5-1990

Coordinating Committee Meeting, San Juan, Puerto Rico, May, 1990

Long Term Ecological Research Network

DRAFT PROTOCOL

INTERSITE FINE LITTER DECOMPOSITION EXPERIMENT

NOVEMBER 25, 1989

Mark E. Harmon

Jerry Melillo

INTRODUCTION

The need for long-term, intersite experiments was aknowledged at the 1989 Decomposition Workshop held at Wood's Hole, Massachusetts. These studies will play a major role in advancing our understanding of the general principles underlying decomposition and nitrogen cycling processes. This understanding will be crucial to address the effects that global climate will have upon detrital storage and productivity of ecosystems.

Study plans for a number of long-term studies were developed by the workshop participants and these were reviewed by the National Science Foundation Staff. Of those plans, the intersite fine litter exchange was selected for installation. It must be emphasized that this study is the first step toward a general class of intersite comparative experiments. We anticipate the other planned studies will be funded in the future.

The following draft protocol describes the rationale and methods of the study and is based upon the plan developed at the Wood's Hole Decomposition Workshop.

OBJECTIVES AND HYPOTHESES

The primary objective of this study is to examine the control that substrate quality and climate have on patterns of long-term decomposition and nitrogen accumulation in above- and below-ground fine litter. Of particular interest will be to examine the degree these two factors control the formation of stable organic matter and nitrogen after extensive decay.

The decomposition of fine litter is a complicated process controlled by a number of factors including substrate quality, size, decomposer species, edaphic conditions, and climate. We offer the following working hypotheses concerning long-term litter decomposition dynamics fully aware that factors and interactions not considered may also be important.

Decomposition of fine litter can be divided into three phases (Figure 1). In the first phase, the labile or **fast** fractions is lost via rapid microbial assimilation or leaching. This stage dominates the first months of decomposition. The second phase is dominated by the loss of structural or **slow** carbon, which are primarily cell wall polymers such as cellulose. The third and final phase is the **stable** or metastable in which is there is a very slow net decrease in mass or nitrogen content. Given the short-term nature of most prior studies of fine litter

decomposition, the primary focus of this study will be the examination of changes (or lack of) just prior to and during the stable phase.

In order to develop the hypotheses if is helpful to have an undrlying model. As a first approximation, long-term decomposition of fine litter can be described by the following model:

$$Mass_{(t)} = Fast\ exp(-k_{fast}t) + Slow\ exp(-k_{slow}t) + Stable\ exp(-k_{stable}t)$$

where k_{fast}, k_{slow}, and k_{stable} are the rate constants for the fast, slow, and stable fractions, t is time, and fast, slow and stable are the fraction of the litter in each of these classes. On the time scale of 10 years, the rate constant of the stable fraction can be assumed to approach zero. While a more mechanistic model of long-term decomposition should be developed that includes Q_{10} temperature effects and degradation of specific carbon fractions, this simple model suggests the following working hypotheses would be useful to test:

Hypothesis 1) The fraction of fine litter in the fast, slow, and stable phases is primarily determined by the initial chemical composition of the litter.

The amount of litter disappearing in the fast phase would be expected to be proportional to the water/alcohol extractable fraction. The fraction occuring in the stable phase should be proportional to the original lignin content. This hypothesis would be supported if the amount of in each of these three fractions remained constant for a species amoung the sites.

Hypothesis 2) The time spent in each phase is primarily determined by the climatic conditions of a site.

This assumes that the primary effect of climate is to influence the rate constant (k_{fast}, k_{slow}, and k_{stable}) of each phase. Of the three phases we hypothesize that the slow phase will be most sensitive to climatic control, whereas the fast and stable phases will be least sensitive. This hypothesis would be supported if the rate constants for a species were dependent upon site climate, whereas the proportion in each phase for a species was constant between sites.

EXPERIMENTAL DESIGN

Regression will be the primary form of statistical analysis used to examine results of the long-term decomposition experiment.

The objective will be to provide a response surface using climatic and substrate quality factors as independent variables. Independent variables will include the mass remaining, nitrogen content, and rate constants for total mass, fast, slow, stable, lignin, and cellulose.

The major factors to be considered will be site, species of litter, and time. Twentyone sites, representing a wide array of moisture and temperature conditions, will be used for litter incubations (Table 1). Climatic independent variables will include mean annual temperature, degree days, total precipitation, and actual evapotranspiration. Nine types of "standard" litters will be sent to each site (Table 2). These include three types of fine roots (graminoid, hardwood, and conifer) and six types of leaf litter (which range in lignin/nitrogen ratio from 5 to 75). The primary independent variable used to characterize substrate quality will be the lignin/nitrogen ratio, although other variables such as C/N ratio, and extractive content will be examined as well. Samples will be collected ten times; the time between samples will depend on the site, but for most sites it will be one year. There will be four replicates for each species, site and time.

In addition to the standard litters, each site will be represented by a "wildcard" litter which appears at one randomly choosen site for each sample collection. The purpose of the wildcard species is to verify the predictions from the standard

species. There will be four replicates for each species, site and time.

To examine the effects of invertebrates on the early stages of decomposition the decomposition the first two years of two broadleaf species and one grass will be examined at a subset of sites choosen to represent each major biome. The experimental design of this study will be a split-plot, with six sites (arctic, temperate conifer, temperate hardwood, grassland, desert, and wet tropical), two mesh sizes (1 mm versus 7 mm), and three species (wheat, sugar maple, rhododendron). Samples will be collected at two times. There will be four replicates for each mesh size, species, site and time.

METHODOLOGY

Collection of Litter

Each site is responsible for collecting the litter to be used in the experiments. Whenever possible the leaf litter should be collected directly from senesecent plants or as freshly fallen litter. In cases where "green" material is used this should be noted. Litter will be air dried prior to shipment to the Central Processing Laboratory.

Fine roots will be collected by three methods. Tropical hardwood fine roots will be collected fron recently windthrow root systems. Graminoid roots will be collected from exposed stream cut banks. Pine roots will be collected by excavating surface roots in sandy soil.

Bag Design

All bags will be 20- by 20-cm and filled with 10 g of air dried litter. Each bag will be identified with a unique number embossed on an aluminum tag. The initial air dry weight, calculated oven dry weight, species, site, replicate number for each litterbag will be recorded prior to placement in the field. Subsamples of litter material will be taken to determine a air dry to oven dry conversion factor and the initial chemistry of the litter.

Three types of bags will be used in this experiment. For the long-term leaf litter experiment the bags will have a top mesh of 1 mm and a bottom of 55 micron mesh. The bags used for fine roots will be entirely of 55 micron mesh. The bags used in the invertebrate effects study will have a top mesh of 7 mm and a bottom of 55 micron mesh.

Sample Placement

Samples will be placed in the field during spring of 1990 by each of the participating sites. It will be the responsibility of each site to choose a location or locations that is typical of the major ecosystem represented by their site. If possible locations should be choosen to be near climatic stations and in areas protected from disturbances that could destroy the litter bags. For example, sites with frequent fire should be avoided, but areas prone to grazing would be suitable. The locations should also be typical of areas that other intersite decomposition experiments might be conducted.

The exact method for placement will vary from site to site, but the following standards should be applied if possible. Deviations from these suggestions may be neccessary at some site; these alterations will be important to note in the study establishment report.

1) Four separate locations should be selected (Figure 2). These can be in the same stand if access is limited, but it would be preferrable if each replicate is placed in a similar but different area to avoid pseudo-replication problems.

2) Each set of bags to be collected will be connected by a cord; these sets of bags should be laid out in parallel lines in a random order.

3) Leaf litterbags should be placed so that contact with the underlying litter layer is made. Fine root litterbags will be inserted into the upper mineral soil (humus layer for histosols). A vertical cut with a shovel, the bag inserted the correct depth, and another cut should be used to press the soil against the bag (Figure).

Sample Collection Schedule

A list of the litterbags, and prelabeled bags will be sent to each site prior to sample collection.

For temperate zone sites the fine litter samples will be collected on an annual basis for a 10 year period. For tropical sites (LaSelva and Luquillo) sampling will be conducted every three months. For the boreal and Arctic sites, samples will be collected annually unless decomposition rates are too slow for the stable fraction to be formed within 10 years.

Sample Processing

The final oven dry weight (55^{o}C for 4 days) will be determined at the Central Processing Laboratory (Andrews LTER). Samples will be pooled by species, site, and time for grinding and archiving. Each sampling time will result in 210 of these pooled samples.

Chemical analyses will be performed using two methods. Each pooled sample from each species, site, and time (N=210) will be analysed for total nitrogen, lignin, and cellulose using near infrared reflectance spectoscopy (Wessman et al. 1988). Internal variablility of samples will be estimated by running replicates of high and low lignin species. Twenty five percent of the pooled samples (N=50) will also be sampled for Kjeldahl nitrogen, lignin, cellulose, water extractive, non-polar extractive, and ash content using traditional wet chemical methods. Wet chemical methods will be used to calibrate the near infrared reflectance spectoscopy methods.

Sample Archiving/Access

Ground, dried material from each species, site, and time will be archived for future reference at Andrews LTER. Ideally, >10 g of this material would be on hand to repeat questionable analyses or for other/new analyses. Material will be stored in sealed plastic vials at room temperature. Access to samples will be provided after a short description of the new study has been approved by an oversite commitee.

Data Storage/Access

All information/data concerning these experiments will be stored in the Forest Science Data Bank/Andrews LTER. Along with the data, an abstract describing the experiment, the formats and variable definitions of the data, and the programs used to process the data will be stored.

An annual report summarizing the results will be sent to each participating site. In addition, data files will be made available to all sites that make a request.

RESPONSIBILITIES

Collection of Samples

It will be the responsibility of each of the participating sites to collect the litter samples on the agreed schedule and to prepare the samples for shipping to the central processing laboratory. Preparation for shipping will include removal of samples from the litter bags, oven drying and recording the dry weight. It will also be the responsibility of the site to provide mean temperature and total precipitation data for the appropriate site and year.

Dry Weight and Other Preparation

Dry weight determination, grinding, and archiving of litter samples will be conducted at the Central Processing Laboratory (Andrews LTER, Oregon State University). It will be the responsibility of the Central Processing Laboratory to send samples to the appropriate laboratories for chemical analysis. Another responsibility will be to enter, store, and ensure the data are freely accessable to all participating sites.

Chemical Analysis

Near infrared reflectance spectoscopy analysis for nitrogen, lignin, and cellulose will be conducted by Dr. John Aber's

laboratory (Harvard Forest LTER). Wet chemical analysis for Kjeldahl nitrogen, lignin, cellulose, water extractive, non-polar extractive, and ash content will be conducted by Dr. John Pastor's laboratory (Cedar Creek LTER). Material will be available for other analyses upon request and approval of the Oversight commitee.

References

Wessman, C. A., J. D. Aber, D. L. Peterson, and J. M. Melillo. 1988. Foliar anlysis using near infrared reflectance spectroscopy. Can. J. For. Res. 18:6-11

Table 2. Chemical characteristics of species used in the intersite long-term litter decomposition experiment.

SPECIES	LIGNIN	NITROGEN	LIGNIN/N RATIO
	%	%	
LEAF LITTER			
ASPEN	14	0.6	22
BEACH GRASS			
BEECH OR CHERRY			16
BIG BLUE STEM			
BLACK GRAMA			
BLACK LOCUST	18	2.2	8
BLUE GRAMA			
CHESTNUT OAK*	25	1.2	21
CREOSOTE BUSH			
DOUGLAS-FIR	15	0.7	23
DRYPETES*	11	1.6	7
KOBRESIA			
LITTLE BLUE STEM			
PACIFIC RHODODENDRON	20	0.4	50
PACIFIC DOGWOOD	5	1.0	5
RED PINE*			50
SLASH PINE			60
SPARTINA			
SUGAR MAPLE*	17	1.0	17
VOCHYSIA			
WAX MYRTLE			
WESTERN REDCEDAR*	25	0.3	75
WHEAT STALKS*			
WHITE PINE	31	0.9	34
YELLOW BIRCH			16
ROOT LITTER			
GRASS*			
TROPICAL HARDWOOD*			
SLASH PINE*			
RED PINE ROOTS			

* standard species sent to all sites