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BIOLOGY AND ECOLOGY OF NITROGEN

Proceedings of a conference

National Academy of Sciences

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University of California, Davis

November 28 to December 1, 1967

Production Processes Subcommittee

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PREFACE

Under the auspices of the Production Processes Subcommittee of the U.S. National Committee for the International Biological Program, a conference was held at the University of California, Davis, from November 28 to December 1, 1967, to consider the biology and ecology of nitrogen.

The conference attempted to determine what research on the biology and ecology of nitrogen is most urgently needed, considered the extent to which international cooperation could accelerate research activities in this subject area, and examined means by which such international cooperation could be encouraged.

The Subcommittee recognizes that research dealing with the biology of nitrogen is closely related to the problems of food production that confront the world. And because these problems are urgent it is clear that priorities must be strongly influenced by practical considerations. Indeed, if the only motivation for research were scientific curiosity, an attempt to establish priorities would be irrelevant.

The problems of the Western Hemisphere and the possibilities for cooperative work among scientists of this area were emphasized, but the manner in which programs might fit into the global research scene was borne in mind. Means for coordinating work on these problems and for improving communication among scientists involved were carefully considered.

<u>Production Processes Subcommittee</u>

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SUMMARY, FINDINGS, AND RECOMMENDATIONS

Summary

During the first two days of the conference, papers were presented on the status of various phases of research in the biology and ecology of nitrogen. These papers elicited considerable discussion not only about the biology and ecology of nitrogen but also about the role of the International Biological Program and related scientific and sociological problems.

Although an anticipated expansion of the world's population lends urgency to the quest for information about the production of food, the magnitude of the increase is difficult to assess with accuracy and is subject to many social and biological variables. Some conference participants questioned the advisability of attempting to develop food production by growing algae or engaging in other single-cell-protein production. They underscored their belief that a critical element in the production of food is the energy input for nitrogen fixation by either biological or chemical means. In their view, efforts to expand food production by conventional means might well yield impressive results if augmented by judicious input of energy into the nitrogen-fixation process and by improving other management practices.

"Photosynthesis in a test tube" was also cited as an unpromising solution to food problems. While the elucidation of the mechanisms of photosynthesis certainly is a worthwhile scientific endeavor, the industrial application of the reactions seems lacking in potential for feeding the population. It may be possible to develop a process that will substitute for photosynthesis. Should the use of other energy sources in food production in fact become necessary, it is unlikely that photochemical processes would be the mechanisms of choice.

When the concept of farming the sea was examined, it was concluded that, while the efficiency of harvesting sea products needs to be improved, efforts to increase useful production will become economically sound only after a much more thorough exploitation of land areas has been achieved. This does not mean that research on biological nitrogen cycling in the sea is unimportant. On the contrary, much must be learned about the sea before it can be harvested effectively and wisely.

The sea must be looked upon primarily as a protein rather than a carbohydrate source. Thus, problems relating to nitrogen are of particular significance.

Where farming the sea is concerned, it will be necessary to pay closer attention to problems of conservation than is now the case. The complete ecological system must be examined, and since nitrogen is one of the principal limiting factors, its cycling must be better understood.

The group felt that biological fixation can be a significant means of meeting the need for fixed nitrogen in food production but that, for many areas, industrial fixation is probably a more economical means. In areas where transportation difficulties would interfere with the use of industrially fixed nitrogen, biological fixation would be competitive. Where food production per unit area is critical, however, the utilization of other energy sources for nitrogen fixation probably will make possible a more direct and efficient application of solar energy to the production of both carbohydrate and protein.

The role of organic nitrogen sources was examined. Use of organic sources must be considered in relation to the problem of solid waste disposal, which is part of the problem of maintaining the quality of our environment. Acceptance of such sources would depend largely on transportation costs as weighed against the need for disposal.

The energy required for nitrogen fixation is key to many problems. The energy required for the fixation of nitrogen to go into a given quantity of food is probably the equivalent of about 10% of the caloric value of the food produced. This estimate, which includes inefficiency in distribution and conversion, is applicable regardless of the source of energy for nitrogen fixation. When the area available for photosynthetic production of food becomes limited, the use of other energy sources for nitrogen fixation will be encouraged.

Consideration of the potential role of nitrogen transformation reactions was handicapped by lack of information about the biology and ecology of nitrogen. Decisions on even the most fundamental points could not be made. These gaps in information led the conference to recommend a broader, more coherent program of research on transformation reactions.

International cooperation was felt to be desirable in some areas of research, almost mandatory in others. The group agreed that the immediate challenge is to find the means of increasing production of food, fiber, and other basic commodities and that this endeavor would be facilitated by international cooperation in biological research. With respect to nitrogen research, the most immediate need is to define the present status of entire ecosystems and from this base to devise methods for their improvement.

Decisions or recommendations were made in accordance with certain guidelines. First, the conference tried to distinguish between studies that should constitute a United States program and those that need international cooperation for their accomplishment. Implementing mechanisms have been suggested, although it is unlikely that the recommendations of this committee will in themselves cause scientists to conduct research along the lines suggested. Since funds available to the U.S. National Committee and to the International Committee are limited, only limited recommendations were made in regard to funding. In making recommendations for the action phases of the research, the group recognized that funds are necessary if results are to be achieved.

Even under the most favorable circumstances, inadequate manpower and funds will for some time limit the rate at which this research can progress. Therefore an attempt was made to place priorities on the various tasks to be performed. These priorities are flexible and are intended only to assist in planning.

Findings

1. Two groups of problems are likely to arise in outlining required research on nitrogen. The first group relate to meeting the need for increased production of food. The second consist of environmental problems resulting from a greatly changed system of nitrogen inputs. In both areas, solutions will depend on increased study of practically every facet of nitrogen input, output, and transformation processes.

2. The increasing use of high-analysis nitrogen fertilizers are aggravating already serious problems, including nitrogen losses from soil and nitrate movement into subsoils, and are increasing the difficulty of maintaining water quality in lakes and streams.

3. Quantitative information on the nitrogen economy of temperate and tropical soils under different management systems is lacking.

4. There is need for improved methods of studying nitrogen transformation processes. The increased use of N^{15} and the refinement of techniques for tracer and nontracer analysis are desirable.

5. More information on biological fixation by symbiotic

and nonsymbiotic processes is required. These reactions contribute significantly to the nitrogen economy of many ecosystems, especially in humid tropical areas. Several nonsymbiotic nitrogen-fixing organisms seem unique to the tropics, but their practical significance has not been established.

6. A shortage of trained personnel seriously limits research on the input-output of nitrogen and on nitrogen transformations in soil, plants, and water. Stimulation of training is essential.

7. There is a need for better communication among Western Hemisphere workers engaged in research on the biology and ecology of nitrogen. Communication would be improved by holding conferences and by circulating a newsletter.

Recommendations

Research and training needs that the Subcommittee considers worthy of high priority at this time are listed below. Opposite the needs are recommendations for meeting them. Some of the needs can be met by expanding existing research. Others require new effort.

The Subcommittee is aware that a number of needs are omitted from the list. They are omitted not because they lack importance but because, in the Subcommittee's opinion, the needs listed should be dealt with first.

Needs	Recommendations
Communications	
Increased, faster exchange of views and research results among workers	Issue quarterly newsletter, to be edited initially by C. C. Delwiche, University of California, Davis; hold annual or biannual conferences
Training	
Opportunity for training in concepts, needs, and special skills	Conduct training programs in institutions now engaged in work on nitrogen biology and geobiology; upon request, provide training grants to institutions qualified to provide training
Analytical capability	
Standardized methods and improved equipment capability in participating laboratories	Publish and distribute manual of standardized methods; provide support for equipment needs; continue to encourage methods development
Increased availability of N^{15} at lower cost	Establish program for procuring N^{15} in quantity and make commitment to justify production
Nitrogen balances	
Study nitrogen balances in agricultural ecosystems	Expand existing programs of study. Arrange for cooperative studies, particularly on tropical soils
Study nitrogen balances in native ecosystems	Expand program by including model watershed systems. Invite parallel studies in different environments

Needs	Recommendations
Nitrogen in freshwater bodies	
Study to determine extent of nitrogen pollution and its potential	Expand existing studies on nitrogen pollution
Study to develop methods of controlling nitrate levels in freshwater bodies	Expand existing programs
Study to find methods for handling nitrogenous wastes	Expand existing programs
Nonsymbiotic fixation	
Evaluate nonsymbiotic fixation in temperate climates	Expand activity of U.S. workers by providing support funds. Encourage expansion of work in England, Australia, Canada, and South America
Evaluate nonsymbiotic fixation in tropical climates	Extend U.S. work to subtropical areas of Florida, California, and Texas. Invite South American scientists to participate; encourage expansion of work in tropical countries in South America and elsewhere
Evaluate nonsymbiotic fixation in arctic climates	Expand U.S. work. Invite scientists in England and northern Europe to participate
Catalog and make available known fixing strains	Establish center for cataloging species and listing availability (to be implemented by U.S./PP Subcommittee). Invite cooperative effort by international PP Subcommittee

Needs	Recommendations
Symbiotic fixation	
Study strain and species specificity with legumes	Expand U.S. studies; establish catalog of *Rhizobium* strains and provide for exchange (to be implemented by U.S./PP Subcommittee). Provide for exchange of species of *Rhizobium* and legumes
Evaluate legumes not now used for agricultural purposes	Encourage U.S. participation in programs of Brazil, Chile, and Argentina. Encourage expansion of programs in several South American countries
Examine nonlegume symbiotic systems	Expand work being done by several U.S. investigators. Encourage participation in tropical areas
Evaluate production potential of fixing legumes, N-fertilized legumes, and other crops	Initiate programs
Economic and social factors	
Evaluate biological nitrogen fixation in comparison with industrial sources of fixed nitrogen	Encourage interdisciplinary study in institutions that are strong both in biology and in the social sciences
Evaluate factors determining food acceptance	Encourage interdisciplinary projects

Needs	Recommendations
Impact on environment	
Evaluate effect of intensive use of nitrogen fertilizers on quality of groundwater and streams	Initiate program
Study mechanisms for optimizing effectiveness of nitrogen fertilizers	Expand management studies; expand study of nitrification inhibiting agents; encourage study to determine effect of nitrification inhibitors on food quality
Evaluate effect of nitrate on food quality (establish permissible levels)	Expand studies
Estimate long-term aspects of increased nitrogen inputs	Expand geochemical investigations; initiate study of public health aspects (oxides of nitrogen in atmosphere, water quality, disposing of nitrogenous organic wastes)
Disciplinary studies	
Obtain on a selective basis the disciplinary information needed to support the programs recommended here	Provide general support

After establishing a number of areas in which research or training is needed, the Subcommittee drafted a prospectus for research in one of the areas: evaluating biological nitrogen fixation in comparison with industrial sources of fixed nitrogen. The purpose was to outline a typical program of study and possible ways of implementing it. The prospectus is presented in Appendix A.

THE BIOGEOCHEMISTRY OF NITROGEN

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It is unnecessary to treat in great detail the subject of the biogeochemistry of nitrogen since members of this conference are generally familiar with those facets of the subject which pertain directly to their areas of interest.

This paper will therefore cover broad generalities to provide background for this conference, and will present an interpretation of this group's function in analyzing the status of research concerning the biology and ecology of nitrogen, making recommendations for the advancement of this research, and devising a coherent research program and suggesting means for its implementation.

Presently, an atmosphere of urgency prevails in which means for increasing the world's potential food and fiber production must be expanded greatly to meet rapidly expanding needs.

It is meaningless to cite any single element as the most important one necessary for the production of food, since every element essential to the growth of plant or animals must be considered important. Nevertheless, it must be recognized that from a practical standpoint the production potential of most agricultural areas is more likely to be limited by the supply of fixed nitrogen in available forms than by any other element required by plants or animals. We are probably justified in assuming that an understanding of the role of nitrogen in production processes and the study of means for increasing its availability and enhancing the efficiency of its use probably are keys to increasing the world's food supply.

Shown in Figure 1 is a diagrammatic representation of the nitrogen cycle. All elements can be thought of as cycling in the biological world, but the nitrogen cycle holds a special significance as the processes whereby nitrogen is lost to the atmosphere as nitrogen gas and subsequently, by various processes, reconverted to combined organic or inorganic forms. This part of the cycle is a relatively slow one in terms of the major nitrogen reservoir of the world, the atmosphere. Quantitative data with which an estimation of this cycling rate can be made are limited. Most probable figures, however, suggest that atmospheric nitrogen has been cycled in the biosphere at least once and perhaps not more than 10 times since the evolution of life on earth.

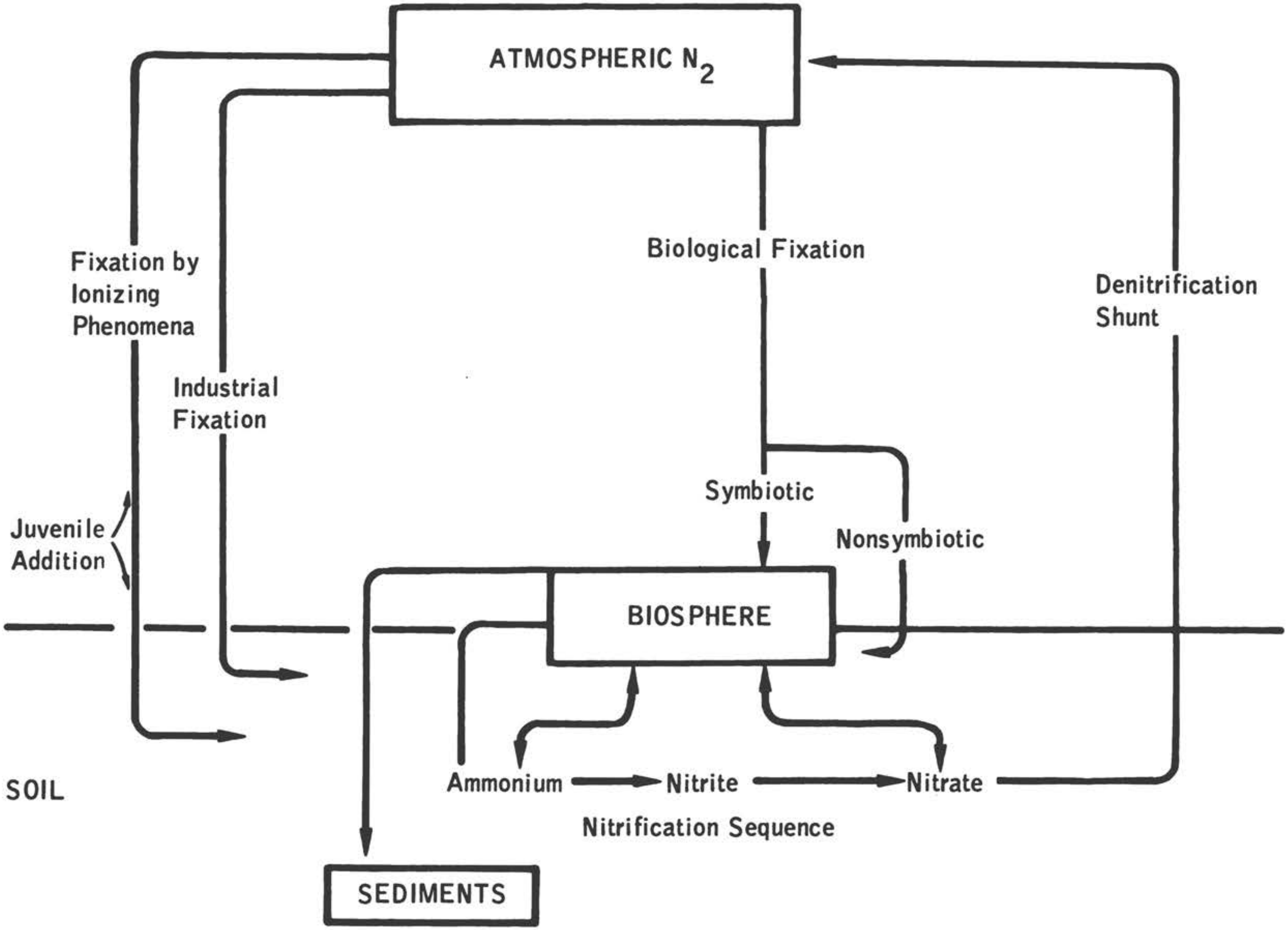

FIGURE 1 The nitrogen cycle.

Let us consider as a starting point nitrogen as ammonia, either from processes of biological or industrial nitrogen fixation, microbial degradation of organic compounds, or other sources. It can be taken up directly by plants, or it can serve as an energy source for autotrophic nitrifiers which oxidize it successively to nitrite and nitrate in the process normally referred to as nitrification. Nitrification can also be accomplished by various heterotrophic organisms; although it has not been established that ammonium serves as an energy source in this process, and the comparative significance of heterotrophic nitrification in contrast to the autotrophic process is not known.

Since the soil colloid normally has more negative absorption sites than positively charged ones, nitrate ion has more mobility than ammonium in the soil and will tend to move downward more rapidly under normal conditions of rainfall or irrigation. It is thus carried into the root zone and, if not absorbed by the plant, will pass through the root zone where it eventually appears in ground waters or streams.

Alternatively, if conditions become anaerobic, nitrate may serve as a hydrogen acceptor for various heterotrophic organisms which reduce it either to nitrous oxide or to nitrogen gas whereupon it is lost to the atmosphere. This is the process of denitrification.

Atmospheric nitrogen can be fixed by a number of processes including various ionizing events in the upper atmosphere, by electric discharge, by deliberate industrial processes of man, or by biological fixation reactions either in free-living organisms or in symbiotic plant-microbial associations.

Before the intervention of man into agricultural practices and industrial processes, the distribution of nitrogen between the atmosphere, the biosphere, the lithosphere, and the hydrosphere represented something approaching a steady state between the various processes for the interconversion of nitrogen and its transport from one phase to another. It is of interest to observe here parenthetically that although the processes of nitrogen fixation and denitrification were recognized at almost the same period in history, the observation of denitrification reaction did at one time cause a flurry of anxiety over the possible irreversible loss of the world's supply of fixed nitrogen to the atmosphere. I mention this to emphasize the need to consider all processes concerning the transformation of nitrogen, rather than to be stampeded in our thinking by one practice or process which may appear either desirable or undesirable at the time with the limited state of our knowledge.

As scientists we are interested in these reactions from a purely theoretical standpoint, but we cannot afford the luxury of considering them simply because they are there; instead, we must plan our investigations and coordinate our actions to gain the information necessary in solving some of our immediate and anticipated problems. I will attempt to keep this concept of the need for a national approach before us and to emphasize that the sometimes-encountered view that research work must not be tainted with any potential usefulness to be fundamental or basic represents a certain professional immaturity.

Dealing briefly with our efforts here, we plan, as the program indicates, to spend two days examining various important aspects of the biology and ecology of nitrogen through presentations by various members of the group. The succeeding two days will be devoted to an analysis of some of the problems developed in these presentations, some of the methods for attacking these problems, and possible mechanisms for making our efforts more efficient and more effective. I would ask you, therefore, to put behind you your natural and laudable loyalty to your particular area of investigation and interest. I ask you to view the problem as a whole, and where your particularly favorite series of biochemical reactions does not appear to be the best solution to some of the woes of mankind do not let it disturb you. Indeed, to evaluate the usefulness or lack of usefulness of these reactions may be ample justification in itself for studying them.

I would also ask that you give some inquiring thought to several concepts that might be considered scientific clichés. I believe you have several of these in mind, but I will cite a few questions that relate to them:

1. Just what is the potential significance of nitrogen fixation by free-living organisms in meeting the nitrogen requirements for an increased production? What is the relationship of direct counts of Azotobacter or other nitrogen-fixing population counts as related to nitrogen fixation capacity of the soil?

2. To what extent can legumes or other symbiotic systems be depended upon to meet nitrogen requirements; does the legume's requirement for solar energy to accomplish nitrogen fixation contribute to a comparative decrease in potential yield as contrasted with the potential yield of protein or caloric energy foods when grown with exogenously supplied nitrogen of industrial sources?

3. To what extent can industrially fixed nitrogen (or other nitrogenous materials) be applied to agricultural soils

without seriously degrading the quality of food grown thereon or otherwise degrading the quality of the environment?

4. To what extent can the production of single-cell protein be depended upon to make a significant contribution to protein needs, now and in the foreseeable future; under what circumstances, if any, might these processes have an advantage over more conventional agricultural practices?

5. Where does the concept of "photosynthesis in a test tube" fit into the picture? Is there any likelihood that the industrial application of photosynthetic reactions will exceed in overall efficiency (including the economics of industrial plant construction) the potential efficiency of more conventional agricultural means?

6. At what point in these biological research endeavors is international cooperation desirable to accomplish overall objectives, and where does a more casual coordination meet the requirement for efficiency and speed in accomplishing the objectives?

7. Just what are the real deficiencies in communication of scientific results among workers in the field, and how extensive are the mechanisms necessary for the correction of these deficiencies?

In asking these questions, I do not intend in any way to inhibit consideration of the related problems. On the contrary, I believe they should be examined critically in order that some of our preconceptions do not lead us to incorrect conclusions. As is usually the case in matters of this sort, the answers are probably not clear-cut, and our conclusions undoubtedly will entail a certain amount of intuitive judgment. We must not permit our uncertainty to prevent us from making specific recommendations. It is essential that we make recommendations in our best judgment and then let time determine the wisdom of these recommendations. Should time prove our judgment wrong, we must have the flexibility to alter our opinions and adopt a new course, keeping our objectives always in sight.

I expect that we will have a most productive conference. I anticipate some disagreement; I hope for much debate, and I trust that any idea, trivial though it may seem, will be expressed, given serious consideration, and then adopted or discarded as our judgment suggests. During the final two days, as I indicated earlier, we will be preparing our conclusions and recommendations. For the latter, it is essential that we concentrate on the most important items and if possible establish some sort of priority. It is pointless to make the virtuous recommendation that all scientific research be advanced posthaste, since this attitude is already assumed to be part of any scientist's thinking. Let us instead produce a program that has some boundaries and has practical possibilities of implementation.

I repeat Professor Stout's welcome to the University and the Davis campus and anticipate that we will have a productive session.

THE NITROGEN CYCLE IN THE SEA

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The nitrogen cycle in the sea appears to be similar qualitatively in most respect to that in the soil. However, oceanic nitrogen atoms are involved in geographical translocations that are large in comparison with those of nitrogen atoms trapped temporarily in the terrestrial productivity system. The major differences between the nitrogen regimes of the two environments lies in the species composition of the micro- and macro-organisms involved and in the relatively great mobility of nitrogen dissolved in sea water. Vacarro (1965) has reviewed the subject.

The complexity of the marine nitrogen cycle has made it difficult to measure rates along the various pathways. During the past five years, my colleague, Dr. John Goering, and I have used N^{15} as a tracer to measure ammonia and nitrate uptake and to find whether nitrogen fixation and denitrification take place in the sea. The bulk of combined nitrogen in the sea occurs as nitrate in deep water. Nitrate is probably formed primarily in the winter at high latitudes, but it appears that direct measurements of nitrification rates have not been made.

Most activity in the nitrogen cycle takes place in the upper several hundred meters. In the uppermost, or euphotic zone, phytoplankton take up nitrate and ammonia; the ammonia arises from local regeneration by zooplankton feeding on the phytoplankton and from the activities of bacteria. N^{15} measurements (Dugdale and Goering, 1967) show that nitrate accounts for about 30% of the inorganic nitrogen uptake in productive regions, and for as little as 1% in the tropical "desert" regions of the sea.

Algae appear to show Michaelis-Mention type kinetics for the uptake of major nutrients with a preliminary estimate of $K_t = 1$ µg-At N/l for ammonia (MacIsaac, unpublished). The low level and small range of ammonia concentration in the sea (0.2-5 µg-At NH_4^+N/l) is apparently linked to this ability of phytoplankton to absorb ammonia rapidly at low concentrations (Dugdale, in press). Isotope-dilution measurements of ammonification rates show that they are in balance with ammonia uptake rates. The turnover time for ammonia in tropical surface waters is on the order of 40 hours. The turnover of nitrate in surface waters is determined by the rate of transport from deep water; a mean figure of about 50 hours appears to be reasonable for productive waters. In the tropical deserts nitrate is often undetectable and the turnover time may be as high as a year or more.

Nitrogen fixation can provide new combined nitrogen to the phytoplankton, supplementing the nitrate supply, which may be low in tropical regions. Heavy nitrogen-fixing blooms of a blue-green alga, *Trichodesmium* spp., occur in the tropical ocean (Dugdale, Goering, and Ryther, 1965). Nitrogen-fixation rates have been measured with N^{15} (Goering, Dugdale, and Menzel, 1966). The highest result in an estimate of 0.32 kg N_2/ha/da fixed under bloom conditions. Blooms have been observed over 50,000 km^2 of sea surface and could add as much as 10^3 metric tons/da to the combined nitrogen pool at the maximum observed rate.

Balance sheets for the marine nitrogen cycle show a large residual loss that suggests substantial denitrification. The oxygen level in the ocean is sufficiently low to provide a suitable environment for denitrifiers in only a few regions associated with coastal upwelling. Thomas (1966) examined phosphate-nitrate ratios (normally almost constant in the deep water of a given ocean) in the oxygen minimum zone off the west coast of Mexico. He found nitrate deficiencies associated with a nitrite maximum and concluded that denitrification had taken place. Goering (unpublished), working in the same region and using N^{15}, measured denitrification rates of about 0.3 µg-At/l hr. Reduction to nitrite occurred simultaneously, and the rates of denitrification were strongly influenced by oxygen concentrations of less than 0.3% saturation.

The dissolved organic nitrogen fraction is relatively large and undoubtedly considerable activity occurs within it. Uptake of labeled urea and glycine has been shown (Goering and Dugdale, unpublished), and some algae are able to use a variety of organic nitrogen sources. However, the data with which to compute turnover and rates of uptake in the sea do not exist.

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NITROGEN-FIXING PLANTS AND THEIR SYMBIONTS

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Agriculturalists have used the legumes and their symbionts, the Rhizobia, to the extent that nonlegumes are almost ignored. Two good reviews of the literature on this subject have been written in the past five years. Professor G. Bond of the University of Glasgow has a particularly fine paper in the Society for General Microbiology symposium volume, Symbiotic Associations. I have drawn heavily from his paper and also from Dr. W. D. P. Stewart's book Nitrogen Fixation in Plants. I have also looked at about 30 original research papers and have drawn most of my data from about 15 of these.

In assessing the contribution of nitrogen-fixing plants to the soil store of nitrogen, it is quite easy to see the role of alfalfa in rotation or as a green-manure crop. The contribution of a plant such as the buffaloberry growing in stony ground or a bog myrtle is a good deal more difficult to assess. When it is apparent that good soil-forming processes include the growth of plants, it can be seen that the process of soil formation probably proceeds quite slowly until the soil will support a certain number of plant species. In a very young soil the amount of nitrogen in a form available to plants is quite low. The autocatalytic nature of nitrogen brought in from the atmosphere by pioneering plants on soil formation is obvious.

It is difficult to understand the maintenance of soil N over centuries of cropping in many areas of the world without taking into account some method of returning N to the soil from the atmosphere. In trying to determine the past, the present, and the potential value of N_2 fixation in soil formation and productivity, it is essential that we learn all we can about these apparently slow but persistent nitrogen-fixing plants and their symbionts.

The Plants Involved

About a dozen genera of nonleguminous plants are able to form a symbiotic association with a microorganism, after which the two organisms are able to utilize molecular nitrogen. In some genera all species can do this, and in others only selected ones can. These plants are not grouped in one order but are found in six or seven orders that are not closely related in any other way. One genus, *Pinus*, is a monocotyledonous plant, but the rest are dicots. A rather primitive plant, *Coriaria myrtifolia*, is in the order *Coriariales*. This is the only genus of plants in this order, according to Hutchinson's system of classification. Species of this plant are found in tropical and semitropical climates. Hutchinson shows them distributed from northern Mexico or southwestern United States through Central America and down the west coast of South America to about latitude 30° S. They are also found in Mediterranean countries of southern Europe and northern Africa and from the Himalayas across southern Asia down to the northern tip of Australia and New Zealand and associated lands. Bond lists 10 species.

The myrtles (order *Myricales*), such as the bog myrtle (*Myrica gale*), are widely distributed in both temperate and tropical regions. They are found growing near bodies of water and streams. Forty-five species have been described. The genus *Alnus*, order *Fagales*, contains 25 species that are all nitrogen-fixing. These species are the alder trees, which are found in wet soils mainly in temperate regions of North and South America, Europe, and Asia.

A fourth order of plants that contains nitrogen-fixing species is *Casuarinales*. Among these is the beefwood tree, or forest oak, of India, Malaya, and Australia. This has been called an old but not necessarily a primitive order of plants.

Using Hutchinson's statement of the plant orders, as Bond has done, we find in the order *Rhamnales* five more genera of plants that are able to grow symbiotically with what appears to be a Streptomyces endophyte and fix nitrogen. The silverberry and Russian olive are two species in the genus *Elaeagnus* (*E. ommutata* and *E. augustifolia*). Also in the order *Rhamnales* is the sea buckhorn (*Hippophae rhamnoides*) and the buffaloberry (*Shepherdia argentea*) of North America. These plants live in dry, rather poor soils. Delwiche *et al.* (1965) tested 12 species of *Ceanothus* and found that excised nodules from all 12 were able to fix significant quantities of N^{15} labeled N_2. They conclude that " . . . all species examined were found to fix nitrogen at rates which were ecologically significant."

A tropical plant *Phychotria bacteriophila* is found in the order *Rubiales*. This plant has nodules on the leaves in which *Klebsiella* or *Aerobacter* cells grow in a symbiotic association that is able to use N_2.

Professor J. H. Becking of Wageningen in the Netherlands has studied a species of pine that with a fungal endophyte appears to utilize molecular nitrogen. Richards and Voight (1963) concluded that *Pinus rodiata* seedlings in association with a fungus fix N_2. Their data were obtained by using N^{15} labeled N_2 but are not conclusive since their uninoculated controls sometimes fixed nitrogen, perhaps from contamination.

As can be seen from the foregoing data, these plants are distributed throughout most of the world. I suspect that the apparent absence of these plants from Africa and a few other areas is due to a lack of knowledge about them. In habitat, they vary a great deal, but when we look carefully it can be seen that each of these habitats would be expected to be nitrogen poor: bogs for the myrtles, dry, stony ground for the buffaloberry, and dunes and sandy areas for the *Casuarinales*.

The Endophyte

Only for *Phychotria bacteriophilia* has the species or genus of the endophyte been identified. Becking *et al*. (1964) did commendable work, using electron microscopy, and showed the nature of the endophyte in *Alnus glutinosa*. They found the organism in three forms in the parenchyma cells of nodules on the roots of this alder. They found hyphal, vesicular, and bacteria-like structures that they assume to be three stages of the same streptomycete endophyte. The filamentous nature, lack of a nuclear membrane, and presence of "plasmalemmosomes" in the hyphal structures lead them to this conclusion. Silver (1964) has made a similar study of *Myrica cerifera*. These studies concur with the work of a number of others on the basis of light-microscope studies. Inability to cultivate the micro-symbiont outside of the host cells prevents further characterization.

Centifanto *et al*. (1964) isolated the bacterial microsymbiont from the leaf nodule of *Phychotria bacteriophilia*, identified it in the genus *Klebsiella*, and proposed the name *Klebsiella rubiacearum*. They were able to show that the bacterium is able to fix nitrogen in the absence of the plant. The fact that the suspected organism is the one causing nodulation and thereby nitrogen fixation could be proved by applying Koch's postulates; that is, isolate the organism from the nodule, grow it in a pure culture, and produce the nodule and obtain N_2 fixation by inoculation of an endophyte-free plant. This has been done with the *Phychotria-Klebsiella* system, but no one has isolated the streptomycete endophyte. Plant seedlings that appear to be free of endophytes have been inoculated with crushed nodule material or soil from an area where the plants have been grown. *Phychotria bacteriophilia* seed seem to carry the *Klebsiella*.

Cross-inoculation between species of the genus *Alnus* has been accomplished. In the family *Elaeagnaceae*, cross-inoculation has been carried out between *Elaeagnus* and *Hippophae* and *Shepherdia*. Bond states that this is the only family in which cross-inoculation of genera has been shown.

Physiology

Isotopic studies with N_2^{15} have shown that the nodule is the site of fixation and that newly fixed nitrogen is transported rapidly to the rest of the plant. This is not the case in the pine and fungus system studied by Becking (1965); here, the isotope did not move into the plant from the nodule in 24 hours. Work in Dr. Bond's laboratory showed that members of the genera *Alnus*, *Casuanina*, and *Myrica* require cobalt for optimum nodulation and nitrogen fixation. It was also proved that molybdenum stimulates the plants when fixing N_2. In the same laboratory it was observed that increasing O_2 up to about 20% in the atmosphere stimulated N_2^{15} uptake in excised root nodules of four genera, whereas levels above this depressed nitrogen fixation. Hydrogen was shown to be a specific inhibitor of fixation. Glutamic acid seems to be an early product of fixation in *Alnus* and amides in *Myrica*. In general it may be concluded that the fixation mechanism in these plants seems to be similar to that found in legume-*Rhizobium* and nonsymbiotic systems.

Questions That Remain

To assess the value of these plants in adding nitrogen to the soil, we need to answer several questions:

1. How much of the plant's requirement for nitrogen can be met by nitrogen fixation in their habitats?

2. What plant density in an area would be required to add significantly to the soil nitrogen?

3. Is any fixed nitrogen excreted into the soil by perennial nitrogen-fixing plants during growth? (Data for Ceanothus will be found in Delwiche *et al.*, 1965.)

In the interest of further understanding and utilization of these plants, we should know:

1. What is the endophyte, and how is it grown?

2. How can the endophyte be inoculated effectively into the plant?

3. Could we effectively increase N_2 fixation by these plants through selection and breeding?

Whether we can domesticate and use these plants remains to be seen, but there is no doubt in my mind that it is important to learn more about these nitrogen fixers and search for others that may now escape observation.

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INPUTS FOR MAXIMUM PRODUCTIVITY

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Competition for space and resources has imposed on us the necessity to optimize our exploitation of cultivated and wild lands. Furthermore, new dimensions are being added to such problems at an accelerating rate. Economic constraints change rapidly as technology introduces new tools and materials, new distribution patterns for products, and increased availability of inputs such as water, nitrogen, and improved genetic materials.

In agriculture, much of our increased production during the past 100 years has been through the limiting-factor approach, repeated in a cyclic, iterative manner, usually with rather long-time constants. For example, a serious limiting condition in the culture of a particular crop may be fertility, moisture, or salinity. When research leads to a way of changing the limitations, the new circumstances then cause us to adjust plant spacings, water management, and, finally, the genetic capabilities of the plants. The total effort has been an empirical one because of the necessary specialization of the scientists and innovators involved and a lack of clear principles and theories of productivity. That is not to say, though, that certain specialized aspects have not been approached in an elegant and rational manner.

Clearly, progress would be more efficient and rapid if we could integrate our manipulation of controllable inputs so that new varieties and new cultural practices were developed concomitantly. Jensen (1967) has called to our attention that this end would be reached more quickly if the ideas of modern systems analysis could be applied to agricultural and managed natural ecosystems. In such analysis, a major component of the system would be the structure of the production processes in the plant community. The chain of events leading to harvestable game, fish, or timber from wild systems, and fruits, grains, or animal products from cultivated lands, while obviously complex, always begins with photosynthesis in green plants. Thus, if the approach is to optimize output, it is obvious that we should consider first the capture of radiant energy and CO_2 by the producer plants, then, stepwise, the conversion of photosynthate to usable product, and, finally, efficiency in product utilization (i.e., do we consume it directly or are there intermediate agents, such as livestock?).

I shall deal only with the photosynthesis aspects in terms of energy captured or dry matter produced. One should keep in mind that maximizing photosynthate production is not always the desired condition for optimizing output for utilization by man. Further, while optimizing output relative to the input of radiant energy, and as other limiting factors are reduced, we must keep in mind that there will always be regions and climates where we cannot afford to eliminate restrictions imposed by other variables. It is true that a discussion of light limits on productivity applies most directly to intensive agriculture and to prime forests and grasslands where water and nutrients are not limiting or can be supplied easily, but such discussion nevertheless provides a standard against which to gauge the benefits of removing such constraints in other environments.

An analysis of the productive structure of a forest or crop can start with assessment of the photosynthetic capabilities of individual leaves. Data from Hesketh (1963) in Figure 1 show that species differ greatly in response to increasing illumination and that some plants (such as sugar cane and maize) are far more efficient than others (clover and beet). When canopies are constructed with such leaves, the differences are still evident and thus are important in accounting for variations in production. This can be shown by measuring photosynthesis in the field or by simulating the result with a suitable mathematical model on an electronic computer. The latter approach is illustrated in Figure 2, where maize is shown to have a far greater productivity than clover with the same amounts and arrangements of leaves (Duncan *et al*., 1967; Loomis *et al*., 1967). The amount of leaves in space and time has long been recognized as important. Also critical, we have recently learned, is the manner of leaf display relative to the direction of the incoming light. Figure 2 illustrates this for simple cases with vertical or horizontal leaves. The basis for this influence of leaf angle lies in the nonlinear response to increasing illumination, as shown in Figure 1.

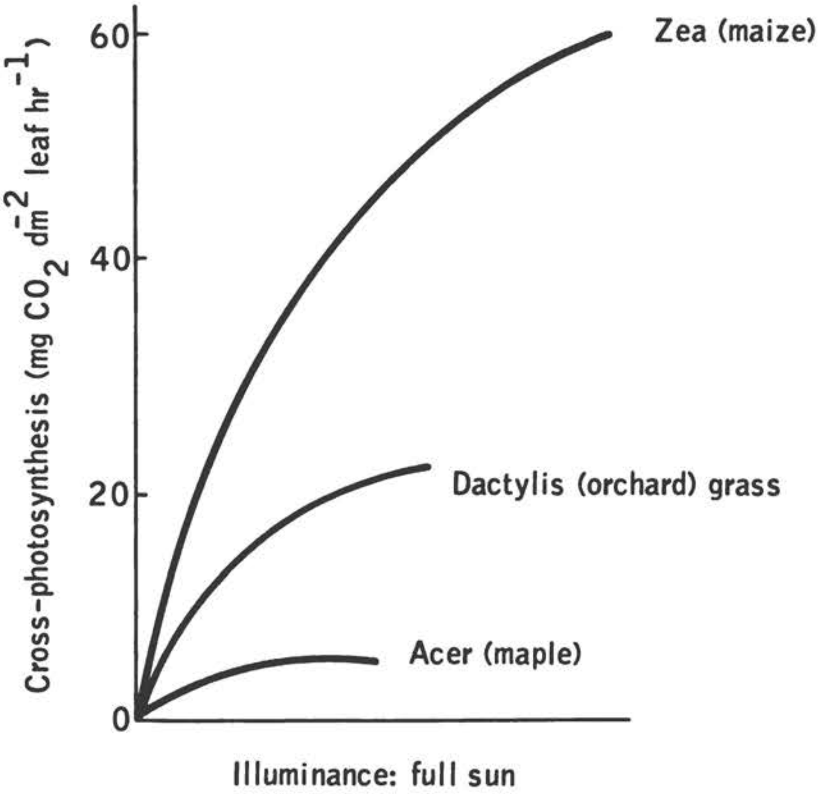

FIGURE 1 Leaf photosynthesis for several species. Chart illustrates wide differences in responses to increasing amounts of light. (After Hesketh, 1963.)

Other important parameters include the flux of radiant energy, transfer of CO_2, and plant respiration. No one has yet attempted to optimize the relationships of these variables within the limits set by the real world, but certain of the basic principles for maximum production are evident from Figure 2.

Productivity has real limits imposed by the nature of the photosynthetic process. With sunlight and a requirement of 10 quanta of light absorbed for each molecule of CO_2 reduced, the upper limit is reached near 5% energy conversion, or at about 14 µg of CH_2O production per calorie of solar energy (Loomis and Williams, 1963).

A high efficiency of capture of net energy in the producing community does not necessarily lead to maximum exploitation. With crops and forests, only a part of the plant is harvested, and the absolute yield and quality of this part may be greatest at plant densities too sparse for maximum photosynthesis. Thus, consideration of canopies capable of rapid photosynthesis must be coupled with selection for plant types that convert larger proportions of the total biomass to useful products. For many crops, as with the new rice and wheat varieties, this might mean growing dense stands of sparsely leafed plants with an erect habit of leaf display and high photosynthetic capability.

A key feature of the new types of cereals is their tolerance to an abundance of nitrogen, and the greatest yields are obtained with nitrogen supplies that would have lowered the yields of older varieties. It is clear that many aspects of nitrogen management must be reconsidered with the increase of biomass and nutrient removal.

It seems particularly important that we provide plant breeders with simple means for measuring nitrogen use so that selection for high efficiency can go hand in hand with selection for large response. It is also important that we consider carefully the best tactics for supplying nitrogen inputs, particularly the extend of reliance on biologically fixed nitrogen. Energy from organic carbon compounds is used in fixation, and considerable difference results from proper choice of the point in a scheme of energy flow through an ecosystem at which we tap the flow to support nitrogen fixation. Symbiotic fixation depends upon the current photosynthate within the producer plant and thus represents a direct drain on productivity. Suppose that 30 mg N can be fixed per g sucrose consumed; then a modest annual fixation rate of 20 kg N ha^{-1} would divert about 670 kg CH_2O ha^{-1} from primary productivity. Further, if low-value legumes are rotated with high-value crops, the loss in efficiency of production can

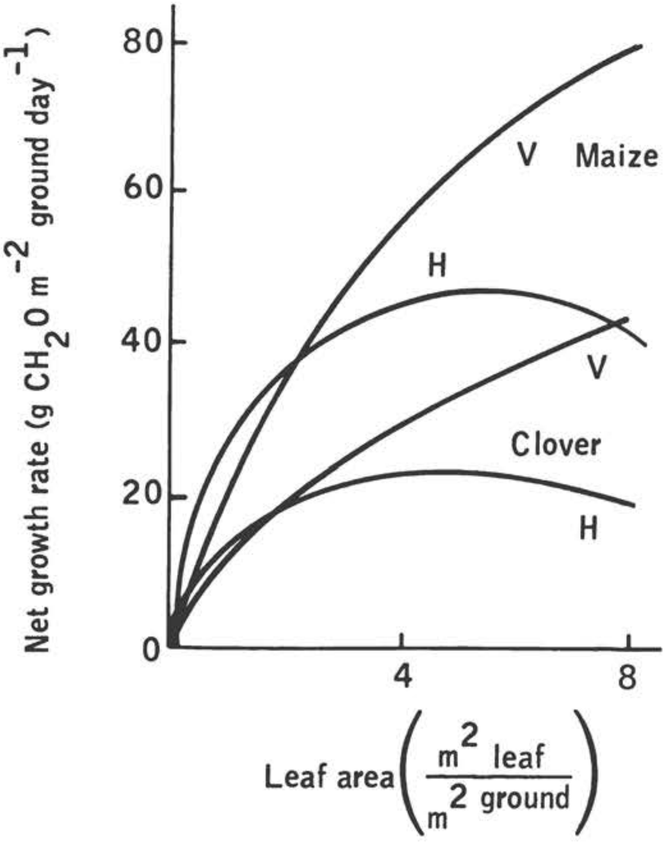

FIGURE 2 Rates of dry-matter accumulation by maize and clover communities with various foliage densities at two orientations of leaves. Computer simulations for July 1 and latitude 40°N.

be very great. In fact, there may be very few places in the world where the increased value of the food produced by continuous cropping of good lands would not compensate for the costs of chemical nitrogen. If, in contrast, nitrogen fixation is accomplished by nonsymbiotic bacteria scavenging on crop residues, there is no direct drain on productivity. Another scavenger operation that does not tax the productive system is that of returning animal manures, though this usually involves considerable labor.

Another type of concern may arise when the desired output of the ecosystem is not closely coupled to primary productivity. At this conference, Garst outlined an approach that would maximize the flow of nitrogen rather than carbon through an agricultural food chain, thus emphasizing the production of food protein for areas with low dietary protein. Dugdale's paper reveals a similar view for nitrogen-limited fisheries where nitrogen flow can be equated with the production of fish. In these circumstances, a systems analysis based on nitrogen could be coupled with an analysis of the carbon flow.

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SIGNIFICANCE OF THE NITRIFICATION REACTION IN NITROGEN BALANCES

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Nitrification is usually defined as the biological oxidation of ammonium to nitrate with nitrite as an intermediate in the conversion. This definition has some limitations (see Alexander, 1965), but it is adequate here. Since the primary purpose of this paper is to outline the present state of knowledge concerning the significance of the nitrification reaction in nitrogen balances, we will not discuss the nitrification reaction in any detail or attempt to review the literature on the biochemistry and microbiology of this subject (for review, see Alexander, 1965).

The nitrification reaction has three obvious effects on the recovery of soil and fertilizer N by crops. First, it affects the nitrogen nutrition of crops because the rates of assimilation by plants of the substrate and the products of nitrification (ammonium and nitrate) can be quite different. Second, it promotes nitrogen loss by leaching because it converts a slowly leached, cationic form of N to a readily leached, anionic form. Third, it brings about oxidized forms of nitrogen that are susceptible to volatilization by microbial and chemical processes in soils, nitrate, and nitrite N through denitrification and nitrite N through chemodenitrification.

Forms of Nitrifiable N in Soils

Two points should be emphasized in any discussion of this subject. The first is that most of our knowledge of nitrification in soils has been obtained from studies of this reaction in natural ecosystems or in other systems that differ considerably from the soil systems encountered in the field under modern conditions of soil management and N fertilization. Few attempts have been made to study nitrification and its significance in soils treated with current methods of N fertilizer, and very little is known about the nitrogen transformations that occur at the surfaces or in the immediate vicinity of N fertilizer granules in soils. The need for research on such transformations is obvious, but there are serious experimental difficulties in studies of nitrogen transformations at microsites in soils, and new techniques must be developed for such studies.

The second point that deserves emphasis is that the amount of chemically fixed N being used to increase crop production is increasing rapidly and is steadily approaching the amount of biologically fixed N utilized for crop growth. The consumption of fertilizer N in the United States during 1966 exceeded 5 million tons, and by 1980 it is expected to exceed 12 million tons. The current annual world consumption of fertilizer N is about 18.4 million tons, and within 5 years it is expected to increase to about 49 million tons. The patterns of nitrogen fertilizer consumption in the United States and the world are similar, but there is a marked difference in the form and method of application that affects the role of the nitrification reaction in determining the fate of the applied nitrogen. In the United States, almost 70% of the N used is applied directly rather than in fertilizer mixes. Anhydrous NH_3 represents 43% and aqua NH_3 an additional 5% of all direct applications.

Use of anhydrous NH_3 in Europe will increase sharply during the next few years. In Asia, the increase in N fertilizer use will result largely in increased use of solid materials (mainly urea and calcium ammonium nitrate) for direct application (less ammonium sulfate will be used). In Latin America, N sources will go primarily into mixed nitrogen-phosphorus-potassium (NPK) fertilizers. These sources will include the ammonium phosphates, sodium nitrate, and calcium cyanamide.

The fact that the amount of chemically fixed N being used for crop production is increasing relative to the amount of biologically fixed N has considerable significance because nitrification of the latter is closely associated with mineralization of legume and microbial residues, and the eventual reactions of the nitrifiable N are different from those associated with the nitrification of N fertilizers. The N source and its form and method of application affect nitrification in both a qualitative and a quantitative way.

Most solid N fertilizers are water-soluble compounds that readily hydrolyze in soil and upon dissolution form a local environment at the point of application that is acid, alkaline, or neutral, depending on the kind of material, form of application, amount applied, various soil factors, and disposition in the soil. Urea, ammonium carbonate, diammonium phosphate, and urea ammonium phosphate form alkaline solutions upon hydrolysis. Ammonium sulfate, nitrate and chloride, and monoammonium phosphate form acid solutions. Potassium, sodium, and calcium nitrate do not hydrolyze, and solutions of these salts are essentially neutral.

Soil microorganisms are immediately affected by the dissolving and hydrolyzing fertilizer granule. The effects on activities of the nitrifying organisms are easiest to observe. Nitrification of an N source can be stimulated or repressed by adjusting the ammonium concentration and pH at the granule applications site to a favorable or an unfavorable level. The optimum pH for nitrification is about 7.5 to 9.0. Therefore, ammonium from urea, which forms an alkaline hydrolysis solution, is nitrified more readily than ammonium from acid-hydrolyzing ammonium sulfate, especially in acid soils. These differences tend to decrease in neutral to alkaline soils.

One result of unequal nitrification rates is that different relative amounts of ammonium and nitrate are available for crop uptake. Another result may be nitrite buildup around certain N sources. Nitrite usually does not accumulate in soils, because of its rapid biological oxidation to nitrate. However, Nitrosomonas is less affected by environmental stresses imposed by high pH and salt concentrations than Nitrobacter. When large amounts of materials that form an alkaline solution are added to soil, the activity of Nitrobacter is temporarily repressed to a greater extent than that of Nitrosomonas, and nitrite builds up around the fertilizer reaction zone. Nitrite accumulation has, therefore, been associated with high ammonium concentrations at high pH and low soil buffer of nitrifying capacities. High concentrations of urea, diammonium phosphate, and anhydrous ammonia will lead to nitrite accumulation in both acid and alkaline soils, as will ammonium sulfate added at high rates to alkaline soils.

The accumulation and persistence of nitrite in the soil can reduce N fertilizer efficiency by both phytotoxicity and N loss. Root damage to seedling plants from applied urea has been reported, but it is not always clear whether the toxic agent is nitrite or free ammonia formed by urea hydrolysis. Nitrogen deficits ranging up to 55% of the nitrogen applied have been reported for acid soils that accumulate nitrite, and recent work has provided considerable indirect evidence that significant gaseous loss of fertilizer N can occur through chemical decomposition of nitrite formed by nitrification of ammonium or ammonium-yielding fertilizers in soils.

Volatilization of Ammonium

The conversion of ammonium to nitrate by the nitrifying organisms in soils may reduce gaseous loss of N under certain circumstances because ammonium is susceptible to volatilization as ammonia.

It is well established that a significant gaseous loss of nitrogen from soils can occur under certain conditions through volatilization of ammonium (see Allison, 1955, 1965, 1966). The conditions promoting loss of N as ammonia are fairly well defined and include high pH, low cation-exchange capacity, high temperatures, drying of the soil surface, and surface application of ammonium or ammonium-yielding fertilizers. The last factor named deserves emphasis because there is abundant evidence that significant gaseous loss of N as ammonia is likely to accompany any surface application of fertilizer N that results in high ammonium concentration and high local pH (e.g., broadcast application of ammonium salts to neutral or calcareous soils, shallow injections of anhydrous ammonia, or surface applications of urea on bare or grass-covered soils). Loss of N as ammonia from ammonium or ammonium-yielding fertilizers can be greatly reduced simply by incorporating these fertilizers into the soil, and it is difficult to understand why this is not standard practice in application of fertilizer N that is susceptible to volatilization as ammonia. The much-discussed losses of urea N as ammonia are negligible if the urea is mixed with the soil.

Chemodenitrification

Until a few years ago it was generally accepted that gaseous loss of nitrogen from soils resulted largely, if not entirely, from volatilization of ammonium and denitrification of nitrate or nitrite. Recent work, however, has provided strong presumptive evidence that significant gaseous loss of fertilizer nitrogen can occur through chemical decomposition of nitrite formed by nitrification of ammonium or ammonium-yielding fertilizers in soils (for reviews, see Clark, 1962; Allison, 1965, 1966; Broadbent and Clark, 1965; Broadbent and Stevenson, 1966; Hauck, 1967). The processes whereby nitrite is decomposed chemically in soils resemble denitrification in that they lead to formation of N_2 and N_2O. Recent N^{15}-tracer studies have shown that these processes involve fixation and volatilization of nitrite N through reaction of nitrite with soil organic matter (see Bremner and Nelson, 1967). For the purposes of this paper, the term "chemodenitrification" will be used to describe the chemical reactions in soils leading to volatilization of nitrite N.

Recent tracer studies at Iowa State University have shown that substantial gaseous loss of nitrogen can occur from urea-treated soils under conditions that do not lead to volatilization of ammonium or denitrification of nitrate, and there seems little doubt that this loss can be attributed to chemodenitrification. These studies have also shown that nitrite decomposition in soils is controlled largely by soil pH and organic-matter content and that it leads to evolution of NO_2 as well as N_2 and N_2O (see Bremner and Nelson, 1967). Current evidence indicates that NO_2 is formed by self-decomposition of nitrous acid and that fixation of nitrite N and volatilization of nitrite N as N_2 and N_2O observed on treatment of soils with nitrite occur through the reaction of nitrite with phenolic constituents of soil organic matter (a nitrosation reaction). Further research is needed to elucidate the mechanisms of nitrite decomposition in soils and to identify the soil conditions that promote fixation and volatilization of nitrite N during nitrification of ammonium. It is clearly important to encourage research on chemodenitrification and nitrite transformations in soils because there is good reason to believe that nitrite decomposition in soils has considerable practical significance under modern conditions of soil N fertilization. It is noteworthy in this connection that nitrite is formed in soils both by oxidation of ammonium when oxygen is in adequate supply and by reduction of nitrate when oxygen is lacking. The possibility that gaseous loss of nitrate N from soils as N_2 and N_2O may involve microbial reduction of nitrate to nitrite and subsequent chemical reduction of nitrite to N_2 and N_2O by soil organic matter clearly deserves consideration in view of recent findings concerning nitrite transformations in soils.

Denitrification

Although it has long been known that soil microorganisms can reduce nitrate and nitrite to N_2 and N_2O, it is only within the last 15 years that serious attempts have been made to study the factors affecting microbial denitrification in soils and the economic significance of this process. The literature on this subject is confusing and contradictory, but much of the divergence in results and conclusions can now be attributed to deficiencies in the analytical techniques used in several investigations and to the occurrence of chemodenitrification. For example, recent work leaves very little doubt that gaseous loss of N previously attributed to aerobic denitrification of nitrate was actually due to chemodenitrification of nitrite.

The factors affecting denitrification in soils are now fairly well defined, and there is good evidence that this process is controlled largely by the availability of oxygen and readily decomposable organic matter (energy supply) to the microorganisms responsible for denitrification. It is also well established that denitrification is markedly affected by soil temperature and pH and is likely to occur most extensively in fine-textured, neutral, or calcareous soils during warm, rainy weather.

Leaching

Although it is well known that leaching of nitrate constitutes one of the main channels of nitrogen loss from soils, it is difficult to obtain reliable data concerning the magnitude of this loss under different cultural and climatic conditions. Many laboratory studies of leaching of nitrate through soil columns have been reported, but these have little practical significance because the conditions of these studies usually bear little resemblance to those encountered in field soils. The literature on loss of nitrate from soils by leaching has recently been reviewed by Allison (1965, 1966), and it suffices here to note two points emphasized in his reviews. One is that leaching of nitrate beyond the plant-root zone usually does not occur to any marked extent in cultivated, medium-textured soils in the United States during the main plant-growing season unless the annual rainfall exceeds about 50 inches. In addition, loss of nitrogen through leaching can often be appreciably reduced through improved farm-management practices, and appropriate practices vary widely with the soil, climate, amount of rainfall, extent of irrigation, cropping system, and type and amount of fertilizer used.

Control of Nitrification

Several methods of controlling nitrification in order to increase crop recovery of fertilizer N are being explored (see Nelson and Hauck, 1965). One approach is to develop nitrogen fertilizers that release nitrogen slowly because of their limited water solubility or slow rate of degradation by soil microorganisms. Several fertilizers of this type are now available commercially (e.g., magnesium ammonium phosphate, crotonylidenediurea, isobutyridenediurea, and ureaforms), but their high cost has limited their use largely to turfs, gardens, and specialty crops.

Another approach is to coat soluble nitrogen compounds with some material that will delay or slow down the release of nitrogen. Many types of coating materials have been tested (waxes, paraffins, acrylic resins, vinyl acetate, latex, gums, oils, sulfur, etc.), and it has been demonstrated that coatings can be effective. However, it is costly and difficult to coat fertilizer particles uniformly in large-scale plant operations, and unless this is achieved coatings are of little value.

Natural organic materials such as processed sewage or garbage and ammoniated brown coal or lignite exhibit slow-release properties, but their use is restricted by their high cost per pound of N compared with conventional nitrogen fertilizers. Research to develop slow-release nitrogen fertilizers is based on the hope that use of such fertilizers will minimize loss of N by leaching and volatilization, reduce damage to plants, and permit closer matching of the plant's nitrogen needs with the rate and amount of N release from the fertilizer. However, the high cost of these products and the lack of any convincing evidence that they have potential agronomic value make it difficult to justify the effort being devoted to their development.

A more sophisticated approach to the problem of increasing the recovery of fertilizer N is through development of nitrification inhibitors. Several intensive surveys have been conducted in recent years to find nonphytotoxic chemicals that can selectively inhibit the nitrification process in soils and thereby reduce loss of N through leaching and volatilization of nitrate or nitrite. A large number of chemicals, including thiourea, cyanamide, dicyandiamide, mercapto compounds, and pyridine, pyrimidine, and triazine derivatives, have been tested, and several have been patented for use with fertilizers containing ammonium or other reduced forms of nitrogen. The compound that has aroused most interest is 2-chloro-6-(trichloromethyl)-pyridine, which is being marketed by Dow Chemical Company under the trade name of "N-Serve." Studies using this compound indicate that it has the ability, at least in certain circumstances, to retard nitrification and decrease loss of fertilizer N from soils through leaching and volatilization. A continued search for specific inhibitors of nitrification in soils is clearly desirable, but attention should also be given to the possibility that such inhibitors may have undesirable effects. For example, the possibility that use of nitrification inhibitors may lead to increased fixation or volatilization of ammonium deserves attention. The basic assumption in the use of nitrification inhibitors, that crop plants can utilize ammonium at least as effectively as nitrate, requires evaluation because existing knowledge of the inorganic nitrogen nutrition of plants does not permit a valid appraisal of this assumption. However, the question of ammonium versus nitrate nutrition cannot be studied satisfactorily without development of nonphytotoxic chemicals that can block nitrification of ammonium in soils.

Research Needed

To summarize, an appraisal of present knowledge concerning the significance of the nitrification reaction in the nitrogen economy of soils indicates that the following lines of research deserve special attention:

1. Development of closed-systems techniques and analytical procedures for meaningful investigations of denitrification and chemodenitrification.

2. Development of methods of characterizing microsite environments and of following nitrogen transformations at microsites.

3. Definition of conditions that promote volatilization and fixation of nitrite N, and elucidation of the mechanisms of nitrite decomposition in soils.

4. Development and evaluation of compounds that inhibit nitrification and retard ammonification of urea in soils.

5. Comparison of ammonium and nitrate as N sources for crop production.

The need for research to obtain more detailed information about the nitrification process in soils is also readily apparent. As Clark (1962) has pointed out, one approach to the problem of eliminating gaseous loss of N through nitrite decomposition in soils is to achieve rapid and efficient nitrification under conditions that favor neither nitrite accumulation nor nitrite dismutation.

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NITROGEN STORAGE OF SEVERAL CALIFORNIA FOREST SOIL-VEGETATION SYSTEMS

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The balance of nutrient elements in a forest has been a matter of interest for more than a century. The removal of materials such as leaf litter from the forest was found to have depressing effects on the productivity of the site. Extensive studies of the composition of such materials relative to the other parts of the forest were reviewed by Ebermayer (1876); recent work in the context of the ecosystem concept has been reviewed by Ovington (1962). This paper deals with nitrogen storage in some soil-vegetation systems typical of California forests: the microforest of chaparral, the mixed conifer forests in which ponderosa pine is a major species, and the redwood forest. This variety of vegetation was chosen for study to show the range of nitrogen quantities found in various types of forests.

The Chaparral Microforest

Chaparral is a woody brush cover typical of a large area of the California landscape. It is similar to the maquis vegetation of the Mediterranean countries. This cover can be considered a dwarf forest and was described as such by Fultz (1923). It might be thought of as a model of larger forest ecosystems, or a microforest. It is usually less than 20 feet tall, yet it may be more than 50 years old. A lysimeter study using some of the species typical of chaparral has been under way at the San Dimas Experimental Forest, U.S. Forest Service, in southern California. This installation and its history have been described by Sinclair and Patric (1959). Nitrogen balances were made of the vegetation in several of these lysimeters and are presented in Table 1.

TABLE 1. Nitrogen balance of chaparral stands in San Dimas lysimeters, expressed as grams of nitrogen per square meter [a]

Part of system	Type of cover						
	Original soil	Barren	Af	Eff	Qd	Ccr	Cp
Vegetation							
Leaves	0	0	2.4	1.6	1.9	11.5	--[b]
Stems	0	0	2.8	9.0	4.2	18.1	--[b]
Total	0	0	5.2	10.6	6.1	29.6	--[b]
Leaf litter	0	0	3.2	30.0	17.9	32.4	6.8
Mineral soil to 122 cm.	318.0	283.8	278.5	318.8	343.6	320.1	320.4
TOTAL	318.0	283.8	286.9	359.3	367.5	382.0	---
Change from original condition		-34.2	-31.1	+41.3	+49.5	+64.0	---

[a] To convert to lbs./acre multiply by 8.9.

[b] Not measured.

The stands of chaparral for which the nitrogen balances were determined were grown in lysimeters constructed by digging pits 7 feet deep and 17.5 feet square. These pits were filled with the same soil mixed in a homogeneous manner. They were vegetated temporarily from 1940-1946 with a Bromus mollis annual grass cover. The chaparral species sampled in this study were planted in the lysimeters in 1946, and the sampling for this study was carried out in 1959. Pure stands of the following species were maintained in the lysimeters: Adenostoma fasiculatum H & A (abbreviated Af in tables), Eriogonum fasiculatum Benth. (Eff), Ceanothus crassifolius Torr. (Ccr), and Quercus dumosa Nutt. (Qd). Also a stand of Pinus coulteri Don. (Cp), a species often associated with chaparral, was grown in one of the lysimeters. The soil was kept barren in one lysimeter for the entire period, and it was also used in this study. However, it was of different construction in that it was bordered by concrete walls and was 10.5 by 21 by 6 feet deep. The nitrogen balances were determined for each of these lysimeters. Samples of the original soil were used to estimate the initial nitrogen content, and the subsequent changes were induced by the various species.

The nitrogen balances of these vegetation stands and their associated soil and leaf litter were obtained from samples taken from various parts of the vegetation and soil related to a unit area of the lysimeter. The vegetation stands, which ranged from 3 to 12 feet in height, were sampled by cutting a uniform area of the vegetation. Known areas of the leaf litter were sampled, and the soil was sampled in uniform depth increments to 4 feet. The nitrogen determinations were made by the Kjehdahl method. These data, related to a unit area of the ecosystem, are presented in Table 1.

The nitrogen storage in the stands of chaparral in the lysimeters indicate that there may be a gain or loss in nitrogen from the original conditions, depending upon species, and that the proportional distribution of the total nitrogen in the ecosystem between its various components may vary with the species. Thus it is to be noted that the nitrogen content had dropped considerably in the lysimeter with the stand of chamise and that this drop was nearly as much as in the soil that had been maintained bare of cover. The other species showed gains in total nitrogen in the soil-vegetation system. The ceanothus vegetation showed the largest gain, consonant with its reputation as a host plant for symbiotic nitrogen-fixing organisms. A large proportion of the gain in ecosystem nitrogen associated with the ceanothus is in the above-ground vegetation and in the surface leaf litter. In contrast, the Quercus dumosa stand showed its major nitrogen gain to be in the mineral soil with some addition in the leaf litter part of the system. The gain in the *Eriogonum* stand was mainly in the leaf litter, with some gain shown in vegetation above ground. It is important to know whether these results, obtained under the controlled conditions of a lysimeter study, correspond to those obtained with variables inherent in the natural setting.

A sampling of chaparral microforests has been made throughout California; in the parts of this work for which the nitrogen analyses are available, these findings from the lysimeter vegetation are substantiated. Table 2 shows the nitrogen content of the vegetation and soil of stands of *Adenostoma fasiculatum* and *Quercus dumosa* that were sampled on two climatic sequences of increasing precipitation, one in San Diego County and one in Los Angeles County. The sampling sites were selected in such a manner that the variables of geology, topography, and age of vegetation were kept similar while the climate varied and the species contrast was made. This is in the manner proposed by Jenny (1941) for soil studies, and the data represent climo-functions of the vegetation part of the ecosystem as described by Major (1951). Uniform areas (usually 35 sq. ft.) of vegetation were cut into increments of uniform height. The foliage was picked off and grouped into one sample. The leaf litter was sampled in unit areas, and the data reported combine separate analyses of the L and F horizons. Soil samples were taken to the depth of rock, which in chaparral was 61 cm. At each site separate series of samples were obtained in pure clumps of the species being compared.

The comparison between *Adenostoma fasiculatum* and *Quercus dumosa* shown in Table 2 supports the conclusion based on the lysimeter that there is less nitrogen in association with *Adenostoma* in the desert than anywhere else. Also to be noted is that generally there will be an increase in the nitrogen content of this microforest ecosystem with increasing precipitation, but that the effect of other climatic factors, such as maritime climate, needs to be assessed. Thus the species composition may be used to assess the nitrogen status of the ecosystem according to the relative abundance of the species, and this is related to the climate in which the vegetation formation occurs.

TABLE 2. The nitrogen content of Adenostoma fasiculatum (Af) and Quercus dumosa (Qd) vegetation and leaf litter and soil along two sequences of climatic change (N as grams per square meter) [a]

SAN DIEGO COUNTY SEQUENCE

Location	Scissors Junction		Descanso		Sunrise		Vista	
Precipitation	11"		25"		38"		12"	
Species	Af	Qd	Af	Qd	Af	Qd	Af	Qd
Part of ecosystem								
Vegetation								
Leaves	4.2	3.6	1.7	7.8	4.3	15.5	1.8	11.3
Stems	5.6	10.3	6.5	13.7	15.6	19.2	4.8	10.0
Total	9.8	13.9	8.2	21.5	19.9	34.7	6.6	21.3
Leaf litter	1.9	18.9	6.6	46.8	49.5	28.4	17.0	23.5
Soil to 61cm. (2')	196.9	134.1	218.2	321.2	290.9	702.0	337.9	428.8
Total vegetation and soil	208.6	166.9	233.0	389.5	360.3	765.1	361.5	473.6

LOS ANGELES COUNTY SEQUENCE

Location	Hollywood		El Prieto Can.		Tanbark Flat	
Precipitation	14"		24"		35"	
Species	Af	Qd	Af	Qd	Af	Qd
Part of ecosystem						
Vegetation						
Leaves	4.2	10.2	5.3	9.2	10.0	15.0
Stems	5.6	15.0	5.6	21.6	16.2	15.6
Total	9.8	25.2	10.9	30.8	26.2	30.6
Leaf litter	5.0	75.0	10.1	38.4	9.2	18.7
Soil to 61cm.	145.9	217.3	201.7	250.2	215.9	246.1
Total vegetation and soil	160.7	317.5	222.7	319.4	251.3	295.4

[a] Both sequences were obtained on areas having soils derived from granitic rocks with west to southwest exposures and moderate slopes (10% to 15%), and having vegetation unburned for more than a decade. Precipitation is annual average in inches.

The Mixed Conifer Forest

The estimation of nitrogen storage in a mature, mixed, conifer forest soil-vegetation system, as is typical of the western slope of the Sierra Nevada in California, can be accomplished by determining the nitrogen content of large standing trees and the volume of soil that they occupy. Knowledge of the nitrogen content of individual trees of the various species and the species composition makes possible an estimate of the nitrogen storage for the forest. This has been achieved in the analysis of the nitrogen content of the stem, leaf litter, and the soil associated with large mature ponderosa pine (*Pinus ponderosa* Laws.), incense cedar (*Libocedrus decurrens* Torr.) and Douglas fir (*Pseudotsuga menziesii* Mirb.) Franco. The soil was sampled to a depth of 4 feet, the leaf litter was sampled in a unit area, and the stem of the tree was extracted by means of a core, and a plaque of bark was taken from the tree at 4 feet. Total nitrogen content was determined by the Kjehldahl method and related to a unit area in the forest by means of physical measurements of the trees, leaf litter, and soil. Since the tree sample was a single core taken from the base of the tree, the data presented here are only approximations.

As in the chaparral microforest, it was noted here that the species being compared differed in the distribution of nitrogen in the soil-vegetation system. Thus, as seen in Table 3, if comparisons between species are made of the distributions of nitrogen in stem, leaf litter, and soil, it is apparent that Douglas fir maintains a higher percent of the site nitrogen in the tree. With ponderosa pine the proportion of nitrogen on the site is greater than in the soil, and incense cedar is intermediate in this respect. This is partly due to the fact that the nitrogen content of Douglas fir is usually higher at given sites than is that of ponderosa pine. It was noted that in comparing sites with soils developed in granite to those with soils developed on andesite under similar climatic conditions, a higher proportion of total site nitrogen was in the vegetation of the ecosystems based on andesite. This may be related to the additional storage of soil moisture available for vegetative growth on these sites. The present study excludes foliage nitrogen content that is probably similar in magnitude to leaf litter nitrogen.

TABLE 3. The nitrogen balances of parts of ecosystems in which comparisons are made between mature trees of *Pinus ponderosa* (Y), *Libocedros decurrens* (I), and *Pseudotsuga menziesii* (D) at two locations differing mainly in geology (N as grams per square meter)

Location	Viola, Lassen Cnty.			Chinquapin, Mariposa Cnty.		
Precipitation	55"			55"		
Soil	Gray brown podzolic			Gray brown podzolic		
Parent Rock	Andesite			Granite		
Species	Y	I	D	Y	I	D
Part of ecosystem						
Tree stem (wood + bark)	202	157	522	94	142	348
Leaf litter	49	46	55	32	54	63
Mineral soil to 122 cm.	542	373	601	717	572	709
Total	794	576	1178	843	768	1120

The total forest nitrogen was found to increase with increase in precipitation when other factors, such as age of tree, soil, parent-rock type, and exposure were held as invariable as possible. Thus, as seen in Figure 1, mature ponderosa pine in conditions of sequentially increasing rainfall from Susanville to Mineral in Lassen County, California, gradually increased in total nitrogen (excluding foliage and large roots) from 439 g/m^2 at the dry end of the sequence (15 inches per year) to 1,360 g/m^2 at the high rainfall end of the sequence (55 inches per year). A nearly constant proportion of 70% of the nitrogen on the site was in the mineral soil when stem, leaf litter, and soil nitrogen were considered.

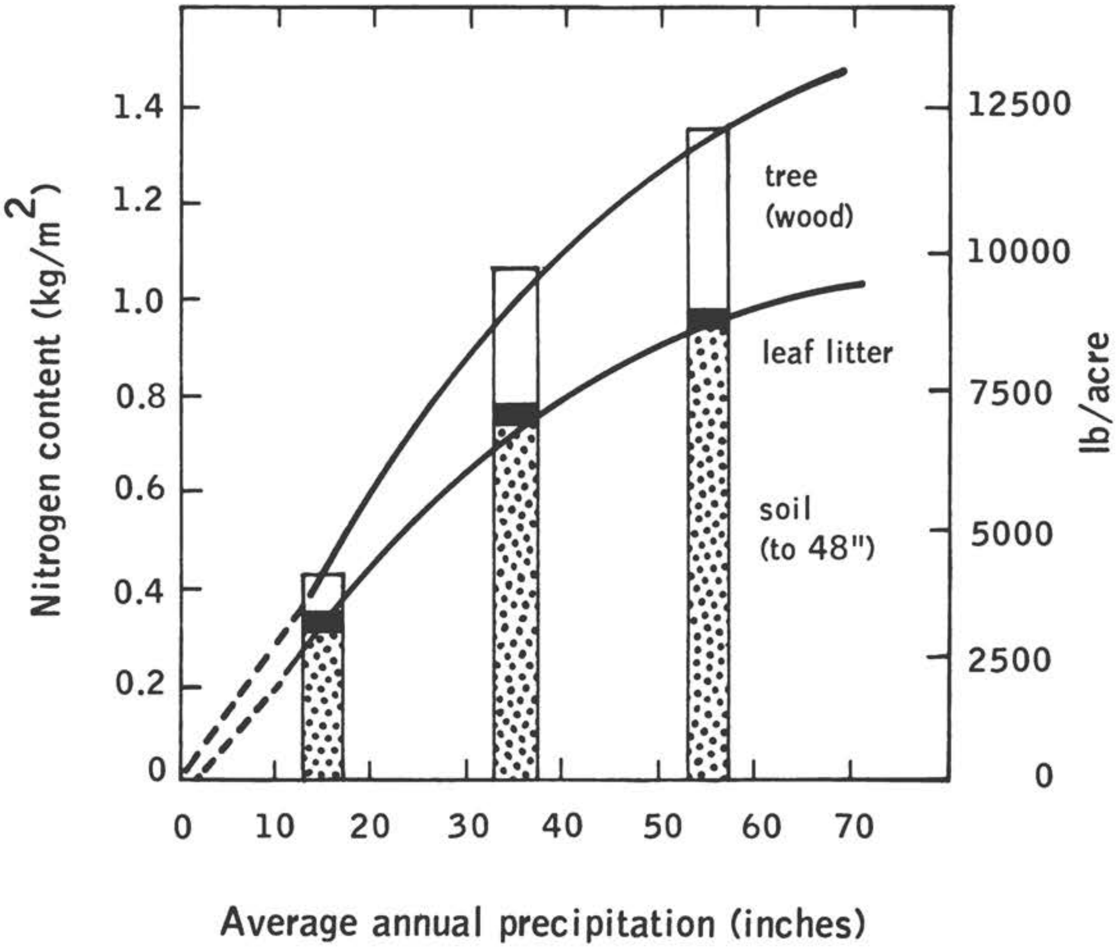

FIGURE 1 Nitrogen content of mature ponderosa pine tree stems (wood and bark) and associated leaf litter and mineral soil to a depth of 122 cm as a function of precipitation.

It was observed that site quality or productivity increased with the increase in nitrogen. Site quality in forest vegetation can be expressed by the site index, or height of dominant trees at a given age, and in the case of mixed conifer forests in the Sierra, it is the height at 300 years of age (or maturity). A previous paper showed that total soil nitrogen content to a depth of 4 feet could be correlated with site index (Zinke, 1960). By using 23 mature ponderosa pines of varying site index throughout California, the following relationship was obtained:

$$\text{Height in meters at 300 years} = 49.4 (\log Q_N) - 90$$

The correlation coefficient for this relationship is 0.71, and the standard error of estimate is 8.7 m. The main conclusion is that it may be possible to estimate ecosystem nitrogen by some field measure of the forest, such as site index, if appropriate quantitative data are obtained.

The Redwood Forest

An estimation of the nitrogen storage of a large redwood forest on an area such as is found at Bull Creek flat is of considerable interest because it represents a possible maximum. In this area, the trees grow to a height of nearly 100 m. Advantage was taken of a tree that fell as a result of erosion by Bull Creek in Rockefeller Redwood Forest. This tree fell from the top of a 35-ft bank on which a complete analysis of soil nitrogen had been made in a previous study. The tree was slightly less than a thousand years old, 96 m (320 ft) tall, 3 m in diameter at a height of about 1 m (10 ft), and had a root platform 8.7 m in diameter and 3 m deep. The wood, bark, and foliage of the tree were sampled at intervals to the top. The wood samples obtained were sawdust from a complete sawed cut of the tree at the sampling points. Bark samples were taken at the same locations. Foliage samples were taken beginning with the lower foliage at a height of 37.6 m (125 ft), and at intervals of about 15 m (50 ft) to the top of the tree. Roots were not sampled. It was noted that there was an increasing nitrogen content of the wood with height as has been observed in other woody plants, ranging from a low of 0.06% N to 0.087% N toward the top of the tree. The bark nitrogen amounts ranged from 0.157% N to 0.392% N, increasing with height on the tree. Measurements of the tree included bark thickness, which is considerable in redwood, being 25 cm (10 in) at the base of this tree. The estimated nitrogen balance sheet for an area of redwood forest such as is represented by this tree is seen in Table 4. This balance is to a soil depth of 3 m. It is of interest to note that about 11% of the nitrogen on the site is in the tree, not including the roots. It is also to be noticed that the redwood forest is relatively frugal in its use and storage of nitrogen even though storage is high. If an estimate is based on the amount in the trees at age 1,000 years, the rate is about 1/3 g per m^2 per year (1.5 to 3.0 lb per acre per year).

TABLE 4. Approximation of the nitrogen balance for the redwood forest on Lower Bull Creek Flat based on the sampling of one large tree and the soil profile associated with it (exclusive of large roots)

Part of ecosystem	Nitrogen Content (g/m^2)
Vegetation	
Foliage[a]	121
Wood + bark	190
Total	311
Leaf litter[b]	21
Soil (to 3 m)	2,421
Total	2,753

[a] Based on an estimated foliage weight derived from yearly leaf litter collections.

[b] 0.62% of 3,400 g of leaf litter per m^2.

Conclusions

Nitrogen-storage data have been presented for chaparral microforest, mixed conifer forest, and redwood forest in California to represent the range of nitrogen storage that may be expected in forests. There are principles that seem to apply to each of these types. There is usually a difference in the total nitrogen of the system, the difference depending on species variation in the forest. Also, there may be a different distribution of the total nitrogen among the various parts of the ecosystem, the distribution depending on the species present; some species may bring about a higher proportion of the ecosystem nitrogen in the vegetation, some a higher proportion in the soil. Generally, the forest ecosystem nitrogen will be higher with increasing precipitation within the climatic ranges in this study. The total amounts of nitrogen present ranged from a low of 200 to 300 g per m^2 in the chaparral microforest to a high of 2,753 g per m^2 in the redwood forest ecosystem. The mixed conifer forest ecosystem of the Sierra Nevada has amounts of nitrogen intermediate in this range. The productivity of the forest as indicated by site quality may be related to total ecosystem nitrogen content. The redwood forest studied had about 11% of the ecosystem nitrogen in the trees. Much more work is needed with large forest ecosystems, because of the diversity of these forests and the magnitude of the weights of materials involved and their inherent variability.

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THE NITROGEN ECONOMY OF GRASSLAND AND DUNE SOILS*

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Interest in quantitative formulation of the nitrogen cycle, including microbial transformations and the status of reserves, is important because so many nutritional aspects of production are limited by some step in the economy of this element of protein. Quality as well as quantity of foodstuffs, wild forage, and natural return of litters to soils depends on nitrogen transfer rates, not merely on content in the soil.

In the vast grassland region of central North America a better understanding of nitrogen-climate relationships can now expand the early formulations of Jenny (1928, 1930b). Elucidation of major nitrogen inputs and outputs of the ecosystem, quantification of microbial transformations, determination of internal nitrogen turnover (e.g., ^{15}N experiments of Jansson, 1958), and extensive application of low-cost nitrogen fertilizer are facets of the nitrogen economy that still need study. Problems that require special attention include deficits in balance sheets (Allison, 1955), nitrate contamination of ground-water supplies, and the changing status of carbon-nitrogen relationships and availability as influenced by decreases or regional variations in soil organic matter (e.g., Klemmedson and Jenny, 1966). Effort needs to be directed toward better definition and quantification of those steps in the nitrogen cycle that are closely related to rates of mineralization and immobilization (Jansson, 1958). The resulting turnover fractions for any given income rate will regulate both the availability and the ultimate reserves of nitrogen.

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In areas of intensive agriculture, it is now economically feasible to provide most of a crop's nitrogen needs in the form of artificially produced fertilizer input. In other native and managed systems, much of the total nitrogen and organic matter is a product of many ecosystem variables and climatic factors. Shortage of available nitrogen commonly occurs because of the slow turnover of older organic residues.

An extensive amount of Russian literature beyond the scope of this review has become available in summary form (Rodin and Bazilevic, 1965, 1966); see also Bazilevic and Rodin (1966). Grassland parameters that are especially useful for our reference and later calculations are summarized in Table 1 from Bazilivich (1958). It seems that an especially useful function of the International Biological Program would be the organization of extensive vegetational information on total and available nitrogen and the inventory of other elements for the same ecosystem. Collection of the information on animal consumption and microbial turnover based on the same unit areas for which total vegetational, soil data, and other ecosystem data are known or obtainable is also necessary. This especially concerns domesticated and wild animals because they make effective use of native and planted grasslands. Degrees of use vary widely from overgrazing (ecosystem degradation) to underutilization. What is the system's optimum balance in terms of nitrogen as well as energy?

To understand and manipulate or perhaps design an optimum ecosystem economy from basic principles requires systems analysis. Therefore, the historic foundation for a state variable (analysis of grassland soil economy) is reviewed in the next section. Integrating other items of disconnected information into such an analysis is a third need for the International Biological Program; this is perhaps the greatest challenge, short of the more general goal of reassessing the whole earth's nitrogen cycle (Figure 1).

TABLE 1 Mass (g/m^2 = metric tons/km^2) and chemical indices of western Siberian steppes and steppe meadows, after Bazilevich (1958). Fractions expressed may not correspond to turnover ratios.

Index	Meadow-steppe soil formation				Steppe soil formation		
	Meadow-chernozem soil, Steppe meadow	Weakly solonetz-like meadow-chernozem soil, Steppe meadow	Weakly solonetz-like meadow-chernozem soil, Steppe meadow	Leached chernozem, Grassy-grain meadow-steppe	Typical chernozem, Rich grassy-tipchak feather grass steppe	Southern chernozem, Grassy-tipchak-feather grass steppe	Chestnut soil, Wormwood feather grass-tipchak steppe
	Novosibirsk region				Altay region		
	Mikhaylovskly rayon	Kargatskly rayon	Ubinskly rayon	Ordynskly rayon	Shupunovskly rayon	Rodinskly rayon	Slavgorodskly rayon
Total dry organic mass, cntr/ha	250	100	113	230	240	210	98
Including:							
green assimilating part, cntr/ha	80	60	33	80	45	30	14
green assimilating part, %	32	32	29	35	19	14	14
roots,[a] cntr/ha	170	130	80	150	201	180	84
roots, %	68	68	71	65	81	86	86
Included in litter: cntr/ha	137	103	60	130	112	90	42
Fraction of whole organic mass, %	55	54	53	56	46	43	43
Including:							
green assimilating part, cntr/ha	80	60	33	80	35	30	14
Fraction of whole organic mass, %	58	58	55	62	40	33	33
roots, cntr/ha	57	43	27	50	67	60	28
Fraction of whole organic mass, %	42	42	45	38	60	67	67
Total N, kg/ha	161	121	111	161	147	93	45
Green assimilating parts, kg/ha	104	81	75	107	65	42	16
Fraction of total N, %	65	67	68	66	44	45	35
Roots, kg/ha	57	40	36	54	82	51	29
Fraction of total N, %	35	33	32	34	56	55	65
Average content of N in litter, %	1.18	1.20	1.80	1.24	1.04	1.03	1.06
Total ash, kg/ha	944	834	423	829	671	572	215
Green assimilating parts, kg/ha	603	537	262	557	224	172	100
Fraction of total ash, %	65	65	62	67	33	30	46
Roots, kg/ha	341	297	161	272	447	400	115
Fraction of total ash, %	35	35	38	33	67	70	54
Average ash content of litter, %	7.3	8.1	7.1	6.4	6.0	6.4	5.1
Litter/ash ratio	14:1	12:1	14:1	16:1	16:1	16:1	19:1
Total amt. Ca-K-P-S in ash, kg/ha	381	484	172	141	172	151	68
Fraction of total ash, %	40	58	41	17	26	26	31
Total amt. Ca-K-P-S in ash of green part, kg/ha	201	310	118	91	62	53	23
Fraction of total Ca-K-P-S, %	53	64	69	65	36	35	34
Total amt. of biohalogens (Na-Cl-excess S), kg/ha	10	55	11	26	14	12	6
Biohalogen fraction of total ash, %	1	7	3	3	2	3	3
Biohalogens in green part, kg/ha	9	47	9	22	10	8	2
Fraction of total biohalogens, %	90	85	82	85	72	67	33
Biohalogen fraction of litter, %	0.08	0.53	0.18	0.20	0.13	0.13	0.14
Total amt. of ash, kg-eq/ha	30.5	28.5	21.2	27.0	22.0	18.5	7.2
Excess of cations, in kg-eq/ha	9.4	10.3	2.9	1.8	3.9	3.5	0.9
Cation excess in green part, %	53	57	58	42	19	18	20

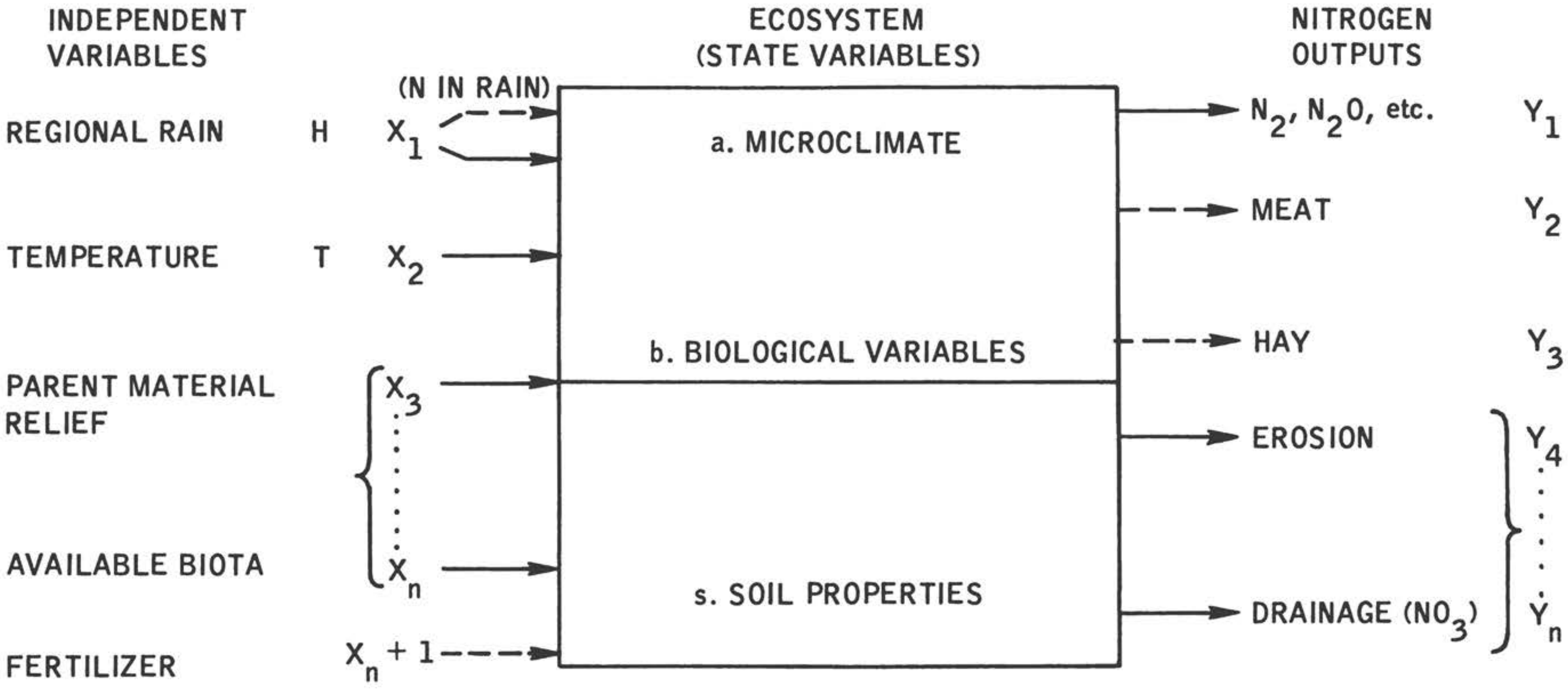

FIGURE 1 Relationship between ecosystem properties, independent variables (inputs), and nitrogen outputs.

Some History of the State Variable Approach Applied to Grassland Soil Nitrogen

Pedological equations of state discussed in detail by Jenny (1941) show surprising geographical regularity in accumulations and equilibria under native prairie conditions. These functional relationships between soil properties (s), biological components (b), and the ecological system's microenvironment (a) are state variables that in turn were influenced by moisture (H), temperature (T), and other independent variables or inputs (called X_i in Figure 1). Jenny immigrated to Missouri from Zurich, seeking a "viewpoint of a general theory of state (allgemeine Zustandslehre) in which soil properties, soil processes, and soil-forming factors are united into a comprehensive system" (1930b, page 1053). He had examined in considerable detail the exponential decline of surface (0 to 15 or 18 cm) soil nitrogen percentage as a function of temperature (1928, 1929). He reconsidered the same data (1930a) in relation to various measures of moisture (H = precipitation/absolute saturation deficit = Niederschlag Sättigungsdefizit Quotient = N. S. Q. of Meyer and also the simpler precipitation/temperature quotient P/T or "rain factor" of Lang and the precipitation/evaporation quotient, P/E, of Transeau). Jenny (1961) summarized this and much more relevant material.

The combination of both variables can be restated somewhat differently by:

$$N = f(T, H \text{ given other soil-forming factors nearly constant}) \qquad (1)$$

Deviations (dN) from any standard condition are just a sum of contributions due to each relevant independent variable:

$$dN = \left(\frac{\partial N}{\partial T}\right)_H dT + \left(\frac{\partial N}{\partial H}\right)_T dH + \sum_{i_{3\ldots n}} \left(\frac{\partial N}{\partial X_i}\right)_{T,H} dX_i + \text{extra terms} \qquad (2)$$

Here the summation sign includes influences of factors other than temperature and moisture multiplied by the differentials of these other factors from the chosen standard reference condition. The condition described in the case study below includes near-native grassland biotic factors, moderate drainage and exposure, potentially loamy substrate, and sufficient time for ecological succession and soil formation to equilibrate through developmental processes. The extra terms may represent quadratic and higher order terms in a Taylor series expansion of the function f, plus a sum of random variables inherent in either the inputs to the ecosystem or the sampling properties of the system. Naturally, standard deviations increase with the local heterogeneity of the system. Standard or mean errors of a mean can be diminished if expanded sampling does not trap us into including too much heterogeneity of local independent variables.

Jenny's east-west loamy grassland soil transects Colorado through central Missouri to Ohio and Texas to Mississippi (1930a, b). He demonstrated that the total soil nitrogen content followed the general form

$$N_T = a\ (1 - e^{-k_2H}) \qquad (3)$$

for nearly constant temperature. This means essentially that soil N (Table 2) fits a pattern of rapid increases with each centimeter of rain (or unit of N.S.Q.) in dry regions, but progressively less per unit in wetter grasslands. The constants for the respective transects mentioned were

$$N_{11}o = 0.320\ (1 - e^{-0.0034H}) \qquad (3a)$$

$$N_{19}o = 0.110\ (1 - e^{-0.0073H}) \qquad (3b)$$

In accounting for the exponentially rising equilibrium values northward (as decay rates declined with decreasing temperature), equation 3 was modified to

$$N = Ce^{-k_1T}(1 - e^{-k_2\ H}) \qquad (4)$$

$$= 0.55e^{-0.08T}(1 - e^{-0.005H}) \qquad (4a)$$

A three-dimensional plot of the data available in the 1920's (Table 2) is shown in Figure 2. These and newer data need to be restated on the basis of nitrogen per unit area (g/m^2 = $tons/km^2$ = 10 x kg/hectare = 8.9 x lb/acre) both for the surface soil and for the remaining horizons that are normally included in the more careful pedological analyses.

Unfortunately, good sequences of grassland soils of known age are rare. The chronosequence described by Olson (1958) happens to be based on much sandier soils than those of Jenny--probably even less silty than the sand hills of Nebraska and other Great Plains states. Olson's paper follows earlier examples of Jenny <u>et al</u>. (1949) in suggesting a formula for nitrogen buildup as a function of time t. The increase toward a limiting value N_{lim} is similar to that of equation 3 as a function of moisture H:

TABLE 2 Observed and calculated values of the soil nitrogen-temperature-humidity factor equation[a]

Annual Temperature (C°)	Annual Humidity Factor (N.S.Q.)	Average Total Nitrogen Content of Soil (%)		Regions
		Observed	Calculated	
0.0	350	0.47 ± 0	0.45	South-central Canada (Saskatchewan, Manitoba)
2.2	200	0.29 ± 0.050[b]	0.29	Southwestern Saskatchewan and northwestern North Dakota
	380	0.34 ± 0.017	0.38	Southeastern Saskatchewan and northeastern North Dakota
4.4	220	0.24 ± 0.015	0.26	Southwestern North Dakota
	350	0.30 ± 0.017	0.32	Southeastern North Dakota
5.6	380	0.27 ± 0.018	0.30	West-central Minnesota
6.7	350	0.27 ± 0.012	0.27	South-central Minnesota
	420	0.30 ± 0.028	0.29	West-central Wisconsin
7.8	420	0.26 ± 0.011	0.26	Southwestern Wisconsin
8.9	320	0.21 ± 0.014	0.21	Northwestern Iowa
	380	0.28 ± 0.016	0.23	Northern Illinois
10.0	230	0.19 ± 0.012	0.17	South-central Nebraska

	350	0.17 ± 0.005	0.20	Southern Iowa
11.0	75	0.08 ± 0.009	0.07	Southeastern Colorado
	125	0.11 ± 0.007	0.11	East-central Colorado and Northwestern Kansas
	275	0.20 ± 0.005	0.17	Northeastern Kansas and Northwestern Missouri
	325	0.19 ± 0.005	0.18	North-central Missouri
	375	0.22 ± 0.008	0.19	Central Illinois
13.3	350	0.16 ± 0.020	0.16	Southern Illinois
14.4	150	0.09 ± 0.006	0.09	Texas Panhandle
16.7	350	0.12 ± 0.016	0.12	Northern Mississippi
19.0	150	0.08 ± 0.007	0.06	Central Texas
	250	0.095 ± 0.006	0.08	Eastern Texas
	350	0.10 ± 0.006	0.10	Central Mississippi
22.2	200	0.075 ± 0.004	0.06	Southern Texas

[a] Jenny, 1930b.

[b] Mean error.

$$N = N_{lim} (1 - Ge^{-K_3 t}) \qquad (5)$$

$$= 0.087 (1 - 0.997e^{-0.003t}) \qquad (5a)$$

Both the turnover fraction -0.003 and the asymptotic value for soil nitrogen (here for 0-10 cm of soil only) were expected to vary, even locally, as a function of topographic and parent-material factors (including siltiness due to residual loess in the oldest dune soils) and, of course, as a function of input rates of nitrogen. Here $G = 1$ minus a fraction of the equilibrium value that is already present in parent material or in the preexisting soil that was entering a new system of management. If $G > 1$, equation 5 describes decrease from an initial value to a lower limiting value (Jenny, 1941, page 149; Bartholomew and Kirkham, 1960). We suggest that a term like that in parentheses in equation 5 could simply be multiplied by equation 4 to give a still more general expression that would encompass changes in the nitrogen accumulation naturally through time or through depletion due to cultivation (if insufficient crop residues are left in the soil):

$$N = ce^{-k_1 T}(1 - e^{-k_2 H}) \quad (1 - Ge^{-k_3 t}) \qquad (6)$$

Additional terms like those in equation 2 could be added to account for any significant soil-forming factors besides climate and time and for stochastic variation that is not merely an artifact of sampling.

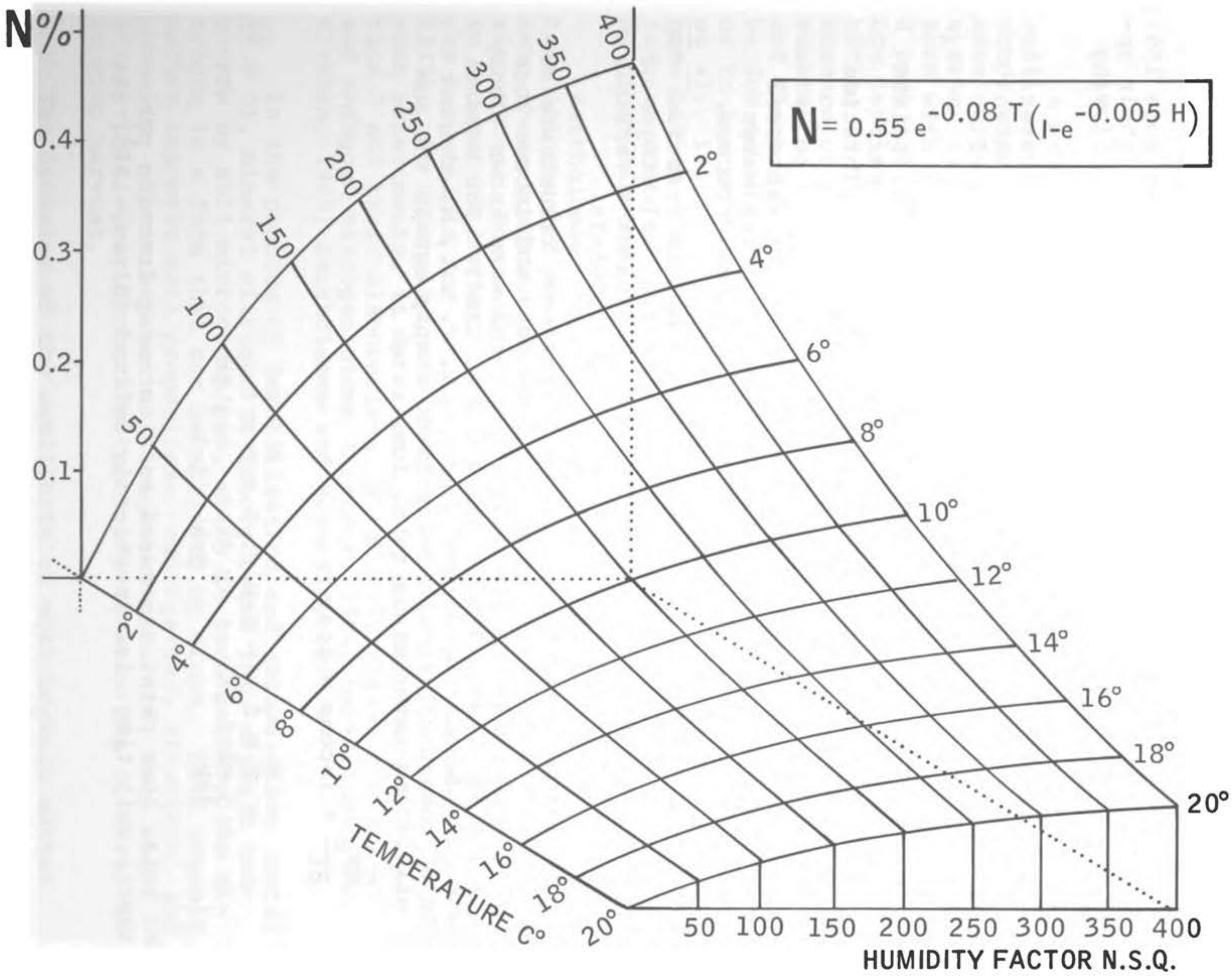

FIGURE 2 The nitrogen content of loamy grassland soils in the United States as a function of annual temperature and humidity.

Income-Loss Balances

As explained more fully elsewhere (Olson, 1958, page 159), the sand dunes at the southern tip of Lake Michigan (their approximate age is known) may be compatible with the following simple hypothesis:

1) Income indicated by the early accumulation rates in 0-10 cm of soil is only 4 kg/ha (0.4 g/m^2), which seems compatible with commonly cited estimates of rainfall income of 4.6 to 9.2 kg/ha.

2) Fractional loss rates (the exponential coefficient of time in equation 4 are close to 0.003 per year, which implies loss equal to income after equilibration toward N_{lim}.

3) Lag time during the accumulation can be expressed in various ways, but 3/k = 1,000 years approximates the time to 95% of equilibration in overall process.

If there were somewhat different income and loss fractions for the several persistent, intermediate, successional stages of ecosystem development, succession would represent a variable approaching a variable, i.e., a shifting asymptote.

Equations 5 and 6, or their elaborations, illustrate solutions that could be deduced from such data and income-loss equations if pertinent plus and minus terms were known (Jenny <u>et al</u>., 1949; Olson, 1958, 1963 a and b, 1964). For several forms of N denoted by either j or k = 1, 2, ..., m, the net rate of change of the kth compartment simply equals the sum of all income rates minus total loss rate:

$$\frac{dN_k}{dt} = \text{income} - \text{loss}$$

$$= \sum_{\substack{j=1 \\ j\neq k}}^{m} N_j f_{jk} - (-N_k f_{jk})_{j=k} = \sum_{j=1}^{m} N_j f_{jk} \qquad (7)$$

The total loss rate, expressed as a turnover fraction per (short) unit time, is conveniently defined (Olson, 1965) as:

$$\underset{j=k}{f_{jk}} = f_{jj} = f_{kk} = -\sum_{\substack{k=1\\k\neq j}}^{m} f_{jk} \tag{8}$$

$$= -k \text{ (in terminology of Olson (1958) and Jenny)}$$

A major difference between grassland soils and those of cultivated land is that the former are always occupied by the crop, whereas the latter are periodically disturbed by alternate cultivation and cropping. Under the extensive grass-root system, the major fraction of soil nitrogen is in an organic form with low levels of ammonium-nitrogen (seldom more than 8 ppm) and even less nitrate (Harmsen and van Schreven, 1955). Low levels of nitrate in grassland soils may reflect an inhibition of the nitrifying microorganisms either through an absence of substrate (i.e., ammonia) or as a result of toxic substances excreted by the grasses (Theron, 1951; Soulides and Clark, 1958). Alternatively, the absence of nitrate may be due to the chemical or biological decomposition of nitrate or to its precursor nitrite in the grassland soil (see Clark et al., 1960). A total ecosystem model would emphasize the possibility that nitrate or ammonium is absorbed by very extensive roots or microorganisms as quickly as it is generated by other microorganisms, rain, or other inputs.

Bartholomew (1956 et seq.), Jansson (1958), and others have illustrated ways by which ^{15}N tracers have helped estimate these transfer and total turnover fractions in agronomic situations. However, a great deal of work is still required to understand the soil economy of the native prairie, which was responsible for creating rich humus reserves (White et al., 1945). In untangling the simultaneous incomes and losses in such experiments, it is essential to use methods like equation 7 and their elaborations, which distinguish the tracer and untagged nitrogen atoms (Kirkham, 1956; Bartholomew and Kirkham, 1960; Bartholomew and McDonald, 1966).

In the process of immobilization and accumulation (until $\frac{dN}{dt} = 0$), mineral nitrogen is incorporated into organic compounds by soil microorganisms, which in turn release the nitrogen in a form that can be utilized by crops. Soil organic matter improves soil properties: aggregation, structure, and porosity. There may be real long-term benefits, even where trial yield experiments are too crude to show big differences in crop harvest.

The dynamics of the equilibria of soil organic matter (nitrogen) have been considered in further detail from several points of view. Periodic organic inputs to a steady-state system may balance with decomposition or other losses. The turnover fraction is the sum of all losses (equation 8) expressed as a proportion of the quantity of organic matter present (Jenny et al., 1949; Bartholomew and Kirkham, 1961). One decomposition rate (Jenny, op cit.; Greenland and Nye, 1959; Bartholomew and Kirkham, 1960) or several (Woodruff, 1949; Russell, 1964; Gore and Olson, in press) may be estimated for materials of varying resistance.

Crop residues have been turned into the soil with before-and-after measurements of soil nitrogen to evaluate new equilibria (Albrecht, 1936; Bartholomew and McDonald, 1966). By conducting the experiments in lysimeters it has been possible to compile a balance sheet showing nitrogen gains, losses (Brown, 1965), and distribution in different components of the cropping system. Deficits occur in most instances (Chapman et al., 1949; Pratt et al., 1960) and suggest that denitrification can be important even in fairly well aerated soils.

The recovery rate for fertilizer nitrogen applied to grassland systems is seldom more than 60%. Dilz and Woldendorp (1960) concluded that nitrogen losses were the result of the action of denitrifying bacteria in the rhizosphere of plants. Considerable quantities of gaseous products (N_2 + N_2O) were formed when ryegrass and pea roots were incubated with nitrate (Table 4). With ammonium-nitrogen or in the presence of killed roots, nitrogen losses were slight (Table 5).

In natural systems such as grassland communities, low rates of nitrogen fixation (up to 1 g/m^2 per year) would be adequate to maintain the nitrogen status with no harvested output such as meat products or hay. Where large quantities of nitrogen are removed in crops, as in intensive farming, more than 5 g/m^2 per year may be required. Parker (1957) presented evidence of significant nitrogen accumulation in grassland soils and attributed this to the activity of free-living bacteria. Estimates of nitrogen gains in grass-soil systems under field and laboratory conditions are presented in Tables 6 and 7.

Herbaceous systems (usually grass) have been established and maintained for extended periods of time, and the resulting nitrogen changes are measurable (Richardson, 1938; McHenry and Newell, 1947; Goedewaagen and Schuurman, 1950; Haas and Evans, 1957; Clement and Williams, 1964). Fresh inputs of carbon and nitrogen from grass roots directly to the undisturbed soil matrix account for an appreciable accumulation of nitrogen.

TABLE 3 Distribution of fertilizer nitrogen between herbage, roots, and soil (240 mg KNO_3-N added per sod)[a]

	mg N per sod			
	Herbage	Soil + roots	Roots	Total
Sandy soil	155	45.6	22.9	201
Clay soil	142	46.0	25.0	188
Peat (95% W.H.C.)	100	43.0	4.5	143
Peat (80% W.H.C.)	137	57.1	5.0	194

[a] Dilz and Woldendorp, 1960.

TABLE 4 Release of gaseous nitrogen products (N_2 + N_2O) by plant roots[a b]

Treatment	Nitrogen gas released (mg)
Control (no plants)	0.1
4-day perennial ryegrass	1.0
14-day perennial ryegrass	1.7
8-day peas	3.2
21-day peas	3.6

[a] 10 mg of nitrate-nitrogen added to vermiculite in the presence or absence of plants.
[b] Woldendorp, 1963.

TABLE 5 Effect of living and dead root systems on nitrogen losses[a]

	Nitrogen Source (%)	
	NO_3^-	NH_4^+
Living root system	20	7
Killed root system	7	0

[a] Woldendorp, 1962.

TABLE 6 Nitrogen gains in grass-soil systems (field experiments)[a]

Description	Nitrogen gain (lb/A/yr)	Reference
Bluegrass plots, previously cultivated 35 years (Bethany, Mo.)	102	Whitt (1941)
Solonized soil under *Lolium rigidum* (Merredin, W. Australia)	22	Parker (1957)
Latosolic soil under grass (*Cynodon plectostachyus*) (Ibadan, W. Nigeria)	80	Jaiyebo and Moore (1963)
Grass cut periodically and allowed to lie (New York)	40	Lyon and Wilson (1928)
Kentucky bluegrass, miscellaneous native grasses and weeds (no legumes) (Pennsylvania)	15	White *et al.* (1945)
Native grass sod on desurfaced Blackland soil (a few legumes included) (Texas)	30	Smith *et al.* (1954)

[a] Moore, 1966.

TABLE 7 Nitrogen gains in grass-soil systems (laboratory experiments)[a]

Description	Nitrogen gain (lb/A)	Period (days)	Reference
Yolo soil incubated with decaying, inverted grass sod in desiccator with N-15-labeled atmosphere (California)	12	31	Delwiche and Wijler (1956)
Grass sod grown under low light intensity in Yolo soil with N-15-labeled atmosphere (California)	0	31	Delwiche and Wijler (1956)
Lolium perenne grown in fertile alluvial sandy loam (England)	46	42	Ekpete and Cornfield (1964)

[a] Moore, 1966.

More important is the fact that the C-N organic form is not subject to leaching and denitrification losses. Maintenance of nitrogen reserves in this manner is suitable for grazing or park land but is impractical under intensive cropping systems.

Missouri Prairie: A Case Study

Complementing the regional analysis approach of Jenny, we analyzed the nitrogen cycle of a native grassland in greater detail. The area near Columbia, Mo., probably represents the precultivation nitrogen content in this ecosystem. Basic principles of the nitrogen cycle are exemplified because of the grassland community's primeval condition and the absence of prior agricultural manipulation. Information obtained there can serve as a reference for future comparison with other agricultural systems found in the vicinity and native grasslands elsewhere. A large quantity of organic matter (4% in the A_1 horizon) and corresponding nitrogen (0.21%) have accumulated in this (Dahlman and Kucera, 1965) and associated (Weaver et al., 1935) soil.

The nitrogen content of a peak standing crop of shoots (3.6 g/m^2) and roots (6-12 g/m^2) is shown in Figure 3. A comparison of the nitrogen distribution in plant biomass, litter, and soil compartments indicates that the major portion of nitrogen is present in the soil. Only a small part of the total ecosystem budget is associated with recent organic production (plant parts and microorganisms). Because most of the nitrogen is in the reserve category and only a small fraction is present in biological parts, it is possible that the transfer rates, residence time, and interrelationships among the different components contribute greatly to the economy of the ecosystem. Preliminary analysis of this system suggests that new production is supported by the available nitrogen pool, much of which arises from rapid turnover of biological materials, which is weekly for microorganisms (Jansson, 1958) and 4 years for roots (Dahlman and Kucera, 1965) and shoots (Koelling and Kucera, 1965a). Field studies with ^{15}N would reveal more precisely the major source of the nitrogen assimilated into the new biomass.

Appreciable growth above ground--at least 500 g/m^2 (Koelling and Kucera, 1965b)--and only negligible consumption in areas reserved from grazing produces a sizable cover of standing dead grass and litter. Distinctive litter layers are not apparent, however, in contrast to the situation of several mulch stages of decay described by Dyksterhius and Schmutz (1947). This implies a nearly complete decomposition

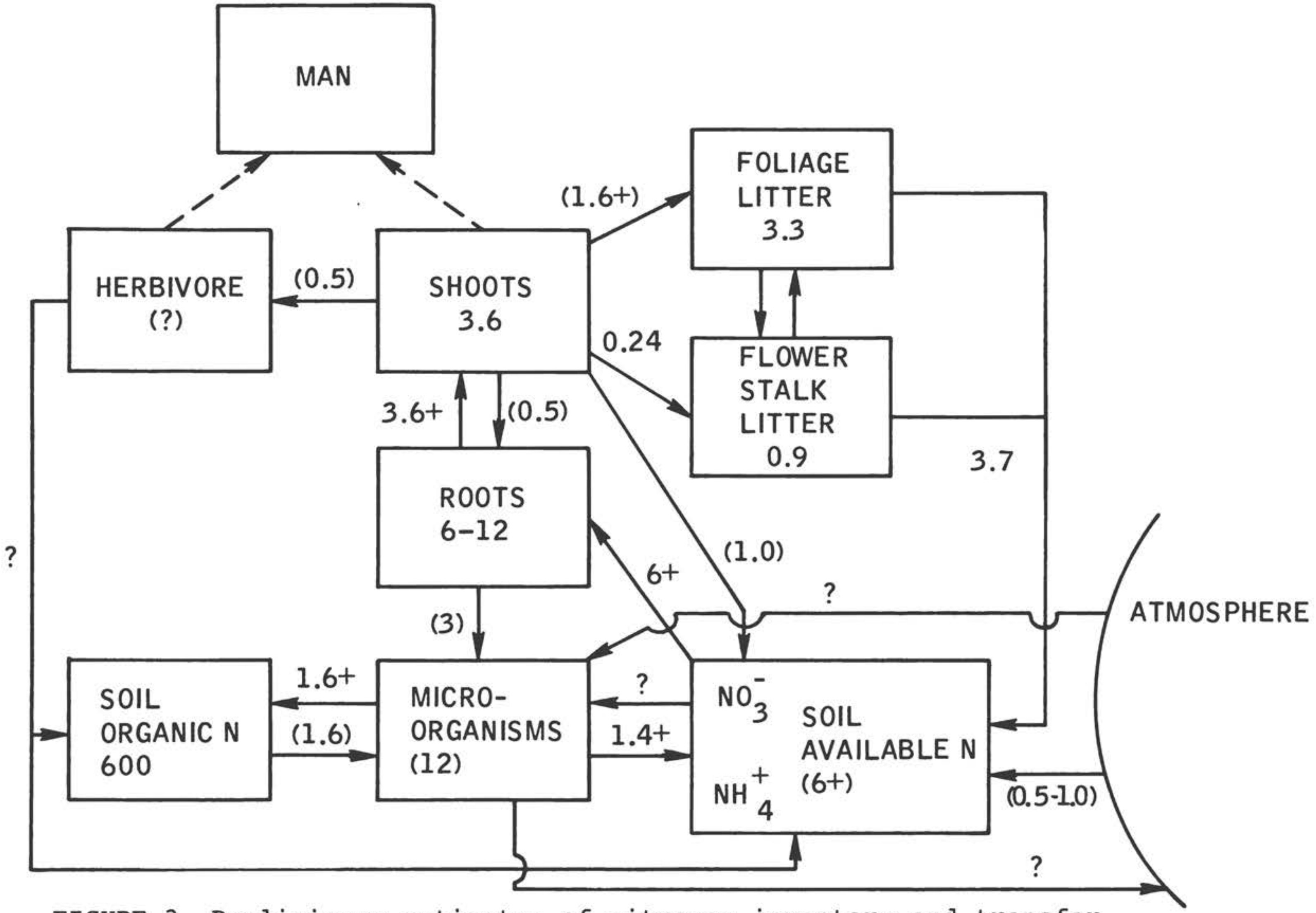

FIGURE 3 Preliminary estimates of nitrogen inventory and transfer relationships for different compartments of a native prairie ecosystem. Contents and rates are expressed in grams per square meter per year; numbers in parentheses are first-order approximations.

of organic materials above ground and a neglibible input of residue to the soil. It is more likely that during litter breakdown nitrogen returns to the available pool, thus maintaining the balance of the system. The magnitude of this return (3.7 g/m^2) contributes appreciably to the supply of available nitrogen. This value is based on a summation of nitrogen release from different age fractions over a 4-year period. It is less than the inventory of both foliage and flower stalk litter because some interchange between these compartments probably occurred, and a small quantity of nitrogen was prevented from returning to the available pool.

The fate of nitrogen in the litter compartment is presented in Table 8 for a 2-year period. A close relationship between carbon and nitrogen is illustrated in the dead plant biomass. In the foliage litter compartment, nitrogen was initially a limiting factor for microorganisms as they oxidized the carbon to CO_2, causing a reduction in the C/N ratio but not allowing significant transfer of nitrogen to the available nitrogen pool. There was essentially no change in the nitrogen content in this compartment after 1 year, and 25% of that which was present had disappeared after 2 years. Presumably, it was returned to the available nitrogen pool of the soil. Even loss of nitrogen at the C/N of 50/1 is somewhat surprising because mineralization usually occurs after the ratio approaches 20/1 (Jansson, 1958). Particulate losses of organic nitrogen from the litter containers probably accounted for some of the observed nitrogen decrease.

The flower-stalk compartment initially possessed an extremely high C/N ratio (300/1), which was an indication of an even greater shortage of nitrogen for the microbial populations. It is possible that this demand is partially satisfied by transfer from foliage to flower-stalk litter since there was an increase in nitrogen content of the decomposing flower stalks (0.24 to 0.42 g/m^2) in the 2-year period. Assuming heterogeneity in the physical matrix of these two compartments (foliage and flower-stalk), the small loss of nitrogen from foliage could transfer directly to the flower stalks. Thus, some mineralization decreases may be retained in the aboveground component and reduce that available for incorporation into new growth. After 2 years the C/N ratio was still 100/1, a level that is not likely to release much nitrogen for use by other components of the ecosystem. Other parts of the ecosystem probably contribute significantly to the nitrogen economy because the quantity from litter accounts for about half of the inventory in higher plants.

Then, let us consider root system turnover and dynamics in relation to nitrogen. By using a ^{14}C label, it was possible

TABLE 8 Carbon-nitrogen relationships in grass foliage and flower stalk litter[a] in Missouri prairie[b]

Decomposition Time (years)	Organic Content of Biomass[c] (g)		Nitrogen Content (%)	C/N	Nitrogen Balance for Litter Component[e] (g/m^2)	Net Change in Nitrogen (g/m^2)
	O.M.	C[d]				
			Prairie Grass Foliage			
Initial	45.6	23.1	.41	110/1	1.4	
+ 1	33.0	16.5	.51	70/1	1.3	-0.1
+ 2	16.6	8.3	.80	50/1	1.1	-0.3
			Prairie Grass Flower Stalks			
Initial	48.9	24.5	.15	300/1	0.24	
+ 1	40.9	20.5	.26	185/1	0.33	+0.1
+ 2	28.7	14.4	.47	100/1	0.42	+0.2

[a] Litter in mesh containers to permit recovery and measurement.
[b] Koelling and Kucera (1965a).
[c] Based on initial quantity of 50 g.
[d] Based on 50% carbon in the plant organic material.
[e] Assuming that foliage constitutes 70% and flower stalks 30% of total production.

to assess carbon transformations between roots and soil (Dahlman and Kucera, 1967). In the first year following ^{14}C assimilation by the plants, at least 45% of the ^{14}C lost from roots was measured in soil organic matter. Four months later about 10% of the root loss was present in the soil (Table 9). In effect, over 90% of the new root organic input to the soil was oxidized in this rather short period--16 months. On the basis of an annual organic contribution from roots of at least 430 g/m^2 (Dahlman and Kucera, 1965) and the carbon (40%) and nitrogen (0.7%) content of roots, the input of root nitrogen to the soil was calculated as 3 g/m^2. Because of the rapid turnover, a corresponding prompt reduction of the C/N ratio from 55/1 to 6/1 would be expected, assuming the nitrogen is retained in some organic C-N complex. Obviously, mineralization would occur as the C/N approached 11/1, the normal relationship in this soil. While this discussion greatly oversimplifies the nitrogen mineralization-immobilization phenomena, there are evidently important relationships between root turnover and the nitrogen economy of the native grassland ecosystem.

On the basis of carbon loss greater than that required to maintain the normal C/N ratio, organic nitrogen would be mineralized and released to the available nitrogen pool. This quantity was estimated to be about half (1.4 g/m^2) of that present in the annual root input (3 g/m^2). The rest (1.6 g/m^2) probably remained as organic nitrogen and was added to the large quantity already present in the soil. It is recognized that this approach provides only an approximation of nitrogen distribution between the organic and available fractions as the result of mineralization of roots. Field experiments with ^{15}N would permit better quantification of this differential.

The quantity of available nitrogen in the soil varies seasonally, and the annual inventory is not likely to exceed 1% to 2% of the organic nitrogen or 6 to 12 g/m^2. The annual input to the available nitrogen pool from different sources was calculated as 3.7 g/m^2 from litter decomposition, 1.4 g/m^2 from root turnover, and 0.5 to 1.0 g/m^2 from rainfall. Undetermined inputs would be biological fixation and mineralization of the microbial tissues. The estimated inputs accounted for the minimum quantity that probably was present in the available nitrogen pool on an annual basis.

Utilization of available nitrogen by higher plants was at least 6 g/m^2 on the basis of the inventory of the season's root and shoot growth. This value agrees well with the estimated annual available nitrogen pool. Although the demand of microorganisms for nitrogen is unknown, it probably is as great as that of higher plants. Under steady-state conditions, it

TABLE 9 Turnover of root and soil carbon following a single organic input of 450 g/m^2 to the root-soil matrix[a]

Decomposition time (months)	Single organic input from roots (g/m^2)	Fractional carbon transfer to soil organic matter (g/m^2)	Quantity of C remaining in soil organic matter[b]		Implied turnover (g/m^2)
			(%)	(g/m^2)	
14	430	170	9	15	155
26	-	-	3	9	330

[a] Nitrogen content of roots was 0.7%.

[b] Based on ^{14}C analysis (Dahlman and Kucera, 1967).

is likely that microorganisms maintain the pool at a relatively constant level because of the equilibria established between immobilization and mineralization. For the most part, microbial inputs to the pool would be compensated for by corresponding utilization. Consequently, the nitrogen demand of higher plants would largely be satisfied from nitrogen released from mineralized organic inputs (roots and shoots), assuming negligible input from other sources.

Information was not available concerning the nitrogen inventory and transfer rates related to the microorganism compartment of the system. The standing crop nitrogen content of this compartment was estimated to be 2% of organic nitrogen (Russell, 1961), which as a first-order approximation was 12 g/m^2. Transfers to soil organic nitrogen and available nitrogen, as shown in Figure 3, reflect the fate of nitrogen from roots following decomposition. Distinguishing between plant-nitrogen and microorganism-nitrogen relationships is a formidable task, and the resulting informational void hinders interpretation of this part of the nitrogen cycle.

Another part of the nitrogen cycle concerns the immediate fate of the nitrogen metabolites in the shoots at the end of the growing season. That considerable redistribution probably occurs is indicated in the results of Koelling and Kucera (1965a), where the content in the current season's litter was significantly less than that in the mature standing crop (0.41% vs. 0.73%). This decrease was attributed to retranslocation within the intact plant and to leaching losses from standing dead material. For illustrative purposes here, the retranslocation was estimated at 0.5 g and leaching from dead standing crop at 1.0 g/m^2. Further investigation with a ^{15}N tracer would determine the magnitude and fate of nitrogen metabolites as the shoots mature and death ensues.

Summary

A preliminary model of a nitrogen cycle was presented for a native grassland ecosystem. Quantitative inventory and transfer rates are suggested for several compartments. On the basis of incomplete data, it was concluded that appreciable nitrogen was returned to the available nitrogen pool by means of shoot and root organic turnover. Mineralized nitrogen from these sources would nearly satisfy the minimal nutritional demand by higher plants. Enumeration of the nitrogen inventory and transformation of microorganisms was incomplete or at best a first-order-of-magnitude estimate because the cycling parameters are very difficult to measure in the field. Further progress in system analysis of the nitrogen cycle requires additional field experiments concerning microbial transformations. Perhaps an outcome of the International Biological Program's planning meeting on nitrogen processes will be the design of investigations that consider all aspects of the nitrogen cycle quantitatively and simultaneously.

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EFFECTS OF NITROGEN FROM LEGUMES AND CROP RESIDUES ON SOIL PRODUCTIVITY

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For many centuries, man has recognized that the nitrogen fixed by legumes is a contribution to the store of nitrogen available in the soil. Only recently, however, has knowledge developed concerning some of the interactions between the soil environment and both leguminous and nonleguminous plant materials, and their influence on soil productivity. This paper recounts experimental evidence bearing on the effect of the aeration status of soil on the immobilization of nitrogen contained in incorporated plant materials and the effect on the soil's physical condition of the nitrogen concentration in plant material incorporated into soil. The applications of the results of both types of interaction to soil productivity are illustrated, and promising leads for further investigation are identified.

Nitrogen Fertility Aspects under Aerobic and Anaerobic Soil Regimes

Materials with Low Nitrogen Concentrations

Under an aerobic regime, fresh organic material containing less than an average concentration of 1.5% nitrogen generally immobilizes much of the available soil nitrogen. Actually, this value is an average of the net immobilization percentages, ranging from 1% to 2%, that are needed to satisfy the microorganisms of decomposition. This range of net immobilization percentages results from variation in the chemical and physical nature of the organic material, soil pH, soil moisture, and amount of nitrogen and other nutrients in the soil (Bartholomew, 1965).

Anaerobic conditions also have received some study in laboratory investigations (Acharya, 1935; Sircar *et al.*, 1940; Mitsui, 1954), and the results have indicated that the nitrogen requirement is much lower than for aerobic conditions. We have endeavored to test this information under field conditions in a rice paddy culture and to establish the net immobilization percentage for rice straw returned to the anaerobic soil (Williams et al., in press). In our experiments, the yield response of rice to the incorporation of rice straw was a function of the nitrogen concentration in the straw. This is shown in Table 1, where seven comparisons are made among various lots and amounts of rice straw. A highly significant linear regression results, $Y = 57.6\ N - 31.4$, and it is clear that nitrogen immobilization and mineralization, as reflected in rice yield response, is associated directly with the nitrogen level in the straw. Yields were increased by straws with nitrogen concentrations above 0.6% and reduced by straws with less than 0.5% nitrogen. When evaluated in terms of field response, the net nitrogen immobilization percentage in rice straw (percent of original dry material) can be calculated from the regression equation to be 0.54%. How well the regression fit ($p < 1\%$) confirms the importance of the nitrogen percentage in the straw. Effects were insignificant from other confounding variables, such as the amount of straw (2 to 4 tons/acre) and length of exposure to weathering (1 to 6 months of the rainy season).

TABLE 1 Effect of nitrogen concentration of rice straw incorporated in the soil on the yield increment of rice grain relative to control (no straw)

Straw Lot No.	Straw Dry Weight (ton/acre)	Straw N (%)	Yield Increment over Control (cwt/acre)
1	3	0.64	+7.4
2	3	0.70	+6.7
3	4	0.55	+1.4
3	2	0.55	-1.4
4	4	0.46	-3.4
5	3	0.45	-4.7
4	2	0.46	-5.6

It is evident that the nitrogen requirement for the decomposition of rice straw in flooded soils is one-third of the average concentration of nitrogen required for organic residue decomposition in an aerobic soil environment (0.54% vs. 1.5%). Practically, this means that rice crop residues produced at moderately high nitrogen fertility conditions will not immobilize nitrogen but will release nitrogen during decomposition in a flooded soil environment.

Materials with High Nitrogen Concentration

Investigations of the use of farm-grown organic sources of nitrogen in a California rice culture have also considered materials with high nitrogen content. The high-nitrogen materials are typified by vetch grown as a green-manure crop during the winter season. Abundant evidence has been accumulated showing that vetch containing 3% to 4% nitrogen, as it normally does at plow-down time, is a highly effective (and often inexpensive) source of nitrogen for the succeeding rice crop (Williams *et al.*, 1957b; Williams and Finfrock, 1962).

The depth of incorporation of a high-nitrogen organic material may influence the subsequent path of chemical change in the nitrogen that it contains. The oxidation status of a rice soil soon after flooding varies in depth from an oxidizing condition at the soil-water interface to a strongly reducing state at a depth of 1 inch or more. The oxidative pathway leading to nitrate production causes nitrogen loss because of subsequent denitrification to molecular nitrogen and nitrogen oxides in the reducing zone of the flooded soil. Moreover, prior to the flooding of a field for rice culture, conditions may be favorable for aminization, ammonification, and nitrification if soil temperature, moisture, aeration, etc., are appropriate. Hence, loss of effectiveness of the green-manure nitrogen is a possibility if incorporation precedes flooding of the field by a long period. Vetch was incorporated at various intervals prior to flooding and sowing the rice. Under conditions favorable to nitrification during preflooding, the shorter the period the greater was the effectiveness of the organic nitrogen source. Under dry soil conditions, no loss of effectiveness occurred.

Nitrogen contained in leguminous green manures is usually considered less efficient than fertilizer nitrogen as a result of tests with crops grown on well-drained soils. However, the experiments cited above show that when green manure high in nitrogen is applied well into the reducing zone of a flooded soil, efficiency of utilization is equal to that of the best mineral source of nitrogen. Other experiments have shown that the response of rice to green manure nitrogen can greatly exceed the response to mineral nitrogen that has been placed improperly on the soil surface or in the flood water.

The following problem areas are worth further investigation:

1. The influence on the net immobilization of nitrogen of other environments in which anaerobic conditions occur, e.g., flooding during cold weather, intermittent flooding, and continuous and intermittent high water tables.
2. The behavior of low-nitrogen-containing residues of alternate crops commonly grown in rotation with rice relative to their net immobilization of nitrogen.
3. The species of organisms and the nature of the chemical reactions that occur under anaerobic conditions as related to the redox potential of the soil.
4. Recycling rates of immobilized nitrogen under the various environmental variations common in flooded soils. Variables of particular interest are source of available energy and system pH.

Nitrogen Concentration in Incorporated Plant Materials and Structural Improvement of Aerobic Soils

In many areas of California, irrigation water infiltrates the soil very slowly. The cause is poor soil structure resulting from soil compaction or from an inherent condition of aggregate instability common in many soils of semiarid lands. A recognized practice for maintaining the productivity of intensively cropped soils is the frequent incorporation of organic materials, usually crop residues. A major benefit expected from this is maintenance of a satisfactory soil structure (Martin *et al.*, 1955). In a series of field experiments, we have studied the effect of the incorporation of various kinds of plant materials on the infiltration rate of water into irrigation furrows, a sensitive measure of structural status of a soil *in situ*. In several of these experiments we were able to study the relationship between infiltration rate and the nitrogen concentration in the material incorporated. A comparison was made of corn crop residue, cotton crop residue, and cowpea green manure covering a range of nitrogen concentrations from 0.7% to 2.5%. The result was a marked inverse association between nitrogen concentration and infiltration rate (Table 2). Cowpea green manure (tops contained 2.5%N) was ineffectual, whereas corn crop residue (tops contained 0.7%N) doubled infiltration rate as averaged for two conditions, tractor-disturbed and undisturbed furrows.

TABLE 2 Effect of nitrogen concentration in incorporated plant material on rate of infiltration of water in the field

Plant Material	Dry Matter (Tops) (ton/acre)	N (%)	Infiltration (gpm/100 ft)	
			Tractor Disturbed	Undisturbed
Corn crop residue	3.0	0.7	1.0	1.7
Cotton crop residue	3.6	1.6	0.8	1.1
Cowpea green manure	2.5	2.5	0.7	0.7
None	-	-	0.7	0.7
LSD 5%	0.6	0.2	0.2	0.3

Another field experiment compared three green manures producing equal amounts of above-ground plant material: annual ryegrass (tops contained 1.82% N), soft chess (tops contained 1.86% N), and mustard (tops contained 3.43% N). The resulting infiltration rates were inversely related to nitrogen, 1.3, 1.1, and 0.9 inches per hour respectively (LSD 5%, 0.18). In an experiment with barley made in green manure at different stages of growth, a significant inverse relationship between nitrogen concentration and infiltration rate was again observed, although in this case an additional variable was the amount of material incorporated (Williams and Doneen, 1960).

In order to ascertain the effect of low-nitrogen crop residue on crop yield, a test crop of sugar beet was preceded by strips of barley. The residue from the barley crop (tops) amounted to 2.9 tons/acre and contained 0.25% nitrogen. Sugar beet was planted, and an overlay of several rates of ammonium nitrate was applied. Not only was the rate of infiltration markedly improved by the barley crop residue (+ 54%), but the beet crop's sugar production also improved. An average increase of 7.4 cwt sugar per acre ($p < 0.01$) resulted from the residue treatments in which nitrogen was not a limiting factor, i.e., 100 to 200 lb nitrogen per acre (Williams, 1966). Thus, sugar production was associated strongly with infiltration rate enhanced by residue incorporation. This demonstrates that the potential for nitrogen tieup even by materials containing very little nitrogen can be counteracted by fertilizing the crop with sufficient nitrogen to permit the benefit to soil structure to be realized (see Williams *et al.*, 1957a, for additional experimental evidence).

The longevity of the enhanced infiltration rate from low-nitrogen organic applications was studied in other experiments. In undisturbed soil the effects persisted through trial periods of up to 28 months (Williams, 1966). From the evidence obtained in these investigations and others, we may infer that the benefits to soil structure are the results of the activities of decomposing microorganisms and the resulting organic adhesive substances, mycelia, etc. These in turn may also be degraded microbially (Harris *et al.*, 1966). Hence, the effects are time-dependent. Materials that decay very rapidly, e.g., high-nitrogen materials, have only a short-lived effect and hence are of little practical benefit to the crop that follows incorporation.

Areas that need further study are:

1. Interactions between nitrogen and other constituents contained in organic additions to soil and the kinds of orgaisms and their products that are effective in aggregation in the field.
2. The time course and longevity of soil-structure enhancement as a function of nitrogen concentration of organic materials incorporated in the field.
3. Retention of nitrogen by the decomposing organisms, and fertility management practice to ameliorate the effect.

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AN INVESTIGATION OF THE NITROGEN-FIXING ABILITY OF NATIVE CHILEAN RHIZOBIA STRAINS AND SOME PROBLEMS RELATED TO THEIR SYMBIOSIS WITH SEVERAL LEGUMES

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The inoculation of legume seeds with specific and effective strains of *Rhizobia* has become a common practice of enormous importance. The legume-*rhizobia* association facilitates the establishment of artificial pastures and provides, in addition, a higher nitrogen content for the plants. It is a well-known fact that the amount of atmospheric nitrogen that can be fixed by these associations may reach large amounts (Alexander, 1964).

Rhizobia have been intensively studied in connection with their biochemical and symbiotic properties (Allen and Allen, 1950; Burton, 1965) and their serological structure (Bushnell and Sarles, 1939; Graham, 1963), although several fundamental facts about the infection, nodulation, and fixation itself remain to be elucidated. Some environmental factors affecting nitrogen fixation in nature have also been studied (de Mooy and Pesek, 1966; Tanner, 1964).

The importance of nitrogen fixation in agriculture has led to the development of research programs in several countries in order to attain highly infective and effective strains of *Rhizobia* for agricultural use. This is particularly important in Chile and in most of the Latin American countries in which production of meat and milk is not sufficient.

According to the information obtained from government institutions (Dirección de Estadísticas y Censos, 1961), alfalfa and clover are cultivated in artificial pastures in about 450,000 ha; 74% of this is red and white clovers and the remaining 26% is alfalfa. Inoculation of leguminous seeds has not become a generalized practice and, actually, few hectares have received this treatment in recent years.

A limiting factor in the establishment of pastures in our country is the low amount of available phosphorus in certain soils, mainly those located near the Andes mountains in the central part of the country, caused by phosphorus fixation (Bleiholder, 1965). Lack of nodulation has been demonstrated in alfalfa grown in these soils when phosphorus fertilization has not been applied at adequate levels (Rodríguez, 1962). Phosphorus plays a very important role in nitrogen fixation (de Mooy and Pesek, 1966), but its primary effect seems to be exerted on the plant host rather than on the microsimbiont (Alexander, 1964).

There are, then, two principal subjects to be studied in relation to the legume-*rhizobia* association in Chile as a basis for further research: *first,* the nitrogen-fixing ability of native strains that belong to several important cross-inoculation groups and, second, the influence of some soil-fertility conditions that may affect the symbiosis. This paper is a summary of the research conducted at the Department of Microbiology of the University of Concepción on the basis of these two approaches.

General Methods Used in These Experiments

Native strains were collected from nodules of different species of *Medicago* and *Trifolium* by the usual bacteriological techniques *(Allen,* 1957). Plants were grown in jars according to the glazed jar method (Allen, 1957). The previously disinfected seeds were planted in quartz sand or in soil with and without phosphorus fertilization. A nitrogen-free nutrient solution was used in irrigating. Plant weight and nitrogen content (micro-Kjeldahl) were determined and results were expressed as plant weight (g per 100 plants), percentage of nitrogen or total nitrogen (mg N per 100 plants). The effectiveness of the various strains was evaluated by comparing the results with those obtained in two sets of control plants: noninoculated plants and plants inoculated with an efficient strain (a commercial inoculant). Those strains behaving in a similar way to the effective control strain but giving results significantly higher than those from noninoculated plants were considered to be effective. Strains giving results not significantly different from those of noninoculated plants were considered to be ineffective, and intermediates were considered to be moderately effective. This nomenclature is similar to that used by Burton (1952).

Experiments Conducted with *Rhizobium meliloti* and *Medicago sativa*

Alfalfa is cultivated in Chile on about 120,000 ha, and efforts are being made to establish it in other parts of the country. However, serious problems have arisen and the efforts have been unsuccessful in most instances. The problems seem to be related to the inefficiency of rhizobia strains and, probably, to the low level of available soil phosphorus.

Five native strains of *Rhizobium meliloti* were tested (Zemelman *et al.*, 1964) according to the methods described above. Three experiments were conducted in parallel: (1) plants grown in quartz sand and inoculated with native strains; (2) plants grown in a phosphorus-fixing type of soil without phosphorus fertilization but inoculated with an effective strain of *Rhizobium meliloti*; and (3) plants grown as in 2 but with the addition of phosphorus in large amounts (10,000 U/P205/ha). Results are shown in Tables 1 and 2.

Several facts were observed in these experiments. First, none of the native strains under test showed an effective nitrogen-fixing property (Table 1) when plants were grown in quartz or soil with phosphorus fertilization. This was true regardless of whether the results were evaluated by plant weight or by total nitrogen content.

Table 2 shows the typical behavior of one of the five native strains in comparison with the effective control strain. When plants were grown in quartz sand, the native strain proved to be ineffective with respect to plant weight, percentage of nitrogen, and total nitrogen. There were very large differences between the results obtained with the native strain and those obtained with the commercial inoculant. In plants grown in soil without phosphorus there was no nodulation, growth was evidently inhibited, and, although the nitrogen percentage in the plant tissues was high (more than 4%), total nitrogen content was quite low. This nitrogen was supposedly obtained only from the soil and the seeds. No appreciable difference was found between the native strain and the commercial inoculant. When the same experiment was conducted in soil with phosphorus fertilization, it was observed that plant weight as well as the total nitrogen content increased, and a notable difference once again appeared between the two strains. In addition, the percentage of nitrogen diminished to values similar to those recorded in the quartz sand experiment. Hence, when phosphorus fertilization was not employed, the effective control strain was not able to establish symbiosis with *M. sativa*, and it behaved similarly to the native strain.

TABLE 1 Classification of five native strains of *Rhizobium meliloti* according to their effectiveness (host plant: *Medicago sativa*)

Experiment	Results expressed as	Percentage of Ineffective	Percentage of Effective
Greenhouse (quartz sand)	Plant weight	100	0
	Total nitrogen	100	0
Greenhouse (soil with P)	Plant weight	100	0
	Total nitrogen	100	0

TABLE 2 Plant weight, percentage of nitrogen, and total nitrogen in plants of alfalfa grown under different conditions and inoculated with a native strain of *Rhizobium meliloti* and a commercial inoculant

Experiment	Strain	Results Expressed as Plant weight	% Nitrogen	Total nitrogen
Greenhouse (quartz sand)	Native	1.95	1.14	22.3
	Commercial inoculant	19.34	2.47	477.9
Greenhouse soil (no P)	Native	4.04	4.66	188
	Commercial inoculant	3.13	4.50	141
Greenhouse soil (with P)	Native	49.79	1.73	860
	Commercial inoculant	68.86	2.43	1,674

It is concluded from these results that none of the five strains of Rh. meliloti tested were effective. Adequate levels of phosphorus are necessary for the establishment of symbiosis between Rh. meliloti and M. sativa, even when the seeds have been inoculated with strains of known effectiveness.

Experiments Conducted with Rhizobium trifolii and Trifolium pratense

Red clover is being cultivated in many areas of the country where artificial pastures have been established. In general, problems in growth and nodulation have not been observed.

Eighteen strains of Rh. trifolii were collected from nodules of red and white clover that had not received any previous inoculation. The native strains were tested in the greenhouse with quartz sand. Later, selected strains were used to inoculate seeds in a field experiment (Herrera et al., 1965). T. pratense was used as host plant throughout. Results are presented in Tables 3 and 4.

These results show that there was a relatively high frequency of isolation with effective strains. However, when the experiment was performed with selected strains in the field, this frequency was considerably decreased. In fact, no effective strains were detected when plant weight was used to evaluate effectiveness, and only 18.2% of the strains were considered to be effective when total nitrogen was used. The different behavior of the strains exhibited in the two experiments could be explained by the influence that several environmental factors exert on the activity of Rhizobia and on the symbiosis (Burton, 1965; Hely et al., 1957; Tanner and Anderson, 1964). This fact emphasizes the importance of performing the final test of effectiveness of selected strains under natural field situations.

It was also observed (Table 4) that when using T. pratense as the host plant, the strains isolated from T. repens exhibited a higher frequency of effectiveness. It should be kept in mind that white clover is the most abundant clover naturally growing in Chilean pastures. From these results it is concluded that the frequency of effective strains of Rh. trifolii normally present in Chilean soils might explain the lack of difficulties in establishing forage pastures with this legume.

TABLE 3 Classification of 18 native strains of *Rhizobium trifolii* according to their effectiveness (host plant: *Trifolium pratense*)

Experiment	Results expressed as	Percentage of	
		Ineffective	Effective
Greenhouse	Plant weight	61.1	38.9
	Total nitrogen	66.7	33.3
Field (11 strains)	Plant weight	100	0
	Total nitrogen	81.8	18.2

TABLE 4 Classification of 18 native strains of *Rhizobium trifolii* isolated from nodules of *Trifolium pratense* and *Trifolium repens* according to their effectiveness (host plant: *Trifolium pratense*)

Strains isolated from	Results expressed as	Percentage of	
		Ineffective	Effective
Trifolium pratense	Plant weight	69.2	30.8
	Total nitrogen	76.9	23.1
Trifolium repens	Plant weight	40	60
	Total nitrogen	40	60

Experiments Conducted with *Rhizobium trifolii* and *Trifolium subterraneum*

Several efforts have been made to establish the legume *T. subterraneum* in various parts of the country (coastal mountains) where the amount of rain is usually low and where there is no artificial irrigation. The difficulties observed seem to be due to the ineffectiveness of native strains, since nodulation has been reported to be deficient. The following experiment was conducted in order to determine the ability of those strains to associate with *T. subterraneum* and to fix atmospheric nitrogen (Zemelman *et al.*, 1967). Twenty-six strains were isolated from nodules of red and white clover and tested in the greenhouse; quartz sand was used. Results are shown in Table 5.

TABLE 5 Classification of 26 strains of *Rhizobium trifolii* according to their effectiveness (host plant: *Trifolium subterraneum*)

Experiment	Results expressed as	Percentage of Ineffective	Percentage of Effective
Greenhouse	Plant weight	88.5	11.5
	Total nitrogen	88.5	11.5

Similar results were obtained when plant weight and total nitrogen were used to evaluate the activity of each strain. Frequency of effective strains establishing symbiosis with *T. subterraneum* is low; hence, that failure to establish this forage legume may be due to the lack of active nitrogen-fixing strains in the soil. According to Strong (quoted by Vincent, 1945), there seems to be some relationship between the original host and the performance of the strain in the association with other clovers. Results reported by Vincent (1945) do not support this idea, but the present results clearly indicate that the frequency of isolation of strains from red or white clovers effective with *T. subterraneum* differs substantially from that reported for *T. pratense*.

Experiments Conducted with *Medicago sativa*, *Trifolium pratense*, and *Trifolium subterraneum* Grown under Different Levels of Phosphorus Fertilization in a Soil Exhibiting a High Phosphorus-Fixing Ability

It has already been stated that some Chilean soils, mainly those located near the Andes mountains, show a high phosphorus-fixing property. This characteristic has become a limiting factor in the establishment of some forage legumes (Bleiholder, 1965; Rodríguez, 1962), particularly alfalfa. The following experiments were carried out to determine how phosphorus fertilization influences the *rhizobia*-legume association and nitrogen fixation (Zemelman *et al.*, 1965).

Plants of *M. sativa*, *T. pratense*, and *T. subterraneum* were grown in a phosphorus-fixing type of soil (showing no available phosphorus in the absence of adequate fertilization). Seeds of each legume were planted in sets of jars containing increasing amounts of calcium monophosphate ($CaH_2PO4_2 \cdot H_2O$). These amounts were equivalent to: 10, 100, 316, 1,000, 3,160, and 10,000 U/P205/ha. All seeds were inoculated with an effective strain of specific rhizobium.

The following results were recorded:

1. No nodulation was observed in the plants grown under phosphorus levels below 316 U/P205/ha. In these instances, plant weight and total nitrogen were low, growth was inhibited, and nitrogen percentage was quite high (about 4%).

2. Nodulation appeared at 316 U/P205/ha, although this did not seem to represent a well-established symbiosis according to the appearance of the nodules.

3. At 1,000 U/P205/ha, nodulation was satisfactory, and plant weight and nitrogen content increased significantly. Nitrogen percentage abruptly dropped to values similar to those observed in plants grown in jars with phosphorus fertilization in the first experiment (Table 2).

4. At higher levels of phosphorus fertilization, plant weight continued to increase but at a higher rate than that of nitrogen content.

5. These results were similar in three species tested.

It is concluded that in this type of soil, phosphorus should be supplied in great amounts to allow the establishment of an effective association between *Rhizobia* and the host plant.

Summary

A series of experiments were conducted in order to determine the relative effectiveness of native strains of *Rhizobium meliloti* in symbiosis with *Medicago sativa* and strains of *Rhizobium trifolii* in symbiosis with *Trifolium pratense* and *Trifolium subterraneum*. In addition, the influence of different levels of phosphorus fertilization on symbiosis between specific and effective *Rhizobia* and the host plants mentioned above was studied.

No effective strains of *Rh. meliloti* were found when tested in the greenhouse and in the field. More than 30% of the native strains of *Rh. trifolii* were found to be effective with *T. pratense* in the greenhouse, and a low frequency of effective strains of *Rh. trifolii* with *T. subterraneum* (11.5%) was encountered.

It was found that more than 300 U/P205/ha were needed for the establishment of symbiosis between *rhizobia* and their specific host plant (*M. sativa*, *T. pratense*, and *T. subterraneum*) in a soil with high phosphorus-fixing ability. A significant increase in plant weight and nitrogen content was obtained by using 1,000 U/P205/ha.

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INOCULATION OF LEGUMES IN BRAZIL

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The practice of inoculation of legumes in Brazil is quite old. However, the manufacture of commercial inoculants began less than 20 years ago on a small scale at the Instituto Biológico do Estado de São Paulo. Soon after, in 1950, the Seção de Microbiologia Agrícola da Secretaria da Agricultura do Estado do Rio Grande do Sul also began to manufacture inoculants. The latter group has developed efficient Rhizobium strains for diverse groups of leguminous crops. The Instituto Agronômico do Estado de São Paulo and the Instituto de Pesquisas e Experimentação Agropecuários Centro Sul do Ministério da Agricultura, Rio de Janeiro, is now conducting research on strain selection and is manufacturing inoculants on a small scale. There is also a private firm in Rio Grande do Sul that namufactures inoculants on a large scale (Freire, 1964a), a small private laboratory in the state of São Paulo, and a state-supported institute in Paraná that produces a moderate amount.

The state of Rio Grande do Sul, which is one of Brazil's principal agricultural regions, has a subtropical climate. Extensive areas are devoted to leguminous crops for the production of grain (e.g., soybeans, French beans, and peas) and forages (e.g., alfalfa, various clovers, birdsfoot trefoil, cowpea, and serradela). For the most part, these legumes were adapted to soils rich in calcium, and therefore are highly dependent on soil pH for maximum yields and are also highly specialized relative to Rhizobia (Norris, 1965). Soybeans and possibly serradela are somewhat tolerant to soil pH variations. In the northern part of the state and in the central region of

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Brazil, tropical legumes predominate that are adapted to acid soils of low fertility and exhibit a high degree of Rhizobia promiscuity. The French bean that is cultivated throughout Brazil and many other Latin American countries belongs to the first group mentioned and is grown exclusively on fertile land rich in organic matter. Peanuts are cultivated widely in the state of São Paulo. However, legumes are probably of greatest importance in Rio Grande do Sul, both for human consumption and animal feed. Although legume research, production of inoculants, and their use in this state have rapidly advanced, the pace in other regions has been restricted because of the belief, even among many technicians, that tropical legumes do not benefit from nodulation or that inoculation is not necessary since effective nodules will form naturally.

Selection of Efficient Rhizobium Strains

Research has been conducted in Rio Grande do Sul to obtain more efficient Rhizobium strains for many crops, including soybeans, French beans, clover, alfalfa, birdsfoot trefoil, peas, and lentils. The first two have received the most attention because of their economic importance and also, in the case of the beans, because of the problem of effective nodulation of this plant.

In selecting more efficient strains, isolates are first screened in the greenhouse of the growth chamber and then evaluated in the field with the more important crop varieties grown in this state. The results of the most recent field experiment (Freire, 1965b) with soybeans are presented in Table 1. Only the variety Amarela comom did not benefit appreciably from inoculation with the strains being tested. Although this soybean variety has been popular in the past, it is rapidly being replaced in Rio Grande do Sul by more productive varieties. Dobereiner (1965, 1966) and Sampaio (1967), working in the state of Rio de Janeiro, have reported similar information relative to strain evaluation for soybeans. They have also characterized certain environmental and nutritional factors for this crop relative to nitrogen-fixation rates.

TABLE 1 Effect of inoculation with selected *Rhizobium* strains on the yield of four soybean varieties (kg/ha of grain) in the field at Guaiba, R.S., 1966[a]

Treatment No.	Strain	Variety and % Increase over No-Inoculation Treatment							
		Hood	%	Majos	%	Hill	%	Amarela	%
1	No inoculation	1576	-	1467	-	1467	-	1569	-
2	519 Re	1976	25	1572	21	1615	10	1629	4
3	506	1967	25	1995	36	1867	27	1506	0
4	509	1842	17	2004	37	1733	18	1608	3
5	532c	2180	38	2115	44	1907	30	1585	0

[a] Seção de Microbiologia, Secretaria da Agricultura, R.S.

Benefits of Inoculations

The difference in the ability of various plant varieties to form an effective symbiotic relationship with Rhizobia is evidenced in the following example. Table 2 presents the effect of a multistrain inoculant on the yield of eight soybean varieties. Table 3 shows the effect of four multistrain inoculants on the yield of six soybean varieties. The effect of a fertilizer nitrogen treatment is included. It is observed from this and other experiments that certain plant varieties nodulate and effectively fix atmospheric nitrogen with a great number of strains under a wide range of environmental and soil conditions, whereas others are much more specific and extremely sensitive to the external atmosphere and to the extreme of physiological alteration, lack of nodulation, or nitrogen fixation (Dobereiner, 1964, 1965, 1966; Franco, 1967; Sampaio, 1967).

With certain other leguminous crops, inoculation has been highly effective. For example, in field experiments with birdsfoot trefoil and subterranean clover, inoculation resulted in yield increases of 100% and 340%, respectively (Freire, 1964b, 1964c, 1965a). However, to date the field studies with French beans have not been very satisfactory, especially on the highly weathered acid soils.

Effect of Lime and Fertilizer on Nodulation and Production of Soybeans

Most of the soils of Rio Grande do Sul and Brazil are acid, extremely deficient in phosphorus, and often deficient in certain trace elements and sulfur. There are also many that contain relatively high levels of aluminum or manganese or both. The objective of experiments now in progress is to learn more about these soils and to determine the factors limiting nitrogen fixation for soybeans and French beans.

Table 4 presents the effect of lime and fertilizer applied to a soil low in pH, low in available phosphorus, and high in potassium. Inoculation was generally much more effective in the cases where lime was applied alone or with nitrogen or phosphorus. Potassium appeared to depress the effect of inoculation, a result that agrees with those obtained by other researchers, including Dobereiner. Table 5 presents further data concerning the effect of lime and fertilizer on the nodulation of soybeans at two field sites. The soils at the two sites were acid (5 kg/ha) and high in potassium (300 kg/ha).

TABLE 2 Effect of *Rhizobium* inoculant on the yield of soybeans (kg/ha of grain) in the field at Encruzilhada, R.S., 1958

Treatment No.	Variety	Yield (kg/ha) No inoculation	Yield (kg/ha) Inoculation	% increase over no-inoculation treatment
1	Mariloxi	1,110	1,903	71.4
2	Acadian	1,160	1,750	50.8
3	Amarela	860	1,432	65.0
4	LA 41-1219	1,013	1,080	6.6
5	Abura	670	1,106	65.1
6	CNS	477	1,206	152.8
7	Jackson	712	860	20.7
8	Lee	453	572	26.2
	Averages	807	1,239	53.2

TABLE 3 Effect of selected *Rhizobium* inoculants on the yield of six soybean varieties (kg/ha of grain) in the field at Guaiba, R.S., 1966[a]

Treatment No.	Inoculant	Variety L-571	L-2006	IAC-1	Hill	Bienville	Majos
1	No inoc.	1800	1383	1794	2272	1788	1947
2	No inoc.+N*	1028	1178	1813	1639	1688	2050
3	S.Micro.A.	1928	2322	2708	2363	2950	2933
4	S.Micro.B.	1492	1500	2366	1900	2228	2841
5	USA	1480	1278	2439	1991	2378	2972
6	IPEACS	2239	1922	2908	2091	2272	2878
% increase over no inoculation		26	67	43	4	65	52

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[a] Seção de Microbiologia Agrícola, Secretaria da Agricultura, R.S.

* Applied as ureia, 50 kg of N/ha.

TABLE 4 Effect of fertilizer, lime, and inoculation on the yield of soybeans (kg/ha of grain) in the field at Veranopolis, R.S., 1958[a]

Treatment No.	Fertilizer[b]	No lime: No inoculation	No lime: Inoculation	No lime: % increase	Lime (4,000 ton/ha): No inoculation	Lime (4,000 ton/ha): Inoculation	Lime (4,000 ton/ha): % increase
1	Check	1,726	1,966	10	2,136	2,853	33
2	N	1,623	1,780	9	2,350	2,826	22
3	P	1,403	1,493	6	1,853	2,416	30
4	K	1,726	1,586	-8	2,053	2,230	8
5	NPK	1,730	1,840	6	2,493	2,583	3

[a] Seção de Microbiologia Agrícola, Secretaria da Agricultura, R.S.

[b] Fertilizer was applied at the rate of 10 kg/ha of N as $NaNO_3$, 50 kg/ha P_2O_5 as superphosphate, and 40 kg/ha K_2O as KCl.

TABLE 5 Effect of lime, phosphorus, and potassium on the total weight of soybean nodules (g. over dry wt.) from four replicate treatments (10 plants/replicate) in the field at two localities, 1967

Treatment No.	Treatment	Girua, R.S.: Broadcast	Girua, R.S.: Row	Nova Prata, R.S.: Broadcast	Nova Prata, R.S.: Row
1	Check	0.17	0.13	0.70	0.67
2	Lime	0.24[a]	0.24[a]	0.82[a]	0.89[a]
3	K	0.10	0.18	0.58	0.55
4	K lime	0.31[a]	0.28	1.08	1.08
5	P	0.16	0.24[a]	0.84[a]	0.72
6	P lime	0.27	0.40	1.39	1.05
7	PK	0.09	0.21	0.78	0.72
8	PK lime	0.32	0.32	1.06	1.37

[a] Statistically significant difference at the 5% level from the check treatment.

The application of lime and certain phosphorus treatments significantly increased the weight of the nodules at both sites. Although not statistically significant, the potassium reduced nodule weight in most cases. In another experiment, presented in Table 6, the application of phosphorus significantly increased both nodulation and yield. The beneficial interaction between proper soil fertility levels (and lime) and symbiotic nitrogen fixation is evident from these results. For investigators studying fertility interactions with leguminous crops, this also shows that the influence of symbiotic nitrogen fixation cannot be overlooked in critically evaluating the results.

TABLE 6 Effect of phosphorus fertilization on the nodulation and yield of soybeans (kg/ha of grain) in the field at Girua, R.S., 1966

Treatment No.	P_2O_5 (kg/ha)	% increase in number of nodules over control	Yield (kg/ha)	% yield increase over control
1	0	-	1,174	-
2	50	25[a]	2,183[a]	86[a]
3	100	41[a]	2,228[a]	89[a]

[a] Statistically significant increase over the check treatment at the 5% level.

Effect of Lime, Sulfur, and Trace Elements on Nodulation, Nitrogen Fixation, and Yields of French Beans (Phaseolus Vulgaris)

The French bean is cultivated extensively in Latin American countries and constitutes in many the basic dietary staple. However, since it obtains little or no benefit from Rhizobium phaseoli, it is cultivated exclusively in soils high in organic matter that supply adequate nitrogen or in other soils where fertilizer nitrogen is applied. The cause of the lack of benefit is probably the extreme sensitivity of the Rhizobia to low pH values, high levels of aluminum or manganese, and high plant variety specificity.

The soils of the Planalto Riograndense and of central southern Brazil have tremendous agricultural potential, but in general they are old and highly weathered with numerous nutrient deficiencies, including trace elements and areas of aluminum and possibly manganese toxicity.

In greenhouse experiments conducted with three soils of Rio Grande do Sul, the effect of applying lime, phosphorus, potassium, boron, molybdenum, manganese, and zinc was studied. The results of these experiments, expressed as dry weight of the aerial portion of the plants, are presented in Figures 1-3. There was a strong lime and inoculation effect in two soils. In fact, in the three soils tested, nodulation did not occur when lime was not applied. There was not a significant difference between the trace elements relative to yield or nodulation.

This project is being continued to evaluate further the necessity of liming and to determine the problem of element deficiencies or toxicities relative to effective nitrogen fixation in the soils of Rio Grande do Sul.

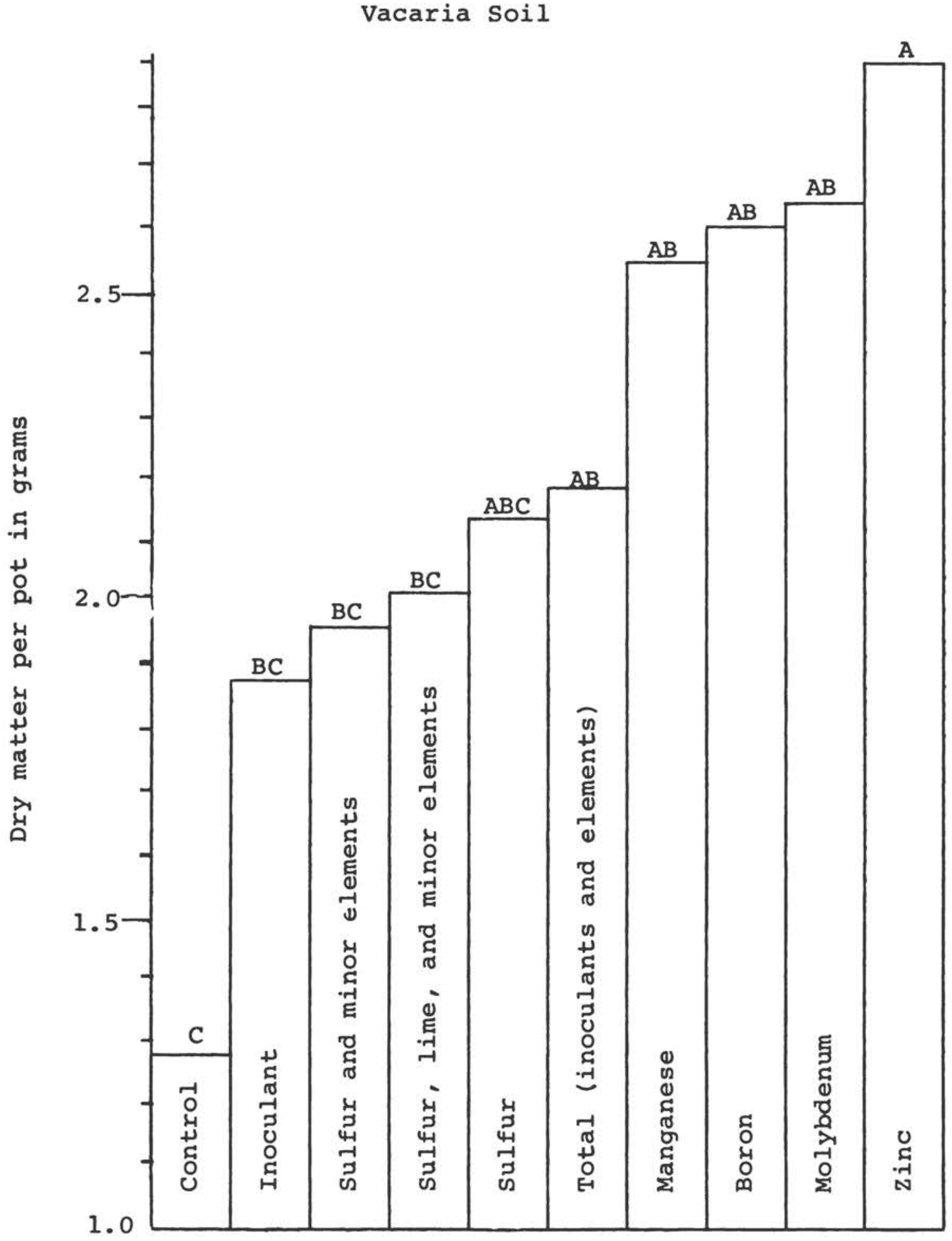

FIGURE 1 Effect of lime, potassium, phosphorus, sulfur, and minor elements on the dry matter of French beans in Vacaria soil.

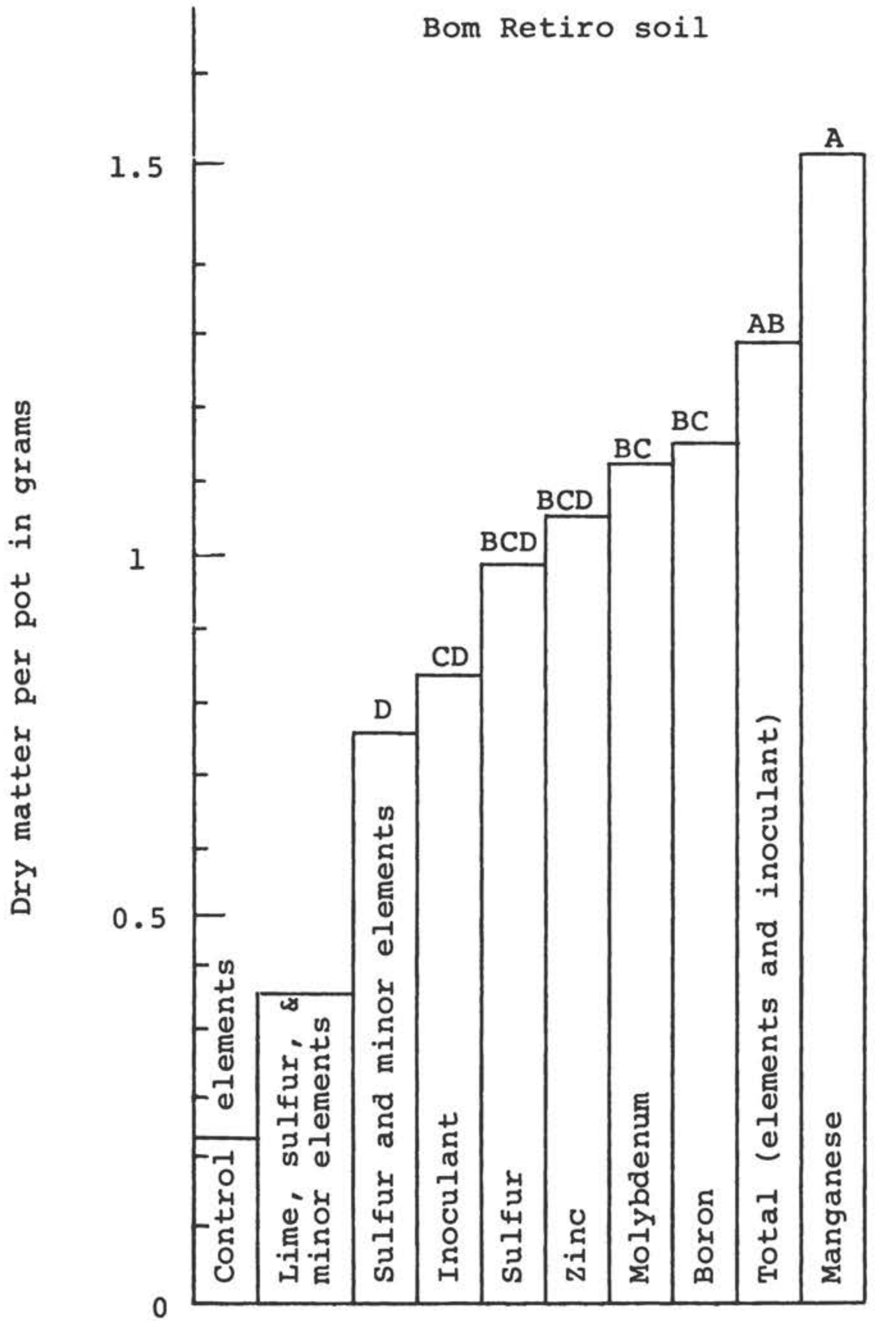

FIGURE 2 Effect of lime, potassium, phosphorus, sulfur, and minor elements on the dry matter of French beans in Bom Retiro soil.

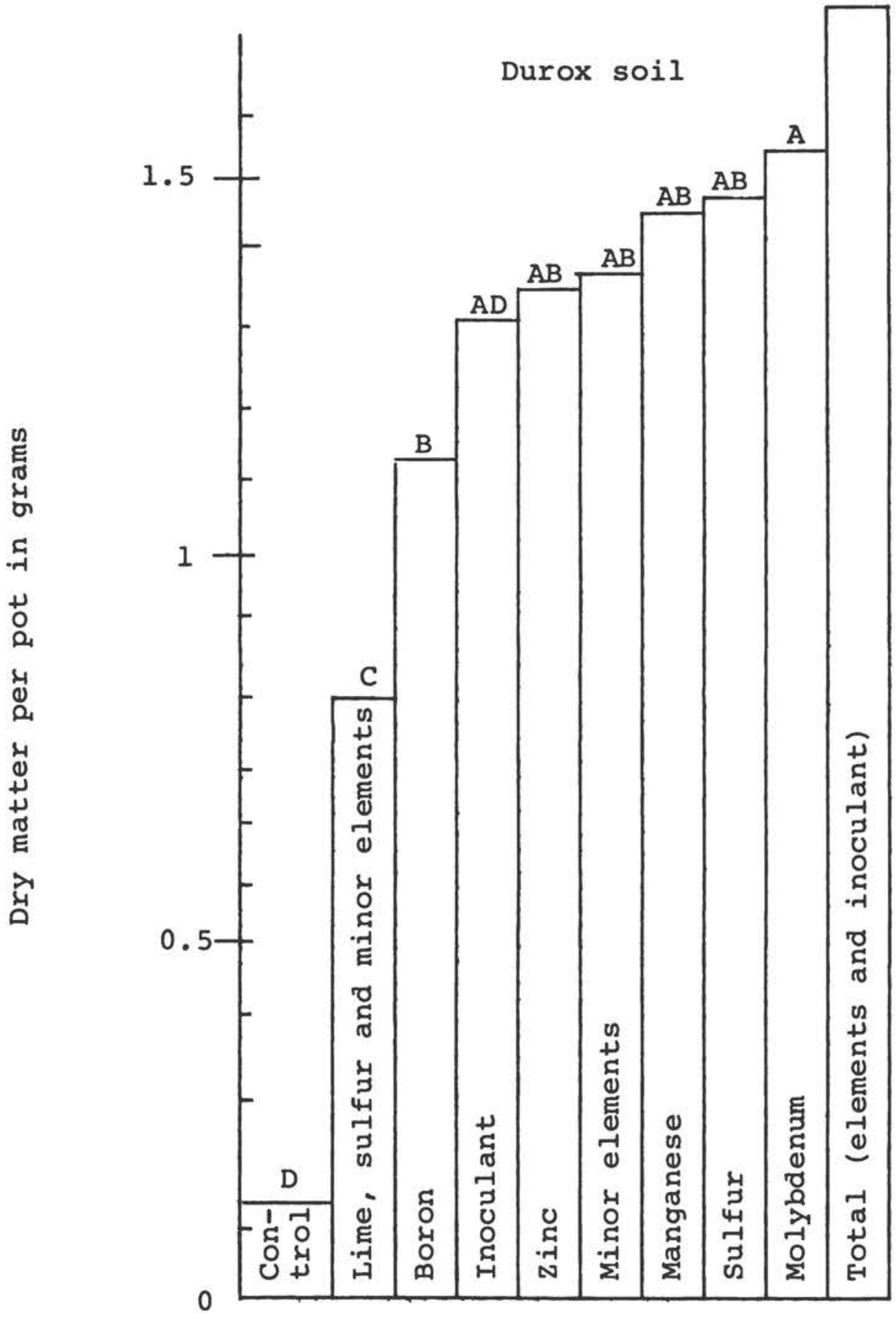

FIGURE 3 Effect of lime, potassium, phosphorus, sulfur, and minor elements on the dry matter of French beans in Durox soil.

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NONSYMBIOTIC NITROGEN FIXATION IN TROPICAL SOILS

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The problem of nonsymbiotic nitrogen fixation has been studied by innumerable scientists since the description of Azotobacter chroococcum and Azotobacter agilis by Beijerinck in 1901. These studies seem appropriate because proof of nitrogen fixation in amounts of economic importance could explain the lack of plant response to nitrogenous fertilizer, the recuperation of soils left under fallow, and the origin of the large reserves of nitrogen in soils where legumes cannot be the main source of this nutrient.

The innumerable papers dealing with Azotobacter in temperate regions do not show more than 10 to 15 kg of N/ha/year fixed by this organism, and even these quantities were dependent on available carbon sources (Henzell and Norris, 1962). The same authors mention the main limiting factors of Azotobacter distribution in the soil: low pH, lack of carbon sources, and the presence of nitrate that stimulates other microorganisms in the soil. These organisms then compete with Azotobacter for energy material.

The available carbon source requirement for nonsymbiotic nitrogen-fixing bacteria in the soil could provide a solution to the problem of these organisms dominating the microbial equilibrium in the rhizosphere. In this case, the plant would furnish energy material for nitrogen fixation. The legume symbiosis, in which all energy sources for nitrogen fixation are furnished by the plant, illustrates the feasibility of this solution. Since the biochemical mechanism of symbiotic and nonsymbiotic nitrogen fixation are very similar, there is no reason why a loose association of nonsymbiotic nitrogen-fixing bacteria developing along the roots could not use the carbon substances excreted by the plant to fix nitrogen that would then be directly or indirectly (after mineralization) available to the plant. If one continues comparing this type of association with the legume symbiosis, it becomes evident that such a relationship is possible only if there is a close interchange between plant and bacteria and if the bacteria in question find good growth conditions in the rhizosphere that permit them to compete successfully with the great bulk of other soil microorganisms.

The search for such an association between higher plants and the conventional *Azotobacter* species led to many papers that have been reviewed by Allison (1947) and more recently by Macura (1966) and Rovira (1965). Peas, maize, tomatoes, oats, and wheat were observed to stimulate *Azotobacter* in their rhizosphere but only under certain conditions. It is known that older plants with roots already in decomposition stimulate *Azotobacter*, whereas younger plants do not, and that the rhizosphere within a certain distance from the root seems to be more appropriate for *Azotobacter* growth than the root surface proper. Therefore, there does not seem to be a close and specific relationship between *Azotobacter chroococcum* and *A. agilis* and higher plants. There may be casual associations that lead to a depression of the great bulk of soil microorganisms in favor of *Azotobacter* because of a deficiency of mineral nitrogen in the rhizosphere. The main difficulty seems to be the inability of *Azotobacter* to compete with other microorganisms for energy material.

We can conclude that the economic importance of nonsymbiotic nitrogen-fixing bacteria under temperate climatic conditions is doubtful for the following reasons:

1. *Azotobacter chroococcum* and *A. agilis* were seldom found to occur in soil so abundantly that they were able to compete successfully with other soil microorganisms for carbon substances.

2. Acid soils strongly restrict the development of these organisms.

3. Optimal growth temperatures for these organisms occur during a limited period of the year.

4. No constant, close relationship between these two *Azotobacter* species and higher plants has been demonstrated.

If one compares this situation with that found under tropical conditions, it will be noted that few of these arguments remain. The purpose of this paper is to emphasize the differences that seem to exist in nonsymbiotic nitrogen fixation under tropical conditions--differences that suggest an immense field of worthwhile research. The well-established contribution of blue-green algae to the nitrogen economy of rice fields will not be considered in this paper.

Nonsymbiotic Nitrogen-Fixing Bacteria That Seem Restricted to Tropical Environment

Six species of nitrogen-fixing bacteria have been described and accepted but not found in soils of temperate regions. They will be discussed here in terms of characteristics that are relevant to the purpose of this paper.

Beijerinckia indica

This organism was isolated in 1959 by Starkey and De from Indian soils, by Becking (1961a) from African, South American, South European, and North Australian soils, and by Döbereiner (1959a) from soils all over central and northern Brazil. The occurrence of *Beijerinckia* in tropical and subtropical soils of the world is summarized in Table 1. It is remarkable that in addition to the three negative samples reported by Becking (1961a), there does not seem to be one report in the literature of *Beijerinckia* occurring in the United States or Central America.

Beijerinckia indica was found to be tolerant of soil pH from 4.9 to 7.4 (Becking, 1961a) and was found to grow well in a culture medium with pH from 2.9 to 8.4. It is tolerant of much higher aluminum and manganese concentrations than *Azotobacter* (Becking, 1961b), and its optimal growth temperature in a culture medium was 20° to 37° C (Becking, 1961b). Nitrogen fixation of *Beijerinckia indica* reached 17.2 mg N/g sucrose and 31.2 mg N/g glycerol (Döbereiner, unpublished data). Dommergues and Mutaftschief (1965) demonstrated an increase in nitrogen fixation when *B. indica* was cultivated in mixed cultures with *Lipomyces starkeyi*, a yeast frequently found associated with *Beijerinckia* in tropical soils.

Beijerinckia fluminensis

This organism was described by Döbereiner and Ruschel in 1958. It was found less abundantly than *B. indica* in tropical soils from four Brazilian states (Döbereiner and Ruschel, 1958) and in the Congo (Y. Dommergues, personal communication). Its tolerance of an acid culture medium is almost equal to that of *B. indica* (pH 3.5 to 9.2), and the pH range of the soils from which it was isolated was 4.3 to 5.2. The optimal growth temperature in a culture medium was 26° to 33° C. The amount of nitrogen fixed reached 12.9 mg N/g sucrose (Döbereiner and Ruschel, 1958). As in the case of *B. indica*, this organism showed increased nitrogen fixation in mixed culture with *Lipomyces starkeyi* (Dommergues and Mutaftschief, 1965).

TABLE 1 Distribution of Azotobacter and Beijerinckia in temperate, subtropical, and tropical soils[a]

Region	Number of samples	Beijerinckia (positive %)	Azotobacter (positive %)
Temperate			
Europe	110	0	28
Australia (south)	30	0	17
Subtropical			
Europe (south)	45	4	98
Australia	3	0	-
Africa (north)	8	0	75
Africa (south)	40	38	13
North America (Florida)	3	0	100
South America (Brazil)	19	21	10
Asia (Japan)	8	63	0
Tropical			
Africa (central)	53	57	21
Asia (south)	43	53	37
Australia (north)	45	35	15
South America	191	56	14

[a] Becking, 1961; Tchan, 1953; Döbereiner, 1959.

Beijerinckia derxii

This species was described by Tchan (1957), and his description was later confirmed by Hilger in 1965. It was isolated from Australian soils (Tchan, 1957), from Indian and Central African soils (Hilger, 1965), and from Brazilian soils (Ruschel, unpublished data). It seems more sensitive to pH changes; pH 5.8 to 7.4 are the limits for good growth (Hilger, 1965). Efficient nitrogen fixation took place only at pH 6.7 (Tchan, 1957). Maximal nitrogen fixation was 13.5 mg N/g glucose (Tchan, 1957).

Derxia gummosa

This organism, described in 1960 by Jensen *et al.*, was isolated from a West Bengal soil. It was recently isolated from 20 samples of rhizosphere soil of pasture grasses collected in Rio de Janeiro State (Döbereiner, unpublished data). Colonies of the organism on silica-gel plates were seldom found to occur in large numbers. It is, however, a peculiarity of the bacteria that only a few develop in nitrogen-free media on plates inoculated with streaks of thousands of organisms (Jensen *et al.*, 1960). Some growth substances or vitamins may be essential for colony growth. When soil containing high numbers of *Derxia* is inoculated into silica-gel plates, probably only a small fraction of the bacteria are able to start a colony. Therefore, actual numbers of this bacteria may be many times higher than have been estimated by plate counting. Once the colonies start growing, they develop to immense size (up to 3 cm in diameter and 0.5 cm in height).

Derxia was found in soils with pH ranging from 5.0 to 6.2. In a culture medium, growth occurred between pH 5.5 and 9.0 (Jensen *et al.*, 1960), and nitrogen fixation took place between pH 5.7 and 8.9. Efficiency in this organism's nitrogen fixation reached 25 mg N/g glucose (Jensen *et al.*, 1960). Optimal temperatures were 25° to 37° C, the latter being better for isolation than 34°.

Derxia indica

This species was described by Roy and Sen (1962). It was isolated from partially rotted jute plants in India. The optimal temperature for growth was 33° to 34° C, and the pH range for growth in a culture medium was 4 to 9. Efficiency in nitrogen fixation reached 30 mg N/g glucose.

Azotobacter paspali

This species was isolated from the rhizosphere of *Paspalum notatum* and *Paspalum plicatum*, where it occurs in large numbers (Döbereiner, 1966). Since the organism has not been found in soil without *P. notatum* (Bahia grass) or in the rhizosphere of other plants, and since *Paspalum* is a grass that occurs only under subtropical and tropical conditions, it is probable that the organism will not be found in temperate regions. *Azotobacter paspali*, as well as the other species of this genus, is sensitive to low pH; 5.7 to 8.4 is the limit for growth in a liquid culture medium (Döbereiner, 1966). Recent observations showed an even narrower pH range when only small amounts of inoculum were used (pH 6.3 to 6.8) (Machado and Döbereiner, unpublished data). However, the organism was found in great numbers in the rhizosphere of *P. notatum* grown in soils with a pH between 4.9 and 7.8. This was explained by a buffering effect of the plant roots.

Higher temperatures are optimal for isolation of *Derxia* than for the other *Azotobacter* species (Table 2). Nitrogen fixation of *Azotobacter paspali* in a culture medium reached 30.4 mg N/g sucrose.

Association of Nonsymbiotic Nitrogen-Fixing Bacteria with Tropical Plants

Stimulating effects of higher plants on nonsymbiotic nitrogen-fixing bacteria have been reported in tropical environments since 1959. Sugar cane, rice, and pasture grasses, all in the family *Gramineae*, seem to be the most important ones.

Sugar Cane

During the studies of Beijerinckia occurrence in Brazilian soils (Döbereiner, 1959a, b), it became evident that most soils planted with sugar cane for more than 1 year showed an abundant population of this organism (Table 3). The stimulating effect of sugar cane on *Beijerinckia* development was explained by the excretion of substances rich in sucrose, which is the preferred carbohydrate for this organism. Such substances, excreted by sugar cane leaves and stems, were washed into the soil by rain. Plants grown in pots protected from rain also stimulated *Beijerinckia* growth (Döbereiner and Alvahydo, 1959). Under field conditions in a sandy soil, cropping with sugar cane increased the number of microcolonies of *Beijerinckia* per gram of soil from 20 to more than 1,000 in

TABLE 2 Effect of incubation temperature on the development of colonies of *Azotobacter paspali* and *Derxia* sp. on silica-gel plates with calcium citrate as carbon source[a]

[Data represent number of microcolonies per gram of root-surface soil from *Paspalum notatum*]

		Incubation time	
Temperature	Species	4 days	5 days
29° C	*A. paspali*	50	140
	Derxia sp.	30	30
34° C	*A. paspali*	250	1,000
	Derxia sp.	30	30
37° C	*A. paspali*	600	1,000
	Derxia sp.	50	60

[a] Döbereiner, 1966.

the root-surface soil and to 500 in the rhizosphere soil (Döbereiner, 1961). This stimulation of *Beijerinckia* in the rhizosphere of sugar cane was accompanied by a reduction in the number of bacteria that require amino acids, actinomyces, and fungi. This indicates a dislocation of the microbial equilibrium in favor of *Beijerinckia* (Table 4).

TABLE 3 Effect of sugar cane crop on the occurrence of *Azotobacter* and *Beijerinckia* in soil[a]

	No sugar cane	Sugar cane
Number of samples	124	131
Azotobacter positive	10	9
Number of microcolonies per gram (mean of pos. samples)	66 ± 19	37 ± 17
Beijerinckia positive	77	125
Number of microcolonies per gram (mean of pos. samples)	67 ± 14	331 ± 110

[a] Döbereiner, 1959b.

TABLE 4 Changes in microbial equilibrium in the rhizosphere of sugar cane in the field[a]

[Data represent relative increase in relation to check samples taken between rows; means of eight replicates]

Months after planting	Microorganism	Ratio (rhizosphere/ check)	Ratio (root surface/ check)
2	*Beijerinckia*	4.7	-
12	do.	22.7	17.2
18	do.	22.3	55.8
18	bacteria in egg albumin agar	0.4	0.2
18	actinomyces	1.0	0.1
18	fungi	0.7	0.7

[a] Döbereiner, 1961.

Rice

Under field conditions rice also stimulated *Beijerinckia* development, although not to the same degree as sugar cane. Results of this experiment are summarized in Table 5. When rice was inoculated with *Beijerinckia* in a greenhouse experiment, the plant effect became more pronounced (Table 6). It became apparent that inoculation of the seeds (i.e., the introduction of small amounts of *Beijerinckia* into the soil) brought about a marked change in the rhizosphere equilibrium, stimulating *Beijerinckia* and reducing the number of microorganisms that require amino acid.

Forage Grasses

The occurrence of *Beijerinckia* and *Azotobacter* in the rhizosphere of *Hiparrhenia rufa*, *Panicum maximum*, and *Paspalum notatum* was studied by Ruschel and Britto (1966), and the occurrence of *Digitaria decumbens*, *Panicum purpurescens*, *Cynodon dactylon*, *Setaria sphacelata*, and *Melinis multifloris* was studied by Ruschel and Döbereiner (1965). With the exception of *Melinis multifloris*, which constantly reduced *Beijerinckia* occurrence, all grasses showed stimulating effects on this organism. *Azotobacter* was found to be extremely abundant in the rhizosphere of *Paspalum notatum* (Ruschel and Britto, 1966).

Paspalum notatum

Recently it has been shown that the association of *Azotobacter* with *Paspalum notatum* seems to be a specific one. A new *Azotobacter* (*Azotobacter paspali*) was characterized; it occurred in practically all samples from the rhizosphere of *Paspalum notatum* and always in extremely high numbers (Döbereiner, 1966). Table 7 shows that *A. paspali* occurred on only two out of seven *Paspalum* species and on none of the other plants, including 27 species of *Gramineae*, eight legume species, and a number of other unidentified plants. It occurred in much higher numbers at the root-surface soil than in samples including the whole rhizosphere. Root-surface soils from other plants grown in between the *Paspalum* sward contained the organism, but it was less abundant there than in the corresponding rhizosphere samples (Machado and Döbereiner, unpublished data).

The dependence of *A. paspalis* growth in soil on a higher plant indicates a close relationship that affords a completely new approach to the question of nonsymbiotic nitrogen-fixing bacteria in tropical soils. Furthermore, this dependence may

TABLE 5 Effect of rice on *Beijerinckia* development in the field[a]

[Data represent number of microcolonies per gram of soil; means of six replicates]

	Beginning of vegetation cycle	Blooming	Ripening
Between rows	195	565	777
Rhizosphere	101	736	738
Root surface	282	770	1250

[a] Döbereiner and Ruschel, 1962.

TABLE 6 Effect of inoculation of rice seeds with *Beijerinckia* on its establishment in the rhizosphere (means of four replicates)[a]

	Inoculated		Not inoculated	
	Rhizosphere	Root surface	Rhizosphere	Root surface
Beijerinckia (number of microcolonies per gram)	86	357	6	7
Bacteria in egg albumin agar x 1,000	272	184	418	364

[a] Döbereiner and Ruschel, 1961.

TABLE 7 Occurrence of nitrogen-fixing bacteria in the root-surface soil of pasture plants[a]

Plant and number of samples	Positive samples (%)		
	A. chroococcum *A. agilis*	*A. paspali*	*Derxia* spp.
Paspalum notatum 76	17	98	15
Paspalum plicatum 3	33	66	0
Paspalum spp.[b] 27	7	0	0
Other grasses[c] 63	26	0	0
Legumes[d] 12	16	0	8
Other plants 10	40	0	20

[a] Döbereiner, 1966.

[b] *P. convexum*, *P. vaginatum*, *P. clandestinum*, *P. coloratum*, *P. maritimum*, *P. erianthum*.

[c] More than 28 species of *Gramineae*.

[d] More than eight species of *Leguminosae*.

lead to speculations on evolutionary hypotheses concerning nonsymbiotic nitrogen fixers and the symbiotic relationship of the legumes.

Nitrogen Fixation in Tropical Soils

An evaluation of the amounts of nitrogen fixed under bacteria-plant systems is awaiting further research. The final proof of atmospheric nitrogen being fixed by nonsymbiotic organisms and then absorbed by the plant should arise from experiments with N^{15}, which, to the best of our knowledge, have not been carried out with any of the described systems. However, there are some experimental data that indirectly suggest nitrogen fixation in the rhizosphere of subtropical or tropical plants. Rouquerol (1963) and MacRae and Castro (1967) observed significant increases of nitrogen in rice soils that were attributed to heterothrophic nonsymbiotic bacteria. In a greenhouse experiment, Döbereiner and Ruschel (1962) demonstrated nitrogen fixation in rice plants inoculated with one *Beijerinckia* strain, but other strains of this organism did not affect plant growth (Table 8). Unfortunately, in this experiment algae were not eliminated, and there is still the possibility of certain *Beijerinckia* strains having stimulated algal growth that contributed to nitrogen fixation. Recent inoculation of *Beijerinckia* in sand cultures in the greenhouse resulted in significant increases of the total nitrogen content of elephant grass (*Pennisetum pupureum*) (Souto and Döbereiner, 1967).

TABLE 8 Effect of inoculation of rice seeds with *Beijerinckia* on rice yield (means of four replicates)[a]

	Straw (g/pot)	Grain (g/pot)
Strain A	23.3	6.2
Strain B	15.6	2.7
Strain C	15.5	3.8
Strain D	15.0	3.2
Check (no inoculation)	18.4	4.0
Check (strain C + nitrogen)	30.1	6.0

[a] Döbereiner and Ruschel, 1961.

Conclusions

In conclusion, we can now summarize the following distinct features of tropical soil-plant systems as related to nonsymbiotic nitrogen fixation:

1. At least six species of nonsymbiotic nitrogen-fixing bacteria occur that have not been found in temperate climate soils. All these species were shown to be able to fix 12 to 30 mg of N/g of carbon source.

2. All six species have been found to occur in acid soils.

3. All *Beijerinckia* species require for optimal growth temperatures in the mesophylic range like the classical *Azotobacter* species. *Azotobacter paspali* and *Derxia* spp. seem to need higher temperature for optimal development (33° to 37° C). In tropical regions, optimal temperatures for these organisms occur practically during the whole year.

4. Stimulating effects of higher plants on three of these species have been demonstrated; the association of *Beijerinckia indica* with sugar cane and of *Azotobacter paspali* with *Paspalum notatum* are the most remarkable cases.

It is evident that these points eliminate, for tropical soils, most of the arguments that contradict the economic importance of nonsymbiotic nitrogen fixation in temperate soils. However, the importance of nonsymbiotic nitrogen fixation in tropical soils must still be demonstrated and is therefore a research area of considerable importance.

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IMPORTANCE OF NONSYMBIOTIC ORGANISMS IN THE NITROGEN ECONOMY OF TROPICAL SOILS

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The nitrogen content of the soil depends on several factors, such as climate, topography, organisms, type of vegetations, and kind of land use. They may be considered independent variables (59), and the nitrogen content may be considered a dynamic equilibrium or the tendency to reach it. Any change in these variables creates a change in the nitrogen to fit the new conditions. The well-known nitrogen cycle is an estimation of the nitrogen losses and gains in the soil that are determined by the factors mentioned. The analysis of nitrogen economy is based on these losses and gains and seems to be fruitful to some extent in tropical soil explorations.

Tropical climate conditions oscillate from desert to perhumid, and although the temperature may reach extremes, cold winter is generally absent. On high mountains in the Tropics, the climate may be similar to that in temperate regions but with variations in amount of daylight. For the present discussion, an area was selected that presents average conditions in temperature and rainfall and is well suited for agricultural purposes. This area is the state of São Paulo, Brazil, which covers 247,900 km^2 and represents similar regions constituting 1,290,000 km^2 of the country. It is located between 20° and 25° latitude south and 44° and 53° longitude west.

Soil-Nitrogen Conditioning Factors in the State of São Paulo

Climate

The climate is defined by an average annual rainfall of 1,000 to 1,700 mm (40 to 67 inches) except on the seacoast, where it may reach 4,000 mm (157 inches) (112). The mean annual temperature is 12° to 18° C (54° to 64° F) in the mountains (15% of the territory) and 18° to 23° C (64° to 73° F) in most of the region (104). In this area, the climate is of the continental type, tropical with a wet, hot summer and a dry and somewhat cold winter with deficits of water reaching up to 150 mm (6 inches) (19).

Microorganisms are very active in summer as can be inferred from work on nitrate levels, but their activity is restricted in winter mostly by low water content in the soil (20). Losses of nitrates by percolation water is high.

The climate-nitrogen function for soil in temperate zones (59) suggests that tropical soils should have low percentages of nitrogen. Some exceptions may be cited that represent local conditions, but in general this is true for the state of São Paulo. The content there is 0.03% to 0.09% nitrogen in sandy soil and 0.12% to 0.18% in clay soil, all well drained and at plow depth.

Soils

The dominant soils are the red-yellow Podzolic and those belonging to the suborder Latosol (red-yellow Latosol, terra roxa, dark-red Latosol, etc.) (32, 101). In the scheme of the seventh approximation, these soils are classified as Ustisols and Oxisols (114). They have strong structure for medium and high clay textures, high porosity, and low to high acidity, except for some with low alkalinity. These properties affect the activity of microorganisms, accelerating the decomposition of organic matter and probably selecting the types of microorganisms.

It was shown (118) that nitrogen content is related to soil texture and composition and that sandy soil has almost half the nitrogen of clay. Those soils with large amounts of iron and aluminum oxides have the highest nitrogen content. High content does not mean necessarily a better capacity to supply nitrogen to plants, as was demonstrated in field experiments for several crops (Table 1) and fertility levels obtained for cotton crops in the state of São Paulo as follows:

	Sandy Soil	Clay Soil
Low--strong response to nitrogen fertilization	0.04	0.12
Medium--low to medium response	0.04-0.09	0.12-0.16
High--very low or no response	0.09	0.16

TABLE 1 Crops and their response to nitrogen, phosphorus, and potassium under tropical conditions in the state of São Paulo, Brazil

Crop	Trials (No.)	Soil N Content (%)	Response to Nitrogen (No.)		Response to Phosphorus (No.)		Response to Potassium (No.)	
			Positive	Negative	Positive	Negative	Positive	Negative
Banana	3	0.19-0.30[a]	2	1	0	3	2	1
Barley	1	0.19	0	1	--	--	--	--
Cabbage	3	0.06-0.30	3	0	3	0	0	3
Cassava	19	--	2	17	11	8	0	19
Castor beans	15	0.06-0.17[b]	4	11	6	9	4	11
Coffee	12	0.07-0.19	12	0	0	11	8	4
Corn	17[c]	0.05-0.90	13	4	--	--	--	--
	23[d]	0.05-0.17	5	18	20	3	6	17
Cotton	39[c]	0.05-0.24	9	30	--	--	--	--
	308[d]	0.04-0.24	51	257	130	178	25	283
Dry beans	3[c]	0.07-0.16	3	0	--	--	--	--
	56[d]	0.05-0.31	23	33	36	20	2	54
Murakami ramie	4	--	1	3	3	1	0	4
Oat	1[c]	0.19	0	1	--	--	--	--
Onion	1	--	0	1	1	0	0	1
Orange	1	--	1	0	1	0	1	0
Papaya	1	0.07	0	1	1	0	0	1
Peanuts	2	0.07-0.09	0	2	2	0	0	2
Potato	12[e]	0.29-1.20	9	3	--	--	--	--
	18[f]	0.31-1.20	13	5	9	9	5	13
	36[c]	0.40-0.19	16	21	--	--	--	--
	15[d]	0.05-0.10	8	7	8	7	4	11
Rice:								
Paddy	1[c]	0.10	1	0	--	--	--	--
	1[d]	0.09	1	0	0	1	0	1
Upland	9[c]	0.05-0.06	2	4	6	0	2	4
Soybeans	6	0.01-0.38	2	4	6	0	2	4
Sugarcane	4[c]	0.21-0.23	2	2	--	--	--	--
	67[d]	0.06-0.25	22	21	19	18	39	9
Sweetpotato	30	0.05-0.28	2	28	7	23	7	23
Tomato	1[c]	0.13	1	0	--	--	--	--
Wheat	12[d]	0.06-0.13	4	8	12	0	2	12

[a] Alluvial soils.

[b] Some soils without N analysis.

[c] Experiments to study forms of N without P or K responses.

[d] Experiments with NPK.

[e] Alluvial and bog soils, as experiments to study forms of N without P or K responses.

[f] Alluvial and bog soils; trials with NPK.

Organisms

The primitive land was covered by forest, a special type of savanna called "cerrado," and small areas of grassland. The cerrado is a consequence of low soil fertility, and generally the nitrogen content is low. In the forested soils the amount is higher and in equilibrium with the conditions prevailing in the region. The forest floor is small when compared with the temperate regions and indicates that the decomposition velocities of organic matter are intense (116). Few studies were made of microorganism activity. Some data were obtained from other states but in the same ecological region. The dominant nonsymbiotic fixing organisms belong to the genus *Beijerinckia*, mostly *B. indica* derx., followed by *B. indica var. alba* and *B. fluminensis*, a new species (36, 43). The bacteria *Azotobacter chroococcum* is found in soils with low acidity or under certain conditions (36, 64, 103). The other microorganisms were not studied, but it has been reported that the fungus *Aspergillus flavus* is able to promote nitrification in the soil (67). The symbiotic fixation seems to be similar to that in other regions (30, 49, 50, 88).

Topography

The accumulation of water in depressions creates anaerobic conditions and formation of bog, half-bog, and humic gley soils (122). The nitrogen in them is inactive, and soils with as much as 1% nitrogen need fertilization, as was shown by field experiments with rice, bananas, and potatoes (15, 33, 34, 53, 54, 111).

Land Use and Its Management

Nitrogen content depends on the use of soil and on the management level that is employed. Some practices are destructive (e.g., burning crop residue), but others are ameliorative. Some of these relationships may be inferred from Table 2, which shows results obtained at the Ribeirão Preto Experiment Station in a very homogeneous soil called terra roxa.

Practically all factors that influence soil nitrogen in tropical regions are conducive to low content in soil. There are exceptions, discussed by Jenny (61), but they appear to be results of local conditions.

TABLE 2 Average soil content in carbon and nitrogen according to land uses in the Ribeirão Preto Experiment Station, São Paulo, Brazil

Land use	Samples (No.)	Average content (%)		C/N ratio
		C	N	
Explored forest	19	2.23	0.25	9.1
Pasture	29	1.55	0.16	9.5
Coffee plantations	85	1.71	0.18	9.3
Other crops	86	1.39	0.14	9.7

The Nitrogen Cycle in Tropical Soils

Any understanding of the nitrogen economy must be gained through analysis of nitrogen gains and losses; such an analysis will be carried out for the state of São Paulo. The gains are all factors that contribute to increased soil nitrogen, and losses are those that determine its movement outside, ending at the atmosphere. The accumulative factors are: rainfall, gas exchange between soil and atmosphere, symbiotic and non-symbiotic nitrogen fixation by microorganisms, nitrogen fertilization, and some special forms like mulching with residues from the outside. Crop residues left on the field are not considered either a loss or a gain, but a temporary translocation, and the same is true for a forest. The losses are determined by removal of crops (or human and animal feeding or burning to clean the field), lixiviation by water that percolates through the soil profile, gas exchange between soil and atmosphere, denitrification, and erosion. Other types of losses and gains that represent local conditions are not discussed here.

Soil Gains

The most important factors that promote soil nitrogen in the state of São Paulo are described below.

Rain Data from several periods between 1890 and 1951 indicate that the amount of ammonium and nitric nitrogen added by precipitation varies between 3 and 9 kg/ha/year (2.7 to 8.1 lb/acre) (123). These small amounts would be enough to replenish nitrogen in soils covered by forest where losses are minimal. They would not cover the losses that occur in land used for agriculture--losses increased by leaching, removal of plant parts, and other factors.

Exchange of Gases between Soil and Atmosphere Studies to determine the amount of nitrogen involved in this exchange have not been made, but the amount is generally thought to be very small and unimportant for these considerations.

Nitrogen Fertilization When the soil's ability to supply a crop's nitrogen demand is low, fertilization must be used. A study of 722 field experiments is presented in Table 1 for 22 crops of São Paulo (1-18, 20-28, 31, 33, 34, 44, 46-48, 51-58, 63, 66, 68-87, 89-101, 104, 107, 109-111, 113, 124-128).

The most important plant nutrient required by these crops was phosphorus, and the second was nitrogen. Soils of São Paulo have high total phosphorus content. The high phosphorus needs of these soils can be explained by the large amount of iron and aluminum oxides that fix the phosphorus (120). Nitrogen availability is high, but the content is low (119). The response to nitrogen fertilization is not in agreement with availability and soil content because the intense transformation would exhaust the soil in a short period. Each crop has a different nitrogen demand and response.

Nitrogen Fixation by Microorganisms The nonsymbiotic fixation by *Azotobacter chroococcum* is limited to soils of neutral or slightly acid reaction, with some exceptions (35). In the generally acid soils of São Paulo, the genus *Beijerinckia* is dominant, including *B. indica* var. *alba*, *B. fluminensis*, and mostly *B. indica* derx. with the same capacity for fixation as *A. chroococcum* (34, 43, 109). The study of microorganisms associated with plants of *Gramineae* indicates that *Beijerinckia* is found at the rhizosphere and in greater numbers at the rhizoplan (root surface) of grasses, certain crops, or native plants (38, 39, 42, 105, 106). A new species of *Azotobacter* (*A. paspali*) was found to occur in large numbers on the rhizoplan and less abundantly in the rhizosphere of two related native grasses, *Paspalum notatum* and *Paspalum plicatum* (42). Döbereiner (35) indicated that the anaerobic bacteria *Bacillus amylobacter* (syn. *Clostridium pasteureanum*) that is also able to fix nitrogen is found in the soils of the region. These studies indicated a new microflora for nonsymbiotic fixing organisms in the Tropics that have not been thoroughly studied.

Symbiotic fixation in the state of São Paulo seems to have the same magnitude as in other areas. The association of *Rhizobia centrosema* (*Centrosema pubens*) with cowpea (*Vigna sinensis* L. Sav), sword beans (*Canavalia ensiformis* D. C.), soybeans (*Glycine max*), crotalaria (*Crotalaria juncea* L.), and velvet beans (*Stizolobium deeringianum* Bart.) showed a nitrogen fixation from 43 to 204 kg/ha (39 to 184 lb/acre) (30, 49, 50, 88). The amount fixed depends on the legume crop, *Rhizobium* strain, etc. For pasture (generally only grass) and agricultural land crops, this fixation is of little importance since green manure is not established in São Paulo as a normal rotation crop. Other types of nitrogen fixation were not studied.

Losses of Nitrogen

Nitrogen Exported in Crops Under normal conditions the removal of nitrogen from the field can be evaluated at 16 to 55 kg/ha (15 to 50 lb/acre) (29). A sandy soil with 0.06% nitrogen, without response to fertilizer at the beginning, would theoretically support 24 to 82 crops. Losses resulting from burning the residues and leaching out were not computed. The experiments have shown that the nitrogen needed as fertilizer is related to soil content, but some factor is diminishing the need of this element (Table 3).

Soil Leaching Only a few studies shed light on the problem of leaching. The nitrification power of the soil was studied in field conditions and showed at times the presence of 425 kg/ha of NO_3^- (126 kg/Nha or 113 lb/N/acre) at plow depth (119). The presence of nitrates was related to rainfall in summer, indicating the leaching of the nutrient. In the lysimeter, data indicate intense leaching for several soil types (65).

The lack of nitrogen response in early field trials was at first attributed to an abundance of the element, but now all experiments with fertilizer recommend using split applications and top dressings to avoid the losses by leaching. Experiments on the time of nitrogen application for cotton (90, 91), potatoes (54, 55, 92, 94), coffee (44), sugarcane (4, 7), dry beans (73, 79), and corn (75, 127) helped explain

TABLE 3 Nutrient exported by some crops under tropical conditions of São Paulo State, Brazil (19)

Crops and parts removed	Amount removed (ton/ha)	Exported nutrients from soil (kg/ha)		
		N	P_2O_5	K_2O
Banana (clusters)	15.00	26	6	95
Cassava (roots)	25.00	55	11	48
Cocoa (fruits)	4.68	16	3	27
Coffee (berries)	2.00	30	5	39
Corn (ears)	4.80	47	9	32
Cotton (seeds, fibers, branches and leaves)[a]	3.20	44	14	40
Dry beans (beans)	1.00	31	8	8
Rice (grains)	3.75	22	12	32
Potato (tubers)	8.00	28	5	39
Sugarcane	60.00	62	9	56
Tobacco (leaves)	1.25	28	18	58

[a] By law, all plant residues must be burned to help control insect pests.

the early lack of response to nitrogen when this element was applied only at growing time.

Exchange of Gases between Soil and Atmosphere Some forms of nitrogen can be transferred from the soil to the atmosphere. No determinations were made, but it is admitted that these nitrogen exchanges are negligible.

Denitrification Although no studies were made in the state of São Paulo, it may be assumed that denitrification occurs at the same rate as in other countries.

Erosion Loss through erosion has not been studied as thoroughly as it deserves to be, since in tropical areas the intensive erosion that follows the clearing of the land for agriculture may remove the surface layer. The intensity of this phenomenon as presented by Verdade and others (121) is shown in Table 4. The amount of nitric and ammonium nitrogen introduced by rainwater far exceeds the losses through runoff for the same compounds. For other nitrogen forms, mostly organic, rainfall does not compensate for erosion. The amount lost depends on soil conservation practices, rainfall, and the crop itself.

TABLE 4 Amounts of nitrogen received in rainwater and lost by erosion under different soil-conservation practices in cultivated cotton fields, São Paulo, Brazil

Soil-Conservation Practices, by Year	Nitric and Ammonium N (kg/ha) In Rainwater[a]	Nitric and Ammonium N (kg/ha) In Runoff	Other Nitrogen Losses in Runoff (kg/ha) Water	Other Nitrogen Losses in Runoff (kg/ha) Soil
1951-52				
Alternated cultivation	1.88	0.16	0.83	5.78
Narrow strips of sugarcane	1.35	0.04	0.25	-
Contour planting	2.44	0.37	1.60	40.38
Downslope planting	2.85	0.76	2.69	55.64
1952-53				
Alternated cultivation	7.97	0.14	0.10	1.30
Narrow strips of sugarcane	0.78	0.02	0.47	-
Contour planting	4.53	0.66	1.51	1.22
Downslope planting	8.22	0.47	0.47	0.23

[a] Determined only for rains that produced runoff.

Discussion

Under the tropical conditions prevailing in São Paulo, the interactions of factors that influence soil nitrogen tend to produce a low content in well-drained soils. Nitrogen content varies from very low in sandy soils to medium in clay when compared with the content found in temperate countries. The activities of microorganisms are very high, and a rapid degradation of organic matter occurs in the soil, which takes care of the nutrient demands of plants.

The balance of gains and losses in the nitrogen cycle shows that the contribution by rainfall is relatively small and cannot overcome the losses except in a forest soil. The rapid transformation of nitrogen in summer and the heavy rains that fall in the same period lead to great nitrogen losses. Jenny studied the high nitrogen content of certain tropical soils and attributed this fact to additions by rainwater and both nonsymbiotic and symbiotic fixation of atmospheric nitrogen. He stressed the last factor because of the large number of legume trees present in the forested soils studied. It is not possible to accept Jenny's suggestion that symbiotic fixation is responsible for maintenance of nitrogen fertility in São Paulo. In this area, forest plantations are composed of Eucalyptus or Pinus, pastures are almost entirely grasses, and only a small number of legume crops are used.

Vasconcellos (116) studied the nitrogen economy of orange trees during a 10-year period and found that the crop exported 1.7 kg (3.7 lb) of nitrogen per tree and received only 0.26 kg (0.16 lb) from fertilizer. The plants were showing symptoms of excess of nitrogen at the end of the period. The nitrogen difference was supplied by rain, soil nitrogen, and green manure.

Vageler (115) proposed a photochemical fixation to explain the limited response of plants to nitrogen fertilization in São Paulo. The difficulties that nitrogen has in combining with hydrogen or oxygen and the lack of experimental evidence do not permit us to accept his hypothesis.

The use of soil for agricultural purposes in tropical regions like the one described modifies the condition of the primitive forest and reduces the nitrogen content gradually until equilibrium with new conditions is attained. Experimental data show that during the first part of the evolutionary process, there is an excess of soluble nitrogen for the plants. After a period of time as yet undetermined, available nitrogen is no longer formed at the rate needed to fulfill the plant requirements, and fertilizer must be added. This phase has been reached in some agricultural areas of the state of São Paulo but generally later than expected. Furthermore, in certain types of terra roxa there is still no response to nitrogen fertilizers in spite of long periods of cultivation. It is therefore evident that some factor is acting in these soils that balances the losses and compensates partially for the expected needs of nitrogen; this factor is the non-symbiotic fixing organisms. Studies on these organisms are very promising for tropical conditions. Present knowledge is only part of the whole picture, and the determination or microorganisms involved, their life cycle, the possibility of inoculation, etc., will permit better soil management for food production with bacteria of the genus *Beijerinckia* in rice (42), sugar cane (41), and grasses (105). This was found feasible through experiments with crops that occupy large tropical areas.

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PHYSIOLOGY OF THE SYMBIOSIS BETWEEN RHIZOBIUM AND LEGUMINOUS PLANTS

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Symbiotic nitrogen fixation occurs through an intimate association of bacteria of the genus Rhizobium with leguminous plants. As a result of this symbiosis, atmospheric nitrogen is converted to an available form. This process is of considerable economic and geochemical importance since it makes N_2 available to higher plants that are not known to be capable of using molecular nitrogen. Moreover, as a result of symbiotic fixation, a large part of the nitrogen that has been volatilized by biological and chemical denitrification returns to the terrestrial cycle.

Two possible approaches stand out in investigations of the symbiosis of Rhizobium with leguminous plants: (1) an inquiry into the relationship between macrosymbiont and microsymbiont as related to the fixation of molecular nitrogen, and (2) an investigation of the infection process in relation to the onset and development of the symbiosis. The bacteria-plant association is of general biological interest because it is a classic and well-documented instance of obligate symbiosis (both organisms are required for the nitrogen to be assimilated) and because of the marked specificities of the two symbionts for their respective partners.

Vincent (1967), in a clear presentation of problems of symbiotic specificity, has listed eight major steps in the establishment of an effective nodule. These are as follows: (1) occurrence and proliferation of the specific Rhizobium near the root of the host; (2) root hair curling and entry of the bacterium; (3) infection-thread establishment and advance; (4) encounter of the host tetraploid cell with the infection thread and growth of the teraploid and diploid nodule issue; (5) release of the bacteria from the infection thread and formation or persistence of the membrane envelope; (6) multiplication of the released bacteria; (7) conversion to the bacteriod condition, and (8) establishment of a functional symbiosis involving the reduction of molecular nitrogen to the fixed form and the assimilation of nitrogen thus fixed.

Our research has been largely concerned with the specific substances, formed by the plant and the bacterium, that are required for infection, nodule initiation, and the symbiosis. Valera and Alexander (1965) reported that nodulation of excised roots of alfalfa plants was enhanced by an extract of alfalfa seeds and that coconut water exerted a comparable effect on nodule formation on excised roots of several legumes. Yatazawa and Yoshida (1965) also noted that extracts of soybean cotyledons promoted root-nodule formation on soybean seedlings. We have obtained more direct evidence for a plant-synthesized factor involved in nodule formation by showing that nodules failed to appear on bean explants derived from hypocotyls taken from ungerminated bean seeds, although bean seedlings that developed from embryos devoid of cotyledons nodulated when exposed to a Rhizobium population. Such a nodulation factor was also observed in a dialyzable portion of coconut water.

More recently, we have purified the legume nodulation factor present in coconut water, making use of a biological assay procedure involving the nodulation of adventitious roots growing from segments of bean hypocotyl tissue. By purification of the coconut water material on activated charcoal, a 70-fold purification was obtained; the final preparation still contained at least 10 different compounds. On the other hand, a fraction of coconut water that enhanced the growth of carrot root explants was also active in the nodulation bioassay test, and this material represented a 4,000-fold purification of the diffusible fraction of coconut water. The active substance in nodule morphogenesis appears to be effective probably at about a concentration of 1 part in 1 million or 1 part in 10 million, and it may thus be considered as a growth hormone or growth regulator (Schaffer and Alexander, 1966, 1967a, 1967b).

Another possible approach to establishing the identities of components that contributed to the symbiosis by either of the two participating organisms involved the use of antimetabolites. If an antimetabolite antagonizes the action of a substance necessary for some stage of the infection or nodulation process, this antagonism might be observed as an inhibition of nodule formation. On the other hand, if the metabolic analogue antagonized the action of a biologically formed repressor, it would tend to increase nodule abundance. Molina and Alexander (1967) examined the effect of a large number of antimetabolites of amino acids, vitamins, auxins, purines, and pyrimidines on nodule formation of birdsfoot trefoil and excised bean roots. Most of the antimetabolites had no detectable specific effect on the nodulating test system, and they frequently had a marked toxic influence on root or top growth. Of particular interest, however, is the fact that α-picolinic acid, a niacin analogue, prevented nodule formation on birdsfoot trefoil although the antimetabolite had no detectable effect on the root or top growth or on proliferation of the infective microorganism when tested in vitro. Further, benzenehexachloride and α-methylglutamic acid inhibited nodulation of excised bean roots without apparently inhibiting the development of these roots. Conversely, pyridine-3-sulfonate markedly increased nodule abundance on excised bean plants. Benzene-hexachloride is an analogue of inositol, and pyridine-3-sulfonate is an antagonist of niacin. This influence of niacin and inositol antimetabolites is reminiscent of a comparable effect observed in Saccharomyces carlsbergensis in which niacin reverses the growth-promoting influence of inositol on an inositol-requiring strain of the yeast. Moreover, Weir (1960) and Raggio, Raggio, and Burris (1959) reported also that inositol stimulates nodulation of either intact plants or excised bean roots.

Recently, we have been looking for mutants of Rhizobium that have lost either their effectiveness or their infectiveness, mutants derived from cultures that are both effective and infective. A number of ineffective strains of R. trifolii and R. meliloti have been obtained; Kanamycin resistance was used to select for mutants. By contrast with the reported work of Mueller (1963), nitrogen fixation of uninoculated pea plants or of pea plants inoculated with the ineffective mutants was not promoted by adding vitamin B_{12} either to the roots or to the foliage of the pea plants. Preliminary evidence indicates that the ineffective bacteria produced more extracellular polysaccharide material than the effective strain, and studies of the correlation between the symbiotic patterns of the Rhizobia and the chemistry of the bacterial polysaccharides are in progress. Preliminary evidence also indicates some difference in amino acid excretions between two effective R. meliloti strains and ineffective mutants derived from them. No evidence was found, however, for a correlation between the quantity of vitamin B_{12}, niacin, and pantothenic acid excreted and effectiveness.

Assuming that the polysaccharide gum elaborated by the root-nodule bacterium might be related to the specificity in nodulation, a streptomycin-resistant, phage-sensitive, nodulating strain of R. trifolii was irradiated and non-gum-producing survivors were tested for nodulation. One of the mutants thus selected proved to be nonnodulating. This isolate was antigenically deficient by comparison with the parent culture. Except for the antigenic deficiency and symbiotic performance, the mutant appears to be identical to the wild type. Revertants to the characteristics of the parent were obtained from these nonsymbiosing mutants; these revertants were gummy, capable of inducing nodules, and serologically indistinguishable from the parent. Biochemical studies of these cultures are now in progress.

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Appendix A

PROSPECTUS: EVALUATING BIOLOGICAL NITROGEN FIXATION IN COMPARISON WITH INDUSTRIAL SOURCES OF FIXED NITROGEN

Abstract

Nitrogen fixation in tropical, temperate, and arctic climates will be studied in both natural and agricultural environments to determine the extent to which this process contributes to the total nitrogen economy in food production. Factors affecting nonsymbiotic nitrogen fixation will be evaluated, and the extent to which this process can be exploited in the production of food will be estimated. An effort will be made to determine (1) the areas of the world where dependence on biologically fixed nitrogen would be logical and desirable and (2) the areas where industrially prepared nitrogen fertilizers would be the main source of nitrogen. An effort will be made to determine the effect that intensive use of nitrogen fertilizers at high levels would have on the quality of the environment and on food quality. Consideration will be given to mechanisms whereby the contamination of the environment with excessive quantities of nitrogen can be avoided and to mechanisms for dealing with situations in which inordinately high levels of nitrate appear in ground water, lakes, and streams. An effort will be made to determine means for resolving possible conflict between (1) the desirability of maintaining high nitrogen-fertilizer levels for maximum food production and (2) the desirability of maintaining tolerable levels of nitrate in ground water and foods. It is anticipated that important information about the biogeochemistry of nitrogen and about the so-called nitrogen cycle will come out of this study. Such information will give a clearer understanding of the evolution of the atmosphere of the earth and of other planets.

Introduction

Research on the biology and ecology of nitrogen began long before the advent of organized scientific endeavor. The methods were empirical. Stories about exploitation of biological nitrogen cycling come down to us from the distant past--for example, stories about the use of fish as fertilizer for maize. We see these efforts as crude, but our own understanding of biological nitrogen cycling is inadequate. Because of the need to expand food production, and because food production on most of the world's agricultural land is limited primarily by nitrogen input, it is urgent that these biological processes be understood.

The ability of the United States to sustain a luxury level in food production is due largely to the use of nitrogenous fertilizers and the exploitation of biological processes of nitrogen fixation. Yet we do not have the information necessary to judge the extent to which production might be increased throughout the world by proper nitrogen husbandry, and we do not know just how an increased and more intensive use of industrially fixed nitrogen would affect the quality of the environment or the quality of the food produced.

We also need more information about the nitrogen economy of the world. We understand the fundamentals of some of the processes involved, and we have reason to believe that the present atmosphere of nitrogen had its origin in some reduced form and that this early form was changed through a sequence of biological reactions. But we do not have a clear understanding of how these processes took place or of their significance in the broader question of the evolution of planetary atmospheres.

Increased use of industrially fixed nitrogen will result in questions about the relation of this nitrogen and its management to the quality of ground water, streams, and lakes. It is commonly argued that phosphate is the limiting factor in maintaining the biotic level of freshwater lakes. This argument is based on the assumption that blue-green algae will arise to provide a bloom in these lakes, provided adequate phosphate is present. But we know from experience that this does not occur in many cases and that nitrate contributes significantly to lake pollution.

Several tentative conclusions can be drawn:

1. The demand for an increase in world food production will require a greatly increased input of fixed nitrogen.

2. In many areas of intensive agriculture, industrially fixed nitrogen will be used at much greater rates. (In this country the use of nitrogenous fertilizers has increased about fivefold in the last two decades.)

3. The extent and significance of nonsymbiotic nitrogen fixation as a nitrogen source for plants are not understood. In temperate climates, particularly where nitrogen fertilizers are used extensively, nonsymbiotic fixation has a relatively insignificant role. There is some suggestion, however, that in tropical climates nonsymbiotic fixation might be considerably greater, and there are many areas where lack of transportation makes it difficult to introduce nitrogen fertilizers. It is possible that nonsymbiotic fixation reactions may be of greater importance in such areas, particularly where production per unit of area is not critical.

4. The comparative efficiency of nitrogen-fixing legumes and industrially fixed nitrogen applied to crops as means of producing protein requires further examination.

5. The nitrogen economy of arctic soils has not been explored in detail. Although the growing season is short in arctic areas, the photosynthetic day is long. Fertilizer studies on the tundra have demonstrated that high photosynthetic production can be attained with the judicious application of nitrogen.

6. Increased use of nitrogenous fertilizers and the need for efficiency in their use have led to the use of nitrification-suppressing agents. Means for the control of nitrification and the relation of these means to food quality and to the quality of the environment have not been studied systematically.

Objectives

Information on the following subjects is needed:

1. The nitrogen status of the world, both in isolated natural ecosystems and in broader socioeconomic structures.

    a. The significance of symbiotic nitrogen fixation, both by legumes and by nonleguminous plants.

    b. The significance of microbial associations in natural ecosystems.

    c. The extent of nonsymbiotic nitrogen fixation in tropical, temperate, and arctic climates.

2. The protein and caloric yield of leguminous plants utilizing atmospheric N_2 as a nitrogen source as compared with yields of (a) leguminous plants grown on exogenously supplied nitrogen and (b) other plants, particularly cereals, utilizing exogenously supplied nitrogen at optimal levels.

3. The optimum comparative balance between the dependence on biological systems for nitrogen fixation and the use of industrially fixed nitrogen fertilizers.

4. The effect that the use of nitrogen fertilizers may have on the quality of the environment.

   a. The contribution of nitrogenous fertilizers to nitrate in ground water, levels of nitrate in ground water that can be tolerated over a long period, and means for minimizing this contamination.

   b. The effects of high levels of nitrogen fertilization on the quality of food products.

   c. The effect of nitrification inhibitors on the quality of food products.

5. The extent to which an energy input for the industrial fixation of nitrogen in a given social unit can, by the increased production of food in that unit, result in increased efficiency and a higher standard of living.

6. The processes of nitrogen transformation, with emphasis on the processes of nitrogen fixation, nitrification, denitrification, and the movement of nitrogen in soils and ground water.

Although information is necessary in all these areas, the present proposal is concerned only with the following:

1. An evaluation of nonsymbiotic nitrogen fixation in tropical, temperate, and arctic climates, in both natural and agricultural environments.

2. A comparative study of symbiotic nitrogen fixation, nonsymbiotic nitrogen fixation, and the use of industrially supplied nitrogen fertilizers as sources of nitrogen for efficient food production.

3. The nitrification reaction as carried on by autotrophic organisms and heterotrophs in relation to agricultural practices.

4. The significance of nitrates in ground water, streams, and lakes and mechanisms for maintaining the quality of these waters.

5. Systems of nitrogen loss, including leaching and denitrification reactions.

Research Projects

The following research projects are proposed:

1. Evaluation of the complete nitrogen economy of various agricultural ecosystems, including a comparison between the direct input of industrially fixed nitrogen and the input of nitrogen by biological fixation, symbiotic and nonsymbiotic.

2. Evaluation of the complete nitrogen economy of natural ecosystems, including freshwater lakes and streams.

3. Evaluation by direct methods of the extent of nonsymbiotic nitrogen fixation in temperate soils under various conditions of soil, climate, and vegetation.

4. Evaluation by direct methods of the extent of nonsymbiotic nitrogen fixation in tropical soils under various conditions of soil, climate, and vegetation.

5. Evaluation by direct methods of nonsymbiotic nitrogen fixation in arctic soils under various conditions of soil, climate, and vegetation.

6. Investigation of (a) mechanisms for controlling the process of nitrification and (b) the effect of such mechanisms on the quality of food.

7. A complete study of nitrate nitrogen in profile in agricultural areas that have a geologic history of evapotranspirative loss exceeding rainfall to determine the distribution of nitrate nitrogen in the profile, to determine the extent to which the introduction of an irrigation regime will transport this nitrogen to fossil ground water, to determine the extent to which such a transport of nitrate nitrogen to fossil ground water would influence local or remote economies, and to explore means by which any such deleterious phenomena could be avoided.

8. Investigation of mechanisms whereby the adverse effects of excessive quantities of nitrates in fresh waters might be avoided or countered.

Study Methods

1. Studies will frequently entail tracer techniques in which the heavy stable isotope of nitrogen, N^{15}, are used. Means should be found for improving the precision with which N^{15} can be determined.

2. Because of the expensive equipment involved and the special training necessary for its operation, it is desirable to consolidate methodology and N^{15} analysis in a few laboratories and to facilitate means whereby scientists without these facilities can obtain necessary analytical assistance.

3. Methods for the field evaluation of nitrogen fixation need further refinement and standardization. Present limits of isotopic nitrogen technique make possible the detection of fixation rates of approximately 25 kilograms per hectare per annum. The acetylene-eltylene method is much more sensitive but calibration of this method is needed. Another order of magnitude of precision would be desirable both in determining the significance of the fixation reaction in agricultural regimes and in refining biogeochemical information.

Special Needs

1. Exchange of information among workers in the field should be improved. This need can be met in part by a quarterly newsletter.

2. Communication would also be improved by holding annual or biannual conferences at which workers would exchange views and information.

3. An inexpensive source of isotopically labeled nitrogen is necessary. Present experiments involving the use of tracer nitrogen are limited in scope and number by the present high cost of N^{15}. It is possible that by consolidating existing techniques, this cost could be reduced by 90% or more.

4. Provision must be made for training scientists in the concepts, needs, and special skills required for carrying out an expanded program of study on the biology and geobiology of nitrogen.

Coordination and Administration

With proper coordination, the program should yield results beyond those that could be expected from a program in which workers communicated only through the journals or through meetings. It would be desirable to obtain coherence, coordination, and direction through an administrative structure. It might be desirable to have a program coordinator who would have overall responsibility and would perform these duties in addition to his regular research duties. He could have an advisory committee consisting of either the Production Processes Subcommittee of the IBP National Committee or a group selected by the Production Processes Subcommittee. He would need assistance with such matters as communication, budgeting, and publications.

Budget

Project	Year 1	 2	 3	 4	 5	 6-10
	------Thousands of dollars-------					
Nitrogen economy of agricultural ecosystems	96	96	120	120	120	600
Nitrogen economy of natural ecosystems	80	80	100	100	100	500
Nonsymbiotic fixation in temperate climates	48	90	90	90	45	-
Nonsymbiotic fixation in tropical climates	-	35	53	53	53	30
Nonsymbiotic fixation in arctic climates	-	-	35	53	53	73
Control of nitrification effects on environment	-	50	66	66	66	660
Nitrogen in an arid profile	30	48	48	48	30	-
Effects of nitrates in fresh waters	60	96	96	96	66	660
Newsletter publication	1	1	1	1	1	5
Research conferences	5	5	5	5	5	25
Training	60	60	120	120	120	300
Totals	380	561	734	752	659	2,853

Appendix B

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