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Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage

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ABSTRACT

Functional indicators of stream health have the potential to provide insights into stream condition that cannot be gained by traditional structural indices. We examined breakdown of leaves, wood, and cotton cloth strips at 18 sites along a gradient of effects of drainage from coal mines in New Zealand to determine the usefulness of these methods as functional indicators of stream health. The pH varied from 2.7 to neutral across the streams, and the more acidic streams typically had higher concentrations of aluminum, iron, zinc, and other metal ions. Precipitates of metal (mainly iron) hydroxides were present in most streams affected by mine drainage, especially in those with a pH of 4–5. Breakdown rates of all organic matter types were highest in several reference streams with neutral pH and lowest in sites with high rates of metal hydroxide deposition. Breakdown was relatively fast in the most acidic streams (pH < 3), in some cases as fast as at reference sites; these sites also had elevated nutrient concentrations. Shredding invertebrates were absent in litterbags from acidic streams and common at only 2 reference sites; their presence contributed to fast breakdown of leaves in the field and in lab microcosms. Microbial respiration was closely related to breakdown rates of leaves and wood; it was high at neutral and highly acidic streams, but lower at sites with pH 4–5, where metal hydroxides were precipitating onto solid surfaces. In these metal hydroxide-stressed streams, leaf and wood breakdown was slower, and associated biota, including microbes, were more affected than by water chemistry stressors (pH, dissolved metals) associated with mine drainage. Litter breakdown and microbial respiration provide insight into the functioning of streams, yielding different responses than traditional structural measures based on macroinvertebrates, which did not accurately distinguish impacts from acid mine drainage.

1. Introduction

Organic matter from terrestrial vegetation provides an important energy source for most streams (Wallace et al., 1997). This organic matter, primarily leaves and wood, supports and is broken down by both microbial and animal communities. Given the importance of this energy source, organic matter breakdown has been studied in many streams and recently was proposed as a cost-effective, synthetic, and functional indicator of stream health (Bunn et al., 1999; Gessner and Chauvet, 2002; Niyogi et al., 2003; Young et al., 2008). Functional measures of stream health such as organic matter breakdown directly integrate microbial assemblages, energy conversion, and animals. Consequently, they provide insights into stream processing and energy transfer that traditional structural measures, mostly fish and invertebrate diversity, do not provide.

We examined organic matter breakdown as a functional measure of the effects of acid mine drainage (AMD) in New Zealand streams. Many sites throughout the world are affected by drainage from active or abandoned mines. Biota in streams receiving mine drainage must deal with multiple stressors (Kelly, 1988; Niyogi et al., 2002a). The weathering of sulfide minerals upon exposure of ore-bearing rocks to air and water can produce acidity (from sulfuric acid) and release dissolved metals. Some metals, especially iron and aluminum, can precipitate as hydroxides or other compounds when AMD enters a stream and is diluted by circum-neutral or buffered water from upstream; metal hydroxides precipitate out of solution as the pH of metal-rich water increases beyond their solubility thresholds (McKnight and Feder, 1984; Kelly, 1988). These metal hydroxides often coat streambeds, as well as biota and organic matter (Letterman and Mitsch, 1978; Sode, 1983; Niyogi et al., 1999, 2009).

Several studies have examined litter breakdown in streams affected by mine drainage (e.g., Gray and Ward, 1983; Maltby and Booth, 1991; Bermingham et al., 1996; Carlisle and Clements, 2005; Medeiros et al., 2008), including streams in New Zealand (Barnden

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and Harding, 2005). These studies found slower breakdown in AMD-impacted streams, mediated by the effects of multiple stressors on shredding invertebrates and microbial communities. Many shredding invertebrates are sensitive to elevated concentration of dissolved metals; however, some are relatively tolerant of low pH (McKnight and Feder, 1984; Ledger and Hildrew, 2000; Simon et al., 2009). Some invertebrate taxa in New Zealand, including stoneflies and caddisflies, can be found at relatively low pH (<4) (Winterbourn and McDiffett, 1996; Hogsden and Harding, 2012). Slower breakdown of leaves is often related to lower shredder abundance or biomass (Niyogi et al., 2001), or the loss of key shredder taxa in acidified streams (Griffith and Perry, 1993; Dangles and Chauvet, 2003; Simon et al., 2009). Streams with minor effects of mine drainage that do not limit shredding invertebrates can have breakdown rates comparable to unimpacted sites (Nelson, 2000), but sublethal effects of mine drainage may still limit shredder activity in some cases (Medeiros et al., 2008).

Microbial activity on leaves can be depressed in some streams of low pH (Thompson and Bärlocher, 1989; Simon et al., 2009). However, microbes can remain active in some acidic streams, especially when competition with or consumption by shredders is reduced (Niyogi et al., 2002b). Additionally, some stream fungi can be relatively tolerant of dissolved metals (Miersch et al., 1997), although other studies have reported negative effects (Duarte et al., 2004). Furthermore, the deposition of metal hydroxides in streams affected by mine drainage can limit microbial activity (Schlief, 2004; Schlief and Mutz, 2005; Niyogi et al., 2009).

In order to determine the suitability of litter breakdown as a functional indicator of impacts by AMD, we measured breakdown rates of leaves, two types of wood, and cotton cloth strips at 18 sites along an AMD gradient on the West Coast of the South Island, New Zealand, in areas with abandoned and active coal mines (Harding and Boothroyd, 2004). Biotic mechanisms behind differences in breakdown rates were explored with field observations and microcosms. We hypothesized that multiple stressors from mine drainage affect microbial activity and shredding invertebrates, which would result in lower breakdown rates for the four types of organic matter.

2. Materials and methods

2.1. Study sites

The West Coast of the South Island of New Zealand has been mined for coal for over 160 years and has many abandoned and active mines. We focused on three main areas, Reefton, Denniston, and Stockton, within a single ecoregion (Westland Forest; Harding et al., 1997) where intensive coal mining has affected many streams. Within each of these areas, both unimpacted reference sites and sites affected by mine drainage were examined (Table 1). Mine drainage was the only observed anthropogenic stressor at almost all sites; some sites were downstream of houses or other buildings, but there was no significant urbanization, agriculture, or obvious influence of sedimentation, nutrient enrichment, or other stressors. Furthermore, there was no active mining within 1 km of any of our sites, so effects of mining are mainly due to effects of mine drainage, as opposed to effects from mining trucks, explosives, and other direct impacts.

2.2. Stream characteristics

Discharge at each site was measured following standard protocols of width, depth and velocity measures with a Marsh McBirney current meter (Gore, 1996) with the exception of one site (Ngakawau River) that was not wadeable; we visually estimated

Table 1
Characteristics of sites along West Coast of South Island, New Zealand. Sp. Cond. is specific conductivity, Zn is dissolved zinc concentration, Al is dissolved aluminum concentration, MH is metal hydroxide amount on streambed. DIN is dissolved inorganic nitrogen, and SRP is soluble reactive phosphorus. Values for discharge, pH, Sp. Cond., DIN, and SRP are averages from 5 readings over the course of incubations.

Site	Region	Discharge ($L\,s^{-1}$)	Temperature (°C)	pH	Sp. Cond. (μS/cm)	MH ($mg\,cm^{-2}$)	Fe (mg/L)	Al (mg/L)	Zn (mg/L)	DIN (μg/L)	SRP (μg/L)
Lankeys	Reefton	51.4	12.9	7.38	101	0.05	0.07	0.051	0.001	14	25
Murrays	Reefton	88.2	11.6	7.17	77	0.05	0.05	0.11	0.002	12	9
Bradleys	Stockton	11.4	13.5	7.16	280	0.08	<0.02	0.015	0.001	51	5
Twins	Stockton	16.7	12.2	6.74	103	0.05	<0.02	0.069	0.006	24	2
Ngakawau	Stockton	8000[a]	14.6	5.71	133	0.66	<0.02	0.034	0.072	134	2
Denniston Road	Denniston	6.9	13.9	5.04	22	0.05	0.03	0.076	0.002	40	8
Devils – lower	Reefton	24.1	11.3	4.65	144	0.44	0.10	0.37	0.026	24	5
Devils – upper	Reefton	11.3	11.9	4.52	115	0.72	0.13	0.94	0.036	17	7
Burnetts Face	Denniston	66.8	13.0	4.14	62	0.14	0.12	0.86	0.047	41	6
Garveys	Reefton	61.4	13.1	3.99	449	0.85	0.02	2.3	0.150	1872	2
Whareatea Mine	Denniston	15.4	12.2	3.85	82	0.49	0.14	0.41	0.010	11	3
Wellman	Reefton	55.2	12.9	3.26	849	2.73	0.89	8.3	0.89	189	6
Granity	Stockton	316	13.1	2.94	705	0.14	7.4	23	0.30	764	16
Mine	Stockton	140	15.5	2.87	989	0.05	9.5	41	0.58	1345	45
Portal	Stockton	7.4	15.4	2.87	1079	0.09	12	46	0.44	1124	58
Sullivans West	Denniston	54.2	11.1	2.80	949	0.68	11	14	0.52	112	76
Packtrack	Stockton	5.1	15.2	2.77	1174	0.12	13	34	0.40	984	45
Old Bathhouse	Stockton	36.6	18.4	2.76	1399	0.46	23	56	0.64	1831	340

[a] Discharge was visually estimated for this river.

discharge for this site (based on estimates of width, depth, and velocity). Stream temperature, specific conductivity, and pH were measured on each of five visits with a meter (Fisher Scientific Accumet AP85). Stream temperature was also recorded at most sites on hourly intervals using dataloggers (Onset temperature loggers). Average temperature was calculated over the course of organic matter incubation (23 December 2008 to 29 April 2009). Dissolved nutrients (ammonium-N, nitrate-N, and soluble reactive phosphorus SRP) were measured by standard protocols (APHA, 1998) on filtered (Whatman GF/F) water samples collected on each visit. Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonium-N and nitrate-N. The concentrations of four dissolved metals (aluminum, iron, nickel, and zinc) were measured at each sampling date on filtered (0.45 μm cellulose nitrate filters) water samples by ICP-MS by Hill Laboratories (Hamilton, New Zealand).

The amount of metal hydroxides on the streambed was also measured. Cobbles from areas of moderate stream velocity (10–30 cm s^{-1}) were carefully retrieved (without disturbing accumulated deposits) and placed in plastic bags for analysis in the laboratory. Metal precipitates on the cobbles were brushed into weigh boats, and dry and ash masses were determined. The ash (inorganic) mass of the deposited material was used to calculate mass of metal hydroxides per rock area, estimated from rock dimensions. Most of the sites affected by mine drainage had visible deposition of metal hydroxides, indicated by orange (iron hydroxides) or white (aluminum hydroxides) fine deposits on the streambed. There was no visible evidence of other inorganic deposits, such as clay or sand from erosion, on the cobbles during sampling.

2.3. Litter breakdown

Bags of three types of litter were placed in the streams in December of 2007 (austral summer). Bags contained either 1 g dry mass (DM) of red beech (*Nothofagus rubrus*) leaves, a piece of red beech wood (an untreated tongue depressor of about 1.7 g DM), or a piece of birch (*Betula* sp.) wood (an untreated coffee stirrer of about 1.4 g DM). Three replicate bags of leaves were retrieved on each of four sampling times: 21, 49, 84, and 128 days after placement. Wood of both types was retrieved after 49, 84, and 128 days incubation at the stream sites. The mesh size of the plastic bags was 8 mm to allow invertebrate colonization. Additionally, we deployed cotton cloth strips (10 cm × 120 cm), a commonly used standard surrogate for litter (Boulton and Quinn, 2000; Clapcott et al., 2010). Strips were placed in the stream sites for 1 week at the end of the other organic matter incubations in late April 2008. Strips were analyzed for tensile strength followed the methods described by Clapcott et al. (2010) using a tensometer.

After collection, leaves and wood were rinsed with distilled water to remove invertebrates and inorganic debris. Standard protocols (Benfield, 1996) were followed for ash-free dry mass (AFDM) determination of remaining organic matter. A high percentage (20%) of the initial leaf mass was leached from leaves upon incubation in deionized water in the laboratory for 3 days. We excluded this abiotic loss from estimates of breakdown coefficients by setting the initial mass of litter to 0.80 g per litterbag. In some cases, significant amounts of metal hydroxides remained on the leaves and wood after rinsing in water. We added a correction factor for organic material that was adsorbed onto metal hydroxides (McKnight et al., 1992) by subtracting an estimated AFDM of these organic compounds from the AFDM of remaining leaves or wood. For this estimate, the ash mass of the remaining leaves or wood was calculated, and the ash mass of unincubated leaves or wood then subtracted. This difference in ash mass, which was treated as the inorganic mass of metal hydroxides, was multiplied by the ratio of AFDM to ash mass for metal hydroxide deposits collected at each site (see above). An exponential decay model (Petersen and Cummins, 1974) was used to estimate breakdown coefficients (k) for the leaves and wood, where AFDM data were log-transformed and regressed versus time.

2.4. Biotic communities

Macroinvertebrates were separated from leaves in the litterbags and sorted to genus following Winterbourn et al. (2000). Macroinvertebrates were not found in litterbags containing wood. We calculated the number of invertebrates per mass of litter by dividing counts by the AFDM of remaining leaves and estimated the number of shredding invertebrates per mass of litter by including only taxa identified as shredders. We also calculated a structural index based on the invertebrate communities at each site. We collected a kicknet sample (500 μm mesh), and calculated the Macroinvertebrate Community Index (MCI), which is specific to New Zealand. The MCI weights the presence of taxa by specific indices of tolerance to organic pollution and is the most commonly used and sensitive structural indicator in New Zealand streams (Boothroyd and Stark, 2000). The MCI ranges from 20 to 200; scores below 80 indicate severe pollution, and sites with scores above 120 are considered unimpacted.

Microbial respiration was measured on decomposing leaves and wood as described by Niyogi et al. (2001). Three 1.3-cm diameter discs of leaves or small sections of wood veneers were placed into 26-mL vials. The vials contained stir bars that allowed mixing of the water during incubations at 15 °C for between 8 and 18 h. Dissolved oxygen was measured in the vials at the end of the incubation with a YSI Model 85 meter. Respiration rates were calculated from declines in dissolved oxygen over time in vials with organic matter compared to vials with only water. Oxygen consumption rates were corrected for the AFDM of the leaf discs or wood pieces.

2.5. Experimental trials

We used separate laboratory experiments to assess the leaf shredding ability of invertebrates and the pH-sensitivity of microbial communities on leaves. Red beech leaves from litterbags were exposed to two invertebrate shredder taxa. Leaves were conditioned at one of the reference sites (Lankey Creek) for 7 weeks prior to experimentation. About 0.3 g AFDM of litter was held in 100 mL aerated stream water in jars maintained at 15 °C. Invertebrate treatments included three replicates each of a control (no invertebrates), an obligative shredder (*Austroperla cyrene*, Plecoptera), and a facultative shredder (*Olinga feredayi*, Trichoptera). These taxa were selected as they are common shredders in streams in this region. One *Austroperla* and three *Olinga* individuals were used in each replicate to have a similar amount of invertebrate biomass. After five days, the AFDM of remaining leaves collected on a 1 mm sieve was compared among the three treatments. The fine particulate organic matter (FPOM) of the remaining material was collected by filtering the material that passed through the 1 mm sieve onto filters (Whatman GF/F) and measuring its AFDM.

Leaves conditioned at three different sites were tested in shredding trials with the caddisfly *O. feredayi*. Red beech leaves were incubated for 3 weeks at 3 sites: Portal Stream (very low pH, high dissolved metals), Garvey Creek (low pH, moderate metals, high hydroxide deposition), and Lankey Creek (circum-neutral pH, low metals), representing the range of water chemistry and influence of mine drainage at our sites (Table 1). Three discs of leaves (1.3 cm diameter) were kept in jars with 100 mL aerated water from Lankey Creek, and three caddisfly larvae. After 5 days, the AFDM of remaining leaves collected on a 1 mm sieve was compared to leaves incubated in a control jar without shredders.

An additional experimental trial was run to test the effects of pH on microbial activity on decomposing leaves. Leaf discs from litterbags at two sites, Portal Stream and Lankey Creek, were incubated in 100 mL water in flasks on a rotary shaker at 15 °C. Treatments included water from the two streams, and water from Lankey Creek where the pH was lowered (to pHs of 5, 4, 3) by addition of sulfuric acid. An additional treatment of pH 2 water was tested for leaves from Lankey Creek. Microbial respiration on the leaves was measured after 3 days of incubation.

2.6. Data analyses

Pearson correlations were calculated among variables at the sites, including pH, concentrations of dissolved metals, metal hydroxide amounts, and nutrient concentrations. Regression analysis (multiple stepwise and best subsets) was used to determine the effects of the abiotic variables on rates of litter breakdown and other biological responses. Similarly, simple and multiple linear regressions were used to examine the effects of invertebrate abundance and microbial activity on breakdown rates. Results from the experimental trials were compared using analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Variables were log-transformed as necessary to meet assumptions of parametric statistics. Statistics were performed with SigmaStat software (version 2.03). Results were considered significant if P was < 0.05.

3. Results

3.1. Site characteristics

Mean stream discharge at the study sites ranged from about 5 to 8000 L s^{-1}; however, most sites were between 10 and 100 L s^{-1} (Table 1). Generally, sites averaged a temperature of 11–13 °C over the course of the study (December to April). However, several sites in the Stockton area had higher average temperatures of around 15 °C, and one site, Old Bathhouse, had an average temperature of 18.4 °C (as the source of discharge probably passed through an underground burning mine).

The 18 sites had a wide range of water chemistries (Table 1). Streamwater pH ranged from 2.7 to 7.4, with highly acidic (pH < 4) sites found in all three geographical areas. Specific conductivity and dissolved metals (Fe, Al, Ni, Zn) were positively correlated with each other, and negatively with pH (P for all comparisons <0.05). Highly acidic sites (pH < 4) generally had the highest concentrations of SRP and DIN. Sites of intermediate pH (4–6) tended to have the highest amounts of metal hydroxides on the stream bottom; these sites also usually had low SRP concentrations compared to the highly acidic sites.

3.2. Leaf breakdown and associated biota

Breakdown rates of red beech leaves varied by an order of magnitude from 0.0019 to 0.0219 d^{-1}. Breakdown rate varied with pH, but not linearly (Fig. 1A). The highest rates occurred at circumneutral sites, and lowest rates occurred at sites with pH 4–5. The highly acidic sites (pH < 4) had a wide range of breakdown rates, from 0.0029 to 0.0110 d^{-1}, some of which were similar to rates at reference sites. The amount of metal hydroxides deposited on the stream bottom accounted for almost 50% of the variation in leaf breakdown rates (Fig. 2A). Somewhat surprisingly, other variables, including pH and dissolved metals, did not account for significant variation (P > 0.2 for all variables when added to regression with metal hydroxides) in leaf breakdown rates after considering effects of metal hydroxides. Similarly, temperature also did not account for significant variation (P = 0.82) in breakdown after considering the

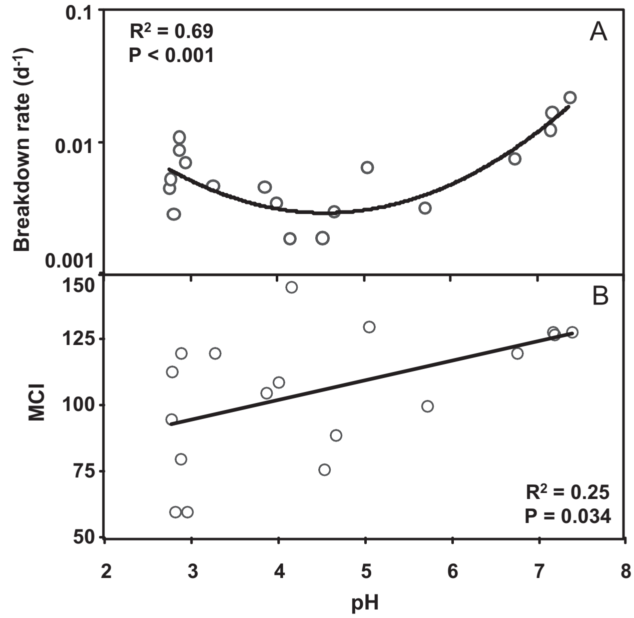

Fig. 1. (A) Breakdown rate of red beech leaves versus pH for 18 sites. Regression line and statistics are for polynomial (2nd order) fit. (B) Macroinvertebrate Community Index (MCI) versus pH for 18 sites. MCI scores are intended to reflect water quality, and scores indicate the following "stream health" conditions: >120 = clean, 100–120 = mild pollution, 80–100 = moderate pollution, <80 = severe pollution.

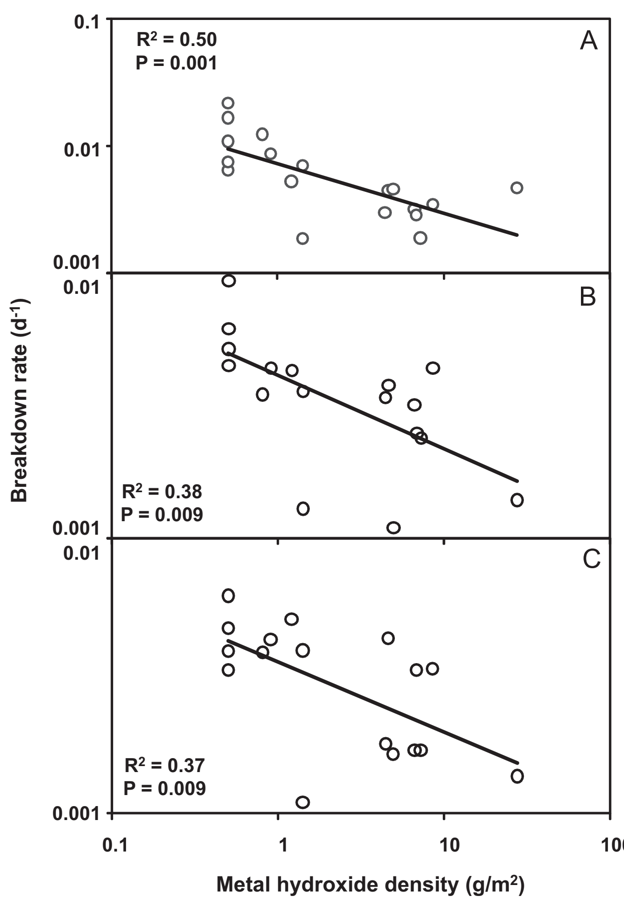

Fig. 2. Breakdown rate of red beech leaves (A), beech wood (B), and birch wood (C) versus metal hydroxide density on streambed for 18 sites.

Table 2
Results of multiple regression analyses. Degrees of freedom are 2, 15 for both results. SRP is soluble reactive phosphorus.

Dependent variable	R^2	Overall *p*-value	Independent variable	*p*-Value
Microbial respiration	0.48	0.008	SRP	0.020
			Metal hydroxides	0.043
Leaf breakdown	0.55	0.002	Microbial respiration	0.016
			Shredder density	0.057

effects of metal hydroxides. Analysis of breakdown rates based on degree days (data not shown) yielded similar patterns to analysis of breakdown rates per day.

Invertebrates were found in litterbags at only 7 of the 18 sites. Two reference sites in the Reefton area, Lankey and Murray Creeks, had the highest densities (32.2 and 7.6 individuals g $AFDM^{-1}$, respectively). These sites were also the only sites with shredding invertebrates in litterbags. Both sites had *Austroperla cyrene* stoneflies, and Lankey Creek also had the caddisfly *Olinga feredayi*. These two sites also had the highest leaf breakdown rates in our study.

Microbial respiration on leaves varied from 0.098 to 0.280 $\mu g\, O_2\, mg\, AFDM^{-1}\, h^{-1}$. Respiration was positively related to SRP concentrations and negatively to metal hydroxide deposition, which together accounted for 48% of the variation in respiration rates (Table 2). Leaf breakdown rates were closely related to rates of microbial respiration (Fig. 3), and microbial respiration and shredder density together accounted for 55% of the variation in breakdown rates in the streams (Table 2).

3.3. Experimental trials of leaf breakdown

The two presumed shredders (*Austroperla cyrene* and *Olinga feredayi*) readily consumed red beech leaves that had been colonized by microbes in a reference stream (Lankey Creek, neutral pH, low metals). After 5 days, one *Austroperla* caused a 50% reduction in AFDM of leaves relative to a control treatment, whereas three *Olinga* caused a 38% reduction (ANOVA $F = 11.6$, $df = 2, 6$, $P = 0.009$; difference was significant between both shredders and control; shredder treatments were not different from each other). Most of the leaf mass lost during the experiment (57% for *Austroperla* and 59% for *Olinga*) could be accounted for in the mass of FPOM collected in the experimental containers. FPOM generated in the experiment appeared to be primarily fecal pellets from the shredding invertebrates, and few discarded whole fragments were observed.

A second trial was conducted to measure the capacity of *Olinga* to consume leaves conditioned at sites differing in pH and metal hydroxide deposition. Incubation site had a significant effect on leaf consumption ($F = 9.3$, $df = 2, 9$, $P = 0.007$). *Olinga* consumed over 50% of leaf mass from the reference site (Lankey, pH 7.4) over 5

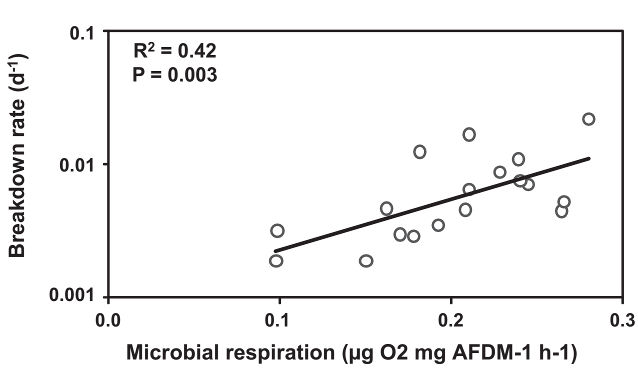

Fig. 3. Breakdown rate of red beech leaves versus microbial respiration rate on leaves for 18 sites.

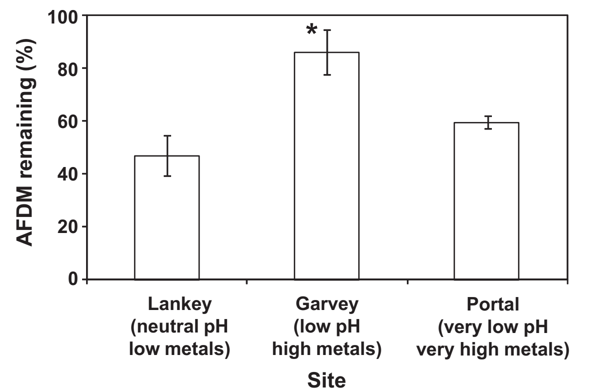

Fig. 4. Mass of leaf litter remaining from three sites after incubation with *Olinga* caddisflies in lab microcosms. *Significant difference ($P < 0.05$) from other sites by Tukey comparison after ANOVA.

days (Fig. 4), and 40% of leaf mass from a highly acidic site (Portal, pH 2.9) (Tukey comparison was not significant, $P = 0.4$). Leaves from an acidic, high metal hydroxide site (Garvey, pH 4.0), had significantly lower consumption than the neutral site (Tukey comparison $P = 0.006$) and the highly acidic site (Tukey comparison $P = 0.046$). Leaves from Garvey Creek had significant metal hydroxide coatings after colonization in the stream.

Laboratory experiments revealed a differential effect of pH on the microbes from reference and highly acidic streams. The microbial respiration rate on leaves from a reference site (Lankey, pH 7.4) declined several fold when incubated in stream water from the same stream with artificially lowered pH (Fig. 5, ANOVA $F = 13.6$, $df = 5, 18$, $P < 0.001$). Respiration rate was also lower when leaves were incubated in water from a highly acidic stream (Portal, pH

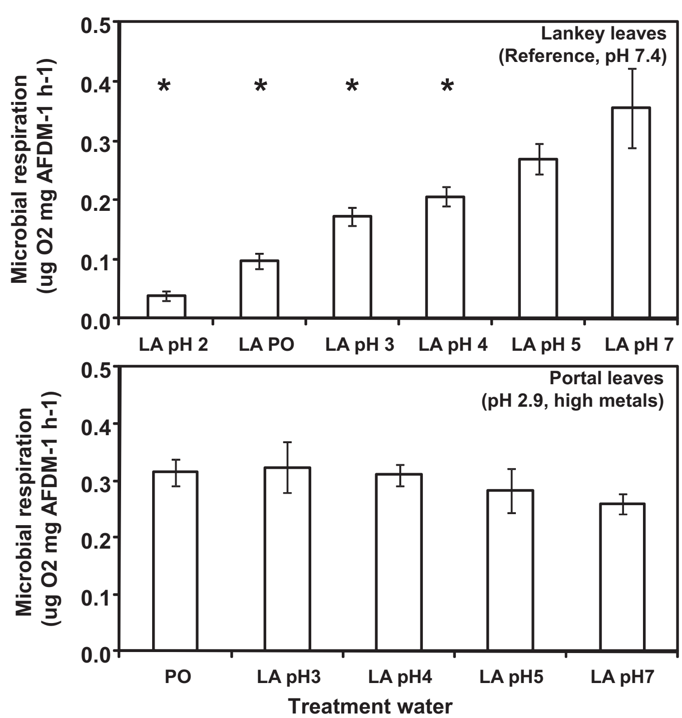

Fig. 5. Microbial respiration rate on leaves from Lankey Creek (top panel) and Portal Stream (bottom panel) after incubation in different treatment waters. LA is Lankey Creek water, pH indicates treatment pH after acidification. PO is Portal Stream water. *Significant difference ($P < 0.05$) from control treatment (LA pH 7) by Tukey comparison after ANOVA.

2.9). In contrast, leaves from this highly acidic site had a more consistent respiration rate across pH, with a slight but not significant decline at high pH (ANOVA $F = 0.73$, $df = 4, 15$, $P = 0.58$).

3.4. Wood breakdown

Wood breakdown rates varied from 0.00011 to 0.00098 d^{-1} for beech, and 0.00011 to 0.00062 d^{-1} for birch. Breakdown rates of beech and birch wood were closely related to each other ($R = 0.89$, $P < 0.001$), and had very similar responses to abiotic and biotic characteristics at the sites. Much like leaves, wood breakdown declined with increasing metal hydroxide deposition on the streambed (Fig. 2B and C). Other abiotic variables did not account for significant variation in breakdown rates of both types of wood after considering the effects of metal hydroxides.

Microbial respiration rates accounted for most of the variation in breakdown rates for both types of wood across all sites. Respiration accounted for 63% ($P < 0.001$) of the variation in beech wood breakdown rates, and 71% ($P < 0.001$) of the variation in birch wood breakdown rates. As with breakdown, microbial respiration on both types of wood was negatively related to metal hydroxides ($R^2 = 0.34$, $P = 0.013$ for beech; $R^2 = 0.45$, $P = 0.003$ for birch).

3.5. Cotton tensile strength

Cotton strips were placed at all 18 stream sites for 1 week prior to tensile strength measurement. Decay of tensile strength was positively related to red beech leaf breakdown rates ($R = 0.56$, $P = 0.016$). However, unlike in experiments with leaves and wood, abiotic variables were not strongly related to tensile strength, with only SRP concentrations showing a weak relationship ($R = 0.40$, $P = 0.09$).

3.6. Macroinvertebrate community index

The MCI, a measure of macroinvertebrate stream health, varied from 60 (indicating poor health) to 145 (indicating excellent health). The MCI was related to pH (Fig. 1B), but the relationship was weaker and different than the pattern for leaf breakdown rate (Fig. 1A) as pH only explained 25% of the variation in MCI among sites. Some highly acidic sites had the lowest MCI scores (<80), but other acidic sites had high scores (>100). MCI scores were not significantly related to breakdown rates of leaves or wood ($P > 0.10$).

4. Discussion

4.1. Leaf breakdown

Leaf breakdown in streams has been proposed as a measure of ecosystem health (Gessner and Chauvet, 2002; Young et al., 2008), and can either decrease if affected by stressors such as acidity or increase if affected by subsidies such as nutrients. As expected, we found that the breakdown rates of red beech leaves were usually lower at sites affected by mine drainage than unimpacted references sites. Along a gradient of leaf types, red beech leaves have intermediate breakdown rates (Linklater, 1995; Parkyn and Winterbourn, 1997), which ranged from 0.0065 to 0.0219 d^{-1} at our reference sites. In our sites affected by mining, red beech breakdown rates ranged from 0.0019 to 0.0088 d^{-1}, showing some overlap between stressed and reference sites.

The strongest effect of AMD on leaf breakdown was from metal hydroxide deposition, which was highest at sites with moderately low pH (4–5). At the highly acidic sites, metal hydroxides often remain dissolved in solution, and do not pose a physical stress on biota through smothering of habitat, food resources, or respiratory structures of biota. Other studies have noted the negative effects of metal hydroxides on leaf breakdown (Gray and Ward, 1983; Niyogi et al., 2001; Schlief and Mutz, 2005; Ehrman et al., 2008). We did not find a significant effect of dissolved metals, including Al and Zn, after accounting for the effects of metal hydroxide deposition. Dissolved metals can influence breakdown and associated biotic communities in some streams (Niyogi et al., 2001; Carlisle and Clements, 2005; Baudoin et al., 2008; Hogsden and Harding, 2012) and experimental microcosms (Duarte et al., 2004, 2008), but their effects here were less significant than metal hydroxides.

The relatively high breakdown rates at our highly acidic sites may also be influenced by high nutrient concentrations. Thus, the stresses from acidity and dissolved metals in mine drainage were associated with subsidies from nutrients, and the net effect on breakdown rates could be neutral at the most acidic, most nutrient-rich sites. High nutrients in mine drainage can occur if the exposed ore tailings have P minerals in addition to metals. High N in mine drainage can originate from mining chemicals or geologic sources of N (e.g., Morford et al., 2011). Precipitation of P with metal hydroxides (Tate et al., 1995) may induce microbial P limitation and slow litter breakdown rates (Simon et al., 2009), but this should not occur at very low pH where metals remain in solution. Indeed, in the most acidic sites (pH < 3), SRP concentrations were high.

4.2. Biotic communities

For sites affected by mine drainage, the associated stressors (pH, dissolved metals, deposited metals) can affect shredding invertebrates, microbial communities, or both (Niyogi et al., 2001; Carlisle and Clements, 2005). Although shredding invertebrates have been found at mining-impacted sites in our study (Harding, unpublished data) and in our kicknet samples, they were common in the litterbags at only two reference sites. The paucity of shredders (both shredder taxa and densities) in New Zealand is well documented (Winterbourn et al., 1981), although they can be locally abundant. In West Coast streams they are rarely common, which may in part be due to the flashy hydrographs in these systems.

Laboratory experiments with the two presumed shredder taxa in our study revealed both their potential importance to leaf breakdown and their susceptibility to AMD effects. Red beech leaves were readily consumed by both *Austroperla* and *Olinga* in the trials. *Austroperla* is moderately tolerant to acidic conditions and elevated metal concentrations, has been found at acidic sites in low numbers, and is widespread throughout the West Coast region (Harding, unpublished data). Thus, it could play a role in leaf breakdown in some streams affected by mine drainage.

The differential effects of AMD stressors on leaf consumption were seen during the shredding trial when leaves from three sites were differentially consumed by caddisflies. Leaves incubated in Garvey Creek, which had a pH of 4 and high mass of metal hydroxides on the streambed, were consumed at a significantly lower rate than leaves from both the circum-neutral site (Lankey Creek) and a highly acidic stream (Portal Stream). Microbial respiration was high at both of these sites, and this activity probably led to leaf conditioning that allowed consumption by the shredding caddisfly. Dangles and Chauvet (2003) found that low pH did not have a large effect on fungal biomass or the palatability of leaves to the shredding amphipod *Gammarus fossarum*, but Gonçalves et al. (2011) found differences in leaf palatability to shredders between uranium-mine and reference streams. The effects of mining on leaf palatability are determined by effects on microbial activity and conditioning, with results varying depending on the predominant stressor.

The lack of a strong shredder influence and similar abiotic effects of physical abrasion among sites on litter breakdown in our streams suggests this ecosystem function was largely driven by microbial processes. Microbial activity was restricted by the deposition of metal hydroxides on the leaves, as others have found in similar cases (Schlief, 2004; Schlief and Mutz, 2005). Surprisingly, microbial activity was not significantly related to pH or dissolved metals at our sites after considering the effects of metal hydroxides. Other studies on aquatic fungi and dissolved metals have reported conflicting results, with some finding negative effects (Duarte et al., 2004) and some indicating tolerance (Miersch et al., 1997; Gonçalves et al., 2011). Microbial activity was also related to P concentrations at our sites, and nutrients commonly increase microbial activity on organic matter (Suberkropp and Chauvet, 1995; Gulis and Suberkropp, 2003). As stated above, our most acidic sites also had high concentrations of P and N, and subsidies of nutrients may have counteracted stress from low pH or dissolved metals.

Microbial activity on leaves from two sites was tested in a variety of waters to examine the influence of acidity. Leaves from a circum-neutral stream had highest activity at neutral pH, while leaves from a stream with very low pH had microbes that were active across a range of pH from 2.9 to 7. Not surprisingly, the microbial communities at the two streams were adapted to their native stream acidity. However, the community from the circum-neutral stream had limited tolerance to acidic conditions. Molecular analyses of the fungal communities have revealed large differences between the sites (Niyogi, unpublished data), so different taxa at the sites likely have different sensitivities to pH.

4.3. Wood and cotton as indicators

Wood breakdown is generally slower than leaves, in part because few shredders consume it. Thus, wood breakdown should be largely driven by microbial processes. Stress from metal hydroxides appeared to impair microbial activity and the rate of wood breakdown, whereas low pH and dissolved metals were less significant. Tank and Winterbourn (1995) compared microbial activities on wood from two brown-water acidic (as low as pH 3.7) and two neutral streams in our study area. They reported little evidence for impairment of microbial activity from low pH in their streams, supporting our finding that microbial activity can remain high even in highly acidic streams.

Cotton strips have been proposed as a standard type of organic matter that can be used for functional assessment of streams over relatively short incubation periods (Boulton and Quinn, 2000; Clapcott et al., 2010). The decay of cotton tensile strength over the 1-week period was similar to the leaf breakdown rates at the same streams. However, it was not significantly related to any of the three stressors from mine drainage, suggesting that it has limited utility in defining the mechanisms that suppress litter breakdown. However, the lack of effect of metal hydroxides on the cotton decay may be related to the short time period of incubation. After only 1 week in the streams, there was little evidence of metal hydroxides coating the cotton strips, whereas leaves at some sites would have visible accumulation of hydroxides after a month or more.

Of the three types of organic matter (leaves, wood, and cotton), we suggest that breakdown of leaves is the most integrative measure of stream functional health, given that it incorporates the role of animal shredders. Wood breakdown over long incubation times may be useful in providing a measure that integrates varying water quality based on hydrologic events. Although cotton decay was not closely related to AMD stressors in our study, it was related to leaf breakdown and has the advantage of being a simple, short-term measure of functional health.

4.4. Implications for stream health and remediation

Many monitoring agencies use macroinvertebrate measures of stream health, and the MCI index is commonly used in New Zealand. The MCI was positively related to pH, but there was high variability in MCI scores under stress from acidity (Fig. 1B). The lack of sensitivity of the MCI to stress from acid mine drainage is because the taxa scores are largely based on tolerance to organic pollution. Some invertebrates found in streams affected by AMD, including Plecoptera and Trichoptera taxa, have high MCI values but are tolerant of low pH and dissolved metals (Hickey and Clements, 1998), and this can lead to high MCI scores at impacted sites. In the case of AMD, breakdown of organic matter, a functional indicator, can be more sensitive than structural measures such as the MCI, especially when metal hydroxides are present and impacting microbial communities involved in breakdown.

Organic matter breakdown has been proposed as an alternative measure of stream health, given its ease of measurement, integration of roles from varying biota, and importance to the food webs of streams (Gessner and Chauvet, 2002; Young et al., 2008). We endorse this approach and believe that breakdown of leaves and wood served as an important measure of ecosystem functioning and food web support in our study streams affected by mine drainage. A key argument for using such a functional indicator is that it incorporates the effects of stressors on multiple biotic communities, including shredders and microbes for the case of litter breakdown. Furthermore, given that energy pathways in streams rely on microbial or algal production, animal communities are dependent on ecosystem functions such as litter breakdown and primary production, and these functions can respond differently from animal metrics.

Most of our highly acidic sites had an active microbial community that could tolerate the low pH and elevated dissolved metals. However, as pH increases either downstream or with remediation of mine drainage, stream functioning may decline as pH increases to the 4–5 range where metal hydroxides can be the main stressor for breakdown or other measures of health, including primary production (Niyogi et al., 2002a) and macroinvertebrates (McKnight and Feder, 1984). This nonlinear pattern of stream health response to pH in cases of mine drainage (as seen for litter breakdown in Fig. 1A) is important to consider in stream rehabilitation. Streams may undergo a decline in health in space or time going from highly acidic conditions where microbes persist to moderately acidic conditions where metal hydroxides limit their function.

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