Response of Southern Shrub Peatland Phenolics and Carbon Dioxide Flux to Drought and Nitrogen Additions

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Introduction

Peat forms when wetland conditions prevent oxygen from entering the soil and, in turn, dramatically decrease the rate of plant litter decomposition (Bragazza *et al.* 2006). Peatlands only cover three percent of land area worldwide, yet they store one third of all terrestrial carbon due to thwarted decay (Bragazza *et al.* 2006). The lack of oxygen also results in decreased phenol oxidase which allows the wetland to accumulate phenolics (Fenner and Freeman 2011). Phenol enters the soil from within plant litter or as a leachate from plant parts (Hattenschwiler and Vitousek 2000). Phenol compounds are referred to as the "enzymic latch" because they further reduce decomposition and lock in the stored carbon (Fenner and Freeman 2011). Phenol also inhibits nitrification and digestive enzyme activity (Hattenschwiler and Vitousek 2000). The presence of polysaccharides, carbohydrate molecules that provide energy for microbes, in peat soil aids in decomposition (Zaccone *et al.* 2008). However, the ability of polysaccharides to adsorb to organic soil may reduce the decomposition rate and enzyme activity (Martin 1971). In more recent years, drought and nitrogen additions are interfering with existing chemical interactions and threatening the effectiveness of peat as a carbon sink.

At high latitudes, where most peat is located, drought frequency and severity is predicted to increase (Fenner and Freeman 2011). Drought impacts a saturated peatland by introducing oxygen and triggering a biogeochemical cascade (Figure 1) (Fenner and Freeman 2011). Oxygen stimulates bacteria which grow at an increasing rate as drought proceeds (Fenner and Freeman 2011). Then phenol oxidase activity increases which reduces the presence of phenolic compounds (Bragazza *et al.* 2006). This process promotes microbial growth which breaks down organic matter and releases carbon dioxide (CO_2) (Fenner and Freeman 2011). The overall response also decreases dissolved organic carbon (DOC) and ammonium and releases nitrate (Fenner and Freeman 2011). Furthermore, drought raises the soil pH and stimulates anaerobic decomposition (Fenner and Freeman 2011). Comparatively, drought may not affect unsaturated peatlands because they contain controlling factors other than anoxia, such as polyphenols, to resist carbon decomposition and CO_2 emission (Wang *et al.*).

Under increased drought, the decline of CO_2 emission may be attributable to gradually exhausting the labile carbon pool with time or the build-up of decomposer inhibitors (Wang and Richardson in preparation). Yet, when peat becomes re-wetted, enzymes are considerably more active and accelerate carbon loss (Fenner and Freeman 2011). Another important factor is that long-term hydrologic management regimes greatly affect peatland CO_2 emissions (Wang and Richardson in preparation). In addition, phenol oxidase activity may only occur within a range of ideal soil moisture levels (Wang and Richardson in preparation).

Nitrogen inputs through agricultural runoff and atmospheric deposition reduce phenol and enhance peat decomposition rates (Bragazza *et al.* 2006). Therefore,

nitrogen loading also releases the “enzymic latch” and contributes to a positive feedback loop with climate change (Fenner and Freeman 2011). Ultimately, this study hypothesizes that the biogeochemical reaction caused by drought or nitrogen input decreases the storage of dissolved organic carbon in shrub peatland and elevates atmospheric carbon dioxide.

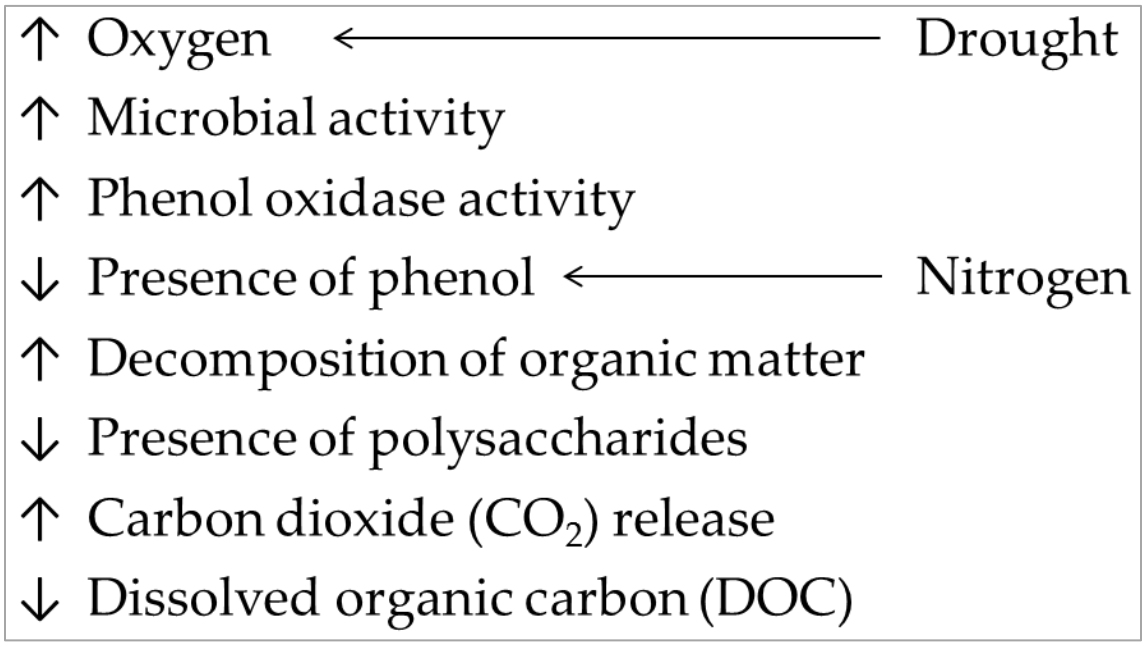

Figure 1: An illustration of the biogeochemical cascade. The cascade occurs in order from the top of the list to the bottom. The arrows pointing up indicate an increase while the arrows pointing down indicate a decrease. Drought triggers the cascade by increasing oxygen in the peat while nitrogen inputs trigger the cascade by decreasing phenol. Both triggers ultimately result in the release of CO_2 and a decrease in DOC. (Figure modified from Fenner and Freeman 2011)

Objectives

This project explores the effects of drought and added nitrogen on shrubland peat from the Pocosin Lakes region of Eastern North Carolina. Based on existing literature, the introduction of drought or nitrogen to peat in an experimental setting should trigger several chemical responses. The anticipated outcome of the drought and nitrogen treatments is for the amount of phenol to decrease. Additionally, the experimental samples should release dissolved organic carbon (DOC) and carbon dioxide (CO_2), thus decreasing the amount of carbon stored in the soil. This study predicts that by encouraging peat decomposition, the amount of polysaccharides will decrease as well. During the experiment, the amount of ammonium should decrease and the soil should release nitrate. Moreover, an evident increase in enzyme activity should occur.

While current literature provides enough evidence to predict the biogeochemical impacts, most of the research focuses on sphagnum and grassland peat. This research on shrub peatland will determine the effects of drought and nitrogen additions on the Pocosin Lakes region as well as other comparable ecosystems.

Methods

Experiments

Peat was collected from the Pocosin Lakes region in North Carolina by shoveling the soil from one area into a bucket. The peat sample was extracted from one location so that the soil components remained fairly constant throughout the analyses. Then the peat was placed into a plastic trash bag to maintain the original moisture content. In the lab, the sample went through a 4.75mm sieve. The amount of sieved soil was enough to fill 6L of container space. NH_4NO_3 fertilizer was added to half of the sieved peat. In the short term experiments, 3 grams of NH_4NO_3 fertilizer were added per 508.0 grams of dry soil. In the long term experiments, 10 grams of NH_4NO_3 fertilizer were added per 1069.7 grams of dry soil.

12 1L jars were obtained for the experiment with only drought and nitrogen treatments. 6 jars were filled halfway with the original sample and 3 of them were covered with Parafilm. The other 6 jars were filled halfway with the nitrogen enriched sample and 3 of them were covered with Parafilm. The mass of soil was determined for all 12 jars. The jars rested for a selected amount of time such that each experiment would simulate the drought and nitrogen effects in question. The soil was weighed again at the end of the experimental period to calculate the amount of moisture loss.

The temperature variable experiment was conducted by placing the remaining contents from the 12 jars into sealed plastic Ziploc bags and storing them in a 4°C cold room. The samples were allowed to rest for an amount of time that would simulate a cold season, approximately three months. All of the above analyses were repeated to examine the effect of reduced temperatures on the existing drought and nitrogen treatments.

The time variable experiment was conducted by using the original peat extracted from the field to repeat the 12 jar experiment. The jars were allowed to rest for a greater amount of time and then were analyzed according to the same methods. These values were compared to the original results to determine if an increase in time exaggerated the impact of drought and nitrogen additions.

Chemical Analyses

Soil moisture analysis was conducted by weighing 11-12g of sample from each of the 12 jars. The soil was placed in a non-air forced oven until the soil was completely dry. The soil was weighed again and the wet weight/dry weight ratio was calculated.

The inorganic nitrogen content was analyzed by extracting 3-4g of wet soil from all 12 jars with 20mL of 2M KCl solution. Next, the samples went into a shaker followed by a centrifuge and then were filtered to obtain a clear extract. A flow-injection analyzer was used to measure the amount of ammonium (NH_4) and nitrite (NO_2^-) + nitrate (NO_3^-) in each sample.

For gas analysis, an airtight cap was placed on each jar. The air was evacuated out of the jars to create a vacuum and then the jars were filled using a pressurized air tank. This process was repeated one additional time. The samples were incubated at room temperature for at least 4 hours and the length of the incubation period was recorded. A syringe was used to transfer one sample from each jar to an airtight glass vial. A Gas Chromatograph was used to analyze the samples and compare them to blank and standard vials.

To analyze phenolics, 3-4 grams of wet soil were extracted from all 12 jars with 40mL of deionized (DI) water. The samples went into a shaker followed by a centrifuge and then were filtered to obtain a clear extract. Standards of 2, 4, 6, 8, and 10 µg/mL vanillic acid solution were prepared. A 2.5mL aliquot of sample extract or standard was pipetted into a test tube. 2.5mL DI water, 0.75mL 20% w/v Na_2CO_3 solution, and 0.25mL Folin-Ciocalteau phenol reagent were all added to the aliquot. The samples were mixed by inversion and stood for 1 hour at room temperature (20-25°C). The absorbance was read at 750nm against a blank of DI water. A calibration curve was prepared and the water soluble phenolics were calculated in µg/g of vanillic acid equivalent.

Polysaccharide analysis was conducted using the DI water extracts from the phenolics method. Standards of 3.2, 6.4, 9.6, 32, and 64 µg/mL glucose solution were prepared. A 1.5mL aliquot of sample extract was placed in a test tube. 6mL 0.5M H_2SO_4 solution was added and the samples were autoclaved for 90 minutes at 103kPa (15psi). 1.0mL of standard or autoclaved sample was pipetted into a test tube. 0.5mL 5% w/v phenol solution and 2.5mL 96% w/w H_2SO_4 were added to the samples. Each tube was capped with an acid-resistant stopper and mixed by inversion. Then, the tubes rested until the temperature of the liquid was approximately 25-30°C. Absorbance was read at 490nm. Zero absorbance was set with a reagent blank prepared using 1.0mL of DI water in place of a standard. A calibration curve was prepared and labile polysaccharide was calculated in µg/g of glucose equivalent.

The DI water extracts from the phenolics method were used for dissolved organic carbon (DOC) analysis. A total carbon analyzer was used to determine the amount of dissolved organic carbon (DOC) in each sample as the difference between total carbon and inorganic carbon.

To conduct enzyme analysis, centrifuge tubes were used to weigh 5g of soil from each jar. The tubes were sealed and placed in a cold room for storage prior to analysis. The 5 grams of soil were combined with 50mL of 5mM bicarbonate ($NaHCO_3$) buffer in

a vessel large enough to fit an 8-channel pipette. The mixture was stirred rapidly with a stir bar for at least 1 minute or until the solution looked homogenous. The multi-channel pipette was filled with 100µL of the soil solution while stirring. The solution was dispensed from each soil sample into the columns of a clear 96-well plate. Each column of the plate represented a different soil sample. 100µL of 5mM L-DOPA (dihydroxyphenylalanine) was added to 6 of the 8 rows. 100µL of the bicarbonate buffer was added to the remaining 2 rows, these wells contained the controls. The samples were incubated at 27°C and the increase in absorbance was measured at 460nm every 5 minutes for 1 hour.

Statistical Analyses

The response values indicated on each figure represent the means of triplicate samples. The error bars represent standard error. This study utilized The SAS System Version 8 to conduct statistical analysis. Specifically, this software analyzed the data using Duncan's test with two-way ANOVA.

Results and Discussion

Soil Moisture

As expected, soil moisture (Figures 2-3) decreased in both drought conditions and the long term drought treatment (Figure 3) experienced more significant drying. The nitrogen addition did not show a significant impact for either nitrogen condition in the short term or long term experiment. Soil moisture also had a statistically significant interaction with drought in both the short term and long term experiments (short term: p = <.0001, long term: p = <.0001).

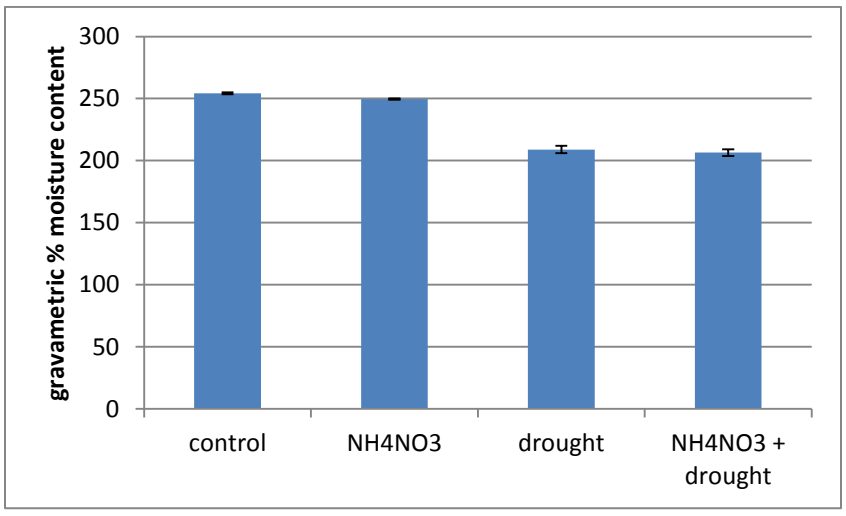

Figure 2: Soil moisture response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

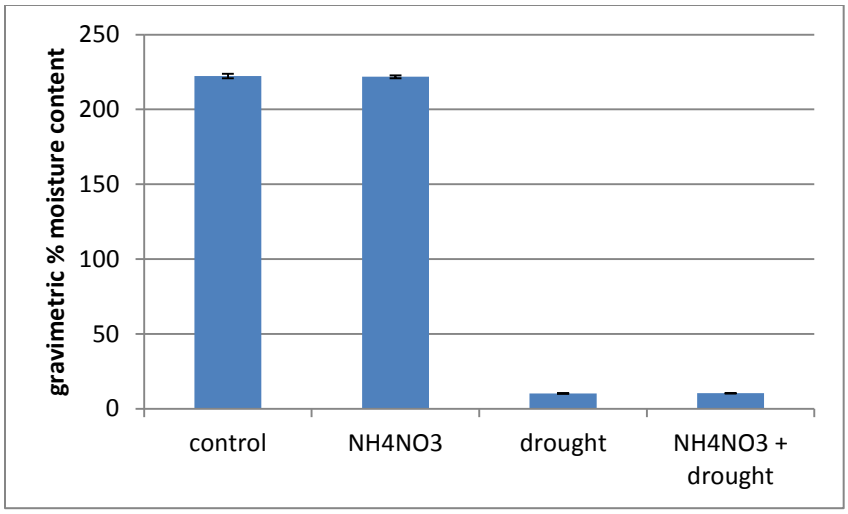

Figure 3: Soil moisture response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Inorganic Nitrogen Analysis

In the short term treatment (Figures 4-5), NO_x-N and NH_4-N increased significantly in both nitrogen conditions as expected. The values for NO_x-N and NH_4-N remained similar to the control samples in response to the short term drought treatment alone (Figures 4-5). In the long term drought and nitrogen treatment, NO_x-N (Figure 6) decreased in response to nitrogen additions, drought, and nitrogen and drought combined. In response to the long term drought and nitrogen treatment, NH_4-N (Figure 7) increased significantly in the nitrogen condition, decreased slightly in the drought condition, and increased slightly in the nitrogen plus drought condition. A possible explanation for the long term decrease in nitrogen is that the nitrogen application caused microbial growth and greater nitrate uptake in the long term experiment, while the community did not have enough time to produce a growth response in the short term experiment. In the long term control samples, bacteria likely acted on the nitrogen by first converting ammonium to nitrate and then producing nitrous oxide gas. Both the short term and long term experiments had statistically significant interactions with drought (NO_x: short term $p = 0.002$, long term $p = <0.0001$; NH_4: short term $p = 0.0005$, long term $p = <0.0001$), nitrogen (NO_x: short term $p = <0.0001$, long term $p = <0.0001$; NH_4: short term $p = <0.0001$, long term $p = <0.0001$), and the interaction term drought*nitrogen (NO_x: short term $p = 0.0021$, long term $p = <0.0001$; NH_4: short term $p = 0.0024$, long term $p = <0.0001$).

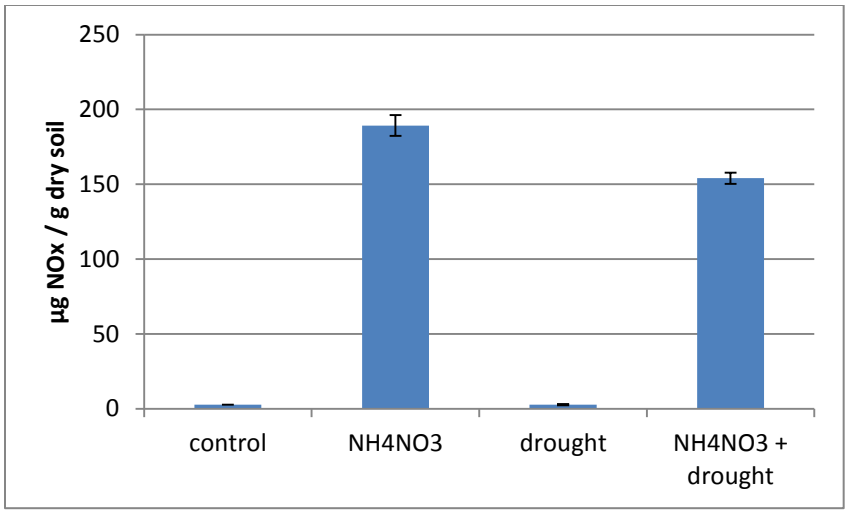

Figure 4: NO_x response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

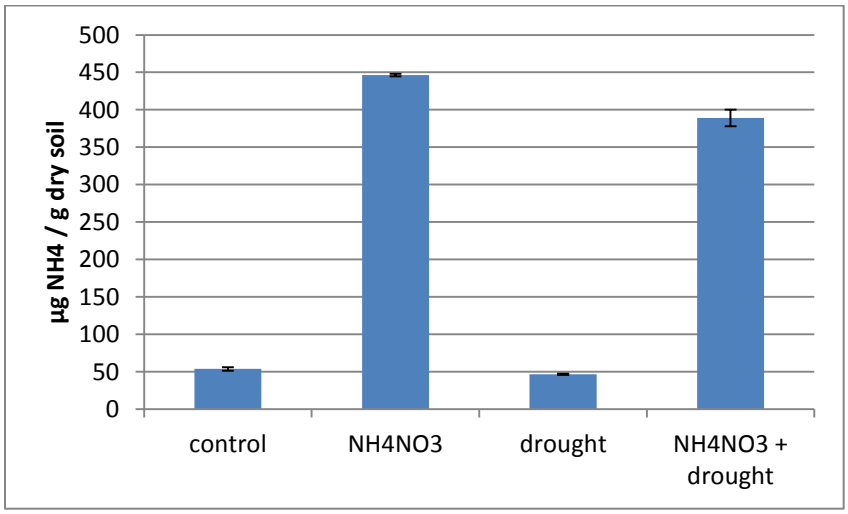

Figure 5: NH_4 response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

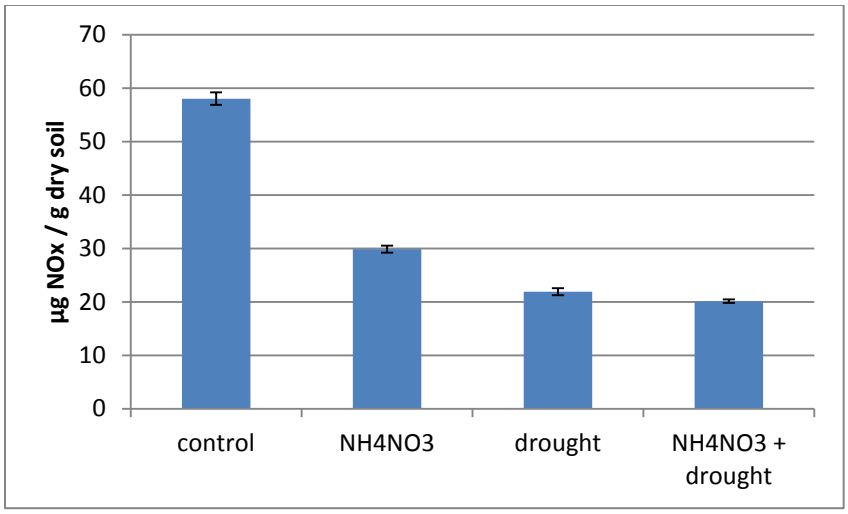

Figure 6: NO_x response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

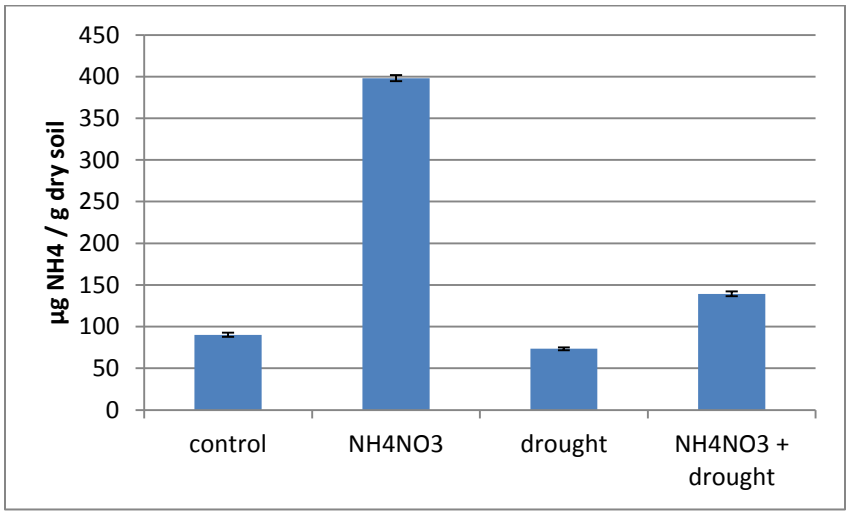

Figure 7: NH_4 response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Gas Analysis

In the short term drought and nitrogen treatment (Figure 8), carbon dioxide emissions increased slightly with nitrogen additions, decreased with drought, and decreased slightly with nitrogen and drought combined. The long term drought and nitrogen experiment (Figure 9) had a different response where carbon dioxide emissions increased slightly with nitrogen additions alone but decreased significantly with drought as well as drought and nitrogen combined. Furthermore, the long term drought and nitrogen treatment had a statistically significant interaction with nitrogen ($p = .0088$), drought ($p = <.0001$), and the interaction term drought*nitrogen ($p = .0287$). While nitrogen additions and drought were expected to increase carbon dioxide

emissions, a possible explanation for the long term results is that severe drought limits microbial activity and, in turn, reduces carbon dioxide emissions regardless of whether nitrogen is added or not.

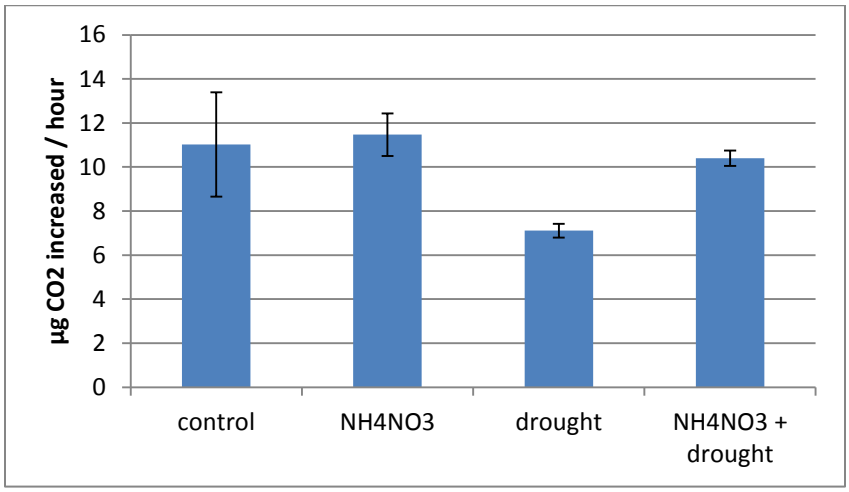

Figure 8: CO_2 response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

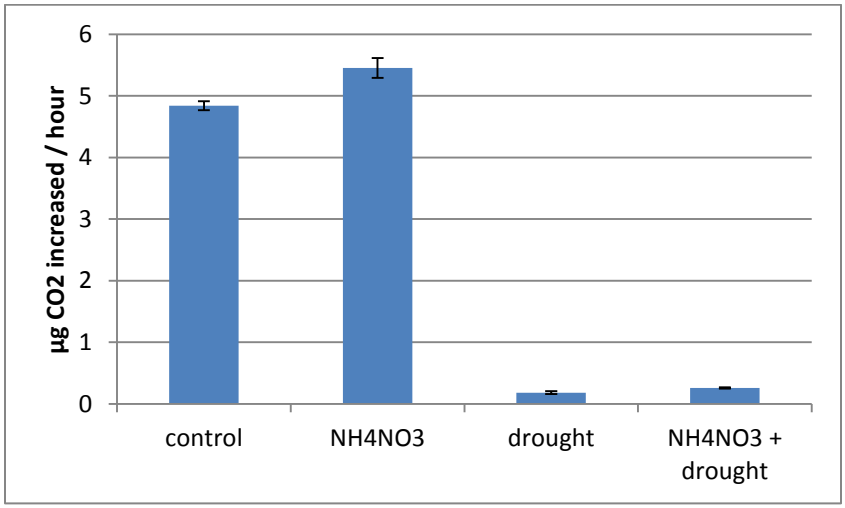

Figure 9: CO_2 response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Phenolics

The amount of phenol in the short term drought and nitrogen samples (Figure 10) decreased substantially in both nitrogen conditions and deceased slightly with the drought treatment alone. In the long term drought and nitrogen treatment (Figure 11), phenolics increased by a considerable amount with nitrogen additions alone, decreased slightly with drought, and increased slightly with nitrogen plus drought. Perhaps the soil contained lignin which is high in phenol. Therefore, in the long term samples, the

nitrogen application increased phenol by increasing lignin decomposition while drought decreased phenol by reducing lignin decomposition. Furthermore, phenol had a statistically significant interaction with nitrogen additions in both the short term and long term experiments (short term: p = <.0001, long term: p = 0.042).

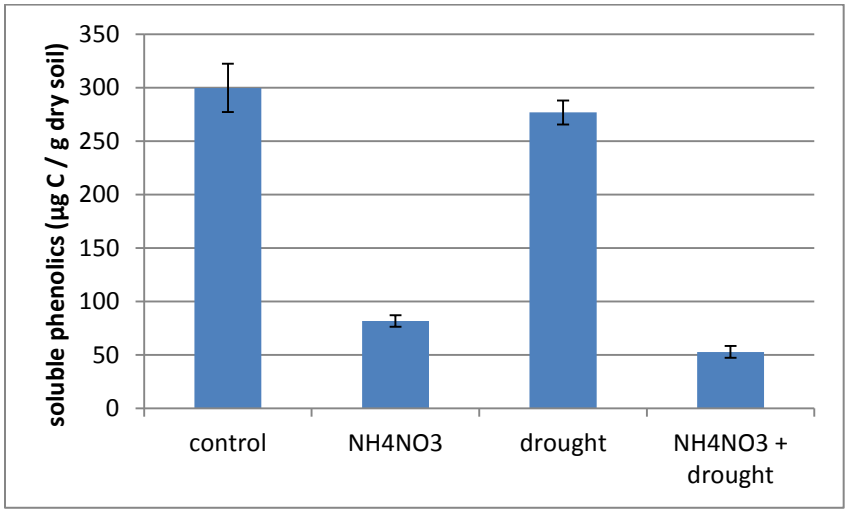

Figure 10: Phenol response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

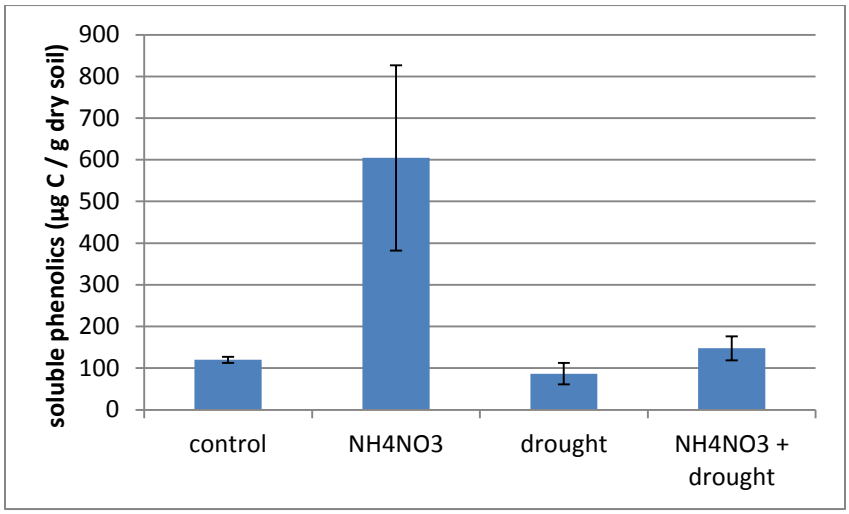

Figure 11: Phenol response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Polysaccharide Analysis

In the short term drought and nitrogen treatment (Figure 12), polysaccharide decreased most significantly with the drought treatment but also decreased considerably in both nitrogen conditions. Perhaps this decrease occurred because microbes in the soil were carbon limited and therefore, they used carbon and nitrogen

together for energy. The short term impacts on polysaccharide had a statistically significant interaction with drought ($p = 0.0151$) and the interaction term, drought*nitrogen ($p = 0.034$). In the long term drought and nitrogen treatment (Figure 13), polysaccharide increased with nitrogen additions alone but decreased in both drought conditions. A likely explanation for this response is that the microbial activity in the soil was mainly drought limited.

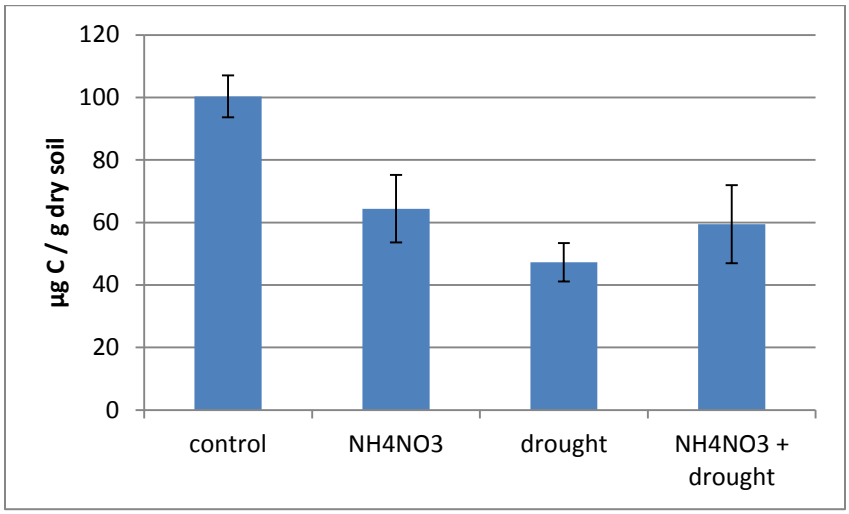

Figure 12: Polysaccharide response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

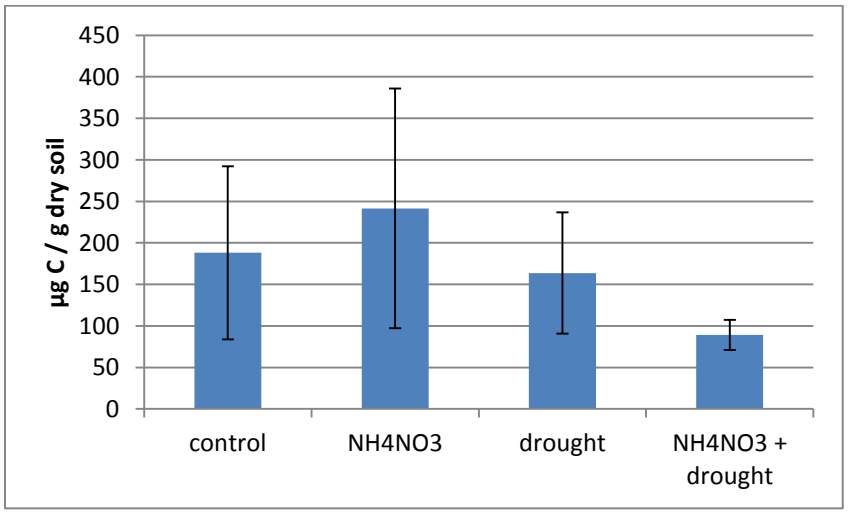

Figure 13: Polysaccharide response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Dissolved Organic Carbon (DOC) Analysis

The amount of dissolved organic carbon in the short term drought and nitrogen treatment (Figure 14) had a significant decrease with nitrogen additions, a slight

decrease with drought, and the most notable decrease with nitrogen plus drought. A likely explanation for the response to nitrogen addition is that dissolved organic carbon decreased through microbial uptake and the microbes released carbon dioxide through respiration. The long term drought and nitrogen treatment (Figure 15) caused dissolved organic carbon to increase significantly with nitrogen additions, decrease slightly with drought, and increase with nitrogen plus drought. Both the short term and long term experiments had a statistically significant interaction with drought (short term: p = 0.0119, long term: p = 0.0052) and nitrogen (short term: p = <.0001, long term: p = <.0001). The interaction term drought*nitrogen was also statistically significant for the long term samples (p = 0.0165).

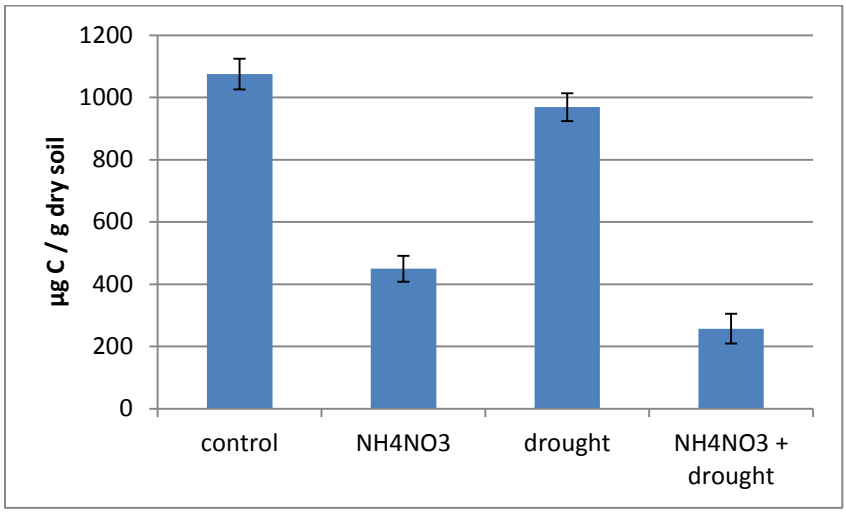

Figure 14: DOC response of Pocosin peat soils to short term impacts of nitrogen and drought treatments.

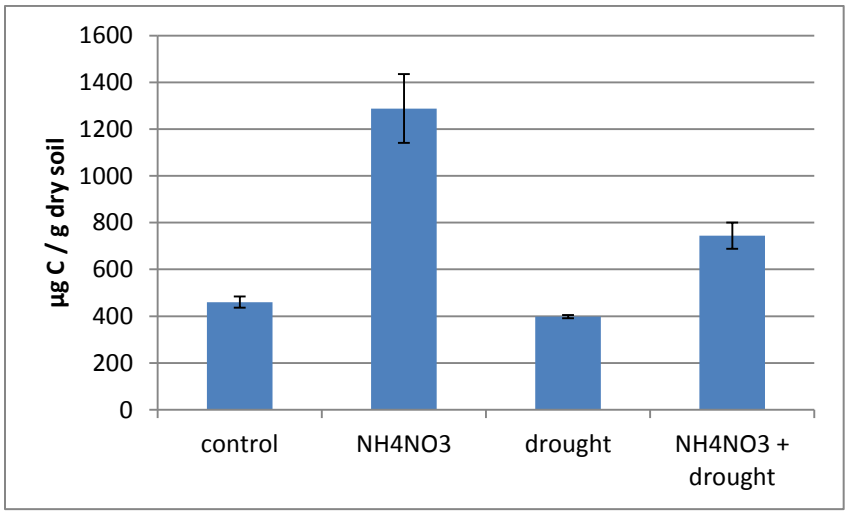

Figure 15: DOC response of Pocosin peat soils to long term impacts of nitrogen and drought treatments.

Enzyme Analysis

This method showed success in previous studies that analyzed mineral soil and sphagnum peat. Upon completion of the enzyme analysis on shrubland peat in this study, the data were not consistent enough to produce clear insights into the responses of southern peat to nitrogen additions and extended drought versus short term drought. Further research needs to be conducted on additional samples over extended periods of treatments to see if patterns can be discerned. Clearly, short term drought and long term drought produce different responses, which will require detailed microbial and enzymatic analysis to help determine if the enzymic latch theory is different in southern shrub peats from those reputed in the northern peatlands by Fenner and Freeman (2011).

Summary

Peat is an essential global carbon sink and the introduction of drought and nitrogen to the soil can trigger a biogeochemical cascade which results in the release of stored carbon. This research shows that the length of exposure to drought and nitrogen additions may alter the expected impacts on shrub peatland. Soil moisture responded as expected in the short and long term experiments by decreasing in both drought conditions. NO_x-N and NH_4-N responded as anticipated in the short term experiment by increasing in both nitrogen conditions. However, in the long term experiment, NO_x-N and NH_4-N did not increase in both nitrogen conditions. Perhaps nitrogen decreased in the long term experiment because the added nitrogen stimulated the microbial community, which increased nitrogen uptake. In the long term control samples, bacteria likely converted ammonium to nitrate and then to nitrous oxide gas. Although nitrogen additions and drought were expected to increase carbon dioxide emissions, severe drought in the long term experiment limited microbial activity and reduced carbon dioxide emissions. Phenol was predicted to decrease in response to nitrogen and drought treatments, but in the long term samples the nitrogen application increased phenol by possibly increasing lignin decomposition and drought decreased phenol by feasibly reducing lignin decomposition. The presence of polysaccharide in the soil decreased as expected in response to the short term nitrogen and drought treatments. In the long term experiment, the microbial activity was mainly drought limited and polysaccharide increased in the condition with nitrogen alone. In most of the samples, dissolved organic carbon decreased in response to the nitrogen and drought treatments as expected. Yet, in both long term nitrogen conditions dissolved organic carbon likely decreased through microbial uptake. Ultimately, the findings in this study, with samples from the Pocosin Lakes region of Eastern North Carolina, provide implications for peat storage and loss in shrub peatland ecosystems.

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