Microorganisms and Soil Fertility

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Editor's Preface

Sharp-eyed readers will at once notice what appears to be a consistent typographical error throughout this MONOGRAPH. Since the word "microbology" is not one commonly found in dictionaries, it requires a note of explanation. As used here the term *microbology* refers to that segment of life that may be generally classified as microbes. It is *microbe-ology* rather than *micro-biology.*

Dr. Ward Giltner, late Professor of Bacteriology and Hygiene, Michigan State College, in his Elementary Textbook of General Microbology (P. Blakiston's Son & Company, Philadelphia, 1928) emphasized that *microbology* and *microbologist* are more correctly descriptive terms. *Microbiology* and *microbiologist,* strictly defined, mean "small biology" and "small biologist," respectively. However, while terms are important and should be precise before becoming fixed in the literature, they are not as important as facts and ideas.

Microorganisms and Soil Fertility

Soil is a basic treasure. Soils produce good yields and keep on doing so if they are well managed. The management of soil is among the oldest of the arts, but none is changing more rapidly than it. We know more about taking care of the soil than our fathers and grandfathers did. There is much more that we should know.

—EZRA TAFT BENSON

The soils of the United States produced bumper crops in 1958, and produced them on fewer acres. During the past 10 years our soils have become more and more productive. With the yield per acre index for 1947-49 taken as 100%, for 1957 and 1958 it increased to 127% and 142% respectively. Under the Agricultural Act of 1958 federal economists expect farmers to offset continuing declines in farm prices and increases in farming costs by continuing increase in farm production. Such steadily improving production is due in large measure to more and better machinery, improved seed and varieties, and increased use of pesticides and fertilizers. Fertilizer use, especially of nitrogen, has increased markedly during the past decade (Figure 4, page 18); nevertheless, the fertility of cropped soils is declining (Table 1).

Producing more and more from less and less acreage is a demand characteristic of our economy. The population is rapidly increasing and exerting on available food supplies a pressure that poses a grave problem in the coming decade. Not only have we reached the limits of land available for farming, but agricultural space is shrinking at the rate of a million acres annually due to expansion of our cities, building of industrial sites, and construction of highways. We should therefore regard our soil with profound concern. Harvested crops remove plant food and the fertility of cropped soils is declining. Despite the use of fertilizers and crop residues, how long can improved farm practices and the pressure for increased crop yields continue without deleteriously affecting beneficial soil organisms, without developing some hidden hunger or depleting some aspect of soil fertility that must be restored by means now unknown? Future research will provide the answer and it is likely that some phase of soil microbology will assist in finding it.

Microorganisms in the soil bear a peculiar relation to soil fertility. Soil fertility is the ability of the soil to supply nutrients to plants; it is essentially the crop-producing power of the soil under given climatic conditions. The crop produced, or the productivity, is determined not by the crop-producing power alone but by a combination of climatic factors, crop factors, and cultural practices. Two forms of soil fertility are recognized—active and potential. Active fertility is immediately available; potential fertility becomes available by chemical or microbial action on minerals and organic matter. *The function of soil microorganisms is to render potential fertility available.* Thus

Table 1. ANNUAL BALANCE OF PLANT NUTRIENTS IN SOILS OF THE UNITED STATES, 1930.

Changes	Nutrients					
	N	P	K	Ca	Mg	S
Losses—*in thousand tons* (Harvested crops, grazing, erosion, leaching)	22,900	4,221	50,109	68,186	24,558	12,044
Additions—*in thousand tons* (Fertilizers and liming materials, manures and bedding, rainfall, irrigation waters, seeds, nitrogen fixed)	16,254	1,448	5,151	12,562	4,041	9,030
NET ANNUAL LOSS	6,646	2,773	44,958	55,624	20,517	3,014

J. G. Lipman and A. B. Conybeare, 1936, from New Jersey Agricultural Experiment Station Bulletin 607.

the gaseous nitrogen of the atmosphere represents a vast store of potential fertility. It is not directly available to plants. Nitrogen-fixing bacteria, however, absorb this gas from the soil solution and convert it to cell protein. When the cells die, other microbes attack the protein and convert the nitrogenous constituents to ammonium, which then becomes an available nutrient. Similarly, microbial action on plant and animal residues releases the many combined nutrients from their unavailable forms. In many instances microbial action results in indirect as well as direct production of active fertility. An example of this is the oxidation of flour sulfur, sometimes applied to soils deficient in this essential element. Bacteria of the genus *Thiobacillus* oxidize elemental sulfur to sulfuric acid. The sulfate ion is directly available to plants, while the acid has a solvent action on complex minerals and releases potassium, phosphorus, and other elements in available forms.

Higher plants and microorganisms grow in close relationship and are mutually dependent in many ways. Large numbers of bacteria and molds in the soil use plant and animal residues as food and are active in transforming them to humus and available plant nutrients. Through the production of carbon dioxide (CO_2), (which with water forms carbonic acid), and other acids such as nitric and sulfuric, they are also responsible for a gradual liberation of available food from the insoluble soil minerals and from unavailable fertilizer materials. Some species change elemental sulfur and sulfides to the assimilable sulfate; some are active in the production of ammonia and nitrates from protein material. Other species utilize atmospheric nitrogen, building it into compounds which eventually become incorporated with humus, thus adding to the supply of nitrogen which is so often a limiting factor in soil fertility. The nitrogen-fixing root-nodule bacteria of leguminous plants are especially important. Inoculated plants draw much less nitrogen from the soil, while if turned under as green manure they may add as much as 200 pounds of fixed nitrogen per acre. In Idaho, a soils technologist recently has estimated that if the nitrogen fixed by legumes in that state in one year were to be purchased in the form of commercial fertilizer it would cost the farmers over 16 million dollars.

Since bacteria capable of inoculating a specific leguminous plant are not always present in the soil, artificial inoculation may be necessary. Elucidation of this phenomenon and the development of cultures for legume inoculants are major contributions of microbology to soil science.

The soil contains a vast number of microorganisms including—in the usual order of abundance—bacteria, actinomyces, molds, algae, and protozoa. Each kind produces chemical changes which influence the development of all other organisms. Many of their activities are essential to the development of higher plants and animals. While extremely minute in size, the total number of microbes in a fertile soil comprise a mass of hundreds of pounds per acre furrow slice, considered to be 6⅝″ deep, and equivalent to 2,000,000 pounds of mineral soil (Table 2). This mass of organisms, including insects and worms, is highly active and brings about changes that develop soils and create and maintain fertility.

Table 2. Living Organisms in Fertile Soil

Organisms	Live weight per acre 6⅝″	Relative numbers
	Pounds	*Percent*
Bacteria	1,000	60-90
Actinomycetes	1,000	10-40
Molds	2,000	1-10
Algae	100	1
Protozoa	200	2
Total	4,300	
Dry Weight	1,000	
Nematodes	50	
Insects	100	
Worms	1,000	
Roots (dry weight)	2,000	

The soil microbologist is usually not concerned with earthworms, although there is some evidence that soil microorganisms may be a source of food for the worms and that certain enzymes, especially cellulase and chitinase, may be produced by organisms living in the intestine. Since 1837 when Charles Darwin wrote his first paper on the effects of earthworm activity, important influences on the soil have been recognized. It has been demonstrated, at least qualitatively, and usually under laboratory conditions, that earthworms can decay plant remains, aggregate soil particles, improve drainage and aeration, and conserve soil moisture. Earthworm numbers vary in relation to soil type and field history, grassland populations generally being higher than in old arable land. Population densities up to several million per acre have been recorded. The biomass of 2,000,000 earthworms per acre has been estimated at about 1,000 pounds.

Animals that live in the soil in many places are almost as important in the development of soil profiles as the vegetation of the region. Worms facilitate the conversion of raw organic matter to humus and mix humus with soil minerals. Ants probably produce a greater effect on soils than do earthworms. They transport sandy and gravelly soil materials and incorporate fragments of vegetation in their mounds. Clearings made by ants leave the soil exposed to erosive effects of wind and rain. Termites, especially in the tropics, construct larger mounds than ants and the mound material is distinctly higher in calcium, pH, and fertility than is the surrounding soil. Wood lice, centipedes, millipedes, and spiders consume organic matter of various kinds and help convert it to humus.

Crabs and crawfish are active in soils of certain regions where the water table is near the surface. In making tunnels to contact the water they deposit chimney-like structures at the soil surface. These animals thus move and mix enormous amounts of earth, perhaps up to 10 tons per acre in a single season. In addition, their activity influences soil aeration and water movement. When they occur, their effects on soil profile development are pronounced. Prairie dogs, gophers, and other rodents, active in grasslands, semideserts, and deserts, move tons of soil material per acre in building burrows and mounds. An example in Corvallis is currently to be found on the old irrigation plots just south of the railroad and east of 35th Street; one cannot walk across this area without continually stepping on huge gopher mounds, and it appears reasonable to estimate that these rodents have turned up at least 10% of the surface foot during the past season. Burrowing animals in forests have similarly great, though less noticeable, effects. Many other kinds of burrowing animals add their wastes and dead bodies to the soil organic matter and thus influence soil fertility and profile development.

The soil is a natural body of definite layers or horizons physically, chemically, and biologically derived from the earth's mantle. These horizons have characteristic morphological, constitutional, and physiological features determined by nature of parent material, climate, biosphere, and topography. Every well-developed soil has its own distinctive profile characteristics. It is a mineral-biological complex of organic and inorganic substances composing a dynamic polyphase physical-chemical system in unstable equilibrium with vital phenomena and factors of environment. Its complexity cannot be adequately described or realized. As designated by Sante Mattson, an outstanding Swedish soil scientist, it is the sum and also the product of the intermingling of the four spheres of nature: atmosphere, hydrosphere, lithosphere, and biosphere (Figure 1). As indicated in Figure 1, various combinations of these spheres occur in nature, but only where all are combined is there a soil. To these should be added the radiosphere as an additional factor of environment.

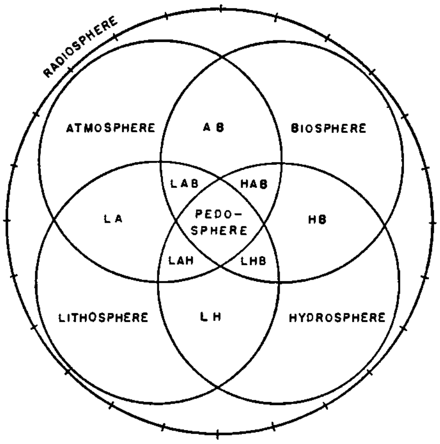
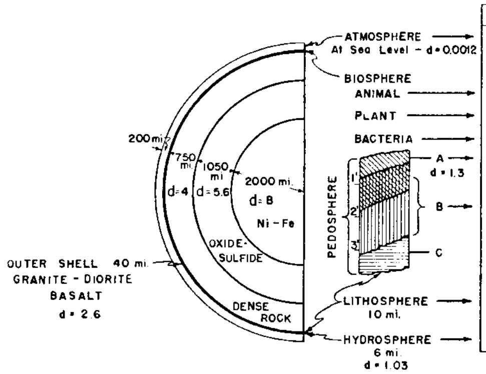


Figure 1. **Constitution of the Pedosphere (W. B. Bollen, 1958)**

It is revealing to compare the composition of these spheres and the soil, or pedosphere, with the composition of organisms, which must wrest substance from this environment (Figure 2). With respect to carbon—their major constituent—plants, animals, and microbes are dependent primarily upon the extremely dilute supply of 0.04% by weight of carbon dioxide in the atmosphere.



ELEMENTAL COMPOSITION
WEIGHT % DRY BASIS

	C	O	H	N	Si	Al	Fe	C/N
ATMOSPHERE At Sea Level - d = 0.0012	0.01	23.15		75.51	0.00	0.00	0.00	
BIOSPHERE								
ANIMAL	60.95	10.83	7.40	8.93	0.003	0.003	0.03	7/1
PLANT	43.62	44.48	6.24	1.46	1.17	0.11	0.09	30/1
BACTERIA	50.0	30.0	7.0	10.0	0.01	0.00	0.09	5/1
A	1.15	45.00	1.0	0.09	35.65	5.29	2.52	13/1
B	0.46	43.50	0.5	0.05	35.0	6.24	4.01	9/1
LITHOSPHERE 10 mi.	0.09	47.46	0.14	0.00	27.61	8.07	5.06	
HYDROSPHERE 6 mi. d = 1.03	0.00	85.79	10.67	0.00	0.00	0.00	0.00	

Figure 2. **Composition of the Spheres of Nature in Relation to Organisms and their Environment (W. B. Bollen, 1946)**

The Soil as a Culture Medium

Soil microorganisms live chiefly in the colloidal complex of organic and inorganic materials more or less saturated with water and supported by the soil particles, mainly mineral grains—the whole serving as a culture medium. They impart to the soil characteristics of a living body. Various branches of soil science resemble those of biology since areas are devoted to anatomy, physics, chemistry, physiology, taxonomy, and evolution. Physiology of the soil is a primary concern of soil microbology.

Microorganisms growing in the soil are influenced by seven factors of environment: moisture, temperature and other radiant energy factors, aeration, pH, food supply, biotic factors, and inhibiting factors. Each organism has its own cardinal values for each of these factors. For most saprophytes the minimum-maximum range is rather wide. Optimum values are not sharp unless all the other factors are rigidly controlled. In natural media, and especially in the soil, a change in one factor immediately induces changes in all the others. In effect, therefore, an optimum range rather than an optimum point exists for each factor (Table 3).

Control of these factors insofar as possible by cultural practices helps to maintain and increase soil fertility, due in no small part to favoring activity of beneficial soil microorganisms. It must be recognized, however, that the interactions of organisms and environments are reciprocal. While the environment determines the conditions under which life develops and exists, the organisms influence conditions prevailing in their environments.

Table 3. Factors of Environment and Their Approximate Cardinal Values for General Microbology Activity in Soil

Factors	Minimum	Optimum	Maximum
Moisture	5%*	50%*	80%*
Temperature	2° C.	28° C.	40° C.
Aeration	varies	at 50%* H_2O	varies
pH	4	7	10
Food supply	varies	balanced, C/N = 25/1	varies
Biological	–	symbiosis; limited antibiosis	–
Inhibiting	Positive or negative extremes of other factors.		

* of moisture capacity

In dealing with the soil, and especially in attempting to gain the greatest advantage of its potential fertility, it is necessary to bear in mind its two fundamental characteristics; the soil is biologically alive, and its colloidal clays and organic matter have cation exchange properties which govern the release of plant food.

Oxygen and hydrogen are abundantly available. Nitrogen in available form is often limiting. Nitrogen gas is abundant in the atmosphere but is unavailable to organisms except to the few species of nitrogen-fixing bacteria and algae. Although the rainwater which falls on the land surface of the earth brings with it annually about a billion tons of dissolved oxygen and almost an equal amount of dissolved carbon dioxide, it carries down only 5 to 6 pounds of nitrogen in the form of ammonium and nitrate per acre per year. The ultimate source of carbon for the biological world is carbon dioxide. This emphasizes the ecological significance of the minute, though continually present, supply of carbon dioxide of the air, and of the organic nitrogen in soil and marine humus.

The outstanding common characteristic of the total flora and fauna of the soil is the power to continually transform matter and energy. As a result the various food elements are dynamically transformed in cycles which maintain circulation of these elements in nature and prevent their permanent isolation in organisms after death. Bacteria play a large part in these transformations for two reasons:

(1) They grow and transform matter more rapidly than other organisms.

(2) They perform reactions not possible for other organisms.

Two basically distinct types of nutrition separate all living things into two classes from the standpoint of natural economy. The first class are strictly mineral feeders. They synthesize organic tissue from carbon dioxide; they are producers—constructive and independent of other organisms. These are the autotrophes and they include all chlorophyll-containing plants, photosynthetic bacteria, and certain chemosynthetic bacteria. The other class are the heterotrophes. These are biological feeders, requiring organic food previously synthesized by some other organism. In nutrition they are consumers—destructive and dependent. They embrace all animals, the fungi, and most bacteria. The ecological significance of these two groups can be appreciated from a brief consideration of the cycles of carbon and nitrogen.

The Carbon Cycle

In the carbon cycle (Figure 3), carbon dioxide from the atmosphere is converted by autotrophes into organic compounds of high energy content. Photosynthetic organisms obtain energy for this transformation from the

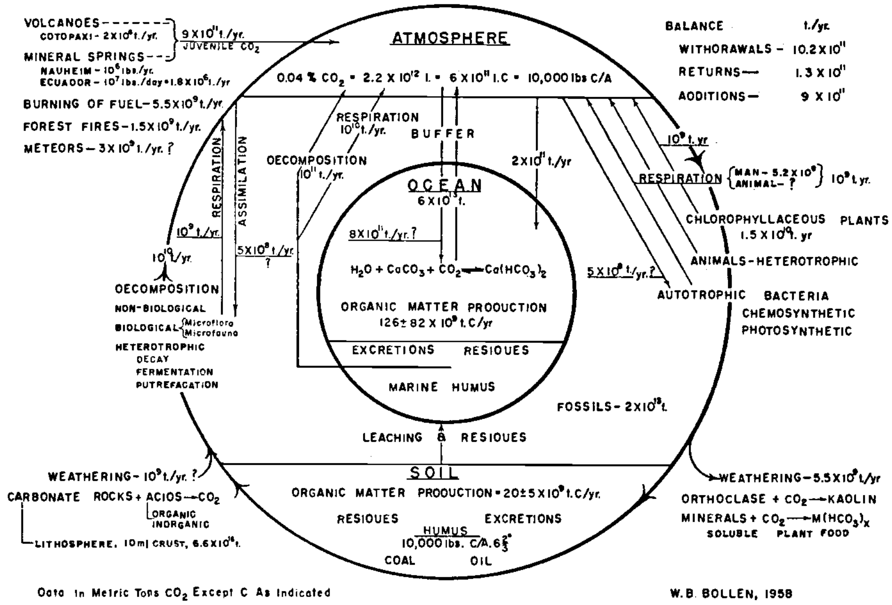


Figure 3. **The Carbon Cycle (W. B. Bollen, 1958)**

sun's rays. The autotrophic bacteria derive energy from oxidation of certain elements, such as sulfur or hydrogen, or from oxidation of simple mineral compounds such as ammonia, hydrogen sulfide, or carbon monoxide. Heterotrophes consume organic substance previously elaborated by autotrophes and other heterotrophes. This biological material is utilized for both structure and energy, the greater proportion being oxidized for energy and therefore yielding more or less carbon dioxide, which returns to the cycle.

Return of this carbon dioxide to the atmosphere by decomposition and respiration on land and in the sea is often considered vital to growth of plants. The atmosphere over one acre contains enough carbon dioxide to produce 200 bushels of corn, including the stover. A 30-bushel corn crop grown on all the earth's arable acres each year would exhaust all the atmospheric carbon dioxide in 100 years were it not for replenishment. The total return, about 1.3 x 10^{11} tons per year (Figure 3), from all decomposition and respiration accounts for only about 5% of the remarkably constant content of the "Spiritus Vitalis" (CO_2) found in the air. Volcanoes and mineral springs, as pointed out by Dumas and Boussingault in 1841, are the main sources of supply. Such CO_2, in addition to being mineral, is also juvenile.

Organic Matter and Nitrogen

Soil organic matter is a product of environment and differs from soil to soil. In kind and amount it varies with the type of vegetation, temperature, rainfall, drainage, soil population, and management.

The nitrogen cycle and carbon cycle are biologically bound together and proceed simultaneously, as also do the cycles of sulfur, phosphorus, and other nutrient elements. Organic matter in the soil is necessary to supply carbonaceous food for microorganisms and to maintain the humus. Humus is essential for good tilth and also serves as a store of nitrogen, phosphorus, sulfur, and other nutrients rendered slowly available by microbial action. Nearly all of the nitrogen and sulfur and much of the phosphorus in humid agricultural soils is derived from the soil organic matter. Organic matter, especially in its humified forms, improves soil physical conditions, has favorable effects on aeration and moisture capacity, and acts as a buffer against pH changes. Good soil management aims to adjust the additions of crop residues and fertilizers, the sequence of crops, and the losses through biological activity in such a way that profitable crops may be harvested without reducing the humus supply of the soil below a definite level. This equilibrium level varies with climate and soil type.

Bacterial protein in large part resists decomposition and accumulates in the soil. It is sufficiently reactive to combine with lignins, the most resistant residues left in decomposition of plant materials. This combination forms the humus nucleus which absorbs iron, aluminum, and silicon compounds to become the complex of rather indefinite residual substance commonly referred to as humus, and termed by Sante Mattson the "Alfesic complex." The nitrogen content of this complex is high; although the carbon:nitrongen ratio (C/N) commonly is near 10/1, humus is only slowly decomposable, and only by specialized bacteria. For this reason it represents a store of slowly but continually available nitrogen in the soil as long as crop residues are returned. If additional plant residues were excluded, the final decomposition product of the humus would be an accumulation of dead bacterial cells, with a C/N ratio of 5/1.

Analysis of a sample of Walla Walla silt loam soil from northeastern Oregon showed 1.35% carbon and 0.11% nitrogen, giving a C/N ratio of 12.3/1. The equivalent in soil organic matter per acre plow depth (2,000,000 pounds) is approximately 50,000 pounds, while the nitrogen represents nearly 14,000 pounds (dry basis) of dead bacterial protein roughly equivalent to 110,000 pounds of previously living cells. For the total number of living microbes in this acre plow slice of soil, 100,000 trillion is a reasonable figure, and their dry weight would approximate 1,000 pounds. Of this the bacteria would contribute 250 pounds, about one-half of which is resistant protein. Even after making due allowance for periods of rapid multiplication and death of bacteria in the soil, and for slow decomposition of their protein, it becomes apparent that any appreciable store of humus must represent years of accumulation.

It is thus evident that soil organic matter in the usual sense, meaning more or less humified material, is a complex mass resulting from microbial action on dead organisms of all kinds. Partially decomposed or partially humified organic matter can be built up, but it is relatively unstable and more difficult to maintain than is the original humus content. Living microorganisms require food, and the supply must be maintained. For cultivated soils this involves use of crop residues and often the use of fertilizers.

Nitrogen fertilizers, by stimulating decomposition, deplete rather than conserve soil carbon, although carbon in adequate amount and form of organic matter is required to conserve nitrogen. Stabilized organic matter buildup in cultivated soils is always limited by moisture, temperature, and aeration. These same factors control the total carbon and C/N ratio characteristic of the virgin soils of different climates. High nitrogen is always associated with a wide C/N ratio; cultivation lowers carbon more than nitrogen, so the ratio narrows. Northern soils are higher in organic matter and nitrogen, and have a wider C/N ratio.

Decomposition of plant and animal remains is an essential feature of life and the circulation of nutritional elements in nature. It constitutes a mineralization of organic matter. Carbon is returned to circulation as carbon dioxide, nitrogen is again made available as ammonia and nitrate, sulfur is liberated as sulfide and converted to sulfate, and other essential constituents reappear in the forms required by plants. Since organic remains are of mixed composition they are acted upon by various species of microorganisms and exhibit several well-defined stages of decomposition. In these stages, different groups of microorganisms predominate as part of the substrate is more or less rapidly and completely decomposed, part is reassimilated, and part is resistant and very slowly decomposed. Depending on the frequency with which fresh remains appear on or in the soil, the several stages of decomposition are more or less concomitant.

Starting with fresh material, there is first a stage of rapid decomposition in which the readily available substances are utilized by many heterotrophic microorganisms. Molds and spore-forming bacteria are especially active in consuming the proteins, starches, and cellulose. The relatively large amount of carbon dioxide liberated is important for its solvent action on soil minerals. Development of free-living, nitrogen-fixing bacteria is stimulated by the supply of carbohydrate, which they utilize chiefly for growth energy. Byproducts which are formed include ammonia, hydrogen sulfide, hydrogen and organic acids, alcohols, and other incompletely oxidized substances. In the second stage these substances are reassimilated in two phases: an autotrophic phase, wherein autotrophic bacteria oxidize the ammonia, hydrogen sulfide, and hydrogen; and a heterotrophic phase in which the organic byproducts liberated in the first stage are utilized by a wide variety of microorganisms.

A strong solvent action on soil minerals results from the nitric and sulfuric acids produced in the autotrophic phase of reassimilation. Development of the heterotrophes in this stage is influenced not only by the energy materials but also by the nitrogen compounds available. There is competition for nitrogen between higher plants and microorganisms carrying on the decomposition, the balance being determined by the carbon-nitrogen ratio of the original plant or animal residue. If this material has a nitrogen content of about 1% or less, all the nitrogen is consumed by the microorganisms, and in addition they compete with higher plants for more available nitrogen from the soil as long as oxidizable carbon compounds remain. An extended nitrogen starvation may result, which may be corrected by addition of inorganic nitrogenous fertilizers. When the nitrogen content is from 2% to 2.5%, only a temporary nitrogen starvation occurs and is followed by liberation of ammonia. With a higher percentage of nitrogen the requirements of the organisms active in the decomposition are more than satisfied and ammonia is liberated throughout the process.

The final stage of decomposition is the stage of humification. This is characterized by the formation and gradual continual decomposition of the humus complex. Nitrogen assimilated by microorganisms is reassimilated by succeeding generations and repartitioned until much of it accumulates as protein of dead bacterial cells. Bacterial protein is resistant to decomposition and most of the nitrogen of humus is in this form. The nonnitrogenous portion of humus is composed largely of lignin, hemicellulose, and various other resistant substances, but the lignin is of peculiar importance because it exerts a specific effect in nitrogen conservation by binding proteins. The amount of protein bound, whether of bacterial or other origin, depends upon the supply, but the bound protein is always more resistant to decomposition. Only actinomyces and certain nonsporeforming bacteria which comprise an autochthonous or continually present and active native microflora can attack ligno-proteinate and other humus complexes. As a result, the nitrogen is only slowly but continuously liberated, maintaining for higher plants a supply of available nitrogen that bridges the intervals between additions of fresh organic residues.

Soil microorganisms are active in four zones of decomposition: surface debris, turned-under residues, root envelopes, and humus. Each zone is of peculiar significance. Infiltration of the end products of surface decomposition influences soil formation and morphology. This is strikingly shown in development of the podsol profile, characterized by a bleached horizon below a fermenting layer of organic residues. Such a horizon is readily visible below the organic layer produced by the lush vegetation on the edges of the yellow sandstone seacliffs along the Oregon coast. Artificial incorporation of crop residues and manures by cultural practices distributes and hastens mineralization to the immediate advantage of plant growth. In soils upon which plants are growing, a large proportion of the microflora is confined to a narrow zone about the roots. This zone, the rhizosphere, is of major importance in the nutrition of higher plants for here are impressed a series of relationships ranging from symbiosis, mutualism, and stimulation to inhibition, toxicity, and parasitism. Humus, under natural conditions, is distributed from the soil surface downward in a decreasing concentration and to a depth characteristic of the soil type. This affects accordingly the distribution and activity of the autochthonous microflora.

As a whole the soil microbes are a population of workers capable of multiplying when decomposable material is present. This illustrates the importance of organic matter in the soil. The microbes themselves are subject to death and decomposition and their remains make up a large part of humus, thus constituting a considerable reserve of plant food slowly becoming available. It is thus readily seen why the soil is sometimes regarded as a corporation of three factories: a manufacturing plant, a disposal plant, and a storage plant.

Next to water, oxygen, and carbon dioxide, nitrogen in suitable form is the major nutrient demanded in largest quantity by microorganisms as well as by crops; it is most often the limiting food element in soil fertility. World agriculture now uses nearly 10 million tons of fertilizer nitrogen annually. In 1957 North American farmers used 2.8 million tons. Much of this is lost from the soil by leaching and denitrification before it can be utilized by plants. This is a major problem in soil fertility, especially from the standpoint of soil microbology. Seriousness of the problem is apparent from the fact that in the United States, of 52 Agricultural Experiment Station projects presently assigned to soil microbology, 42, or approximately 80%, are concerned with the nitrogen problem, chiefly studies on transformations and fixation. A summary of our agricultural nitrogen situation is shown in Figure 4. The uptake by crops of nitrogen from fertilizer sources commonly indicates 40% to 80% recovery. This recovery varies with the amount and form of nitrogen added, with kind and age of plant, and with environmental factors, including moisture, pH, organic matter, and C/N ratio, and other elements of fertility. Some of the nitrogen remains in the soil for succeeding crops; some, on the average about 15%, is denitrified. Luxury nitrogen especially is subject to extensive loss. Moisture availability is important in determining the rate of nitrogen fertilization required for maximum crop yields since fertilization must be increased as moisture stresses decrease.

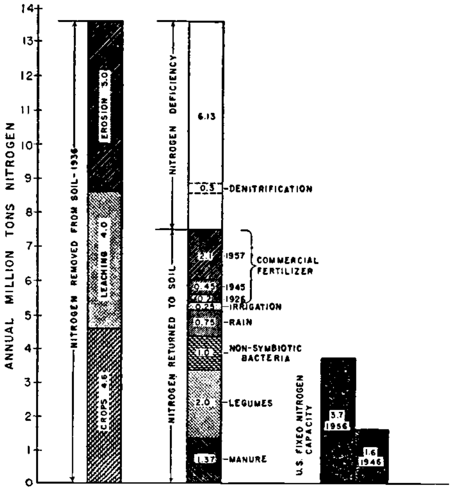


Figure 4. **Annual Total Agricultural Nitrogen Situation in the United States in Tons of 2,000 Pounds, 1957 (W. B. Bollen, 1958)**

Ammonium (NH_4^+) and nitrate (NO_3^-) salts differ in their relative merits as sources of nitrogen for higher plants. In general, NH_4^+ is generally more suitable for grains and grasses. Some plants can absorb both forms at equal rates under certain conditions. Peas in solution culture show inhibition when all the nitrogen is supplied as NH_4^+. Absorption of cation nitrogen is maximum during seedling phase of growth, while nitrate absorption is usually greater near blossoming. pH of the growth medium exerts a marked effect: below pH 6, NO_3^- is absorbed more and more rapidly than is NH_4^+; above pH 6, NH_4^+ is absorbed to a much greater extent. While these conclusions are derived in large part from solution culture studies, they nevertheless emphasize that various forms of nitrogen differ in fertility value, affecting plant composition as well as crop yield.

The problem of nitrate utilization by plants as well as by microorganisms is complicated by a dual function. Nitrate serves not only as a source of nitrogen for synthesis of nitrogenous tissue, but also functions as an oxidizing agent in certain energy-yielding reactions when O_2 is limited. Under anaerobic conditions nitrate may thus serve as an oxygen fertilizer. In either case, it is reduced to NH_4^+ or NH_2^-, and for protein synthesis nitrate must be reduced before it can be assimilated.

Organic matter, nitrogen fertilizers, and microorganisms are reciprocally affected during microbial transformations. The microbes have essentially the same food element requirements as do higher plants; they respond to nitrogen additions in much the same manner. Organic residues low in nitrogen decompose slowly unless additional nitrogen is available from the soil or added fertilizer. Lack of nitrogen limits the crop of microorganisms as well as higher plants. Bacteria and molds decomposing organic matter of wide C/N ratio will compete with growing plants for available nitrogen. Material with a C/N ratio near 30/1 has only sufficient nitrogen for rapid decomposition, while a ratio nearer 20/1 carries an excess which is liberated as NH_4^+. The microbial tissue thus synthesized becomes subject to decomposition on death of the organisms. Although much bacterial protein is resistant, mold tissue is readily decomposed and the nitrogen soon becomes available again as NH_4^+.

The Nitrogen Cycle

The significance of soil microorganisms in the conservation and use of nitrogen and organic matter is evident from Figure 5, illustrating the nitrogen cycle.

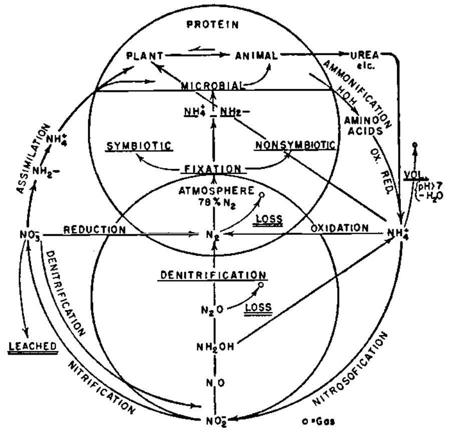


Figure 5. **The Nitrogen Cycle (W. B. Bollen, 1956)**

Ammonification

Most of the soil's nitrogen is derived from protein of plant, animal, and microbial residues. A variety of bacteria and molds rapidly transform protein material and liberate the nitrogen as NH_4^+, which is then assimilated by plants and microorganisms. Any excess over immediate requirements is stored on the cation exchange complex.

An additional phase of ammonification is provided by the urea bacteria, which liberate ammonia from urea and uric acids excreted by animals. These organisms are remarkable for their tolerance of high alkalinity, and also for their extreme rate of metabolic activity. One species, *Micrococcus ureae,* can ferment six times its own weight of urea in one hour.

Nitrification

As long as any ammonia remains on the exchange complex it is held against leaching but is available to plants and to nitrifying bacteria, which oxidize it to nitrite and nitrate to obtain energy for the autotrophic reduction of carbon dioxide. Their activity is favored by good aeration and available calcium and phosphate; it is retarded by excessive concentrations of ammonia and by extremes of pH outside the range 5.6-8.2. Certain species or strains of nitrifiers, however, are active in forest soils even at pH 4.

Nitrification may be regarded as a nonessential and perhaps harmful stage of the nitrogen cycle. While plants utilize NO_3^-, they can often use NH_4^+ to equal or better advantage for reasons already mentioned. Even though NO_3^- is used at no physiological disadvantage, its presence in the soil means nitrogen in a form always subject to some loss by leaching and by denitrification. A soil with a good supply of nitrate or a high nitrifying power is generally a fertile soil because the supply of bases, phosphate, and other factors favor crop growth as well as nitrification. If the production of NO_3^- from NH_4^+ could be eliminated without altering these favorable conditions, soil fertility could well remain the same. At the same time, it could be better maintained because there could be no nitrogen losses by denitrification and leaching.

NITROGEN FIXATION

A phase of outstanding importance in the nitrogen cycle is nitrogen fixation. This is the assimilation of elemental nitrogen (N_2) and is an ability possessed by only a few bacteria, most of which are heterotrophic, and certain blue-green algae. The nitrogen is converted to ammonium or hydroxylamine (NH_2OH), then to amino acids, and finally to cell protein; on death of the cell this reenters the cycle and is subject to ammonification. Under favorable conditions ammonium and amino acids are produced more rapidly than protein is synthesized. As a result, some soluble nitrogen compounds may be excreted from the cell and become available to other organisms.

Species of *Rhizobium* carry on fixation only when living symbiotically in nodules on roots of leguminous plants, much of the fixed nitrogen being immediately available to the host. Of the total nitrogen fixed by *Rhizobium* as well as by the nonsymbiotic *Azotobacter* and *Clostridium,* a considerable part is liberated in soluble extracellular organic form during the life of the cell and excreted to the soil. Some ammonia is also liberated. Thus nitrogen-fixing bacteria convert the generally unavailable gaseous nitrogen into immediately available compounds as well as into nitrogenous tissue which must later be decomposed by other organisms before becoming active in fertility. The extent of nitrogen fixation, with the exception of *Rhizobium* in association with leguminous plants, is difficult to evaluate. *Azotobacter* are generally distributed in soils but are often limited in number and activity by low phosphate, potash, lime, molybdenum, or pH. If these factors are favorable, *Azotobacter* may fix 20 to 40 pounds of N_2 per acre per year.

More important in nonsymbiotic nitrogen fixation is *Clostridium butyricum.* It is present in all soils and is much less sensitive to the unfavorable factors affecting *Azotobacter.* Moreover, its nitrogen-fixing activity is not retarded by appreciable amounts of available nitrogen, while *Azotobacter* fixes little or no nitrogen in the presence of much more than 2 ppm N as NH_4^+ or NO_3^-, these forms being assimilated preferentially instead of N_2. *Clostridium* under favorable conditions may fix 30 to 40 pounds of N_2 per acre per year.

Nonsymbiotic nitrogen-fixation in the field, however, is generally so small that it is difficult to determine by soil analysis. On the average, it is probably less than 10 pounds per acre per year. Usually it need not be considered in planning nitrogen fertilizer requirements for farm crops. From the practical standpoint nonsymbiotic fixation in agricultural soils not treated with nitrogen fertilizers may be considered as approximately equal to losses by leaching and denitrification. Symbiotic fixation by *Rhizobium,* on the other hand, is considerable and can be utilized to help maintain or increase soil nitrogen.

Nitrogen fixation by the bacteria in nodules of leguminous plants may exceed 100 pounds per acre in a good growing season. Maximum fixation is attained under soil and climate conditions favoring crop growth, but with a minimum of fixed nitrogen available in the soil. Like *Azotobacter, Rhizobium* uses NH_4^+ or NO_3^- in preference to N_2. Nitrogen fertilizers, therefore, diminish fixation by these bacteria. A light application, however, is often helpful in giving a leguminous stand an early start. On the other hand, a greater supply of ammonium or nitrate fertilizer may even prevent nodule formation.

Free nitrogen fixed by bacteria becomes available to other organisms in two ways:

(1) Under optimum growing conditions more N_2 is fixed and converted to NH_4^+ or NH_2^- more rapidly than the cell can assimilate it to form new protein. The excess is then excreted as NH_4^+ or amino acids. This is the basis of benefit derived by leguminous plants from their symbionts. It can occur also with *Azotobacter* and *Clostridium*. Nonleguminous plants in association with inoculated legumes frequently derive some fixed nitrogen; the nodule bacteria may carry on fixation at a rate in excess of requirements of the host, and this excess diffuses from the nodules or roots into the soil.

(2) After death of nitrogen-fixing bacteria, their protein, containing the fixed nitrogen, is subject to ammonification by proteolytic microorganisms in the soil. Similarly, the protein of leguminous residues or green manures becomes ammonified. Microorganisms carrying on this process utilize the nitrogenous tissue mainly for energy, liberating CO_2 and NH_4^+. As long as the C/N ratio of the material undergoing decomposition is about 20/1 or less, nitrogen is in excess of the microbial requirements and the excess is liberated in the soil.

Denitrification

Microbial reduction of nitrate is a property possessed by many facultatively anaerobic bacteria. Physiologically it enables them to grow in the absence of free oxygen (O_2) by utilizing NO_3^- as an alternative agent for oxidizing carbohydrate or other substrates to obtain energy. Although this oxidation is anaerobic, it can be more or less extensive in normally aerated soils. Isolated microregions or atmospheres devoid of O_2 exist in any soil not water free. Most soil organisms are aerobic by choice and consume O_2 with great avidity, thus often depleting the supply more rapidly than diffusion and solution can replenish it. Thus obligate anaerobes can thrive in a symbiosis with organisms that consume O_2 and create anaerobosis in proximity. This explains why *Clostridium butyricum*, an obligate anaerobe, can be important as a nitrogen fixer in all soils. It also explains why bacteria having the ability to use NO_3^- instead of O_2 cause denitrification even in well-aerated soils. These denitrifiers would use O_2 by preference if it were available. It is impossible to saturate the soil solution, unless sterile, with O_2 by any practical means. In a water-saturated soil no dissolved O_2 can exist, except at the surface. Under such conditions denitrification is most rapid and complete.

There are two stages of denitrification:

(1) A great many kinds of bacteria reduce NO_3^- to nitrite (NO_2^-). This causes no loss, and the NO_2^- may again be nitrified to NO_3^-.

A likely detriment is that NO_2^- in concentrations of 10 to 100 ppm N becomes toxic to plants, especially if the soil is acid. In properly balanced solution cultures, however, NO_2^- can support good growth.

(2) Other, more specialized bacteria utilize NO_2^- as well as NO_3^- for an oxidant. In the process they produce several successively more reduced compounds of nitrogen. Among these are hydroxylamine (NH_2OH), which may spontaneously yield NH_4^+ by reacting with water; nitrous oxide (N_2O), and free nitrogen (N_2). The last two named are gases and escape from the soil. As mentioned earlier, the extent of this loss is considerable. The minimum is likely to be not less than 20% of the nitrogen applied or returned to the soil. Organic nitrogen is ammonified and nitrified, while ammonium fertilizers are subject to nitrification. Considering the extensive use of nitrogen fertilizers, it becomes evident that these losses from denitrification run into millions of dollars annually.

While it is unlikely that any NH_4^+ produced in denitrification will be lost as such, loss of this form can occur from either anhydrous or aqua ammonia and from ammonium fertilizers. Volatilization may occur only when evaporation of moisture is rapid where the pH exceeds 7. Unless these conditions exist, NH_4^+ ions are held by negative charges of clay particles in the cation exchange complex; they can be removed only by living microbes and plant roots, or by replacement with other positively charged ions. Humified organic matter also has cation exchange capacity; in many soils it accounts for 25% to 50% of the total exchange capacity. Plant roots must compete not only with microorganisms for available nitrogen, but also with the exchange complex for nutritionally important bases.

The exchange complex of soils can sorb NH_4^+ and hold it against leaching, at the same time not retarding its availability to plants or to nitrifying bacteria. These bacteria are, in fact, most abundant and active at the sorption sites. A stronger type of NH_4^+ fixation primarily attributable to micaceous minerals is common in many Western soils. This fixed NH_4^+, equivalent to as much as 200 pounds of N per acre, is largely unavailable to plants and to nitrifiers.

It may be repeated now that denitrification occurs also in assimilation of nitrate by plants. This probably causes no loss in nitrogen but may reduce efficiency of assimilation.

Assimilation

Assimilation of ammonium and nitrate, and their conversion to protein by plants completes the nitrogen cycle. Protein metabolism by animals extends the cycle without greatly altering the fundamental mechanism.

In connection with assimilation of NO_3^-, it recently has been found that molybdenum (Mo) is a constituent element of the enzyme, nitrate reductase, involved in the transformation to NH_2^- or NH_4^+, prior to amino acid and protein synthesis. On Mo-deficient soils, plants cannot use NO_3^- to advantage; although they absorb it through the roots, it accumulates. NH_4^+ can be assimilated without Mo. Even with NH_4^+, however, plants require lesser traces of the metal ion for functions not associated with nitrogen nutrition.

Denitrifying bacteria also require traces of Mo; NO_3^- is not reduced without it, although to a certain extent it can be replaced by vanadium. Nitrogen-fixing bacteria do not fix N_2 without even greater amounts of Mo than required for denitrification.

Soils deficient in Mo can be made productive by application of a few ounces of a molybdenum salt per acre, equivalent to less than one ppm Mo. Lime and phosphate release Mo from minerals in some soils. Sulfate, manganese, and acidity decrease the availability. Thus minor element deficiencies and fertilizer practices inducing them can influence soil nitrogen availability and losses.

Soil Fertility and Management

In supplying nutrients, the soil may be considered as a table at which the soil organisms and plant roots feed. Food at this table will not appear in sufficient amount, however, until the exchange complex is well supplied. Then, figuratively speaking, the microbes eat at the first table set in the soil; the crop or other growth on the soil eats at the second table. The food supply may suffer not only a shortage of any element, but may also suffer imbalances in regard to combinations of many of the available nutrients. These imbalances may occur naturally or may result from injudicious fertilizer applications or cultural practices. Fortunately, microbes in the soil tolerate any shocks of imbalance in soil treatments better than such imbalances are tolerated by crops. For good plant growth, therefore, it is necessary to build up the soil in organic matter as well as in mineral nutrients. Such procedure feeds the soil first, then the crop.

Fertilizer practice and soil management must consider all these biocolloidal phenomena. Microbes are the soil's primary crop, and they must be provided a balanced nutrition, particularly with respect to their major requirements for carbon and nitrogen. Of the remaining food elements, none is likely to be limiting except phosphorus. With heavy applications of straw, sawdust, and other highly carbonaceous residues of wide C:N ratio, nitrogen always limits development of bacteria and molds. These organisms then compete with plant roots for available nitrogen and the plants will accordingly suffer from nitrogen starvation. If fertilizer nitrogen is added, the resulting enhanced microbial increase may be limited by lack of sufficient available phosphorus. In some soils molybdenum is lacking as a micronutrient, and certain bacteria, particularly *Azotobacter*, will not grow. On such soils the addition of a few ounces of molybdate per acre will supply the necessary 5 to 10 parts per billion of molybdenum required not only by these bacteria but also for good forage crops.

Ecology of Soil Microorganisms

The soil microbologist investigates the soil as a complex dynamic and versatile system composed of physical, chemical, and biological components. The ecology of microorganisms in this system is largely a matter of microbial physiology. The bacteria are of particular interest because from the evolutionary standpoint their minute uncellular structure has strictly limited their morphological development. Physiologically, however, they exhibit a wide evolution in nutritional level resulting from development of enzyme systems capable of utilizing various products in increasing variety.

Autotrophic bacteria present the most primitive nutritional level. They require only mineral nutrients, synthesizing complex organic compounds from carbon dioxide and water. For this reason they could have been the first organisms to develop on the primitive earth. Oparin's hypothesis that evolution of organic compounds must have preceded evolution of discrete organisms is based upon the thesis that carbon dioxide is entirely a biological product, a tenent long unaccepted by most geochemists. Autotrophic bacteria and algae are able to grow on bare rock and contribute to the formation of soil and accumulation of organic matter. As a result they pave the way for development of heterotrophes. Possibly after developing widely enough in the dawn of biology to create an appreciable store of complex organic substances, they provided the original opportunity for the evolution of heterotrophes as new types of organisms deriving energy and structural materials from these sources.

An intermediate or transitional nutritional level is exhibited in the facultative autotrophes, typified by certain sulfur-oxidizing and hydrogen-oxidizing bacteria which can metabolize either the mineral carbon dioxide or biological carbon compounds. This faculty is probably more widespread than is apparent. Recently it has been discovered that *Escherichia coli,* the colon bacterium heretofore regarded as a typical dependent heterotrophe, is able to grow autotrophically and obtain energy by oxidizing hydrogen. Facultatively autotrophic bacteria may be looked upon as representatives of the first physiological types which arose by virtue of the nutritional possibilities offered by the abundance of organic matter synthesized by the autotrophes. Another intermediate group is represented by the nitrogen-fixing bacteria and certain myorrhizal fungi, which require complex carbon compounds but can use nitrogen in elemental form. Nitrogen-fixing bacteria, such as *Desulfovibrio* growing autotrophically on hydrogen, and nitrogen-fixing blue-green algae are prototrophes or mineral feeders *par excellence.*

The obligate heterotrophes present a series of higher levels of nutrition. Opportunity provided by the great variety of carbon compounds available from dead organic matter probably incited the development and use of new reactions for utilization of these materials for both energy and structure. Numerous species have been evolved on the basis of these nutritional possibilities. Further differentiation has taken place with respect to nitrogen source; the level has raised from ammonia and nitrate to amino acids. Many of the saprophytic bacteria are facultative in this respect, while some of the pathogens are highly exacting and require specific amino acids.

The nitrogen-fixing bacteria present extreme examples which, while they require carbon compounds synthesized by other organisms, can elaborate protoplasm from elemental nitrogen. The symbiotic nitrogen fixers (*Rhizobium*) have evolved to a higher nutritional requirement since they have lost the ability, possessed by free-living forms, to synthesize an essential respiration coenzyme. At the opposite extreme are certain blue-green algae which not only fix nitrogen but also assimilate carbon dioxide by photosynthesis.

Restriction of synthetic ability leads finally to parasitism and dependence upon living tissue for necessary growth requirements, including growth factors or "bacterial vitamines." The highest nutritional level is thus exemplified by the exacting requirements of such pathogens as the influenza bacillus (*Hemophilus influenzae*) and the gonococcus (*Neisseria gonorrhoeae*).

An apparent anomaly is presented by this evolution in adaptation to available food. The most highly evolved bacteria have the most complex food requirements. By virtue of this they must possess the simplest metabolic mechanism. Autotrophes, in contrast, being capable of utilizing the most simple foods, must possess the most complex nutritional mechanism. Thus have microorganisms made possible the soil and its crops essential to man for his food, clothing, and shelter. Ultimately, the metabolic activities of bacteria and other microbes in the soil control the destinies of man.

Bibliography

Direct citation to original literature is not feasible. Summary papers, textbooks, and general articles which apply are listed below.

1. Allison, F. E., 1954, The Enigma of Soil Nitrogen Balance Sheets. Proc. Soil Microbiology Conference. Lafayette, Indiana.
2. Bollen, W. B., 1951, The Nature of Organic Pollutants in Relation to Stream B.O.D. Water and Sewage Works. *98*:277-281.
3. Clarke, F. W., 1925, The Data of Geochemistry. U. S. Geol. Survey Bul. 770. Washington, D. C.
4. Dumas, M. J. and J. B. D. Boussingault, 1844, The Chemical and Physiological Balance of Organic Nature. An essay. Ed. by D. P. Gardner. Saxton and Miles. New York.
5. Hopkins, C. G., 1910, Soil Fertility and Permanent Agriculture. Ginn and Co. Boston.
6. Hutchinson, G. E., 1944, Nitrogen in the biogeochemistry of the atmosphere. Amer. Scientist. *32*:178-195.
7. Joffe, J. S., 1949, The ABC of Soils. Pedology Publications. New Brunswick, N. J.
8. Katznelson, H., A. G. Lochhead, and M. I. Timonin, 1948, Soil Microorganisms and the Rhizosphere. Bot. Rev. *14*:543-587.
9. Lipman, J. G., and A. B. Conybeare, 1936, Preliminary Note of the Inventory and Balance Sheet of Plant Nutrients in the United States. New Jersey Agr. Exp. Sta. Bul. 607.

10. Lyon, T. L., H. O. Buckman, and N. C. Brady, 1952, The Nature and Properties of Soils. Macmillan. New York.
11. Mahan, J. N., and C. A. Graham, 1958, The 57-58 Fertlizer Situation. Commercial Fertilizer. *97*:23-45.
12. Mattson, S., 1948, The Constitution of the Pedosphere. Annal. Agr. College Sweden. *5*:261-276.
13. McElroy, W. D., and B. Glass, 1956, Inorganic Nitrogen Metabolism. Johns Hopkins Press, Baltimore.
14. Mehring, A. L., J. R. Adams, and K. D. Jocob, 1957, Statistics on Fertilizers and Liming Materials in the United States. Statistical Bulletin No. 191. U.S.D.A. Washington, D. C.
15. Meiklejohn, J., 1955, Soil Microbiology: Some Microscopical Methods and Results. J. Quekett Micr. Cl., Ser. 4 V. *4*:171-184.
16. Nikiforoff, C. C., 1959, Reappraisal of the Soil. Science. *129*:186-196.
17. Page, H. J. 1956, An Annual Review of World Production and Consumption of Fertilizers. Food and Agricultural Organization of the United Nations. Rome.
18. Prianishnikov, D. N., 1951, Nitrogen in the Life of Plants. Kramers Business Service, Inc. Madison, Wisconsin.
19. Rabinowitch, E. I., 1948, Photosynthesis. Sci. Amer. *179*(2) :25-35.
20. Redfield, A. C., 1958, The Biological Control of Chemical Factors in the Environment. American Scientist. *46*:205-221.
21. Riley, G. A., 1944, The Carbon Metabolism and Photosynthetic Efficiency of the Earth as a Whole. Amer. Scientist. *32*:129-134.
22. Russell, E. J., 1953, Soil Conditions and Plant Growth. 8th Ed. Longmans, Green & Co. London.
23. Satchell, J. E., 1958, Earthworm Biology and Soil Fertility. Soils and Fertilizers. *21*:209-219.
24. Stefferud, A. Editor. Soil. The 1957 Yearbook of Agriculture. U.S.D.A., Washington, D. C.
25. Stoklasa, J., and E. G. Doerell, 1926, Biophysikalische und Biochemische Durchforschung des Bodens. Parey. Berlin.
26. Thorp, J., 1949, Effects of Certain Animals that Live in Soils. Scientific Monthly. *68*:180-191.
27. Waksman, S. A., 1953, Soil Microbiology. Wiley, New York.
28. Winogradsky, S. I., 1949, Microbiologie du Sol: Problems et methodes. Masson, Paris.
29. Plass, G. N., 1959, Carbon Dioxide and Climate. Scientific American. *201* (*1*) : 41-47.

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