# THE MICROFLORA OF GRASSLAND

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## I. Introduction

The role of microscopically small organisms in numerous soil processes, particularly those affecting plant productivity, is well recognized. Less well known is a quantity of recently gathered information concerning microorganisms as components of major plant communities and the extent to which they participate in the total energy flow therein. Macfadyan (1963) has calculated that, of ecosystem gross productivity, rough-

ly 14% is respired by higher plants, 28% is consumed by herbivores, and 56% is metabolized by the decomposer organisms, namely, the soil and plant microflora and fauna. Similarly, Golley (1960) has estimated that the decomposers as a group use 70% of net production.

Although the soil microflora is the single most important group in the annual turnover of energy trapped by photosynthesis, to the authors' knowledge no broad general review of the microflora of grassland has heretofore been compiled. Indeed this review itself achieves little more than fragmentary coverage of the existing literature on the microflora present in grassland soils or associated with either the living or dead vegetation thereon. It makes no attempt to discuss those influences on the ecosystem that the bacteria and fungi may exert in their role as causative agents of plant or animal disease. In several instances in which review discussions do exist on specific aspects of the grassland microflora, citation of such literature is used in lieu of duplicate discussion. In other instances, data are cited for nongrassland soils or communities. This may be either for comparison with similar data for grassland or, if comparable data are not available for grassland, to point up the need for such data. In the context of this review, grassland denotes any landscape which supports mainly grasses as its native vegetation or if exploited by man, is used mainly for graminous plants.

## II. The Microflora of the Living Plant

There is a rich microflora associated with the surfaces of living plants that is either nonpathogenic or at most functioning at an extremely marginal level of pathogenicity. This microflora feeds primarily on exudates and sloughed cellular material. It commonly is divided into two categories: (a) the aboveground, shoot-associated microflora, or broadly the phyllosphere; and (b) the belowground, root-associated microflora, broadly, the rhizophere.

### A. Phyllosphere

Although earlier workers (Beijerinck, 1888; Winkler, 1899) had isolated bacteria from plant leaves, Burri (1903) first showed that a variety of plants support an abundant leaf-surface microflora. Bacterial populations of from several millions to more than a hundred million per gram of fresh leaf material were observed. Legumes and vegetable crops carried appreciably higher phyllosphere populations than did grass leaves. A year later Duggeli (1904) reported essentially similar observations. Both Burri and Duggeli, as well as other workers of the era (Huss, 1907; Gru-

ber, 1909; Lohnis, 1910; Wolff, 1913), recognized that the phyllosphere microflora of plants differed qualitatively from the microflora in soil.

Phyllosphere studies during the past half century can be grouped into three categories according to their emphasis on (a) population numbers; (b) specific or generic identifications of microorganisms in the phyllosphere; and (c) characterization of the kinds and amounts of materials in leaf exudates. Summation of representative information within each of these areas can be accomplished most conveniently in tabular form.

Table I summarizes phyllosphere populations as determined by diverse investigators. It shows that great variability exists in the phyllosphere populations not only for different plant species but also for an individual species sampled on different dates or in different locations.

TABLE I
Phyllosphere Populations Reported for Grass and Other Plants

| Investigator | Plants studied | Microbial population fresh material basis |
|---|---|---|
| Burri (1903) | Grass | $1–10 \times 10^6$ bacteria/g |
| | Clover | $113 \times 10^6$ bacteria/g |
| Duggeli (1904) | Grass | $0.1 \times 10^6$ bacteria/g |
| | Clover | $30 \times 10^6$ bacteria/g |
| Wolff (1913) | Grass | $0.7–2.4 \times 10^6$ bacteria/g |
| Allen *et al.* (1937) | Grass | $1.6–43 \times 10^6$ bacteria/g |
| Kroulik *et al.* (1955) | *Dactylis glomerata* | |
| | 4/28/51 | $3.3 \times 10^6$ bacteria/g |
| | 5/11/51 | $0.3 \times 10^6$ bacteria/g |
| | 7/05/51 | $173 \times 10^6$ bacteria/g |
| Doxtader (1969, personal communication) | *Bouteloua gracilis* | $0.1 \times 10^6$ bacteria/g |
| Thomas and McQuillin (1952) | Grass | $10^7$ Aerobacter/g |
| Di Menna (1959) | *Lolium perenne* | $2 \times 10^6$ yeasts/g |
| Leben (1961) | *Cucumis sativus* | $5–50 \times 10^6$ bacteria/cm$^2$ of leaf surface |
| Ruinen (1961) | *Coffea, Eria, Qualea, Peoliona,* and *Cacao* spp. | $10–20 \times 10^6$ bacteria/cm$^2$ of leaf surface |

Such variable populations as encountered by Kroulik *et al.* (1955) (Table I) on leaves of orchardgrass *(Dactylis glomerata)* make it difficult to draw any conclusions regarding possible differences between species. In general, however, the data suggest that broad-leaved plants support more microorganisms in their phyllospheres than to the grasses.

Table II summarizes observations concerning the occurrence of certain bacteria and yeasts in the phyllosphere. Gram-negative, yellow-pigmented bacteria appear to be highly characteristic of this particular environment. However variously these bacteria are named (see Table II), many workers (Mack, 1936; James, 1955; Billing and Baker, 1963; Dye, 1964; Graham and Hodgkiss, 1967) believe that they comprise a relatively homegeneous group, possibly even a single species.

Yeasts are also common inhabitants of grass leaves. Some species

TABLE II
The Occurrence of Certain Grass-Negative Bacteria and Yeasts in the Phyllosphere

| Investigator | Plants studied | Microbial species reported |
|---|---|---|
| *The "herbicola-trifolii" complex* | | |
| Duggeli (1904) | *Dactylis glomerata, Trifolium repens* | *Bacterium herbicola aureum* |
| Huss (1907) | Clover | *Pseudomonas trifolii* |
| Wolff (1913) | Grass | *Pseudomonas* spp. |
| Mack (1936) | Grass | *Flavobacterium herbicola* |
| Clark *et al.* (1947) | *Gossypium hirsutum* | *Xanthomonas* spp. |
| James (1955) | Cereal grains | *Xanthomonas trifolii* |
| Kroulik *et al.* (1955) | *Dactylis glomerata* | "Gram negative, yellow-pigmented bacteria dominant" |
| Dye (1964) | Grass | *Erwinia herbicola* |
| *Yeasts and yeastlike fungi* | | |
| Smit and Wieringa (1953) | *Amelanchier* sp., *Forsythia* sp. | *Pullularia pullulans* |
| Last (1955) | *Triticum vulgare, Hordeum vulgare* | *Sporobolomyces roseus, Tilletiopsis minor, Bullera alba* |
| Di Menna (1957, 1958a,b,c, 1959) | *Lolium perenne, Anthoxanthum odoratum, Agrostis tenuis* | *Torulopsis seria, T. ingeniosa, Candida humicola, C. curvata, Cryptococcus albidus, C. terreus, C. diffluens, C. laurentii Rhodotroula graminis, R. flavus, R. mcucilaginosus, R. marina, Schizobastosporion starkeyihenricii.* |
| Ruinen (1963, 1966) | *Aloe* spp., *Sanseviera* spp. | *Cryptococcus laurentii, Rhodotorula glutinis, Candida* spp. |
| Crosse (1959) | *Prunus cerasus* | *Pullularia pullulans* |

found thereon are listed in Table II. The yeasts dominant in the phyllosphere are not the same species that are dominant in the underlying soil. Di Menna (1959) has suggested that the yeasts inhabiting the phyllosphere may be species especially vulnerable to microbial antagonisms and therefore unable to grow in soil wherein they would be exposed to the antibiotic activities of a much more varied microflora. Perhaps the simplest explanation for the specificity of the phyllosphere microflora is that sugars and organic acids are prominent components of leaf exudates and therefore yeasts and other fast-growing, sugar-utilizing microorganisms are preferentially encouraged.

Several early investigators of the phyllosphere reported that aerogenic or coliform bacteria and lactobacilli were heavily present on grass leaves. Allen *et al.* (1937) stated that fresh grass contains millions of lactobacilli along with predominance of coliform bacteria. Stone *et al.* (1943) believed that all normal green plants utilized for silage contain a plentiful supply of desirable lactic acid bacteria. Later investigators, however, have found the coliforms and lactobacilli to constitute a relatively minor portion of the phyllosphere microflora. Kroulik *et al.* (1955) observed relatively few lactobacilli on fresh grass leaves, and none were typical of the lactobacilli present in silage. Keddie (1959) found that freshly cut grass rarely showed more than a thin seeding of lactobacilli. *Lactobacillus plantarum,* the species usually dominant in silage, was never encountered on fresh grass. *L. fermenti* was not found to occur in silage but did occur on grass. Coliform bacteria, particularly *Aerobacter* spp., are usually present on green plants but according to some workers, constitute only a minor portion of the total microflora thereon (Taylor, 1942; Prescott *et al.,* 1946; Clark *et al.,* 1947; Graham and Hodgkiss, 1967). Others ascribe to them a much greater dominance, with counts as high as $10^7$ per gram fresh grass (Thomas and McQuillin, 1952).

Information on the qualitative composition of the phyllosphere microflora beyond that given above is scanty. Bacteria variously reported to inhabit leaf surfaces may in some instances be true colonizers and in others represent nothing more than dust-born contaminants. They may also represent epiphytic growth of plant pathogens. *Phytomonas morsprunorum* is known to be capable of multiplying on the leaves of cherry trees (Crosse, 1959, 1963) and *Xanthomonas vesicatoria,* on the leaves of tomatoes (Leben, 1963), prior to actual invasion of the plant tissues. Other microorganisms variously encountered in the phyllosphere include the following: aerobic sporeformers, clostridia, micrococci, streptococci, *Beijerinckia* spp., *Azotobacter* spp., spirilla, actinomycetes, fungi, lichens, and protozoa (Wolff, 1913; Allen *et al.,* 1937; Ruinen, 1956, 1961).

Table III summarizes some observations on the nature and quantity of phyllosphere exudates. Unfortunately, the available information is largely limited to nongraminous plants and the most meaningful is that

TABLE III
The Nature and Quantity of Phyllosphere Exudates

| Investigator | Plant species studied | Observations reported |
|---|---|---|
| Greenhill and Chubnall (1934) | *Lolium perenne* | Glutamine is guttated |
| Schweizer (1941) | *Hevea brasiliensis*, *Coffea* sp. | Sugar content in dew on leaves measured as 115 to 244 mg/l |
| Dalbro (1956) | *Malus sylvestris* | Precipitation washes down 100 g carbohydrates/$m^2$/yr |
| Schnitzer and DeLong (1955) | *Populus grandifolia* | Experimentally prepared leaf leachates contain acidic polysaccharides |
| Long *et al.* (1956) | *Phaseolus vulgaris* | Leaf leachates contain carbohydrates, principally galactan |
| Tukey *et al.* (1957) | *Phaseolus vulgaris* | 7.5 mg carbohydrate/24 hr was obtained by continued artificial leaching, equivalent to 4.8% of the leaf weight |
| Carlisle *et al.* (1966) | *Quercus petraea* | Precipitation washes down 130 g/$m^2$/yr of dissolved organic matter; 90 g of this total was carbohydrate, mainly glucose, fructose, melezitose |
| Ruinen (1966) | *Aloe* sp., *Sanseviera* sp. | Of fatty acids excreted by leaves, 60% by weight is acetic acid; 5–15.5% palmitic; 7–29% oleic; smaller amounts of myristic, stearic, linoleic, linolenic acids |
| Malcolm and McCracken (1968) | *Quercus falcata*, *Q. virginiana*, *Pinus palustris* | Precipitation washes down 2 g organic matter/$m^2$/yr; 1 g or more is organic acids; 400 mg, reducing sugars; and 100 mg, polyphenols |

concerning the amount of organic matter in the throughfall of tree canopies. Even this information is quite inconsistent—the three values tabulated range from 2 to 130 g/$m^2$/yr. The organic matter measured in the throughfall almost certainly does not represent the total exuded or lost from leaves. It represents only that which is not metabolized by microbes in the phyllosphere. Data on the amount so metabolized are not available; however, a rough calculation is possible. If there are $10^7$ microorganisms per square centimeter of leaf surface (Ruinen, 1961) in vegetation whose

leaf area index equals 2, if $10^{12}$ bacteria weigh 0.2 g, dry weight basis, and if bacteria assimilate into cell substance one-tenth of the amount of organic substrate metabolized (Alexander, 1961), then the standing microbial crop of 0.04 g biomass/$m^2$ represents 0.4 g of substrate metabolized. If one assumes that 10 standing microbial crops are produced annually and ignores cryptic growth, then the amount of phyllosphere substrate metabolized is 40 g/$m^2$/yr. This is less than the 130 g/$m^2$/yr reported in throughfall by Dalbro (1956) but much more than the 2 g/$m^2$/yr reported by Malcolm and McCracken (1968). An estimate of 40 g/$m^2$/yr as the amount metabolized by leaf-associated microorganisms is undoubtedly too high for grass communities and certainly too high for the Pawnee National Grassland in Colorado. At that site, the phyllosphere population has been measured as $10^5$ bacteria/$cm^2$ of leaf surface and the leaf area index has been estimated as 0.5. Substitution of these values in the above calculation indicates that only a negligible amount (0.01 g/$m^2$/yr) of organic matter is metabolized by microbes in the phyllosphere. Further studies are needed concerning the magnitude of the energy flow from the plant to decomposers in the phyllosphere.

## B. Rhizosphere

Following discovery of symbiotic nitrogen-fixing bacteria in leguminous root nodules (Hellreigel and Wilfarth, 1888) and recognition of the economic importance of this symbiosis, interest in the microbiology of plant roots became widespread. It was soon realized that in addition to rhizobia forming symbioses with legumes, a great many heterotrophic bacteria were associated with plant roots. There are numerous reviews dealing with the quantitative and qualitative composition of the rhizosphere microflora (Katznelson *et al.*, 1948; Clark, 1949; Starkey, 1958; Katznelson, 1965; Rovira and McDougall, 1967; Parkinson, 1967; Gams, 1967). Inasmuch as several of these are current and give adequate coverage of the literature—that by Gams (1967), for example, contains 481 citations—it appears unnecessary to duplicate their coverage here. It will suffice simply to point out that while most rhizosphere studies have been concerned with plants commonly grown as cultivated or intertilled crops, such studies as have been conducted on grasses show that this group of plants does not possess any greatly dissimilar or unique root-associated microflora. The total number and the kinds of bacteria determined for several grasses *(Poa pratensis, Phleum pratense, Anthoxanthum odoratum, Deschampsia flexuosa)* by Gyllenberg (1955) are not strikingly different from determinations by other workers for a variety of nongraminous plants.

Gams (1967) has compiled information provided by a number of workers concerning identity of the organic material exuded from roots of wheat. Table IV was contructed from his review and lists the organic

TABLE IV
Organic Acids, Sugars, and Amino Acids Reported to Occur in Root Exudates of Wheat[a]

| *Organic acids* | *Sugars* |
|---|---|
| Acetic acid | Arabinose |
| Propionic acid | Fructose |
| Butyric acid | Galactose |
| Valerianic acid | Glucose |
| Maleic acid | Mannose |
| Oxalic acid | Maltose |
| Lactic acid | Raffinose |
| Tartaric acid | Ribose |
| Succinic acid | Sucrose |
| Fumaric acid | Xylose |
| Glycolic acid | |
| Citric acid | |
| *Amino acids* | |
| Leucine | Lysine |
| Isoleucine | Tyrosine |
| Valine | Threonine |
| Glutamine | Phenylalanine |
| Serine | Proline |
| Cysteine | Methionine |
| Glycine | Arginine |
| Asparagin | Histidine |
| Aspartic acid | Cystathionine |
| Glutamic acid | Cysteic acid |
| $\alpha$-Alanine | Hydroxypipecolinic acid |
| $\beta$-Alanine | $\alpha$-Aminobutyric acid |
| | $\gamma$-Aminobutyric acid |

[a]Compiled after Gams (1967).

acids, sugars, and amino acids that are encountered in root exudates of seedling wheat plants grown aseptically. Other compounds known to be exuded but not listed in Table IV include a diverse array of microbial growth stimulators and inhibitors, mineral salts, enzymes, nucleotides, and aldehydes. Essentially the same range of compounds as given for wheat roots can be expected to occur in the root exudates of grasses.

Further discussion of the rhizosphere in this review will be limited to the relatively few papers that have been concerned with the quantity of organic material coming from the root and serving as the energy source for the root-associated microflora. Such material is commonly divided into two categories, the sloughed root debris and the exuded or diffused soluble organic material. As roots grow through the soil, the root-cap cells slough off. Root hairs and cortical cells, as well as small rootlets in the course of the self-pruning activity of the root system, variously become senescent and also slough off. This sloughed material might well be considered as root litter, together with the major diebacks or self-prunings of root systems. Rhizosphere microbiologists, as yet unable to differentiate microbial responses due to sloughed root hairs or cells and those due to solubles exuded from the root, commonly consider the cellular debris from roots as partly responsible for the rhizosphere phenomenon.

The energy contribution represented in sloughed cellular detritus is not easily estimated. Measurements made on young plants growing in solution or container cultures are very probably underestimates because of difficulty in recovering microscopically small particulate materials, such as root hairs. Also involved are the probabilities that there is less root abrasion in such cultures than in field soil and a much greater production of cellular debris by roots as they become older.

Rovira (1956) reported that 31.5 mg of cell debris was sloughed by the roots of 50 pea plants grown in sterile sand culture for 21 days, and 14.8 mg by the roots of 50 oat plants. Such loss is of the order of 0.02 mg per plant per day. If one multiplies by a factor of 10 to correct for the more extensive root system and the more active sloughing that can be expected for older plants, by a factor of 100 for days in the growing season, and by 200 for the number of plants/$m^2$, the resultant extrapolation amounts to 4 g/$m^2$ during the growing season.

Measurements on the amount of soluble exudates show that these exceed the particulate debris. Harmsen and Jager (1963) found that for vetch grown in a synthetic soil, exuded carbon ranged from 1.6 to 2.9% of the carbon in the roots at harvest. Lyon and Wilson (1921) found that for maize grown 49 days in sterile nutrient solution, organic matter in the solution was 1.18% of that in the harvested plants. Rovira and McDougall (1967) have pointed out that measurements of exudates in sterile root cultures may be too low, inasmuch as root exudate patterns may be changed by microorganisms in several ways. These include altering the permeability of root cells, modifying the root metabolism, and microbial assimilation of substances exuded by roots. Factors other than micro-

organisms are known to influence the nature and quantity of root exudates. High light intensities and temperatures and temporary moisture stresses favor exudation (Rovira, 1959; Burstrom, 1965).

An interesting and possibly a more reliable approach than that of using axenic cultures for measuring loss of organic matter from roots is that of using radioactive carbon, as done recently by Shamoot *et al.* (1968). Their method involved the growth of plants within closed chambers containing a $^{14}C$-enriched atmosphere. Following harvest of the plants, including meticulous removal of all obvious root material, determinations were made for total and tagged organic carbon remaining in the soil, and the quantity of root-derived organic matter in the soil was calculated. Table V summarizes part of their data. Their values for plant-derived organic matter are sharply higher than those just cited for axenic cultures showing exfoliation and exudation values of the order of 1 or 2%.

TABLE V
Rhizo-Deposition of Organic Debris during Plant Growth[a]

| Plant | Top growth g/pot | Root growth g/pot | Plant-derived organic debris | | |
|---|---|---|---|---|---|
| | | | g/pot | g/100 g of tops | g/100 g of roots |
| Bermudagrass | 22.5 | 12.2 | 1.83 | 4.7 | 8.6 |
| Sudangrass | 19.0 | 5.7 | 2.36 | 8.3 | 27.7 |
| Fescue | 4.9 | 3.7 | 1.74 | 19.6 | 25.9 |
| Alfalfa | 21.8 | 5.0 | 2.31 | 7.0 | 30.6 |
| Ladino clover | 21.5 | 4.4 | 2.21 | 6.7 | 32.5 |
| Sweet clover | 5.8 | 1.9 | 1.51 | 12.6 | 38.4 |
| Lespedeza | 4.7 | 2.3 | 1.75 | 20.6 | 42.2 |
| Fallow | – | – | 0.78[b] | – | – |

[a] Compiled after Shamoot *et al.* (1968).
[b] Algal growth occurred in the fallow soil.

In contrast, Table V shows root-derived organic matter to average 11.4% of the weight of the top growths obtained. Inasmuch as the fallow soil showed appreciable algal growth and $^{14}CO_2$ fixation, possibly the measured rhizo-deposition should be adjusted downward to some extent to correct for possible algal growth in the cropped soils. However, no mention was made by Shamoot *et al.* of such growth therein. More likely, the differing values obtained by tracer and axenic techniques reflect the extent to which the tracer technique more fully measures the microscopic debris not recovered by manual techniques as well as measuring that

portion of root-derived organic matter which in transformed into microbial tissues or metabolites during the time that the plants are being grown.

McDougall (1968) employed tracer methodology to determine the time required for $^{14}C$ fixed by photosynthesis to be translocated to and exuded from wheat roots. Seedlings were grown for 5 or 6 days under aseptic conditions and then given pulse exposure to $^{14}CO_2$, after which photosynthesis was allowed to continue in ordinary air. The time required for transfer of $^{14}C$ assimilates to and down the root is shown in Table VI. Exudation of $^{14}C$ into the liquid substrate was detected within

TABLE VI
Distribution of Tagged Assimilates In Root System after Pulse Exposure to $^{14}CO_2$[a]

| Time after exposure (min) | Radioactivity (counts/100 sec) in root segments at differing distances down root from base: | | | |
|---|---|---|---|---|
| | 0–1 cm | 1–3 cm | 3–5 cm | 5–7 cm |
| 45 | 0 | 0 | 0 | 0 |
| 60 | 5320 | 5070 | 0 | 0 |
| 75 | 15,200 | 4150 | 0 | 0 |
| 90 | 8680 | 18.800 | 12,630 | 3540 |

[a] McDougall (1968).

3–4 hours after $^{14}C$ was first supplied to the tops. The amount of radioactivity in exudates collected for 12 hours after pulse exposure represented about 1% of that in the ethanol-soluble fraction extracted from the same roots at the end of this period. McDougall also observed that radioactive substances exuded mainly from the basal regions of the root whereas $^{14}C$ within the root accumulated primarily in the apical sections. Her observation on apical accumulation of assimilates is in agreement with an earlier, nonquantitative study by Williams (1964).

### III. The Microflora of Grassland Litter

In the following discussion litter will be considered as all nonliving plant material morphologically recognizable as of plant origin. In the published literature, aboveground litter is often divided into standing dead vegetation and surface litter, and the latter may be further subdivided into loose litter and compressed litter. These last-named terms are synonymous to fresh mulch and humic mulch as used by some writers. In this review, the term humus will be reserved for the polydisperse

organic material derived or synthesized from litter by the activity of microorganisms. Such material will be discussed separately in a following section. In this section, attention will be given to the quantity of litter present in grassland, to the microorganisms occurring on litter, and to the rate of litter decomposition.

### A. Quantitative Considerations

In most natural vegetation, the amount of organic matter in the system remains approximately constant from year to year. A rough estimate of the amount of material being decomposed annually can be obtained by estimating the annual litter fall and the annual death of plant roots (Burges, 1967). For grassland, these in turn can be equated roughly to the living plant biomass in the standing crop at the end of the growing season. Such statements are of course only approximations. As Wiegert and Evans (1964) have pointed out, the peak standing crop can equal net primary production only if all vegetation stops growing at a single instant in time, with all mortality of plants or plant parts in the post-growth period.

Although total yearly decomposition approximates annual productivity, this does not imply that each standing crop is fully decomposed at the end of the following year. The major portion of decomposition accomplished during a given year is usually at the expense of the preceding year's crop, and successively smaller fractions, at the expense of progressively smaller remnants of earlier crops. Likewise, the fact that the yearly transfer of living biomass to litter approximates litter decomposition does not imply that the actual quantity of litter in the system bears any constant ratio to the quantity of living biomass. Although in grassland the quantity of litter at times exceeds that present as living biomass by a factor of two or more, the actual ratio varies greatly between and within sites. Table VII illustrates seasonal variations observed by Kelly and Opstrup (1968) in Andropogon and Festuca communities.

Published values on the quantity of aboveground litter observed on grasslands in the central and northern Great Plains in the United States generally fall within the range of 100–1000 g/m$^2$. Values for ungrazed sites largely reflect the season and annual productivity patterns at the individual sites. Increasing intensity of grazing usually decreases the amount of both standing dead and surface litter. Rhoades *et al.* (1964) measured litter on nongrazed and on lightly, moderately, and heavily grazed sandy range sites as 952, 498, 439, and 274 g/m$^2$, respectively. Others (Hopkins, 1954; Lewis *et al.*, 1956; Rauzi and Hanson, 1966) variously have noted litter reductions due to grazing as from 10% to more than 90%. In addition to grazing intensity, other site factors influence the

TABLE VII
Seasonal Values for Live Plant, Standing Dead, and Surface Litter Biomass in Andropogon and Festuca Communities[a]

| Time of sampling | Live plant biomass ($g/m^2$) | Standing dead ($g/m^2$) | Surface litter ($g/m^2$) |
|---|---|---|---|
| Andropogon community | | | |
| Early April | 13 | 824 | 218 |
| Early June | 96 | 591 | 161 |
| Early August | 300 | 519 | 162 |
| Mid-September | 373 | 527 | 198 |
| Late October | 313 | 633 | 184 |
| Late December | 20 | 806 | 163 |
| Festuca community | | | |
| Mid-January | 75 | 300 | 105 |
| Mid-March | 52 | 245 | 107 |
| Mid-April | 122 | 354 | 119 |
| Mid-May | 194 | 408 | 121 |
| Mid-June | 199 | 355 | 108 |
| Late July | 257 | 333 | 117 |
| Late September | 302 | 356 | 100 |
| Late November | 258 | 309 | 132 |

[a]Compiled from data of Kelly and Opstrup (1968).

quantity of grassland litter. Rauzi *et al.* (1968) noted less litter on heavy than on light soils. Beetle (1952) noted up to 4-fold differences in quantities of litter associated with topographical and drainage variations within a shortgrass area in Wyoming. Rauzi *et al.* (1968) reported litter quantities in three different rainfall belts to be as follows: 25–35 cm belt, 74 $g/m^2$; 36–50 cm belt, 117 $g/m^2$; and 51–70 cm belt, 260 $g/m^2$. Fire drastically reduces or eliminates surface litter at the time of burning, but the prefire level of litter is usually reestablished within a few years (Dix and Butler, 1954; Daubenmire, 1968).

The amount of root-derived or belowground litter is less easily measured than the aboveground litter. Using root productivity and turnover measurements, Dahlman and Kucera (1965) estimated 25% of the total root dry matter was turned over annually and hence the equivalence of the total root weight would be turned over every four years. Accordingly, root biomass measurements divided by four can be used as estimates of the amount of root-derived material available for decomposition annually. The amounts of root biomass encountered on some grassland sites in the central-western United States are shown in Table VIII. Profile data pre-

sented in the same table show that four-fifths of the total root biomass is found in the upper 25–30 cm of soil. Additional data of Dahlman and Kucera (1965) show that 48–60% of the roots are found in the upper 5 cm of soil.

TABLE VIII
Amount of Root Biomass on Some Grassland Sites in the Central-Western United States

| Reference | Site and grass species | Profile depth and Root biomass ($g/m^2$) | |
|---|---|---|---|
| Schuster (1964) | Colorado, foothills area; | 0– 30 cm: | 443 |
| | *Muhlenbergia montana,* | 30– 60 cm: | 79 |
| | *Festuca arizonica,* | 60– 91 cm: | 17 |
| | *Bouteloua gracilis* | 91–183 cm: | 9 |
| | | 0–183 cm: | 548 |
| Wiegert and Evans (1967) | Michigan, swale area; *Poa pratensis* | 0– 90 cm: | 1018 |
| | Michigan, upland area; *Poa compressa, Aristida purpurascens* | 0– 90 cm: | 685 |
| Dahlman and Kucera (1965) | Missouri, humid prairie; | 0– 25 cm: | 1575 |
| | *Andropogon gerardi,* | 25– 56 cm: | 214 |
| | *Andropogon scoparius,* | 56– 86 cm: | 112 |
| | *Sorghastrum nutans* | 0– 86 cm: | 1901 |

## B. The Microflora of Aboveground Litter

Microbial invasion of aging and senescent plant tissues starts before the death of the plant parts themselves. Of the primary fungal invaders, some species appear capable of growth on widely dissimilar plant species, while others are almost litter and site specific. *Cladosporium herbarum* is representative of the more ubiquitous invaders. It has been encountered on *Dactylis glomerata* and *Agropyron repens* by Webster (1956, 1957), on *Saccharum officinarum* by Hudson (1962), on *Eucalyptus regnans* by Macauley and Thrower (1966), on *Fagus sylvaticus* by Hogg and Hudson, (1966), on *Pinus sylvestris* by Kendrick and Burges (1962), on *Andropogon sorghum* and *Triticum vulgare* by Lal and Yadav (1964), on *Musa sapientum* by Meredith (1962), and on *Carex paniculata* by Pugh (1958). These workers have described the course of the fungal successions in the senescent and dead vegetation with which they were concerned.

Inasmuch as the fungal successions of different litters are sufficiently individualistic that they do not lend themselves to a generalized discussion, the work of Webster (1956, 1957) on the fungal succession in stems of *Dactylis glomerata* for the two-year period following flowering has been chosen for summary presentation here. Webster subdivided the fungal species encountered into five groups. Group 1 fungi *(Cladosporium herbarum, Alternaria tenuis, Epicoccum nigrum, Pleospora vagans,* and *Leptosphaeria microscopica)* were the first to appear on stems and culms as they became moribund, and there was fungal progression of this group from the basal leaves in the early summer to successively higher leaves as they senesced. By late summer, group 2 (*Acrothecium* sp.) was present on the lower internodes. In the following spring and early summer, group 3 fungi *(Leptosphaeria nigrans, Mollisia palustris)* replaced the primary invaders on the basal internodes, and group 5 *(Mycosphaerella recucita* and *Selenophoma donacis)* on the upper internodes. Later in the summer season, group 4 *(Tetraploa aristata, Helminthosporium hyalospermum, Menispora ciliata, Microthyrium culmigenum)* joined group 3 on the basal internodes, after which groups 3 and 4 spread to the upper internodes to replace group 5 as the stems collapsed during the second winter. Subsequently, Hudson and Webster (1958) reported that differing water contents in the upper and lower internodes primarily determined which fungi were able to grow at the two internode levels. Only fungi capable of resisting sharply fluctuating water contents normally grew on the upper internodes, but if the stems were laid on the ground, the basal internode colonizers rapidly spread to the upper internodes. Hudson and Webster (1958) and Webster and Dix (1960) also showed that anatomical and nutritional differences in the litter of *Agropyron repens* and *Dactylis glomerata* were influential in determining which species of fungi became successful colonizers. Consequently, certain fungi were to be found on one of the two grasses but not on the other. Different plant parts on a single species, such as leaf blades, petioles, bud scales and floral parts, may also favor different sequences of microorganisms (Burges, 1968).

The initial bacterial invaders of senescent tissues are usually the superficial forms already present in the phyllosphere. With the death of plant tissue and development of the typically low humidity in plant parts becoming cured into standing dead vegetation, bacterial activity in such litter usually becomes negligible and the fauna and fungi, rather than bacteria, function as the principal agents of decay. At such time as standing or loose litter becomes compressed into surface litter, either because of animal trampling or meterological events or because of comminution

by the soil fauna, typically there occurs a sharp increase both in the moisture content and in the number of bacteria associated with the litter. Precipitation can cause the same effects in standing litter.

The bacterial population of moist litter exceeds that of the phyllosphere. Whereas phyllosphere counts of bacteria are of the order of $10^5$ to $10^7$ per $cm^2$ of leaf surface (Table III), the number of bacteria in litter is commonly of the order of $10^7$ to $10^9$ (Stout, 1960; Minderman and Daniels, 1967). When grass is mown for hay, the bacterial populations of the leaves rapidly increase by as much as 10-fold and at the same time there is a marked qualitative change in the bacterial flora (Kroulik *et al.*, 1955). There is limited information concerning the succession of bacterial species in litter. Stout (1960) has noted that the bacteria in grass litter are predominantly species of Flavobacterium and Micrococcus. As with the fungi, it can be expected that the individual species of bacteria will vary according to the origin and nature of the litter and the microclimate under which it is exposed in the field. Numerous workers have observed the successional and interlocking roles of the soil microflora and the soil fauna in litter decomposition (Doeksen and van der Drift, 1963; Graff and Satchell, 1967). Some initial attack by fungi and bacteria apparently is necessary before litter becomes palatable to the majority of the soil invertebrates. The fauna in turn, by comminution or fragmentation and by gut passage of the litter, greatly accelerates further microflora activity. The interaction of the microflora and fauna in the decomposition of forest litter is nicely shown in recent work by Will (1968).

### C. The Microflora of Belowground Litter

The microflora of belowground, root-derived litter can be construed, on the one hand, to include that associated with the cellular debris and exudates of living roots and on the other to include the microflora of the particulate or "fines" fraction of soil organic matter and more commonly studied in association with the soil humic component. A further complication in discussing the microflora of belowground litter is that at one moment a plant fragment may be on the surface of the soil and in the next moment, beneath the surface, because of earthworm or other transport activity. As in the discussion of aboveground litter, again it appears preferable to cite individual studies rather than to undertake a generalized discussion.

Waid (1957) studied the fungal succession in aging ryegrass roots as encountered in white, light-brown, and dark-brown roots collected in the field. *Trichoderma viride, Gliocladium roseum,* and *Cladosporium herbarum* were inhabitants of root surfaces, and as decay progressed were

initial invaders of the outer cortex. These species, however, were uniformly low on a percentage of occurrence basis and appeared unable to invade the inner cortex to any significant extent. Fungi with sterile hyaline hyphae initiated the breakdown of the inner cortex and were the dominant fungi in the interior of decaying roots. Fusarium was abundant on healthy roots and in the course of senescence and root decay was the genus most frequently encountered in the outer tissues of decaying roots. The occurrence of Fusarium as a rhizosphere inhabitant and as a primary decomposer is in agreement with work of Samuel and Greaney (1937) and Sadasivan (1939), who found that numbers of *F. culmorum* increased on the roots and stubble of wheat after harvest. Kreutzer (1969) has recently reported that *Fusarium* spp. constitute the dominant fungal biomass on the roots of grasses. Species of Agropyron, Bromus, and Poa invariably yielded *F. solani, F. roseum,* and *F. oxysporum.* These several studies show not only that Fusarium is a dominant colonizer of roots, but also that the fungal successions in aboveground and belowground litter are dissimilar. Detailed studies are lacking on the bacterial successions in root-derived litter. Iswaran and Harris (1968) buried cereal straws in potted soil and noted an early flush of bacterial growth. Individual species encountered were *Enterobacter cloacae, Erwinia herbicola, Flavobacterium* spp., *Alcaligenes denitrificans,* and *Bacillus megaterium.*

### D. Rate of Litter Decomposition

Litter decomposition has been measured both in the laboratory and in the field. The laboratory approach has been informative in showing the comparative rates of decomposition of different litters and of individual plant constituents, such as cellulose and lignin. It has been particularly useful in permitting one experimental variable to be altered singly in standardized experiments. Although excellent data are thus recorded, unfortunately they are largely unrealistic insofar as applicability to field conditions is concerned. The laboratory experiment usually provides a uniform and highly optimized environment with the litter finely fragmented and mixed into the soil. Consequently the onset of decomposition is rapid. In the field, litter may become cured into standing dead vegetation, the ambient microclimate may be quite unfavorable for decomposer organisms, and the interval of time necessary for invertebrate activity or other forces to achieve litter fragmentation may be quite variable.

Among the methods for measuring rates of litter decomposition in the field are the following: (a) gravimetry; (b) quantitative chemistry (including radiochemistry) of the decaying litter or of specific end products

of decomposition; and (c) direct observation, macroscopic and microscopic. Simple gravimetry on quantity of litter per unit area shows only net change in the amount of litter. If litter accretion is excluded so that only disappearance or decomposition is measured, the resulting data do not fully reflect what might happen in the natural plant community. In order to mark specific litter in a field environment, the mesh-bag and the string-tie techniques have been developed. Advantages and disadvantages of these two methods are discussed by Witkamp and Olson (1963). Entrapment and measurement of $CO_2$ is especially suitable for measuring decomposition rates in the laboratory. Portable $O_2$ and $CO_2$ analyzers permit gaseous measurements in the field without unduly disturbing the natural litter environment. Production and use of $^{14}C$-labeled plant material is a useful technique in that it requires minimum disturbance of the field environment during the period of decomposition. Direct observational techniques are essentially qualitative rather than quantitative in nature.

Table IX shows some rates of litter decomposition in terms of the number of days required for one-half of an initially added or marked substrate to disappear. The data were chosen as a cross section of differing experimental procedures and materials, and they represent only a small part of a very extensive literature. That literature permits several generalizations. Buried litter usually decomposes more rapidly than does litter on the soil surface. Individual plant constituents such as cellulose and lignin vary in their rates of decomposition. Soil and meteorological conditions greatly affect rates of decomposition. The decomposition of natural litter is more rapid and more complete in the presence of soil animals than in their absence. For given materials, for example cellulose or wheat straw, given optimal or near optimal incubation conditions in the laboratory, comparable rates of decomposition are measured by different investigators. For a given natural litter under field conditions, differing experimental techniques, such as gravimetry *vs.* tracer chemistry or the litter bag *vs.* the string tie method, may indicate sharply differing rates of decomposition.

Natural plant litter does not show a constant rate of decay even if given a constant environment. Initially, there is rapid breakdown of sugars and cellulose. In part these materials may be resynthesized into microbial tissues or products that are much more resistant to decay than the initial constituent. Because of this resynthesis, simple compounds such as dextrose do not uniformly lose all their carbon as carbon dioxide. Nor can any uniform value be taken as the amount of the initial carbon that becomes resynthesized. Depending on the nature of the soil microflora and on the amount of preformed enzymes and microbial cells, the amount of

TABLE IX
Rates of Decomposition of Plant Constituents and Litter as Reported by Various Investigators

| Reference | Nature of experiment | Organic material studied | Days required for loss of half the added or marked material |
|---|---|---|---|
| B. A. Stewart *et al.* (1966) | $CO_2$ measurement in the laboratory | Glucose | 3 |
| | | Cellulose | 15 |
| Minderman (1968) | Approximations based on forest litter studies | Lignin | 360 |
| | | Waxes | 900 |
| | | Phenols | 2400 |
| Hayes *et al.* (1968) | $CO_2$ measurement in the laboratory | Ryegrass leaves | 140 |
| Parker (1962) | Weight loss in mesh-bag litter: | | |
| | On the soil surface | Maize stover | 140 |
| | Covered with soil | | 75 |
| Witkamp and Olson (1963) | Weight loss in: | | |
| | Mesh-bag litter | Maple leaves | 357 |
| | | Oak leaves | 360 |
| | | Pine needles | 502 |
| | String-tie litter | Maple leaves | 125 |
| | | Oak leaves | 152 |
| | | Pine needles | 250 |

substrate carbon used for cell synthesis may vary from 10% to 70%.

Minderman (1968) has shown graphically that the summation of the decay rates of the individual components of litter does not equal that actually found (Fig. 1). However, if Minderman's data on the individual components are corrected for resynthesis of secondary metabolites which break down at a slower rate than do the primary components initially involved (L. H. Sorensen, 1967, 1969), the summation curve closely ap-

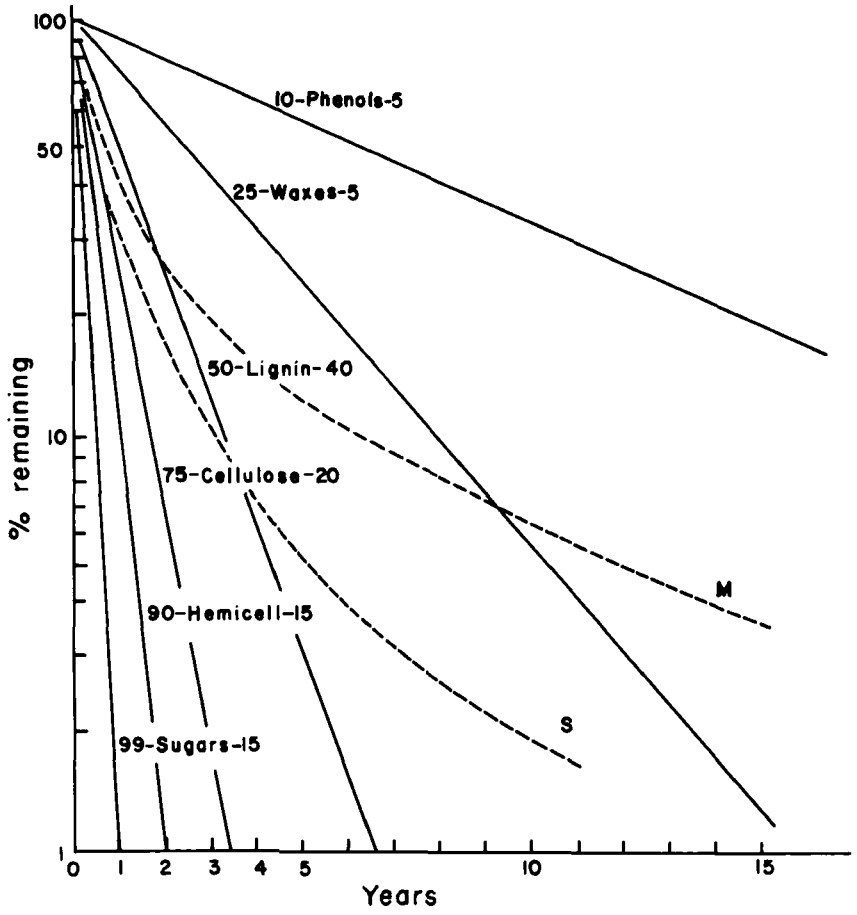


FIG. 1. Decomposition curves of constituent groups in litter, if their decomposition could be represented logarithmically by straight lines from the starting point. The number in front of the constituent indicates loss in weight after one year; the number following, the approximate percentage composition by weight in the original litter. The line *S* is a summation curve obtained by calculation from the residual values of the separate constituents. The line *M* is an approximation based on some analyses of the probable course of decomposition in a mor-type forest litter. (Redrawn from Minderman, 1968.)

proximates that actually found. The phenomenon of masking of cellulose by lignin and the accumulation of polymerized polyphenols which may have very slow decomposition rates also must be taken into account.

Paul (1970) has recently summarized much of the existing information on rate of turnover of particulate soil organic matter. He emphasized that rather slow rates of decomposition were to be found after the initial flush of microbial activity. For example, when $^{14}C$-labeled immature oat residues were added to a chernozemic soil, approximately one half the carbon was lost during the degradation of an easily decomposable fraction having a half-life in the soil of 24 days. A second fraction of moderately resistant material showed a half-life of 325 days, and a third fraction of the residues, a half-life of 802 days. Extrapolation to field conditions indicated that the two more resistant fractions combined would have a half-life of approximately ten years under the semiarid cool conditions of Saskatchewan.

Fewer investigators have been concerned with the decomposition of root-derived than of shoot-derived litter. Greater difficulty is experienced in marking specific root material and in following its decomposition without disruption of the normal soil environment. Data have been secured by excavation and marking studies on individual root systems, by excavation and removal of soil monoliths with their contained roots and study of the decomposition of those roots after killing the crown and shoot growth, and by use of $^{14}C$ methodology. Decomposition data obtained by these several techniques show quite good agreement. Using soil monoliths, Weaver (1947) concluded there was little weight loss during the first year. During the second year, root weights in big bluestem, little bluestem, and blue grama monoliths decreased 35, 53, and 54% respectively. By the end of the third year, cumulative losses were 83, 80, and 66% respectively. Using root productivity and turnover measurements, Dahlman and Kucera (1965) estimated 25% of the total root dry matter was turned over annually and hence the equivalence of the total root weight would be turned over every four years. Subsequently Dahlman (1968), using $^{14}C$-labeled root material, confirmed the occurrence of root system turnover every four years. Cumulative transfer of $^{14}C$ from the roots to the soil was observed to peak 10–14 months after the plants were tagged. At this time 45% of the $^{14}C$ lost from the roots was recovered in the humus fraction of the soil.

## IV. The Microflora of Grassland Soils

Microbiologists have long recognized that individual soils may have sharply dissimilar microfloras, depending upon climate, soil, physical and

chemical properties, and the type and amount of plant cover. Approaches used by individual workers to study soil microfloras have varied widely. Collectively, soil mycologists have placed a major emphasis on the preparation of floristic lists of the fungi in soil, while soil bacteriologists were for many years concerned largely with the total bacterial count or with the enumeration of physiological groups, such as the cellulolytic, denitrifying, or nitrogen-fixing bacteria. It appears difficult or impossible to arrange the formidable body of data collected by plate count procedures into any comprehensive picture of the microflora in grassland soil. In the following paragraphs, discussion will be centered almost entirely on the question of whether or not there are qualitative differences in the bacterial and fungal floras of grassland and nongrassland soil.

### A. Bacteria and Actinomycetes

The bacteria, the most numerous of the free-living microorganisms in soil, vary in size from cells invisible or barely visible in the light microscope to clubbed, stalked, and branching cells and filaments many microns in length. Although some half dozen or more orders of bacteria are recognized, a great majority of the commonly occurring soil forms are placed in the orders Eubacteriales and Actinomycetales. In the soil microbiological literature, these two orders are usually referred to as the bacteria and the actinomycetes, respectively.

In extended study of the soil bacteria in New Zealand tussock grassland, Stout (1958,1960,1961) found that the majority of the bacteria encountered were species of *Pseudomonas*. The aerobic spore-forming bacilli were also a relatively common group. A portion of Stout's data is shown in Table X. Inasmuch as soil bacteriologists in other countries

TABLE X
Percentile Distribution in Different Genera of 415 Bacterial Isolates from New Zealand Soils[a]

| | Native tussock soils | Sown pasture soils | Cropped soils |
|---|---|---|---|
| *Pseudomonas* spp. | 53.1 | 48.1 | 70.3 |
| *Achromobacter* and *Flavobacterium* spp. | 15.4 | 25.9 | 12.7 |
| *Bacillus* spp. | 20.4 | 21.5 | 17.0 |
| *Aerobacter* spp. and miscellaneous spp. | 11.1 | 4.5 | 0 |

[a] Compiled from data of Stout (1960). Percentages in first vertical column based on study of 162 isolates, and in second and third columns, on 135 and 118 isolates, respectively.

had reported that the predominant bacteria in soils generally were members of the genus Arthrobacter (Jensen, 1933; Topping, 1937; Gibson, 1939; Lochhead, 1940; Clark, 1940), the possibility was raised that there might be a different microflora dominant in grassland, or at least in New Zealand tussock grassland. To investigate this possibility, Robinson and MacDonald (1964) studied the bacterial flora of such grassland. They concluded that the bacterial flora therein was essentially the same as that commonly encountered in most soils. In an independent study, Loutit and Loutit (1966) found that species of Arthrobacter, Nocardia, and Mycobacterium formed the major portion of the bacterial flora in New Zealand grassland. Their work, a part of which is summarized in Table XI, confirmed observations of Robinson and MacDonald (1964).

TABLE XI
Bacterial Types Encountered in New Zealand Grassland[a,b]

| Bacterial type | Hastings site | | Napier site | |
|---|---|---|---|---|
| | January | June | January | June |
| Gram-negative bacteria | 19.5 | 22.5 | 18.5 | 30.0 |
| Gram-positive bacteria | 2.0 | 5.5 | 3.5 | 5.0 |
| Pleomorphic types | 36.5 | 45.0 | 33.5 | 37.0 |
| Spore-forming bacilli | 17.5 | 13.5 | 12.5 | 13.5 |
| Actinomycetes | 23.0 | 13.0 | 31.0 | 13.0 |
| Undetermined types | 1.5 | 0.5 | 1.0 | 0.5 |

[a] Compiled from data of Loutit and Loutit (1966).

[b] Values represent the frequency distribution (%); the distribution within each vertical column is based on study of 400 random isolates picked from the plating media employed.

Taylor (1938) surveyed 90 soils selected from widely separated parts of Canada for the occurrence of *Arthrobacter globiformis*. In prairie, woodland, garden, orchard, and mixed crop soils not strongly acid, *A. globiformis* was invariably present in large numbers. The pasture and grassland soils surveyed did not group themselves apart from the other soils in percentages of *A. globiformis* colonies found in total plate counts. Vandecaveye and Katznelson (1938) compared the microflora of forested and grassland soils developed from the same parent material under similar climatic conditions in the northwestern United States. There appeared to be no distinct association between kinds of bacteria and type of vegetation. Aerobic cellulose-destroying bacteria and anaerobic nitrogen-fixing bacteria were sparsely encountered in both soils. Ross (1958,1960), studying the nonsymbiotic nitrogen-fixing bacteria in tussock grassland,

found numbers of Clostridium comparable to those reported for other temperate soils. Similar findings for other groups of bacteria are common in the literature. However, there is evidence that nitrifying bacteria are less abundant in grassland than in nongrassland soils. Discussion of this evidence will be deferred until a later section dealing with nitrogen transformations. In brief, and with possible exception for the nitrifying bacteria, the existing literature on the bacterial flora of grassland does not show it to be greatly different from that in soils generally.

It has frequently been emphasized that the conditions often existing in grassland, namely, low soil moisture content, warm temperature, a neutral or alkaline reaction, and a good supply of organic matter, such as that provided by grass roots, preferentially stimulate the growth of the actinomycetes over that of the bacteria (Alexander, 1961; Kuster, 1967; Kutzner, 1956; Mishustin, 1956) and that consequently the actinomycetes constitute a greater proportion of the total count in grassland than in nongrassland soil. Orpurt and Curtis (1957) and Vernon (1958), working in Wisconsin prairie and New Zealand tussock sites, respectively, observed that actinomycetes accounted for half the total plate count. In contrast, there are also numerous reports that grassland soils contain no higher percentages of actinomycetes than do adjacent cultivated or forested soils (Vandecaveye and Katznelson, 1938; Timonin, 1935; Sandon, 1928). Data of Robinson and MacDonald (1964) given in Table XII show the percentage incidence of actinomycetes to be lower in undisturbed grassland than in nearby cultivated soil. In view of the conflicting literature, it does not yet appear possible to state whether actinomycetes are proportionately more or less numerous in grassland than in nongrassland soil.

### B. Fungi

Knowledge about fungi in grassland soils is limited and parallels that for fungi in soil generally. Warcup (1967) has stated that although long floristic lists of soil fungi have been compiled, one is still unable to give an adequate picture of the fungal flora in a soil. While the importance of specific substrates for fungal growth has become recognized, there remain major problems in mycological studies, such as determining what organisms are present on a substrate in soil, differentiating between dormant and active portions of a fungus or of different fungi, and with measuring, in some sense, the activity of fungi in natural substrates.

During the early years of the present century, descriptive studies of fungi took precedence over eclogical investigations concerning their distribution in soil. The early literature on soil fungi has been adequately

TABLE XII
Actinomycete Populations Encountered in New Zealand Soil as Shown by Three Different Plating Media[a]

| Plating agar employed | Sampling site | Total count (millions/g) | Actinomycetes (millions/g) | Actinomycetes/total count (%) |
|---|---|---|---|---|
| Nutrient agar | Native grassland | 9.9[b] | 4.8 | 48.5 |
| | Cultivated (fallow) | 8.9 | 5.0 | 56.2 |
| | Cultivated and limed | 14.4 | 7.7 | 53.5 |
| Fortified soil extract agar | Native grassland | 16.5 | 4.3 | 26.1 |
| | Cultivated (fallow) | 12.8 | 5.7 | 49.5 |
| | Cultivated and limed | 19.2 | 6.6 | 34.4 |
| Soil extract agar | Native grassland | 11.1 | 3.4 | 30.6 |
| | Cultivated (fallow) | 9.6 | 4.0 | 41.7 |
| | Cultivated and limed | 17.0 | 5.7 | 33.5 |

[a]Compiled from data of Robinson and MacDonald (1964).
[b]Each value given represents mean of two sampling dates: November 3 and December 14.

reviewed by Waksman (1916). The literature was sufficiently fragmentary to cause Waksman (1917) to ask whether there was a specific fungus flora of the soil, or whether the species present therein were only occasional invaders. After study of soils from several widely separated geographical areas, he concluded there was a distinct soil fungus flora, with the species occurring in any particular soil dependent on a number of soil and climatic conditions. His conclusions were generally supported by other soil mycologists of the era (Werkenthin, 1916; Brown, 1917; Brierly, 1923; LeClerg and Smith, 1928; Jensen, 1931). Although some of these workers included an occasional meadow or pasture soil in their studies, for the most part they were concerned with nongrassland soils, and it remained for a later group of workers to ask whether there was a specific fungus flora for grassland soil.

Paine (1927) observed that *Mucor* spp. were not as abundant in grassland as in forest areas, whereas Hormodendron and Cladosporioides and *Aspergillus fumigatus* were more numerous in pasture. In a study of virgin and cultivated profiles in Manitoba, Bisby and co-workers (1933, 1935) found *Aspergillus* spp. relatively rare, and *Penicillium* spp. relatively common. Trichoderma, although present in both grassland and forested soils, was more numerous in the latter. Fusarium was especially common in prairie soils, and *F. oxysporum* was the species most frequently encountered, usually in the A horizon. They observed that *Monotospora daleae (Mycogone nigra)* was isolated frequently from grassland, but never from forested soil. As this species was also encountered in wheat fields, they concluded that it was commonly associated with the Gramineae.

In a study of five English grasslands, Warcup (1951) found that such soil factors as acidity and temperature were of overriding importance in determining species distribution. He concluded that different fungal populations occur in different grassland soils. England and Rice (1957) compared the soil fungi of a tall grass prairie with that of an adjacent abandoned field in central Oklahoma. With soil sampling conducted throughout the year, at no time did species common to both plots exceed 50%; the average was 35.2%. There were 9 species in both plots at all sampling periods; namely, *Aspergillus fumigatus, Pencillium notatum, Mucor globosus, M. sphaerosporus, Monotospora brevis, Fusarium decemcellulare, F. lateritium, F. nivale,* and *F. orthoceras.*

Orpurt and Curtis (1957) determined the soil microfungi for 25 prairie sites in Wisconsin. Whereas *Fusarium* spp. were encountered only infrequently in forested soils, they were among the most prominent and characteristic members of the prairie soil flora. Likewise, *Aspergillus*