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Nonmetric multidimensional scaling (NMDS) is an ordination technique which is often used for information visualization and exploring similarities or dissimilarities in ecological data. In principle, NMDS maximizes rank-order correlation between distance measures and distance in the ordination space. Ordination points are adjusted in a manner that minimizes stress, where stress is defined as a measure of the discordance between the two kinds of distances. Before and After Control Impact (BACI) is a classical analysis of variance method for measuring the potential influence of an environmental disturbance. Such effects can be assessed by comparing conditions before and after a planned activity. In certain ecological applications, the extent of the impact is also expressed relative to conditions in a control area, after a particular anthropogenic activity has occurred. In this paper, two statistical techniques are employed to investigate the effects of stream nutrient addition on a riverine benthic macroinvertebrate community. The clustering of sampling units, based on multiple macroinvertebrate metrics across pre-determined river zones, is explored using NMDS. BACI is subsequently used to test for the potential impact of nutrient addition on the specified macroinvertebrate response metrics. The combination of the two approaches provides a powerful and sensitive tool for detecting complex second-order effects in river food chains. Statistical techniques are demonstrated using eight years of benthic macroinvertebrate survey data collected on an ultra-oligotrophic reach of the Kootenai River in Northern Idaho and Western Montana downstream from a hydro-electric dam.

Introduction

Nonmetric multidimensional scaling (NMDS) is an ordination technique which is often used for information visualization and exploring similarities or dissimilarities in ecological data (Rabinowitz 1975). It is commonly used to compare groups of samples based on phylogenetic or count-based distance metrics. The objective of NMDS analysis is to maximize rank-order correlation between distance measures and distance in the ordination space. Ordination points are adjusted in a manner that minimizes stress, defined as a measure of the discordance between the two kinds of distances. It was developed by Shepard (1962) and Kruskal (1964) for psychological data and was first applied in ecology by Anderson (1971).

Before and After Control Impact (BACI) is an analysis of variance technique for measuring the potential influence of an environmental disturbance (Smith 2002). Impacts are assessed by comparing conditions before and after a planned activity. In some cases, the extent of the impact is also expressed relative to conditions in a control area.

In this paper, both NMDS and BACI statistical methods are utilized to investigate the effect of river nutrient supplementation on a benthic macroinvertebrate community. The clustering of sampling units, based on multiple macroinvertebrate metrics across river zones is explored using NMDS. BACI is then used to test for the potential impact of nutrient supplementation on a set of specific macroinvertebrate response metrics. Applications are demonstrated using eight years of replicated benthic macroinvertebrate data collected on the Kootenai River in Northern Idaho and Western Montana.

Methods

NMDS is a multivariate analysis ordination technique used primarily for data clustering in exploratory data analyses. It is based on a fundamentally different approach than the eigenanalysis of sum of squares and cross-product methods common to Principle Component Analysis (PCA), Correspondence Analysis (CA), and Detrended Correspondence Analysis (DCA) (Kenkel and Orloci 1986; Cox and Cox, 2001). Axes of NMDS are not rotated axes of a high-dimensional space; rather, NMDS works in a space with a specified small number of dimensions (e.g., 2 or 3). Of main interest, is the clustering of the points in this ordination space.

The data on which NMDS operates are the elements of the dissimilarity matrix among all pairs of objects, for example, Bray-Curtis dissimilarities, computed from community data. Let D_{ij} be the dissimilarity between objects i and j, and let δ_{ij} be the Euclidean distance between objects i and j in the ordination space. The objective is to produce an ordination such that:

$$D_{ij} < D_{kl} \Rightarrow \delta_{ij} \leq \delta_{kl} \text{ for all i, j, k, l} \qquad (1)$$

That is, if any given pair of objects has dissimilarity less than some other pair, then the first pair should be no further apart in the ordination than the second pair. A scatter plot of ordination distances, δ, against dissimilarities, D, is known as a Shepard diagram.

The degree to which distances agree in rank-order with dissimilarities can be determined by fitting a monotone regression of the ordination distances between the ith and jth observations, δ_{ij} , onto the dissimilarities *D.*

Badness of fit (Kruskal's Stress Test), S, is defined as:

$$S = \sqrt{\frac{\sum_{i=2}^{n}\sum_{j=1}^{i-1}\left(\delta_{ij} - \hat{\delta}_{ij}\right)^2}{\sum_{i=2}^{n}\sum_{j=1}^{i-1}\delta_{ij}^{\;2}}} \tag{2}$$

where $\hat{\delta}_{ij}$ is the estimated dissimilarity. Stress decreases as the rank-order agreement between distances and dissimilarities improves. The aim is therefore to find the ordination with the lowest possible stress.

The BACI approach is a classic method for measuring the potential environmental impact of an activity, i.e., discharge, disturbance, nutrient addition, etc, on the ecosystem. Such affects can be analyzed by measuring conditions before a planned activity and then comparing the findings to those conditions measured after; an approach that is applicable for comparing the affects of anticipated future activities as they apply to the ecosystem under consideration.

Several variations of the BACI model are proposed in the literature (see for example, Stewart-Oaten et.al 1986; Roberts, E.A. 1993; Smith 2002). The BACI model used in this study took the form:

$$Y_{ijk} = \mu + \alpha_i + \tau_{k(i)} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \tag{3}$$

where, Y_{ijk} is the response being measured, e.g. abundance or biomass, μ is the overall mean, α_i is the effect of period (i= before or after), $\tau_{k(i)}$ represents times within period (k = 1, 2, . . . , τ_A, for i = after; k= 1, 2, . . . , τ_B for i = before), β_j is the effect of location (j = control or impacted), $(\alpha\beta)_{ij}$ is the interaction between period and location, and ε_{ijk} is the remaining residual under the standard linear model assumptions.

A mixed model repeated measures design was specified assuming a compound symmetric (CS) correlation structure with times (years) nested within the period as the repeated measures effect. Period and Locations were assumed to be fixed effects. The restricted maximum likelihood (REML) was employed for model estimation. Statistical computations, graphics, and diagnostics were carried out using SAS Ver. 9.3.

Demonstration

Data Description

The Kootenai River is located along the junction of the Idaho, Montana and Canadian borders. The river runs south and west from Canada to Montana and Idaho, then returning back north to Canada. A large hydro-electric facility, Libby Dam, impedes the river flow near the town of Libby, MT and has resulted in oligotrophic conditions downstream. In 2002, fourteen biomonitoring sites were established along the river to monitor water quality, primary production, benthic invertebrates, and fish populations (Figure 1). Of the variables measured, the benthic macroinvertebrate data for ten selected sites will be considered for analysis here. As a means of mitigating the biological impacts due to operation of the dam, a nutrient addition (phosphorus) program was initiated at the ID – MT border in 2005 and has continued during the June-September time frame of each subsequent year (Holderman, et al. 2009). Based on this nutrient addition program, three river zones, encompassing the ten selected sites, were designated as: the Upper River Zone (URZ, sites KR12, KR11, and KR10: an untreated control region above the nutrient addition point); the Nutrient Addition Zone (NAZ, sites KR9, KR7, and KR6: a region immediately adjacent and downstream of the nutrient addition point), and; the Lower River Zone (LRZ, sites KR4, KR3, KR2, and KR1: a river section located further downstream from the nutrient addition point).

Benthic macroinvertebrate sampling was typically carried out multiple times per year, although the exact timing and number of samples varied depending on river conditions and project requirements. In order to establish a common sampling timeframe across years, the months of July through November were selected for analysis. These months were the most populated with available data and, because nutrient addition was initiated approximately in June of each year, these months were considered as the most biologically relevant for the benthic invertebrate communities of interest. The years spanning 2003 through 2010 were selected for analysis as they encompassed several years of both pre and post-nutrient addition periods. A matrix of the available sampling times for all years and months is shown in Table 1.

At each sampling event, 5-6 replicates (random samples) were taken at each site and date. Each sample was sorted and identified to the species level or the nearest taxonomic grouping of benthic macroinvertebrates. From this taxonomic information, additional responses such as total abundance, total biomass and community diversity metrics were determined. From a large collection of potential measures, a set of seven metrics were determined to be important for the assessment of the effect of nutrient addition on the benthic community. These metrics were: Total Abundance, Total Biomass, EPT Abundance, Filterer Abundance, Chironomidae Abundance, Baetidae Abundance, and EPT Richness. For each response, the average value was then computed for each year - site combination and these data were further classified according to the nutrient addition period (Pre: 2003-2005; or Post: 2006-2010) and river zone as defined above.

NMDS Analysis

Prior to analysis, the seven benthic invertebrate responses were double square root transformed in order to mitigate issues of scale and heterogeneity. Bray-Curtis distances were then computed among the site-year values of each metric. Based on the resulting distance matrix, several NMDS scenarios of varying dimensions were fit. The stress of each model, Eq. 2, provided assessments of the model fit for each dimension. A plot of stress (badness of fit) values against model dimensions is shown in Figure 2. A dimension of 2 described the system well. Higher dimension models did not substantially decrease stress values or improve the model fit. The transformed and observed distance pairs for the 2 dimensional model showed a high level of correspondence (r=0.99), also indicating adequate model fit (Figure 3).

The correlations between the two axes and the seven component invertebrate metrics are given in Table 2. The first axis was strongly correlated with abundance measures, biomass, and EPT richness. The second axis was highly correlated with the abundance of taxa Chironomidae. All significant correlations were negative, suggesting an inverse relationship between NMDS axes and individual invertebrate responses.

Plots of the two axes were made to assess any clustering or patterning of the transformed data. While notable aggregations or clusters of axis data points were observed, they did not directly correspond to the Pre and Post nutrient addition periods (Figure 4A). Examination of River Zones, however, did show some correspondence with the main clustering of data points (Figure 4B). The Lower River Zone (LRZ) was clearly separated from the remaining data. This segregation of data points is likely due to the substantial differences in the river substrates, physical habitat features, and invertebrate communities of the sites within the LRZ. For the remaining data, however, distinct grouping was not clear, as indicated by overlapping points for both the Nutrient Addition Zone (NAZ) and Upper River Zone (URZ). This may be expected as the sites in these two zones share similar invertebrate habitats and communities.

Further coding of the data points by both Period and River Zone, however, helped provide a better definition of structure among the groups (Figure 5A). Assuming bivariate normality, 95% confidence regions were also added to these plots to help discern differences among clusters. In the LRZ (Figure 5B), there was an increase in variability along the second NMDS axis during the post nutrient addition period. This axis was shown earlier to have a high correlation with Chironomidae abundance, suggesting a change in the community contribution of that taxa group. The post nutrient addition data grouping also had a positive shift along the first NMDS axis, which, based on component correlations with the axes, indicated that the metrics decreased in abundance, biomass, and richness following nutrient addition. A plot of NMDS axes for the NAZ (Figure 5C) showed a clear separation of pre and post nutrient addition periods (Figure 5B). A negative shift along both NMDS axes was evident after nutrient addition, suggesting that all abundance, biomass and richness measures increased at those sites. In contrast, the pre and post nutrient addition clustering of the URZ (Figure 5D) was completely overlapping (Figure 5C). While some increase in variability was present along both axes during the post nutrient addition period, the negligible change in data patterns suggested that no substantial alteration in the seven invertebrate community measures occurred in that zone.

BACI Analyses

Average response trends over sample sites are demonstrated for three responses, EPT Abundance, Total Biomass, and EPT Richness (Figures 6A-C). These metrics are representative of the river ecosystem and the remaining four responses, while not shown here for brevity, showed similar trends. The response trends were consistent with the NMDS results with the LRZ sites showing notably different responses than those of the NAZ and URZ. The NAZ showed a consistent and stronger positive change in metric values during the post nutrient addition period than either the LRZ or URZ, where post treatment changes in responses were either negligible or inconsistent.

In order to quantify and examine these differences more thoroughly, each of the seven responses was considered in repeated measures BACI analysis as outlined in Eq. 3. Because of the notable differences in site morphology and community structure of the Lower River Zone, the BACI analyses considered only the Nutrient Addition Zone and Upper River Zone (treated and control sites, respectively). All abundance and biomass responses were logarithmically transformed prior to analysis to meet the assumptions of ANOVA.

Results for the BACI analyses are given in Table 3. Overall, a strong Period effect was seen. In most responses, the interaction was also highly significant. That is, the effect of Period was not consistent across Zones. Interaction means for EPT Abundance, Biomass and Richness are given in Figures 7A-7C and show a substantial increase in response values relative to the control sites in the post nutrient addition period. Similar interaction patterns were also seen for the other responses, even when non-significant. Post nutrient addition treatment effects were stronger and positive for the NAZ sites, while the untreated control zone sites of the URZ showed little to no response.

While the pre and post nutrient addition differences in the interaction plots are notable, the effects of nutrient addition are more pronounced when displayed as the relative site changes across periods, e.g. the percentage of the total response due to the Post treatment period, (Post – Pre) / (Post + Pre). Charts for these relative changes are shown for the three demonstration responses in Figure 8. In all cases, sites located within the Nutrient Addition Zone had strong positive changes, while those sites in the untreated Upper River Zone were smaller, or even negative.

Concluding Remarks

NMDS and BACI provide an effective combination for assessing environmental change. NMDS was able to describe patterns in river benthic macroinvertebrate communities spanning several years and different geomorphic conditions in relation to nutrient augmentation. BACI analysis provided the inferential tools necessary for discerning significant changes in individual community metrics before and after nutrient addition. Together, these analyses have shown improvements in benthic macroinvertebrate communities relative to overall river health after nutrient addition. These results are consistent with those of the primary and tertiary trophic communities and have provided project personnel with essential tools for the continued management and implementation of the nutrient addition program.

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		Month									
Zone	**Year**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Lower	2003		7	5	19	15		19	6	16	
	2004		24	24	24	24	24	24	24		
	2005		12								
	2006		5			25					22
	2007		24			10	12			19	5
	2008		26			8			23		
	2009		18			24			23		
	2010		18			19					
NAZ	2003		12				17	14	18		
	2004		18	18		6	6	6	19		
	2005		18					18	18		
	2006		18			12			18		
	2007							18	18		
	2008	18							18		
	2009		18			18				18	
	2010		10				10			10	
Upper	2003		18				6	17	18		
	2004		6	18		6		6	20		
	2005		18					12	18		
	2006		18			6			18		
	2007							18	18		
	2008	18							18		
	2009		18			18				18	
	2010		5				5			5	

Table 1. The monthly macroinvertebrate sampling schedule for the Lower River Zone, Nutrient Addition Zone (NAZ), and Upper River Zone indicating the number of samples collected.

	NMDS Axis 1		NMDS Axis 2	
Response	Correlation	p > 0	Correlation	p > 0
EPT Abund	-0.95	0.0001	0.1002	0.40
Filterers Abund	-0.91	0.0001	0.0307	0.80
EPT Richness	-0.86	0.0001	0.1132	0.34
Biomass	-0.85	0.0001	0.0288	0.81
Baetidae Abund	-0.84	0.0001	0.0268	0.82
Total Abundance	-0.84	0.0001	-0.3806	0.00
Chironomidae Abund	-0.37	0.0013	-0.9177	0.00

Table 2. Correlations of benthic macroinvertebrate metrics with NMDS axes.

			p > F					
Source	df	Error df	Abundance	Biomass	EPT Richness	Filterer Abun.	Chiro. Abun.	EPT
Period	1	4	0.0010	0.0103	0.0006	0.0028	0.0153	0.0086
Year(Period)	6	27	0.0012	0.1347	0.0001	0.3206	0.0001	0.1684
Zone	1	4	0.3277	0.1742	0.4979	0.4310	0.3767	0.4400
Period*Zone	1	7	0.0024	0.0072	0.0004	0.0200	0.0639	0.0070

Table 3. BACI results for the seven benthic macroinvertebrate responses.

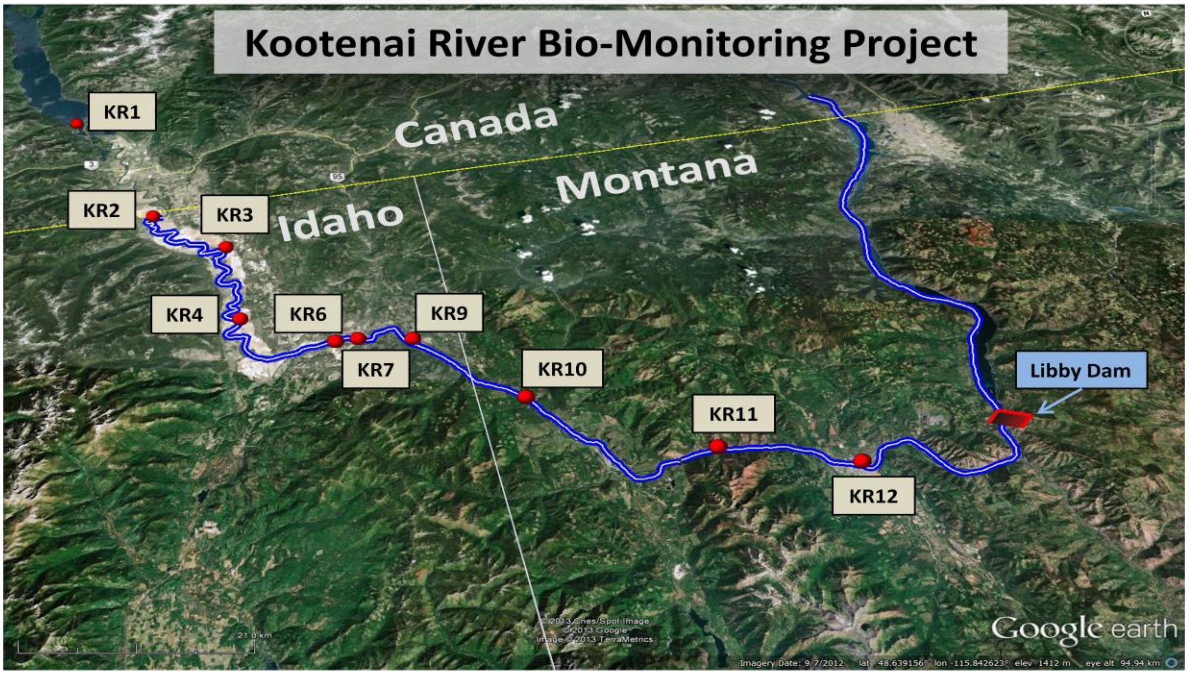

Figure 1. The ten biomonitoring sites along the Kootenai River.

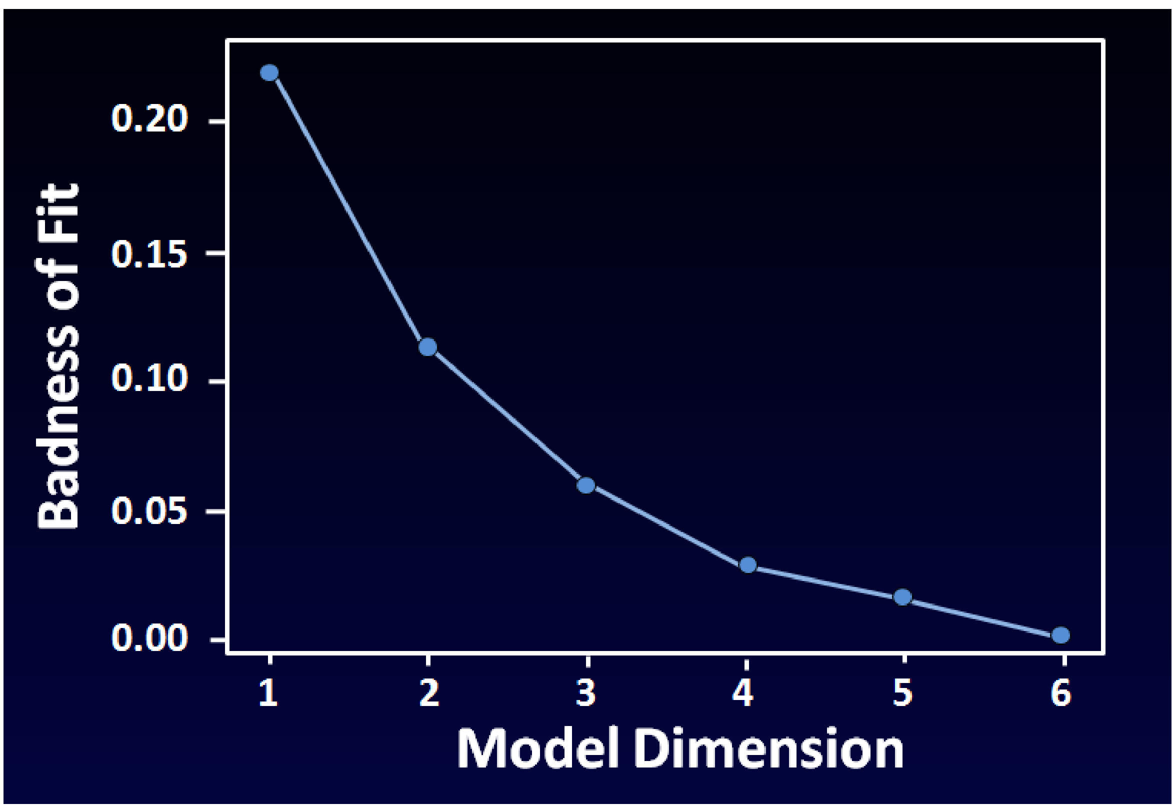

Figure 2. Change in badness of fit (stress) with model dimension.

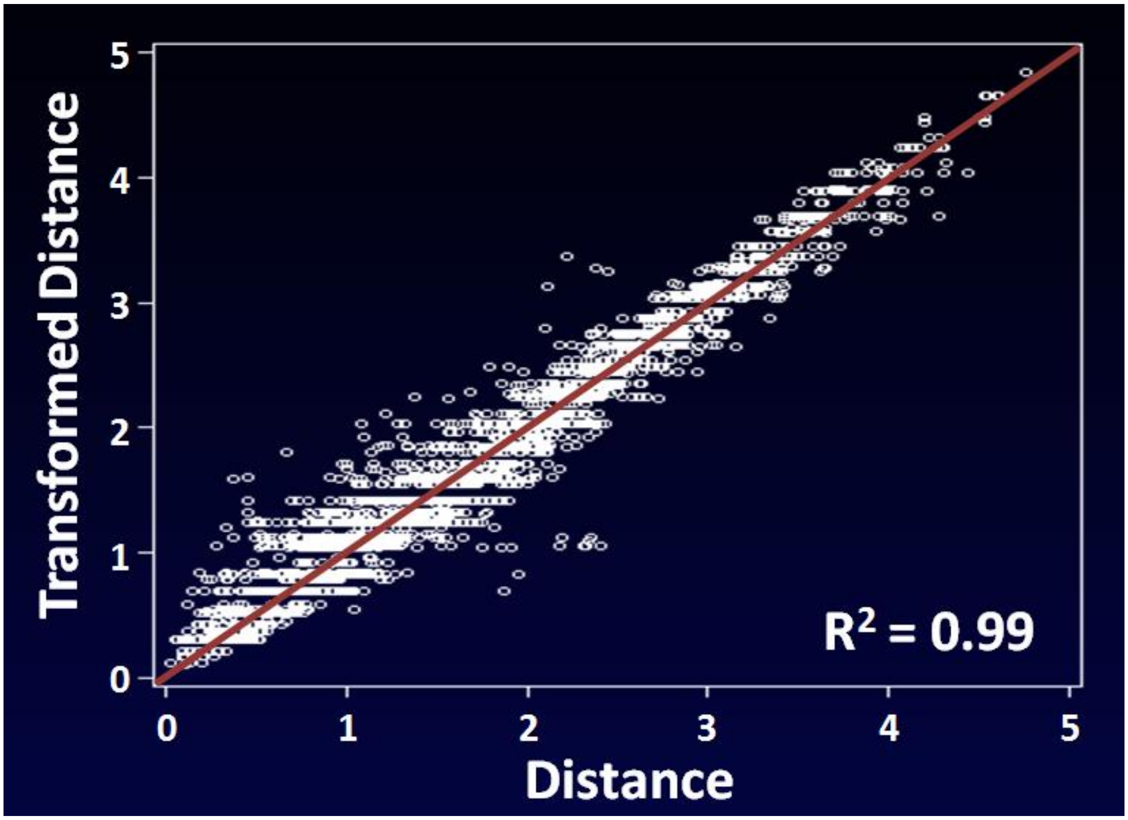

Figure 3. The correspondence between the observed and transformed distances of the two dimensional NMDS model.

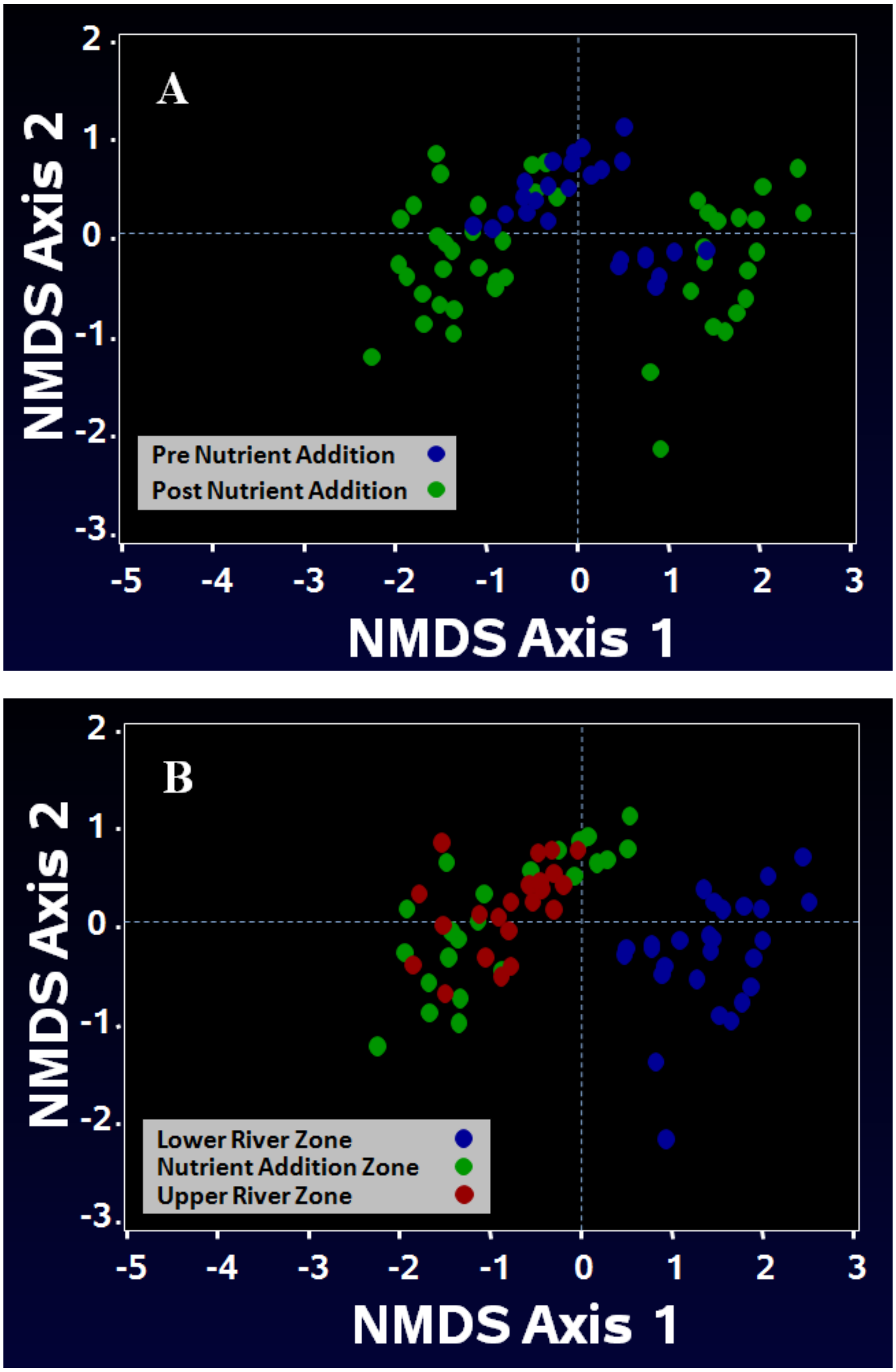

Figure 4. NMDS plots color coded by A) nutrient addition (Blue=Pre, Green=Post).

and B) River Zone (Blue=Lower River, Green=Nutrient Addition, Red=Upper River).

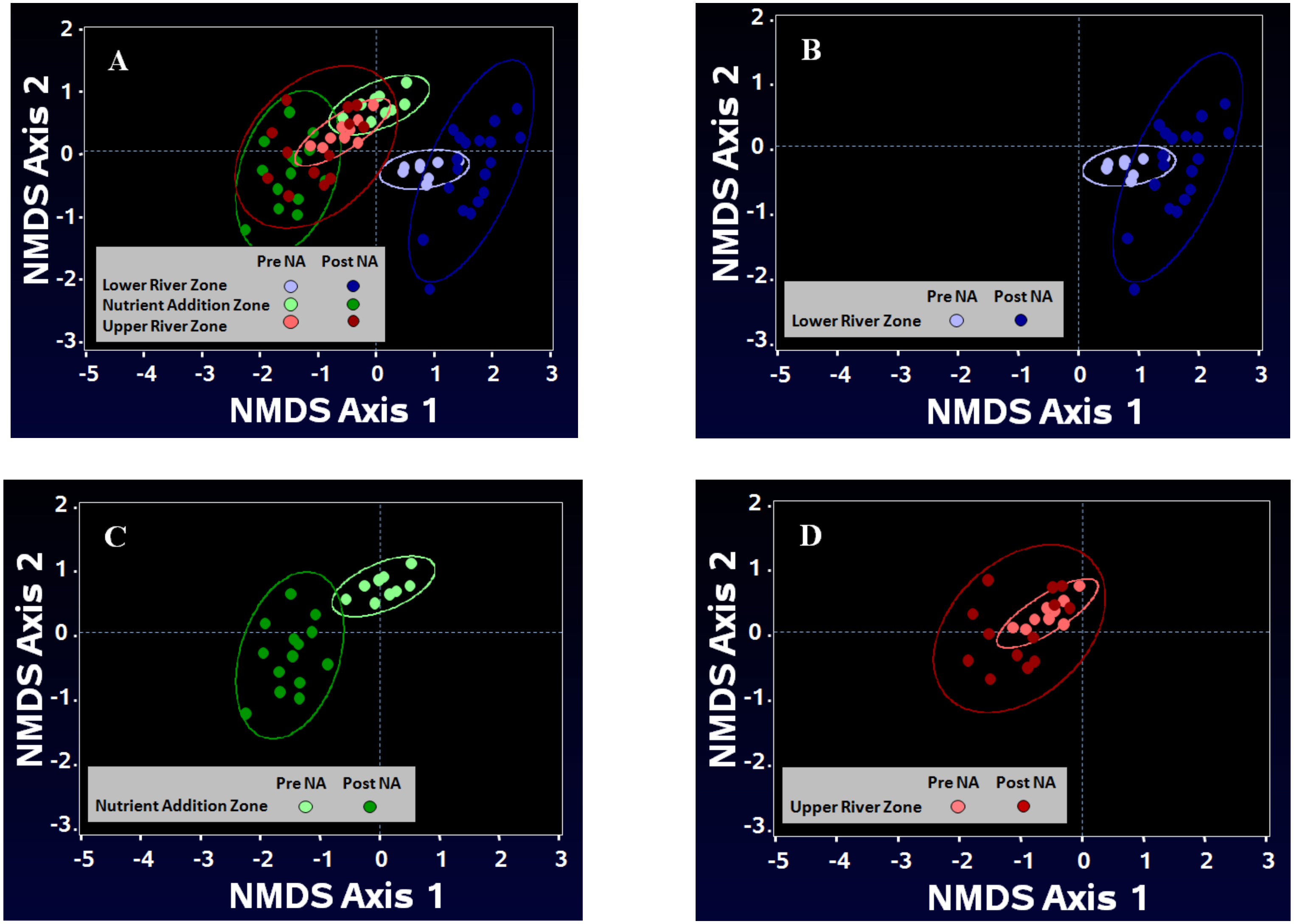

Figure 5. NMDS plots color coded by pre-post nutrient addition (light color-=Pre, dark color=Post) for A) all river zones, B) the Lower River Zone (Blue), C) the Nutrient Addition Zone (Green) and D) the Upper River Zone (Red). Ellipses represent 95% confidence regions.

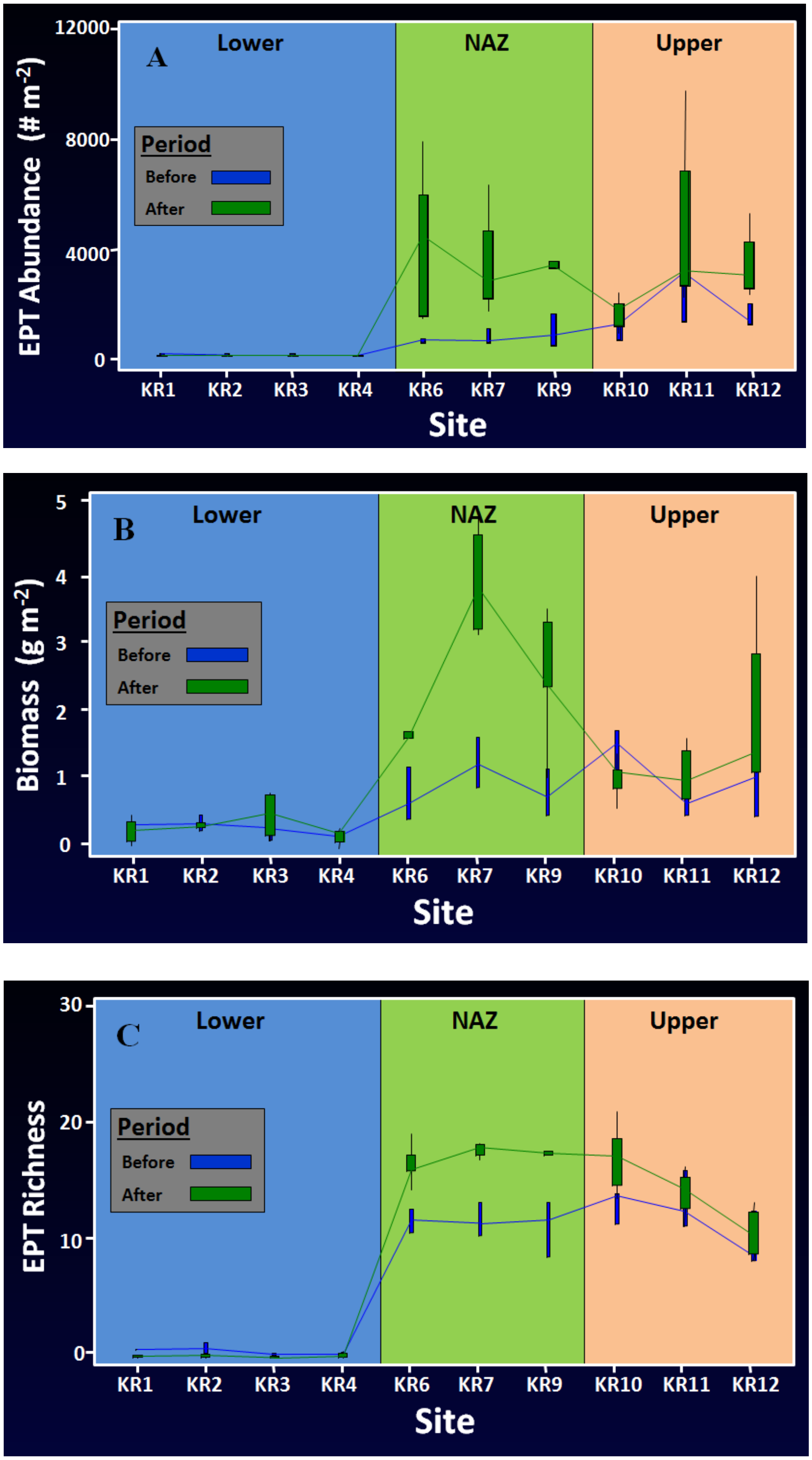

Figure 6. Average trends for A) EPT Abundance, B) Biomass, and C) EPT Richness across sample sites.

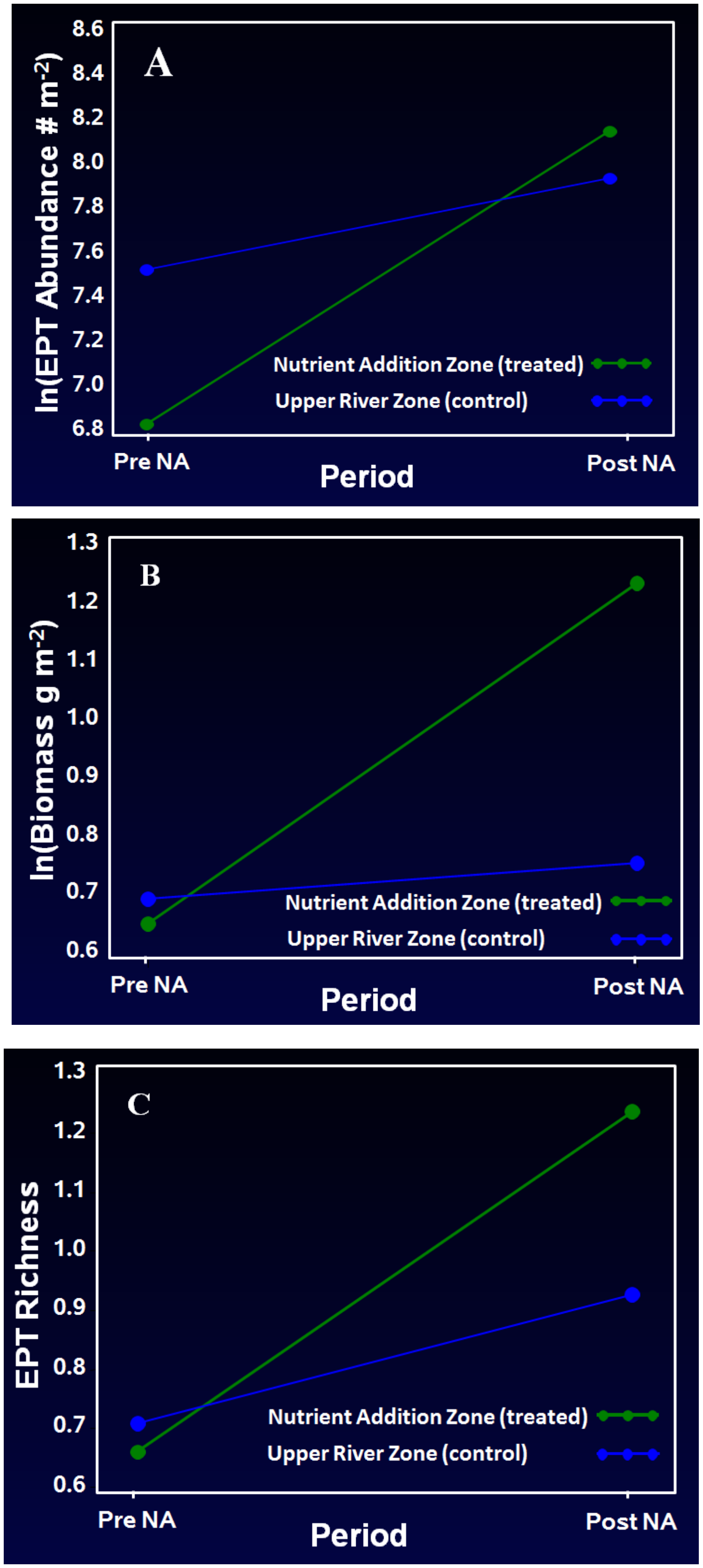

Figure 7. BACI interaction plots for A) EPT Abundance, B) Biomass, and C) EPT Richness.

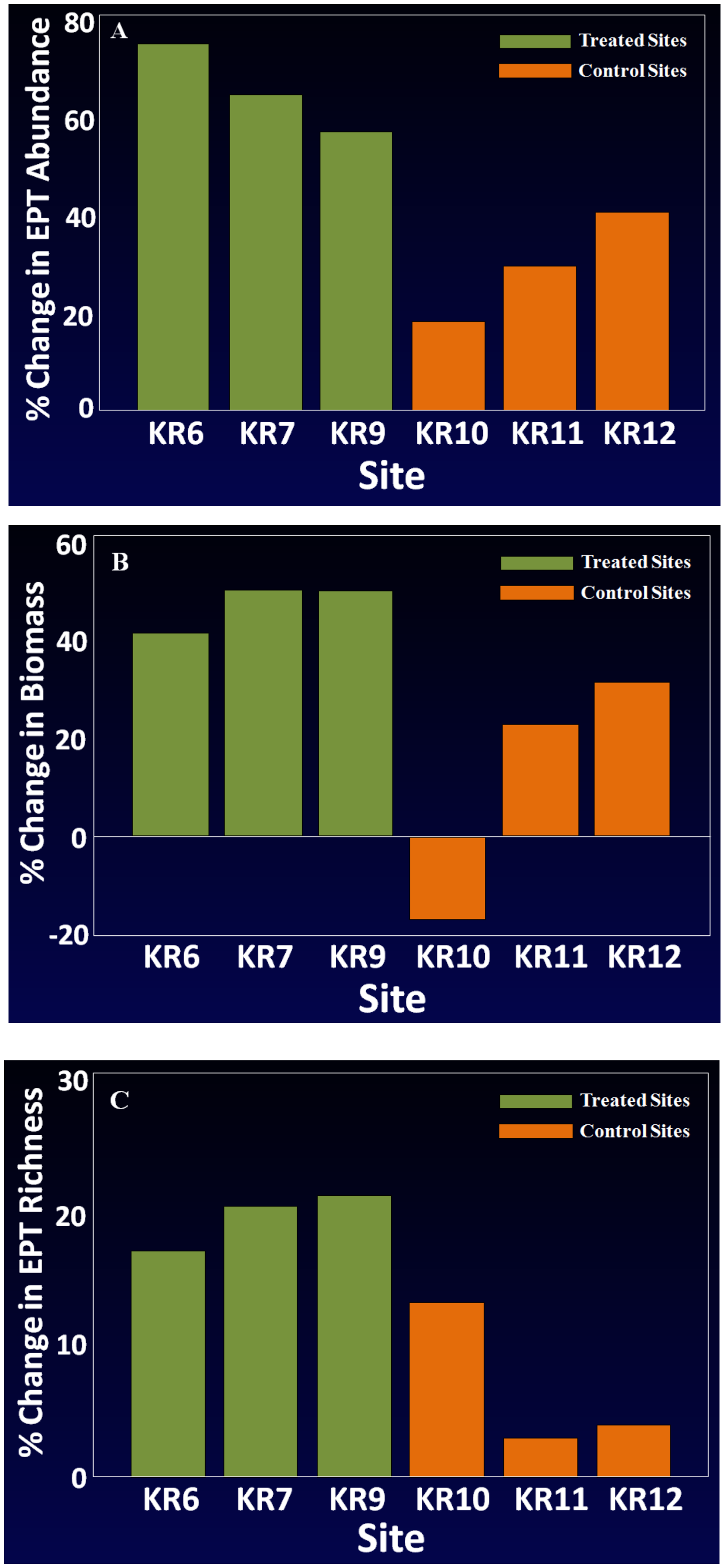

Figure 8. Relative response changes for A) EPT Abundance, B) Biomass, and C) EPT Richness.