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Determination of the Relationship Between Wetland Vegetation Nutrients and Overall Wetland Nutrient Levels

Nutrients are a major cause of poor water quality in our nation's wetlands. In excess, nutrients can cause hyper-eutrophication, destroying a sensitive aquatic environment. Quantifying these nutrient levels is time consuming due to changing ecological conditions. Vegetation directly uptakes nutrients from the surrounding soil and water within a wetland ecosystem. This wetland vegetation maintains relatively constant nutrient levels through regular periods of growth. This study attempted to determine if nutrient levels in wetland vegetation act as an effective method of assessing the nutrient status in a wetland ecosystem.

Vegetation, litter, water and soil samples from 74 wetlands throughout the Southeastern United States were analyzed for the nutrients phosphorus, nitrogen and carbon using standard Kjedahl digestion techniques and gas chromatography procedures. Correlations between phosphorus, carbon and nitrogen levels in these samples were determined using the JMP 4 Statistical Analysis Program. Logarithmic transformation was performed on the data to achieve a normal distribution. A significant relationship between all wetland vegetation nutrient levels and overall wetland nutrient quantity was not found to exist. Several relationships between nutrient conditions that considered vegetation species and wetland ecosystem type were found to exhibit statistically significant correlations with R^2 values greater than 0.5 and p values <0.05. These relationships were found within the non-riverine swamp and non-riverine marsh sampling areas, and within the vegetation species *Pontedaria cordata*, *Panicum spp.*, *Taxodium* spp., and *Nyssa sylvatica*.

A second trial of this research was conducted using a 10 wetland sampling area in the Carleton Reserve of Venice, Florida. The methods that were used to analyze nutrient levels in this second trial did not diverge from the methods used in the previous trial of this research. A significant relationship was not found to exist between overall vegetation nutrient levels and soil, litter and water nutrient levels. A significant relationship did occur between the phosphorus levels in *Fraxinus caroliniana* vegetation samples and the phosphorus levels found in non-riverine Pop Ash swamps. The results of the two separate trials in this research indicate that local or specific species of vegetation are useful in assessing wetland water quality. However, This project was unable to conclude that wetland nutrient assessment techniques can be accurately performed through quantifying the nutrient levels in wetland vegetation.

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Introduction

Developing quantitative water quality standards for nutrients in a wetland is a daunting task due to frequently changing ecological conditions. An accurate method of determining the nutrient levels in an aquatic site, which can include wetlands, is to collect samples throughout an entire flow year ("Nutrients: Frequently asked questions", 2003). This method has become an ineffective process that requires an overabundance of manual labor and data collection (M.W. Clark, personal communication, June 17, 2004).

The U.S. Environmental Protection Agency (EPA) defines a wetland as "areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions" (2003). Wetlands are ecosystems where prolonged or frequent water presence creates an environment that can support aquatic life (U.S. EPA, 2003).

Vegetation takes up nutrients from the surrounding soil and water within a wetland ecosystem. This research attempts to establish a method of assessing the nutrient conditions in a wetland by quantifying nutrient levels in wetland vegetation. Koerselman and Meuleman (1996) found that the nitrogen to phosphorus ratio in wetland vegetation could predict the limitation of wetland nutrients. Other studies have also indicated that algae and other forms of vegetation can indicate the nutrient conditions in a wetland (U.S. EPA, 2002). These studies suggest that there is a relationship between nutrient levels in a wetland and nutrient levels in the vegetation surrounding and within a wetland. If a favorable relationship does exist, then it can potentially be used as a tool for effectively determining wetland water quality.

The Clean Water Act was passed in 1972 by the United States' federal government to help protect the nation's wetlands and waterways (Carriker & Rahmani, 1994). Responsibility of enforcement was given to the EPA, which created various new requirements on industrial discharges, city wastewater treatment facilities and state waterways (Williams, 2002). The EPA also required states to assign a specific purpose to each wetland, labeling them as aquifers, wildlife harbors or recreation areas. (M.W. Clark, personal communication, June 17, 2004). Each wetland and waterway designation was matched with certain toxin and pollutant level guidelines (M.W. Clark, personal communication, June 17, 2004). These guidelines were created by the EPA to protect organisms that obtain water from these sources ("Nutrients: Frequently asked questions", 2003).

The Clean Water Act has had a substantial impact on our nation's water quality. Over the past 30 years the number of the nation's waterways that support fishing, swimming and other activities has risen by over 30% (Williams, 2002). In 1997, Vice President Gore and the administrator of the EPA developed a comprehensive action plan that called for enhanced control of pollution runoff and the establishment of nutrient level guidelines for assessing impacted wetlands (Cantilli, 1998). Nutrients, such as phosphorus and nitrogen, are a major cause of poor water quality in our nation's waterways (Childers *et al*., 2003). Excessive nutrients in a waterway can lead to hyper-eutrophication, which creates a wide range of negative ecological impacts for aquatic life ("Nutrients: Frequently asked questions", 2003).

Nutrients are essential for the successful growth and development of vegetation. The abundance of a nutrient in the soil influences the amount that can be absorbed by that plant (Sinnott, 1931). As long as the nutrient is not limiting, the rate of uptake remains steady over long periods of growth (Arbol, 1990).

The objectives of this study were to investigate the relationship between wetland vegetation nutrients and overall wetland nutrient levels, and to determine what factors influence this relationship. It was hypothesized that a relationship exists between soil, water and litter nutrient levels and vegetation nutrient levels in a wetland ecosystem. Since all plant species have different nutrient absorption properties, this relationship can be strengthened if species type is taken into account. The correlation between wetland nutrient levels and vegetation nutrient levels will also strengthen if the researcher only uses vegetation samples that have nitrogen to phosphorus ratios higher than or equal to a standard ratio. A plant with a low N:P ratio has nitrogen as a limiting nutrient, and will not absorb standard quantities of other nutrients, such as carbon or phosphorus. According to Koerselman and Meuleman (1996), a vegetation sample with a N:P ratio less than 14, has nitrogen as a limiting nutrient .

Methods

Southeastern Wetland Biogeochemical Survey Samples

Soil, water, vegetation and litter samples were collected and documented by graduate students attending the University of Florida. Samples were collected during the time period from March 2003 to October 2003, and obtained from multiple impacted and un-impacted wetlands in Florida, Georgia, Indiana, Alabama, and South Carolina. The wetlands consisted of riverine swamps, riverine marshes, non-riverine swamps and non-riverine marshes. Every sample was labeled with sample ID, collection site number, and collection date. The vegetation and litter samples were dried and milled using a Willy mill. Samples that did not have a homogeneous consistency were ball milled into a finer grain. Soil samples were dried in a drying oven at 40º C for three days, and then put through a 2mm filtering device. The vegetation samples were transferred into small plastic storage containers. One hundred and forty vegetation samples from fourteen species were used. The distribution of species included twenty-four *Acer rubrum*, seven *Salix caroliniana*, eight *Taxodium ascendens*, twenty *Panicum spp.*, nine *Pontedaria cordata*, one *Itea virginica*, two *Sambuscus Canadensis*, one *Lyonia lucida*, two *Persea palustris*, sixteen *Nyssa sylvatica variant biflora*, twenty-one *Myrica cerifera*, fifteen *Cephalanthus occidentalis*, eleven *Sagittaria latifolia* and two *Orontium aquaticum*.

Analysis of Samples

Graduate students, prior to the researcher's involvement with the project, analyzed the soil and water samples for phosphorus, nitrogen, and carbon levels. The levels of nitrogen and carbon in the soil, vegetation, and litter samples were quantified using the Carlo Erba 1500 Nitrogen/Carbon Analyzer. A three to four milligram vegetation, soil, or litter sample is needed to use to nitrogen/carbon analyzer. This mass of sample is obtained using a Sartorius Microbalance model 1500 P. The samples were encased in 3.5 mm by 5-mm tin capsules. These tin capsules were placed in individual 2 mL polyethylene cups.

The nitrogen/carbon analyzer uses gas chromatography to analyze individual samples. First the capsule with the sample is placed into an auto-sampler that revolves every three minutes to the next sample. Once a sample is ready to be analyzed, it drops into a combustion chamber where it is ignited at 1,020ºC in the presence of oxygen. The tin helps make a violent reaction that causes the complete oxidation of the samples. The products of this combustion reaction are sent into a reduction column where nitrogen oxides are reduced to elemental nitrogen at 650ºC. The nitrogen and carbon dioxide gases are then passed through a container of magnesium per chlorate filter where excess water is absorbed. The gases are then sent into a chromatographic column where the elemental nitrogen gas is separated from the carbon dioxide gas. These gases are separately passed through a thermal conductivity detector that generates an electrical signal proportional to the concentration of carbon and nitrogen in the sample. A Hewlett Packard 3390A Reporting Integrator charts the electrical charge from the thermal conductivity detector and integrates the chart to find the amount of nitrogen and carbon in the sample. Standard samples were also analyzed to ensure quality control. Data from each sample was then put into a Microsoft Excel template that was used to calculate the percentage of nitrogen and carbon in each sample.

Water samples were analyzed for total nitrogen using Kjedahl digestion techniques. Total phosphorus in water samples was quantified using sulfuric acid and potassium persulfate digestion techniques.

Analyzing the Nutrient Data and Creating Correlations for Specific Wetlands

The JMP Statistical Discovery Software V4 was used to determine relationships between nutrient levels of the soil, water, vegetation and litter samples. Data distributions were tested for normality using the statistical program. Data that was not normally distributed was logarithmically transformed to improve its normality.

Results

The vegetation samples showed levels of total carbon ranging from 38.12% to 50.02% and nitrogen levels from 0.27% to 4.64%.

Tests for statistical correlations between all of the vegetation samples and water samples showed that no significant relationships occurred between the nutrient levels of these sample types. To be considered statistically significant, the regression correlation between the two data types must have an R^2 value of 0.5 or greater, and a probability factor of 0.05 or less.

No relationship between the vegetation nutrient levels and water nutrient levels had these statistical characteristics.

Significant statistical regression correlations between the soil samples’ nutrient levels and the vegetation samples’ nutrient levels could not be established. Significant statistical regression correlations could also not be established between the litter samples’ nutrient levels and the vegetation samples’ nutrient levels.

Vegetation samples were divided into four categories based on wetland type type. The means and standard errors of total carbon, total nitrogen and total phosphorus in the soil, litter, water and vegetation samples for each wetland type are displayed in Tables 1 through 4. In non-riverine swamps, the total phosphorus (TP) levels in vegetation samples had a significant statistical relationship with the TP levels in water samples. On Figure 1 the graph displays an exponential relationship between these two data types. The R^2 value for this relationship is 0.589 and $p<.0001$. In this data set for non-riverine swamps, no other significant statistical relationships exist.

In non-riverine marshes, the total nitrogen (TN) in vegetation samples displayed a significant exponential relationship with the total carbon (TC) of litter samples. This relationship has an R^2 value of 0.547 ($p=.0006$) and can be seen in Figure 3. No other obvious statistical correlations existed within non-riverine marshes. There were no significant statistical relationships between vegetation and soil, water or litter samples in either riverine marshes or riverine swamps.

Vegetation samples were divided into species types in order to determine relationships between nutrient levels. In the species *Acer rubrum, Cephalanthus occidentalis, Myrica cerifera, Nyssa* spp. and *Sagittaria latifolia,* no significant correlations existed between vegetation nutrient levels and soil, water, or litter nutrient levels. In the *Nyssa sylvatica variant biflora* samples, a statistically significant relationship occurred between vegetation TP and litter TP. This linear relationship had an R^2 value of 0.669 ($p<0.0001$) and can be seen in Figure 3. In the *Panicum* spp. samples, a significant relationship was evident between vegetation TN and soil TC. As seen in Figure 4, this relationship is expressed using a natural logarithmic function and had an R^2 value of 0.498 ($p<0.0001$). In the *Pontedaria cordata* samples, a statistically significant relationship was determined between vegetation TC and litter TN. This relationship had an R^2 value of 0.669 ($p<0.0001$) and can be expressed exponentially, as seen in Figure 5. In the *Taxodium* spp. population, a significant relationship with an R^2 of 0.608 ($p=0.055$) occurred between vegetation TN and water TN. This exponential relationship is displayed in Figure 6.

The vegetation samples were also divided by the ratio of nitrogen to phosphorus levels. The vegetation samples with N:P ratios less than 14 were removed from the sample population, and the remaining samples were correlated with levels of nutrients in the soil, water and litter samples. This method proves unreliable in increasing the statistical significance of the correlations between nutrient levels.

All of the vegetation, litter, water and soil samples were grouped by collection location. Correlations between nutrient levels of vegetation samples and nutrient levels of soil, water, and litter samples were investigated for each specific wetland location. According to a pairwise correlation test, no significant relationships occurred between these nutrient levels.

Discussion

The original hypothesis was not supported in its entirety by this study. No direct statistical relationship existed between all wetland vegetation types and the overall nutrient levels in the wetlands surveyed. However, when vegetation species type and wetland type were considered, statistical relationships did develop. As predicted, certain species were found that exhibited relationships between vegetation and soil, water and litter nutrient levels. These vegetation species might be able to act as nutrient level indicators. It was also found that the nitrogen to phosphorus ratio in vegetation samples does not significantly affect the correlation of nutrient levels between sample types.

These results seem to signify that an overall quantitative relationship between wetland vegetation nutrient levels and overall wetland nutrient levels is either nonexistent or hard to express due to multiple abiotic and biotic factors. The genetics of wetland vegetation species may play a larger role in nutrient uptake than the actual nutrient content of a wetland ecosystem. This would suggest that an efficient quantitative nutrient indicator must be entirely specific for a wetland type and eco-region. Other factors in an eco-region such as sunlight intensity, human impact, or precipitation may also have larger role in nutrient acquisition than the surrounding nutrient concentrations (Sinnot, 1931).

In this study there were multiple factors that may have affected the results of the research. The samples were collected at different times of the year. This suggests that the season or position in the flow year had an affect on the nutrient levels on the soil, water, vegetation and litter samples; however this was not further investigated. The number of samples collected from each wetland was not similar, so the overall data analysis may have been biased towards a particular wetland type.

The relationships that were determined in non-riverine marshes and swamps and within the vegetation species *Nyssa sylvatica variant biflora*, *Panicum* spp., *Taxodium* spp., *Pontedaria cordata* could be eventually used as tools for assessing water nutrient levels. In non-riverine swamps there appears to be a substantial exponential relationship between vegetation and water TP. Nutrients in a non-riverine swamps are relatively constant since no new nutrients are brought in by flowing water (Schafale,1999). Vegetation would have a long exposure time to nutrient concentrations in a non-flowing water environment, allowing more time for nutrient uptake. This extended period of time for nutrient uptake might explain the exponential relationship that occurs between vegetation and water TP in non-riverine swamps. The relationship that was found in non-riverine marshes, while statistically significant may not be reliable due to a clustering pattern of the data points observed during data analysis. To define this relationship more accurately, more samples are needed.

The vegetation species *Nyssa sylvatica variant biflora* is a large woody tree that grows in wooded wetland communities. In these vegetation communities, peat or litter buildup has a major impact on hydrology and nutrient concentrations (Mitsch & Gosselink, 1993). With the large impact that litter has on available nutrients in this ecosystem, it is likely that there is an actual linear relationship that exists between vegetation and litter TP in *Nyssa sylvatica variant biflora* populations. *Taxodium* spp., while often found in wooded communities, did not display a relationship between vegetation nutrient levels and litter nutrient levels. The relationship that was formed between water TN and vegetation TN in *Taxodium* spp. samples, while statistically significant is questionable due to a small population size.

A unique, natural logarithmic relationship was found in the *Panicum* spp. sample population. *Panicum* spp. is a herbaceous species that can be found living in the poorly drained soils of wetlands (Harris & Hurt, 1999). This logarithmic relationship between vegetation TN and soil TC most likely forms because of limitations of other nutrients in the soil ("The Concept of Limiting Nutrients", 2001). If this relationship is governed by other nutrient factors, it may not be an effective method to determine the water quality of a wetland ecosystem.

Pontedaria cordata, another herbaceous species which grows in fresh water wetlands, displays a statistical relationship between vegetation TC and litter TN. *Pontedaria cordata* plants have high levels of nitrogen in their leaves, and therefore low C:N ratios (Schafale,1999). When these leaves fall onto the marsh floor and become litter, the litter has high levels of nitrogen. When comparing the litter TN to vegetation TC in wetlands that have a large population of *Pontedaria cordata*, the correlations that arise might be due to the C:N ratios originally found in the vegetation. This relationship might be more useful in determining the nutrient levels in this vegetation species rather than assessing wetland nutrient levels.

The relationships that were determined in this research, with more data collection and analysis, might make useful tools in assessing wetland nutrient levels. Also, this research could serve as a model for future studies that would wish to establish quantitative nutrient level relationships for a specific eco-region. A single eco-region, rather than the entire Southeastern United States, may show less diverse ecological conditions and therefore fewer variables may be present.

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Tables

Table 1

Soil Nutrient Data For Each Wetland Type

Wetland Type	Number of observations	Mean Total Carbon (%)	Std. Error	Mean Total Nitrogen (%)	Std. Error	Mean Total Phosphorus (mg kg-1)	Std. Error
Non-riverine Marsh	54	8.70	2.01	0.58	0.15	389.17	66.96
Non-riverine Swamp	73	13.94	1.77	0.54	0.05	286.86	26.29
Riverine Marsh	56	9.38	2.21	0.66	0.12	514.45	57.98
Riverine Swamp	224	9.65	0.71	0.70	0.11	388.01	20.49

Table 2

Litter Nutirent Data For Each Wetland Type

Wetland Type	Number of observations	Mean Total Carbon (%)	Std. Error	Mean Total Nitrogen (%)	Std. Error	Mean Total Phosphorus (mg kg-1)	Std. Error
Non-riverine Marsh	54	44.32	0.60	1.38	0.12	983.08	151.58
Non-riverine Swamp	73	45.21	1.57	1.00	0.04	1038.67	82.48
Riverine Marsh	56	40.39	0.76	1.47	0.04	2247.08	222.46
Riverine Swamp	224	40.39	0.76	1.68	0.34	2086.43	109.29

Table 3

Water Nutrient Data For Each Wetland Type

Wetland Type	Number of observations	Mean Total Nitrogen (%)	Std. Error	Mean Total Phosphorus (mg kg-1)	Std. Error
Non-riverine Marsh	54	2.40	0.25	0.15	0.04
Non-riverine Swamp	73	2.34	0.13	0.12	0.02
Riverine Marsh	56	1.73	0.11	0.11	0.01
Riverine Swamp	224	2.38	0.21	0.09	0.01

Table 4

Vegetation Nutrient Data For Each Wetland Type

Wetland Type	Number of observations	Mean Total Carbon (%)	Std. Error	Mean Total Nitrogen (%)	Std. Error	Mean Total Phosphorus (mg kg-1)	Std. Error
Non-riverine Marsh	54	43.13	0.39	2.04	0.08	1553.72	74.73
Non-riverine Swamp	73	45.81	0.29	1.79	0.06	1382.00	127.24
Riverine Marsh	56	45.00	0.31	1.91	0.11	1781.77	125.93
Riverine Swamp	224	44.23	0.18	2.29	0.06	1885.45	80.60

Figures

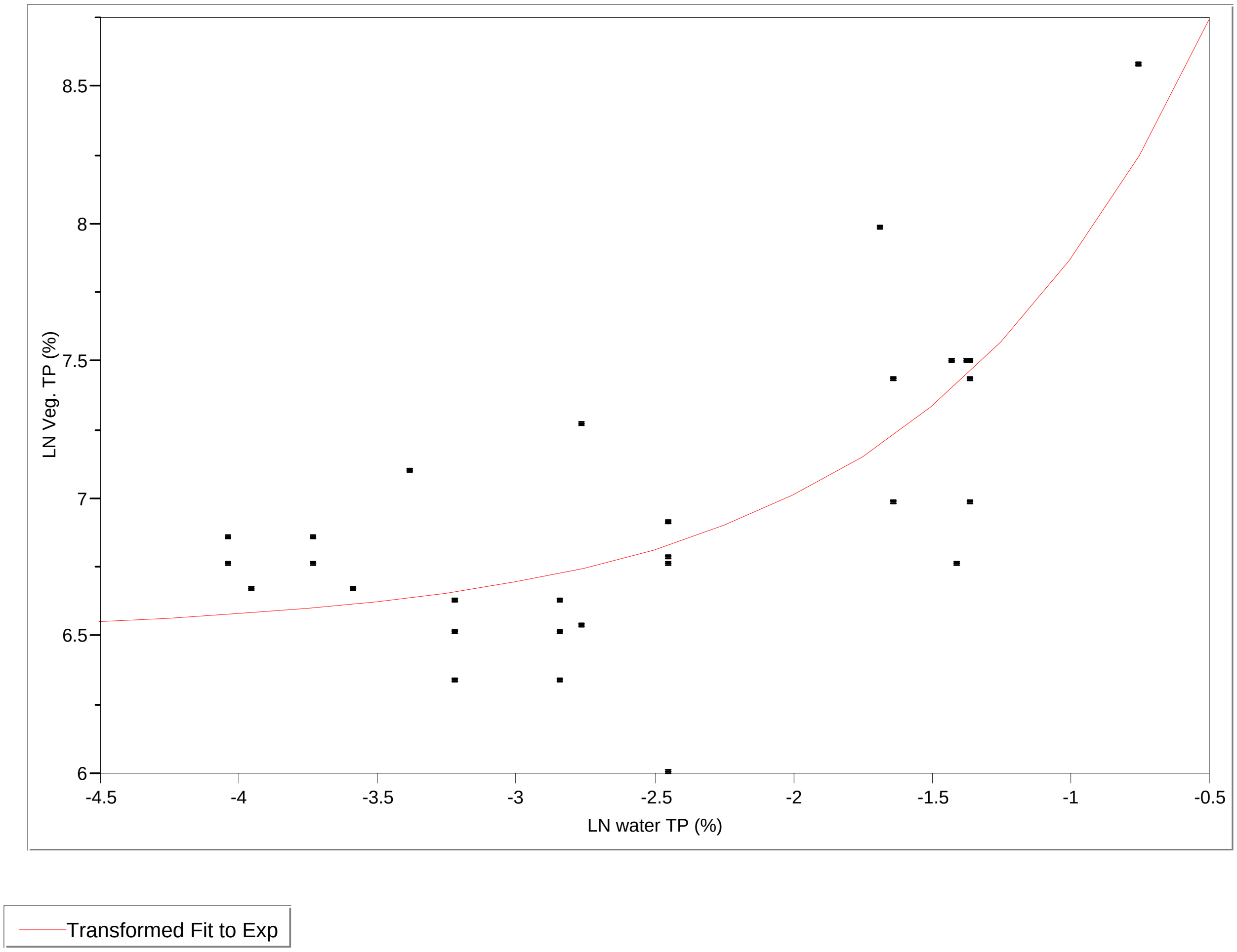

Figure 1. Relationship between the log of the percent of total phosphorus (TP) in water and vegetation for non-riverine swamps.

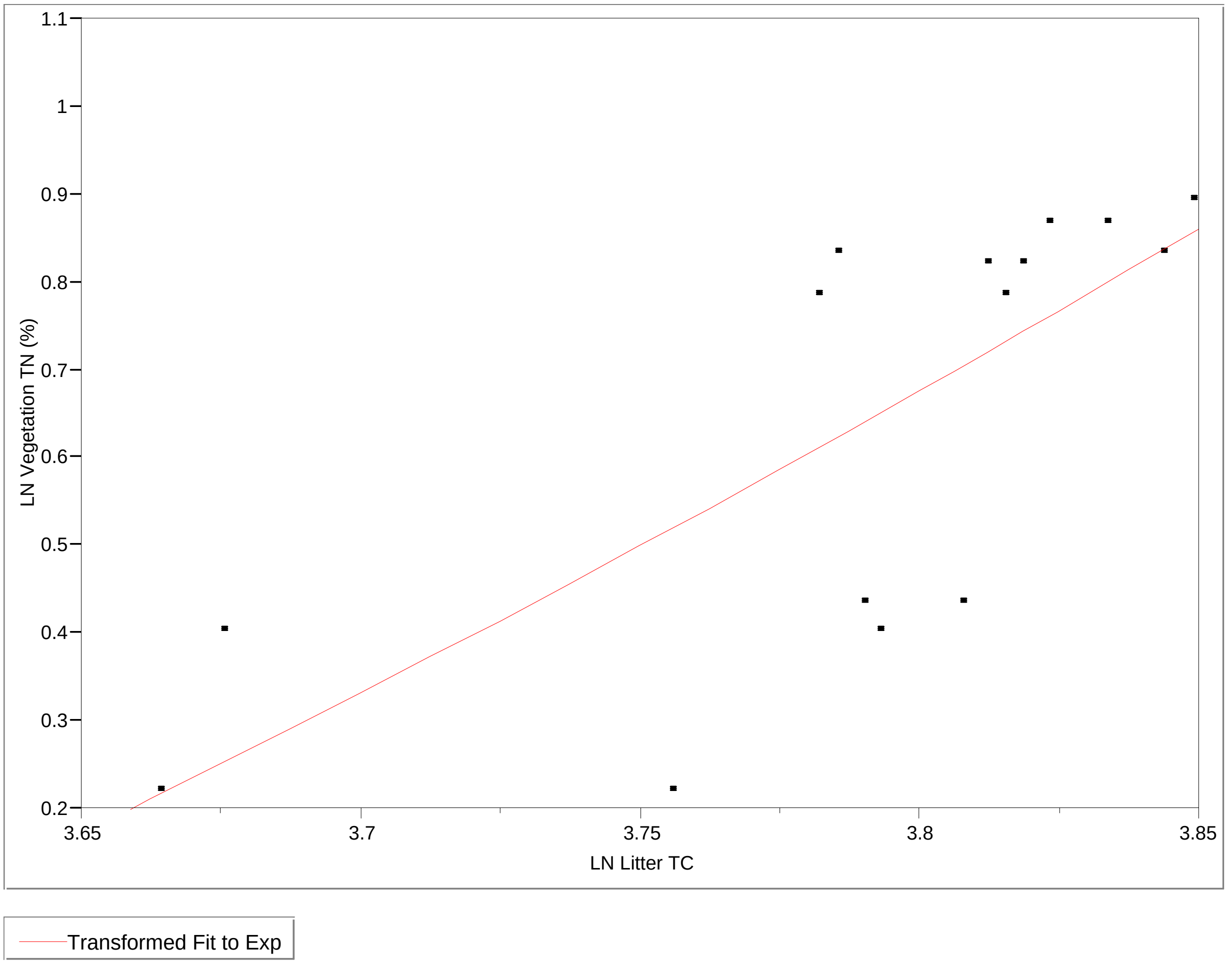

Figure 2. Relationship between the log of the percent of total carbon (TC) in litter and the log of the percent of total nitrogen (TN) in vegetation for non-riverine marshes.

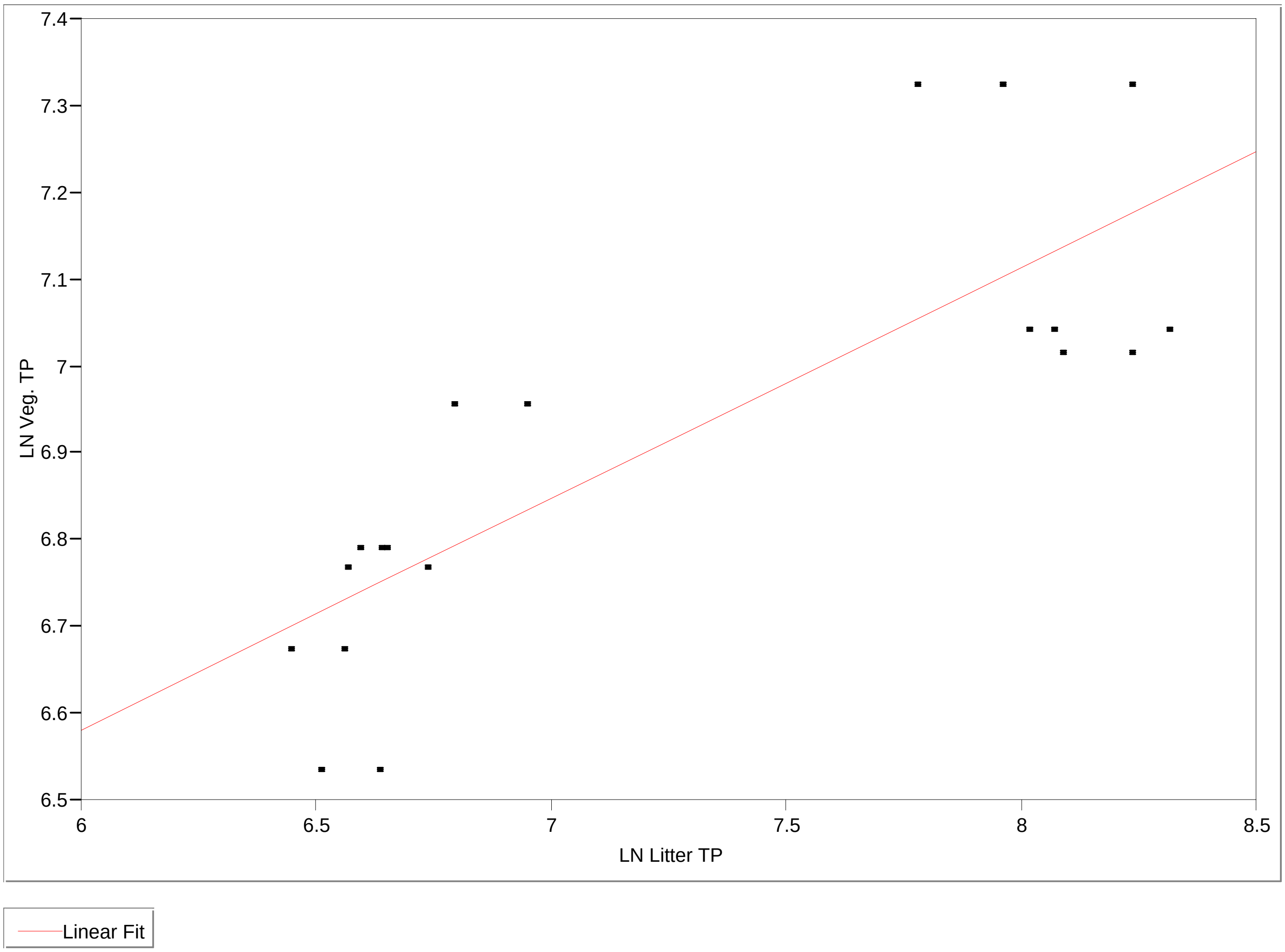

Figure 3. Relationship between the log of the percent of total phosphorus in litter and vegetation in *Nyssa sylvatica variant biflora* samples.

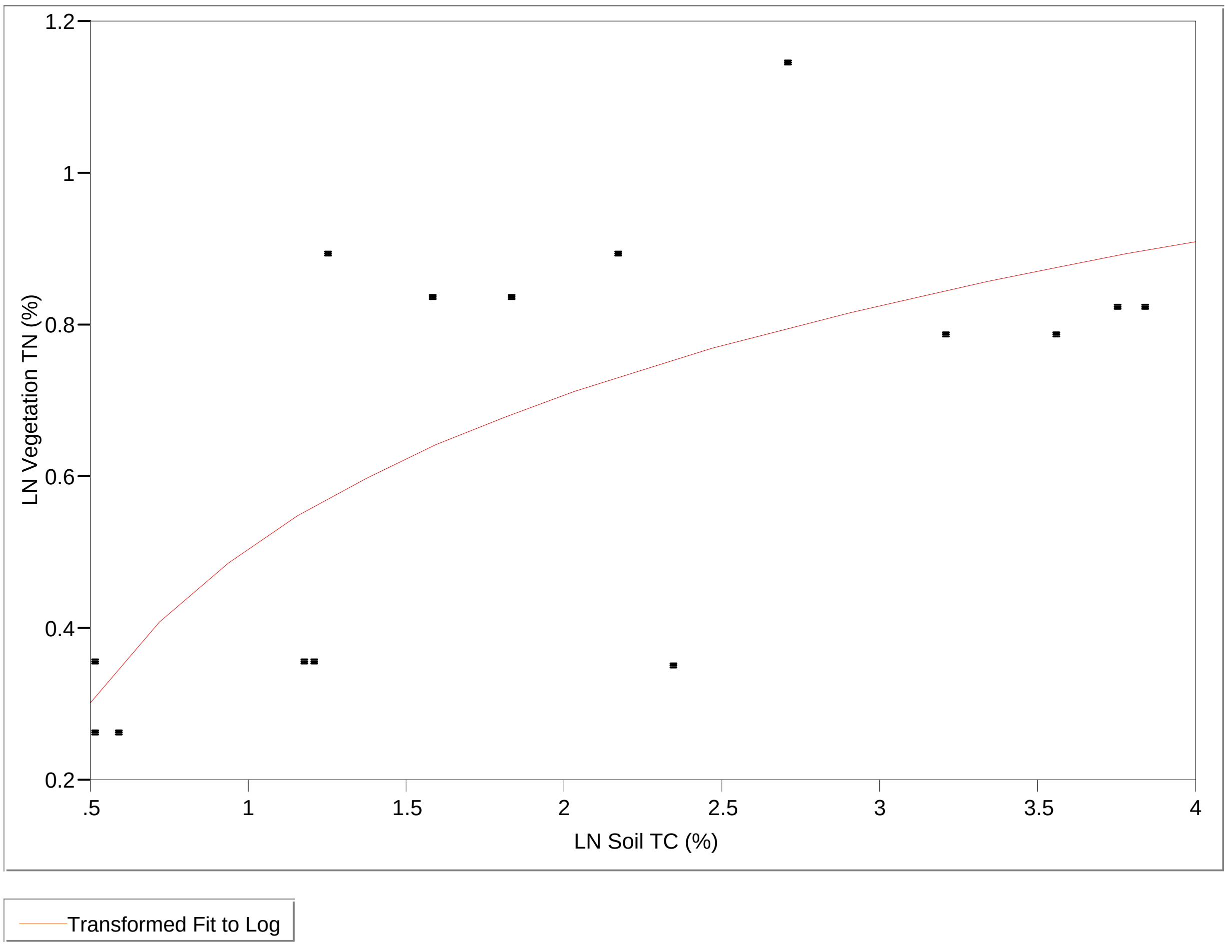

Figure 4. Relationship between the log of the percent of total carbon in soil and the log of the percent of total nitrogen in vegetation in *Panicum* spp. samples.

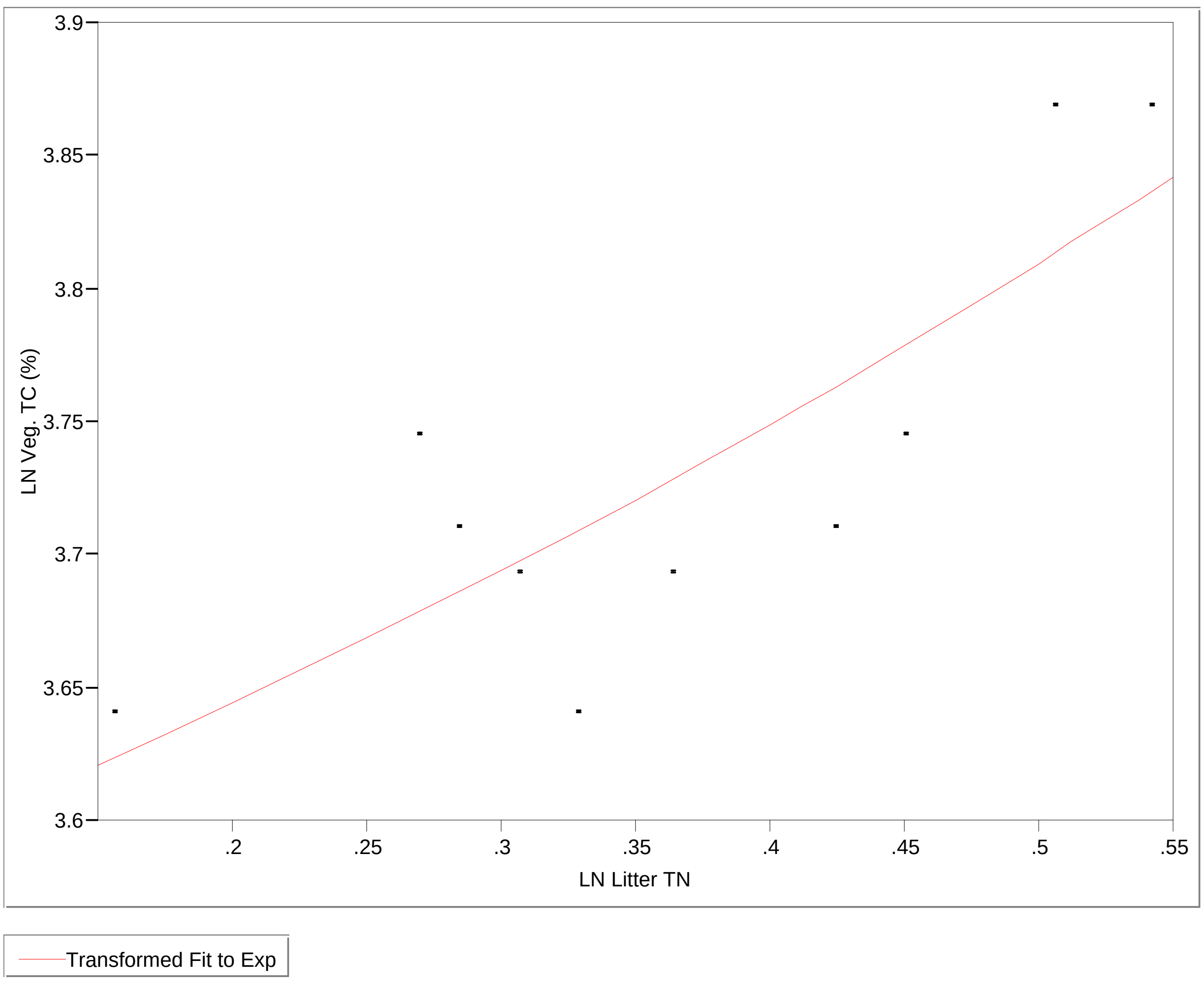

Figure 5. The relationship between the log of the percent of total nitrogen in litter and the log of the percent of total carbon in vegetation in *Pontedaria cordata* samples.

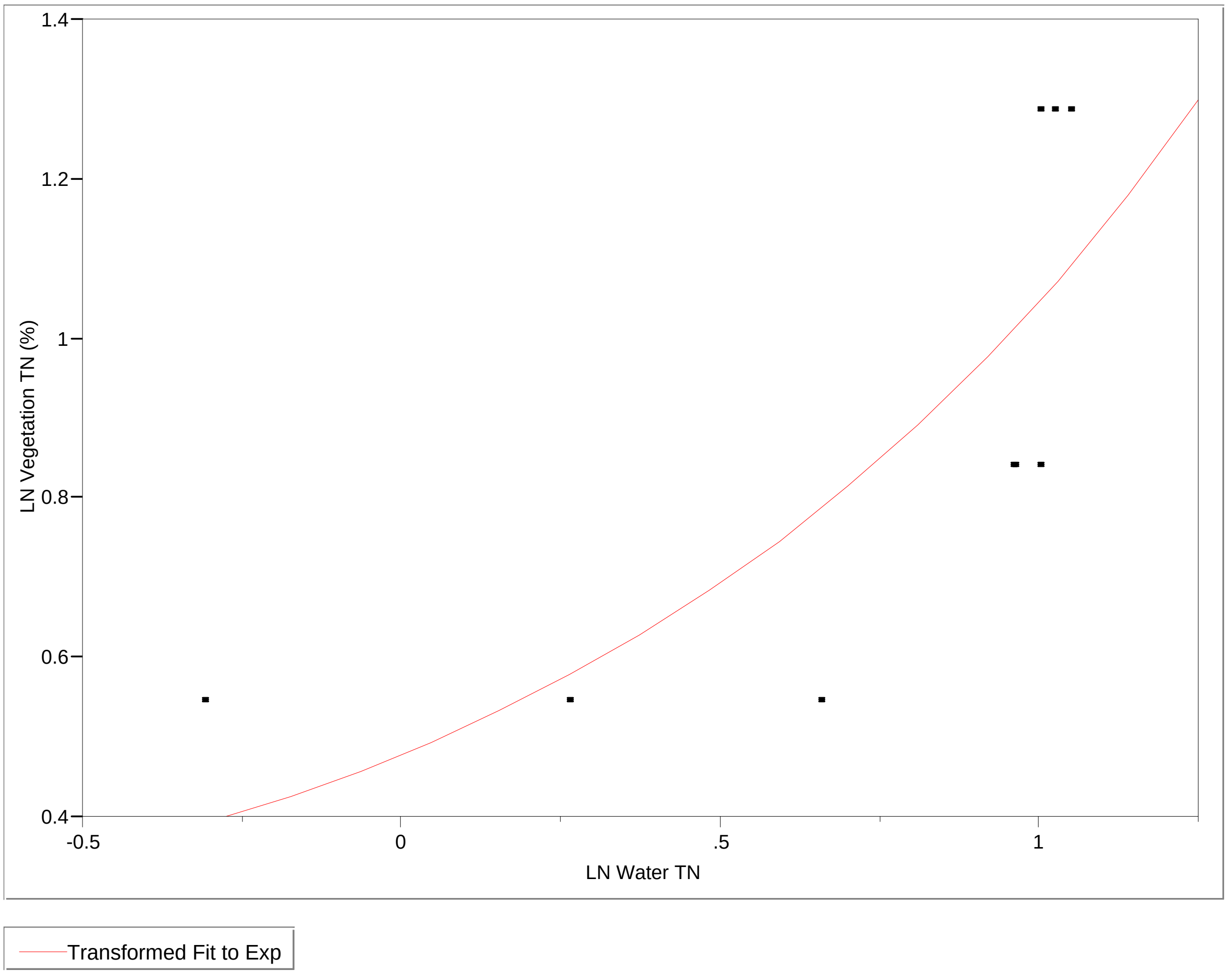

Figure 6. The relationship between the log of the percent of total nitrogen in water and vegetation in *Taxodium* spp. samples.