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The Effects of Acid, Nitrate and Sulphate Treatments on Soil Microflora and on the Cellulolytic Fungal Community.

Kathy Svatek

A Thesis

in

The Department

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ABSTRACT

The Effects of Acid, Nitrate and Sulphate Treatments on Soil Microflora and on the Cellulolytic Fungal Community.

Kathy Svatek

Solutions of sulfuric and nitric acids, and sodium sulfate and sodium nitrate were applied during a two year period to simulate some of the effects of acid rain. Cellulose decomposition was examined by burying strips of cotton cloth in treated plots, and measuring tensile strength after burial. The community of cellulolytic fungi was monitored by isolating species from the cotton. Bacterial cell numbers and total length of fungal hyphae were also measured. Soil analyses were performed after the final treatment to measure levels of five cations, sulphates, nitrates, and pH in each sub-plot. No treatment effects were found on fungal and bacterial abundance. The fungal species survey, soil chemistry, and cotton decomposition showed significant but inconsistent effects. Seasonal effects on the abundance of bacteria and fungi, degree of cotton decomposition, and isolation of certain fungal species were found. The occurrence of T. koningii correlated most consistently with tensile strength loss of the cotton strips. This species, as well as Penicillium thomii and Chrysosporium pannorum were isolated most frequently.

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Above all, my husband and children have helped directly in many ways, by hauling heavy solutions uphill into a pathless forest wilderness, and by acting as intermediaries between myself and various computers. This study would have been much more difficult without their encouragement.

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INTRODUCTION

A great deal of attention and research has been devoted to the question of forest die-back since the mid-1970's, when it was noticed that trees in Scandinavia and elsewhere in Europe had begun to show serious signs of damage. Concern has since intensified in the northeastern United States and Canada, where high-elevation forests are showing fifty percent losses in some species, and annual tree-ring growth has dropped sharply in the last twenty years (Mohnen, 1988). Many causes have been suggested to account for forest die-back including ozone damage to leaves, excessive fertilization, insect and fungal pathogens, drought, and heavy metal pollution, as well as the effects of acid precipitation, and many studies have been conducted to investigate various aspects of these problems, as reviewed by Hinrichsen (1987). Comparatively little effort has been spent on studying the effects of acid rain on biological processes in the soil, perhaps due to the difficulties inherent in studying such a complex system. However, as forest dieback appears to be related to every aspect of the ecosystem, research emphasis must be increasingly directed to areas which are still little understood, especially the soil ecosystem, in which the critical recycling of nutrients takes place (Dessureault, 1985).

Among the studies which have been undertaken, many

involve laboratory examinations of single processes in artificial or sterilized substrates, in strictly controlled conditions, and with a minimum of organisms, in order to avoid interactive effects between them. In preparing for such experiments, the soil must be disturbed to some degree. For instance, soil cores may be removed and tested under laboratory conditions (Lang and Jagnow, 1986; Stroo and Alexander, 1986; Bitton and Boylan, 1985), horizons may be separated for analysis (Bewley and Parkinson, 1986), and sod may be removed (Bienkowski, 1986). Samples are often sieved and air-dried (Matzner et al., 1986; Weir and Gilliam, 1986), and stored for various lengths of time (Bewley and Stotzky, 1983). Such manipulations undoubtedly disrupt delicate relationships within the soil matrix, which may have been established through adsorption of microorganisms or enzymes on surfaces, or through hydrogen bonding or other linkage mechanisms. Competitive or synergistic interactions between different species may also be affected (Dahm and Strzelczyk, 1987; Dighton et al., 1987; Widden and Hsu, 1986). In cases in which a small selection of organisms or processes is being investigated in isolation (Entry et al., 1987; Bewley and Parkinson, 1984), results may be dramatic, but may not have direct relevance to natural circumstances. The effects of seasonal and climatic conditions, variable substrate and nutrient availabilities, inputs from other systems, such as leaching from leaves or animal incursions, among other

factors, may alter biological responses. For instance, it has been observed that organisms which are unable to degrade cellulose in pure culture may indeed be cellulolytic in mixed culture with other non-cellulolytic organisms, and that decomposition often occurs more rapidly in mixed than in pure cultures, indicating possibly that organisms with an incomplete complement of the cellulase enzyme system may work in cooperation with other such organisms to complete the system (Alexander, 1977). Other studies have shown that toxicity of sulphuric acid to soil microbes may be decreased in the presence of certain clays (Bewley and Stotzky, 1982).

In consideration of such problems associated with laboratory studies, the conclusion was reached that an experimental design in the field could provide the most realistic information about biological effects on the soil from acid precipitation. A study was proposed in which the soil of a sensitive area could be treated in situ with acid solutions, and small soil samples could subsequently be removed for laboratory analysis, preserving as much as possible the normal interactions between all the components of the ecosystem until analyses could take place.

In a field experiment such as this, a true control area is nonexistent, since the site has already been exposed, in this case for many years, to acid rain. Also, the addition of treatment solutions assumes that a quantity of water must be applied to the soil along with the acid. These factors were

considered in the design of the study.

A sugar maple stand was chosen for this project since this species is currently suffering serious losses in Quebec. It is located at the southern edge of the Laurentian Mountains, and is thus exposed to weather systems originating in the eastern United States, where there are dense concentrations of coal-powered electricity generating plants, other heavy industries, and high populations producing pollution from transportation related sources. Surveys of the ions found in wet and dry deposition along the eastern seaboard have shown that sulphate and nitrate are the predominant anions, with chloride and phosphates appearing in lower concentrations. Protons occur in the same general quantity as sulphates and nitrates, but ammonium, calcium, magnesium, and potassium cations are also deposited in small amounts (ERDA, 1980). Relative concentrations of each component may change considerably depending upon the season, weather systems, wet or dry deposition, snow or rain precipitation, fog conditions, and so on (Dasch, 1987; Isaac and Daum, 1987). Some of the deposition takes place in the form of salts of these ions with an associated possibility of increasing acidification (Richter, 1988). In addition to location, elevation has also been found to be a factor in the degree of damage caused by such weather systems, since mountain ranges have been shown to remove acidic pollutants from air masses (Likens et al., 1979), and forests at higher

elevations appear to suffer damage before those situated at lower altitudes (Binns, 1985). Because of its location, and the lack of neutralizing capacity in the podzolic soil of the granitic Canadian Shield, the study site is at high risk of developing serious pollution related problems (Singh et al., 1987). Recent surveys of the area have placed these trees in the "lightly damaged" category, with loss of foliage between eleven to twenty-five per cent (MERQ Survey, 1987). Signs of stress already evident include loss of foliage at the crown, reduced size of leaves, bare branches in the live trees, and many dead trees, roughly twenty per cent of the total number. However, as this damage is not yet considered severe, it was hypothesized that applications of acid to the soil in such a forest could provide useful information by increasing the rate of possible effects from acidification on the soil, thus giving some indication of future responses of such soils to continued acid loading.

The microbial population in the soil consists of a balance between fungi, actinomycetes, bacteria, algae, protozoans, and viruses (Alexander, 1980). Processes in the soil such as mineralization, nitrification, and denitrification are the results of the metabolism of various combinations of these organisms. A shift in the proportion of bacteria and actinomycetes which are acid-sensitive in general, to fungi, which are much more acid-tolerant, could be an indicator of soil acidification (Alexander, 1980; Lohm,

1980, cf. McFee and Cronan, 1982), and could result in a disruption of crucial soil processes. For instance, a disturbance of the nitrogen cycle in which bacteria are responsible for nitrification, denitrification, and nitrogen-fixation could produce deficiencies of this critical nutrient. Although fungi are known to be more tolerant of high hydrogen ion levels than bacteria, they may be adversely affected by the elevated metal concentrations sometimes associated with acid conditions (Entry, et al., 1987) or inhibited by a lack of nutrients produced by cation leaching resulting from proton loading. Fungal biomass has been shown to be highly correlated with cellulase activity in soil samples (Kanazawa and Miyashita, 1987). In addition, fungal cellulases have been measured to be six times the quantity produced by bacteria in soils (Rhee et al., 1987), thus indicating the relative importance of soil fungi in cellulose decomposition. As well as being responsible for nutrient cycling, soil microorganisms are also involved in maintaining soil texture, water retention capabilities, aggregate formation, and humus accumulation (Fenwick and Knapp, 1982; Alexander, 1977). Since a healthy soil is necessary for the existence of the forest community, microbial biomass may serve as an indicator of the extent of acid rain damage sustained by the ecosystem.

The process of cellulose decomposition is crucial for normal nutrient cycling in the biosphere, for the release of energy in the form of carbon, and for making available other

essential nutrients previously immobilized. Organic matter decomposition, along with phosphatase activity and the nitrification process are known to be particularly sensitive to chemical stress (Domsch, 1984). Several techniques for measurement of cellulose decomposition are commonly used in the field. These include indirect methods such as litter bag weight loss measurements (Berg, 1986), or more direct methods such as the recording of carbon dioxide evolution (Trofymow et al, 1983), or measuring the weight loss of filter paper, or lens paper as cellulose substrates. Dyed cellophane, which allows monitoring of weight loss as well as sugar production may also be used (Khan and Frankland, 1984). In order to provide data which would correspond as closely as possible to natural cellulose decomposition, a substrate was sought which would be in the form of untreated crystalline cellulose, needing the full complement of cellulase enzymes to degrade it, and which could be easily measured for decomposition.

The fungal community of a northern hardwood forest such as that at Ste Hippolyte consists of characteristic species in typical frequencies of isolation. The normal shifting amongst species of fungi from season to season reflects changes in temperature, and moisture levels, but soil properties such as pH and certain cation concentrations have also been shown to be significant factors affecting the fungal community (Widden, 1986a and 1986b; Bissett and Parkinson, 1979; Christensen, 1969). This balance could be undermined by a changing soil

environment caused by increased acidification. Studies of aquatic systems reveal a decrease in the number of species found at every level of the ecosystem, with concomitant deleterious effects on decomposition and nutrient cycling associated with increased proton concentrations (Conway and Hendrey, 1982). Such effects may also appear in the soil. The presence or absence of particular fungal species could have a consequence in terms of the cellulolytic capacities of the microflora, although it is possible that more tolerant cellulolytic species may simply become more successful competitors (Alexander, 1980). While bacteria are less important than fungi in cellulose decomposition, it must be remembered that there is evidence of synergism between these two communities in cellulolysis, and that the majority of bacterial species do not tolerate acid conditions well.

Increased loading by hydrogen ions may result in a decreased cation exchange capacity in the soil as well as increased solubility of aluminum, iron and heavy metals. Base saturation may be lowered and nutrient cation availability may be decreased due to increased leaching (Aber et al., 1982; McFee and Cronan, 1982). On the other hand, an input of nitrates can be beneficial due to their fertilizing properties. Amounts which represent a substantial percentage of the total annual nitrogen turnover are currently being deposited in this part of North America in precipitation (McFee and Cronan, 1982).

While a decrease in mineralization as a result of increased acidity could lower the availability of sulphur to plants (Alexander,1980), the deposition of sulphates could tend to offset the lack of availability of this essential nutrient. Although some sulphur-oxidizing bacteria such as Thiobacillus thiooxidans and T. ferrooxidans are able to convert various forms of sulphur to sulphates in extremely acid environments, most bacteria in this group prefer neutral or alkaline conditions (Alexander, 1977). The major proportion of sulphur input from pollution falls in the form of sulphate, which may affect cation leaching and Al^{3+} solubility, depending on the mobility of the sulphate ion, this mobility being increased by mineralization and oxidation processes (Wainwright and Nevell, 1987). Sulfur oxidations also lead to increased acidification through the formation of sulphuric acid, and consequently inputs of reduced forms of inorganic sulphur such as that falling in particulate deposition could indirectly increase the acidity of the soil. Sulphates are often found adsorbed to colloids, and this can be dependent upon pH, associated cations, and the concentration of Al and Fe oxides, which are themselves subject to variation as a result of increased H^+ loading (Tabatabai, 1987). Much of the sulphate in the soil is immobilized in the microfloral biomass, especially by fungi, which require a considerable quantity, and this process may balance the increased input from pollution (Wainwright and Nevell, 1987). In general,

however, the sulphate introduced into the soil from acid rain is not strongly held in the soil, but is leached readily into the soil solution bound to a cation such as Mg^{2+} or Ca^{2+}, a process which may contribute to a depletion of these nutrients (Binns, 1985).

Changes in pH levels may be detected in response to acid treatments, although such results are seldom expected in a short-term field experiment, where the buffering capacity of the organic constituents of the soil is high (McFee and Cronan, 1982). Lowering of soil pH levels has been recorded in Scandinavia where records have been kept for up to fifty years (Falkengren-Grerup et al., 1987; Tamm and Hallbacken, 1986), but it is unlikely that a two-year in situ experiment would result in detectable pH changes. Rather, the measurement of pH is a further means of monitoring the soil environment. In fact, the leaching of cations from the soil as well as anions such as SO_4^- and NO_3^- are better indicators of acidification in a short-term in situ study (De Vries and Breeuwsma, 1987).

There is evidence to indicate that cellulose breakdown by fungi proceeds more slowly as pH levels fall below 5.0 (Alexander, 1977). Less well-known are the effects of leached base cations or heavy metals on the cellulose degrading system. These elements may be found in significant concentrations in the soil solutions of acidified areas. Aluminum, which is present locally in very substantial amounts

in the soil, but bound tightly in clays and mineral compounds, is leached into the soil solution more readily in acid conditions (Havas and Jaworski, 1986; Bache, 1980). This metal has been shown to be associated with detrimental effects on mycorrhizal formation (Entry, et al., 1987), maple development (Thornton et al., 1986; Glatzel and Kazda, 1985), and many agriculturally important species (Havas and Jaworski, 1986). Other cations which may become leached under conditions of increased acidity are trace metals such as lead, zinc, copper, iron, cadmium, nickel, silver, and manganese. As the pH of the soil drops, there is a corresponding increase in the concentrations of metal ions in the leachate (Kabata-Pendias and Wiacek, 1986; Zoettl and Huettl, 1986). The abundance of soil and leaf microflora has been reported to be negatively correlated with these metal concentrations (Arnebrant et al., 1987; Yamamoto et al., 1985; Hadar et al., 1984; Bewley, 1979). Even calcium has been shown to inhibit fungal growth when complexed with montmorillonite clay (Hadar et al., 1984). The toxicity of mercury and copper to fungi has been the basis of many important fungicides, in the form of copper sulphate, mercuric chloride, and phenyl mercuric acetate (Newby and Gadd, 1986).

Fungi possess varying abilities, inherent in each species, to break down cellulose. In some cases, the entire complement of cellulases is present, as in several Trichoderma species. In others, activity is limited to parts of the

cellulase complex. Years of laboratory analyses have produced data concerning the cellulolytic abilities of a great range of common microfungi. However, the ability to degrade cellulose substrates *in vitro* may not reflect the activity of a species in the field, where competitive interractions may be significant. Temperature, water stress, litter and soil type may influence the success of one species over another (Dix and Frankland, 1987; Widden and Hsu, 1986; Widden and Abitbol, 1980). Surveys of northern maple forest soils have revealed the common fungi native to this general area, but the role of each species in cellulose decomposition is less understood (Kuter, 1986; Widden, 1986c). The relative frequencies of fungal isolations from cellulose substrates in the field may differ substantially from their abundance in the soil, regardless of their performance as cellulose degraders in laboratory experiments (Gillespie et al., 1988). By measuring the abundance of individual fungal species found growing on a cellulose substrate in the field a more realistic picture of the most successful competitors as well as those species responsible for cellulose breakdown in the soil should emerge. Measurement of the degree of decomposition of the substrate may also be related to the occurrence of individual species, providing an indication of the cellulolytic abilities of the individual species (Gillespie, et al., 1988; Widden et al., 1986; Widden and Howson, unpublished data).

In consideration of all the factors outlined above, an

experiment was designed to examine some effects of acid input on the soil from several different perspectives. Solutions were prepared to simulate both acid and salt components of the local acid precipitation. Both nitric and sulfuric acids were included as these make up the greatest proportion of the acid input. Since salt effects could be responsible for fertilization as well as acidification, nitrate and sulphate salt solutions were applied to the soil in separate experimental plots. Another set of sub-plots was included which received only distilled water as a treatment to serve as the control, with the understanding that a strict control in which unaffected soil could be examined was unfeasible. The concentration of the acid and salt solutions was three times the natural loading, calculated to be strong enough to elicit a detectable response from the soil without burning the microflora. Hyphal lengths and numbers of bacterial cells were measured at regular intervals throughout the frost-free months in each treated sub-plot to monitor microbial abundance in response to the applied solutions. The cellulose decomposition system was chosen as a critical and sensitive soil process to be studied for its reaction to the simulated acid rain. Cotton strips were selected as the cellulose substrate since they consist of crystalline cellulose and can be easily and quickly tested on a tensiometer for loss of tensile strength, a reflection of decomposition. Isolations of fungal species from the same test strips were performed in order to monitor the

microfungal cellulolytic populations under the different treatment conditions. In addition, a survey of the fungal species was made from soil samples in order to compare the species found in soil from the apparent cellulolytic species found on the cotton cloth. Species of microfungi found on the cotton could also be related to the tensile strength loss of the individual strips, to look for any association between a particular species and cellulose degradation.

Analyses of the soil were conducted at the conclusion of the second summer's simulated acid rain treatments. Measurements were made of concentrations of four basic cations and Al^{3+} in each of the treatment sub-plots, as well as nitrates, sulphates, and pH levels.

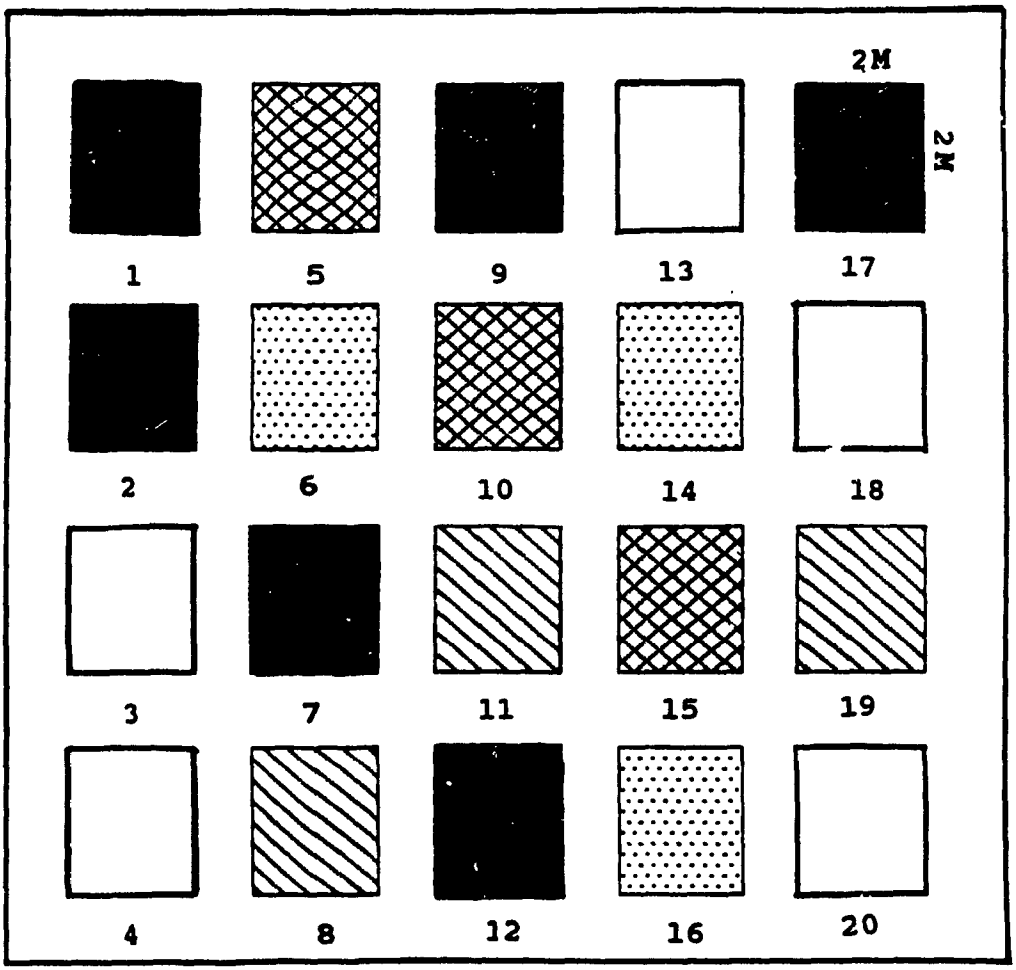
METHODS AND MATERIALS

The study area was a sugar maple (Acer saccharum Marsh) stand approximately eighty years old located in the Biological Research Station of the University of Montreal at Ste Hippolyte in the Laurentian Mountains. The mature trees were sugar maples, but there was an understory of Fagus grandifolia Ehrh. saplings, and an occasional Tsuga canadensis (L.) Carr. Spring ephemerals, such as Erythronium americanum Ker-Gawl and later-flowering species such as Smilacina racemosa (L.) Desf. covered the forest floor (Peterson and McKenny, 1968). The soil was a sandy ferro-humic podzol with an average pH of approximately 4.3. An LFH layer approximately eight centimeters deep had developed above a thin Ah layer of approximately two centimeters. Below this was a Bf_1 horizon also about eight centimeters deep, which was chosen to be the experimental horizon due to the abundance of fine root growth. The lower horizons, beyond ten centimeters in depth, were not sampled for this study. They consisted of Bf_2, Bf_3, and BC horizons to a depth of about 70 centimeters, followed by a mineral C horizon. The area sloped downward slightly,and was a little uneven topographically. Mature trees were spaced approximately fifteen feet apart or in irregular clumps of two or three.

The study area was a 13 X 16 meter plot, sub-divided into twenty 2m x 2m squares. Each square was separated from the

others by a one metre wide buffer zone, which allowed easy access for treatments while avoiding disturbance to the soils. A one metre border around the entire area was also marked out (Figure 1).

Figure 1. Design of the Experimental Field Plot at Ste Hippolyte, Quebec



ACID AND SALT TREATMENTS

Five treatment solutions were applied individually to the soil at two-week intervals beginning in June, 1986, and continuing until late October, then resuming at the beginning of May, 1987, and ending at the end of August of the same year. Fifteen sub-plots were randomly chosen from the total number of twenty sub-plots, with sub-plots 1 to 4 at the lowest side of the very slight incline. In the case of plot three, in which a large rock was located, and plot thirteen, in which a mature tree occupied most of the area, the adjacent higher numbered sub-plots were used. Solutions were prepared in washed plastic "jerry cans" equipped with a nozzle from a watering can to spread the spray out and applied in such a way as to cover the surface soil of each sub-plot as evenly as possible.

Four different solutions were applied, as well as a control of distilled water. Each application was replicated three times, making a total of fifteen treated sub-plots every week. Each application consisted of ten litres of solution or distilled water, which represented about one-tenth of the average local rainfall. Concentrations were approximately three times the natural proton loading, or its equivalent salt concentration (Table 1).

Table 1

Concentrations and pH Levels of Treatment Solutions

	concentration	pH
H_2SO_4	9.465×10^{-4} M SO_4^{2-}	2.7
	1.893×10^{-3} M H^+	
	(0.517 ml acid/10 liters H_2O)	
HNO_3	1.893×10^{-3} M NO_3^-	2.6
	1.893×10^{-3} M H^+	
	(1.18 ml acid/10 liters H_2O)	
Na_2SO_4	9.465×10^{-4} M SO_4^{2-}	5.7
	(1.344 g/10 liters H_2O)	
$NaNO_3$	1.893×10^{-3} M NO_3^-	5.8
	(1.893 g/10 liters H_2O)	
Distilled Water	10 liters	5.7

NUMBERS OF BACTERIAL CELLS

Before each application of solutions, two soil cores from the Bf_1 horizon were taken from each treatment sub-plot. These were kept in a cooler until being returned to the lab, and were then stored at 5 degrees C. for twenty-four hours. This soil was pooled into one sample per sub-plot.

Dilution plates were then prepared, using peptone-yeast agar.

PYE Agar

5 g.	Difco "Bacto" peptone
1 g.	Difco yeast extract
15 g.	Oxoid agar technical grade #3
1000 ml	distilled water

Ten grams from the pooled soil sample were mixed with 90 mls of sterilized, distilled water and shaken on a wrist-action shaker for twenty minutes. A dilution series was then prepared up to a dilution of 10^{-8}. One ml of each dilution was plated with twenty mls of peptone-yeast agar, allowed to solidify, and incubated for one week. Five replicate plates were made of each dilution from each sub-plot. After incubating, the number of colonies on the plates from the dilution which had produced between thirty and three hundred bacterial colonies were counted. To measure moisture content, approximately ten grams of soil were weighed, oven-dried for twenty-four hours, at 90 degrees C. and reweighed. This data

was used to relate measurements of bacterial cell numbers and hyphal lengths to gram dry weight of soil.

MEASUREMENT OF LENGTHS OF FUNGAL HYPHAE

Three grams of soil from the same pooled soil samples were used to make Jones and Mollison slides for direct mycelial measurement (Parkinson et al., 1965; Jones and Mollison, 1948). The soil was ground with a mortar and pestle in fifty mls of distilled water. Fifty mls of water agar were then added, and the mixture was pipetted onto haemocytometer slides with wells of a known depth. Cover slips were dropped onto each well on the slide, and weighed down until the agar hardened. The solidified squares of agar incorporating the soil suspensions were floated onto standard microscope slides, mounted with a drop of glycerine jelly, and stored for later measurement. Four slides were made from the soil of each treatment sub-plot. The mycelial fragments from ten random fields per slide were drawn using a camera lucida projection and measured with a map-measuring device. The total length of mycelium was then calculated using the known dimensions of the microscope field, the volume of the agar squares, and adjusting for the magnification of the microscope and the dilution factor.

CELLULOSE DECOMPOSITION

Cotton strips were used as the cellulose substrate. These were unbleached, unsized strips cut into 25cm x 10cm pieces from bolts of cotton provided by Dominion Textile of Canada. The cotton cloth had a tensile strength of about 40 kg, was about 92 per cent holocellulose with a minimal starch content, and had a plain weave of 60/60 threads (Cunningham, unpublished report). No other additives were present.

The cotton strips were autoclaved after cutting into large strips, and stored under sterile conditions until used. Two different regimens were followed in the two years of the field study. In 1986, two sets of strips were buried, each set totalling two hundred and twenty-five strips, with fifteen strips buried in each sub-plot. The strips were inserted into the soil by folding approximately two centimeters of cloth over the blade of a rectangular garden spade and treading the blade into the soil until only a short end of the strip remained above the soil surface. The spade was sterilized by rinsing with ethanol after each cloth was planted. The location of each strip was marked with a small plastic rod to avoid losing them under the litter. The first set of strips were buried at the beginning of June, 1986. Five strips were removed from each sub-plot after three weeks, another five strips were removed after six weeks, and the remaining five strips were taken from the soil after nine weeks. The second

set of strips was buried in mid-August and removed as before, after three, six and nine weeks.

Since the cotton strips had decomposed more quickly than expected in 1986, the length of burial time was decreased in the second year. Five strips were buried in each sub-plot at the beginning of each month from May to September 1987, and removed after two weeks.

Five strips were buried in each sub-plot at the conclusion of the study in order to obtain measurements of strips which had undergone the physical stress of insertion and removal without any soil incubation. These field controls were immediately removed, processed and measured for tensile strength loss in the same manner as the test strips.

After removal from the soil, the cotton strips were placed in a cooler for transporting back to the lab where they were stored at five degrees for twenty-four hours maximum. Several threads were then removed from each strip to be used for fungal community analysis. The strips were then washed in cold running tap water to remove soil and debris and sterilized by soaking in 95% ethanol for one minute. The strips were dried for twenty-four hours at 40 degrees C. and stored in a desiccator to prevent further deterioration until tensile testing could be performed. At this point, each strip was cut to 5 x 2.5 cm lengthwise across the warp in the area of the strip which had been incubating in the Bf_1 horizon. They were then frayed 0.5 centimeters from each edge to fit

the dimensions of the tensiometer clamps. Tensile strength was measured using an Instron Tensiometer Model TTBM, Ser. #248, and the Instron Tensiometer Model 1123 at the Pulp and Paper Research Institute of Canada. The strips were allowed to acclimatize to a temperature of 22 degrees C. and humidity of 50% before tearing across the warp. The speed of the clamp movement was 2 centimeters per minute.

FUNGAL COMMUNITY STRUCTURE

Species of Microfungi Isolated from Cotton Strips

Several threads were taken from each strip of cotton as soon as they had been brought to the laboratory. In 1986, the threads from each sub-plot were pooled, but in 1987, threads from each strip were processed separately. The threads were washed ten times in a soil-washing apparatus to remove spores and debris (Bissett and Widden, 1975), dried for twenty-four hours on sterilized filter papers in autoclaved glass petri dishes, and cut aseptically into segments approximately one millimeter long. In 1986, these were plated, four to a plate, onto twenty-five acidified Czapek-Dox agar plates per sub-plot (pH 4.5), giving a total of 100 segments per sample.

Czapek-Dox Agar

2.0 g.	Sodium nitrate
0.5 g.	Potassium chloride
0.5 g.	Magnesium glycerophosphate
0.01 g.	Ferrous sulphate
0.35 g.	Potassium sulphate
36.0 g.	Sucrose
12.0 g.	Oxoid agar #3

In 1987, fifty-two thread segments from each strip were plated, four to a plate, on a total of thirteen plates per

strip. The mycelium incorporated in the threads was allowed to grow until an identifiable colony developed at which time each species was recorded. If immediate identification was not possible, the plates were stored in a five degree incubator until they could be identified.

Species of Microfungi Isolated from the Soil

Before starting the treatment applications in the spring of 1986, two soil samples were taken from each subplot, and pooled. Several grams of this soil were washed in the soil-washing apparatus, dried on sterilized filter paper in sterile petri dishes for twenty-four hours, and plated on acidified Czapek-Dox plates, with four soil particles per plate.

Twenty-five plates with one hundred fungal cultures in total from each sub-plot were incubated until the growth was sufficient for the fungal species to be identified.

SOIL ANALYSES

Measurement of Cation Concentrations

Cation concentrations were measured by the method of Hendershot and Duquette (1986). Two soil samples were taken from each sub-plot at the conclusion of the second year of treatments in the fall of 1987. These were sieved through a #10 two-millimeter mesh to remove small rocks and coarse organic material. Two three-gram sub-samples from each sample were weighed out (to 3.000±.001g) and 15 mls of a 0.1 M $BaCl_2$ solution were added to each sub-sample. This mixture was shaken for two hours on a rotary shaker, and then centrifuged for ten minutes at 3500 rpm. The supernatant was then filtered through a Whatman #41 filter and analyzed using a Perkin-Elmer Atomic Absorption Spectrophotometer model #2380. For analysis of Ca^{2+} and Mg^{2+}, one ml. of 2,000 ppm. lanthanum (La^{3+}) was added. The cations measured were Al^{3+}, Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}. From these data, the cation exchange capacity and per cent base saturation of the soil samples could be calculated.

Measurement of Nitrate and Sulphate Concentrations

Two ten-gram (10.000±0.001g) samples were weighed from each of the two soil samples taken from each sub-plot. The samples were dried, sieved, and mixed with 25 mls. of ultrapure distilled water and shaken on a rotary shaker overnight. The soil mixtures were then centrifuged for ten minutes at 3500 rpm and filtered through a #41 Whatman filter.

The filtrate was then passed through a Nuclepore 0.4 micrometer filter, and placed in vials for analysis. Two blanks of ultrapure distilled water were prepared in the same way as the soil sample mixtures. Nitrate and sulphate concentrations were determined in a Millipore Waters model 510 Autoanalyzer.

Measurement of pH

The pH levels of the soil were measured using the same soil samples which were used for the other chemical analyses (Chapman, 1976). A water-to-soil mixture of roughly 2:1 was prepared, stirred, and allowed to settle for about twenty minutes. The pH of the supernatant was then measured with a Fisher Accumet Selective Ion Analyzer model 750.

Statistical Analyses

In order to detect differences in tensile strength, fungal and bacterial biomass, and the frequency of isolation of the most common fungal species as a result of time of sampling, treatment, and sub-plot, a series of nested ANOVAs were run using the SPSS^{x} sub-routine MANOVA (SPSS Inc., 1984) after checking that the assumptions of normality were satisfied. *A posteriori* Tukey tests were performed on those data which showed significant effects of time and/or treatment. The relationship of tensile strength loss of each

cotton strip in September, 1986 and 1987, to the frequency of individual fungal species occurring on each strip was analyzed using the SPSS Pearson correlation analysis. Correlation analysis of the environmental factors of soil moisture and average daily air temperature with tensile strength, fungal length and bacterial numbers data was also performed.

RESULTS

Environmental Monitoring

Figure 3 indicates temperature and ambient precipitation for the months from May to September, 1987, as recorded at the permanant weather station of the Universite de Montreal at Ste Hippolyte, and the means of the fungal and bacterial data, and tensile tests on the cotton strips from each sampling time. Table 2 gives the means of the monthly precipitation data and air temperatures for 1986 and 1987, averaged from readings taken twice daily at the weather station.

Soil Analyses

Results from the soil analyses are presented in Tables 3 and 4. Mg^{2+} and Ca^{2+} showed significantly reduced levels in response to nitric acid treatments and significantly higher levels in the soils treated with sodium nitrate. No significant ($P<0.05$) changes were seen in the other parameters measured.

Table 2

Means of the Monthly Precipitation and Air Temperature

Maxima recorded at the Meteorology Station - Ste Hippolyte

Year	May	June	July	August	September
			Precipitation (mm)		
1986	144.1	114.3	108.6	104.6	154.8
1987	96.7	180.6	69.1	54.1	150.2
			Air Temperature (°C)		
1986	17.2	18.1	21.0	18.5	13.7
1987	15.8	20.5	23.3	20.7	16.1

Table 3
Exchangeable Cations, Cation Exchange Capacity, and Base Saturation

Treatment	Exchangeable cations (cmol(+)/kg)					CEC (cmol(+) kg^{-1})	BS %
	Al	Ca	Mg	Na	K		
H_2SO_4	2.5 ±.37	0.88 ±.17 ad	0.084 ±.02 ac	0.033 ±.01	0.039 ±.01	3.47 ±.59	29.4 ±1.6
HNO_3	1.7 ±.34	0.57 ±.16 bd	0.049 ±.01 bc	0.042 ±.01	0.028 ±.004	2.36 ±.38	28.1 ±3.8
Na_2SO_4	2.0 ±.38	0.85 ±.31 ad	0.066 ±.02 ac	0.052 ±.01	0.029 ±.01	2.97 ±.71	32.7 ±3.5
NaNO3	2.5 ±.34	1.19 ±.25 a	0.102 ±.02 a	0.058 ±.06	0.043 ±.01	3.89 ±.61	35.9 ±3.4
dH_2O	2.2 ±.43	0.77 ±.12 cd	0.063 ±.01 ac	0.028 ±.002	0.033 ±.01	3.13 ±.54	28.7 ±3.5

Error terms represent 95% confidence limits.
Means which are significantly different in Ca^{2+} and Mg^{2+} data have no common suffix letters.
No significant differences were found in the other data.

Table 4

Nitrate, Sulphate, and pH Levels

Treatment	NO_3^- (μeq/L)	SO_4^{2-} (μeq/L)	pH
H_2SO_4	49.95 ±10.5	117.72 ±28.8	4.54 ±.68
HNO3	86.70 ±42.0	68.55 ±21.6	4.50 ±.13
Na_2SO_4	74.92 ±37.0	117.48 ±22.2	4.64 ±.07
$NaNO_3$	82.30 ±9.1	90.63 ±16.1	4.55 ±.10
dH_2O	49.43 ±7.3	57.80 ±11.0	4.45 ±.14

Error terms indicate 95% confidance limits.
No significant differences between means were found in these data.

Cellulose Decomposition

Results from the tensile testing of the cotton strips from 1986 are shown in Table 5. Because tensile strength was negligible after nine weeks, only the data from the three-week and six-week batches are shown.

Results for 1987 are shown in Table 6 and Figure 2. Since decomposition was so rapid in 1986, a fixed two-week period was used in 1987.

No significant differences were found between treatments in 1986. In 1987, there was a significant decrease in decomposition in the sodium sulphate treated plots in August. Differences between the first and second three-week batches, and the first and second six-week batches were significant in 1986. In 1987, the August sampling time was significantly lower than the May and September samplings (Table 6).

Table 5

Tensile Strength of Cotton Strips
1986

(kg at maximum load)

Sampling time	Treatments					Overall Mean
Batch 1	H_2SO_4	HNO_3	$NaNO_3$	Na_2SO_4	dH_2O	
June 27 mean 3 weeks	20.61 ±3.78	22.38 ±4.98	20.94 ±4.77	23.01 ±3.18	21.97 ±4.12	21.78[a] ±1.15
July 18 mean 6 weeks	6.94 ±1.42	7.21 ±1.53	6.04 ±1.54	9.13 ±2.79	7.68 ±2.48	7.40[c] ±1.31
Batch 2						
Aug. 29 mean 3 weeks	10.56 ±3.45	11.25 ±2.27	10.03 ±2.96	12.15 ±3.41	11.65 ±2.96	11.13[b] ±0.97
Sept.19 mean 6 weeks	2.83 ±1.64	3.05 ±1.94	2.05 ±1.61	1.06 ±1.07	1.93 ±2.16	2.18[d] ±0.91
Field Controls	39.56 ±1.59					

Error terms represent 95% confidence limits.
Means with different letter suffixes are significantly different.

Table 6

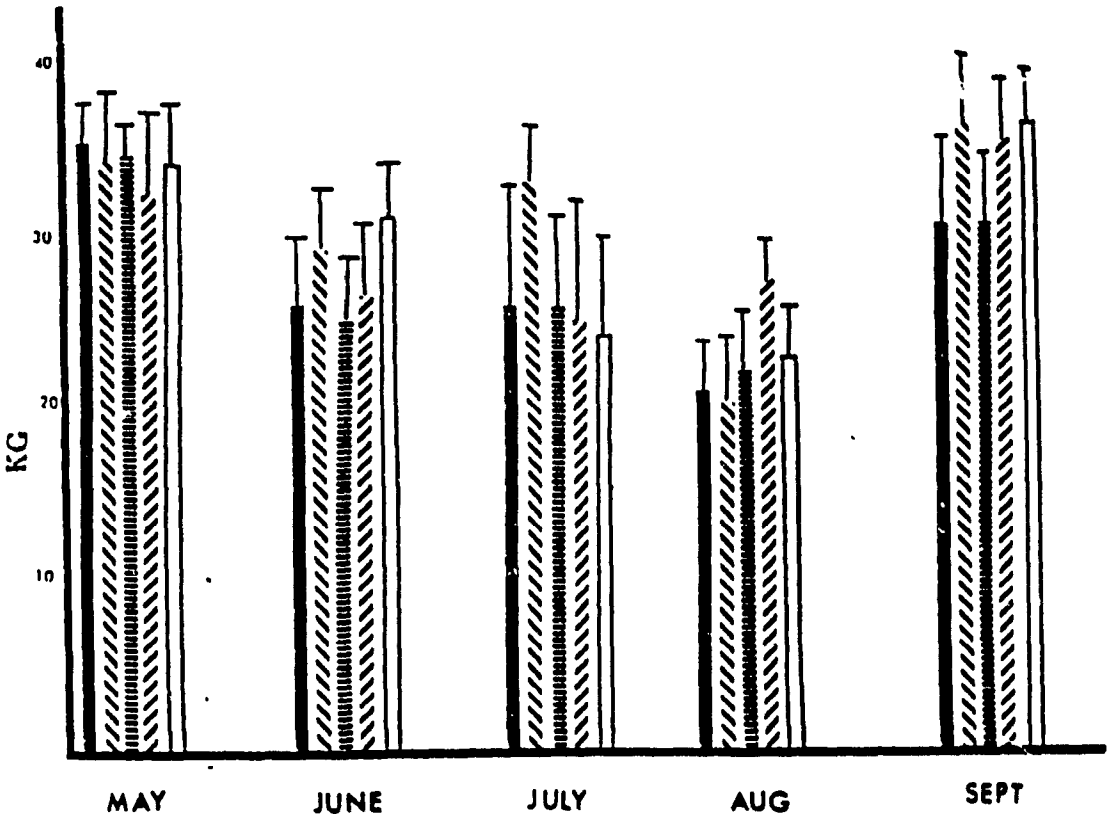
Tensile Strength of Cotton Strips
1987

(kg at maximum load)

Sampling Time	Treatment H_2SO_4	HNO_3	$NaNO_3$	Na_2SO_4	dH_2O	Overall Mean
May						
mean	35.31 ±2.60	35.71 ±3.14	34.54 ±2.10	34.34 ±3.09	35.69 ±1.92	35.12^{a} ±0.74
June						
mean	25.86 ±3.90	29.17 ±3.41	25.70 ±3.50	26.68 ±4.18	30.16 ±3.41	27.51^{ab} ±2.33
July						
mean	29.88 ±4.90	33.17 ±3.36	27.82 ±3.82	26.66 ±6.31	26.15 ±4.40	28.74^{ab} ±3.29
August						
mean	20.91 ±2.87	20.55 ±3.45	23.67 ±2.36	27.49* ±2.25	22.82 ±3.27	23.09^{bc} ±3.20
Sept						
mean	30.81 ±4.80	36.68 ±1.90	32.11 ±2.21	35.87 ±2.33	37.04 ±2.73	34.50^{a} ±3.27
Field Controls	39.58 ±1.59					

Error terms represent 95% confidence limits.
Means which do not share any letter suffixes indicate significant differences.
* indicates significant ($P<0.05$) effect of the sodium sulphate treatment in August.

Figure 2. Tensile strength of cotton strips after two weeks soil incubation, measured in kilograms at maximum load - 1987.



H_2SO_4
HNO_3
$NaNO_3$
Na_2SO_4
H_2O

Total Lengths of Fungal Hyphae

Results of hyphal measurements for 1987 are indicated in Table 7. A posteriori Tukey tests showed a significant decline from the third to the fourth sampling. No treatment effects were significant.

Measurement of Bacterial Cells

Numbers of bacteria were not altered significantly by the treatments, but the initial sampling on May 1 showed significantly higher abundance of bacterial cells than the subsequent samplings, as indicated in Table 8.

Influence of Environmental Factors on Total Hyphal Lengths, Bacterial Numbers, and Tensile Strength

Pearson Correlation Analysis

Significant positive associations were found between soil moisture and total hyphal lengths and bacterial numbers, and between temperature and bacterial numbers, as presented in Table 9.

Table 7

Hyphal Lengths of Soil Fungi
(M/gm dry weight of soil).

Date	Treatments					Overall Mean
	H_2SO_4	HNO_3	$NaNO_3$	Na_2SO4	dH_2O	
May 5 mean	502.9 ±123.2	394.5 ±90.3	341.4 ±155.0	585.6 ±142.9	450.1 ±75.4	454.9^{ab} ±109.0
May 28 mean	519.6 ±162.8	512.4 ±132.7	748.0 ±126.7	534.1 ±137.6	544.9 ±124.9	571.8^{ab} ±114.2
June 9 mean	664.1 ±213.5	475.2 ±137.9	704.6 ±114.2	631.3 ±189.6	677.8 ±137.9	630.6^{a} ±104.4
June 22 mean	360.9 ±106.6	450.2 ±99.1	421.8 ±142.0	387.0 ±122.6	489.6 ±66.9	421.9^{b} ±58.4
July 9 Mean	501.5 ±170.4	449.4 ±163.7	520.1 ±155.3	525.1 ±112.2	560.3 ±132.3	511.3^{ab} ±46.0
July 22 mean	413.9 ±139.2	646.5 ±188.0	439.1 ±136.6	567.8 ±137.2	564.8 ±143.1	526.4^{ab} ±112.0
August 5 mean	573.3 ±120.2	529.6 ±132.2	430.3 ±92.3	649.2 ±121.2	707.5 ±124.9	578.0^{ab} ±123.4
August 19 mean	566.5 ±140.9	470.2 ±183.0	388.3 ±90.0	450.4 ±197.2	548.9 ±117.1	484.9^{ab} ±84.3

Error terms indicate 95% confidence limits.
Means with no common suffix letters are significantly different.

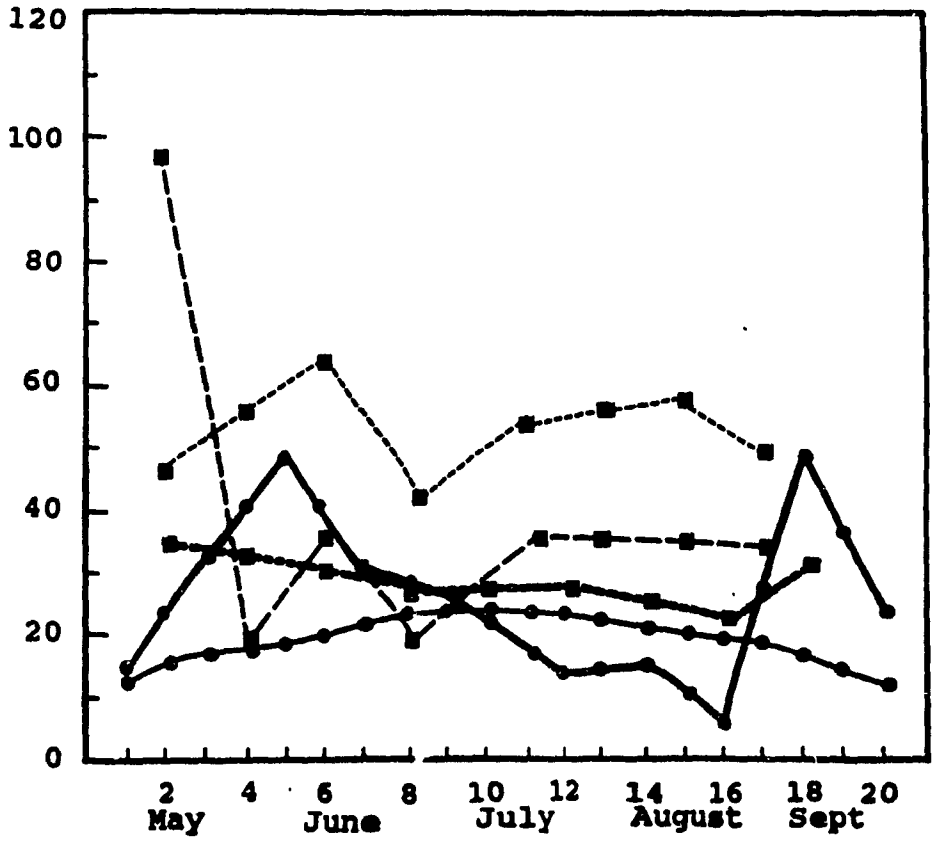
Table 8

Soil Bacteria
(cells x 10^5/gm dry weight of soil)

Date	Treatment					Overall Mean
	H_2SO_4	HNO_3	$NaNO_3$	Na_2SO_4	dH_2O	
May 5 mean	10.58 ±2.09	6.11 ±1.26	13.06 ±3.70	7.18 ±2.83	10.97 ±4.01	9.58[a] ±3.3
May 28 mean	1.84 ±.48	2.11 ±.72	2.20 ±.81	2.05 ±.81	1.39 ±.52	1.92[b] ±.37
June 9 mean	4.45 ±.78	3.59 ±.73	3.90 ±.60	3.42 ±.84	4.19 ±.62	3.91[b] ±.48
June 22 mean	1.69 ±.56	3.06 ±.67	1.64 ±.46	2.40 ±.43	1.94 ±.50	2.14[b] ±.68
July 9 mean	3.25 ±.66	2.02 ±.65	3.28 ±.90	4.13 ±1.14	2.91 ±.53	3.12[b] ±.88
July 22 mean	3.45 ±.69	3.30 ±.45	3.45 ±.66	3.75 ±1.26	4.21 ±.68	3.63[b] ±.41
August 5 mean	4.25 ±.44	3.05 ±.47	3.61 ±.72	3.13 ±.38	2.94 ±.96	3.40[b] ±.62
August 19 mean	4.50 ±.95	3.12 ±.62	2.61 ±.44	3.47 ±.82	2.90 ±.74	3.32[b] ±.84

Error terms represent 95% confidence limits.
Different suffix letters indicate significant differences between means.

Figure 3. Numbers of bacteria, total hyphal lengths, and tensile strength measured biweekly; mean maximum air temperature and precipitation, measured weekly - 1987.



Air Temperatures (°C) ———

Precipitation (mm) ———

Numbers of Bacteria ($\times 10^5$) — — —

Hyphal Lengths (M x 10) - - - - -

Tensile Strength (kg) . . . . . .

Table 9
Relation of Soil Moisture and Air Temperature to Total Hyphal Lengths, Bacterial Numbers and Tensile Strength of Cotton Strips - 1987.
Pearson Correlation Analysis

	Hyphal Lengths	Bacterial Numbers	Tensile Strength
Soil Moisture	P=.000*	P=.000*	P=.056
Air Temperature	P=.062	P=.000*	P=.098

* indicates significance to P<0.01.

All associations were positive except those between air temperature and hyphal lengths, and between temperature and tensile strength.

Treatment Effects on Fungal Species Isolated from Cotton Strips

Only one species, Mucor hiemalis, showed a weakly significant (P<0.05) difference in 1986 between the nitric acid-amended plots in which the abundance was reduced, and the sodium nitrate-treated plots, in which its occurrence increased. No treatment effects were found for this species in 1987. The light-coloured Cylindrocarpon showed significant treatment effects in 1987, increasing its frequency of isolation in the sodium nitrate and nitric acid amended plots, and decreasing in the sulphuric acid and sodium sulphate plots. No treatment effects were noted for this species in 1986 (Table 10).

Seasonal Occurrence of Fungal Species From Cotton Strips

Per cent frequencies of the individual species were found by calculating the ratio of numbers of isolations of a particular taxon to the total number of isolations of all species during each sampling period (Table 11). Several of the fungi did show highly significant effects related to the time of sampling or to the source of the inoculum, either soil sample or cotton threads. Only in the case of Trichoderma hamatum did there appear to be a pattern of decreased isolation frequency as the cotton cloth degradation progressed, but this result was not statistically significant.

The species among the most abundant fungi which occurred with significantly greater frequency in the soil than on the cotton were Paecilomyces carneus, T. hamatum, and Trichocladium opacum.

Fungal species which were found in significantly greater frequency on the cotton strips than in the soil were a sterile black species, Chrysosporium merdarium, Chrysosporium pannorum, a sterile pink isolate, Penicillium thomii, a light-coloured Cylindrocarpon species, P. spinulosum, P. glabrum and P. citrinum. Among those fungi which had significantly different frequencies related to time of sampling, Gliocladium roseum, Oidiodendron echinulatum, P. citrinum, and the white and pink sterile fungi were irregular in their occurrences, and did not follow discernible patterns related to a seasonal

progression. Frequently isolated species which did not show any significant effect of sampling time were Fusarium spp., Paecilomyces farinosus, Trichoderma virens, Mortierella ramanniana var angulispora, and Penicillium janthinellum. Twenty-four fungal species isolated from one per cent or more of the soil samples or cotton threads on at least one sampling date were included in statistical calculations.

Table 10
Fungi Showing Significant Treatment Effects

Year	Species	Significance Level
1986	Mucor hiemalis	.049*
1987	Cylindrocarpon (light sp.)	.016*

* Significant to $P<0.05$.

Table 11
% Frequencies of Most Abundant Species
Isolated from Cotton Strips - 1986

Species	Soil	First Batch			Second Batch		
	Samples	3 wks	6 wks	9 wks	3 wks	6 wks	9 wks
Cylindrocarpon (dark)	2.5	2.0	3.5	4.3	0.8	1.0	0.2
Gliocladium roseum Bain.	1.0	0.7	0.5	0.9	2.8	0.3	1.6
Paecilomyces carneus (Duche and Heim) A.H.S. Brown and G. Sm.	4.0	0.0	0.0	0.3	0.8	0.3	0.3
Oidiodendron echinulatum Barron	0.0	0.5	0.0	0.0	0.4	0.7	1.0
Sterile black	0.5	1.7	2.6	1.2	4.1	8.8	7.1
Trichoderma koningii Oudem.	10.8	14.6	9.1	7.7	8.7	10.9	13.0
T.hamatum (Bonard) Bain.	10.9	7.7	2.7	1.9	5.3	1.5	2.9
Chrysosporium merdarium (Link ex Grev.) Carm.	0.5	0.4	0.0	0.7	3.3	1.4	0.6
C. pannorum (Link) Hughes	0.4	4.9	9.6	11.9	2.9	2.8	2.3
Sterile white	1.8	0.3	0.8	0.5	2.5	2.3	0.5
Sterile pink	0.3	2.0	0.9	5.0	0.3	0.0	0.1
Trichocladium opacum (Corda) Hughes	1.9	0.1	0.1	1.4	1.0	0.5	0.1
Penicillium thomii Maire	2.5	13.4	15.6	22.1	5.0	6.1	7.5

Table 11, cont'd.

Species	Soil Samples	First Batch 3 wks	First Batch 6 wks	First Batch 9 wks	Second Batch 3 wks	Second Batch 6 wks	Second Batch 9 wks
Cylindrocarpon (light)	1.7	6.1	6.3	7.5	1.9	1.3	0.1
P.glabrum	0.5	0.1	2.1	2.1	1.5	2.5	1.5
P.spinulosum Thom.	0.4	0.1	0.1	0.6	0.7	1.1	1.7
P. citrinum Thom.	0.5	1.1	2.2	0.5	1.5	2.7	1.7

T. koningii and P. citrinum show significant differences due to sampling time ($P<0.05$)
All other species show significance to 0.01 probability level.

Relation of Tensile Strength to Frequency of Isolation of Fungal Species - Pearson Correlation Analysis

The two Cylindrocarpon isolates were associated with tensile strength loss in alternate years, and M. hiemalis and P. citrinum showed a significant correlation with decomposition but only in 1987. T. koningii was the only species of microfungus to be associated in both years with loss of tensile strength in the cotton strips (Table 12).

Table 12
Relation of Tensile Strength to Frequency of Isolation of Fungal Species - September 1986 and 1987

Pearson Correlation Analysis

Species	1986 r	sign.	1987 r	sign.
Cylindrocarpon (dark)	o.3843	.000**	-0.0569	.314
Fusarium sp.	-	-	-0.0693	.277
Mucor hiemalis Wehmer	0.0014	.495	-0.3301	.002**
P.farinosus(Holm ex Gray) A.H.S. Brown and G. Sm.	0.0425	.359	-0.0791	.250
M. Ramanniana (Moller) Linnem.	-0.0558	.317	-0.1710	.071
O. echinulatum Barron	0.0140	.452	-0.0231	.422
Sterile black	-0.0055	.481	-0.0837	.238
T. koningii Oudem	-0.2421	.018*	-0.3414	.001**
T. hamatum (Bonard) Bain	-0.0849	.234	-0.0970	.204
C. merdarium (Link ex Grev.) Carm	0.1336	.127	-0.1527	.095
C. pannorum(Link) Hughes	0.0965	.205	-0.0168	.443
P. thomii Maire	-0.0568	.314	-0.2185	.030*
Cylindrocarpon (light)	0.2819	.007**	-0.0752	.261
P. glabrum (Wehmer) Wrestling	-0.0199	.433	-0.0945	.210
P. janthinellum Biuorge	-0.0711	.272	-0.0392	.369
P. spinulosum Thom	-0.0423	.359	-.1157	.161
P. citrinum Thom	-0.1601	.035	-0.2853	.007**
T.virens Miller, Giddens and Foster	0.1462	.105	-0.1768	.065
P. brevicompactum Dierckx	-0.0753	.260	0.0082	.472

* indicates significant correlation between tensile strength and frequency of isolation from a cotton strip (P<0.05)
** indicates P<0.01.

DISCUSSION

The reduction in magnesium and calcium cation concentration found in the soil solutions of the nitric acid treated sub-plots may be indicative of a leaching effect produced by an increased input of protons. Such results have been noted elsewhere (Kelly and Strickland, 1987; Bergkvist, 1986). The hydrated basic cations are normally adsorbed by hydrogen bonding to the surfaces of clay particles. Ca^{2+} and Mg^{2+} are the base cations most strongly held in this manner (Fenwick and Knapp, 1982). Hydrogen ions are able to displace the base cations at exchange sites, allowing micronutrients such as Ca^{2+}, Mg^{2+}, and K^{+} to move into the soil solution and leach away. Calcium ions are lost in the greatest amounts, since this is most often the dominant cation in soils (Bache, 1982), and it was in fact reduced to a highly significant degree in the nitric acid treated soils, suggesting that a large proportion of the adsorbed cations had been leached out of the soil. Mg^{2+} ions also showed significant reductions in the nitric acid treated sub-plots. These results may indicate that the acid treatments are increasing the rate of cation leaching to the point at which replenishment of the cation pool from mineralization processes in the organic horizons cannot keep pace with the depletion, resulting in deficiencies. Such a pattern of topsoil depletion of Ca^{2+} and

Mg^{2+} has been coincidental with complete die-back of spruce in Sweden (Paces, 1986). However, sub-plots treated with sulphuric acid showed no significant reductions in the concentrations of the cations measured, although other studies have reported significant cation reductions using treatments of this acid (Brown, 1987; McClenahen, 1987; Skeffington and Brown, 1986). Calcium, magnesium, sodium, and potassium ions have been shown to be better adsorbed in soils when applied with sulphate, as opposed to nitrate salt anions (Wiklander, 1982), and such an effect may be a factor in the higher concentrations of every cation except Na^{+} in the sulphuric acid treated plots compared to the nitric acid treated plots. However, the soils treated with sodium nitrate had higher concentrations of all the cations in comparison with those treated with sodium sulphate. Applications of weak salt solutions containing the sulphate anion have been shown to increase acidity in acid forest soils (Richter et al., 1988). This effect may produce leaching which could account for the lower level of cations found in the sulphate-amended plots in comparison to those in which nitrate salts were applied.

The addition of nitrate in the nitric acid plots could stimulate plant growth and uptake of nutrients and thus explain a reduced level of cations in these soils. However, the sub-plots which were treated with sodium nitrate had the highest levels of calcium and magnesium, suggesting that a highly stimulated flora consuming micronutrients in these sub-

plots has not developed.

Sodium is even more powerful as a replacement cation than H^+, since protons are readily consumed in other chemical reactions, and for this reason a leaching effect in the salt-amended plots might have been expected. However, the sodium nitrate plots showed significantly higher levels of both calcium and magnesium, while the sodium sulphate plots did not. Since a leaching effect does not appear to be consistent in all treatments, this phenomenon cannot explain the experimental results satisfactorily.

Cation exchange capacity is a measure of the potential of a soil to adsorb cations, and thus a low CEC may indicate loss of adsorption sites due to acid effects on clay and colloid conformations. Acid conditions have been shown to be involved in the dissolution and mobilization of fulvic acids in podzols, as well as the aggregation of humic acids (Schnitzer, 1982). Since these are central components of the organic fraction of podzols, such disruption in their form and concentration could have a deleterious effect on the CEC. No significant differences were found in the mean CEC levels between treatments in this experiment, however. Base saturation was highest in both salt-amended soils, and lowest in the acid treated sub-plots, but differences were very slight and not significant.

The importance of several base cations as micronutrients is well-known. In addition, these ions are required for such

processes as the flocculation of clays in soil (Fenwick and Knapp, 1982). Other cations, such as Al^{3+}, Fe^{3+}, and Mn^{2+} may produce toxic effects as they become more available. Aluminum in particular is directly toxic to roots, and disrupts the uptake of phosphorus (Rorison, 1982). However, in this study, no effects of treatments on Al^{3+} levels were detected.

The moisture level has a direct effect on microorganisms, not only influencing such processes as aerobic respiration and denitrification, but also affecting competitive abilities, for instance, between species of Penicillium (Kroeckel and Stolp, 1986; Hocking and Pitt, 1979). The distilled water treatment was therefore established as a control, so that differences found in the treatment plots could not be attributed to additions of water. A true control was impossible to find, since the study area has already been exposed to acid precipitation for many years. Further control plots having no distilled water or other treatments would have possibly indicated the effects that the water amendments may have been producing. However, since the added water represented only about one-tenth of the average monthly rainfall, this was not considered necessary. No significant effects on the soil chemistry were found in the water treatments in comparison with the others although the concentrations of all the cations in these sub-plots tended to be low.

The level of sulphates was higher in the plots which had

been treated with sulphuric acid and sodium sulphate, as opposed to the plots in which no sulphate anion had been added, indicating that at least some of the added anion had become adsorbed in the soil matrix, or bound in organic sulphur forms (David and Mitchell, 1987). However, the results were not statistically significant due to the high degree of variability between sub-plots within each treatment, and between soil samples within each sub-plot. Sulphates are not strongly adsorbed in soil (Foster et al., 1986) or readily taken up by plants, and this may lead to high concentrations at root surfaces. Such a situation may result in increased local acidification if the roots are releasing protons, but deleterious effects from such a scenario have not been reported (Foster et al., 1986; Rorison, 1982).

The concentration of nitrates, though elevated, was not significantly higher in the nitric acid and sodium nitrate treated soils than in the sulphuric acid and distilled water sub-plots, again probably due to high variability between samples. Nitrate levels were highest in the nitric acid plots, in which the higher acidity may have inhibited the microflora involved in immobilization of this nutrient anion. Distilled water treatments showed the lowest anion concentrations in each case, but the means were not significantly different from the treatment means.

pH levels appeared to be entirely unaffected by the two years of treatments, since no pH differences were found among

the treatments. Such a result is entirely predictable in such a field experiment in which there is a buffering effect from the LFH horizons, and in which the soil is already acidified (Chang and Alexander, 1984). Long-term records in Scandinavia have demonstrated that pH levels can decrease significantly over a period of several decades (Falkengren-Grerup, 1987; Tamm and Hallbacken, 1986), but unfortunately no such data are available for this study site.

Variances in the data from soil samples within the same sub-plot were sometimes significant ($P<0.05$) and almost always considerable. Variability in the soil chemistry between samples taken from the same sub-plot reflected the great heterogeneity among microsites, and prevented a clear analysis of the treated soils from emerging. Differences between texture, colour, and organic content of soil samples was often pronounced, although taken from areas in close proximity to each other. Although obviously unusual samples were avoided, such as sites of recently rotted logs, this heterogeneity was still reflected in most soil chemistry measurements.

Measurements of bacterial numbers and fungal hyphae did not appear to be affected by the acid or salt treatments, although it is known that many soil bacteria are sensitive to increases in acidity. Negative effects on numbers of bacteria and actinomycetes (Bewley and Parkinson, 1986 and 1984; Mancinelli, 1986) and on processes such as nitrogen fixation (Alexander, 1977; Hallgren and Huss, 1975), nitrification

(McColl and Firestone, 1987; Mancinelli, 1986; Lettl, 1985; Wang et al, 1980), denitrification (Christensen, 1985; Alexander, 1980), sulphate reduction (Alexander, 1977), and lignin and cellulose degradation (Pometto and Crawford, 1986) are well known. However, no decrease in the numbers of bacterial cells in the acidified treatment plots was found. Since the research site has been receiving acid rain for many years, it is possible that an acid-tolerant bacterial population may have developed, or that some organisms may have become acclimatized to the increased acidity (Alexander, 1980). A shift in populations to spore-formers has been reported (Bååth et al., 1980) or to acidophilic strains of bacteria and actinomycetes (Hagedorn, 1976), with no overall decrease in cell numbers. Conversely, no detectable stimulation of the populations of bacteria or fungi was produced by the salt treatments. The very high bacterial numbers recorded in the first spring sampling reflect the high moisture levels and nutrient flush of the time directly following snowmelt.

Soil moisture and air temperature were associated with the abundance of hyphae and bacteria. A highly significant positive correlation was demonstrated between soil moisture and total hyphal lengths, and soil moisture and bacterial numbers as expected for this crucial variable (Alexander, 1977). Air temperature was also highly positively correlated with increased bacterial counts, although the fungi did not

seem to be as affected by this variable. Bacteria appeared to respond to the higher temperatures in the summer months, in spite of the decreased precipitation at this time.

The medium used to prepare the dilution plates was peptone yeast extract agar. The choice of a single medium and standard growth conditions, aerobic at room temperature, necessarily will select for bacterial species which are at least facultative aerobes, and which will grow with the nutrients provided and at this temperature. Anaerobic species, species with more extreme temperature optima, or those which may be inhibited by the nutrients provided or which require specific nutrients which are not available in PYE agar will not be represented in these dilution plates. As well, no indication of bacterial groups, such as nitrifiers, is possible with this method. Therefore, population shifts in response to the treatments would not have been detectable.

Measurement of fungal hyphae using the modified Jones and Mollison method provides a rough estimate of the total mycelium in the soil, but does not indicate the state of viability of the hyphae encountered. In order to measure only hyphae which are metabolically active, a fluorescent stain such as fluorescein diacetate or similar vital dye must be used (Cooke and Rayner, 1984). The Jones and Mollison technique, which involves incorporation of a soil suspension into an agar film results in the measurement of living and non-living hyphae and in this case may have provided

misleading data. In fact, increases in total mycelium may actually indicate a decrease in the decomposition rate of the non-viable hyphae, due to a decrease in activity of soil microorganisms or microarthropods (Bååth et al., 1980). In a study in which both total hyphae and fluorescent-stained hyphae were measured, it was found that active hyphae decreased whereas the total mycelium actually increased after seven years of simulated acid rain treatments (Bååth et al., 1980). Nevertheless, the Jones and Mollison method was a fast and convenient technique for analyzing the great numbers of samples prepared in this study, and for detecting environmental and treatment effects.

An increase in fungal biomass may result from a decrease in the bacterial and actinomycete populations which are competitive or injurious to some soil fungi (Rosenzweig and Stotzky, 1979), although in the present study there was no indication of deleterious effects to the soil bacteria or a consistent change in total fungal lengths due to any of the treatments. Soil processes which are carried on in neutral or alkaline soils by acid-sensitive species may be carried out by more acidophilic organisms in acidified soils. Since nitrifying bacteria such as Nitrosomonas and Nitrobacter are known to oxidize nitrogen at pH levels above 5.0 only, an undetected population shift away from these bacterial species may result in a high proportion of soil nitrification being carried out by heterotrophic fungi, such as Verticillium

lecanii which will nitrify optimally at pH 3.5. This species did occur in these soils, but with less than one per cent frequency. The importance of fungal nitrification in acid forest soils has been observed in Scandinavia (Lang and Jagnow, 1986).

Negative effects on mycorrhizae due to low soil pH have been noted from studies with Scots Pine seedlings (Dighton and Skeffington, 1987) and culture studies of ericoid mycorrhizae (Cooke and Rayner, 1984). Similar effects from metals in solution, a possible product of increased acid loading, have been seen on different species of ectomycorrhizal fungi (Jones and Hutchinson, 1988). The presence of vesicular-arbuscular mycorrhizae has been noted in the sugar maple roots of this research site (Cooke, unpublished data), but pH effects on these symbiotic fungi remain to be explored.

Conversion of hyphal lengths and bacterial numbers to microbial biomass was avoided due to the confusion inherent in the conversion factors. For instance, changes in the water tension of the soil can produce significant variations in results (van Veen and Paul, 1979).

The cotton strips tested in 1986 showed significant effects of the burial times, but no treatment effects. This suggests a seasonal effect on decomposition rates due either to rate changes in the activity of the cellulolytic community, or to changes in the cellulolytic population to more effective cellulose degraders as the summer progresses. Rainfall in

September, 1986, was approximately fifty per cent higher than in the previous two months (Table 2), a factor which may be related to the greater decomposition of the six-week strips in September compared to the six-week strips of July, since higher moisture levels tend to be associated with increased microbial activity. Temperature changes may tend to increase or offset the effect of precipitation. If there is adequate moisture, higher temperatures will increase rates of microbial metabolism, peaking for the predominant mesophiles around 35° C. However, higher temperatures are often associated with dry conditions, resulting in an inhibition of microbial processes.

The effects from the treatments in 1987 were inconclusive. Although there was a weakly significant ($P<0.05$) decrease in decomposition of the cotton in the sodium sulphate-treated plots in August, no such pattern was found in the other months. Other studies have demonstrated reduced carbon mineralization after acid treatments (Gallagher et al., 1987; Berg, 1986a and b; Bewley and Stotzky, 1983a and b; Hovland, 1981; Baath et al., 1980), as well as decreased rates of respiration (Hovland, 1981), nitrification (McColl and Firestone, 1986; Stroo and Alexander, 1986; Weier and Gilliam, 1986; Bitton et al., 1985), nitrogen fixation (Sigal and Johnston, 1986), protease activity (Bitton et al., 1985) and other soil processes. However, many researchers have reported no effects on many processes from simulated acid rain treatments (Stroo and Alexander, 1986; Bitton and Boylan,

1985). In fact, stimulation of microbial processes (McColl and Firestone, 1987; Mancinelli, 1986; Chang and Alexander, 1984; Killham et al., 1983) and tree growth (Tveite and Abrahamson, 1980) have been reported in some cases. Since the amount of available nitrogen is known to increase the rate of cellulose decomposition (Alexander, 1977), inputs of nitrogen in the form of nitrates, either as a sodium salt, as in this experiment, or in nitric acid, could have been expected to result in a lowering of tensile strength in those treatment plots. This effect was not evident from the experimental data.

The treatments had significant effects on the occurrence of only two species of microfungi. Mucor hiemalis was more frequent in the sub-plots treated with sodium nitrate than in the other soils. This species has been reported to be positively associated with nitrate levels (Widden, 1986b). However, its decrease in the nitric acid soils, which were receiving the equivalent nitrate loading, may reflect an intolerance to the increased acidity which has been reported for this species elsewhere (Widden, 1986b). The positive association between the Cylindrocarpon species and nitrate is slightly stronger, but since there was no equivalent stimulation of this species in 1986, this result may be unreliable. Again, the increase was not duplicated in the nitric acid plots.

One difficulty inherent in the cotton strip assay as a measure of decomposition rates was demonstrated by the

frequent appearance of a single highly decomposed area on an otherwise relatively undegraded strip. Tensile testing gave a low tensile strength for the strip in these cases, since the cloth tore almost immediately at such a site, whereas strips which had been uniformly colonized by a large number of mildly cellulolytic species tore at a higher maximum load. A particularly effective cellulolytic colony was commonly found to leave a distinctive yellow stain on the cotton, a pigment associated with Trichoderma species (Danielson, unpublished thesis) and to grow in a clearly defined small circle. Strips having this yellow pigment always tore directly from the centre of this colony site, at a particularly low kilogram load, leaving a possibly deceptive impression of substantial decomposition where there may have been little. Many other pigments were left on the cotton after they had been soaked in ethanol and washed. Besides the yellow circles, more irregular black and pink stains were very common. A survey conducted in England related fungal species with pigments left on cotton strips (Gillespie, 1982). Yellow was associated with Trichocladium opacum, Chrysosporium merdarium, a Cylindrocarpon and an Oidiodendron species, and a Penicillium isolate, as well as Trichoderma, pink with C. pannorum, and black with T. opacum. Many of the isolates found at Ste Hippolyte were not examined in the English survey, however. Although insufficient data are available to allow identification of species from pigments on the cotton, further

examination of this phenomenon could aid in the study of the fungal community found on the cloth.

The most abundant groups of microfungi found in these soils were Trichodermas and Penicillia, as expected (Widden, 1979). High frequencies of sterile fungi, Chrysosporium, Gliocladium, Paecilomyces, Fusarium, and Cylindrocarpon were also found. The most numerous twenty-four species were analyzed, out of an overall total of more than one hundred species.

The appearance of Paecilomyces carneus, Trichocladium opacum, and Trichoderma hamatum in significantly greater frequencies from the soil samples than from the cotton strips could indicate species which are not particularly cellulolytic. Although this is known to be the case for Paecilomyces, there are numerous reports of the cellulolytic abilities of T. hamatum and T. opacum (Gillespie, 1988; Kuter, 1986; Widden and Hsu, 1986; Domsch et al., 1980), and their scarcity may be more indicative of a lack of competitive success based on other factors. In fact, although T. hamatum was found in significantly greater abundance in the soil samples than on the cotton in most samplings, its frequency of isolation from the cotton was still high, although reduced with time. These results indicate that this fungus is both cellulolytic and abundant in this soil, but is not a highly successful competitor on crystalline cellulose. None of these fungi, including T. hamatum, showed a strong correlation with

tensile strength loss. Conversely, the two Chrysosporium taxa, and four Penicillium species were found to be scarce in the soil isolations, but more commonly found on the cellulose substrate, suggesting that these fungi have cellulolytic capabilities which may be stimulated by a readily available carbon source. These taxa are known to be cellulolytic to varying degrees except for C. merdarium (Domsch et al., 1980) which may have been surviving as an opportunistic species absorbing sugars released by the extracellular enzymes of neighbouring fungi. Of the Penicillia, P. thomii and P. citrinum were both significantly correlated with loss of tensile strength in 1987, and strongly, though not significantly in 1986. Both these species are known to be common decomposers of litter and textiles (Pitt, 1979). The other four species did not demonstrate a strong association with decomposition of the cotton. P. thomii has been found associated with maple forest soils elsewhere, although not in such abundance as in the cotton strip isolations. Penicillia common in other maple soils which were frequently isolated at the Ste Hippolyte site were P. spinulosum, P. brevicompactum, P. janthinellum (Kuter, 1986; Widden, 1979).

The increasing frequencies of certain fungi may be a response to seasonal changes, some being naturally more abundant later in the summer than in early spring when the soil samples were taken. Species progressions have been reported as cotton breaks down in the soil (Widden and Howson,

unpublished data). For instance, P.glabrum has been found to occur typically in a later stage of succession. Such a phenomenon was not demonstrated clearly in this study. However, distinct seasonal shifts in frequency of isolation were seen. In the cases of the two species of Cylindrocarpon, Chrysosporium pannorum, P. thomii, and the sterile pink isolate, the populations increased gradually from the initial sampling to the fourth in early August, after which there was a definite decrease in occurrence of these fungi. Although C. pannorum has been identified as a cold weather species (Widden, 1986a), its cellulolytic capacity (Domsch et al., 1980) has perhaps provided a competitive edge on the cotton strip, and allowed it to flourish throughout the summer, reaching very high levels by early August. P. thomii has been associated with the summer months in southern Quebec, and this may account for its relative scarcity in the spring soil samples (Widden, 1986a). There is evidence that P. thomii is highly cellulolytic in various soils in comparison to other Penicillia, such as P. spinulosum and P. glabrum (Gillespie, 1980; Pitt, 1979), neither of which demonstrated an association with tensile strength loss in this study. Other studies conducted using dried and lyophilized inoculum have reported a lack of cellulolytic ability in P.thomii, while noting cellulose breakdown by P. spinulosum, P. brevicompactum, and P. janthinellum (Gochenaur, 1984), results which were not observed in this survey. Some species such as

T. koningii, P. spinulosum, and the sterile black isolate increased steadily as the season progressed and were most abundant in the final samplings in October. Several factors are probably involved in these population shifts. Competition between species and within species is well-known and documented (Brown et al., 1987; Summerbell, 1987; Widden and Hsu, 1986; Vajna, 1985). T. koningii and T. hamatum both appeared in the soil in equal frequencies, and were the most common fungi found in this soil. The prominence of these two microfungi in soils has been previously reported (Danielson and Davey, 1973), although a study of maple litter in Wisconsin based on unwashed and sometimes frozen leaf samples found them to be scarce and unimportant species (Kuter, 1986). T. koningii appeared on the cotton cloths much more often than did T. hamatum throughout the study. Some strains of T. hamatum have been reported to be sensitive to the lower moisture levels occurring in midsummer (Danielson, 1971), but T. koningii has been associated with a high temperature preference, as well as being a particularly effective overall competitor which is characteristically abundant in the summer (Widden, 1986a; Widden and Abitbol, 1980), factors which may contribute to its prominance. Since this study only followed a single cotton strip for a maximum of nine weeks in 1986, and two weeks in 1987, it is possible that the dominance of T. koningii might not have continued through a longer incubation period. This fungus has been observed to be the stronger

initial decomposer of cellulose, although a strain of T. hamatum was shown to be more cellulolytic on dogwood leaves after three months growth (Danielson, 1971).

T. koningii was most consistently associated with tensile strength loss, demonstrating this significant correlation in both 1986 and 1987 (Table 12), whereas T. hamatum was not, although it is known to degrade CMC and cellobiose (Domsch et al., 1980). In studies with maple litter, T. koningii was shown to penetrate the substrate at a consistently higher rate than T. hamatum (Widden and Hsu, 1986), suggesting a competitive advantage for the former species at this site. It is known to possess the complete array of cellulases (Domsch et al., 1980), while strains of T. hamatum have been reported which show low enzyme activity.

Environmental factors, such as climate and soils as well as cellulolytic ability, will combine to favor certain species over others at various times, resulting in competitive success or failure. For instance, T. opacum, though known to be cellulolytic and fairly abundant in this soil, does not occur frequently on the cotton, an observation also made in an English cotton strip study (Widden et al., 1986). This fungus degrades cellulose well in alkaline soils, but is less cellulolytic in acid conditions (Gillespie, 1988), suggesting that a fungus may be a successful competitor in one soil but not in another.

The most abundant fungi were T. koningii, T. hamatum, and

P. thomii, all of which are known to be capable degraders of crystalline cellulose. Although the importance of Trichoderma species have sometimes been overlooked when discussing cellulose decomposition in the field, Trichoderma koningii was consistently and highly significantly associated with tensile strength loss of the cotton strips, and was also the most frequently isolated species overall, although other researchers have reported much lower incidences of T. koningii on sugar maple litter (Kuter, 1986), and on other deciduous species (Gochenaur, 1984; Kjoller and Struwe, 1980). This study found it to be a good competitor in the spring, summer and fall, both in the soil and on native cellulose.

Both the dark and light species of Cylindrocarpon were significantly associated with decreased tensile strength loss in 1986, but not in 1987. The 1986 result supports reports of certain species of this genus being cellulolytic (Domsch et al., 1980). As well, although the data is not significant, the results from 1987 associate the two Cylindrocarpon species with tensile strength loss.

The association of Mucor hiemalis with tensile strength loss in the 1987 data is unexpected as this species is not known to be cellulolytic (Gochenaur, 1984). However, an endo-1,6-beta-glucanase has been shown to be produced, and hemicellulose is degraded (Domsch et al., 1980). Although Trichoderma koningii is reported to inhibit Mucor hiemalis (Domsch et al., 1980), the quick growth and vigour of this

species possibly allows it to grow in association with a more strongly cellulolytic population by taking advantage of sugars produced by the external enzymes of those fungi. No significant association was found in 1986 between Mucor hiemalis and tensile strength loss.

CONCLUSIONS

In spite of the fact that the phenomenon of forest die-back is becoming increasingly worrisome, field studies which can demonstrate a distinct cause-and-effect relationship between tree damage and acid rain are scarce. One of the chief reasons for this is the difficulty involved in studying a complex and spatially heterogenous system such as a maple forest, in which many groups of organisms interact with each other and with the abiotic environment. Experimental controls must be arbitrary to some extent, and minor topographical variations may be very influential. Destructive effects from pollutants can be masked in the short-term by the buffering of the soil, or the shifting of microbial species to tolerant strains. The great variability between juxtaposed soil samples also increases the possibility of overlooking significant local effects. Nevertheless, some effects of the acid and salt treatments on the soil chemistry, tensile strength of cotton strips, and fungal taxa began to appear after one year, and increased by the end of the second summer of treatments. Although the data were inconclusive, they indicated the initiation of possible nutrient leaching and changes in the cellulolytic community resulting from the treatments. A continuation of the treatments might indeed demonstrate more consistent changes in the chemical and microbiological constituents of the soil.

In the first year, there was a weak stimulating effect on Mucor hiemalis in the nitrate-amended plots, and at the end of the second year a Cylindrocarpon isolate was shown to be not only increasing in the nitrate salt and acid plots, but decreasing in the sulphuric acid and sulphate treated soils. Since this area of southern Quebec has been exposed to acid precipitation for decades, a tolerant community of bacteria and fungi has possibly already evolved and thus the solutions applied to the soils would have to be more concentrated or copious to cause detectable changes in the soil within the limited time available.

The appearance of Trichoderma koningii as the most consistent species to be significantly associated with decomposition of the cotton cloth is in accord with recent evidence concerning the cellulolytic ability and competitiveness of this species during the summer in a deciduous forest of southern Quebec. Although T. hamatum was equally abundant in the soil, its competitive ability on the cotton cloth was inferior to that of T. koningii throughout the trial period. Penicillium thomii, P. citrinum, Mucor hiemalis, and two unspecified Cylindrocarpon species were also significantly associated with cotton decomposition, but t correlation was not as consistent over the two years of the study as was that of Trichoderma koningii. The Penicillium results agree with previous reports from England, but are at variance with others. The presence of the Mucor may simply reflect the

ability of this fungus to take advantage of by-products of cellulolytic neighbours, and to grow extremely rapidly.

Because of the great variance between the soil samples, many more samplings in every parameter tested were probably needed to provide more consistent and significant results. Although the pH of the acid treatments could not be lowered due to the danger of burning the soil microorganisms, an increase in the quantity of the solutions might have provided sufficient acid and salt loading to produce more consistent effects. Moreover, short-term experiments such as this one are less likely to demonstrate effects than the long-term studies which have shown forest damage to be associated with acid rain in Europe and Scandinavia.

The cotton strip technique appears to be a highly useful method for detecting changes in cellulose decomposition rates. The ease with which cellulolytic soil microfungi can be isolated from the cotton strips before tensile testing of the cotton makes this method a valuable tool in the study of cellulolytic fungal communities in the soil.

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