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# 2 Nitrogen

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## 2.1 DETERMINATION OF ESSENTIALITY

Discovery of the essentiality of nitrogen is often credited to de Saussure (1–3), who in 1804 recognized that nitrogen was a vital constituent of plants, and that nitrogen was obtained mainly from the soil. De Saussure noted that plants absorb nitrates and other mineral matter from solution, but not in the proportions in which they were present in solution, and that plants absorbed substances that were not required for plant growth, even poisonous substances (2). Other scientists of the time believed that nitrogen in plant nutrition came from the air. The scientists reasoned that if it was possible for plants to obtain carbon from the air, which is a mere 0.03% carbon dioxide (by volume), then it would be easy for plants to obtain nitrogen from the air, which is almost 80% nitrogen gas. Greening was observed in plants that were exposed to low levels of ammonia in air, further suggesting that nitrogen nutrition came from the air. Liebig (1–3) wrote in the 1840s, at the time when he killed the humus theory (the concept that plants obtain carbon from humus in soil rather than from the air), that plants require water, carbon dioxide, ammonia, and ash as constituents. Liebig supported the theory that plants obtained nitrogen as ammonium from the air, and his failure to include nitrogen in his “patent manure” was a weakness of the product. Plants will absorb ammonia at low concentrations from the air, but most air contains unsubstantial amounts of ammonia relative to that which is needed for plant nutrition.

The concept that nitrogen was acquired from the air or from soil organic matter was dismissed in the mid-1800s, as it was shown that crop yields rose as a result of fertilization of soil. Using laboratory methods of de Saussure, Boussingault (1), in field research of 1838, developed balances of carbon, dry matter, and mineral matter in crops. Boussingault established a special position for legumes in nitrogen nutrition, a position that Liebig did not support (1). Other research also showed that different nitrogen fertilizers varied in their effectiveness for supporting crop production, with potassium nitrate often being a better fertilizer than ammonium salts (1). Microbial transformations of nitrogen in the soil made it doubtful as to which source was actually the best and which form of nitrogen entered into plants. Studies made with sterile media and in water culture demonstrated that plants may utilize nitrate or ammonium and that one or the other might be superior depending on the species and other conditions. At the time when much of this research was performed, organic fertilizers (farm manures) and gas-water (ammonia derived from coal gases) were the only ones that were cost-effective, considering the value of farm crops and the cost of the fertilizers. With the development of the Haber process in 1909 for the synthesis of ammonia from hydrogen and nitrogen gases, ammonia could be made cheaply, leading to the development of the nitrogen fertilizer industry.

The recognition of the importance of nitrogen in plants predates much of the relatively modern-day research of de Saussure and others. It was written as early as the 1660s and 1670s (1,3) that plants benefitted from nitre or saltpeter (potassium nitrate), that plants accumulated nitre, and that the fertility of the land with respect to nitre affected the quality of crops for storage and yields of sugar.

## 2.2 NITROGEN METABOLISM AND NITROGENOUS CONSTITUENTS IN PLANTS

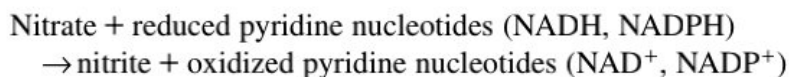
Nitrogen has a wide range of valence states in compounds, which may be used in plant metabolism. Although some compounds have oxidation–reduction states of +7, as in pernitric acid, plant

metabolites have oxidation–reduction states ranging from +5 (nitric acid, nitrate) to –3 (ammonia, ammonium) (4). Organic, nitrogen-containing compounds are at the oxidation–reduction state of nitrogen in ammonium (–3). Biologically important organic molecules in plants include proteins, nucleic acids, purines, pyrimidines, and coenzymes (vitamins), among many other compounds.

### 2.2.1 NITRATE ASSIMILATION

Nitrate and ammonium are the major sources of nitrogen for plants. Under normal, aerated conditions in soils, nitrate is the main source of nitrogen. Nitrate is readily mobile in plants and can be stored in vacuoles, but for nitrate to be used in the synthesis of proteins and other organic compounds in plants, it must be reduced to ammonium. Nitrate reductase converts nitrate into nitrite in the nonorganelle portions of the cytoplasm (5,6). All living plant cells have the capacity to reduce nitrate to nitrite, using the energy and reductant (NADH, NADPH) of photosynthesis and respiration in green tissues and of respiration in roots and nongreen tissues (5). Nitrite reductase, which is located in the chloroplasts, reduces nitrite into ammonium, utilizing the energy and reductant of photosynthesis (reduced ferredoxin).

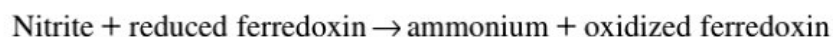
#### 2.2.1.1 Nitrate Reductase



Nitrate reduction requires molybdenum as a cofactor. A two-electron transfer takes place to reduce nitrate (N oxidation state, +5) to nitrite (N oxidation state, +3). Respiration is the likely source of reduced pyridine nucleotides in roots and also, along with photosynthesis, can be a source in shoots.

The conversion of nitrite into ammonia is mediated by nitrite reductase, which is located in the chloroplasts of green tissues and in the proplastids of roots and nongreen tissues (5,7,8).

#### 2.2.1.2 Nitrite Reductase



In leaves, nitrite reduction involves the transfer of six electrons in the transformation of nitrite to ammonium. No intermediates, such as hyponitrous acid ( $\text{H}_2\text{N}_2\text{O}_2$ ) or hydroxylamine ( $\text{HONH}_2$ ), are released, and the reduction takes place in one transfer. The large transfer of energy and reducing power required for this reaction is facilitated by the process being located in the chloroplasts (8). In roots, a ferredoxin-like protein may function, and the energy for producing the reducing potential is provided by glycolysis or respiration (9,10).

In plants, roots and shoots are capable of nitrate metabolism, and the proportion of nitrate reduced in roots or shoots depends on plant species and age, nitrogen supply, temperature, and other environmental factors (11–15).

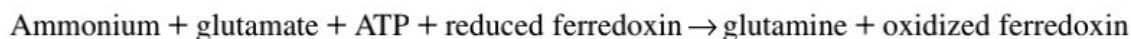
The assimilation of nitrate is an energy-consuming process, using the equivalent of 15 mol of adenosine triphosphate (ATP) for each mole of nitrate reduced (16). The assimilation of ammonia requires an additional five ATP per mole. In roots, as much as 23% of the respiratory energy may be used in nitrate assimilation compared with 14% for ammonium assimilation (17). However, nitrate can be stored in cells without toxic effects, but ammonium is toxic at even low concentrations and must be metabolized into organic combination. Consequently, ammonium metabolism for detoxification may deplete carbon reserves of plants much more than nitrate accumulation.

### 2.2.2 AMMONIUM ASSIMILATION

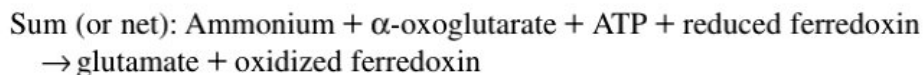
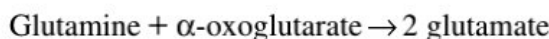
The metabolism of ammonium into amino acids and amides is the main mechanism of assimilation and detoxification of ammonium. Glutamic acid formation is a port of entry of nitrogen into organic compounds and occurs in the chloroplasts or mitochondria. Ammonium assimilation in root

mitochondria probably uses ammonium absorbed in high concentrations from nutrient solutions. One enzyme is involved in ammonium assimilation in mitochondria: glutamic acid dehydrogenase. Ammonium assimilation in chloroplasts utilizes the ammonium that is formed from the reduction of nitrite by nitrite reductase and that which is released in photorespiration. Two enzymes are involved in chloroplasts, glutamine synthetase and glutamate synthase. Glutamine synthetase forms glutamine from ammonium and glutamate (glutamic acid). Glutamate synthase forms glutamate from glutamine and  $\alpha$ -oxoglutarate ( $\alpha$ -ketoglutaric acid). These enzymes are also active in roots and nodules ( $N_2$  fixation). These enzymes assimilate most of the ammonium derived from absorption from dilute solutions, reduction of nitrate,  $N_2$  fixation, or photorespiration (18–25). Further discussions of glutamine synthetase, glutamate synthase, and glutamic acid dehydrogenase follow.

### 2.2.2.1 Glutamine Synthetase

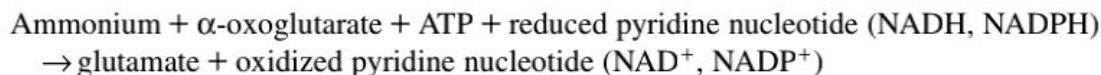


### 2.2.2.2 Glutamate Synthase



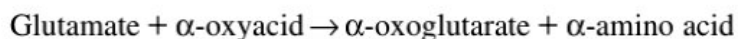
Glutamine synthetase has a high affinity for ammonium and thus can assimilate ammonium at low concentrations, such as those that occur from the reduction of nitrate. If this enzyme is inhibited, however, ammonium may accumulate to phytotoxic levels. Ammonium accumulation to toxic levels from the inhibition of glutamine synthetase is the mode of action of the herbicide glufosinate ammonium (26,27).

### 2.2.2.3 Glutamic Acid Dehydrogenase



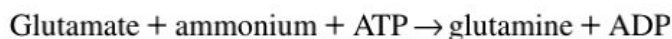
Another pathway for ammonium assimilation into organic compounds is by glutamic acid dehydrogenase, which is located in the mitochondria (28). Glutamic acid dehydrogenase has a low affinity for ammonium and becomes important in ammonium assimilation at high concentrations of ammonium and at low pH in growth media (15).

### 2.2.2.4 Transamination



Ammonium that is assimilated into glutamate from mitochondrial or chloroplastic assimilation can be transferred by aminotransferases (transaminases) to an appropriate  $\alpha$ -oxyacid ( $\alpha$ -ketoacid) to form an  $\alpha$ -amino acid. The transfer can also be to other keto-groups on carbon chains to form, for example,  $\gamma$ - or  $\delta$ -amino acids. The keto acids for the synthesis of amino acids are derived from photosynthesis, glycolysis, and the tricarboxylic acid cycle, among other processes.

### 2.2.2.5 Amidation



Amides are formed by the amidation of carboxyl groups. Amides are nitrogen-rich compounds that can store or transport nitrogen. Common amides are glutamine (5C, 2N) and asparagine

(4C, 2N). Glutamine is formed from amidation of glutamic acid (glutamate), and asparagine is formed by amidation of aspartic acid (aspartate). Often, when the external supply of ammonium is high, asparagine, a metabolite unique to plants, will dominate among the amides, as plants respond to conserve carbon in the detoxification of ammonium.

### 2.2.3 PROTEINS AND OTHER NITROGENOUS COMPOUNDS

Unlike animals, plants do not eliminate nitrogen from their bodies but reuse nitrogen from the cycling of proteins and other nitrogenous constituents. Nitrogen losses from plants occur mainly by leaching of foliage by rain or mist and by leaf drop (29). Nitrogen in plants is recycled as ammonium. In the case of hydrolysis (breakdown) of proteins, the amino acids of proteins do not accumulate, but rather nitrogen-rich storage compounds (amides, arginine, and others) accumulate as reserves of nitrogen at the oxidation–reduction level of ammonium. These compounds are formed from the catabolism of proteins. The carbon and hydrogen of proteins are released as carbon dioxide and water. These nitrogen-rich products also accumulate if accumulation of nitrogenous compounds occurs in excess of their conversion into proteins. The amino acids that enter into proteins are not mingled with the storage reserves or translocated products but are made at the same site where protein synthesis occurs. The carbon framework (carbon skeletons) remaining after the donation of nitrogen (ammonium) for amino acid synthesis for incorporation into proteins is metabolized into carbon dioxide and water. Thus, the products of protein catabolism are ammonium, carbon dioxide, and water. Protein turnover (breakdown and resynthesis) may occur in plants in a diurnal cycle, with synthesis occurring in the light and breakdown occurring in the dark, or anabolism and catabolism of proteins may proceed in different compartments of the same cell at the same time (29–31). In a 24-h period, one quarter of the protein in a healthy leaf may be newly synthesized as a result of protein turnover. Most authors indicate a protein turnover of 0.1 to 2% per hour (32,33). With *Lemma minor*, Trewavas (34,35) measured turnover rates of 7% per day. In an excised leaf, protein synthesis does not proceed after protein hydrolysis, and soluble nitrogenous compounds accumulate. In a nitrogen-deficient plant, the nitrogen will be translocated to a site of need. Also, under normal conditions, leaves will donate some of their nitrogen in leaf proteins to fruits and seeds.

Amino acids are assimilated into proteins or other polypeptides (28). Although plants contain more than 100 amino acids (1,29), only about 20 enter into proteins (Table 2.1). Hydroxyproline may be formed after incorporation of proline into proteins. Cystine is the dimer of cysteine and is formed after incorporation of cysteine into protein. Animal proteins occasionally contain amino acids other than those listed in Table 2.1.

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**TABLE 2.1**  
**Amino Acids Occurring Regularly in Plant Proteins**

Alanine	Glutamic acid	Leucine	Serine
Arginine	Glutamine	Lysine	Threonine
Asparagine	Glycine	Methionine	Tryptophan
Aspartic acid	Histidine	Phenylalanine	Tyrosine
Cysteine	Isoleucine	Proline	Valine

*Source:* From McKee, H.S., *Nitrogen Metabolism in Plants*, Oxford University Press, London, 1962, pp. 1–18 and Steward, F.C. and Durzan, D.J., in *Plant Physiology: A Treatise. Vol IVA: Metabolism: Organic Nutrition and Nitrogen Metabolism*, Academic Press, New York, 1965, pp. 379–686.

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**TABLE 2.2**  
**Approximate Fractions and Common Ranges of Concentrations of Nitrogen-Containing Compounds in Plants**

Compound	Fraction of Total Nitrogen (%)	Concentration ( $\mu\text{g/g}$ Dry Weight)
Proteins	85	10,000 to 40,000
Nucleic acids	5	1000 to 3000
Soluble organic	<5	1000 to 3000
Nitrate	<1	10 to 5000
Ammonium	<0.1	1 to 40

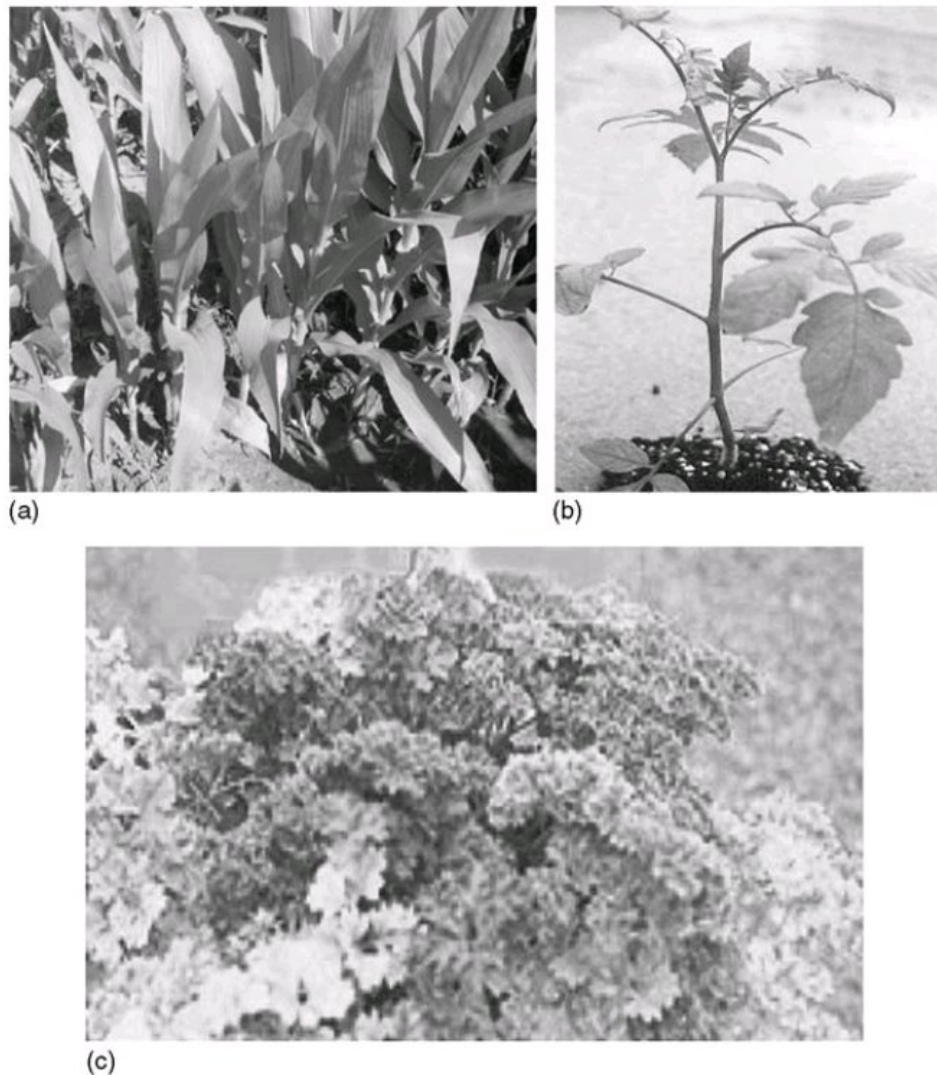
The major portion of nitrogen in plants is in proteins, which contain about 85% of the total nitrogen in plants (Table 2.2). Nucleic acids (DNA, RNA) contain about 5% of the total nitrogen, and 5 to 10% of the total nitrogen is in low-molecular-weight, water-soluble, organic compounds of various kinds (36).

Some of the low-molecular-weight, water-soluble, organic compounds are intermediates in the metabolism of nitrogen. Some have specific roles in processes other than intermediary metabolism. Amides and amino acids have roles in transport and storage of nitrogen in addition to their occurrence in proteins. Ureides (allantoin and allantoic acid) are prominent in xylem sap and transport nitrogen fixed in root nodules of legumes (15,29). Amines (ethanolamine) and polyamines (putrescine, spermine, spermidine) have been assigned roles or have putative roles in the lipid fraction of membranes, as protectants, and in processes involved in plant growth and development (15,37–43). Putrescine accumulation in plants may be a physiological response to stresses such as the form of nitrogen supplied and the nutrient status of plants (39,44–46). Simple nitrogen bases, such as choline, are related to alkaloids in plants and to lipids (29). Analogs of purines and pyrimidines have functions in growth regulation (29). Various amino acids other than those in proteins exist in plants. Often, the nonprotein amino acids are related to those occurring in proteins.  $\beta$ -Alanine, homoserine, and  $\gamma$ -aminobutyric acid are common examples of these amino acids (1,29). Accumulation of amino acids such as ornithine and citrulline is generally rare in plants, but they may be the major soluble nitrogenous constituents of some species (1). Nonprotein amino acids may be natural products or metabolites, but their functions are generally unclear.

## 2.3 DIAGNOSIS OF NITROGEN STATUS IN PLANTS

### 2.3.1 SYMPTOMS OF DEFICIENCY AND EXCESS

A shortage of nitrogen restricts the growth of all plant organs, roots, stems, leaves, flowers, and fruits (including seeds). A nitrogen-deficient plant appears stunted because of the restricted growth of the vegetative organs. Nitrogen-deficient foliage is a pale color of light green or yellow (Figure 2.1). Loss of green color is uniform across the leaf blade. If a plant has been deficient throughout its life cycle, the entire plant is pale and stunted or spindly. If the deficiency develops during the growth cycle, the nitrogen will be mobilized from the lower leaves and translocated to young leaves causing the lower leaves to become pale colored and, in the case of severe deficiency, to become brown (firing) and abscise. Until the 1940s crops received little nitrogen fertilizer (a typical application of N was 2 or 3 kg/ha), and when the light green color and firing appeared, farmers assumed that the soil was droughty (47). Sometimes under conditions of sufficiency of nitrogen, leaves, especially the lower ones, will provide nitrogen to fruits and seeds, and symptoms of deficiency may develop on the leaves. These symptoms, which develop late in the growing season, may not be evidence of yield-limiting deficiencies but are expressions of transport of nitrogen from old leaves to



**FIGURE 2.1** Photographs of nitrogen deficiency symptoms on (a) corn (*Zea mays* L.), (b) tomato (*Lycopersicon esculentum* Mill.), and (c) parsley (*Petroselinum crispum* Nym.). (Photographs by Allen V. Barker.) (For a color presentation of this figure, see the accompanying compact disc.)

other portions of the plant. For additional information on nitrogen-deficiency symptoms, readers should consult Cresswell and Weir (48–50), Weir and Cresswell (51,52) or Sprague (53).

At least 25%, more commonly more than 75%, of the nitrogen in leaves is contained in the chloroplasts (29,54). Most of the nitrogen of chloroplasts is in enzymatic proteins in the stroma and lamellae. Chlorophyll and proteins exist in lamellae as complexes referred to as chlorophyll proteins or holochromes (55–59). Nitrogen-deficient chloroplasts may be circular in profile rather than elliptical and may appear swollen. Nitrogen deficiency generally brings about a decrease in protein in chloroplasts and a degradation of chloroplast fine (lamellar) structure (60). Almost all membranous structure may be disrupted. Grana are often reduced in number or are indistinguishable. The loss of membranous structures is associated with the loss of proteins (61). A loss of chlorophyll occurs simultaneously with the loss of membranes and proteins, leading to the loss of green color from nitrogen-deficient leaves.

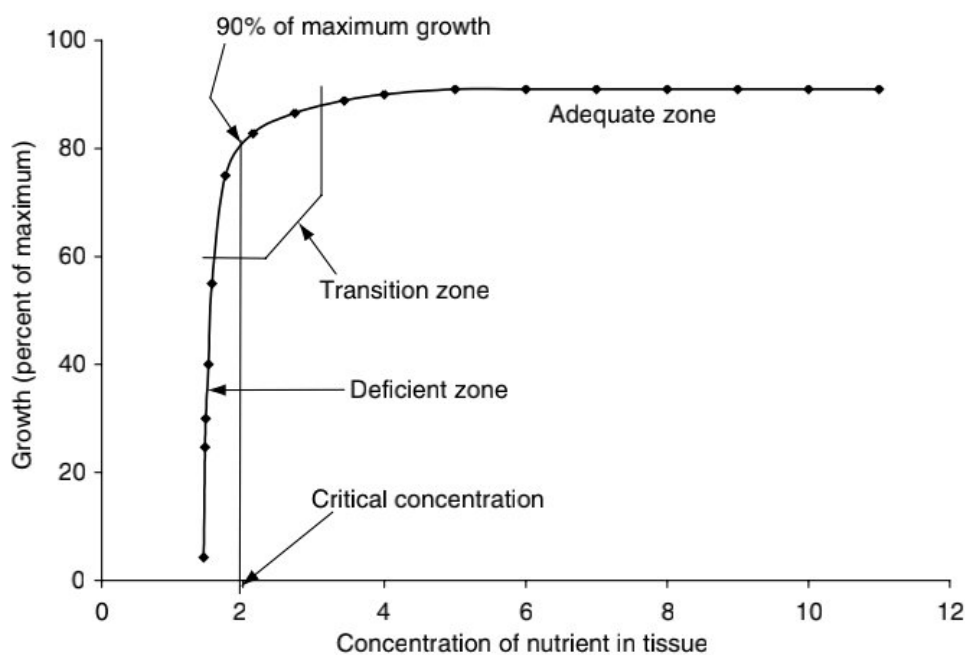
The loss of fine structure in chloroplasts during nutrient deficiency is not unique to nitrogen deficiency. Association of chloroplast aberrations with specific nutritional disorders has been difficult because of similarities in appearance of nutrient-deficient chloroplasts (62,63). The similarities are due to the effects that the deficiencies have on protein or chlorophyll synthesis (64,65). Elemental toxicities can also impart structural changes that resemble elemental deficiencies in chloroplasts (66).

### 2.3.2 CONCENTRATIONS OF NITROGEN IN PLANTS

Many attempts have been made to relate yields of crops to nutrient supply in media and to accumulation in plants. Deficiency of nitrogen or another nutrient is associated with suboptimum development of a plant, as reflected by the appearance of symptoms of deficiency, the suppression of yields, or to the response of plants after the accumulation of the deficient nutrient following its application as a fertilizer. Plant analysis (tissue testing) is used in the diagnosis of nutritional deficiency, sufficiency, or excess. Generally, the concentrations of nitrogen in plants reflect the supply of nitrogen in the root medium, and yields increase as internal concentration of nitrogen in plants increases. The use of information on internal concentrations of nitrogen in plants should not be directed toward forecasting of yields as much as it should be used in assessing how yields can be improved by fertilization.

Various models have been developed to describe the response of plants to nutrient supply and accumulation (67). Pfeiffer et al. (68) proposed a hyperbolic model in which plants approached an asymptote or maximum value as nutrient accumulation increased. Linear models have been proposed to describe growth responses to nutrient accumulation (67). Other researchers identified a three-phase model (69–71) (Figure 2.2). In this model, growth curves describe a deficient level of nutrient accumulation, region of poverty adjustment, or minimum percentage where yields rise with increasing internal concentrations of nitrogen. In the second zone of the growth curve, a transition from deficiency to sufficiency occurs followed by a region known as luxury consumption in which internal concentration of nitrogen rises but yield does not rise. The concentration of nitrogen at the transition from deficiency to sufficiency is known as the *critical concentration*. Eventually, nitrogen accumulation will rise to excessive or toxic levels.

Nitrogen concentrations in plants vary with species and with varieties within species (72,73). Nitrogen accumulation in plants also varies among families. Herbaceous crops from fertilized fields commonly have concentrations of nitrogen that exceed 3% of the dry mass of mature leaves. Leaves of grasses (Gramineae, Poaceae) (1.5 to 3.5% N) are typically lower in total nitrogen concentrations



**FIGURE 2.2** Model of plant growth response to concentration of nutrients in plant tissue. Units of concentration of nutrient in tissue are arbitrary. The model shows the critical concentration of nutrient at a response that is 90% of the maximum growth obtained by nutrient accumulation in the tissue. Deficient zone, transition zone, and adequate zone indicate concentrations at which nutrients may be lacking, marginal, or sufficient for crop yields.



**TABLE 2.3**  
**Concentrations of Total Nitrogen in Plant Parts**

Plant Part	Concentration of Total Nitrogen (% Dry Weight)	
	Range	Optimum
Leaves (blades)	1 to 6	>3
Stems	1 to 4	>2
Roots	1 to 3	>1
Fruits	1 to 6	>3
Seeds	2 to 7	>2

than those of legumes (Leguminosae, Fabaceae) (>3% N). Leaves of trees and woody ornamentals may have <1.5% N in mature leaves. Genetic differences attributable to species or families are due to many factors affecting absorption and metabolism of nitrogen and plant growth in general.

The concentrations of nitrogen in leaves, stems, and roots changes during the growing season. In the early stages of growth, concentrations will be high throughout the plant. As plants mature the concentrations of nitrogen in these organs fall, and is usually independent of the initial external supply of nitrogen. Mobilization of nitrogen from old leaves to meristems, young leaves, and fruits leads to a diminished concentration of nitrogen in old, bottom leaves of plants. Whether a plant is annual, biennial, or perennial affects considerations of yield relations and the state of nutrient accumulation in organs (leaves) during the season. If the development of a plant is restricted by low levels of external factors, such as other nutrients, water, or temperature, internal concentration of nitrogen may rise. Root structure and metabolism can lead to differential accumulation of nitrogen. Assimilation and transport of nitrogenous compounds in plants can lead to differential accumulation among species and within the plants. Nitrogen sources can have large effects on total nitrogen concentrations in plants. Plants grown on ammonium nutrition can have twice the nitrogen concentrations in vegetative parts as plants grown on nitrate nutrition.

The choice of tissue for plant analysis is important in plant diagnosis (Table 2.3). Generally, leaves are the most satisfactory plant part to use for diagnosis (69,72,74). Blades are used more frequently than leaf petioles or whole leaves. Blades are chosen as the diagnostic part if total nitrogen is to be assessed, whereas petioles may be selected if the nitrogenous component is soluble, such as nitrate. Total nitrogen quantity in tissues is the most commonly measured fraction, although some researchers believe that nitrate contents reflect the nutritional status better than total nitrogen.

### 2.3.2.1 Concentrations of Nitrogen in Plant Parts

With a nutrient supply in which all elements except nitrogen are held at a constant high level, the concentration of nitrogen in a plant will be expected to rise, along with growth and yields, with increases in nitrogen supply. Nitrogen concentrations in leaves are often not correlated with increased growth and yields. Shortages of other nutrients or stresses imposed by growth-limiting temperatures or water supply can cause concentrations of total nitrogen or nitrate to increase, along with a suppression of yield (75). The age of plant tissues is important in diagnosis of nitrogen sufficiency. In the early stages of plant growth, the concentration of nitrogen in plants will be higher than at the later stages. Increased external concentrations of nitrogen will increase the concentration of nitrogen in plant organs, but the trend is for nitrogen concentrations to fall in leaves, stems, and roots as plants mature. These changes will vary with whether the plant is annual, biennial, or perennial (67). It is important to sample plants for nitrogen determinations at a given time of the year or stage of plant development. Some researchers recommend that samples be taken at a certain time of the day, since light intensity and duration can

affect the amount of nitrate in tissues (76). Nutrient concentrations in leaves can vary by as much as 40% during a diurnal period (67). Nitrate can vary with time of day, with lower concentrations occurring in the afternoon than in the morning.

Analysis of whole shoots may be the best index of the nutritional status of plants even though each organ of a plant will vary in nitrogen concentrations. Since organs of plants vary in composition and since the proportions of organs vary with the nitrogen status of plants, a particular organ of a plant is usually chosen for analysis. Conducting tissue, such as that of stems or petioles, may provide the best index of the response of plants to nutrient applications or the best index of the nutrient status at a given time in growth. Nitrate concentrations in corn (*Zea mays* L.) stalks are usually several times higher than those of leaves (77). Measurement of nitrate in the lower stalk of corn is valuable in the diagnosis of the nitrogen status of the crop (78–80). Brouder et al. (79) noted that analysis of grain for total nitrogen was as good as the stalk test in determining sufficiency or deficiency of corn. Leaf petioles as conducting tissues are often analyzed to assess the nutritional status of vegetable crops (81). Leaves are often taken as samples for nitrogen determinations since they are the organs of active assimilation and hence likely to be the best for analysis to reflect the nutrient status of the whole plant. Leaf samples can be taken conveniently in nondestructive harvests of plants, and leaves can be identified by position or stage of development on plants. Random sampling of leaves is not as good a technique as sampling based on position on plant, size, and age. Nitrogen is a mobile element in plants; hence, it moves from lower leaves to upper leaves, and analysis of lower leaves might be a better index of deficiency than analysis of upper leaves. Sometimes, young leaves or the first-fully expanded leaves are chosen for analysis because of convenience in identifying the sample and because the lower leaves might be dead or contaminated with soil. Deficient, sufficient, and high concentrations of nitrogen in the leaves of plants are reported in Table 2.4.

**TABLE 2.4**  
**Concentrations of Nitrogen in Leaves of Various Crops Under Cultivated Conditions**

Type of Crop	Diagnostic Range (% Dry Mass of Leaves)		
	Low	Sufficiency <sup>a</sup>	High
<b>Agronomic Crops</b>			
Grass grains	<1.5	1.8 to 3.6	>3.6
Legume grains	<3.6	3.8 to 5.0	>5.0
Cotton	<3.0	3.0 to 4.5	>5.0
Tobacco		4.1 to 5.7	>5.7
Rapeseed		2.0 to 4.5	>4.5
Sugarbeet		4.3 to 5.0	>5.0
Sugarcane	<1 to 1.5	1.5 to 2.7	>2.7
<b>Bedding Plants</b>		2.8 to 5.6	
<b>Trees</b>			
Conifers	<1.0	1.0 to 2.3	>3.0
Broadleaf	<1.7	1.9 to 2.6	>3.0
<b>Cut Flowers</b>	<3.0	3.1 to 4.7	>5
<b>Ferns</b>		1.8 to 2.9	
<b>Potted Floral</b>		2.5 to 4.2	
<b>Forage Crops</b>			
Grasses	<1.5	2.0 to 3.2	>3.6
Legumes	<3.8	3.8 to 4.5	5 to 7
<b>Tree Fruits and Nuts</b>			
Nuts	<1.7	2.0 to 2.9	>3.9

**TABLE 2.4** (*Continued*)

Type of Crop	Diagnostic Range (% Dry Mass of Leaves)		
	Low	Sufficiency <sup>a</sup>	High
Citrus	<2.0 to 2.2	2.3 to 2.9	>3.3
Pome	<1.5 to 1.8	2.1 to 2.9	>3.3
Stone	<1.7 to 2.4	2.5 to 3.0	>3.8
Small Woody	<1.5	1.5 to 2.3	>4.5
Strawberry	<2.1	2.1 to 4.3	>4.3
Banana		3.0 to 3.8	
Pineapple		1.5 to 2.5	
<b>Foliage Plants</b>		2.2 to 3.8	
<b>Herbaceous Perennials</b>	<2.2	2.2 to 3.2	>4.0
<b>Ornamental Grasses</b>	<1.6	1.6 to 2.5	>3.0
<b>Ground Covers</b>			
Herbaceous-broadleaf	<2.0	2.0 to 3.9	>4.0
Herbaceous-monocot	<1.5	1.6 to 2.4	>4.0
Woody		1.5 to 2.5	
<b>Turfgrasses</b>		2.6 to 3.8	
<b>Vegetables</b>			
Broadleaf	<2.6	3.5 to 5.1	
Sweet corn		2.5 to 3.2	
<b>Forest and Landscape Trees</b>	<1.9	1.9 to 2.6	
<b>Woody Shrubs</b>			
<b>Palms</b>		2.1 to 3.2	

*Note:* Values with few exceptions are mean concentrations in mature leaves. 'Low' is value where symptoms of deficiency are showing. 'Sufficiency' is mean range of lower and upper concentrations commonly reported in healthy plants showing no deficiencies. 'High' is a concentration that might represent excessive accumulation of nitrogen.

<sup>a</sup>Optimum or sufficient values for maximum yield or for healthy growth of plants will vary with species, age, and nutrition of plant, position of organ on plant, portion of plant part sampled, and other factors.

*Source:* Adapted from Chapman, H.D., *Diagnostic Criteria for Plants and Soils*, HD Chapman, Riverside, Cal., 1965, pp. 1–793; Mills, H.A. and Jones, J.B. Jr., *Plant Analysis Handbook II*, MicroMacro Publishing, Athens, Ga., 1996, pp. 155–414; Goodall, D.W. and Gregory, F.G., Chemical composition of plants as an index of their nutritional status, Technical Communication No. 17, Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England, 1947, pp. 1–167; Weir, R.G. and Cresswell, G.C., *Plant Nutrient Disorders 1. Temperate and Subtropical Fruit and Nut Crops*, Inkata Press, Melbourne, 1993, pp. 1–93; Weir, R.G. and Cresswell, G.C., *Plant Nutrient Disorders 3. Vegetable Crops*, Inkata Press, Melbourne, 1993, pp. 1–104; Walsh, L.M. and Beaton, J.D., *Soil Testing and Plant Analysis*, revised edition, Soil Science Society of America, Madison, Wis., 1973, pp. 1–491; and from other sources cited in references.

### 2.3.2.2 Ratios of Concentrations of Nitrogen to Other Nutrients in Plants

The *critical concentration* (see Section 2.3.2) of nitrogen is the value in a particular plant part sampled at a given growth stage below which plant growth and yield are suppressed by 5 or 10% (82). The responses of plants to nutrient additions are essentially independent of the source of nutrients; hence, the symptoms and nutrient concentrations of affected tissues, and relationships to growth and yields, are identical regardless of the growth medium or location. Therefore, the critical concentration is proposed to have universal application to media and geographic locations (82). However, since leaf (tissue) composition varies with age, the critical concentration can vary and be insensitive

or inflexible to diagnosis of nutrient deficiency (83). For example, if a leaf sample is taken at an early plant-growth stage, the concentration of nitrogen may exceed the critical concentration that was determined for tissue at a later stage of growth. Likewise, a sample taken at a late stage of growth might mistakenly be diagnosed as indicating a deficiency of nitrogen. To deal with the problem of variable critical concentrations with plant age, several sets of critical values are needed, one for each growth stage. Determinations of critical concentrations are difficult because of the many observations that must be made of growth and yield in response to nutrient concentrations in leaves. Hence, few critical concentrations have been determined at one growth stage, not considering that multiple stages should be assessed. Applications of sufficiency ranges, such as those reported (Table 2.4), are often too wide to be used for precise diagnoses.

The Diagnostic and Recommendations Integrated System (DRIS) was developed to assess plant nutrition without regard to variety, age, or position of leaves on plants (83,84). The DRIS method considers nutrient balance and utilizes ratios of nutrient concentrations in leaves to determine the relative sufficiency of nutrients (85). The DRIS method differs from standard diagnostic methods in the interpretation of analytical results based on the concentrations of individual elements. Instead of considering each nutrient concentration independently, DRIS evaluates nutrient relationships that involve ratios between pairs of nutrients and evaluates the adequacy of a nutrient in relation to others. Generation of the DRIS index yields positive and negative numbers, which are deviations from a norm and which sum to zero for all nutrients considered. DRIS norms are standard values suggested to have universal application to a crop. Norms are determined by research and have been published for several crops (86).

The optimum range for plant DRIS indices is  $-15$  to  $15$ . If the index is below  $-15$ , that element is considered to be deficient. If the index is above  $15$ , that element is considered to be in excess. DRIS indices must be interpreted in comparison with other nutrients. A negative number does not indicate that a nutrient is deficient, but it may be used to compare relative deficiencies among nutrients. DRIS may be useful in identifying hidden hunger or imbalances. For example, if nitrogen had an index of  $-12$ , phosphorus an index of  $-8$ , and potassium an index of  $6$ , the order of likely growth-limiting effects would be nitrogen  $>$  phosphorus  $>$  potassium. Variations in DRIS (M-DRIS or modified DRIS) consider dry matter in generation of indices (87,88).

## 2.4 NITROGEN IN SOILS

### 2.4.1 FORMS OF NITROGEN IN SOILS

The total nitrogen of the Earth is about  $1.67 \times 10^{23}$  g (89,90). Stevenson (89,90) reported that about 98% of the nitrogen of the Earth is in the lithosphere (rocks, soil, coal, sediments, core, sea bottom). About 2% of the nitrogen is in the atmosphere, with the portions in the hydrosphere and biosphere being insignificant relative to that in the lithosphere and atmosphere. Most of the nitrogen of the Earth, including the nitrogen in the rocks and in the atmosphere, is not available for plant nutrition. The nitrogen in soils, lakes, streams, sea bottoms, and living organisms is only about 0.02% of the total nitrogen of the Earth (89,90). Plants obtain most of their nitrogen nutrition from the soil. The nitrogen in the soil is about  $2.22 \times 10^{17}$  g, most of which is in soil organic matter and which is a negligible component of the total nitrogen content of the world (89,90). Living organisms (biosphere) contain about  $2.8 \times 10^{17}$  g of nitrogen. The nitrogen of living organisms and of the soil is in a constant state of flux, with some forms of nitrogen being readily transformed in this group and some forms being inactive over a long time (91). Transformations are insignificant in the lithosphere and atmosphere. The amount of interchange of nitrogen among the lithosphere (not including soil), atmosphere, and living organisms is very small.

The total amount of nitrogen in the soil to the depth of plowing is considerable relative to the amounts required for crop production, often above 3000 kg/ha but ranging from 1600 kg/ha in sands through 8100 kg/ha in black clay loams to 39,000 kg/ha in deep peats (Table 2.5) (92). Note that the nitrogen in the atmosphere above a hectare of land exceeds 100 million kg at sea level. When land is

**TABLE 2.5**  
**Estimated Content and Release of Nitrogen from**  
**Various Soils**

Type of Soil	Nitrogen in Soil (kg/ha)	
	Total <sup>a</sup>	Annual Release <sup>b</sup>
Sands	1400	28
Yellow sandy loam	2200	44
Brown sandy loam	3100	62
Yellow silt loam	2000	40
Grey silt loam	3600	72
Brown silt loam	5000	100
Black clay loam	7200	144
Deep peats	39,000	780

<sup>a</sup>From Schreiner O. and Brown B.E., in *United States Department of Agriculture, Soils and Men, Yearbook of Agriculture, 1938*, United States Government Printing Office, Washington, DC, 1938, pp. 361–376.

<sup>b</sup>Estimated at 2% annual mineralization rate of soil organic matter.

put for crop production, the nitrogen content of soils declines to a new equilibrium value (90,92). Crop production that relies on the reserves of nitrogen cannot be effective for long, as the reserves become exhausted. Most plants cannot tap into the large reserve of nitrogen in the atmosphere, although biological nitrogen fixation is a means of enhancing the nitrogen content of soils. Biological nitrogen fixation is the principal means of adding nitrogen to the soil from the atmosphere (89). More than 70% of the atmospheric nitrogen added or returned to soils is by biological fixation, and can exceed 100 kg of nitrogen addition per year by nitrogen-fixing legumes. Most of this nitrogen enters into the organic fraction of the soils. Unless nitrogen-fixing legumes are grown, the addition of nitrogen to soils by biological fixation, averaging about 9.2 kg/ha annually, is too small to support crop production. The remainder is from atmospheric precipitation of ammonium, nitrate, nitrite, and organically bound nitrogen (terrestrial dust). The amount of nitrogen precipitated is normally too small to support crop production but might be of significance in natural landscapes (90). Virtually no interchange of nitrogen occurs between rocks and soils.

#### 2.4.1.1 Organic Nitrogen in Soil

The concentrations of nitrogen range from 0.02% in subsoils to 2.5% in peats (93). Nitrogen concentrations in soils generally fall sharply with depth, with most of the nitrogen being in the top one-meter layer of soils (89). Surface layers (A-horizon, plow-depth zone) of cultivated soils have between 0.08 and 0.4% nitrogen. Well over 90%, perhaps over 98%, of the nitrogen in the surface layers (A-horizon, plow-depth zone) of soil is in organic matter (93,94). Since most of the nitrogen in soil is organic, determination of total nitrogen has been a common method of estimating organic nitrogen. The Kjeldahl method, a wet digestion procedure (93,95,96), provides a good estimate of organic, soil nitrogen in surface soils, even though some forms of nitrogen (fixed ammonium, nitrates, nitrites, some organic forms) are not determined by this analysis. In depths below the A-horizon or plow zone, although the amounts of total nitrogen are small, inorganic nitrogen, particularly fixed ammonium, is a high proportion of the total, perhaps 40%, and results from Kjeldahl analysis should be treated with some caution as this fraction would not be determined (93). The Dumas method, a dry digestion procedure, is seldom used for determination of nitrogen in soils but

generally gives results in close agreement with Kjeldahl determinations, if certain precautions are taken in the analysis (93).

Soil organic matter is a complex mixture of compounds in various states of decay or stability (97). Soil organic matter may be classified into humic and nonhumic fractions, with no sharp demarcation between the two fractions. The partially decayed or nonhumic portion is the major source of energy for soil organisms. Depending on the nature of the plant materials, about half of fresh plant residues added to soil decompose in a few weeks or months (98,99). Humus, or humic substances, are the degradation products or residues of microbial action on organic matter and are more stable than the nonhumic substances. Humus is classified into three fractions, humin, humic acids, and fulvic acids, based on their solubilities. Humin is the highest molecular weight material and is virtually insoluble in dilute alkali or in acid. Humic acids are alkali-soluble and acid-insoluble. Fulvic acids are alkali- or acid-soluble. The humic and fulvic fractions are the major portions, perhaps 90%, of the humic soil organic matter and are the most chemically reactive substances in humus (100). Humus is slow to mineralize, and unless present in large quantities may contribute little to plant nitrogen nutrition in most soils. About 60 to 75% of the mineralized nitrogen may be obtained by a crop (99). The turnover rate of nitrogen in humus may be about 1 to 3% of the total nitrogen of the soil, varying with type of soil, climate, cultivation, and other factors (93,99). The mineralization rate is likely to be closer to 1% than to 3%. Bremner (96) and Stanford (101) discussed several methods to assess availability of organic nitrogen in soils. Among these procedures were biochemical methods (estimation of microbial growth, mineral nitrogen formed, or carbon dioxide released) and chemical methods (estimation of soil total nitrogen, mineral nitrogen, and organic matter and application of various extraction procedures). The chemical methods are applied more commonly than the biological methods in the estimation of mineralization. Correlation of crop yields to estimations of mineralization generally have not been satisfactory in the assessment of the potential for soils to supply nitrogen for crop growth.

Most studies on the fractionation of total soil organic matter have dealt with the hydrolysis of nitrogenous components with hot acids (3 or 6 M hydrochloric acid for 12 to 24 h) (Table 2.6). The fraction that is not hydrolyzed is called the *acid-insoluble nitrogen*. The acid-soluble nitrogen is fractionated into *ammonium*, *amino acid*, *amino sugar*, and *unidentified* components. The origins and composition of each of the named fractions are not clear. The absolute values vary with soil type and with cultivation (94). All of these forms of nitrogen, including the acid-stable form, appear to be biodegradable and, hence, to contribute to plant nutrition (94,102). Organic matter that is held to clays is recalcitrant to biodegradation and increases in relative abundance in heavily cropped soils (94,103,104). This fraction may have little importance in nitrogen nutrition of plants.

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**TABLE 2.6**  
**Fractions of Nitrogen in Soil Organic Matter**  
**Following Acid Hydrolysis**

Nitrogen Component	Fraction of Total Organic Nitrogen (%)
Acid insoluble	20 to 35
Ammonium	20 to 35
Amino acid	30 to 45
Amino sugar	5 to 10
Unidentified	10 to 20

*Source:* From Bremner, J.M., in *Soil Nitrogen*, American Society of Agronomy, Madison, Wis., 1965, pp. 1324–1345 and Stevenson, F.J., *Nitrogen in Agricultural Soils*, American Society of Agronomy, Madison, Wis., 1982, pp. 67–122.

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Cultivation reduces the total amount of organic matter in soils but has little effect on the relative distribution of the organic fractions in soils, suggesting that the results of acid hydrolysis are of little value as soil tests for available nitrogen or for predicting crop yields (94). Humic substances contain about the same forms of nitrogen that are obtained from the acid hydrolysis of soils but perhaps in different distribution patterns (94). Agricultural systems that depend on soil reserves do not remain productive without the input of fertilizer nitrogen.

#### 2.4.1.2 Inorganic Nitrogen in Soil

Soil inorganic nitrogen is commonly less than 2% of the total nitrogen of surface soils and undergoes rapid changes in composition and quantity. Inorganic nitrogen varies widely among soils, with climate, and with weather. In humid, temperate zones, soil inorganic nitrogen in surface soil is expected to be low in winter, to increase in spring and summer, and to decrease with fall rains, which move the soluble nitrogen into the depths of the soil (105). Despite being small in magnitude, the inorganic fraction is the source of nitrogen nutrition for plants. Unless supplied by fertilizers, inorganic nitrogen in soil is derived from the soil organic matter, which serves as a reserve of nitrogen for plant nutrition. Plant-available nitrogen is released from organic matter by mineralization and is transformed back into organic matter (microbial cells) by immobilization. Absorption by plants is the chief means of removal of inorganic nitrogen from soils, although nitrate leaching and denitrification, ammonium volatilization and fixation, and nitrogen immobilization lead to losses of inorganic nitrogen from soils or from the soil solution (105).

Detectable inorganic nitrogen forms in soil are nitrate, nitrite, exchangeable and fixed ammonium, nitrogen ( $N_2$ ) gas, and nitrous oxide ( $N_2O$  gas) (106). Nitrate and exchangeable ammonium are important in plant nutrition. The other forms are generally not available for plant nutrition. Fixed ammonium, entrapped in clays, is a principal nitrogenous constituent of subsoils and is probably derived from parent rock materials; however, the fixed ammonium in surface soils may be of recent origin from organic matter (106). Fixed ammonium is resistant to removal from clay lattices and has little importance in plant nutrition. The gaseous constituents diffuse from the atmosphere or arise from denitrification and have no role in plant nutrition, other than in considerations of losses of nitrogen from soils (107).

Exchangeable or dissolved ammonium is available to plants, but ammonium concentrations in soils are low, usually in a magnitude of a few mg/kg or kg/ha. In well-aerated soils, ammonium is oxidized rapidly to nitrate by nitrification, so that nitrate is the major source of plant-available nitrogen in soil (108,109). Nitrite, an intermediate in nitrification, is oxidized more rapidly than ammonium (109). Hence, little ammonium or nitrite accumulates in most soils. Ammonium and nitrite are toxic to most plants (110). Toxicity of ammonium or nitrite might occur if the concentration of either rises above 50 mg N/kg in soil or in other media, especially if either is the principal source of nitrogen for plant nutrition (110,111). Nitrification is sensitive to soil acidity and is likely to be inhibited in soils under pH 5; this acidity may lead to ammonium accumulation (108).

## 2.5 SOIL TESTING FOR NITROGEN

Testing for plant-available soil nitrogen is difficult. This difficulty arises in part because most of the nitrogen in soil is in organic forms, which have varying rates of microbial transformation into available forms. Also, nitrate, the main form of plant-available nitrogen, is subject to leaching, denitrification, and immobilization. Many attempts have been made to develop availability indexes for release of nitrogen from organic matter and to correlate yields with tests for inorganic nitrogen in soils (93,101,112–114). Biological tests are time consuming and may give variable results if the methodology is not standardized among researchers. Chemical tests for estimating plant-available nitrogen have been empirical in approach and have had low correlations with production of mineral nitrogen and crop accumulation of nitrogen.

### 2.5.1 DETERMINATIONS OF TOTAL NITROGEN

The determination of nitrogen by the Kjeldahl method gives an estimation of the total nitrogen in soils (93,113). This test, often considered a chemical index, is essentially a test for total soil organic matter, since the nitrogen concentration of soil organic matter is relatively constant. This measurement does not estimate the rates of transformations of organic nitrogen into inorganic forms that are available for plants; hence, many irregularities in predicting available nitrogen occur in its use. However, considering that transformations depend on the type of organic matter, temperature, aeration, water supply, acidity, and other factors, total nitrogen is likely as informative as determination of other availability indexes. Nevertheless, determinations of availability indexes have been investigated extensively (96).

### 2.5.2 BIOLOGICAL DETERMINATIONS OF AVAILABILITY INDEXES

Aerobic incubation of soil samples for 2 to 4 weeks under nearly optimum conditions of microbial decomposition of organic matter and measurements of nitrogen mineralization is an extensively employed *biological procedure* for the development of an availability index (96,101,112–114). Incubated samples are tested for the amounts of nitrate, ammonium, or both forms released. Since determinations are run under nearly optimum conditions, only an estimate of the potential for mineralization is provided. Results may differ from mineralization in a field in a particular year. Determinations of indexes by anaerobic incubation involve estimations of ammonium released (115). Other biological tests involve bioassays of microbial growth or pigment production (116), chlorophyll production by algae (117), and carbon dioxide production (118).

#### 2.5.2.1 Determination of Inorganic Nitrogen

These determinations are considered to be chemical indexes of availability of nitrogen soil organic matter. The utility of chemical indexes depends on their correlation for a broad range of soils with biological criteria, such as crop yields, nitrogen accumulation in plants, and biological indexes (101). Inorganic nitrogen is determined in an extraction of soil with water or solutions of acids, bases, chelating agents, or salts at differing concentrations and temperatures (101). Severe extractants, such as moderately concentrated (4.5 to 6 M) boiling mineral acids or bases, generally give nitrogen releases that correlate well with total soil nitrogen. However, total soil nitrogen as such is not a reliable index of nitrogen availability in soils. Also, release of nitrogen by moderate extraction procedures, such as alkaline permanganate, sodium carbonate, and molar solutions of mineral acids and bases, generally are poorly correlated with biological measurements (96,101). Relatively mild extractions with cold, hot, or boiling water or solutions of cold dilute (0.01 M) acids, bases, or salts have been used with the premise that these methods determine nitrogen of which a high proportion is derived from microbial action on the soils (101). Ammonium or nitrate may be determined in the extracts (96,105,106).

##### 2.5.2.1.1 Ammonium

The rate-controlling step in nitrogen mineralization is the conversion of organic nitrogen into ammonium. The conversion of ammonium into nitrate is a rapid step, as a result ammonium generally does not accumulate in well-drained mineral soils. Ammonium in soil, initially present in soils at sampling, is correlated weakly with nitrogen accumulation in plants (113). Temperatures in handling and storage of soil samples are important in judging the correlation between ammonium in soils and accumulation in plants (119). Waterlogging, high acidity (pH < 5.0) or alkalinity (pH > 8.0), or use of nitrification inhibitors can lead to mineralization that stops with the formation of ammonium and hence to accumulation beyond that occurring in well-drained, mineral soils. Determination of ammonium present in soil without any manipulation generally gives better correlations with biological processes than the correlation of ammonium that accumulates with manipulation of processes that lead to ammonium accumulation.



### 2.5.2.1.2 Nitrate

Nitrate is the form of nitrogen that is used most commonly by plants and that may accumulate in agricultural soils. In combination with other factors, such as soil water, nitrate concentrations in soils have been used in assessments of soil fertility since the early 1900s (113,120–122). Ozus and Hanway (123) reported that nitrogen accumulation in crops during early growth was related to nitrate content in soils. Early workers related nitrate in soils to crop yields. Nitrate in soil was shown to be a reliable evaluation of soil nitrogen that is residual from previous fertilization (124–126). Recent work has related tests for nitrate in soils to prediction of the needs of crops for nitrogen fertilization. These tests are commonly called *preplant nitrate tests* and are conducted in the early spring to a soil-sampling depth of 60, 90, 120 cm, or greater.

Nitrate is a soluble form of nitrogen that is subject to downward movement in soils in humid temperate climates (105). Sometimes, soil tests for nitrate in the top 15 or 30 cm of soils have not been well correlated with crop yields because of depletion of nitrate in these zones by leaching in humid regions (113). Good correlations between soil nitrate tests and crop yields have been noted with soil samples taken from 120- to 180-cm depth in the profile. Roth and Fox (125) reported nitrate concentrations that ranged from 36 to 295 kg N/ha in the 120-cm profile following the harvest of corn. Soils fertilized with nitrogen applied at economically optimum amounts had nitrate concentrations ranging from 41 to 138 kg N/ha. Soils with more than 169 kg nitrate-N/ha in the 120-cm profile did not show an increase in corn yields in response to nitrogen fertilization. Jokela and Randall (124) reported that nitrate concentrations in a 150-cm profile ranged from 150 to 500 kg N/ha over a range of fertilizer treatments after corn harvest in the fall but fell by 50 to 70% by the following spring.

Nitrate concentrations vary among soils and among seasons of the year for a given soil and climate (105,127). In humid temperate climatic areas, nitrate in soils is low in the cold of winter, rises in spring and through the summer with warming of soils and falls in the fall with the rains. In unfertilized fields in the winter, nitrate in topsoil (top 30 to 60 cm) is less than 5 or 10 mg N/kg (105). The concentration can rise to 40 to 60 mg nitrate-N/kg in spring and summer. Depending on the permeability of soil, the depletion of nitrate from topsoil can be rapid with fall rains. Tillage of land can bring about an increase of nitrate, as mineralization and nitrification are increased by aeration of the soil due to tillage. Generally, the more intensive the tillage, the greater the nitrate concentrations in the soil (128–130). For example, in the 120-cm-deep soil profile, following a crop of corn, the nitrate in conventionally tilled soils (100 to 120 kg N/ha) was twice that in the profile of soils cropped in a no-tillage system (129). In dry seasons, soil nitrate can be very low due to low microbiological activity, perhaps less than 10 mg N/kg, but increases as rain falls and mineralization and nitrification result in the wetted soil. In some cases, if the subsoil contains nitrate, nitrate may rise with capillary action and accumulate in dry surface soils. Absorption by plants is a principal path of removal of nitrate from soils. Removal is unique with various soils and crops (105). Perennial crops having a developed root system can absorb nitrate as soon as conditions are favorable for plant growth. Grassland soils generally are low in nitrate throughout the year. However, annual crops do not absorb much nitrate from soils until the root systems are developed.

Many soil test recommendations for correlation of soil nitrate with crop yields require soil sampling to a minimum depth of 60 cm (113). Sampling to this depth involves considerable costs, and attempts have been made to develop a test based on shallower sampling. Alvarez et al. (131) developed prediction equations that related nitrate in the top 30 cm stratum to that in the top 60 cm stratum. Recent research has shown good correlations between crop yields and concentrations of nitrate in the surface 30 cm layer of soils early in the growing season (132–135). Determination of the amount of nitrate in the upper stratum of soil early in the season has led to the development of a test called the *early season nitrate test* or *pre-sidedress soil nitrate test* (PSNT).

The basis of the PSNT is the concentration of nitrate in the surface 30 cm of soils at the time that a crop starts rapid growth, for example, when corn is 30 cm tall (133,134). The amount of nitrate in the soil at this depth at this time is an assessment of the amount of nitrogen available for

crop growth for the remainder of the season and of the need for nitrogen fertilization. The critical concentration of soil nitrate for the PSNT is the concentration above which yields are not expected to increase with additional nitrogen fertilization. For corn production, Sims et al. (135) in Delaware reported that the PSNT test identified nitrogen-deficient or nitrogen-sufficient sites with about 70% success. Binford et al. (132) in Iowa determined that the critical concentration of nitrate for corn was 23 to 26 mg N/kg for a 30 cm depth. Sampling 60 cm deep improved correlations between corn grain yields and soil nitrate, but it was felt that the improvement did not justify the additional costs of deep sampling. The critical concentration for the 60 cm depth was 16 to 19 mg N/kg soil. Other research has given similar results. Meisinger et al. (136) in Maryland determined a critical nitrate concentration of 22 mg N/kg with the PSNT successfully identifying nitrogen-sufficient sites across a range of textures, drainage classes, and years. Including ammonium in the analysis slightly improved the predictive use of the test (136). Heckman et al. (137) in New Jersey reported a critical nitrate concentration at the 30 cm depth to be 22 mg N/kg for corn. Evanylo and Alley (138) in Virginia reported critical nitrate concentrations of 18 mg N/kg for corn and noted that the PSNT was applicable to soils without regard to texture or physiographic region. Also for corn, Sainz-Rozas et al. (139) in Argentina reported a critical nitrate concentration of 17 to 27 mg N/kg at the 30 cm depth. They also reported that there was no improvement in reliability if the test was done on samples to 60 cm depth or with the inclusion of ammonium in the determinations. Critical concentrations, similar in magnitude to those for corn have been reported for sweet corn (*Zea mays rugosa* Bonaf.) (140), lettuce (*Lactuca sativa* L.), celery (*Apium graveolens dulce* Pers.) (141), cabbage (*Brassica oleracea capitata* L.) (142), and tomato (*Lycopersicon esculentum* Mill.) (143).

If the concentration of nitrate is below the critical concentration, fertilization of the crops is necessary. However, the need to collect soil samples during the growing season has limited the usage of the PSNT. Fertilization is delayed until the results of the PSNT are obtained, and bad weather can delay applications of nitrogen.

#### 2.5.2.1.3 Amino Sugars

Fractionation of soil hydrolysates has been used to determine a labile pool of organic nitrogen in soil and to relate this fraction to crop responses to nitrogen fertilizers (102,144). The results of most of these studies have shown little variation among soil types or cultivation patterns in the partitioning of hydrolyzable soil nitrogen into various nitrogenous components and the capacity of soil organic matter to form nitrate. The uniformity among soils was attributed in part to errors in analysis (145,146). Mulvaney and Khan (147) developed a diffusion method for accurately determining amino sugar nitrogen in soil hydrolysates. Mulvaney et al. (145) noted that hydrolysates (6 M HCl) of soils in which crops were nonresponsive to nitrogen fertilization had higher concentrations of amino sugars (e.g., glucosamine, galactosamine, mannosamine, muramic acid) than did hydrolysates of soils in which crops responded to nitrogen fertilization. They reported no consistent differences among the total nitrogen, the ammonium nitrogen, or the amino acid nitrogen fraction of the soil hydrolysate. The amounts of amino sugars were related to mineralization of soil organic nitrogen, since production of inorganic nitrogen upon aerobic incubation of the nonresponsive soils was much greater than that in the responsive soils (145). Concentrations of amino sugars were correlated with response to fertilizer nitrogen applied. Mulvaney et al. (145) classified soils with more than 250 mg amino sugar nitrogen per kg as being nonresponsive and those with less than 200 mg amino sugar nitrogen per kg as being responsive to nitrogen fertilization. Khan et al. (146) developed a simpler test for determining amino sugar nitrogen than the processes involving soil hydrolysis. The simpler test involved soil being treated with base (2 M NaOH), followed by heating (50°C) to release ammonia, and then determining the amount of ammonia releases by volumetric methods. This method determined ammonium and amino sugar nitrogen without liberating substantial nitrogen from amino acids and none from nitrate or nitrite. Test values for soils nonresponsive to nitrogen fertilization were 237 to 435 mg N/kg and for responsive soils were 72 to 223 mg N/kg soil.

Amino sugars may constitute 5 or 6% of the humic substances in soils (148). Variations in kind and amount of amino sugars have been noted with climate and with cultivation of soils (149,150).

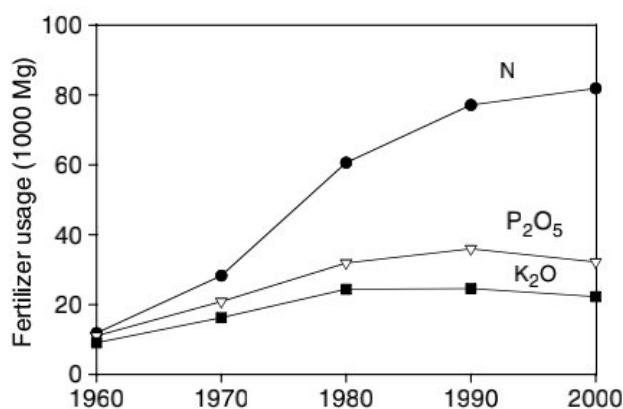
## 2.6 NITROGEN FERTILIZERS

Soils have little capacity to retain oxidized forms of nitrogen, and ammonium accumulation in soils is small; consequently, most of the soil nitrogen is associated with organic matter. Release of nitrogen from organic matter is slow and unpredictable. If soil organic matter is depleted, as occurs in cultivated soils, nitrogen for plant growth is limited. Nitrogen is usually the most deficient nutrient in cultivated soils of the world, and fertilization of these soils with nitrogen is required. To maintain or increase productivity of soils, worldwide consumption of nitrogen fertilizers continues to increase with time (Figure 2.3). However, the consumption of phosphorus and potassium fertilizers has leveled.

Anhydrous ammonia ( $\text{NH}_3$  gas) is the starting product for manufacture of most nitrogen fertilizers. Anhydrous ammonia is manufactured from hydrogen and nitrogen gases by the Haber process (Haber–Bosch process). The reaction is performed at high temperature (400 to 500°C) and high pressure (300 to 1000 atm) in the presence of a catalyst (iron or other metal) (151–153). The nitrogen gas is obtained from the air, which is about 79% nitrogen by volume, and the hydrogen is obtained from natural gas (methane), oil, coal, water, or other sources.

Jones (152) and Moldovan et al. (154) describe the production of other nitrogen fertilizers from ammonia. A brief summary of these processes follows. Nitric acid, produced from ammonia, is another basic material in the manufacture of nitrogen fertilizers. To produce nitric acid, compressed ammonia and air are heated in the presence of a catalyst and steam. The nitric acid can be reacted with ammonia to produce ammonium nitrate. Sodium nitrate is the product of the reaction of nitric acid with sodium bicarbonate. Sodium nitrate also is produced from caliche (Chilean saltpeter), which is a mineral that contains sodium nitrate and various salts of sodium, calcium, potassium, and magnesium. Sodium nitrate, sometimes called Chilean nitrate, is one of the earliest commercial nitrogen fertilizers marketed. Until 1929, all of the sodium nitrate marketed was extracted from Chilean saltpeter (154). Urea is manufactured chiefly by combining ammonia with carbon dioxide under high pressure. Ammonium sulfate is manufactured by the reaction of ammonia with sulfuric acid, gypsum, or sulfur dioxide.

The merits of nitrate and ammonium fertilizers have been researched and reviewed extensively (155–166). Many manufactured fertilizers and most organic fertilizers are ammoniacal; however, the ammonium that is inherent in the fertilizer or that is released upon contact with soils is soon oxidized to nitrate, unless nitrification is inhibited (167–171). Nitrification inhibitors may be employed with ammoniacal fertilizers to restrict losses of nitrogen from soils by leaching or denitrification.



**FIGURE 2.3** Worldwide consumption of nitrogen, phosphorus, and potassium in fertilizers for the period 1960–2000. Units of Mg are 1000 kg or one metric ton. (Adapted from <http://www.fertilizer.org/ifa/statistics/indicators/tablen.asp>.)

## 2.6.1 PROPERTIES AND USE OF NITROGEN FERTILIZERS

The nitrogen concentrations of the following fertilizers have been rounded to values of commonly marketed grades.

### 2.6.1.1 Anhydrous Ammonia (82% N)

Anhydrous ammonia is the most-used nitrogen-containing fertilizer for direct application to land in the United States (152). Worldwide, consumption of anhydrous ammonia is ranked fourth or fifth among nitrogen fertilizers (Table 2.7). In agriculture, anhydrous gaseous ammonia is compressed into a liquid and is applied under high pressure with a special implement by injection at least 15 cm deep into a moist soil. The ammonia gas reacts with water to form ammonium ions, which can be held to clay or organic matter. If the ammonia is not injected deeply enough or soil is too wet or dry, ammonia can be lost by volatilization. Anhydrous ammonia is usually the cheapest source of nitrogen, but equipment and power requirements of the methods of application are specific and high.

### 2.6.1.2 Aqua Ammonia (21% N)

Aqua ammonia is ammonia dissolved in water under low pressure. Aqua ammonia must be incorporated into land to avoid losses of nitrogen by ammonia volatilization; however, it needs not be incorporated as deeply as anhydrous ammonia.

### 2.6.1.3 Urea (46% N)

Urea is the most widely used dry nitrogen fertilizer in the world (Table 2.7). After application to soils, urea is converted into ammonia, which can be held in the soil or converted into nitrate. Ammonia volatilization following fertilization with urea can be substantial, and if urea is applied to the surface

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**TABLE 2.7**  
**Worldwide Nitrogen Fertilizer Consumption in the**  
**Year 2000**

Nitrogen Fertilizer	Nitrogen Fertilizer Usage (Metric Tons)
<i>Straight N Fertilizer</i>	
Urea	41042
Ammonium nitrate	5319
Calcium ammonium nitrate	4768
N solutions	3812
Anhydrous ammonia	3581
Ammonium sulfate	2738
Other	7907
Total straight	69168
<i>Mixed N Fertilizer</i>	
NPK-N	6347
Ammonium phosphate	4631
Other NP-N	1656
NK-N	74
Total mixed	12708
Total N fertilizer	81880

Source: Compiled from <http://www.fertilizer.org/ifa/>

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of the land, considerable loss of nitrogen can occur (172,173). Hydrolysis of urea by urease produces ammonium carbonate. With surface-applied urea, alkalinity of pH 9 or higher can develop under the urea granule or pellet, and ammonia will volatilize into the air. Volatilization occurs on bare ground, on debris, or on plant leaves. Urea is readily soluble in water, and rainfall or irrigation after its application move it into the soil and lessens volatilization losses. Use of urease inhibitors has been suggested to lessen the volatilization losses of ammonia from surface-applied urea (174). Manufactured urea is identical to urea in animal urine.

Calcium nitrate urea (calurea, 34% N, 10% Ca) is a double-compound fertilizer of calcium nitrate and urea to supply calcium and nitrogen (152).

Several derivatives of urea are marketed as slow-release fertilizers (175,176). Urea formaldehyde (ureaform, 38% N) is a slow-release fertilizer manufactured from urea and formaldehyde and is used for fertilization of lawns, turf, container-grown plants, and field crops (177–180). Urea formaldehyde is also a glue and is used for the manufacture of plywood and particle board (181,182). Dicyandiamide (cyanoguanidine) (66% N) is a nitrogen fertilizer but is used most commonly as an additive (2% of the total N fertilizer) as a nitrification inhibitor with urea (153,183–185). Sulfur-coated urea (186,187) is a slow-release formulation (30–40% N) used as a fertilizer for field crops, orchards, and turfgrass (175,177,188–191).

Isobutylidene diurea (IBDU) is similar to urea formaldehyde, but contains 32% nitrogen. However, utilization of IBDU is less dependent on microbial activity than urea formaldehyde, as hydrolysis proceeds rapidly following dissolution of IBDU in water (175). Nitrogen is released when soil moisture is adequate. IBDU is used most widely as a lawn fertilizer (176,192). Its field use is to restrict leaching of nitrogen (181).

Methylene ureas are a class of sparingly soluble products, which were developed during the 1960s and 1970s. These products contain predominantly intermediate chain-length polymers. The total nitrogen content of these polymers is 39 to 40%, with between 25 and 60% of the nitrogen present as cold-water-insoluble nitrogen. This fertilizer is used primarily in fertilization of turfgrass, although it has been used with other crops on sandy soils or where leaching of nitrate is an environmental concern (176,191,193).

#### **2.6.1.4 Ammonium Nitrate (34% N)**

Ammonium nitrate is a dry material sold in granular or prilled form. It can be broadcasted or sidedressed to crops and can be left on the surface or incorporated. It does not give an alkaline reaction with soils; hence, it does not volatilize readily. However, incorporation is recommended with calcareous soils. Ammonium nitrate is decreasing in popularity because of storage problems, e.g., with fire and explosion.

Calcium ammonium nitrate (ammonium nitrate limestone, about 20% N and 6% Ca) is a mixture of ammonium nitrate and limestone. This fertilizer is not acid-forming and is used to supply nitrogen and calcium to crops (152).

#### **2.6.1.5 Ammonium Sulfate (21% N)**

Ammonium sulfate is marketed as a dry crystalline material. It is recommended for use on alkaline soils where it may be desirable to lower soil pH. Nitrification of ammonium is an acidifying process. Ammonium sulfate can be broadcasted or sidedressed. It can be left on surfaces or incorporated, although on calcareous soils watering in or incorporating is recommended to avoid ammonia volatilization (176).

#### **2.6.1.6 Nitrogen Solutions (28–32% N)**

These fertilizers are mixtures of ammonium nitrate and urea dissolved in water. In the solutions, half of the nitrogen is supplied as urea, and half is supplied as ammonium nitrate. Because of the difficulties in handling, urea and ammonium nitrate should not be mixed together in dry form. The

solution acts once the dry materials are applied to the soil. Ammonia volatilization may be substantial during warm weather, especially with surface application. The solutions should be watered into the soil and should not be applied to foliage.

### 2.6.1.7 Ammonium Phosphates (10–21% N)

Ammonium phosphates are important phosphorus-containing fertilizers because of their high concentrations of phosphorus and water solubility. Diammonium phosphate (commonly 18% N, 46%  $P_2O_5$ ) is a dry granular or crystalline material. It is a soil-acidifying fertilizer and is useful on calcareous soils. It should be incorporated into the soil. It is a common starter fertilizer and is a common component of greenhouse and household fertilizers. Monoammonium phosphate (commonly 11% N, 48%  $P_2O_5$ ) has uses similar to those of diammonium phosphate. Ammonium polyphosphate (10% N, 34%  $P_2O_5$ ) is marketed as a solution. Its use is similar to that of monoammonium phosphate and diammonium phosphate. Ammonium phosphates are made by reaction of ammonia with orthophosphoric acid (mono- and diammonium salts) or with superphosphoric (pyrophosphoric) acid (152).

### 2.6.1.8 Other Inorganic Nitrogen Fertilizers

Many other nitrogen-containing fertilizers include double-salt mixtures such as ammonium nitrate sulfate (30% N), ammonium phosphate nitrate (25% N), urea ammonium phosphate (25–34% N), nitric phosphate, and ammoniated superphosphate (8% N) (152). These materials are used in the manufacture of mixed N-P-K fertilizers or for special needs in soil fertility.

### 2.6.1.9 Organic Nitrogen Fertilizers (0.2–15% N)

Although naturally occurring, sodium nitrate may not be recognized as an organic fertilizer. Most organic fertilizers are derived from plant and animal sources and are proteinaceous

**TABLE 2.8**  
**Representative Nitrogen Concentrations and Mineralization**  
**of Some Organic Fertilizers**

Fertilizer	% N (Dry Mass) <sup>a</sup>	Mineralization <sup>b</sup>
Feather meal, hair, wool, silk	15	Moderate–Rapid
Dried blood, blood meal	12	Rapid
Fish scrap (dry)	9	Moderate–Rapid
Tankage, animal	8	Moderate–Rapid
Seed meals <sup>c</sup>	6	Rapid
Poultry manure	2–3	Moderate–Rapid
Livestock manure	1–2	Slow
Sewage biosolids	1–4	Slow
Bone meal, steamed	1	Moderate–Rapid
Kelp	0.7	Slow
Compost	0.5–1	Slow

<sup>a</sup>Concentrations will vary from these representative values, depending on the handling of the products, nutrition of livestock, and source of materials.

<sup>b</sup>Mineralization rate will vary with the products. Rapid mineralization is more than 70% of the organic N expected to be mineralized in a growing season; moderate is 50 to 70% mineralization; and slow is less than 50% mineralization.

<sup>c</sup>Includes by-products such as cottonseed meal, soybean meal, linseed meal, corn gluten meal, and castor pomace.

materials. The fertilizer industry started with meat and other food processors, who wanted to dispose of and find a use for wastes and by-products (152,194). Around 1900, about 90% of nitrogen fertilizer was derived from proteinaceous wastes and by-products, but today usage has declined to less than 1%. Organic materials range from less than 1 to about 15% N compared with the chemical sources described above, which range upward to over 80% N. Costs of handling, shipping, and spreading of the bulky, low-analysis organic materials have led to their decline in usage with time. Also, many of the proteinaceous by-products of food processing have higher value as feeds for poultry and livestock than as fertilizers (194,152). Nevertheless, demand for organic fertilizers remains, as organic farmers require these products in the maintenance of soil fertility on their cropland (195).

The value of organic nitrogen fertilizers depends on their rate of mineralization, which is closely related to their nitrogen concentration (152,195,196). Generally, the more nitrogen in the fertilizer, the faster the rate of mineralization. Some common organic fertilizers are listed in Table 2.8.

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