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Response of macroinvertebrates to calcium addition in the Adirondack Mountains, USA

Abigail Cahill '07

Biology

Acid deposition is a well-studied phenomenon in the lakes and streams of the northeastern United States and in northern Europe, but the effect of acid deposition on terrestrial communities is less well-known. I examined the effects of large-scale calcium addition on the communities of macroinvertebrates present in the litter layer of maple-beech forests in the Adirondacks, which are strongly affected by acidic deposition due to poor buffering capacities in the soils. Lime was added to five treatment plots over the course of a year. Untreated deciduous and coniferous forests in Madison County, which has a well-buffered soil, were used as reference sites, and untreated plots in the coniferous Earlville State Forest were used as outgroups. Invertebrates were sampled in both Madison County and the Adirondacks. Discriminant analysis showed differences in calcium-rich invertebrate groups (Diplopoda, Arionidae, and Isopoda) between the three regions. All were more abundant in the calcium-rich Madison County sites, indicating that the need for calcium to form external structures (millipedes and isopods) or eggs (slugs) may be a factor in their distribution. One year after liming, invertebrate communities did not measurably differ between limed and control plots in the Adirondacks. Physical factors of the litter (pH, percent organic matter, elemental composition, etc.) were measured to correlate invertebrate communities to their microhabitats.

Acid deposition is a well-studied disturbance of lakes and streams of the northeastern United States and in northern Europe (Stoddard et al. 1999; Tipping et al. 2002). However, the effects of decreased pH on the terrestrial fauna are less well-known (Bouwman et al. 2002). Earthworms are known to be particularly vulnerable, in part due to the increased toxicity of aluminum at low pH levels, resulting in lower growth rates and less cocoon formation (van Gestel and Hoogerwerf 2001). Snails and millipedes have been shown to increase in size and density when exposed to calcareous dust at roadsides (Kalisz and Powell 2003) or calcium addition on the forest floor (Johannessen and Solhoy 2000). The decline of snail shells and other macroinvertebrate calcium sources in forests affected by acid deposition has been shown to cause a decline in the reproductive success of birds (Graveland et al. 1994; Ramsay and Houston 1999). However, there has been very little work to date examining whole-community responses of forest-floor macroinvertebrates to acid deposition.

Macroinvertebrates are an integral part of a forest ecosystem, in particular as part of the forest-floor community, but they also affect above-ground trophic levels and interactions (Miyashita et al. 2003). In British

Columbia, Cárcamo et al. (2000) showed that a large millipede species can consume approximately 36% of the annual litter fall in deciduous forests. This has drastic implications for the importance of invertebrates in processes like leaf fragmentation and nutrient leaching, both of which have subsequent impacts on the biota of the forest. Invertebrate fecal production is important for moving elements like magnesium and calcium through the forest-floor ecosystem, and is crucial for making nitrates more available to other organisms (Teuben and Verhoef 1992, Dangerfield 1994, Cárcamo et al. 2000).

Litter invertebrates are frequently used as ecological indicators in determining the health of a system (Geissen and Kampichler 2004; Cassagne et al. 2005; Langor and Spence 2006). This is due in part to the large sizes of their populations, their immovability (preventing them from escaping the effects of disturbance), and the ease and cost-effectiveness with which they are sampled (Hilty and Merenlender 2000; Langor and Spence 2006). Many taxa of invertebrates are well-studied and ubiquitous (e.g. carabid beetles), thus providing a background from which to determine ecosystem health (Langor and Spence 2006). Collembolans also are increasingly used as bioindicators because they are wide-ranging as a group, but vary on a local scale (Cassagne et al. 2005). As decomposers, they represent an important participant in the cycling of both organic matter and nutrients (Geissen and Kampichler 2004; Cassagne et al. 2005; Partsch et al. 2006). Dipteran larvae are another ubiquitous, well-studied group. Although larvae usually are not affected by acidification, Frouz (1999) discovered that fewer adult flies emerge in acidified soils, indicating that acidification was affecting life processes of these larvae.

Invertebrate distribution in terrestrial systems is correlated with factors in the soils that create microhabitats, including moisture and temperature (Krivtsov et al. 2006), or distribution of vegetation (Ettema and Wardle 2002). In turn, the services provided to the ecosystem by the invertebrates, such as decomposition and nutrient recycling, can influence the distribution of vegetation in these systems (Ettema and Wardle 2002). Krivtsov et al. (2006) found moisture to be the most important factor in determining distribution of micro and mesoinvertebrates in a Scottish forest. However, nutrient availability also is important to invertebrates. Collembolans and orbatid mites have been shown to be more abundant in soils with a greater proportion of organic matter (Hasegawa 2001), and isopod distribution varies across a forest relative to total litter mass (Gongalsky et al. 2005). An important patch resource in forests is dead logs; invertebrates have been found to be more dense closer to these resources, which also correlate with a high C:N ratios (Evans et al. 2003).

The current study took place as part of a four-year, ongoing study in the Adirondack Mountains of New York State that aims to measure the effects of calcium addition on soil chemistry and biota in an area heavily affected by acid deposition. This study compared macroinvertebrate communities in the Adirondacks to forest-floor communities in Madison County, a region with a highly buffered soil that is therefore not measurably changed by acid deposition. The effects of lime addition to the Adirondack communities also were studied. Microhabitat characteristics of the litter were used as covariates potentially affecting macroinvertebrate community composition.

Materials and Methods

Study Area

Five sites in the Adirondack region were used in this study: 4 near Old Forge (43°44'N 74°58'W) and one on private property near Eagle Bay: (43°48'N 74°51'W). Three sites in Madison County, New York (42°48'N 75°37'W, 42°49'N 75°32'W, 42°49'N 75°25'W) also were used as deciduous reference sites, as well as three coniferous sites in the Earlville State Forest (ESF). Each Adirondack and Madison County site was divided into two plots (45 m in diameter); each ESF site was comprised of one plot. A web of reference markers was constructed on each plot (Figure 1). One plot from each site in the Adirondacks was chosen at random to be the limed plot. Seven and a half metric tons of lime were added to the sites by hand during the fall of 2005 and spring of 2006 at a rate of 9.4kg/m^2.

Field Sampling

We collected samples during late June and early July 2006 at all plots. Ten sampling stations were randomly selected at each web, with samples collected 1.0 m from the station in a random direction. A vacuum sampler (Harper and Guynn 1998) was used to remove all of the litter from the 625-cm^2 quadrat at each sample point. We used a Hormelite® leaf blower (Model 69, 30 cc displacement, 5 HCPS), reconfigured for suction and connected to a plastic tube 15.5 cm in diameter. Following Harper and Guynn (1998), a metal box (25×25×25 cm) was placed over the quadrat to be sampled, allowing for standardization of quadrat size and preventing the escape of flying insects. The litter within the quadrat was vacuumed from the box and into a cheesecloth bag, then removed from the sampler and returned to the lab.

Invertebrate Extraction

Samples were sorted using Berlese funnels (Southwood 1966) over 75% ethanol. The funnel system used 40-watt light bulbs inside 15 cm metal cans, with the sample placed underneath in 12.5 cm cans. Wire mesh with 5 mm apertures was placed in the bottom of the smaller cans and over funnels 14 cm in diameter. Organisms > 2 mm long were hand-picked from the material that fell into the collecting vials. Samples were left in the funnels for five days, and invertebrates were stored in 75% ethanol. Specimens were then identified to the lowest practical taxonomic level using Bland et al (1978), Chu (1992), and Dindal (1990). Most organisms were identified to the order or family level.

Physical Analysis

One litter sample from each plot was randomly selected for microhabitat analysis (22 in all). Stem count per plot was determined during sampling for the Adirondack and Madison County sites. Total mass of the litter samples was measured following Berlese drying. These samples were ground using a Wiley Mill and stored in a drying oven. Loss on ignition, used to measure organic matter content, was determined by ashing 0.5 g of ground leaf material in a muffle furnace at 500°C to burn off organic compounds. The pH of each of the 22 samples was determined using a modified protocol from Hendershot et al. (1993). Samples of litter weighing ~1.0 g were placed in

20 ml of water; pH measurements were taken using a NEED MAKE meter. Soil pH was measured using the same protocol (Hendershot et al. 1993), using ~2.0 g of soil in 20 ml of water.

Samples were analyzed for calcium, magnesium, aluminum, and manganese content using block digestion, following Parkinson and Allen (1975) and Berendse et al. (1989). Digested solutions were analyzed concentrations using Inductively Coupled Plasma (ICP) spectrometry (Perkin Elmer – Optima 3000 ICP-AES). A matrix solution of known calcium, magnesium, and manganese concentrations was used as a standard. Apple leaves (NIST SRM 1515) of known calcium, magnesium and manganese concentrations (1.526% ± 0.015, 0.271% ± 0.008, and 0.054% ± 0.003 respectively) were used to analyze the success of digestion and to standardize elemental concentrations. Lignin was analyzed using the acetyl bromide digestion method adapted from Morrison (1972) and Iiyama and Wallis (1990).

Data Analysis

I used discriminant analysis and MANOVA to examine differences in macroinvertebrate communities between the limed and unlimed sites, as well as the differences among the Adirondack, Madison County, and ESF sites. Richness was determined using Margalef's Index at each site, and the diversity determined using Shannon Index (Ludwig and Reynolds 1988).

ANOVAs were performed to compare diversity and richness among the three regions and between limed and unlimed sites within the Adirondack region. ANOVAs were performed to look at differences across regions and between limed and unlimed sites in the Adirondacks with relation to these factors. Physical factors of the litter were also analyzed using one-way ANOVAs. Limed and unlimed sites from the Adirondack sites were compared, as were unlimed sites from all three regions. All analyses were performed with SPSS.

Results

Community Composition

Overall, sixty-nine different invertebrate taxa were found in the litter samples (Table 1). There was a significant difference in the number of macroinvertebrates found in the three regions ($F_{2,19}=3.652$, p=0.045). The fewest macroinvertebrates were found in the ESF sites (mean=225.8 ± 83.8 SD), with the most found in Madison County sites (mean=316.8 ± 62.0). The major ecological groups found were decomposers (e.g., Annelida, Diplopoda, Collembola) and predators (e.g., Araneae, Carabidae, Chilopoda).

MANOVA showed a clear difference between the three regions (T=2.574, $F_{114,320} = 3.612$, p<0.001). There was also a clear difference in macroinvertebrates between Madison County, the Adirondacks, and the Earlville State Forest as shown by discriminant function analysis (eigenvalue of function 1= 0.895; eigenvalue of function 2 = 0.804) (Figure 2). Seventeen groups entered the analysis as important to the differences between the three regions and are summarized with their canonical function values in Table 2.

MANOVA showed no difference between limed and unlimed sites (T=0.768, $F_{40,59} = 1.133$, p=0.326). Discriminant analysis showed that Sminthuridae (Collembola) were different between the limed and unlimed sites, but there were only five individuals in the 10 analyzed samples. Therefore, based on the eigenvalue (0.065), discrimination between the two communities was not possible.

Richness and Diversity

The Madison County sites had an average taxonomic richness of 4.185 ± 0.576 (SD), while the Adirondack sites had an average taxonomic richness value of 3.267 ± 0.642 (SD). The ESF sites had an average taxonomic richness of 3.280 ± 0.160 (SD). Single-factor ANOVA showed a significant difference among the three groups ($F_{2,19}=6.291$, p=0.008) (Figure 2); however, linear contrasts showed no significant differences between the Adirondack sites and the reference sites.

Madison County sites had a Shannon taxonomic diversity of 2.682 ± 0.281 (SD), while the Adirondack sites had a slightly higher taxonomic diversity of 2.818 ± 0.511 (SD). The ESF sites had an average diversity of 2.229 ± 0.180 (SD). Single-factor ANOVA showed a significant difference among the three groups ($F_{2,19}=4.353$, p=0.028) (Figure 3). There was a significant difference between the Adirondack sites and the reference sites (p=0.043), but not between the Madison County and ESF sites.

Limed sites had an average Margalef's richness value of 0.130 ± 0.01 (SD); whereas unlimed sites had an average Margalef's value of 0.091 ± 0.0004 (SD). There was no difference in taxonomic richness between the groups ($F_{1,8}=1.030$, p=0.34, df=9). Average Shannon Index values at limed sites were 2.408 ± 0.17 (SD), and at unlimed sites averaged 2.867 ± 0.34. Results of the single-factor ANOVA were insignificant ($F_{1,8}=2.076$, p=0.19, df=9).

Physical factors

The percent organic matter differed between limed and unlimed sites in the Adirondacks ($F_{1,8}=149.353$, p<0.001). Limed sites had an average value of 79.67% ± 0.02% (SE); unlimed sites averaged 94.27% ± 0.02% (SE).

There were significant differences with respect to pH values between the regions ($F_{2,14}=4.033$, p=0.041). The highest pH was found in the ESF sites, which averaged 6.645 ± 0.141 (SE); the lowest pH was found in the Adirondacks, averaging 6.270 ± 0.154 (SE).

Soil pH values were not significantly different among the three regions ($F_{2,14} = 1.430$, p=0.272). However, soil pH significantly differed between limed and unlimed sites ($F_{1,8}=9.566$, p=0.015); limed sites had a higher mean pH (mean = 7.922 ± 0.144) than the unlimed sites (mean = 6.720 ± 0.144).

Litter mass was significantly different among the three regions ($F_{2,19}=5.565$, p=0.013). Linear contrast showed that the Adirondack sites were different from the Madison County and ESF sites ($F_{1,19}=11.020$, p=0.004), but the latter two regions did not differ from each other.

Elemental analysis showed no significant difference among unlimed sites in the three regions ($F_{2,14}=1.490$, p=0.259). Magnesium, however, was different among the regions ($F_{2,14}=4.406$, p=0.033). Calcium did significantly differ between limed and unlimed sites ($F_{1,8}=23.614$, p=0.001), as did magnesium ($F_{1,8}=11.582$, p=0.009).

The trees-per-plot counts (stem counts) did not vary significantly either between the Madison County and Adirondack regions ($F_{1,14}=1.698$, p=0.259) or between limed and unlimed sites ($F_{1,8}$=1.776, p=0.219). P-values for all ANOVA tests are summarized in Table 3.

Discussion

There were higher numbers of invertebrates found in Madison County and the Adirondacks than in the ESF sites. This is probably because the ESF sites are dominated by conifers, and the other two regions are dominated by broadleaf trees, which often support a more diverse community than coniferous forests (Ammer et al. 2006). This also explains the lower values for richness and diversity at these sites.

There was a distinct difference between the Madison County and Adirondack sites in terms of invertebrate composition and abundance at the two sites. Notably, gastropods, diplopods, and isopods were all more abundant in Madison County, where calcium levels were higher. These three taxa all have high calcium demands. Millipedes and isopods have a calcareous exoskeleton, especially compared to the chitinous exoskeleton of insects, and snails have a calcareous shell (Ruppert et al 2004). Although most of the gastropods found in Madison County were slugs, the calcareous eggs of all terrestrial gastropods dictate that calcium is a crucial nutrient for slugs as well as snails (Tompa 1975; Johannessen and Solhoy 2001).

All of these invertebrates are important constituents in the diets of larger animals (Graveland et al 1994; Miyashita et al 2003), but the calcium provided in the exoskeleton of millipedes and isopods may be particularly important to insectivorous birds and mammals. Graveland et al (1994) showed that decreased calcium in diets of birds reduced reproductive success.

Decomposers were more abundant in Madison County; both millipedes (Diplopoda) and isopods (Isopoda) entered into the discriminant analysis as important to the differences between the two regions. These groups are responsible for the cycling of nutrients and organic matter through the ecosystem, and can improve plant growth, diversity, and function (Cassagne et al 2006; Partsch et al 2006). It is therefore likely that decomposition is slower in the Adirondacks than Madison County and that nutrients are mineralized more quickly in Madison County, improving overall ecosystem health. Earthworms, another important decomposer group (van Gestel and Hoogerwerf 2001; Partsch et al 2006), were also included in the discriminant analysis as one of the groups contributing to regional variation. Earthworms are noticeably scarce in the Adirondack regions as compared to the other two (Table 1).

There were no observable differences between the limed and unlimed sites in the Adirondacks in any of the statistical analyses. Although Sminthuridae (Collembola) were more abundant in the limed plots, their small sample size makes it impossible to draw conclusions about their importance in ecosystem function. If this trend continues with further sampling, it could indicate that collembolans, which are decomposers, are responding to the lime treatment. This could lead to greater plant diversity and productivity in the forest (Partsch 2006).

It is possible that there was no observable response to the liming treatment because it is too soon after the onset of the treatment for populations to show changes. This would seem to contradict Johannessen and Solhoy (2001), who found that calcium affected populations of snails within a five-week time period, but the authors specify that these effects were probably due to migration and not a reproductive increase. Since the plots in the current study were much larger than those in Johannessen and Solhoy (2001), short-distance migration effects may not be observable.

The significant difference between the limed and unlimed sites in relation to percent organic matter is probably due to the powdered lime that is present on the samples. It did not burn off in the muffle furnace, and so these samples showed a lower mass loss than the unlimed samples. Although the limed and unlimed sites could not be compared with respect to pH data, the limed sites showed higher pH values due to the dissolved lime.

The relatively high pH values in the ESF sites are probably the result of the large volume of pine needles in those samples. Since pH was determined using only hand-crushed litter samples (not ground in a Wiley mill), the pine needles were more intact and therefore less likely to leach organic acids. The low pH found in the Adirondack samples corresponds to the high level of organic matter found there; decomposition is more prevalent in that system than in the other two. Interestingly, there were no differences in soil pH among regions, indicating that the pronounced effects of acidic deposition in the Adirondacks are due to something else, such as lack of buffering calcium in the soil.

However, there were also no significant differences among unlimed sites in the three regions regarding calcium content. It seems that magnesium is a more important element to the differences among the sites. More study is needed to determine the effects of the liming treatment on the macroinvertebrates in the community. More work also is recommended to determine the effects of calcium depletion or addition in the diets of insectivores.

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Table 1: Total invertebrates identified in Madison County (6 webs), the limed sites of the Adirondacks (5 webs), the unlimed sites of the Adirondacks (5 webs), and the Earlville State Forest (6 webs).

Class	Order	Family	Madison County	Limed	Unlimed	ESF
Worms			331	47	41	35
Diplopoda			324	40	105	23
Chilopoda	Lithobiomorpha		69	7	19	39
	Geophilomorpha		30	29	41	34
	Scutigeromorpha		0	0	1	0
	Scolopendromorpha		1	2	0	0
Arachnida	Araneae		90	96	123	91
	Pseudoscorpionida		70	25	66	136
Crustacea	Isopoda	Oniscidae	17	0	0	20
		Trichoniscidae	2	1	0	0
		Ligiidae	12	1	4	0
Gastropoda	Geophila	Arionidae	18	1	1	3
		Other (snails)	10	4	4	5
Insecta	Collembola	Isotomidae	162	88	163	86
		Entomobryidae	100	17	61	24
		Sminthuridae	4	1	13	2
	Thysanoptera		2	0	0	0
	Lepidoptera		7	0	0	0
	Hemiptera	Tingidae	1	0	0	2
		Antocoridae	1	0	0	0
		Cicadellidae	1	0	0	0
		Cixiidae	1	0	0	0
		Delphacidae	2	0	0	0
		Other	15	1	2	2
	Hymenoptera	Formicidae	90	48	54	19
		Braconidae	3	0	0	0
		Ichneumonidae	1	0	0	0
		Scelionidae	1	2	2	1
		Platygastridae	0	1	1	0
		Sphecidae	0	1	0	0
	Diptera	Rhagionidae	7	3	3	0
		Mycetophilidae	0	1	1	0
		Chironomidae	6	3	2	3
		Psychodidae	0	2	3	1
		Sciaridae	0	0	1	0
		Pipunculidae	2	0	0	0
		Heleomizidae	1	0	0	1
		Tabanidae	0	0	1	0
		Cecidomyiidae	2	2	0	0
		Dolichopodidae	2	0	0	0
		Syrphidae	0	0	1	0
		Tipulidae	0	0	0	3
	Coleoptera	Staphylinidae	20	8	10	31
		Carabidae	13	5	15	1
		Curculionidae	86	1	1	5
		Chrysomelidae	1	1	0	0
		Scaphilidiidae	3	1	8	0
		Scarabidae	1	0	0	0
		Mycetophagidae	2	0	0	7
		Cantharidae	2	1	0	0
		Bostrichidae	2	0	0	0
		Dytiscidae	0	0	1	0
		Silphidae	0	1	0	0
		Rhipiceridae	0	0	0	1
		Scolytidae	0	0	0	1
		Other	0	2	1	0
Insect Larva	Coleoptera		151	163	189	119
	Diptera		154	123	154	80
	Mecoptera		2	0	0	0
	Lepidoptera		20	31	36	47
	Hymenoptera		31	1	5	0
Insect Pupa	Diptera		23	4	4	4
	Lepidoptera		1	1	0	0
	Hymenoptera	Formicidae	4	0	0	0

Table 2: Canonical discriminant function coefficients for the 17 significant taxa in the discriminant analysis.

Taxon	Function 1	Function 2
Worm	0.149	0.679
Diplopoda	0.312	0.397
Lithobiomorpha (Chilopoda)	0.285	-0.151
Oniscidae (Isopoda)	0.108	0.376
Trichoniscidae (Isopoda)	0.359	-0.099
Ligiidae (Isopoda)	0.329	-0.167
Arionidae (Mollusca)	0.290	-0.085
Coleopteran larvae	-0.518	-0.066
Dipteran larvae	-0.276	-0.064
Lepidoptera	0.444	-0.058
Braconidae (Hymenoptera)	0.241	-0.193
Staphylinidae (Coleoptera)	0.091	0.269
Carabidae (Coleoptera)	0.049	-0.290
Curculionidae (Coleoptera)	0.525	-0.352
Scarabidae (Coleoptera)	0.112	-0.248
Mycetophagidae (Coleoptera)	0.096	0.236
Cantharidae (Coleoptera)	0.251	-0.098

Table 3: Summary of p-values of ANOVAs for physical analyses among the Madison County, Adirondack, and ESF sites and between limed and unlimed plots. Stem count numbers were only taken in Adirondack and Madison County sites. (*)

Factor	3 Regions	Limed/Unlimed
Richness	0.008	0.269
Diversity	0.028	0.684
% Organic		<0.001
Litter pH	0.041	<0.001
Soil pH	0.272	0.015
Calcium	0.259	0.001
Magnesium	0.033	0.009
Aluminum		
Stem count	0.214*	0.219
Litter mass	0.014	0.001

Figure 1. Arrangement of invertebrate sampling quadrats to be used at six sites in the Adirondack Mountains (adapted from McCay et al 2004).

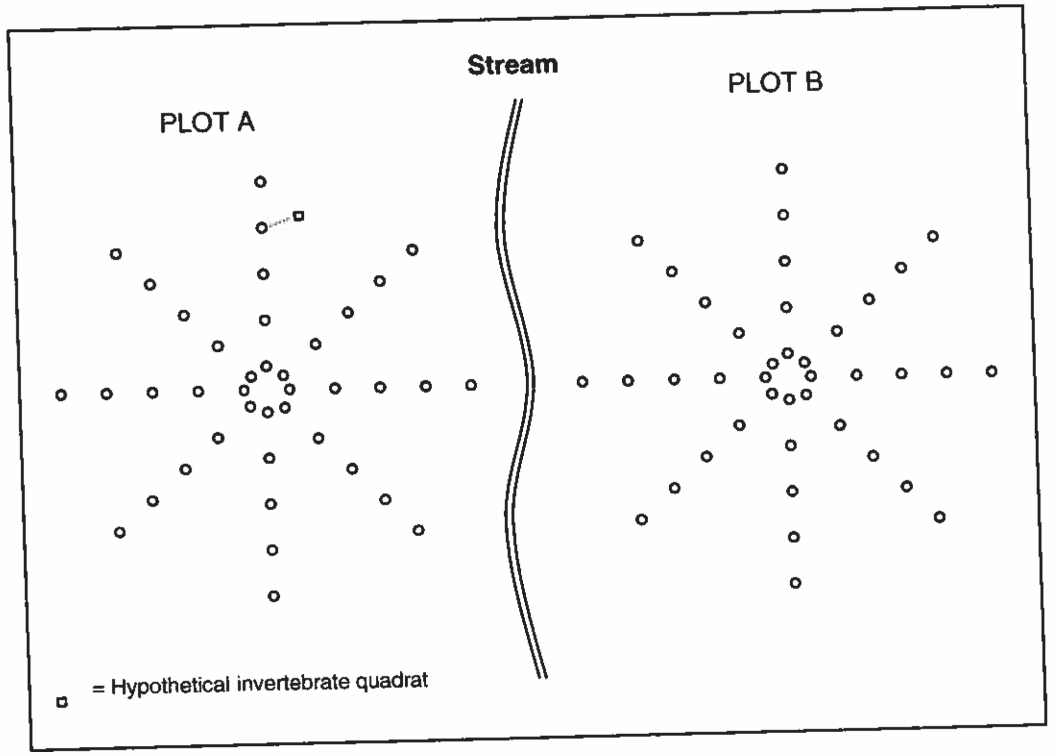


Figure 2: Taxonomic richness scores among the three regions according to the Margalef Index: Madison County (MC), Adirondacks (AK), and Earlville State Forest (ESF). ANOVA showed a significant difference among the three groups ($F_{2,19}=6.291$, $p=0.008$).

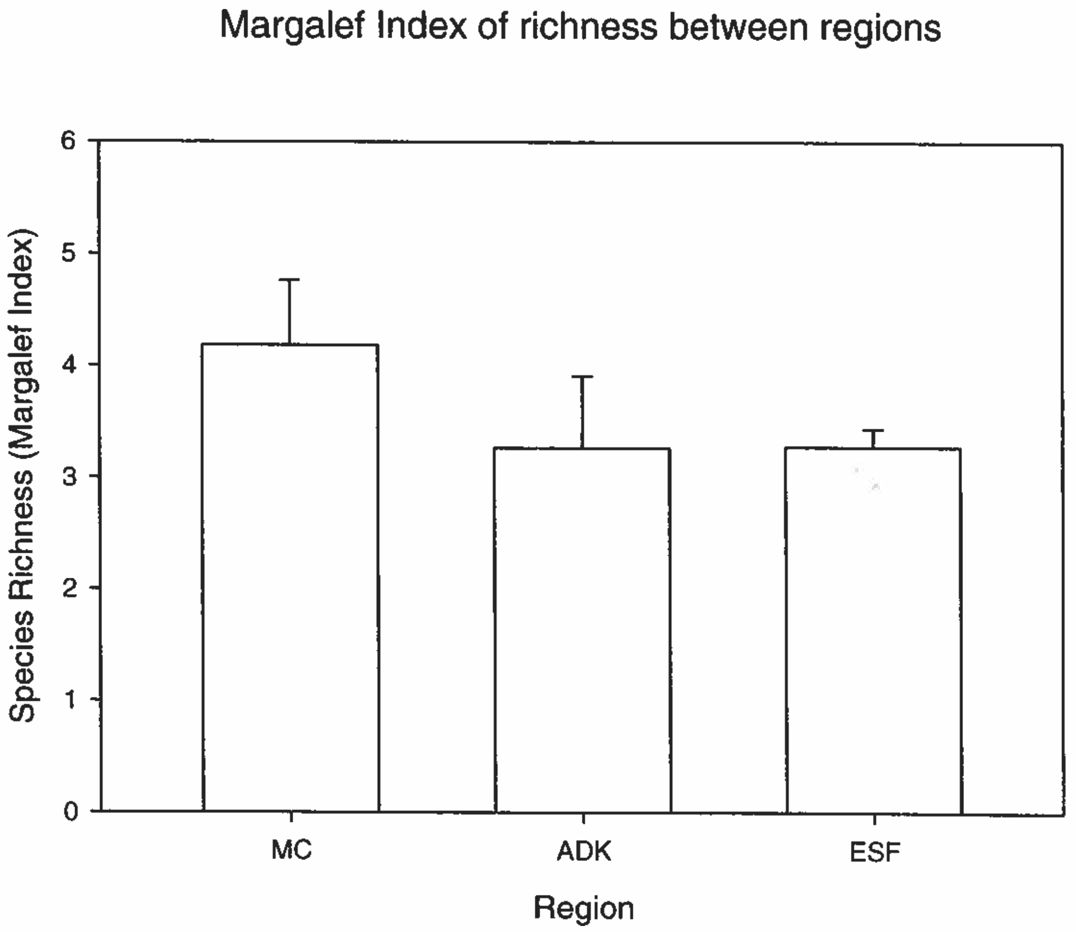
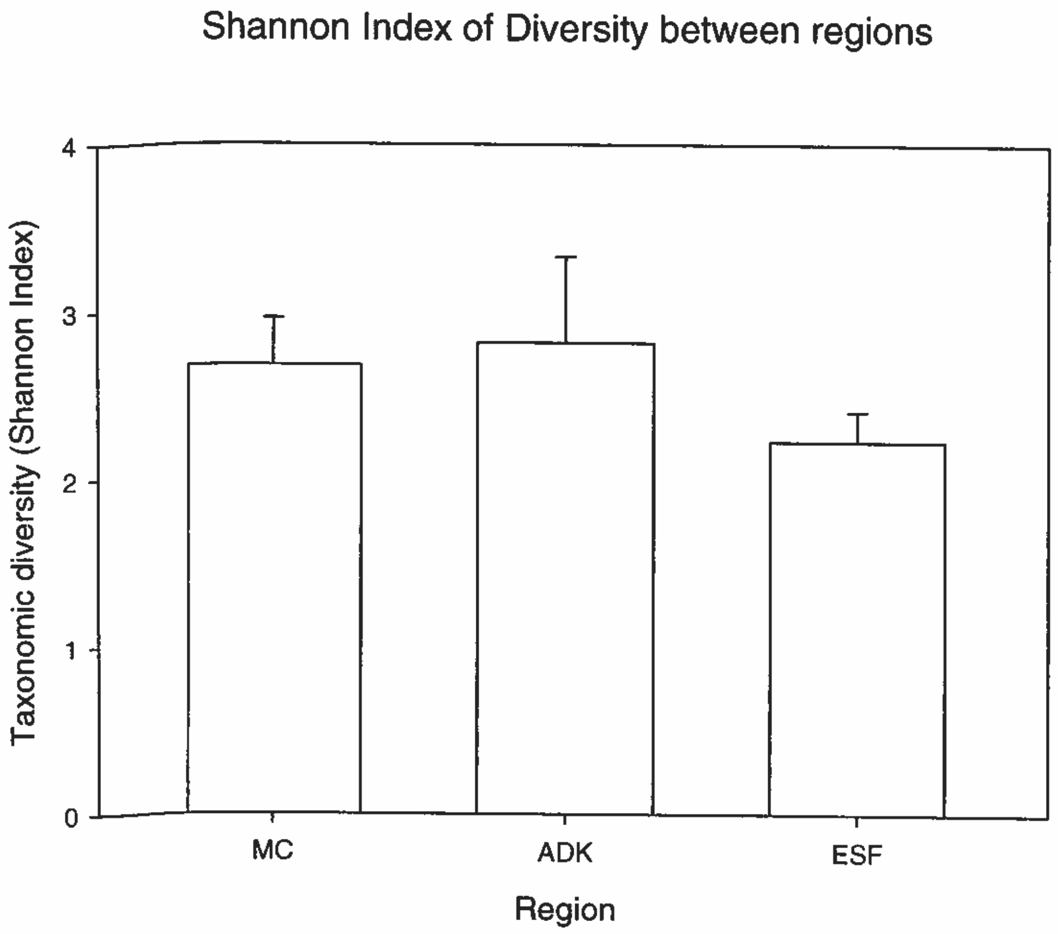


Figure 3: Taxonomic diversity scores among the three regions according to the Shannon Index: Madison County (MC), Adirondacks (AK), and Earlville State Forest (ESF). ANOVA showed a significant difference among the three groups ($F_{2,19}$=4.353, p=0.028).



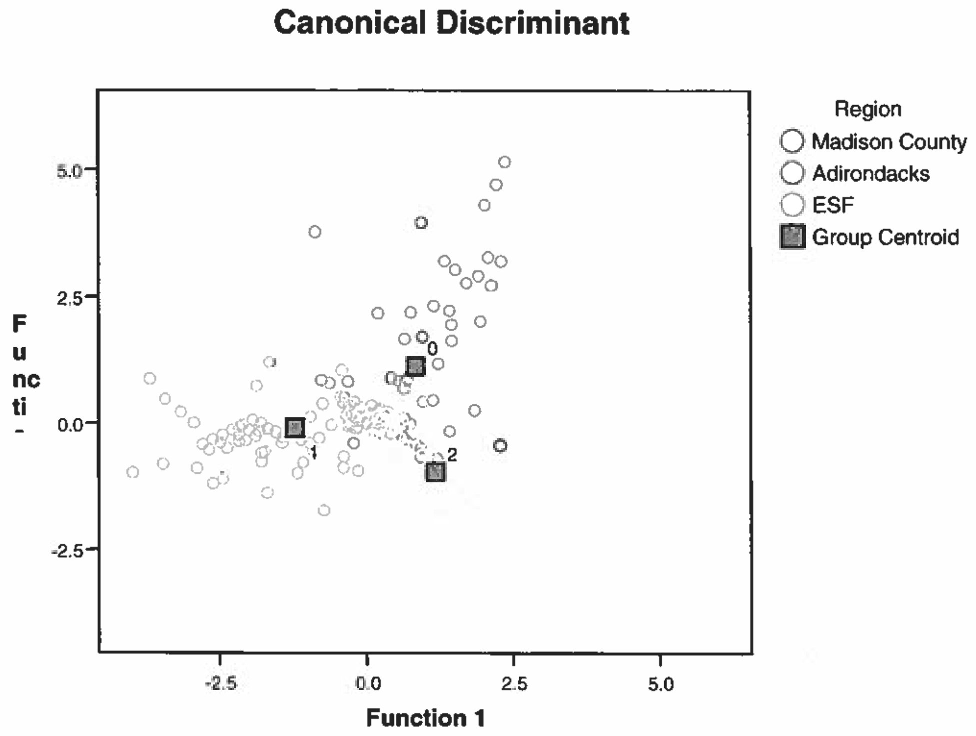


Figure 2: Plot of discriminant function scores showing strength of differences between Madison County, Adirondack, and Earlville State Forest invertebrate communities.