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Decomposition and Mineralization in an Artemisia tridentata Community in Northern Nevada

P. L. Comanor

D. C. Prusso

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DECOMPOSITION AND MINERALIZATION IN AN *Artemesia tridentata* COMMUNITY IN NORTHERN NEVADA

P. L. Comanor, Project Leader

and

D. C. Prusso

University of Nevada

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ABSTRACT

Litter fungal analysis through July, 1972, produced a list of 37 isolates. Identification was made to species when possible. *Aureobasidium pullulans* occurred with the greatest frequency in the spring. Preliminary observations revealed the presence of a successional pattern of fungi on the litter. Preliminary growth studies revealed the potential for understanding of the role of fungi in the decomposition of sagebrush litter.

During major decomposition activity there appeared to be a correlated increase in leaf nitrogen content. The rates of increase were similar regardless of the sample age. Several possible explanations for this increase are discussed briefly.

Carbon dioxide production showed a direct correlation with weekly rainfall. Higher rainfall resulted in greater CO_2 evolution until the onset of cold temperature, which presumably restricts microorganism activity. Maximum evolution recorded was on October 18 at 4500 mg $CO^2/m^2/24$ hr.

Weight loss of bagged litter samples showed variable results. There was an initial loss of weight of all 2 g subsamples after placement in the field. The greatest weight loss occurred in those samples placed in the field from July to September. Monthly weight loss was greatest for all samples during the August-October period, in some cases being about 0.4 g.

INTRODUCTION

The purpose of this project was to provide data on rates of decomposition and mineralization of *Artemisia tridentata* Nutt. under field and laboratory conditions in order to establish mineral cycling rates through the leaf litter compartment by microbial decomposition and leaching. Rates of turnover of N, P, K, Ca, lignin and cellulose, were to be determined in an attempt to correlate them with decomposition. Fungi isolated from the litter were to be used to establish successional patterns, unifungal decomposition rates, and rates of growth under a range of environmental conditions representative of field conditions. In mid-1972, at the request of the microbiological studies Coordinator, the objectives of the study were altered to better fit into the research objectives of the Biome. The continuation of the project into 1973 will emphasize the rate of organic matter breakdown expressed in terms of controlled variables, as well as the rate of C and N release from organic matter, chiefly *A. tridentata* material.

OBJECTIVES

The primary objectives of the original proposal were:

1. Analysis of fresh litter for N, P, K, Ca, lignin and cellulose content.
2. Analysis of decomposing litter of known age for N, P, K, Ca, lignin and cellulose content.
3. Rates of release of minerals from leaf litter under controlled laboratory conditions.
4. Weight loss of decomposing leaf litter on a monthly basis.
5. Isolation and identification of fungi from fresh and decomposing leaf litter.
6. Determination of fungal succession on decomposing leaf litter.
7. Rates of decomposition of leaf litter by mixed and unifungal cultures under controlled laboratory conditions.

In July, 1972, the project direction was changed to eliminate the analysis of P, K, Ca, lignin and cellulose and the isolation of fungi for further laboratory work. The primary objectives for 1972/1973 became:

1. Determination of the rate of litter break-down of *Artemisia tridentata* as a function of the time of initial placement in the field and meteorological variables.
2. Determination of rates of transfer of carbon and nitrogen from litter to ammonia, carbon dioxide, organic nitrogen and organic carbon, and, when possible, their disposition into the several compartments or pools of the system including soil, atmosphere and decomposer accumulation.

3. The rate of organic matter break-down in terms of controlled variables, as well as the rate of C and N release from organic matter, chiefly *Artemisia tridentata*.

The three objectives above constitute continuing ones for the duration of the project. The types of litter analyzed during 1973 will be expanded.

METHODS

Experimental design

Artemisia tridentata is an evergreen shrub which loses leaves throughout the year (Mack, 1970). The experimental design incorporates a monthly collection of leaves to be bagged, returned to the field, and retrieved at monthly intervals for analysis.

A site was selected upon the recommendation of USDA personnel as being very typical of the big-sage habitat. The site was 24 miles north of the University of Nevada at the junction of Highways US 395 and California State Route 70 at Hallelujah Junction, Plumas County, California, elevation 4950 ft. The site is adjacent to an ARS enclosure, providing access to some long-range meteorological data as well as better security for field equipment, in an area classed as sagebrush steppe (Kuchler, 1964).

A plot 12 x 12 m was arbitrarily selected and staked into meter squares for ease of placement and recovery of randomly-placed litter samples (Fig. 1). Adjacent areas provided 3 secondary or auxiliary plots used for additional studies (i.e. soil pits, litter traps, monitoring of soil and litter relative humidity, etc.).

Once a month a 12-channel YSI telethermometer was used to monitor soil and litter conditions hourly over a 24-hour period in identified micro-habitats in the main plot, the data located on Data Set A3UCH02. Additionally, 2 max/min thermometers, 2 hygro-thermographs (of the latter, one was placed under a shrub, the other in a standard weather shelter) and rain gauge were installed in adjacent areas.

Monthly collections of fresh leaves were made, encompassing collection and placement for 12 consecutive months and retrieval lasting for 24 months. Short terminal branches were the source of the leaves. These are cut from shrubs in the area adjacent to the research plots and taken to the laboratory for drying, weighing and bagging.

In an adjacent plot 4 litter traps were constructed, 2 with open tops and 2 completely enclosing shrubs. Monthly collections are being made of litter either by hand or using a 12-volt automotive vacuum.

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Fresh litter collected in the field was air-dried at room temperature, weighed into 2 g units (sub-samples) and sewn into 1 dm^2 polyester bags with 1 mm^2 mesh. The bags were placed in the research plot in groups of 4 (a sample) in randomly-selected locations, except that areas without shrubs were avoided. Each month a suite (12 samples consisting of 48 subsamples) was placed in the field and a sample from each previously-placed suite was returned to the laboratory for analysis.

During the first part of the study 1 subsample from each sample returned to the laboratory was placed into a sterile container and used for fungal isolation and identification. Leaves from these bags were placed on 2 types of agar, Water Agar (H_2O) and fortified Corn Meal Agar (CM+). During the earlier months of the year they were incubated at both 12 C and 23 C. As colonies appeared on the agar they were isolated and, where possible, identified, then maintained as a pure culture for further work. Some indication was made of their frequency on the leaves; however, no attempt was made to make plate counts, etc. This portion of the study was discontinued in July, 1972.

The 3 remaining subsamples were kept separate and treated as follows. Each bag was weighed as it was brought into the laboratory (bag plus leaves and any foreign material). Leaves were then removed from the bag and, as much as possible, all foreign material removed, and fresh weight of the leaves recorded. The leaves were then allowed to air dry at room temperature and weighed, after which they were oven-dried at 45 C for 48 hr and reweighed. The weight data are on Data Set A3UCH01.

Total nitrogen content of the leaf litter was determined by the micro-Kjeldahl method as presented by Jackson (1958) and Bremner (1965), with modifications being made for plant material. Oven-dried (45 C) leaves were ground sufficiently to pass a 0.4 mm screen and weighed into approximately 0.5 g portions, then wrapped in cigarette paper. After drying a minimum of 2 days these samples were digested using copper (Cal-Pak Powder #2-Gunning Method) and a selenium catalyst (Hengar Selenized Granules). Distillation was carried out using a boric acid trap with the distillate being titrated with a 0.01 N sulfuric acid standard using N-point indicator.

Litter respiration in the field was monitored using bagged samples in order that respiration could be correlated with litter weight loss. The bags were placed in sealed plastic containers along with a vial containing a static 20 ml NaOH trap. Six samples (24 bags) and 12 controls were monitored for a 1-2 day period each week. Laboratory analysis of the NaOH uses titration with standard HCl after fixing with $BaCl_2$ and using thymolphthalein as the indicator (Coleman, 1971).

RESULTS

Figure 1 shows the distribution of the cover within the main 12 x 12 m plot. Most cover is provided by big sagebrush with some *Tetradymia canescens* DC (gray horsebrush). Several grasses are also present, but not mapped.

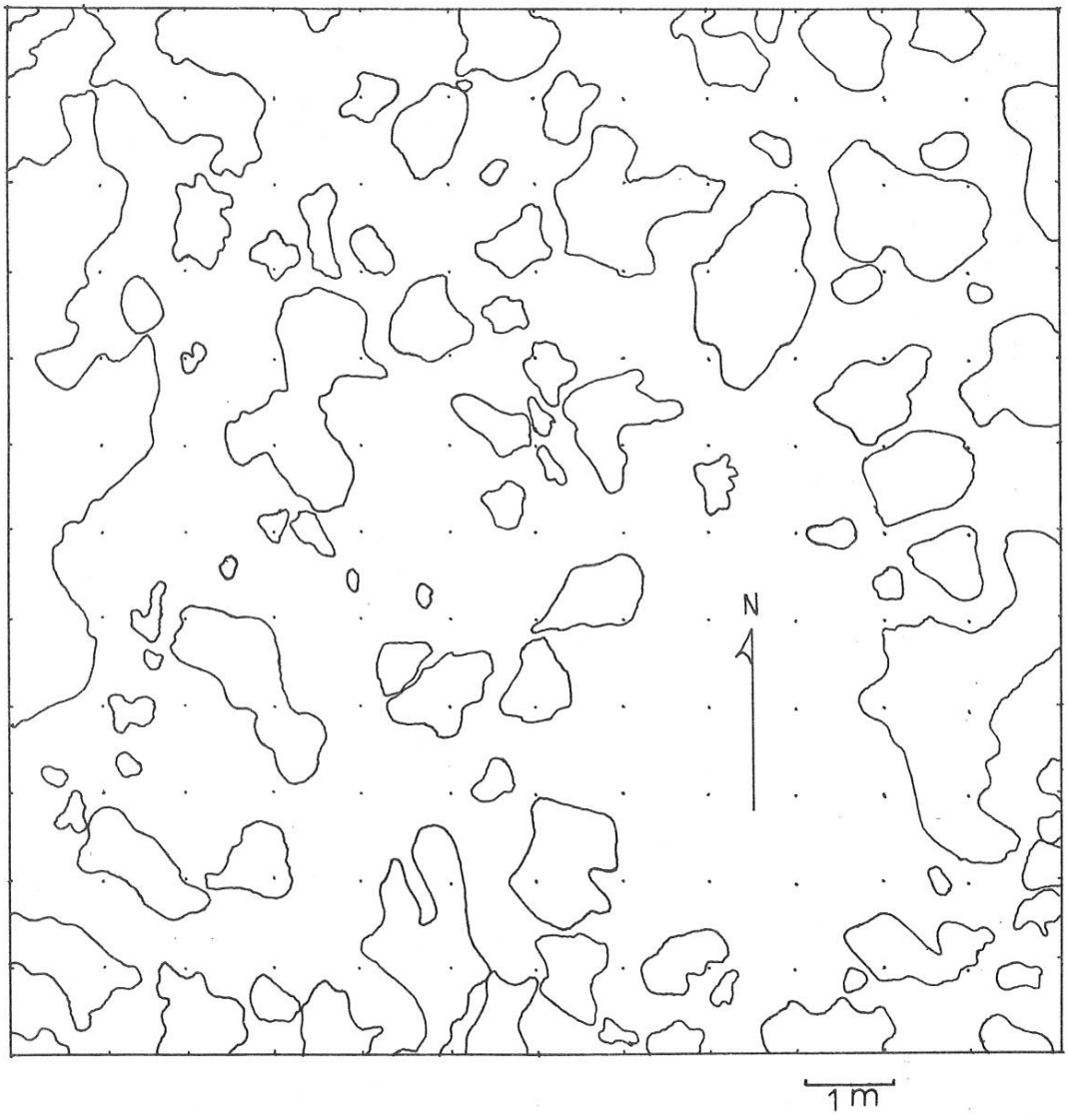

Figure 1. Map of the main 12 x 12 m plot showing shrub crown outlines plus the location of grid stakes.

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Fungal analysis

Following is a list of the fungi isolated up to June, 1972, when the primary objectives of the project were modified.

Division Mycota
Subdivision Eymycotina
Class Zygomycetes
Order Mucorales
Family Mucoraceae
Mucor sp.
Family Thamnidiaeceae
Thamnidium anomalum Hess & Ander.

Class Ascomycetes
Order Spaaeriales
Melanomma Subcatum Ell.
Unknown 1 sp

Class Basidiomycetes
Order Uredinales
Family Pucciniaceae
Puccinia absinthii (Hedwig.) DC

Fungi Imperfecti
Order Sphaeropsidales
Chaetodiplodia sp.
Diplodina tridentatae Cooke & Shaw
Phoma sp. (2 isolates)

Order Moniliales
Family Moniliaceae
Beauvaria sp. (2 isloates)
Penicillium sp. (6 isolates)
Trichoderma viride Pers.

Family Dematiaceae
Alternaria sp. (5 isolates)
Aureobasidium pullulans (de Bary) Arnaud
Cladosporium sp. (6 isolates)
Humicola sp.
Nigrospora sp.
Paecilomyces sp.
Stemphylium sp.

Family Tuberculariaceae
Epicoccum nigrum Link
Fusarium sp. (2 isolates)

Although no attempt was made to quantify the numbers of each species present, notes were kept on the number of leaves on which each appeared. The most obvious change involved *Aureobasidium pullulans*. Every leaf plated from the April collection of the March suite produced l-several colonies of *A. pullulans*. The May retrieval of the March suite showed about 50% of the leaves with *A. pullulans*. The June retrieval showed no colonies of this fungus, but no other species seemed to be dominant. Cursory examination of the July retrieval of the March sample showed a different, unidentified species appearing more frequently on all leaves. This pattern was also noted in the collections of the samples of other suites brought in during May, June and July.

Preliminary growth studies were undertaken on some of the fungal isolates utilizing a small incubator lacking adequate light, lighting control and temperature control. Variations in colony growth, production of reproductive structures, and by-products of metabolism appearing in the agar were observed.

During the study the presence of other fungi in or near the research plot were noted. The following fungi were recorded and collected for more positive identification:

Class Basidiomycetes
 Subclass Heterobasidiomycetidae
  Order Ustilaginales
   Family Ustilaginaceae
    Ustilago gayophyti Hark on *Gayophytum ramosissimum* T & G
 Subclass Homobasidiomycetidae
  Order Agaricales
    Unidentified sp. 2
  Order Lycoperdales
    Geastrum sp.
    Tulostoma sp.
    Unidentified sp. 2
  Order Nidulariales
   Family Nidulariaceae
    Unidentified sp. 1
   Family Sphaerobolaceae
    Sphaerobolus sp.

Sagebrush litter weight loss

Figure 2 presents the weight loss of bagged *Artemisia tridentata* leaves during the study period; Data Set A3UCH01. In all cases there was an initial loss of weight of the 2 g subsamples after they were placed in the field plot. The slopes of weight loss during the first month in the field were variable; the loss ranged from 2-16% of the initial weight. The slopes were lowest for the March and April samples, and greatest for the samples placed in the field from July-September. Weight losses for the second and following months were also variable.

The slopes of the curves may also be considered by monthly time intervals during the year. The May-June period shows greater weight loss than either of the adjacent monthly periods. The most consistently large weight loss, for all bags regardless of time of placement, occurs during the August-October period. For the August and September (fresh) samples this loss exceeded 0.3 g. For those samples which had been in the field for some time the loss was variable; two "older" samples also showed a weight loss equal to those placed in the field in the August-September period. Anomalous weight gains are evident for some of the samples.

Figure 3 presents the bagged litter weight loss during the study period on an oven-dried weight basis; Data Set A3UCH01. Weight losses during the first month in the field (calculated on this basis) are highly variable, showing an anomalous weight increase in several instances. Consistent weight losses are evident for all samples

during the May-June period. With one exception, the greatest weight loss occurred during the August-October period. Weight loss on a fresh weight basis (Fig. 2) was the greatest during that period also. Weight losses were greatest for those samples which had been in the field for some time. Oven-dried weight equivalents of the 2 g air dry bagged litter ranged form 1.72 - 1.82 g.

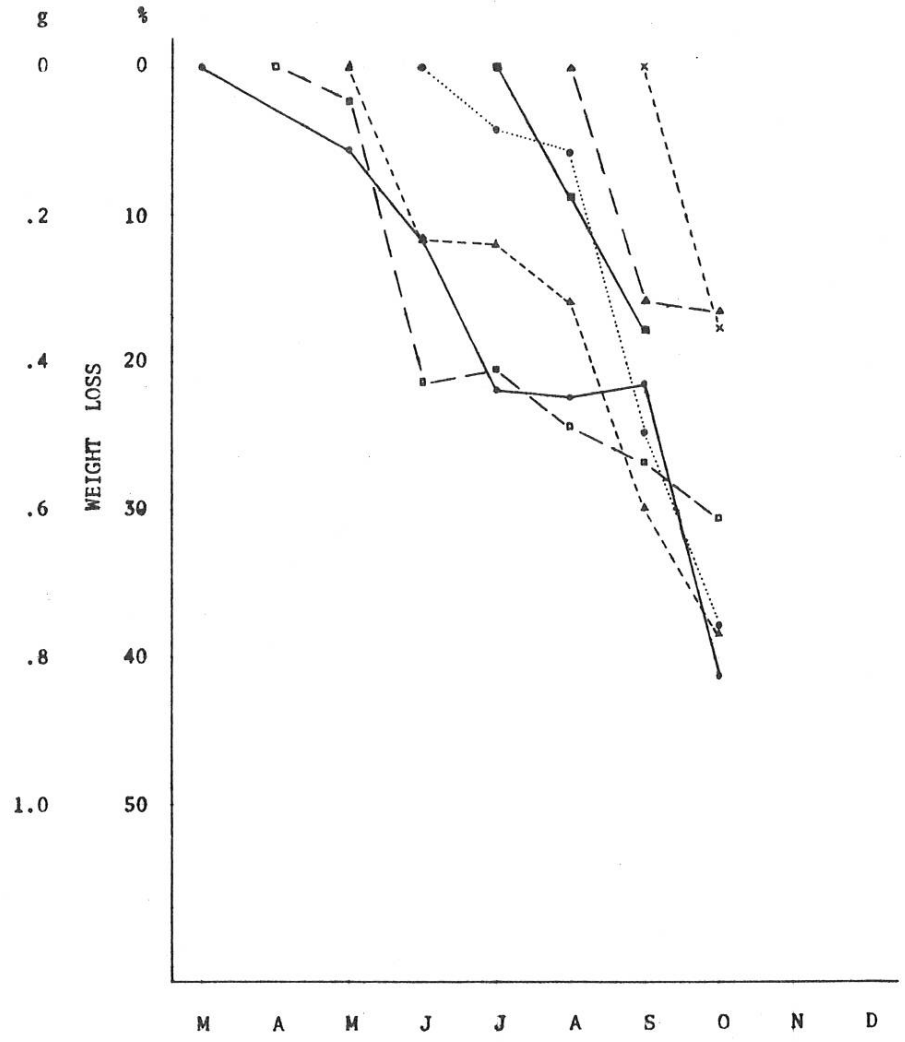

Figure 2. Weight loss of bagged *Artemisia tridentata* leaves on the soil surface as a function of time. Weight loss is expressed in grams and as a percent. Starting points for each curve represent initial placement of sample in field. Data based on initial air-dry weights of 2 g/sample. DSCODE A3UCH01

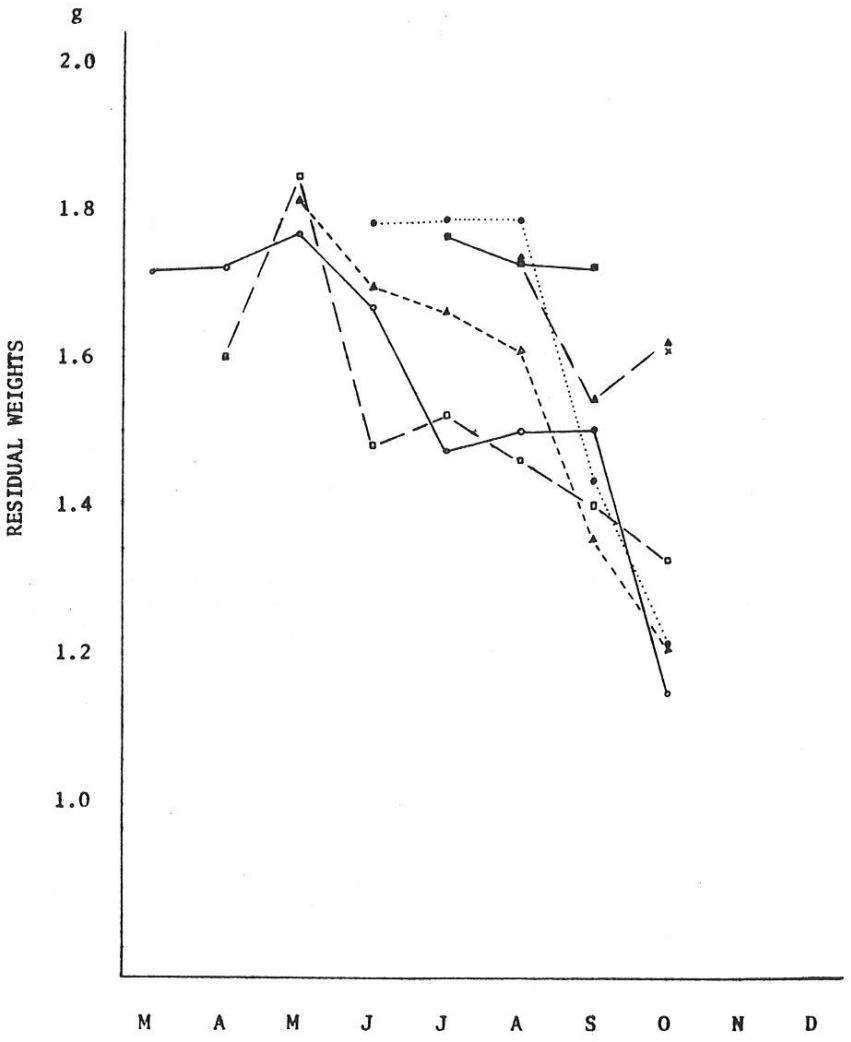

Figure 3. Residual weights of bagged *Artemisia tridentata* leaves on the soil surface as a function of time. Starting point for each curve represents initial placement of sample in field. Data based on oven-dry weight calculations. DSCODE A3UCH01

Litter nitrogen content

Although fluctuating, there was little variation in nitrogen content of the samples until the August-September period, when a sharp increase took place (Fig. 4). Samples placed in August and September show an immediate increase in N content. The increase in N content in this period corresponds with the increase in weight loss of the bagged litter (Fig. 2). The slopes of the curves of N content are very similar during this period, regardless of the time of placement of the samples into the field.

Carbon dioxide evolution from bagged litter

As shown in Fig. 5, weekly carbon dioxide evolution during the months of October and November, 1972, correlates directly with weekly precipitation, which was measured for the previous week on the date the CO_2 monitoring apparatus was set up. The week prior to the 18 October sampling period had the highest rainfall of the study period and also showed the greatest 24-hr CO_2 evolution from the bagged *Artemisia* leaves. The following two weeks the rainfall was only a trace and the CO_2 evolution was markedly reduced. In the week prior to 8 November an increase in rainfall again related directly to an increase in CO_2 evolution. Although precipitation the week prior to the 15 November sampling period was higher, it was in the form of snow, with associated lower temperatures. Low CO_2 evolution was recorded on 30 November corresponding with very little precipitation and below freezing temperatures.

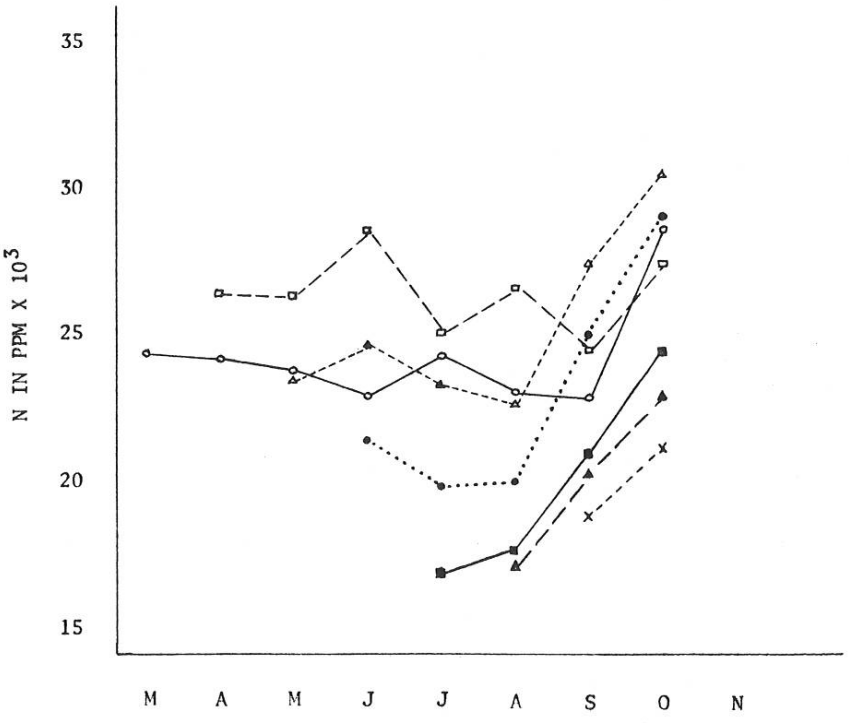

Figure 4. Total N content of bagged *Artemisia tridentata* leaves on the soil surface as a function of time.

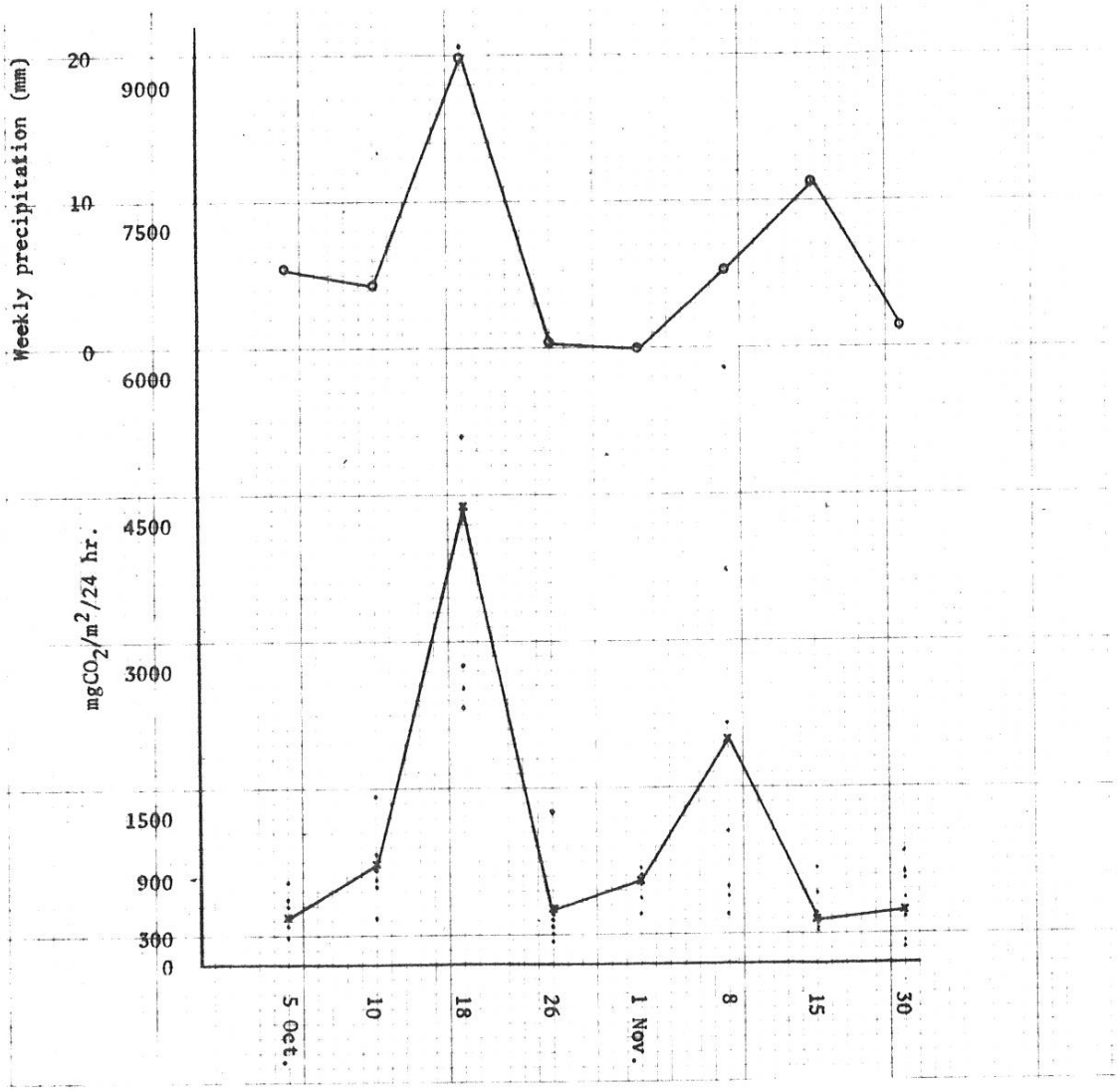

Figure 5. CO_2 evolution from bagged *Artemisia tridentata* leaves on the soil surface plotted against total weekly precipitation.

DISCUSSION

Fungal analysis

The study of successional patterns of fungi on decomposing leaves, and those fungi responsible for decomposition, was terminated in the early stage of the project. The results available are not sufficient to warrant the formation of any conclusions or lead to any meaningful discussion.

The preliminary growth studies showed the necessity of utilizing an incubator equipped with adequate light and diurnal controls for both light and temperature, should this portion of the study be resumed at a later time.

Litter weight loss, nitrogen content, and CO_2 evolution

It appears that during periods of major decomposition activity there is a correlated increase in leaf N content. Possibly, rather than being deposited into the soil or atmosphere during decomposition some of the N is being retained in association with the leaves. It is possible that as the decomposer organisms break down the leaves, this N is being concentrated by fungal action (and possibly the bacterial cells) so that when nitrogen analysis is carried out it is being detected in both leaves and decomposer organisms. Also, if relatively little nitrogen is utilized by the microorganisms or converted to other products (i.e., gas) a relatively high amount of N may still be present in the leaf tissue even though the weight of the remaining leaf has been reduced. Thus, the nitrogen content of a 0.5 g sample of decomposed litter would be higher than in a 0.5 g sample of fresh litter. Only additional study of both microorganism utilization of nitrogen and products of decomposition would help to provide the answer to the increase in N. The role of leaching still has to be investigated since the increase in nitrogen takes place during major moisture periods, indicating that leaching may not have too much of an effect.

EXPECTATIONS

The experimental design establishes the pattern of placement of samples for 1 year and retrieval for a 2-year period. The analysis of litter weight loss, N content and CO_2 evolution will continue for that period. It is hoped that some of the variations and unexpected phenomena will be explained as the picture of year-round decomposition becomes clearer.

Bagged litter samples will be expanded to include both root and stem litter, with the root samples being monitored *in situ*. Also, bagged litter will be buried to investigate the rate of decomposition in that type of environment.

Plans include the development of automatic micrometeorological recording instrumentation. The key emphasis will be on combining the results of detailed micrometeorological measurements with weight loss, C loss, N transformation, etc. Correlations obtained will be very specific. As an example, identified sub-samples are monitored weekly in the field for CO_2 evolution. These samples will be analyzed afterwards for weight loss and total carbon content.

Limitations to the decomposition process will also be studied and will involve the monitoring of decomposition in the incubator as well as the field, controlling the variables of temperature, relative humidity, substrate, and microorganism composition, which should allow a better understanding of the role of climatic conditions and the microorganisms in the overall decomposition process.

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