Saskatchewan at Saskatoon where he specialized in entomology. His doctoral degree in 1961 at Glasgow University in Scotland was also in entomology. For 13 years following his undergraduate degree, Zacharuk was employed as a research scientist with a research branch of the Canadian Department of Agriculture on their Prairie Research Station. Since 1963, he has been professor and chairman of the Biology Department of the University of Saskatchewan at Regina. He was coordinator for consumer research in the Canadian IBP Matador Grasslands Project. Thus, the authors of Chapter III represent producer, consumer, and decomposer viewpoints toward the design of research studies and the establishment of research teams.

F. H. Bormann and G. E. Likens have been successful in applying ecosystem concepts to studying nutrient cycles on forests and watersheds in the Northeast. This team, too, represents different disciplines. Bormann received his undergraduate degree in botany from Rutgers University in 1948 and his doctorate from Duke University in 1952. Subsequently he has served as assistant professor at Emory University, professor of biology at Dartmouth College, and, since 1966, professor of forest ecology at the School of Forestry at Yale University. His major interests have been experimental ecology of plants, especially the pines, and ecosystem dynamics. Likens received his undergraduate training in biology at Manchester College in 1957; subsequently his master's and doctorate degrees were obtained, respectively, in 1959 and 1962 in zoology at the University of Wisconsin. His major interests are concerned with nutrient cycles from a biochemical approach and circulation and heat budgets of lakes and lake sediments. Their intriguing ecosystem study, described in Chapter IV, is another example of a team effort needed for comprehensive field research. Several other investigators, representing different agencies or institutions, have also been involved in this highly coordinated study.

A. M. Schultz brings to Chapter V the rationale, design, and some results of a long-term study of the arctic ecosystem. These studies, too, have involved persons of varied disciplines and have focused in recent years on nutrient cycling studies as an aid to the unraveling of the age-old problem of fluctuations of arctic microtine populations. Schultz has training in both plant and animal ecology. His bachelor's degree and master's degree were in zoology and animal ecology, respectively, at the University of Minnesota. He, too, obtained his Ph.D. under J. E. Weaver at the University of Nebraska. Since his doctorate degree, Schultz has held various positions in the School of Forestry at the University of California. His work and interests have covered grassland, brushland, forest, and tundra ecology. His present teaching is concerned primarily with ecology and the introduction of ecosystem concepts to students from a diversity of fields.

Chapter III Procedures for Study of Grassland Ecosystems

*R. T. COUPLAND, R. Y. ZACHARUK, and E. A. PAUL**

I. INTRODUCTION

The concept of the ecosystem has been employed by biologists for several decades as a teaching technique to emphasize the interdependence of various strata of organisms within the same habitat and their relation to the environment (Odum, 1959). Intriguing hypotheses have been developed and theoretical models constructed to explain these relationships (Olson, 1964). However, research on ecosystems has not yet provided sufficient data to test these models.

The efficient management of renewable resources depends on a knowledge of the interrelations of organisms at various levels of activity and

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their relationships to abiotic subsystems. Most of our understanding of terrestrial ecosystems is limited to the relationship of a dominant plant species or vegetation to an edaphic situation, of a consumer to vegetation, or of one consumer to another. This knowledge has been applied to the management of systems in which such pairs of components exist, with the assumption that for each system the significant relationships have been studied. Perhaps we have been satisfied with this situation, because it was felt that research facilities were not available for more comprehensive studies.

The complexity of ecological systems dictates that a highly organized and integrated systematic approach be applied to their study. This chapter is devoted to a consideration of the procedures used and some of the problems that can be encountered in the organization and initiation of an integrated study of a grassland ecosystem.

II. AIMS OF AN ECOSYSTEM STUDY

The ecosystem approach is particularly well suited to the study of the biological basis of productivity that is being conducted under the International Biological Program (IBP). The objective in this instance is to develop a model of energy flow and nutrient transformations. Quantitative measurement is a preliminary aim, but will lead rapidly into the study of processes. Both are necessary for an understanding sufficient to synthesize a model that approaches reality and that can be tested by examining the effect of manipulation of components on the function of the system as a whole. Such studies are underway in a grassland at a field station established in 1967 at Matador, Saskatchewan, by the Canadian Committee on the International Biological Program (CCIBP) (Fig. 1). In the United States, in a program initially planned by the National Committee for the IBP, the concentrated effort is at the Pawnee Site in northeastern Colorado. Studies were initiated in 1968. This chapter is concerned primarily with the approach in the Matador study.

While it is of considerable interest to observe the magnitude of the pathways of energy and of nutrients through a single ecosystem, an important consideration in studying grassland is the comparative aspect. Interpretation of data relative to metabolism and nutrient transformations will require experimental manipulation and comparison of ecosystems that are basically similar. Such experiments are essential also in understanding processes. At first it will be necessary to manipulate one major factor at a time. In the Matador Project, manipulation initially will be through provision of different types of vegetative cover.

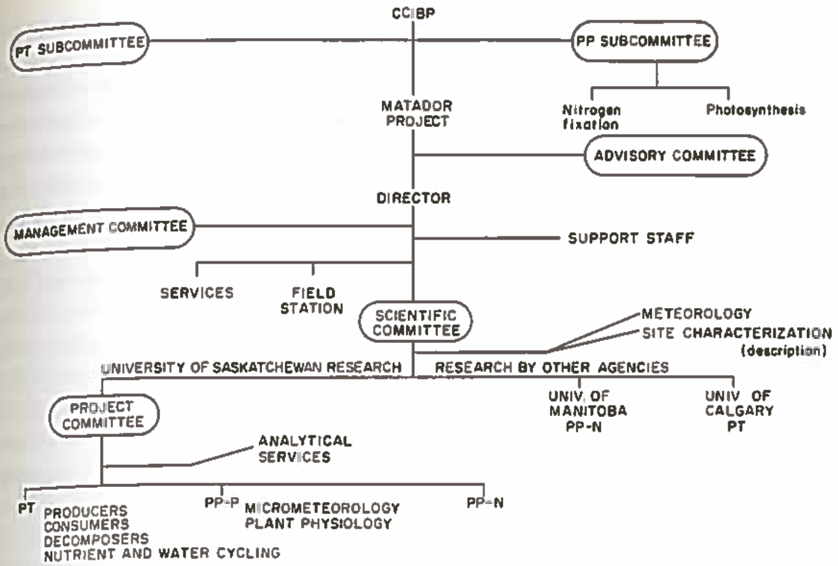

FIG. 1. An organization chart for the Matador Project, the grasslands study in the Canadian International Biological Program. CCIBP = Canadian Committee for the IBP; PT = Productivity Terrestrial; PP = Production Processes.

III. CHARACTERISTICS OF A STUDY SITE

The choice of a suitable location for such a study must be considered in relation to the objectives that have been defined. If only one ecosystem is to be studied, then emphasis must be given to homogeneity. If more than one ecosystem is involved, the comparisons that are to be made must be considered with respect to biotic or abiotic influences. If the project aims to compare ecosystems differing in biotic populations, then it is important that they be located in the same soil and climate. It is also important that each ecosystem be sufficiently extensive to permit the separation of biotic populations and environmental influences. The degree to which these principles can be followed depends upon the nature of the ecosystems involved. For example, a great difference in the height of vegetation may complicate the comparison of herbaceous and forest areas. Where cultivated and natural ecosystems are compared, the extent of the latter must be sufficient to provide a buffer zone that will assure isolation.

In the Matador study it was decided to compare cultivated and natural herbaceous ecosystems in an unforested region where tillage did not begin until the present century. The criteria that were established for site selection stressed the need for (1) a large enough area of relatively homogeneous native grassland that had not been modified much by agriculture and with a buffer zone of sufficient extent to provide a high degree of future protection; (2) absence of physiographical features that would affect environmental or biological characteristics of the site; (3) cultivated crops that had been grown on the same soil and physiographical type (as the native grassland) for at least 20 years within a reasonable distance of the natural area; and (4) reasonable proximity to a scientific establishment. The site that was selected is judged to fulfill these requirements and has the added advantages of being located in an area of soil highly suited for crop production and being representative of the most extensively cultivated situation in the region (Mitchell *et al.*, 1944). Initially 500 ha (1250 acres) of the natural system under study will be controlled. Another 2500–3000 ha (6200–7500 acres) of the same system, which protects the site from encroachment by tillage, is available for studies requiring larger sampling areas than are available on the controlled site. This allows for future manipulations.

The choice of site may be criticized because of emphasis on the natural ecosystem, when artificial ones also are to be studied. The agronomist may suggest that consideration be given to finding a convenient location for study of the arable ecosystems, with the expectation that a comparable natural system will be available nearby. Such a procedure will almost inevitably result in the choice of a natural system that has been avoided by tillage because of an unfavorable topographical or edaphic feature. The alternative is to seek out a natural system that survived within a tilled region because of some unusual historical development. The resulting choice then, of course, will be open to criticism as a relict not representative of any appreciable existing area of natural vegetation. However, it will represent conditions that previously existed in tilled land. The Matador site is the last known, large, surviving area of grassland that previously existed in 36% of the land that is now tilled in the brown soil zone of Saskatchewan. The choice of such a rare, natural system can be supported further in that, for economic reasons, it is less liable to future survival than more plentiful natural systems that have been avoided by tillage. Therefore, its study should not be delayed.

IV. ASSEMBLING THE RESEARCH TEAM

To assemble a sufficient number of competent and enthusiastic scientists knowledgeable of grassland from the required disciplines to conduct an ecosystem study is a major undertaking. It is almost certain that such

workers are already engaged in full-time activities and are distributed among a number of university and government research groups. Even if funds were available to support them on a long-term project offering tenured positions, it is unlikely that a sufficient number of them could be attracted. The alternative is to encourage and facilitate their part-time participation.

In the Canadian IBP grassland study, we found that the direct approach, with a view to attracting specialists from various research groups before a plan was prepared, was not successful. In addition to being fully involved with their own programs, they were individually of the opinion that group research is not sufficiently rewarding to the individual or that adequate funds to support such a complex study would not be available. This approach, therefore, was abandoned. The alternative was found to lie in a request for assistance in the production of a hypothetical plan. The resulting discussions indicated not only the feasibility but also the applicability of the ecosystem approach to the investigator in his own field. When the project became a reality, the potential participants had the required background and enthusiasm to become thoroughly involved in the project.

The success of an ecosystem study will depend in large measure on the degree to which participants operate as a team. Individual initiative must be encouraged within a framework that includes sharing of basic data and collection of data, at least partially, as a service to others. The activities that are of greatest value to the group may be routine for the individual. However, opportunity also must be provided for related studies in which the individual may make more independent contributions.

Some full-time professional associates will be desirable in key positions to work in collaboration with the part-time participating specialists and to assure continuity of study. The need for support in different areas of activity will depend on the extent of participation of the specialist and on the degree of dependence of others on the data from that portion of the project. For example, meteorological measurements are of importance to almost every participant. They thereby warrant full-time professionals responsible to the project as a whole. Obviously coordination of activities is an important requirement at all levels and support staff will be needed for this purpose.

In a project of this type there will be a number of workers who wish to participate, but who are not attuned to working on field projects. Substitution of laboratory projects for essential field projects must not occur. Where laboratory studies are essential, these should not be divorced from field participation. This is a basic requirement to an interpretation of data collected in the real world. Another danger is that difficulties in recruitment may result in the decision to fit the project to the readily available scientific pool. It seems unlikely that a total ecosystem approach would be successful under these circumstances.

The total number of workers involved in an ecosystem study is likely to be considerable (see Fig. 1). In the Matador Project it is expected that the number will be between 80 and 100. Of these, about 30 will be professionals (usually Ph.D.'s), 20–30 will be graduate assistants, and the remainder will be summer assistants (4–5 months) and technical assistants.

V. PREPARING THE SCIENTIFIC PLAN

The plan must be produced by the people who will do the work. Moreover, participation in an international program, such as the IBP, requires that it must conform to agreed standards. We have been able to reconcile these matters in Canada by providing for planning within the project, but with the guidance of minimum standards that have been set up by IBP working groups. Planning, as well as research, must be coordinated; consequently, each participant should consider the plan of every other participant. In the Matador Project this is done through membership on the Scientific Committee (Fig. 1).

The participants will give much thought to the kind of research that should be conducted. In the beginning this will seem to include all disciplines of biological and environmental research. However, consideration of needs in relation to the theme (e.g., productivity) of a particular study will keep its scope within reason. The success of the activities related to this theme will depend largely on the extent to which erosion of their support can be avoided by inclusion of unrelated research.

Some difficulty will be experienced in planning because of differences of opinion concerning the intensity to which each aspect of the study should be conducted. Uniformity of treatment is vital. No guide can be given as to how uniformity in this respect can be achieved. It is a matter for frequent review by the participants throughout the period of study and for continuous consideration by the coordinators. Inevitably funds will become limiting, so pressure will always be present for uniform intensity of treatment. The decision concerning the number of ecosystems among which to share the funds may involve a consideration of whether all ecosystems will be studied in equal depth, but care should be taken to assure that all investigators apply the same priorities.

At all biotic levels in the ecosystem it will be possible to consider the activities of individual species only when these comprise a major proportion of the activities within a biological subsystem. This is most likely to

be possible with the dominant producers and vertebrates, much less with the invertebrates, and probably not at all feasible with microorganisms. Consequently, this is not a feature upon which uniformity of treatment of different groups can be based.

Intensive planning by each interest group is necessary to consolidate and coordinate the plans of individual researchers, but integration of research into a master framework will only be possible by intensive intergroup discussions. The attendance in these sessions of chemists, data processors, and systems analyists will increase the effectiveness of the plan.

A particular problem with priorities may be experienced in attempting to keep the abiotic studies in balance with the biotic ones. Studies of the soil system have progressed much further than those of the total biotic system, and procedures are already well established for the former. Consequently, the soil workers are in a position to offer an initial plan that is complete; however, the biologists, who are working within a new framework, will find that additions to their plan are required as the study proceeds. As a result, if the full support is allocated at an early stage, treatment of the organismal portion of the system will suffer.

The project will seek to develop a model. Consideration in planning must be given from the outset to make certain that all significant information is collected to produce that model. It seems essential that the system be analyzed theoretically at an early stage to provide for this. However, opinions vary concerning the effectiveness of detailed system analysis before much preliminary data have been accumulated. At this time, opinions range from the desirability of developing a detailed theoretical model in the planning stage to that of working with a generalized model until some data have been collected. As knowledge of ecosystems increases, the feasibility of early analysis presumably would increase.

VI. ORGANIZING THE RESEARCH

In a productivity study it seems reasonable to organize the activities into four subsystems: producers, consumers, decomposers, and abiotic components. Superficially this seems to subdivide, respectively, green plants, animals, and microorganisms from environmental factors. However, this separation will be modified in application. Using microorganisms as an example, the decomposers will probably be investigated by the same personnel using the facilities that are employed in studies of nondecomposers (e.g., algae and nitrogen fixers) and parasites of producers and consumers. Similarly, while measurements of energy fixation and carbon assimilation by producers may be made by both biomass and photosynthesis approaches, these two activities will be less closely related than those of the physiologist and the micrometeorologist who will need to share equipment. Consequently, the organization of the activities at Matador (Fig. 1) consists of (1) biomass studies of producers by vegetationists; (2) consumer research by entomologists, ornithologists, and mammalogists; (3) microorganism research by bacteriologists, mycologists, and algologists; and (4) abiotic research by physicists, pedologists, micrometeorologists, chemists, and physiologists.

The following discussion of research procedures in a productivity study is concerned primarily with the organismal portions of the system. The methodology and concepts concerning the abiotic portion have been reasonably well developed elsewhere.

A. Producers

In a productivity study, research on producers must provide a reliable estimate of the amount of input (energy and carbon) into the system. This is important to interpretations of various other data. If methodology was sufficiently developed, gross measurements by physical or physiological techniques could be depended upon to furnish this estimate. At present, however, it seems necessary to use, as base measurements, net values obtained by biomass (harvest) studies. However, gross productivity measurements of CO_2 and energy by physicists using CO_2 flux and energy transfer techniques may be useful to the biomass determinator in considering the reliability of his data; these are considered together in a later section. The acquiring of accurate primary production data will provide a basis for comparison of the photosynthetic efficiency of various kinds of vegetative cover.

In the following discussion, the measurement of production by producers in terms of weight of plant tissue has been stressed, since past measurements of this parameter in herbaceous crops have only recently been shown to be deficient. Conversion of these measurements to calorific values is assumed, but other measures of quality also are desirable to provide reasonable comparisons between ecosystems. Carbon and nitrogen analyses usually will be minimum requirements. Westlake (1963) has discussed exhaustively the methods and standards to be applied and the means of presentation of comparative production data.

1. STEMS AND LEAVES

The main source of information concerning the net productivity of herbaceous vegetation is an estimate of the standing crop of stems and

leaves at the end of the growing season. This measure has serious shortcomings. Let us consider in detail some of the problems that are experienced in measuring net productivity in herbaceous communities.

The peak standing crop does not always occur at the time when growth appears to have been completed. For example, Wiegert and Evans (1964) report that by clipping 20 times during one season the peak standing crop of aboveground parts in a Michigan old field was found to be 13% greater than the value at the time of cessation of growth (270 g/m^2 vs 238 g/m^2).

The peak standing crop and the net primary production are identical only when the vegetation is composed of individuals that stop growing at a single instant in time (Wiegert and Evans, 1964). Thus, the peak standing crop might be expected to be a reasonable method of estimating the yield of an annual crop which germinates and develops uniformly or of a perennial grass sward consisting of one species, but it cannot be validly applied to a mixed stand of species (such as native grassland) that grow at different periods. The peak standing crop must be obtained for each species, if this method is to estimate accurately production in a mixed stand. Wiegert and Evans (1964) obtained a 26% higher estimate of peak standing crop in prairie by separation into only three categories (340 g/m^2 vs 270 g/m^2). This value was 43% above the value (238 g/m^2) that would have been obtained by a single clipping at maturity. These factors do not appear to have been considered in comparisons of the yield of native grassland with seeded, introduced grasses (Smoliak *et al.*, 1967) and with annual crops (Ovington *et al.*, 1963).

One of the major criticisms of the practice of equating total annual growth with peak standing crop of the current season is that mortality occurring during the sampling period is not measured. Because grass grows and dies continuously throughout the season, the error introduced by replacing annual growth with peak standing crop is greatest where grass (as opposed to forbs and shrubs) forms a high proportion of the cover. Wiegert and Evans (1964) used the following relationships to estimate mortality in Michigan:

(a) Change in standing crop of green material = growth − mortality
(b) ∴, Growth = change in standing crop of green material + mortality
(c) But mortality cannot be measured directly
(d) However, change in standing crop of dead material = mortality − material disappearing
(e) ∴, Mortality = material disappearing + change in dead standing crop

They measured change in standing crop of dead material (standing dead and litter) and rate of disappearance during 14 periods in 1959 and during 10 in 1960. Calculations indicated that growth was 2.4–2.5 times peak standing crop (on the basis of 10 and 8 samplings of green material) on upland and 4.5–4.9 times in swales. This disparity is probably much greater in prairie than in cropland (where one fast-growing species is harvested at the time of its maximum standing crop). It should be noted that, in a system that has reached equilibrium, the amount disappearing during the entire year should equal the net annual growth. In the present case, mean standing crop of dead material (195 g/m^2) × annual instantaneous rate of disappearance (1.72 g/g/year) = 335 g/m^2.

Repeated harvesting of the same plots is possible in stands of herbaceous plants, but it will provide a different result than if a sequence of plots is used. If the purpose is to measure the capacity of undisturbed vegetation to fix energy, then a sequence of plots must be used, with sampling becoming a greater problem. In order to avoid the possible differential effect, in subsequent years, of harvest at different seasons, the plot locations must be moved from year to year. This would seem to be the proper procedure to use in a project where the purpose is to compare the productivity of native grassland with a cereal crop or a seeded perennial hay crop. In ecosystems where harvest of grazing animals is the ultimate objective, comparisons are more legitimate if harvests are made at intervals on a sequence of plots that have been protected from grazing only for the period since the previous harvest, but the same plots may be sampled from year to year.

Measurements of primary productivity must be related to the proportion of shoots that are being removed by consumers. In a project that aims to compare the rate of production of native grassland and a cereal crop, it would seem logical that the native grass should receive the same amount of protection from herbivores during growth as does the crop. Since a considerable proportion of the energy fixed is removed from the cropland at harvest, consideration should be given also to the removal of a portion of the matured stems of the grassland. However, it would seem inadvisable to place the grassland under continuous grazing by domesticated livestock in such a comparison, for the resultant data would then involve a comparison between primary productivity in the crop and primary plus secondary productivity in the grassland.

2. UNDERGROUND PARTS

Another major shortcoming of many productivity studies is the failure to account for the photosynthates that are transferred to roots, rhizomes, and stem bases below the level of clipping. There is not great difficulty in sampling to obtain weights of these structures, but for perennial species it is difficult to make reliable estimates of annual increment. A minimum estimate has been obtained (Wiegert and Evans, 1964) by finding the difference in weight of underground parts between the high and low values for the year (143 g/m^2 for upland, 358 g/m^2 for swales). A more refined method has been to divide the soil profile into layers and to determine an increment for each layer, which may be different for different periods. Dahlman and Kucera (1965) obtained a value of 510 g/m^2 for Missouri prairie using 3 layers (452 g/m^2 when corrected for mineral matter) or 452 when the profile was taken in its entirety and not corrected, giving about 13% increase by profile division. Kucera *et al.* (1967) have attempted to separate underground parts of the current season from those of previous seasons and have obtained a similar value for production in the same prairie in the same year (179 g/m^2 of rhizomes and 369 of roots = 548 g/m^2 total). In both of these studies (Michigan and Missouri) the rate of decay of underground material approximated 25% per year. In an Indian study (Singh, 1967), biomass determinations were confined to the upper 30 cm of soil, being justified on the basis of the high proportion (95% or more) of the underground plant parts occurring near the surface.

B. Consumers

The studies of consumers should measure quantitatively and qualitatively: (1) the intake of primary production by herbivores (primary consumers); (2) the proportional transfer of this intake to carnivores (secondary consumers) and from all consumers to the environment as excreta, secreta, and dead organic materials; and (3) the losses from the ecosystem through consumer metabolism. A tremendous and costly task presents itself if the relations within the ecosystem of all consumer and trophic levels are to be considered. Establishment of priorities and levels of intensity for research will be necessary for each system.

Of primary importance is intensive research on herbivores, but this may have to be restricted to major or key species only. It may be necessary also to study intensively one or two key species of carnivores, if these may drastically affect intake of primary production through predation on herbivores. Also of primary importance will be an accurate estimation of standing crop of all consumer species within the ecosystem, minor as well as major, even if no initial, detailed research of their productivity or energetics is possible. Omnivorous animals, such as certain soil invertebrates, will present special problems in proportioning their activities as herbivores, carnivores, and decomposers. Portions of the studies on such animals will require close integration with the studies outlined in the following section on microorganisms. Thus, major animal groups that will require investigation in all grasslands are invertebrates (particularly selected insects and perhaps spiders), mammals, and birds.

A preliminary survey of the fauna in each ecosystem will be necessary to obtain a general idea of number and abundance of species in each group and at each trophic level. From this survey, the key species can be identified for initial, intensive study. Representative specimens of each species, perhaps three of each sex, should be preserved appropriately at this time for a reference collection. This survey undoubtedly will be concentrated on the study site. It is desirable, however, to include an appropriate zone surrounding the study site in an initial survey. Future problems involving wide-ranging species that visit the site sporadically, such as ungulates, and migrations to the site of animals with population centers nearby, such as grasshoppers, could thus be defined in the planning stage.

Faunal studies will be required above, at, and below the soil surface. Individual animal species will live in one, two, or all three layers at the same time, at different times, or in different life stages. In a systems study such as the Canadian one at Matador, where soil and aboveground invertebrates are being investigated by different groups of workers, careful integration and coordination of effort is imperative for completeness of coverage and accuracy. Further coordination of studies on soil animals, soil microorganisms, and primary productivity (root systems) is desirable and may even be necessary in such operations as sampling procedures and determinations of soil metabolism.

Primary productivity in grasslands could be appreciably affected by the feeding activities of sucking insects such as aphids and plant bugs. The effect, through removal of primary production by such consumers, may be minor in comparison with their effect on primary production processes. Some investigations on the total effect, while not without difficulty, should be initiated. Desroches (1958), Andrzejewska (1961), and Ricou and Duval (1964) evaluate the importance of, and give some methodology for, such studies in grasslands.

1. Standing Crops

Estimates of standing crops are required, not only for determinations of productivity in terms of biomass (weight/unit area), but also for relating to the system data from all subsequent studies on population dynamics and energetics (calories/unit area). Accuracy in the estimation of standing crops is, therefore, basic to the design of the model for the energetics of the system. Suitable quantitative sampling procedures and

techniques for recovery of animals from samples must be devised for each ecosystem. Size, number, and pattern of samples must be considered in relation to type of soil, relief, vegetation, and other factors affecting animal distributions. The need for information on seasonal and annual fluctuations and age and stage structure of the populations also will require appropriate, periodic sampling programs. Recovery of the organisms during surface sampling or from soil samples could introduce further substantial errors in standing crop estimates, particularly in invertebrate studies. In the Matador Project the "quick-trap" method of Turnbull and Nicholls (1966) with vacuum suction recovery will be used for aboveground insects. Soil invertebrates will be sampled by a hydraulic core sampler, or the power-operated sampler of Burrage *et al.* (1963), with recovery to be devised for the heavy lacustrine soils using soil washing, temperature differential funnels, and/or hand-sorting techniques.

Researchers on consumers must consider the possible effect of sampling on reduction of populations, directly through removal of individuals, or indirectly through removal of breeding stock. Presumably this problem will increase with the size of the animal and its reduced numbers (the concentration of standing crop in a few large individuals). It may also arise in small organisms, such as wireworms or grasshoppers, if removal through intensive sampling coincides with a periodic concentration of breeding individuals. Such destructive sampling must be restricted to areas where other subsystems are not being studied.

2. Population Dynamics and Energetics

In order to interpret data on standing crops in terms of rate of transformation of products of photosynthesis, detailed studies will need to be made on production through growth and reproduction, food ingestion, excretion, secretion, defecation, and metabolism. These would be expressed as caloric contents or losses. The energy budget for any consumer could be defined as:

$$I = Pg + Pr + E + M$$

where I = caloric equivalent of material ingested, Pg = production through growth, Pr = production through reproduction (Pg and Pr expressed as caloric equivalent of protoplasm produced), M = metabolic energy or heat loss through respiration and intraorganismal conversion, and E = caloric equivalent of material egested through secretion, excretion, and defecation.

Assimilation (A), the caloric equivalent of the material retained by an organism, could be defined as $A = Pg + Pr + M$. Secondary productivity, the rate of production per unit time, would be: $(Pg + Pr)/T$. From the information derived from such studies, the assimilation/ingestion ratio (A/I) and the secondary production/assimilation ratio $(Pg + Pr)/A$ also could be calculated. The former is a measure of the efficiency with which a consumer extracts energy from the material ingested, and the latter measures the efficiency with which the energy assimilated by the consumer is transformed into products useful as energy sources for other organisms in the ecosystem.

The studies on growth and reproduction will provide a basis for conversion of the data on age and stage structure, derived through sampling for standing crop, to a clearer understanding of population dynamics of each species selected for detailed investigation. Measurements of ingestion will require studies on food preferences and rate of intake of different types of food by age, stage, and season. Caloric measurements on materials excreted, secreted, and defecated will indicate the energy that is ingested and not utilized by the consumer, but passed on for use by other organisms in the system. Energy lost to the ecosystem through consumer metabolism may be calculated by $M = I - Pg - Pr - E$. However, direct measurements of metabolic rates also should be made for greater accuracy in the calculation of energy budgets. While respirometry will give a measure of energy losses through respiration, adiabatic calorimetry will also be necessary if the energy loss through intracellular interconversions (e.g., fats to carbohydrates to proteins) are to be measured.

In most of the above studies, rearing in confinement will be necessary, but this should be coupled with field studies wherever possible. Such field studies can be accomplished through enclosures and/or exclosures in some instances. In other instances a taxon may be so active in comparison with other consumers, that food intake may be measured directly from clipping of herbage without manipulation of population. Radioactive tracer techniques are applicable to some food intake studies. It will be necessary to relate many of the above measurements to different temperature and activity regimes.

Budgetary, space, or manpower restrictions will limit studies on population dynamics and energetics of many minor species of consumers. Where such information is available in the literature for the species in question, or a related species, even if from a different ecosystem, it may be applied to the standing crop data that will be obtained to increase the accuracy of estimation of total energy budget for the system under study.

Many details on concepts and methodology for researchers on consumers in grassland ecosystems are available in the proceedings of the Symposium on Secondary Productivity held at Warsaw in 1966. Noteworthy are those by Macfadyen (1966), Petrusewicz (1966), and Wiegert and Evans (1967), among others.

3. PREDATION AND PARASITISM

The essential need to stress herbivore activities in a study of a grassland ecosystem will limit the resources available for research on carnivores. The importance of the latter in the system, however, should be under continuous surveillance. In some situations, herbivore and predator and/or parasite relationships may be so important that it will be necessary to establish a high priority for their more intensive study.

C. Microorganisms

Microorganisms, because of their small size and diversity, present a special challenge to the ecologist. Except for specialized studies, such as those of the rhizosphere (Macura, 1965; Rovira, 1965) and root nodule formation (Stewart, 1966), microorganisms have usually been studied with reference to a specific chemical reaction they carry out. The organisms that have received the most study do not constitute a significant portion of the microflora present in the soil–plant system at any one time (Burges, 1958). The role that the general soil population plays in energy transformations, in nutrient cycling, and in the breakdown of pollutants makes it imperative that microorganisms be studied in the detail accorded larger plants and animals.

The contributions that are expected of a microbiologist in a productivity study of grassland involve measurement of the degree of activity and functional role of microorganisms in energy flow and nutrient transformations. The methods developed for the study and the knowledge gained will be applicable to many other systems, for microorganisms tend to be ubiquitous in nature. Research in this area is complicated by the difficulty in the separation of the kinds of organisms present and in the determination of their biomass. Because changes in activity of the soil microflora take place after sampling, the samples must be utilized immediately, preferably in a field laboratory, as close to the point of sampling as possible.

1. INITIAL SURVEYS

The microbial population occurs in the soil, on the surfaces of, and within, the tissues of producers and consumers, and in the excreta of consumers. Microbial communities differ both qualitatively and quantitatively among these discontinuous microhabitats (Garrett, 1956; R. M. Jackson, 1965).

Sampling for microorganisms on a microhabitat basis is similar to that already described for soil animals, in that seasonal effects and vertical distribution will be measured using soil samples obtained with hydraulically operated soil samplers. In the case of microorganisms, sampling to the depth of root penetration is necessary.

The microhabitats to be considered are plant litter, rhizosphere, phylloplane, animal excreta, living animals, and animal remains. Detailed characterization of the taxa constituting these populations is probably impossible, even with a fairly large group of specialists working in their own field. However, concepts such as those utilized in numerical taxonomy (Brisbane and Rovira, 1961) and biochemical ecology (Alexander, 1964), plus the traditional classification procedures, should make general estimates feasible. The saprophytic fungi, actinomycetes, and bacteria, as well as the parasitic fungi and bacteria, will have to be considered in some detail. The protozoa and chemo- and photoautotrophs, if they are not covered in the producer or consumer groups, should be considered.

The goal of the initial surveys will be to estimate the relative importance of these major groups in terms of numbers and biomass in order to determine the microbiological homogeneity of the ecosystem. Synecological studies provide a means of assessing homogeneity and background information for studies of carbon and nitrogen turnover.

Photosynthesis and nitrogen fixation are being stressed in the current IBP program. Nitrogen fixation and the input of available nitrogen into the system by abiotic means, such as rainfall, are some of the major factors affecting both the quantity and the quality of primary productivity in many grassland areas.

The extent of nitrogen fixation by asymbiotic and symbiotic microorganisms is to be measured under field and laboratory conditions. Isotopic labeling (^{15}N) work is slow and expensive. The acetylene reduction technique, if used with appropriate standardization, may prove to be a very useful tool (Stewart *et al.*, 1967), both in assessing the level of field fixation and in laboratory studies. The sensitive ^{13}N technique also can be used for laboratory investigations.

2. Biomass Measurements

Measurements of microbial biomass are essential to an understanding of quantitative changes associated with the cycling of carbon and other nutrients. Some techniques are available for estimating the standing crop of microorganisms in soil samples, but few are available for assessing microbial cell production and turnover in a particular microhabitat. Methodology investigations must, therefore, continue to play an important role in the overall research design. Parkinson (1969) has recently reviewed the techniques available for estimating the biomass of microorganisms.

Conversion of microscopic and plate counts to biomass can be made only after chemical characterizations of the organisms. This, in turn, necessitates culturing the organisms in a simulated soil environment. Calorific and carbon and nitrogen measurements are of importance. Special components such as RNA, DNA, specific enzyme fluorescence, and diaminopimelic acid (el Shazily and Hungate, 1966) may provide effective complementary measurements (Rotman and Papermaster, 1966; Skujins, 1967).

3. Decomposer Cycle

Measurements of respiration in the field are necessary to interpret energy flow. The equation that was described above for consumers cannot be applied directly to microorganisms because of the diversity of the population and the disparity between the total biomass of microorganisms and the respiration they actually carry out, either under field or laboratory conditions (Clark, 1967).

Preliminary investigations at the Matador site have indicated a population of approximately 2×10^9 cells/g soil in the top 16 cm, with half of this amount being found at the 75-cm depth. Clark has stated that on the basis of 2 billion bacteria/g soil and measured bacterial respiration rates of 0.004 mg/hour/billion cells, the calculated activity of bacteria would be at least 10 times that normally found under field conditions. This indicates that a great majority of the soil organisms, although viable, must be present in a resting state. Calculation of the potential respiration of soil bacteria counted using the plate technique approximates field conditions much more closely than does the microscopic count.

Absorption systems incorporated into pits and infrared absorption spectrophotometers are applicable to field CO_2 measurements. Absorption techniques, such as alkali which removes the CO_2 from the system, may, however, alter the soil microbial activity and can yield artificial results if they act as a CO_2 sink. Paramagnetic and polarographic oxygen measuring systems are now available for field use. The polarographic technique is, however, very sensitive to temperature differentials and compensation techniques are required.

The roots of a grassland system not only produce large amounts of primary caloric materials, but also act as heterotrophs in gross soil CO_2 measurements. Therefore, it is essential that the decomposer cycle studies be closely associated with measurements of the growth rates of roots and of the amounts of organic materials excreted by the root systems (Harmsen and Jager, 1963; Rovira, 1962).

Tracers such as ^{14}C, ^{13}C, ^{32}P, ^{15}N, and ^{35}S utilized under canopies or incorporated into various substrates, give a measure of nutrient cycling and the metabolism of the products of primary producers (Paul *et al.*, 1969). The humic materials contain the largest source of potential calories in the ecosystem and a measure of their turnover rate must be obtained. Mathematical models describing the turnover rate have been developed from data obtained using both tracer and nontracer techniques (Bartholomew and Kirkham, 1961; Jenkinson, 1965). These mathematical models can be readily made to contain the whole decomposer and consumer cycle in the soil. This information could be integrated relative to the aboveground consumers and the data from the primary producers and could be used to describe the overall turnover process.

D. Abiotic Studies

Abiotic studies pertinent to an ecosystem study of productivity must consider the environment in the soil and lower atmospheric layers in relation to the activity of producers, consumers, and microorganisms. To facilitate the common usage of equipment, investigations of photosynthesis, respiration, and transpiration must be integrated with micrometeorological investigations.

1. PHOTOSYNTHESIS

In recent years, physiological ecologists have become interested in the measurement of photosynthesis in the field. Elaborate, air-conditioned canopies have been devised to permit a reasonably accurate simulation of external conditions within the canopy (Musgrave and Moss, 1961; Eckardt, 1966). The utilization of CO_2 by producers is measured by the use of infrared gas analyzers, with corrections being made for respiration. A continuous record of photosynthesis throughout the growing period is most desirable. However, the nature of the gas sampling equipment used and the necessity for moving the canopy at frequent intervals will interfere with the continuous record. Perhaps sufficient data can be obtained by intensive measurements during representative periods. The objective of photosynthesis measurements is to obtain an estimate of gross primary productivity. This will serve as a basis for comparison with estimates that are obtained by biomass and micrometeorological methods.

2. METEOROLOGY AND MICROMETEOROLOGY

Meteorological instrumentation should be set up to provide a continuous record of temperature, humidity, wind, rainfall, evaporation, and radiation. The purpose of the installation is twofold. First, it will provide participants with a detailed record of conditions that occurred near the sample plots at the time of sampling and in the intervals between sampling. Second, it will serve as a means of comparing the climate in the study area with that of standard weather stations where long-term records are available. To satisfy these requirements it will be necessary to employ standard instruments in addition to specialized ones. For example, a cup-counter anemometer is used as a standard to measure the daily run of wind, but in addition, a continuous record of speed and direction at a standard height also is required. The measurements of radiation will be the most sophisticated and costly.

Micrometeorological instrumentation should provide measurements of gradients of temperature, humidity, CO_2, and wind speed in the lower atmosphere; radiation components and spectral composition; temperatures and heat flux in soil; and fluxes of heat and moisture above the soil surface. The application of Bowen ratio, aerodynamic, and eddy correlation methods will provide measures of the activity of organisms in the ecosystem (Lemon, 1960, 1965, 1966; Tanner, 1963). Because of the high cost of setting up data acquisition facilities for this purpose, it may be desirable to install them in portable shelters.

3. WATER CYCLING

The amount of water available to organisms is reflected in the floristic composition of producers and in the magnitude of primary production. This relationship is exaggerated in native grasslands that owe their existence to a dry, warm climate. It is important, therefore, to consider the physics of soil water and the efficiency of its use by producers. The main objective is to examine the moisture flux in the soil, the air, and the plant and to determine its relationship to growth. It will be necessary to characterize plant environment as it changes with time. Soil parameters that are of consequence include rate of gaseous diffusion, porosity, resistance to penetration, moisture retention, and permeability. Where laboratory measurements of these variables are made, they must be related to moisture contents in the field.

To obtain data on the rate of water use by plants, a lysimeter should be provided. Additional characterization of transpiration in the producers can be obtained by measuring leaf water potentials at various times of the day and during the growing season, in as many species as practicable. Such measurements can be related to soil and atmospheric water fluxes.

4. SOIL NUTRIENTS

The fluxes of mineral elements, especially nitrogen and phosphorus, in an ecosystem are closely related to the flow of carbon in that system. Measurement of these nutrients, therefore, yields information on the quality of primary production. In addition, it can aid in the interpretation of the extent and relevance of the energy transformations involved. Determination of the total amounts of nutrients present is relatively straightforward (M. L. Jackson, 1958; Black *et al.*, 1965). However, these nutrients occur in a number of different forms, both in the organisms and in the soil (Bear, 1965). In an intensive study, the various forms must be measured. In addition, nitrogen undergoes a complex series of biological cycles, some of which can lead to losses from the system, making the determination of a balance sheet difficult (Bartholomew and Clark, 1965). The extent to which nutrient cycling is studied will depend on the resources available.

An essential feature of analyses, especially where the systems approach is used, is a requirement for consistency of measurements of all components. To measure phosphorus in some components, nitrogen in others, and dry matter in still others could result in a great deal of meaningless data.

The other aspect of nutrient cycling pertains to the possibility that one or more of the nutrients could be in short supply. They would then be one of the abiotic factors limiting either primary production or the energy and carbon transformations by decomposers and consumers. Analyses of grassland soils have shown that nitrogen, phosphorus, potassium, copper, zinc, molybdenum, boron, and sulfur fluctuate considerably. Some of these, as well as a few others, such as selenium, magnesium, and sodium, can be present in such concentrations that toxicity factors limit production.

The initial soil nutrient assessment can best be correlated with a detailed soil survey. This indicates the variability of the site and yields basic information required in establishing the experimental design. It is also useful for interpreting the data at a later stage. The scope of the routine survey depends on the site and on the aims of the research. The routine survey analyses are pH, conductivity (soluble salts), cation exchange capacity, exchangeable bases, mechanical analysis, total carbon, inorganic carbon, and total nitrogen (Black *et al.*, 1965; Stelly, 1967).

The manipulation of comparative sites requires an assessment of (1) available nutrient levels in the soil, and (2) the need for extra nutrients to achieve optimum growth. The losses through leaching, erosion, and volatilization and the input of nutrients into the system by rainfall, excreta, or soil management will affect the interpretation relative to nutrient cycling and abiotic factors controlling plant growth (Fried and Broeshart, 1967).

VII. CONCLUSION

This discussion of procedures involved in a productivity study of a grassland ecosystem has been shaped by the experience of the authors in the planning and initiation of such a project. Many lessons are still to be learned concerning the processing and interpretation of data on an integrated basis and the most effective means of presenting the results. Plans are being prepared concurrently by a number of IBP groups and will almost immediately become available to supplement the above treatment. An example is the handbook, "Methods for Estimating the Primary Production of Grasslands, Arid Lands and Dwarf Shrublands," by R. E. Hughes, C. Milner, R. O. Slayter, and G. H. Giningham sponsored by Section PT.

Hopefully, a large number of ecosystem studies established in many countries during the IBP will result in detailed understanding of the organization of systems which are being depended upon for sustained production of food crops. They will undoubtedly result in important advances in techniques of study.

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