Fire ash influences on aquatic primary producers through changes in water quality.

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Introduction

Fire is an important ecological factor in many terrestrial ecosystems, resetting the growth of the ecosystem to an early successional stage. However, current climate models predict increased frequencies of forest fires, potentially altering ecosystem function as return intervals are reduced (Fontaine et al. 2015). In addition to decreases in fire return intervals, fires can have short term impacts on water quality as ash is transported into waterways and reservoirs with most of the ash being mobilized within a year of the fire event (Reneau et al., 2007). However, while the dynamics of ash mobilization are difficult to predict (Bodí et al., 2014; Ferreira et al., 2008), there are severe implications when post-fire ash flows enter waterways. While major impacts of post-fire flows are increased erosion resulting large quantities of sediment entering waterways (Ryan et al., 2011), there are also changes in water quality measurements such as increased conductivity and decreased dissolved oxygen concentrations (Dahm et al,. 2015; Earl and Blinn, 2003).

Other than physical and chemical changes which may occur post-fire, aquatic organisms can also be impacted by fluctuations in water quality. While much of the literature focuses on the impacts of ash on invertebrate communities, there is relatively little information on impacts to algal communities (Caldwell et al., 2013). Algal assemblages are the basis of many food chains and are frequently used for biomonitoring of natural waters. A highly responsive and universal group of algae that are frequently used for bio-monitoring are diatoms (Stevenson, 2014). A previous study in New Mexico found that diatom assemblages in the Gila River, N.M. decreased in diversity with a focused loss of larger-bodied diatoms with a shift towards smaller taxa, in particular, *Cocconeis placentula*, a small prostrate growth form (Earl and Blinn, 2003). More recent work in the East Fork of the Jemez River (EFJR) in the Valles Caldera National Preserve (VCNP) found that epiphytic diatom assemblages followed a similar pattern following the Las Conchas wildfire in 2011 of decreased diatom diversity and loss of large taxa with an increase in smaller taxa (Clark et al., 2014). Water quality sensors that were deployed in the EFJR at the time recorded increases in conductivity with decreases in both pH and dissolved oxygen during these post-fire ash flows that may account for these diatom community changes.

Due to the complexity of water quality changes that accompanies ash flows, it is difficult to determine the cause of diatom community changes. Because of this complexity, it is necessary to explore and isolate individual chemical parameters that may be impacting diatom growth post-fire. One possible scenario is that diatom assemblages are impacted by decreases in pH that has been previously observed in other ecosystems (Hlúbiková et al. 2014). The objective of this study is to experimentally determine the impact that pH changes and leachate compounds produced by wildfire ash have on diatom populations with implications on food resources and ecosystem health.

For this research four diatoms were chosen based off of the VCNP observational study due to specific growth characteristics. The diatoms are *Cocconeis placentula* var. *lineata*, an epiphytic diatom which became dominant following the Las Conchas ash flow, *Staurosira construens*, an episammic diatom that is readily scoured by flooding but prevalent in the EFJR, *Rhopalodia gibba*, a large motile growth form that is capable of fixing atmospheric nitrogen due to its cyanobacterial endosymbiont, and *Gomphonema truncatum*, which grows on polysaccharide stalks and is considered an upper canopy taxon for aquatic biofilms. These diatoms were grown in monoculture experiments to isolate specific changes in growth due to the presence/absence of ash. This project looked at the hypothesis that increased pH would cause a positive fertilization effect on diatom growth compared to the control which will partially explain a larger fertilization effect caused by fire ash leachate.

Methods

Ash production-

Tree branches were harvested from the Valles Caldera National Preserve (VCNP) in December 2014 and May 2015 (for Aspen). Six tree species were collected and identified using vouchers from the Museum of Southwestern Biology Herbarium: Ponderosa Pine (*Pinus ponderosa*), Gambel Oak (*Quercus gambelii*), Blue Spruce (*Picea pungens)*, Rocky Mountain Douglas Fir (*Pseudotsuga menziesii* var. *glauca*), Rocky Mountain Juniper (*Juniperus scopulorum*), and Trembling Aspen (*Populus tremuloides*). These trees were selected because they are commonly found in the VCNP and were expected to have been burned in the Las Conchas wildfire in 2011. Tree branches were dried for a minimum of 48 hours at room temperature before ashing. Each tree was ashed separately from the others due to the small size of the muffle furnace and to prevent cross contamination by each of the other tree taxa. Tree material was burned for 2 hours at 400 °C in a muffle oven. Ashed materials were removed from the oven and further burning was minimized by encasing the ash between two Pyrex glass trays to inhibit oxygen. Large particulates were then ground down using a mortar and pestle and stored for later use in an airtight container.

Water quality-

Changes in water quality due to the ash produced by the above procedure were examined in several ways. To determine a concentration of ash similar to that of the East Fork of the Jemez River during the Las Conchas fire flows, a batch experiment was run and conductivity was measured to match a post-fire chemistry of the river. Ash from Rocky Mountain Juniper was weighed and added to 500 mL of deionized water that had been aerated by stirring for 48 hours prior. The final concentrations of ash (in g ash/L water) were 1, 2, 4, 8, 12, 16, 20, 24, and 28 g/L. The ash mixtures were then measured for conductivity, salinity, and dissolved oxygen using a YSI multi-meter for 8 hours with an interval of an hour between readings after the first 4 hours. Alkalinity was measured using colorimetric titration of phenolphthalein indicator dye for pH 8.3 and mixed indicator of bromocresol green and methyl red for pH of 4.5. Samples were prepared by weighing 0.3g of each species of tree ash into a double layer of coffee filters and leached in 100mL of deionized water. After one hour after mixing the ash, 50 mL aliquots were removed and titrated using 0.02N sulfuric acid in the presence of the indicator dyes.

Diatoms-

The diatoms that were experimented with were *Gomphonema truncatum*, *Cocconeis placentula* var. *lineata*, *Staurosira construens*, and *Rhopalodia gibba*. Diatom cultures and the media they were grown in (CR1-S diatom media for *G. truncatum*, and CR1 diatom media for the other 3 taxa) were purchased from the University of Texas collection of algae (UTEX). The diatoms were then grown in the lab until a sufficient density was reached before being subsampled for different experimental conditions. Complications with *G. truncatum* cultures led to only three of the species being included in the first experiment. CR1 diatom medium was homogenized and then 250 mL of media was measured into three 500 mL volumetric flasks. The control was diluted with 250 mL of deionized water. The pH-adjusted treatment was filled with 200 mL of D.I. water and then the pH was modified using sodium hydroxide and hydrochloric acid until a pH of 8.5 was reached and then diluted to a final volume of 500 mL. The ash leachate for the experiment was made by creating 500 mL of a 12 g/L Ponderosa Pine ash solution which was mixed for 48 hours and then filtered just before use. The ash leachate treatment had 125 mL of 12 g/L ash leachate added as well as 75 mL of DI water. Subsequently the pH of the leachate treatment was adjusted the same as the pH adjusted treatment (Figure 1). Each replicate consisted of 50 mL of the treatment solution and 5 mL of diatoms from the stock culture. Pre-experiment values were determined by taking 5 mL of the stock culture and saving in a labeled tube throughout the inoculation process so that any changes in starting conditions would be recorded. All of the samples were fixed with 2.5 mL of 10% formalin to prevent further diatom growth until the samples were counted.

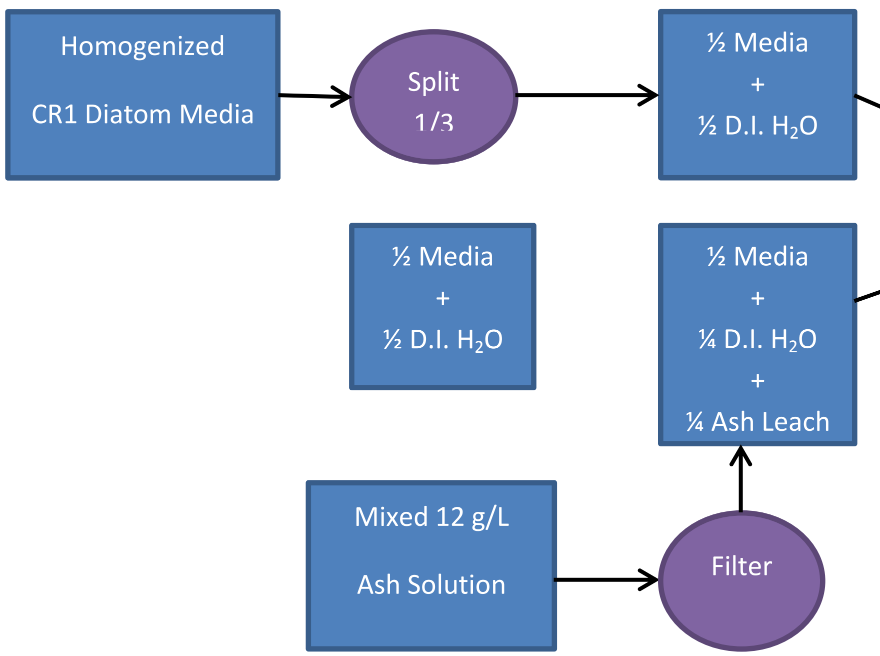

Figure 1: Preparation of treatments for the diatom experiments. Each ash solution was mixed for 48 hours prior to being filtered.

After 8 days of growth, each culture was sonicated for 10 minutes to loosen the diatoms' attachment to the beaker walls and then 5 mL subsamples were removed to determine the percent live/dead counts. The remaining 50 mL of experimental culture were then boiled in 30% hydrogen peroxide for one hour to remove organic matter. The samples were serially rinsed with deionized water after 8 hours of settling and then the volume was reduced to 20 mL. Cell counts were done until a minimum of 300 cells were counted and completion of a transect or a maximum count of 4 transects of a Palmer counting cell (0.1 mL) for all samples and then total numbers were calculated for each culture.

For the multiple ash species experiment, ash of all six tree species was used to create six ash leachate treatments. Each leachate used was kept at a 3 g/L final concentration of leachate and a final pH of 8.3. This experiment was run due to previous data which indicated a variation in the tree ash alkalinity between tree species. In addition, the same method for creating the control and the pH adjusted media were employed in this experiment. Cultures consisted of 50mL of treated media and 5 mL of diatoms and were grown for eight days in four replicates. *Rhopalodia gibba* was the only diatom that was grown and counted for this experiment.

<u>Data Analysis-</u>

Data were analyzed using R statistical software (2015) and graphed using the ggplot2 package within the same software. Diatom growth data was analyzed using one-way analysis of variance (ANOVA) models by species. Tukey post-hoc analysis was run for multiple comparisons to analyze between group variations. Significant differences were determined at the p-value = 0.05 level.

Results

Data from the concentration experiment was plotted as a function of time. As the conductivity data did not have much variation across the experiment, they were averaged across all post-ash addition values and plotted as a function of concentration (Figure 2). These data then allowed for the determination that 3 g ash/L of water was the best concentration to match previous data from the VCNP for comparison. The data from this experiment also indicated an increasing chemical oxygen demand across the concentration gradient, despite noise in the data (Figure 3). Alkalinity was found to vary for the ash of all tree species (Figure 4).

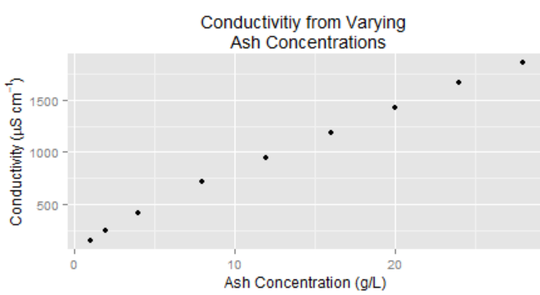

Figure 2: Plot of mean conductivity values following addition of the fire ash to deionized water. The resulting values exceed the conductivity reading from the previous study in the East Fork Jemez River.

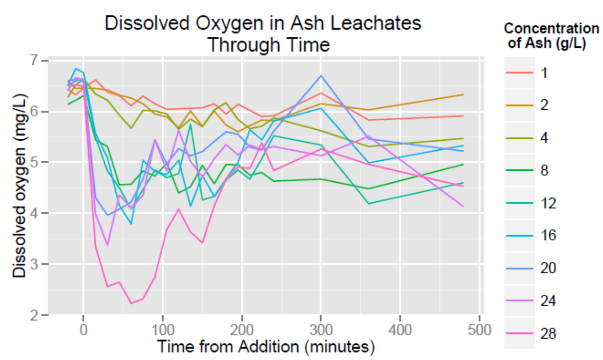

Figure 3: Plot of dissolved oxygen following addition of the fire ash to deionized water. The immediate consumption of oxygen is due to chemicals present in the Juniper ash that readily bind to dissolved oxygen. The chemical oxygen demand will be further studied with Drs. Cerrato and Blake of UNM

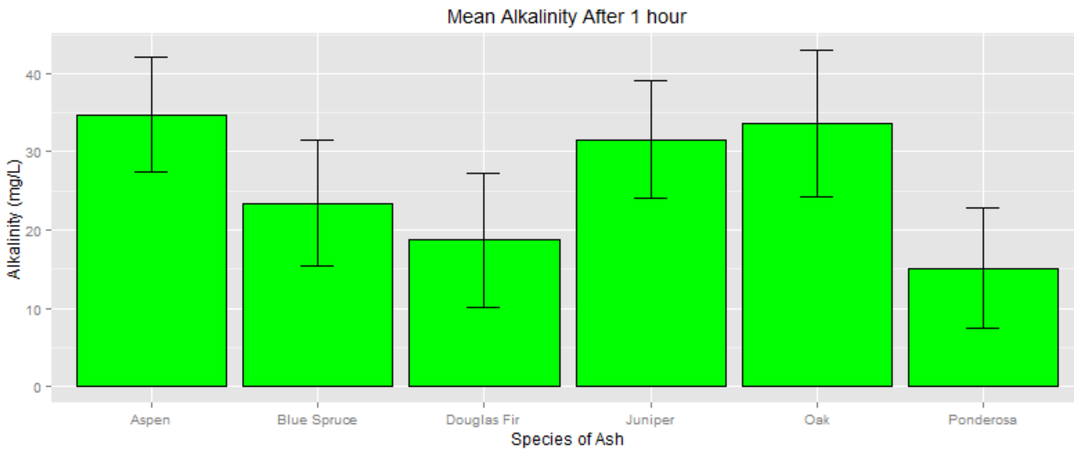

Figure 4: Mean alkalinity of fire ash leachate after one hour. The alkalinity of fire ash can vary between species of tree, though a forest fire will have ash from multiple sources and so will vary from the measured values.

Diatoms from the first experiment were counted and plotted through time and growth rates were analyzed using one-way ANOVAs. Analysis was done on the final cell number counts and found that between species there was no significant difference among treatments for *C. placentula* ($p = 0.380$), *G. truncatum* ($p = 0.538$), or *S. construens* ($p = 0.540$). However, when the growth rates of each diatom were calculated and compared between species there was a significant difference ($p < 0.0001$) with *R. gibba* having the slowest growth rate and *C. placentula* having the fastest growth rate. Counts for *R. gibba* were also completed for the 6 ash species experiment and further analyzed (Figure 5). Between treatments there is a significant difference in the final counts ($p = 0.00174$), however, the difference between the pH-adjusted treatment and the control is not significant based off of Tukey post-hoc tests ($p = 0.618$). The significant differences between ash are all compared to the pH-adjusted treatment and are for the ash species of Aspen ($p = 0.016$), Fir ($p = 0.049$), Oak ($p = 0.009$), and Spruce ($p = 0.004$).

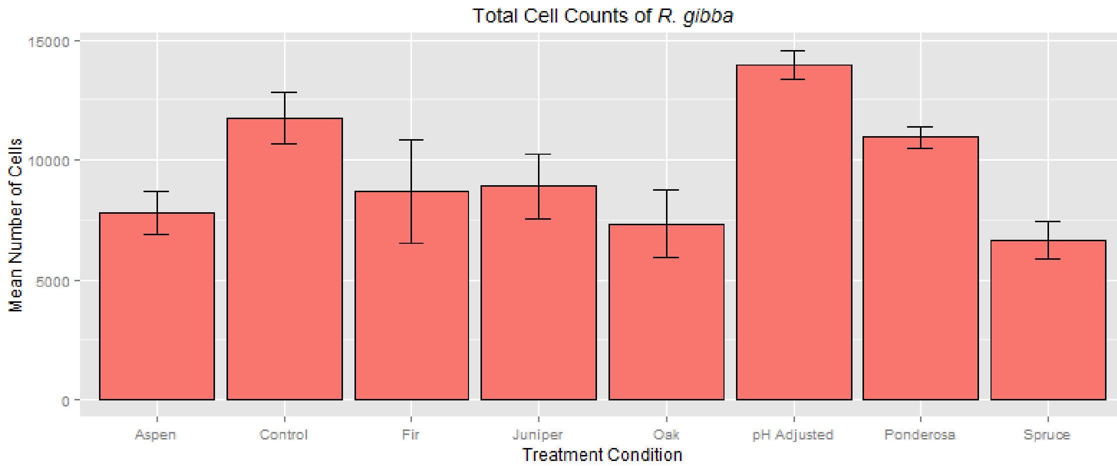

Figure 5: Total cell counts of *R. gibba*. While the control and pH-adjusted treatments have relatively similar magnitudes, the addition of fire ash lowers the productivity of *R. gibba* by varying amounts.

Conclusion

Water quality can be greatly impacted by the increased terrestrial matter provided by forest fires. The increases in ash leachate conductivity with increasing concentration seen in this experiment are an example of this impact(Figure 2). Large quantities of ash may enter water ways and carry with them varying concentrations of salts and organic matter which can impact stream function. While the dissolved oxygen has been found to consistently decrease in natural water instances following a post-fire flow (Dahm et al,. 2015; Earl and Blinn, 2003), the results of the batch experiment are interesting because both the ash and deionized water are sterile, indicating a chemical rather than biological change as an explanation of the observed changes in the reduction-oxidation states of the water. Further study into this change and determination of an approximate concentration of ash entering water ways during natural fire events will allow land managers to better predict when an ash flow event may cause fish kills due to hypoxic or anoxic conditions. The alkalinity data are not surprising due to the alkaline nature of ash; however the interspecies variation is worth examining for possible causes of the observed differences in growth rate.

Diatoms are important as both bio-indicators and sources of food for higher organisms. Because of their species-specific response to changes in water quality, they are important study organisms which many states have used to develop pollution indices. The diatoms that were experimented with are found naturally in the EFJR, which is a relatively undisturbed grassland stream. The observed differences in the diatom growth rate may therefore be a primary driver in the recolonization of a stream population following a scouring event, such as a post-fire sediment flow.

Throughout both of the diatom experiments there was a decrease in diatom growth from the pH adjusted (pH = 8.3) data to the control (pH = 7.8) data. Though this difference is not statistically significant there are possible explanations for the observed change. One possible explanation for the lack of significance is that the diatoms were not grown long enough to be impacted by the pH change. Another explanation may be that the difference in pH readings may not have been extreme enough to induce a noticeable response in the diatom cell densities.

The multiple ash species experiment also looked at multiple species of tree's ash and different ashes' impact on *R. gibba*. Although the ash was all prepared in similar manners, there was decreased growth across all of the treatments compared to the pH-adjusted and control treatments. While a forest may not be a single vegetation type, by understanding how individual types of tree ash impact diatom growth, it may allow for further understanding when mixed stands of trees are burned and enter waterways following rainstorms. Due to the presence of a nitrogen fixing endosymbiont in *R. gibba,* the variable growth across multiples species of ash may be due to possible toxicity from increased nitrate concentrations which were leached from the ash, though additional chemical analysis will be required to examine this explanation.

Additional counts and analysis will need to be done to understand how diatoms with different growth morphologies are impacted by the presence of fire ash leachate. The remaining three diatoms (*C. placentula*, *S. construens,* and *G. truncatum*) will need to be analyzed to determine if the toxic nature of the ash to *R. gibba* is replicated across all species. Additionally, statistical modeling will need to be applied to look for correlated increases in chemical quantities with the decline of *R. gibba*.

Further analysis into the chemical properties of the ash will allow researchers and land managers to understand the implications of fire ash flow into waterways. Ash from this project is undergoing additional characterization in collaboration with UNM Department of Civil Engineering. This collaboration will help to better understand fire ash influences on water quality. Additionally the methods for creating fire-ash in the laboratory are not standardized and so further work will allow the production of ash to be more reproducible.

Acknowledgments

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Presentations of this project

Clark, A., and R. Bixby. 2014. Fire ash influences on aquatic primary producers through changes in water quality. New Mexico Water Resources Research Institute Annual Conference, Santa Fe, 19 November 2014.

Presentations and papers in progress

Clark, A., and R. Bixby. 2015. Fire ash influences on diatom populations through changes in water quality. North American Diatom Symposium, Beaver Island, Michigan, 9-13 September 2015.

Clark, A., R. Bixby, J. Blake, and J. Cerrato. (In Preparation). Methods for production of wildfire ash in a laboratory for experimental understanding of water quality changes.

Clark, A. (In preparation) Changes in diatom growth in culture due to the presence of wildfire ash. University of New Mexico Biology Department Honors Thesis.

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