

Technical Report No. 146
BASIC SAMPLE COLLECTION AND HANDLING PROCEDURES
FOR THE GRASSLAND BIOME
1972 SEASON

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GRASSLAND BIOME
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ABSTRACT

This report details sample collection requirements at all Grassland Biome sites. Methods and frequencies of collection of each type of sample material are explained along with instructions for handling and shipping of sample material to the Natural Resource Ecology Laboratory. In addition, the operations of the three laboratory facilities utilized in sample analysis are outlined.

INTRODUCTION

Samples collected in the Grassland Biome study are broken into three categories:

1. Samples, other than soil samples, for which chemical analyses are required. These analyses are performed by the Analytical Laboratory located in the Natural Resource Ecology Laboratory.
2. Diet and botanical samples for which species composition analyses are required. These samples are analyzed by the Colorado State University Diet Analysis Laboratory on a contract basis.
3. Soil samples. All analyses on soil are performed by the Colorado State University Soil Testing Laboratory on a contract basis.

All samples, regardless of type, should be sent to the Natural Resource Ecology Laboratory. Do not send samples directly to either of the two contract laboratories mentioned.

ANALYTICAL LABORATORY

Analyses Available

Chemical analyses on sample materials other than soil samples are performed in the Analytical Laboratory located in the Natural Resource Ecology Laboratory. Presently, this laboratory is equipped to do the following types of analyses:

1. phosphorus,
2. total nitrogen (Kjeldahl),
3. ash,
4. gross energy,
5. cell wall constituents (neutral detergent fiber),
6. acid detergent fiber,
7. lignin (72% H_2SO_4), and
8. in vitro digestibility (modified one-stage Telly and Terry technique--no pepsin digestion).

In addition, carbohydrate analysis is being considered because a number of investigators have shown an interest in it. If it is decided to begin performing carbohydrate analyses, all Pawnee and Comprehensive Network investigators will be informed.

Sample Preparation, Packing, and Shipping

Plant material submitted to the analytical lab should be dried at 60°C and then ground to 20 mesh in a Wiley mill. At least 5 g of material are required for each sample. The ground material for each sample should be placed in a 20- or 40-dr plastic vial (available from Unab Bokhari, Natural Resource Ecology

Laboratory). Do not use glass vials as they often break in transit. Samples should be labeled on the *side* of the vial with the following information as a minimum: *site, investigator's specimen or identification number, investigator's initials, treatment, and replicate*. Pack the vials carefully in boxes to avoid breakage during shipment.

Each sample or group of samples sent to the Natural Resource Ecology Laboratory must be accompanied by NREL-60 "Request Form for Laboratory Analysis." Please use the new green version of this form and not the obsolete dark blue version. Incompletely filled out request forms will cause a delay in sample processing.

Sample Handling at the Natural Resource Ecology Laboratory

Samples arriving at the Natural Resource Ecology Laboratory are brought to the sample preparation room where they are verified, processed, and stored until analyses can be performed.

Verification consists of checking the samples against the accompanying request form and checking the form for completeness. If no request form is received, the samples are stored pending receipt of the form. If the request form is incomplete, the investigator making the request is contacted to obtain the missing information.

When verification is made, each sample is given a unique number which is recorded on the side of the sample container and on the analysis request form. The numbers are taken from a sequential list of numbers generated by the Grassland Laboratory Information Management System (GLIMS), an information storage and retrieval system written by Bob Robinson of the Natural



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REQUEST FORM FOR LABORATORY ANALYSIS

Routine Sample

Special Sample _____

Date of Request

Name of Investigator

[illegible]

Resource Ecology Laboratory and implemented on the Colorado State University CDC 6400 computer. The request forms are keypunched, and the information on them is stored by GLIMS. At this time the investigator is sent a post-card verifying receipt of his samples.

Processing of samples prior to analyses consists of grinding and preparing duplicate samples when required. If samples are not uniformly ground, they are reground. Investigators who do not have a micro-Wiley mill available for grinding small samples should notify laboratory personnel so that their samples may be scheduled for grinding.

A subset of samples received is selected at random by GLIMS to be divided in half and to have duplicate analyses performed on each half to check the precision of the analytical procedures. This is done in such a way as to prevent the technicians from distinguishing between normal and duplicate samples. Any sample selected by GLIMS for duplication is poured onto a piece of paper, mixed, and divided into four parts. Diagonal parts are pooled together making two samples. One is put back in the original vial; the other is placed in a new vial and labeled with the laboratory number generated for it by GLIMS. As needed, GLIMS generates for each analysis a list of lab numbers of samples requiring that analysis. These lists, which provide space in which the technicians write the result and intermediate calculations, are given to the technicians who get the samples from the storage room, perform the analysis, and record the results. The results are keypunched and then stored by GLIMS.

GLIMS performs a variety of functions in addition to those mentioned above. It produces reports on the number of samples requiring each type of analysis and the number of each type of analysis which have been performed.

Using the paired results from duplicated analyses, GLIMS calculates statistics relative to the precision of the various analyses. The system also retrieves analysis results in a selective fashion, recognizing any parameters on NREL-60 as selection criteria.

At present, results are retrieved and sent to investigators only upon request. GLIMS is currently being modified, however, to permit automatic, periodic retrieval of results on a site-by-site basis and to generate reports of the results which will be sent to the sites.

Samples Required

The following sample collections are required at all sites in the Comprehensive Network. When submitting these samples, mark the accompanying NREL-60 to show that they are "routine samples."

Aboveground samples. At six dates during the year, a sample of (i) live material, (ii) standing dead material, and (iii) crowns will be collected for each major species (i.e., those that contribute more than 5% of the total season-long production) and for each group of minor species (i.e., miscellaneous grasses, forbs, etc.). These samples can come from material clipped during aboveground biomass sampling and should be composited by replicate.

The samples should be taken

1. at the start of the growing season,
2. between the start of the growing season and the time of peak standing crop,
3. at the time of peak standing crop,
4. between the time of peak standing crop and the end of the growing season,
5. at the end of the growing season, and
6. during the middle of the non-growing season.

Each of the three samples of each species or group should be ground separately and placed in a separate vial. When filling out NREL-60 to accompany these samples, request ash, nitrogen, phosphorus, total available carbohydrates, lignin, and CWC determinations. Major plant species should be coded using codes found in *National List of Scientific Plant Names* (U.S. Department of Agriculture, 1971). Miscellaneous grasses, forbs, shrubs, succulents, sedges or others should be coded as MIGR, MIFO, MISH, MISU, MISE, OR MIOT.

Litter samples. On the same dates that the aboveground samples are collected, a sample of litter should be collected, composited by replicate, ground to 20 mesh, and divided into two samples of 5 g each. One sample should be accompanied by NREL-60, requesting nitrogen, phosphorus, and ash determinations. The other sample should be accompanied by NREL-63, "Request Form for Diet or Botanical Composition Analysis." This form is explained in the Diet Laboratory section of this report.

Belowground samples. Three times during the year, *hand separated, unwashed* root samples should be collected. Take five or six cores per replicate to a depth sufficient to account for at least 90% of the root material in the soil profile. Divide the cores into 10-cm segments and composite the segments by replicate. Separate the root material from each composite segment, grind it to pass through a 20-mesh screen, and send a sample of at least 5 g from each segment to the Natural Resource Ecology Laboratory. Accompany these samples with NREL-60 requests for nitrogen, phosphorus, ash, total available carbohydrates, lignin, and CWC determinations. These samples should be taken at the start of the growing season, at peak standing crop, and at the end of the growing season.

Twice during the year, very clean, *washed* samples should be taken, segmented, composited, and ground as above. Accompany these samples with NREL-60 requesting an ash determination.

Special Samples

When submitting samples other than those discussed above, accompany samples with NREL-60 requesting the analysis you desire and indicating that these are "special samples." Also include a statement explaining the purpose, justification, source, project, desired priority, completion date, and any special handling required.

DIET LABORATORY

Analyses Available

Analyses of botanical composition and dietary analyses are performed by the Diet Laboratory using micro-techniques. A low power technique (10-80X) is used to identify fragments of arthropods. A high power technique (100X) is used to identify plant fragments.

The low power technique is employed on samples such as the stomach contents of small mammals. The stomach is dissected, and its contents are placed in a Petri dish which is marked in the center with an X. The material is agitated and the particle on the X is identified. This is normally repeated 40 times. This technique provides an estimate of the types and quantities of arthropods in the sample. The contents of small mammal stomachs are subsequently subjected to high power analysis.

High power analysis is used to estimate botanical composition of litter samples and to estimate proportions of various plant species found in dietary samples (e.g., stomach contents and fecal material). Fragments of arthropods cannot be identified using the high power technique.

Material to be examined under high power is ground over a 20-mesh screen. One or more slides are made from this material. A variable number of fields are observed on each slide. A field is an area of the slide delimited by the field of the microscope at the selected power of magnification. All fragments of epidermal material in the field are identified and recorded. A more complete description of this technique can be found in Hansen (1971). These frequency data are then used to estimate percent relative density and percent dry weight (Hansen, 1971; Sparks and Malechek, 1968).

Sample Preparation, Packing, and Shipping

Plant material to be analyzed for botanical composition should be dried at 60°, ground in a Wiley mill over a 20-mesh screen, and placed in a plastic vial labeled with the following information: *site, investigator's specimen or identification number, investigator's initials, treatment, and replicate*. Vials should be packed carefully in boxes for shipment to the Natural Resource Ecology Laboratory. *Do not send samples directly to the Diet Analysis Laboratory.*

Stomachs of small mammals to be analyzed for diet composition should be placed in plastic vials with 70% ethanol. Vials should be labeled as above and shipped to the Natural Resource Ecology Laboratory. Alternatively, stomachs may be dissected and the contents placed in the vial. In either case, the animal should be collected at the time it is feeding, and digestion should be stopped rapidly by freezing or by forcing 70% ethanol into the gut. For a more complete discussion see French (1971).

Samples of either type should be accompanied by a completed copy of NREL-63, "Request Form for Diet or Botanical Composition Analysis." This form supersedes the old NREL-60 for this purpose. On NREL-63, column 66 is labeled "Analysis." Completion of this item by the investigator is optional. If completing this item, use the following codes:

- 1 = botanical composition
- 2 = diet-microscopic, low power
- 3 = diet-microscopic, high power
- 4 = diet-microscopic, high and low powers

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U.S. INTERNATIONAL BIOLOGICAL PROGRAM

REQUEST FORM FOR DIET OR BOTANICAL COMPOSITION ANALYSIS

Routine Sample _____
Special Sample _____

IBP



Date of Request _____ Name of Investigator _____

Data Type	Site	Initials	Date Collected		Replicate	Treatment	Source	Non-taxonomic Identification	Class	Order	Family	Genus	Species	Sub-species	Sex	Age/Lifestage	Investigator's Specimen or Data Number	NREL Laboratory Number	Composite Sample	Number in Sample	Analysis	Comments	
			Day	Month																			Year
1-2	3-4	5-7	8-9	10-11	12-13	14	15	16-17	18-24	25-28	29-32	33-36	37-39	40-42	43-45	46	47-48	49-55	56-61	62	63-65	66	67-80
63																							
	Site																						
	01 ALE																						
	02 Bison																						
	03 Bridger																						
	04 Cottonwood																						
	05 Dickinson																						
	06 Hays																						
	07 San Joaquin																						
	08 Jornada																						
	09 Osage																						
	10 Pantex																						
	11 Pawnee																						
	12																						
	Treatment																						
	1 Ungrazed																						
	2 Lightly grazed																						
	3 Moderately grazed																						
	4 Heavily grazed																						
	5 Ungrazed current year only																						
	A Diet light																						
	B Diet moderate																						
	C Diet heavy																						
	D ESA - 0																						
	E ESA - W																						
	F ESA - N																						
	G ESA - WN																						
	Sex																						
	0 Unknown																						
	1 Male																						
	2 Female																						
	Composite Sample																						
	0 No																						
	1 Yes																						
	Age/Lifestage																						
	00 Undetermined																						
	01 Juvenile																						
	02 Sub-adult																						
	10 Adult																						
	20 Pupae																						
	30 Egg																						
	40 Nymph or Larva, early																						
	41 Nymph or Larva, middle																						
	42 Nymph or Larva, late																						
	43 Nymph or Larva, 1st																						
	50 Instar, 1st																						
	52 Instar, 2nd																						
	53 Instar, 3rd																						

Source

01 mouth & cheeks
02 esophageal
03 rumen
05 stomach
06 crop

08 caecum
09 colon
11 fecal
13 caches & stores
15 hand-clippings

16 hand-plucks
17 animal clips & wastes
18 mechanical harvested
19 litter & detritus
20 aboveground plants (live + dead)

21 roots (live + dead)
22 reproductive
23 standing dead
24 standing live
25 roots (live)

26 roots (dead)
27 crowns

Analysis

1 Botanical composition
2 Diet--microscopic, low power
3 Diet--microscopic, high power
4 Diet--microscopic, high and low power

Sample Handling at the Natural Resource Ecology Laboratory

Samples received at the Natural Resource Ecology Laboratory are logged in, assigned an NREL lab number, and if NREL-63 is complete, sent to the Diet Lab for analysis.

A computerized system to store, retrieve, and manage these data is under development and should be completed by July 1972.

Samples Required

Litter. Six times per year, when aboveground samples are collected, litter should be collected, composited by replicate, and ground to 20 mesh. At least 5 g of this material per replicate should be sent to the Natural Resource Ecology Laboratory accompanied by NREL-63, requesting analysis of botanical composition.

Small mammal stomachs. Stomachs or stomach contents of up to 10 individuals of each species should be collected on each small mammal sampling date, prepared as explained above, and sent to the Natural Resource Ecology Laboratory accompanied by NREL-63. *Do not composite these samples.*

SOIL TESTING LABORATORY

Analyses Performed

A variety of physical and chemical analyses are available at the Colorado State University Soil Testing Laboratory. At present, the laboratory provides us with the following analyses on a contract basis: texture; pH; organic matter; exchangeable Ca, Mg, Na, and K; cation exchange capacity, soil water retention curves; ammonia; nitrate; and bicarbonate phosphorus.

Sample Preparation, Packing, and Shipping

After collection soil samples should be air-dried slowly in a pan or other suitable container in a clean, warm room free from contaminating dusts and gasses. Samples intended for NO_3 analysis should be air-dried immediately to prevent further loss of NO_3 . If necessary, other wet samples may be stored in plastic bags in a refrigerator for 1 or 2 weeks before drying.

The Soil Testing Laboratory recommends grinding samples to pass through a 10-mesh (2-mm) screen. If you do not have grinding facilities, this will be done at the Natural Resource Ecology Laboratory. Mechanical grinding which reduces the mineral crystals to a fine powder should be avoided. Such grinding releases nutrients held in minerals which were probably unavailable to plants.

Soil samples should be placed individually in the paper soil-sample bags sent to each site. Bags should be packed in cardboard boxes for shipping to the Natural Resource Ecology Laboratory. Take care in packing so that bags will not break or come open during shipping. *Label each sample bag with site, investigator's*

initials, investigator's specimen or data number, treatment, and replicate.

Accompany each sample or group of samples with a completed copy of NREL-61, "Request Form for Laboratory Analysis of Soils." Use the new, yellow version of this form, not the old raspberry colored version. Copies of the old version should be destroyed. Samples collected pursuant to this report should be shown as "regular samples" on NREL-61. Other samples for which analyses are requested should be shown as "special samples" and must be accompanied by justification.

Sample Handling at the Natural Resource Ecology Laboratory

Soil samples and the accompanying request form are checked when they are received at the Natural Resource Ecology Laboratory. If the samples appear adequate and undamaged and if the form is properly completed, they are logged in and assigned an NREL lab number.

A modification is being made to the Grassland Laboratory Information Management System (GLIMS), so that requests for and results of soil analyses can be entered into that system. Information on the request form will be keypunched and entered into the system. GLIMS will produce a work and result form which will be sent with the samples to the Soil Testing Laboratory. Analysis results will be recorded on these forms at the Soil Testing Laboratory, and they will be returned for keypunching and inclusion in GLIMS. For a further discussion of GLIMS, see the section on the Analytical Laboratory in this report.



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REQUEST FORM FOR LABORATORY ANALYSIS OF SOILS

Routine Sample _____
Special Sample _____

Date of Request _____ Name of Investigator _____

Data Type	Site	Initials	Date Collected			Treatment	Replicate	Series Name	cm to Top	cm to Bottom	Horizon	Investigator's Specimen or Data Number	NREL Laboratory Number	Analyses Requested															
			Day	Month	Year									Exchangeable Ca	Exchangeable Mg	Exchangeable Na	Exchangeable K	pH	Cation Ex. Cap.	Tension Curve	Texture								
1-2	3-4	5-7	8-9	10-11	12-13	14	15	18-24	25-27	28-30	31-32	33-36	39-44	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
61																													
Site 01 ALE 02 Bison 03 Bridger 04 Cottonwood 05 Dickinson 06 Hays 07 San Joaquin 08 Jornada 09 Osage 10 Pantex 11 Pawnee 12																													
Treatment 1 Ungrazed 2 Lightly grazed 3 Moderately grazed 4 Heavily grazed 5 Ungrazed current year only A Diet light B Diet moderate C Diet heavy D ESA - O E ESA - W F ESA - N G ESA - WN																													
Horizon 1 A0 2 A 3 B 4 C																													

Samples Required

The following soil samples are required from all Grassland Biome sites for two types of soil analyses.

Descriptive soil analyses. Samples for descriptive analysis need to be taken at one time only.

On each replicate take (i) three samples from the 0- to 5-cm layer and composite them, (ii) three from the A horizon and composite them, and (iii) three from the B horizon and composite them. Record the depth to the top of the B horizon on NREL-61. The composited samples should weigh at least 150 g and preferably 1 lb. Ship these samples to the Natural Resource Ecology Laboratory with NREL-61 requesting analysis of texture and pH.

In addition, take three cores per replicate to a depth of 60 cm. Divide the cores into 10-cm segments and composite by depth within each replicate. Request analysis for exchangeable Ca, Mg, Na, and K; total N and P; cation exchange capacity; and soil water retention curves for these samples.

Dynamic soil analyses. Whenever root samples are taken, take at least three cores per replicate to the same depth as the root cores. Divide these cores into 10-cm segments, composite them by depth within replicate and send 400 g from each composited sample to the Natural Resource Ecology Laboratory. Accompany these samples with NREL-61 requesting analysis for ammonia, nitrate, and bicarbonate phosphorus.

LITERATURE CITED

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