

Is Taxonomic Diversity Enough?
The Role of Phylogenetic and Functional Diversities in Drought Tolerance

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Abstract:

The benefits associated with increased taxonomic diversity in a community have been well established. However, phylogenetic and functional trait diversities frequently outperform taxonomic models. To investigate the relationship between phylogenetics, functional traits and ecological performance, a water addition experiment was conducted in a short-grass prairie ecosystem to test if functional diversity improves drought tolerance in the plant community. The study measured taxonomic, phylogenetic and functional diversity in experimental plots. Plots were paired by functional diversity and one plot in each pair received water addition. Analysis weakly demonstrated a positive relationship between phylogenetic and functional diversity within the community. Pre-treatment biomass was not significantly related to taxonomic, phylogenetic, or functional diversity. Drought tolerance, defined by a proportional change in biomass from July to August, displayed a moderate trend with functional diversity, but was not related to taxonomic or phylogenetic diversity. This inability of taxonomic indices to predict ecological performance could have important implications in restoration decision making. Further investigation is needed to understand alternative approaches to diversity.

Introduction:

The magnitude and severity of expected climatic change mandates a greater understanding of the mechanisms underlying ecosystem stability. Across the Great Plains, rising temperatures and increases in extreme weather will have important ecological consequences (Shafter et al. 2014). Seasonal droughts are expected to increase throughout most of the region. (Georgakakos et al. 2014). Bunchgrass prairie of the northern Great Plains is of particular conservation concern, as the remaining bunchgrass landscape occupies only 1% of the historical range (Johnson and O'Neil 2001). Warming in the northern Great Plains is expected to exceed 2.0°C and annual increases in precipitation of 20% are expected over the next century (IPCC

2007). These changes will also be seen with an expected increase of 3 to 4 days in the number of consecutive dry days that the region experiences (Shafter et al. 2014). Although precipitation levels are predicted to increase, the increase will be seen in infrequent heavy precipitation events, straddled by longer dry periods (IPCC 2007). These changes will significantly intensify the drought stress on ecological communities of the region.

The predicted increase in drought stress has important concerns for ecological conservation and restoration. A large body of ecological research exists investigating habitat stability. Species diversity is thought to promote efficient use of limited resources as well as create ecological redundancy which promotes stability. While a few species might dominate a habitat, a larger species pool increases the climate and disturbance tolerance of the overall community (Diaz and Cabido 2001). Increasing species number also increases the likelihood of containing species that are able to migrate in response to change climate (Diaz and Cabido 1997). This trend has been observed previously in response to drought stress. More diverse plant communities exhibited a greater variety of water use strategies which facilitated drought tolerance (Fry et al. 2013). However, species diversity is not always a reliable measure of ecological performance (Diaz and Cabido 2001).

Functional diversity and phylogenetic diversity have been proposed as superior methods to measure the ecological potential of communities. Functional diversity can be defined broadly to include physiological processes, life history traits, performance and fitness. Functional traits are traits related to growth, reproduction and survival that affect fitness (Violle et al. 2007). Functional traits can differ both intra and interspecifically, an important difference with species richness (Jung et al. 2014). The collection of functional traits in a community can greatly affect ecosystem function. It has been shown that a loss of functional traits can shift a community from

a carbon sink to a carbon source (Kuiper et al. 2014). Evaluating communities on the basis of functional traits offers a powerful tool for analysis which does not rely on taxonomic structure.

Functional traits have been shown to contribute to drought tolerance of plant communities. Under short-term drought stress, intraspecific functional variability can be a stronger predictor of community response than interspecific differences (Jung et al. 2014). These stresses can also induce population level shifts in functional attributes (Ravenscroft et al. 2014). Drought-resistant species frequently share particular functional traits. Simulated drought experiments found that drought-tolerant species possessed shorter root lengths (Fort et al. 2013). Investigations of functional diversity also offer opportunities to observe relationships not seen with species diversity. Community-level functional traits can have a significant relationship with above-ground biomass when species diversity is insignificant (Cantarel et al. 2013). In particular, resource limited habitats demonstrate a stronger relationship between functional traits and performance (Sherrard et al. 2009).

The applicability of functional diversity extends into many ecological fields. An understanding of functional discrepancies between invasive and native species greatly facilitates understandings of invasion biology. Species diversity can be misleading, as invasive species increase the region's species count. However, invasive and native plants often have distinct collections of functional traits that better highlight the shift from native to invasive communities (Daneshgar et al. 2013). Functional approaches can also be used effectively for restoration planning efforts, as incorporating the functional identity of sown species promotes habitat stability (Roberts et al. 2010). Functional diversity is most efficiently used when research criteria select uncorrelated traits that contribute to the stress response of interest (Laughlin et al. 2014,

Voltaire et al 2014). Models incorporating functional traits have been shown to be as effective at explaining variability as species identity models (Clark et al. 2012).

Phylogenetic diversity also offers another avenue of research to explain ecological response to stressors. Phylogenetic diversity is strongly related to species identity as a system of classification for a community. Higher species diversity correlates with higher phylogenetic diversity. However, phylogenetic diversity is unique in that it incorporates essential understandings of evolutionary dynamics and community assembly properties. It also presents a stronger environmental significance than species counts. A pair of closely related species and a pair of distantly related species are equivalent in species identity models, although they can differ greatly in ecological role.

Phylogenetic models incorporate phylogenetic distance as a method of measuring diversity. This reflects the greater likelihood of more distantly related species differing functionally. As a result, phylogenetic models possess advantages similar to functional diversity models, as genetic traits demonstrate critical functional differences within a community. Phylogenetic methods have been shown to more accurately predict community response than species incidence or functional trait approaches (Cadotte et al 2009). However, phylogenetic analyses require extensive genetic information and can be more resource intensive than functional-based approaches. This study investigated whether functional and phylogenetic approaches can be used to successfully predict drought tolerance in a bunch grass prairie community.

Methods:

Study Site:

The study occurred within the National Bison Range in Moiese, Montana. Established in 1908 as a refuge for declining bison populations, the National Bison Range contains a bunchgrass prairie ecosystem containing primarily C₃ grass species, especially wheatgrass, fescue, and bluegrass species. The experimental plots were located within a fenced portion of the range which excludes big game such as bison. The fence also separates the region from all roadways.

Field Collection:

Data were collected from a series of 1m² plots located in the Triangle region of the National Bison Range. Plots were placed along a series of four transects spaced 5m apart. Each transect contained five plots placed every 10 meters, giving a total of 20 plots. Within each plot, species incidence was measured through identifying and counting individuals of all plant species present in each plot. Biomass prior to experimental manipulation was recorded for each plot utilizing a hand-held radiometer. Radiometer calibration consisted of measuring five 0.1m² areas near the experimental plots and calculating a regression with dried biomass values of those plots obtained from clipping (Belovsky and Slade 2000). Three nails were arranged in each plot to form a 0.5 m² area in which three radiometer readings were taken 1.5m from ground level. Upon completion of the experimental manipulations, final biomass was recorded utilizing the same hand-held radiometer procedure.

Treatment Assignment:

Upon completion of field measurements, a literature search was completed to gather functional trait data on the species present in each plots. Information was gathered primarily through the Encyclopedia of Life and USDA PLANTS online databases for the ten traits listed in

Table 1. The functional traits were used to create a matrix of proportional trait abundances for all species observed in each plot. This trait matrix was then analyzed through a principal component analysis followed by a hierarchical clustering technique to create pairings between plots. The results of this cluster analysis can be seen in Figure 1. The two plots with the greatest similarity were grouped into a pair and removed from a subsequent round of clustering. This was repeated until 10 pairings were created.

One plot in each pairing was randomly assigned to receive a water addition manipulation. Water addition occurred once a week from July 18th, 2014 until August 7th, 2014 and consisted of providing 20% of the previous week's rainfall. Precipitation data were obtained from the KC7MRQ Weather Station #1 in Ronan, Montana (N 47° 31' 36", W 114° 5' 30") accessed via the Weather Underground webpage. Over the course of the experiment, each plot in the addition treatment received 3810 mL of water.

Calculating Phylogenetic and Functional Diversity:

Calculations of phylogenetic and functional diversity were mirrored of the approach used in Cadotte et al. (2009). Phylogenetic diversity required the calculation of a maximum likelihood phylogeny of the present plant species. The phylogeny was constructed utilizing sequences of the maturase K (matK) gene obtained from the Genbank online database. MatK is a protein-coding chloroplast gene commonly used for angiosperm phylogenies due to its relatively high substitution rate (Barthel and Hilu 2007, Hilu et al. 2003). MatK sequences could not be obtained for four species (*Linaria dalmatica*, *Pascopyrum smithii*, *Filago arvensis*, and *Lithospermum ruderale*) and sequences from members of the appropriate genus were instead substituted into the analysis. Using the UGene bioinformatics software, matK sequences were aligned using

MUSCLE to produce the phylogeny seen in Figure 2 (Okonechnikov et al. 2012). Phylogenetic diversity was defined as the sum of the branch lengths of all species present in a plot.

Calculations of functional diversity required the creation a functional diversity dendrogram. A trait matrix was compiled for each individual species incorporating the traits listed in Table 1. Utilizing the R software package “vegan”, non-metric multidimensional scaling was performed on the trait matrix and hierarchical clustering was then used to produce the dendrogram seen in Figure 3. Similar to phylogenetic diversity, a plot’s functional diversity was defined as the sum of the branches lengths of all species contained within it.

Statistical Analysis:

For each plot, species count, Shannon diversity and Simpson diversity were calculated to represent taxonomic diversity. Linear regression analyses were performed on pre-manipulation plot biomass and the following in order to determine effects on ecological productivity: species count, Shannon diversity, Simpson diversity, phylogenetic diversity and functional diversity. A linear model with a continuous predictor was constructed to elucidate the relationship between phylogenetic diversity and functional diversity across plots.

After final biomass estimates were obtained, the proportional change in biomass was calculated for each plot. To determine any effect of the manipulation, a paired Student’s t-test was performed incorporating proportional change across water treatment groups. Within treatment groups, regression analyses were performed with Shannon diversity, Simpson diversity and phylogenetic diversity to determine any contributions to the change in plot biomass. To test the role of functional diversity, a linear model was constructed including mean functional diversity of a plot pairing as a predictor for the difference between biomass changes in the two plots. For the

above analyses, the significance level was adjusted to 0.0036 after applying the Bonferroni correction. Non-metric multidimensional scaling was performed on the final biomass measures as an ordination technique to look for clustering due to treatment or diversity levels. All analyses were conducted using the R version 3.0.2 software package.

Results:

The results of the regression analyses including pre-treatment biomass are summarized within Table 2. Each of the five predictor variables displayed a downward trend, as biomass decreased as diversity measured increased, but none of these results reached statistical significance ($0.05045 \leq p \leq 0.1111$). The trend was consistent across taxonomic, phylogenetic and functional diversity metrics. The linear model of phylogenetic diversity across plots appeared to have a positive relationship with functional diversity, approaching significance ($F = 4.201$ on 1 and 18 DF, $p = 0.05527$) as depicted in Figure 4. Water treatment appears to have affected proportional change in biomass (see Figure 5) as watered plots decreased by $24.4 \pm 8.38\%$ (1 S.E.) while non-watered plots decreased by $32.9 \pm 3.20\%$ (1 S.E.). This relationship was not significant at the Bonferroni-corrected level ($p = 0.02684$).

Mean functional diversity of plot pairs showed a positive relationship with the differences between biomass changes in paired plots (see Figure 6). This relationship did not reach statistical significance ($F = 4.261$ on 1 and 8 DF, $p = 0.07289$). Within both treatment groups, Shannon diversity, Simpson diversity and phylogenetic diversity had statistically insignificant relationships with proportional change in biomass, as shown in Figure 7. The ordination plots (seen in Figure 8) depict plots by water treatment and quartiles in diversity. There is no apparent clustering patterns based on these criteria, which does not support strong intragroup similarity.

Discussion:

The results of the above statistical analysis do not offer strong evidence in support of the importance of functional diversity in community response to environmental stressors. There were slight, but consistent, trends across diversities indices that are important to consider. The relationships between diversity indices and pre-treatment plot biomass were inverse relationships. This result was unexpected, as species diversity has been established as a positive influence on the productivity on most habitat types (Tucker and Cadotte 2013). Plot heterogeneity likely contributed to this distribution. The plots highest in biomass tended to be dominated by one or two species, especially *Bromus tectorum* and *Symphoricarpos albus*. Many species were found as only a handful of individuals across the plots, in particular where the dominant species were absent and where bare ground cover was higher. Therefore, the trend may be more representative of particular species interactions rather than the broader concerns the study meant to address.

The relationship between phylogenetic diversity and functional diversity measured across all plots is also worthwhile to consider. While statistically insignificant, there is noticeable positive trend between the two indices. It is important to note that the phylogenetic tree was constructed with four genus-substitute species. Also, the patterns of relationships between phylogenetic and functional diversity are highly variable across ecosystem type. Phylogenetic and functional diversity are often highest shortly after community establishment and decrease over time as organisms and traits undergo selection and other forms of habitat filtering (Horner-Devine and Bohannan 2006). This trend has been observed in a variety of habitats, including grasslands and montane forests (Gerhold et al. 2003 and Kunstler et al. 2012). Similarly, more productive habitats often allow for a greater number of functionally-similar organisms than resource limited habitats. Therefore, productive habitats often have a lower ratio of functional

diversity to phylogenetic diversity. These findings contribute to the understanding of this study's results in that it would be unlikely to find a single trend across all plots. Plots displayed heterogeneity in invasion state and shrub encroachment. Increasing sampling size in further investigations in the topic will be important to help elucidate whether such factors confound results.

Likewise, there was a lack of strong evidence supporting an effect of water addition treatment in the plots. Water addition reduced percentage of biomass lost and was significant at $\alpha = 0.05$, but not the Bonferroni-corrected value. This relationship could also be due to plot heterogeneity and variation between paired plots. The differences in functional diversity between plotted pairs ranged from 4.5% up to 71%. Therefore, the sample size of 20 plots may have been inadequately robust against this variation. The timing of the experiment may have also contributed to the results. Water addition occurred from mid-July until early August, a period after much of the growing season for the study species. Therefore, water addition would likely have a different effect than what was seen in this study.

These concerns need also to be addressed in the relationship between functional diversity of plot pairs and the change in biomass per pair. Similar to the initial pre-treatment regressions, the positive trend seen across plot pairings was unexpected. Increased difference in biomass implies that less functionally diverse pairs exhibit stronger drought tolerance. This can be a product of the species that compose the plots. Of the 19 species identified in total, there could be significant differences in drought tolerance. Furthermore, the timing of the study could have removed the contributions of species that had undergone senescence prior to experimental manipulation. While the clustering procedure was designed to minimize the differences between plots in pairs, inter-plot variation existed in all pairings. This could have been a source

of significant variance in the calculated relationship. No significant trends were found when taxonomic and phylogenetic diversity were related to change in biomass within treatment. The ordination plots revealed no strong clustering when incorporating treatment and diversity. The results present no definitive conclusions between phylogenetic and functional diversities and community climate sensitivity.

Local variability likely contributed significant influence to the outcome of this study. Further studies can address the concern in a number of ways. A similar field experiment can be completed using a larger sample size and completing water addition during the growing season. It will also be important to expand to other ecosystems and successional states, due the variable nature of phylogenetic and functional diversity. Methodologies for calculating diversity can be expanded upon. A larger number of functional traits could provide a better basis for a functional diversity dendrogram. Phylogenetic approaches can be expanded to multiple genes, to whole genome sequence and transcriptome investigations. The contributions of plant functional diversity to other trophic levels is also an area of further study. An understanding of the phylogenetic and functional nature of a community holds great potential in habitat conservation and restoration. Many opportunities exist to apply the concepts addressed by this study into a broader ecological and evolutionary context.

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Functional Traits Included	
Plant Type	Nitrogen Fixation
Life Cycle	Root System
Plant Shape	Leaf Shape
Growth Form	Bloom Period
Drought Tolerance	Seed Period

Table 1: Functional traits used to calculate functional diversity

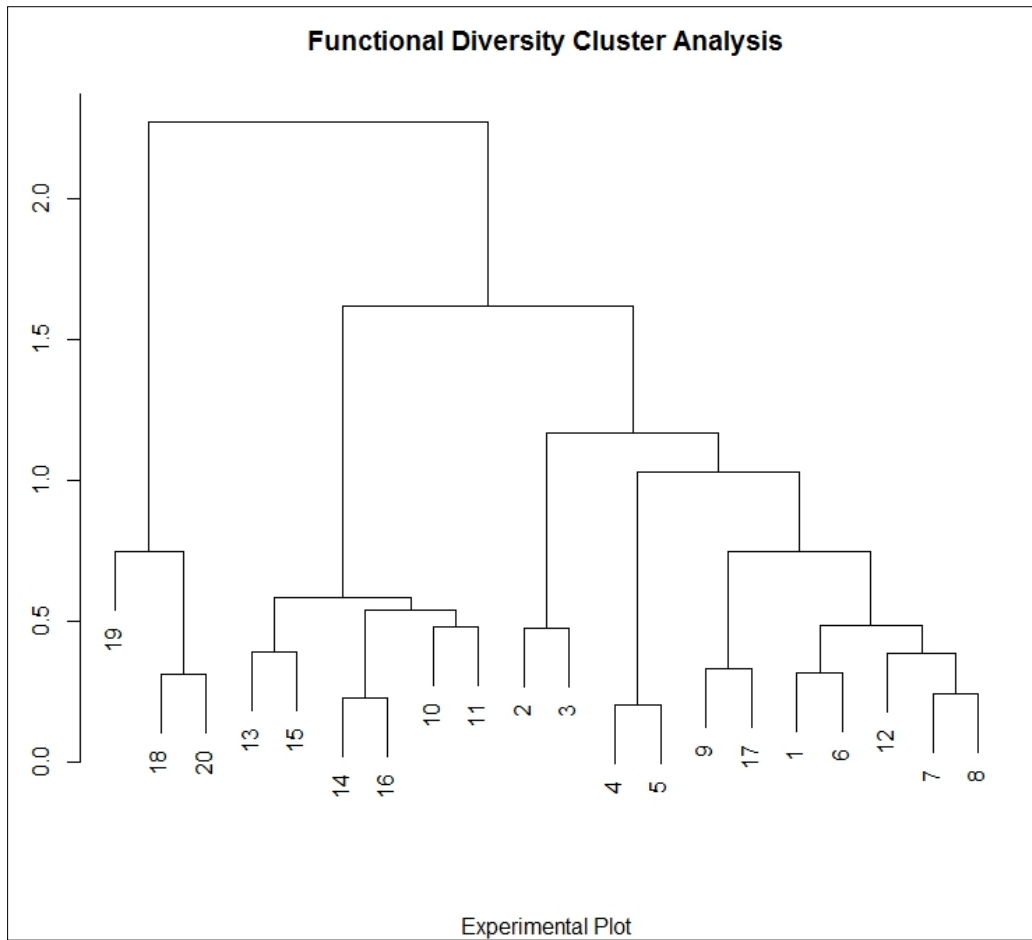


Figure 1: Cluster analysis used to assign plots into experimental pairs

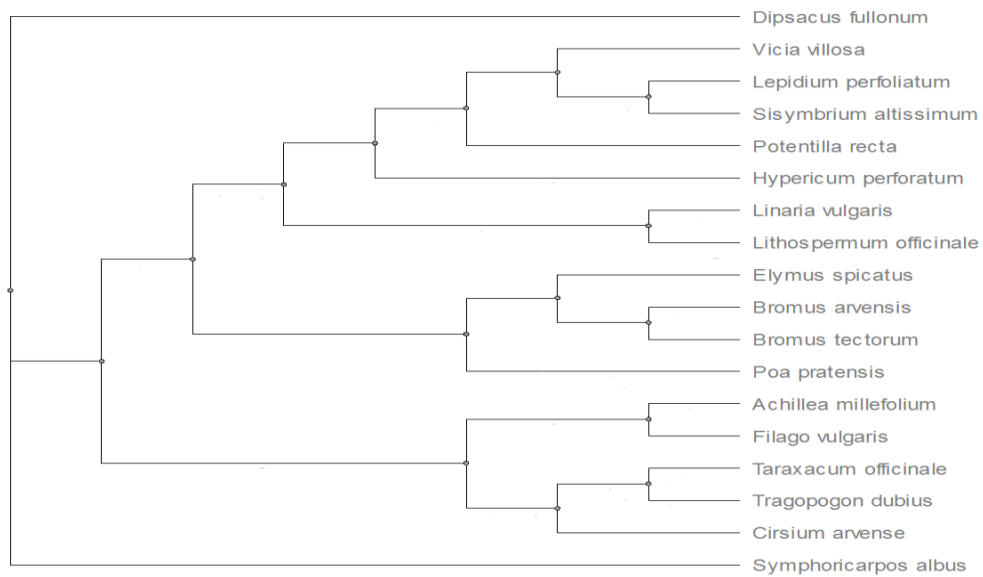


Figure 2: Phylogenetic tree of study species based on *matK* gene sequences

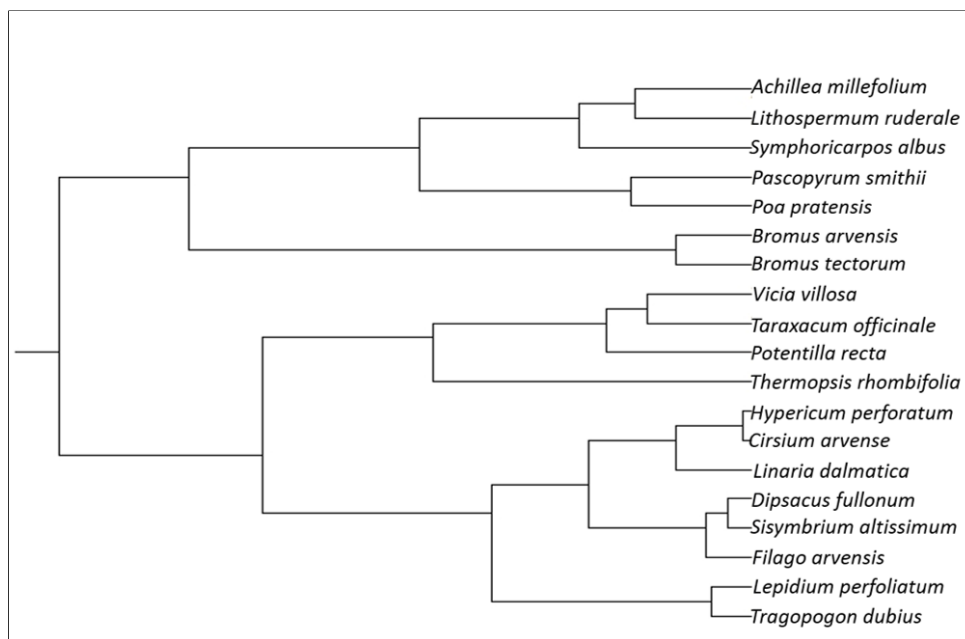


Figure 3: Functional dendrogram of study species based on functional trait matrix

Predictor	Regression	R ²	p
Species Count	$y = -2.457x + 42.679$	0.1385	0.05947
Shannon Diversity	$y = -4.555x + 43.612$	0.1516	0.05045
Simpson Diversity	$y = -4.433x + 40.178$	0.08686	0.1111
Phylogenetic Diversity	$y = -10.053 + 39.312$	0.1031	0.1031
Functional Diversity	$y = -2.912x + 45.251$	0.09479	0.09479

Table 2: Results of regression analysis with pre-treatment plot biomass

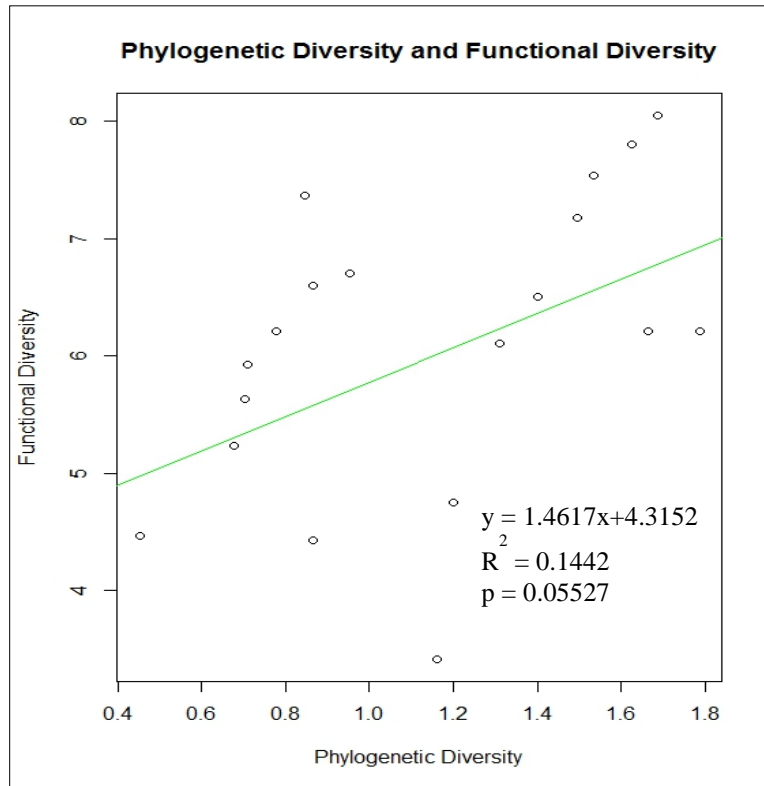


Figure 4: The relationship between phylogenetic and functional diversity across all plots

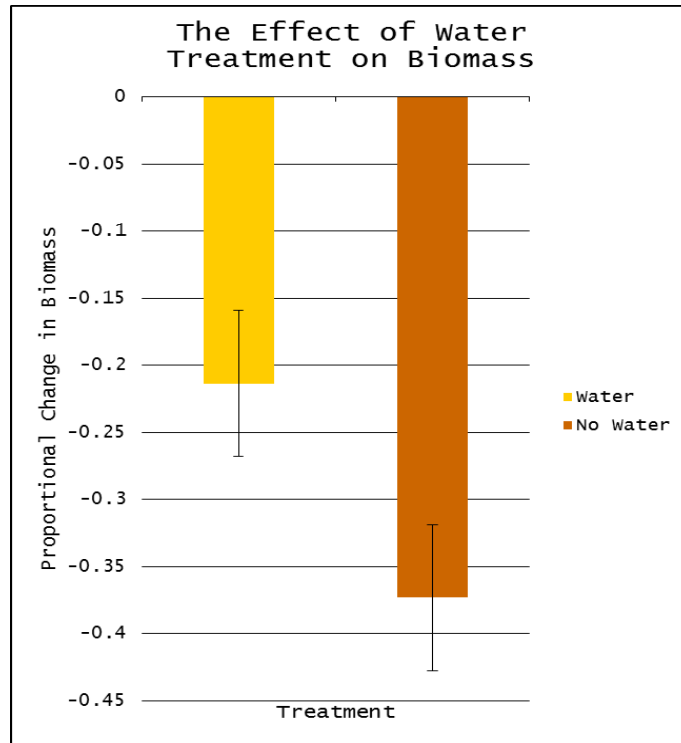


Figure 5: The proportional change across water addition (t -value = 2.6417, p = 0.02684)

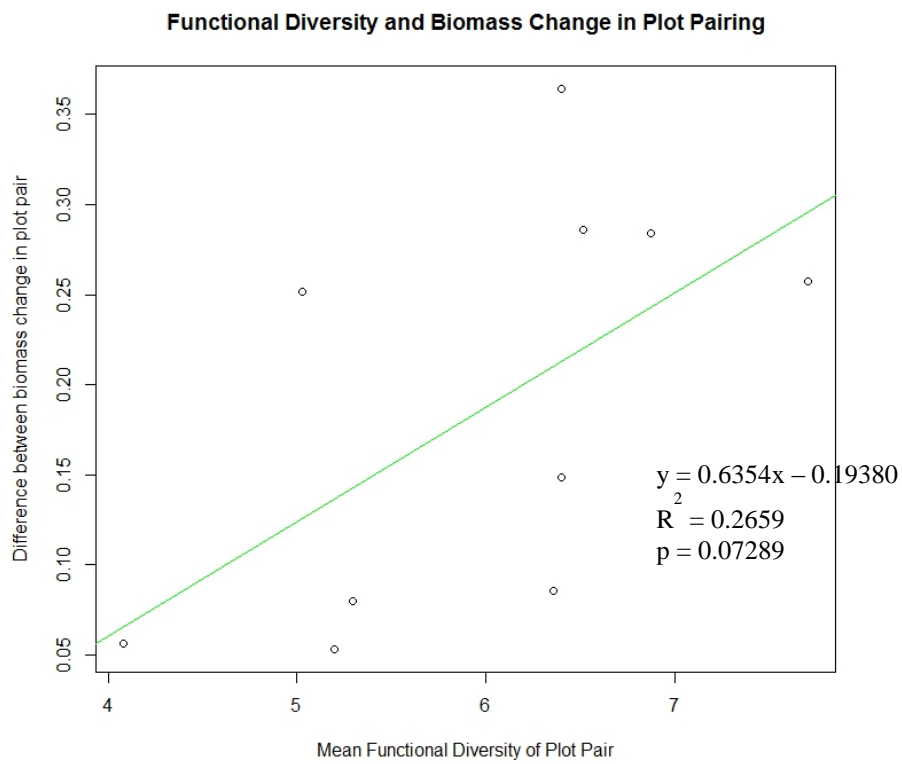


Figure 6: Relationship between functional diversity and biomass change across treatment in paired plots

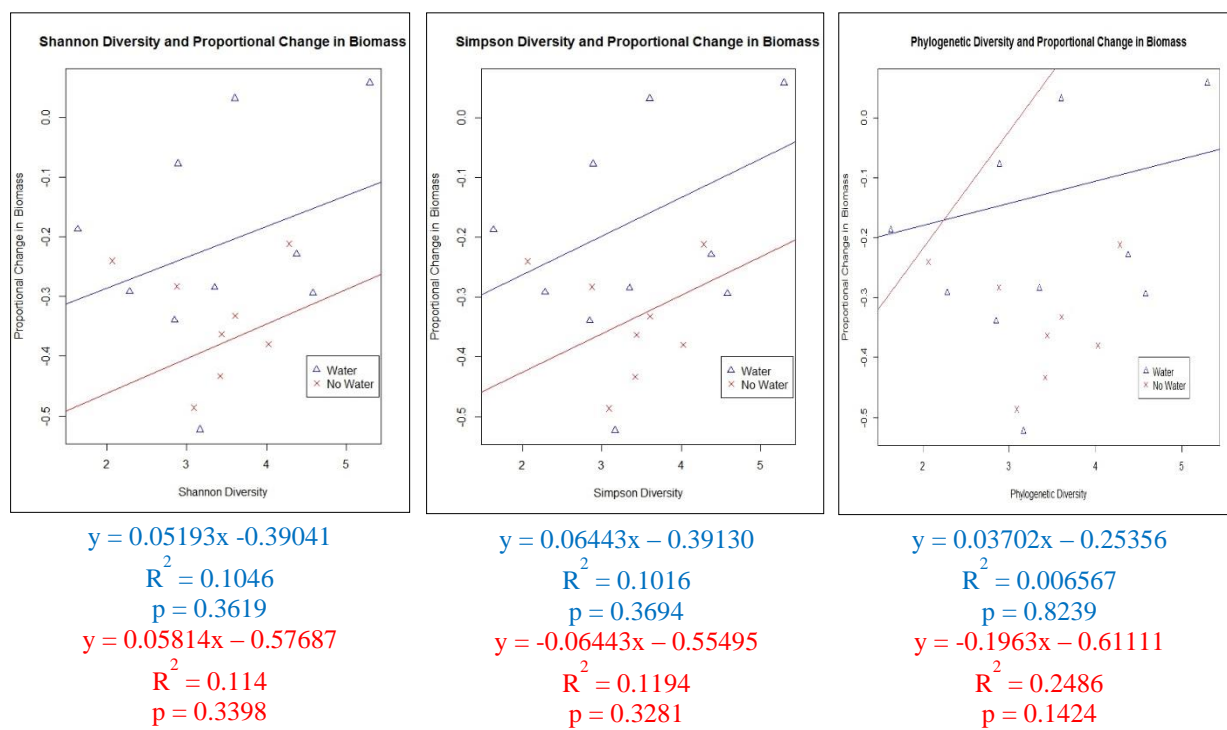


Figure 7: Results of regression analysis within treatment groups

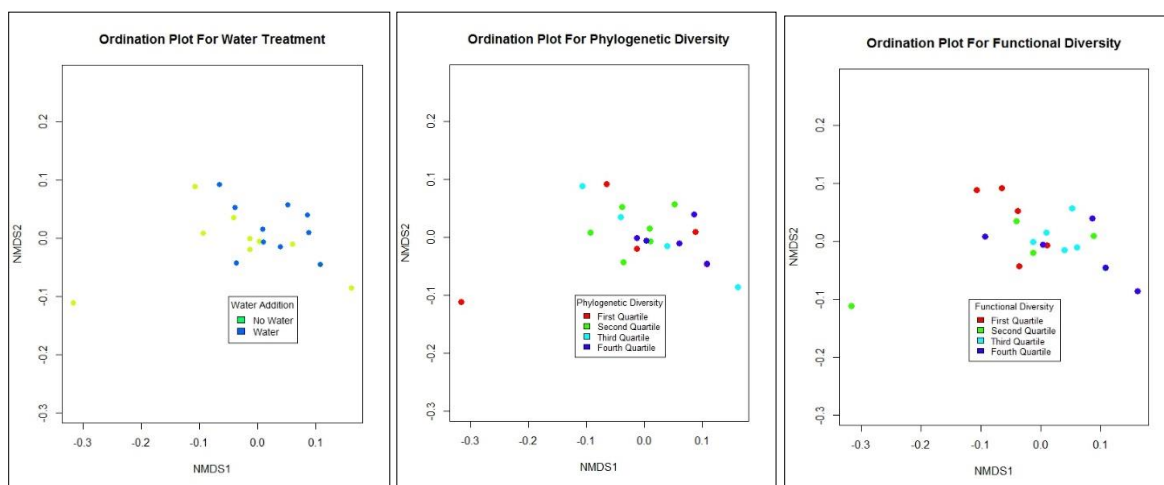


Figure 8: Post-treatment non-metric multidimensional scaling ordination plots