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Land Use Influences Along Elevation Gradient on Macroinvertebrate Communities

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Land Use Influences Along Elevation Gradient on Macroinvertebrate Communities

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Abstract

Land use activities have caused disturbances that affect the quality of freshwater ecosystems worldwide. How the influences of land use along an environmental gradient and the associated environmental variables that may influence stream diversity and function is unclear. We address these issues by studying biodiversity, abundance, and functional diversity of macroinvertebrates across different land types along a gradient in Colorado, USA. We also address how diversity may change along an elevation gradient by analyzing previously published macroinvertebrate research. We found evidence that land use and disturbance are stronger explanations of changes in macroinvertebrate communities, rather than elevation. Functional trait patterns of macroinvertebrates also differ from biodiversity and community composition measurements. Our research highlights the importance of land use, the influence on environmental variables, and the use of functional traits for characterizing communities.

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Chapter One: Influence of land use along an elevation gradient on benthic macroinvertebrates

Introduction

Freshwater ecosystems are endangered across the world, with significant losses in biodiversity due to overexploitation, water pollution, flow modification, species invasion, and habitat degradation (Reid et al. 2019; Dudgeon et al. 2006). Benthic macroinvertebrates have been widely studied and are often used globally as indicators of freshwater stream quality (Merritt and Cummins 1996; Cummins 1973). These bottom-dwelling aquatic animals are found in freshwater systems during their larval stages and include many orders of insects, but also non-insect species such as mollusks, annelids, nematodes, and platyhelminths. Macroinvertebrate surveys have proven to be an effective method for evaluating the health of streams or monitoring changes to biodiversity within the watershed (Wallace 1996; Poff et al. 2006), because they are sensitive to pollution and other habitat changes (Collier et al. 2016). These surveys have typically focused only on biodiversity measures while overlooking the relationship between traits of insects and environmental characteristics of the system, such as elevation (Menezes et al. 2010; Carter and Resh 2001). Although the influence of elevation and land use on benthic macroinvertebrates have been studied individually, there is a gap in research in studying land use along an elevation gradient. In this study, we address this gap by investigating which environmental variables influence not only community composition, and biodiversity, but also functional traits among high elevation streams in Colorado. Specifically, we test how diversity and functional traits respond to anthropogenic land uses and elevation gradients in the Front Range of Colorado.

Macroinvertebrate surveys have allowed ecologists to understand how a community responds to abiotic or biotic changes, such as seasonality, gradients of disturbance, and relationships between in stream biota and riparian biota (Jackson and Fuereder 2006). Natural disturbances such as wildfires and floods have been found to cause short-term changes to macroinvertebrate food source availability, nutrient availability, and other environmental variables that decrease overall biodiversity, while increasing densities of genera tolerant of disturbance in streams (Scrimgeour et al. 2001; Mi-Jung et al. 2014). Macroinvertebrate taxa are typically split into two categories, tolerant insects as members of the order dipteran or sensitive taxa as ephemeroptera, plecopteran, or trichopota (EPT) taxa. However, on a long-term scale, it has been observed that the invertebrate community, regardless of sensitivity, can recover the original biodiversity lost from natural disturbance and return to a previous state if stream chemistry recovers (Mi-Jung and Park 2009; Jackson and Fuereder 2006; Minshall 2003).

Recovery following anthropogenic disturbances may be less likely, since these impacts tend to be irreversible and constantly occurring with no rest period for habitat recovery (Arzina et al. 2006; Wantzen 2006). Mining activity can cause irreversible damage to streams and macroinvertebrate communities by decreasing pH, introducing metal ions, and covering the natural substrate with layers of toxic sediment (Pond et al. 2014; MacCausland and McTammany 2007). Similarly, ranching can also cause chemical changes such as pH reduction, nutrient fluctuations, complete removal of canopy cover, and sediment disturbances (Allan 2004; Freilich et al 2003). However, there has been a lack of knowledge of how such anthropogenic disturbances may impact environments that are already facing a natural stressor, such as the harsh physiological conditions at high elevation. As impacts of climate change become more apparent, it is especially urgent to understand ecological systems in vulnerable areas such as those along gradients exposed to anthropogenic influences.

How elevation impacts aquatic invertebrates is not well resolved (Chapter 2). Elevation is a common environmental variable in aquatic studies because it is believed to exert a physiological pressure upon invertebrates, but the severity of its impact appears variable (Chapter 2). Some researchers have found biodiversity or abundance decreases with increased elevation (Fiellheim 2000; Füreder 2006; Pringle and Ramirez 1998), but others have found no effect of elevation within their study streams (Allan 1975; Jacobsen 2003). One proposed reason is that at high elevations, although stream temperatures are much colder, which would be expected to result in higher solubility of oxygen, the atmospheric pressure is much lower and thus causes a decline in oxygen solubility at high elevations, negatively affecting macroinvertebrates (Jacobsen et al. 2003). However, those who have found no impact of elevation hypothesize that high altitude taxa are able to acclimate their respiration rate while maintaining a higher metabolism and growth rate when exposed to oxygen-deficient environments, such that they do not experience physiological stress from high elevation environments (Rostgaard and Jacobsen 2005). Thus, although physiology points towards a negative interaction, this has not always been observed as the primary influence on invertebrate communities.

Anthropogenic impacts could be more severe at high elevations since these influences change many components of stream hydrology and chemical composition. For example, streams at high elevation should have colder water temperatures, but if ranching or agriculture practices have removed natural canopy cover, the water temperature may actually be warmer than usual (Hepp et al. 2010). The lack of canopy cover will also remove natural reinforcements which prevent erosion or changes to the sediment and hydrology of streams (Pond et al. 2014). In systems impacted by high disturbances or pollution, land use may be the driver of diversity, but the added strain of elevation could increase these effects or mitigate them.

Ecosystems at high altitudes are also especially vulnerable to climate change (McGregor et al. 1995; Harper and Peckarsky 2003; Domisch et al. 2011) as the ecosystems may be further physiologically strained and will become even more stressful with unpredictable weather and changes to precipitation or snowmelt. Climate change has been documented to cause changes to permafrost, annual precipitation, and increased water temperatures, which have the capacity to influence stream diversity and has been observed in several studies (Burgmer and Pfenninger 2007; Ashmore and Church 2001; Smith and Riseborough 1996). Climate change effects have been documented to also be responsible for changes in phenology, such as causing late insect emergence times (Ohmura 2012). Climate change poses further disturbance and threat to macroinvertebrate communities along elevation gradients which may already be experiencing a decline from anthropogenic effects, so an analysis on current communities under stress must be thoroughly understood.

Most aquatic research has explored environmental impacts on insects by measuring biodiversity or community composition, such as abundance or richness of the community (Heino 2009), but ecological studies within the past few years have begun to use a functional trait approach towards identifying the biodiversity or roles of aquatic macroinvertebrates (Vandewalle et al. 2010; Tullos et al 2009). Functional traits are characteristics of organisms that represent their roles within an environment, rather than a taxonomic approach of measuring biodiversity or community richness. Measuring traits have also been helpful in understanding how ecological function is impacted by human disturbances, leading to stronger conservation and land management practices (Mayfield et al. 2010; Baraloto et al. 2010). Using functional traits as an ecological tool has similarly been applied in other systems to study the effects of invasive species, forest structure, and microbial communities (Matzek 2012; Martiny et al. 2013). Within the realm of aquatic invertebrates, functional traits can include diet, feeding mechanisms, sensitivity to pollution, body size, or any specific characteristic that allows taxon to function. Although some functional trait research has been done on freshwater aquatic systems, there is a gap in studying ecological function of benthic macroinvertebrates in disturbed streams at high elevations. Use of functional trait guilds will allow us to determine if these traits respond to environmental gradients in the same way as biodiversity or abundance, or if they differ.

Functional groups can also be used to observe the traits that separate tolerant and sensitive taxa. For example, if all groups of sensitive taxa are herbivores, their sensitivity to environmental changes could relate to the destruction of canopy cover, which is typical for agriculture or ranching land use. Current taxonomy and biodiversity indices do not reveal this detail since they are quantitative descriptors of the community. The taxonomic approach of grouping organisms relies on shared anatomical traits or ancestry (Bailey et al. 2001) while the functional trait grouping relies on functional roles, life history, and morphology that is not typically used in taxonomy (Ding et al. 2017; Poff 1997). Although taxonomic and functional trait groupings may overlap, this has not always found to be the case (Normandin et al. 2017; Sechi et al. 2015). Functional groups and biodiversity indices can be used together to further our understanding of macroinvertebrate systems. Typically, identification of sensitive species has been done by taxonomic guild; while EPT taxa typically require specific habitats and diets, require oxygen-rich water, and are sensitive to water pollution (Klemow 2000; Sweeney & Vannote 1984;Wielgolaski 1975) it is possible that other, non-EPT genera are functionally similar. By creating guilds based on traits, we can confirm or dispute the traditional use of EPT (a taxonomic grouping) as indicator species. Since specific functional guilds represent an ecological role, we can use them to investigate the relationship between traits and environment. For example, EPT taxa include both sediment dwellers and those that require fast flowing water; this trait could critically distinguish between different anthropogenic stressors. A guild able to live within the sediment and use their tegument to breathe may thrive in agricultural areas because [fill in here], whereas other so-called sensitive insects that instead swim within fast-flowing, vegetation rich environments may be excluded. In this way, functional trait guilds can allow us to identify the actual traits that make an insect tolerant or sensitive to an environment. This is an important and different metric than the response of taxonomic diversity.

We have studied streams within the Rocky Mountains to observe macroinvertebrate communities and their functional guilds along an elevational gradient with various anthropogenic disturbances. This study addresses three questions regarding the benthic macroinvertebrate community in Colorado streams: 1) Do different types of anthropogenic land use, such as recreation, residential, ranching, or mining, impact biodiversity metrics, abundance, and functional trait guilds along an elevational gradient? Based on studies done in lowlands, we predicted that high disturbance land uses such as mining and ranching will be associated with decreased diversity in community structure and biodiversity, relative to less disturbed land areas. We also predict that increased elevation will negatively influence invertebrate diversity, regardless of land use, but we cannot predict how these may these variables interact, given the potential number of environmental factors involved. 2) What are the functional trait guilds of this system, and how do they differentially respond to elevation and land use? 3) If diversity or functional groups are impacted by land uses and/or elevation, what environmental variables may explain these relationships? We predict that land uses with the most pollutants or disturbance will impact many environmental variables, such pH or substrate type. We expect that increasing elevation will influence decrease both water temperature and dissolved oxygen.

Methods

Study Locations

The research area was located within the Rocky Mountains in the South Platte Basin located in central and northeast Colorado, in Park and Clear Creek counties with altitudes ranging from 7218 - 11,548 ft (Figure. 1.1). The climate in this area consists of up to 14 inches in annual precipitation, up to 90 inches annual snowfall, and temperatures between -5 to 20 ºC for the year sampled. The ecosystems are dominated by coniferous forest and classified as montane at 5,600-9,500 ft, subalpine at 9,000-11,000 ft, and alpine tundra at above 11,000 ft. We sampled 16 first or second order streams along this elevation gradient between April and June of 2019 before annual emergence of adult invertebrates occurred. Streams at the lowest elevations were sampled from low to high elevation, to account for the seasonal differences. We selected sample locations at each stream through a collaboration with Mountain Area Land Trust, which granted us access to private properties and the Rocky Mountain National Park.

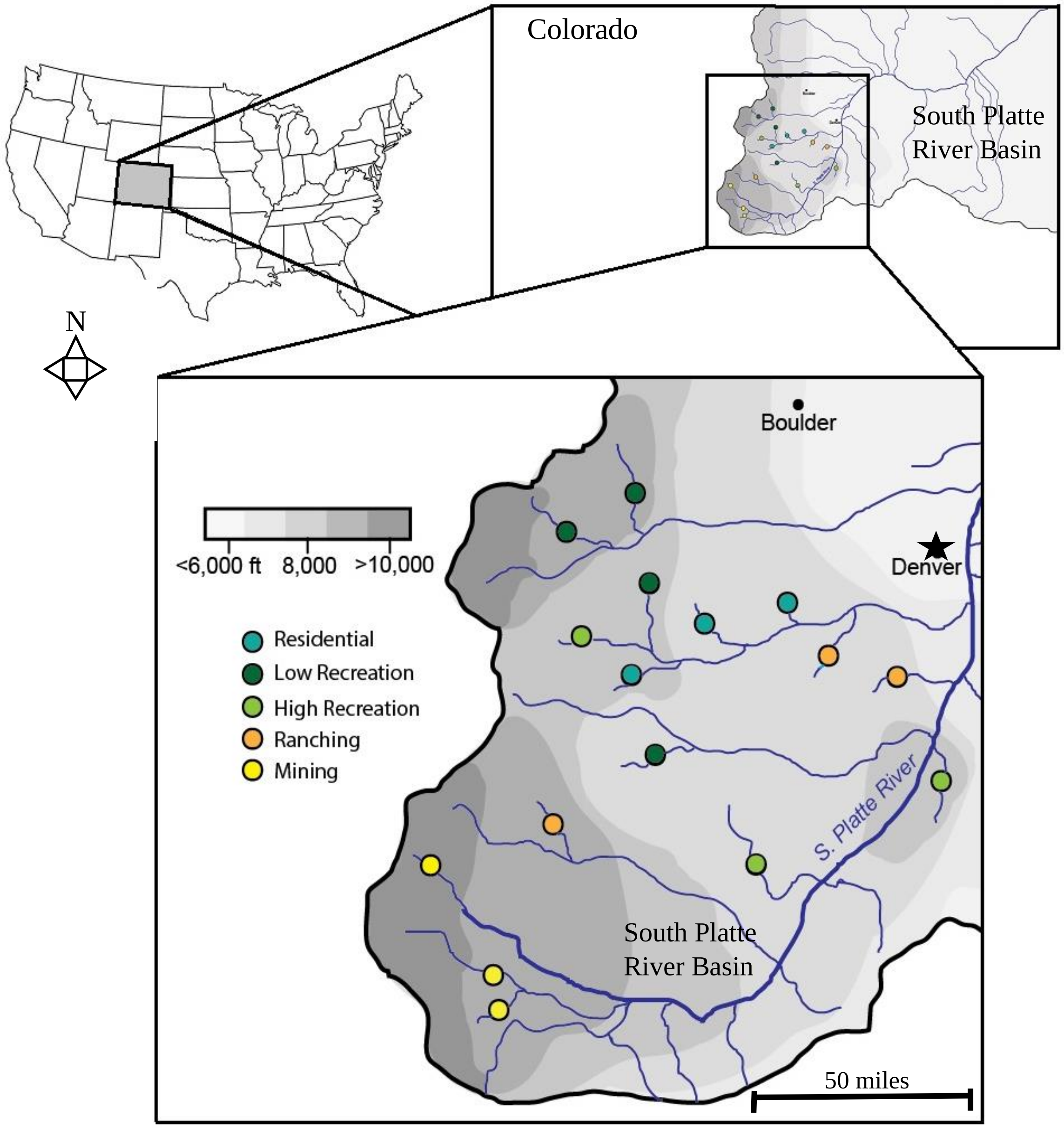

Figure 1.1. Study sites located within tributaries (thin blue lines) of the South Platte River (thick blue line) within the South Platte watershed (thick black line). Elevation topography, watershed boundaries, and stream/river locations based on data collected by the United States Geological Survey (USGS).

We sampled streams with varying land uses which were occurring upstream or within our sampling area. Based on their location and/or human use, we categorized sites as residential, low recreational, high recreational, ranching, and mining. Areas of water recreation were ranked as low if they were near trails, roads, or camping areas while recreational areas were ranked high if they received direct human interactions such as fishing, water sports, or other continual disturbances. Locations were considered residential, ranching, or mining if the collection area in the stream took place in properties where these activities were the dominant land use by the owners. Ranching and mining activities were active within the last five years in streams with those land use categories.

Field Collection

Sampling methods were designed to ensure randomness and so that all features of the stream were sampled, including riffles, pools, and substrate microhabitats (such woody debris, organic materials, large boulders, cobble, etc.) so as to represent the variability of the aquatic community. For each site, we first measured a 100m stretch of stream to represent the stream as a whole, including pools, riffles, and substrate types. We randomly selected 3-5 replicates along the100m stretch to sample. We selected replicates by marking 10m segments within the 100m, and then randomly selecting which would be sampled using the last digit of a running stopwatch. Although we planned to collect 5 replicates for each stream, some of the randomly selected 10m segments were not able to be sampled if heavy brush or low clearance bridges blocked the net for sampling, leading to a few streams with just 3 replicates. After we determined the replicates, collection started at the most downstream site to avoid upstream disturbances from impacting the sample. For each replicate, we collected benthic invertebrates with a standard D-frame kicknet with a 500 µm mesh. We collected each sample by kicking into the net for 2 minutes total (following Poff et al. 2006), splitting this time among the different microhabitats to sample all substrate types. Our time spent kicking at each substrate type was proportional to how common each habitat was within the 10m replicate stretch of stream. Habitat types included: woody debris, leaf pack, silt, sand, gravel, cobble, boulder, and bedrock. For example, if 50% of the stream was gravel, 25% was sand and 25% was woody debris, then 1 minute would be spent kicking in gravel, with 30 seconds spent in the other two habitats (1 + 0.5 + 0.5 = 2 minutes). After collection in the net, all invertebrates and debris were transferred to a container and preserved in 95% ethanol.

We also collected water chemistry data from each stream from the most downstream replicate before any invertebrate collection took place. We used a Vernier probe to collect pH, conductivity, water temperature, and dissolved oxygen measurements. We also recorded degree day, elevation, canopy cover, stream depth, stream width, and land usage for each site.

Lab Processing

We placed each sample into a 500 µm sieve to be rinsed, which separated small debris, dirt, and microorganisms from the macroinvertebrates. After rinsing and removing larger items such as rocks or sticks, we sorted the remaining invertebrates and removed them from the debris using forceps. We identified all larval stage invertebrates found in the sample using a 60x stereo microscope and *An Introduction to the Aquatic Insects of North America* for identification. They were identified to genus, except for those in the Chironomidae family and Oligochaetes due to the difficulty of accurate identification (Hannaford & Resh 1995; Rabeni & Wang 2001). The abundance of individuals was recorded for each taxon.

Functional Traits

We researched functional traits for all taxa found within the 16 sampled streams. We selected the following traits for inclusion: trophic category, trophic feeding group, trophic diet, pollution tolerance, average bod size, mobility type, risk of drift, rheophily (preference to flowing water), voltinism, respiration, habitat preference, development, ability to exit stream, swimming ability, and crawling ability. These 15 traits have been identified as important for measuring the ecological function of streams (Cummins et al. 2005; Poff et al. 2006; Tullos et al. 2009). We identified functional traits for each genera by using peer-reviewed literature and using a taxonomic resource published by several sources (Aspin et al. 2018; Canobbio et al. 2010; Clarke et al. 1997; Colas et al. 2014; Dewalt et al. 2012; Hieno 2005; Melody et al. 2004; Merritt and Cummins 2008; Phillips 2011; Stewart and Stark 2011; Tolonen et al. 2000; Usseglio-Polatera et al. 2000; Vieira 2003; Wang et al. 2018;).

Statistical Analysis

We first performed a cluster analysis in R-3.6.2 with qualitative data in order to place each taxon into one of four guilds based on shared functional traits. This was done by applying hierarchical group average clustering to the 14 functional traits (Appendix A, Figure. S1) (Legendre 2012) and created a Gower dissimilarity matrix with the optimal number of groups set at four (Le & Ho 2005). This gave us four distinct functional groups with similar traits which were used as dependent variables, which we named as follows: motile clingers (guild 1), swimmers (guild 2), sessile clingers (guild 3), and tolerators (guild 4).

Next, we calculated the dependent variables of species diversity in terms of Simpson's diversity, Shannon's diversity, abundance, and taxa richness (Appendix A, Table S1). For the independent variables, we first ran a correlation matrix on all environmental measurements taken and then used a Principle Components Analysis (PCA) to reduce highly correlated substrate variables into two new variables: PC1 ("Substrate type 1") explained 33.1% variability and a high value represented leaf pack, boulder, and bedrock while low amounts indicated woody debris, silt, sand, and gravel. PC2 ("Substrate type 2") explained 23.6% of the variability and high values indicated content of woody debris and silt while low values of PC2 represented sand and cobble (Appendix A, Table S2). The environmental measures in this study included elevation, land use, conductivity, pH, stream width, dissolved oxygen, water temperature, and the two substrate types (PC 1 & 2 and all other variables were tested for normality, and log-transformed where necessary).

We used a mixed model to determine how land use impacts communities along an elevation gradient. Our dependent variables were Shannon's index, Simpson's index, abundance, taxa richness, and the four functional guilds, and our independent variables were land use type, elevation, and an interaction of land use and elevation with site as a random variable.

Finally, to investigate the association of land use and/or elevation with environmental variables, we performed another mixed model with dissolved oxygen, pH, stream width, conductivity, water temperature, and substrate type as dependent variables, with site as random variable, and land use, elevation, and the statistical interaction of land use and elevation as independent variables. We first performed this analysis on the full range of elevations and a two-way ANOVA on a subset of sites located between 7,750 – 10,000 ft to observe land use influences without confounding effects of elevation

Results

Anthropogenic Land Use impacts

We collected 6,198 individuals from 37 different taxa from the 16 streams sampled. There was a significant statistical interaction between elevation and land use for Shannon's index, abundance, taxa richness, and all four guild measurements (Table 1.1). High recreation sites showed a positive relationship between elevation and diversity as measured by Shannon's index, Simpson's index, and taxa richness, whereas all of these diversity measures plus abundance decreased with increasing elevation in low recreation sites (Figure. 1.2). Sites with low recreation and ranching showed a negative relationship between elevation and Shannon's Index, Simpson's Index, taxa richness, and abundance.

Mining did not change biodiversity or abundance measures along an elevation gradient, but values were lower than residential, low recreation, and some high recreation locations. However, at residential land use sites, elevation had a negative relationship with Shannon’s Index, Simpson’s, and abundance, but a positive relationship with taxa richness. At the lowest elevations, 8,500 ft and below, there were only residential, low recreation, and ranching sites. Together, this meant that at lower elevations, highest diversity was found in residential sites, but highest abundance and taxa richness was seen in low recreation sites. At mid elevations, from 8,501 – 10,000 ft, there were only high recreation, ranching, and mining sites where high recreation had the highest diversity and community composition. At the highest elevations, above 10,000 ft, there were only mining sites, which generally had lower diversity than residential or recreation sites. Ranching generally had the lowest values of biodiversity or abundance along the gradient. Overall, the lowest diversity for measurements were associated with ranching or mining, except in the case of abundance, which also had low values in high recreation sites.

Table 1.1. Influence of elevation and land use on diversity measures used in this study, as measured with a mixed model with site as random variable and elevation, land use and the statistical interaction of these as fixed variables.

	R^2	Elevation * Land use		Elevation		Land use	
		F Ratio	**P**	**F Ratio**	**P**	**F Ratio**	**P**
Shannon's Index	0.66	5.44	**0.001**	16.60	**0.001**	4.74	**0.003**
Simpson's Index	0.53	2.82	0.035	2.60	0.113	2.86	**0.033**
Abundance	0.69	18.68	**0.001**	30.65	**0.001**	9.60	**0.001**
Taxa Richness	0.60	6.51	**0.001**	1.39	0.244	2.11	0.094
Motile clingers	0.57	2.68	**0.043**	0.14	0.706	3.95	**0.008**
Swimmers	0.54	6.67	**0.001**	12.67	**0.001**	2.29	0.074
Sessile clingers	0.71	16.48	**0.001**	27.11	**0.001**	7.06	**0.001**
Tolerators	0.49	13.71	**0.001**	7.06	**0.001**	8.74	**0.001**
		df = 4,48		df = 1,48		df = 4,48	

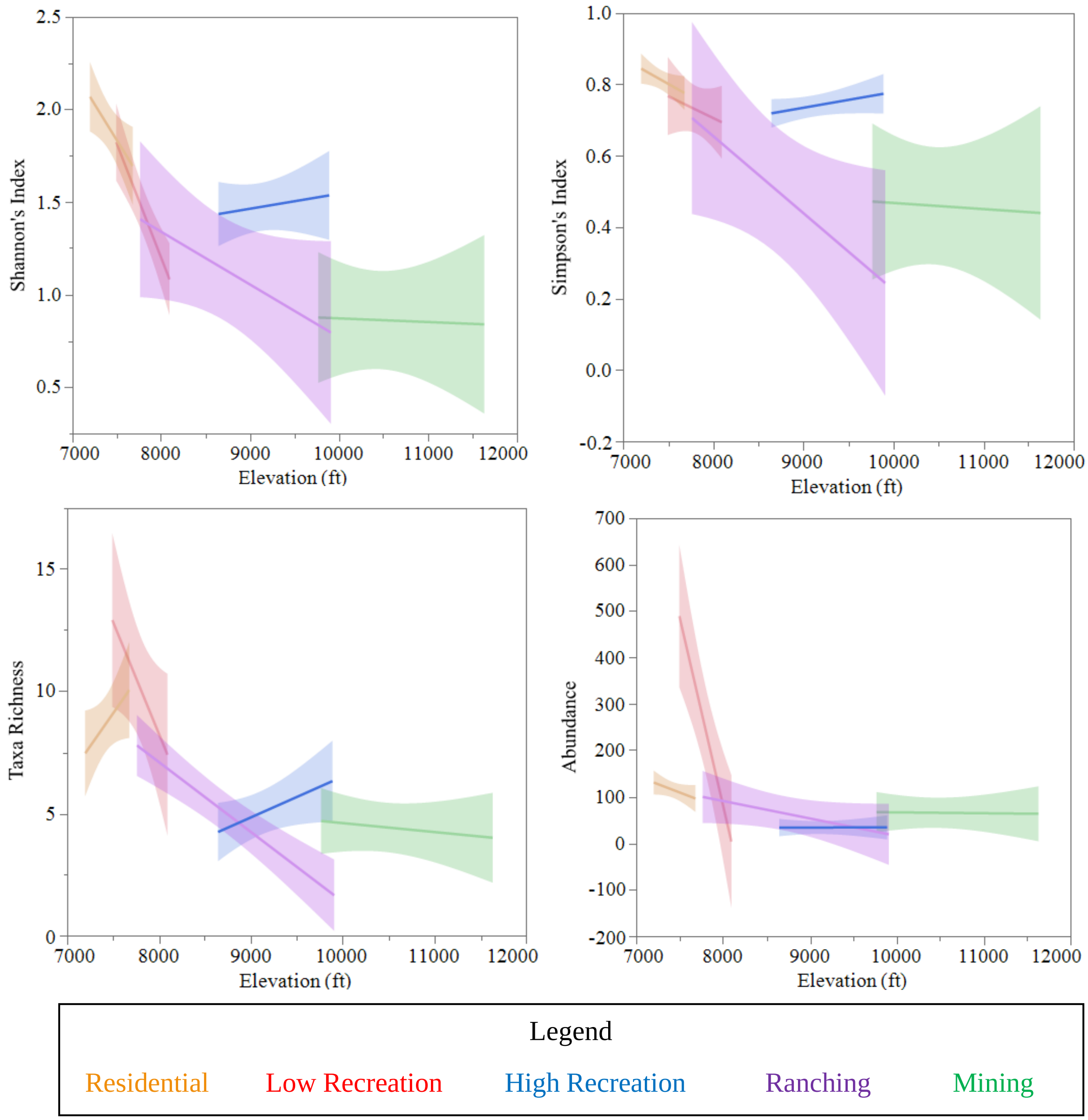

Figure 1.2. Changes to the diversity measures in each land use type along an elevation gradient. In each graph, lines are best fit created with data from replicates of each site. Confidence interval is shown by the shading around lines.

Functional Guilds

Within the four functional guilds, motile clingers were predominantly mayfly taxa, which shared traits in feeding groups, mobility, rheophily, voltinism, respiration, and swimming abilities (Table 1.2, See Appendix B Table S 3 for taxonomic identification of guilds). Swimmers were comprised of mayfly and stonefly taxa and shared mobility, preferred habitat, and stream exiting traits. Sessile clingers had several orders of insect, but mostly caddisflies, and shared mobility, voltinism, preferred habitat, development speed, and swimming abilities. Tolerators were the most diverse guild with many genera of dipterans which all shared feeding category, preferred habitat, risk of drift, and crawling ability. We also found significant interactions between elevation and land use for functional group response (Figure. 1.3). Low recreation was associated with significantly lower abundances of swimmers ($F = 7.30$, $df = 4,48$, $p < 0.05$), sessile clingers ($F = 4.76$, $df = 4,48$, $p < 0.05$), and tolerators ($F = 16.91$, $df = 4,48$, $p < 0.01$) most of which decreased with elevation (Table 1.1). At low elevations below 8,500 ft, low recreation had the highest abundance of each guild which decreased with elevation. At mid elevations, high recreation and ranching had the highest values of motile and sessile clingers and swimmers.Tolerators had the highest abundance in mining sites at mid elevations, however, this abundance slightly decreased with elevation in the high elevation zones above 10,000 ft. Motile clingers also increased in abundance in high elevation mining sites, while the other two guilds were relatively low in abundance. Among all land types, ranching and mining had the lowest abundance of all guilds except the tolerators.

Table 1.2. Of the fourteen functional traits identified for the insects in this study, twelve were found to be shared among the insects in at least one of the four guilds. If no specific trait is shared, the trait is listed as variable.

Functional trait	Explanation	Motile clingers	Swimmers	Sessile clingers	Tolerators
Trophic feeding group	Refers to the food chain, may be predators, herbivores, detritivores, or a variation of several.	Herbivore and detritivore	*Variable*	*Variable*	*Variable*
Feeding category	The mechanism organisms use to find food by collecting, gathering, or scraping.	*Variable*	*Variable*	*Variable*	Scraping
Mobility	Organisms may move by clinging to nearby sediments, swimming, or by burrowing.	Cling	Swim	Cling	*Variable*
Rheophily	Preference for fast flowing riffles, which can occur in despositional or erosional areas.	Despositional	*Variable*	*Variable*	*Variable*
Voltinism	The number of generations per year.	Univoltine	*Variable*	Univoltine	*Variable*
Respiration	Insects may respire through tegument or gills.	Gills	*Variable*	*Variable*	*Variable*
Preferred habitat	Some insects require a certain habitat for food sources or to avoid predators.	*Variable*	Woody debris	Cobble	Sediment
Risk of drift	Possibility of moving downstream within a life cycle to avoid predators or via catastrophic event.	*Variable*	*Variable*	*Variable*	Common
Development speed	A general measurement of how quickly larva develop and exit the stream.	*Variable*	*Variable*	Slow	*Variable*
Swimming ability	The ability to swim in open water.	Weak	*Variable*	None	*Variable*
Crawling ability	Speed and ability to crawl across the streambed.	*Variable*	*Variable*	*Variable*	Very low
Ability to exit as larva	Some larva may be able to leave the stream under certain circumstance while still in the larval state.	*Variable*	Absent	*Variable*	*Variable*

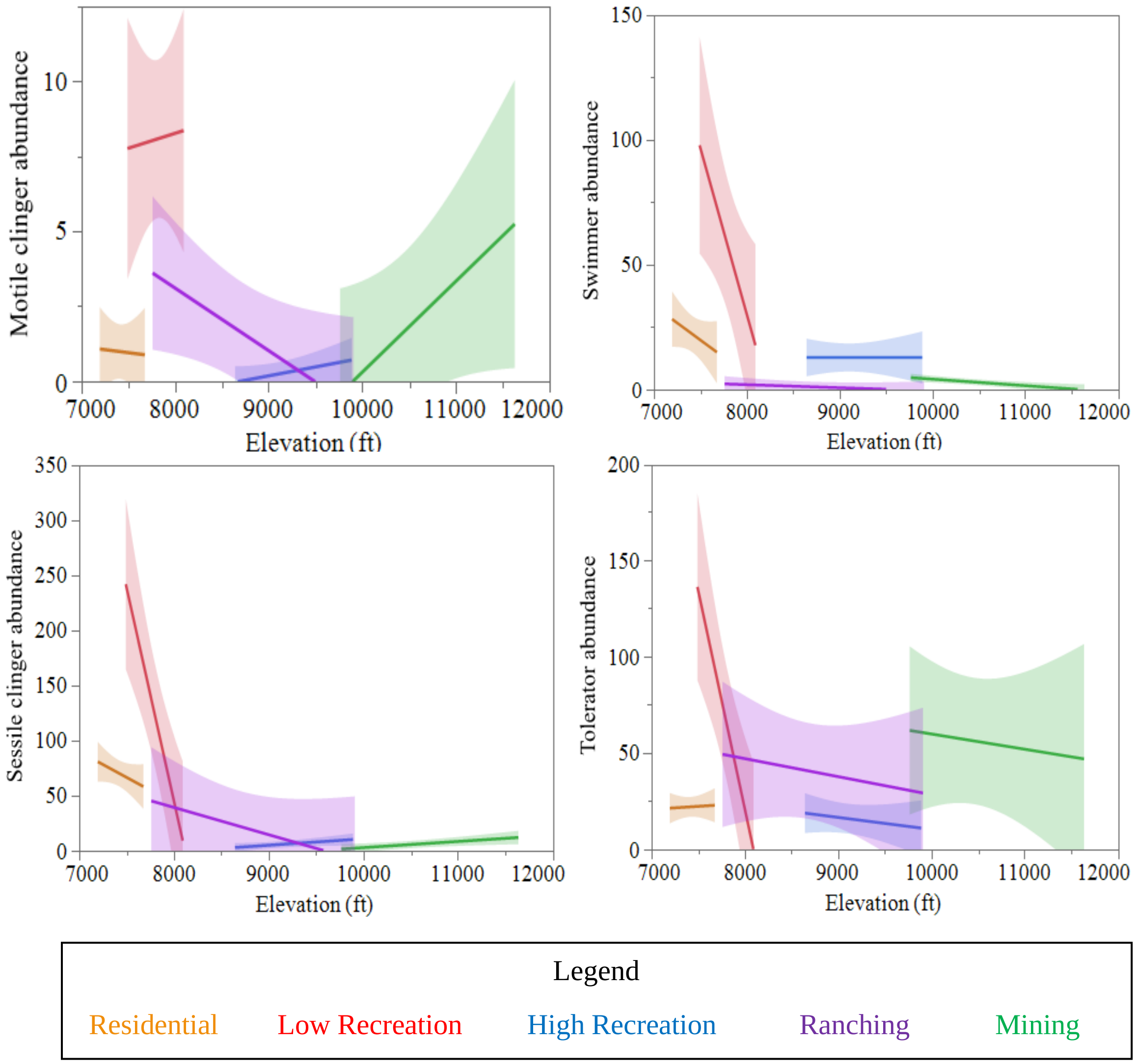

Figure 1.3. Changes to the guild communities in each land use type along an elevation gradient. In each graph, lines are best fit created with data from replicates of each site. Variance of site is explained by the shading around lines.

Since elevation was confounded with land use to some extent (e.g., all residential sites were lower elevation and all mining sites were higher elevation), we also performed a mixed model to determine if differences between land use types were still significant among those with similar elevations. We used the elevational range of 7,750 to 10,000 ft, which included every land use type except and residential (Figure 1.4). Shannon’s and Simpson’s indices both had highest diversity at high recreation and lowest diversity at mining sites. Taxa richness was highest at low recreation sites while the other three land types were similar. Motile clingers, swimmers, and sessile clingers had highest abundance at low recreation sites while tolerators were highest at mining sites. However, mining sites had the lowest abundance of motile clingers, swimmers, and sessile clingers. Tolerators were significantly less abundant at both types of recreational sites.

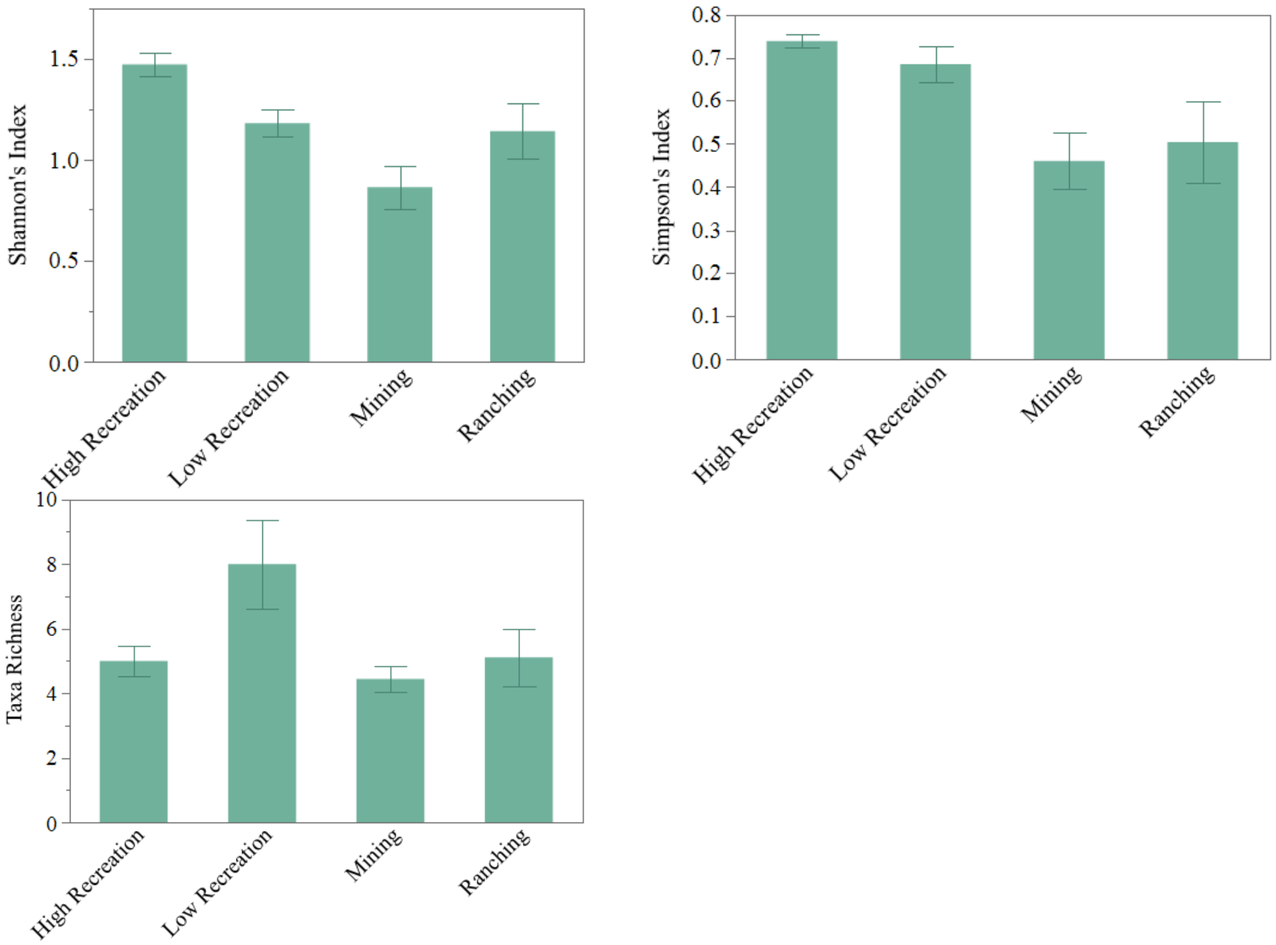

Figure 1.4. Influence of land use types on diversity measures and functional guilds only within elevation ranges 7,750 – 10,000 ft.

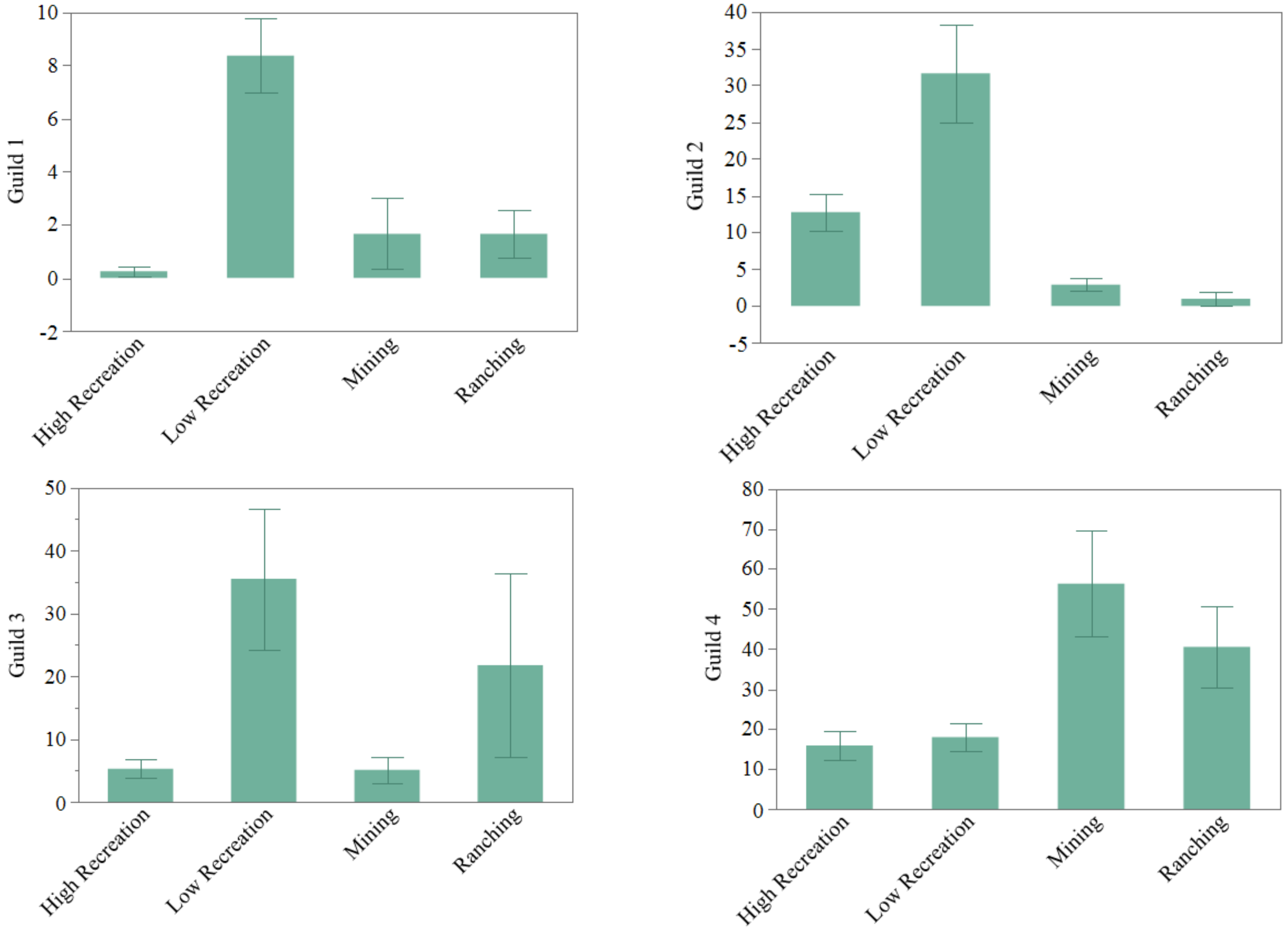

Figure 1.4 (Continued).

Environmental Variables

We found that the statistical interaction of land use and elevation was significant for explaining all tested environmental variables (Table 1.3). Residential land use caused a negative relationship along an elevation gradient with conductivity, pH, and canopy cover, and had a positive relationship with stream width, dissolved oxygen, and both substrate types (Figure 1.5). Low recreation sites had a negative relationship with water temperature, dissolved oxygen, and substrate type 2, and a positive relationship with conductivity, width, and substrate type 2. High recreation had a negative relationship with conductivity, width, water temperature, dissolved oxygen, and substrate type 1, and a positive relationship with pH, canopy cover, and substrate type 2. Ranching had a negative relationship with pH, dissolved oxygen, and substrate type 2, with no noticeable relationship with other variables. Mining had a negative relationship with width, pH, canopy cover, and substrate type 1, and a positive relationship with water temperature and substrate type 2.

Table 1.3. Influence of elevation and land use on commonly measured environmental variables as measured with a mixed model with site as random variable and elevation, land use and the statistical interaction of these as fixed variables.

	R^2	Elevation * Land use		Elevation		Land use	
		F Ratio	**P**	**F Ratio**	**P**	**F Ratio**	**P**
Conductivity	0.43	5.93	**0.001**	13.89	**0.001**	8.61	**0.001**
Water Temperature	0.81	24.44	**0.001**	27.87	**0.001**	38.20	**0.001**
Stream Width	0.37	4.61	**0.003**	2.71	0.106	8.22	**0.001**
pH	0.94	96.20	**0.001**	97.54	**0.001**	71.66	**0.001**
Dissolved oxygen	0.39	4.76	**0.003**	0.11	0.743	7.92	**0.001**
Substrate type 1	0.29	4.74	**0.003**	0.01	0.974	4.73	**0.003**
Substrate type 2	0.30	3.42	**0.015**	0.01	0.980	4.59	**0.003**
Canopy Cover	0.41	3.81	**0.009**	0.84	0.363	2.78	**0.037**
		df = 4,48		df = 1,48		df = 4,48	

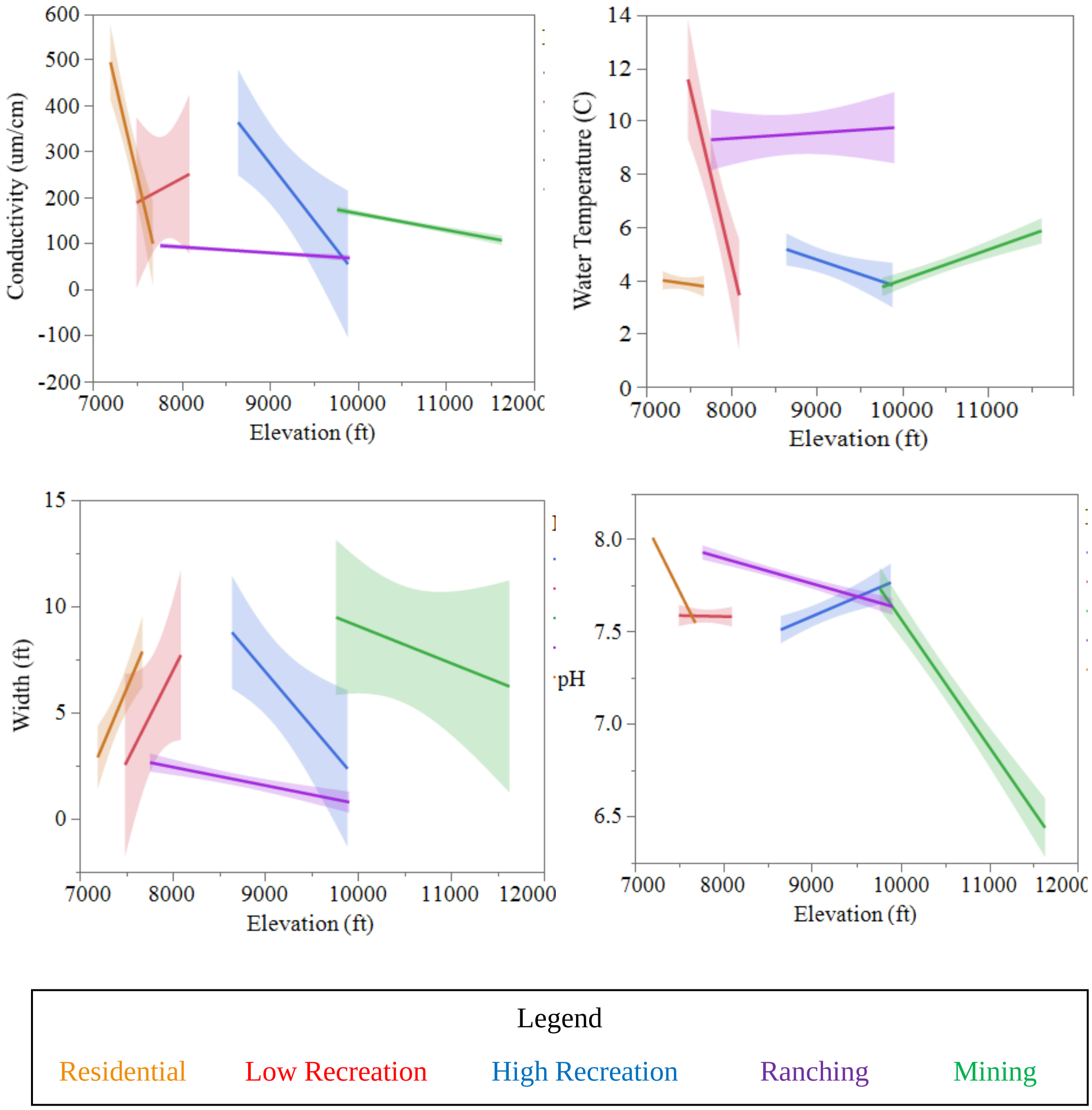

Figure 1.5. Changes to the environmental variables along an elevation gradient in each type of land use. In each graph, lines are best fit created with data from replicates of each site. Variance of site is explained by the shading around lines.

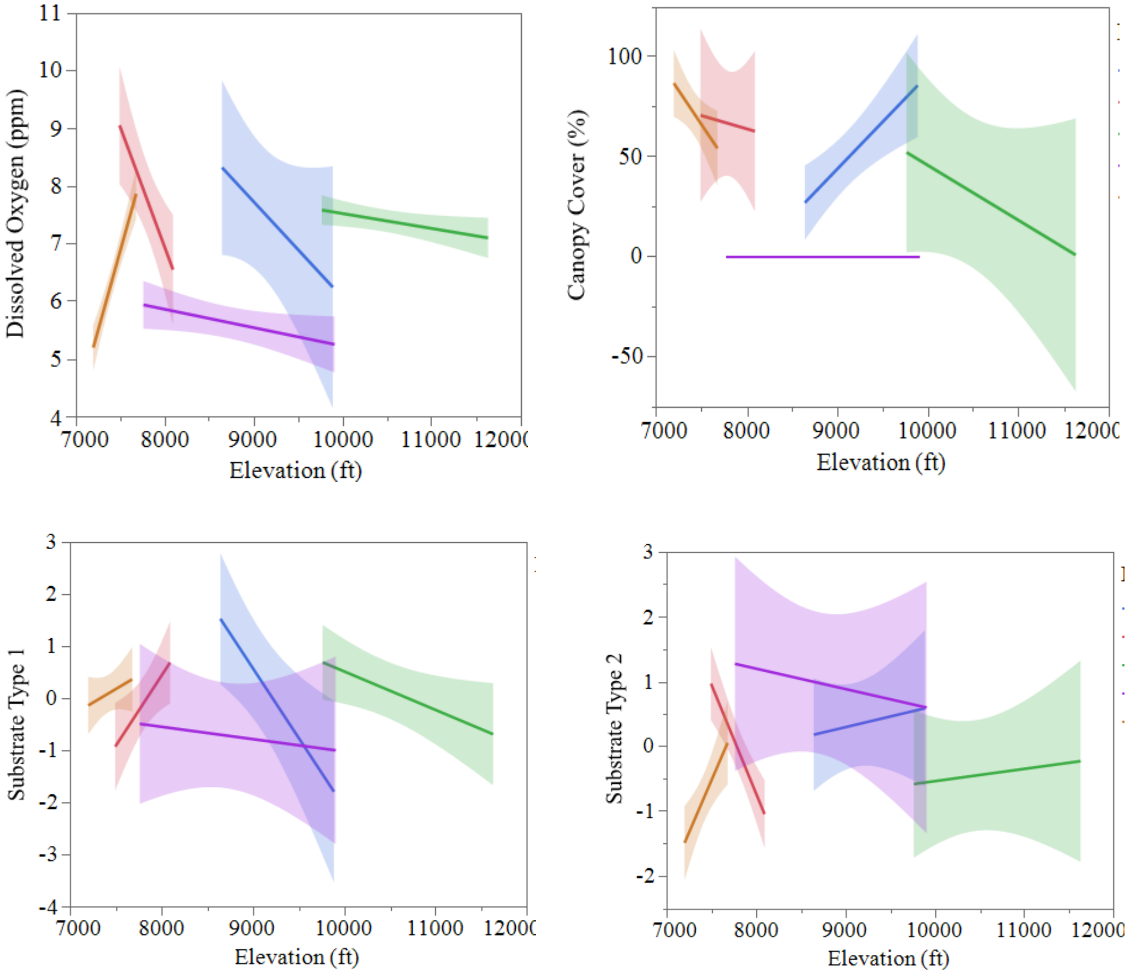

Figure 1.5 (Continued).

Legend

Residential Low Recreation High Recreation Ranching Mining

Discussion

Anthropogenic Land Use and Functional Guilds

We found that different types anthropogenic land uses influence benthic macroinvertebrate communities in different ways along an elevation gradient in their abundance, biodiversity, and functional guilds. This is consistent with other worldwide research on macroinvertebrate communities that has shown that anthropogenic influences cause both short-term and long-term disturbances to streams (Baumgartner & Robinson 2017; Manfrin et al. 2013; Murphy & DavyBowker 2005). As we predicted, ranching and mining land use had the lowest abundance for most guilds, except tolerators and motile clingers, and lowest overall abundance and biodiversity measures. Less disturbed land uses such as low recreation and residential sites had the highest diversity, abundance, and abundance of guilds. Low disturbance land use, such as recreational areas, typically have short-term impacts if any on stream invertebrates, since the chemical and physical composition does not change (Escarpinata et al. 2014; Ikomi & Arimoro 2014), as opposed to ranching and mining land uses. These sites were also used for fishing activities, which would require a strong community of macroinvertebrates as part of the food web for fish (González-Bergonzoni et al. 2014). However, we must also remember that low disturbed sites were located at low elevations while highly disturbed sites were located at high elevations. Our results have indicated that the statistical interaction of land use and elevation are important for understanding the influence on macroinvertebrate communities. But, in our analysis to reduce elevation confounding land use, we observed once again that highly disturbed land uses such as ranching and mining had the lowest biodiversity and abundance (Figure 1.4).

The functional trait guilds we used allowed us to identify how functionally similar taxa responded to the land uses we tested. It was unexpected that the functional guilds followed taxonomic categories as closely as observed (Appendix B Table S3) since functional traits include ecological roles, life histories, and morphology characteristics that are not always used in taxonomic categorization. It has been stated that taxonomic assessments of macroinvertebrates are not as descriptive as other means, such as functional assessment (Jones 2008). Although the results may be similar, a functional approach with descriptive results may improve freshwater monitoring and understanding, when used with other biodiversity or abundance measures. The use of function and biodiversity allows deeper understanding of ecological roles and trends, which can improve communication from scientists to land managers or legislators on the issue of conserving freshwater systems (Menezes et al. 2010).

One interesting result is that ranching at its highest elevations had somewhat lower biodiversity and abundance values for most indices or functional guilds than mining land use. This is interesting since ranching land use is usually categorized as agriculture land uses and viewed as a lesser pollutant than mining (Biggs et al. 2002; Freilich et al. 2003; McDowell & Magilligan 1997). Mining influences without preventative measures are known to dislodge sediments, disrupt substrate habitats, and cause toxic buildup on certain sediments, which will disrupt pH and substrate types (Brim & Mossa 1999; Jong-Yoon et al. 201 7), hence why diversity would be lowest at these sites. However, our results lead us to believe that ranching can be more detrimental than mining in some instances, although both have the lowest diversity of all land uses. It should be noted that the ranching sites were all active within the last year, with cattle ranching and other livestock activities, while mining activities were active within the last five years. The timing of activities may be an explanation for the differences in severity on the community, however, the impacts of mining last for many decades (Gray 1997), so it is unlikely that the community would be able to recover within five years of possible inactivity (Akcil & Koldas 2006). It is more likely that there is one or several environmental variables influenced by ranching which negatively influence the community.

However, not all functional guilds responded as expected to areas of mining and ranching. We found that motile clingers, which are comprised of sensitive EPT taxa actually increased with elevation along mining sites. Our other two EPT groups, swimmers and sessile clingers, declined in mining conditions as expected. Of the EPT guilds, motile clingers are the only group to lack a preferred habitat type (Table 1.2). It is possible that since mining changes sediment and causes build up on the natural substrate, EPT taxa reliant on certain habitats are not able to survive while motile clingers are able to survive in variable habitat types. It should also be noted that of all guilds, motile clingers were the least abundant overall, so the small sample size may mask the true response of these insects. However, we can also see tolerators respond positively to ranching and mining, with highest abundance in those streams regardless of elevation. Taxa such as midges, worms, and other dipterans in our tolerators group are known to be tolerant to pollution or disturbance (Young-Seuk et al. 2003; Compin & Céréghino 2003) which allows them to fill the unwanted niches in disturbed environments (Nussle et al. 2015).

Environmental Variables

We observed sediment changes, and shifts in pH, conductivity, dissolved oxygen, and water temperature with both use and elevation. While the effect of elevation or stream disturbance individually on these has been previously documented, we believe we are the first to identify the interactions between the two. This suggests that the negative influences on biodiversity and function of insect communities from mining and ranching are likely due to their strong influences on chemical and physical properties of the environment. Previous research on mining and ranching at low elevations (below 5,000 ft) that found that high disturbance land uses such as ranching or mining changed chemical properties and microhabitats of streams (DeNicola et al. 2016; Steinman et al. 2003), similar to our results at high elevations.

Ranching may cause low abundance, biodiversity, and functional guild abundance because it completely lacks canopy cover at all sites and replicates (Figure 1.5). Canopy cover has been found in recent research to be a strong variable in influencing high elevation macroinvertebrate communities (Gutiérrez et al. 2018). Within our own research, we also found that ranching had no canopy cover, low dissolved oxygen which decreased with elevation, and higher water temperatures than other sites. This same trend can be observed with mining but not as obvious, as canopy cover decreases in mining sites, dissolved oxygen decreases and water temperatures increase, even at high elevations. These three variables may be related to each other since a lack of canopy cover removes a food source from macroinvertebrates and removes natural temperature regulation for streams. Streams with removed canopy cover are vulnerable to erosion, hydrology changes, and increased temperatures (Brooks et al 2005; Logan & Brooker 1983) which then leads to less dissolved oxygen, since warm water carries less oxygen than cold water. The lack of canopy cover, oxygen, and increased temperatures pose a threat to sensitive EPT taxa, such as motile clingers and swimmers which rely on organic material as a food source or habitat (Table 1.2). Dissolved oxygen is also capable of slowing development of macronvertebrate taxa (Connolly et al 2004; Lowell & Culp 1999), especially those who are univoltine such as sessile clingers, which already develop slowly in comparison to other taxa, which would further hinder these taxa from emerging on time for reproduction (Harper & Peckarsky 2006; Flannagan & Lawler 1972).

We also found that substrate type 1 may be influential of stream biodiversity and functional abundances. Ranching had generally low values of substrate type 1, which indicated it was comprised of fine sediment. Mining and high recreation sites also decreased from course and organic sediments to fine sediments. Not only does this indicate higher elevations might have finer sediments, it could be a reason why ranching has low abundance of certain guilds and low biodiversity. Organic and course materials are required for motile clingers, swimmers, and sessile clingers, all three EPT guilds, as a food source or habitat, which likely explains why these guilds were less abundant in these land uses. Degradation or lack of course substrate types have been found to negatively influence macroinvertebrate assemblages (Buss et al. 2004), since taxa rely on organic materials to hide from predators or use as a food source (Culp et al. 1983). However, residential land use had increased dissolved oxygen and high percentage of fine and organic substrates over the elevation gradient.

We also found that oxygen responded to an interaction between elevation and land use. With previous literature there was disagreement about the relationship between elevation and dissolved oxygen, our research has shown that dissolved oxygen is not just dependent on elevation, but also land use or disturbance. An important implication of this research is that macroinvertebrate research in high elevation environments must consider not only elevation gradients, but also land use or disturbances (Chapter 2).

We found that the interaction of land use and elevation causes changes to certain environmental variables such as canopy cover, dissolved oxygen, water temperature, substrate, and many more variables, which may be the cause of changes in the diversity and function of macroinvertebrate communities. The effect of land use is influenced by elevation, through different associations of changing environmental variables, which may be beneficial or disadvantageous for diversity or function of invertebrates. As the influences of climate change occur and advance, sensitive systems such as those at high elevations will need to be monitored. The unpredictability of climate change and its effects on freshwater systems make it important? to collect baseline data and understand current influences and how we can mitigate those changes. With this research, we have found that streams impacted by ranching land uses require further protection and rebuilding of canopy cover. This information is important for land managers in understanding how to restore or conserve the freshwater systems on their properties.

Chapter Two: Analysis of macroinvertebrate diversity and study design along elevation gradients

Introduction

Understanding how freshwater ecosystems respond to environmental gradients, such as elevation, is important because stream habitat and water quality are key elements of ecosystem health. Sampling benthic macroinvertebrates in streams has become a common method for researchers and governments to effectively study freshwater systems for stream health, conservation, and recovery (Resh and Rosenberg 1993). However, macroinvertebrate communities face a decline in diversity due to many factors such as pollution, invasive species introduction habitat degradation, and other disturbances (Dudgeon et al. 2006). These disturbances are known to directly influence stream conditions such as dissolved oxygen, pH, water temperature, and substrate type, all of which have found to negatively influence biodiversity of macroinvertebrates (Sharifinia 2016; Azrina et al 2006; Whiles and Wallace 1995). Environmental variables can also change with association to environmental gradients such as elevation (Jacobsen et al. 2003; Sandin and Johnson 2000).

Increased elevation is assumed to decrease diversity of macroinvertebrates because it influences many aspects of the environment, which may in turn affect diversity (de Mendoza et al 2017; von Fumetti et al 2017). For example, if the water temperature is colder at high elevations, it may cause metabolism and development decrease relative to warmer streams (Beracko and Revajová 2019; Fraley 1979), which causes some invertebrates to avoid this condition (White et al 2017). Dissolved oxygen is also influenced by water temperature; dissolved oxygen usually increases as water temperature decreases; however, dissolved oxygen decreases with elevation due to lower atmospheric pressure (Jacobsen 2020; Null et al. 2017). A decrease of oxygen in streams can decrease biodiversity, as some taxa require high oxygen levels to develop (Galic et al 2019; Chessman 2018). Elevation also introduces changes to the riparian habitat of streams, which then changes the substrate content. For example, streams above tree line will lack organic matter, which is what many macroinvertebrates rely on for their herbivorous or detritivorous diets (Cheney 2019). Since elevation has the capacity to influence so many aspects of habitat (Cárcamo et al. 2019; Alther 2019; Nieto Peñalver et al. 2017), it would make sense that macroinvertebrate diversity decreases as these resources become less preferable to invertebrates, and such comparisons have been made many times in the literature. It has been found in several studies that diversity does decrease with high elevation (Füreder 2006; Pringle and Ramirez 1998) but to our knowledge, before now there has not been a systematic review of this literature to determine if macroinvertebrate diversity consistently decreases with increased elevation.

High elevation environments are less likely to be influenced by human activities such as urban activity, highways, or agriculture than low elevation environments (Eisenlohr et al. 2013; Littell et al. 2010; Pedersen 2003). Unlike elevation, land uses that disturb freshwater ecosystems can influence more than just water temperature or dissolved oxygen. Land uses such as agriculture, mining, and damming can cause long-term or short-term changes to the hydrology and chemical composition of streams, including changes to pH, dissolved oxygen, nutrient availability, organic matter, substrate, and the addition of toxic materials (Burdon et al. 2019; Vishnivetskaya et al. 2011; Pardo et al. 1998). Therefore, any investigation of elevation differences must also account for any confounding disturbances in the area.

How studies are designed can also influence results. Approaches to studying macroinvertebrate assemblages in streams has changed over time. In the 1990's and early 2000's, government stream sampling and some large-scale research studies, rapid bio-assessments for invertebrates was often used (Carter et al. 2017). Rapid assessments allow researchers to collect macroinvertebrates, identify, count, and release macroinvertebrates while in the field, which allows for quick data collection but may lead to higher error in accuracy of identification and count of macroinvertebrates (Gillies et al. 2009; Hunnaford and Resh 1995). In recent decades, it has become more common to bring macroinvertebrate samples back to a laboratory for identification under high-powered microscopes, which allows for higher accuracy in macroinvertebrate identification and abundance counts (Moulton et al 2000; Blackwood 2007). Although laboratory analysis has become the norm, there is currently no standard for the number of streams surveyed, number of sites per stream, or replication per site, as these are determined by the researcher and could vary widely among studies. Well-replicated experiments can also be used to test local and regional effects, such as elevation (Underwood and Petraitis 1993; Hurlbert 1984). Biodiversity indices are especially dependent on sample size, thus replication, number of sites, and number of streams is important for comparing diversity among macroinvertebrate communities (Cao et al 1997; Downes and Hindell 2000). Although it is well known that replication is necessary, it is still overlooked in many different types of ecological studies (Ries et al. 2017).

Considering the variability in environmental influences and study methods, we conducted a systematic review of the literature to address the following questions: 1) Does diversity of invertebrates change along elevation gradients? Based on frequent assertions in the literature that elevation negatively influences biodiversity and presents a possible explanation for this pattern, we hypothesize that diversity will decrease with increased elevation. 2) Are there any other features of the study, such as human disturbance, that explain the observed diversity patterns? 3) How are studies on macroinvertebrates along an elevation gradient designed? Where are they taking place, how well replicated are they, and what diversity measures are being used?

Methods

In January 2020, we collected literature for our survey using the following databases: Biological Abstracts, Web of Science, Academic Search Complete, GreenFILE, and Aquatic Sciences and Fisheries Abstracts. We used the following search terms in each database: macroinvertebrat* AND benthic AND insect* AND (altitude OR elevation) AND (stream* OR river* OR riparian) AND (assemblage* OR communit* OR diversity). We did not limit the search to any date or time, exported all literature available, and found approximately 1,050 publications that we downloaded. We first eliminated those that were not focused on macroinvertebrates, elevation gradients, or diversity measurements, based on the title and abstract. This preliminary review left us with approximately 350 papers that appeared to be relevant and required in-depth reading of the methods and results to extract the data we needed. We then did a more in-depth sorting of the papers, removing studies from the review if they did not specify their elevation gradients or diversity metrices for each elevation or if the data were not clearly represented. We also excluded studies that lacked an elevational gradient more than 20m, since this is not a gradient large enough to observe the mechanisms which act on biodiversity with increased elevation (Hodkinson 2005). After this second round of sorting, we were left with 21 publications for the review. Many of these had measured more than one diversity metric, which allowed us to extract more than one case for each publication. We thus had 70 cases from the 21 publications. For each case, we recorded the paper author, year, country, mountain range, lowest and highest elevations in meters, number of streams samples, sites per stream, the diversity measure used, and the diversity at low vs. high elevations. When there were multiple elevations sampled, the lowest and highest elevation sites were used. For each diversity measure, we recorded whether diversity was higher at low elevation, high elevation, or if there was no change.

Most of these studies did not include standard error or variance, so a meta-analysis could not be used to analyze the data; instead we conducted a vote-count. However, we did use statistical analyses to investigate patterns in the literature, in which each observed pair of values (diversity in the high and low sites) from a case was used as an independent replicate; in most cases they did actually represent a single set of measurements (hence the lack of standard error). We normalized the diversity measurement variable with a logarithmic transformation. We then used paired t-test to determine whether there was a significant change in diversity between low and high sites. We also used a Chi-square goodness of fit with the native stats package in R-3.5.3 and RStudio to determine if our data differed from the expected hypothesis that diversity is higher in low elevations. We did a chi square test to see if pairs of sites considered natural (i.e., undisturbed by major human activity such as mining) were more likely to have lower diversity at high elevation, and also a general linear model to determine whether the dependent variable of taxa richness (the most common measure of diversity) was explained by individual sites being natural vs. disturbed (according to the authors), high or low elevation, or the interaction of these two independent variables. We did a logistic regression to determine if difference in elevation predicted the probability of lower diversity at the high-elevation site. Finally, we performed a chi-square to determine if high elevation sites were more likely to be undisturbed than low elevation sites.

Results

Studies that fit our criteria for inclusion took place across the globe with many different approaches taken (Figure. 2.1 A and B). Fourteen different diversity measures were used across the studies, with all but two studies using more than one diversity measure. Studies varied in sampling method, including number of elevation zones and replication: within sites, among sites, and number streams sampled (Figure 2.1 C, D, E, and F). Only 48% of studies had replication within their sites. The most common range of number of sites sampled were 6 – 10 (29%) or over 20 (29%) while the most common range of number of streams sampled was 1 – 5 (38%).

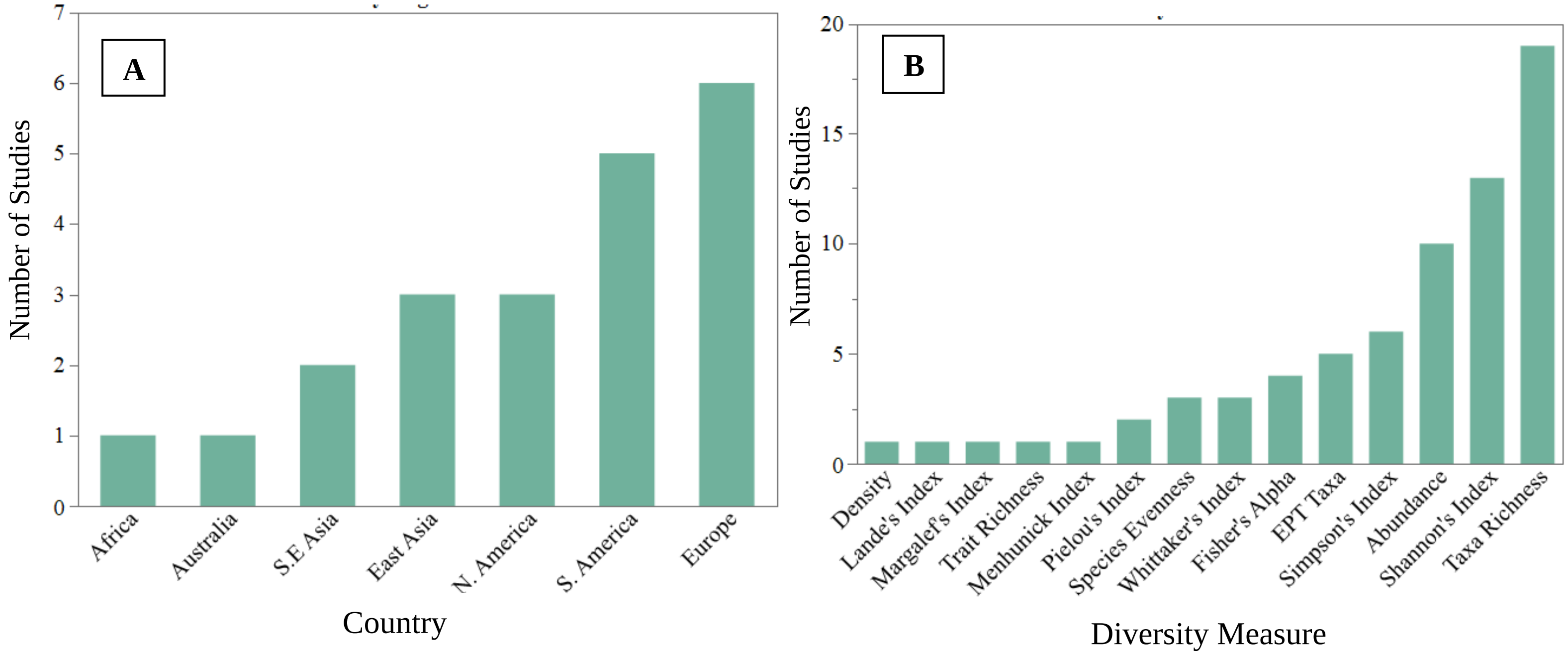

Figure 2.1. Number of studies for each of the following aspects: the study location (**A)**, diversity measure used in each study (**B**), distinct elevation zones (**C**), replication of sites (**D)**, sites sampled (**E**), number of streams sampled (**F**), and if the site was disturbed or natural at high and low elevations (**G**).

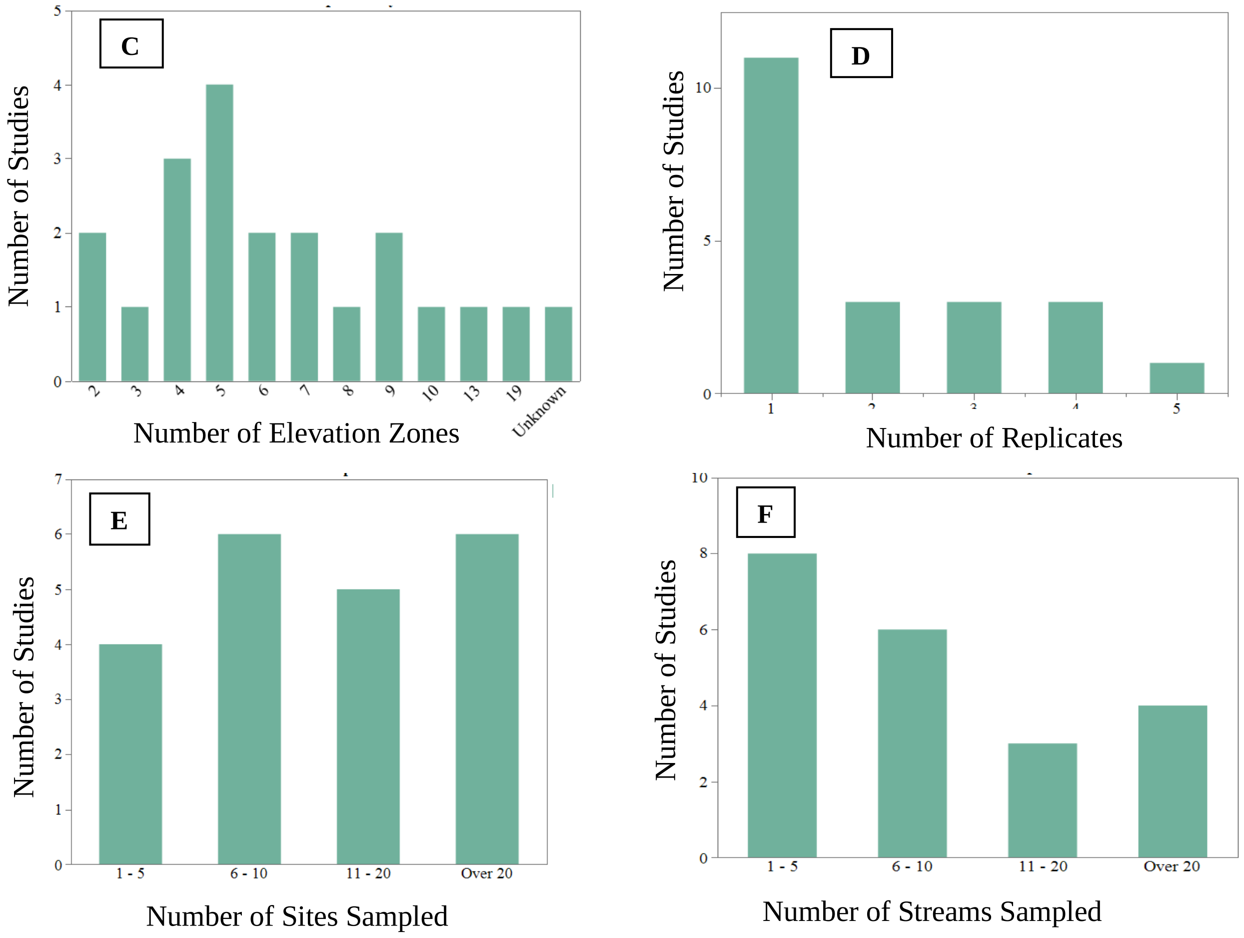

Figure 2.1 (Continued).

We found that the difference in diversity observed between the high and low elevation sites was not different from what you would expect by random chance ($t = -1.25$, $n = 68$, $p > 0.21$). We expected diversity to be highest at the lowest elevation sites, but this was statistically untrue ($x^2 = 87.68$, df = 2, $p < 0.001$) (Figure 2.2 A). High elevations included sites from 575 – 4500 m and low elevations included sites from 1 – 2965 m. However, difference in elevation between the high and low site did not explain likelihood of low diversity being found at the high elevation site ($x^2 = 2.43$, df = 2, $p = 0.30$).

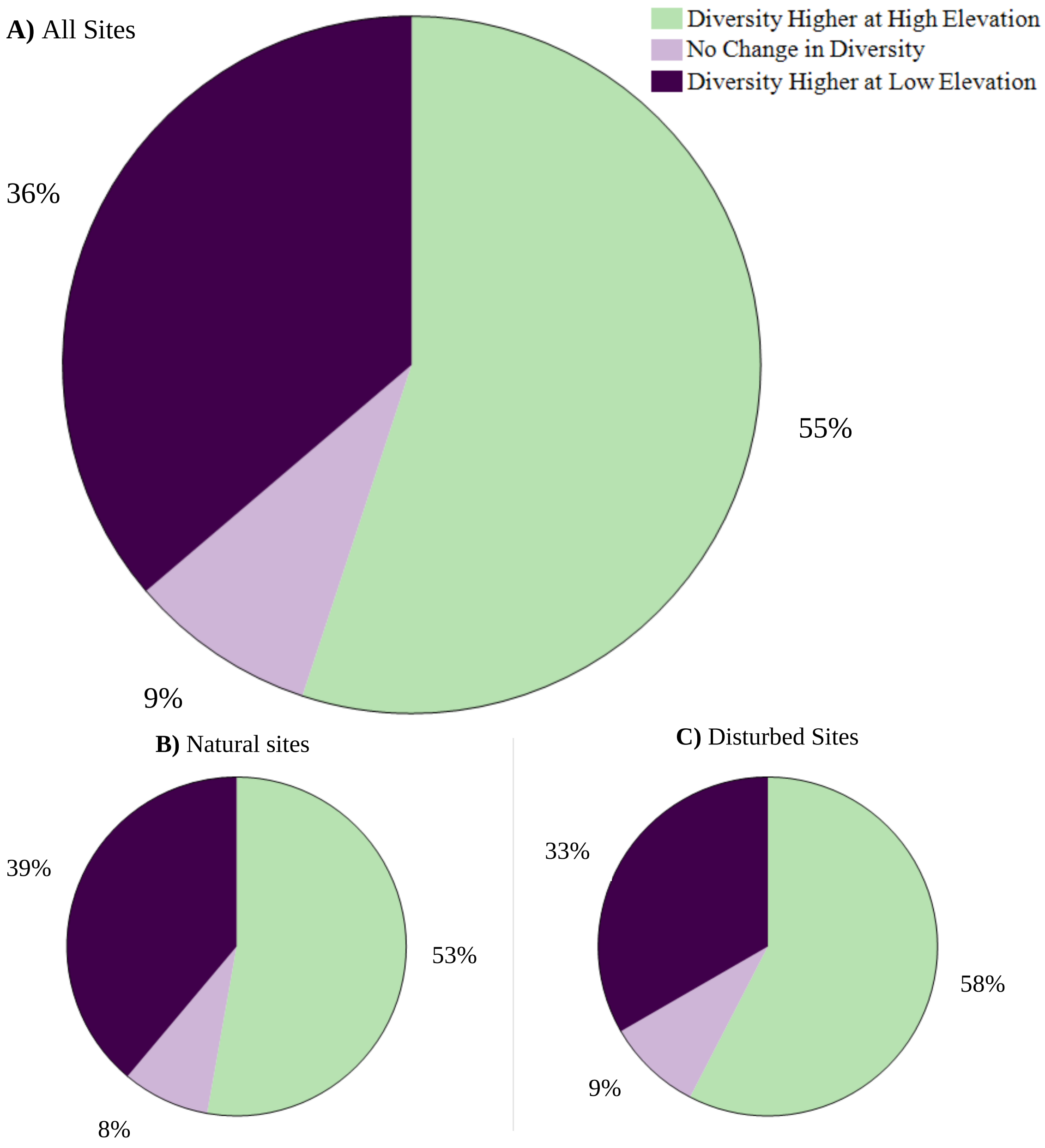

Figure 2.2. The percent of sites refers to which point, high or low, had the highest diversity among 70 different diversity measurements. Graphs refer to the following: (**A)** all sites, **(B)** only natural sites, **(C)** sites with one or both disturbed sites.

There were 4 types of disturbance in our studies, urban, damming, agriculture, and wastewater discharge, and while it was common for only one or the other to be disturbed, the incidence of disturbance did not differ between high and low sites (Figure 2.3). Diversity did not significantly differ between studies where both high and low sites were considered natural vs. those studies where one or both were considered disturbed (Chi Square = 0.23, n = 69, df = 2,69, p = 0.89) (Figure 2.2 B and C). However, when high and low sites were identified individually as natural vs. disturbed, taxa richness was found to be significantly (28%) higher than diversity at disturbed sites. Elevation did not explain species richness in that test. No other measurement of diversity could be tested in this way for the impact of disturbance because of low sample size.

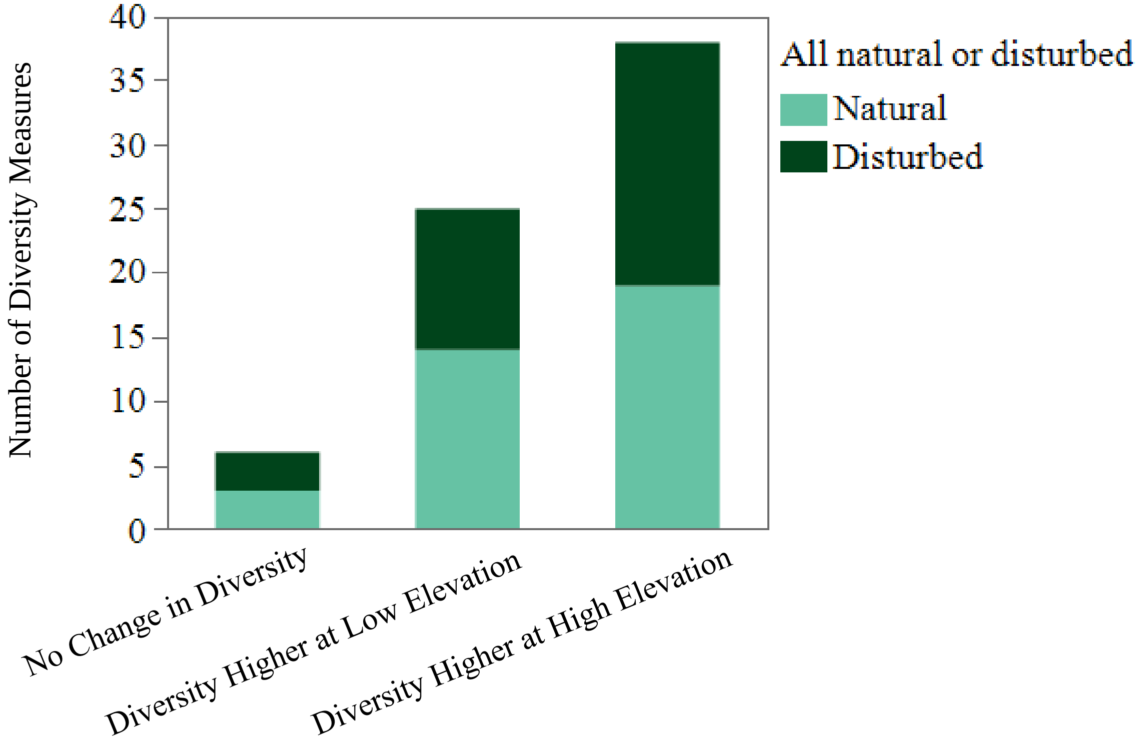

Figure 2.3. Number of disturbed or natural sites for each diversity measure and the change in diversity for each incident.

Table 2.1. Mixed model results for taxa richness (n = 18).

	F Ratio	**P**	**df**	**Mean**
Environmental condition	7.33	**< 0.01**	1,17	
Natural				3.48
Disturbed				2.72
Elevation	0.48	0.49	1,17	
High Elevation				3.35
Low Elevation				3.16
Environmental Condition * Elevation	1.14	0.29	1,17	
	R^2 = 0.17			

Discussion

It has been assumed that biodiversity of macroinvertebrates is lower at high elevation, but we did not find support for this hypothesis in our review. Our result was unexpected since elevation influences other variables, such was water temperature, which are known to make streams uninhabitable for some macroinvertebrate taxa. Instead, we found evidence that presence or lack of disturbance was more important than elevation for predicting species diversity. We found that study design varied, with many different types of diversity measures were used, and high variability in the number of elevation zones, sites, and streams sampled within research, but there was generally low replication within sites. This review thus both challenges a widely held idea and illuminates limitations of most previous studies.

Diversity

Effects of elevation on macroinvertebrate diversity appear to not be as direct or as strong as traditionally thought (Figure 2.2). It is possible that there were not enough studies in our sample where the lowest and highest study sites were there was a big enough difference for elevation to influence water temperature or dissolved oxygen. Depending on the season, water temperatures will increase by 0.3 – 1.2º C for every 1000 meters (Küry et al. 2017; Ficklin et al 2013) and so warm-water macroinvertebrate taxa will not survive at higher elevations where temperature decreases (Verberk et al. 2008; Batz and Marks 2005; Dudgeon 1993). However, the difference in elevation did not significantly predict the likelihood that the higher elevation site was lower diversity in our study, making this explanation less likely.

Our finding that disturbances such as those caused by dams, agriculture, or urban activities mattered more than elevation was consistent with our well-replicated research that found that disturbance significantly affects macroinvertebrate communities at high elevation (Chapter 1). Unlike elevation which indirectly influences dissolved oxygen and temperature, disturbances can have both direct and indirect effects on streams, with a plethora of chemical and physical changes to stream habitats on a small or large scale, depending on the type of disturbance (Rosser and Pearson 2018; McCabe and Gotelli 2000; Richards and Minshall 1992). Not only do these disturbances change the hydrology, but they also change the chemical properties of streams, nutrient content, pH, conductivity, and water temperature can also be negatively influenced (Neupane and Kumar 2015; Sharma and Wilson 2015; Al-Shami et al. 2011; Chowdhary 2011; Chapter 1).

Study Design

The studies we used have a wide global spread, but with only one study per continent in several cases. Different regions will have their own traits and differences in tolerance to environmental changes (Buss et al. 2015), however without multiple studies, it is difficult to say whether geographical differences may be important in influencing biodiversity results. Lack of replication for geographical location and within studies may also be the reason we did not find support for a strong relationship between diversity and elevation. We found that replication was neglected in over half of the studies used, which could explain our unexpected results. Replication is necessary for precisely and accurately measuring stream biota and should be modified based on initial field collection or observations and modified to ensure each sample is representative of the community (Elliott 1977; Resh 1979; Stark 1993). Although the importance of replication within sites is well-known (Hurlbert 1984), we found little evidence that replication is happening in high altitude studies on benthic macroinvertebrates within the literature. It is also possible that psuedoreplication is occurring in macroinvertebrate research since there is so much variability in stream or study sites while actual replication per site is lacking.

Although replication was lacking in most studies, all studies used at least two different diversity measures to analyze macroinvertebrate communities. This assures us that researchers are measuring different aspects of the community, since each index will differ slightly, increasing our knowledge of how the community responds to the environment. We also noticed that some research may be focused on studying many different sites or streams while neglecting per site replication. Standardizing replication within sites is the most valuable improvement for macroinvertebrate research in the future. Without replication, accurate biodiversity statistics are vulnerable to statistical errors.

To conclude, our results suggest that macroinvertebrate communities do not typically have lower diversity at high elevations in comparison to low elevations and that the large role of disturbance may be the reason. Based on what we found with these 21 studies, macroinvertebrate research should expand on natural or disturbed sites separately along elevation gradients when possible, to avoid having one or both sites influenced by variables other than those impacted by elevation. Replication within study sites must also be a priority along any elevation gradient. The lack of replication per site among our studies may have influenced our own results on the difference in diversity between high and low elevations. We argue that researchers should favor greater replication within sites over sampling many different streams or sites (Heino et al. 2003).

There have been widely accepted ideas that macroinvertebrate diversity within streams is lowest at high elevations due to environmental changes such as temperature or dissolved oxygen, but we did not find this. Instead, we found that disturbance is the force which changes diversity of freshwater communities. We also found that poor replication may be limiting our understanding of these communities. Replication is a basis of scientific understanding and it is a practice that should be a priority in all diversity studies, to ensure accuracy of results. We also must realize that elevation is not always an explanation for diversity, but disturbance can. We must continue to study different types and degrees of disturbance in order to understand how to conserve and restore threatened freshwater communities.

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Appendices

Appendix A: Supplementary materials in methods

Table S1. Diversity values and elevation of each stream.

Elevation (ft)	Stream Name	Simpson's Index	Shannon's Index	Taxa Richness	Abundance
8709	N. Beaver Brook	0.72	1.34	4.61	280
8065	Vance Creek	0.76	1.19	12.24	515
7218	Big Gulch	0.72	1.53	7.62	640
8700	Willow Creek	0.65	1.38	4.00	49
7858	Blue Creek	0.62	1.22	7.71	319
8424	Unnamed 1	0.80	1.83	5.72	184
7519	Last Resort Creek	0.57	1.17	13.23	2453
7867	Rock Creek	0.75	1.44	7.00	233
9832	Cascade Creek	0.79	1.63	5.33	83
9659	Ute Creek	0.28	0.60	7.00	126
9950	Sacramento Creek	0.65	1.14	5.31	315
9850	Middlefork Creek	0.25	0.82	4.00	94
9810	Unnamed 2	0.79	1.70	2.00	82
7500	Unnamed 3	0.80	1.80	9.00	349
7656	Unnamed 4	0.45	0.86	10.00	281
11548	Pennsylvania Creek	0.72	1.53	4.00	185

Table S2. PCA eigenvectors for each of the two substrate types.

	PC 1	PC 2
Woody debris	-0.18	0.60
Leaf pack	0.51	0.21
Silt	-0.19	0.56
Sand	-0.27	-0.07
Gravel	-0.26	0.16
Cobble	0.08	-0.44
Boulder	0.50	0.07
Bedrock	0.53	0.24

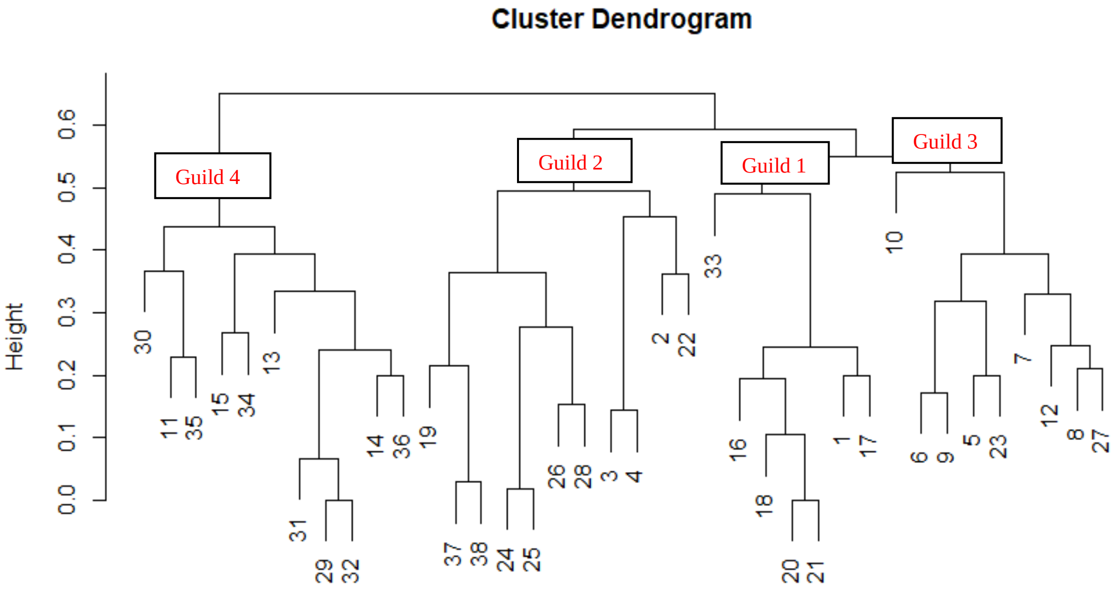

Figure S1. Within R, the cluster dendrogram was produced from the hierarchical cluster analysis. Each taxon of benthic invertebrate is indicated by a number from 2-38 which was used to identify which invertebrate taxa belonged to which guild.

Appendix B: Functional trait guilds taxonomic classification

Table S3. Taxa categorized to each functional trait guild by genus and order classifications.

	Guild 1	Guild 2	Guild 3	Guild 4
Genera	Ephemerella, Epeorus, Drunella, Cinygmula, Heptagenia, Rhithrogena, Asellus	Baetis, Podmosta, Triznaka, Sapada, Amphinemura, Sweltsa, Swala, Malenka, Pteronarcella, Ostrocerca, Prostoia	Isoperla, Actropsyche, Hydropsyche, Micrasema, Tipula, Optioservus, Odontomyia, Rhyacophila, Brachycentrus	Chironomidae (family), Oligochaeta (order), Planarian, Ceratopogonidae, Pericoma, Hexatoma, Antocha, Dicranota, Simulidae, Gammarus, Ferrissia
Orders	Ephemeroptera (Mayflies) and Isopoda	Ephemeroptera and Plecoptera (Stoneflies)	Plecoptera, Trichoptera (Caddisflies), Coleoptera	Diptera, Oligochaeta, Tricladida, Peracarida, Gastropoda