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Stream Macroinvertebrate Community Affected by Point-Source Metal Pollution

key words: acidic stream, community structure, Ephemeroptera, metal pollution, mountain stream, Plecoptera

Abstract

The impacts of mining activities on aquatic biota have been documented in many stream ecosystems. In mining streams, point-source heavy metal pollution often appears in the stream. We hypothesize that this pollution is toxic to macroinvertebrates owing to high concentrations of metals and therefore affects macroinvertebrate community structure. We investigated macroinvertebrate community structure in mountain streams, including heavy metal-polluted sites and neutral-pH streams, to determine the relationship between community structure and environmental factors such as low pH and heavy metal concentrations. Based on multidimensional scaling ordination, the macroinvertebrate community at heavy metal pollution sites was remarkably different from that at the other sites. Inductively coupled plasma mass spectrometry revealed high concentrations of aluminum and iron in surface water at the polluted sites. Macroinvertebrate community structure at the metal pollution sites was significantly different from that at other sites in the same stream and in neutral-pH streams. Thus, point-source metal pollution may reduce the density and diversity of *in situ* macroinvertebrates.

1. Introduction

The impacts of mining activities on water quality and aquatic biota have been documented in a number of river systems, and the relationship between metal pollution and aquatic communities in streams is well established (Clements *et al.,* 2000; Courtney and Clements, 2002). A number of studies have documented the tolerance of aquatic macroinvertebrate communities to low pH and high concentrations of heavy metals (e.g., Townsend *et al.*, 1983; Bukaveckas, 1993; Clements and Kiffney, 1995; Clements *et al.*, 2000; Courtney and Clements, 2000; Guerold *et al.*, 2000), and several have reported a significant relationship between pH and macroinvertebrate community composition (e.g., Townsend *et al.*, 1983; Clenaghan *et al.*, 1998; Courtney and Clements, 2000). In addition, Hirst *et al.*, (2002) concluded that metal pollution most clearly influenced macroinvertebrate community structure through changes in diversity.

Most studies examining the relationship between the macroinvertebrate communities and the pH and heavy metal pollution have been conducted at one site in streams ranging from acidic to neutral pH (e.g., Clements *et al.*, 1989; Courtney and Clements, 2000; Guerold

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et al., 2000). However, some acidic mining streams contain "point-source metal pollution sites" with high concentrations of heavy metals in the stream water. Areas of high metal concentration are observed in streambeds near mining areas owing to stream erosion. Point-source metal pollution is predicted to affect *in situ* macroinvertebrate community structure, although this relationship has not been studied. To fully understand the effects of mining activities on water quality and aquatic biota in stream ecosystems, the effects of point-source metal pollution must be determined. Case studies at point-source metal pollution sites are appropriate for estimating these effects.

In the Honzawa, a mining stream in Japan, metal pollution from abandoned lignite pits, including heavy metals and organic matter, was observed in the stream (Doi *et al.*, 2005). We hypothesised that this point-source metal pollution is toxic to macroinvertebrates as a result of high metal concentrations and therefore affects macroinvertebrate community structure. In this study, we investigated macroinvertebrate community structures in streambeds, including at point-source metal pollution sites and in neutral-pH streams. We determined the relationship between the pH and metal concentration and the macroinvertebrates in the streams. We also compared macroinvertebrate community structure in the mining stream with that in low- and neutral-pH streams.

2. Methods

2.1. Study Sites

The study sites were located on a small mountain (Aoba-yama) near Sendai, in northeast Honshu, Japan (38°15′ N, 140°51′ E), at five first-order headwater streams, the Honzawa Stream (low pH, 5.3–5.7; includes point-source metal pollution sites), Sankyozawa Stream (neutral pH, 6.6), and three unnamed steams (neutral pH, 6.6–6.8; include iron-rich sites). Eight research stations were established: four at the Honzawa (Stations A1–A4), one at the Sankyozawa (Station D), and one at each of the unnamed steams (Stations B, C, and E; Fig. 1). The distance between Stations A1 and A4 was about 0.8 km. In the Honzawa, metal precipitation from abandoned lignite pits, including heavy metals and organic matter, was observed in the streambed, approximately 5 m upstream from Station A3. Point-source metal pollution, including iron oxide, was also observed in the streambed, approximately 5 m upstream from Station D. The substrates of these streams were dominated by gravel/sand, and the majority of the stream habitats were riffles and runs.

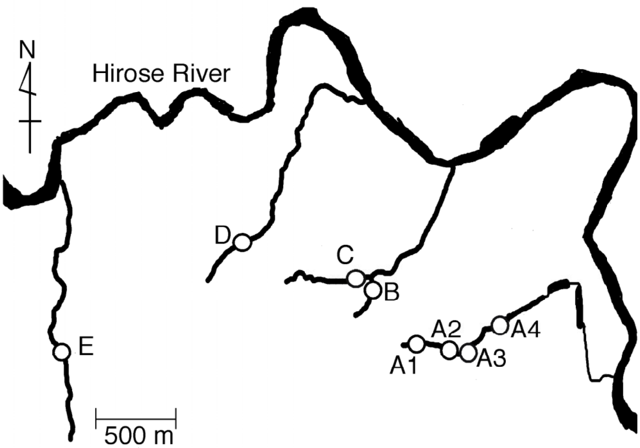

Figure 1. Map showing the sampling sites.

2.2. Collection of Macroinvertebrates

Benthic macroinvertebrates were collected at the eight stations on 18–24 December 2002. Four replicate samples were taken with a 25 · 25 cm^2 Surber sampler (250 μm mesh), respectively. We randomly selected the four Surber sampling points at each site, in areas with stream width greater than 25 cm. Streambed surface samples were collected at depths of 0 to 5 cm. The macroinvertebrates were preserved in 5% formalin and separated from other material. Invertebrates were identified to the lowest feasible classification level (most to genus) under a binocular stereoscopic microscope.

2.3. Analysis of Environmental Factors

Water temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO) of streambed surface waters were measured in triplicate at the sites, using a multiple water quality sensor (U-22, Horiba Co.). Current velocity close to the streambed was measured in triplicate with a digital current meter (UC-3, Tamaya Co.). Stream width and water depth were measured in triplicate at each site. For chlorophyll *a* measurements, epiphyton samples were collected in triplicate by brushing a 100 cm^2 surface area of randomly selected stones. Chlorophyll *a* was extracted from the samples using N,N-dimethylformamide, filtered through GF/F glass filters, and measured with a fluorometer (10-AU, Turner Designs Co.). Samples of fine particulate organic matter (FPOM, 250 to 500 μm) were collected in triplicate from the streambed, by sieving; the samples were dried at 60 °C and weighed.

The concentrations of heavy metals in the streambed surface waters were measured using an inductively coupled plasma mass spectrometer (ICP-MS; ELAN 7000, PerkinElmer Co.). The blank used for the heavy metal measurements was based on sulfide instead of the standard nitrate because of the high concentration of sulfate in the stream water.

2.4. Statistical Analyses

Macroinvertebrate density was compared among sites using multiple comparison Holm tests with R-project 2.0.1 software ($\alpha = 0.05$). To compare community structure among sites, we used non-metric multidimensional scaling (MDS) ordination of the Bray-Curtis similarity using PC-ORD version 4.0 for Windows (MCCUNE and MEFFORD, 1999); log-transformed macroinvertebrate densities were used for the Bray-Curtis similarity.

3. Results

3.1. Environmental Factors

The environmental factors recorded at the sampling stations are shown in Table 1. Stations A1–A4 were low-pH sites (5.3–5.7) owing to pollution from abandoned lignite pits around Honzawa Stream (Table 1). In contrast, Stations B–E had a neutral pH (6.6–6.8) that was significantly higher than the pH at Stations A1–A4 (multiple comparison Holm test, $P < 0.01$, $n = 3$). EC was significantly lower at Stations B–E (25.6–30.8 mS m^{-1}) than at Stations A1–A4 (33.0–37.4 mS m^{-1}; Holm test, $P < 0.01$, $n = 3$). Stations A3, A4, and D had high concentrations of aluminium, manganese, and iron (Table 1, DOI *et al.* 2005).

The Mn concentrations at Stations A3, A4, and D were much higher than the criterion value of manganese (Mn) for aquatic organisms, 50 μg L^{-1} (EPA 1986). The criterion values of aluminum (Al) and iron (Fe) for aquatic organisms are 150 and 1000 μg L^{-1}, respectively (EPA 1986); thus the Al concentration at Station A3 was higher than the criterion value. Copper (Cu) and zinc (Zn) were present at low concentrations, below their respective criterion values for aquatic organisms, 50 and 12 μg L^{-1} (EPA 1986; Table 1). The cadmium concentrations were below 0.1 μg L^{-1} at all stations and were not measureable by ICP-MS.

Table 1. Stream water quality (mean and 1 SD in parentheses, $n = 3$) of the sampling sites. EC and DO indicate electrical conductivity and dissolved oxygen. Chl-*a* indicates chlorophyll *a*. FPOM indicates fine particulate organic matter.

Characteristics	St. A1	St. A2	St. A3	St. A4	St. B	St. C	St. D	St. E
Width (cm)	23 (2.9)	37 (7.6)	38 (5.8)	40 (5.0)	35 (5.0)	42 (11)	38 (7.6)	58 (18)
Water depth (cm)	4.0 (2.0)	6.0 (1.0)	3.7 (0.6)	6.3 (2.5)	9.0 (0.0)	7.3 (0.6)	5.0 (1.0)	6.3 (2.5)
Velocity (cm s^{-1})	46 (0.4)	37 (0.2)	23 (0.6)	30 (0.0)	22 (0.1)	12 (0.1)	28 (0.0)	32 (0.2)
PH	5.7 (0.0)	5.3 (0.1)	5.4 (0.0)	5.5 (0.1)	6.8 (0.0)	6.6 (0.1)	6.6 (0.0)	6.6 (0.0)
EC (mS m^{-1})	36.9 (0.1)	37.4 (0.3)	36.6 (0.3)	33.0 (0.0)	25.6 (0.1)	30.8 (0.0)	30.3 (0.5)	26.0 (0.1)
DO (mg L^{-1})	5.7 (0.0)	5.5 (0.1)	5.3 (0.1)	5.3 (0.0)	6.7 (0.2)	6.7 (0.3)	7.1 (0.0)	7.0 (0.2)
Temperature (°C)	4.0 (0.0)	4.7 (0.0)	5.0 (0.0)	4.8 (0.0)	3.8 (0.0)	6.3 (0.1)	6.5 (0.0)	5.0 (0.0)
Chl-*a* (μg cm^{-2})	871 (51)	515 (236)	737 (279)	660 (53)	848 (156)	998 (154)	483 (151)	262 (202)
FPOM (mg cm^{-2})	1.1 (0.1)	1.3 (0.5)	0.4 (0.2)	0.7 (0.5)	2.3 (1.1)	2.0 (0.9)	2.0 (0.9)	3.0 (0.9)
Al (μg $L^{-1)}$)	12.1 (0.2)	15.6 (0.3)	151 (26)	1.6 (0.2)	0.6 (0.7)	1.4 (0.4)	0.9 (0.1)	3.2 (0.5)
Mn (μg L^{-1})	18.2 (2.7)	10.2 (7.0)	101 (13)	67.1 (4.7)	3.9 (4.2)	2.8 (3.0)	73.6 (3.1)	6.6 (6.7)
Fe (μg L^{-1})	15.1 (0.5)	3.9 (6.1)	76.8 (7.5)	26.8 (3.2)	8.9 (2.5)	12.5 (0.5)	27.5 (1.4)	13.8 (1.0)
Cu (μg L^{-1})	0.9 (0.1)	0.8 (0.8)	0.8 (0.7)	0.5 (0.0)	0.5 (0.6)	0.7 (0.0)	0.8 (0.0)	1.6 (0.1)
Zn (μg L^{-1})	12.4 (0.8)	2.3 (0.9)	10.0 (6.7)	6.0 (0.3)	2.1 (1.5)	4.0 (0.1)	3.7 (0.4)	1.9 (0.3)
Cd (μg L^{-1})	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

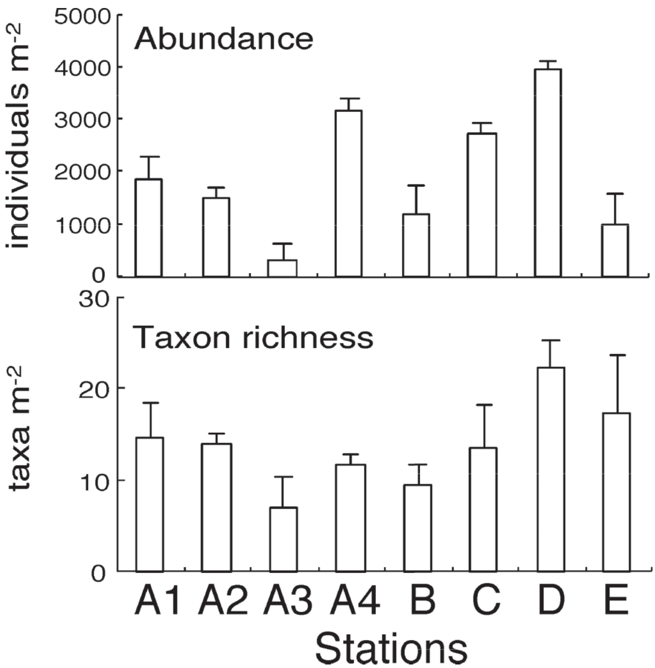

Figure 2. Overall density and taxon richness at each sampling site (mean ± 1 SD, $n = 4$).

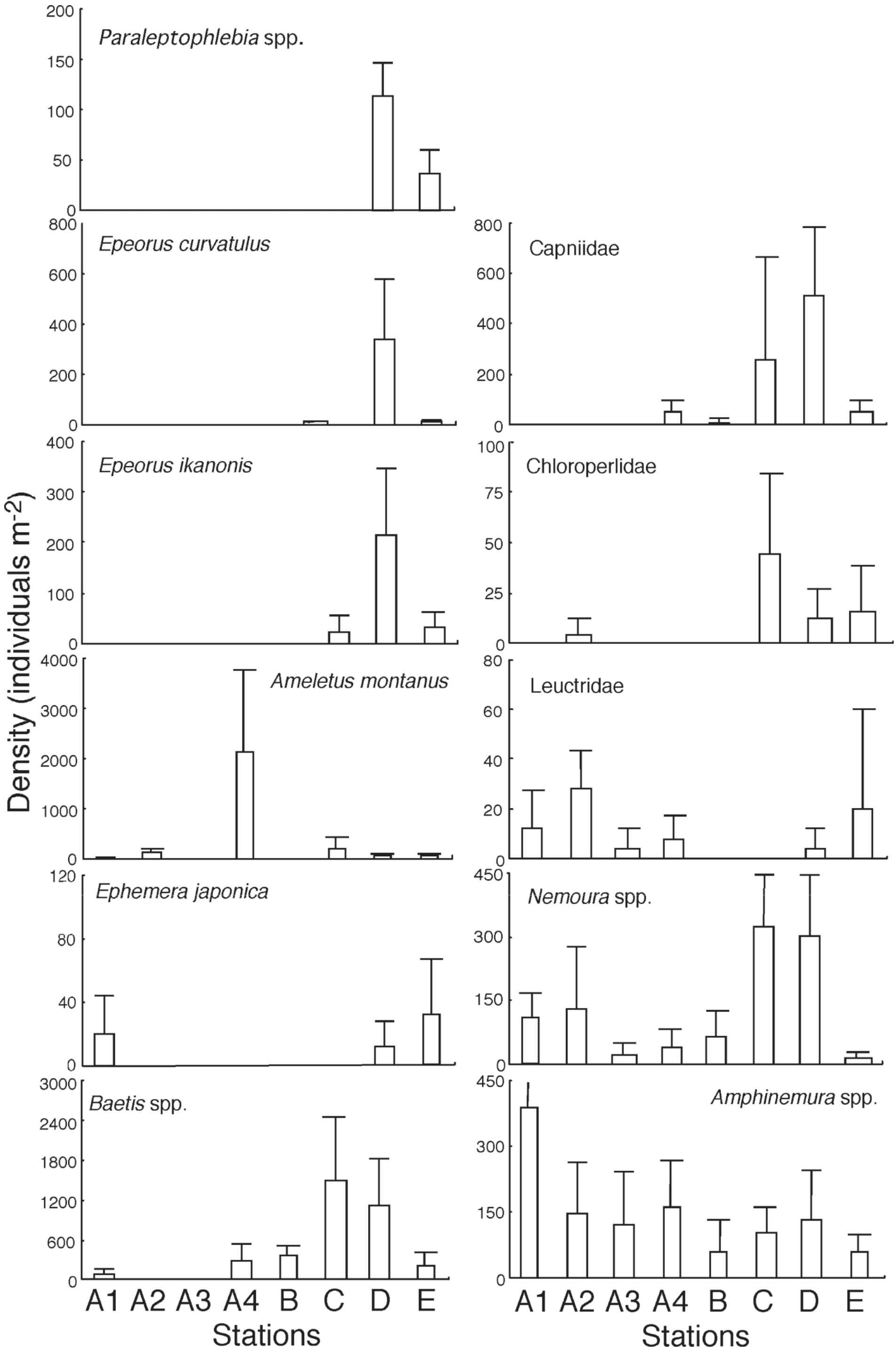

Figure 3. Density of Ephemeroptera and Plecoptera at each sampling site (mean ± 1 SD, $n = 4$).

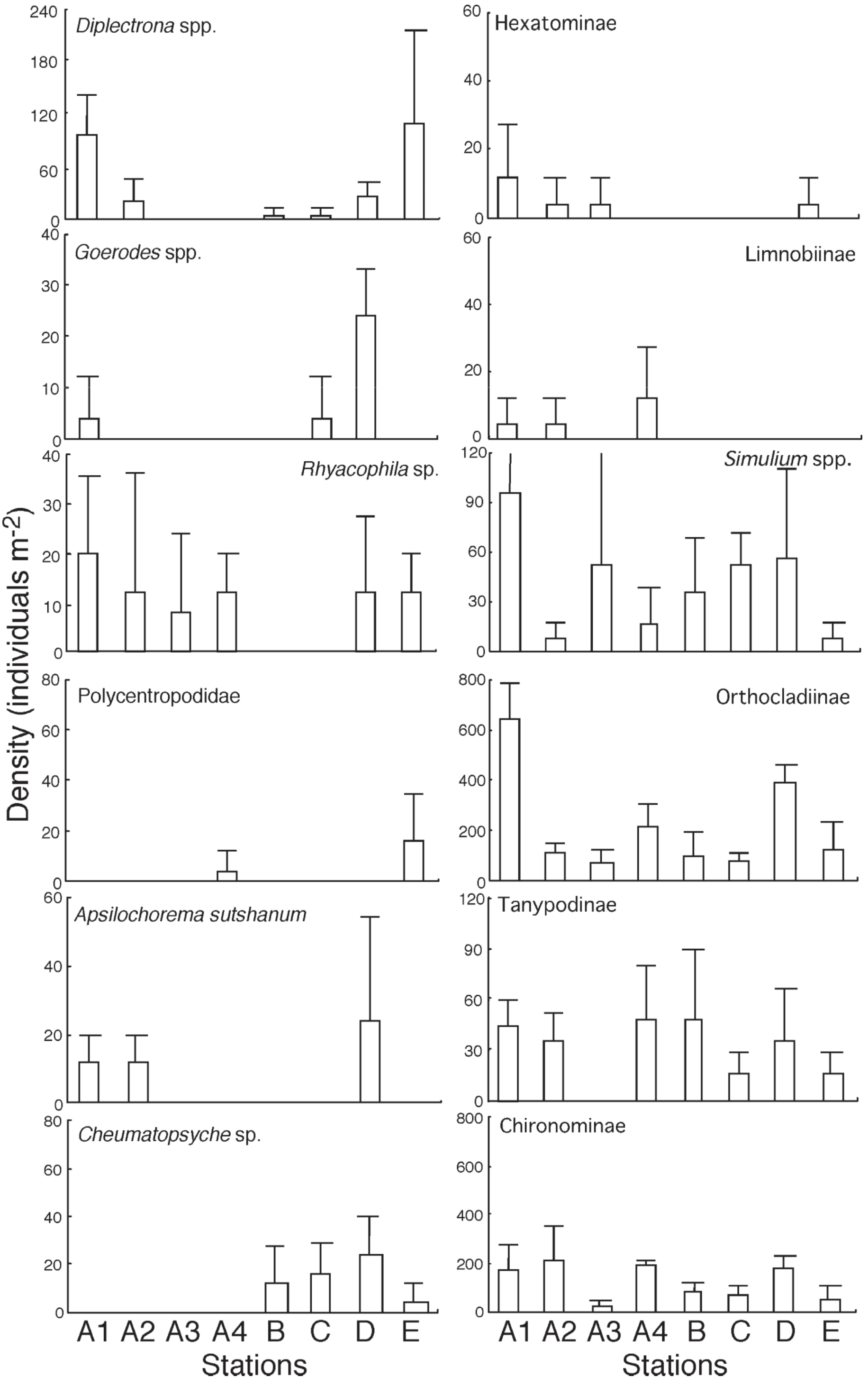

Figure 4. Density of Trichoptera and Diptera at each sampling site (mean ± 1 SD, $n = 4$).

3.2. Abundance and Taxon Richness of Macroinvertebrates

The abundance of macroinvertebrates ranged from 348 to 3972 individuals m^{-2} (Fig. 2). Macroinvertebrate abundance at Station A3 (point-source metal pollution site) was significantly lower than that at the other sites (Holm test, $P < 0.01$, $n = 4$). Abundance at Stations A1–A3 was significantly lower than that at Station A4, and abundance at Stations A1–A4 was significantly lower than that at Stations C and E (Holm test, $P < 0.05$, $n = 4$). The taxon richness at Station A3 was significantly lower than that at the other sites (Holm test, $P < 0.01$, $n = 4$), and taxon richness at Stations D and E was significantly higher than that at the other sites (Holm test, $P < 0.05$, $n = 4$).

3.3. The Distribution of Macroinvertebrate Species

Epeorus curvatulus, *E. ikanonis*, and *Paraleptophebia* spp. (Ephemeroptera) were not found at Stations A1–A4 in the low-pH stream, but *E. curvatulus* and *E. ikanonis* were most abundant at Station D (Holm test, $P < 0.05$, $n = 4$, Fig. 3). The Plecoptera families Capniidae and Chloroperlidae (Plecoptera) were not found at Stations A3 and A4, respectively (Fig. 3). *Amphinemura* spp. and *Nemoura* spp. inhabited all of the sampling sites. Trichoptera, except for *Rhyacophila*, were rarely found at Station A3. *Cheumatopsyche* was found at Stations B–E (neutral-pH streams) but not at the low-pH sites at Stations A1–A4. *Diplectrona* spp. and *Apsilochorema sutshanum* were dominant at the low-pH stream, but had lower densities at Stations A3 and A4 than at Stations A1 and A2 (Holm test, $P < 0.05$, $n = 4$, Fig. 4). Hexatominae and Limoniidae (Diptera) were restricted, for the most part, to Stations A1–A4 in the low-pH stream. Members of the dipteran family Chironomidae were found at all sites.

3.4. MDS Results

MDS ordination revealed community-level differences over time among the sites (Fig. 5). MDS stress was 0.10, indicating that the MDS ordination was significant. The macroinvertebrate communities at Stations A3 and A4 were largely different from the communities at

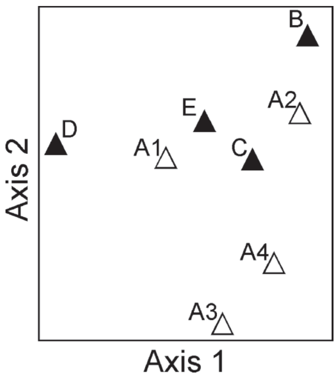

Figure 5. Non-metric multidimensional scaling (MDS) ordination of Bray-Curtis similarity of log-transformed macroinvertebrate densities. The symbol and labels indicate each site. MDS stress is 0.10.

the other stations (Fig. 5). Moreover, the community at Station D was remarkably different from the communities at the other stations. The communities at Stations A1 and A2 (acidic sites) were similar to the communities at Stations C and E (neutral sites; Fig. 5).

4. Discussion

Our results suggest that the macroinvertebrate community structure at point-source metal pollution sites is significantly different from that at other sites in the same stream and in other neutral-pH streams. Thus, point-source metal pollution may reduce the density and diversity of *in situ* macroinvertebrate taxa.

The point-source metal pollution sites have high concentrations of heavy metals such as Mn, Fe, and Al, and the macroinvertebrate community at these sites differs from that upstream of the sites, although the distance between Stations A2 and A3 is only 10 m. Thus our study shows that, in small streams, point-source metal pollution is a major cause of the loss of macroinvertebrate diversity. Owing to proton excess, the occurrence of toxic Fe and Al, combined with a severe calcium deficiency, makes acidic waters toxic for most aquatic organisms (GUEROLD *et al*., 2000). We found large numbers of macroinvertebrates upstream of the metal pollution sites. As a result, point-source acidification might lead to stream ecosystems with altered structure and function.

As a result of low pH and relatively high heavy metal concentrations, many Ephemeroptera were absent from the point-source metal pollution sites, and Trichoptera species, expect for *Rhyacophila*, were at low abundance at Station A3. Thus, Ephemeroptera and Trichoptera were most strongly affected by metal pollution. Our results are similar to previous findings (TOWNSEND *et al*., 1983; ALLAN, 1995; GUEROLD *et al*., 2000, HIRST *et al*., 2002) and suggest that a pH slightly below neutral has detrimental effects on some species of Ephemenoptera and Tricoptera in mountain streams. Among the Plecoptera, *Nemoura* spp. and *Amphinemura* spp. were found at all sites, and their abundance was not strongly correlated with pH or EC. The abundance of these plecopteran shredders was not positively correlated with pH (TOWNSEND *et al*., 1983), and they reached rather high densities at low pH sites (GUEROLD *et al.,* 2000). Thus, the shredder plecopterans, including *Nemoura* spp., *Amphinemura* spp., and Leuctridae, may have a greater tolerance for low pH. In contrast, the shredder family Capniidae tended to be less dense at low-pH sites, especially at Station A3, and their abundance was strongly correlated with pH because of the additional effects of higher heavy metal concentrations (Table 1). Thus, at Station A3, the concentrations of heavy metals, especially aluminum, in addition to low pH might be detrimental to many species of macroinvertebrates, including Ephemeroptera, Plecoptera, and Tricoptera species.

Our results indicate that the macroinvertebrate community structure is affected by point-source metal pollution. Most relationships between the macroinvertebrate community structure and the pH and heavy metal concentration have been documented in streams that are polluted along their entire length. However, some mining and neutral-pH streams have point-source metal pollution, as in the present study site. Thus, metal pollution should be considered at the microhabitat level, in addition to the whole stream level, for macroinvertebrate communities affected by point-source metal pollution.

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