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Influence of Two Common Bryophytes on Acidity and Divalent Cation Concentrations in Standing Spring Water

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INFLUENCE OF TWO COMMON BRYOPHYTES ON ACIDITY AND DIVALENT CATION CONCENTRATIONS IN STANDING SPRING WATER

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ABSTRACT

This laboratory experiment examines the influence of two common mosses on the pH and solute dynamics of water from a spring brook. Bivariate analysis of variance tests (MANOVA) revealed significant changes in concentrations of H^+ and the combined variable, divalent cations (Ca^{++} & Mg^{++}) over a three week incubation period in microcosms containing *Thuidium delicatulum* and *Brachythecium rivulare*, mosses commonly found in low order woodland streams. Divalent cation concentrations in the presence of moss were 36% higher, on average, than in similar microcosms with moss absent. In microcosms containing decomposing wood, H^+ concentrations were 15% lower in the presence of moss. There were approximately 7 mg of divalent cations in every gram of moss tissue (AFDM), while a gram of wood contained 1-2 mg of divalent cations, values similar to those reported elsewhere in the literature. I suggest reversed cation exchange is the mechanism responsible for elevated divalent cation concentrations and changes in solute dynamics. A hypothesis concerning expected responses of fungal enzymes to the observed changes in solute dynamics is discussed.

Key words: Bryophytes, calcium, magnesium, MANOVA, pH streams.

INTRODUCTION

Mineral inputs to streams come from four general sources: subterranean inputs from groundwater, directly from the atmosphere, overland flow from run-off, and from within the stream itself, through biological and abiological dissolution of minerals in substrata and litter that falls into the stream. This experiment focuses on in-stream biological processing of dissolved ions.

Catchment surface area and elevation have been shown to affect mineral concentrations in streams (Johnson et al. 1997) and lakes (Kratz et al. 1997). Aquatic systems at high elevation typically have low levels of salts and nutrients and tend to be poorly buffered, simply because groundwater routes and overland run-off routes are short. Under such conditions, in-stream biological processes are likely to have an important effect on solute dynamics.

Calcium dynamics in streams affect decomposition rates (Rosset & Barlocher, 1985), in part because calcium is a cofactor of the enzyme pectin lyase (Vitali et al. 1998), a key enzyme in the decomposition of leaf litter (Suberkropp & Klug, 1980; Jenkins & Suberkropp, 1995). This may explain why leaf decomposition rates in hardwater streams are generally higher than in softwater streams (Chamier, 1985). Thus, in low order, high elevation woodland streams, calcium concentrations are likely to significantly impact what is generally considered the foundation of stream energy webs (Minshall, 1967; Kaushik & Hynes, 1971).

Natural buffers such as HCO_3^- are subject to the same principles of catchment size which influence mineral concentrations. This is aptly demonstrated by Kratz et al. (1997) who found that alkalinity was negatively correlated with elevation in lakes of similar size

in northern Wisconsin. Resulting pH fluxes may impact the stream community, either by fluctuating beyond the tolerance limits of stream organisms, or possibly via trophic interactions resulting from microbial enzyme reactions to changes in pH (Kok & Van der Velde, 1991; Polis & Strong, 1996).

Low order woodland and alpine streams are often colonized by bryophytes (Hynes, 1971; Stream Bryophyte Group, 1999; Suren & Ormerod, 1998). These organisms are known to have a profound effect on solute dynamics in bog communities via the mechanism of ion exchange (Clyme, 1963). Sphagnum mosses in bog communities preferentially adsorb cations to cell-wall surfaces, displacing H^+ ions. In this manner they greatly increase the acidity of the environment. H^+ ions produced by the plant are loosely bound to galacturonic acids on the external surface of the cell wall, such that they can readily be exchanged with cations in solution in accordance with LeChatelier's principle (Clyme, 1963; McQueen, 1990). Although bound H^+ are typically exchanged for cations in solution, it is theoretically possible that the reverse may occur under the right physical conditions, for instance when most exchange sites are bound with cations and solution pH is suddenly lowered. To my knowledge, there are no studies of cation exchange among stream bryophytes in the literature (Stream Bryophyte Group, 1999). This is understandable, because stream pH conditions in the absence of anthropogenic interference tend not to be acidic like bogs.

This paper examines the influence of the mosses *Thuidium delicatulum* L., Hedw. and *Brachythecium rivulare* B. & S. on solute dynamics in standing water from a spring-brook. These mosses are commonly found in, or very near, cold running waters throughout the northeastern United States (Palmer & Fowler, 1974). Specifically, I was

interested in their effect on the dynamics of pH and the divalent cations (DC) calcium and magnesium. I hypothesized that under the proper conditions, low pH and low concentrations of DC's, cation exchange would occur in reverse.

METHODS

Field Collection

Mixed colonies of free-floating and attached (cobble and wood) *B. rivulare* and *T. delicatulum* were collected on 3/19/99 from Stream 6 in Allegany State Park, NY (42.0 ^{0}N, 78.8 ^{0}W), a first-order spring-brook at 2000 feet above sea level (Brady, 1993). Bare cobbles and 4 liters of stream water were also collected. Mosses, stream water and substrata were transported on ice to the Water Quality Laboratory at the State University of New York College at Brockport (SUNY Brockport) within three hours of collection. Stream water was stored at 4 ^{0}C while mosses and substrata were stored in a tank (15 ^{0}C) containing 2 liters of circulating distilled water. Microscopic examination revealed no evidence of cell damage due to the low osmotic pressure of distilled water. The following morning, stream water was filtered (0.45 um pore-size) and mosses, wood and cobbles individually received two one-hour rinses in about 4 liters of fresh distilled water.

Experimental Design

Fifty-four microcosms were constructed in glass jars (Qorpak ^{TM}) with tight sealing lids holding 200 ml of filtered stream water. Twenty seven jars had moss present and 27 had moss absent. One third of each treatment's microcosms had no substrate, one third had a cobble substrate and one third had a wood substrate. For the wood substrate

treatment, nine pieces of wood with moss growing on one end but absent from the other were selected and cut in half. Microcosms containing no substratum and no moss served as controls.

Two representatives from each treatment were systematically distributed to one of three laboratory carts. Microcosms on each cart were then randomly distributed for incubation at room temperature and naturally occurring light conditions (average of 12.5 hours daylight; 410 langleys/day). Carts were turned periodically, in order to equalize exposure to sunlight from a nearby window. Once weekly, over a period of three weeks, the microcosms were subjected to a 12 hour incubation inside a cardboard box (dark) and a 12 hour incubation with a 150 watt incandescent light on either side of each cart (light). After each incubation, pH was measured and 10.0 ml of water were filtered for Ca^{++} and Mg^{++} analysis. A distilled water blank was prepared after every 18 replicates. A single spiked sample was prepared to test for matrix effects.

Divalent Cation Concentrations

Calcium and magnesium analyses were conducted on a Perkin-Elmer 3030 Atomic Absorption Spectrophotometer (APHA, 1998). Linear calibration curves were constructed using five standard concentrations ranging from 2.50 - 20.00 mg Ca^{++}/L and 1.00 - 12.00 mg Mg^{++}/L with a minimum acceptable correlation coefficient of 0.995. Quality control (QC) standards made from separate stock at concentrations roughly corresponding to the mid-point of calibration curves were used to ensure accuracy of

measurements. Laboratory standards, practices and equipment are monitored biannually through the New York State Department of Health's Environmental Laboratory Approval Program through which the Water Quality Laboratory at SUNY Brockport is certified (ELAP #11439).

pH Analysis

The pH of microcosm waters was measured with a Beckman ^{R} 45 pH meter equipped with a temperature compensating electrode. The instrument was calibrated after every 5th measurement with two standard buffers of pH 4.00 and 9.00. Once every 18 measurements a QC buffer of pH 6.86 was measured. In the event this measurement deviated more than 0.12 pH units from 6.86, the preceding 18 measurements were repeated. Calibration and QC buffers were incubated with the microcosms in order to minimize temperature differences.

Dissolved Oxygen

Following the three week incubation period, two of the replicates from each treatment type (microcosms with the highest and lowest concentrations of divalent cations) were transferred to clean mason jars holding 500 ml of freshly filtered stream water and were, again, subjected to a dark and light incubation. Percent saturation of

dissolved oxygen was measured as an indicator of photosynthetic and respiratory activity with a dissolved oxygen meter (Yellow Springs Instruments Model 58) and a temperature compensating dissolved oxygen probe (YSI 5739) following each incubation. The probe was stirred manually in order to prevent entanglement of mosses in motorized stirring devices. Values remaining constant for one minute were recorded as % saturation.

Cations Adsorbed to Microcosm Walls

At the end of the three week incubation period, water in those microcosms not tested for photosynthetic and respiratory activity was analyzed for divalent cation concentrations. After contents were removed, an acid rinse (0.05N HNO_3) to remove DCs from container walls was analyzed. Adsorbed cations were calculated as the difference in concentration between these final two measurements.

Determination of Ash Free Dry Mass (AFDM)

Dry weight of moss, wood and cobbles was determined after constant mass was achieved at 50-60 ^{0}C (5-6 days). Moss ash content was determined from ground composite samples, with a minimum of three replicates per treatment, weighing approximately 250 mg each in ashed, pre-weighed aluminum pans. Wood shavings, representing the surface and interior of substrata, were similarly analyzed but the samples

were not composites. Samples were weighed after heating in a muffle furnace at 550 +/- 20 ^{0}C for 30 minutes. The organic proportion of samples (O) was determined as:

$$O = (D - A)/D \quad (1)$$

where O represents the proportion of organic matter by mass, D represents the sample dry mass and A represents the mass of the sample's ash. AFDM of the moss and substrata in each microcosm was estimated as the product of the dry mass and the appropriate O.

Extraction of Divalent Cations from Plant Tissue

Ashed samples of moss and wood were transferred to glazed silica crucibles for digestion. Prior experimentation determined, on average, 1.1% of the ash was lost upon transfer. Ash was brought to a boil in 20.0 ml 6 N HNO_3 and the solution was filtered through ashed, pre-weighed 25 mm glass fiber filters (Whatman GF/F) into a 50 ml graduated centrifuge tube (Likens & Bormann, 1976). The crucible and filter-funnel were rinsed with distilled water and this too was passed through the filter. Volume was brought up to 40 ml and analyzed for DCs (APHA, 1998). The glass fiber filters were dried at 104 ^{0}C until constant mass was achieved. Filter mass was measured to test for complexes resistant to acid digestion (Likens & Bormann, 1976).

Statistical analyses

Calcium and magnesium concentrations were separately converted from mg/L to meq/L and then combined to produce the single variable DC. H^+ meq/L was calculated as the antilog of pH data. These two variables, H+ meq/L and DC meq/L, were analyzed using a multivariate analysis of variance (MANOVA). Analyses were performed on two time-scales using two separate tests: a "long-term" scale spanning the entire three week incubation period, and the diel cycle. Alpha levels were reduced to 0.025 for multiple test procedures (Zar, 1999). The MANOVA examined the effects and interactions of two factors: Factor 1 (Substrate) had three levels (none, cobble, wood) and Factor 2 (Moss) had two levels (present, absent). The Pillai's Trace statistic was used to determine MANOVA F values because this statistic is recommended when data are skewed (Zar, 1999). For post-hoc analyses, two-factorial univariate analysis of variance tests (ANOVA) were employed, followed by the Tukey test when there were more than two levels in a significant treatment effect.

For the long-term analysis, a mean for each variable was determined at weeks 1, 2 and 3 by averaging the values measured following dark incubations and the values measured following light incubations. For example, H^+ values for Week 1 were calculated from the following equation:

$$H^+ \text{ (Week 1)} = (1/2)\,[H^+(\text{dark}) + H^+(\text{light})] \qquad (2)$$

The long-term change in H^+ molarity was calculated for each microcosm as the difference between Week 3 and Week 1. Change in DCs were calculated similarly, but Week 3 included the cations desorbed from chamber surfaces. Microcosms with the

highest and lowest DC values were not included in this analysis, so the statistic analyzed by MANOVA was the trimmed mean. Five other values were missing from this data set due to breakage during handling.

Diel variation was assessed by calculating a mean dark incubation value for each variable, and a mean light incubation value for each variable. The change in each dependent variable was then calculated as the difference between values from light incubations and dark incubations.

Regression analysis was performed for each treatment from both data sets for descriptive purposes, with the change in H^+ concentration as the independent variable and change in DC concentration as the dependent variable.

Data were root transformed as necessary to satisfy the assumptions of normality and homoscedasticity. For both variables the mean was skewed 0.30 standard deviations to the right of the median, but MANOVA is reported to be robust under these circumstances (Zar, 1999). All statistical analyses were performed using SYSTAT statistical software.

Calcite Modeling

The computer program PHREEQ was employed to model calcite deposition in Stream 6. Input values were obtained from data collected every two weeks over a period of two years (Brady, 1993).

RESULTS

Tissue extract

The concentration of DCs in moss was over threefold that in wood (Figure 1). Mosses also had a very high fraction of ash that was insoluble in boiling 6N HNO_3, and a low organic fraction (Table 1). Fractions of insoluble complexes in poikiliohydric mosses and algae have been reported elsewhere (Kroken et al. 1996; quoted in Stream Bryophyte Group, 1999). The insoluble fraction of wood ash was less than 1% of its dry mass, similar to the values reported by Likens & Bormann (1976) for a wide variety of plant tissues.

Divalent cation concentrations in non-colonized wood tissue were slightly higher than concentrations in moss-colonized wood (Figure 1). However, this was not a consistent pattern. Of the three paired samples analyzed, two of the bare-wood samples had higher divalent cation concentrations than their moss colonized siblings. In the remaining pair, this trend was reversed, with approximately the same magnitude of difference.

Three Week Incubation Dynamics

There was a significant change in both H^+ and DC concentrations over the three week incubation period (Table 2). Post-hoc analyses (Table 3) revealed the change in

DC concentrations was attributable to the presence of moss, while changes in H^+ concentrations were attributable to wood substrata (Figure 2). DC concentrations increased significantly in the presence of moss but did not change significantly in the absence of moss. H^+ concentrations decreased slightly in all microcosms, but the only significant decrease occurred in the wood substrate treatment (Figure3). Controls had an average 10% decrease in H^+ meq/L and an average 1.9% decrease in DC meq/L.

There was a marginally significant interaction of H^+ dynamics between the moss and substrate treatments ($P = 0.029$). The change in H^+ meq/L in the wood substrate treatment was smaller in the presence of moss. In cobble substrate treatments moss had no effect while in no substrate treatments moss was associated with augmented H^+ dynamics.

Regression analyses of H^+ dynamics *vs.* DC dynamics for the three week incubation period are summarized in Table 4. Controls had a positive slope with a weak coefficient of determination. All slope values were positive in the absence of moss. In the presence of moss, slope values were either very close to zero or negative. Only the wood substrate treatment with moss present had a reasonable coefficient of determination ($R^2 = 0.87$) and a reasonably large negative slope (-2.23).

The concentrations of H^+ and DCs over the three week incubation period are presented graphically in Figure 3. Note that these values do not include cations desorbed from container surfaces. For the no-substrate and cobble-substrate treatments, both H^+ and DC concentrations were higher in the presence of moss, with DC concentrations increased by 30% (non-transformed data). In the wood substrate treatment however, H^+

concentrations were lower in the presence of moss while DC concentrations were 49% higher on average.

Dissolved Oxygen

All but one of the mason jars containing moss reached 100% O_2 saturation following 12 hours incubation under incandescent light, while only one achieved 100% saturation in the absence of moss. On average, water in the presence of moss was at 71% saturation following 12 hours incubation in the dark, rising to 87% saturation following 12 hours incubation under incandescent light. Water in which moss was absent reached a mean low of 80% saturation and rose to a mean high of 86% saturation. Thus, the change in dissolved oxygen concentration was greatest in the presence of moss (Table 5). Two of the mason jars containing a wood substratum (one with moss present, the other with moss absent) actually lost dissolved oxygen during the incubation under incandescent light, indicating respiratory activity from decomposers outweighed photosynthetic activity. However, I observed gas production (bubbles) in the wood substratum colonized by moss, which had a very small change in dissolved oxygen concentration following the light incubation. Also, over 3 cm of new growth was observed in some mosses.

Diel time scale

The bivariate analysis of diel dynamics is summarized in Table 6. The substrate treatment had a significant effect on diel DC dynamics. The no-substrate and cobble-substrate treatments behaved similarly, with a greater magnitude of change than the wood-substrate treatment (Figure 3). The moss treatment also had a significant effect on diel DC dynamics. Diel changes in DC concentrations were consistently augmented in the presence of moss. There was no interaction for diel DC dynamics between the moss and substrate treatments.

Neither treatment had a significant effect on diel H^+ dynamics, however there was a significant interaction between the moss and substrate treatments. Inspection of Figure 4 reveals this resulted from contrasting effects of moss within the no substrate treatment and the wood substrate treatment. Within the no substrate treatment, there was significantly greater diel variability in H^+ concentrations in the presence of moss, while changes in H^+ concentrations within the wood substrate treatment were smaller in the presence of moss. The mean diel change in H^+ meq/L is close to zero for controls. Variability is high, with a coefficient of variation of 590% (Figure 4).

Regression analyses of diel changes in H^+ and DC^{++} meq/L revealed no treatment with slopes approaching negative two. The no substrate treatment groups had very low correlation coefficients and slopes close to 0.1, some negative and some positive. Wood and cobble substrate treatments had slopes close to positive one. Correlation coefficients were poor (data not shown).

Concentrations of H^+ and DC's within the diel cycle were similar to long-term concentrations. DC concentrations were consistently augmented in the presence of moss. H^+ concentrations were augmented within the wood substrate treatment, except where moss was present.

DISCUSSION

By far the most striking effect of *T. delicatulum* and *B. rivulare* on microcosm waters was the large observed increase in DC concentrations (Figure 3). The effect of moss on DC concentrations was consistent, whereas that of wood was inconsistent, probably influenced by the age and specie of wood, the amount of time spent in the stream, and the manner in which the tissue died (senescence or breakage). I found no evidence that the DCs in moss tissue were extracted from the growth substrate. DC concentrations in regions of wood colonized by moss were the same as in regions from the same piece of wood where there was no moss colonization. In addition, DC concentrations in wood substrata were similar to those reported elsewhere for a wide variety of plant tissues (Likens & Bormann, 1976), while DC concentrations in moss were threefold to fourfold higher (Figure 1).

Controls exhibited H^+ and DC fluctuations in both long-term and diel analyses. Changes in these variables were either positively correlated or not correlated at all, and are likely due to both physical and biological processes. The physical processes which likely affected solute dynamics are changes in water volume and temperature. H^+ concentrations decreased in all microcosms, including controls, over the entire incubation period (Figure 3). This was probably due, in part, to a 36% decrease in water volume over the incubation period. Increased surface-area-to-volume ratios in microcosms are reported to lead to increased ion adsorption to container surfaces (Stream Solute Workshop, 1990). Increases in temperature, on the other hand, usually lead to decreased adsorption to container surfaces. Because microcosms were always warmer following

incubations under incandescent light than they were following incubations in darkness, sometimes by as much as 6^0C, diel H^+ dynamics in control microcosms was counteracted and error was high (Figure 4). These physical effects were probably exacerbated by the presence of moss, due to the large surface area inherent in moss architecture. Thus, the dynamics attributed to moss in this experiment were probably greater than would occur in nature, where changes in volume and temperature are neither so drastic or predictable. The design of this experiment could have been improved by the introduction of artificial moss in control treatments (Suren, 1998).

Controls were also host to a microbial community. Water from two of the control microcosms were diluted and subjected to the test for photosynthetic activity. In both cases, slight increases in dissolved oxygen were observed following the incubation under incandescent light. I am reasonably certain, therefore, that a planktonic community of phototrophic microorganisms was active in controls, and probably an attached community as well. We did not sterilize microcosms and, in any case, microorganisms would have been introduced to controls by the syringe used for sampling. These microorganisms almost certainly contributed to diel H^+ dynamics. Here again, an artificial moss would have been instructive. Suren (1998) found evidence of grazing upon moss epiphytes by aquatic invertebrates. It seems likely that this is a common occurrence in nature. Since aquatic invertebrates were not observed in our microcosms, some of the photosynthetic activity attributed to mosses was probably performed by epiphytic microorganisms. Thus, photosynthetic activity of mosses per unit mass was probably overestimated. However, the density of moss in microcosms was a small fraction of the

density found in Stream 6 in the Allegany State Park, so that photosynthetic and respiratory activity of mosses per unit area were probably underestimated.

It is tempting to suspect that sediments adsorbed to moss plants contributed to elevated DC concentrations, especially considering the high inorganic fraction of moss AFDM (Table 5). However, I feel the parent material of inorganic sediments (i.e. cobbles) was well represented in this experiment and led to no change in DC concentrations (Figure 3). Plants may, indeed, have been contaminated by sediments, but there is no evidence that these sediments contributed to elevated DC concentrations. Kroken et al. (1996) reported bryophyte cell-wall components that were insoluble in acid digestion, but these were not quantified. It is possible that these compounds contributed to the inorganic fraction of moss AFDM.

The discussion above begs the question, where then, did the high levels of DCs found in moss tissue come from? Ruling out extraction from the growth substrate and sediment contamination, we are left with two alternatives, neither of which are mutually exclusive: cation exchange and deposition of calcite.

Diel variation of DC concentrations in microcosms was consistent with calcite deposition. As H^+ mM decreased and temperature increased (data not shown), the probability of calcite deposition would have increased (Jacobson & Langmuir, 1974) and, indeed, a slight decrease of DCs in solution was observed. Computer programs which model the solubility of calcite and $CaHCO_3^+$ under given conditions of pH, alkalinity, Ca^{++} concentrations and temperature are available. I did not measure alkalinity so we cannot state with any degree of certainty whether deposition contributed to diel variation in microcosm DC concentrations. However, I was able to model calcite solubility based

on Brady's two years of field data for Stream 6 (1993). Using all combinations of minimum, maximum and average recorded values for the pH, alkalinity and Ca^{++} molarity, at 8 ^{0}C, the program PHREEQ modeled calcite and $CaHCO_3^+$ concentrations below saturation. CO_2 was consistently super-saturated. Thus, there is no evidence that calcite deposition occurred in the field during the two years (1987 - 1989) of Brady's study (1993). Never the less, calcite deposition cannot be discounted as a possible source of DCs in moss tissue. For one thing, a decade elapsed between the end of Brady's study and the time at which we collected mosses from Stream 6. Some of the variable parameters may have changed in the interim. Secondly, the model does not account for laminar boundary layer effects on ion solubility. These effects are poorly understood (Hart et al. 1999) but may enhance calcite deposition around mosses due to localized uptake of CO_2. Therefore, I still consider calcite deposition as a possible source of DCs in moss tissue.

Long-term H^+ and DC dynamics in microcosms with moss present on wood substrata were consistent with the changes I expected via the cation exchange mechanism. I hypothesized that throughout most of a given year, mosses would exchange internally generated H^+ for DCs in solution. At the time of leaf fall, H^+ concentrations in solution would rapidly increase, due to microbial respiration, when most exchange sites would be occupied by DCs. According to the principle of mass action, I predicted cation exchange would proceed in the opposite direction, once some unknown threshold of H^+ concentration was exceeded. This would result in a lower net increase of H^+ meq/L accompanied by an increase in DC meq/L. This is, in fact, what is observed in the wood substrate treatments with moss present. Changes in H^+ and DC

concentrations were negatively correlated with a reasonable coefficient of determination (Table 2). The test for photosynthesis indicated respiratory activity in wood substrate treatments was higher than in all other treatments. H^+ concentrations were also significantly higher in the wood substrate treatment (Figure 2). However, when moss was present among wood substrata, H^+ concentrations were lowered while DC concentrations were augmented (Figure 3).

The changes observed in the wood substrate treatments with moss present are expected to favor fungal decomposition of leaf litter. The fungal enzyme pectin lyase is thought to govern initial leaf decomposition activities (Suberkropp & Klug, 1981; Chamier & Dixon, 1983), and has been shown to be stimulated by calcium at concentrations up to 1.0 mM (Ayers et al. 1966; Chamier & Dixon, 1983; but see Suberkropp, 1998). Functional conformation is achieved at pH 6.5, with a pH optimum of approximately 8.5 (Rexova-Benkova & Markovic, 1976; Chamier & Dixon, 1983). It is probably for this reason that leaf litter decomposition rates have been shown to proceed at faster rates when at or above the pH 6.5 threshold in laboratory chemostats (Kok & Van der Velde, 1991) and in streams (Mulholland et al. 1987; Griffith et al. 1995; Jenkins & Suberkropp, 1995). Fungal xylanase and cellulase enzymes are also influenced by pH, but have much lower pH optima (Rexova-Benkova & Markovic, 1976; Griffith et al. 1995; Jenkins & Suberkropp, 1995). Given that most low order woodland streams contain large quantities of decomposing wood, the presence of moss may increase leaf litter decomposition rates while simultaneously decreasing wood decomposition rates. This would be advantageous for mosses. Because wood acts as a substrate, it is in the interest of mosses to preserve it. Leaf litter, however, is best removed quickly because it

obscures light and its decomposition is a source of CO_2. This relationship may also benefit aquatic hyphomycete fungi, which typically invest a large portion of the energy derived from leaf litter in sporulation, but apparently invest a larger portion of their energy budget in vegetative hyphae when growing on substrata that decompose slowly (Maharning & Barlocher, 1996).

In summary, mixed colonies of *T. delicatulum* and *B. rivulare* significantly affected the pH and concentration of DCs in microcosm spring water, in a manner that is consistent with reversed cation exchange. Whether this effect is significant under natural conditions of flow and temperature remains to be tested.

FUTURE RESEARCH

This experiment has provided evidence that cation exchange in T. delicatulum and B. rivulare is subject to the principle of mass action, and may proceed in either direction depending on environmental conditions and conditions within the plant's cells. I hypothesize that conditions favoring reversed cation exchange will occur in nature soon after leaf fall. The next phase of research should examine this hypothesis.

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LIST OF TABLES AND FIGURES

Table 1: Mass characteristics from composite samples of plant tissues. Values represent means and standard deviations.

Tissue	n	Insoluble ash As % dry mass	Organic Proportion
Moss	3	6.67 +/- 3.17	0.85 +/- 0.025
Bare wood	3	0.48 +/- 0.42	0.98 +/- 0.007
Wood with moss	5	0.36 +/- 0.42	0.99 +/- 0.004

Table 2: Summary of MANOVA for H^+ meq/L and divalent cation meq/L dynamics over the three week incubation period. Data analyzed were the differences in concentrations between Week 3 and Week 1. Cations desorbed from container walls were included in values for Week 3. Five values are missing from the data set due to breakage. Level of significance = 0.25. Asterisk denotes significance.

Source of Variation	**Numerator *df***	**Denominator *df***	**Pillai-Trace *statistic***	**F**	**P**
Substrate	4	62	0.382	3.659	0.01*
Moss	2	30	0.629	25.389	<0.0005*
Substrate x moss	4	62	0.315	2.899	0.029

Table 3: Summary of *post-hoc*, univariate analyses of variance for H^+ and DC^{++} dynamics over the three week incubation period. Data analyzed were the differences in concentrations between Week 3 and Week 1. Cations desorbed from container walls were included in values for Week 3. Five values are missing from the data set due to breakage. Asterisk denotes significance.

Source of Variation	Variable	F	P
Substrate	H^+	7.42	0.002*
	DC^{++}	1.51	0.24
Moss	H^+	0.37	0.55
	DC^{++}	52.32	<0.0005*
Moss x Substrate	H^+	1.58	0.22
	DC^{++}	5.57	0.009*

Table 4: Summary of regression analyses on H^+ and DC^{++} dynamics over the three week incubation period. The change in H^+ meq/L between Week 3 and Week 1 was treated as the independent variable. The change in DC^{++} meq/L between Week 3 and Week 1 were treated as the dependent variable. Week 3 DC^{++} meq/L values include cations desorbed from container surfaces.

Substrate	Moss	n	Slope	R^2
None	Present	7	-0.15	0.002
	Absent	6	2.91	0.49
Cobble	Present	7	0.58	0.04
	Absent	5	1.57	0.16
Wood	Present	5	-2.23	0.87
	Absent	7	0.22	0.03

Table 5: Dissolved oxygen as % satturation in microcosm waters before and after incubation under incandescent light. Two microcosms from each treatment were chosen for this experiment. Numbers represent the actual value recorded for each microcosm.

Substrate	Moss Present		Moss Absent	
	Dark	**Light**	**Dark**	**Light**
None	82/78	100/100	94/92	96/95
Cobble	79/83	100/100	89/83	100/95
Wood	83/21	100/21	80/42	93/35

Table 6: Summary of MANOVA for diel variation. Data analyzed were the differences in meq/L of H^+ and DC^{++} between incubations under incandescent light and incubations in darkness. Level of significance = 0.025. Asterisk denotes significance.

Source of Variation	Numerator df	Denominator df	Pillai-Trace	F	P
Substrate	4	96	0.469	7.35	<0.0005*
Moss	2	47	0.688	51.7	<0.0005*
Substrate x Moss	4	96	0.263	3.64	0.008*

Table 7: Summary of *post-hoc* analyses for diel H^+ and DC^{++} dynamics, eight degrees of freedom. Asterisk denotes significance.

Source of Variation	Variable	F	P
Substrate	H^+	2.72	0.076
	DC^{++}	17.0	<0.0005*
Moss	H^+	1.96	0.168
	DC^{++}	91.8	<0.0005*
Moss x Substrate	H^+	5.66	0.006*
	DC^{++}	3.20	0.050

FIGURE LEGENDS

Figure 1: Results of tissue analysis for Ca^{++} and Mg^{++}. Bar height represents the average mg of calcium plus magnesium in one gram of ash free tissue from three composit samples of moss and from eight individual samples of wood. Error bars represent one standard deviation.

Figure 2: Changes in H^{+} and DC^{++} meq/L in microcosm waters over the three week incubation period. Columns represent the mean change in values from Week 3 and Week 1 for seven microcosms. Cations desorbed from container surfaces were included in values for Week 3. Error bars represent 95% confidence intervals. Five microcosms were not included in this analysis due to breakage.

Figure 3: Concentrations of H^{+} and DC^{++} in microcosm waters over the three week incubation period. Weekly values were the average of one incubation in darkness and one incubation under incandescent light. Points represent the mean of nine microcosms. Error bars represent 95% confidence intervals.

Figure 4: Diel solute dynamics. Columns represent the difference in solute concentrations between the mean of three incubations in darkness and the mean of three incubations under incandescent light for nine microcosms. Error bars represent 95% confidence intervals.

Figure 1

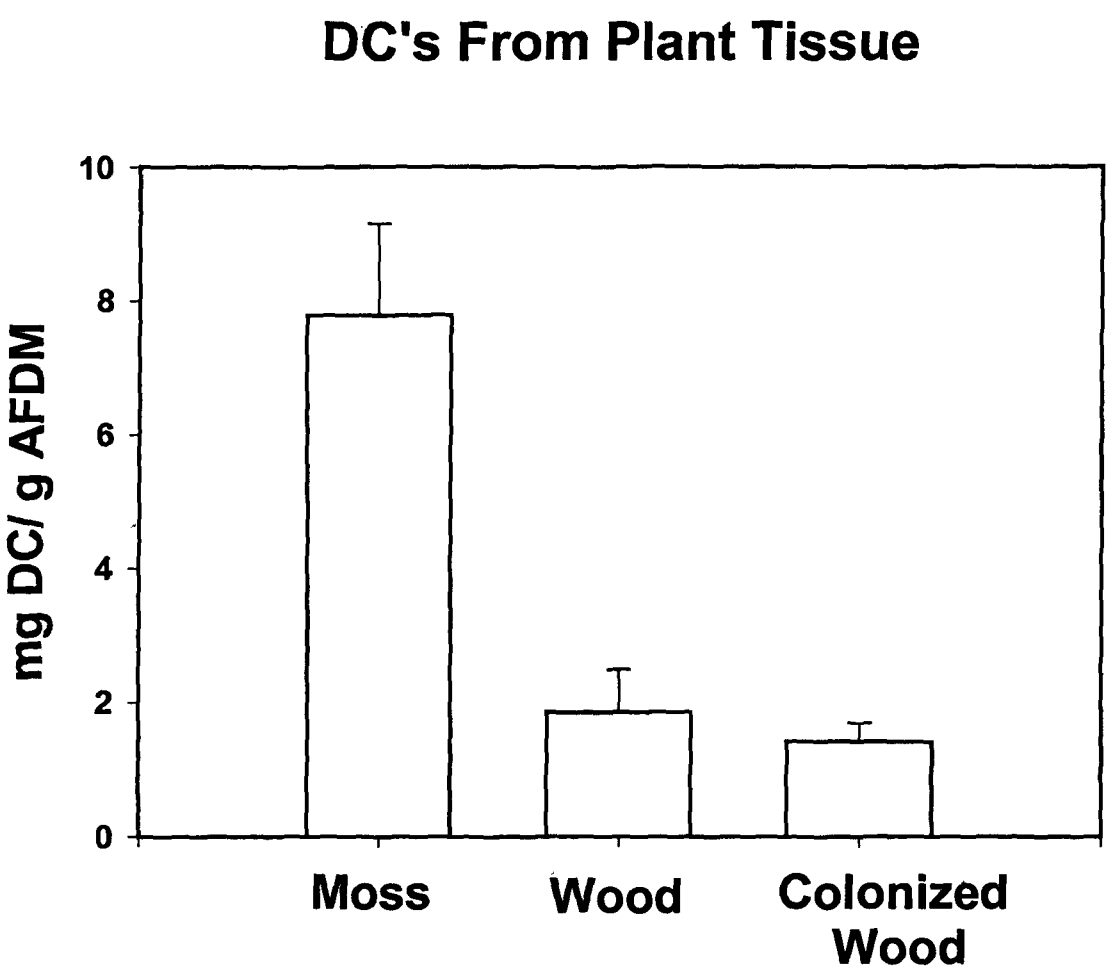

DC's From Plant Tissue
mg DC/ g AFDM
10
8
6
4
2
0
Moss
Wood
Colonized Wood

Figure 2

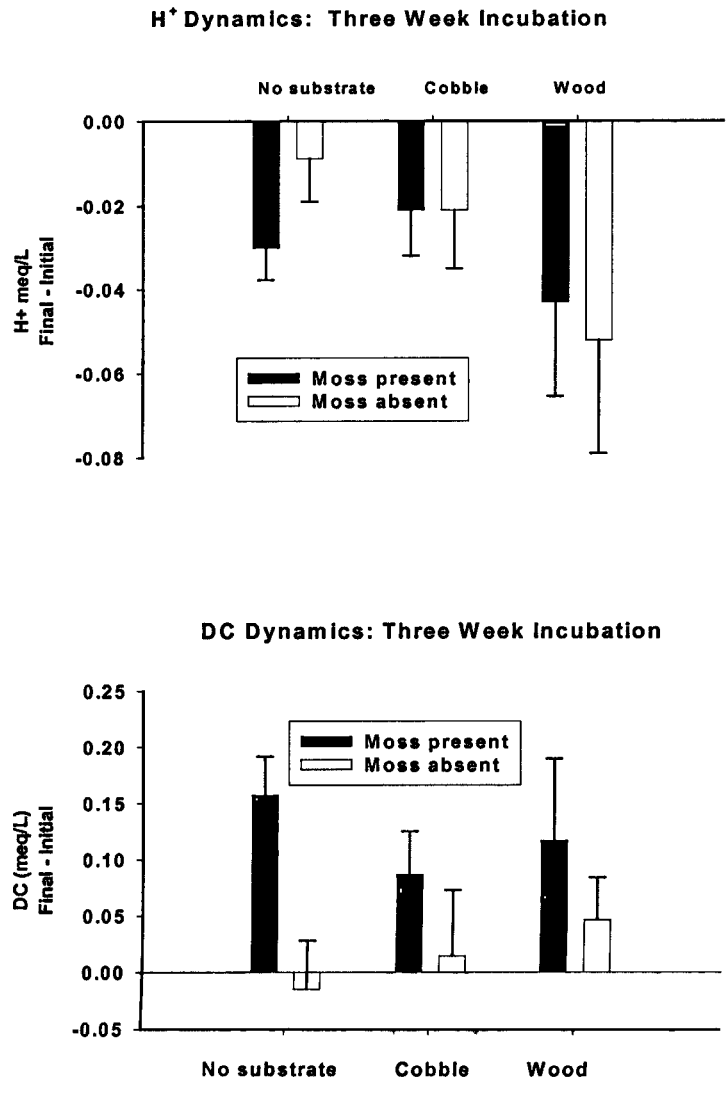

H+ Dynamics: Three Week Incubation
No substrate
Cobble
Wood
0.00
-0.02
-0.04
-0.06
-0.08
H+ meq/L
Final - Initial
Moss present
Moss absent
DC Dynamics: Three Week Incubation
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
DC (meq/L)
Final - Initial
Moss present
Moss absent
No substrate
Cobble
Wood

Figure 3

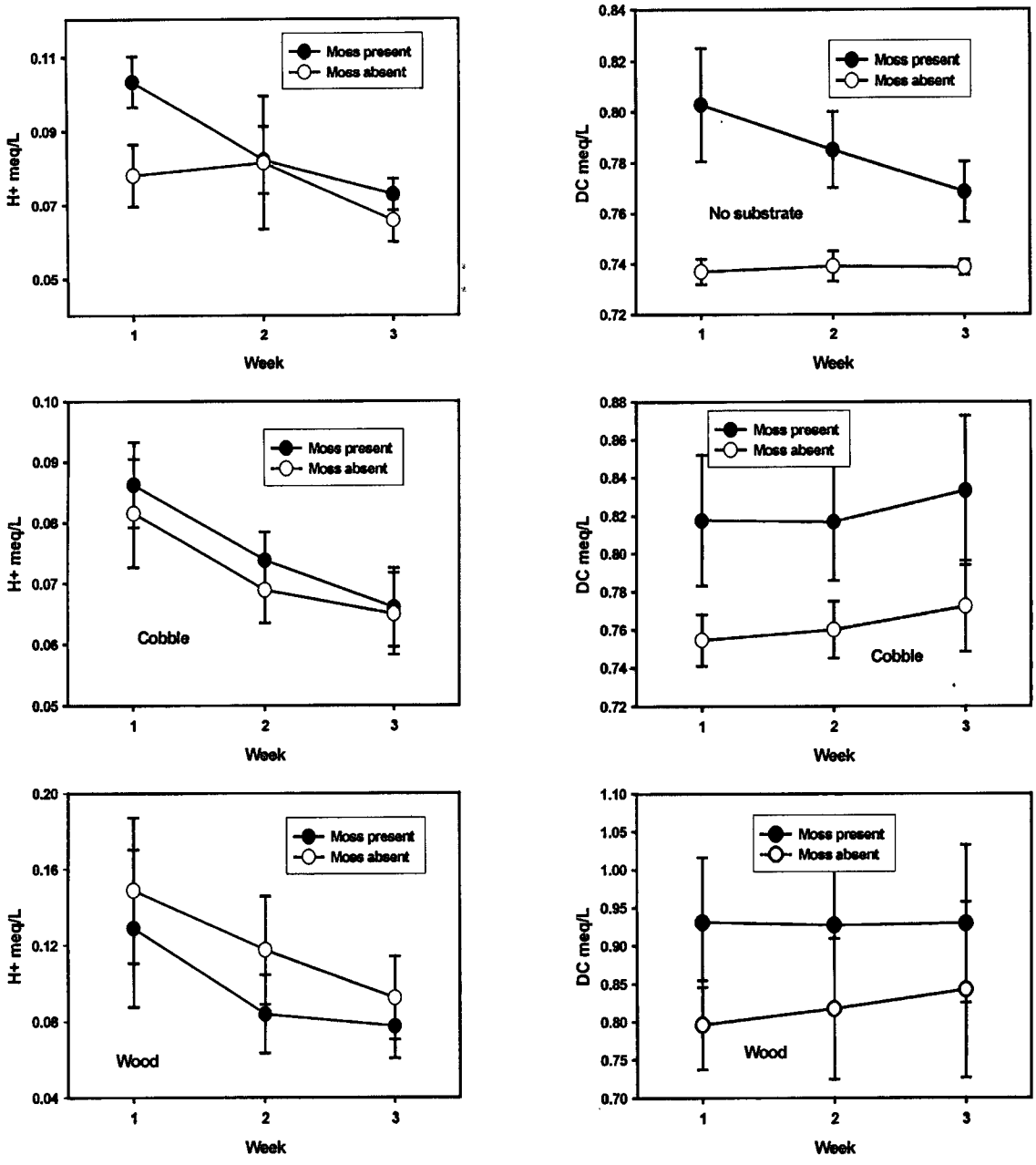
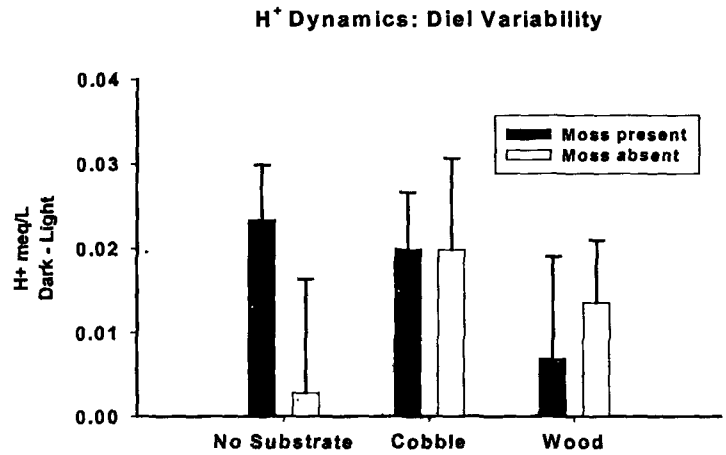
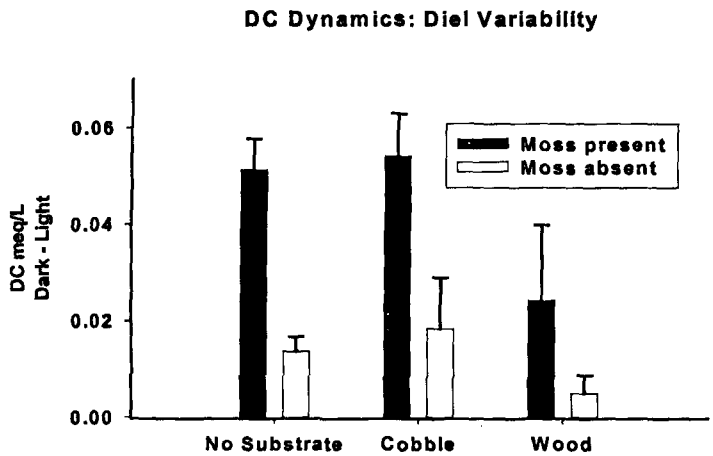

Moss present
Moss absent
H+ meq/L
DC meq/L
Week
No substrate
Cobble
Wood

Figure 4


H+ Dynamics: Diel Variability
H+ meq/L
Dark - Light
0.04
0.03
0.02
0.01
0.00
Moss present
Moss absent
No Substrate
Cobble
Wood


DC Dynamics: Diel Variability
DC meq/L
Dark - Light
0.06
0.04
0.02
0.00
Moss present
Moss absent
No Substrate
Cobble
Wood