naturally acidic stream food webs. In particular, mining streams had smaller webs (i.e. five times fewer species) and 92% fewer links than those in naturally acidic streams. Notably, fish and crayfish commonly found in nearby naturally acidic waters were absent from mining streams. Many links were absent because fish were not found in mining streams due to the extreme water chemistry that exceeded the known pH and metal tolerance ranges of fish species in Westland (Greig et al., 2010). As larger species found higher in the food web, fish often add more links than invertebrates (Cohen et al., 2003). In New Zealand, almost all fish are predatory and feed generally on diverse invertebrate communities. Where present in naturally acidic streams, fish contributed 7 – 30 links per species whereas invertebrates contributed only 5 – 12 links on invertebrate
prey. In mining streams, the number of links between invertebrate predators and prey items was further reduced to 1 – 2 per species. This represented a significant reduction and narrowing of energy pathways in these species-poor, sparsely populated mining streams. The abundance of large-bodied invertebrate predators often increases in the absence of fish in both acidic and heavy metal contaminated waters (e.g. Hildrew, 1992; Rutherford and Mellow, 1994). However, this was not observed in our mining streams, where low abundances of small-bodied chironomids (Tanypodinae) and a free-living caddisfly (Psilochorema spp.) assumed the top food web position.

The absence of fish, smaller and less diverse invertebrate communities, and a reduced number of links resulted in naturally high metal streams sharing key structural properties with mining streams. Nickel is not highly mobile between the water and sediment at the pH levels observed in our naturally high metal streams (Table 1; Garrett, 2000). While the dissolved nickel concentrations in these streams exceeded the known tolerance range of most New Zealand fish (Greig et al., 2010), they are not considered lethal for other fish taxa (Pyle et al., 2002; Pane et al., 2002). Nevertheless, some studies have shown that fish can be sensitive to chronic exposures (35 µg L⁻¹; Nebeker et al., 1985). Reduced prey availability may also contribute to the absence of fish in these naturally high metal streams. The less diverse and reduced invertebrate prey base may compromise fish feeding as reported in metal-contaminated lakes (Köveces et al., 2005). This bottom-up energetic deficiency may stem from low levels of basal resources available to primary consumers in these streams. Our naturally high metal streams did occur at higher elevations than all other streams in this study and as many fish species are migratory, fish diversity does tend to decline with elevation in New Zealand. However, it
seems unlikely that elevation limits fish distribution in this area, as brown trout, long fin eel, and several species of galaxiids are found in low metal streams at similar elevations (760 – 1150 m ASL) in nearby ranges (New Zealand Freshwater Fish Database, 2009).

![Fig. 6 Simplified food web diagrams in a) mining, b) naturally high metal, c) naturally acidic, and d) circumneutral streams. Links are based on gut contents analysis. Each node represents a combination of several species (e.g. stonefly represents multiple predatory invertebrate species).]

**Key to nodes:**
- Resources (e.g., algae, detritus, plant material)
- Primary consumers (e.g., mayfly, caddisfly, blackfly, shredding stonefly)
- Secondary consumers (predatory stonefly, crayfish)
- Terrestrial insects
- Top consumers (fish)
Similarity between naturally acidic and circumneutral food webs

We observed no negative effect of natural acidity on food web structure. In fact, webs were highly comparable between naturally acidic and circumneutral streams. Both webs were species-rich, included fish as a top predator, and had many links overall. Consumer community-level patterns in our study were consistent with previous work that has shown similarities in invertebrate and fish community composition down to about pH 4.5 in New Zealand (Collier et al., 1990; Greig et al., 2010). It is likely that adaptation to naturally low pH over evolutionary time partially accounts for these diverse communities in naturally acidic waters (Collier et al., 1990; Petrin et al., 2008). In addition, monomeric Al mobilized in naturally acidic streams is made biologically unavailable by dissolved organic carbon, so organisms are less likely to be metal-stressed (Collier et al., 1990). Our work builds on these earlier studies by identifying links between species and constructing food webs based on gut content analysis. Having many of the same predator and prey species resulted in similar patterns in connectance, number of links overall, and linkage density in these food webs.

Naturally acidic and circumneutral streams food webs have not been compared before, although one of the best described food webs in the ecological literature is for an acidic stream (Broadstone Stream; Hildrew, 2009). Food web properties in our naturally acidic streams were similar to those reported in early descriptions of Broadstone Stream (pH 4.5 – 6.6) in south-east England (Lancaster and Robertson, 1995; Woodward and Hildrew, 2001). Furthermore, these food web properties were comparable to properties reported in similar-sized circumneutral stream webs constructed with similar taxonomic resolution for basal species (e.g. Tavares-Cromar and Williams, 1996; Mantel et al.,
The structural similarities between naturally acidic and circumneutral stream food webs suggest that key ecosystem processes differ little between these stream types.

**The importance of metals to stream food webs**

Conductivity was identified as a key chemical variable explaining the differences in food web properties between stream types. As conductivity reflects in part the concentration of dissolved metals, which is a function of pH, it can be considered a more effective indicator of mining contamination than pH alone (Winterbourn and McDiffett, 1996). The similarities between key food web properties of mining and naturally high metal streams highlight the potential importance of metals, regardless of source, as a driver of food web structure. This suggestion is consistent with that of Greig et al. (2010) who found that dissolved metal concentrations (Al, Zn, Mn, and Ni), rather than pH, explained fish diversity, density, and biomass in New Zealand streams with natural and mining-induced acidity. Limited information is available for metal tolerances of aquatic invertebrates in New Zealand (Hickey and Clements 1998) but evidence from elsewhere indicates metals are an important force driving community structure and species interactions (e.g. Gower et al., 1994, Clements, 1999, Clements et al., 2000). More work is needed to understand the direct and indirect effects of metals in streams receiving either anthropogenic or natural inputs. In particular, very little research has been done to examine the effects of naturally elevated metal levels on aquatic biota. Understanding the role of metals in stream food webs is important because metals can bioaccumulate and transfer up the food chain, although this varies considerably between metals, species, and
feeding pathways (e.g. Woodward et al., 1995, Beltman et al., 1999, Winterbourn et al. 2000b).

Conclusions

We found small and extremely simplified food webs in chronically stressed mining streams indicating that the combination of anthropogenic acidity and metals has serious implications for stream food webs. Stressors outside the natural range for a region clearly have negative impacts on food webs due to substantial changes at each trophic level. Our comparison of stream food web structure revealed the importance of source (anthropogenic vs. natural) on food web size, food chain length, and interactions between species. Furthermore, the notable differences between mining and naturally acidic stream food web structure suggests that knowledge of food webs in naturally acidic streams is not necessarily useful for understanding and predicting food web patterns in streams acidified by mine drainage. It may be more useful to compare food web structure and function in mining impacted streams with those reported in other metal-stressed streams.

Acknowledgements

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Chapter 2 – Acidity, metals & food web structure

Applications 10, 626-638.


Risk Assessment 6, 945-963.


Chapter 2 – Acidity, metals & food web structure


Chapter 2 – Acidity, metals & food web structure


Supplementary Material. Northing and easting coordinates and location of each of the twenty streams sampled on the South Island, New Zealand.

<table>
<thead>
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<th>Stream type</th>
<th>Stream name</th>
<th>Location</th>
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Chapter 3

Leaf breakdown, detrital resources and food webs in streams affected by mine drainage

An obligate shredder, Austroperla sp., coated in iron hydroxide precipitates.
Chapter 3 – Leaf breakdown & energy flow

Abstract

Breakdown of leaf litter is essential for providing detrital food resources for food webs but can be impaired by anthropogenic activities. We explored the relationships between breakdown rates, detrital resources, and food web structure in streams receiving acid mine drainage and compared them with those in naturally acidic and circumneutral streams. Streams affected by mining were of two types; those with highly acidic water (pH < 3) and those with metal precipitates. Breakdown rates of leaves were significantly slower in mining-affected streams than circumneutral streams (by almost 50%) but not naturally acidic streams and were driven primarily by microbial activity, as shredding invertebrates were often absent. Metal precipitates suppressed microbial activity further. Detrital resource availability was comparable among stream types but was highly variable. Mining-affected stream food webs were small, simplified structures with fewer species (particularly intermediate species) and links than those of naturally acidic or circumneutral streams. Overall, our results indicate that mine drainage disrupts mechanisms responsible for leaf breakdown and energy flow within food webs. Faster breakdown rates were associated with larger food webs. Nevertheless, leaf breakdown in mining-impacted streams was sufficient to support and maintain primary consumers and simplified food webs.

Keywords: leaf breakdown; acid mine drainage (AMD); iron precipitates; ecosystem function; food web structure; detritus
Chapter 3 – Leaf breakdown & energy flow

Introduction

Detritus is a dominant basal food resource supporting food webs, particularly in forested headwater streams (Wallace et al., 1999; Woodward et al., 2005). The breakdown of leaf litter, which represents the main terrestrial input to streams, is an important process that provides energy as dissolved or fine particulate organic carbon (Fisher and Likens, 1973; Wallace et al., 1999). This organic matter is incorporated by microbes and consumers, and in turn, is transferred up the food web (Allan, 1995). As a process, leaf litter breakdown integrates multiple trophic levels from microbes to invertebrates and fish. In particular, the activity of microbes, particularly hyphomycete fungi, and shredding invertebrates contributes directly to the conditioning and breakdown of leaf litter in combination with physical abrasion and leaching of soluble compounds from the leaves (Webster and Benfield, 1986; Gessner et al., 1999). Fish can also mediate the breakdown process and transfer of detrital energy through food webs by controlling the abundance or activity of shredders and indirectly suppressing breakdown rates (Greig and McIntosh 2006; Woodward et al., 2008).

Accumulation of significant amounts of undecomposed or slowly decomposing leaf litter in polluted streams may indicate that the breakdown process is disrupted (e.g., Schlief and Mutz, 2005) and the capacity to support diverse consumers and complex food webs may be reduced. Anthropogenic inputs of acidity and metals from atmospheric deposition, road runoff and mine drainage are known to impair leaf breakdown in streams by reducing the abundance and activity of either microbes or shredding invertebrates, or a combination of both (Maltby et al., 1995, Schultheis et al., 1997; Niyogi et al., 2001; Woodcock and Huryn, 2005; Simon et al., 2009). Alterations to detrital processing in
acid- or metal-stressed streams can potentially limit food quantity and quality, nutrient fluxes, and energy flow to other stream consumers within the food web and further downstream (e.g., Groom and Hildrew, 1989; Woodcock and Huryn, 2007). For example, Carlisle and Clements (2005) linked decreases in litter breakdown with low secondary production in streams along a zinc pollution gradient. However, despite the wider negative effects of impaired leaf breakdown, and the importance of this process to stream food webs, links between this key process and food web structure have rarely been reported (Young et al., 2008; Gessner et al., 2010; but see Woodward et al., 2005; Hlaydz et al., 2011).

Species interactions, which affect the transfer of energy and matter in food webs, are often disrupted or reduced by anthropogenic stress. This in turn affects ecosystem processes and has the potential to generate energetic bottlenecks (Sherwood et al., 2002; Woodward, 2009). The loss of species that drive ecosystem processes can impair function. For example, low production of a shredding stonefly (*Taenionema pallidum*), a primary contributor to leaf litter breakdown, was linked to significantly reduced breakdown rates in zinc-contaminated streams in Colorado, USA (Carlisle and Clements, 2005). However, Ledger and Hildrew (2005) found that detritivores consumed algae and maintained herbivore-algal interactions in acidic streams lacking specialist grazers. Therefore, shifts in feeding strategies or functional redundancy among species can enable streams to maintain processes or retain some limited ecological function, despite species losses and food web simplification.

We examined the relationship between leaf breakdown, detrital resources, and food web structure in streams affected by acid mine drainage (AMD). Acidity, elevated
concentrations of dissolved metals, and metal hydroxide precipitates are the primary stressors associated with AMD and influence the structure and function of benthic communities in different ways (Hogsden and Harding, 2012a [Chapter 1]). Slow leaf breakdown rates are well documented in AMD-impacted streams due to the reduced activity of microbes and abundance of shredding invertebrates (e.g., Niyogi et al., 2001, Schlief and Mutz, 2005). Breakdown rates can be further reduced in streams with metal precipitates that coat leaves, slow leaf softening, and limit microbial colonisation of the litter (Gray and Ward, 1983; Barnden and Harding, 2005). However, the accumulation of slowly decomposing litter could be viewed as increased substrate for microbial activity in the absence of precipitates if fungi or bacteria are tolerant of low pH and dissolved metals (e.g., zinc, Niyogi et al., 2001). In contrast, the structure of food webs has only recently been described in streams receiving mine drainage (Hogsden and Harding, 2012b [Chapter 2]). That study reported small, simplified food webs (i.e., few links overall, short food chains) with few tolerant invertebrate consumers and no fish in streams affected by mining, but did not distinguish between the potential effects of elevated dissolved metals and metal precipitates on food web structure. These substantial modifications in food web structure were expected to limit energy flow throughout the stream. However, while AMD-impairment of leaf litter processing will likely affect primary consumers and upper trophic level consumers, reduced function may be sufficient to support the small webs found in mining streams. We compared leaf litter breakdown in streams receiving mine drainage with rates in nearby naturally acidic and circumneutral streams and related differences in breakdown rate to food web structure.
Methods

Study Sites

The study was conducted in 12 streams located in a region with a long history (> 150 years) of coal mining on the northern west coast of the South Island, New Zealand (Appendix B). We selected streams receiving mine drainage from active or abandoned mines, naturally acidic streams, and circumneutral streams near Reefton, Stockton, and Denniston in the Westland Forest ecoregion (Harding et al., 1997). Streams affected by mine drainage had either highly acidic pH (< 3) and elevated concentrations of dissolved metals (Al, Fe) or moderately acidic pH (3.5 – 4.5) and iron hydroxide precipitates coating the stream substrata. We therefore had four stream types; “acidic mining”, “precipitate mining”, naturally acidic and circumneutral. Natural acidity in streams in this region arises from the leaching of organic acids from soil and decomposing vegetation in the surrounding forests (Collier et al., 1990). All streams were selected to be as similar as possible with respect to other physical factors and were in relatively isolated catchments dominated by native vegetation and unaffected by urbanisation or agriculture. The study was conducted from January to March 2011 (austral summer), streams (and leaf packs) being sampled on four occasions (days 8, 15, 32, and 60).

Stream characteristics – Physicochemical, organic matter & invertebrate community

On each sampling occasion, pH, specific conductivity, and flow rate were measured in-stream using standard meters (YSI 63; YSI Environmental Incorporated, Ohio, USA; and Flo-mate 2000; Hach Company, Colorado, USA) and a water sample was taken for nutrient analysis. Water samples were filtered in the field through a 0.7-µm
glass microfiber filter (Whatman GF/F) and stored at -4°C until analysis for nitrate and soluble reactive phosphorus with an Easychem Plus (Systea Scientific, Italy). Samples for analysis of dissolved metals (Al, Fe, Zn) were collected once in each stream at the start of the experiment (day 0). These samples were filtered through a 0.45-µm mixed cellulose ester filter, acidified with nitric acid, and stored at -4°C until analysis by ICP-MS (Hills Laboratory, Hamilton, New Zealand). Physical disturbance of the streambeds was assessed using the channel stability index (Pfankuch, 1975). Finally, three Surber samples (0.06 m², 250 µm mesh) were collected from a riffle section of the stream, with cobble-sized substrata, on each sampling date to assess in-stream biomass of coarse particulate organic matter (CPOM; > 1000 µm), fine particulate organic matter (FPOM; 250 – 1000 µm) and to characterise invertebrate community composition and biomass. CPOM, FPOM, and invertebrate samples were dried, separately (60°C), weighed, ashed (550°C for 4 h), and re-weighed to determine ash-free dry mass (AFDM).

Leaf litter breakdown - field experiment

Leaves of mahoe (*Melicytus ramiflorus*), a common riparian tree species in New Zealand, were collected on a single occasion. Mahoe leaves have a relatively fast breakdown rate and have been used in previous studies of leaf decomposition (Linklater, 1985; Hicks and Laboryrie, 1999; Young and Collier, 2009). Leaves were weathered outdoors for 14 days, dried for 48 h at 60°C, and placed in mesh bags (5 g/pack; 5 mm mesh). Twelve replicate leaf packs were deployed at each site and were secured to the substrate with a cable tie and pegs. A water temperature logger (Hobo Pendant, Onset Computer Corporation, Massachusetts, USA) was deployed in each stream to calculate
degree-days. Three leaf packs were retrieved on each sampling date. Each pack was placed into a separate plastic bag, stored on ice in the dark, and returned to the laboratory for processing.

Leaf litter – sample processing

In the laboratory, the remaining leaf material in each pack was rinsed and sorted to remove debris and invertebrates. All leaves remaining in leaf packs (including pieces used in respiration assays – see below) were dried, weighed, ashed, and re-weighed to determine AFDM. We corrected the initial leaf mass to exclude mass loss due to leaching by soaking five pre-weighed leaf packs in circumneutral stream water for 2 days. After leaching, mean AFDM of leaf packs was 71% of the initial mass (range 70-72%). Leaf litter breakdown rates (-k) were calculated by regressing the natural log (ln) of the percentage leaf mass remaining (AFDM) against time expressed as experiment days (d) and degree-days (dd).

Microbial respiration was measured using small pieces of leaf (~ 2 cm) from each leaf pack. Leaf pieces were placed in 30-mL glass vials that contained stream water from the test stream. The vials were sealed and incubated at 15°C for 8-12 h. Vials were gently shaken during the incubation. Respiration rates were calculated as the difference in dissolved oxygen concentration between vials with leaves and vials containing only stream water per unit leaf mass (g AFDM) per hour. Dissolved oxygen was measured with an electronic meter (YSI550A; YSI Environmental Incorporated, Ohio, USA). Leaves were also cross-incubated in water from each of the four stream types for 12 h to determine whether microbes were adapted to water chemistry by comparing respiration
rates with those obtained in their native streams. Cross incubations were done on leaves and water from one stream from each type on the final day of the experiment (day 60).

Invertebrates collected from the leaf packs were preserved in ethanol (70%), identified following Winterbourn et al. (2000) and counted under a dissecting microscope. The density of total invertebrates and shredding invertebrates found in leaf packs are reported as number of individuals per g AFDM of leaf litter remaining.

**Food web structure & properties**

Food webs were constructed using in-stream data collected during the experiment and a cumulative binary matrix that identified links between predators, prey, and basal resources based on gut contents analysis for streams in our study area. The cumulative matrix was originally compiled by Hogsden and Harding (2012b) and included additional links identified in analysis of recent samples from the same streams (K. Hogsden, unpublished data). Fish and invertebrate consumers used to construct food webs are listed in the Supplementary Material. We calculated several food web properties for each stream, including: web size (S); number of links (L); proportions of basal, intermediate, and top species; predator-prey ratio (Cohen, 1977); linkage density (L/S); and connectance (L/S²).

**Data analysis**

Water chemistry, physical stream characteristics, and leaf litter breakdown rates were compared among the four stream types using analysis of variance (ANOVA) with mean values from each stream as replicates (N = 3 per type). ANOVAs were followed by
Tukey’s post-hoc tests, if significant differences were found. Differences in microbial respiration rates among stream types and over time were examined using repeated-measures ANOVA. We used the Huynh-Felt corrected significance level to address violation of the sphericity assumption ($P = 0.05$). Microbial respiration rates from the cross-incubations were examined using a 2-factor ANOVA with water source and leaf source as factors. Invertebrate and shredder density on leaf packs over time were compared among stream types using the Friedman Test (non-parametric equivalent to repeated-measures ANOVA) and Wilcoxin Signed Rank post-hoc tests with Bonferroni-adjustment for multiple comparisons where significant effects were observed among stream types. Non-parametric tests were used as invertebrate data was highly skewed and could not be normalised to meet normality assumptions. The relationships between both microbial respiration and shredder colonisation with breakdown rate were determined using linear regression. Non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM), both conducted on a Bray-Curtis similarity matrix, were used to establish differences in food web properties among stream types. Cluster analysis was used to confirm groupings in NMDS. Similarity percentages (SIMPER) were then calculated to determine which properties contributed most to the differences between stream groups.

Variables were log-transformed as needed to meet assumptions of normality for parametric tests. Food web properties were square-root transformed prior to analysis. Statistical analyses were performed using SYSTAT 10 (SPSS, 2000), Sigmaplot 11.0 (Systat Software Inc., 2008) and Primer 6.1.12 (Clarke and Gorely, 2006).
Results

Stream characteristics

Stream groups had markedly different water chemistries, particularly for variables related to the presence of mine drainage (i.e., pH, conductivity, Al, Fe), but were similar in terms of physical disturbance (e.g., Pfankuch channel stability index; Table 1). Acidic mining streams were significantly warmer over the course of the experiment and had generally higher concentrations of nitrate and phosphate than all other streams (Table 1). Precipitate mining streams were differentiated from acidic mining streams based on water chemistry and the presence of iron hydroxides. Aside from having a lower pH, water chemistry in naturally acidic streams was comparable to circumneutral streams.

The mean amount of CPOM standing stock (biomass) did not differ between stream types (Table 1), and was highly variable in all types (CV: acidic mining = 62%; precipitate mining = 31%; naturally acidic = 55%; circumneutral = 24%). Similarly, FPOM biomass was comparable among all stream types (Table 1) and highly variable within types, particularly in precipitate mining streams (CV: acidic mining = 107%; precipitate mining = 245%; naturally acidic = 92%; circumneutral = 68%). In contrast, in-stream invertebrate biomass differed significantly among stream types (Table 1). This difference was driven by lower invertebrate biomass in both acidic and precipitate mining streams compared to circumneutral streams.

Leaf litter breakdown

Breakdown rates of mahoe leaves ranged from 0.01 – 0.09 d⁻¹ among all streams. Breakdown rates were significantly slower in acidic mining, precipitate mining, and
### Table 1

Physicochemical characteristics, standing stocks of CPOM and FPOM, and invertebrate biomass in the four stream types over the 60-day experiment. Values shown are group means with ranges below in parentheses. Results of one-way ANOVAs testing for differences among stream types for each variable are also shown. Stream type differences are indicated by different superscript letters (*N* = 3 streams per type). * *P* < 0.05, ** *P* < 0.001

<table>
<thead>
<tr>
<th></th>
<th>Acidic mining</th>
<th>Precipitate mining</th>
<th>Naturally acidic</th>
<th>Circum-neutral</th>
<th>ANOVA</th>
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<td><strong>pH</strong></td>
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<td>3.9</td>
<td>5.0</td>
<td>6.8</td>
<td>10.1*</td>
</tr>
<tr>
<td></td>
<td>(2.5 - 2.9)</td>
<td>(3.5 - 4.9)</td>
<td>(4.1 - 5.6)</td>
<td>(6.5 - 7.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Specific conductivity (µS&lt;sub&gt;25 cm&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>1717&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.5**</td>
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<tr>
<td></td>
<td>(896 - 2230)</td>
<td>(76 - 529)</td>
<td>(26 - 75)</td>
<td>(34 - 64)</td>
<td></td>
</tr>
<tr>
<td><strong>Al (mg l&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>84.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>149.0**</td>
</tr>
<tr>
<td></td>
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<td>(2 - 4)</td>
<td>(0.23 - 0.81)</td>
<td>(0.02 - 0.05)</td>
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</tr>
<tr>
<td><strong>Fe (mg l&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>30.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.1**</td>
</tr>
<tr>
<td></td>
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<td>(1 - 3)</td>
<td>(0.19 - 0.29)</td>
<td>(0.02 - 0.06)</td>
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</tr>
<tr>
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<td>14.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(8.6 - 37.1)</td>
<td>(8.4 - 33.2)</td>
<td>(9.1 - 35.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Pfankuch index</strong></td>
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<td>68</td>
<td>67</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(64 - 110)</td>
<td>(58 - 75)</td>
<td>(59 - 72)</td>
<td>(63 - 108)</td>
<td></td>
</tr>
<tr>
<td><strong>NO&lt;sub&gt;3&lt;/sub&gt;⁻ (µg l&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>209.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>26.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>31.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8*</td>
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<tr>
<td></td>
<td>(102 - 466)</td>
<td>(4 - 232)</td>
<td>(4 - 57)</td>
<td>(11 - 63)</td>
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<tr>
<td><strong>PO&lt;sub&gt;4&lt;/sub&gt;³⁻ (µg l&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td>101.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.3&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>8.5*</td>
</tr>
<tr>
<td></td>
<td>(23 - 232)</td>
<td>(0 - 16)</td>
<td>(0 - 10)</td>
<td>(2 - 78)</td>
<td></td>
</tr>
</tbody>
</table>

CPOM standing stock (g AFDM m<sup>-2</sup>)
- 2.0 (0.6 - 2.9)
- 1.8 (0.5 - 3.3)
- 1.5 (1.0 - 2.5)
- 1.8 (1.7 - 1.9)
- 0.7

FPOM standing stock (g AFDM m<sup>-2</sup>)
- 0.2 (0.004 - 0.4)
- 2.0 (6.2)
- 0.2 (0.02 - 0.5)
- 0.04<sup>a,b</sup> (0.03 - 0.4)
- 4.7<sup>i</sup>

Invertebrate biomass (g AFDM m<sup>-2</sup>)
- 0.0007<sup>a</sup> (0.0001 - 0.001)
- 0.0003<sup>a</sup> (0.001 - 0.001)
- 0.05<sup>b</sup> (0.04 - 0.07)

Sampling notes: Temperature was logged hourly throughout the experiment. Al and Fe samples and Pfankuch scores were taken on day 0. All other variables are means for 5 sampling dates.

<sup>i</sup>Indicates H value for Kruskal-Wallis ANOVA on ranks rather than parametric ANOVA (df = 3).
naturally acidic than circumneutral streams ($F_{3,11} = 23.47, P < 0.001$; Fig. 1a). The slowest rates were observed in precipitate streams, but these rates were not statistically different from acidic mining or naturally acidic streams. When breakdown rates were corrected for temperature (i.e., using degree-days), the patterns remained as above but the difference in rates between naturally acidic and circumneutral streams was no longer significant ($F_{3,11} = 6.80, P = 0.01$; Fig. 1b). The remainder of analyses including breakdown rates will be reported in degree-days.

Microbial respiration

Microbial respiration on leaves differed significantly among the four stream types over the course of the experiment (RM-ANOVA, type x time, $F_{6,12} = 3.02, P = 0.05$; Fig. 2). Overall, respiration rates of leaves from acidic and precipitate mining streams were ~40 and 60% lower, respectively, than those from circumneutral streams whose rates were always highest. Mean respiration rates were highest on leaves from all streams early in the experiment (day 15), except in naturally acidic streams where rates were slightly higher on day 60. Leaves from precipitate streams had accumulated precipitates on their surfaces and showed a consistent decline in respiration rates over time (Fig. 2). We also found a significant effect of leaf source ($F_{3,15} = 6.82, P = 0.01$) but not water source ($F_{3,15} = 2.93, P = 0.09$) on respiration in the cross-incubation experiment. Respiration rates tended to be lowest on leaves from streams with iron hydroxide precipitates (Fig. 3), however, respiration was only significantly lower on precipitate leaves when compared with leaves from circumneutral streams regardless of water source ($P < 0.05$). Finally,
microbial respiration was positively related to breakdown rate ($R^2 = 0.71, P < 0.001$; Fig. 5a).

**Fig. 1** Breakdown rates of mahoe leaves after 60-days exposure in acidic mining, precipitate mining, naturally acidic, and circumneutral streams reported by (a) days and (b) degree days. Data shown are group means + standard error ($N = 3$ streams per type).
**Fig. 2** Microbial respiration rates measured on leaves collected from acidic mining, precipitate mining, naturally acidic, and circumneutral streams on days 15, 32, and 60 of the leaf breakdown experiment. Data shown are group means + standard error. ($N = 3$ streams per type).

**Invertebrates**

Total invertebrate and shredder colonisation of leaf packs differed significantly among stream types (invertebrate: $\chi^2 = 25.6$, df = 3, $P < 0.001$; shredder: $\chi^2 = 15.9$, df = 3, $P = 0.001$) but not over time (Fig. 4a, b). Significant pairwise differences were observed between all types, except acidic and precipitate AMD streams (Bonferroni-adjusted $P = 0.08$). These differences were driven by the low number of invertebrates (1-4 individuals/pack) found on leaf packs in any AMD-impacted stream. On average, three times more invertebrates and shredders were found in circumneutral than naturally acidic
stream leaf packs. However, there was considerable variation within streams and on different sampling dates. Only four shredder taxa were identified in leaf packs, the stonefly *Austroperla cyrene* and the caddisflies *Oeconesus* sp., *Olinga feredayi* (both facultative shredders), and *Triplectides* sp. (an obligate shredder); they made up, on average, less than 25% of the invertebrate community in any stream (Fig. 4b). As most streams had few shredders in the leaf packs, there was no relationship between shredder colonisation and breakdown rate (Fig. 5b).

Fig. 3 Microbial respiration rates measured on leaves from a representative stream (leaf source) in each type cross-incubated in water from representative streams (water source) in all other types. Leaves were collected from the streams on day 60 ($N = 3$ replicates per cross).
Fig. 4 Mean (a) total invertebrate and (b) shredder colonisation (as density) on leaf packs in acidic mining, precipitate mining, naturally acidic, and circumneutral streams during the 60-day leaf breakdown experiment. Data shown are group means + standard error ($N = 3$ streams per type). Significant post-hoc differences ($P < 0.05$) are indicated by different letters above each group of bars.
Fig. 5 Relationships between breakdown rate and (a) microbial respiration rate and (b) shredder density in leaf packs. Open triangles represent acidic mining streams, open inverted triangles precipitate mining streams, closed squares naturally acidic streams, and closed diamonds circumneutral streams. Data shown are means for each stream over the 60-day experiment (error bars not shown).

Food web properties

Streams were clearly separated into three clusters based on food web properties, which differed significantly among stream types (ANOSIM: Global $R = 0.77$, $P = 0.03$;
Due to low replication within types, it was not possible to test for pairwise differences. However, the first cluster included only acidic mining streams, the second cluster included all precipitate mining streams and one naturally acidic stream, and the third cluster included the remaining two naturally acidic and all circumneutral streams. These clusters (at 75% similarity) were confirmed using hierarchial clustering analysis (Clarke and Gorley, 2006). Some streams within each cluster could not be significantly differentiated from each other based on food web properties (data not shown). Differences in the number of links (L) and web size (S) contributed most to the dissimilarity among food webs of the different stream types (minimum contribution 22%; Table 2). These two properties were highly and positively correlated in our streams ($r = 0.97$, $P < 0.001$).

**Fig. 6** NMDS ordination of streams based on 10 food web properties. Clusters at similarity levels of 75% have been superimposed on the ordination diagram. Symbols for stream types as in Fig. 5.
Acidic mining streams had the smallest food webs with the fewest links overall (Table 2). Basal resources (i.e., coarse detritus (CPOM), amorphous detritus (FPOM), algae, filamentous green algae, diatoms) made up the greatest proportion of ‘species’ in acidic mining food webs; more than twice the proportion found in precipitate mining streams (Table 2). Of all stream types, precipitate mining food webs had the highest proportion of top species, which were primarily predatory hydrobiosid caddisflies, dobsonflies and tanypod chironomids. The proportion of intermediate species, which play an important role in energy transfer to upper trophic levels, was at least 2.5 times higher in the more species-rich naturally acidic and circumneutral stream food webs relative to both mining stream groups (Table 2) and reflected the presence of diverse primary and secondary (invertebrate) consumer communities. Fish were absent from all mining streams and one naturally acidic stream but were present in all other streams (Supplementary Material).

\textit{Linking breakdown with food web structure}

Our results indicate that leaf breakdown in our study streams, particularly mining and naturally acidic streams, was primarily driven by microbial activity. Therefore, we considered the relationship between breakdown and the two food web properties (i.e., L and S), which contributed most to the differences between stream groups, from a bottom-up perspective rather than from a top-down (i.e., indirect food web mediated) perspective (e.g., Hlyadz et al., 2011). As the number of links (L) and food web size (S) were highly correlated, we have only presented results for S. Food web size was significantly related to breakdown rate, which was highest in species-rich circumneutral stream food webs \( (R^2) \).
Precipitate mining streams had slower breakdown rates but supported larger food webs with more links than acidic mining streams (Fig. 7).

**Fig. 7** Relationships between food web size (S) and leaf breakdown rate. Data shown are mean breakdown rates for each stream (error bars not shown). S values were calculated once for each stream. Symbols for stream types as in Fig. 5.
Table 2 Food web properties for each stream group: web size (S); number of links (L); proportions of basal, intermediate, and top species; predator-prey ratio (Cohen, 1977); linkage density (L/S); and connectance (C = L/S²). Values shown are group means with ranges in parentheses (N = 3 streams per group).

<table>
<thead>
<tr>
<th></th>
<th>Acidic mining</th>
<th>Precipitate mining</th>
<th>Naturally acidic</th>
<th>Circumneutral</th>
</tr>
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<tr>
<td>S</td>
<td>6</td>
<td>16</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(6 – 7)</td>
<td>(10 – 20)</td>
<td>(17 – 42)</td>
<td>(41 – 55)</td>
</tr>
<tr>
<td>L</td>
<td>7</td>
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</tr>
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Discussion

Leaf breakdown – rates & mechanisms

Rates of leaf breakdown were slow in mining-impacted streams, compared to rates in circumneutral, but not naturally acidic streams. Breakdown rates of mahoe in this study extended the range reported across a land use gradient in New Zealand (-k = 0.04 – 0.07 d⁻¹; 0.002 – 0.005 da⁻¹; Young and Collier, 2009) and were considered ‘fast’ relative to other species used in AMD-impacted (American sycamore, Barnden and Harding, 2005; red beech, Niyogi et al., 2013) and naturally acidic streams (kamahi, Collier and Winterbourn, 1987) in this region of New Zealand.

Slow rates of leaf litter breakdown in mining streams were driven primarily by microbial activity, and are consistent with the results of other studies (e.g., Siefert and
Mutz, 2000; Niyogi et al., 2001). In fact, respiration rates mirrored patterns in leaf breakdown across all stream types suggesting that microbial activity played a primary role in breakdown in all streams, particularly as shredder abundance was consistently low or variable in leaf packs. Experimental work has shown that microbes are adapted to ambient pH and can effectively contribute to organic matter breakdown in anthropogenically acidified (Simon et al., 2009) and naturally acidic waters (Collier and Winterbourn, 1987; Tank and Winterbourn, 1995). However, microbes in naturally acidic streams are more likely to be consumed by invertebrates compared to those in mining streams where they are released from intense grazing pressure. In addition, elevated water temperatures and nutrient (N & P) concentrations may have enhanced microbial activity in acidic mining streams. Low groundwater inputs may have contributed to the warmer temperatures of these forested streams relative to others in the study. Niyogi et al. (2013) also reported high N and P concentrations in highly acidic streams affected by mine drainage and suggested these two nutrients, which can be derived from geologic sources, mineral tailings, or mining chemicals, may have negated the stress effects of acidity and dissolved metals on microbial respiration. In contrast, respiration declined over the course of our in-stream experiment as iron precipitates accumulated on leaves, likely inhibiting fungal colonization of leaf surfaces (Gray and Ward, 1983) and negatively affecting bacterial activity (Niyogi et al., 2003). Our finding that leaves coated in precipitates always had the lowest respiration rates in our cross-incubation experiment regardless of ambient water chemistry provides further support for that suggestion. The lack of relationship between shredder colonisation and breakdown rates in our study indicated that shredders are not always important contributors to the breakdown
process; this may be expected, as there are few specialist shredders in the New Zealand stream fauna (Thompson and Townsend, 2000). Nevertheless, evidence of fragmented leaves in some circumneutral streams (K. Hogsden, personal observation) indicated that where locally abundant, shredders could contribute to faster breakdown. Furthermore, the presence of more shredders in circumneutral streams may have contributed to faster breakdown compared to naturally acidic streams, as shown in other streams in the Westland Forest ecoregion (Collier and Winterbourn, 1987; Collier et al., 2006).

Leaf breakdown and food webs

Leaf breakdown rates were slower and food webs were small and simplified in mining-impacted streams compared to non-impacted streams in our study. Inputs of mine drainage disrupted mechanisms regulating leaf breakdown (i.e., microbes and shredders), caused substantial loss of consumers, and resulted in declines in overall interactions between species. Despite the impairment of detrital processing, it appears that sufficient resources are contributed to the detrital pool to support a small number of tolerant consumers in AMD-affected streams. However, by reducing the rate at which leaf litter is converted to smaller, edible size fractions, AMD might limit the abundance and biomass of consumers that can be supported. The energetic constraints from reduced detrital processing, which can lead to low secondary production (e.g., Carlisle and Clements, 2005), will likely be more pronounced in recently or moderately-affected mining streams with larger and more complex food webs, particularly where fish are present, as there would be insufficient prey resources available. The correlational data presented here for the four stream types, shows that streams with faster breakdown rates also had larger
food webs with more links among species. This suggests the more rapid conversion of
detrital energy in naturally acidic and circumneutral streams has the capacity to support
larger food webs, because energy is more readily available to transfer to higher trophic
levels. The similar patterns of food web structure and function between naturally acidic
and circumneutral streams is consistent with findings of strong similarities in community
composition and leaf breakdown rate reported by a number of authors (Collier et al.,
1990; Petrin et al., 2008; Hogsden and Harding, 2012b [Chapter 2]).

Interestingly, precipitate mining streams had slower breakdown rates but
supported food webs with more than twice as many consumers as acidic mining streams.
It is usually assumed that precipitates make leaves unavailable or inedible for consumers,
or cause food quality to decline corresponding with reduced microbial conditioning of
leaves. For example, Niyogi et al. (2013) recently reported that consumption by a
shredder (O. feredayi) of beech leaves coated in iron hydroxides was significantly
reduced relative to leaves conditioned in circumneutral or highly acidic mining-affected
waters. In contrast, Schlief and Mutz (2006) found that hydroxides did not influence the
palatability of leaves for Gammarus pulex. Thus, precipitates may serve as a substrate for
the Fe-loving bacteria, and perhaps a site for the adsorption of carbon compounds that
provide new or different foods for consumers (Schlief and Mutz, 2006). Consequently,
the presence of precipitates on decaying leaves may benefit consumers other than
shredders (e.g., collector-grazers; Hlaydz et al., 2011) as more detached bacteria, fungal
hyphae and conidia are released with FPOM from the decomposing leaves (Gessner et
al., 1999). More detailed dietary analysis is needed to confirm the presence and
significance of these proposed microbial links. These additional resources may partially
explain the greater number of consumers and larger food webs that were found in precipitate mining compared to acidic mining streams.

*Detrital resources and energy flow*

Despite leaf breakdown being impaired in mining streams, we did not observe accumulations of undecomposed or slowly decomposing litter (as CPOM standing stock) in them. Furthermore, FPOM standing stock was comparable across all streams. While our four stream types did not differ based on physical disturbance, the West Coast region receives frequent heavy, intense rainfall events, which scour stream beds, flush organic material downstream, and can cause variability in standing stocks of detrital resources (e.g., as CPOM, FPOM; Kitto, 2009). The high variability observed in CPOM and FPOM standing stock in most streams likely reflects this high level of physical disturbance rather than differences in water chemistry and suggests the streams have highly disturbed and volatile resource bases.

Most ecological interactions in our mining stream food webs occurred between consumers and amorphous detritus (FPOM) and there were few predatory interactions. Layer et al. (2010) reported a similar pattern in streams across a pH gradient (5.0 – 8.4), although weak predatory interactions between fish and invertebrates were maintained in their most acidic streams. Gut content analysis has revealed that FPOM is the dominant basal resource in the diet of tolerant consumers in mining streams (Hogsden and Harding 2012b [Chapter 2]), suggesting that consumer-detritus linkages drive energy flow in these webs, as in other acidic streams (Ledger and Hildrew, 2005; Hildrew, 2009; Layer et al., 2010). However, the transfer of energy was disrupted at the primary consumer level in
our mining streams, as there were few intermediate species to provide direct or indirect links between detrital resources and higher trophic levels (Woodward, 2009). The proportion of intermediate species in mining stream food webs was considerably smaller than in naturally acidic and circumneutral webs in our study, which were comparable to those in other, similarly resolved webs (see Mantel et al., 2004). The absence of fish and the presence of only a few predatory invertebrates in mining stream webs meant that little energy was required to support higher trophic levels. Therefore, as energy flow pathways were disrupted by species loss at intermediate trophic levels, any unused processed energy or nutrients were likely exported downstream.

**Conclusions**

Leaf breakdown and food web structure are both effective indicators of mine drainage impacts on streams (Gessner and Chauvet, 2002; Hogsden and Harding, 2012b [Chapter 2]). We found that the processing of detritus was suppressed by inputs of mine drainage, which limited the presence and activity of shredding invertebrates and microbes, but that the few tolerant consumers present in the small mining stream food webs maintained consumer-detritus interactions. Together, our results indicated that mine drainage disrupted mechanisms responsible for a key ecosystem function and links for energy flow within a food web. However, while less detrital energy from leaf breakdown is available to be transferred up the food web, little was needed to support the small number of higher trophic level consumers. In addition, our results highlight differences in food webs and leaf breakdown in highly acidic and precipitate mining streams, which
suggests the importance of metals, both in dissolved and precipitated forms, as drivers of structure and function in streams affected by mine drainage.

Acknowledgements

I thank Ian Reeves and Phil Jellyman for field assistance, Milen Marinov, Haley Stoddart, and Linda Morris for help in the laboratory, and Mike Winterbourn for useful comments that improved this manuscript. The Brian Mason Scientific & Technical Trust and the Foundation for Science Research & Technology (Grant CRLX0401) funded this research. I was supported by scholarships from the Natural Sciences and Engineering Council of Canada (NSERC) and the University of Canterbury.
References


Pfankuch, D.J., 1975. Stream reach inventory and channel stability evaluation, USDA Forest Service Northern Region, Montana.


**Supplementary Material.** Fish and invertebrate consumers used to construct food webs and the number of streams in which they were found for each stream type. (− = absent)

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<th>Precipitate mining</th>
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<th>Circum-neutral</th>
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Chapter 4

Does food quantity and quality affect food webs in streams polluted by acid mine drainage?

Deposited metal hydroxides coat stream substrata.
Abstract

Food influences the structure of all consumer communities, however, in polluted streams food resources may be severely reduced and act as an additional, though often ignored, stressor. We examined the quantity and quality of basal resources and prey items for invertebrate consumers and characterised their diets using stable isotope and gut content analyses in 12 streams along a gradient of acid mine drainage (AMD) pollution (pH range: 2.7 – 7.1). Algal and detrital resource quantity (biomass) and quality (C: N ratio) did not differ significantly along the gradient, except for algal C: N, which was lower in highly stressed and circumneutral streams. Furthermore, prey availability, size, and diversity decreased significantly with increasing stress. Most primary consumers fed on and assimilated algal and detrital resources, regardless of AMD stress, but algae became increasingly common in their diets as stress increased, even where detrital resources were abundant. Predators were opportunistic, consuming prey items that reflected the taxa local abundant. Generally these were low densities of small-bodied chironomids in highly stressed streams, whereas increasingly abundant, larger-bodied prey (mayflies, caddisflies, and stoneflies) were most common in moderately stressed and circumneutral streams. Our results indicate that food quantity was unlikely to be limiting for primary consumers, but may be an additional stressor affecting predators in streams receiving AMD. Declines in energy flow to higher trophic levels may also stress food webs downstream or in adjacent ecosystems.

Keywords: acid mine drainage (AMD), basal resources, C: N ratio, stable isotopes, filamentous green algae, prey
Chapter 4 – Invertebrate food quantity & quality

Introduction

Mining activities, including the release of acid mine drainage (AMD) from historic and contemporary mines, exert significant pressures on freshwater biota worldwide. Typically, AMD is highly acidic, heavy metal-rich drainage and if exported downstream can cause widespread contamination along river networks and potentially in receiving coastal zones. Much ecological research on AMD in streams has focused on its impacts on benthic invertebrate communities, particularly species richness and abundance, and on shifts in communities to those dominated by acid or metal tolerant species (e.g., Koryak et al. 1972; Winterbourn 1998; Gerhardt et al. 2004; Battaglia et al. 2005; MacCausland and McTammany 2007). Such community-based changes are most often attributed to low pH or toxic metal concentrations (e.g., Al, Cu, Fe, Zn). Additionally, metal hydroxides, especially those of Al and Fe, may precipitate out of solution as stream water pH increases (Kelly 1998). Metal precipitates can also coat animal bodies, gill surfaces, gut linings, and clog digestive tracts reducing activity and survival (Gerhardt 1992; Soucek et al. 2001). Furthermore, the precipitates on stream substrata can limit algal growth and slow leaf litter breakdown, thereby potentially reducing food available for primary consumers (e.g., McKnight and Feder 1984; Schlief and Mutz 2005).

Changes in the quantity and quality of food resources may act as stressors influencing the structure of invertebrate communities in AMD-impacted streams (McKnight and Feder 1984; Wellnitz and Sheldon 1995; Niyogi et al. 2002). The availability of basal resources (e.g., algae, detritus) to primary consumers and invertebrate prey to predators may be affected in streams receiving inputs of AMD, but
has received limited attention. Algal biomass can vary substantially in impacted streams, with low biomass often reflecting the dominance of diatoms, and high biomass the presence of large-celled filamentous green algae (Verb and Vis 2000; Winterbourn et al. 2000a; Bray et al. 2008). However, even if algal resources are abundant, they may not be available as food for invertebrates due to limitations in their feeding strategies (e.g., grazers among filamentous algae). Similarly, terrestrial food subsidies in the form of leaf litter (detritus) may be abundant, but the conversion of leaf litter to smaller (edible) size fractions may be limited due to the reduced activity of microbes and shredders, which impairs the breakdown process in AMD-affected streams (e.g., Niyogi et al. 2013). In contrast, consistently low invertebrate abundance and diversity suggests that prey availability is often reduced in the presence of AMD (Schultheis et al. 1997; Gerhardt et al. 2004; Battaglia et al. 2005). Furthermore, invertebrates are likely to consume contaminated foods since metals accumulate in periphyton, plant material, bacteria, fungi, and invertebrate body tissues (Bermingham et al. 1996; Winterbourn et al. 2000a; Besser et al. 2001); however, other aspects of food quality (e.g., C: N) have not been documented in AMD-affected streams. Several factors such as microbial colonisation, particle size, and nutrient concentrations that can influence the elemental composition of basal resources in streams (Cross et al. 2005) may be affected by inputs of AMD. For example, several studies have shown that metal precipitates reduce microbial colonisation and slow the breakdown of leaf litter in streams receiving AMD (Gray and Ward 1983; Schlief and Mutz 2005; Niyogi et al. 2013). They may also limit increases in food quality (i.e., decreases in C: N) that typically accompany litter breakdown (Quinn et al. 2000). A shift to a periphyton assemblage dominated by acid-tolerant taxa may also affect food
quality, as nutrient ratios can differ with the composition and biomass of organisms present (Frost et al. 2005). Therefore, food resources have the potential to mediate changes in invertebrate community structure in streams receiving AMD.

The feeding habits of tolerant taxa in acidic or metal-polluted streams may reflect food availability and quality. When resources are limited, consumers may be forced to adapt and feed more generally, or opportunistically, and may also include a greater proportion of lower quality foods in their diets. For example, Layer et al. (2012) showed that herbivore-detritvores consumed less algae and more detritus as acidity increased in 20 streams along a pH gradient (pH 5.0 – 8.4). Similarly, Ledger and Hildrew (2000) reported that the diet of a detritivorous stonefly (*Nemurella pictetii*) shifted between algae and fine particulate organic matter (FPOM) in an acid stream over the course of one year depending on resource availability. Diets of some primary consumers have also been shown to track acid-induced changes in algal community composition following experimental acidification (Junger and Planas 1993). The C: N ratios of resources might also influence food choice, as foods with lower C: N are of higher quality (e.g., algae) and are preferred by most consumers (Sterner and Elser 2002). Furthermore, evidence from feeding studies in acidic streams suggests that some tolerant taxa can adjust their ingestion rates to compensate for lower quality food (e.g., a leptophlebiid mayfly, Collier and Winterbourn 1990; a nemourid stonefly, Ledger and Hildrew 2001). The feeding habits of predatory invertebrates may also be constrained in polluted streams by the availability, body size, and composition of their prey communities. For example, metal-tolerant simuliids and orthoclad chironomids dominated the diets of predatory invertebrates in streams moderately polluted by zinc, as the availability of prey items
typical of nearby unpolluted streams (e.g., hepatgeniid mayflies) was low (Carlisle 2001). Factors that affect food quantity and quality translate directly into effects on energy flow and can limit production at higher trophic levels in stressed stream food webs (Shieh et al. 2002; Carlisle and Clements 2003).

Recent work on AMD-impacted stream food webs in New Zealand has indicated that most primary consumers are generalist feeders (gut content analysis; Hogsden and Harding 2012 [Chapter 2]) but less is known about the assimilation of resources or dietary links between secondary consumers and their prey. The composition of food assimilated by consumers is reflected by their stable carbon isotope signatures ($\delta^{13}$C) and the trophic position of consumers can be estimated from the nitrogen isotope signatures ($\delta^{15}$N) of body tissues. Together, gut content and stable isotope analyses can provide complementary information on food ingestion and assimilation, respectively, and have been used to identify food resources, feeding links and trophic interactions (i.e., basal resource – consumer, prey - predator) in streams. Despite their combined value, these techniques have not often been used together in stream food web studies (but see Mantel et al. 2004; Layer et al. 2012).

In the present study, we focused on the quantity and quality of algae, detritus, and invertebrates as foods available for invertebrate consumers in streams. Our objectives were to determine: 1) whether food quantity and quality (C: N ratio) differed in streams along an AMD-impact gradient; and 2) whether the diets of invertebrate consumers reflected any changes. We expected that the quantity but not quality of basal resources would be comparable across the gradient and that fewer prey items of similar quality
would be present in mining-impacted streams and that predatory consumer feeding habits would reflect these differences.

Methods

Study sites

The study was conducted in the Buller-Grey region in the north-west of the South Island, New Zealand. The region is part of the Westland Forest ecoregion, has relatively uniform climatic conditions, geology and freshwater biota, and a long history of coal mining (Harding et al. 1997; Harding and Boothroyd 2004). We selected twelve streams ranging from strongly affected by acid mine drainage to non-impacted, based on known water chemistry (i.e., pH, conductivity, metal concentrations) (Winterbourn et al. 2000a; Greig et al. 2010; Hogsden and Harding 2012 [Chapter 2]). The AMD gradient included nine streams receiving inputs from active or abandoned coal mines and three reference streams (Appendix C). Fieldwork was undertaken in December 2009 (austral summer) under baseflow conditions with all samples being collected along a 20 m reach within each stream. Stream water pH and conductivity (at 25°C) were measured in the field using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA) to confirm the degree of AMD impact on each stream. Spot water samples were also collected for dissolved metal analysis. These samples were filtered in the field (0.45 μm mixed cellulose ester filter), acidified with nitric acid, and kept cool prior to analysis using an ICP-MS at Hill Laboratories, Hamilton, New Zealand. Metal hydroxide precipitates present on stream substrata were quantified by scrubbing the upper surfaces of five randomly selected stones of known area from each stream. The resulting slurry
was dried, weighed and ashed (550°C for 4 h) in the laboratory to determine the amount of metal hydroxide (i.e., inorganic or ash mass) per area of stone. Because the mass of metal hydroxide precipitate did not differ significantly between streams (ANOVA: $P > 0.05$) it is reported as present or absent. All streams were of similar size (first or second order, wetted width 1.9 – 5.4 m), had cobble-gravel substrates or bedrock, and were surrounded by either native podocarp and/or beech forest, or scrub. Shading was estimated visually in the middle of each stream near the sampling reach using a densiometer (Robert E. Lemmon Forest Densiometer Model-A, Oklahoma, USA).

Food quantity - Sampling and analysis

Periphyton was removed from the upper surfaces of 15 randomly selected cobbles (length 3 – 13 cm) using a hard-bristled brush, rinsed with filtered stream water, and pooled into three representative samples. The surface area of each cobble was estimated from length measurements of the two main axes. Each sample was filtered (0.7 µm-GF/F) in the field and frozen prior to determining algal biomass (as chlorophyll $a$). In the laboratory, algal pigments were extracted in ethanol (90%) for 24 h, at 4°C in the dark. Tubes were centrifuged and dilutions, if required, were made using 90% ethanol. Extracts were read on a Shimadzu model UV-1700 Pharmaspec spectrophotometer (Shimadzu, Kyoto, Japan) at 665 nm and 750 nm wavelengths using a 1 cm path-length quartz cell. Extracts were then acidified with 0.1 mL of 0.1 N HCL, gently agitated, held for 90 s and re-read at the above wavelengths to correct for pheophytin, if present. Chlorophyll $a$ was calculated following Biggs and Kilroy (2000) and is expressed per area of upper cobble surface. Benthic organic matter was collected in five Surber samples (0.06 m$^2$; 250 µm
mesh) and divided into two size fractions: fine particulate organic matter (FPOM; 250 – 1000 μm) and coarse particulate organic matter (CPOM; > 1000 μm). FPOM and CPOM samples were dried, weighed, ashed (550°C for 4 h) and re-weighed to determine ash-free dry mass (AFDM).

Benthic invertebrates were picked from the five Surber samples to characterise the availability and diversity of prey items. Samples were preserved in ethanol (70%) and processed under a dissecting microscope in the laboratory. Taxa were identified and counted to the lowest possible taxonomic level according to Winterbourn et al. (2000b) and unpublished keys (NIWA, Hamilton, New Zealand). All invertebrates were classified into functional feeding groups following Winterbourn et al. (1984) and Chadderton (1988), dried, weighed, ashed (550°C for 4 h), and re-weighed to determine AFDM.

**Food quality and diet - sampling and analysis**

We estimated food quality based on carbon to nitrogen (C: N) ratios and evaluated the sources of food assimilated by invertebrates (algae vs allochthonous detritus) using stable carbon ($^{13}$C: $^{12}$C) and nitrogen ($^{15}$N: $^{14}$N) analyses. To complement the stable isotope work, gut content analyses were conducted on invertebrates following Hogsden and Harding (2012). All invertebrates used for stable isotope and gut analyses were medium-large individuals of their respective species. Food items from gut contents were identified (animal prey, CPOM, FPOM, algae, filamentous algae and diatoms), their relative abundance in the gut estimated visually by scanning whole prepared slides and categorised as: rare (<5%), common (5 – 20%) or abundant (>20%). Aquatic bryophytes, filamentous green algae, and periphyton (epilithon) were collected (when present) from
the surfaces of submerged rocks, and rinsed to remove any invertebrates, detritus, or sediment before freezing. Detritus (primarily decomposing leaf litter) was collected from the streambed, hand-picked to remove invertebrates, rinsed to remove biofilm, and frozen until further analysis. Fine particulate organic matter, which can have multiple origins, was not collected for isotope analysis, but its allochthonous component can be expected to have a $\delta^{13}$C signature similar to that of dead leaves taken from the study streams (Rounick et al. 1982; Winterbourn et al. 1984). For each stream, replicate samples of each basal resource (if available) were pooled to ensure sufficient material was available for analysis. Additional invertebrates for gut and isotope analysis were collected with a kicknet (500 µm mesh) sorted into taxa, placed in vials in the field, and frozen.

In the laboratory snails and caddisfly larvae were removed from their shells and cases, respectively, and guts were removed from most invertebrates. They were then oven dried at 60°C for at least 48 h and ground to a fine, homogeneous powder. Basal resource and prey samples were pooled for each stream. Invertebrate samples for analysis comprised material from 2-40 individuals, depending on their size. Samples of dried powder (c. 3 mg for plants, 1 mg for invertebrates) were packed into tin capsules and analyzed at the Cornell Isotope Laboratory (Cornell University, Ithaca, New York) on a Therma Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer to obtain total C and N masses and isotope signatures. C: N ratios were calculated based on total C and N masses. Isotope signatures are expressed in delta ($\delta$) notation defined as parts per thousand (‰) deviation from a standard reference material (i.e., $\delta^{13}$C = ($R_{\text{sample}}/R_{\text{standard}} - 1$) x 1000). Standard reference materials were PeeDee belemnite limestone for $\delta^{13}$C and atmosphere nitrogen for $\delta^{15}$N. Experimental precision
was 0.19‰ (δ^{13}C) and 0.17‰ (δ^{15}N) based on the standard deviation of replicates of an internal standard. We corrected for lipid effects on δ^{13}C signatures using the formulae of Post et al. (2007) prior to data analysis.

**Statistical analysis**

Relationships between water chemistry variables (i.e., pH, specific conductivity, dissolved Al and Fe concentrations) were examined using Pearson correlations. Principal components analysis (PCA) was used to combine these key chemical variables into an AMD stress gradient. PCA axis 1 included pH, conductivity and Al concentration and explained 88.1% of the variance among streams. Pearson correlations between all chemical variables indicated that Fe concentration was highly correlated (r > 0.9) with Al concentration, so Fe was excluded from the PCA. Conductivity and Al concentration were log_{10} (x +1) transformed prior to analysis. Relationships between the AMD gradient and basal resource quantity (chl \( a \) biomass, CPOM and FPOM biomass), prey quantity (invertebrate density, biomass/density, and richness) and food quality (C: N; periphyton, CPOM, prey pooled as primary and secondary consumers) were examined using regression analysis. Different models (linear, polynomial, exponential) were run for each dependent variable to test for best-fit patterns. C: N ratios of periphyton and CPOM in all streams were compared using the Mann-Whitney U test. The isotopic composition (δ^{13}C and δ^{15}N) of all food resources along the AMD stress gradient was also examined using regression. Pearson correlations were calculated between both δ^{13}C and δ^{15}N of basal resources, primary consumers, and secondary consumers, and in-stream metal concentrations (Al, Fe). For metals that were below detection limits, we used a value of zero in calculating correlations. We used false discovery rate (FDR) corrected \( P \) values to
account for the multiple comparisons made in this study (Pike, 2010). Our FDR adjusted $P$ value for the regressions and the correlations was 0.03. Analyses were conducted in Primer 6.1.12 (PCA; Clarke and Gorley 2006) and Sigmaplot 11.0 (correlations and regressions; Systat Inc., 2008).

**Results**

*Water chemistry*

Stream water ranged from highly acidic (pH 2.7) to circumneutral (pH 7.1) along the AMD gradient (Table 1). pH was below 5.0 in all AMD-impacted streams, and above 6.8 in reference streams. Stream water pH was strongly correlated with specific conductivity ($r = -0.69$, $P < 0.01$), and the concentrations of total dissolved Al ($r = -0.60$, $P = 0.04$) and Fe ($r = - 0.87$, $P < 0.001$). Metal hydroxide deposits were present on the substrata of six streams, all with specific conductivities between 100 and 600 $\mu$S$_{25}$ cm$^{-1}$ (Table 1). The 12 streams were ordered along the gradient from circumneutral reference streams to highly stressed acidic streams with elevated metal concentrations. Low and moderately stressed streams were grouped closely together due to similarities in water chemistry at the time of sampling and will be referred to as moderately stressed streams.

*Food quantity*

The biomass of algal and detrital basal resources did not change significantly with AMD stress along the gradient ($P > 0.05$; Fig 1a –c; Table 2). Higher mean periphyton biomass in three AMD-impacted streams (Miller, Mine, and Burnetts Face) reflected the presence of filamentous green algae, which also contributed to considerable variability in biomass among samples within those streams (Fig. 1a). In contrast, periphyton biomass
was consistently low in circumneutral streams. Across all streams, periphyton biomass was not associated with shading ($R^2 = 0.14$, $P = 0.66$). The distribution of CPOM was highly variable within all streams (Fig. 1b). FPOM biomass was generally low in all streams, except at one moderately acidic site (pH 4.1, Alborns) where it was much higher, although very variable in distribution (Fig. 1c). This stream differed from all others in having high-suspended sediment loads in the water column.

**Table 1** Key chemical and physical characteristics of 12 streams forming an AMD gradient in the north-west of the South Island, New Zealand. Values are from single samples collected on 4 – 8 December 2009. * non-AMD streams.

<table>
<thead>
<tr>
<th>Stream name</th>
<th>pH</th>
<th>Conductivity (µS$_{25}$ cm$^{-1}$)</th>
<th>Al (mg l$^{-1}$)</th>
<th>Fe (mg l$^{-1}$)</th>
<th>Metal hydroxides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mine</td>
<td>2.7</td>
<td>1267</td>
<td>43</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>Miller</td>
<td>2.8</td>
<td>1088</td>
<td>34</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>Wellman</td>
<td>3.7</td>
<td>558</td>
<td>4.6</td>
<td>3.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Coal</td>
<td>3.7</td>
<td>240</td>
<td>3</td>
<td>2.5</td>
<td>Yes</td>
</tr>
<tr>
<td>Page</td>
<td>4.5</td>
<td>240</td>
<td>2.4</td>
<td>1.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Coalbrookdale</td>
<td>3.7</td>
<td>132</td>
<td>1.3</td>
<td>1.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Alborns</td>
<td>4.1</td>
<td>128</td>
<td>1</td>
<td>1.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Garvey</td>
<td>4.9</td>
<td>267</td>
<td>0.76</td>
<td>1.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Burnetts Face</td>
<td>4.1</td>
<td>69</td>
<td>0.62</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>Kiwi*</td>
<td>6.8</td>
<td>58</td>
<td>0.2</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>Lankeys*</td>
<td>7.1</td>
<td>40</td>
<td>0.27</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>Italian*</td>
<td>6.9</td>
<td>18</td>
<td>0.23</td>
<td>-</td>
<td>No</td>
</tr>
</tbody>
</table>

"-" indicates concentration was below the level of analytical detection (< 0.40 mg l$^{-1}$)
Mean invertebrate density was lowest in streams impacted by AMD and increased significantly as stress decreased \( (P = 0.002; \text{Fig. 2a}; \text{Table 2}) \). Richness followed a similar pattern, with an increasing number of taxa present as stress decreased \( (P < 0.001; \text{Fig. 2b}; \text{Table 2}) \). Similarly, the mean size of invertebrates, expressed as the ratio of biomass to density, decreased with AMD stress \( (P = 0.02; \text{Fig. 2c}; \text{Table 2}) \). Low biomass and low densities of AMD-tolerant invertebrate taxa, particularly larval Chironomidae (\textit{Eukiefferiella} and unidentified orthoclads), were typical of the highly stressed streams. In contrast, more larger-bodied mayflies, caddisflies, and stoneflies were present in moderately stressed and circumneutral reference streams (see Supplementary Material). Primary consumers were numerically dominant in all streams along the gradient and included collector-browsers, filter feeders, and shredders (Fig. 2d; Supplementary Material). However, with the exception of a shredding scirtid beetle larva, shredders and filter feeders were absent from all AMD-impacted streams (Supplementary Material). Similarly, predatory invertebrates were often absent from acidic streams or were represented by very few species at low densities in contrast to circumneutral streams (Fig. 2e).
Fig. 1 Mean basal resource biomass in streams along the AMD stress gradient ± SE. a) periphyton biomass (chlorophyll $a$), b) coarse organic particulate matter (CPOM) biomass, and c) fine particulate organic matter (FPOM) biomass. $N=12$ streams. Note different y-axis scales for all panels. pH range for streams along AMD gradient also shown.
Fig. 2 Mean (± SE) values of invertebrate a) density, b) richness, and c) individual size (biomass/density) at 12 sites along the AMD stress gradient. Fitted lines show significant regressions. Note different y-axis scales for all panels. Mean invertebrate d) density and e) richness of primary and secondary consumers along the gradient. pH range for streams along AMD gradient also shown.
Table 2 Regression analyses of food quantity and quality parameters with the AMD stress gradient (PCA Axis 1 scores). Significant relationships are highlighted in bold (FDR-corrected $P = 0.03$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>$R^2$</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periphyton biomass</td>
<td>1,11</td>
<td>0.10</td>
<td>2.25</td>
<td>0.17</td>
</tr>
<tr>
<td>CPOM biomass</td>
<td>2,11</td>
<td>0.21</td>
<td>2.43</td>
<td>0.14</td>
</tr>
<tr>
<td>FPOM biomass</td>
<td>1,11</td>
<td>0.00</td>
<td>0.83</td>
<td>0.38</td>
</tr>
<tr>
<td>Invertebrate density</td>
<td>2,11</td>
<td>0.59</td>
<td>14.52</td>
<td>0.002</td>
</tr>
<tr>
<td>Invertebrate richness</td>
<td>1,11</td>
<td>0.75</td>
<td>34.60</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Invertebrate size</td>
<td>2,11</td>
<td>0.61</td>
<td>9.83</td>
<td>0.006</td>
</tr>
<tr>
<td>C: N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periphyton quality</td>
<td>2,7</td>
<td>0.5</td>
<td>11.41</td>
<td>0.01</td>
</tr>
<tr>
<td>CPOM quality</td>
<td>1,7</td>
<td>0.00</td>
<td>0.05</td>
<td>0.59</td>
</tr>
<tr>
<td>Primary consumer</td>
<td>2,9</td>
<td>0.00</td>
<td>0.60</td>
<td>0.57</td>
</tr>
<tr>
<td>Secondary consumer</td>
<td>1,8</td>
<td>0.21</td>
<td>3.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Isotopic signature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta^{13}$C periphyton</td>
<td>2,7</td>
<td>0.13</td>
<td>1.52</td>
<td>0.30</td>
</tr>
<tr>
<td>$\delta^{15}$N periphyton</td>
<td>2,7</td>
<td>0.31</td>
<td>2.55</td>
<td>0.17</td>
</tr>
<tr>
<td>$\delta^{13}$C CPOM</td>
<td>2,11</td>
<td>0.03</td>
<td>1.18</td>
<td>0.35</td>
</tr>
<tr>
<td>$\delta^{15}$N CPOM</td>
<td>1,11</td>
<td>0.00</td>
<td>0.95</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Food quality and isotopic composition*

C: N ratios of periphyton but not CPOM were significantly related to AMD stress (Fig 3a-b; Table 2). C: N ratios were lower in periphyton from two highly stressed and a circumneutral stream compared to moderately stressed streams, however the differences were relatively small (Fig. 3a). Overall, periphyton had a significantly lower C: N ratio
(i.e., higher quality) than CPOM (Mann-Whitney U test, \( P < 0.001 \)). For all sites, mean C: N ratios for periphyton and CPOM were 15.1 and 83.2, respectively. The C: N ratios of primary and secondary consumers were not related to AMD stress (\( P > 0.03 \); Fig. 3c; Table 2) and the range of C: N ratios for primary consumers (3.95 – 6.22) overlapped the range for secondary consumers (3.91 – 5.23).

Neither the \( \delta^{13}C \) nor \( \delta^{15}N \) signatures of periphyton differed significantly across the AMD gradient (\( P > 0.03 \); Table 2). Similarly, the isotopic signatures of CPOM did not change along the AMD gradient (Table 2). In all streams, periphyton resources were depleted in \( ^{13}C \) relative to CPOM (i.e., they had lower \( \delta^{13}C \) signatures). Finally, neither the \( \delta^{13}C \) nor \( \delta^{15}N \) signatures of periphyton, CPOM, primary or secondary consumers were significantly correlated with key water chemistry variables (pH, conductivity, dissolved Al or Fe concentration; \( P > 0.03 \)).

**Diet**

The relative importance of algal and allochthonous detrital resources to consumers was inferred from a combination of isotopic data and gut analyses. The \( \delta^{13}C \) signatures of detritus ranged from -28.2 to -32.0‰ in the 12 streams and those of algae ranged from -23.0 to -34.1‰ in the 8 streams from which all types of samples could be collected. Despite overlap in their ranges over the full complement of streams, signatures of the two resources were distinct at individual sites. The \( \delta^{13}C \) values of collector-browsers, which might be expected to feed on a mixture of detritus and algae, fell between those of the two resources in three streams (Alborns, Garvey, Kiwi), indicating
Fig. 3 C: N ratios of a) periphyton, b) CPOM, and c) invertebrate primary and secondary consumers in streams along the AMD stress gradient. Single values for pooled samples from each stream are given for periphyton ($N = 8$) and CPOM ($N = 12$), and means ($\pm$ SE) for primary and secondary consumer taxa ($N = 10$ and $N = 8$, respectively). Fitted lines show significant regressions. pH range for streams along AMD gradient also shown.
assimilation of both resources. However, they fell outside the isotopic ranges of algae and detritus in another four streams, although more closely resembling algal signatures in three of them (data not shown). Gut content analysis indicated a mixture of algae (filamentous greens, diatoms) and fine detritus was ingested by numerically dominant primary consumers, which were mainly collector-browsers in all streams regardless of AMD stress level (Table 3). Where present, shredders commonly consumed CPOM (Table 3). Interestingly, almost half the primary consumer guts examined from acidic streams were empty, whereas those from circumneutral streams always contained some food.

The trophic positions of consumers, inferred from $\delta^{15}N$ signatures assuming a fractionation rate of 2.3 to 3.4‰ (see McCutchan et al. 2003; Kilham et al. 2009), indicated that groups of primary and secondary consumers were more distinct in circumneutral streams. In contrast, the $\delta^{15}N$ signatures of some species that are normally considered to be either primary or secondary consumers (Winterbourn et al. 1984) did not differ as expected in some acidic streams. For example, the collector-browser stonefly, Spaniocercoides had similar $\delta^{15}N$ values to the predatory caddisfly, Psilochorema and the predatory stonefly, Stenoperla. Gut contents revealed that in circumneutral streams, predators, such as Stenoperla, Hydrobiosis, and Archichauliodes had often fed on the numerically most abundant primary consumer (i.e., the mayfly, Deleatidium). In contrast, small chironomid larvae were the most abundant invertebrates in stressed acidic streams and had been ingested by the principal predator Psilochorema, which was only present in stressed streams with pH > 3.0.
Chapter 4 – Invertebrate food quantity & quality

Table 3 Basal resource use by numerically dominant primary consumers as indicated by gut content analysis. Data from individual streams are combined into stress classes: high (pH < 3.0), moderate (pH 3.7 – 4.9) and reference (pH 6.8 – 7.1) streams. R = rare, < 5%; C = common, 5 – 20%; A = abundant, > 20%. N = 98 guts examined.

<table>
<thead>
<tr>
<th>Stress level</th>
<th>Consumer taxon</th>
<th>FFG</th>
<th>CPOM</th>
<th>FPOM</th>
<th>Algae</th>
<th>Filamentous algae</th>
<th>Diatom algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Chironomidae</td>
<td>CB</td>
<td>-</td>
<td>A</td>
<td>R</td>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>Moderate</td>
<td>Chironomidae</td>
<td>CB</td>
<td>-</td>
<td>C</td>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Deleatidium</em></td>
<td>CB</td>
<td>-</td>
<td>C</td>
<td>R</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Spaniocercoides</em></td>
<td>CB</td>
<td>-</td>
<td>C</td>
<td>R</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Reference</td>
<td>Chironomidae</td>
<td>CB</td>
<td>-</td>
<td>A</td>
<td>C</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td><em>Coloburiscus</em></td>
<td>FF</td>
<td>R</td>
<td>A</td>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Deleatidium</em></td>
<td>CB</td>
<td>-</td>
<td>C</td>
<td>C</td>
<td>-</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td><em>Olinga</em></td>
<td>SH</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>-</td>
<td>C</td>
</tr>
</tbody>
</table>

Discussion

Effects of AMD on food quantity and nutritional quality

Both algal and detrital resources were present in comparable quantities regardless of AMD stress, whereas invertebrate populations were severely reduced in acidic AMD-streams compared to circumneutral streams. Algal biomass did not change in a predictable manner across the stress gradient as reported in other studies (e.g., Ledger and Hildrew 2005, pH 4.6 – 6.4) and varied considerably in abundance within and between AMD stressed streams. This variability was due, at least in part, to the presence of
filamentous algae, which increased algal biomass at some highly stressed sites, and to metal precipitates, which coated stones in other streams, reducing potential sites for algal colonization and limiting primary production (McKnight and Feder 1984; Verb and Vis 2000; Bray et al. 2008). In contrast, high abundances of collector-browsers and unstable flow regimes were likely to have been responsible for the low algal biomass found in all circumneutral streams. All streams received inputs of terrestrial leaf litter but standing stocks of detritus did not differ significantly along the AMD gradient, despite AMD being known to slow leaf litter breakdown (e.g., Gray and Ward 1983; Niyogi et al. 2013). Again, the comparable (if variable) amounts of detritus found across all streams likely reflect their poorly retentive nature (Kitto 2009). However, invertebrate populations became much less abundant and diverse as streams became more acidic and could support only tolerant species. Furthermore, invertebrates in stressed streams tended to be smaller-bodied taxa, which supported very small predator populations.

Basal resource quality, as indicated by C: N, did change for periphyton but not for CPOM (leaf litter) along the AMD stress gradient. C: N ratios of periphyton were higher than those previously reported in New Zealand (5.8-8.5) in all but one stream although the ratios of leaf litter were generally similar to those reported in other New Zealand streams (15.8 - 130; Hicks and Laboyrie 1999; Quinn et al. 2000; S.E. Graham, University of Canterbury, unpublished data). However, in freshwaters elsewhere, periphyton has been found to vary substantially in C: N (4-280) around a mean of about 10 (Cross et al. 2005). This variation can reflect the proportions of algae, bacteria, fungi, fine detritus, or microfauna embedded in the periphyton assemblage, or associated with detritus (Cross et al. 2005). The higher quality periphyton we observed may reflect algal
community composition (e.g., presence of N-fixing cyanobacteria in the circumneutral stream; Bray et al. 2008) or additional inputs of N (e.g., elevated nitrate in highly acidic mining-impacted streams; Niyogi et al. 2013) but this requires further investigation. In contrast, lower periphyton quality in the moderately impacted streams may be due in part to the presence of iron hydroxide precipitates. Interestingly, we did not observe consistently lower quality CPOM (i.e., high C: N) in the presence of the precipitates, which were expected to slow microbial conditioning that drives improvements in quality (i.e., decrease in C: N). Also, metal precipitates did not accumulate substantially on stony substrates or fine and coarse debris in contrast to the findings of other studies (e.g., Al, Niyogi et al. 2001; Fe, Schlief and Mutz 2005), however additional chemical analysis (e.g., ICP-AES) could have been completed to confirm that the deposits were actually metal oxides. Instead, the lowest quality CPOM (C: N > 100) observed in our study was likely associated with the presence of wood and unconditioned dead leaves. We recognize that C: N is only one measure of resource quality and a combination of other measures, including elemental ratios and concentrations of structural compounds such as waxes and lignin will affect the attractiveness of leaves (e.g., Hladyz et al. 2009). Our results indicate that algal C: N can differ in AMD-polluted stream and this may be an additional factor affecting the algal food quality.

The C: N ratios of primary and secondary consumers overlapped in many of our streams along the pH gradient, but the ranges were narrower than those collated by Cross et al. (2005) for freshwater primary (5.0 – 9.0) and secondary consumers (4.6 – 7.0). Although nutrients tend to be more concentrated in predators than primary consumers belonging to other functional feeding groups (e.g., 7 -16 % higher N in predators, Cross
et al. 2003), opportunistic feeding on a range of food resources may weaken relationships between C: N and FFGs (Dang et al. 2009). This may be particularly true in AMD-impacted streams where food is limited and many consumers are feeding on similar resources.

**Consumer diets in streams along the AMD stress gradient**

Primary consumers in all streams appeared to be generalist feeders and predators fed opportunistically on whatever prey was available. Algae became increasingly common in the diets of collector-browsers (i.e., chironomids, *Deleatidium* mayflies) as stress decreased. In contrast, detritus (as FPOM) was an important food resource in the diet of primary consumers regardless of stress as in many other New Zealand streams (Rounick et al. 1982; Winterbourn et al. 1984; Ledger and Winterbourn 2000). Similarly, Carlisle and Clements (2003) found fine detritus was the most important food resource in a stream strongly contaminated by zinc and that the contribution of algae to secondary production was low. The very few shredders found in our AMD-affected streams, reflects their general paucity in freshwaters in the region (Hogsden and Harding 2012 [Chapter 2]). Our δ¹³C data provided some support for these dietary links between basal resources and primary consumers and indicate the assimilation of carbon from both algal and detrital food sources in some streams. Animal prey items found in the streams and in predator guts differed substantially between AMD stressed and circumneutral streams, but ingested prey reflected taxa that were locally abundant. Comparable changes in the feeding habits of fish, in response to reduced prey availability and altered prey
community composition, have been found in AMD-impacted streams (Scullion and Edwards 1980).

Interestingly, we found that the trophic position of primary consumers (based on δ\textsubscript{15}N signatures) increased as stress from AMD increased and in some instances matched the trophic position of secondary consumers in stressed streams. Quinn et al. (2003) also reported enriched δ\textsubscript{15}N in some primary consumers (e.g., baetid and heptageniid mayflies, nemourid stonefly) from an AMD-impacted stream relative to those collected from a nearby circumneutral reference stream. Starvation and the nutritional quality of food may increase δ\textsubscript{15}N enrichment (Hobson et al. 1993; Adams and Sterner 2000). We found that many invertebrates from acidic streams had empty guts, suggesting reduced ingestion and possible starvation. This finding suggests that invertebrates may have been putting more energy into detoxifying metals than consuming food for energy and growth (Rainbow 2002). Alternatively, higher δ\textsubscript{15}N values of primary consumers in stressed acidic streams could indicate a degree of omnivory, although invertebrate fragments were not found in our gut analyses of stonefly and chironomid larvae.

**Consequences for energy flow in AMD-affected streams and adjacent ecosystems**

Our work has demonstrated that energy flow pathways are altered in streams receiving AMD. The quantity of basal resources available in these streams supported a small community of tolerant primary consumers, which consumed both algae and detritus. However, the low abundance and biomass of primary consumers in AMD-impacted streams meant limited energy was available to support higher trophic levels. Feeding interactions between invertebrate predators and prey in AMD-polluted streams
were restricted by the low availability of prey items and the energetic benefits of foraging were further constrained by the numerical dominance of small-bodied prey (e.g., chironomids). Therefore, energy flow pathways to predators were limited or disrupted, and at least partially explain the very low abundance of predators in the AMD-affected streams. Reductions in the abundance and biomass of aquatic invertebrates in AMD-impacted streams, especially insects with terrestrial adult stages, can also be expected to reduce the availability of prey to riparian predators such as spiders and birds (e.g., Burdon and Harding 2008; Paetzold et al. 2011). Therefore, acid mine drainage entering tributary streams can also have negative effects on biological communities downstream not only through direct chemical means but also through reduced colonization by insects from upstream and reduced invertebrate drift on which fish may depend.

Acknowledgements

I thank Ian Reeves and Dev Niyogi for help in the field. My research was financially supported by the Brian Mason Scientific & Technical Trust and the Foundation for Science Research & Technology (Grant CRLX0401).
Chapter 4 – Invertebrate food quantity & quality

References


Chapter 4 – Invertebrate food quantity & quality


**Supplementary Material** Benthic invertebrates recorded in the 12 study streams grouped according to AMD stress. Functional feeding group classification (FFG: CB = collector-browser; FF = filter-feeder; SH = shredder; P = predator) and the number of streams in each stress group in which each taxon were found are also shown.

<table>
<thead>
<tr>
<th>AMD stress (pH range; ( N = ) streams)</th>
<th>FFG</th>
<th>High (&lt;3.0;2)</th>
<th>Moderate (3.7-4.9;7)</th>
<th>Reference (6.8-7.1;3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ephemeroptera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deleatidium spp.</td>
<td>CB</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ichthybotus hudsoni</td>
<td>CB</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Neozephlebia scita</td>
<td>CB</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nesameletus ornatus</td>
<td>CB</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Oniscigaster distans</td>
<td>CB</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Zephlebia spp.</td>
<td>CB</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Coloburiscus humeralis</td>
<td>FF</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Ameletopsis perscitus</td>
<td>P</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>Plecoptera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaniocercoides philpotti</td>
<td>CB</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Zelandobius furcillatus group</td>
<td>CB</td>
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<td>2</td>
<td></td>
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<tr>
<td>Zelandobius spp.</td>
<td>CB</td>
<td>-</td>
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<td>2</td>
</tr>
<tr>
<td>Zelandoperla spp.</td>
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<td>2</td>
</tr>
<tr>
<td>Austroperla cyrene</td>
<td>SH</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Stenoperla macellani</td>
<td>P</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Stenoperla prasina</td>
<td>P</td>
<td>-</td>
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<tr>
<td><strong>Trichoptera</strong></td>
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</tr>
<tr>
<td>Beraeoptera roria</td>
<td>CB</td>
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</tr>
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<td>Helicopsyche spp.</td>
<td>CB</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Oxyethira albiceps</td>
<td>CB</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Pycnothecelus ruensis</td>
<td>CB</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Pycnothecelus spp.</td>
<td>CB</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Rakiura vernale</td>
<td>CB</td>
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<td>Zelolessica meizon</td>
<td>CB</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Hydropsyche sp.</td>
<td>FF</td>
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</tr>
<tr>
<td>Oeconesus sp.</td>
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<td>-</td>
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</tr>
<tr>
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<td>SH</td>
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<td>SH</td>
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<td>2</td>
</tr>
<tr>
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<td>P</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Costachorema spp.</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Hydrobiosella mixta</td>
<td>P</td>
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</tr>
<tr>
<td>Hydrobiosella spp.</td>
<td>P</td>
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<td>3</td>
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<tr>
<td>Hydrobiosis frater</td>
<td>P</td>
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<td>1</td>
</tr>
<tr>
<td>Hydrobiosis umbripennis group</td>
<td>P</td>
<td>-</td>
<td>-</td>
<td>1</td>
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Table 4.1 Invertebrate food quantity & quality

<table>
<thead>
<tr>
<th>Invertebrate</th>
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<td></td>
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<tr>
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<tr>
<td>Kokoria miharo</td>
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</tr>
<tr>
<td>Neurochorema confusum</td>
<td>P</td>
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<tr>
<td>Plectrocnemia maclachlani</td>
<td>P</td>
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<tr>
<td>Psilochorema embersoni</td>
<td>P</td>
</tr>
<tr>
<td>Psilochorema tautoru</td>
<td>P</td>
</tr>
<tr>
<td>Psilochorema sp.</td>
<td>P</td>
</tr>
</tbody>
</table>

**Megaloptera**

Archichauliodes diversus | P | - | 1 | 3 |

**Diptera**

Chironomidae (non-predatory) | CB | 1 | - | 3 |
Eriopterini | CB | - | - | 1 |
Eukiefferiella spp. | CB | 2 | 2 | - |
Hexatomini | CB | - | - | 1 |
Mischoderus sp. | CB | - | 1 | - |
Muscidae | CB | - | - | 1 |
Neocurupira hudsoni | CB | - | - | 1 |
Orthocladiinae sp A | CB | 2 | 3 | - |
Orthocladiinae sp B | CB | 1 | - | - |
Orthocladiinae sp C | CB | 1 | 1 | - |
Austrosimulium spp. | FF | - | - | 2 |
Aphrophila spp. | P | - | - | 2 |
Ceratopogonidae | P | - | 1 | 2 |
Empididae | P | - | - | 2 |
Paralimnophila skusei | P | 1 | 1 | - |
Tanypodinae: Macropelopiini | P | - | 2 | 1 |
Tanypodinae: Pentaneurini | P | - | 1 | - |

**Mollusca**

Potamopyrgus antipodarum | CB | - | - | 2 |

**Coleoptera**

Homalaena sp. | CB | - | - | 2 |
Hydora sp. | CB | - | - | 3 |
Hydrophilidae | CB | - | 1 | - |
Ptilodactylidae | CB | - | 1 | 1 |
## Supplementary Material (continued)

<table>
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<tr>
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<th>AMD stress (pH range; ( N = \text{streams} ))</th>
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<tr>
<td></td>
<td>FFG High (&lt; 3.0; 2)</td>
<td>Moderate (3.7 - 4.9; 7)</td>
<td>Reference (6.8 - 7.1; 3)</td>
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</tr>
<tr>
<td>Scirtidae</td>
<td>SH 1</td>
<td>2</td>
<td>-</td>
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<tr>
<td><strong>Oligochaeta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligochaeta indet.</td>
<td>CB -</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Crustacea</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Amphipoda</td>
<td>CB -</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Playthelminthes</strong></td>
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<td></td>
</tr>
<tr>
<td>Neppia sp.</td>
<td>P -</td>
<td>-</td>
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</tr>
</tbody>
</table>
Chapter 5

Isotopic metrics as a tool for assessing the effects of mine pollution on stream food webs

Highly acidic waters (pH < 3) flow in Miller Stream, north of Westport.
Abstract

Most tools used to assess pollution impacts are based on structural, or less frequently, functional aspects of biotic communities. However, the application of measures which take a food web approach to understand the effects of stress on stream ecosystems offers a new perspective and promising insights. We assessed quantitative isotopic metrics, which describe characteristics of food web structure, as indicators of acid mine drainage (AMD) in 12 streams along a pollution gradient and compared these metrics with traditional structural and functional metrics. The gradient ranged from highly stressed (pH < 3) streams with elevated concentrations of dissolved metals (Fe, Al) to moderately acidic streams (pH 3.6 – 4.9) with substrata coated in metal hydroxide precipitates, to circumneutral reference streams. Key differences in food web structure were detected by the isotopic metrics. Specifically, fewer trophic levels (δ¹⁵N range) and reduced trophic diversity (area enclosed by species in δ¹³C- δ¹⁵N space) characterized food webs in all mining impacted streams but these differences were not significant along the gradient. Furthermore, the metrics indicated that food webs were more sparsely and unevenly packed with species as pollution impacts increased. In contrast, most structural and functional metrics were significant predictors of AMD stress. Our results suggest that isotopic metrics offer little advantage over traditional metrics in terms of detecting impacts for biomonitoring purposes. However, they do provide additional insights into how whole food webs are disrupted, and are likely to be more useful for guiding stream management and rehabilitation strategies.

Keywords: acid mine drainage (AMD), stable isotopes, metrics, gradient, food web
Introduction

Biomonitoring tools are commonly used to assess the impacts of pollution on streams. Traditionally, metrics that describe community structure (e.g., taxonomic richness, diversity) have been widely used, while attempts to identify specific effects of pollution have resulted in the proliferation of single and multi-metric indices and predictive models. Many of these approaches are derived from species tolerance scores and have been developed for a particular type of pollution (e.g., organic) or region (Downes et al. 2002). Metrics utilising algae, benthic invertebrates, and fish communities have all been used as indicators of pollution, but efforts are typically focused on only one community, usually invertebrates, despite the importance of links between trophic levels for understanding ecosystem effects of stress (Attrill and Depledge, 1997; Woodward, 2009). Furthermore, while individual structural metrics can detect the loss of sensitive species or reductions in diversity, it is not always clear how community-based changes affect ecosystem function. More recently, several functional measures have been proposed as useful indicators of stream health (e.g., Young et al., 2008; Niyogi et al., 2013). Functional measures are likely to better identify how ecological processes are affected by pollution and can include direct in-stream measurements of key ecosystems processes (e.g., leaf litter breakdown; Gesser and Chauvet, 2002) or the assignment of biological traits to species that allow for rapid, indirect assessment of function (e.g., feeding habits, body size; Dolédec et al., 1999). Analysis of invertebrate functional feeding groups has also been used as an indirect functional measure, as they reflect changes in resource availability with human impacts (Palmer et al., 1996; Harding et al., 1999). Functional approaches can be used to complement structural measures and provide
a more complete understanding of the effects of anthropogenic stress on ecosystems (Young et al., 2008; Friberg et al., 2009; Woodward et al., 2012). However, a food web approach to assessing stream health might offer several advantages by building on the strengths of traditional metrics for detecting impacts, connecting responses among species and trophic levels, and integrating aspects of structure and function provided by other measures. Although food web structure is increasingly being investigated in order to better understand and predict human impacts on aquatic ecosystems (e.g., Layer et al., 2010; Rawcliffe et al., 2010; Woodward et al., 2010), it has not yet been used as a biomonitoring or rehabilitation tool.

Food webs are often constructed using stable isotope analysis due to the valuable dietary ($\delta^{13}C$) and trophic ($\delta^{15}N$) information integrated into consumers’ isotopic signatures (Peterson and Fry, 1987; Post, 2002). Recently, Layman et al. (2007a) proposed several quantitative isotopic metrics, which depict food webs based on the relative spacing of consumer species in isotopic space, and thus condense food web complexity into single values that describe different aspects of food web structure (e.g., extent of basal resource use, trophic diversity). An advantage of such metrics is that they can be compared across “similarly-defined” (e.g., standardised sampling methodology, level of taxonomic resolution, link criteria) food webs that lack common species, which is typical across wide environmental gradients. Their value as a tool for examining food web responses to human impacts has been demonstrated in fragmented tidal creeks (Layman et al., 2007b), eutrophic ponds (Rawcliffe et al., 2010), hydrologically-disturbed marshes (Sargeant et al., 2010), and invasive lake macrophyte beds (Kovalenko and Dibble, 2011) but not in streams receiving chemical pollutants. Recent work has shown
radical re-structuring of food webs in streams receiving acid mine drainage (AMD; Hogsden and Harding, 2012a [Chapter 2]), indicating that these isotopic metrics may be useful for identifying and interpreting impacts of mine pollution. The only other food web study we are aware of that has used stable isotopes in AMD streams was by Quinn et al. (2003) who traced metal accumulation using $^{15}$N-inferred trophic positions of invertebrates.

Streams polluted with AMD are often highly acidic and contain elevated concentrations of dissolved metals (e.g., Al, Fe, Zn), resulting in marked declines in species diversity and impairment of some ecosystem processes (see review by Hogsden and Harding, 2012b [Chapter 1]). Furthermore, AMD can be diluted when it enters circumneutral waters and acidity can be reduced to the point that the solubility of some metals is exceeded. This results in the precipitation of metal hydroxides onto stream substrata imposing an additional physical stressor (Kelly, 1998). The negative effects of AMD on stream ecosystems have been widely assessed using structural metrics or biotic indices based on benthic invertebrate community composition and less often using direct (e.g., nutrient uptake, Northington et al., 2011; organic matter breakdown, Niyogi et al., 2013) or indirect (e.g., FFGs, Gerhardt et al., 2004) functional measures. While invertebrate species richness and EPT richness appear to be the most useful structural indicators and complement AMD-specific indices, other metrics may not provide robust assessments within streams affected by AMD (e.g., %EPT; Gray and Delaney, 2010; Gray and Harding 2012). Furthermore, the relationships between structural and functional measures in mine-polluted streams are not always clear (Northington et al., 2011; Niyogi et al., 2013).
In this study we assessed the potential use of isotopic metrics as indicators of mine drainage pollution in streams. To do this, we compared isotopic metrics with traditional structural and functional metrics used for monitoring stream health and evaluated these metrics along a pollution gradient from circumneutral reference streams to highly impacted AMD streams.

Methods

Study area & stream characteristics

The study was conducted in the Buller-Grey region, an area with a long history of coal mining, on the north-west of the South Island, New Zealand (Appendix C). The region, part of the Westland Forest ecoregion, has spatially consistent climatic conditions, geology, and freshwater biota (Harding et al., 1997). We selected twelve streams that were distributed along an AMD gradient from unimpacted reference to highly impacted streams based on known water chemistry (e.g., pH, conductivity, dissolved Al, Fe concentrations) from previous studies (Winterbourn et al., 2000a; Greig et al., 2010; Hogsden and Harding, 2012a [Chapter 2]). The gradient included three reference streams and nine streams receiving AMD inputs from historical or current coal mining activities. All streams were of similar size (first or second order), had cobble-gravel substrates or bedrock, and were surrounded by native podocarp forest or scrub vegetation.

All sampling was conducted in December 2009 (austral summer) under baseflow conditions. Stream water pH and conductivity (at 25°C) were measured in the field using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA) to confirm the degree of AMD impact on each stream. Water samples were also collected for dissolved metal analysis. These samples were filtered in the field (0.45 µm mixed
Chapter 5 – Isotopic metrics as indicators of mine pollution

cellulose ester filter), acidified with nitric acid, and kept cool prior to analysis using an ICP-MS (Hill Laboratories, Hamilton, New Zealand). Metal hydroxide precipitates present on stream substrata were quantified by scrubbing the surfaces of five randomly selected stones of known area from each stream. The resulting slurry was dried, weighed, and ashed (550°C for 4 h) in the laboratory to determine the amount of metal hydroxide (i.e., inorganic or ash mass) per area of stone. The difference in metal hydroxide biomass between all streams was not significant (ANOVA: \( P > 0.05 \); range: \( 1.4 \times 10^{-4} - 4.4 \times 10^{-3} \) mg cm\(^{-2} \)) so we have excluded metal hydroxides from further analysis. This physical variable/stressor of AMD-affected streams is reported as present or absent.

**Stable isotope sampling & analysis**

Our study streams contained a diversity of invertebrates and fish, although the same species were not present in all streams. Therefore, we focused on common taxa found in earlier studies (Winterbourn, 1998; Greig et al., 2010; Hogsden and Harding, 2012a [Chapter 2]) to provide a robust representation of consumers. Invertebrates for stable isotope analysis were collected by kicknet (500 \( \mu \)m mesh) and sorted by taxa, placed in vials in the field, and frozen. Fish and crayfish were captured using a backpack electrofishing machine (Kainga 300 EFM), euthanized with 2-phenoxyethanol and frozen until further analysis. A maximum of 10 fish per species was collected from each stream for stable isotope analysis.

In the laboratory, all samples were oven dried at 60°C for at least 48 h and ground to a fine, homogeneous powder. Whole body samples of 3 – 40 individual invertebrates per species (with guts removed) were processed together to attain sufficient biomass for
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isotopic analysis. Snail shells and caddisfly cases were removed prior to grinding to avoid contamination from non-dietary sources of carbon. Dorsal muscle tissue was taken from each fish \( (N = 91) \) and abdominal muscle from crayfish \( (N = 15) \). Samples of dried powder \( (1 \, \text{mg}) \) were packed into tin capsules and analyzed at the Cornell Isotope Laboratory on a Therma Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer. Isotope ratios are expressed in delta \( (\delta) \) notation defined as parts per thousand \( (\text{‰}) \) deviation from a standard reference material:

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where \( X \) represents \( \delta^{13}C \) or \( \delta^{15}N \) and \( R \) is the corresponding ratio of \( ^{13}C/^{12}C \) or \( ^{15}N/^{14}N \), respectively. Standard reference materials were PeeDee belemnite limestone for \( \delta^{13}C \) and atmosphere nitrogen for \( \delta^{15}N \). Experimental precision with our samples was 0.19‰ \( (\delta^{13}C) \) and 0.17‰ \( (\delta^{15}N) \) based on the standard deviation of replicates of an internal standard. We corrected for lipid effects on \( \delta^{13}C \) signatures using the mathematical formulae of Post et al. (2007) prior to data analysis.

Invertebrate community sampling

Benthic invertebrates were collected from riffle and run habitats with a Surber sampler \( (0.06 \, \text{m}^2; 250 \, \mu\text{m mesh}) \). Five samples were taken from each stream, preserved in ethanol \( (70\%) \) and identified under a microscope \( (10 - 20 \times \text{magnification}) \) in the laboratory. Taxa were identified and counted to the lowest possible taxonomic level using the keys of Winterbourn et al. (2000b) and a unpublished keys (NIWA, Hamilton, New
Zealand). All invertebrates were classified into functional feeding groups following Winterbourn et al. (1984) and Chadderton (1988).

**Metrics**

A total of 15 metrics, five isotopic, five structural, and five functional were calculated. Our predictions for how each metric will change and potential mechanisms driving these patterns along the AMD stress gradient are shown in Table 1. Sample sizes and species used for calculation of isotopic metrics differed from those used for structural and functional metrics because only a subset of taxa used to compute these metrics was collected for stable isotope analysis (see methods).

**Isotopic metrics**

The five isotopic metrics were: nitrogen range (NR), carbon range (CR), trophic area (TA), mean nearest neighbour distance (NND), and standard deviation of NND (SDNND). NR represents vertical structure in the food web, whereas CR describes the extent of basal resource use by consumers. Both metrics are calculated from the spacing of consumer (invertebrate and fish) species in $\delta^{13}\text{C} - \delta^{15}\text{N}$ biplot space. TA is a measure of total trophic diversity in the food web and was estimated by calculating the total convex hull area encompassed by the smallest polygon containing all species in biplot space using R (R Development Core Team, 2011). When only two species were present, it was not possible to calculate an area but we used a small value (0.001) to represent the space taken up by the species. NND and SDNND were derived from the spacing of species
Table 1 Predicted change in 15 metrics as stress increases across an AMD stress gradient and potential mechanisms.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Predicted trend</th>
<th>Potential mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isotopic metric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen range (NR)</td>
<td>Decrease</td>
<td>Loss of top predators (fish)</td>
</tr>
<tr>
<td>Carbon range (CR)</td>
<td>No change</td>
<td>Algal and detrital resources present and available for consumers</td>
</tr>
<tr>
<td>Trophic area (TA)</td>
<td>Decrease</td>
<td>Loss of top predators; reduced diversity of primary and secondary consumers</td>
</tr>
<tr>
<td>Nearest neighbour distance (NND)</td>
<td>Increase</td>
<td>Loss of sensitive species leads to fewer functionally similar consumers present</td>
</tr>
<tr>
<td>SD nearest neighbour distance (SDNND)</td>
<td>Increase</td>
<td>Greater variability in species position relative to each other in isotopic space</td>
</tr>
<tr>
<td><strong>Structural metric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invertebrate richness</td>
<td>Decrease</td>
<td>Loss of sensitive species</td>
</tr>
<tr>
<td>Shannon – Wiener diversity</td>
<td>Decrease</td>
<td>Reduced abundance and loss of sensitive species</td>
</tr>
<tr>
<td>EPT richness</td>
<td>Decrease</td>
<td>Loss of sensitive EPT taxa</td>
</tr>
<tr>
<td>% Chironomidae</td>
<td>Increase</td>
<td>Invertebrate community numerically dominated by tolerant chironomids</td>
</tr>
<tr>
<td>AMDI</td>
<td>Decrease</td>
<td>Presence of tolerant species with low indicator scores</td>
</tr>
<tr>
<td><strong>Functional metric</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional feeding group richness</td>
<td>Decrease</td>
<td>Loss of sensitive groups</td>
</tr>
<tr>
<td>% collector-browsers</td>
<td>No change</td>
<td>Collector-browsers present and numerically dominant</td>
</tr>
<tr>
<td>% predators</td>
<td>Increase</td>
<td>Increased % of invertebrate predators in the absence of fish</td>
</tr>
<tr>
<td>% shredders</td>
<td>Decrease</td>
<td>Loss of sensitive shredder species</td>
</tr>
<tr>
<td>% filter-feeders</td>
<td>Decrease</td>
<td>Loss of sensitive filter-feeder species</td>
</tr>
</tbody>
</table>
relative to each other. NND is a measure of trophic redundancy in the web as functionally similar species are located close to each other in isotopic space, whereas SDNND is a measure of the evenness of species distribution relative to each other. Due to very low abundance and richness of invertebrates in some streams it was not possible to calculate isotope metrics for two streams (Garvey and Wellman). Furthermore, it was not possible to calculate SDNND for three further streams (Alborns, Page, and Miller) because sufficient biomass of only two invertebrate species could be collected from each of them. As an example, a stable isotope biplot (Fig. 1) shows the position of consumers in $\delta^{13}$C-$\delta^{15}$N isotopic space for two streams at extremes of the AMD stress gradient encompassed by this study. The calculation of the isotopic metrics is based on the distribution of species in this space.

**Structural metrics**

Total invertebrate taxonomic richness, invertebrate diversity (Shannon-Wiener index), EPT richness, %Chironomidae, and the Acid Mine Drainage Index (AMDI) were calculated for each stream. The AMDI is a biotic index, based on benthic invertebrate tolerance scores, that was recently developed to assess the impacts of AMD on streams in New Zealand (Gray and Harding, 2012).

**Functional metrics**

The total number of FFGs (FFG richness) and the percent abundance of four FFGs (i.e., collector-browsers, predators, shredders, and filter-feeders) were calculated as functional metrics.
Data analysis

Pearson correlations were calculated among the key chemical variables associated with AMD, including pH, specific conductivity, and concentrations of dissolved metals (Al, Fe). Principal components analysis (PCA) was used to combine these key chemical variables into an AMD stress gradient (Chapter 4). PCA axis 1 included pH, conductivity and Al concentration. Pearson correlations between all chemical variables indicated that Fe concentration was highly correlated ($r > 0.9$) with Al concentration, so Fe was excluded from the PCA. Conductivity and Al concentration were $\log_{10} (x +1)$ transformed prior to analysis. Regression analysis was then used to separately test for best-fit patterns in 15 metrics across the AMD gradient using different models (linear, polynomial, exponential). Residuals were examined to ensure that assumptions of regression were met and normality was examined with the Shapiro-Wilks test. Percent abundance of chironomids was arcsine square root transformed and percentages of functional feeding groups (collector-browsers, predators, filter-feeders, and shredders) were rank-transformed. Other variables were $\log_{10} (x +1)$-transformed if necessary prior to analysis. Finally, we used Spearman rank correlations to determine the relationship between isotopic, structural, and functional metrics. To account for the multiple comparisons we made, we used false discovery rate (FDR) corrected $P$ values (Verhoeven et al., 2005; Pike, 2010). Our FDR adjusted $P$ value for the regressions was 0.03 and for the correlations among metrics it was 0.04. Analyses were conducted in Primer 6.1.12 (Clarke and Gorley, 2006), SYSTAT 10 (SPSS, 2000) and Sigmaplot 11.0 (Systat Software Inc., 2008).
Fig. 1 Stable isotope biplot of $\delta^{15}$N and $\delta^{13}$C values for consumers in two streams on either end of the AMD gradient in this study. White circles represent consumers from a highly stressed stream (pH 2.8) and black circles represent consumers from a circumneutral stream (pH 7.1). Each symbol represents the mean value of 2-16 individuals for a particular consumer species. Lines enclose the convex hull area in isotopic space used to calculate the isotopic metrics (e.g., NR = $\delta^{15}$N range, CR = $\delta^{13}$C range, TA = area enclosed in polygon, etc.).

Results

Stream water chemistry

Stream pH across the gradient ranged from highly acidic (pH 2.7) to circumneutral (pH 7.1) (Table 2) and was below 5.0 in all AMD-impacted streams and above 6.8 in reference streams. Stream pH was strongly correlated with specific conductivity ($r = -0.69, P < 0.01$), and the concentrations of total dissolved Al ($r = -0.60, P = 0.04$) and Fe ($r = -0.87, P < 0.001$). Metal hydroxide deposits were present on the substrata of six of
the 12 streams (Table 2). The 12 streams were ordered along the gradient from circumneutral reference to highly stressed acidic streams with elevated metal concentrations. Low and moderately stressed streams were grouped closely together along the gradient due to similarities in water chemistry at the time of sampling and will be referred to as moderately stressed streams.

Response of metrics along the AMD stress gradient

Isotopic metrics

Nitrogen range (NR), which represents vertical structure in food webs, was not related to AMD stress (Fig. 2a; $P = 0.04$). However, NR was consistently smaller in all AMD-impacted streams (range: 0.9 – 5.0), compared to circumneutral streams (range: 6.9 – 10.8). Lower NR values indicated the presence of two or fewer invertebrate consumer trophic levels based on a fractionation rate of 2.3 to 3.4‰ (See McCutchan et al., 2003 and references therein; Kilham et al., 2009; Table 3). The $\delta^{13}$C carbon range (CR) was unrelated to the AMD stress gradient (Fig. 2b; $P = 0.32$). This suggests that consumers were using a consistent range of basal food resources regardless of AMD. Although variation in CR was greater for the AMD-impacted than reference streams (mean ± CV %: 4.01 ± 74.24 vs. 6.36 ± 17.05), the difference in mean CR was not significant between stream types ($t = 1.13$, $P = 0.23$). Trophic area (TA) was not related to stress (Fig. 2c; $P = 0.05$) reflecting reduced consumer species richness, loss of top predators, and low functional diversity (Table 3).
Table 2 Key chemical and physical characteristics of the 12 study streams on the West Coast of the South Island in order of increasing AMD stress along the gradient.

<table>
<thead>
<tr>
<th>Stream name</th>
<th>Code</th>
<th>pH</th>
<th>Conductivity</th>
<th>Al</th>
<th>Fe</th>
<th>Metal hydroxides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian</td>
<td>ITA</td>
<td>6.9</td>
<td>18</td>
<td>0.23</td>
<td>*</td>
<td>No</td>
</tr>
<tr>
<td>Lankeys</td>
<td>LAN</td>
<td>7.1</td>
<td>40</td>
<td>0.27</td>
<td>*</td>
<td>No</td>
</tr>
<tr>
<td>Kiwi</td>
<td>KIW</td>
<td>6.8</td>
<td>58</td>
<td>0.2</td>
<td>*</td>
<td>No</td>
</tr>
<tr>
<td>Burnetts Face</td>
<td>BF</td>
<td>4.1</td>
<td>69</td>
<td>0.62</td>
<td>*</td>
<td>No</td>
</tr>
<tr>
<td>Garvey</td>
<td>GAR</td>
<td>4.9</td>
<td>267</td>
<td>0.76</td>
<td>1.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Alborns</td>
<td>ALB</td>
<td>4.1</td>
<td>128</td>
<td>1</td>
<td>1.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Coalbrookdale</td>
<td>CBD</td>
<td>3.7</td>
<td>132</td>
<td>1.3</td>
<td>1.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Page</td>
<td>PAG</td>
<td>4.5</td>
<td>240</td>
<td>2.4</td>
<td>1.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Coal</td>
<td>COA</td>
<td>3.7</td>
<td>240</td>
<td>3</td>
<td>2.5</td>
<td>Yes</td>
</tr>
<tr>
<td>Wellman</td>
<td>WEL</td>
<td>3.7</td>
<td>558</td>
<td>4.6</td>
<td>3.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Miller</td>
<td>MIL</td>
<td>2.8</td>
<td>1088</td>
<td>34</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>Mine</td>
<td>MIN</td>
<td>2.7</td>
<td>1267</td>
<td>43</td>
<td>10</td>
<td>No</td>
</tr>
</tbody>
</table>

* indicates concentration is below the level of analytical detection (< 0.40 mg l⁻¹)

Trophic area (TA) was not related to stress (Fig. 2c; \( P = 0.05 \)) reflecting reduced consumer species richness, loss of top predators, and low functional diversity (Table 3).

Trophic area was very small in some moderately and highly stressed streams, which had low richness (e.g., 2-3 species). The mean TA of highly stressed streams was less than 75% of that for reference streams. The wide range in TA observed in circumneutral reference streams (17.7 – 47.66) was driven primarily by the number of fish species present (2 – 7) and the functional diversity of invertebrate prey in their diets. Mean distance to the nearest neighbour (NND) was greater in highly stressed streams and decreased as stress decreased along the gradient; however, this relationship was not significant (Fig. 2d; \( P = 0.06 \)) due to the influence of one AMD-impacted stream.
Fig. 2  Relationships between (a) nitrogen range, (b) carbon range, (c) trophic area, (d) nearest neighbour distance, and (e) standard deviation NND and AMD stress. Fitted lines show significant regressions. $N=10$ for all except (e) where $N=7$. pH range for streams along the gradient is also shown.
Table 3 Taxonomic richness by trophic level and functional feeding group (FFG) for stable isotope samples used to calculate isotope metrics. Values in adjacent parentheses are taxa richness for samples that were used in the calculation of structural and functional metrics. The values differ for each stream because only a subset of taxa was collected for stable isotope analysis. Streams codes are listed in Table 2.

<table>
<thead>
<tr>
<th>Trophic level</th>
<th>Stream type</th>
<th>Circumneutral reference</th>
<th>Decreasing pH →</th>
<th>Highly acidic AMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FFG</td>
<td>LAN</td>
<td>ITA</td>
<td>KIW</td>
</tr>
<tr>
<td>4° consumer</td>
<td>Fish</td>
<td>Predator</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3° consumer</td>
<td>Fish</td>
<td>Predator</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>2° consumer</td>
<td>Invertebrate</td>
<td>Predator</td>
<td>5 (10)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>1° consumer</td>
<td>Invertebrate</td>
<td>Collector</td>
<td>browser</td>
<td>7 (13)</td>
</tr>
<tr>
<td></td>
<td>Invertebrate</td>
<td>Shredder</td>
<td>1 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td></td>
<td>Invertebrate</td>
<td>Filter feeder</td>
<td>1 (2)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>16 (29)</td>
<td>19 (44)</td>
</tr>
</tbody>
</table>
(Alborns; Table 2). This stream differed physically from all other streams because of its relatively high-suspended sediment load, which may have affected invertebrate colonization or sample collection. The relationship was considerably stronger when this outlier was removed ($P = 0.002$). The distribution of species relative to the nearest neighbour (SDNND) was significantly related to AMD stress (Fig. 2e; $P = 0.01$). The wide range of SDNND in streams receiving AMD inputs suggests they were colonized by an uneven distribution of species performing different functional roles.

Structural metrics

Taxonomic richness of invertebrates ranged from 2 to 42 among streams and was related non-linearly to AMD stress (Fig. 3a; $P < 0.001$). Invertebrate richness in AMD-impacted streams was consistently low (< 11) and reflected the loss of many species (Table 3). Both invertebrate diversity and richness of EPT taxa were negatively related to stress (diversity: Fig. 3b; $P < 0.0001$; EPT richness: Fig. 3c; $P = 0.0001$), but percent abundance of Chironomidae was not (Fig. 3d; $P = 0.08$). This metric showed considerable variation with stress and the highest value was reported in a highly stressed (pH 2.8) stream where chironomids accounted for 69% of all invertebrates present. AMDI scores ranged from 0 (severely impacted) to 59 (unimpacted) and were significantly related to AMD stress (Fig. 3e; $P < 0.0001$). According to the AMDI, all the AMD streams would be classified as ‘severely impacted’ (score < 20; Gray and Harding, 2012).
Functional metrics

The number of invertebrate functional feeding groups was significantly and negatively related to AMD stress (Fig. 4a; \( P = 0.004 \)). The majority of invertebrates present in all streams were collector-browsers and percent abundance of this group was not related to the stress gradient (Fig. 4b; \( P = 0.54 \)). In highly stressed streams (pH < 3), collector-browsers were orthoclad chironomids, which were numerically dominant, however, in moderately stressed and circumneutral streams (pH > 3.7) collector-browsers were mostly mayflies, in particular Deleatidium sp. A negative relationship was found between percent predators and the AMD gradient (Fig. 4c; \( P = 0.03 \)). Predators were absent from highly stressed streams, but the relative abundance of predators increased in moderately stressed streams. Thus, the proportion of predatory, acid-tolerant caddisflies (Psilochorema tautoru) and tanypod chironomids in one moderately stressed stream was comparable to the proportion of all predatory acid-sensitive hydrobiosid caddisflies and the dobsonfly (Archichauliodes diversus) in a circumneutral stream. Percent abundance of shredders and filter-feeders increased, in a non-linear manner with stress (Fig. 4d-e; \( P = 0.03 \) and \( P < 0.001 \), respectively) but made up only a small fraction of the invertebrate community in circumneutral reference streams. However, with the exception of a few shredding scirtid beetles found in one highly stressed stream, shredders and filter-feeders were absent from AMD-impacted streams (pH < 5.0).
Fig. 3 Relationships between (a) invertebrate richness, (b) invertebrate diversity, (c) EPT richness, (d) %Chironomidae, and (e) AMDI and AMD stress. Fitted lines show significant regressions. $N=12$. pH range for streams along the gradient is also shown.
Figure 4. Relationships between (a) functional feeding group richness, (b) % collector-browsers, (c) % predators, (d) % shredders, and (e) % filter-feeders and AMD stress. Fitted lines show significant regressions. \( N = 12 \). pH range for streams along the gradient is also shown.


Relationships among isotopic, structural, and functional metrics

Spearman rank correlations indicated strong relationships among several isotopic, structural, and functional metrics (Supplementary Material). Of the 105 correlations, 36 were significant (FDR adjusted \( P = 0.04 \)). However, there were fewer strong, significant relationships with isotope metrics than with structural and functional ones. CR and NND were only correlated with isotope metrics (TA and SDNND, respectively), whereas NR was most strongly correlated with diversity and the percentage of shredders and filter feeders \( (r > 0.68, \ P < 0.05) \). TA was strongly related to several other metrics, including several structural ones (diversity, EPT richness, AMDI), two isotopic ones (NR, CR) and one functional metric (% filter feeders). Most relationships with NND were negative and weak \( (r < 0.34) \), except with functional feeding group richness \( (r = - 0.52) \). Finally, SDNND was strongly and negatively related to diversity and percentage of predators and was significantly and positively related with NND \( (r = 0.93, \ P < 0.001) \) but less strongly with most other metrics (Supplementary Material).

Overall, the strongest correlations \( (r = 0.70 – 0.94) \) were found among metrics within each type \( (\text{e.g., structural-structural}) \) but some strong \( (r = 0.75 – 0.85) \) relationships also existed among metrics of different types \( (\text{e.g., structural-functional}) \). Consumer taxonomic richness, invertebrate diversity, AMDI, functional feeding group richness, % filter-feeders, and trophic area were most frequently correlated with other metrics at \( r > 0.60 \).
Discussion

Isotopic metrics as indicators of mine drainage pollution

Food web structure is increasingly being used as an indicator of the wider effects of stress on stream ecosystems by focusing attention on interactions among species and among trophic levels (Layer et al., 2010a; Sargeant et al., 2010; Woodward et al., 2010). In our study, several aspects of food web structure, as measured by isotopic metrics, changed predictably (Table 1) but not significantly along the mine drainage pollution gradient. Specifically, the TA, NND, and NR metrics identified small, sparsely populated food webs with short food chains in all AMD-impacted streams. These food web properties are expected in stressed food webs, due to the loss of sensitive species or entire trophic levels (Odum, 1985; McCann, 2000) and have been reported in mining-impacted streams food webs in New Zealand (Hogsden and Harding, 2012a [Chapter 2]).

Consumers were sufficiently affected by AMD to change trophic structure as indicated by metric values even at moderate stress in our study. The NR metric indicated that fewer trophic levels were present in all AMD streams compared to circumneutral streams, and reflected a shift from fish to invertebrates as top predators. Furthermore, the $\delta^{15}$N values revealed that some “top” consumers in these streams fed directly on basal resources. Sargeant et al. (2010) also showed reduced NR following disturbance (i.e., drying) in the Florida Everglades as fish fed at lower trophic levels due to reduced invertebrate and fish (prey) availability. The dietary and trophic information extracted from stable isotope analysis can indicate whether species have changed their feeding interactions in response to stress and in what ways (e.g., Vander Zanden et al., 1999; Kövecses et al., 2004). Additionally, stable isotope analysis could be used to detect cross-
system subsidies if consumers shifted to feed on terrestrial resources due to reduced in-stream prey availability (as in Scullion and Edwards, 1980). Our AMD-impacted streams lacked fish and the presence of few functionally different invertebrate consumers (Table 3), led to low trophic diversity, measured by TA. The use of cross-ecosystem subsidies as invertebrate prey (i.e., terrestrial insects) was not apparent from our isotope data, but primary consumers were feeding on FPOM of terrestrial origin. However, if a stressor does not eliminate top fish predators, reductions in TA could be used to track declines in prey availability (e.g., Layman et al., 2007b).

The range of carbon used by consumers in our study did not differ along the gradient indicating that consumers were able to use the full range of basal food resources. This is corroborated by earlier findings (based on gut contents analysis) that invertebrate primary consumers are generalist feeders in AMD streams (Hogsden and Harding, 2012a [Chapter 2]). However, our isotope data indicated that the extent of the carbon range in AMD streams was more likely to be maintained by an individual species rather than a group of species. This highlights the fragility of food webs in AMD-impacted streams and the susceptibility of their energy flow pathways to additional stressors.

Comparison of isotopic with traditional structural and functional metrics

Structural measures of invertebrate communities are accepted as effective indicators of AMD impact on streams and are commonly used in stream assessments (e.g., Winterbourn, 1998; Gerhardt et al., 2004). The structural metrics used in this study revealed typical and expected community-level responses to mine drainage, including reduced taxonomic richness, EPT richness, and diversity (Table 1). The AMDI was also
effective at discriminating between AMD and circumneutral streams. Our results provide support for studies that have compared the effectiveness of different structural metrics and suggest that taxonomic richness, EPT richness, and AMD-specific indices best detect impacts (Gray and Delaney, 2010; Gray and Harding, 2012).

We found strong relationships between several of the structural and isotopic metrics suggesting that isotopic metrics may be largely redundant in terms of detecting impact (Supplementary Material). For example, AMD impacted streams with low invertebrate diversity had fewer trophic levels (i.e., low NR) and reduced trophic diversity (i.e., small TA). In contrast, weak relationships between other structural and isotopic metrics suggest that each provide different information, such as NND, which was weakly related to all structural metrics and provided new information on the position and distribution of species within the food web. As AMD stress increased, the food webs were occupied by fewer consumer species, the distance between them was greater in isotopic space, and therefore fewer species were performing a similar function. Low functional redundancy is of particular importance in systems where consumers rely on distinct resource pools (Kilham et al., 2009). Therefore, the isotope metrics can further our understanding of how some stream biota are affected by mine drainage.

Functional indicators have been used less frequently than structural ones in assessment of AMD impacts. Some direct measures of function are promising indicators, particularly when the process involves several biotic communities (e.g., organic matter breakdown; Niyogi et al., 2013). Indirect approaches using FFGs to add functional value to species data have also been useful in some AMD-impacted systems (Schultheis et al., 1997; Gerhardt et al., 2004). In these studies predators tended to numerically dominate
invertebrate communities, whereas other FFGs (filter-feeders, shredders, collector-gatherers, scrapers) decreased in relative abundance. Our findings partially support this pattern, with the exception that most species were collector-browsers. The loss of shredders and filter feeders may represent an important disruption in energy processing pathways. However, the use of FFGs as metrics may not be appropriate in these streams as many invertebrates are generalist feeders, best classified as ‘herbivore-detritivores’ rather than into more specific groups as commonly done in North America or Europe (e.g., Winterbourn, 2000). Thus, a metric that relies on differences in food source or feeding mode would be ineffective. Moreover, it appears that metrics based on percent abundance (e.g., FFGs) are not effective when taxa richness is so low that the presence of one or two individuals in a particular group can inflate the result. We did find some strong relationships between functional and isotope metrics (e.g., % FF – NR, TA; %SH – TA; % P - SDNND) but most were weak (Supplementary Material). However, we believe most of these strong relationships reflect inflated percent abundance (as above) rather than a true pattern. However, the isotope data can provide new functional insights into energy transfer through defining feeding relationships, rather than indicating what might be expected from FFGs.

Practicalities and challenges of using isotopic metrics as tools

Our aim was to test the suitability of isotopic metrics as biomonitoring tools by comparing them to more traditional structural and functional metrics. An ideal monitoring or bioassessment tool should effectively discriminate impacts, be relatively simple to use, be time- and cost-effective, and provide easy to interpret results (Norris
and Hawkins, 2000; Bonada et al., 2006). The stable isotope metrics were effective at discriminating between mine-polluted and circumneutral streams, in our study, but did not detect significant differences as AMD stress increased along the gradient, as key structural changes in food webs were common to all AMD streams. Furthermore, they are relatively simple to use. In-stream samples are collected using standard equipment, and methods for processing samples for analysis are easy to follow and replicate. However, the equipment used to run the analysis (i.e., mass spectrometer, elemental analyzer) is expensive and requires specialized skills to use. In addition, it is more time-consuming to assemble and process the appropriate data and they would be unlikely to be cost-effective to produce. That said, isotopes do provide a time-integrated and relatively quick means of constructing an overview of food webs compared to traditional food web approaches (i.e., gut content analysis; Fry, 1991). The isotopic metrics themselves are easy to calculate using δ\(^{13}\)C and δ\(^{15}\)N data following Layman et al. (2007a). However, interpretation of the metrics requires an understanding of stable isotope fractionation patterns (e.g., stepwise enrichment of δ\(^{15}\)N) and consideration of the influence of watershed process (i.e., biological and chemical) along with trophic interactions on isotope values (Layman et al., 2007a; Layman et al., 2011). In addition, interpretation of isotopic metrics can be more challenging than for other metrics, which generate “scores” or “values” that are assigned to different levels of pollution (e.g., Biological Monitoring Working Party (BMWP) biotic score; Wright et al., 1988).

As with all tools, there are limitations in the use of the isotopic metrics particularly in streams with few consumers. Firstly, it can be difficult to collect sufficient biomass of consumers for stable isotope analysis when overall species abundance and biomass is
very low and most tolerant species are small-bodied (e.g., chironomids). As a result, some species are excluded or may have to be pooled at a higher level of taxonomic resolution prior to analysis. This limits the inclusion of species in the calculation of metrics. All isotopic metrics we calculated were strongly correlated with the number of taxa used for isotope analysis at a site \((P < 0.04)\). In particular, NR, CR, and TA increased, while NND decreased with an increase in the number of taxa included in analysis. While this strong relationship may represent a sampling issue or cause problems with interpretation (see Jackson et al., 2011), it reflects the true pattern of substantial species loss and altered trophic structure in AMD-affected streams in New Zealand (Winterbourn et al., 2000a; Greig et al., 2010; Hogsden and Harding, 2012a [Chapter 2]) and probably elsewhere. Despite these challenges and caveats relating to the application of isotope metrics (Layman et al., 2007a; Layman et al., 2011), we believe they are valuable tools to include in comparisons of food webs across wide environmental gradients.

**Informing management and rehabilitation efforts**

The best way to assess and restore ecosystem health is to understand the response to human impacts of an entire aquatic community (Karr, 1991; Lake et al., 2007). A high level of understanding can be achieved by a combination of structural community metrics and direct functional measures of processes, or by using isotopic metrics, which integrate function as feeding relationships into structural aspects of a food web. Stable isotopes connect responses of multiple trophic levels to stress (e.g., Sargeant et al., 2010; Rawcliffe et al., 2010) and provide a rapid and more cost-effective means for
constructing food webs than extensive gut content analysis required to resolve a connectance web, as potentially more information is gained from fewer samples. Isotopic metrics provide new insights and can be valuable tools for stream managers with ecosystem-based management goals. They may also be a useful starting point for a rehabilitation program and could be applied to measure the success of management strategies. For example, the lack of relationship between the carbon range and the AMD gradient observed in this study indicates that similar basal resources are available to all consumers, and suggests that energetic bottlenecks occur at higher trophic levels.

Acknowledgments

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Chapter 5 – Isotopic metrics as indicators of mine pollution

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Chapter 5 – Isotopic metrics as indicators of mine pollution

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Supplemental Material  Spearman rank correlations of the relationship between structural, functional, and isotopic metrics (N = 12 for all except, NND where N = 10 and SDNND where N = 7). Significance levels are * P < 0.04, ** P < 0.01, and *** P < 0.001.

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General Discussion

As the global demand for energy increases coal mining will continue to expand worldwide. This increased pressure to mine, in conjunction with more widespread concerns about the environmental consequences of mining has created a pressing need to understand the complex ecological effects of AMD on stream ecosystems in order to predict or mitigate future impacts and develop effective management and rehabilitation techniques (Gray, 1997; Palmer et al., 2010; Bernhardt and Palmer, 2011). I believe a useful way to begin to understand these complex effects is to understand how mining impacts affect the structure and functioning of stream food webs. This approach provides a holistic perspective, connects responses among species and trophic levels, and integrates structural and functional aspects of these high stressed ecosystems. To date, much ecological research into the effects of mining on streams has focused on individual benthic communities. This approach tells us much about how diversity and community composition are affected but contributes little to our understanding of energy flow throughout the ecosystem or how these systems might need to be “repaired” to function as natural systems. By conducting a literature review and using a combination of survey and experimental approaches, I assembled the first complete pictures of food webs in AMD-affected streams and examined how changes in community and food web structure influenced how energy was transferred in these highly stressed ecosystems in New Zealand. Although I focused on impacted systems on the northern west coast of the South Island, it is apparent from the literature that patterns, processes and mechanisms explored in my research have relevance to mine-affected streams worldwide.
The structure of food webs in AMD streams

In streams receiving inputs of AMD, food webs were consistently small and simplified, with low densities of a few tolerant invertebrates, no fish and few interactions among species (Chapters 2, 3 & 5). This was consistent with patterns of reduced size and complexity reported in other stressed freshwater food webs (e.g., Layer et al., 2010a; Rawcliffe et al., 2010). The substantial changes in the structure and function of benthic communities (i.e., food web components) in AMD-impacted streams have often been attributed to direct toxicity of pH, dissolved metals, and accumulation of metal precipitates on invertebrate and fish gills or reduced habitat caused by precipitates which coat stream substrata (Chapter 1 and references within). My results highlighted the importance of metals, both in dissolved and precipitated forms, as important drivers of food web structure, rather than pH alone (Chapter 2). Furthermore, my data also supports the suggestion that reduced prey (e.g. abundance, diversity, and body size of invertebrates) may act as an additional stressor limiting the number of predatory consumers and higher trophic levels that can be supported in streams polluted by mine drainage (Chapter 4; Scullion and Edwards, 1980). As a result of substantial species loss, food chain length was reduced and less links were possible between the few consumers and basal resources. However, primary consumers fed generally on almost all algae and detrital resources resulting in highly connected food webs (Chapter 2; Schmid-Araya et al., 2002). High connectance is indicative of web stability (Dunne et al., 2002), which suggests that the base of these simplified webs may be relatively stable and robust; however, overall there are very few species present to buffer against further environmental change. The negative effects of anthropogenic acidity and metals from mine drainage for the structure of stream food webs were increasingly apparent when
compared with webs in streams receiving natural inputs of acidity and metals. Mining-impacted food webs had far fewer species, shorter food chains, and less links between species than those in streams receiving acidity and metals from natural sources or those with circumneutral water chemistry (Chapter 2). However, I found that mining-impacted food webs were structurally most similar to those in naturally high metal streams in terms of web size, number of links, and food chain length.

The structure of food webs is increasingly being studied across stress gradients, in order to predict human impacts on aquatic ecosystems and to identify potential thresholds of change (Woodward et al., 2010; Layer et al., 2010b; Sargeant et al., 2010). The identification of thresholds along stress gradients imposed by mine drainage are poorly understood and can be complicated by the different structural and functional responses of biota to chemical and physical stressors exerted by AMD (e.g., primary producers, Niyogi et al. 2002). Using stable isotopes of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N), I constructed and compared twelve replicate food webs along an AMD-stress gradient (Chapter 5). Interestingly, I found that key changes in food web structure, specifically the presence of fewer trophic levels and reduced trophic diversity (reported as isotopic metric values), were comparable across all AMD impacted sites (pH range: 2.7-4.9). The vertical structure of the food webs tended to be smaller in AMD-affected streams, regardless of impact level, compared to webs in circumneutral streams due to the shift from fish to invertebrates as top predators. This suggests that a threshold separates streams that are impacted from those that are not impacted as there is not a gradual transition in key structural attributes that corresponds to increasing stress. This result is also consistent with findings that intense and frequent stress maintains short food chains
(Post, 2002). Furthermore, these results highlighted that the differential responses of consumers to the chemical (acidity, elevated concentrations of dissolved metals) and physical (metal precipitates) stresses imposed by AMD can contribute to similar patterns in food web structure and refined our proposed food webs (Chapter 1, Fig. 2). For example, I expected that food web size would be considerably larger in mildly impacted streams (i.e., far less acidic, lower dissolved metal concentrations) relative to highly impacted streams due to increased consumer diversity and presence of fish. However, invertebrate diversity did not change predictably along the pH gradient and fish were absent from mildly impacted streams despite the fact that water chemistry (i.e., pH, dissolved metals) was within tolerable levels for some New Zealand fish taxa (Greig et al., 2010). This concurs with other stream studies that found predictability declines with decreasing AMD stress along a gradient as other factors (biotic and abiotic) become more influential in shaping community structure (Verb and Vis, 2005; Bray et al., 2008). Furthermore, although there often was a greater diversity of consumers in mildly or moderately (i.e., precipitate) impacted streams (Chapters 3 & 5), isotope analysis revealed that they were performing similar functions resulting in comparable values of trophic diversity (Chapter 5, Fig. 2). Clearly, important changes in the structure and function of food webs can occur even at low levels of AMD impact.

**Disrupted energy flow and ecosystem function**

The consequences of modified food web structure will have serious implications for energy flow in human-impacted ecosystems (Woodward, 2009), particularly where the transfer of energy is constrained by the loss of species and trophic links (e.g., Sherwood et al., 2002; Rawcliffe et al., 2010). In AMD-impacted streams, energy flow in
the radically re-structured food webs, with low abundances of few species responsible for
function, was disrupted and limited by altered interactions among species at multiple
trophic levels. I resolved species links using a combination of complementary techniques
(i.e., gut content and stable isotope analyses) and found that most interactions occurred
between primary consumers and basal resources, particularly amorphous detritus
(FPOM), and that there were few predatory interactions (Chapters 2, 3 & 4), which has
been shown in other acidic stream food webs (Layer et al., 2010b). Although detritus
formed the basis of diets for primary consumers, they also consumed some algae and by
using both basal resources maintained multiple pathways to support energy flow to higher
consumers in the food web. Few intermediate species were present to continue the
transfer of energy but the disproportionate loss of top consumers (invertebrate predators
and fish) meant that little energy was required to support higher trophic levels. This
highlighted the potential for energetic bottlenecks to occur at the primary consumer level
in mining-affected streams with energy flow pathways disrupted by species loss at
intermediate and higher trophic levels. The short chains through which energy flowed
through AMD-impacted food webs starkly contrasted with the longer, more complex and
reticulate pathways found in unimpacted naturally acidic and circumneutral stream webs,
where large top consumers (predatory fish) were present to integrate energy pathways
(Chapters 2 & 3). The declines in energy flow to higher trophic levels will also have
consequences for food webs in adjacent ecosystems, with significantly reduced subsidies
of adult insects available for riparian predators (Burdon and Harding, 2008; Paetzold et
al., 2011).
Despite species loss and food web simplification, AMD-impacted streams can retain some limited ecological functions, such as primary production (Niyogi et al., 2002) and the processing of detrital resources through microbially-mediated leaf breakdown (Chapter 3). The impairment of the latter process, owing to the reduced abundance and activity of either microbes or shredding invertebrates, or a combination of both, is typical of streams receiving inputs of mine drainage and is suppressed to a greater degree in the presence of metal hydroxide precipitates (Chapters 1 & 3; Niyogi et al., 2013). Most studies that relate leaf breakdown with structure do so at the community-level with a focus on primary consumers (e.g., Schultheis et al., 1997, Niyogi et al., 2001). However, my work built on these studies by tracing detrital energy flow through the food web. With few species present to process leaf litter and transfer detrital resources, it was clear that inputs of AMD disrupted both the mechanisms responsible for leaf breakdown and links for energy flow within stream food webs (Chapter 3). Nevertheless, leaf breakdown was sufficient to support and maintain primary consumers and simplified food webs in mining-impacted streams and the quantity of detrital resources was comparable to nearby unimpacted streams (Chapter 4).

**Food web patterns and interpretation**

Processes operating at different spatial and temporal scales can potentially influence patterns reported in stream food web structure (e.g., species dispersal, predator to prey interactions; Woodward and Hildrew, 2002 and references therein). However, most food web studies are conducted on small spatial scales and provide a ‘snapshot’ of food web structure. This is true of the food webs described in my thesis, which are based on one-off surveys conducted over a 3-year period in stream reaches (20 m) during
summer. Few studies address seasonal or inter-annual variation in food webs despite distinct changes in basal resource availability or consumer size structure that may influence links between species (but see Tavares-Cromar and Williams, 1996; Schmid-Araya et al., 2012). Strong seasonal effects were not expected in my study streams as basal resources are in continuous supply and there is a lack of seasonal energy pulses (e.g., significant inputs of leaves in autumn) typical of Northern Hemisphere streams. In addition, many New Zealand aquatic invertebrates are univoltine and non-seasonal (i.e., individuals of several ages are present at all times; Scarsbrook, 2000). Finally, while snapshot or summary webs can generate higher values for web size (S), links (L), and linkage complexity (Tavares-Cromar and Williams, 1996), temporal analysis of food web structure from six streams that I sampled in two different years (2008 & 2011; Chapters 2 & 3) indicated no significant pairwise differences in 12 food web properties between streams over time ($T$-tests, all $P > 0.60$).

Spatially, the food webs in my thesis were sampled from 20 m reaches, which is in the range of reach length (20 – 30 m) for studies based on several (> 10) streams. This length was chosen for logistical reasons but with the knowledge that streams contain species with very different distributions and mobility (i.e., fish). To partially address this, spot electro-fishing was conducted up to 100 m on either side of the sampling reach to assess if any taxa were missed. Most stream sites in my thesis were relatively well spread out across the study region (Appendix A) except for the naturally high metal streams, which were clustered close together, inland and some distance (minimum 80 km) from the other streams (Chapter 2). The selection of these streams was limited to the Red Hills area with the unique ultramafic geology that contributed to elevated Ni levels in the
stream water. The close spatial proximity of these sites may have meant less variability in food web structure, although considerable differences can exist between stream patches within a single stream (Thompson and Townsend, 2005). The distance inland and elevation of the naturally high metal sites may have caused fish access issues but fish distribution data indicates several species were found in nearby ranges at similar elevation (see Chapter 2) and streams were selected after assessment of the absence of potential barriers (e.g., waterfalls, dams). If fish were present but not detected in the naturally high metal streams, several food web properties would have changed (e.g., food chain length, links, predator: prey ratio, connectance) and overall web structure may have been more similar to naturally acidic than mining-impacted streams (Chapter 2). Finally, cross-ecosystem subsidies of resources can contribute to patterns in stream food webs (Woodward and Hildrew, 2002). The contribution of allochthonous resources (primarily leaf litter) from adjacent forests was a key factor structuring the detritus-based food webs reported in my thesis. In contrast, I found very little evidence (Chapter 2, 3, & 4) to suggest the importance of terrestrial insects in the diets of predatory invertebrate consumers or fish in any study stream. In other mining-impacted streams where in-stream resources are substantially reduced or inaccessible, predatory consumer diets have been shown to rely almost primarily on terrestrial insects (Scullion and Edwards, 1980), so this cross-system subsidy can clearly be important in structuring food webs in stressed streams.
New insights for restoration and management

Adopting a food web approach, which provides a more complete understanding of ecological processes linked to rates and flow of energy and matter based on feeding relationships, has the potential to provide a better understanding of how to assess, restore and effectively manage stream ecosystems affected by mining (Ryder and Miller, 2005; Lake et al., 2007). Currently, restoration or management of AMD-impacted streams often begins with passive or active treatment to improve water chemistry (i.e., increase pH; Johnson and Hallberg, 2005) and may also include structural stream channel alterations (e.g., reshaping channel dimensions or sinuosity; Bernhardt and Palmer, 2011) and riparian vegetation planting with the expectation that once in-stream conditions improve biota will naturally recolonise and food webs can reassemble (Palmer et al., 1997). However, increasing stream pH can lead to additional issues (i.e., precipitated metals) and is not necessarily enough to restore functional stream communities, as biological recovery is often delayed or incomplete (Cravotta et al., 2010; Gunn et al., 2010; Northington et al., 2011; DeNicola et al., 2012). Findings from my research imply that trophic bottlenecks, especially for predatory invertebrates and fish, will need to be addressed to enable healthy and functioning food webs. The successful re-colonisation of fish, whether naturally or by human intervention, will have to be preceded by the establishment of an abundant benthic invertebrate community, which includes functionally diverse species (e.g., predators, filter feeders). Similarly, inputs of organic matter will need to resume in order to support consumers in areas where mining activities have removed substantial tracts of riparian vegetation (e.g., Gunn et al., 2010). Although detrital resources were not limiting for consumers in my study streams, amorphous
detritus formed the basis of the diet for primary consumers and consumer-detritus linkages were essential for transferring energy up the food web (Chapter 3). Alternatively, the establishment of food web structure would be a useful starting point for restoration or management plans. This could effectively be achieved using food web metrics derived from consumer isotope signatures that provide insights into how AMD disrupts food web structure and energy flow (Chapter 5). This holistic assessment could be used to focus goals, identify necessary manipulations (e.g., re-introduction of key species that drive ecosystem processes) and track success with repeated samplings that detect the establishment of links between species.

Conclusions

In conclusion, my thesis has contributed to our understanding of the complex ecological effects of AMD on stream ecosystems by identifying interactions among species that structure the small and simplified food webs and maintain limited energy flow and ecosystem function. My results also highlight the importance of metals, in both dissolved and precipitated forms, for both the structure and function of stream food webs. It is my hope that these results will be of interest in New Zealand and internationally, because as mining activities continue to increase, so too will the need to be able to predict and manage impacts, which will benefit from a more holistic understanding of interactions among species and trophic levels in these highly stressed ecosystems.
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Appendices
Appendix A. The location of study sites where I compared food web structure from streams with either anthropogenic or natural sources of acidity and metals or circumneutral water chemistry on the South Island, New Zealand (Chapter 2). Streams were classified into the following four types: mining (i.e., those affected by AMD); naturally acidic; circumneutral, and naturally high metal.
Appendix B. The location of study sites where I examined the relationships between leaf breakdown, detrital resources, and food webs on the West Coast, South Island, New Zealand (Chapter 3). Streams were classified as acidic mining (pH < 3), precipitate mining (pH 3.5 – 4.9; metal hydroxide precipitates present on substrata), naturally acidic (pH 4.1 – 5.6), and circumneutral (pH 6.5 – 7.0).
Appendix C. The location of study sites along an AMD-impact gradient, where I examined the quantity and quality of food resources (Chapter 4) and investigated the use of isotopic metrics to detect the effects of mine drainage pollution on stream food webs (Chapter 5) on the West Coast, South Island, New Zealand.
Appendix D. In this section, I have provided more detailed methods for field sampling and laboratory-based techniques than were presented in individual chapters.

Chapter 2

*Water chemistry & physical characteristics of streams*

Stream water pH, specific conductivity, temperature, and dissolved oxygen were measured using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA). Water samples (125 mL) collected for analysis of dissolved metals were filtered in the field through a 0.45 µm mixed cellulose ester filter, acidified with nitric acid, and kept cool until analysis by ICP-MS (Hill Laboratories, Hamilton, New Zealand). Wetted channel width, depth, and velocity were recorded at five points along three randomly selected transects in each 20 m reach. Substrate size was estimated by measuring 30 randomly selected particles from the streambed. Vegetation cover in the middle of the reach was estimated visually using a densiometer (Robert E. Lemmon Forest Densiometer Model-A, Oklahoma, USA).

*Basal resources – Algae & detritus*

Samples of algae and organic matter were collected to quantify basal food resources. Attached algae were removed from the surface of 15 randomly selected stones using a hard-bristled brush, rinsed with filtered stream water, and pooled into three 200 mL representative samples (i.e., 5 stones per representative sample). The surface area of each stone was estimated from length measurements of the two main axes. In the field, a 50 mL subsample was filtered (0.7 µm-GF/F) and frozen for chlorophyll a analysis to determine algal biomass. In the laboratory, algal pigments were extracted in buffered
ethanol (90%) and quantified using fluorometry (Trilogy Fluorometer, Turner Designs, California, USA). Benthic organic matter was collected in five Surber samples (0.06 m²; 250 μm mesh) and divided into two size fractions using sieves: FPOM (250 – 1000 μm) and CPOM (> 1000 μm). Suspended organic matter (seston) was collected from 2 L of filtered (0.7 μm-GF/F) stream water. All FPOM, CPOM, and seston samples were dried, weighed, ashed (550°C for 4 h) and re-weighed to determine ash-free dry mass (AFDM).

**Consumer samples - Benthic invertebrates and fish**

Benthic invertebrates were collected in five Surber samples (0.06 m²; 250 μm mesh) from riffle and run habitats. They were fixed in ethanol (70%) and returned to the laboratory for processing. Taxa were identified and counted under a dissecting microscope to the lowest possible resolution according to Winterbourn et al. (2000a) and unpublished keys for hydrobiosid caddisflies and other invertebrate groups (NIWA, Hamilton, New Zealand). Following identification, molluscs and cased caddisflies were removed from their shells and cases, respectively. All invertebrates were classified into functional feeding groups, dried, weighed, ashed (550°C for 4 hr), and re-weighed together by group to determine AFDM. Kick net samples were collected at each stream to gain a more complete taxonomic list and collect individuals for gut contents analysis. Specimens for gut analysis were fixed in ethanol (70%) in the field. Fish and crayfish were sampled using quantitative electrofishing techniques in the 20 m reach. Stop nets were placed at the top and bottom of the reach and fish were removed during three successive passes. The fork length of the captured fish was measured in the field and wet weights were measured using a portable balance or determined from length-weight
regressions *(unpublished data, P.G. Jellyman, D.J. Jellyman, M.L. Bonnet, NIWA, Christchurch, New Zealand)*. Spot-electrofishing was conducted up to 100m on either side of the sampling reach to confirm the absence of taxa not captured in the 20 m reach. Fish were returned to the stream except for a subset (up to 10 per species) that was retained for gut analysis. These fish were euthanized in a large container using 2-phenoxyethanol and preserved in ethanol (100%). Crayfish were collected for gut content analysis but were not included in invertebrate biomass estimates.

*Food web structure & properties*

Gut contents analysis was used to identify feeding links and construct food webs for each stream. Invertebrate guts were removed, mounted on slides in lactophenol-PVA stained with lignin pink, and examined under a microscope (400x magnification). Invertebrates found in gut contents were identified to the lowest possible taxon and counted. Otherwise, food was itemised as animal prey, filamentous algae, diatoms, other algae, plant detritus (CPOM), and amorphous detritus (FPOM). The relative abundance of food items was visually estimated by scanning the whole slide and categorised as: rare (< 5%), common (5 – 20%), or abundant (> 20%). These established abundance categories were obtained from (Hershey et al. 2006). Stomach contents of fish were examined under a dissecting microscope. Prey items were identified to the lowest taxonomic resolution possible and counted or estimated as described above. All terrestrial insects found in guts were grouped into a single basal resource category (i.e. terrestrial insect) and counted.
Because many species were present at low densities in our streams, particularly the mining streams, we were only able to collect a limited number of individuals of each species for gut analysis. Frequently, this was less than the minimum of 10 individuals per species recommended by Thompson et al. (2001). In order to standardise our effort and generate comparable food webs across the four stream groups, we identified gut contents from several different species in each functional feeding group in all streams to get dietary information on a wide range of species. The number of guts analysed for each species ranged from 2 – 47. We then compiled a cumulative binary matrix (Cohen et al., 1990) for predators and prey and included links found in all 20 streams. Based on this cumulative matrix, we constructed individual food webs for each stream and were able to infer links for species where gut content data was missing but the species was present in samples for a particular stream. This approach assumes the presence of a feeding interaction between two species in all streams in which they are found but does not allow for differences in prey selection or the strength of interactions between streams (Havens, 1992). However, the procedure limited bias due to inadequate or unachievable sample size. We assessed invertebrates and fish in each stream for evidence of all six basal resources (algae, filamentous green algae, diatoms, FPOM, CPOM, terrestrial insects). If one or more was absent, all links with this resource were excluded for a particular stream. Links between predators and algal basal resources were also excluded to avoid links that were due to prey within prey (Thompson et al., 2001). However, detrital resource links were retained due to evidence of detritus in predator guts lacking prey. The constructed food webs did not incorporate meiofauna, bacteria, or fungi, and therefore provide a conservative estimate of overall food web structure.
The following food web properties were calculated for each stream: number of species, including basal resources (or web size, $S$), number of links ($L$); fraction of basal species (species with predators but no prey); fraction of intermediate species (species with predators and prey); fraction of top species (species with prey but no predators); maximum food chain length (number of links from a basal species to a top species, excluding feeding loops); predator – prey ratio (Cohen, 1977); linkage density ($L/S$; Pimm et al., 1991); and connectance (fraction of all possible links that are realised within a food web; $L/S^2$). This commonly used measure of connectance is less likely than other measures to be affected by differences in web size.

Chapter 3

Stream characteristics – Physicochemical, organic matter & invertebrate community

On each sampling occasion, pH, specific conductivity, and flow rate were measured in-stream using standard meters (YSI 63; YSI Environmental Incorporated, Ohio, USA; and Flo-mate 2000; Hach Company, Colorado, USA) and a water sample was taken for nutrient analysis. Water samples were filtered in the field through a 0.7-µm glass microfiber filter (Whatman GF/F) and stored at -4°C until analysis for nitrate and soluble reactive phosphorus with an Easychem Plus (Systea Scientific, Italy). Samples for analysis of dissolved metals (Al, Fe, Zn) were collected once in each stream at the start of the experiment (day 0). These samples were filtered through a 0.45-µm mixed cellulose ester filter, acidified with nitric acid, and stored at -4°C until analysis by ICP-MS (Hills Laboratory, Hamilton, New Zealand). Physical disturbance of the streambeds was assessed using the channel stability index (Pfankuch, 1975). Finally, three Surber samples (0.06 m², 250 µm mesh) were collected from a riffle section of the stream, with cobble-
sized substrata, on each sampling date to assess in-stream biomass of coarse particulate organic matter (CPOM; > 1000 µm), fine particulate organic matter (FPOM; 250 – 1000 µm) and to characterise invertebrate community composition and biomass. CPOM, FPOM, and invertebrate samples were dried, separately (60°C), weighed, ashed (550°C for 4 h), and re-weighed to determine ash-free dry mass (AFDM).

*Leaf litter breakdown - field experiment*

Leaves of mahoe (*Melicytus ramiflorus*), a common riparian tree species in New Zealand, were collected on a single occasion. Mahoe leaves have a relatively fast breakdown rate and have been used in previous studies of leaf decomposition (Linklater, 1985; Hicks and Laboryrie, 1999; Young and Collier, 2009). Leaves were weathered outdoors for 14 days, dried for 48 h at 60°C, and placed in mesh bags (5 g/pack; 5 mm mesh). Twelve replicate leaf packs were deployed at each site and were secured to the substrate with a cable tie and pegs. A water temperature logger (Hobo Pendant, Onset Computer Corporation, Massachusetts, USA) was deployed in each stream to calculate degree-days. Three leaf packs were retrieved on each sampling date. Each pack was placed into a separate plastic bag, stored on ice in the dark, and returned to the laboratory for processing.

*Leaf litter – sample processing*

In the laboratory, the remaining leaf material in each pack was rinsed and sorted to remove debris and invertebrates. All leaves remaining in leaf packs (including pieces used in respiration assays after incubations were completed—see below) were dried,
weighed, ashed, and re-weighed to determine AFDM. We corrected the initial leaf mass to exclude mass loss due to leaching by soaking five pre-weighed leaf packs in circumneutral stream water for 2 days. After leaching, mean AFDM of leaf packs was 71% of the initial mass (range 70-72%). Leaf litter breakdown rates (-k) were calculated by regressing the natural log (ln) of the percentage leaf mass remaining (AFDM) against time expressed as experiment days (d) and degree-days (dd).

Microbial respiration was measured using small pieces of wet leaf (~2 cm) from each leaf pack that had been kept cool and in the dark. Stream water containers were shaken prior to pouring into the vials to re-saturate the water. Leaf pieces were placed in 30-mL glass vials that were full of stream water (i.e., no air space in vial) from the test stream. The vials were sealed and incubated at 15°C in a temperature-controlled room for 8-12 h. Vials were gently shaken during the incubation. Respiration rates were measured under low light and calculated as the difference in dissolved oxygen concentration between vials with leaves and vials containing only stream water per unit leaf mass (g AFDM) per hour. Dissolved oxygen was measured with an electronic meter (YSI550A; YSI Environmental Incorporated, Ohio, USA). Leaves were also cross-incubated in water from each of the four stream types for 12 h to determine whether microbes were adapted to water chemistry by comparing respiration rates with those obtained in their native streams. Cross incubations were done on leaves and water from one stream from each type on the final day of the experiment (day 60).

Invertebrates collected from the leaf packs were preserved in ethanol (70%), identified following Winterbourn et al. (2000) and unpublished keys for hydrobiosid caddisflies and other invertebrate groups (NIWA, Hamilton, New Zealand) and counted.
under a dissecting microscope. The density of total invertebrates and shredding invertebrates found in leaf packs are reported as number of individuals per g AFDM of leaf litter remaining.

Food web structure & properties

Food webs were constructed using in-stream data collected during the experiment and a cumulative binary matrix that identified links between predators, prey, and basal resources based on gut contents analysis for streams in our study area. The cumulative matrix was originally compiled by Hogsden and Harding (2012b [Chapter 2]) and included additional links identified in analysis of recent samples from the same streams (K. Hogsden, unpublished data). Fish and invertebrate consumers used to construct food webs are listed in the Supplementary Material. We calculated several food web properties for each stream, including: web size (S); number of links (L); proportions of basal, intermediate, and top species; predator-prey ratio (Cohen, 1977); linkage density (L/S); and connectance (L/S^2).

Chapter 4

Stream water pH and conductivity (at 25°C) were measured in the field using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA) to confirm the degree of AMD impact on each stream. Spot water samples were also collected for dissolved metal analysis. These samples were filtered in the field (0.45 µm mixed cellulose ester filter), acidified with nitric acid, and kept cool prior to analysis using an ICP-MS at Hill Laboratories, Hamilton, New Zealand. Metal hydroxide
precipitates present on stream substrata were quantified by scrubbing the upper surfaces of five randomly selected stones of known area from each stream. The resulting slurry was dried, weighed and ashed (550°C for 4 h) in the laboratory to determine the amount of metal hydroxide (i.e., inorganic or ash mass) per area of stone. Because the mass of metal hydroxide precipitate did not differ significantly between streams (ANOVA: \( P > 0.05 \)) it is reported as present or absent. All streams were of similar size (first or second order, wetted width 1.9 – 5.4 m), had cobble-gravel substrates or bedrock, and were surrounded by either native podocarp and/or beech forest, or scrub. Shading was estimated visually in the middle of each stream near the sampling reach using a densiometer (Robert E. Lemmon Forest Densiometer Model-A, Oklahoma, USA).

**Food quantity - Sampling and analysis**

Periphyton was removed from the upper surfaces of 15 randomly selected cobbles (length 3 – 13 cm) using a hard-bristled brush, rinsed with filtered stream water, and pooled into three representative samples (i.e., 5 stones per representative sample). The surface area of each cobble was estimated from length measurements of the two main axes. Each sample was filtered (0.7 \( \mu \text{m}-\text{GF/F} \)) in the field and frozen prior to determining algal biomass (as chlorophyll \( a \)). In the laboratory, algal pigments were extracted in ethanol (90\%) for 24 h, at 4°C in the dark. Tubes were centrifuged and dilutions, if required, were made using 90% ethanol. Extracts were read on a Shimadzu model UV-1700 Pharmaspec spectrophotometer (Shimadzu, Kyoto, Japan) at 665 nm and 750 nm wavelengths using a 1 cm path-length quartz cell. Extracts were then acidified with 0.1 mL of 0.1 N HCL, gently agitated, held for 90 s and re-read at the above wavelengths to
correct for pheophytin, if present. Chlorophyll $a$ was calculated following Biggs and Kilroy (2000) and is expressed per area of upper cobble surface. Benthic organic matter was collected in five Surber samples (0.06 m$^2$; 250 µm mesh) and divided into two size fractions using sieves: fine particulate organic matter (FPOM; 250 – 1000 µm) and coarse particulate organic matter (CPOM; > 1000 µm). FPOM and CPOM samples were dried, weighed, ashed (550°C for 4 h) and re-weighed to determine ash-free dry mass (AFDM).

Benthic invertebrates were picked from the five Surber samples (each Surber a replicate) to characterise the availability and diversity of food items for invertebrate predators. Samples were preserved in ethanol (70%) and processed under a dissecting microscope in the laboratory. Taxa were identified and counted to the lowest possible taxonomic level according to Winterbourn et al. (2000b) and unpublished keys for hydrobiosid caddisflies and other invertebrate groups (NIWA, Hamilton, New Zealand). All invertebrates were classified into functional feeding groups following Winterbourn et al. (1984) and Chadderton (1988), dried, weighed, ashed (550°C for 4 h), and re-weighed to determine AFDM.

Food quality and diet - sampling and analysis

We estimated food quality based on carbon to nitrogen (C: N) ratios and evaluated the sources of food assimilated by invertebrates (algae vs allochthonous detritus) using stable carbon ($^{13}$C: $^{12}$C) and nitrogen ($^{15}$N: $^{14}$N) analyses. To complement the stable isotope work, gut content analyses were conducted on invertebrates following Hogsden and Harding (2012). All invertebrates used for stable isotope and gut analyses were medium-large individuals of their respective species. Food items from gut contents
were identified (animal prey, CPOM, FPOM, algae, filamentous algae and diatoms), their relative abundance in the gut estimated visually by scanning whole prepared slides and categorised as: rare (<5%), common (5 – 20%) or abundant (>20%). Aquatic bryophytes, filamentous green algae, and periphyton (epilithon) were collected (when present) from the surfaces of submerged rocks, and rinsed to remove any invertebrates, detritus, or sediment before freezing. Detritus (primarily decomposing leaf litter) was collected from the streambed, hand-picked to remove invertebrates, rinsed to remove biofilm, and frozen until further analysis. Fine particulate organic matter, which can have multiple origins, was not collected for isotope analysis, but its allochthonous component can be expected to have a δ13C signature similar to that of dead leaves taken from the study streams (Rounick et al. 1982; Winterbourn et al. 1984). For each stream, replicate samples of each basal resource (if available) were pooled to ensure sufficient material was available for analysis. Additional invertebrates for gut and isotope analysis were collected with a kicknet (500 µm mesh) sorted into taxa, placed in vials in the field, and frozen.

In the laboratory snails and caddisfly larvae were removed from their shells and cases, respectively, and guts were removed from all invertebrates. They were then oven dried at 60°C for at least 48 h and ground to a fine, homogeneous powder. Basal resource and prey samples were pooled for each stream. Invertebrate samples for analysis comprised material from 2-40 individuals, depending on their size. Samples of dried powder (c. 3 mg for plants, 1 mg for invertebrates) were packed into tin capsules and analyzed at the Cornell Isotope Laboratory (Cornell University, Ithaca, New York) on a Thermo Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer to obtain total C and N masses and isotope signatures. C: N ratios were
calculated based on total C and N masses. Isotope signatures are expressed in delta ($\delta$) notation defined as parts per thousand (‰) deviation from a standard reference material (i.e., $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$). Standard reference materials were PeeDee belemnite limestone for $\delta^{13}\text{C}$ and atmosphere nitrogen for $\delta^{15}\text{N}$. Experimental precision was 0.19‰ ($\delta^{13}\text{C}$) and 0.17‰ ($\delta^{15}\text{N}$) based on the standard deviation of replicates of an internal standard. We corrected for lipid effects on $\delta^{13}\text{C}$ signatures using the formulae of Post et al. (2007) prior to data analysis.

Chapter 5

All sampling was conducted in December 2009 (austral summer) under baseflow conditions. Stream water pH and conductivity (at 25°C) were measured in the field using standard meters (YSI 63 and YSI 550A, YSI Environmental Incorporated, Ohio, USA) to confirm the degree of AMD impact on each stream. Water samples were also collected for dissolved metal analysis. These samples were filtered in the field (0.45 µm mixed cellulose ester filter), acidified with nitric acid, and kept cool prior to analysis using an ICP-MS (Hill Laboratories, Hamilton, New Zealand). Metal hydroxide precipitates present on stream substrata were quantified by scrubbing the surfaces of five randomly selected stones of known area from each stream. The resulting slurry was dried, weighed, and ashed (550°C for 4 h) in the laboratory to determine the amount of metal hydroxide (i.e., inorganic or ash mass) per area of stone. The difference in metal hydroxide biomass between all streams was not significant (ANOVA: $P > 0.05$; range: $1.4 \times 10^{-4}$ - $4.4 \times 10^{-3}$ mg cm$^{-2}$) so we have excluded metal hydroxides from further analysis. This physical variable/stressor of AMD-affected streams is reported as present or absent.
Stable isotope sampling & analysis

Our study streams contained a diversity of invertebrates and fish, although the same species were not present in all streams. Therefore, we focused on common taxa found in earlier studies (Winterbourn, 1998; Greig et al., 2010; Hogsden and Harding, 2012a [Chapter 2]) to provide a robust representation of consumers. Invertebrates for stable isotope analysis were collected by kicknet (500 µm mesh) and sorted by taxa, placed in vials in the field, and frozen. Fish and crayfish were captured using a backpack electrofishing machine (Kainga 300 EFM), euthanized with 2-phenoxyethanol and frozen until further analysis. A maximum of 10 fish per species was collected from each stream for stable isotope analysis.

In the laboratory, all samples were oven dried at 60°C for at least 48 h and ground to a fine, homogeneous powder. Whole body samples of 3 – 40 individual invertebrates per species (with guts removed) were processed together to attain sufficient biomass for isotopic analysis. Snail shells and caddisfly cases were removed prior to grinding to avoid contamination from non-dietary sources of carbon. Dorsal muscle tissue was taken from each fish \( (N = 91) \) and abdominal muscle from crayfish \( (N = 15) \). Samples of dried powder \( (1 \text{ mg}) \) were packed into tin capsules and analyzed at the Cornell Isotope Laboratory on a Thermo Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer. Isotope ratios are expressed in delta (δ) notation defined as parts per thousand (‰) deviation from a standard reference material:

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]
where \( X \) represents \( \delta^{13}C \) or \( \delta^{15}N \) and \( R \) is the corresponding ratio of \(^{13}C/^{12}C\) or \(^{15}N/^{14}N\), respectively. Standard reference materials were PeeDee belemnite limestone for \( \delta^{13}C \) and atmosphere nitrogen for \( \delta^{15}N \). Experimental precision with our samples was 0.19‰ (\( \delta^{13}C \)) and 0.17‰ (\( \delta^{15}N \)) based on the standard deviation of replicates of an internal standard. We corrected for lipid effects on \( \delta^{13}C \) signatures using the mathematical formulae of Post et al. (2007) prior to data analysis.

Invertebrate community sampling

Benthic invertebrates were collected from riffle and run habitats with a Surber sampler (0.06 m\(^2\); 250 \( \mu m \) mesh). Five samples were taken from each stream, preserved in ethanol (70%) and identified under a microscope (10 - 20 x magnification) in the laboratory. Taxa were identified and counted to the lowest possible taxonomic level using the keys of Winterbourn et al. (2000b) and unpublished keys for hydrobiosid caddisflies and other invertebrate groups (NIWA, Hamilton, New Zealand). All invertebrates were classified into functional feeding groups following Winterbourn et al. (1984) and Chadderton (1988).

References

*Note - only one new reference added to Appendix D is listed below.