hydrobiology

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# Effects of Soil, Light, and Temperature on Freshwater Tannin Concentrations

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**Abstract:** Tannins are plant secondary compounds that leach into soil and water. Tannin concentrations can be higher in lentic freshwater than in other aquatic ecosystems, which can result in toxicity to freshwater organisms. While the amount of plant material and the plant tissue concentrations affect aqueous tannin concentrations, little is known about which environmental conditions alter the removal and breakdown of tannins in freshwater, altering the exposure of freshwater organisms. We investigated the effects of soil, light, and temperature on aqueous tannin concentrations. Tannins degraded faster in the presence of and/or sorbed to wetland soils, silt, and clay but not sand. It is unclear whether finer soil particles or the chemical makeup of soils facilitated sorption and/or degradation. Contrary to previous work, we found no effect of ambient light over a 14-day period or sunlight over a 7-day period on tannin degradation, but tannins degraded faster in warmer water. Warmer freshwater ecosystems with greater silt or clay sediments are likely to have lower aqueous tannin concentrations with lower toxicity to aquatic organisms. This research used tannic acid, which contains gallotannins. Further research is needed to determine if similar patterns are found with other tannin types and the complex mixtures of different tannins found in most plants.

**Keywords:** tannic acid; wetlands; water chemistry; soil; photodegradation



Academic Editor: Cláudia Pascoal

Received: 3 December 2024
Revised: 6 January 2025
Accepted: 8 January 2025
Published: 10 January 2025

**Citation:** Earl, J.E.; Aubert, J.F.; Michael, E.O.; Edwards, D.J. Effects of Soil, Light, and Temperature on Freshwater Tannin Concentrations. *Hydrobiology* **2025**, *4*, 2. https://doi.org/10.3390/hydrobiology4010002



## 1. Introduction

Tannins are a group of water-soluble polyphenols that bind to proteins and chelate metal ions [1]. Tannins can be divided into three main groups: condensed tannins, hydrolysable tannins, and phlorotannins. Condensed and hyrdolyzable tannins are found in plants, while phlorotannins are found in marine algae. Condensed tannins can be subdivided into procyanidins and prodelphinidins, while hyrdrolyzable tannins can be subdivided into simple gallic acid derivatives, gallotannins, and ellagitannins. Plants contain a complex mixture of different tannic compounds that vary through time and by species and plant tissue type [1]. Plants produce tannins to deter herbivores with a bitter, astringent taste and protect against plant pathogens through antimicrobial and antifungal activity [2]. Both wetland and terrestrial plants produce tannins and these compounds are retained in the leaves after senescence [3] because they do not contain limiting elements like nitrogen or phosphorus. When leaves fall into aquatic ecosystems (a substantial carbon input to many systems [4–6]), tannins in the leaves affect decomposition rates [3] and organisms that process leaf material [7]. Tannins also leach out of the leaves into the water to alter water chemistry and affect aquatic organisms, including microbes [8,9], phytoplankton [10,11], invertebrates [12], tadpoles [13,14], and fish [15,16].

Tannins dissolved in freshwater can affect organisms in several different ways, most of which result in toxicity but depend greatly on the chemical structure of the particular tannin molecule. While tannins can have antioxidant properties (depending on the reaction conditions) [2] that improve growth in livestock and aquaculture [17], tannins can also promote oxidation that can cause toxicity in organisms [1]. Tannins are defined by their ability to bind to proteins. Binding to proteins can reduce protein availability in the diet of herbivores, interfere with proteins in the gut and other tissues [12], and degrade gills reducing their function. Tannins such as eucalyptus tannins, tannic acid, and epigallocatechin gallate are known to block protein synthesis and lower chlorophyll content in algae [11]. These tannins, as well as spruce bark tannins, are also known to bind to gills in fish, lowering survival through reduced oxygen uptake [15,16]. Neutral to acidic pH conditions are generally required for tannins to precipitate proteins [1]. These conditions are very common in freshwater systems and in the digestive systems of many organisms. Tannins can also bind to metals including iron, zinc, and cobalt [18], essential for the synthesis of certain enzymes, which limits phytoplankton growth [10]. However, tannin complexation with metals can reduce some metals' toxic effects on plants, which has been suggested to be a natural method for detoxification [19]. These various mechanisms result in changes in the developmental rates, growth, and survival of different aquatic organisms [11,20,21] and are affected by the type of tannin.

Despite the many negative effects of tannins, some aquatic animals are attracted to ponds with higher concentrations of tannins. Female frogs are more likely to oviposit in water with higher tannic acid and phenolic concentrations [13,22,23], and some adult aquatic beetles are more likely to colonize ponds with higher phenolic concentrations (Edwards and Earl, unpubl. data). This suggests that at least some phenolics and tannins provide benefits to these animals. Phenolics may allow animals to hide from predators through darker water or by interfering with predator chemosensory activity [23], reducing predation risk. There is also evidence that some tannin-containing leaf extracts inhibit amphibian pathogens and parasites [24,25], but more work is needed to determine whether tannins are responsible. However, some invasive plants (e.g., Chinese tallow [*Triadica sebifera*], purple loosestrife [*Lythrum salicaria*]) may have tannin or phenolic concentrations much higher than aquatic species in the United States evolved with, which may make this cue maladaptive in some circumstances [13,26–28].

With these diverse effects of tannins on freshwater organisms, it is important to understand what conditions lead to higher aqueous concentrations of tannins in freshwater habitats, though all studies have actually measured phenolics that contain tannins instead of tannins separately. While many studies examine tannins in leaf tissues [29,30], we know very little about natural variability in tannin concentrations in freshwater and what environmental factors alter those concentrations. Pond phenolic concentrations (including tannins) have been found to range from 0 to 8 mg/L in closed canopy ponds in Missouri [14] and as high as 14.7 mg/L in a puddle and 18.8 mg/L in a tree hole in Louisiana (Earl et al., unpubl. data). Total phenolic concentrations have been found as high as 11 mg/L in ponds invaded by purple loosestrife in New York [28] and 14 mg/L in temporary ponds in Spain [31]. Biological effects have been found in this range of concentrations [14], though many experiments leach compounds out of leaves but do not measure tannins or phenolics in water (though some do measure them in leaves added to water). It is clear that leaves with higher phenolic concentrations leach more phenolics into water [22,32].

Phenolic concentrations in natural ponds increase with the proportion of riparian trees that are oaks (*Quercus* spp.), but this only accounts for about 18% of the variation in the data [14], suggesting that other environmental variables also affect tannin and phenolic concentrations in ponds.

Although little is known about the factors that regulate tannin concentrations in freshwater, more research is available for brackish ecosystems, particularly in mangroves. Aqueous tannin concentrations are controlled by both physical and chemical processes [33], though microbial and fungal degradation also sometimes occurs in the water [34–36], soil [9,37], or guts of animals [38]. The physical processes include aggregation and adsorption to sediments and organic matter. Chemical processes include a structural change like polymerization and depolymerization after leaching, as well as coprecipitation out of the water column by binding to protein [33], metal ions [39], and polysaccharides [40]. These processes are affected by light through photodegradation and by salinity through aggregation. A large portion of mangrove tannins are processed quickly, with a half-life of less than one day [33], resulting in very low aqueous tannin concentrations in estuarine wetlands [41,42]. In freshwater, phenolic concentrations can be much higher (see above), suggesting lower rates of adsorption, degradation, and/or coprecipitation. It is unclear whether this is due to differences between freshwater and estuarine environments or to differences in tannins found in mangroves versus those common in freshwater (e.g., from tree species or wetland plants), though tidal changes are likely important.

We focused on assessing how environmental factors affect tannin concentrations in freshwater. We conducted a series of experiments examining the effects of soil, light, and temperature on aqueous tannin concentrations using commercially available tannic acid, which is a mixture of gallotannins. For soil, we examined the effects of soil presence and different soil mineral components. For light, we examined indoor ambient light and sunlight during summer in Louisiana, USA. We predicted that tannins would be removed from the water column when soil was present and that tannins would have a faster rate of degradation with higher light levels and warmer temperatures. Together, this information will help predict what types of aquatic ecosystems are likely to have stronger effects of tannins on aquatic organisms.

## 2. Materials and Methods

### *2.1. General Experimental Methods*

To examine the effects of soil, light, and temperature on tannin concentrations, we performed four experiments in the laboratory and one experiment outside. In all experiments, we placed 800 mL of DI water in glass jars containing 8 mg/L of dissolved, powdered tannic acid (Alfa Aesar, Ward Hill, MA, USA) that we monitored through time. We chose 8 mg/L because it represented a high but realistic concentration of tannins in freshwater [14]. In all experiments, we also included 2–3 control replicates, containing 800 mL of DI water with 0 mg/L tannic acid to assess whether contamination took place. Tannic acid is commercially available and contains a mixture of gallotannins that can vary by manufacturer. Jar locations were randomly assigned on the laboratory bench or shelves, and jars were covered loosely with a paper towel to prevent dust contamination unless otherwise specified. Samples were taken over time by removing 60 mL of water from each jar into plastic sample bottles on each sampling date. Samples were frozen until analysis. Tannin concentrations were estimated with the Tyrosine method [43] using the Tanniver reagent (Hach, Ames, IA, USA) and a portable spectrophotometer (Hach DR 3900). These reagents also bind to phenol and other hydroxylated aromatic compounds, so while it is typically expressed as mg/L tannins, our estimates may be considered a proxy of total phenolic concentrations. In our experiment, we did not add other hydroxylated aromatic compounds, but the tannic acid breakdown products may bind differently to the reagents, which could affect the accuracy of our results. Previous studies with similar questions have also used proxies for tannins, such as dissolved organic carbon [33]. In laboratory experiments, we also recorded temperature with a benchtop thermometer recording the average, minimum, and maximum air temperature next to the experimental replicates.

### *2.2. Soil Experiments*

#### 2.2.1. Wetland Soils

To examine the effects of wetland soil on tannin concentrations, we established two treatments of grass and forest wetland soil. We collected soil on 21 March 2019 from Wafer Creek Ranch in Ruston, Louisiana (LA) in the southeastern United States of America, which is a shortleaf pine (*Pinus echinata*) oak (*Quercus* spp.) hickory (*Carya* spp.) forest restoration site. To collect soil from wetlands, we dug out the top layer of sediment (~5 cm in depth) and removed large invertebrates (>2 mm) and plant material (>5 mm). Both wetlands had standing water at the time of soil collection, but these wetlands dry in summer. In both areas, we measured pond size, canopy cover, and water quality, including conductivity, pH, dissolved oxygen, and water temperature (Table S1). Soil samples were dried at 45–50 °C for 5 days.

Four subsamples of each soil type were sent to the Louisiana State University Agriculture Center's Soil Testing and Plant Analysis Lab and were measured for percent organic matter, pH, and the following element concentrations (in ppm): calcium, copper, magnesium, phosphorus, potassium, sodium, sulfur, and zinc. The organic matter was determined via acid-dichromate oxidation read by a dip-probe colorimeter (Brinkmann PC 900, Riverview, FL, USA), and the element concentrations were determined by a Mehlich 3 ICP soil test [44] using a Spectro Arcos II ICP-OES (Ametek, Berwyn, PA, USA).

For the experiment, we established three treatments with four replicates each. These consisted of no soil, grass wetland soil, and forest wetland soil. For each replicate, 800 mL of water with 8 mg/L tannic acid was added to a glass jar. We added 8 g of the appropriate dried soil to each replicate receiving soil. We additionally had two control replicates, for a total of 14 total replicates. Water samples were taken on days 0, 3, 7, 10, and 14 for later tannin analysis. Water samples were filtered through 0.7 μm pore size (AP40 filters, Millipore Sigma, Burlington, MA, USA) glass fiber filters. Air temperature ranged from 21.5 to 24.8 °C during the experiment, and the experiment used a natural photoperiod (~12.5:11.5 h day/night).

#### 2.2.2. Soil Mineral Components

To examine the effects of mineral soil components on water tannin concentration, we established four replicates of five treatments: clay, silt, coarse sand, fine sand, and no soil. We added 800 mL of water with 8 mg/L of tannic acid to each replicate. We also established two controls with 0 mg/L tannic acid for a total of 22 replicates. We obtained soil from Ward's Science (Rochester, NY, USA), and added 8 g of the appropriate soil type to each replicate. Water samples were taken on days 0, 1, 2, 4, and 7. This experiment is shorter in duration because the previous study showed little change in tannin concentration after 7 days. Water samples were filtered through 0.7 μm pore size (AP40 filters, Millipore Sigma, Burlington, MA, USA) glass fiber filters. Air temperature ranged from 20.9 to 23.4 °C, and the experiment had a natural photoperiod (~12.25:11.75 h day/night).

Three samples of each soil type were sent to LSU as above and measured for the same variables as the previous study plus carbon and nitrogen, but not % organic matter due to the focus on soil mineral components. Carbon and nitrogen were determined using a LECO CN analyzer (St. Joseph, MI, USA).

### 2.3. Light Experiments

#### 2.3.1. Ambient Light

To examine the effects of ambient light on tannin concentrations, we established two treatments (light and dark) containing five replicates each in addition to two controls for a total of 12 jars. Jars were placed inside on a lab bench across from windows, so the light received was not direct sunlight but did vary naturally with a realistic photoperiod (~14.0:10.0 h day/night) from 9 to 23 July 2019 in Ruston, LA. We measured the light levels using a light meter (model 401027, Extech, Boston, MA, USA) during full sunlight in the afternoon (i.e., the highest light level during the experiment) at 7 locations on the bench, which were an average of 59.9 footcandles (range 57.3–63.3 fc). The dark treatment was established by wrapping the appropriate jars completely with aluminum foil [45]. All jars were covered from above by aluminum foil to prevent dust, but light treatment jars could receive light from the sides. Water samples were taken on days 0, 1, 3, 7, and 14. Temperatures during this experiment ranged from 22.0 to 23.1 °C.

#### 2.3.2. Sunlight

To examine the effects of sunlight on tannin concentrations, we established three treatments (light, shade, and dark) containing five replicates each in addition to three controls (no tannins added) for a total of 18 jars. The dark treatment was established by wrapping the appropriate jars completely with aluminum foil, and the shade treatment was established by wrapping the jars in a gray window screen, including the top. The light and shade treatment jars were also covered in plastic wrap and secured with a rubber band to prevent dust, insects, or rainwater from entering the jars but allow light through. Jars were placed outside in a wading pool at a randomly selected location. The wading pool was filled with about 10 cm of tap water, which helped to regulate the temperature of the water to keep the temperature of the light treatment jars from getting warmer than the dark treatment jars. We added water to the wading pool as needed to compensate for evaporation. The jars received direct sunlight during the middle of the day for several hours but were shaded by nearby buildings in the morning and evening. Sunlight also varied naturally with the weather and photoperiod (~14.0:10.0 h day/night) from 11 to 25 July 2022 in Ruston, LA, which has a humid subtropical climate. Water samples were taken on days 0, 1, 3, and 7 and frozen for tannin analysis. We were unable to continue the experiment through day 14 due to the summer heat causing rapid evaporation of water in the wading pool and altering the temperature among the treatments. Temperatures in the jars during days 0 through 7 ranged from 30.3 to 33.0 °C and did not differ by treatment.

### 2.4. Temperature

To examine the effects that increasing temperature has on the degradation of dissolved tannins, we established two temperature treatments, 24 °C (room temperature) and 30 °C, using 10-Watt YukiHalu Submersible Aquarium Heaters (Shenzhen, China). We have previously found ponds with temperatures in these ranges in summer in Louisiana, USA. There were four replicates each for the two temperature treatments as well as four control jars for a total of 12 jars for the experiment. Each jar was filled with 800 mL of water with 8 mg/L tannic acid and was sampled on days 0, 1, 3, 7, and 14. Water levels were marked after sampling, and prior to sampling DI water was added to each jar to compensate for differences in evaporation with different water temperatures. Temperature was also measured in each jar at each sampling time to document the actual temperatures produced by heaters. The experiment received a natural photoperiod (~11.1:12.9 h day/night).

### 2.5. Statistical Analysis

Repeated-measures ANOVAs were used to examine changes in water tannin concentrations through time for each experiment and changes in temperature for the temperature experiment only. The sphericity assumption was violated in the wetland soil and sunlight experiments. When the sphericity assumption was violated, we reported the adjusted degrees of freedom and *p*-values based on the Huynh–Feldt correction [46], which corrects for the sphericity violation and is more efficient and has greater power than the Greenhouse-Geiser correction [47]. Differences in soil characteristics among soil types were analyzed using one-way ANOVAs. For the mineral soils, the carbon, nitrogen, and sulfur values were log-transformed to meet assumptions of normality and homoscedasticity. All statistics were performed in SPSS version 26 (IBM, Armonk, NY, USA).

## 3. Results

### 3.1. Wetland Soils

Tannin concentrations were affected by an interaction between treatment and time ($F_{13.5,43.9} = 71.73$, $p < 0.001$; Figure 1). In all treatments, tannin concentration decreased over time. By the end, the soil treatments had significantly lower concentrations of tannins than the no soil treatment, indicating that tannins likely adsorbed to soil particles or that soil increased degradation rates. Tannin concentrations were 26% and 66% lower in the grassland and forest wetland soil treatments, respectively, in comparison to the DI water treatment with no soil added after 14 days. It is unclear what may have caused the difference between the soil types because the soils were different across many different variables. Forest wetland soils had higher percent organic matter and higher concentrations of calcium, copper, magnesium, potassium, sulfur, and zinc than grassland wetland soil, while grassland wetland soils had higher pH and a higher concentration of phosphorus than forest wetland soils (Table 1). The two soil types were not different in sodium concentrations.

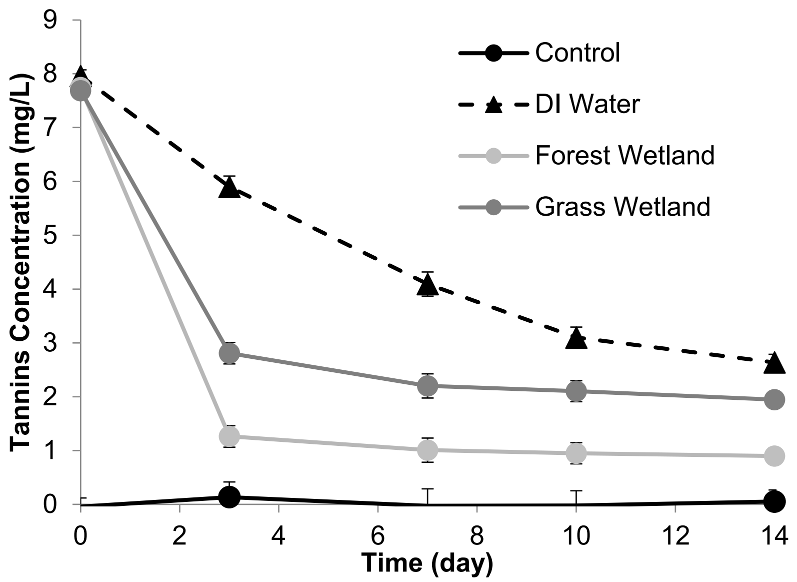


**Figure 1.** Effects of two types of wetland soil and DI water with no soil added on tannin concentrations in water. Error bars are standard errors, some of which are smaller than the data symbol.

### 3.2. Soil Mineral Components

Tannin concentrations were affected by an interaction between treatment and time ($F_{20,64} = 63.78$, $p < 0.001$; Figure 2). Tannin concentrations decreased linearly in the DI water, fine sand, and coarse sand treatments and were not different from each other at the end of the experiment. Tannin concentrations in the silt treatment decreased rapidly during the first day of the experiment and then gradually for the following six days. Unlike the other soils, the clay treatment had low tannin concentrations near the beginning of the experiment, which increased through day two and then decreased through day seven (Figure 2). We suspect the initial low concentrations were due to fine clay particles smaller than the filter pore size (0.7 μm) suspended in the water that interfered with spectrophotometer measurements during tannin analysis. On day seven, there was no significant difference in water tannin concentration between the clay and silt treatments, and the clay treatment and silt treatment were 89% and 82% lower than the DI water treatment, respectively, after 7 days.

**Table 1.** Characteristics of grassland and forest wetland soils ($n$ = 4 for each type) collected in Ruston, Louisiana, USA, showing mean ± standard deviation.

| Soil Variable | Grassland | Forest |
|---|---|---|
| % Organic Matter ** | 1.96 ± 0.14 | 2.47 ± 0.16 |
| pH * | 4.55 ± 0.06 | 4.74 ± 0.09 |
| Calcium (ppm) *** | 241.79 ± 3.12 | 293.59 ± 13.36 |
| Copper (ppm) ** | 0.48 ± 0.02 | 0.57 ± 0.04 |
| Magnesium (ppm) *** | 39.94 ± 1.55 | 60.00 ± 0.79 |
| Phosphorus (ppm) * | 18.84 ± 0.55 | 16.66 ± 1.24 |
| Potassium (ppm) *** | 32.26 ± 1.05 | 49.66 ± 1.61 |
| Sodium (ppm) | 19.60 ± 2.36 | 22.32 ± 1.75 |
| Sulfur (ppm) ** | 20.88 ± 0.50 | 22.59 ± 0.82 |
| Zinc (ppm) *** | 1.38 ± 0.11 | 2.26 ± 0.11 |

* indicates the two soil types are significantly different at $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

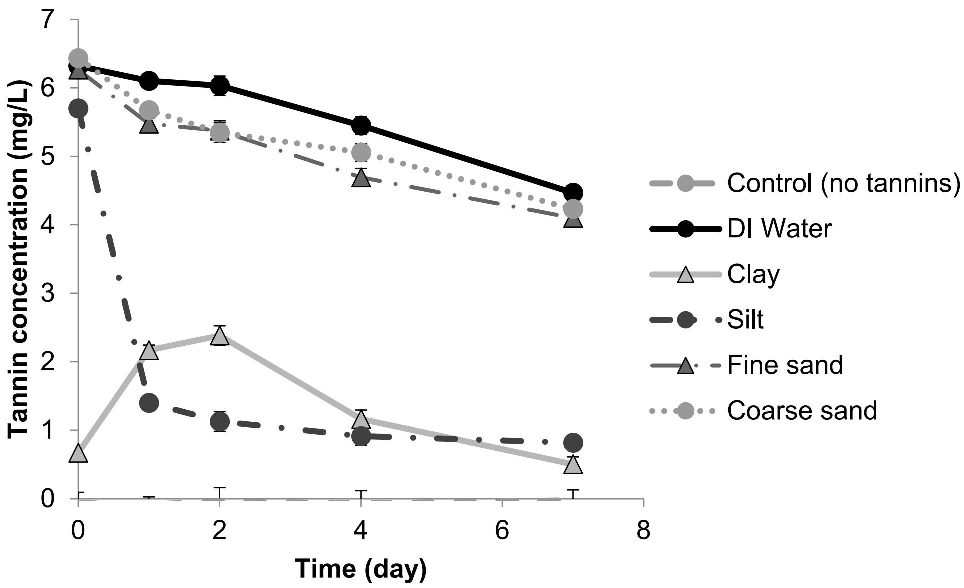


**Figure 2.** Effects of soil components on tannin concentrations in water. The Control line is covered by the x-axis, because the values are right at zero, so that only the top of the error bars are visible. Error bars are standard errors, some of which are smaller than the data symbol.

Most elements were more abundant in silt than the two types of sand with clay intermediate (Table 2), including carbon, copper, magnesium, nitrogen, potassium, sodium, and zinc. Sulfur showed a similar pattern except that fine sand had higher concentrations than coarse sand. Calcium and soil pH were highest in clay and silt with fine sand intermediate and coarse sand the lowest. Phosphorus was highest in silt with all other soil types not significantly different from each other.

**Table 2.** Characteristics of mineral soils ($n$ = 3 for each type), showing mean ± standard deviation.

| Soil Variable | Clay | Silt | Fine Sand | Coarse Sand |
|---|---|---|---|---|
| pH *** | 8.01 ± 0.01 A | 7.65 ± 0.28 A | 5.66 ± 0.36 B | 4.88 ± 0.13 C |
| Calcium (ppm) *** | 4,635 ± 164 A | 5,171 ± 121 A | 47 ± 11 B | 26 ± 2 C |
| Carbon (%) *** | 0.91 ± 0.08 A | 6.79 ± 1.24 B | 0.02 ± 0.02 C | 0.02 ± 0.01 C |
| Copper (ppm) *** | 0.88 ± 0.07 A | 4.74 ± 0.03 B | 0.10 ± 0.01 C | 0.08 ± 0.00 C |
| Magnesium (ppm) *** | 152.71 ± 3.21 A | 315.89 ± 9.44 B | 10.70 ± 0.54 C | 7.56 ± 0.39 C |
| Nitrogen (%) *** | 0.14 ± 0.00 A | 0.47 ± 0.03 B | 0.03 ± 0.00 C | 0.02 ± 0.01 C |
| Phosphorus (ppm) *** | 2.87 ± 0.66 A | 181.14 ± 1.22 B | 2.00 ± 0.18 A | 1.85 ± 0.04 A |
| Potassium (ppm) *** | 101.90 ± 1.94 A | 894.61 ± 14.34 B | 5.92 ± 0.42 C | 8.54 ± 0.27 C |
| Sodium (ppm) *** | 45.56 ± 1.13 A | 64.71 ± 4.47 B | 18.51 ± 0.20 C | 17.79 ± 1.97 C |
| Sulfur (ppm) *** | 168.93 ± 14.16 A | 63.03 ± 0.84 B | 8.36 ± 0.37 C | 3.70 ± 0.14 D |
| Zinc (ppm) *** | 0.59 ± 0.13 A | 10.80 ± 0.14 B | 0.17 ± 0.03 C | 0.06 ± 0.01 C |

*** indicates the two soil types are significantly different at $p \leq 0.001$; different letters indicate significant differences among soil types with Tukey's multiple comparison.

### 3.3. Light

In the ambient light experiment, tannin concentrations did not differ at any time point between the light and dark treatments ($p$ = 0.63). There was a significant interaction between time and treatment ($F_{8,36}$ = 13.06, $p$ < 0.001) that showed no change in tannin concentration through time for the control and a steady decrease in concentration through time for the light and dark treatments (Figure 3).

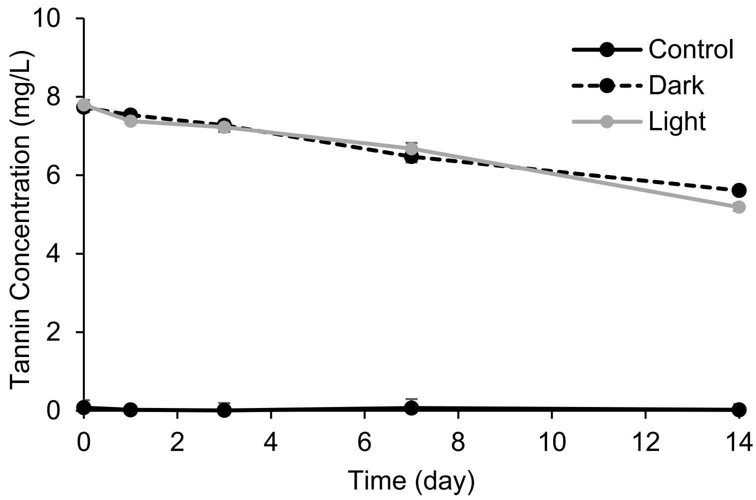


**Figure 3.** Effects of ambient light on tannin concentrations in water. Error bars are standard errors, some of which are smaller than the data symbol.

In the sunlight experiment, tannins did not change through time ($F_{1.7,25.1}$ = 1.14, $p$ = 0.33; Figure 4), and there was no effect of the sunlight treatment on tannin concentrations (all $p$ > 0.69).

### 3.4. Temperature

While temperatures fluctuated slightly, the temperature remained different between the two temperature treatments throughout the experiment ($F_{2,9}$ = 338.43, $p$ < 0.001) with an average of 23.7 °C in the 24 °C treatment and an average of 28.8 °C in the 30 °C treatment (Figure S1). There was a significant interaction between time and treatment on tannin concentrations ($F_{8,36}$ = 43.04, $p$ < 0.001). Tannin concentration was 27% lower in the 30 °C treatment than in the 24 °C treatment after two weeks (Figure 5).

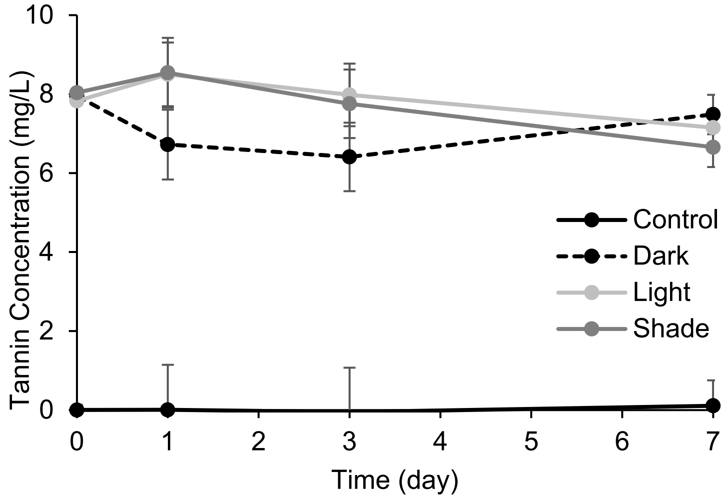


**Figure 4.** Effects of sunlight and shading on tannin concentrations in water. Error bars are standard errors.

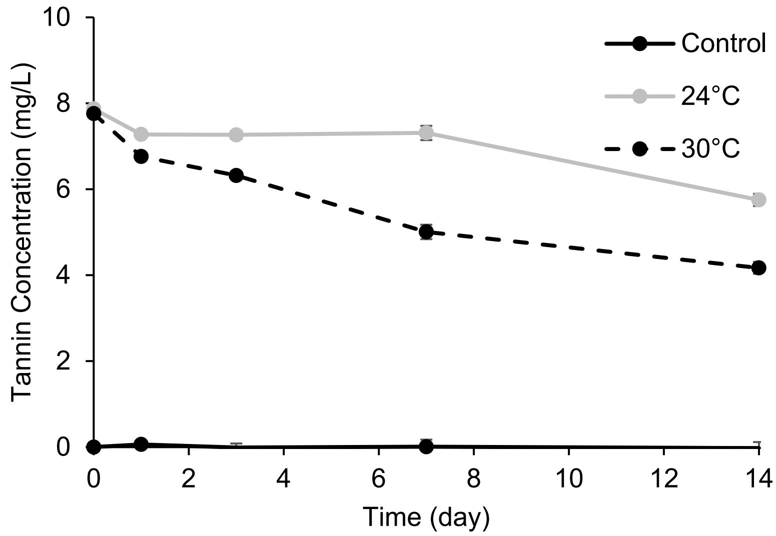


**Figure 5.** Effects of temperature on tannin concentrations in water. Error bars are standard errors, some of which are smaller than the data symbol.

## 4. Discussion

Aqueous tannin concentrations were affected by soil and temperature but not light. While the vegetation in and around aquatic ecosystems will always be important as the source of aqueous tannins [14,28,31], our results show that other environmental variables alter the fate of tannins after leaching into water. Assuming similar vegetation and water volume, warmer aquatic ecosystems with silt or clay sediments will likely have lower tannin concentrations due to adsorption to sediment and faster degradation under higher temperatures. Our results did not show photodegradation as an important process, but greater sun exposure will likely decrease tannin concentrations through warming. These aquatic ecosystem differences will affect organisms through changes in the length and concentration of exposure to tannins with associated positive (e.g., lowered pathogen risk) and negative effects (e.g., toxicity).

We found the largest decreases in aqueous tannin concentrations with soil additions. Soil type was critical to these differences. The presence of sand did not affect tannins concentrations, but tannins either adsorbed onto clay and silt particles or degraded more quickly in their presence, resulting in aqueous tannin concentrations more than 80% lower than no soil after 7 days. It is unclear whether this was due to soil particle size or the chemical composition of these different soil types used in this experiment. Schmidt et al. [48] found positive relationships between tannin sorption and the percent clay, silt, and sand in pasture soils, suggesting that it may not be particle size. We also found that tannins adsorbed or degraded more readily with forest wetland soil than the grassland wetland soil, but there were also many differences between these two soil types. In both of our experiments, soil types differed in the concentration of many different elements, but there were some common trends that could be examined. Across both experiments, soils that resulted in lower tannin concentrations had higher pH, calcium, copper, magnesium, potassium, and zinc. Prior work shows that tannins can bind to zinc [10], copper [19], and with higher soil pH [48]. Though we did not measure iron in the soil, tannins can bind to iron, mobilizing it from deeper sediments in reservoirs [18]. Further work is needed to separate out the relative importance of these different soil variables.

Organic matter and nitrogen are other components in soil likely important for tannin adsorption to soil. Forest wetland soils were higher than grassland wetland soils in organic matter, previously shown to adsorb tannins. Maie, Pisani, and Jaffé [33] found that more than 80% of tannins were adsorbed onto peat. Similarly, more tannins sorb to forest and pasture soil layers with higher organic matter [49]. However, Schmidt, Halvorson, Gonzalez, and Hagerman [48] found no relationship between tannin sorption and the organic matter of six different pasture soils. These different findings may result from differential tannin sorption to different types of organic matter depending on the nitrogen content, polarity, and degree of humification [50].

Another important factor is that we used tannic acid, consisting of an unknown mixture of gallotannins. Gallotannins (a type of hydrolysable tannin) sorb to soil more readily than condensed tannins [49,50], possibly enhancing the differences found in our study. Tannins in aquatic ecosystems include a diverse mixture [1], which could affect tannin dynamics in these systems. The molecular weight and polarity of tannin molecules affect the sorption to soil with higher sorption for nonpolar tannins with higher molecular weights [48]. The percentage of tannins sorbed in soil is also affected by whether tannins are in mixtures or not. It appears that some tannins compete for binding sites on soils resulting in less sorption, while other tannins create new soil-binding sites increasing sorption [48]. Most plants produce a variety of tannins that change based on environmental variables [51–53], and most freshwater habitats have different combinations of tannin-producing terrestrial and aquatic plants. This added complexity will make it difficult to understand tannin sorption to soils in both aquatic and terrestrial ecosystems, but repeating these experiments with tannins leached from leaves and more precise chemistry methods (e.g., HPLC) will determine if similar patterns are observed.

Warmer temperatures also facilitated tannin degradation. Tannin concentrations were 27% lower at 29 °C than 24 °C after 14 days. These temperatures are environmentally relevant, as surface water temperatures often reach 29–30 °C during summer in areas with a hot climate [54–56] and can exceed 35 °C in south Florida wetlands [57]. Freshwater ecosystems that are shallow and unshaded have warmer water, which will likely facilitate tannin degradation. Submerged vegetation also warms water by absorbing light and reflecting heat into the water [57]. Further warming is expected with climate change which will likely enhance tannin degradation. Alternatively, shallower water will have decreased hydroperiods, and increased evaporation may concentrate tannins. In colder environments, tannins may persist longer, though data are needed at a broader range of temperatures.

In the light experiments, we found that ambient light did not affect tannin degradation over two weeks, and sunlight did not affect tannin degradation over 7 days. Our replicates were covered with plastic to prevent contamination and evaporation, but that may have blocked some of the UV, potentially affecting our results. Previous work on the photodegradation of tannins is mixed [33,36] likely due to different methods and possibly different types of tannins and/or the length of exposure. Similarly to our results, Ghosh and Bhadury [36] found no effect of light exposure in the laboratory over 2 weeks using tannic acid. Maie, Pisani and Jaffé [33] found a 30% decrease in tannin concentration with light exposure using a solar simulator equivalent to 25 days of unobstructed sunlight at solar noon in Miami, FL condensed into a 7-day period. This is more sunlight exposure and a higher sunlight intensity than used in our experiment, which may be necessary for rapid photodegradation. It is also likely more sun exposure than many freshwater ecosystems receive due to shading from canopy cover, emergent vegetation, and cloud cover. Maie, Pisani and Jaffé [33] also used mangrove tannin concentrations about twice as high (~16.5 mg/L) as those in our experiment. Mangrove tannins may be more susceptible to photodegradation than tannic acid. Further study will be necessary to separate out these different effects.

## 5. Conclusions

Our results show that freshwater tannic acid concentrations are affected by soil and temperature but not light. This suggests that warmer freshwater systems with more exposed sediment will have lower aqueous tannin concentrations and lower negative effects on aquatic organisms. It also suggests that experiments examining the impacts of leaves may have artificially high tannin concentrations if soils are not added, though several previous studies were still around the high range of phenolics found in ponds and small pools [32,58] (Earl et al., unpubl. data). Many studies on leaves in freshwater ecosystems do not measure tannin or phenolic concentrations or measure them only in the leaves and not in the water. This information would be helpful to compare to natural freshwater, though additional data on concentrations in ponds, streams, and wetlands would aid in this comparison. Some invasive plants (e.g., Chinese tallow, purple loosestrife) found in North America have much higher leaf tannin concentrations than most native species [22,28,59] inducing toxicity in some aquatic organisms [26,27]. It would be helpful to better understand what environmental factors regulate the aqueous tannin concentrations from invasive plants in particular.

Our research started to identify what environmental characteristics affect aqueous tannin concentrations to help predict the effects of tannins on freshwater organisms and ecosystems. Other environmental variables not included in our study are also likely important to tannin concentrations as well, including salinity [33], concentrations of other compounds (e.g., soil minerals), and pH. Examining the effects of these different environmental factors should be combined with the examination of different types of tannins and more accurate analytical chemistry like HPLC for a more holistic view of their role in ecosystems. This work will enhance our understanding of the complex chemistry of tannins and how they affect a variety of organisms and environmental processes.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/hydrobiology4010002/s1, Figure S1: Temperature during the temperature experiment examining effects on tannin water concentrations. Error bars are standard error; Table S1: Characteristics of grassland and forest wetland ponds where soil was collected.

**Author Contributions:** J.E.E.: Conceptualization, Investigation, Writing—original draft, Funding acquisition, Project administration, Supervision. J.F.A.: Investigation, Writing—original draft, Writing—reviewing and editing. E.O.M.: Investigation, Writing—original draft. D.J.E.: Investigation, Writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** The Louisiana Tech University College of Applied and Natural Sciences, School of Biological Sciences, and the Louisiana Board of Regents RCS [LEQSF(2021-24)-RD-A-08] also provided funds for equipment used in this research.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available at https://knb.ecoinformatics.org/view/doi:10.5063/F1HD7T52 (accessed on 8 January 2025).

**Acknowledgments:** We would like to thank Johnny Armstrong for funding and for giving access to his property, Wafer Creek Ranch, to collect soil samples. We would also like to thank our colleagues G. Cohenour, D. Johnson, M. Lowry, and K. Smith for assisting with laboratory work. L. Vernon implemented one of the experiments and was supported by the Army Educational Outreach Program. The graphical abstract was created in BioRender.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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