The Response of the Benthic Macroinvertebrate Community to New Step-Pools Sequences in Strawberry Creek

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ABSTRACT

A step-pool system is a common restoration practice for high-gradient streams integrating boulders and cobbles across the channel to form steps, the plunge into pools composed of finer sediments. To understand how benthic macroinvertebrate (BMIs) communities use habitat in a restored step-pool system in Strawberry Creek in Berkeley, CA. l answer the following questions: 1) What benthic macroinvertebrate taxa characterize the different step and pool habitats of the restored stream reach? 2) How do the benthic macroinvertebrate communities in the restoration areas differ from natural step-pool areas? 3) How does seasonality affect the distribution of benthic macroinvertebrate taxa? I collected the macroinvertebrates from 8 step-pool pairs and 3 riffle in fall, and 6 step-pool pairs and 1 riffle in spring. I found no significant difference in most of the bioassessment metrics among steps, pools and riffles except the % contribution of dominant taxon from Kruskal—Wallis test. The bioassessment metrics in the restored step-pool sites were generally similar to the natural step-pool sites from two-sample t-test. To determine how similar of different sampling sites were in terms of benthic assemblage under four variables: habitat types location, seasonality and restoration status, I used a 3-dimesnional non-metric multidimensional scaling analysis. I observed only season (p= 0.001), location (0.005) and restoration status (0.011) were the significant factors that determined the distribution of macroinvetebrate taxa among different sites. The higher similarity of macroinvertebrate taxa between restored sites and the South Fork, and the presence of sensitive EPT species both suggested the degraded habitats have been restored.

KEYWORDS

River restoration, bioassessment, habitat complexity, geomorphology, urbanization

INTRODUCTION

Water covers approximately 71 percent of the Earth's surface, however only 3.5 percent of the total freshwater stored in glaciers, rivers and lakes supports life and is accessible for human use (Shiklomanov 1993). Despite these very limited freshwater resources and the need to protect such essential resources, a recent national assessment determined that less than one-third of the US streams were in good condition (Paulsen et al. 2008) and more than half of the national streams and rivers are in poor condition for aquatic life (EPA 2013). Deforestation of riparian zones, increased impervious surfaces, and altered channel morphology are the main watershed stressors sources that degrade water quality in urban streams and rivers (Walsh et al. 2005, Wenger et al. 2009). These land-use changes result in higher stream temperatures, increased toxins, nutrient inputs and benthic instability, and decreased leaf litter standing stock, which further alters the composition of the original ecosystem and affects species interactions, especially the sensitive macroinvertebrate taxa (Chadwick et al. 2006, Kenney et al. 2009).

River restoration projects have rapidly increased in recent years because of growing public awareness of anthropogenic impacts on natural systems from urbanization (James and Marcus 2006). Restoration has expanded to more river types and evolved to promote channel-floodplain connectivity and process-based restoration (Wohl et al 2015), which focuses more on restoring the physical, biological and chemical drivers of ecosystem function and dynamics than simply engineers channels or habitats (Beechie et al. 2010). Traditional engineering approaches to reduce channel incision and prevent sediment deposition downstream usually results in building check dams and armoring the streambed or streambank with concrete materials (Kauffman et al. 1997, Castillo et al. 2007). But these approaches actually do little to help the stream regain ecological integrity, and often harm the stream environment over the long-term by suppressing or stopping the recovery of riparian vegetation and causing erosion downstream (Kauffman et al. 1997, Castillo et al. 2007). Thus, the naturalistic and ecologically-friendly engineering approaches such as step-pools have become more popular and blend in with the ecosystem aesthetics (Lenzi 2002).

Because bank erosion in high gradient is so problematic, step-pool sequences increasingly use in river restoration. Step-pool sequences integrate boulders across the channel to form steps, followed by plunge into pools. The alternation between step and pool forms a sediment size contrast and a staircase-like longitudinal profile that serves as an energy dissipater and functions as a hydraulic resistance and minimizes the kinetic energy used for erosion and sediment transport (Chin 2003, Chin and Wohl 2005). Apart from dissipating the water flow energy, the development of step-pool system can enhance stream habitat by creating heterogeneous environment that provides greater surface area, more physical refugia from predation or disturbance, and higher or more diverse supplies of limiting resources (Chin et al. 2009). Indeed, the benthic macroinvertebrate abundance in streams with step-pool system is much higher than the streams without step-pool systems due to the increased habitat diversity for the biocommunity (Wang et al. 2009). Hence, step-pool systems have gradually replaced the traditional engineering approaches that only focus on stopping erosion and scour.

The University of California Berkeley recently has restored a reach of Strawberry Creek with a step pool system based on the advantages of the step-pools for high gradient streams. In 2014, a stable bank slope in Eucalyptus Grove, which is the confluence of the North and South forks of Strawberry Creek, was developed by removing two old failing check dams and installing two step-pools and one log drop structure, and revegetating the bank slope (Massell et al. 2014). This restoration approach is expected to bring positive impacts to the habitat and increase stability of the ecosystem. A recent campus study showed the significant improvement to the benthic macroinvertebrate assemblage remained to be determined and required further post-project monitoring (Poniatowski 2015). However, Poniatowski (2015) only focused on the larger downstream changes in the benthic macroinvertebrate assemblages instead of assessing the habitat complexity and species diversity in the restoration site. The taxa that live in the restored step-pool habitats and the similarity of the species diversity in the restored step-pools compared to Strawberry Creek's natural step-pools and similar structures, is currently unknown.

Benthic macroinvertebrates can serve as biological assessment tools to determine the effectiveness of step-pool restorations for creating habitat for aquatic organisms. The various biomonitoring metrics for macroinvertebrate community including richness measures, composition structure, tolerance or intolerance measures and feeding measures can assess aquatic ecosystem health and impairment (Barbour et al. 1999). The advantages of using benthic macroinvertebrates as an indicator are: 1) They are abundant in most stream and responds to environmental perturbations because live on the bottom of streams constantly in contact with pollutants, and receive successive exposure (Beasley and Kneale 2002). 2) Benthic macroinvertebrates have limited migration patterns and long life cycles that cannot escape from the pollutantion events (Griffiths 1991, Lammert and Allan 1999). 3) The large numbers of species resident in streams constitute a wide range of trophic levels and pollutant tolerance (Barbour et al. 1999, Bae et al.2005). 4) Benthic macroinvertebrates are easily sampled and identified (Gerth and Herlihy 2006). Therefore, the biological metrics of benthic macroinvertebrates can be used to assess the water quality and to frame stream regulated management and restoration (Gore et al. 2001)

The objective of this study is to determine how benthic macroinvertebrate communities live habitat in a restored step-pool system in Strawberry Creek in Berkeley, CA. I answer the following questions: (1) What benthic macroinvertebrate taxa characterize the different step and pool habitats of the restored stream reach? (2) How do the benthic macroinvertebrate communities in the restoration areas differ from natural step-pool areas? (3) How does seasonality affect the distribution of benthic macroinvertebrate taxa? I hypothesize that macroinvertebrate communities in the restoration site will be similar to those in natural step-pools sites because the degraded area has been restored. In addition, I expect higher species diversity occurs in steps habitats than in other stream habitats because of the increased habitat complexity in step.

METHODS

Study site

Strawberry Creek is an urban stream approximately 8 kilometers long located at 37.8808 N, 122.2317 W (Hans and Maranzana 2006). Its source begins above the University of California, Berkeley campus, passes through the campus, Berkeley, the city of Berkeley, CA, and debouches into the San Francisco Bay (Purcell et al. 2002). The stream comprises of two branches: the North Fork and the South Fork. These two braches converge and form the main stem in central campus before flowing under the city of Berkeley. Strawberry Creek is a high gradient stream with a slope of 9% in the upstream canyon area and a slope of 3% in the main campus (Charbonneau 1987). Streams with a high velocity and steep gradient are likely to develop streambed incision and subsequent bank erosion easily (Castro 2003).

Strawberry Creek was highly degraded by the end of the 1900s as a result of urbanization, channel alternation and chronic pollution. The first effective management plan and restoration projects dated back to 1987 in response to the deteriorated environmental quality of Strawberry Creek (Charbonneau and Resh 1992). In the past, streambeds and stream banks of Strawberry Creek were covered by concrete to stabilize channels and eroding banks, and reduce channel incision. Check dams were built to prevent further streambed incision and subsequent bank erosion (Charbonneau and Resh 1992). However, recent restorations not only focused on preventing bank erosion, but also focused on improving the ecological condition of the stream. In 2014, the campus removed two failing large check dams near the confluence and installed two new step-pool structures (Massell et al. 2014).

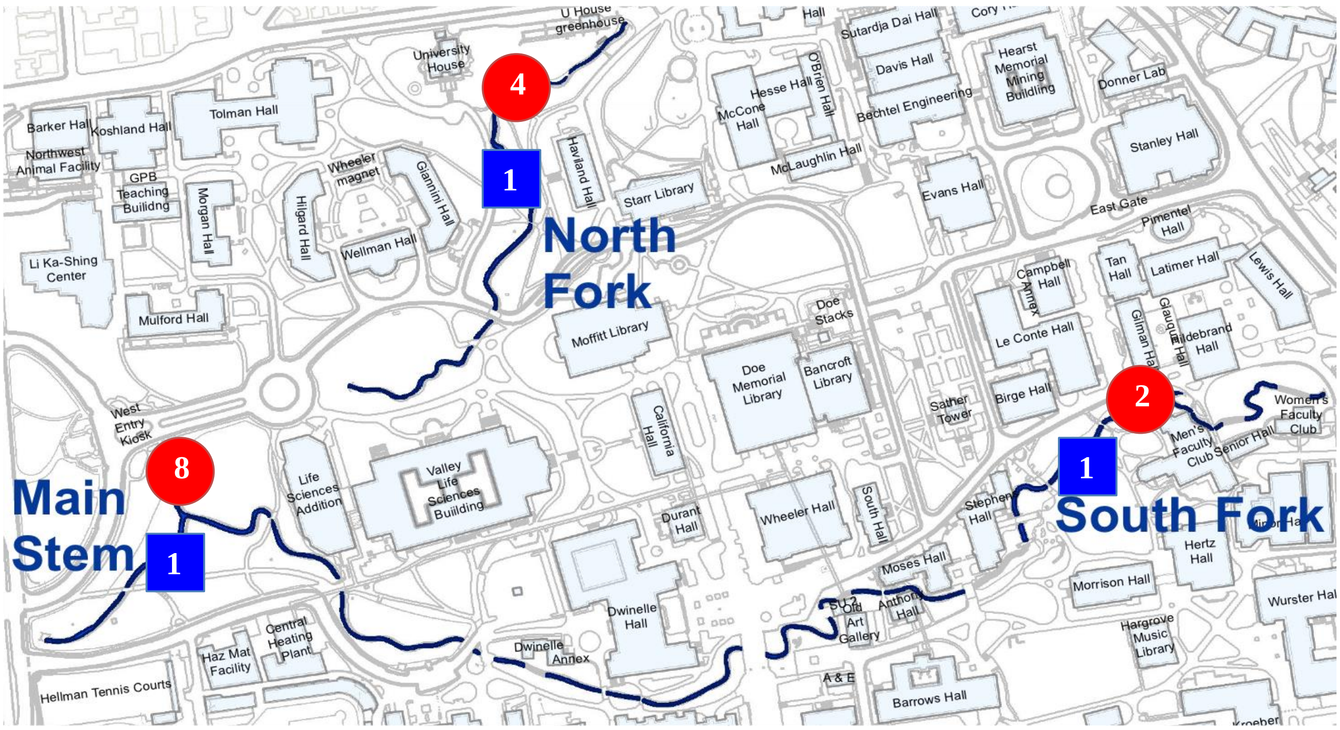


Figure 1. Map of the Strawberry Creek. The 17 sampling sites are shown above: red circles represent the number of step and pool habitats and the blue squares represents riffle habitats

Data collection

Habitat assessment

Before collecting the benthic macroinvertebrates, I did a visual habitat assessment (Barbour et al. 1999) on 6 December 2015 for each sampling site because the physical environment of the habitat and the biological diversity in rivers are closely linked. For 4 reaches longer than 15-20 meters, I completed the Habitat Assessment Field Data Sheet For High Gradient Streams. I evaluated 10 parameters: epifaunal substrate/ available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alternation, frequency of riffles, back stability, vegetative protection and riparian vegetative zone width. I also completed a field data sheet for the microhabitat assessment that only focused on the inorganic substrate components. I rated each habitats assessment metrics from 0 (poor) to 20 (optimal) and recorded the microhabitat assessment condition in detail.

Collecting macoinvertebrates

To determine benthic macoinvertebrate habitat use in restored step-pools systems in Strawberry Creek, I collected the macroinvertebrates from 8 step-pool pair sites and 3 riffle sites using modified protocols from the California Stream Bioassessment Procedure (CDFG 2003) on 16 October 2015. In spring, I resampled the macroinvertebrates from 4 step-pool pairs and 1 riffle site in the main stem on 23 March 2016. I then sampled the macroinvertebrates from the same step-pool pair in North and South forks again on 7 April 2016. Because sampling would disturb the sediment and benthic macoinvertebrate and affect the downstream water quality, I sampled the main stem (downstream location) first, then sampled the North Fork, and last, sampled the South Fork (upstream location). In total, I collected 17 samples along Strawberry Creek in fall: 9 samples from the main stem, 5 samples from the North Fork and 3 samples from the South Fork (Figure 1). In spring, I collected a subset totaling 13 samples: 9 samples from the main stem and 2 samples from North and South forks.

Sampling in riffles

I used a 500-micrometer D-frame net with a long hardwood handle to sample three 1-meter cross-sections of stream at each riffle site (main stem, north fork and south fork). I placed the net at the bottom of the stream and kicked the sediment immediately upstream of the net while wearing rubber boots to disturb the macroinvertebrates to float up into the water and into the net. I repeated the kicking three times per meter and waited for 30 seconds after each kick. As a result, there were a total of 9 kicks per riffle site. After 9 kicks, I rinsed the sample through the 500-micrometer sieve and stored the material in 95% ethanol.

Sampling in steps

I used a 500-micrometer flexible net. Instead of the rigid D-frame, the flexible net had a piece of thick tubing as support that allowed the net to be pressed closely to an irregular rock surface. To sample, I agitated, rubbed and moved the rocks on top of the step by hand for one minute to disturb the water and organisms into the net, while my sampling partner held the net near the downstream side (Velasco 2013, O'Dowd and Chin 2016). We sampled all the parts of the steps to ensure collection of benthic macoinvertebrate from the step. I then rinsed the sample through the 500-micrometer sieve and stored it in 95% ethanol.

Sampling in pools

I used the 500-micrometer D-frame net with a long hardwood handle and swooped through the water for a minute to disturb the pool materials. I then rinsed the sample through the 500-micrometer sieve and stored it in 95% ethanol.

Sorting and identifying the sample

In the laboratory, I rinsed the samples through the 500-micrometer sieve and transferred them into a white sorting tray with water. I sorted the samples by eye and put them into small petri dishes using forceps. I then used McCafferty (1983), a taxonomic key for larval benthic macroinvertebrates, and identified the insects in family level under the microscope. For the noninsects, I used Harrington and Born (1999) and identified individuals to order level. After sorting, identifying and counting the individuals, I labeled all the taxa and stored each taxon separately in a 5-dram vial with 75% ethanol.

Data analysis

To compare the data from riffles, steps and pools, I calculated biomonitoring metrics for each site including diversity and composition, richness measures, composition structure, tolerance/intolerance measures and feeding measures (Barbour et al. 1999). I used boxplots to visualize the biomonitoring metrics in three different types of habitats: step, pool and riffle. To determine if there are significant differences of benthic macroinvertebrate among three in-stream habitat types, I first tested for normality to check whether the data were normally distributed by Shapiro–Wilk test in R Commander (R Development Core Team. 2015). I used the Kruskal—Wallis test to determine which habitat type had higher or lower biometrics (diversity and composition, richness measures, composition structure, tolerance/intolerance measures and feeding measures) for fall sample. I used the two-sample t-test to analyze the difference between steps and pools in spring because I only sampled one riffle site and excluded it from the analysis. Furthermore, I performed two-sample t-tests between restored and the natural step-pool sites for both fall and spring samples to determine whether bioassessment metrics values in the restored step-pool sites were different from the natural step-pool sites.

To determine how similar of different sampling sites were in terms of benthic assemblage under four variables: habitat types (step, pool and riffle), location (South Fork, North Fork and confluence), seasonality (fall and spring) and restoration status (restored and natural), I used a 3-dimesnional non-metric multidimensional scaling (NMDS) statistical analysis in RStudio (RStudio Team 2015) using package vegan and function. The NMDS uses species and counts of species to characterize sites and arrange them in multivariate space. I coded the sites by the four variable arrangements. I examined the correlation between the NMDS axes and species abundance using the envfit function. To assess whether the habitats in the confluence has been restored, I separated the fall and spring samples and conducted another NMDS analysis, and examined the distribution by coding the sites to restoration status.

RESULTS

Physical habitat assessments comparison

When comparing the reaches in different sites using the EPA physical habitat scores, the south fork overall had better physical habitat for the aquatic organisms than the north fork and the confluence (Appendix A). The south fork scored highest overall (156) and the north fork scored lowest (124). The presence of invasive Algerian Ivy and extensive channelization in the North Fork resulted in channel alteration and vegetative protection scores at a suboptimal level (11-15) and (6-8). A year after the restoration, the vegetative protection and riparian vegetative scores in the restored confluence sites were the lowest among 4 sites, which were both at a marginal level (3-5). The confluence and the south fork both had an optimal condition in epifaunal substrate/ available cover (18, 14), organic matters embeddedness (17,16), diverse velocity/depth regime (15,18) and lower formation of sediment deposition (16,17).

As for the microhabitat assessments in the 17 sample sites, the most common inorganic substrate component in steps were boulder and cobbles; in pools were gravels and silts, and in riffles were dominated by gravels and cobbles (Appendix B). In contrast, I found less organic substrates including detritus, muck-mud and marl in restored sites than in the South and North forks.

Bioassessment metrics analysis

In-stream habitats comparison

I found no significant difference in most of the bioassessment metrics (Table 1, Figure 2) among steps, pools and riffles in fall by large p-values (Table 2), except the % contribution of dominant taxon (p= 0.034). Similarly, majority of the bioassessment metrics in spring between steps and pools were not significant different from the two sample t-test (Table 3). Only % predator abundance had a significant difference (p= 0.031) between steps and pools.

Table 1. Bioassessment metrics among 3 in-stream habitats. I calculated the fall and spring bioassessment metrics averages and standard deviations from steps, pools and riffles.

Bioassessment metrics	Season	In-Stream Habitat		
		Step	Pool	Riffle
Diversity and Composition				
Total Abundance	Fall	86.9 ± 82.2	74.7 ± 80.4	141 ± 196.9
	Spring	41.8 ± 40.6	102.5 ± 102.7	NA
Total EPT Individuals	Fall	1.3 ± 3.4	0.42 ±0.79	21 ± 36.4
	Spring	29.5 ± 18.9	13.5 ± 11	NA
Community Structure				
Family Richness	Fall	4.7 ± 2	4.4 ± 2	9 ± 4.6
	Spring	8.2 ± 3	6 ± 2.4	NA
EPT Richness	Fall	0.4 ± 1.2	0.4 ± 0.8	1±1.7
	Spring	2.3 ± 1.4	1.7 ± 0.8	NA
Composition Structure				
% EPT Abundance	Fall	3.7 ± 9.7	0.9 ± 1.6	5.6 ± 9.8
	Spring	37.7 ± 18.3	36.5 ± 7.8	NA
Ratio of EPT to EPT +C Abundance	Fall	0.2 ± 0.6	0.2 ± 0.4	18.1 ± 31.4
	Spring	1.1 ± 0.7	1.5 ± 1.3	NA
% Contribution of Dominant Taxon	Fall	59.1 ± 27.4	84.9 ± 10.3	45.8 ± 21.9
	Spring	51 ± 18.6	42.9 ± 13.2	NA
Tolerance/Intolerance Measures				
Family Biotic Index	Fall	6 ±0.7	6.3 ± 0.8	6.1 ± 0.4
	Spring	5.1 ± 0.8	5.1 ± 0.4	NA
Feeding Measures				
%Collector-Filter Abundance	Fall	7 ± 9.5	2 ± 5.4	1.6 ± 2.1
	Spring	2.4 ± 2.6	0.8 ± 2	NA
% Gatherer-Collector Abundance	Fall	76.6 ± 29.6	72.4 ± 43	71.4 ± 35.3
	Spring	83.4 ± 20.1	74.9 ± 23.4	NA
% Predator Abundance	Fall	11 ± 16	22.8 ± 36.1	17.6 ± 23.3
	Spring	2.7 ± 2.8	12.4 ± 10.9	NA
% Scraper Abundance	Fall	4.9 ± 11.8	2.2 ± 5.8	6.8 ± 11.4

	Spring	0.5 ± 0.8	2.2 ± 3.8	NA
% Shredder Abundance	Fall	0.4 ± 1.1	0.6 ± 1	2.5 ± 4.4
	Spring	11 ± 19.2	8.1 ± 11.8	NA

Table 2. Kruskal—Wallis test results in fall. I used the Kruskal—Wallis test to determine whether there were significant differences of different bioassessment metrics among steps, pools and riffles in fall.

Bioassessment metrics	p-values
	Three In-stream habitats
Diversity and Composition	
Total Abundance	0.963
Total EPT Individuals	0.726
Community Structure	
Family Richness	0.117
EPT Richness	0.777
Composition Structure	
% EPT Abundance	0.825
Ratio of EPT to EPT+C Abundance	0.726
% Contribution of Dominant Taxon	**0.034***
Tolerance/Intolerance Measures	
Family Biotic Index	0.854
Feeding Measures	
%Collector-Filter Abundance	0.390
% Gatherer-Collector Abundance	0.973
% Predator Abundance	0.827
% Scraper Abundance	0.341
% Shredder Abundance	0.726

Table 3. Two Sample T-Test in spring. I used a Two Sample T-Test to determine whether there were significant differences of different bioassessment metrics between steps and pools.

Bioassessment metrics	p-values

	Two In-stream habitats
<u>Diversity and Composition</u>	
Total Abundance	0.896
Total EPT Individuals	0.948
<u>Community Structure</u>	
Family Richness	0.903
EPT Richness	0.836
<u>Composition Structure</u>	
% EPT Abundance	0.558
Ratio of EPT to EPT+C Abundance	0.257
% Contribution of Dominant Taxon	0.798
<u>Tolerance/Intolerance Measures</u>	
Family Biotic Index	0.519
<u>Feeding Measures</u>	
%Collector-Filter Abundance	0.877
% Gatherer-Collector Abundance	0.743
% Predator Abundance	**0.031***
% Scraper Abundance	0.16
% Shredder Abundance	0.62

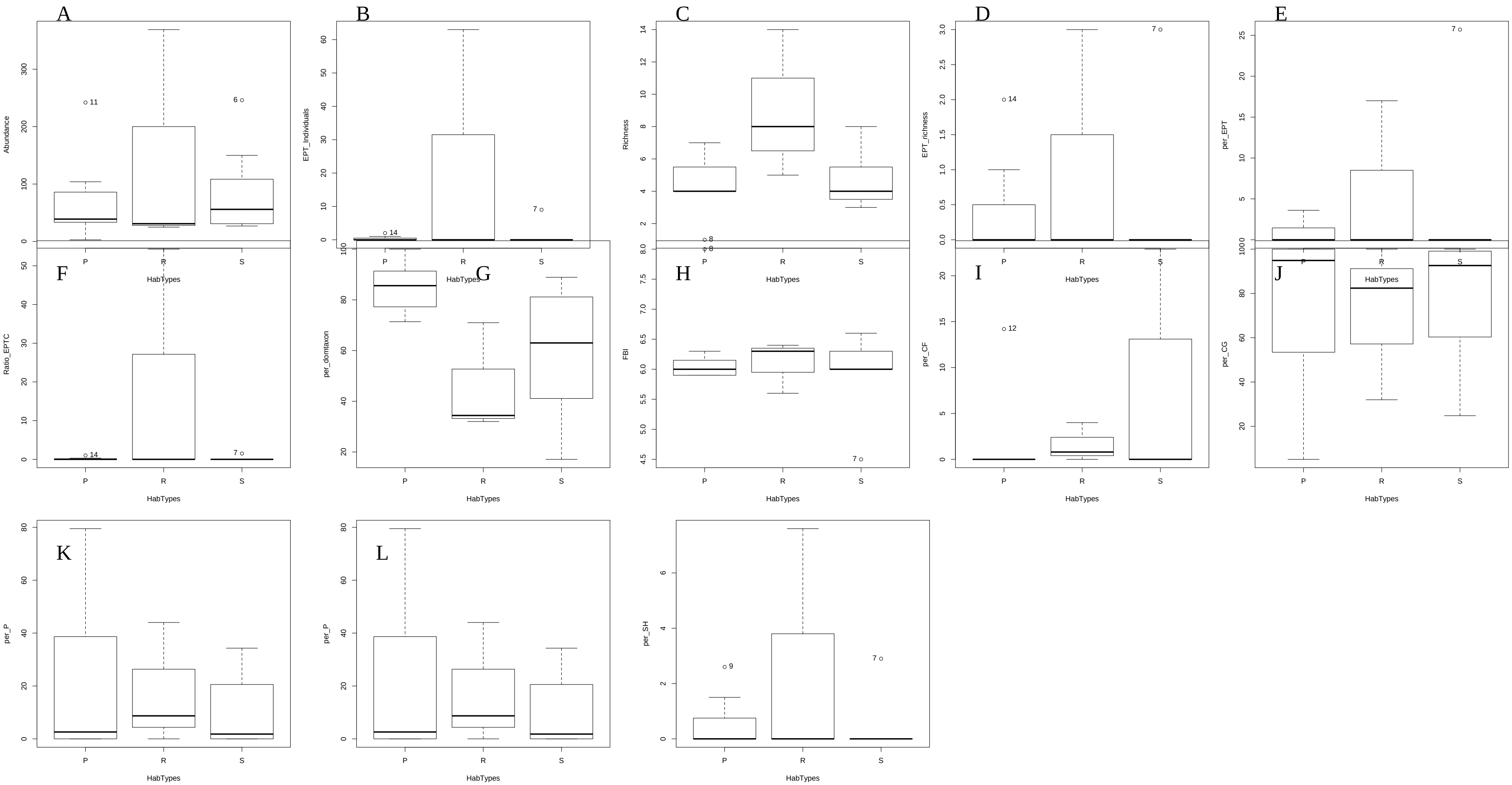


Figure 2. Boxplots of in-stream habitats bioassessment metrics values in fall. I used the boxplots to visualize the mean and the standard deviation of the macroinvertebrates found in step, pools and riffles with different bioassessment metrics. The in-stream habitats are displayed as: P(pools), R (riffles) and S(step). (A) Total Abundance (B) Total EPT Individuals, (C) Family Richness (D) EPT Richness, (E) % EPT Abundance (F) Ratio of EPT to EPT + C Abundance (G) % Contribution of Dominant Taxon, (H) Family Biotic Index, (I) %Collector-Filter Abundance (J) % Gatherer-Collector Abundance (K) % Predator Abundance (L) % Scraper Abundance and (M) % Shredder Abundance.

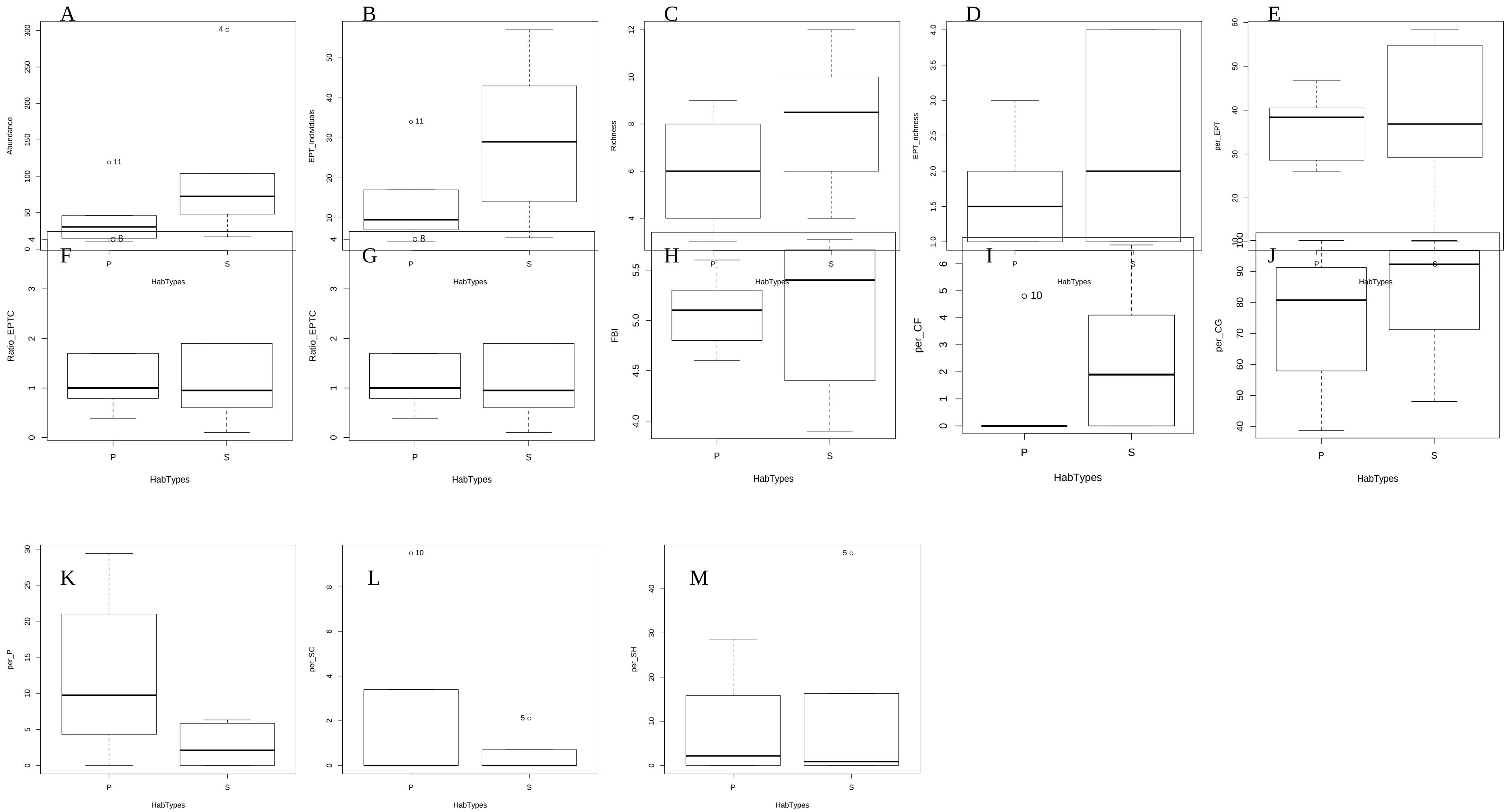


Figure 3. Boxplots of in-stream habitats bioassessment metrics values in spring. I used the boxplots to visualize the mean and the standard deviation of the macroinvertebrates found in step, pools and riffles with different bioassessment metrics. The in-stream habitats are displayed as: P(pools) and S(step). (A) Total Abundance (B) Total EPT Individuals, (C) Family Richness (D) EPT Richness, (E) % EPT Abundance (F) Ratio of EPT to EPT + C Abundance (G) % Contribution of Dominant Taxon, (H) Family Biotic Index, (I) %Collector-Filter Abundance (J) % Gatherer-Collector Abundance (K) % Predator Abundance (L) % Scraper Abundance and (M) % Shredder Abundance.

Restored and natural step-pool sites comparison

The bioassessment metrics in the restored step-pool sites were generally similar to the natural step-pool sites (Table. 4, Figure 4&5). I found a significant difference in family richness (p-value= 0.032), % collector-filter abundance (p-value=0.016) and % predator abundance (0.027) in fall (Table 5), while the other 10 bioassessment metrics were not significant by different between restored and natural step pool sites. Likewise, in spring, only % ratio of EPT+ C abundance (p-value= 0.037) and % predator abundance (p-value= 0.024) in restored sites were significant different to the natural step-pool sites (Table 5). I also served a significant difference in % shedder abundance (p=0.006), where shredders had a higher abundance in natural step-pool sites than the restored sites (Figure 5).

Table 4. Bioassessment metrics between restored and natural step-pool pairs. I calculated the fall and spring bioassessment metrics averages and standard deviations from restored and natural step-pool sites.

Bioassessment metrics	Season	Natural	Restored
Diversity and Composition			
Total Abundance	Fall	161 ± 107.5	162 ± 162.8
	Spring	145 ± 31.1	144 ± 140.9
Total EPT Individuals	Fall	5 ± 7.8	0
	Spring	63 ± 1.4	33.8 ± 22.3
Community Structure			
Family Richness	Fall	8.7 ± 3.2	4.8 ± 1
	Spring	12.5± 3.5	8.8 ± 3.5
EPT Richness	Fall	1.3 ± 1.5	0
	Spring	3 ± 1.4	2 ± 0.8
Composition Structure			
% EPT Abundance	Fall	4.9 ± 7.6	0
	Spring	44.6 ± 10.5	30.7 ± 12
Ratio of EPT to EPT	Fall	0.4 ± 0.6	0
+C Abundance	Spring	1.4 ± 0.4	0.7 ± 0.3
% Contribution of Dominant	Fall	47.7 ± 10.5	51.1 ± 34.4
Taxon	Spring	33.2 ± 2.1	52.9 ± 18.4
Tolerance/Intolerance Measures			
Family Biotic Index	Fall	6.3 ± 0.5	6.1 ± 0.1
	Spring	4.5 ± 0.1	5.5 ± 0.2
Feeding Measures			
%Collector-Filter Abundance	Fall	5.8± 4.1	0
	Spring	2.9± 4	1.6 ± 1.2
% Gatherer-Collector	Fall	53.7 ± 31.7	97.8 ± 3.1
Abundance	Spring	55.2 ± 19.7	94.3 ± 3.9
% Predator Abundance	Fall	27.3 ± 20.3	2.2 ± 3.1
	Spring	14.9 ± 9.5	2.3 ± 2.2
% Scraper Abundance	Fall	10.2 ± 16.8	0
	Spring	1.5 ± 2.1	0.4 ± 0.9
% Shredder Abundance	Fall	0.6 ± 1.1	0
	Spring	25.2 ± 12.6	1.1 ± 1

Table 5. Two Sample T-Test in restored and natural step-pool pairs. I used a Two Sample T-Test to determine whether there were significant differences of different bioassessment metrics between restored and natural step-pool sites.

Bioassessment metrics	p values	
	Natural and restored step-pool sites	
	Fall	Spring
<u>Diversity and Composition</u>		
Total Abundance	0.504	0.497
Total EPT Individuals	0.121	0.077
<u>Community Structure</u>		
Family Richness	**0.032***	0.142
EPT Richness	0.065	0.156
<u>Composition Structure</u>		
% EPT Abundance	0.119	0.121
Ratio of EPT to EPT+C Abundance	0.137	**0.037***
% Contribution of Dominant Taxon	0.562	0.886
Tolerance/Intolerance Measures		
Family Biotic Index	0.163	0.999
<u>Feeding Measures</u>		
%Collector-Filter Abundance	**0.016***	0.283
% Gatherer-Collector Abundance	0.098	0.993
% Predator Abundance	**0.027***	**0.024***
% Scraper Abundance	0.132	0.195
% Shredder Abundance	0.143	**0.006***

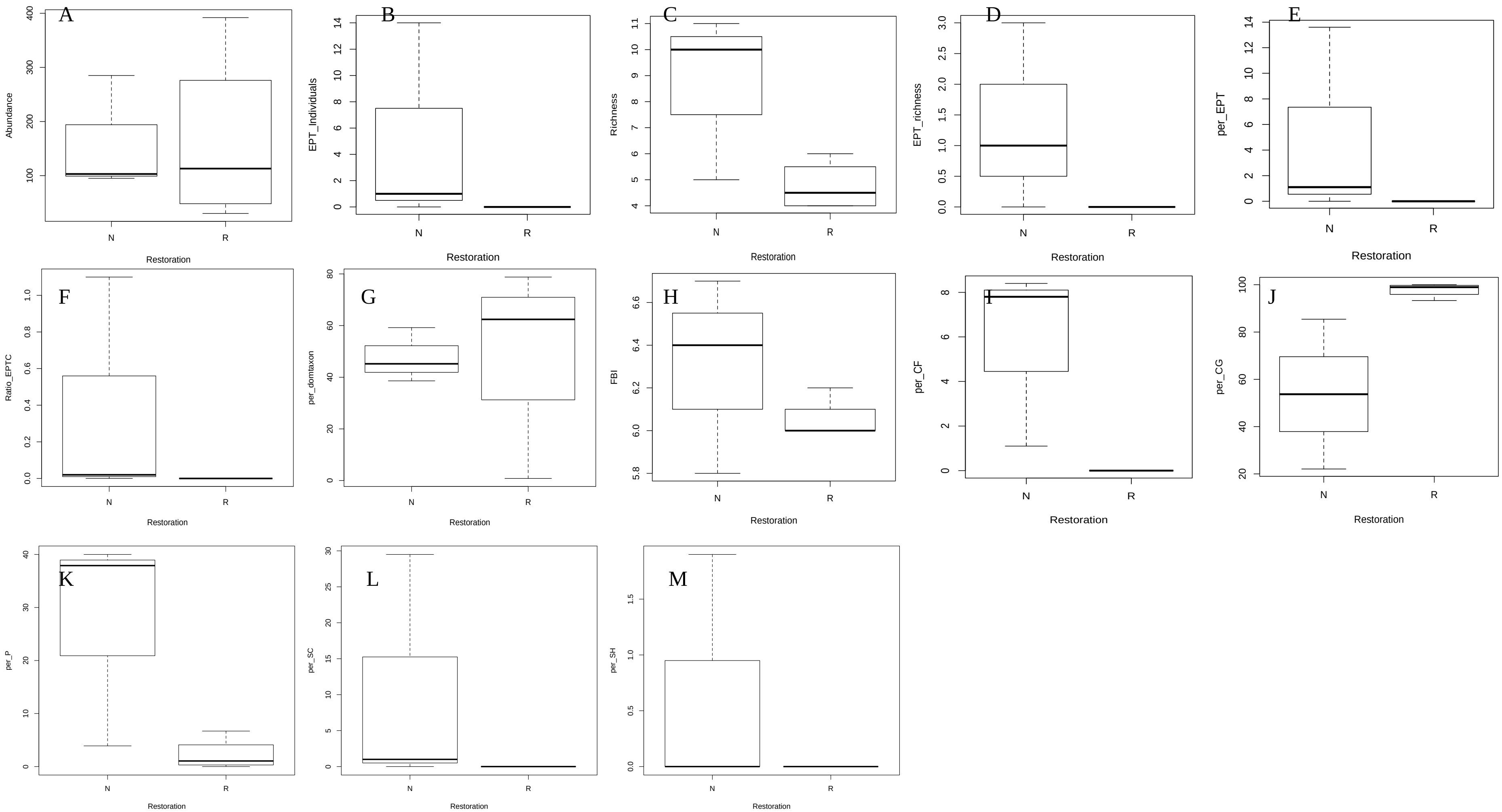


Figure 4. Boxplots of restored and natural step-pool sites bioassessment metrics values in fall. I used the boxplots to visualize the mean and the standard deviation of the macroinvertebrates found in restored and natural step-pool sites with different bioassessment metrics. It displayed as: N(natural) and R(restored). (A) Total Abundance (B) Total EPT Individuals, (C) Family Richness (D) EPT Richness, (E) % EPT Abundance (F) Ratio of EPT to EPT + C Abundance (G) % Contribution of Dominant Taxon, (H) Family Biotic Index, (I) %Collector-Filter Abundance (J) % Gatherer-Collector Abundance (K) % Predator Abundance (L) % Scraper Abundance and (M) % Shredder Abundance.

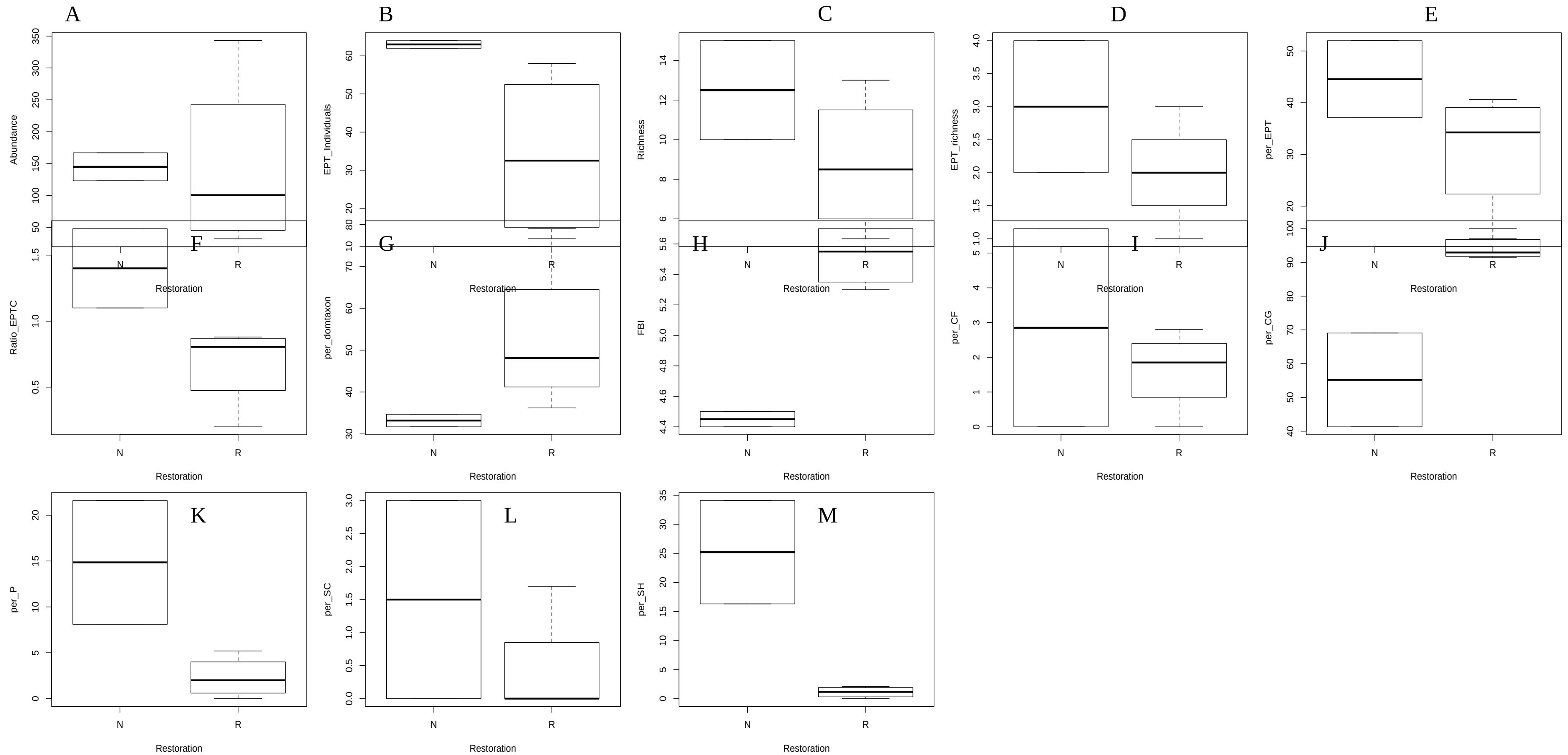


Figure 4. Boxplots of restored and natural step-pool sites bioassessment metrics values in spring. I used the boxplots to visualize the mean and the standard deviation of the macroinvertebrates found in restored and natural step-pool sites with different bioassessment metrics. It displayed as: N(natural) and R(restored). (A) Total Abundance (B) Total EPT Individuals, (C) Family Richness (D) EPT Richness, (E) % EPT Abundance (F) Ratio of EPT to EPT + C Abundance (G) % Contribution of Dominant Taxon, (H) Family Biotic Index, (I) %Collector-Filter Abundance (J) % Gatherer-Collector Abundance (K) % Predator Abundance (L) % Scraper Abundance and and (M) % Shredder Abundance.

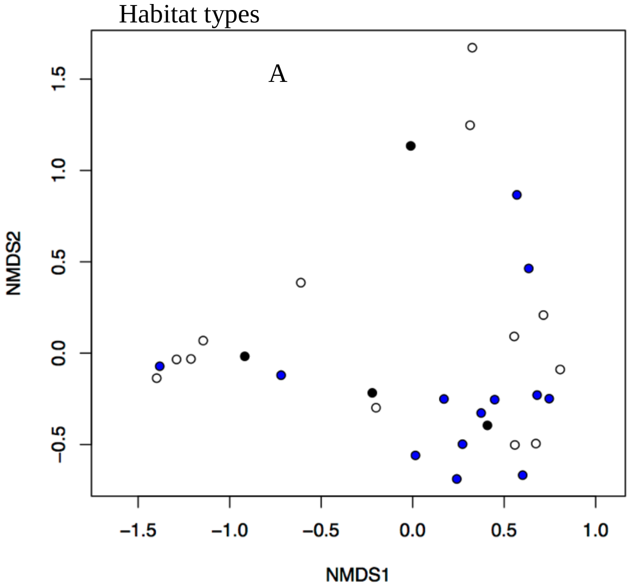
Nonmetric multidimensional scaling (NMDS) analysis

The stress value of the NMDS ordination was 0.078≤ 0.05, which indicated a good fit. The NMDS envfit analysis showed only season (p= 0.001), location (0.005) and restoration status (0.011) were the significant factors that determined the distribution of macroinvetebrate taxa among different sites, while habitat types (p value= 0.168) was not the significant factor (Table 6, Figure 6). Amphipoda and decapoda were the common taxa found in the confluence and pools; Ephemeroptera baetidae and Diptera chironomidae were heavily collected in the South fork and steps, and Odonata coenagrionidae and ancylidae mostly occurred in the North Fork. The fall and spring samples had distinctly differenct macroivetebrate community with a clear break along the x-axis. The macroinvertebrate taxa in the confluence were more similar to the south fork than the north fork whenever in fall or spring. Macroinvertebrate taxa in restored sites were more similar to the south-fork natural sites (Figure 6D).

In fall, most of the species were either permanently aquatic in all of their life stages (decapoda, ancylidae, amphipoda and ostracoda) or pollution-tolerant (chironomidae, coenagrionidae and simuliidae) (Figure 7). Both steps and pools in the restored sites were similar to the step and pools in the south fork. In spring, the pollution-sensitive species such as baetidae and peltoperlidae were found in restored and natural step-pool sites. Steps in the restored sites were similar to the step in the South Fork.

Table 6. NMDS results for fall and spring samples. I used NMDS to analysis whether a significant difference in marcoinvertebrates taxa distribution under 4 factors habita type, location, season and restoration status

Factors	p value
Habitat type	0.168
Location	**0.005 ****
Season	**0.001 *****
Restoration status	**0.011 ***



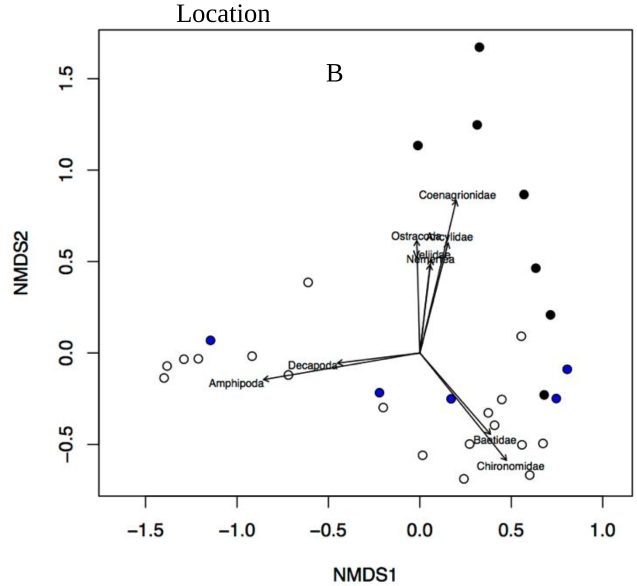
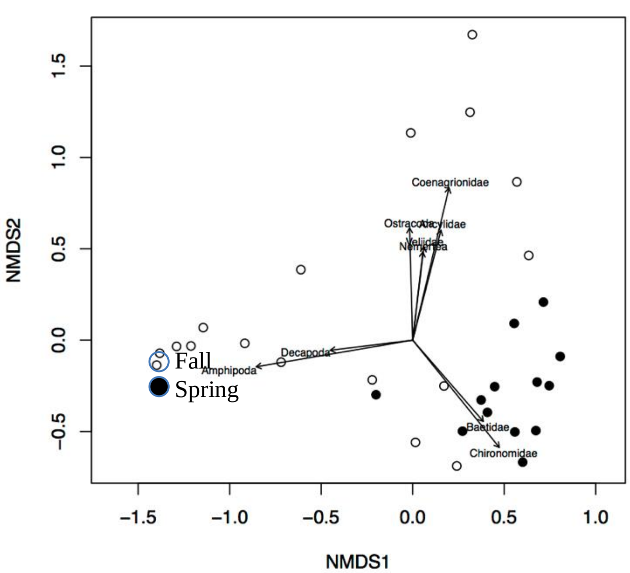
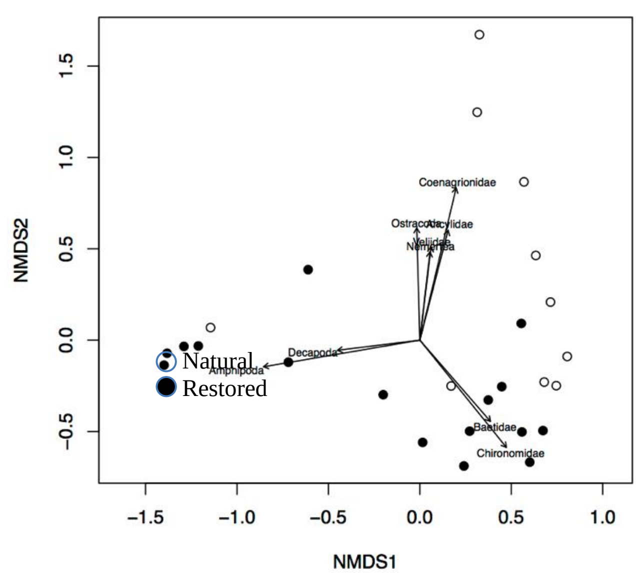


Figure 6. NMDS analysis of macroinvetebrates assemblages distribution group by 4 different factors. I used NMDS analysis to determine how similar different sites are from each other as: underlying habitat types, location, season and resorted status. It displayed N(natural) and R(restored). (A) Habitat types (B) Location (C) Season and (D) Restoration status.





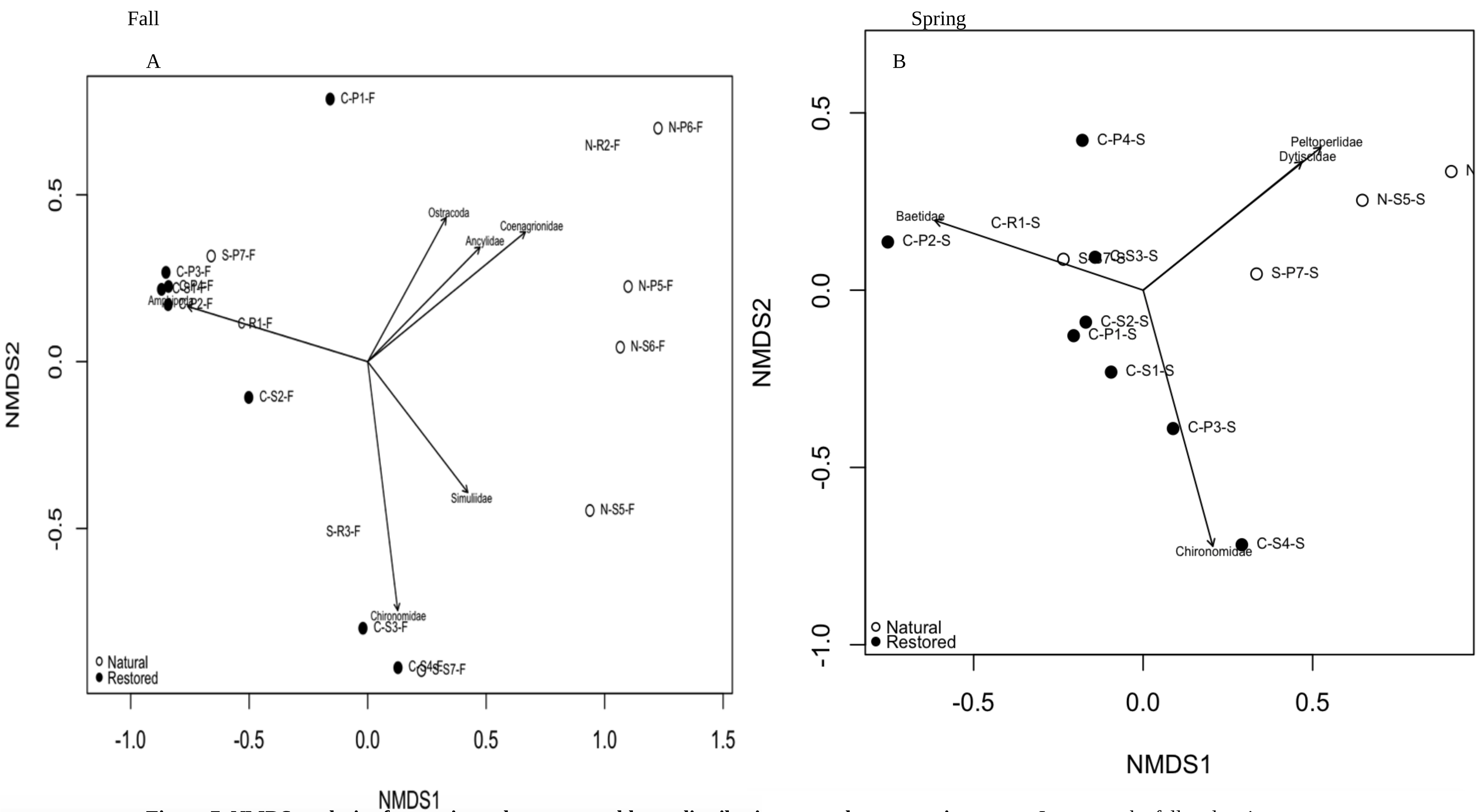


Figure 7. NMDS analysis of macroinvetebrates assemblages distribution group by restoration status. I separate the fall and spring samples to determine how similar the restored and natural step-pool sites were. (A) Fall and (B) Spring

DISCUSSION

Installing a step-pool sequence in high-gradient stream is a novel restoration practice to dissipate energy and enhance stream habitat (Chin 2003, Chin et al. 2009). In this study, I determined how benthic macroinvertebrate communities were affected by different in-stream habitat structure: step, pool, riffle, and assessed whether there was a significant difference between restored sites and natural step-pool sites. I found no significant difference in terms of bioassessment metrics among step, pool and riffle except % contribution of dominant taxon, owing to the small sample size and unequal sampling area. I also hypothesized that macroinvertebrate communities in the restoration site would be similar to those in natural step-pools sites because the degraded area has been restored. I found the macroinvertebrate communities in the restoration sites were similar to the natural step-pool site in the South Fork from the NMDS analysis. I also observed the sensitive group Ephemeroptera: Baetidae started to inhabitat in the restored sites during spring. Amphipoda and decapoda were the common taxa found in the confluence and pools; Ephemeroptera: Baetidae and Diptera: Chironomidae were heavily collected in the South fork and steps, and Odonata: Coenagrionidae and Ancylidae mostly occurred in the North Fork. This study suggests that the habitats in restored sites have recovered.

Habitat complexity

The hydraulic condition and the physical habitat structure greatly affect the formation and distribution of benthic macroinvertebrate assemblages (O'Dowd and Chin 2016). Variation in physical characteristics such as inorganic and organic substrate components and water velocity among steps, pools and riffles is one of the reasons to explain the difference macroinvertebrate taxon found in three in-stream habitat types. Although I found there was no significant difference in terms of bioassessment metrics between steps and pools in spring samples except the percentage of predator abundance, it could be attributed to the small sample size (6 step-pool pairs) and unequal sampling area. In fact, the O'Dowd and Chin (2016) showed steps had greater taxa richness, diversity and percentage of EPT compared to pools. This difference likely results from more complex habitat structure in steps. First, the habitat structures in steps are the most heterogeneous, whereas pools have the lowest habitat complexity. Because steps are formed by cobble and boulder chains, wood or bedrock (Chin 1989), the different size of its inorganic substrates and various spacing between stone substrates not only can create more microhabitats for the macroinvertebrates and other organisms to live, but also provides more available refuge space for the macroinvertebrates (Warfe 2008, Tokeshi and Arakaki 2012). Additionally, the Flecker and Allan (1984) showed the abundance of benthic macroinvetebrates were higher on complex and irregular substrates containing large numbers of interstitial spaces for refugia and trapping detritus than spatially simple substrates.

In contrast, the plunge pools are only consisted of finer sediment (Chin 1989) that provides less interstitial space for the macroinvertebrates to hide from predation and resist disturbance. Macroinvertebrates living in pool have higher risks of being pried and disturbed than those living in steps and riffles. Hence, the increase in usable microhabitats and interstitial spaces result in forming greater taxa diversity and abundance by decreasing predation rates, increasing the amount of living places and food levels (O'connor 1991, Tokeshi and Arakaki 2012).

Restored sites had lower percentages of organic substrates and lower score in riparian vegetative zone than the natural step-pool sites in the North and South Forks. Although the campus had planted the native plants along the bank slope in restored sites last year, the revegetated area have not fully established yet and the campus was still exploring the suitable plant species through continually experiment. The plant density was still low compared to South Fork and North Fork, and might provide less leaf litter and organic matter to the macroinvertebrate communities. The leaf litter and wood debris are the major food supply for many species of macroinvetebrates, either consuming them directly or by eating fine detrital particles (Richardson 1992). Similarly, leaf litter not only provides food and nutrient to the aquatic ecosystem, but it also functions as a shelter and refuge from predators (Richardson 1992). Therefore, the density of macroinvertebrate taxa is often higher in an environment with abundant fallen leaves (Mackay and Kalff 1969). The fewer inputs of litterfall and organic matter and undeveloped algal community may explain the low abundance and diversity of macroinvertebrate taxa in restored sites. Meanwhile, I observed a marked significant different (p value= 0.006) of percentage of shedders abundance between restored and natural step-pool sites. The boxplot (Figure 5L) showed the restored step-pool sites has lower percent abundance of shedders than the natural step-pool sites, which also implied less leaf litter in the restored habitats. It is because shedders feed on leaf litters or other coarse particulate organic matters. The density of shedders was controlled by the availability of organic matters (Graca 2001). Hence, the low density of riparian vegetation cover around the habitats will lead to a decline in the shedder population.

Seasonality

In general, the climate of Mediterranean streams is very fluctuating and intermittent. It is characterized by sequential floods in a cold winter and prolonged drought in the hot summer (Gasith and Resh 1999). The highly variable streamflows both influence the macroinvertebrate assemblages by disrupting or amplifying hydrologic connectivity: droughts decrease the connectivity and floods restore it (Bonada et al. 2006). I detected the average of family richness, and percent of EPT abundance are higher in spring than in fall (Table 1). Meanwhile, the NMDS analysis showed most of the species were either permanently living in aquatic (Decapoda, Ancylidae, Amphipoda and Ostracoda) or pollution-tolerant (Chironomidae, Coenagrionidae and Simuliidae) (Figure 7), whereas the pollution-sensitive species such as Beatidae and Peltoperlidae were commonly found in spring. It is because the Mediterranean-type streams (Strawberry Creek) start drying during the late summer and fall, and it declines habitat availability and deteriorates water quality. Species that can tolerant discharge, warmer water and poor water quality will then dominate the aquatic biota (Gasith and Resh 1999). Therefore, species richness and percent of EPT remained at a low level during the fall. Apart from this, I detected that the macroinvertebrate taxa in the restored sites were similar to the natural step-pool sites in South Fork than the North Fork whenever in fall or spring. This result and the present of EPT species: Beatidae in restored sites indicated the degraded habitat has been improved because South Fork always has higher species diversity and percent of EPT abundance and better water quality than the North Fork (Hans and Maranzana 2008). And the better the water quality is, the more EPT species inhabit.

Drought

Under the low-flow conditions, the shallow regions of the stream such as riffles and runs disappear first and form a series of fragmented pools (Lake 2003; Bonada et al. 2006). Then, the exchange of matter, energy and organisms is constrained between patches, where expected low taxa richness results in disconnected habitats (Bonada et al. 2006). When the stream size shrinks and habitat availability declines by the sharply reduced water flow, the aquatic organisms may be trapped or die in the dried areas such as riffle (Lake 2003). For example, the population of caddisfly *Gumaga nigricula* in Northern California Stream was eliminated by a severe drought in 1977 (Resh 1992). Worse still, the reduced flow and loss of riffles further alters the composition structure of the ecosystem. Shredders become relatively scarce in stream during low-flow period due to less detritus and fine sediment transport and the low decomposition rate of leaves (Lake 2003). That explained why the average of percentage of shedders in steps (0.4 ± 1.1) and in pools (0.6 ± 1) was much lower in fall than in spring (steps: 11 ± 19.2, pools: 8.1 ± 11.8) (Table 1). As the detritus movement stops, the periphyton used by the grazers disappears, and thereby decreasing the abundance of grazers and filters (Lake 2003). Eventually, drought may lead to a drastic change in trophic structure. However, some macroinvetebrate taxa such as the larvae of chironomids are able to withstand dehydration (Butler 1984). The tolerance of water stress may explain the Chironomids were one of the prevalent species in restored sites and natural step-pool sites during ongoing drought.

Although the drought progresses, the pools can function as a refugia for many macroinvetebrates taxa. Because the water gradually dries up in riffles, the macroinvertebrate may drift and move from riffles to pools so as to avoid desiccation (Boulton and Lake 1992). Subsequently, Miller and Golladay (1996) found the density of macroinvertebrate taxa in pools doubled after the riffles dried up in intermittent stream. In Strawberry Creek, in fall, riffles had higher richness and diversity than pools, however it may be attributed by the larger and longer sampling area in riffles than pools.

Urbanization impacts

The campus has put enormous efforts in restoring the Strawberry Creek, yet anthropogenic stresses still threaten the macroinvertebrate communities especially in the North Fork. The low

EPT richness in and high family biotic index in the North Fork's sites and restored sites suggests the water quality is still poor compared to the South Fork. This difference is not surprising because the North Fork was highly polluted that leaded to limited taxon pool formed historically. For instance: The northside residents used to improperly dump oil and other pollutants to the creek. And the effluent directly released from the pipe to the North Fork (Charbonneau and Resh 1992). In 1987, the family richness and percent of EPT in the North Fork was lower than the South Fork, and the water quality was fairly poor (FBI= 6.5-7) (Hans and Maranzana 2008). Moreover, the North Fork is close to and flows under residential and densely populated areas, which has a higher chance to receive anthropogenic pollutants from stormflow (Charbonneau and Resh 1992). I also observed lots of invasive English Ivies grew around the North Fork, which could overwhelm other plant species and understory. And the channelization in the North Fork was more extensive than other sites, which had more concrete embankments and drainage culverts. In contrast, the South Fork has experienced less environmental change and the habitats were less polluted because the water only passes from the source in Strawberry canyon and beyond the UC Botanical Garden to the campus. So, it is expected the water quality is better in the South Fork than the North Fork.

Although the campus already eliminated the significant direct discharge from the point source pollution in 2000 (Hans and Maranzana 2006), the non-point source runoff, spills and illegal dumping still threaten the aquatic ecosystem in the stream. For instance, the storm runoff from the streets directly enters to the North Fork, which includes the oil leaking from motor vehicles, sediments from the construction sites, fertilizers and pesticide residues from residential lawn and garden, and chlorinated water released from sewer pipes and mains and street washing (Hans and Maranzana 2006). In 2011, more than1000 gallons of oils accidentally spilled from Stanley Hall to Strawberry Creek (Karlamangla 2011) that could kill aquatic organism by limiting oxygen exchange, coating their gills and interfering the respiration (Crunkilton and Duchrow.1990). Actually, Mendez (2012) found the population of Sacramento sucker and California roach declined after the spill.

In addition, fine sediment suspension and deposition from constriction sites on campus also affects the macroinvertebrate through blocking the respiratory structures, changes the filter feeding activities, increases drift and altering the substrate composition of the habitat (Wood and Armitage 1997). Nevertheless, the high-volume of sediment in streams actually benefit some macroinvertebrate taxa: Chironomidae prefer sediments and utilize the sediments in making the protective case and tube (Dudgeon 1994). As a result of greater anthropogenic stressors in the North Fork sites, the habitats are more degraded and it was expected that fewer EPT species could be found in the samples because they were very sensitive to the pollutants. Thus, the North Fork's reach was characterized by large numbers of few pollution-tolerant species such as Coenagrionidae and Ancylidae.

Limitation & Future Directions

Restoring Mediterranean-climate rivers are quite difficult because streams are so intermittent with especailly large seasonal fluctuations. One of the limitations of my study was the short time frame. The six-month long data collection only had a fall and spring sampling period could not provide a comprehensive analysis because it did not include all the seasonal variations. Meanwhile, I collected the sample a year after the restoration was completed which may not provide enough time for macroinvertebrate assemblages to completely recover and recolonize after the large-scale construction works. And similar local of restoration study has shown the macroinvertebrate community took 3 years to recovery very the disturbance (Purcell et al. 2002). One of the main confounding factors of my study is the prolonged drought. I collected the samples in the late fall during the 4th year of severe drought in California, and expect that fewer macroinvertebrates were collected from the stream. Another limitation was the observed-based physical habitat assessment. It is better to include measure physical habitat parameters such as measuring the pH, dissolved oxygen levels or the concentration of heavy metals so as to provide a compressive analysis and to identify the stressors.

In the future, a continually long-term monitoring and assessment on the effectiveness of added step-pool sequences in restored sites is needed. Further studies can focus more on the important role of step-pool sequences in extreme weather events and how it helps the macroinvertebrates to recolonize and recover. More importantly, habitat structure is not the only factor affecting the stream biodiversity. Indeed, for many restoration sites, instead of habitat structure, the poor water quality is the main factor that contributes to the loss of species richness and diversity (Palmer 2010). To select an effective and proper restoration approach for the degraded habitats, the first step should identify the biggest stressor that caused the decreasing biodiversity.

Broader implication

As the warming effect resulting from human activities increases, stream biodiversity will be more impacted by climatic varibility and anthropogenic stresses (Tokeshi and Arakaki 2012). To more effectively restore a degraded habitat, one should not only focus on the change in stream morphology and hydrology; instead, it should also focus on regaining ecological integrity. Although these results have in Strawberry Creek not showed significant difference in terms of biomonitoring metrics among steps, pools and riffles, similar study in Northern California showed steps have higher taxa richness, diversity and percent of EPT than pools (O'Dowd and Chin 2016). Meanwhile, the higher similarity of macroinvertebrate taxa between restored sites and the South Fork and the presence of sensitive EPT species both suggested the degraded habitats have been restored. Considering of step pools' unique ecological benefits in enhancing stream habitats and species diversity, and its ecological functions in protecting the aquatic organisms during extreme weather events, step-pools should be a priority for future river management and restoration.

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APPENDIX A: Physical Habitat Data

	Reaches			
Habitat Parameter	Site 1 (NF)	Site 2 (NF)	Site3 (confluence)	Site 4 (SF)
Epifaunal Substrate/ Available Cover	18	13	14	18
Embeddedness	12	13	16	17

Velocity/Depth Regime	10	9	18	15
Sediment Deposition	16	17	17	16
Channel Flow	13	9	16	12
Channel Alteration	10	12	13	13
Frequency of Riffles (or bends)	13	12	15	11
Bank Stability				
Score (LB)	6	9	7	9
Score (RB)	5	7	6	9
Vegetative Protection				
Score (LB)	6	8	4	9
Score (RB)	6	6	4	10
Riparian Vegetative Zone				
Score (LB)	5	6	5	8
Score (RB)	4	5	4	9
Total Score	124	126	139	156

APPENDIX B: Microhabitat Habitat Data

	% Inorganic composition in sampling reach/area (should add up to 100%)					
Substrate Type	S1 (CF)	P1 (CF)	S2 (CF)	P2 (CF	S3 (CF)	P3 (CF)
Bedrock						

Boulder	20		10		5	
Cobble	70	10	85		85	
Gravel	10	50	5	60	10	50
Sand		20		30		30
Silt		10		10		20
Clay		10				

	% Inorganic composition in sampling reach/area (should add up to 100%)					
Substrate Type	S4 (CF)	P4 (CF)	R1 (CF)	R2 (NF)	S5 (NF)	P5(NF)
Bedrock						
Boulder					15	
Cobble	75		40	40	75	
Gravel	25	60	40	50	10	20
Sand		40	10	10		60
Silt			10			10
Clay						10

	% Inorganic composition in sampling reach/area (should add up to 100%)				
Substrate Type	S6 (NF)	P6 (NF)	R3 (SF)	S7 (SF)	P7 (SF)
Bedrock					
Boulder	30			35	
Cobble	50		55	60	5
Gravel	20	60	30	5	65
Sand		20	15		10
Silt		10	5		15
Clay		10			10

	% Organic composition in sampling reach/area (does not necessarily added up to 100%)					
Substrate Type	S1 (CF)	P1 (CF)	S2 (CF)	P2 (CF	S3 (CF)	P3 (CF)
Detritus	10		10	5	10	5
Muck-Mud		5		10		5
Marl		10				5

	% Organic composition in sampling reach/area (does not necessarily added up to 100%)					
Substrate Type	S4 (CF)	P4 (CF)	R1 (CF)	R2 (NF)	S5 (NF)	P5(NF)
Detritus	15	5	15	20	25	20
Muck-Mud		5	5	30		20
Marl						5

	% Organic composition in sampling reach/area (does not necessarily added up to 100%)				
Substrate Type	S6 (NF)	P6 (NF)	R3 (SF)	S7 (SF)	P7 (SF)
Detritus	25	20	30	20	30
Muck-Mud		20	10		25
Marl					