

5-2016

Stream benthic algal relationships with multi-metric indices of sensitivity, exposure, and vulnerability to watershed land use change, with an emphasis on unconventional natural gas development

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**Stream benthic algal relationships with multi-metric indices of sensitivity, exposure,
and vulnerability to watershed land use change, with an emphasis on
unconventional natural gas development**

An Honors Thesis submitted in partial fulfillment of the requirements for Honors Studies
in Biology

By

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Spring 2016

Biology

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i. Abstract

Unconventional natural gas (UNG) is harvested using a unique fossil fuel extraction method that uses horizontal drilling and hydraulic fracturing techniques. The combined methods have expanded the industry both nationally and globally, and development has the ability to transform landscapes and impact freshwater resources. Natural gas wells are often near streams, yet substantial knowledge gaps remain as to how and the extent to which development affects surface waters. Stream algal biomass can respond positively to anthropogenic stressors associated with different types of land use, including agriculture. Benthic algal biomass can also positively correlate with UNG well density and proximity to streams in the Fayetteville shale, but wells are often associated with agricultural land that may confound the relationship.

The first objective of the present study was to determine if stream benthic algal biomass related to previously developed metrics of sensitivity, exposure, and vulnerability to land use change. These metrics incorporated landscape measures of UNG development, an emerging human land use change that may affect stream ecosystems. The second objective was to determine if the relationships among algal biomass and the metrics differed in streams draining lands with and without the presence of UNG wells. Forty stream reaches in the Fayetteville shale were sampled that represented a gradient of vulnerability scores (hereafter, Vulnerability), and analysis of covariance was used to determine if algal biomass, measured as Chlorophyll a, differed based on land use type across a covariate score. The results indicated no relationship between Chlorophyll a and Vulnerability or its two individual metrics, Sensitivity and Exposure ($p > 0.50$ for all scores). There was no difference in Chlorophyll a between sites

with and without UNG wells present. I suggest modifications to the vulnerability index that might yield an overall Vulnerability effect as well as additional considerations for choosing a response metric.

1. Introduction

Ever-increasing technology for extraction of and demand for natural resources threatens the health and condition of many ecosystems. Unconventional natural gas (UNG) extraction, one of the more recently developed fossil fuel extraction methods, has the potential to threaten freshwater resources in the United States and across the globe. UNG development allows the extraction of natural gas from deposits that have previously been inaccessible, and makes this extraction economically feasible, by combining methods such as high-volume hydraulic fracturing (HVHF) and horizontal drilling (Moran et al. 2015). These combined methods require the use of large volumes of water, often withdrawals from nearby streams (Kargbo et al. 2010), and the potential use of chemicals that are not regulated by the U.S. Safe Drinking Water Act (Vidic et al. 2013). Souther et al. (2014) suggested that top research priorities associated with UNG development include studies examining how this development can lead to contamination of freshwater resources.

Extraction of natural gas from shale formations is a developing industry in the U.S. and abroad. With 20 shale plays, the U.S. leads the way in shale gas extraction globally (Malakoff 2014; Brittingham et al. 2014); certainly, natural gas extraction from shale plays in the U.S. is one of the fastest growing trends in domestic onshore energy production (Arthur et al. 2008). New extraction technologies are important in the transition towards renewable energy, and HVHF may help reduce CO₂ emissions compared to conventional energy (Vidic et al. 2013). In addition, other positives of UNG development include the creation of new jobs, increases in economic activity, and the

creation of a more stable and diverse energy base (Burton et al. 2014; Kargbo et al. 2010).

Despite the potential benefits of hydraulic fracturing, there is still much to learn about how it might be affecting freshwater systems (Vengosh et al. 2014). As the quest for sustainable energy continues, landscapes may be transformed before the long-term ecological effects are fully understood (Souther et al. 2014). Many potentially harmful impacts of UNG development are similar to those resulting from activities such as silviculture, agriculture, mining, and urban development (Burton et al. 2014). However, UNG is unusual on a chemical and geographical scale and could potentially lead to effects that are not directly comparable to those from other types of development (Kiviat 2013; Souther et al. 2014). For example, wastewater spills are a unique threat of UNG (Brittingham et al. 2014), and because of its deeper extraction depth, UNG typically results in a larger disturbance footprint than conventional gas drilling (Drohan and Brittingham 2012). UNG development, which is often located near streams, can alter streams through withdrawals, infrastructure development, and land clearing, which can result in sediment runoff, reduced stream flow, altered riparian vegetation, degraded water quality, and the introduction of contaminants (Entrekin et al. 2011; Entrekin et al. 2015). Estimates of water usage include 400-4000 m³ of water to drill an UNG well, and another 7000-18,000 m³ to hydraulically fracture each well (Gregory et al. 2011). Rozell and Reaven (2011) evaluated potential risks of UNG development to surface water and created a model that identified transportation spills, well casing leaks, leaks through fractured rock, drilling site discharge, and wastewater disposal as the top five water contamination pathways.

It is important to study the environmental effects of HVHF to ensure sustainable development and to make informed decisions on how this industry should progress. Using existing data for six productive shale plays in the U.S., Entekin et al. (2015) developed a multivariate vulnerability index of streams that describes vulnerability as a combination of an ecosystem's natural sensitivity to development and the extent of the exposure of an ecosystem to various types of anthropogenic development. A highly vulnerable stream is one with extensive exposure to stressors and high natural sensitivity to such stressors. In theory, streams with a higher calculated sensitivity are more at-risk for alterations due to exposure to anthropogenic stressors. This threat index might be useful as a tool to help predict ecological impacts resulting from UNG development, as well as other existing stressors, and in refining management practices (Entekin et al. 2015).

The index is composed of two multivariate metrics: Exposure and Sensitivity. The exposure metric attempts to quantify human impacts and includes the following variables: road density, %impervious surface cover, %crop cover, %pasture cover, mine density, dam density, vertical well density, and non-vertical well density. HUC12s were used to rank each variable with a score from 0-4 based on calculated quartiles across all HUC12s, with the lowest exposure receiving a score of 0 and the highest a 4. These scores were summed and averaged within each shale play.

The sensitivity metric takes into account natural factors that may make streams more or less susceptible to anthropogenic stressors. The variables included are: thirty-year mean precipitation, catchment slope (in degrees), %wetlands, soil permeability, soil K factor (erodibility), and stream density. Sensitivity scores were assigned similarly to

exposure scores, with the exceptions being precipitation and wetlands, which were assigned inverse scoring (ex: lower precipitation yielded a more highly sensitive ranking). These scores were also summed and averaged, and a vulnerability score was calculated by multiplying each sensitivity score by the corresponding exposure score. The Fayetteville shale in Arkansas was found to have the 2nd highest average Vulnerability out of the six shale plays, and so it is a good place to test these metrics for biological significance.

The effects of human land use, including agriculture, mining, and urbanization, on stream ecosystems have been extensively studied (see review by Allan 2004; Clapcott et al. 2012; Wang et al. 1997). These human actions constitute a significant threat to the ecological integrity and overall health/condition of freshwater systems, and affect these systems through multiple, complex pathways at various scales (Allan et al. 1997). O'Brien and Wehr (2010) demonstrated that streams draining different landscapes (rural versus urban) differed physically, chemically, and biologically, and Taylor et al. (2004) found that large, spatial-scale differences in land use accounted for much more variation in algal communities among study sites than did physical variables such as light and substratum composition. Anthropogenic impacts can occur due to one or a combination of the following: nutrient, pesticide, and sediment runoff, degraded riparian habitat, and altered flow and hydrology associated with development of natural landscapes (Allan 2004; Paul and Meyer 2001; Lenat and Crawford 1994). A gradient approach is often useful in determining the mechanisms and extent to which land use influences stream ecosystems, since changes in land use can vary continuously (O'Brien & Wehr 2010; Taylor et al. 2004; McDonnell and Pickett 1990).

Development of land for HVHF can also lead to habitat fragmentation and degradation; each well installation is comprised of a wellpad, access road(s), storage areas for water, chemicals, sand, and wastewater, a compressor station, and a pipeline (Kiviat 2013). Studies have documented changes to streams that are correlated with UNG development. Williams et al. (2007) found that UNG installations in the Barnett shale in Texas resulted in increased sedimentation to streams for a short time immediately following well drilling, and the disturbed areas around the pad site continued to supply increased levels of sediments for an even longer period of time. Burton et al. (2014) found that stream ecosystems were significantly impacted by UNG development, and linked these impacts to well pad densities, rates of UNG development, distance of development to streams, and proximity to roads and pasture land. Natural gas wells are often associated with agricultural land, so potential threats from UNG development could combine with existing anthropogenic stressors to affect water quality and biological communities, and these cumulative impacts can be difficult to assess and measure (Vidic et al. 2013; Moran et al. 2015). A combination of land use factors may confound the relationship between a single type of land use and stream responses, so one objective of this study was to assess whether the effects of agriculture, a prevalent industry in Arkansas, and natural gas extraction on stream biota could be separated.

Stream algal communities are often sensitive to anthropogenic impacts and respond quickly, so they are commonly used as biological indicators for stream bioassessment studies (Barbour et al. 1999; Evans-White et al. 2013). Human disturbance can result in excess nutrient runoff to streams, which can lead to elevated algal biomass (Delong and Brusven 1998). Stevenson et al. (2006) found that algal biomass positively

correlated with both nitrogen and phosphorus concentrations in streams with varying levels of disturbance. Dodds et al. (2002) demonstrated bottom-up control of benthic algae and found that Total Nitrogen and Total Phosphorus were positively correlated to Chlorophyll a concentrations and explained 40% of the variation for algal biomass in their study streams. In Fayetteville shale streams in particular, it was found that algal biomass and production were positively correlated to nitrogen concentration, which was positively correlated to natural gas activity (Austin 2015). Austin et al. (2015) proposed that unconventional drilling for natural gas might relieve limiting nutrients in streams by runoff, resulting in elevated algal biomass. Johnson et al. (2015) found differences in macroinvertebrate density and community structure in streams draining UNG lands in the Fayetteville shale, and suggested that the increase in algal biomass could instigate these changes.

This study examined benthic algal biomass in Fayetteville shale streams to determine whether the metrics of the recently available vulnerability model (Entrekin et al. 2015) related to the biomass response of these communities to agricultural land use and UNG infrastructure. A gradient approach was used to assess differences in periphyton biomass across streams that drain mostly agricultural land or land with a combination of agriculture and natural gas wells in an effort to separate the effects of each land use on benthic algae.

I hypothesized that benthic algal biomass would relate positively to increasing Vulnerability and Exposure (Figure 1). I expected to find a positive relationship with increasing Exposure due to increased sediment and nutrient runoff and light availability resulting from habitat alterations. The Sensitivity effect would be dependent upon the

exposure of each ecosystem. Together, I expected algal biomass to have a positive relationship with Vulnerability, and predicted a greater slope for the response between algal biomass and Vulnerability for sites in the presence of UNG wells compared to sites without UNG infrastructure; this difference in slope would imply an “UNG effect” above and beyond agriculture.

2. Materials and Methods

2.1 Experimental design

Benthic algal biomass was calculated for 40 streams in north and central Arkansas (Figure 2) during the summer of 2015. Sites were divided into two land-use categories representing one factor based on the presence or absence of UNG wells (+UNG Wells, No UNG Wells). Analysis of covariance (ANCOVA) and regressions were used to test for statistically significant linear associations ($p < 0.05$) of land use and Vulnerability on algal biomass to determine whether the vulnerability index (Entrekin et al. 2015) correctly predicted the response of streams to different anthropogenic stressors. Similar analyses examined the viability of the two individual metrics, Sensitivity and Exposure, used to calculate Vulnerability. Water samples were also analyzed for nutrient concentrations to examine relationships of nutrients with Chlorophyll a, land use, and physical landscape variables.

2.2 Site Selection

Streams sampled for this study are located in the Fayetteville shale play in Arkansas, and are within the Boston Mountain and Arkansas River Valley Ecoregions. Sensitivity, exposure, and vulnerability scores were calculated for all catchments according to Entrekin et al. (2015) using available data for all streams in the Fayetteville shale (Table1). Table 2 shows the mean and range for each of the land-use variables taken into account in the exposure scores, and for each of the variables taken into account in the sensitivity scores. All sites had some percentage of the draining land used for agriculture (measured as percent crop plus percent pasture). Sites were divided into catchments that had UNG development present and those that had no UNG development,

and were chosen across a gradient of exposure to each land use factor (+UNG, No UNG). This resulted in a gradient of calculated vulnerability scores for each category. Sampling took place during May and June of 2015. Study reaches (~200 m) with and without UNG development present in the watershed (N=18 and 22, respectively) were sampled, resulting in 40 experimental units (N=40).

2.3 Sampling Method

2.3.1 Chlorophyll a

Each site consisted of two riffles, and periphyton samples were taken in each riffle by choosing three random cobbles of roughly average size (6 cobbles per site). A stiff utility brush was used to scrape the periphyton from each cobble into a container, and water was added to create a composite slurry for each site. The algal samples were held on ice and frozen if not immediately processed. Frozen samples were held in the dark and allowed to thaw completely before processing.

As a proxy to estimate algal biomass, samples were analyzed for Chlorophyll a (Chl a). The total volume of each algal sample was measured, homogenized, and two subsamples of 5-30 mL were pushed through pre-ashed glass fiber filters into a vial. Next, 5-10 mL of 95% ethanol was added to each subsample filter to extract the Chl a. The solutions were boiled in a water bath at 78°C and then allowed to sit in a dark refrigerator for 24 hours according to Sartory and Grobbelaar (1984). Using a Genesys 10 VIS spectrophotometer (Thermo Fischer Scientific Inc., Waltham, MA), each sample's absorbance was measured at 664 and 750 nm. Then, 100 microliters of 0.1 N HCl was added to acidify each subsample and correct for phaeopigments, and after 90 seconds, the absorbance at 665 and 750 nm was recorded. Chl a values (μg) were calculated according

to linear relationships between absorbance values and Chl a concentrations published in APHA (2005).

Each cobble from which periphyton was scraped was wrapped in aluminum foil, which was then weighed. Foil mass was converted to surface area using a conversion factor based on the weight of a 1-cm² piece of foil, and the values were divided by two to produce an estimate for the upper half of each cobble's surface (the area accountable for the periphyton). These values were used to convert the Chl a values to mass per unit area ($\mu\text{g}/\text{cm}^2$).

2.3.2 Water Column Nutrients

At the top of the more downstream riffle at each site, water samples were taken to analyze for nutrient concentrations. One unfiltered and one filtered sample were taken and stored on ice or frozen until processing. Samples were analyzed for Total Nitrogen (TN) and Total Phosphorus (TP) concentrations. TP concentrations were calculated by first digesting the samples with persulfate, and then analyzed following the molybdate and ascorbic acid Soluble Reactive Phosphorus method found in APHA (2005) using the above spectrophotometer. The TN analysis followed standard methods found in APHA (2005) using a Lachat QuikChem Analysis System (Lachat Instruments, Loveland, CO).

2.4 Statistical Analysis

Chlorophyll a values were log₁₀ transformed to normalize the data. Analysis of covariance (ANCOVA) was used to examine the interactions and individual effects of Vulnerability (covariate) on algal biomass (response variable) for each of the land use categories (1 factor: +UNG, No UNG). If this yielded a significant interaction effect ($\alpha=0.05$), linear regressions were used to examine the relationships between sites with

UNG and Vulnerability and between sites without UNG and Vulnerability separately. If there was no statistically significant interaction effect, a regression was done across all sites to test for a significant linear relationship between Vulnerability and algal biomass. This analysis was repeated with Sensitivity and Exposure as the covariate, respectively. I wanted to determine if any individual measures within each metric were related to benthic algal biomass. So, a Pearson correlation matrix was used to assess univariate correlations among all variables taken into account in the sensitivity and exposure metrics and nutrient concentrations to determine the variables driving Chlorophyll a variation. All analyses were performed in R Statistical Package, Version 3.2.3 (2015, Vienna, Austria).

3. Results

3.1 ANCOVA Results

3.1.1 Vulnerability Analysis

Chlorophyll a spanned a wide range of values (0.22 – 11.66 $\mu\text{g}/\text{cm}^2$), though concentrations were predominately low (mean=1.83, median=1.10 $\mu\text{g}/\text{cm}^2$). The ANCOVA indicated that the algal response, log-transformed Chl a, did not differ based on the presence or absence of UNG wells ($p=0.69$) across a covariate of vulnerability scores. There was not a statistically significant interaction effect of Vulnerability and land use ($p=0.63$). Linear regression indicated that there was no statistical relationship between Chl a and Vulnerability across all sites ($p=0.66$). (Figure 3)

3.1.2 Sensitivity Analysis

ANCOVA suggested no statistically significant difference of Chl a across a gradient of sensitivity scores based on the presence or absence of UNG wells ($p=0.82$), and there was not an interaction effect of Sensitivity and land use type ($p=0.88$). Across all sites, Chl a was not found to relate to sensitivity scores ($p=0.82$). (Figure 3)

3.1.3 Exposure Analysis

ANCOVA results suggested that sites with UNG wells present did not differ in their Chl a density compared to sites without UNG wells ($p=0.51$) across a gradient of exposure scores. The intercepts of these lines did not differ ($p=0.52$), which implies no effect from an interaction of Exposure and land use. Regression found no overall statistically significant relationship between exposure scores and Chl a ($p=0.54$). (Figure 3)

3.2 Pearson Correlation Results

Two of the sensitivity variables, catchment slope and permeability, and two of the exposure variables, UNG Density and %Pasture+%Crop, explained some of the variation in Chl a (Figure 4, Table 3). Pearson correlation showed weak negative relationships between slope ($r=-0.35$, $p=0.02$) and permeability ($r=-0.27$, $p=0.09$) with the log-transformed Chl a values. Contrastingly, two exposure variables, UNG Density and %Pasture+%Crop, had slight positive correlations with Chl a ($r=0.29$, $p=0.07$; $r=0.32$, $p=0.04$, respectively). Slope was negatively associated with UNG Density ($r=-0.34$, $p=0.03$) and %Pasture+%Crop ($r=-0.82$, $p<0.01$), although permeability did not have statistically significant relationships with UNG Density or %Pasture+%Crop.

TN values ranged from $<0.05 - 1.16 \mu\text{g/L}$ and did not show a significant correlation with Chl a. Like Chl a, TN did have negative relationships with slope ($r=-0.49$, $p<0.01$) and permeability ($r=-0.37$, $p=0.01$). There was not a statistically significant relationship between TN and UNG Density, but TN related positively with %Pasture+%Crop ($r=0.57$, $p<0.01$). Overall, TN had positive relationships with Sensitivity ($r=0.42$, $p<0.01$), Exposure ($r=0.38$, $p=0.01$), and Vulnerability ($r=0.43$, $p<0.01$).

TP concentrations ranged from <2 to $66.31 \mu\text{g/L}$. TP was not related to Chl a, permeability, or UNG Density, but was negatively related to slope ($r=-0.37$, $p=0.01$) and positively related to %Pasture+%Crop ($r=0.32$, $p=0.04$). TP also had positive relationships with Exposure ($r=0.56$, $p<0.01$) and Vulnerability ($r=0.51$, $p<0.01$).

4. Discussion

The previously published vulnerability index was not predictive of the observed variation in Chlorophyll a from the present study's stream reaches sampled in May and June 2015. The algal response metric did not relate to any of the three metrics tested: Sensitivity, Exposure, or their product, Vulnerability. Although this multivariate index approach was limited in its ability to detect relationships, some of the variables included in each metric were related to Chl a. It is possible that these weak relationships, such as permeability, slope, UNG Density, and %Pasture+%Crop, were diluted in their respective index scores due to the several other variables included, which did not have statistically significant relationships with Chl a. Additionally, the opposite-direction relationships of sensitivity variables (which predicted a negative relationship) and exposure variables (positive) may have canceled when combined into a vulnerability score. A detectable Vulnerability effect might result if the relationships between Chl a and the two component indices (Sensitivity and Exposure) were unidirectional.

Multi-metric approaches are difficult due to the many confounding factors that influence stream biota and the varying factors that influence different trophic levels at different spatial and temporal scales. The variables included in the sensitivity metric were designed to predict species diversity and not necessarily algal biomass, but algal biomass is a common and economical bioassessment variable that might be helpful in assessing possible UNG impacts to wadeable streams. The goal of the sensitivity metric was not to directly relate to biological response variables. Instead, its purpose was to identify streams that would have a stronger response to human land use changes that would be visible when Sensitivity was combined with Exposure. Specifically, reaches receiving a

high sensitivity score should have a steeper positive or negative relationship with Vulnerability than reaches receiving a low sensitivity score. Modifying this metric to use specifically with algal biomass estimates may yield more intuitive results. For example, replacing percent wetlands with stream order may generate a steeper, positive slope for the relationship between benthic algal biomass and Vulnerability in forested wadeable streams. Light-limitation in low-order streams could influence the density of periphyton (Hill et al. 2009), and runoff from agricultural lands is more likely to result in eutrophication in low-order streams due to the lower dilution in these smaller streams (Montuelle et al. 2010). As reported in this study, Detenbeck (2000) found that slope and soil permeability influence a stream's resilience to stressors, but that observed relationship was influenced by the natural hydrologic regime, including annual precipitation.

I suspect that the hydrologic regime during the timeline of field sampling, May and June 2015, played a large role in the predominately low Chlorophyll a values observed and the lack of correlation with nutrients. During the summer of 2015, Arkansas experienced higher than average rainfall (NOAA Arkansas Yearly Climate Summary) and frequent flooding during the 2015 El Niño events. It was not possible to sample study reaches during a time when they had been at baseflow for long enough that benthic algae could recover from high flow events since the whole year was characterized by an increased frequency of spates. These spates scoured the benthos and likely greatly altered stream biota, including possible decreased benthic periphyton biomass and increased or diluted nutrient concentrations, depending on sources and type of nutrient. Frequent flooding has been linked to decreases in Chl a (Biggs 1995), and such disturbances can

interact or supersede relationships with other factors that might otherwise influence Chl a. Likewise, Lohman et al (1992) found significant decreases in Chl a after flooding, and suggested that frequency and intensity of floods are the dominant factors in explaining variation in Chl a. Their results also suggest that streams that are more exposed to human development rebound more quickly than undisturbed sites because in flood-free periods, nutrient enrichment is able to exert its influences on biomass accrual. Biggs (2000) also found that streams with a higher frequency of floods did not respond to increases in nutrients as strongly as sites that were less disturbed by flooding. During this study's restricted sampling period, streams were continually rising and falling, and it is possible that they did not have time to fully recover and reach maximum standing crop of benthic algae before sampling took place, leading to the observed typically low densities of Chlorophyll a.

To mitigate the effects of temporal variation in climate, which affects light, temperature, disturbance, and grazing, on nutrient concentration and limitation and algal abundance, it is useful to sample sites multiple times seasonally (Francoeur et al. 1999). The relative importance of these factors that influence algal abundance can also vary seasonally (Rosemond et al. 1999). The temporal scale of this study might have limited the efficacy of the vulnerability model to detect changes in algal abundance related to different land uses. During a two-year study, Hoorman et al. (2008) found significant seasonal variations in stream nutrient concentrations from agricultural runoff. Austin et al. (2015) found seasonal variations in Chl a, which affected the strength of their observed relationship between Chl a and UNG development. In that study, algal samples taken during the winter season had less variability and indicated a stronger correlation

with distance to UNG Wells. These results, along with excessive flooding, imply a cautious interpretation of the lack of statistically significant relationships found in this study.

Another possible reason for the lack of detection of an “UNG effect” based on statistically insignificant ANCOVA results is the low power of the vulnerability model. However, while Austin et al. (2015) found a significant positive correlation between Chlorophyll a and UNG Wells, the results of the present study show only a marginally significant correlation, and it was not able to be separated from effects of other land use, such as agriculture, which explained more of the variation in Chl a. There was also no observed relationship between UNG Density and TN or TP in this study. Over time, the initial impact of UNG development might have decreased until streams were able to recover and reach equilibrium. For the Marcellus shale play in Pennsylvania, Brantley et al. (2014) suggested that HVHF incidents involving nutrients and contaminants that impact freshwater resources might be rare and quickly diluted. Rather than nutrient concentrations, sedimentation due to habitat alteration might be a more suitable predictor of lasting UNG effects (as seen in McBroom et al. 2012; Williams et al. 2007).

Algal communities respond not only to in-stream and landscape-scale physical factors but also to land-use, which makes it difficult to detect individual relationships. Despite the fact that Chlorophyll a is widely used in studies and is relatively easy to sample, there is not a clear consensus on which algal metrics are most useful as bio indicators (Black et al. 2011; Morin and Cattaneo 1992). Specifically, some studies have been unable to detect relationships between land-use or environmental variables with Chl a. Liess et al. (2012) sampled streams across a gradient of catchment land use and was

unable to detect a single significant predictive model of Chl a with any of the land-use, nutrient, or in-stream variables studied. Hill et al. (2009) also found high variation in Chl a when studying the interacting effects of light and nutrients on periphyton, and warned that it should be used cautiously as a proxy for algal abundance. Growth rate, ash-free dry weight, or biovolume could be used in conjunction with Chlorophyll a to provide additional accuracy in abundance estimates. Species composition data, such as diversity and taxon richness, would also be useful in sensitivity analyses, since certain pollution-tolerant taxa could be hypothesized to be in greater abundance in more highly vulnerable sites.

Vulnerability classification of freshwater resources can be a useful tool for environmental managers to assist with preventing further degradation of and restoring previously impaired ecosystems (Detenbeck et al. 2000). There are numerous ways to classify an ecosystem's vulnerability to stressors; nevertheless, Paukert et al. (2008) examined published threat indices and found that many classification methodologies, grouped into categories based on how severity and frequency of stressors are calculated, provide similar threat scores, regardless of the exact system used to calculate such scores. They also found that, for each of the studied indices, percent urban land was most highly correlated with vulnerability, and variables such as road density and number of stream crossings greatly influenced the overall vulnerability scores. Whether each of these indices is biologically significant in a given setting is less clear. My results show that a multi-metric analysis can be limited based on the specifics of a study. The variables included in a threat index must be relevant to the response measured, and the response must provide a clear indication of an ecosystem's quality or condition. When examining

algal communities, this approach is made even more difficult due to the variable relative influence of factors that affect benthic periphyton, such as the interacting effects of light and nutrient limitation/saturation. The scale of such studies must also account for temporal and spatial variation in continuously changing lotic systems.

Overall, this study was unable to validate the ability of the recently available vulnerability index to predict a relationship between its multi-metric scores and algal biomass, though the limited temporal scale of this study must be taken into account. Tailoring the metrics to better reflect a true sensitivity relationship might provide more insight into the capabilities of this biotic index. Further, this model was unable to detect differences in periphyton abundance between sites that had UNG wells compared to those without. I suggest that this is due to a combination of the fading effect of the initial impact of UNG development and the low power of the current model to detect changes in biomass. Further studies based on suggested modifications of this vulnerability model may provide guidance for its use as a predictive tool for UNG development.

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6. Figure Legends

Figure 1. Hypothetical relationship between Vulnerability and Exposure scores and Chlorophyll a.

Figure 2. Map of the location of the 40 study sites in the Fayetteville shale play separated by land use, No UNG Wells (red) and +UNG Wells (black).

Figure 3. Scatterplots of Log(Chl a) versus Vulnerability (a), Sensitivity (b), and Exposure (c) scores as calculated by Entekin et al (2015).

Figure 4. Scatterplots of Log (Chl a) versus Slope (degrees), Permeability, UNG Well Density (wells/km²), and %Pasture+%Crop.

7. Figures

Figure 1.

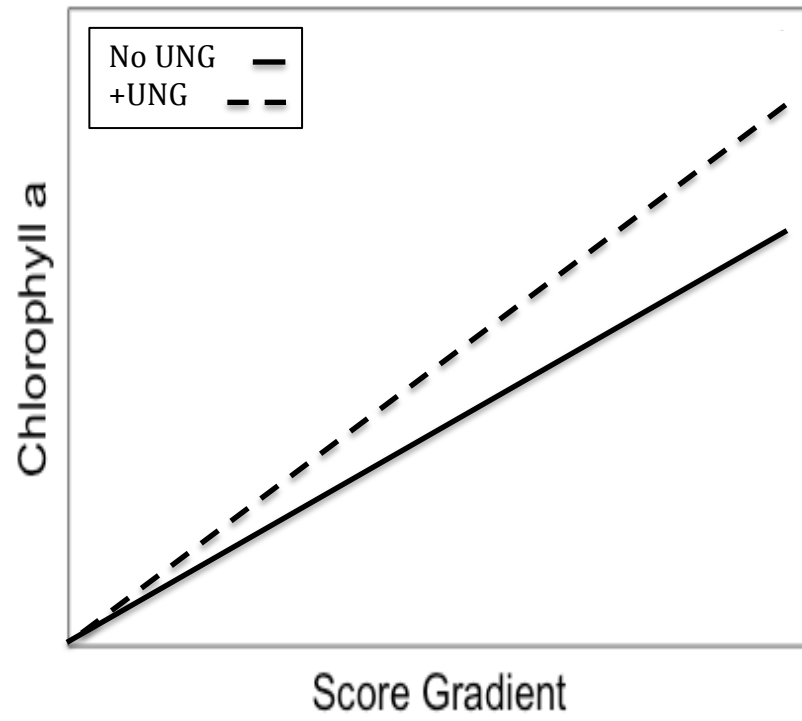


Figure 2.

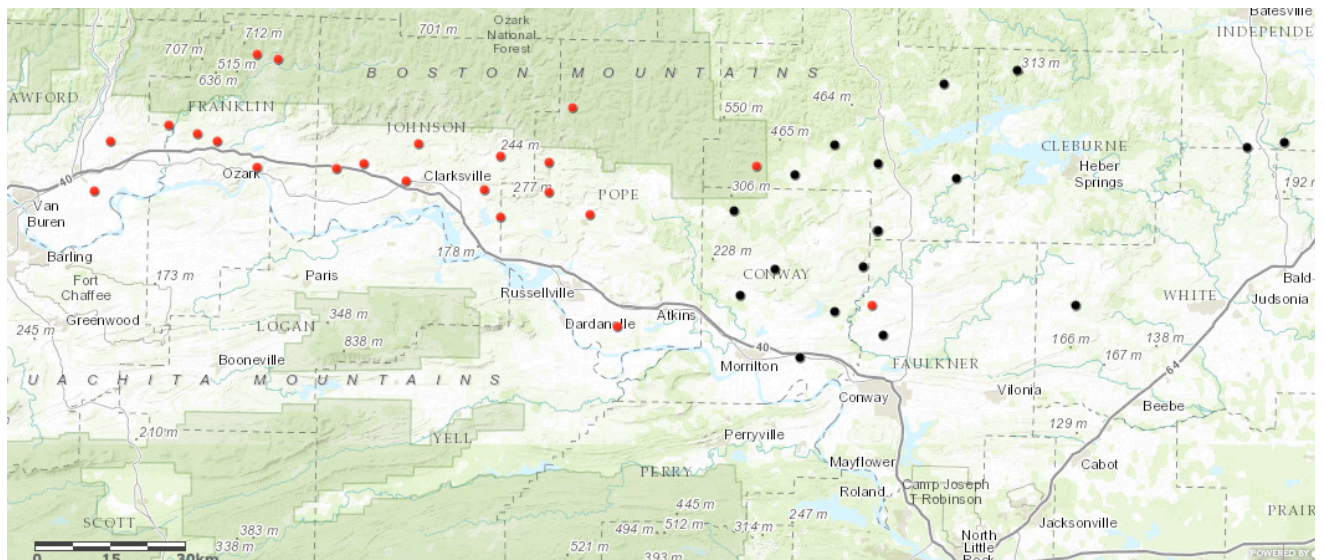
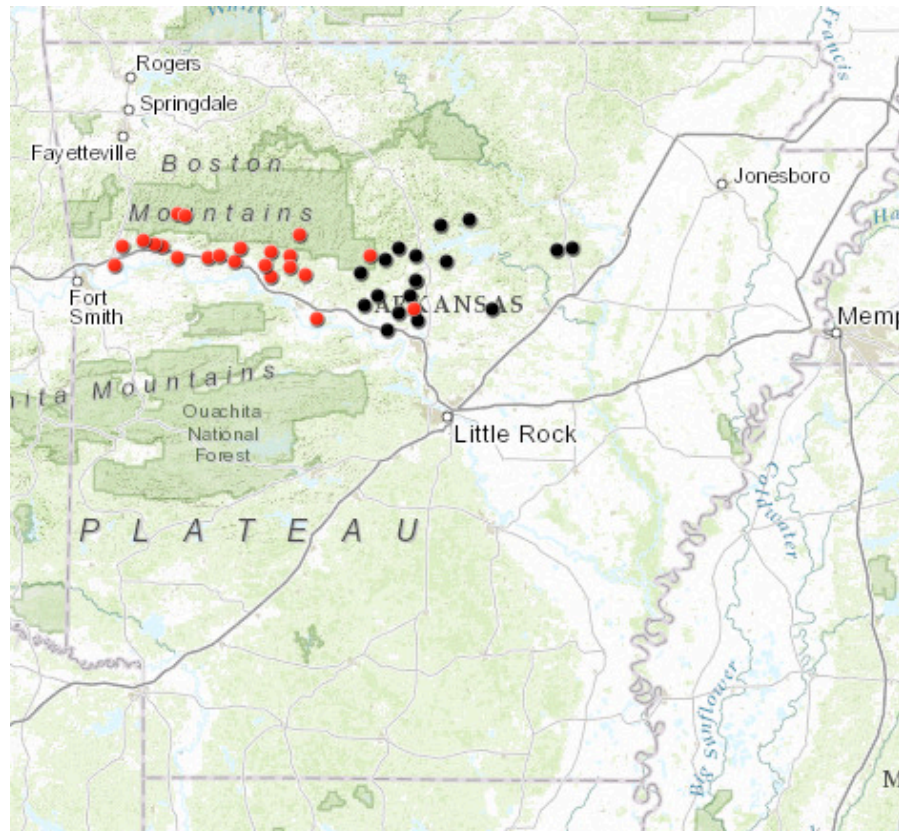
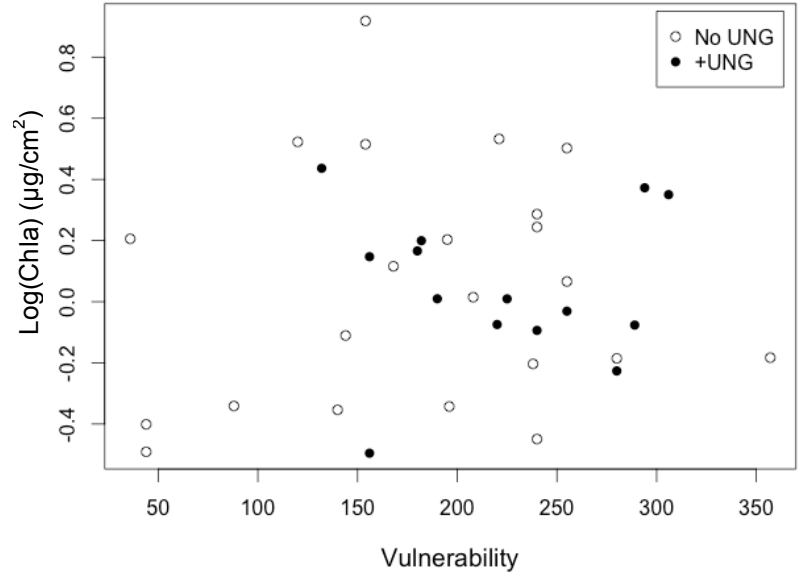
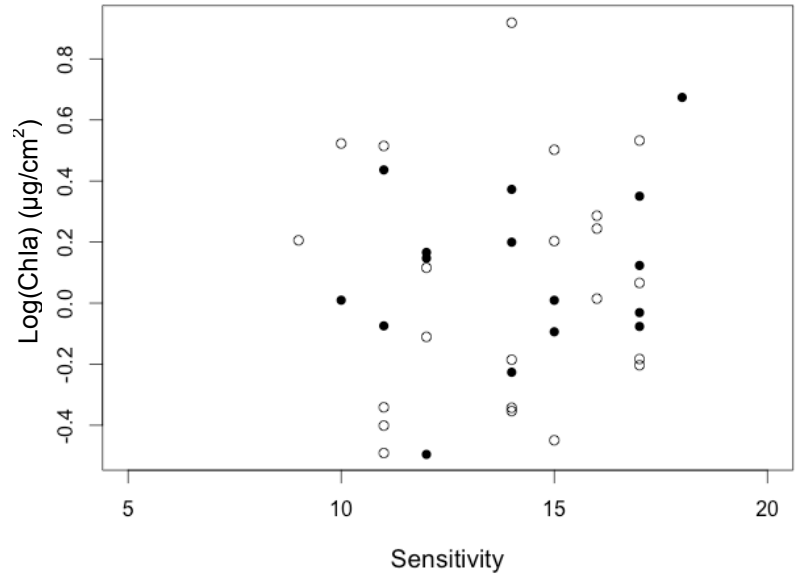


Figure 3.

a.



b.



c.

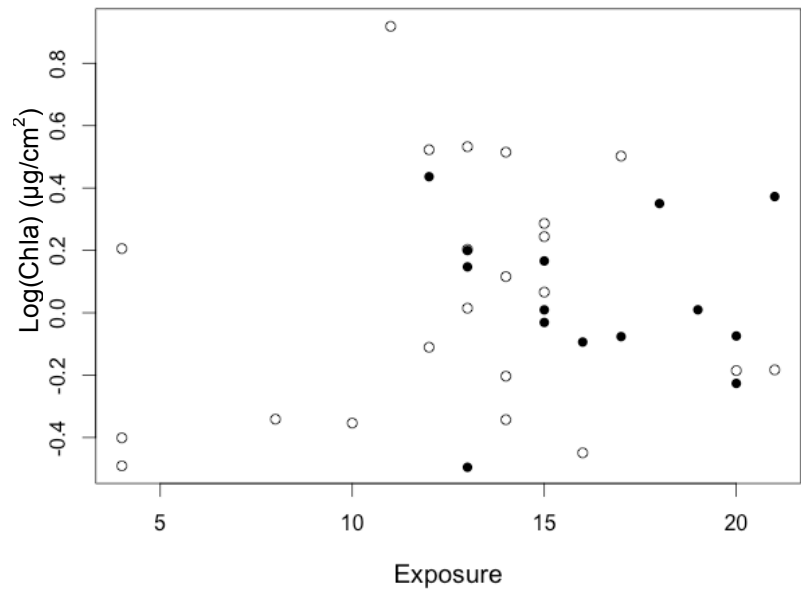
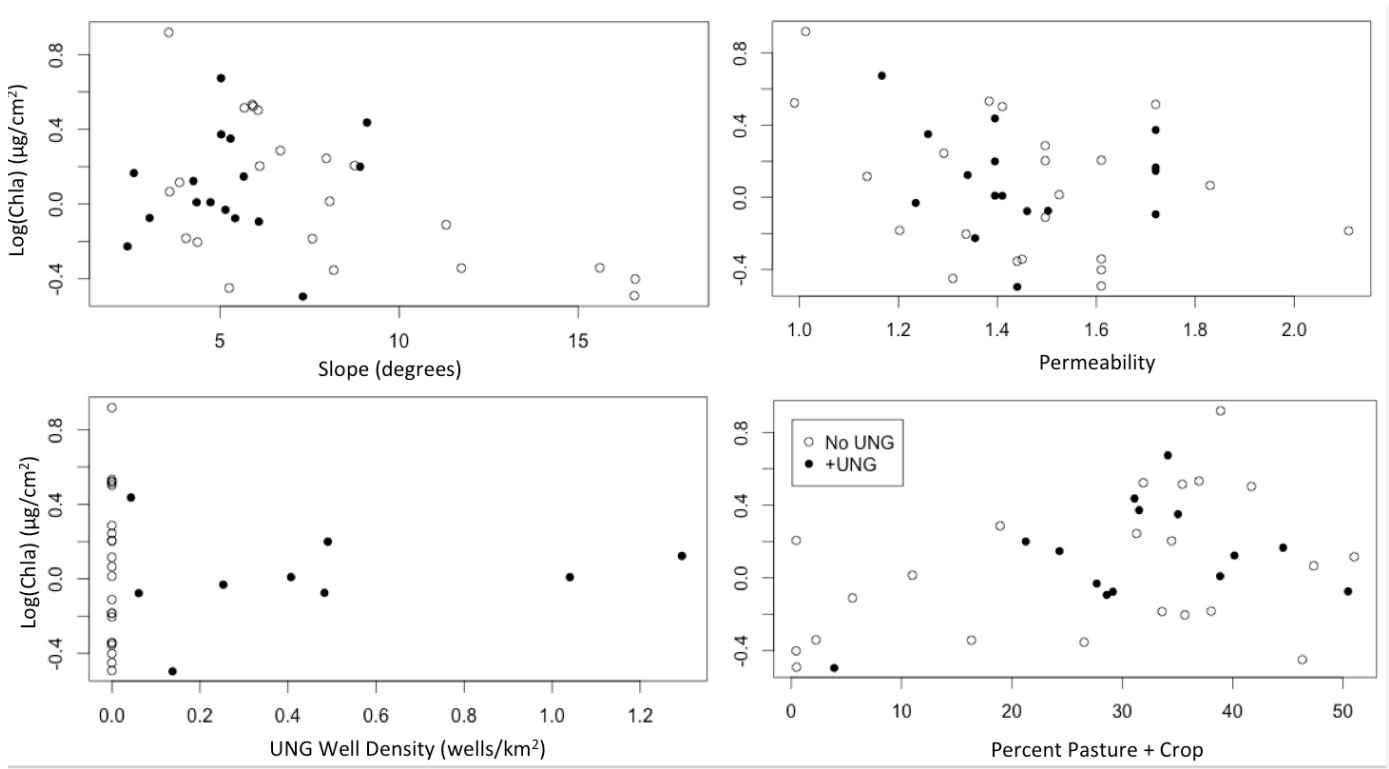


Figure 4.



8. Table Legends

Table 1. Calculated Sensitivity, Exposure, and Vulnerability scores for each of the 40 streams sampled for each of the two land use categories.

Table 2. Mean, minimum, and maximum values for Exposure variables (dark grey) and Sensitivity variables (light grey).

Table 3. Pearson Correlation matrix for each of the scores, sensitivity variables, exposure values, TN, TP, and Log (Chl a).

9. Tables

Table 1.

Stream	Sensitivity	Exposure	Vulnerability	Land Use
Bayou Des Arc	15	15	225	+UNG
Beardy Branch	12	15	180	+UNG
Butler Creek	12	13	156	+UNG
Cedar Big	14	11	154	No UNG
Cedar Cove	17	18	306	+UNG
Choctaw Creek	15	16	240	+UNG
Cravens	11	14	154	No UNG
Creben Creek	17	15	255	+UNG
Departee Creek	10	19	190	+UNG
Dirty Creek	15	17	255	No UNG
Driver Creek	9	4	36	No UNG
EF Horsehead	16	15	240	No UNG
Fane Creek	11	4	44	No UNG
Galla Creek	17	21	357	No UNG
Gap Creek	17	17	289	+UNG
Gar Creek	17	14	238	No UNG
Granny Creek	16	15	240	No UNG
Greenbrier Creek	15	26	390	+UNG
Hill Creek	11	12	132	+UNG
Hogan's Creek	18	22	396	+UNG
Indian Creek	11	4	44	No UNG
Jack's Fork	12	14	168	No UNG
Little Froggy Bayou	17	15	255	No UNG
Little Mulberry	14	20	280	No UNG
Little Spadra	15	16	240	No UNG
Maxie Creek	10	12	120	No UNG
McCoy Creek	17	13	221	No UNG
Mill Creek	16	13	208	No UNG
Mill Mulberry	12	12	144	No UNG
Mountain Creek	11	8	88	No UNG
NF Cadron	14	21	294	+UNG
Pine Creek	12	19	228	+UNG
Pool Hollow	17	24	408	+UNG
Prairie Creek	14	20	280	+UNG
Rock Creek	12	13	156	+UNG
Slover Creek	15	13	195	No UNG
Spadra Creek	14	14	196	No UNG
Tenmile Creek	11	20	220	+UNG
Weaver Creek	14	13	182	+UNG
Wilson Creek	14	10	140	No UNG

Table 2.

Exposure Variables	%Crop	%Pasture	UNG Density	Mine Density	Dam Density	Vertical Well Density	Road Density	Impervious Surfaces
Mean	0.00	30.74	0.62	0.25	0.02	0.24	7.04	0.46
Range	0 – 0.01	0.44 – 64.81	0 – 2.99	0 – 2	0 – 0.09	0 – 1.43	2.47 – 27.39	0.02 – 3.41
Sensitivity Variables	Permeability	Precipitation (inversed)	Stream Density	%Wetlands (inversed)	Slope (degrees)	kfactor		
Mean	1.45	1299.15	1.63	0.47	6.62	0.29		
Range	0.99 – 2.11	1252 – 1395	1.03 – 3.01	0 – 12.69	2.42 – 16.59	0.24 – 0.34		

Table 3.

	Log(Chla)	Slope	Precip	Stream Density	Kfactor	Wetlands	Permeability	Dam Density	Mine Density	UNG Density	Crop	Pasture	Crop +Pasture	Impervious Surfaces	Road Density	Vertical Well Density	TN	TP	
Log(Chla)		-0.35, p=0.02	-0.16, p=0.33	0.16, p=0.31	0.04, p=0.83	0.00, p=0.99	-0.27, p=0.09	-0.06, p=0.70	0.14, p=0.38	0.29, p=0.07	0.00, p=0.98	0.32, p=0.04	0.32, p=0.04	0.17, p=0.29	0.08, p=0.61	-0.10, p=0.53	-0.02, p=0.88	-0.21, p=0.19	
Slope	-0.35, p=0.02		0.8, p=0.00	-0.25, p=0.11	-0.55, p=0.00	-0.16, p=0.32	0.26, p=0.10	-0.23, p=0.15	-0.22, p=0.17	-0.34, p=0.03	-0.12, p=0.46	-0.82, p=0.00	-0.82, p=0.00	-0.17, p=0.29	-0.09, p=0.59	-0.01, p=0.93	-0.49, p=0.00	-0.37, p=0.01	
Precip	-0.16, p=0.33	0.8, p=0.00		-0.16, p=0.32	-0.5, p=0.00	-0.2, p=0.21	0.35, p=0.02	-0.06, p=0.72	-0.26, p=0.11	-0.06, p=0.69	-0.08, p=0.61	-0.62, p=0.00	-0.62, p=0.00	-0.21, p=0.18	-0.12, p=0.44	0.01, p=0.97	-0.52, p=0.00	-0.41, p=0.00	
Stream Density	0.16, p=0.31	-0.25, p=0.11	-0.16, p=0.32		0.33, p=0.03	0.14, p=0.39	-0.26, p=0.10	0.25, p=0.12	-0.2, p=0.22	0.25, p=0.11	-0.15, p=0.35	0.13, p=0.42	0.13, p=0.42	-0.1, p=0.54	0.22, p=0.17	-0.11, p=0.50	0.38, p=0.01	0.17, p=0.29	
Kfactor	0.04, p=0.83	-0.55, p=0.00	-0.5, p=0.00	0.33, p=0.03		0.19, p=0.24	-0.40, p=0.01	0.11, p=0.48	-0.07, p=0.67	0.01, p=0.95	0.31, p=0.05	0.45, p=0.00	0.45, p=0.00	0.28, p=0.07	0.09, p=0.57	0.25, p=0.12	0.60, p=0.00	0.27, p=0.09	
Wetlands	0.00, p=0.99	-0.16, p=0.32	-0.2, p=0.21	0.14, p=0.39	0.19, p=0.24		-0.15, p=0.34	0.43, p=0.00	-0.01, p=0.93	0.07, p=0.64	-0.06, p=0.73	0.16, p=0.32	0.16, p=0.32	0.27, p=0.09	0.66, p=0.00	-0.07, p=0.68	0.05, p=0.74	0.39, p=0.01	
Permeability	-0.27, p=0.09	0.26, p=0.10	0.35, p=0.02	-0.26, p=0.10	-0.4, p=0.01	-0.15, p=0.34		0.06, p=0.70	-0.01, p=0.95	0.14, p=0.38	-0.13, p=0.43	-0.24, p=0.12	-0.24, p=0.12	-0.1, p=0.52	-0.06, p=0.70	-0.2, p=0.21	-0.31, p=0.05	-0.16, p=0.30	
Dam Density	-0.06, p=0.70	-0.23, p=0.15	-0.06, p=0.72	0.25, p=0.12	0.11, p=0.48	0.43, p=0.00	0.06, p=0.70		0.29, p=0.06	0.43, p=0.00	-0.03, p=0.86	0.13, p=0.42	0.13, p=0.42	0.02, p=0.89	0.57, p=0.00	-0.06, p=0.72	-0.05, p=0.76	0.27, p=0.09	
UNG Density	0.29, p=0.07	-0.34, p=0.03	-0.06, p=0.72	0.25, p=0.12	0.11, p=0.48	0.43, p=0.00	0.06, p=0.70	0.43, p=0.00	0.33, p=0.03		-0.17, p=0.29	0.3, p=0.06	0.3, p=0.06	0.01, p=0.93	0.31, p=0.05	-0.28, p=0.07	-0.11, p=0.48	0.04, p=0.80	
Crop +Pasture	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.00, p=0.98	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04	0.32, p=0.04
Impervious Surfaces	0.17, p=0.29	-0.17, p=0.29	-0.21, p=0.18	-0.1, p=0.54	0.28, p=0.07	0.27, p=0.09	-0.1, p=0.52	0.02, p=0.89	0.16, p=0.31	0.01, p=0.93	0.35, p=0.02	0.22, p=0.18	0.22, p=0.18		0.35, p=0.02	0.25, p=0.11	0.09, p=0.60	0.12, p=0.45	
Sensitivity	0.04, p=0.82	-0.37, p=0.02	-0.42, p=0.01	0.48, p=0.00	0.68, p=0.00	0.17, p=0.29	-0.20, p=0.22	0.17, p=0.29	-0.21, p=0.18	0.07, p=0.65	0.21, p=0.19	0.29, p=0.06	0.29, p=0.06	0.17, p=0.28	0.31, p=0.05	0.22, p=0.17	0.42, p=0.00	0.19, p=0.24	
Exposure	0.10, p=0.54	-0.71, p=0.00	-0.50, p=0.00	0.29, p=0.07	0.50, p=0.00	0.31, p=0.04	-0.12, p=0.46	0.61, p=0.00	0.21, p=0.19	0.39, p=0.01	0.36, p=0.02	0.64, p=0.00	0.64, p=0.00	0.19, p=0.23	0.39, p=0.01	0.06, p=0.70	0.38, p=0.01	0.56, p=0.00	
Vulnerability	0.07, p=0.66	-0.63, p=0.00	-0.50, p=0.00	0.43, p=0.01	0.64, p=0.00	0.35, p=0.03	-0.17, p=0.28	0.55, p=0.00	0.02, p=0.89	0.32, p=0.04	0.35, p=0.02	0.54, p=0.00	0.54, p=0.00	0.20, p=0.21	0.46, p=0.00	0.16, p=0.31	0.43, p=0.00	0.51, p=0.00	