Handbook of Plant Nutrition

SECOND EDITION

Edited by
Allen V. Barker
David J. Pilbeam

CRC Press
Taylor & Francis Group

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Preface

In 2007, we edited *Handbook of Plant Nutrition*, a compendium of knowledge at that time on the mineral nutrition of plants. This handbook was inspired by Homer D. Chapman's 1965 book, *Diagnostic Criteria for Plants and Soils*, and had contributions from eminent plant and soil scientists from around the world. Its purpose was to provide a current source of information on the nutritional requirements of world crops, and it covered the uptake and assimilation of elements essential or beneficial for plant growth, the availability of these elements to plants, fertilizers used to enhance the supply of the elements, and diagnostic testing of plants and soils to determine when they are in short supply. Each element considered was given its own chapter, with macronutrients being covered in more detail than micronutrients and beneficial elements.

Since the publication of the first edition, there have been advances in many aspects of plant nutrition, so a second edition is now timely. The second edition seeks to outline recent advances but also to put these advances into the context of our historical understanding of the importance of nutrient and beneficial elements to plants. This action means that inevitably some repetition of ideas that appeared in the first edition will occur, but repetition has been minimized in a variety of ways.

First, most of the chapters are written by different experts, or a group of experts, from those who contributed to the first edition. This change does not reflect on the outstanding contributions made by those earlier authors, some of whom have contributed chapters on different elements in this second edition, but merely gives a stimulus to cover each element in a different manner. Different workers inevitably have different interests in their research and expressions of their knowledge, and these interests and expressions are reflected in the way in which they have written about a particular element.

Second, the chapter sections have been modified to reflect current interests within plant nutrition. For example, the chapters in the second edition have more extensive coverage of the relationship between plant genetics and the accumulation and use of nutrients by plants, but most chapters contain less on the discovery of essentiality or beneficial action, as in most instances this information is covered well in the first edition. The first edition had extensive tables showing elemental composition of different plants and different plant parts. To avoid repetition, such tables are not nearly as extensive in the second edition and concentrate on the summarization of values reported from the period of time between the publications of the two editions. A final difference is that more knowledge about the importance of lanthanides in plant nutrition has become available since the first edition, so these elements are featured in a new chapter in the second edition.

Chapters on the different mineral elements follow the general pattern of a description of the determination of essentiality (or beneficial effects of the element), uptake and assimilation, physiological responses of plants to the element, genetics of its acquisition by plants, concentrations of the element and its derivatives and metabolites in plants, interaction of the element with uptake of other elements, diagnosis of concentrations of the element in plants, forms and concentrations of the element in soils and its availability to plants, and soil tests and fertilizers used to supply the element. These vary slightly depending on whether elements are assimilated, or function unchanged in plants; whether they are macronutrients, micronutrients, or beneficial elements; whether or not we use fertilizers to improve their supply; and whether we know about genetic differences in their plant requirements. There is a color insert of some of the images of plants showing deficiencies or toxicities of different nutrients that appear in black and white in the chapters, some images of subcellular structures, and also some of the diagrams. In addition, there is an introduction in which the editors discuss world population growth, trends in the use of inorganic fertilizers, developments in improving the efficiency of fertilizer use, the ionic composition of plants and its manipulation,

and techniques used in plant nutrition research. The conclusion notes key points discussed in the chapters. This new handbook describes interactions between the elements in plants and highlights areas of rapid change in the study of plant nutrition and in the application of our knowledge.

The editors are grateful to the authors of the chapters in both editions for their detailed and informative coverage of topics in plant nutrition. With the world population, and the number of mouths to feed, increasing rapidly, with pollution from agricultural wastes still an environmental problem in many parts of the world, and with great interest in lowering the energy requirements of agriculture (and the greenhouse gas emissions associated with this), we hope that the second edition will provide a useful stimulus to people working to overcome these problems.

It is with sadness that we note the death of one of the contributors, Dr. Nand Kumar Fageria, during the final stages of preparation of this book. Dr. Fageria, a former senior soil scientist at Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in Brazil, was a leading contributor to work on plant nutrition, with more than 200 publications to his name, including some well-known books on crop nutrition. His chapter on potassium in this book is a detailed and informative account of current ideas on the role of this element in plant nutrition, although in truth he could have also contributed outstanding chapters on many other elements required by plants. We also note the passing of Professor Volker Römheld, who jointly contributed the chapter on iron, and Professor Konrad Mengel, who contributed the chapter on potassium to the first edition. Professor Römheld was professor of plant nutrition at Hohenheim University, and published extensively on acquisition, uptake, and physiology of micronutrients in plants. Professor Mengel was professor of plant nutrition at the Justus Liebig University, Giessen, and not only was an expert on the potassium nutrition of plants but also had a broad interest across the whole of plant nutrition. This interest was put to good use in *Principles of Plant Nutrition* (K. Mengel and E.A. Kirkby), which ran to five editions and was a stimulating introduction to the subject for many students worldwide.

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Editors

Allen V. Barker is a professor at the University of Massachusetts, Amherst, where he has taught organic farming, soil fertility, and plant nutrition for 50 years. His research has addressed nitrogen nutrition of crops with emphasis on ammonium nutrition and on the interactions of nitrogen with other elements in affecting crop growth and nutrient accumulation. He is a member of editorial boards of several journals that publish articles on plant nutrition. He wrote *Science and Technology of Organic Farming*, which is also published by CRC Press, and edited the first edition of *Handbook of Plant Nutrition* with David Pilbeam.

David J. Pilbeam has over 30 years of experience in research and teaching on plant nutrition and physiology at the University of Leeds, United Kingdom. He has published particularly on the physiology of uptake and assimilation of inorganic nitrogen by plants, but also on the accumulation of other elements. Aside from research on the physiological aspects of plant nutrition, Dr. Pilbeam has published on more agronomic aspects of plant growth and nutrition, including work on intercropping, novel crops, and agroforestry. Together with Allen Barker, Dr. Pilbeam edited the first edition of *Handbook of Plant Nutrition* in 2007. He is currently a member of the editorial board of the *Journal of Plant Nutrition*.

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Section I

Introduction

1 Introduction

Allen V. Barker and David J. Pilbeam

CONTENTS

1.1 NEED FOR EFFICIENT CROP PRODUCTION

During the past 30 years, both the size of the world population and the production of crops to feed these people have increased considerably. Based on data of the Food and Agriculture Organization (FAO) of the United Nations, production of the common crop groups increased by 47% between 1985 and 2005, although if all of the 174 crops covered in the FAO reports are considered this increase is approximately 28% (Foley et al., 2011). In this time period, the area of cropland only increased by 2.4% (although taking into account multiple cropping, the decreasing proportion of land left fallow, and decreasing incidence of crop failures, the area of cropland that was harvested increased by 7%). This means that average crop yields per unit land area increased by 20% between 1985 and 2005 (Foley et al., 2011). These yield increases were brought about by advances in crop production techniques, including in the use and application of fertilizers.

Between 1987 and 2012, the world population increased from 5 billion to about 7 billion, and the rate of population growth is such that it is estimated that by 2030 the number of people requiring food will exceed 8 billion (UN Department of Economic and Social Affairs, Population Division, 2013). Very little wilderness can be converted to croplands, and, furthermore, wild land remaining often is poorly suited to agriculture, meaning that extending agriculture across the globe would have only a small impact on overall food production. Although agriculture is responsible for emissions of greenhouse gases (GHGs), through nitrous oxide (N_2O) released from the use of nitrogenous fertilizer, methane (CH_4) from livestock nutrition, CH_4 and N_2O from manure management, CH_4 from rice growing, CH_4 and N_2O from burning of residues, energy consumption in the manufacture of fertilizers and pesticides and their application, and energy consumption in other farm operations, conversion of wildlands would give a greater emission of GHGs than would agricultural intensification (Burney et al., 2010).

Consequently, the increasing population will have to be fed through improved productivity of current cropland. It has been suggested that over the next few decades production of food will have to double, yet at the same time emissions of GHGs from the farming sector will have to fall by at least 80%, the loss of biodiversity and habitats will have to be reduced, the amount of water used in crop production will have to be lowered, and pollution by agrochemicals will have to be reduced (Foley et al., 2011). These requirements certainly produce big challenges, and also big opportunities, in plant nutrition.

1.2 USE OF FERTILIZERS

As global cereal yields increased between 1960 and 2000, matching the increase in use of N and other fertilizers, the nitrogen efficiency of cereal production (the yield of cereals/amount of fertilizer N used) declined (Tilman et al., 2002). This result matches what would be expected from the law of diminishing returns, where supplying one extra unit of nutrient at a current low rate of supply gives a higher increment of extra crop yield than supplying one extra unit of nutrient onto an already high rate (Tilman et al., 2002). This shows us that although we ought to be able to increase the efficiency of fertilizer use in productive areas, there should be big opportunities to increase crop production in areas of the world where productivity is currently low, and increased use of fertilizers will have a big role to play here. World demand for total fertilizer nutrients has been calculated to grow at an annual rate of 1.9% between 2012 and 2016, with the biggest rates of increase being in sub-Saharan Africa, South Asia, Eastern Europe, and Central Asia (FAO, 2012).

Currently, there is a big discrepancy over how much fertilizer is used in different places, and areas where yields are low typically have lower use of agrochemicals in general. In a study of a corn-based system in western Kenya in 2004–2005, inputs of N and P averaged 7 and 8 kg ha^{-1} $year^{-1}$, respectively, and with total outputs of 59 and 7 kg ha^{-1} $year^{-1}$ it can be seen that not only were there low rates of production of nitrogen- and phosphorus-containing metabolites in crops but that the nitrogen balance of the fields was negative (Vitousek et al., 2009). Data for 2009 indicate that consumption of nitrogen, phosphate, and potassium sources (excluding manures) was as low as 1.1 kg ha^{-1} of agricultural land in Angola and only 0.5 kg ha^{-1} in the Democratic Republic of Congo (World Bank, 2013), so increased use of fertilizers here should have a big impact on crop yields. Some crops have better prospects for having their yields increased by more application of fertilizers than others. Whereas global variation in yields of sorghum, millet, and groundnut seem to be explained largely by differences in climate, variability in yields of barley, sugar beet, and palm seem to relate to differences in crop management, in particular application of fertilizers and irrigation (Mueller et al., 2012). Increasing yields of corn in West Africa to 75% of the attainable yield could be achieved by removing current nutrient limitation, whereas increasing corn yields in sub-Saharan Africa to 75% of the achievable yields would require both removing nutrient deficiencies and applying irrigation (although increasing these yields to only 50% of the achievable yields mostly would require only increased application of nutrients) (Mueller et al., 2012).

Indeed, there are some areas of the world where fertilizer application rates are higher than required for maximum crop yields. One area where fertilizer overuse occurs is China (Vitousek et al., 2009; Foley et al., 2011; Mueller et al., 2012), because of the intensive nature of Chinese agriculture and government subsidies for fertilizers (Yang and Zhang, 2006). In the cereal production area of the North China Plain, annual nitrogen fertilizer applications of over 500 kg N ha^{-1} are used, and water leaching at a depth of 1.4 m in the soil profile was found to contain 12–39 mg nitrate-N L^{-1} after maize harvest (Ju et al., 2006). Similarly, in a survey of 916 orchards in northern China the average annual rates of input of nitrogen and phosphorus were 588 and 157 kg ha^{-1}, respectively, two to three times higher than fruit demand (Lu et al., 2012). However, despite the risk of groundwater pollution, and the waste of valuable nitrogen that is not being taken up by the crop plants, it should be borne in mind that the North China Plain is the area of highest agricultural productivity in China, with cereal production commonly being based on growing wheat and corn crops on the same parcel of land in a rotation that can be completed in one calendar year (Ju et al., 2006; He et al., 2010). The high use of fertilizers on both crops gives rise to waste of valuable resources, although the potential leaching of nitrate from this cereal rotation is no higher than the increase in nitrate leaching of 36 kg N ha^{-1} $year^{-1}$ that was estimated to have occurred in the United Kingdom between the early 1940s and the 1990s as annual fertilizer nitrogen use increased from 20 to 190 kg ha^{-1} and wheat yields increased from 2.35 to 5.92 t ha^{-1} (Davies and Sylvester-Bradley, 1995). Although some of the nitrogen and phosphorus applied to crops may be wasted, China has managed to feed a population that has grown

by 9 million per year between 1991 and 2010 (UN Department of Economic and Social Affairs, Population Division, 2013).

In other areas, the use of artificial fertilizers is comparatively efficient. In the United Kingdom, the annual use of fertilizer nitrogen on winter wheat between 2007 and 2011 was 188 kg ha^{-1}, approximately the same as the rate applied in the 1990s, yet wheat yields over this period averaged 7.76 t ha^{-1} (National Statistics, 2011). More than 150 years have elapsed since Liebig published his classic work on plant nutrition (van der Ploeg et al., 1999), and in many countries numerous studies since then on the relationship between crop yields and nutrient supply have enabled agronomists to accurately predict the optimum rate of nutrient supply for different crops in different fields and under different climatic conditions. It is not surprising that only small increases in demand for total fertilizer nutrients are projected for North America and Western Europe between 2012 and 2016 (FAO, 2012).

Over recent years, there has been a trend to use less fertilizer on some farms in North America and Western Europe through adopting extensive agricultural systems as a means of growing crops in a more sustainable and less polluting manner. Saving the energy costs, and preventing pollution at the same time, should give more sustainability to agriculture and horticulture. However, this can have consequences of lowered productivity, and with a world population of 7 billion rising by 80 million per year, that strategy could be risky. Also, although there are decreased GHG emissions from the lower use of fertilizers, life cycle analysis has shown that the efficient use of fertilizers to stimulate crop growth can give higher yields of crops per unit of GHG emission resulting from the manufacture and use of fertilizers and the crop production procedures than in less intensively grown crops (Brentrup and Pallière, 2008; Brentrup and Lammel, 2011). The implication here is that in terms of feeding the increasing population and lowering GHG emissions from the agricultural sector, more intensification of agriculture will be required. However, there will still be a need to lower GHG emissions and pollution arising from agriculture even further.

Another risk associated with increased use of fertilizers is that it may become increasingly difficult to maintain their supplies. Although nitrogen fertilizers may become increasingly expensive as world energy supplies become more expensive, ultimately they can continue to be synthesized indefinitely. The Haber–Bosch process for the synthesis of ammonia from atmospheric N_2 is carried out under high temperature and pressure and so has a very high energy requirement. The recent identification of an iron complex that efficiently catalyzes the conversion under the milder conditions of −78°C at 1 atm of N_2 by scientists at the California Institute of Technology in Pasadena offers possibilities of lowering energy use in the production of N fertilizers (Anderson et al., 2013; editorial, *Nature*, September 5, 2013). This should ensure a reliable supply of nitrogen fertilizers in years to come. However, production of phosphorus and potassium fertilizers relies on mined ores, and in recent years it has been suggested that phosphate supplies may be running out.

The total world reserves of phosphates are currently estimated to be in excess of 300 billion tons (U.S. Geological Survey, 2013), and with worldwide consumption of P_2O_5 in fertilizers expected to be 45.3 million tons in 2016 (U.S. Geological Survey, 2013) and these reserves are sufficient for many years of use in agriculture. Nevertheless, as reserves are utilized the reserves that remain tend to be more difficult to extract, and possibly less pure, making phosphorus fertilizers more expensive. Furthermore, many of the reserves are in parts of the world where political instability could put supplies at risk. A further hazard of using phosphorus fertilizers is that rock phosphate contains the heavy metal cadmium at concentrations between 1 and 200 mg Cd (kg P_2O_5)$^{-1}$, and this cadmium ends up in the commercial fertilizers (Smolders, 2013). Cd^{2+} is sufficiently similar to Zn^{2+} to be taken up by plants and, if ingested in crop products, has the potential to be hazardous to humans. Durum wheat grain (*Triticum durum* Desf., syn. *Triticum turgidum* L. ssp. *durum*), for example, can accumulate cadmium supplied in phosphorus fertilizers (Grant et al., 2013). In European fertilizers, cadmium can be present at up to 120 mg Cd (kg P_2O_5)$^{-1}$, although it is usually at a much lower level, so there have been proposals to limit the amount of cadmium allowed in these fertilizers (Smolders, 2013).

Global reserves of potassium are currently estimated to be 250 billion tons, with mine production of the element running at 34 million tons in 2012 and consumption expected to increase annually by 3% until 2016 (U.S. Geological Survey, 2013). Forty new potash mines are expected to be opened worldwide by 2017 (U.S. Geological Survey, 2013), so potassium supplies appear to be at low risk of diminishing.

One strategy to cope with environmental pollution from the use of fertilizers, to minimize emissions of GHGs arising from their manufacture, and to cope with decreasing availability of essential precursors is to use fertilizers more efficiently, and the wheat–corn system of the North China Plain could have its yield sustained with as little as only half of the current N fertilizer supply (Vitousek et al., 2009). Fertilizer use can be lowered through physical methods such as better forecasting of when (and where) fertilizer application would give worthwhile yield responses, better application methods, and better formulation of fertilizers to release the nutrients at the optimum time for plant growth and biological methods, such as the breeding of plants with a better nutrient-use efficiency.

Improvements in nutrient-use efficiency through better forecasting of when and where fertilizers are required can be achieved in those areas where currently fertilizer recommendations are not well developed. These areas include the productive agricultural systems of China, where work is in progress to formalize recommendations for different crops. For example, Chuan et al. (2013) used data on nutrient responses of wheat in the field between 2000 and 2011 to evaluate a new *Nutrient Expert for wheat* fertilizer decision support system that is site specific. Such systems should enable high yields to be maintained with lower inputs of fertilizers, thereby lowering some pollution problems and lessening the GHG emissions from unnecessary synthesis and application of mineral fertilizers. If there were more energy-efficient production of nitrogen fertilizers, and these were used more efficiently in the field, China could save emissions of between 102 and 357 Tg CO_2 equivalents per year, approximately equal to the emissions reduction targets for 2020 from the entire economies of some of the largest European nations (Zhang et al., 2013).

If efficiencies in fertilizer use can be gained by developing recommendation systems in productive agriculture where traditionally such systems have not existed, the opposite problem is seen in the productive agriculture of Europe. The continent currently has at least 10 soil-P tests currently in use, giving more than threefold differences in fertilizer-P recommendations for similar soil–crop situations (Jordan-Meille et al., 2012). The efficiency of P fertilizer use could be improved on a continent-wide basis through the development of a better model of soil-P availability (Jordan-Meille et al., 2012). The third fertilizer use scenario, subsistence agriculture with low levels of inputs and low yields, would benefit from even small increases in the use of fertilizers. The law of diminishing returns shows that in subsistence agriculture supply of even a small additional increment of fertilizer would give a substantial increase in crop yield.

1.3 IMPROVING CROP QUALITY THROUGH PLANT NUTRITION

As well as trying to produce heavier yields of crops, plant nutritionists are also interested in the production of crops of higher quality. Considerable interest exists currently in the production of crops that are rich in micronutrients that are in low supply in the modern human diet. For example, selenium, zinc (and possibly iron), and other micronutrients seem to be in low supply in the modern Western diet, and there is interest in using plant nutrition to increase concentrations of these elements in crop products. Worldwide, the most prevalent nutrient deficiencies in the human diet are due to shortage of iron, zinc, and iodine, with calcium and selenium also being important and magnesium and copper deficiencies giving problems in some areas and multiple micronutrient deficiencies also occurring (Ramakrishnan, 2002; White and Broadley, 2009; Stein, 2010).

It is possible to give supplements to populations under threat of these deficiencies, but increasing the concentrations of deficient elements in the plant and animal foodstuffs that they consume is a better strategy. One way this can be done is by improving the diet of people and livestock, to increase the consumption of species naturally high in elements otherwise deficient in the diet.

For example, wheat grains are typically low in selenium, whereas *Brassica* species tend to concentrate this element (Watson et al., 2012), so more consumption of brassicas in the Western diet would be of benefit.

The fact that concentrations of different elements vary between plant species has been known about for many years, but more recently research on the extent to which the elemental composition of a species is under genetic control has increased. The mineral elemental composition of plants and other organisms now has its own name, the *ionome* (Lahner et al., 2003; Salt, 2004; Salt et al., 2008). In an analysis of 21 species from 7 plant families grown in 6 plots receiving different fertilizer treatments since 1856, it was possible to distinguish between plants from different families based on their shoot mineral element concentration, irrespective of what fertilizer treatment had been applied to the individuals (White et al., 2012). The elements analyzed, in order of their importance in distinguishing between plant families, were Ca > Mg > Ni > S > Na > Zn > K > Cu > Fe > Mn > P.

This knowledge that there are genetic differences between plants in their accumulation of mineral elements has given a scientific basis to breed increased micronutrient concentration into crop species, thereby generating genetic biofortification of crop species (White and Brown, 2010). For example, Chatzav et al. (2010) investigated the concentrations of micronutrients in grains of ancestral wheat plants and found they contain up to two times the concentrations of zinc and iron of modern bread wheat. This finding suggests the possibility of breeding new wheat cultivars with higher contents of these essential elements. The Consultative Group on International Agricultural Research (CGIAR) runs the HarvestPlus program to breed and distribute developing world staple crop species rich in iron and zinc, along with a high provitamin A content (Welch and Graham, 2005; Nestel et al., 2006; Bouis and Welch, 2010). Breeding crop species with increased concentrations of essential metabolites, including trace elements, is a key policy for improving human health in many countries.

Another plant-breeding approach may be to breed antinutritional compounds out of crop plants. Phytic acid, which is not digestible by humans or monogastric farm animals, chelates calcium, zinc, and iron and is the major store of phosphate in plants. Attempts are being made to breed crops with low concentrations of phytic acid in their grains, an action that will give increased availability of essential nutrients in the diet of humans and livestock and will lower the excretion of phosphate by the farm animals (Raboy, 2009).

Although different plant species may accumulate different nutrients to different extents, there are also effects of environment on their uptake and relative proportions in plants. In a study of one natural clone and five hybrid willow (*Salix* sp.) varieties, concentrations of N, P, S, Mn, and Cu were significantly higher in a treatment with irrigation and multinutrient fertilizer than in control plants, whereas concentrations of Fe, B, Zn, and Al were significantly lower (Ågren and Weih, 2012). K, Ca, Mg, and Na did not show any differences. Despite the current attention to the ionome, in this experiment, environmental conditions (water and nutrient supply) had a bigger effect on elemental composition of the plants than genotype. There are many examples of the influence of environmental conditions on the uptake of individual elements in the following chapters in this book.

As nutrient accumulation in plants is influenced by the environment, it is obvious that low concentrations of essential elements in crop plants can also be tackled by changes in agronomic practices, in particular by changes to plant nutrition that increase the availability of scarce elements. This action has been termed agronomic biofortification (White and Brown, 2010). This practice can involve more growth of species mixtures, where the presence of one species helps make a particular micronutrient more available to a second species or provides a different range of micronutrient concentrations to grazing herbivores, more use of crop residues, farmyard manures, and composts to increase micronutrient levels in the soil, and more analysis of farmgate micronutrient balances so that particular deficiencies can be identified and rectified by the supply of micronutrient fertilizers (Watson et al., 2012).

Work on evaluating micronutrient concentrations in crop plants and then increasing amounts of essential micronutrients in the crop products by supply of micronutrient fertilizers has become

increasingly important. For example, many soils of Scandinavia have low bioavailability of selenium, so crops grown there tend to be low in this element, as are animal products arising from these crops. From 1985, the government of Finland arranged for amendment of NPK fertilizers with sodium selenate to help correct a selenium deficiency in the Finnish diet, and daily Se intake of the population increased considerably (Hartikainen, 2005). Another example is the study of iodine concentrations; iodine is not an essential or beneficial element for plants, but it is essential for humans, so studies have investigated the uptake and accumulation of iodine by plants in order to improve human nutrition (e.g., Smoleń and Sady, 2012).

Another reason for low availability of micronutrients is because soil stocks have become depleted as yields of crops removed from the land have increased following the increased use of NPK fertilizers. This depletion of micronutrients in the soil is often referred to as nutrient mining, and it poses a threat not only to the nutritional quality of the crop products harvested but also to the overall yields of crops (Moran, 2011). Nutrient mining is likely to be more of a problem in old weathered soils (e.g., in Australia and Southeast Asia) than in soils formed more recently (e.g., those formed from volcanic activity or glaciation in Northern Europe and North America) (Jones et al., 2013). Indeed, in a survey of 132 soils in the United Kingdom, there appeared to be no biologically significant decreases in Zn, Cu, and Mn since the National Soil Inventory was drawn up 30 years ago (McGrath et al., 2013). In contrast, although cereal yields have increased markedly in India since 1960, many soils there are now deficient in potassium or sulfur, and also at least one of boron, iron, copper, or manganese (Jones et al., 2013). Where soil concentrations of micronutrients are below a critical threshold, the threat to crop production arises because, as described in Liebig's law of the minimum, a shortage of one nutrient limits the growth of crops even if the other nutrients are available at sufficiency levels (Moran, 2011).

The Law of the Minimum, as originally postulated by Sprengel and promoted by Liebig, indicates that plant growth should be limited by whichever nutrient resource is in most limiting supply for plant growth. However, more recently, the multiple limitation hypothesis (Bloom et al., 1985; Gleeson and Tilman, 1992) has postulated that plant growth is constrained by many limitations simultaneously, so that plants trade off one resource to acquire another resource that is in limited supply. For example, a photosynthesizing plant that grows in nutrient-poor soil is able to use some of its assimilated carbon to support mycorrhizal fungi that make phosphorus available to the plant.

Indeed, Ågren and Weih (2012) pointed out that although there tends to be a linear relationship between internal concentrations of nitrogen or phosphorus (and also sulfur and manganese in their experiments) and relative growth rate (RGR) across the range of concentrations of these elements that plants accumulate, for other essential and beneficial elements plants frequently accumulate amounts above the concentration at which there ceases to be any relationship with RGR. This does not fit the law of the minimum. Further modeling of the relationship between supply of nitrogen and phosphorus to plants and their rate of growth indicates that if both of the elements are limiting the supply of an additional increment of only one of them will increase RGR; resources are presumably used to make the other element more available, and the multiple limitation hypothesis seems to hold (Ågren et al., 2012). According to the model, this situation seems to occur only at concentrations of nitrogen or phosphorus close to the optimum for plant growth, and at other concentrations the Law of the Minimum is more realistic.

Even if some elements can accumulate in plants without stimulating additional plant growth, they can still be beneficial for growth processes to the extent that growth would be limited if they are not available at all. We have known about the essentiality of the most well-known nutrients for some time, and we have a reasonably good idea about why they are essential. This volume delineates the current state of our knowledge in this respect. However, as analytical techniques become more sensitive, and it becomes possible to increase the purity of water for growing control plants in experiments, more elements are found to be essential. In the first edition we included nickel, which had been found to be essential for plant development not long before publication. Various elements had recently been classified as being beneficial, and this relatively new category of plant nutrient

(aluminum, cobalt, selenium, silicon, sodium, and vanadium) was also included. Although these elements are generally regarded as having physiological functions (Pilon-Smits et al., 2009), some authors doubt whether beneficial elements really do give clear benefits for plant growth. Such doubt is clearly discussed for aluminum in Chapter 16. Since the first edition, there has been convincing research showing the beneficial nature of lanthanides (rare earth elements), and these are covered in this second edition.

1.4 METHODS OF RESEARCH IN PLANT NUTRITION

Research in plant nutrition takes advantage of new techniques to move in directions that were not possible in earlier times and to check the accuracy of earlier findings. Detailed discussion of techniques currently used to investigate concentrations of different ions in plants is given by Conn and Gilliham (2010). Many of the techniques used are also listed in the chapters of this book.

Recent methods for following the uptake of ions by plants involve the use of planar optode technology. This is a noninvasive technology that uses reversible changes in fluorescence of fluorophores specific for particular analytes, so by capturing an image of part of a root, a whole root, or a root system and then from measuring change in fluorescence density or fluorescence decay time, a quantitative picture of changes in the amount of an analyte round the root can be built up (Blossfeld, 2013). The technology so far mostly has been used for looking at changes in concentration of rhizospheric oxygen, ammonium, and pH and can give confirmation or rejection of previous studies using microelectrodes (Blossfeld, 2013). This procedure is a quantitative equivalent of the dye techniques used to visualize the uptake of different nutrients and pH changes round roots of plants growing in gel in the laboratory of Horst Marschner in Hohenheim in the 1980s. Another visualization technique, used to measure cytosolic concentrations of micronutrient ions, is that of Förster resonance energy transfer (FRET). A zinc-binding domain flanked by two fluorescent proteins that overlap spectrally was genetically expressed in the root cells of arabidopsis (*Arabidopsis thaliana* Heynh.), and where there was no zinc present the proteins interacted by FRET to give fluorescence, whereas binding to zinc altered their position relative to each other so that the fluorescence was reduced in proportion to zinc concentration (Lanquar et al., 2014).

Earlier studies looked at the uptake of isotopically labeled forms of plant nutrients, and work has inevitably followed to make images of such uptake in real time. Beta (β) particles have high energy and travel some distance through biological samples, so it is possible to monitor the movement of β-emitters such as ^{35}S, ^{45}Ca, ^{55}Fe, ^{32}P, and ^{33}P in whole plant/soil systems in a quantitative manner (Kanno et al., 2012). Other workers have used stable isotopes. For example, Metzner et al. (2011) fed $^{26}MgCl_2$, ^{41}KCl, and $^{44}CaCl_2$ to cut stems of common bean (*Phaseolus vulgaris* L.) and then followed the movement of the stable Mg^{2+}, K^+, and Ca^{2+} ions from the xylem vessels and into the surrounding cells by secondary ion mass spectrometry (SIMS) after freezing the plant material and checking the integrity of the frozen samples by scanning electron microscopy.

A less direct method of observing the uptake of an element is to accurately visualize the microenvironment of the rhizosphere and then to estimate the uptake of an element based on the overall soil concentration of that element and known parameters of its uptake into isolated roots. Accurate pictures of the relationship between root hairs and soil particles could theoretically be made by x-ray microscopy. However, unless x-rays with sufficient energy can be generated, the pictures taken of the changing concentration must have a long exposure and a resolution that is lower than allows for growing root hairs to be seen clearly. This need has led to the use of synchrotrons. A synchrotron source produces radiation that is more intense (it has a greater brilliance) than conventional x-ray tubes. The rays tend to be more parallel (it has high collimation). It has energy ranges from the infrared through high energy x-rays. It is highly polarized and is pulsed (enabling it to be used in studies carried out on a time-resolved basis) (Lombi and Susini, 2009). A synchrotron can generate very high energy x-rays, allowing images to be captured with as short an exposure time of 5 min and giving a resolution of about 1 μm, which is suitable for investigating uptake into growing root hairs.

Keyes et al. (2013) used synchrotron-produced x-rays and a tomographic microscope to visualize the arrangement of soil particles in one plane around root hairs of wheat growing in soil. From this, they were able to model the flux of P across the soil particles and its uptake by the root hairs, based on the P adsorption and desorption characteristics of the soil and uptake kinetic parameters from the literature. Uses of a range of synchrotron techniques in the study of soils, plants, and their interaction in the rhizosphere are discussed by Lombi and Susini (2009).

Observation of uptake of amino acids has been carried out by the use of quantum dots. Different nanoparticle chromophores can be attached to amino or carboxyl groups of organic compounds, so if quantum dots are bound to amino acids and these are taken up, they can then be detected inside the plant. Whiteside et al. (2009) fed arbuscular mycorrhizal annual bluegrass (or annual meadow grass, *Poa annua* L.) grown in sand–vermiculite, and also in a minirhizotron, with glycine labeled with quantum dots on its amino groups, and saw uptake into the fungi and plants. This technique obviously is specialized for investigating the uptake of organic nitrogen into plants, and it is not without problems as Al-Salim et al. (2011) did not see the uptake of quantum-dot-labeled glycine into ryegrass (*Lolium perenne* L.), onion (*Allium cepa* L.), or arabidopsis grown in solution. None of these plants had formed mycorrhizal associations, but arabidopsis never forms mycorrhizal associations yet amino acid uptake has been seen in this species (see Chapter 2).

Not only has it been difficult up to now to quantify the uptake of different nutrients into different parts of root systems growing in solid media, it has sometimes proved problematic to merely visualize morphological changes in root systems in response to nutrient supply. Studies of root responses to deficiencies of specific nutrients have often been carried out on plants grown in agar gel, yet the gelling agent used can contribute nutrients to the plants that make observations on root responses to deficiency of dubious reliability. Recent research has identified suitable agar and agarose sources for use as solid media to study the effects of deficiencies of nine different elements on root characteristics in arabidopsis (Gruber et al., 2013).

One extremely valuable resource in plant nutrition research is the availability of long-term field sites. New techniques can be developed at regular intervals, but the ability to be able to sample plants from plots that have particular nutrient applications over long periods of time is priceless regardless of the techniques used for analysis (Rasmussen et al., 1998; Peterson et al., 2012). Examples include the Park Grass and Broadbalk wheat experiments at Rothamsted, United Kingdom (e.g., Jenkinson et al., 2008; White et al., 2012); the Morrow Plots in Illinois, United States (e.g., Nafziger and Dunker, 2011); and the Rengen Grassland Experiment in Germany (e.g., Hejcman et al., 2010). Long-term experiments are always difficult to finance, as even if they are established by governments or private donors there has to be an ongoing commitment to financial support on an annual basis. The ideal way to manage these experiments is to include storage facilities for plant and soil samples that are taken from the experiment, so that future generations can go back and reanalyze samples in the light of advances in analytical techniques. At the very least, efforts should be made to preserve the actual plots of long-term experiments and to find means of financing their ongoing maintenance, as these are resources that cannot be replaced in a short time once they are lost.

Research in plant nutrition also changes as developments in other disciplines influence the interests of researchers. One of the major areas of research in plant nutrition currently is the genetics of nutrient acquisition and assimilation. The principle of genetic biofortification has been discussed earlier, but as well as increasing concentrations of essential micronutrients in crop products it may be possible to increase the responses of plants to nutrient supply in terms of their overall yields. This possibility has led to considerable interest in understanding the genetic control of expression of genes coding for different transporters and different assimilatory enzymes in plants. As well as following changes in expression of genes for different transporter proteins as a plant is exposed to varying concentrations of a particular nutrient, another approach is to identify quantitative trait loci (QTLs) for broader characteristics, such as nutrient-use efficiency, and then, through searching databases for similar DNA sequences, to identify what the genes in these loci are (e.g., Chardon et al., 2012).

These genes can then be overexpressed or deleted in the target species to further investigate their importance in plant nutrition. These modifications lead to the possibility of breeding similar changes to the gene into crop species in order to improve their agronomic performance. However, many of the traits that could be improved by breeding have multigene control, which makes the problem challenging for the breeders.

Many of the characteristics required to improve the conversion of nutrients into crop yield may not be obvious, so care has to be taken in screening plants for suitable attributes and then breeding them into crops. Research programs on the efficiency of use of individual nutrients by different genotypes of plants, particularly under conditions of low nutrient supply, are currently common and should help refine our views of what genes in plants need to be manipulated so that we can achieve bigger crop yields with lower rates of application of fertilizers.

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Section II

Essential Elements: Macronutrients

2 Nitrogen

David J. Pilbeam

CONTENTS

2.1 INTRODUCTION

Crop plants typically are comprised of C, H, O, N, and then other elements, in that order, so it is obvious that nitrogen is required by plants in large amounts. The fact that nitrogen is taken up by plant roots was shown by de Saussure in 1804, who demonstrated that an inorganic nitrogen source such as nitrate is essential for plant growth (Hewitt, 1966). From these facts, it follows that as plants have a high nitrogen requirement there are many situations in which its supply could limit crop growth, and amending soils and growing media with additional nitrogen can increase yields.

The earliest sources of nitrogen to increase crop growth were animal excreta and plant remains, and analysis of $\delta^{15}N$ in cereal grains and pulse seeds from Neolithic sites across Europe showed that farmers have used manures for at least 8000 years (Bogaard et al., 2013). The Roman writer Cato the Elder, writing a farming manual *circa* 160 BC, gave advice on how to use manure, including which crops needed to be sown in well-manured or naturally strong soil (turnips, kohlrabi, and radish). He also was aware that lupins, beans, and vetch were useful for enhancing the yields of cereals (Hooper and Ash, 1934). We had to wait until the nineteenth century when the German chemists Carl Sprengel and Justus von Liebig realized that the nitrogen in animal manures can be supplied

to plants as ammonium ions in inorganic fertilizers (van der Ploeg et al., 1999) and until later that century when Hellriegel and Wilfarth showed that legume root nodules are able to carry out the conversion of atmospheric dinitrogen to ammonium (Nutman, 1987).

2.2 UPTAKE OF NITROGEN BY PLANTS

Despite the abundance of dinitrogen (N_2) gas in the atmosphere, only plants in the Fabaceae and a few other families are able to utilize this as their N source. For most plant species, the major N forms available to them are nitrate (NO_3^-) and ammonium (NH_4^+).

In experiments where NO_3^- or NH_4^+ is supplied as the sole N source, many plants tend to grow better with the NO_3^-. The exception is plants adapted to acid soils, soils that contain a higher concentration of NH_4^+ than NO_3^- and where NH_4^+ uptake predominates. As acid soils are infrequent in agriculture, it might seem that the NO_3^- ion is the major N source for most crop plants (except rice).

More detailed investigation of this assumption showed that with low N supply to wheat (*Triticum aestivum* L.), the rate of growth was approximately similar, irrespective of whether NO_3^- or NH_4^+ was supplied, or even slightly faster with NH_4^+ than with NO_3^- (Cox and Reisenauer, 1973). However, yields became saturated at concentrations of NH_4–N at which NO_3–N was still giving increased growth, and at even higher NH_4–N supply growth was inhibited. This response usually is referred to as *ammonium toxicity*. The growth rate eventually reached a plateau at higher NO_3^- concentrations, but if the high-NO_3^- nutrient solution was then supplemented with low rates of NH_4^+ there was further stimulation of plant growth (Cox and Reisenauer, 1973). It can be concluded that at low rates of supply either NH_4–N or NO_3–N gives equal amounts of wheat growth, or slightly better growth with NH_4–N; at higher rates of N supply NO_3^- gives noticeably better growth; but the best growth comes from a high rate of supply of NO_3^- supplemented with a small amount of NH_4^+.

These two ions interact for uptake, and the presence of NH_4^+ in the rooting medium slows the uptake of NO_3^- (e.g., Taylor and Bloom, 1998), although the presence of NO_3^- does not affect NH_4^+ uptake. This interaction is affected by external pH, and at acid pH, NO_3^- uptake tends to be much higher than uptake of NH_4^+, whereas at more neutral pH the rates of uptake of both ions are similar (Michael et al., 1965). There is also a temperature effect, and uptake of NO_3^- is depressed more by low temperature than is the uptake of NH_4^+ (Clarkson and Warner, 1979). Uptake of NO_3^- follows a diurnal pattern, with the fastest rate occurring during the light period then declining in the dark (Pearson and Steer, 1977; Matt et al., 2001).

2.2.1 Nitrate

Movement of NO_3^- into roots occurs through different systems that work at different concentrations of the ion in the external medium. Siddiqi et al. (1990), working on barley (*Hordeum vulgare* L.) seedlings, demonstrated that at external concentrations of NO_3^- between 0 and 0.5 mol m^{-3} there were two different uptake systems showing Michaelis–Menten kinetics, and therefore dependent on an energy source to pump NO_3^- into roots against its concentration gradient. In contrast, when the seedlings were supplied with NO_3^- between 1 and 50 mol m^{-3} its rate of uptake showed a linear relationship with external concentration, indicating uptake by means of passive diffusion.

There are transmembrane transporter proteins in plants that act as NO_3^-/H^+ symporters and move NO_3^- ions into and between cells, and even within one plant species there are different forms of these transporters depending on whether they are in the plasmalemma or in the tonoplast of roots or shoots and also on the developmental stage of the plant. The transporters are divided into three families, NRT1, NRT2, and NRT3.

In arabidopsis (*Arabidopsis thaliana* Heynh.), there are two high-affinity nitrate transport systems (HATS) that work at low external NO_3^- concentrations (1 mmol m^{-3} to 1 mol m^{-3}) (Miller et al., 2007). These two systems are a constitutive form (cHATS) that is present all the time, even

when the plant is not exposed to NO_3^-, and a form that is induced when the plant is exposed to the ion (iHATS). There is one low-affinity nitrate uptake system (LATS), which is constitutive and works on external NO_3^- concentrations above 1 mol m^{-3}. Soil concentrations of NO_3^- are typically above this value, at least in agricultural soils.

Generally, LATS is composed of NRT1 transporters, although one of its constituents in arabidopsis, NRT1.1 (a member of the NRT1 family), is a dual-affinity transporter that is active at both low and high external NO_3^- concentrations. This dual activity is regulated by changes to a threonine residue. In a dephosphorylated state, the protein is a low-affinity transporter, but with low nitrate supply the threonine is phosphorylated, and the protein becomes a high-affinity transporter (Liu and Tsay, 2003). NRT2 transporters, such as NRT2.1 in arabidopsis, are high-affinity transporters. The *AtNRT2.1* gene is induced by NO_3^- and is repressed by metabolites of N assimilation, so AtNRT2.1 seems to be involved in iHATS. However, it also appears to have a role in cHATS (Miller et al., 2007). Expression of the *NRT2.1* gene is repressed by NH_4^+ if NO_3^- supply is also high, which may account for the inhibitory effect of NH_4^+ on NO_3^- uptake (Krouk et al., 2006).

The activity of NRT2.1 depends on another protein, NAR2.1 (NRT3.1). Together, they form a complex in the plasmalemma, probably a tetramer comprising two molecules of each (Yong et al., 2010). At least three other transporters are involved in HATS in arabidopsis (NRT1.1, NRT2.2, and NRT2.4), but NRT2.1 seems to make the predominant contribution as mutants with no *NRT2.1* gene expression have as little as 25% of the normal high-affinity uptake and cannot grow when NO_3^- is below 1 mol m^{-3} and is the sole N source (Laugier et al., 2012). Like NRT1.1, this protein is regulated by posttranscriptional control as well as by transcription and represents a point at which the N demand of the whole plant can regulate the rate of NO_3^- uptake (Laugier et al., 2012). Not only can the amount of NRT2.1 transporter protein be regulated by external or internal N concentrations affecting the expression of the *NRT2.1* gene, but activity of existing molecules of the transporter can be regulated very quickly.

These changes to the transporters are important in regulating plant responses to changes in NO_3^- supply. In N-starved barley seedlings, supply of NO_3^- gave immediate uptake (due to constitutive mechanisms), and then there was an induction of uptake that was linked to synthesis of proteins (Siebrecht et al., 1995). Tomato root *NRT1.2* and *NRT2.1* are both upregulated quickly on resupply of NO_3^- to N-deficient plants, an upregulation that lasts for 24 h (Wang et al., 2001). With normal supply to arabidopsis, there is usually high expression of *NRT2.1* in the roots and low expression of the gene for another high-affinity transporter, NRT2.4 (Kiba et al., 2012). Removal of the nitrate source gave a short-term increase in *NRT2.1* expression and then a reversal to the original level by 3 days. However, there was a very large increase in expression of *NRT2.4* in the roots, and it also became detectable in the shoot (Kiba et al., 2012). The NRT2.4 transporter seems to become more important in times of N starvation.

Different parts of the root system express different nitrate transporter genes. The *NRT1.1* gene is expressed in the epidermis at the root tip, allowing synthesis of the NRT1.1 transporter to take up NO_3^- at that point, but is also expressed in the cortex and endodermis further back in the root (Huang et al., 1996). The *NRT1.2* gene in arabidopsis is expressed in the root hairs and epidermis (Huang et al., 1999), allowing low-affinity uptake to occur from the root tip back to the root hair zone. The expression of *NRT2.4* in N-starved plants occurs in the lateral roots, in the epidermal cells, and in young parts of the primary root, whereas NRT2.1 seems to occur in the old parts of the primary root irrespective of whether nitrate supply is abundant or deficient (Kiba et al., 2012). In these more mature parts of the root, the *AtNRT2.1* gene is expressed in the epidermis and cortex (Nazoa et al., 2003). The uptake rate in barley is higher in middle and old zones of the roots than in the tips, an action that could be linked to the fact that the old root cells are vacuolated and have a site to store the NO_3^- taken up (Siebrecht et al., 1995). In corn (*Zea mays* L.), likewise, the uptake rate of NO_3^- is higher in old parts of the root than at the tip (Taylor and Bloom, 1998), although in all parts of the root uptake seems to exceed local demand (Bloom et al., 2012). Up to 60% of the NO_3^- taken up by corn seems to enter through the secondary roots, with much of the remaining

uptake occurring into the basal regions of the primary root; the NO_3^- entering the secondary roots is translocated quickly to the shoot (Lazlof et al., 1992).

In the experiments of Siebrecht et al. (1995), although much of the NO_3^- taken up in the root tips was assimilated locally, some was exported back to the old parts of the roots and was able to induce nitrate uptake mechanisms there. This action gives a role to the root tip as a sensor of NO_3^- in the rooting environment. Further evidence for this response was found in N-depleted maize roots, where rapid induction of iHATS occurred if NO_3^- was supplied, particularly in the tip region, although uptake reverted to the background level after approximately 24 h (Sorgonà et al., 2011). This action seemed to involve induction of the NRT2.1 transporter.

Once NO_3^- is taken up into root cells, some is assimilated there, some is moved across the tonoplast into the vacuoles (in the old parts of the roots, where vacuoles occur), and much of it is moved to adjacent cells and ultimately into the xylem and up to the shoot. The accumulation of NO_3^- in vacuoles (in root and leaf cells) is facilitated through CLCa anion channels in the tonoplast (De Angeli et al., 2006). Although the CLC family were originally named as being chloride transporters, CLCa gives much greater permeability of the tonoplast to NO_3^- than to Cl^- (De Angeli et al., 2006).

The low-affinity NRT1.5 transporter present in the plasmalemma of the pericycle cells around the protoxylem loads NO_3^- into the xylem, although this action is not the only mechanism for nitrate loading (Lin et al., 2008; Dechorgnat et al., 2011). In barley and corn, other xylem-loading systems have been identified, including the general anion channels xylem parenchyma quickly activating anion conductance (X-QUAC) and xylem parenchyma inwardly rectifying conductance (X-IRAC); of these, X-QUAC seems to be the most important in nitrate loading (Köhler and Raschke, 2000; Köhler et al., 2002; Gilliham and Tester, 2005). As well as being expressed in epidermal and cortical cells of roots, the *AtNRT2.1* gene is expressed in the endodermis (Nazoa et al., 2003).

Some crop plants with a high N requirement increase the hydraulic conductivity of their root systems in response to increase in NO_3^- concentration in the root environment (Clarkson et al., 2000). Nitrate is water soluble and moves toward roots in the bulk flow of water through the soil driven by the pull of transpiration, so there could be a link between uptake of NO_3^- and water. The change in hydraulic conductivity is quick, it seems to work on cell membrane hydraulic properties, and it involves aquaporins (Gloser et al., 2007; Górska et al., 2008a). Aquaporin genes were upregulated in roots of N-deficient tomato (*Solanum lycopersicum* L. syn. *Lycopersicon esculentum* Mill.) plants resupplied with NO_3^- (Wang et al., 2001), and under such conditions, the activity of existing aquaporins may increase (Górska et al., 2008b). It is also possible that an increase in hydraulic conductivity arising from a high-nitrate zone in the soil may allow NO_3^- to move between parallel xylem vessels due to differences in water potential breaking down sectoriality and allowing some lateral movement across the root before movement up to the shoot (Thorn and Orians, 2011).

In many plants, hydraulic conductivity of the roots is much higher with NO_3–N than with NH_4–N, and expression of aquaporins seems to be higher in nitrate-supplied plants (Guo et al., 2007). However, in rice hydraulic conductivity is higher with NH_4^+ than with NO_3^- (Gao et al., 2010). It may be that in plants in which nitrate is the main N source, response to localized NO_3^- supply is also a response to availability of water. Increase in root hydraulic conductivity in response to increased NO_3^- supply seems to be higher in fast-growing species, and the ability to use rapid changes in root hydraulic conductivity to make the most of NO_3^- available by bulk flow may be important for plants with a high-nitrate requirement (Górska et al., 2010).

There were shown to be peaks of flux of NO_3^- into roots of dwarf maize (*Zea mays*) from 50 to 250 mmol m^{-3} concentration (within the HATS range), one during early vegetative growth and one just before flowering (Garnett et al., 2013). The rates of flux matched total plant uptake, indicating that HATS can meet requirements of nitrate for growth throughout the life of a plant without involvement of LATS. Of all the *NRT* genes, *ZmNRT2.1* and *ZmNRT2.2* were expressed the most in the roots, and changes in the levels of their transcripts matched the NO_3^- uptake patterns (Garnett et al., 2013).

2.2.2 Ammonium

The pK_a value for the dissociation of ammonia (NH_3) dissolved in water into ammonium (NH_4^+) and hydroxyl (OH^-) ions is 9.25, so at normal soil and cellular pH a plant is exposed predominantly to the NH_4^+ ion. Although NH_3 is able to permeate through membranes, NH_4^+ is taken up by means of specific transporters, the ammonium transporter (AMT) family of proteins. These are split into the AMT1, AMT2, AMT3, and AMT4 subfamilies (Koegel et al., 2013).

In lowland rice (*Oryza sativa* L.), a crop plant that is adapted to anaerobic soils where NH_4^+ is the predominant N species, there are three members of each of the AMT1, AMT2, and AMT3 subfamilies and one member of AMT4 (Gaur et al., 2012). Members of the AMT1 subfamily comprise a high-affinity uptake system (HATS), and as several AMT1 genes are expressed at any one time it seems that NH_4^+ uptake occurs through several transporters (Mota et al., 2011). AMT1.1 gives approximately 30% of the NH_4^+ uptake in arabidopsis roots (Camañes et al., 2012) and works best at low external NH_4^+ concentration. In fact, a period of N starvation gives increased expression of *AtAMT1.1* in the roots and decreased expression of the gene in the shoots (Engineer and Kranz, 2007). Expression of the *AMT1.1* gene also is upregulated by N starvation in rice roots, as are *OsAMT1.2*, *OsAMT3.2*, and *OsAMT3.3* (Li et al., 2012). Higher external ammonium supply leads to the phosphorylation of a key threonine residue in the AMT1.1 protein, giving a slower rate of transport. This phosphorylation seems to be controlled directly by the NH_4^+ itself, so AMT1.1 works as a transceptor (a transporter and a receptor) that detects high soil concentrations of NH_4^+ and lowers its rate of uptake (Lanquar et al., 2009). This act may prevent accumulation of toxic NH_4^+ ions in the roots.

Expression of the *AMT1.1* gene in rice roots is downregulated on the supply of nitrate to N-starved plants, as is *AMT1.2* (Li et al., 2012). In arabidopsis, expression of the gene increases the rate of NO_3^- uptake, and conversely expression of the *NRT2.1* gene increases the rate of NH_4^+ uptake (Camañes et al., 2012). Arabidopsis may grow better with a balanced N supply. AMT1.3 appears to function as a transceptor, and a component of HATS, in rice (Gaur et al., 2012). Its expression is downregulated by N starvation and is upregulated by resupply of NH_4^+ (Li et al., 2012).

There is a low-affinity system that works best at high external pH, and this action could be due to undissociated NH_3 crossing the membranes (and may explain the higher uptake of ammonia/ammonium relative to nitrate at high pH). However, *AMT2* genes seem to be involved in a LATS (Gaur et al., 2012). Ammonium influx occurs at a relatively uniform rate along a corn root from tip to the base of the zone of root growth and is then higher at more basal regions (Taylor and Bloom, 1998), but growth of the tissues at the root tip seems to also rely on NH_4^+ or its precursors taken up further back (Bloom et al., 2012). If NH_4NO_3 is supplied to corn, the NH_4^+ taken up at the root tip is used preferentially there, and the NO_3^- taken up remains unassimilated (Bloom et al., 2012). The *AMT2* gene is upregulated quickly on the resupply of NO_3^- to N-deficient tomato roots, and this upregulation remains for 24 h (Wang et al., 2001).

2.2.3 Organic Nitrogen Forms

It is likely that plants may be able to take up quaternary ammonium compounds as intact molecules (Warren, 2013b). They can also take up urea, in pathways similar to those involved in uptake of NH_4^+ (Mérigout et al., 2008; Witte, 2011). In arabidopsis, there is an active system that transports urea into roots at external concentrations up to 50 mmol m^{-3}, but at concentrations 10 times higher the rate of uptake is related linearly to external concentration and therefore occurs by passive diffusion (Wang et al., 2012b). Urea also is taken up through leaves, and the N derived from its application to leaves of potato (*Solanum tuberosum* L.) was found in the tubers within 48 h (Witte et al., 2002). Genes expressing high- and low-affinity urea transporters have been identified, and detection of ^{15}N-labeled urea in arabidopsis roots after supplying ^{15}N-urea for 5 min seems to confirm that it is taken up as an intact molecule (Mérigout et al., 2008).

One transporter involved is DUR3, a high-affinity transporter with an affinity constant for urea of 3–4 mmol m^{-3} (Kojima et al., 2007). It is expressed in the root epidermal cells, including in root hairs, and also near root xylem and in the shoots, indicating a role in uptake and in transport of urea within a plant (Kojima et al., 2007). Its activity increases under N deficiency (Arkoun et al., 2013) and decreases after resupply of NO_3^- or NH_4^+ (Kojima et al., 2007), and as DUR3 is a high-affinity transporter it seems that its presence is an adaptation to enable plants to use the urea present in soils otherwise deficient in nitrogen but not in soils high in mineral N (Kojima et al., 2007). Supply of NO_3^- with urea seems to increase the rate of uptake of the urea (Garnica et al., 2009). In rice, seedlings grown on urea have less shoot growth than seedlings grown on NH_4–N or even NO_3–N alone (Wang et al., 2013a).

Plants take up amino acids, with several systems being shown to be important in arabidopsis. The lysine histidine transporter 1 (LHT1) is responsible for the uptake of neutral and acidic amino acids, and amino acid permease 1 (AAP1) absorbs the acidic amino acid glutamate, the basic amino acid histidine, and neutral amino acids (Lee et al., 2007), and AAP5 is responsible for the uptake of the cationic amino acids L-lysine and L-arginine (Näsholm et al., 2009; Svennerstam et al., 2011). LHT1 from arabidopsis has K_m values for proline, glutamate, and histidine of 10, 13.6, and 362 mmol m^{-3}, respectively (Hirner et al., 2006). There are other amino acid transporters in plants, although many are doubtless involved with transport within the plants, with 67, 134, and 96 genes involved in amino acid transport having been identified in arabidopsis, black cottonwood (*Populus trichocarpa* Torr. and A. Gray), and rice, respectively (Näsholm et al., 2009). LHT1 is present in the epidermis of emerging roots and lateral roots of arabidopsis, but not in the main root (Hirner et al., 2006). Its expression is induced if plants are grown with amino N rather than inorganic N (Hirner et al., 2006). AAP1 is present in the plasmalemma of the arabidopsis root epidermis and the outer layer of the root cap (Lee et al., 2007).

Arabidopsis plants take up L-amino acids preferentially over D-amino acids, with L-arginine being taken up from a mixture of 10 amino acids and NH_4^+ at two-thirds of the rate of NH_4^+ and with glycine, L-alanine, L-serine, L-valine, and L-isoleucine being taken up at approximately one-third of that rate (Forsum et al., 2008). Uptake of L-glutamine and L-asparagine occurred at a slightly slower rate, but as they contain two NH_2 groups they represented a good source of N for the plants. Indeed, these two amino acids gave the best growth of the plants if the 11 N sources were supplied individually, and they also gave a significantly greater biomass if supplied with NO_3^- than plants grown with NO_3^- on its own (as did L-aspartate) (Forsum et al., 2008).

Many amino acid uptake studies have been carried out at concentrations much higher than those occurring in soil, and there has been little quantification until recently of the extent to which amino acids could meet plant N requirements (Näsholm et al., 2009). This assessment is difficult, as where ^{15}N amino acids are supplied to soils the ^{15}N could be released as inorganic N during mineralization and then taken up in that form. Supply of dual (^{13}C, ^{15}N)-labeled amino acids gives some idea if individual molecules are taken up intact and conjugating glycine with fluorescent quantum dots has enabled the movement of the intact conjugate into hyphae and roots and shoots of annual bluegrass (*Poa annua* L.) to be measured (Whiteside et al., 2009), but it is still difficult to evaluate the potential contribution of all the amino acids in a soil. It was shown that supplying N to perennial ryegrass (*Lolium perenne* L.) in hydroponic culture as an equimolar mixture of NH_4^+, NO_3^-, and glycine gave an increased proportion of the N being taken up as glycine compared with what would have been predicted from supplying the three N forms individually. The rates of uptake of NH_4^+ and NO_3^- were noticeably lower in the mixture, whereas the glycine uptake was only slightly lower (Thornton and Robinson, 2005). Five plant species in a seminatural temperate grassland were shown to take up ^{15}N-labeled ammonium nitrate more than ^{15}N-labeled amino acids (Harrison et al., 2007).

Some plants can take up intact peptides, and wheat has been demonstrated to take up L-trialanine only marginally slower than NH_4^+, although D-tripeptides are taken up more slowly (Hill et al., 2011). In an annual, arabidopsis, and a perennial, angled lobelia (*Lobelia anceps* L. f.), peptides of four amino acid residues in length are taken up (Soper et al., 2011). In arabidopsis, the PTR1

transporter (peptide transporter 1) expressed in the roots seems to carry out the uptake of dipeptides (Komarova et al., 2008). The supply of small peptides in the soil solution also may be increased by plants themselves, as leek (*Allium porrum* L.) seedlings were shown to release into the nutrient solution proteases that degrade proteins to low-molecular-weight peptides (Adamczyk et al., 2009). Some plants seem to use proteins more directly. Arabidopsis, a species from nutrient-rich environments, and mulloway needlebush (*Hakea actites*), a species that grows in nutrient-poor heathland and forms cluster roots, were shown to have proteolytic activity on the root surface and possibly in the root apoplast. They also seemed to have the ability to take up proteins into their root hairs and into cortical cells of roots with root hairs (possibly by endocytosis) (Paungfoo-Lonhienne et al., 2008). However, the plants did not grow nearly as well with protein as the sole N source as with ammonium nitrate, but protein seemed to be a valuable supplemental source of nitrogen to plants grown in mixed protein and inorganic N sources.

The uptake of amino acids by plants may involve mycorrhizas, and several amino acid transporters have been identified in mycorrhizal fungi (Näsholm et al., 2009). In ectomycorrhizal symbioses, the amino acids and peptides have been known for some time to be taken up by the fungus with the N being passed to the plant (Melin and Nilsson, 1953). However, ectomycorrhizal fungi also increase the uptake of NH_4^+ and NO_3^- by their host plant (Plassard et al., 1991, 1994). In a study of 10 ectomycorrhizal fungal species, NH_4^+ was generally a more suitable N source than NO_3^- (Finlay et al., 1992). Some ectomycorrhizal fungal species can live with an N source of either peptides or proteins (Abuzinadah and Read, 1986), and the production of extracellular proteinases has been demonstrated in ectomycorrhizal fungi (Maijala et al., 1991). In ericoid mycorrhizas (symbioses between fungi and plants in Ericaceae in northern hemisphere tundra and heaths and in the understory of boreal forests), the fungi seem to be at least partly responsible for the uptake of not only amino acids into the plants but also NH_4^+ (Rains and Bledsoe, 2007) and NO_3^- (Grelet et al., 2009). Ericoid mycorrhizal fungi also produce extracellular proteinases (Leake and Read, 1990).

Most crop species form arbuscular mycorrhizas (AMs), and being mycorrhizal is their normal state (Smith and Smith, 2011). AM fungal species can take up NO_3^-, NH_4^+, and glycine and pass the N onto plants (Hawkins et al., 2000). However, unlike ectomycorrhizas and ericoid mycorrhizas, AMs are thought to be unable to release the N in complex organic molecules (Smith and Smith, 2011), although in the experiments of Whiteside et al. (2009) annual bluegrass (*Poa annua* L.) plants received glycine from uptake via AM fungi. Ammonium seemed to be the N form taken up most readily into the AM hyphae in the experiments of Hawkins et al. (2000), and in experiments on AM pygmy cypress (*Cupressus pigmaea* Sarg.) trees NH_4^+ was taken up more readily than glycine (Rains and Bledsoe, 2007). In experiments with carrot (*Daucus carota* L.) roots inoculated with AM fungi, the extraradical mycelium was able to take up NO_3^- and NH_4^+ ions, with both being converted in the fungus to glutamine and then to other amino acids and particularly arginine. The arginine moved to the intraradical mycelium, where it broke down with the release of NH_4^+ that was passed into the root cells (Govindarajulu et al., 2005). The *AMT3.1* and *AMT4* genes are expressed in cortical cells of roots of mycorrhizal, but not in nonmycorrhizal, sorghum (*Sorghum bicolor* Moench) plants, and the AMT3.1 protein is localized around the mature arbuscules at the periarbuscular membrane (Koegel et al., 2013). These two transporters therefore seem to be important in the transfer of NH_4^+ from fungus to plant. Another six *AMT* genes were identified in the roots of the nonmycorrhizal plants and in other organs (Koegel et al., 2013), so there appear to be different AMTs involved in the uptake of NH_4^+ directly from the soil.

The extraradical mycelium of AM fungi seems to have both a LATS and a HATS for NH_4^+ (Pérez-Tienda et al., 2012). It is thought that forest trees with ectomycorrhizal associations take up amino acid N more efficiently than inorganic N, whereas trees with AMs take up inorganic N more efficiently than amino acid N (McFarland et al., 2010). In an experiment on durum wheat (*Triticum durum* Desf.), total growth and uptake of N were enhanced by the plants having AM associations, but although the presence of fungi increased soil N mineralization it actually decreased the ability of the plants to acquire N from organic N (Saia et al., 2014). Recent research has indicated that in

N-limited soils of boreal forests the ectomycorrhizal fungi use carbon from the trees but hang on to the N that they acquire, thus exacerbating the effects of N deficiency (Näsholm et al., 2013).

2.2.4 Dinitrogen Gas

Those few plant species that can utilize dinitrogen (N_2) gas as their N source depend on symbiotic microorganisms to fix it. The most commonly studied of these relationships is between gram-negative members of the alpha subgroup of the proteobacteria (rhizobia) that live in root nodules of plants in the family Fabaceae and the nonlegume Parasponia (*Parasponia andersonii*) in the family Cannabaceae (Santi et al., 2013). Other associations within root nodules involve filamentous bacteria (*Frankia* spp., gram-positive actinomycetes), with mostly woody plants in eight different families (Santi et al., 2013).

The nitrogen made available to leguminous plants by the plant–*Rhizobium* symbiosis is also available to other plant species. In pastures, N fixed in the nodules of legumes is passed onto grass species by mycorrhizal fungi, although it is also possible for N in the grasses to be passed to the legume species (Pirhofer-Walzl et al., 2012). Similarly, N has been demonstrated to pass from N-fixing soybean to corn (Bethlenfalvay et al., 1991). It seems likely that in low-input agricultural systems, N fixed by leguminous crops can be passed to nonleguminous weeds by mycorrhizal hyphae connecting the plants, enabling the weeds to grow vigorously (Moyer-Henry et al., 2006). The amount of N passed from N-fixing species to nonfixing species can be up to 80% of the total N in the combined biomass of both species, although in most examples studied it is considerably less, and usually with a small amount of N moving in the reverse direction (He et al., 2009).

A few plant species form symbiotic associations with N-fixing cyanobacteria, either an intercellular association (plants in the Gunneraceae) or an extracellular association (liverworts, hornworts, the aquatic fern azolla (*Azolla* spp. Lam.), and gymnosperms of the Cycadaceae) (Santi et al., 2013) and sphagnum moss (*Sphagnum* spp. L.) (Berg et al., 2013). Although these plants do not include any crop species, such symbioses are potentially possible (Ahmed et al., 2010). Loose associations between N-fixing bacteria (diazotrophic endophytes) and crop plants may certainly be possible, and the Trenton cultivar of wheat inoculated with a strain of klebsiella (*Klebsiella pneumoniae* Trevisan) was shown to receive fixed N from the bacteria present in intercellular spaces of the root cortex and to have improved growth as a consequence (Iniguez et al., 2004). Such associations seem to be common in the family Poaceae, to which most cereal crops belong, and involve a range of alpha- and beta-proteobacteria collectively known as plant growth–promoting rhizobacteria (PGPR) (Santi et al., 2013). The presence of the diazotrophs may enhance crop yields, and yield increases of 13%–22% in rice and 5.9%–33% in corn have been seen in different studies comparing inoculated with uninoculated plants (Santi et al., 2013, and references therein). However, the major stimulation to plant yield may come about not through enhanced N availability but through the PGPRs producing phytohormones such as IAA and cytokinins that modify root architecture.

Understanding uptake of N compounds is a flourishing area of current research, and new discoveries are being made almost on a daily basis. The reader is referred to the reviews of Tegeder and Rensch (2010), Dechorgnat et al. (2011), Kraiser et al. (2011), and Wang et al. (2012c) for further details.

2.3 ASSIMILATION OF NITROGEN IN PLANTS

Nitrate taken up by a plant is reduced to nitrite (NO_2^-) and then to NH_4^+ in reactions that require energy. Ammonium that arises from nitrate, or by uptake from the soil, is added to organic acids to make amino acids. The amino acids are then assembled into proteins or are the starting point for the synthesis of other N-containing molecules such as nucleic acids, chlorophyll, alkaloids, cyanogenic glycosides, and glucosinolates.

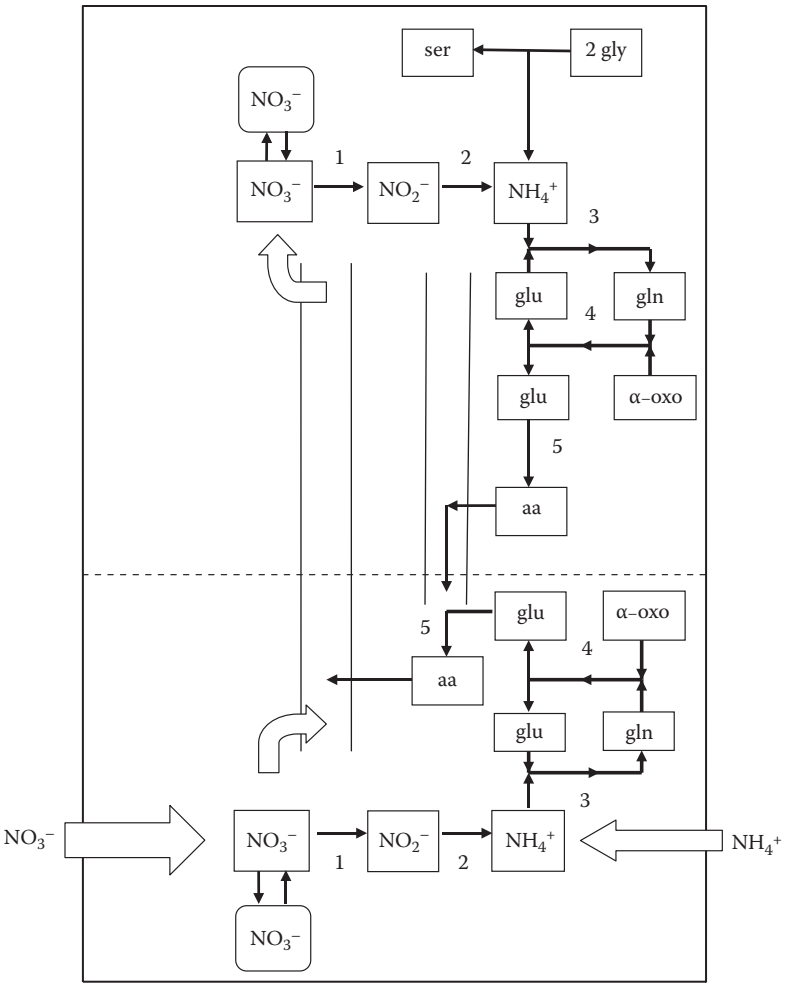

FIGURE 2.1 Assimilation of nitrate and ammonium by plants. *Abbreviations*: α-oxo, α-oxoglutarate; aa, amino acids; glu, glutamate; gln, glutamine; gly, glycine; ser, serine; NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonium. Enzymes: 1 nitrate reductase, 2 nitrite reductase, 3 GS, 4 GOGAT, 5 transaminases.

Where NO_3^- is the form of nitrogen taken up by plants, the first step of assimilation is reduction to NO_2^- (Figure 2.1). This reaction is catalyzed by the enzyme nitrate reductase (NR), located in the cytoplasm (cytosol). The enzyme requires energy to carry out the reduction, with one form of the enzyme using NADH from respiratory reactions in the mitochondria and another using NADPH from photosystem 1. Nitrate reductase uses molybdenum as a cofactor in the reduction of nitrate (Campbell, 1999).

Nitrite produced from NO_3^- moves into the chloroplasts (or the plastids in the roots), where the enzyme nitrite reductase catalyzes its reduction to NH_4^+. This process requires electrons from reduced ferredoxin, which is a product of photosystem 1. Since ferredoxin does not move around the plants, there must be some other reductant in roots for the reduction of NO_2^- arising from nitrate that is reduced there.

The NH_4^+ produced in the chloroplast (or root plastid) joins with a molecule of the amino acid glutamate to make another amino acid, glutamine, in a reaction catalyzed by glutamine synthetase (GS). This enzyme uses ATP as its energy source, and it has a very high affinity for NH_4^+. The glutamine then passes the amino (NH_2) group acquired from the NH_4^+ to the organic acid α-oxoglutarate,

reverting to glutamate itself and generating another molecule of glutamate in the process. This reaction is catalyzed by glutamate synthase (GOGAT). The first molecule of glutamate can then become an acceptor for another NH_4^+ ion, and the second molecule is converted to other amino acids by transaminases. These amino acids are then exported from the site of synthesis to form proteins in other parts of the plant or are converted into N-containing secondary metabolites. GOGAT exists in two forms, one which uses reduced ferredoxin as its reductant and one that uses NADH. The first form is present in chloroplasts, and the second is in roots (especially in root nodules). The α-oxoglutarate comes from the tricarboxylic acid (Krebs) cycle, which is replenished with substrate to prevent the organic acids becoming depleted.

Nitrate reductase is substrate inducible and is subject to feedback inhibition by amino acids (particularly glutamine) (Campbell, 1999). Its activity changes over a 24 h cycle, increasing in the light and decreasing in the dark, and this pattern is general for nitrate assimilation in plants (Pearson and Steer, 1977; Matt et al., 2001). In fact, the pattern closely matches the expression of the *NRT1.5* gene, which is responsible for much of the loading of NO_3^- into the xylem and hence root to shoot nitrate transport (Lin et al., 2008). Light causes the synthesis of NR mRNA and synthesis of more of the enzyme, and dark causes phosphorylation of existing NR molecules, slowing their activity (Campbell, 1999). These then decay during the dark period. Nitrate assimilation in the light occurs at a faster rate than the translocation of NO_3^- from the roots and uses up NO_3^- that is stored in the leaf pools during the dark period (Matt et al., 2001).

The NH_4^+ in the previously mentioned reactions arises from the reduction of NO_3^- (mostly in the leaves, but also in the roots), from the direct uptake of NH_4^+ or in a few plant species from the conversion of N_2 in root nodules. Unlike NO_3^-, NH_4^+ in the root system is nearly all assimilated there, and little moves up to the shoot in the xylem. There is a fourth source of NH_4^+ in plants that is exclusive to the leaves, NH_4^+ released from the formation of one molecule of serine from two molecules of glycine in photorespiration. This NH_4^+ is reassimilated by leaf cytosolic GS (Hirel et al., 2007), and its release and reassimilation seem to represent a greater N flux than the primary assimilation of nitrogen that occurs at the same time (Tcherkez and Hodges, 2008).

Amino acids taken up by plants, rather than being used directly in protein synthesis, are transaminated readily to other amino acids, either in the roots or after transport to the shoots (Näsholm et al., 2009). Urea seems to be assimilated mostly in the roots (through conversion to carbamate and then NH_4^+ by nickel-containing ureases, and then assimilation into glutamine), although some may be translocated to the shoots (Mérigout et al., 2008; Witte, 2011). In arabidopsis and potato, the urease gene is transcribed in all tissues, so urea is certainly likely to be assimilated in shoots in these species (Witte et al., 2002; Witte, 2011). In fact, the concentration of urea in shoots is higher than would be predicted from the rates of potential urease activity, and it is probably stored in vacuoles, whereas the urease is located in the cytoplasm (Witte, 2011).

Regardless of the N form taken up, the ultimate end products of its assimilation are the amino acid constituents of proteins. These amino acids accumulate in developing leaves, roots, and other physiological sinks of a growing plant. A high proportion of plant N is present in leaf proteins, many of which form the enzymes involved in photosynthesis. The enzyme ribulose bisphosphate carboxylase/oxygenase is the major leaf protein, particularly in C3 plants, and it can account for up to 30% of the N in leaves (Lawlor et al., 1989). In the flag leaf of wheat, the concentrations of soluble proteins and chlorophyll reach their maximum at the time of full expansion and then decline to nearly zero by senescence (Lawlor et al., 1989). The amino N remobilized from senescing leaves is partitioned to other processes in the plant, primarily reproductive growth, and is transported mainly to the developing storage organs in the phloem as asparagine and glutamine (Masclaux-Daubresse et al., 2010). In the later stages of growth, typically after flowering in an annual plant, the degradation of proteins in the leaves exceeds the synthesis of new proteins.

In some annual plant species (e.g., oilseed rape, *Brassica napus* L.), N uptake almost ceases once flowering occurs with nitrogen in the developing storage organs coming almost exclusively from senescing leaves (Ulas et al., 2012), but in other species (e.g., corn), a sizeable proportion

of N in the developing grains comes from uptake from the soil, as well as from mobilization (Masclaux-Daubresse et al., 2010). In corn, nitrogen taken up at the later stages of growth is partitioned more to the developing ear than to vegetative structures (Ta and Wieland, 1992), although it may be allocated to the vegetative tissues of the plant first and from there to the kernels as turnover of leaf proteins releases amino acids (Gallais et al., 2006). From 45% to 65% of the N in corn grains comes from mobilization from the leaves, with the rest coming from uptake after silking (Hirel et al., 2007). It has been estimated that 60%–85% of the N in a corn plant at anthesis eventually is located in the cob (Ta and Wieland, 1992).

About 70% of the maximum N content of spring and winter wheat plants has been absorbed by ear emergence, but uptake still occurs after this (Watson et al., 1963). Kichey et al. (2007) found that an average of 71% of winter wheat grain N comes from mobilization. Bogard et al. (2010), following field experiments on cultivars of wheat grown in seven locations in northern France and in a range of environmental conditions, estimated that 84% of winter wheat grain N came from mobilization from the leaves and stems and the remaining 16% from soil uptake. The efficiency of mobilization can be seen from the fact that they calculated that 78% of the N absorbed before anthesis ended up in the grains. Of the N in winter wheat crops grown at recommended fertilizer N rates in the United Kingdom and New Zealand, 14% was structural N, 43% was photosynthetic N, and 43% was reserve N (mainly located in the true stem) at anthesis (Pask et al., 2012). Although most of the grain N came from the leaves, the reserve N appeared to be a major source of N for the grain initially, so that green leaf area was maintained for some time.

Modern *stay-green* cultivars of cereals keep taking N up from the soil for translocation to the developing grains at a time when uptake is slowing in the traditional cultivars, as a longer duration of shoot activity maintains root activity (Borrell et al., 2001). Although much of the N in corn grains already is present in the leaves before silking, most of the total grain dry matter comes from photosynthesis post-silking (Ning et al., 2013). In stay-green cultivars with a larger leaf area and longer leaf area duration than traditional cultivars, total yields were higher than in older cultivars, but a high proportion of the N in the grains still came from mobilization from the leaves, and the extra N taken up after silking was used to maintain leaf area (Ning et al., 2013).

In perennial plants, those that are deciduous tend to scavenge some of the nitrogen present in the leaves before they are shed. In aspen (*Populus tremula* L.) trees, it has been calculated that up to 80% of the N in the leaves is moved to the remaining parts of the tree before leaf fall (Keskitalo et al., 2005).

Other N-containing compounds in plants are formed from amino acids. All plants contain purine and pyrimidine bases (in nucleic acids), chlorophyll, and the indole hormones, and these have amino acid precursors. The distribution of N-containing, nonprotein amino acids, alkaloids, cyanogenic glycosides, and glucosinolates varies between plant species, with some being in only a few species, genera, or families. For example, glucosinolates are restricted largely to the order Brassicales. If amino acids accumulate, they can cause slowing of nitrate uptake as well as nitrate reduction. For example, glutamine represses transcription of the *AtNRT2.1* gene (Nazoa et al., 2003).

2.4 PHYSIOLOGICAL RESPONSES OF PLANTS TO NITROGEN SUPPLY

Plants subjected to nitrogen deficiency tend to be smaller and to have leaves that are paler green than normal (Figures 2.2 and 2.3). This color effect is due to lower chlorophyll concentration.

2.4.1 Effects of Nitrogen Supply on Shoots and Leaves

Not only are plants grown under N deficiency small, but they have a lower shoot:root ratio, as ongoing shortage of nitrogen leads to lowered shoot growth before root growth is slowed (Brouwer, 1962). As well as giving a large shoot and increased plant size overall, higher N supply also gives a larger leaf area. For example, flag leaves of winter and spring wheat grown in sand had a larger area

(a) (b)

FIGURE 2.2 Nitrogen deficiency in soybean (*Glycine max* Merr.). (a) N-deficient plant (note low number of leaves, small area of individual leaves, and paler coloration) and (b) N-sufficient plant.

FIGURE 2.3 **(See color insert.)** Nitrogen-deficient young pepper (*Capsicum* sp. L.) plants showing chlorosis (paler coloration) of oldest leaves. (Photograph by A.V. Barker.)

and a higher fresh and dry mass with high N supply than with low N supply (Lawlor et al., 1989). The high-N plants had more cells in their flag leaf, and furthermore these cells had a larger volume.

Removal of N supply results in existing leaves undergoing cell expansion slowly, and leaves that form later have a slower rate of cell division (Roggatz et al., 1999; Trápani et al., 1999; Broadley et al., 2001). Plants grown with NH_4^+ have a smaller leaf area than plants grown with NO_3^- (Walch-Liu et al., 2000), and as NO_3^- accumulates in the leaves during the light period (Matt et al., 2001) and is an osmoticum, it could be assumed that the NO_3^- ion itself may drive leaf expansion by its effect on leaf water potential. However, direct measurement of turgor pressure in epidermal cells of expanding sunflower (*Helianthus annuus* L.) leaves failed to show any effect of withdrawal of NO_3^- (Palmer et al., 1996). Nitrate makes only a small contribution to changes in the water potential of leaf cells, and organic solutes probably make a much larger contribution to driving leaf expansion (Fricke and Flowers, 1998). An increase in leaf growth rate can be seen upon the supply of NO_3^- to plants previously supplied

NH_4^+, and since this result is apparent with NO_3^- supplied at as low a concentration as 10 mmol m^{-3}, it cannot be an osmotic effect (Rahayu et al., 2005). There is an increased concentration of cytokinins in the xylem and leaves of plants within 4 h of supply of NO_3^-, and that increase appears to control leaf expansion, possibly through affecting cell wall extensibility (Rahayu et al., 2005).

Increased leaf area has to be matched by more translocation of water and nutrients to the shoots, and in rooted cuttings of poplar (*Populus trichocarpa* Torr. and Gray x *deltoides* Bartr. ex Marsh) exposed to high ammonium nitrate, expression of seven aquaporin genes was upregulated in the secondary xylem of the stem. This action may have enabled the inflow of water into areas of xylem cell growth, as the high N supply gave increased xylem cross-sectional area and specific conductivity that matched the increased leaf area seen in these plants (Hacke et al., 2010).

Another effect that nitrogen has on leaves is to maintain leaf area duration, and if plants are subjected to N deficiency leaves tend to senesce early (Spiertz and de Vos, 1983; Lawlor et al., 1989). Nitrogen supply also affects total leaf area by affecting the number of leaves, and under extreme N deficiency the rate of wheat leaf primordia initiation relative to accumulated thermal time is slower (Longnecker et al., 1993). This deficiency also is reflected in the formation of tillers by cereals, with plants receiving high rates of N having more tillers than plants in low N (Tanaka and Garcia, 1965; Spiertz and de Vos, 1983; Wada et al., 1986; Longnecker et al., 1993; Oscarson, 2000; Xue et al., 2013).

Within a leaf, nitrogen is allocated to enable its functions (mainly photosynthesis) to proceed at an optimum rate. The higher the leaf N concentration, the higher the concentration of chlorophyll and proteins (Evans, 1983). In experiments on wheat supplied N fertilizer at 200 kg N ha^{-1}, concentrations of soluble proteins were nearly three times higher, and concentrations of ribulose bisphosphate carboxylase/oxygenase and chlorophyll were more than double those in unfertilized plants (Lawlor et al., 1989). The rate of photosynthesis per unit leaf area was higher in the fertilized plants. However, the relationship between N concentration in the leaf and rate of carbon assimilation (at light saturation) is asymptotic (Evans, 1983). At very high internal N concentrations, less photosynthesis occurs per unit of N present.

Within the canopy, N is allocated differently to the leaves at the top than to those toward the bottom (Kull, 2002; Hirose, 2005). The intensity of photosynthetically active radiation (PAR) declines downward (Gallagher and Biscoe, 1978), so toward the bottom of a dense canopy the amount of energy available is much less than at the top. The lower leaves develop as *shade* leaves, with a higher specific leaf area (the ratio of leaf area to leaf mass), so the N concentration (amount per unit leaf area) is lower as the tissues of the leaf are distributed over a bigger area (e.g., Laisk et al., 2005). This distribution of tissues, and the N in them, optimizes the ability of the plant to benefit from the high irradiance at the top of the canopy. However, this distribution does not match directly the distribution of PAR down the profile of the canopy, with N being at higher concentrations than would be required to give maximum rate of photosynthesis (Kull, 2002; Hirose, 2005). At any one time, photosynthesis usually is downregulated, so although it would be expected that the availability of N in a leaf would affect the ability of the leaf to carry out photosynthesis, it is likely that the rate of photosynthesis determines the concentration of N (Kull, 2002; Laisk et al., 2005).

If N deficiency arises, different crop species react in different ways, with there being a spectrum from maintaining leaf area and losing leaf N content (and the ability to utilize the PAR intercepted efficiently) through to maintaining the assimilatory capacity of existing leaves at the expense of leaf area (Lemaire et al., 2008).

In wheat, the effect of N on leaf initiation and growth gives a bigger leaf area index (and a longer leaf area duration) with N fertilization than in unfertilized crops (Watson et al., 1963; Fischer, 1993). As in other crops, a linear relationship occurs between rate of crop growth and interception of PAR (Monteith, 1977; Gallagher and Biscoe, 1978), so the increased leaf area results in a higher yield. Wheat grain yield is correlated strongly with grain number per unit land area, rather than individual grain size, and was shown to be related linearly to a photothermal quotient (the ratio of PAR intercepted: (mean temperature −4.5°C) over a 30-day period spanning anthesis) (Abbate et al., 1995).

2.4.2 Effects of Nitrogen Supply on Developing Seeds

Grain number is dependent on the number of flower spikes formed, the number of spikelets in each spike, and the number of viable florets in each spikelet. One reason for the increased grain number with N fertilization is that the increased tiller number gives more spikes, and another reason is that the number of spikelets per spike is increased (Longnecker et al., 1993; Ewert and Honermeier, 1999; Oscarson, 2000). However, the increase in number of grains per plant seems to come about more due to the increased number of tillers (and therefore spikes) than through there being more grains per main stem spike (Oscarson, 2000). Within the spikelets, N supply may influence also the development of floret primordia, but Ferrante et al. (2010) found that availability of N does not influence floret initiation in durum wheat. However, many florets that are initiated can degrade, and high N supply prevents degeneration of some of the florets from occurring and gives a larger number of grains than in plants with low N supply (Ferrante et al., 2010). N appears to have an indirect effect, with improved vegetative growth giving more assimilates to lower the extent of floret degeneration that would otherwise occur (Ferrante et al., 2013).

Similar effects are seen in other cereals. In corn, kernel number is also the major determinant of crop yield and is increased by N fertilization. This action occurs because of increased fertilization of ovules or decreased kernel abortion with an adequate N supply, rather than differences in numbers of ovules per cob or cobs per plant, and it arises from the indirect effect of the nitrogen in giving more assimilates for grain filling (Uhart and Andrade, 1995). Some increase in mean kernel weight occurs with increased N supply, although to a smaller extent than kernel number (Uhart and Andrade, 1995; Ciampitti and Vyn, 2011). Rice develops fewer panicles per unit land area and fewer spikelets per panicle, having a smaller leaf area index and lower leaf area duration under nitrogen deficiency (Wada et al., 1986; Xue et al., 2013).

2.4.3 Effects of Nitrogen Supply on Root Systems

As well as affecting the development of leaves, stems, and seeds, N supply also affects the development of the root system. Nitrogen deficiency in corn crops gives rise to increased angles from the horizontal of crown and base roots, particularly in genotypes that normally have shallow angles. This change in morphology gives a deeper distribution of the roots in the soil profile (Trachsel et al., 2013). In addition, N availability affects the proportions of different parts of the root system. It was seen that lateral roots of barley grow into patches of high concentration of NO_3^- and NH_4^+ (Drew, 1975). A similar response occurs in arabidopsis, where lateral roots grow into patches of high NO_3^- when the overall N supply is low, through having a higher density and a faster elongation rate, although lateral root elongation is inhibited with a high, homogeneous supply of NO_3^- (Linkohr et al., 2002). A high C:N ratio in the rooting medium suppresses the initiation of lateral roots, but supply of L-glutamic acid inhibits primary root growth and stimulates the growth of lateral roots (Zhang et al., 2007). It is well known that plants grown in hydroponic culture have smaller roots in NH_4–N than in NO_3–N, and supply of NH_4^+ to arabidopsis inhibits the elongation of primary and lateral roots although the number of lateral roots per unit length of primary root is increased (Li et al., 2010). Contact of arabidopsis shoots with NH_4^+ inhibits the formation of lateral roots (Li et al., 2013).

It seems that uptake of NO_3^- by the NRT1.1 transporter in arabidopsis signals to the plant (through interaction with the *ANR1* gene that helps regulate lateral root growth) that its primary root is growing through a high-nitrate patch (Zhang et al., 2007). The suppression of lateral root initiation by high C:N ratio seems to involve the NRT2.1 transporter, possibly acting as a nitrate sensor. The inhibition of the growth of lateral roots by high nitrate supply overall seems to be signalled by NO_3^- accumulating in the plant, possibly in the shoot, which may inhibit the synthesis or translocation of auxins such as IAA and may cause the accumulation of abscisic acid. The inhibition of formation of lateral roots by NH_4^+ supplied to the shoots is mediated by the formation of ethylene in the shoots, an action that may work by limiting the transport of auxins from the shoot to the roots

(Li et al., 2013). The inhibition of lateral root growth by NH_4^+ supplied to the roots appears to be independent of auxins (Li et al., 2010).

Infection of arabidopsis with the PGPR strain *Phyllobacterium brassicacearum* STM196 antagonizes the inhibition of lateral root development by high extracellular NO_3^- (Mantelin et al., 2006). This effect is thought to be brought about by auxins and cytokinins produced by the PGPR. Both the suppression of lateral root growth and the stimulation of plant growth by the rhizobacteria require increased expression of *NRT2.5* and *NRT2.6* genes in the shoots (Mantelin et al., 2006; Kechid et al., 2013). However, nitrate influx and expression of the *AtNRT1.1* and *AtNRT2.1* genes were decreased by 8 days after inoculation, but the expression of *AMT* genes was not affected (Mantelin et al., 2006). It appears that these plants still are able to absorb the NH_4^+ made available by the rhizobacteria, but they lose the capacity to limit their lateral roots to the high-nitrate patches and expand root growth into other zones and obtain other nutrients that may be in short supply.

Another root system change that occurs with N deficiency is that density and length of root hairs are increased (Foehse and Jungk, 1983). In arabidopsi*s*, N starvation for 2 days gave longer root hairs, and by 5 days root hair number also was increased (Engineer and Kranz, 2007). A similar pattern of increase in length and number of lateral roots occurred. Within the 2 days, the expression of the *AtAMT1.1* gene occurred in the distal parts of the root system, particularly in the new and existing root hairs and in the tips of newly emerging lateral roots and in the junctions of primary and lateral roots. By 5 days of N starvation, this expression was throughout the root system (Engineer and Kranz, 2007).

Growing phosphorus-deficient plants of mulloway needlebush with no nitrogen gave a much higher mass of cluster roots than plants grown with limiting supplies of either ammonium nitrate or glycine (Paungfoo-Lonhienne et al., 2009). No cluster roots were formed if the N supply was nonlimiting, regardless of whether it was ammonium nitrate, glycine, or protein. Although cluster roots are thought of as being involved primarily with the acquisition of phosphate by plants, their formation is regulated partly by the availability of nitrogen, and they have a proteolytic activity that helps make protein N available to plants (see Section 2.2.3).

2.4.4 Effects of Nitrogen Supply on Whole Plant Growth

Within a plant, nitrogen is partitioned to optimize the growth potential, so that proportions of shoot to root vary according to N availability, the proportions of the different parts of the root system vary to optimize the uptake of N from a heterogeneous soil environment, and N is distributed within the canopy to maximize the interception of PAR. As these processes serve to make conditions optimal for the assimilation of carbon, the main contributor to plant dry mass, it can be seen that there must be a critical relationship between N in a plant and its rate of growth. Relative growth rate (RGR) is directly related to the internal concentration of nutrients such as nitrogen, so that

$$\mathrm{RGR} = P_\mathrm{n} \times (C - C_\mathrm{min})$$

where

- P_n is the nutrient productivity (in this case nitrogen productivity, the amount of biomass produced per amount of N it contains per unit time)
- C is the concentration of N in the plant
- C_min is the minimum N concentration below which it has no effect on plant growth (Ingestad and Ågren, 1992)

As C_min is small, P_n is in effect the slope of a linear relationship between RGR and internal N concentration at values well above C_min. At the early stages of vegetative growth, the internal N concentration remains relatively constant, giving a constant value of RGR (and an exponential increase in

mass of the plants). However, as plants age, the proportion of strengthening material (cellulose and lignin) increases, particularly as the stem develops, and as these compounds do not contain N the concentration of N in the whole plant decreases (Ingestad and Ågren, 1992). RGR decreases with this decrease.

If N supply to a plant is interrupted, the plant N concentration decreases, and growth rate slows. However, initially at least, the decline in growth rate is not as great as would be predicted from the relationship between internal N concentration and RGR. In nitrate-grown plants, it seems that the free NO_3^- accumulating in the vacuoles buffers the plants until it is used up, and then RGR decreases dramatically (Walker et al., 2001). Although this effect of free NO_3^- in maintaining RGR can last a few days, it is largely an artificial position brought about by the removal of N supply from plants grown in hydroponic culture. These plants go from a considerable oversupply of N to withdrawal of N very quickly, which would not happen in a field soil. In a mature horticultural crop of tomato grown on rock wool, the size of pools of free NO_3^- may be sufficient to buffer plants against a sudden withdrawal of nitrate for time periods of 2–3 weeks (Le Bot et al., 2001).

The total N content of a crop is related to the mass of the crop according to the equation

$$N_{\text{content}} = aW^b$$

where

N_{content} is the mass of N in the crop on a given land area (kg ha^{-1})
a and b are constants
W is the mass of crop on the same land area

Parameter a is the N content where $W = 1$ t ha^{-1}, and b is the ratio of accumulation of nitrogen to biomass (and has a value lower than 1) (Greenwood et al., 1990; Gastal and Lemaire, 2002). It therefore follows that N concentration is given by the equation

$$N\% = a'W^{1-b}$$

where

$N\%$ is the concentration of N in the crop (as a percentage of dry matter)
a' differs from a to take into account the difference in units between N_{content} and W (Greenwood et al., 1990; Gastal and Lemaire, 2002)

This makes it clear that there is a decline in $N\%$ with increase in W (i.e., a decline in N concentration with crop growth stage) (Figure 2.4), and it follows a similar pattern across a range of crops, with the main difference between groups of crops being that the $N\%$ value is lower at a given W value for C4 plants than for C3 plants; b is relatively constant between the two groups, and a is lower in C4 plants than in C3 plants (Greenwood et al., 1990). However, within these broad groupings, there are differences between individual crop species, although the relationship between $N\%$ and W for any crop seems to be constant across different environmental conditions (Lemaire et al., 2007). The relationship only holds for values of $W = 1$ t ha^{-1} and above. Growth rate under N sufficiency is exponential up to this value of W, but above it becomes linear (Greenwood et al., 1990).

The relationship between $N\%$ and W gives the idea of a critical N concentration, the minimum concentration of N in a crop that generates maximum possible rate of growth of the crop at the value of W at that time (Greenwood et al., 1990). As with accumulation of unassimilated NO_3^- in storage pools, this leads to the concept of luxury consumption of N, with plants accumulating N at higher concentration than is required for maximum growth rate. $N\%$ in excess of the critical value has been demonstrated in a range of crops under different environmental conditions, at least at higher values of W (Lemaire et al., 2007). $N\%_{\text{critical}}$ is constant up to 1 t ha^{-1} and then decreases with further

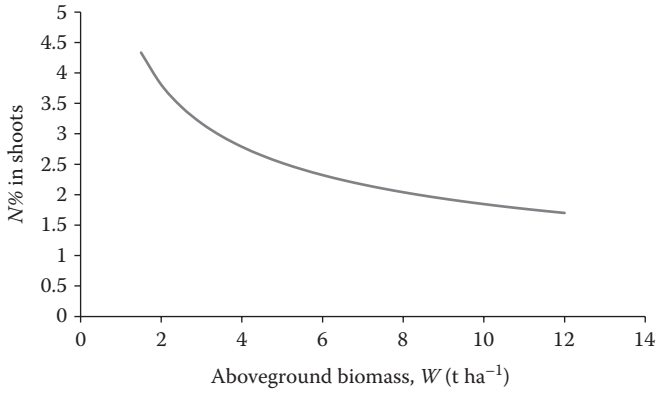

FIGURE 2.4 Change in N concentration in shoot biomass during crop growth. Based on nominal values, $N\% = aW^{1-b}$, $a = 5.2$, and $b = 0.55$, so that $N\% = 5.2W^{-0.45}$. Values for different crops are given in Table 2.1.

TABLE 2.1
Critical Nitrogen Concentrations of Some Crops in Relation to Shoot Biomass (*W*)

Crop	$N\%_{critical}$	Reference
Cereals		
Corn (*Zea mays* L.)	$3.40W^{-0.37}$	Plénet and Lemaire (2000)
Spring wheat (*Triticum aestivum* L.)	$38.5W^{-0.57}$	Ziadi et al. (2010)
Winter wheat (*Triticum aestivum* L.)	$5.35W^{-0.442}$	Justes et al. (1994)
Rice (*Oryza sativa* L.)	$5.18W^{-0.52}$	Sheehy et al. (1998)
Other grasses		
Annual ryegrass (*Lolium multiflorum* Lam.)	$40.73W^{-0.379}$	Marino et al. (2004)
Other crops		
Cabbage (*Brassica oleracea* var. *capitata* L.)	$5.11W^{-0.33}$	Ekbladh and Witter (2010)
Cotton (*Gossypium* spp.)	$4.969W^{-0.131}$	Xiaoping et al. (2007)
	$4.296W^{-0.131}$	
Linseed (*Linum usitatissimum* L.)	$4.69W^{-0.53}$	Flénet et al. (2006)
Winter oilseed rape (*Brassica napus* L.)	$4.48W^{-0.25}$	Colnenne et al. (1998)
Sunflower (*Helianthus annuus* L.)	$4.53W^{-0.42}$	Debaeke et al. (2012)

Note: Values (in g N per 100 g shoot dry mass) for different crops of a and $1 - b$ relative to shoot biomass (W) in the equation $N\% = aW^{1-b}$. The values presented give the relationship shown in Figure 2.4 for each crop species to maintain maximum growth rate at any stage of crop growth once the biomass yield has reached 1 t ha^{-1}.

growth, and any value below the line in Figure 2.4 shows where plants are below this critical value and growth is likely to become limited by shortage of nitrogen. Values for the relationship between $N\%$ and W for a range of crop species are shown in Table 2.1.

2.5 GENETICS OF ACQUISITION OF NITROGEN BY PLANTS

One of the big differences between plant species that could affect N uptake is whether they have tap roots or a fibrous root system, and this difference is controlled genetically. Less substantial differences in root architecture also may have an effect on N acquisition, and with oilseed rape it has been shown that an N-efficient cultivar has a higher root length density and more fine roots than an

older cultivar. Although the efficient cultivar has a slower rate of N uptake during vegetative growth, it continues taking up N once seed formation has begun (Ulas et al., 2012). It outyields the older cultivar with low N supply, so this observation forms the basis of a potential breeding strategy for low-N conditions.

As has already been seen, each of the different transporters for NO_3^-, NH_4^+, amino acids, and urea, and also each of the enzymes of N metabolism, is coded by a different gene, and these genes are expressed at different times and in different tissues. The whole process of acquisition of nitrogen by plants is under genetic control. This fact gives the possibility that differences between species in the genes responsible can be exploited to breed plants that utilize N more efficiently. This advantage also can be achieved by improving the use of N fertilizers, but as fertilizer-use efficiency is influenced strongly by the weather, which in many countries cannot be forecast very reliably more than a few days in advance, genetic improvement offers more reliable prospects (Barraclough et al., 2010).

However, changing expression of an individual gene by breeding does not necessarily improve the agronomic performance of the plant, even if the gene has been chosen carefully. Suppose that you identify an important carrier for NO_3^-; its expression is linked to expression and repression of many other genes, so increasing expression of one does not necessarily increase nitrate uptake. If it does, will the additional ions taken up be assimilated into amino acids? Perhaps a better approach would be to breed for increased expression of nitrate reductase, but as this enzyme is substrate inducible its activity is linked already to how much NO_3^- previously has been taken up by the plant. However, the activity of individual enzymes has been manipulated, and overexpression of NADH-glutamate synthase (NADH-GOGAT) in tobacco (*Nicotiana tabacum* L.) gave bigger shoots at flowering (Chichkova et al., 2001). NADH–GOGAT has an important role in assimilation of NH_4^+ from uptake and mobilization (Quraishi et al., 2011), and increased expression of the enzyme in wheat and barley to improve grain filling is now the subject of a patent (EP2534250 A2). Nevertheless, picking out individual transporters and enzymes such as this approach is problematic, as is discussed clearly by Chardon et al. (2012).

As these authors explain, many of the characters that could be manipulated to improve the acquisition of nitrogen by plants are controlled by more than one gene. A useful approach is to identify quantitative trait loci (QTLs), areas of the DNA that contain genes that give rise to the trait under consideration. Then, by searching databases for similar DNA sequences that already have been identified, it can be seen what those genes are. For example, in considering corn grain yield it was found that the QTLs involved linked to genes for GS (Chardon et al., 2012), showing that this enzyme is very important for grain filling. In winter wheat, GS activity has been found to be correlated strongly with the amount of N mobilized from foliage to grains and to grain yield (Kichey et al., 2007), although other workers found QTLs for GS to colocate with QTLs for grain N content, rather than overall yield (Habash et al., 2007).

In breeding for improved N nutrition, it might be possible to select for nitrogen-use efficiency (NUE), and many studies have identified QTLs for NUE. However, although NUE commonly is defined as the yield per unit of N available to the crop (Hirel et al., 2007), it also can be defined as the amount of N removed in a crop as a proportion of the N supplied in fertilizer or even as the reciprocal of N concentration in the tissues (i.e., biomass/mass of N in the tissues). NUE needs to be defined clearly, and QTLs for the trait as defined can then be investigated under conditions of high or low N supply, to give crops that use N more efficiently in intensive or extensive agriculture, respectively.

Selection of traits to improve needs to be imaginative. It is known that tropical grasses in the genus *Brachiaria* (signalgrass) exude compounds from their roots that inhibit nitrification in the soil, so it has been suggested that this capacity could be bred into cereals in the tribe Triticeae, also in the Poaceae family (Subbarao et al., 2009). This potentially would give barley, rye, and wheat crops that would lower the oxidation of NH_4^+ to NO_3^- in the soil, thereby minimizing the losses of NO_3^- by leaching and making more N available to the plants. As discussed earlier, the abortion of embryos

under N deficiency gives rise to lower yields of corn through causing fewer kernels to develop. Consequently, a good breeding strategy could be to breed corn plants with lower rates of embryo abortion under such conditions so that higher yields can be obtained with low N inputs (Gallais and Coque, 2005). Photosynthesis tends to be downregulated in plants, and as there can be considerable loss of leaf mass without loss of yield, the rate at which crop photosynthesis occurs probably is controlled by sink strength (Greenwood et al., 1986), so breeding for less seed abortion could be a good strategy in all cereals.

The big increases in wheat yields in the second half of the twentieth century came about following the introduction of stem-shortening genes into the germplasm. Less investment of assimilates into the stem automatically gave more investment into other plant parts, including the developing grains, thus giving a higher harvest index and higher yields. An added bonus was that because the stems were shorter, the plants were less prone to lodging, which was particularly common where N fertilizers had been used as the ears were heavy under those circumstances. Wheat varieties with short stems could therefore be grown with high-N application without risk of lodging, so overall yields increased (Barraclough et al., 2010). A breeding modification that has no apparent connection to N metabolism enabled higher yields to occur through more use of N fertilizers.

Perhaps not surprisingly, QTLs for wheat grain yield, grain protein yield, and N harvest index (the amount of N in the grain/total N in the plant) under contrasting conditions of high or low N supply have been linked to the expression of the short allele of the *Rht* dwarfing gene (Laperche et al., 2007; Quraishi et al., 2011). A QTL for NUE has been shown to colocate with two alleles of the *VRN1* (vernalization) gene, *Vrn-A1* and *Vrn-D1* (Quraishi et al., 2011). QTLs for yield traits under contrasting conditions of high and low N supply have been shown to colocalize with alleles of the *PPD1* (photoperiod) gene, in particular *Ppd-B1* and *Ppd-D1* (Laperche et al., 2007). In winter wheat, flowering occurs only in the longer days of spring, after a period of cold weather in the short days of winter. In the comparatively long days of autumn, the *VRN2* gene is expressed and downregulates *VRN3*. *VRN1* is expressed at low level. After a period of cold during the short days of winter, *VRN1* is expressed more, and this action downregulates the expression of *VRN2*. That action in turn allows for the upregulation of *VRN3* and gives more expression of *VRN1* and flowering. The *VRN3* gene also is controlled by *PPD1*, so that in winter wheat, *VRN3* is expressed only after day length has increased (Distelfeld et al., 2009). *Vrn-A1* and *Vrn-D1* are dominant alleles of the *VRN1* gene located in the A and D components of the wheat genome that do away with the requirement for a period of cold (vernalization) in order for flowering to occur (Cockram et al., 2007). *Ppd-B1* and *Ppd-D1* are semidominant alleles of *PPD1* located in the B and D components of the genome involved with the sensitivity of wheat to photoperiod, and the *Ppd-D1a* form gives early flowering in short days or long days (Cockram et al., 2007; Distelfeld et al., 2009). It can be seen that, as with stem shortening, genes controlling developmental pattern rather than aspects of N nutrition can be directly responsible for improving the efficiency with which plants utilize N.

Identification of QTLs for traits associated with efficient uptake and utilization of N should highlight to plant breeders some of the genes that do not otherwise appear to be associated with N nutrition, but the breeders have to be aware of two problems. First, the traits themselves have to be identified precisely. Are we trying to breed crops with the highest possible yields under conditions of abundant N supply, the highest yields with low N supply, highest seed/grain N concentration, highest nitrogen harvest index, or some other characteristic? Second, identification of genes that colocalize with QTLs depends on the environmental conditions where the work is carried out. Manipulation of *Ppd* genes in wheat may appear to offer a chance of improving NUE, but early-flowering *PPD1* genotypes may give higher yields in southeastern Europe where summers are hot and dry, whereas their yields can be lower than later-flowering genotypes in northwestern Europe (Cockram et al., 2007). The *stay-green* trait, which extends the period over which the leaves provide energy to allow uptake of N, may have the potential to increase cereal yields but only in environments where water supply is adequate to maintain a longer period of crop growth.

2.6 CONCENTRATIONS OF NITROGENOUS COMPOUNDS IN PLANTS

Most of the N within a plant is in the proteins, with the highest concentrations being in the seeds, and as protein is important in human and livestock diets there is considerable interest in growing crops with a high protein content. However, as shown earlier, grain filling with carbohydrates from photosynthesis and N from mobilization of foliar amino acids gives an imbalance that leads to grain N concentration (and protein content) varying with environmental conditions and cultivar.

Use of plant N in making grain proteins comes at the expense of further vegetative growth, so there is usually a negative correlation between grain yield and grain protein concentration in wheat crops grown in the field (Bly and Woodard, 2003; Barraclough et al., 2010; Bogard et al., 2010). New stay-green cultivars of corn produce a higher grain yield than older cultivars, as photosynthesis carries on for longer, but the grain N concentration is lower (Ning et al., 2013). In recent times, increases in crop yields have been matched by decreased seed or grain protein concentrations in a variety of crops (Triboi and Triboi-Blondel, 2002), but the negative relationship between wheat grain yield and grain N concentration was even noted by Lawes and Gilbert as early as 1857 (Barraclough et al., 2010). Because of this relationship, there is a negative correlation between concentrations of proteins and the other major component of a seed, so that protein accumulation in cereal grains comes at the expense of starch accumulation, and protein accumulation in oilseed crops such as oilseed rape and sunflower comes at the expense of oil accumulation (Triboi and Triboi-Blondel, 2002).

Where a high seed protein content is required, breeding has given us the potential to select for genotypes that typically have low yields and high seed protein concentration. Wheat cultivars used for bread making have a higher grain protein content and lower grain yields than other wheat cultivars (Barraclough et al., 2010), and cultivar is a more important determinant of milling and baking properties than either level of N fertilization or its timing (Otteson et al., 2008). The major protein present in mature wheat grains is gluten, itself comprising gliadins (protein subunits present as monomers) and glutenins (protein subunits assembled into polymers stabilized by interchain disulfide bonds) in approximately equal amounts (Wan et al., 2013). Gluten forms a viscoelastic matrix in the dough that prevents carbon dioxide from the yeast escaping, with gliadins being the major determinants of dough viscosity and extensibility and glutenins determining dough strength (elasticity) (Wan et al., 2013).

Even allowing for the negative relationship between seed yield and protein concentration, there is some potential to increase seed N content by soil management. In winter wheat, the N harvest index (the proportion of plant N present in the harvested grains) is increased by the application of N at anthesis (Wuest and Cassman, 1992b), and the actual grain N concentration also is increased (Wuest and Cassman, 1992a). In fact, where individual wheat crops varied from a strong negative correlation between grain yield and grain protein concentration, those crops that had a higher protein concentration than was predicted from the yield mostly had a higher uptake of N post-anthesis (Bogard et al., 2010). This feature could mean that stay-green cultivars should have higher grain protein concentration than older cultivars, but in winter wheat, it seems to be less consistently linked to delayed leaf senescence than is grain yield (Bogard et al., 2011).

As N supplied late to wheat plants is partitioned mostly to the developing grain, it is theoretically possible to supply N early in vegetative growth to give maximum yield and then to supply N late to ensure a high grain protein content. Indeed, Bly and Woodard (2003) found that postpollination application of N to bread wheats gave a higher grain protein concentration, particularly in those crops that yielded sufficiently well to be above a target yield. The practical difficulties here include that fact that driving over a high-yielding crop to supply late nitrogen can cause damage to the plants. Also, gliadins are increased more than glutenins, with various γ-gliadin genes being upregulated, so baking quality can be affected adversely (Wan et al., 2013). The N fertilization policy has to match the product that an individual crop is grown for. Bread wheat must have a high grain protein content, so overall yield can be sacrificed, whereas in cereals grown for livestock overall yield may be more important, although protein contents should be as high as possible. Oilseed rape

is grown for its oil content, so seed protein content is less important; corn for human consumption ideally has a reasonable protein content, but if corn is grown for bioethanol, high grain protein is not required (Chardon et al., 2012). Barley used for brewing beer should have low protein and a correspondingly high starch content to optimize the production of monosaccharides to be fermented, so the farmer has to ensure that little N is taken up after anthesis. In order to ensure low soil N at anthesis, fertilizers can be applied only early and at a low rate.

Nitrogen in amino acids, particularly asparagine, can react with reducing sugars in the Maillard reaction, giving rise to the carcinogen acrylamide (Mottram et al., 2002; Halford et al., 2012). This reaction is favored by heat, and as plants contain both precursors plant-based foods may contain acrylamide, particularly when cooked for a long time. Tareke et al. (2002) found concentrations of 150–1000 μg kg^{-1}, or even higher, in carbohydrate-rich foods of plant origin, such as potatoes. This potential for acrylamide formation has led the European Food Safety Authority to monitor the concentrations of acrylamide in foods, with the as yet unsuccessful aim of lowering its concentrations, although epidemiological studies of possible links between acrylamide and cancers have mostly given negative results (Sanderson, 2012). Asparagine can accumulate in wheat or potatoes, often when protein synthesis is low, yet N supply is high, so there is interest in breeding low-asparagine cultivars to help minimize the formation of acrylamide in foodstuffs (Halford et al., 2012). In potatoes, reducing sugars accumulate in the tubers with N deficiency, so maintaining N fertilizer levels not only helps achieve high yields but should also give a product with lower tendency to form acrylamide on cooking (Halford et al., 2012).

Aromatic rice was shown to be more flavored in high-N soil and contained a higher concentration of the imino acid L-proline, which is a precursor of the odiferous compound 2-acetyl-1-pyrroline (Yang et al., 2012). It certainly seems that good availability of N to plants can give high concentrations of nitrogenous secondary metabolites. For example, high N supply can give increased concentrations of alkaloids (Nowacki et al., 1976), and in tobacco a higher rate of N supply gives higher concentrations of the alkaloid nicotine (Matt et al., 2002). However, there is a trade-off with the use of N in primary metabolism, and tobacco genotypes that naturally have higher nicotine contents tend to have lower biomass yields (Matzinger et al., 1972). In the salad vegetable rocket (*Eruca sativa* Mill.), there is a link between the concentrations of different glucosinolates and N supply. Increasing N supply from a low rate gave higher glucosinolate content up to a critical level (Omirou et al., 2012). Increased N supply gave increased concentrations of some volatile aromatic compounds responsible for flavor in tomato (although excess N gave decreased concentrations of some of them) and also gave higher concentrations of soluble sugars and soluble solids that help give taste, along with higher titratable acidity (Wang et al., 2007).

Unassimilated mineral N can accumulate in plants to varying degrees (Table 2.2). While NH_4^+ concentrations are always low in healthy plants, NO_3^- accumulates in the vacuoles. Accumulation of NO_3^- in leaves occurs particularly under conditions of low photon flux density, when there are lower concentrations of soluble photosynthetic products and plants maintain a balance in osmotic potential in the leaves from NO_3^- and soluble organic molecules (Burns et al., 2011).

The accumulation of NO_3^- in plant foodstuffs may give a risk of methemoglobinemia in infants. This malady occurs from the oxidation of ferric (Fe^{2+}) oxyhemoglobin brought about by NO_2^-, which could arise from the NO_3^-, although there is no firm evidence that nitrate alone in the diet would produce such an effect (Hord et al., 2009). In the past, accumulation of NO_3^- in leafy vegetables was thought to give a risk of stomach cancer to consumers, but evidence for such an effect has also not been forthcoming (Santamaria, 2006; Hord et al., 2009). Despite this lack of evidence, nitrate concentrations in leafy vegetables have to be kept low according to legislation enacted in the EU. This regulation leads to attempts by growers to limit nitrate accumulation in salad crops, for example, by increasing NH_4^+ supply to limit NO_3^- uptake, but it can also limit the commercial opportunities to grow leafy salad crops in winter in more northerly latitudes, where photon flux density is low and photoperiod is short, even if the temperature can be kept warm by growing them under glass.

TABLE 2.2
Concentrations of Nitrogenous Compounds in Plants

Compound	Plant	Concentration	Reference
Nitrate	Cabbage crops (*Brassica oleracea* var. *capitata* L.)	500–1000 mg kg^{-1} FM	Santamaria (2006)
	Endive crops (*Cichorium endivia* L.)	1000–2500 mg kg^{-1} FM	Santamaria (2006)
	Leek crops (*Allium porrum* L., syn. *A. ampeloprasum* var. *porrum* (L.) J. Gay)	1000–2500 mg kg^{-1} FM	Santamaria (2006)
	Lettuce (*Lactuca sativa* L.)	Mean 3266 (2291–4833) mg kg^{-1} FM, nutrient film technology Mean 1190 (772–1907) mg kg^{-1} FM, soil grown	Burns et al. (2012)
	Rocket crops (*Eruca sativa* Mill.)	>2500 mg kg^{-1} FM	Santamaria (2006)
	Spinach crops (*Spinacia oleracea* L.)	>2500 mg kg^{-1} FM	Santamaria (2006)
	Tobacco roots (*Nicotiana tabacum* L.)	1500–1750 µmol g^{-1} DM	Camacho-Cristóbal and González-Fontes (2007)
	Corn roots (*Zea mays* L.)	Up to 70 mol m^{-3} NO_3^- in tissues	Bloom et al. (2012)
Ammonium	Tobacco leaf	30–40 µmol g^{-1} DM	Camacho-Cristóbal and González-Fontes (2007)
	Tobacco roots	11–17 µmol g^{-1} DM	Camacho-Cristóbal and González-Fontes (2007)
	Corn roots	Up to 25 mol m^{-3} NH_4^+ (per water volume in tissues)	Bloom et al. (2012)
	Rice shoots (*Oryza sativa* L.)	13.0–75.7 µmol g^{-1} FM	Balkos et al. (2010)
	Rice roots	12.4–37.6 µmol g^{-1} FM	Balkos et al. (2010)
	Rice shoots	0.4–3.0 µmol g^{-1} FM	Wang et al. (2013a)
	Rice roots	0.1–2.5 µmol g^{-1} FM	Wang et al. (2013a)
Urea	Rice shoots	0.1–0.4 µmol g^{-1} FM	Wang et al. (2013a)
	Rice roots	0.04–0.2 µmol g^{-1} FM	Wang et al. (2013a)
Amino acids	Cucumber leaf (*Cucumis sativus* L.)	Free glu 2.653, gln 0.442, arg 0.153 nmol mg^{-1} FM	Borlotti et al. (2012)
	Cucumber root	Free glu 0.904, gln 0.495, arg 0.721 nmol mg^{-1} FM	Borlotti et al. (2012)
	Tobacco leaf	175–200 µmol g^{-1} DM total free amino acids	Camacho-Cristóbal and González-Fontes (2007)
	Tobacco roots	110–130 µmol g^{-1} DM total free amino acids	Camacho-Cristóbal and González-Fontes (2007)
Proteins	Winter wheat flag leaf (*Triticum aestivum* L.)	Soluble proteins, up to 14 g m^{-2} Rubisco, up to 7 g m^{-2}	Lawlor et al. (1989)
	Tobacco leaf	180–200 mg g^{-1} DM	Camacho-Cristóbal and González-Fontes (2007)
	Tobacco roots	175–200 mg g^{-1} DM	Camacho-Cristóbal and González-Fontes (2007)
	Rice shoots	11–19 mg g^{-1} FM	Balkos et al. (2010)
	Rice roots	6–8 mg g^{-1} FM	Balkos et al. (2010)
	Winter wheat grain	6.2%–15.9% DM[a]	Barraclough et al. (2010)
	Corn grain	6.9%–10.7% DM[a]	Ning et al. (2013)
	Rapeseed (*Brassica napus* L.)	16.8%–23.2%	Triboi and Triboi-Blondel (2002)
	Sunflower seed (*Helianthus annuus* L.)	17.1%–21.1%	Triboi and Triboi-Blondel (2002)

(*Continued*)

TABLE 2.2 (*Continued*)
Concentrations of Nitrogenous Compounds in Plants

Compound	Plant	Concentration	Reference
Chlorophyll	Winter wheat leaves	Up to 0.9 g m^{-2}	Lawlor et al. (1989)
	Cucumber leaf	14 mg g^{-1} FM	Borlotti et al. (2012)
	Aspen leaves (*Populus tremula* L.)	Up to 6 mg g^{-1} DM	Keskitalo et al. (2005)

[a] Reported by authors as N concentration, converted to protein concentration using conversion factor from *N*% of 5.7.
DM, dry mass; FM, fresh mass.

2.7 INTERACTIONS OF NITROGEN WITH OTHER ELEMENTS

It is known that there are reasonably constant ratios of tissue concentrations of phosphorus, potassium, calcium, and magnesium relative to nitrogen across a big range of plant species. Adherence to these proportions gives growth that occurs at an optimum rate, although deviation from optimum proportions can be found (Knecht and Göransson, 2004). Local deficiencies of these and other elements can have effects on the internal ratios, but other factors are also important.

As N is the root-acquired element taken up in the largest amounts by plants, whether it is taken up as the NO_3^- anion or the NH_4^+ cation has a big effect on the uptake of other anions and cations. Arnon (1939) showed that barley grown in NH_4^+ contained lower concentrations of the cations Ca^{2+}, Mg^{2+}, and K^+ than plants grown in NO_3^-. Not only is there a general effect in which extensive uptake of one or other of these ions makes it energetically more difficult for a plant to acquire other anions or cations, but it is likely that similarly sized ions may compete with the N forms for binding to the transporter proteins.

This action can be seen in experiments on common bean (*Phaseolus vulgaris* L.), where the rate of uptake of K^+ was much higher with NO_3^- as the N source than with NH_4^+ (Guo et al., 2007). In rice, which has NH_4^+ as its major N source, fresh weight was greatest with seedlings grown in 10 mol NH_4^+ m^{-3} and 5 mol K^+ m^{-3}, but seedlings grown in 10 mol NH_4^+ m^{-3} and 0.02 mol K^+ m^{-3} had lower mass than seedlings grown in lower concentrations of NH_4^+ and different concentrations of K^+ or seedlings grown in 10 mol NO_3^- m^{-3} (Balkos et al., 2010). NH_4^+ was shown to be taken up by K^+ transporters and channels in barley and arabidopsis, and there is direct competition between the two ions for uptake (ten Hoopen et al., 2010).

Cations other than K^+ are less similar in size and properties to NH_4^+, yet there are still interactions between them and nitrogen nutrition because of their importance in nitrogenous compounds. Magnesium is a constituent of chlorophyll, and so could affect photosynthesis. Supplying high concentrations of Mg^{2+} in the hydroponic solution gave sunflower plants supplied NH_4^+ similar shoot dry weight and leaf area to plants supplied NO_3^-, which at low Mg^{2+} supply had much lower shoot dry weight and leaf area (Lasa et al., 2000). The high Mg^{2+} supply increased the rate of photosynthesis per unit leaf area to a similar value to that found in the NO_3-supplied plants, but this result was not an effect due to the role of Mg^{2+} in chlorophyll synthesis as chlorophyll concentrations were not affected by Mg^{2+} supply (Lasa et al., 2000).

Rapid growth of temperate zone pasture crops at temperatures above 14°C following freezing or near freezing temperatures, and after supply of N fertilizers, gives a risk of grass tetany in livestock caused by low Mg^{2+} concentrations in the forage (Robinson et al., 1989). Nitrogen fertilization can increase K^+ concentrations in the forage and also produces more young plant tissues that have a high K^+:(Ca^{2+} + Mg^{2+}) ratio. However, NO_3^- can encourage Mg^{2+} uptake, and a temperature above 14° should encourage oxidation of urea or NH_4^+ to NO_3^-, so the grass tetany risk may come from the increased concentrations of crude protein, fatty acids, and organic acids and decreased concentrations of water-soluble carbohydrates in the forage, all of which decrease the availability of Mg^{2+} to the animals (Robinson et al., 1989). In multispecies pastures, the supply of N fertilizers

encourages the growth of grasses more than forbs and legumes, and as grasses have lower Mg^{2+} concentrations the forage obtained presents a bigger risk to livestock than unfertilized pastures (Robinson et al., 1989).

Supply of N as NH_4^+ rather than NO_3^- also depresses the uptake of Ca^{2+}. Although the calcium-deficiency disorders of horticultural crops, such as blossom-end rot in tomatoes and peppers, are caused by rapid fruit growth outstripping the potential for transpiration to supply Ca^{2+} ions in adequate quantities, supply of NH_4^+ increases the risk of these diseases occurring by lowering Ca^{2+} uptake (Wilcox et al., 1973).

Nitrogen supply affects the uptake of iron (Fe) by plants, principally because uptake of NO_3^- leads to alkalinization of the rhizosphere due to the cotransport of H^+ with the NO_3^- ions, and this alkalinization makes Fe^{3+} less available. Genes for nicotianamine synthase are induced by NO_3^-, thus facilitating the synthesis of nicotianamine, which is involved in uptake and homeostasis of Fe (Wang et al., 2003). However, Fe also affects nitrogen nutrition. For example, Fe-deficient cucumber (*Cucumis sativus* L.) was found to have lower nitrate reductase activity (and a lower level of NR transcript) in the leaves than control plants and slightly lower nitrate reductase activity in the roots. GS and GOGAT activities were increased by Fe deficiency in both organs (Borlotti et al., 2012).

Another divalent cation, Zn^{2+}, had higher concentrations in wheat with N supplied as NO_3^- than with mixed NH_4^+/NO_3^- (Wang and Below, 1998; Drihem and Pilbeam, 2002) and also than with NH_4^+ alone (Wang and Below, 1998). Zinc uptake is enhanced by good N supply, and the rate of uptake and the rate of translocation from roots to shoots of ^{65}Zn were higher in durum wheat seedlings with adequate supply of NO_3^- than in seedlings with low NO_3^- supply, particularly in Zn-deficient seedlings (Erenoglu et al., 2011). There is considerable interest in increasing Zn concentrations in cereal grains, and more accumulates in grains if plant senescence is delayed after anthesis. One way in which senescence can be delayed is by maintaining high-N availability, and use of N fertilizers at anthesis has been shown to increase grain Zn^{2+} accumulation (Kutman et al., 2012).

Nickel (Ni^{2+}) is also taken up more when NO_3^- is the N source. Arabidopsis plants fed NO_3^- had greater expression of the *IRT1* gene (which codes for a transporter of divalent cations including Fe^{2+} and Ni^{2+}) in the roots and contained higher concentrations of Ni^{2+} in roots and shoots than plants supplied NH_4–N (Hu et al., 2013). These high Ni^{2+} concentrations gave the nitrate-fed plants more signs of nickel toxicity. Although Ni^{2+} can be toxic, it is a plant micronutrient as it is a component of ureases (Dixon et al., 1975), and Ni^{2+} deficiency depresses urea uptake (Arkoun et al., 2013).

In the same way that NH_4^+ uptake affects cation uptake, it could be expected that supply of N as NO_3^- might interfere with uptake of another major anion required by plants, SO_4^{2-}. However, interaction at the point of uptake appears to occur more between NO_3^- and Cl^-. Influx of Cl^- in barley is inhibited by NO_3^- in the rooting medium (Glass and Siddiqi, 1985), and there was a negative effect on influx of NO_3^- into barley and carrot root cells from Cl^- ions accumulated in the vacuoles (Cram, 1973). As noted earlier, the CLCa nitrate transporter in the tonoplast has a higher selectivity for NO_3^- than for Cl^- (De Angeli et al., 2006). In *Citrus* species, HATS for NO_3^- uptake is competitively inhibited by external Cl^-, although the LATS that operates at higher external NO_3^- concentrations is not (Cerezo et al., 1997). Within plants, NO_3^- moves more readily through the X-QUAC system (and hence into the xylem in the roots) than Cl^-, which itself moves more readily than SO_4^{2-} (Gilliham and Tester, 2005). There are further interactions between NO_3^- and Cl^- as nitrate reductase is inhibited by Cl^-, which acts on its molybdenum component (Barber et al., 1989). All of these interactions are often masked by the fact that when plants are exposed to excessive Cl^- ions, they may be experiencing salinity, and many of the processes of uptake and assimilation of nitrogen also are affected by the low water potential that occurs.

The effect of Cl^- on molybdenum in nitrate reductase highlights the fact that activity of the enzyme is also dependent on the supply of molybdate (MoO_4^{2-}) to a plant, and its internal

concentration (Shaked and Bar-Akiva, 1967). Another anion whose uptake is affected by the N form supplied is phosphate (Pi), which has been known for a long time to be present at higher concentrations in plants supplied NH_4^+ than in plants supplied NO_3^- (Arnon, 1939). Rice grown with NH_4^+ takes up more Pi than rice grown with NO_3^-, possibly because the low rhizosphere pH generated by NH_4^+ nutrition stimulates the H^+-ATPase that generates the energy for Pi uptake (Zeng et al., 2012).

However, as already discussed, the ratios of N:P in plants are relatively constant at values that give optimum growth (Knecht and Göransson, 2004). Optimum N:P ratios of 11.83:1 have been found in growth-related tissues of many crop species, and average values of 12.65:1 and 13.64:1 occur in terrestrial and freshwater autotrophs (Greenwood et al., 2008). This ratio is not found under all circumstances, and in timothy (*Phleum pratense* L.), higher concentrations of P occurred in the tissues when N supplies were limiting growth (Bélanger and Richards, 1999). In tomato supplied ratios of NO_3–N and P ranging from 18:1 to 2:1, the plants took up N and P at rates that gave internal concentrations closer to 14:1 than to the supply ratios, and where internal homeostasis was unable to give internal values close to 14:1, the plants grew less well (Abduelghader et al., 2011). This occurrence reinforces the idea of there being an optimum N:P ratio for plant growth, although the N:P ratio and the RGR of whole plants decline with age, due to the decline in proportion of tissues involved in growth (Ågren, 2004; Greenwood et al., 2008).

Work on arabidopsis has shown antagonism between uptake of nitrate and phosphate, so that high NO_3^- suppresses phosphate accumulation and high phosphate suppresses N accumulation, although to a lesser extent (Kant et al., 2011). It seems that in plants supplied NO_3^-, low N supply gives rise to responses controlled by different genes, including the *Nitrogen Limitation Adaptation* (*NLA*) gene, which is involved in the regulation of phosphate uptake (Kant et al., 2011). Nitrogen deprivation gave rise to accumulation of inorganic phosphate in corn leaves and down-regulation of genes involved in phosphate homeostasis (Schlüter et al., 2012). In experiments on arbuscular mycorrhizal barrel medic (*Medicago truncatula* Gaertn.) in association with the fungus *Rhizophagus irregularis* C. Walker & A. Schüßler (syn. *Glomus irregulare* Blaszk., Wubet, Renker & Buscot; *Glomus intraradices* G. N. Schenck & G. S. Sm.), plants that were subjected to simultaneous N and P deprivation had more mycorrhizal colonization than plants subjected to low P alone, despite having higher internal P concentrations (Bonneau et al., 2013). More genes were induced in the plants subjected to low N and P together, and N deficiency induced several genes for phosphate transporters.

Sulfur (S) and N nutrition interact at many levels, as the uptake and assimilation of NO_3^- and SO_4^{2-} have much in common, and there are many common products of N and S metabolism (Hesse et al., 2004). Nitrate induces gene expression for sulfate transporters (Vidmar et al., 1999; Wang et al., 2003) and for sulfate assimilatory enzymes (Koprivova et al., 2000; Wang et al., 2003). Ammonium supply to N-deficient plants also increases the expression of a sulfate transporter gene (Vidmar et al., 1999). Conversely, low S depresses the uptake of NO_3^- and NH_4^+ (Clarkson et al., 1989), and there is a negative effect on NR gene expression with S deficiency in tobacco (Migge et al., 2000). This gene repression was caused by an accumulation of glutamine or asparagine following the withdrawal of S. Sulfur-deficient plants accumulate arginine and asparagine in particular, but have lower levels of the sulfur-containing amino acids cysteine and methionine (Hesse et al., 2004). Guinea grass (*Panicum maximum* Jacq.) accumulates asparagine with low S supply, the leguminous pasture crop stylo (*Stylosanthes guianensis* Sw.) accumulates arginine, and both species accumulate free NO_3^- (Schmidt et al., 2013). With low S supply, the N:S ratio can be above 60:1 in these species, whereas for optimal growth a more normal ratio of 20:1 is required (Schmidt et al., 2013). Accumulation of asparagine in wheat grains under S deficiency gives an increased risk of formation of acrylamide when the flour products are cooked (Halford et al., 2012).

Some of the interaction between N and S metabolism comes from *O*-acetylserine, the immediate precursor of cysteine that does not itself contain S. For assimilation of sulfate to occur, plants must contain adequate levels of this precursor, and as it is an amino acid its concentration is dependent on

N nutrition (Hesse et al., 2004). As well as affecting concentrations of S-containing amino acids, S supply affects concentrations of other metabolites that contain S and N. This effect includes alliins (cysteine sulfoxides), concentrations of which were increased considerably in bulbs and leaves of onion and garlic by increased S fertilization but not much by increased N fertilization (Bloem et al., 2005). Concentrations of glucosinolates were high in the heads of broccoli (*Brassica oleracea* L. var. *italica* Plenck.) grown with low rates of N supply, but were low if enough N was supplied to give good yields when S supply was low (Schonhof et al., 2007). Heads with tissue N:S ratios above 10:1 had low glucosinolate concentrations.

Imposition of boron deficiency on tobacco plants led to decreased rates of nitrate uptake within 2 days, and expression of *NRT2* genes decreased (Camacho-Cristóbal and González-Fontes, 2007). This result gave noticeably lower concentrations of NO_3^- in leaves and roots, and the accumulation of carbohydrates in boron-deficient plants could arise from interference with N nutrition.

Soil aluminum is often deleterious to crop production, although there are some plants in which it is a beneficial element. In a comparison of 15 accessions of subspecies *indica* rice, it was found that they preferentially took up NO_3^- as their N source, and they tended to be sensitive to aluminum, whereas 15 subspecies *japonica* accessions were found to take up NH_4^+ preferentially and were aluminum tolerant and even had their growth stimulated by aluminum (Zhao et al., 2013). Although many rice genotypes preferentially acquire their nitrogen as NH_4^+, most other cereal species preferentially take up NO_3^- and are sensitive to aluminum (Zhao et al., 2013).

2.8 DIAGNOSIS OF NITROGEN STATUS IN PLANTS

Plants deficient in N have yellow leaves, due to lowered synthesis of protein and chlorophyll, and traditionally farmers have known when their crops require additional nitrogen by this observation. The logical progression for such observations was the measurement of chlorophyll concentration in crops with small, handheld meters that measure transmissions in the red (R) and near-infrared (NIR) wavelengths (Olfs et al., 2005). Strong negative relationships occurred between the NIR:R reflectance ratio at the prepanicle initiation or the panicle differentiation growth stages of rice and the extra yield that could be obtained by topdressing with nitrogen (Turner and Jund, 1991). Measurement of the reflectance ratio in the uppermost fully expanded leaves of wheat, barley, oats, and rye crops relative to the values with no leaf present gave accurate predictions of the leaf N concentrations and allowed the calculation of critical chlorophyll meter values for fertilizer N requirement (Peltonen et al., 1995). More recently, chlorophyll meters have been replaced by leaf color charts for poor rice farmers (Balasubramanian et al., 1999; Yang et al., 2003).

These methods give a more rapid diagnosis of crop N requirement than laboratory tests. Another quick, field test is to measure NO_3^- concentration in the sap with test strips (Scaife and Stevens, 1983). This test is based on the idea that if NO_3^- concentrations in leaves are low, the potential capacity for the formation of amino acids and proteins will not be realized, so additional N fertilizer should be supplied. It also identifies crops that have accumulated NO_3^- above limits for human consumption. The corn stalk nitrate test can be used to measure N concentration in the stalks of corn at harvest and gives an indication as to whether the crop is N deficient or contains excessive N, and serves as a tool to adjust the N fertilizer supply in following seasons (e.g., Sawyer, 2010).

Initially, chlorophyll (and NO_3^- concentration) measurements were made on individual leaves from representative plants, and it was established if crop yield would be increased by the application of a N fertilizer. Modeling of the relationship between sensor readings and plant yield subsequently became more complex. For example, models were established for wheat to enable farmers to apply N at a 1 m^2 scale in the field to give the maximum grain yield possible at harvest, based on midseason predictions of what the yield would otherwise have been in each square without N application (Raun et al., 2002).

The ratio of reflectance in the NIR wavelengths to the reflectance in the red wavelengths gives a ratio-based vegetation index (VI). From this determination, people developed the normalized

difference vegetation index (NDVI), the ratio of the difference between the NIR and red reflectances and their sum (Jackson and Huete, 1991):

$$\frac{\left(R_{\mathrm{NIR}} - R_{\mathrm{R}}\right)}{\left(R_{\mathrm{NIR}} + R_{\mathrm{R}}\right)}$$

where R_{NIR} and R_{R} are the reflectances in the NIR and red wavelengths, respectively. This index is used for remote sensing of chlorophyll concentration by satellites to estimate plant biomass across areas of land. Other VIs include orthogonal VIs, which have a correction component to account for the background reflectance from the soil, and atmospheric corrected indices, to take into account the spectral properties of the atmosphere (Daughtry et al., 2000; Zhao et al., 2005; Dorigo et al., 2007). Some researchers developed VIs based on derivatives of the reflectance values measured.

Commonly used derivatives include the red-edge effect. Here, the fact that in moving from 680 to 800 nm you pass from the wavelength of maximum absorbance (and minimum reflectance) of chlorophyll (between 660 and 680 nm) to almost zero absorbance (and nearly maximum reflectance) gives the opportunity to plot the gradient of the line between reflectances at the wavelengths chosen in the red and NIR bands to give the chlorophyll concentration (Daughtry et al., 2000; Zhao et al., 2005; Dorigo et al., 2007). Furthermore, if the relationship between reflectance and wavelength is plotted continuously between contiguous wavelengths, it is possible to see at which wavelength the gradient reaches its maximum value, and this value can be used as an indication of the density of vegetation (Daughtry et al., 2000; Dorigo et al., 2007). Determination of the red-edge inflection point is more sensitive than NDVI when biomass is dense, but is less sensitive when biomass is scarce, so when reflectance is used to evaluate N requirements early in wheat crop growth, the extra expense and lower sensitivity of red-edge position measurements make NDVI a more useful technique (Kanke et al., 2012).

Work has been carried out to optimize the choice of NIR and R wavelengths for such models and to evaluate how wide the bandwidth can be for accurate and repeatable measurements. In drawing up an NDVI for the evaluation of N stress in cotton, it was found that although the red channels of most commercial sensors typically operate at 640–660 nm, the best combination of wavelengths to use is between 680 and 730 nm in the red band and between 750 and 850 nm in the NIR (Zhao et al., 2005). However, relationships such as this based on two wavelengths cause problems when an entire canopy is being analyzed, as a dense canopy causes saturation of the NDVI (Wang et al., 2012a). For this reason, indices have been developed based on three different wavelengths (Dorigo et al., 2007). A three-band NDVI has been evaluated on rice and wheat, based on the relationship between

$$\frac{\left(R_{924} - R_{703} + 2 \times R_{423}\right)}{\left(R_{924} + R_{703} - 2 \times R_{423}\right)}$$

and leaf N concentration (Wang et al., 2012a). The regression of leaf N concentration on the result of the three-wavelength term was robust and repeatable for both species, even if relatively broad bandwidths (36 nm for 924 nm, 15 nm for 703 nm, and 21 nm for the 423 nm measurement) were used. This determination facilitated the development of an inexpensive remote sensor for accurate estimation of plant N concentration. In a study on wheat and barley, reflectance in the red, red-edge, and NIR bands (670 nm with a bandwidth of 25 nm, 720 nm with a bandwidth of 10 nm, and 790 nm with a bandwidth of 25 nm, respectively) was measured by satellite, from the air, or by motorbike, and after processing the data through VIs, plant N concentration was plotted at a field scale (Perry et al., 2012).

Other wavelengths can be used, as in studies on the optimal N fertilizer rate in maize (Solari et al., 2008; Sripada et al., 2008; Barker and Sawyer, 2010). The sensors used by these authors

measure in the green wavelengths and in the NIR, and Sripada et al. (2008) found that on testing a range of different VIs based on sensor measurements at the V6 stage of maize growth, the relative green normalized difference vegetation index ($RGNDVI_R$) was the best predictor of the economic optimum N rate (EONR):

$$RGNDVI_R = \frac{GNDVI_{plot}}{GNDVI_{reference\ plot}}$$

$$GNDVI = \frac{(NIR - G)}{(NIR + G)}$$

where

NIR is the reflectance at 880 nm

G is the reflectance at 590 nm

$GNDVI_{plot}$ is the value of the green normalized difference vegetation index (GNDVI) in the trial plot

$GNDVI_{reference\ plot}$ is the value of the GNDVI in a high-N strip (Sripada et al., 2008)

Early sensors were passive, and their accuracy was affected by light conditions in the field. That problem restricted the hours in which sensing could be carried out. With the advent of cheap light-emitting diodes, active sensors have been developed that measure the reflectance of light emitted on to the canopy, and many recent studies have used them. Active sensors allow for the measurement of chlorophyll fluorescence rather than reflectance. They facilitate the measurement of the chlorophyll/flavonoid ratio in plants, which should be more sensitive to the N status as flavonoids increase with N deficiency while chlorophyll decreases (Samborski et al., 2009). In a study on the turfgrasses seashore paspalum (*Paspalum vaginatum* Sw.) and manila grass (*Zoysia matrella* Merr.), the nitrogen balance index (NBI_1) discriminated between six different levels of applied N:

$$NBI_1 = \frac{FRF_G FRF_{UV}}{FRF_R^2}$$

where FRF_G, FRF_{UV}, and FRF_R are the values of fluorescence excited by green, ultraviolet, and red wavelengths, respectively (Agati et al., 2013).

An alternative approach that is cheaper than using specialist sensors is to use digital photography. Measurements of green channel minus red channel values in a rice crop image have been shown to be related to N content (Wang et al., 2013b). A comparison of commercial sensors used for plant N assessment is given by Samborski et al. (2009).

2.9 FORMS AND CONCENTRATIONS OF NITROGEN IN SOILS AND THEIR AVAILABILITY TO PLANTS

In soils that do not receive fertilizers, N occurs mainly in humus, in the proteins of microorganisms, in plant roots, and in soil-living animals. As these organisms die, proteins are hydrolyzed to amino acids, which are then converted to NH_4^+, NO_2^-, and NO_3^- by soil microorganisms. However, there is some input of inorganic N in rainwater as NO_3^- and NO_2^- from the fixation of N by lightning and combustion of fossil fuels, and as N_2 gas that exchanges with N_2 in the atmosphere. This N_2 can be reduced to NH_4^+ by free-living and symbiotic microorganisms.

Microorganisms outcompete plants for mineral N in a natural soil, but if microbial biomass remains constant some of their N content is released as they respire CO_2 from carbon in the organic

matter that they feed on, making N available for plants (Kuzyakov and Xu, 2013). Even where there has been supply of inorganic N in fertilizers, the natural nitrogen cycle operates and N forms other than those applied can arise. Much of the ^{15}N in $^{15}NH_4{}^{15}NO_3$, or ^{15}N urea was immobilized in soil microbes within a month of supplying it as fertilizer (Recous et al., 1988). However, after application of N fertilizer there may be more N present than the soil microbes can utilize, increasing the risk of it being lost (Kuzyakov and Xu, 2013).

The microbial conversion of NH_4^+ to NO_2^- and then NO_3^- depends on there being oxygen available, and in an aerobic soil occurs to the extent that NO_3^- is the major N form present. Under anaerobic conditions, such as waterlogging, these two microbial steps are inhibited, and furthermore, existing NO_3^- is reduced by denitrifying soil microbes and is lost as nitrogen gases (N_2 or N_2O), so NH_4^+ tends to be the major inorganic N form present. Even if it does not give rise to anaerobic soil conditions, heavy rainfall can remove some of the NO_3^- ions by leaching, although NH_4^+ ions are retained in the soil by cation exchange. Soil acidity also inhibits the reactions of nitrification without significantly affecting proteolysis, again giving rise to higher proportions of the soil inorganic N occurring as NH_4^+ and also giving high concentrations of amino acids (Näsholm et al., 2009). Microbial reactions are temperature dependent, and cold conditions such as those in the taiga and tundra tend to slow down many of the steps of the N cycle.

In the seasonally cold soils of the taiga, proteases function well, and amino acids are abundant (Kielland et al., 2007). Amino acid uptake theoretically could account for between 10% and 90% of N requirement in some plant species, although there is adsorption of amino acids to clay minerals and other soil components and there is competition with soil microbes for their uptake (Lipson and Näsholm, 2001; Kuzyakov and Xu, 2013). In recent times, amino acids have come to be regarded as being an important N source for plants and trees in many soils. Measurement of diffusive flux of amino acids to roots of plants in 15 boreal forest soils from northern Sweden showed that in those soils, amino acids supply 74%–89% of the total N flux, with NH_4^+ contributing 5%–15% and NO_3^- 5%–11% (Inselbacher and Näsholm, 2012). This sample included soils that had received mineral N fertilizer. However, the actual concentrations of N forms in the soils differed considerably, with NH_4^+ contributing 79% of the total soil solution N, amino acids 11% and NO_3^- 10% (Table 2.3).

Differences between N flux and concentrations of various N forms can arise because of imbalances between the different steps of mineralization and also because the different N forms are taken up by plants and trees at differing rates. Amino acids may be present mostly in bound form, and in a study of three sites in Sweden with four different land uses, bound amino acids were typically in the concentration range of 20–30 mmol m^{-3}, whereas free amino acids occurred in the range of 0–29 mmol m^{-3} for individual compounds (Jämtgård et al., 2010). These concentrations are within the range of K_m values seen for amino acid transporters. Serine, glycine, and alanine were the major amino acids present. Although bound amino acids may not be instantaneously available to plants, they represent a pool from which free amino acids may arise.

As well as amino acids, soil solution also contains amino sugars (Mulvaney et al., 2001) and quaternary ammonium compounds such as betaine, carnitine, acetyl carnitine, choline, and ergothioneine (Warren, 2013a). In a study of soils from 18 arable sites in Illinois (USA), the concentration of amino sugar N ranged from 31% to 98% as high as amino acid N (Mulvaney et al., 2001). In a subalpine Australian grassland, the quaternary ammonium pool in the soil water was up to 25% the size of the amino acid pool (Warren, 2013a).

Currently, it is thought that in soils of low fertility amino acids are the main N form available to plants, that in soils of high fertility NO_3^- is the predominant form, and that in soils of intermediate fertility it is NH_4^+. The most important N form taken up by plants in each of these different soils matches the form that predominates (Rothstein, 2009). In a study of five forest sites of different levels of fertility in Michigan, USA, the concentration of free amino acids was highest at low fertility and decreased with increase in fertility. The concentration of NO_3^- showed the reverse trend, and the concentration of NH_4^+ was highest in the sites with intermediate fertility. Amino acid concentrations were particularly high in the spring and so could represent an important N

TABLE 2.3
Some Representative Soil N Contents

Amino Acids	Ammonium	Nitrate	Notes	References
3–24 mg N kg^{-1} soil	2–8 mg N kg^{-1} soil	<1.0 mg N kg^{-1} soil	Five forest taiga ecosystems	Kielland et al. (2007)
0.6 mg N kg^{-1} soil (free)	2.8 mg N kg^{-1} soil (free)	0.35 mg N kg^{-1} soil (free)	Unfertilized boreal soil in northern Sweden	Inselbacher and Näsholm (2012)
6 mg N kg^{-1} soil (free + exchangeable)	5 mg N kg^{-1} soil (free + exchangeable)	0.4 mg N kg^{-1} soil (free + exchangeable)		
30 to <5 mg N m^{-2} (September)	80–40 mg N m^{-2} (May)	70–10 mg N m^{-1} (April)	Temperate forest, United States	Rothstein (2009)
150 to <5 mg N m^{-2} (May)	310–70 mg N m^{-2} (September)	400–30 mg N m^{-2} (September)	Soluble peptides 20–150 mg N m^{-2}	
<5 µmol L^{-1} soil solution (free), up to 30 µmol L^{-1} (bound)	Up to 10 µmol L^{-1} soil solution	<5 µmol L^{-1} soil solution	Thinned birch forest, mid Sweden	Jamtgård et al. (2010)
<5 µmol L^{-1} soil solution (free), up to 30 µmol L^{-1} (bound)	Up to 7 µmol L^{-1} soil solution	<5 µmol L^{-1} soil solution	Old grassland, mid Sweden	Jamtgård et al. (2010)
Up to 5 µmol L^{-1} soil solution (free), 30 µmol L^{-1} (bound)	Up to 6 µmol L^{-1} soil solution	5 µmol L^{-1} soil solution	Organic ley, mid Sweden	Jamtgård et al. (2010)
Up to 10 µmol L^{-1} soil solution (free), up to 70 µmol L^{-1} (bound)	Up to 10 µmol L^{-1} soil solution	Up to 2200 µmol L^{-1} soil solution	Organic lettuce, mid Sweden	Jamtgård et al. (2010)
nd	2.0 (range 0–11) mg kg^{-1} soil 0.05–0.8 mol m^{-3} in soil solution	28.7 (range 0–97) mg kg^{-1} soil 0–7 mol m^{-3} in soil solution	256 sites in 4 winter cereal fields, United Kingdom	Lark et al. (2004) and Miller et al. (2007)
Amino Acids	**Ammonium**	**Amino Sugars**		
70–908 mg N kg^{-1} soil (in hydrolysate)	182–604 mg N kg^{-1} soil (in hydrolysate)	116–511 mg N kg^{-1} soil (in hydrolysate)	Maize fields preplanting, Illinois, USA	Mulvaney et al. (2001)
Amino Acids	**Quaternary Ammonium Compounds**	**Peptides**		
4.8 µmol L^{-1} soil solution	1.2 µmol L^{-1} soil solution	0.4 µmol L^{-1} soil solution	Subalpine grassland, Australia	Warren (2013a)

source for the trees, particularly as the trees in the low fertility sites tended to have ectomycorrhizal associations, whereas those in the high fertility sites were more likely to have AM associations (Rothstein, 2009).

In agricultural soils that receive fertilizers and manures, the concentrations of inorganic N forms are higher than in unfertilized soils, and the predominant ion is NO_3^-. However, concentrations vary considerably among soils, among different fields on one soil type, and even within fields. In a study of soil samples taken in October from 4 m intervals along a transect across four fields in a winter cereal rotation in the United Kingdom, concentrations of NO_3^- ranged from below 1 mol m^{-3} to above 7 mol m^{-3} and of NH_4^+ from below 0.1 mol m^{-3} to up to 0.8 mol m^{-3} (Lark et al., 2004; Miller et al., 2007). Although concentrations of both N forms varied considerably between consecutive transect positions, most of the samples contained NO_3^- at concentrations above 1 mol m^{-3},

and so in the range where uptake into plants would occur by LATS rather than HATS. Most of the samples contained NH_4^+ at between 0.1 and 0.2 mol m^{-3}. The significance of urea uptake in relation to crop yields is probably small other than when it is applied to foliage, as the presence of free and microorganism-bound urease means that the urea in a field soil is probably hydrolyzed before much of it is taken up (Engels and Marschner, 1990).

2.10 TESTING SOILS FOR NITROGEN CONTENT

A key requirement for predicting fertilizer N requirements in many North American and European countries is to know the soil mineral nitrogen (SMN) content (the concentrations of NH_4^+ and NO_3^- ions). Laboratory analysis is carried out on representative soil samples (15–20 ha^{-1}) taken within the rooting depth of the crop being grown or about to be grown, typically in 2–3 different soil layers (Olfs et al., 2005). The soil is extracted with a mild extractant such as 1.0 kmol m^{-3} KCl or 0.0125 kmol m^{-3} $CaCl_2$, and the extract is tested for NO_3^- and NH_3 (Olfs et al., 2005). Tests for SMN are typically carried out before planting a crop, so they can be used to predict its N requirement, but can also be carried out during vegetative growth to improve the accuracy of these predictions. An example here is the pre-sidedress nitrate test recommended for corn growing in several U.S. states, where soil cores are collected by the farmer in spring just before rapid crop growth starts and are then sent to a soil-testing laboratory (e.g., Iowa State University, 1997). The pre-sidedress nitrate test has been shown to be more reliable for fertilizer recommendations in corn crops in Argentina than presowing soil nitrate tests (Sainz Rosas et al., 2008). NO_3^- concentration in the soil also can be measured quickly in the field with nitrate test strips (Schmidhalter, 2005).

In addition to measurement of SMN, there can be a requirement to measure the potential for N mineralization in a soil. It is possible to measure the total soil organic N concentration by difference between total N and inorganic N concentrations, but this does not distinguish between organic N that may be mineralized and recalcitrant organic N. The standard method of estimating mineralizable N is to incubate soil for 210 days in an aerobic environment and to measure mineral N at the end of that time (Schomberg et al., 2009). However, if mineralization potential is being measured to predict N fertilizer requirement, results are required quickly. One means of achieving this action is to measure NH_4–N concentration after anaerobic incubation of a soil sample for 7 or 14 days (Schomberg et al., 2009).

Another way of evaluating the N fertilizer requirement from how much mineralizable N there is in a soil is based on the fact that in well-fertilized corn fields amino sugar-N concentrations are high, whereas in poorly fertilized fields they are low (Mulvaney et al., 2001). This measurement is the Illinois Soil Nitrogen Test, where amino sugar-N concentration is measured in soil at 0–15 cm depth before planting corn and the N fertilizer requirement is calculated based on the values obtained. The method involves making hydrolysates by heating soil under reflux in hydrochloric acid and measuring total hydrolyzable N by Kjeldahl analysis, then hydrolyzable NH_4–N, NH_4–N + amino sugar-N, and amino acid-N concentrations in subsequent steps (Mulvaney and Khan, 2001). The test has been used since 2001, but gives a poor level of accuracy in some areas (Laboski et al., 2008).

2.11 NITROGEN FERTILIZERS

Since the amount of crop biomass formed is proportional to PAR intercepted, providing crops with sufficient N to develop and maintain a large leaf area enables maximum interception to occur. Fertilizer policy needs to give sufficient N for RGR to remain constant at its maximum possible value (i.e., growth is exponential) for as long as possible. As N deficiency causes a proportionally larger decline in dry matter accumulation when W (mass of crop per given land area) is small and growth rate under N sufficiency is proportional to W, it is more important to ensure N supplies are sufficient early on in crop growth than when W is larger, and growth rate is approximately linear (Greenwood et al., 1986). Eventually, RGR will decline anyway (due to increased structural material

and self-shading, but also as environmental cues switch on reproductive growth). Prolonging a fast growth rate is advantageous, but it is vital to ensure growth goes as fast as possible to start with.

The relationship between *W* and *N*% is constant for each crop across a range of environmental conditions (Greenwood et al., 1990; Lemaire et al., 2007). Therefore, it is possible to calculate easily how much N already must have been taken up per unit of land area in a crop of a given biomass and to know how much more has to be available to the plants to achieve any particular target yield. This concept is the basis of simple fertilizer recommendations, where subtraction of the value of available N in the soil from the total required gives the value of N that needs to be supplied. As crop growth is reduced in proportion to the ratio of N concentration actually in a crop to the critical N concentration at that growth stage, this ratio can also be used as a nitrogen nutrition index to evaluate the extent of any N deficiency that occurs (Gastal and Lemaire, 2002), and N can be supplied at an appropriate rate.

This N supply can be provided in fertilizers, and the main N fertilizers produced globally are shown in Table 2.4. In commercial horticulture, plants often are grown in nutrient culture (either in true hydroponics, aeroponics, or trickle supply of nutrient solution over an inert support such as rock wool). Here, N is supplied predominantly as NO_3^-, with some NH_4^+ (e.g., in a 23:1 ratio in the work of Le Bot et al., 2001) and at slightly acid pH, ideal for uptake of the NO_3^- ion, thus minimizing competition between the uptake of NH_4^+ and other cations such as K^+ and Ca^{2+}.

In some farming systems, N is provided to farmland in manures and composts. Pig slurry supplies approximately 4.4 kg N per tonne at 6% dry matter (approximately 70% as NH_4–N and 30% as organic N), cattle slurry 2.6 kg N per tonne at 6% dry matter (40% NH_4–N and 60% organic N), fresh farmyard manure about 6.0 kg N per tonne (20% NH_4–N and 80% organic N), and broiler litter 30 kg N per tonne (25% NH_4–N, 65% organic N, and 10% uric acid) (Defra, 2010). Farmland also has some additional input of inorganic N through deposition of N forms that originally entered the atmosphere as pollutants. Although the NOx gases have a harmful effect on the environment, causing acid rain and raising the fertility of natural ecosystems that need to be of low fertility to flourish, they do represent a source of soil mineral N that can be taken up by crop plants. Indeed, total depositions of 117 kg N ha^{-1} $year^{-1}$ have been measured in a wheat–corn cropping system in the North China Plain (He et al., 2010).

Too much N fertilizer application can lead to pollution through leaching of NO_3^-, especially if it occurs at times before crops are growing fast and taking up NO_3^- quickly. Furthermore, excessive use of N on cereals gives lodging and delayed senescence, causing many of the grains to fail to develop completely (Yang and Zhang, 2006). It also gives a risk of foliar diseases developing and silage crops not fermenting properly (Defra, 2010). The ideal is to supply the EONR (N_{opt}, the amount of N supplied that gives the optimum economic return). This optimum fertilization

TABLE 2.4
Annual World Production of Major N Fertilizers

Fertilizer	Production in 2012 (tonnes of N)
Anhydrous ammonia	136,455,000
Urea	74,395,000
Ammonium nitrate	16,030,000
Diammonium phosphate	6,291,000
Ammonium sulfate	4,724,000
Calcium ammonium nitrate	3,783,000
Monoammonium phosphate	2,610,000

Source: Figures from International Fertilizer Industry Association (IFA), www.fertilizer.org/ifa, accessed March 18, 2014. With permission.

varies considerably among fields, because of differences in organic matter content and soil chemical properties and also between years, because of climatic variability (Lory and Scharf, 2003; Olfs et al., 2005; Torres and Link, 2010).

2.11.1 Fertilizer Recommendation Techniques

One way of arriving at N fertilizer recommendations is through an index method, such as the soil nitrogen supply (SNS) index used in the United Kingdom (Defra, 2010). SNS is the SMN + the N already in the crop + the N that will have been made available by mineralization by harvest time. Farmers work out the SNS index value, either by the field assessment method or by measurement of SMN, and use the index value to work out how much N fertilizer to supply (Defra, 2010). The field assessment method requires them to arrive at an SNS value from knowledge of usual rainfall, soil type, and previous crop. The measurement method requires them to take representative soil samples to 90 cm depth for measurement of SMN, to estimate the N already in the crop (from density of cereal shoots or green area index of oilseed rape), and to allow for future mineralization of N in soils with high organic matter content (Defra, 2010).

Measurement of SMN is important in the N_{min} method developed for use on cereal crops in Germany (Wehrmann and Scharpf, 1979). Here a target yield was established, from which the total N requirement of the crop could be deduced, and the SMN measured at the start of the growth period + estimated mineral N that would arise from mineralization was subtracted from this target N requirement to give the amount of fertilizer N required (Olfs et al., 2005).

Another forecasting technique is the balance sheet method (also called a forecast balance sheet), commonly used in the United States, France, and other countries for a variety of arable crops. N_{opt} is estimated as being the total N content of the harvested part of the crop minus all the N coming from sources other than fertilizer and adjusted for the efficiency of the crop in recovering N from the soil (Lory and Scharf, 2003). It is calculated for a selected target yield, so that for a grain crop

$$N_f = \frac{\left(N_g - N_{gs}\right)}{\text{FNUE}}$$

where

N_f is the estimated N_{opt} for a selected yield goal
N_g is the N content of the harvested grain
N_{gs} is the N in the grain that came from the soil rather than the fertilizer
The fertilizer nitrogen-use efficiency (FNUE) is the proportion of fertilizer N applied recovered in the grain (Lory and Scharf, 2003)

It has to take into account the price of the fertilizer:value of grain ratio. It requires information on the availability of N in the soil, a value that can come from an index based on soil properties or previous crop or can come from soil testing.

The problem with models based on testing soil N or NO_3^- content at sowing, or early in the growth period, is that the soil mineralization potential has to be estimated, and as this potential is very weather dependent (Torres and Link, 2010) inaccuracy is introduced into the fertilizer recommendation. Measurement of mineralization potential of the soil by 7-day anaerobic incubation improved the accuracy of fertilizer N recommendations based on the pre-sidedress soil nitrate test in corn (Sainz Rosas et al., 2008) and on soil NO_3^- content at sowing in spring wheat in the important Pampa grain-producing region of Argentina (Reussi Calvo et al., 2013).

Fertilizer recommendations based on soil or plant analysis may be too expensive for small farmers, so recommendation systems that are robust and simple have been produced for Asia and Africa (Chuan et al., 2013). The Nutrient Expert decision support system for cereals uses site-specific,

nutrient management to make field-specific recommendations for N and also for P and K (Pampolino et al., 2012). It uses information given by the farmer to estimate how much N is likely to be available, namely soil color, texture, organic matter content, crop sequence, residue management, water supply, fertilizer inputs, and current yield. The software estimates the natural N supply and what potential yield could be achieved, and makes recommendations of how much fertilizer N should be given (and at which growth stages) to achieve the potential yield. The output is based on experiments in which crops grown in different fields were evaluated for nutrient uptake by comparing a zero-N treatment with a treatment of N supply as in best practice, and yield–response curves were generated (Pampolino et al., 2012). The Nutrient Expert system has given good results for wheat in China (Chuan et al., 2013).

2.11.2 Timing and Amounts of Nitrogen Fertilizer Application

Because of the between-season variability in N availability due to weather conditions, monitoring of soil and plants can remove some of the uncertainty in making N fertilizer recommendations. Monitoring ensures that N is applied only when the plants are responsive to it. That action has given rise to the use of split applications, which shortens the time between N becoming available and being taken up by a crop and minimizes losses due to leaching or volatilization (Torres and Link, 2010). In an experiment on three split applications of N to winter wheat in Germany, the authors found an N-use efficiency (N uptake relative to N supply) of 83% compared with 62% for average yields across the 27 EU member states (Torres and Link, 2010). The normal practice for N fertilization of winter wheat in northern Europe is now for three applications, one early to promote tillering (50–80 kg N ha^{-1}), one at the beginning of stem elongation (50 kg N ha^{-1}), and one at the second node stage (40–50 kg N ha^{-1}) (Hirel et al., 2007). A preplanting N application, with the risks of nitrate leaching during the winter rains, is not required as there is usually sufficient N already in the soil for early growth of the crop. Application of N after the main period of vegetative growth would be wasted for increasing grain yield but could improve crop quality. For example, postpollination foliar application of urea ammonium nitrate to hard red winter wheat and hard red spring wheat crops in South Dakota, USA, increased grain protein concentration (Bly and Woodard, 2003).

Split applications are not universally beneficial, and in winter cereals in Mediterranean climates, the usual practice of a presowing application of N (with P and K) plus a further application at tillering does not seem to give a yield advantage over just one application at tillering (Torres and Link, 2010). Split applications did not give a yield advantage in experiments on barley, wheat, or oilseed rape in western Canada, although there may have been some yield advantages in wetter regions (Grant et al., 2012). Furthermore, while it is possible to drive over cereal crops a few times without causing too much damage, for row crops such as sugar beet and potato you cannot drive over after row closure without yield loss (Olfs et al., 2005).

The normal practice for N fertilization of maize in the United States is an application presowing, with a further application (typically after a pre-sidedress test for soil NO_3^-) during vegetative growth. A split application of 90 kg N ha^{-1} presowing and a further 90 kg ha^{-1} at the V6 or V10 growth stages gives the best results (Walsh et al., 2012). In Colombia, three applications (at sowing and at V6 and V10) have been shown to produce better yields for the same N rate than a double split (Torres and Link, 2011). In China there may be merely one N application, before sowing, but high-yielding farms use a basal application and subsequent applications at V8, V12, and VT growth stages (up to 450 kg N ha^{-1}) (Peng et al., 2013). For silage corn, N often is supplied preplanting as manure, as farmers producing silage are usually doing so for their own livestock and have manure to dispose of.

For lowland (paddy) rice in the tropics, fertilizers are used to supplement biological N fixation. Fixation can be from indigenous organisms, with cyanobacteria supplying on average 30 kg N ha^{-1} per crop and photosynthetic bacteria supplying an average of 7 kg N ha^{-1} (Ladha and Reddy, 2003). Alternatively, it can be supplied either from inoculating azolla as a companion crop or growing

leguminous plants for feed and fodder or as cover crops between subsequent rice crops; azolla can release 20–30 kg N ha^{-1}, with a high proportion of that N coming from atmospheric N_2 (Ladha and Reddy, 2003). Rice in southeast Asia typically is given N fertilizer at sowing (40–100 kg N ha^{-1}), with a topdressing between the formation of the panicle primordia and the late stage of spikelet initiation (15–45 kg N ha^{-1}) (Hirel et al., 2007). The level of supply for the second split can be determined by use of chlorophyll meters or leaf color charts. Because of the interaction between K^+ and NH_4^+, the main N form taken up by rice, potassium should be supplied at the same time (Balkos et al., 2010).

Oilseed rape requires more N than cereals because the oil in the seeds is more expensive for the plant to produce than carbohydrates, and as little N is taken up during seed filling N fertilizer is supplied to winter crops in two splits between sowing and spring (at an optimum level of 180–200 kg N ha^{-1}) (Hirel et al., 2007). Some N also may be supplied presowing (Defra, 2010). Much of the N remains in the crop residue at harvest, so it becomes available for subsequent cereal crops in a typical northern European arable rotation (Hirel et al., 2007).

Split applications have been used for a winter wheat–summer corn rotation in the North China Plain based on a refinement of the N_{min} method. This system receives excessive fertilizer N, with consequent leaching of NO_3^- into watercourses. The improved N_{min} method is based on the changing requirements for N as crops grow, so fertilization is based on the difference between a target value of N that should be present in the shoots of the wheat or corn at three different growth stages to achieve a target yield and the concentrations of SMN measured at 0–30, 0–60, and 0–90 cm depths in the soil at early, middle, and later growth stages, respectively (Chen et al., 2006; Zhao et al., 2006). In a study of the corn part of the rotation across a large number of farms, no fertilizer N was required at planting due to the residual N from the wheat crop, but then supplying N according to the previously mentioned protocol at the 3-leaf stage (V3) and the 10-leaf stage (V10) gave a grain yield of 8.9 t ha^{-1} (compared with 8.5 t ha^{-1} for treatment based on normal farmer practice) yet with a noticeably lower rate of fertilizer supply (157 kg N ha^{-1} compared with 263 kg N ha^{-1}) (Cui et al., 2008). Other workers have suggested a standard soil N_{min} value that should be maintained for the entire growth of maize crops in China or three more accurate values that should be maintained from sowing to the V8 growth stage, from V8 to VT and from VT to R6 (Peng et al., 2013).

2.11.3 Developments in Nitrogen Fertilizer Use

Simple ways of lowering pollution caused by leaching of NO_3^- can be achieved by N fertilizer formulation, with nitrification inhibitors being added to ammonium fertilizers and urea to slow the oxidation of soil-immobile NH_4^+ ions to water-soluble NO_3^- ions. Nitrification inhibitors currently used in this way include nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP), but such use is not common as it is frequently not cost-effective (Subbarao et al., 2009).

Further ways of minimizing losses through formulation come from coating urea fertilizers with protectant coverings (polymer-coated urea). Urea applied to paddy fields is lost rapidly from the system by hydrolysis to volatile ammonia (NH_3) gas, but urea coated with polyolefin was shown to have a lower proportion of the N being lost by volatilization than uncoated urea in the productive rice-growing regions of southern China (Xu et al., 2013). There was a greater accumulation of N in the shoots, due to the slow release of available N matching the plant N demand better. Polymer-coated urea possibly may be used to increase wheat grain protein content as more N should be available for plant uptake later in the growing season than if uncoated urea is supplied, but the slow release may give a shortage of N early in the growing season with a consequent negative effect on overall grain yield (Farmaha and Sims, 2013).

Urea also can be formulated with urease inhibitor, such as phenylphosphorodiamidate (PPD) (Arkoun et al., 2013) or N-(*n*-butyl)thiophosphoric triamide (Agrotain®) (Suter et al., 2013). Field experiments on maize grown on poorly drained claypan soil in Missouri, USA, showed that there

were yield advantages over urea supplied preplanting from urea + urease inhibitor (Agrotain), urea + nitrification inhibitor (nitrapyrin), and most of all with polymer-coated urea (Motavalli et al., 2012).

The application of variable amounts of N fertilizer within and between fields that has been made possible by remote sensing of plant N, coupled with advances in fertilizer formulation, has improved the efficiency of N fertilizer use. However, the biggest gains will come in those countries where models for crop N requirement have not been well developed in the past. This gain could lead to increased crop yields over big areas or to the maintenance of current yields with application of less fertilizer. For example, models of crop N requirement have not been well developed historically in China, and the fact that N fertilizers have been subsidized by the government (Yang and Zhang, 2006) has given rise to excessive N use. This overuse leads to waste of fertilizers (with consequent unnecessary emissions of greenhouse gases in their manufacture) and large-scale nitrate pollution of water courses (Ju et al., 2006). The problem has been made worse by the large amounts of N reaching some agroecosystems through atmospheric deposition. There are highly productive systems in the country that are supported strongly by the use of synthetic N fertilizers, and as techniques for modeling crop yield are perfected even higher yields may be obtained, yet the total amount of N supplied per unit land area will decrease.

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