3

The Biology of Nitrate

The biology of nitrate is to a large extent the chemistry of nitrate implemented by living organisms. Most of the main processes involved occur only in the soil but some of them also occur in completely different parts of the environment, including our mouths (Chapter 9). All form part of the nitrogen cycle, which is shown at the global scale in Fig. 3.1a and at the scale of a field plot in Fig. 3.1b. Practically all the nitrogen in the soil is organic nitrogen, in the very earliest sense of the word 'organic' – that is, produced by living organisms (Box 2.2). The 1–2% of the nitrogen in the soil that is in inorganic or 'mineral' form, as ammonium- or nitrate-N, is most available to plants but causes most of the environmental problems.

Organic Matter – Dead and Alive

Dead – humus

Humus, or dead soil organic matter, comprises the remains of plants, mostly long dead, that grew in the soil. Plant remains do not remain recognizable for long because they are soon 'processed' by the soil population (see next section). Humus often influences the colour of the soil, and there is usually, but not always, more in the topsoil than in subsoil, making the former darker in colour. The soil is the third largest repository of nitrogen on the planet, coming after the atmosphere and the sea (Table 3.1) and the amounts of organic nitrogen in it are huge. Even a soil with a small amount of organic matter, perhaps because it has been cultivated for many decades, will probably contain 2000–3000 kg/ha of nitrogen, that is 2–3 t. Nearly all of this will be in organic forms and most will be in the plough layer, the top 25 cm of soil. More typical arable soils will contain 3–5 t N/ha and a peat soil will contain much more. These figures are for the UK and other countries in the temperate zone. The hotter the climate, the faster organic matter will be decomposed and the less will remain in the soil.

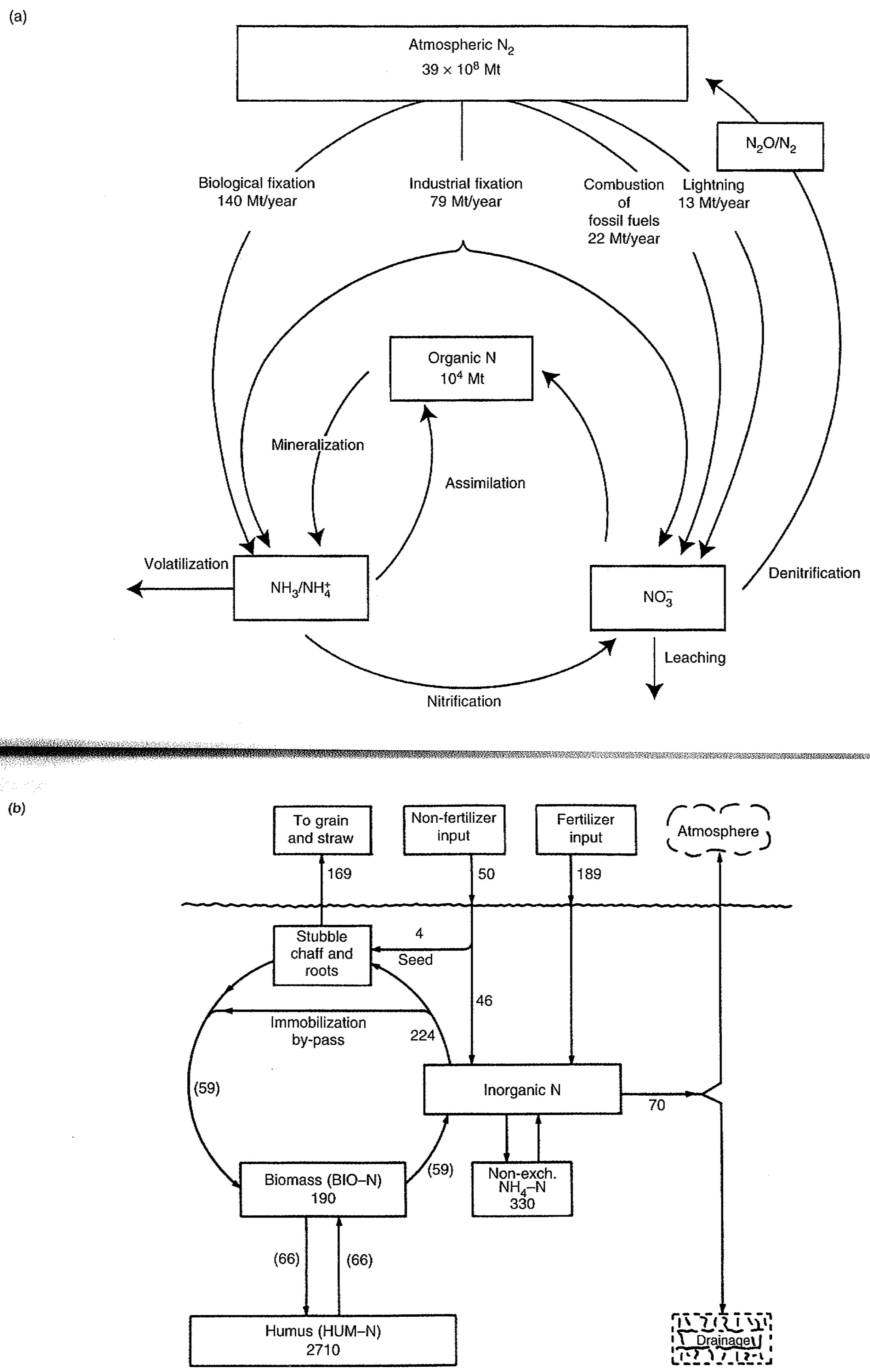

Fig. 3.1. The nitrogen cycle: (a) at the global scale (from L'hirondel and L'hirondel, 2001) and (b) at the scale of a field plot (from Powlson, 1994); the plot was on the Broadbalk Experiment and received 192 kg/ha per year. The amounts of N in (b) are in kg/ha.

Table 3.1. Global distribution of nitrogen. (After Jenkinson, 1990.)

Nitrogen	Amount (t)
The atmosphere	3.9×10^{15}
Soil (non-living)	1.5×10^{11}
Soil microbes	6.0×10^{9}
Plants	1.5×10^{10}
Animals (land)	2.0×10^{8}
People	1.0×10^{7}
Sea (various)	2.4×10^{13}

The quantity of nitrogen in the humus of a soil is 50–100 times greater than the quantity of mineral nitrogen (ammonium-N+nitrate-N), which may be 50–100 kg/ha. It is also much greater than nitrogen in fertilizer applications, which is commonly of the order of 100–200 kg/ha. But these 'small' quantities of nitrogen need to be managed with great care because they are vulnerable to leaching or denitrification, and can have immediate effects on the environment. Humus also needs to be managed, but nothing happens to it rapidly so this is a longer-term project. In recent years there has been great interest in humus as a repository for carbon coming from atmospheric carbon dioxide.

Live – the soil population

The soil contains microbes beyond number. A flock of sheep in a field constitutes a considerable body mass, but there are so many microbes beneath the soil surface that their combined body mass is about the same as that of the sheep above the surface (Jenkinson, 1977). On a global scale, the soil microbes weigh about 30 times as much as the land animals (Table 3.1). The microbes are made up of large numbers of bacteria, fungi, protozoa, algae and – at a slightly larger scale – earthworms and other soil animals. This population beneath the soil surface has a key role in the recycling of plant nutrients on which all other species depend (Edwards and Lofty, 1972; Brookes *et al*., 1982).

When the plants die, the carbon, nitrogen and sulphur they originally absorbed as carbon dioxide or ions (or possibly other small molecules) are entombed in proteins and other macromolecules, while the phosphate, though retaining its identity, is attached to other macromolecules. The cations are bound to varying extents. If nothing further were to happen in a natural ecosystem, the plants would exhaust the supply of nutrients within a few generations, and in time the ecosystem would collapse. In the longer term, the supply of carbon dioxide for photosynthesis would become depleted too. However, the soil population recycles these resources and makes them available to the plants again, thereby playing an important part in the ability of the ecosystem to organize itself. In doing so, it obtains food and energy.

Recycling the residues

Plant, and occasionally animal, remains on the surface may be attacked first by fungi; you can sometimes see a web of white fungal hyphae on dead leaves or cereal straw on the soil. But the first line of attack often comes from soil animals, millipedes, springtails and earthworms. These break up the residues and bring them into closer contact with the microbes that play the main part in the recycling process. During this process they release carbon dioxide and nutrients, leaving the more resistant material. This resistant material usually undergoes further 'processing', with further releases of carbon dioxide and nutrients before it is left as humus. During all these processes, the organisms gain carbon, nitrogen and other elements essential for their structure and metabolism.

Predation speeds up the recycling. Tennyson's reference to 'nature red in tooth and claw' might make you think of an African game-park, but it is equally applicable to the soil beneath your feet. Some species are the scavengers of the soil, akin to vultures or hyenas, feeding on the dead bodies of other organisms. Other soil species are hunters, corresponding to lions or leopards. Protozoa, for example, prey on bacteria. In each case the predator ingests and uses some of the nitrogen from the prey to build proteins and excretes the rest as ammonium, which is usually nitrified. These effects can readily be demonstrated. Eliminating the protozoa from soil decreases the amount of nitrate in it.

Earthworms play an important part in the recycling by pulling leaves and other plant material down into their burrows, leaving the less decomposable parts such as petioles behind (e.g. Edwards and Lofty, 1972). Darwin (1881), who became very interested in earthworms, found that they would even pull small triangles of paper into their burrows. As leaf material passes through an earthworm, the gut microflora begin to break it down, a process continued by microbes in the surrounding soil. The microbes in the gut and in the soil are indistinguishable from each other (Edwards and Lofty, 1972). The end result is that all the nutrients are released from the plant material, albeit in different ways reflecting the extents to which they were bound. The importance of the combined activities of earthworms and soil microbes is shown by the observation that earthworm casts contain appreciably larger concentrations of nitrate and other nutrients (Lunt and Jacobson, 1944; Nye, 1955). Termites play a somewhat similar role in tropical soils, creating mounds which are richer in nutrients than the surrounding soil.

Although much of the plant material is removed at harvest in a cropped ecosystem, the stubble and roots remain and are recycled by essentially the same processes. But these processes, so essential in a natural ecosystem, can become a problem when the soil is cropped and left bare for part of the year, as we shall see in Chapter 5.

Characteristics of the microbes

The significance of the soil microbes was neatly summarized by Jenkinson (1977), who described them as 'the eye of the needle through which all the organic materials must pass'. Despite this key role in ecosystem function, soil microbes are not as well understood as many microbes studied in other spheres of science such as food contamination. Counting microbes on a film of agar in a Petri dish is satisfactory for medical samples, for example, but with soil samples it can give results that differ greatly from other methods, such as selective staining and counting. This difference occurs mainly because the agar is far richer in microbial nutrients than the soil. Selective staining is the more reliable method, but the counting is tedious and makes the method unsuitable for routine use. Modern methods such as PCR assays now make it easier to study individual organisms (e.g. Mendum *et al.*, 1999), but even these methods have their limitations.

The microbes have been studied collectively as *the soil microbial biomass* by three methods, *fumigation–incubation*, *fumigation–extraction* and *adenosine 5′-triphosphate (ATP)*. The first, based on studies by Jenkinson and Powlson (1976a,b) involves incubating the moist soil with chloroform for 24 h and then incubating it for 10 days and measuring the release of carbon dioxide, from which the biomass carbon is estimated by multiplying the release by a constant (Jenkinson and Powlson, 1976b). Fumigation–extraction methods for carbon (Vance *et al.*, 1987) or nitrogen (Brookes *et al.*, 1985) depend on the observation that the amounts of these elements extractable with 0.5 M K_2SO_4 following fumigation with chloroform provide, with the imposition of suitable constants, a good measure of the amounts of biomass carbon and nitrogen in the soil. The same can be done to measure the phosphorus held in microbes, except that $NaHCO_3$ has to be used for the extraction (Brookes *et al.*, 1985). ATP was described in Chapter 1 as the energy currency of living systems. The amount of ATP in the soil therefore gives a measure of the quantity of microbial biomass. It can be extracted by applying ultrasound to the soil to break open microbial cells in the presence of a strong acid such as trichloroacetic acid to suppress unwanted enzyme activity, and assayed with an enzyme from fireflies (Brookes *et al.*, 1985). Another method involves the measurement of respiration induced by a substrate.

Soil organisms can be classified according to size and whether or not they have a nucleus, but the most helpful classification systems in the present context are those based on nutritional requirements and relationship with oxygen. The nutritional system divides organisms into *heterotrophs* and *autotrophs*. Heterotrophs include many species of bacteria and all the fungi. They need carbon in the form of organic molecules for growth. Autotrophs include the remaining bacteria and most algae, which can synthesize organic molecules from carbon dioxide using energy from the sun. There is a subgroup, the *chemoautotrophs*, which use energy obtained by oxidizing inorganic ions or molecules rather than energy from the sun. The oxygen-based system defines three classes. *Aerobes* need to have O_2 as the terminal acceptor of electrons during respiration. *Facultative aerobes* usually need O_2 but can adapt to anaerobic (more specifically anoxic) conditions by using nitrate and similar inorganic compounds as electron acceptors. *Obligate anaerobes* can grow only in the absence of oxygen because O_2 is toxic to them.

Most soil microbes are hardy species adapted well to life under near-starvation conditions in the soil. Indeed they seem to have developed an interesting mechanism for responding to the possible arrival of substrate (or food in our terms). Most soil microbes exist most of the time in a dormant state with just basal metabolism, but they maintain an adenylate energy charge characteristic of microorganisms undergoing exponential growth. This state of 'metabolic alertness' seems to be a survival strategy that enables the organisms to capture substrate before other organisms. It is costly in terms of energy resources, but De Nobili *et al.* (2001), who identified it, suggested that it is an adaptation that gives an evolutionary advantage over microbes that remain dormant in spores.

Mineralization, Nitrification and Immobilization

The release of carbon dioxide, ammonium, nitrate, sulphate and phosphate from soil organic matter as a result of the activity of the soil microbial biomass and the rest of the soil population is commonly known as *mineralization*. For nitrogen, the process involves two stages, *ammonification* and *nitrification*.

Ammonification is the conversion of the more readily decomposable organic nitrogen compounds such as proteins and nucleic acids into ammonium, and can be effected by a wide variety of bacteria and fungi. It is influenced by temperature (e.g. Addiscott, 1983), moisture and other factors that affect such organisms. A hydroxyl ion is formed, so the process makes the soil slightly more alkaline:

$$\text{Organic N} \rightarrow NH_4^+ + OH^-$$

Nitrification of ammonium to nitrate is an oxidation process that has an acidifying effect because hydrogen ions are formed. It is implemented in two stages by chemoautotrophic bacteria, which derive their energy solely from oxidizing ammonium ions. The first stage converts ammonium to nitrite and can be represented by the equation:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O + \text{energy}$$

It was generally believed to be effected by bacteria of the *Nitrosomonas* genera, but recent research using molecular biological techniques (e.g. Mendum *et al.*, 1999) suggests that *Nitrosospira*-type bacteria predominate in arable soils, with *Nitrosomonas* occurring more in ammonium-rich environments such as sewage sludge.

The second stage, for which *Nitrobacter* genera are responsible, is:

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy}$$

Ammonium is the final product of mineralization in very acid soils because the bacteria responsible for the first stage of nitrification are sensitive to low pH, hydrogen ions being a product in the first equation. This could be seen as a consequence of the Law of Equilibrium, but it would be an oversimplification. It is also the final product in permanently waterlogged soils because the bacteria in the first stage are aerobic. Nitrite is toxic to most soil organisms, and it is fortunate that it rarely accumulates in soil. It seems most likely to do so in soils that have become over-enriched with nitrate, probably because *Nitrobacter* functions less well in large concentrations of nitrate, which is a product in the second equation. This again could be seen as a result of the Law of Equilibrium. Nitrification is also sensitive to temperature down to about 2.5°C (Tyler *et al.*, 1959; Addiscott, 1983), below which it is inhibited.

Immobilization

Mineralization, as described above, sounds like a one-way street carrying nitrogen from proteins and nucleic acids to ammonium and then to nitrite and nitrate. As in most traffic systems, there is a parallel street running nearby in the opposite direction. This is *immobilization*, in which soil organisms of a wide range of genera take ammonium and nitrate and convert them into organic forms of nitrogen. It is quite normal for some organisms in the soil to be mineralizing nitrogen while nearby organisms are immobilizing it simultaneously. What conventional measurements of mineralization actually measure is *net* mineralization.

Net mineralization was investigated 20–30 years ago by various researchers and found to show first-order kinetics by Stanford and Smith (1972) and zero-order kinetics by Tabatabai and Al-Khafiji (1980) and Addiscott (1983). The difference probably arose because the first authors used dry soil and the later authors used field-moist soils (see Addiscott *et al.*, 1991, p. 87), and neither is necessarily correct. A textbook of physical chemistry warned long ago that simple kinetic relationships are rarely found in nature (Moelwyn-Hughes, 1957, p. 1090). However, Addiscott found that the changes with temperature in the zero-order rate for net mineralization seemed to obey the Arrhenius relationship. The zero-order model with Arrhenius temperature control on rates was used with leaching and crop uptake models by Addiscott and Whitmore (1987), giving satisfactory statistically assessed simulations of changes during winter in soil mineral nitrogen in soils from seven sites for five seasons.

During these investigations of net mineralization, hardly anybody seems to have asked how large the gross rates of mineralization and immobilization were. This was partly because very few people had thought of the question and partly because the techniques for answering it were not in place. Methods have been developed recently for measuring gross mineralization through a pool-dilution approach in which the soil ammonium pool is labelled with ^{15}N (e.g. Murphy *et al.*, 1998, 1999). (See Box 3.1 for an explanation of a 'pool' and Box 3.2 for information on ^{15}N-labelling.) The pool initially contains ^{15}N at natural abundance, and adding ^{15}N increases the ^{15}N-enrichment. The enrichment and the size of the ammonium pool are then measured as mineralization releases ammonium at natural abundance and dilutes the isotopic abundance of the pool. Gross mineralization can be inferred from the change in ^{15}N abundance over a few days. These new methods have proved valuable, but need to be treated with care because they involve assumptions, such as perfect mixing between labelled and unlabelled N, that may not be fully met.

Box 3.1. A 'pool'.

The term 'pool' is frequently used in the description of flows of organic matter in the soil. It may help to think of an ornamental water garden, in which water enters the highest pool and then flows gently down through a series of pools to the bottom of the system. During this flow some water may be immobilized in a dead-end pool. When the water reaches the bottom of the system, some leaks out, but the rest is re-circulated to the top. If you add dye to one of the pools, you can trace the progress of water from that pool through other pools below it.

For nitrogen in the soil, Jenkinson *et al.* (1985) provided a formal definition of a pool as, 'A compartment containing material that is chemically indistinguishable and equally accessible to plants (or to the soil population)'. This is an exact definition for pools with a clear chemical identity such as ammonium, nitrate or amino acids, but some pools, such as the 'easily decomposable' pool in the SUNDIAL model (Smith *et al.*, 1996a), have a more diffuse identity. The definition can also be applied to nutrients such as sulphur or phosphorus.

The water garden is then an analogy for flows of nitrogen in the soil. Nitrogen enters the system in plant debris and moves through a series of pools with various degrees of chemical identification until it reaches its most oxidized form, nitrate. On the way, some flows into an inert pool of organic matter, where it stays. Some of the nitrogen that becomes nitrate is lost by leaching or denitrification, but the rest is taken up by plants that grow and eventually die. The nitrogen they contain enters the system again in plant debris. If you add ^{15}N-labelled material to one of the pools, you can trace the progress of the material from that pool through subsequent pools. The ^{15}N compound you add must, of course, be chemically indistinguishable from the material in the pool.

Murphy *et al.* (1999) investigated three ways of labelling the pool – adding a mixture of ^{15}N-labelled ammonia and air to the head-space above the soil, injecting the mixture into the soil and adding a solution of ^{15}N-labelled ammonium sulphate to the soil surface. The injection method and the application of the solution gave broadly similar estimates of gross mineralization and showed up the same differences between land uses. The head-space method gave smaller estimates. The rates of gross mineralization were much larger than would be expected from the normal rates of net mineralization. They found gross mineralization rates of 2–3 mg N/kg dry soil per day, roughly 5 kg/ha per day, which must have been countered by comparable rates of immobilization.

Box 3.2. ^{15}N-labelling.

An atom consists of a nucleus surrounded by electrons. The electrons are negatively charged, and their number is the 'atomic number' for the element. The nucleus comprises positively charged protons and uncharged neutrons. The number of protons must be the same as the number of electrons and the number of neutrons is usually, *but not always*, the same as the number of protons. Isotopes of an element are atoms which have the same number of protons but differing numbers of neutrons.

All the isotopes of an element have the same electronic configuration (arrangement of electrons in orbits around the nucleus) and therefore the same chemical properties. Some elements have only one isotope, while others have more. Some isotopes are radioactive. They emit radiation but the strength of the radiation diminishes with time (radioactive decay). This is helpful because the radiation can readily be measured to tell you, if you allow for the lapse of time, how much of the isotope and therefore of the element is present in a sample. But it adds to the problems of the experimenter because radiation is hazardous and there are stringent safety regulations surrounding its use. Other isotopes are not radioactive and are described as stable, but there is no radiation that can be measured and detecting the isotope is much more difficult.

The 'atomic weight' is the sum of the number of protons and the number of neutrons and is often twice the atomic number. Nitrogen is the seventh atom in the periodic table and has an atomic number of 7. Its commonest isotope, ^{14}N, has (as the superscript implies) an atomic weight of 14. Measurements of the atomic weight of nitrogen give a value of 14.008, implying that nitrogen must have a heavier isotope. In fact, nitrogen has both a heavier and a lighter isotope, ^{15}N and ^{13}N, but the former is more common. The ^{13}N isotope is radioactive and ^{15}N stable. Their natural relative abundance is well known, and 0.3663% of all nitrogen atoms are ^{15}N.

The time scale of most agricultural or environmental experiments means that ^{13}N decays far too rapidly to be of use, but it is being used in specialized short-term studies of nitrogen uptake by plants. However, ^{15}N has been a mainstay of nitrogen research for many years. Its stability means that it is not subject to safety legislation and the main limitations on its use are cost of the ^{15}N, which is not usually excessive, and the cost of the equipment and labour needed to measure it, which can be more of a problem. The only thing that differentiates ^{14}N and ^{15}N is atomic weight, and the usual method of discrimination between them is the mass spectrometer, which measures atomic or molecular weight directly. The method is entirely safe, reliable if great care is taken, and tedious. UV spectroscopy has also been used to discriminate between the two isotopes.

Reference was made to this box when the labelling of the soil ammonium pool with ^{15}N was discussed. What this means is that a supply of an ammonium salt has been obtained in which the ammonium ions have been enriched to a specified extent with ^{15}N above the natural abundance of 0.3663 atom%. A fixed amount of this ^{15}N-enriched ammonium has been added to the (chemical) pool of ammonium in the soil, so the soil ammonium pool has been 'labelled' with ^{15}N. The progress of the ^{15}N from this into other pools is followed by sampling those pools and determining the ratio ^{15}N of to ^{14}N. The process is somewhat akin to the tagging of birds and animals. Labelling with ^{15}N has proved invaluable in studies of the fate of fertilizer nitrogen, as we shall see in Chapter 5.

No generalizations can be made about the relation between gross mineralization and net mineralization, except that the latter obviously cannot exceed the former. Net mineralization can be negative, because immobilization may exceed mineralization in some circumstances. Immobilization is often caused by the addition to the soil of cereal straw or other material with a high C:N ratio, and this ratio provides a guide as to whether mineralization or immobilization is likely to predominate in a particular soil (Fig. 3.2). Catt *et al.* (1998b) investigated whether straw incorporation was an effective way of immobilizing nitrate and thereby lessening losses by leaching but their results showed no consistent benefit.

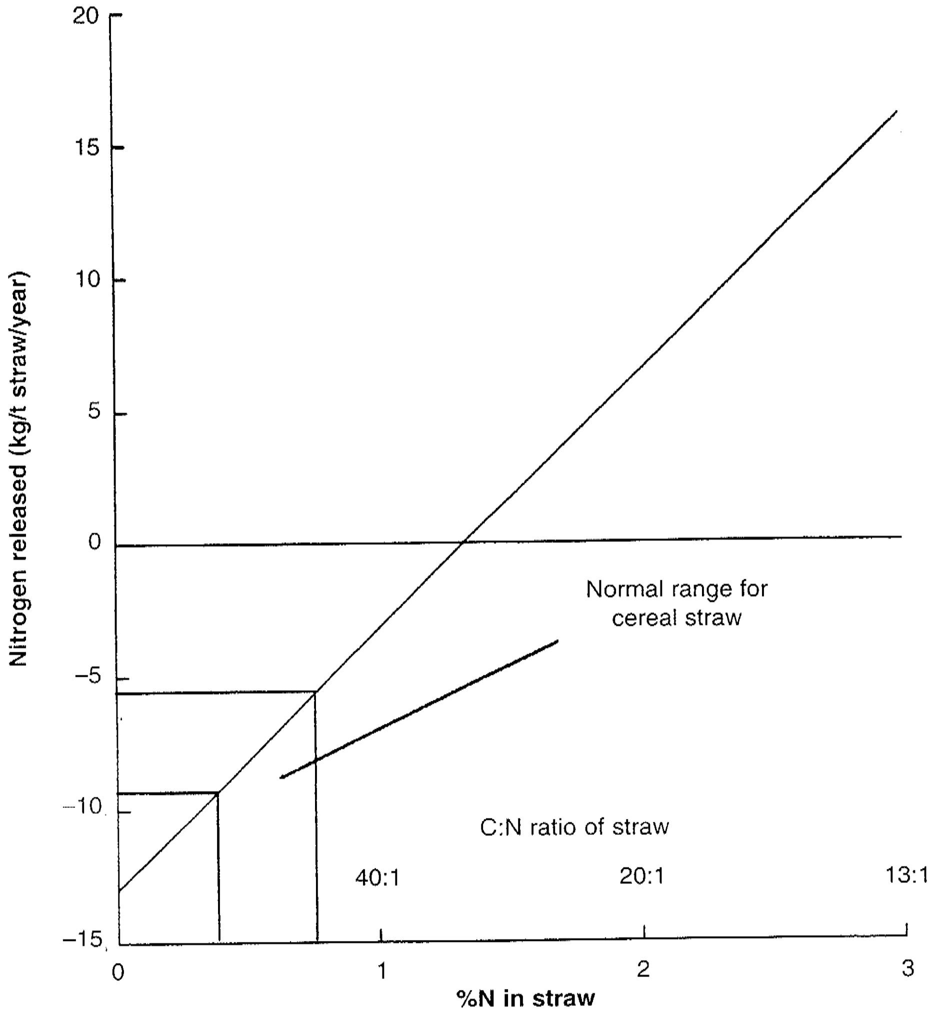

Fig. 3.2. The effect of %N in the straw and thence its C:N ratio on the release of nitrogen from it. A positive release means mineralization and a negative one immobilization. (From Jenkinson *et al.*, 1985.)

Should one measure net or gross immobilization? It depends very much on the purpose of the measurement. If, for advisory purposes, you want to estimate the amount of nitrogen likely to be mineralized in the coming cropping season, a net measurement should be adequate. There is no need for the much greater trouble and expense of a gross measurement. But measuring gross mineralization can tell you far more about the nature of the microbial processes involved. In particular, it can give information on the relation between the nitrogen and carbon cycles in the soil. It is one of the more exciting areas of soil research at the moment.

Denitrification and Related Processes

Nitrogen is the most ephemeral of the major plant nutrients. Nearly 50 years ago, the American soil scientist F.E. Allison published a noted review paper entitled 'The enigma of soil nitrogen balance sheets' (Allison, 1955). The enigma was the failure of so many experiments to balance inputs and outputs of nitrogen to and from the soil. All these experiments showed losses which could not be accounted for by leaching alone. His paper drew attention to denitrification and other processes that release nitrogen gases from the soil. Chemical denitrification is well known in the spontaneous decomposition of ammonium nitrate, in which the nitrate moiety oxidizes the ammonium moiety (Box 4.1). But this would need totally dry soil for it to occur, and the denitrification with which we are concerned happens when the soil is moist and microbes are involved.

We saw above that aerobic microbes need oxygen as a terminal acceptor of electrons. Facultative aerobes can use other molecules, including nitrate, for this purpose. Nitrate is satisfactory as an electron acceptor because it is the most fully oxidized compound of nitrogen, and the reduction of nitrate is the first in a sequence of redox reactions to occur as the soil becomes more anaerobic (Table 3.2). It is carried out mainly by bacteria of the *Pseudomonas* and *Bacillus* species. The equation in Table 3.2 shows the reduction of nitrate proceeding right through to the formation of nitrogen gas, strictly dinitrogen N_2. If this happens, the nitrogen is added to the 78% of nitrogen already in the atmosphere, which is no problem at all. Nitrate has been lost from the pool of plant-available nitrogen, but this is usually a minor problem, except perhaps for paddy rice grown in flooded conditions.

Incomplete denitrification is a major problem. Nitric oxide (NO) and nitrous oxide (N_2O) are formed. The former is a free radical and reacts readily with other free radicals in the atmosphere. Denitrification usually releases only small amounts of nitric oxide from the soil but, when released from larger sources, the gas pollutes the atmosphere by catalysing the formation of photochemical smog and contributing to the formation of ozone in the lower atmosphere (Cotgreave and Forseth, 2002). Nitrous oxide is formed in appreciable amounts and is one of the 'greenhouse gases' as well as facilitating the destruction of the stratospheric ozone layer (Chapter 7).

Denitrification used to be of agronomic concern whether dinitrogen or nitrous oxide was formed, because the nitrate lost would need to be replaced by fertilizer. Today the concern is environmental and it involves the formation of nitrous oxide and its loss to the atmosphere. We are concerned in this chapter with the processes themselves. The assessment of nitrous oxide losses from large areas of land is discussed in Chapter 5, and the role of nitrous oxide in the atmosphere is taken up in Chapter 7.

Table 3.2. Redox reactions in the soil. (After White, 1997.)

Redox reactions occur in the following sequence as the soil becomes more anaerobic:

$2NO_3^- + 12H^+ + 10e^- \leftrightarrow N_2(\text{gas}) + 6H_2O$

$2MnO_2(\text{solid}) + 4H^+ + 2e^- \leftrightarrow 2Mn^{2+} + 2H_2O$

$Fe(OH)_3(\text{solid}) + 3H^+ + e^- \leftrightarrow Fe^{2+}$

$SO_4^{2-} + 10H^+ + 8e^- \leftrightarrow H_2S(\text{gas})$

Compounds or ions not marked as being in the gas or solid phase are in the liquid or solution phase.

Nitrous oxide can also be formed from nitric oxide produced during nitrification because the nitrite (NO_2^-) formed as an intermediate is not stable (Bremner, 1997). This is a key point because the processes of nitrification and denitrification are not subject to the same controls. Denitrification arises from anoxic conditions, nitrification does not. Nitrification uses ammonium as a substrate, while denitrification obviously needs nitrate. It follows that nitrification is the main source of nitrous oxide when ammonium or ammonium-producing (e.g. urea) fertilizers are used, while denitrification predominates with the application of nitrate fertilizers (Bremner, 1997).

The ratio of nitrous oxide to dinitrogen formed depends on the temperature and pH of the soil. At low soil temperatures and pH < 5, at least as much nitrous oxide as dinitrogen is emitted, but at temperatures of 25°C or above and pH > 6, the emissions are nearly all as dinitrogen. Water-filled pore space influences oxygen supply and thence denitrification, and several authors (e.g. Davidson, 1991) have suggested that nitrification was the dominant source of nitrous oxide when this was less than 60%, while denitrification dominated when it was more than 60%.

Denitrification, oxygen and water

The bacteria that implement denitrification are facultative anaerobes whose activity depends on the temperature and the degree of anoxia in the soil. The movement of oxygen in soil is frequently impeded by water in its pores, because oxygen diffuses about 10,000 times more slowly in water than in air. Thus if, as will usually be the case, direct measurements of soil oxygen are not feasible, the percentage of the pore space filled by water provides a useful surrogate measurement of anoxia. But note that anoxia can be caused by enhanced demand for oxygen as well as an impeded supply.

Can denitrification be estimated from soil nitrate, temperature and percentage water-filled pore space? Smith *et al.* (1998) initially found poor correlations, apparently because different factors were limiting denitrification at different times. However, if they included only the sampling events on which two of the three factors were not limiting, the correlations with the third factor became significant. With this observation in mind, Conen *et al.* (2000) developed a model for nitrous oxide emissions from the soil based on what they described as a 'boundary line' approach. This had a threshold value of 10 mg/kg (dry soil) for nitrate below which no emissions occurred. The other two factors were handled by producing a graph with water-filled porosity on the *y*-axis and temperature on the *x*-axis. On this were plotted the nitrous oxide emissions corresponding to combinations of these factors. The resulting pattern enabled two parallel boundary lines to be superimposed. These separated regions in which the emissions were < 10, 10–100 and > 100 g N_2O-N/ha per day. Figure 3.3 shows the boundary lines in relation to the water-filled pore capacity and the temperature but without the data.

This procedure provided an empirical model that could be used to estimate emissions of nitrous oxide from soils, presumably on the assumption that the nitrous oxide came from incomplete denitrification rather than nitrification. The model was neither mechanistic nor exact and the simulations it gave of experimental data were far from perfect, but this is not surprising given that the measurements on which it was based were not made for the purpose. Its great advantage was that it was simple and its workings illustrate clearly the main factors involved in denitrification. A somewhat more mechanistic, but still relatively simple model is that of Rolston *et al.* (1984), which is described in the context of the problem of estimating emissions of nitrous oxide from large areas of land in Chapter 5.

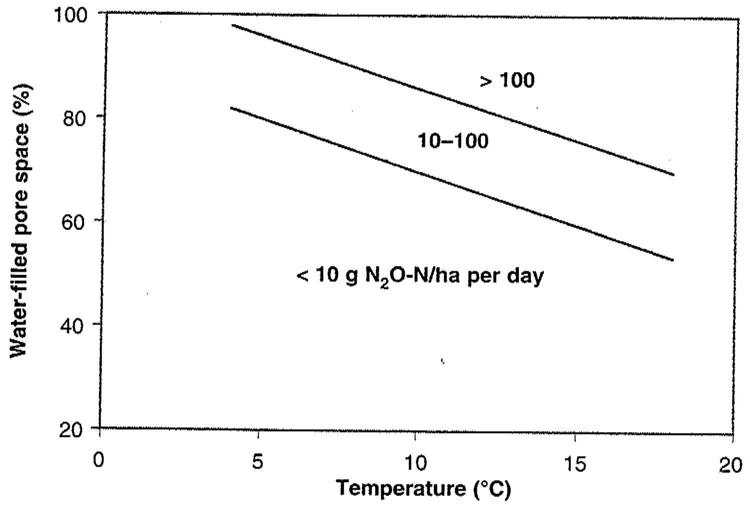

Fig. 3.3. The boundary line approach to emissions of nitrous oxide from soil. For clarity, this diagram shows boundary lines without the data on which they were based. (From Conen *et al.*, 2000.)

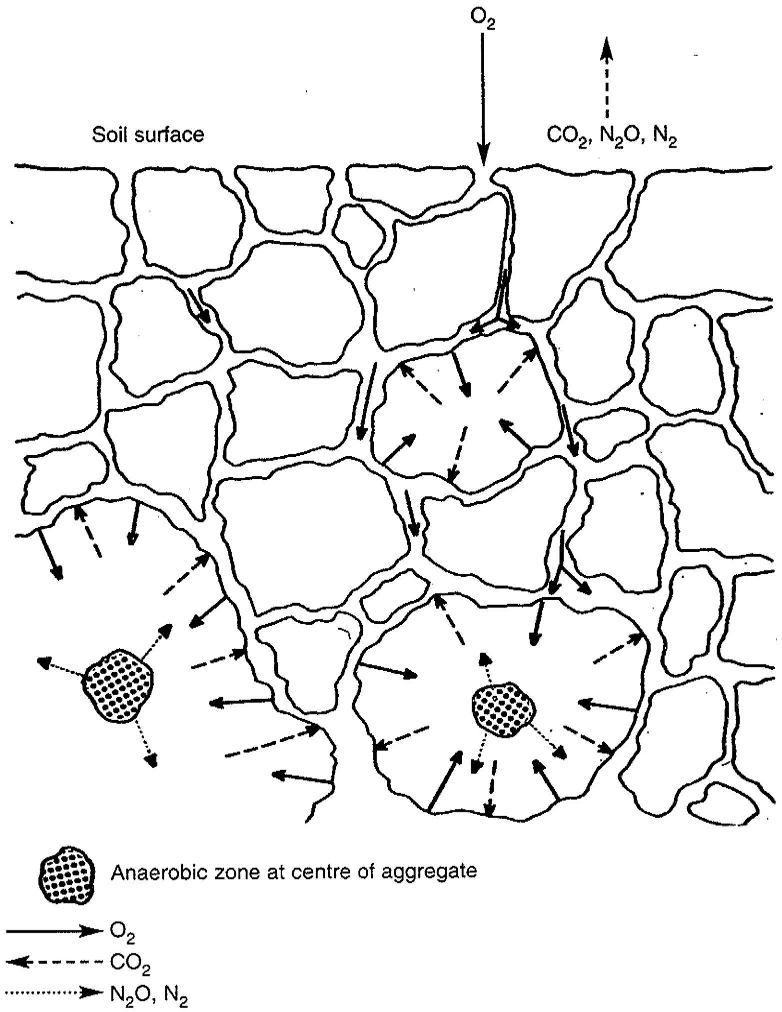

Fig. 3.4. Denitrification in anoxic zones in peds. Oxygen limited by intra-ped diffusion. (From Smith, 1980.)

Even when Conen *et al.* (2000) made allowance for the effects of nitrate, water-filled porosity and temperature, a considerable amount of apparently random nitrous oxide emission remained. This was probably not random but due to localized anoxia caused by the structure of the soil or by 'hot spots' for denitrification (Parkin, 1987). The soil structural effect is likely to arise in heavier, more clayey soils. These tend to have the largest peds (structural units) in which oxygen has to diffuse furthest. As a result, anoxic zones form near the centre of the ped (Fig. 3.4) and denitrification occurs. Smith (1980) described a model for this phenomenon.

Anoxic zones can also occur where there is a strong demand for oxygen that is not met by diffusion. Such a demand could arise from the presence of a substantial fragment of easily decomposed plant material, which could then become the centre of a hot spot for denitrification (Parkin, 1987). Anoxic zones in peds and hot spots both imply great variability in rates of denitrification even in small volumes of soil. As with mineralization and immobilization, nitrification and denitrification can occur simultaneously only a short distance apart in the soil, aerobic and anaerobic processes almost side by side.

The process is not just variable in space; it also varies with time. It happens as a result of a particular set of circumstances and is therefore episodic in nature (e.g. Jarvis *et al.*, 1991). Both forms of variability make it difficult to generalize about the scale of the losses. This is illustrated by some results of Dobbie *et al.* (1999), who reported annual emissions of nitrous oxide ranging from 0.3 to 18.4 kg N_2O-N/ha in a series of experiments run for 3 years with five crops at sites in southern Scotland that had widely varying rainfall and soil types. Total denitrification losses would, of course, have been larger, possibly up to 30–40 kg N/ha. The authors attributed the variations mainly to the number of occasions on which nitrogen fertilizer application was followed by substantial rainfall.

Nitrogen Fixation

Nitrogen gas is chemically inactive. This is just as well, because it makes up 78% of the atmosphere, and life as we know it would not be possible if we were surrounded by a more reactive gas. Imagine living in an atmosphere of chlorine! This inactivity means that nitrogen gas is not directly accessible to plants either, depriving them of a plentiful supply unless something intervenes to help them. That 'something' proved rather elusive when it was sought.

Legumes

Boussingault had shown in 1838 that clover and peas grown in sterilized sand increased the amount of nitrogen in the sand. This was something that crops such as wheat and barley could not do. The legumes must have obtained the nitrogen from the atmosphere, but neither Boussingault nor anyone else could explain how they did so. Lawes and Gilbert showed that the 'Garden Clover' plot at Rothamsted, a small plot of clover which is still maintained in the garden of Rothamsted Manor, obtained huge quantities of nitrogen from somewhere. Around 280 kg/ha were harvested annually in the clover. This was far more than was in the seeds and other inputs, so nitrogen had to be coming from elsewhere. They thought that the nitrogen possibly came from large stores of reactive nitrogen in fertile soil, but if they thought that the clover was using atmospheric nitrogen they did not follow it up, presumably because no one knew how it did so.

Nodules

Curiously enough, neither Boussingault nor Lawes and Gilbert made the connection between this unexplained capacity of leguminous crops to obtain nitrogen and the nodules which could be seen quite clearly on their roots. The issue was not to be resolved until 1886–1888, when two German scientists, Hellriegel and Wilfarth, demonstrated in a series of experiments that the capacity to fix nitrogen involved microbes in the nodules operating in a symbiotic relationship with the plant. A symbiotic relationship is a biological arrangement in which both parties benefit. The plant received nitrogen fixed from the atmosphere, and the microbes in the nodules received a share of the carbohydrates produced by photosynthesis.

But nitrogen gas is dinitrogen, N_2, in which the two nitrogen atoms are held together by a triple bond, N≡N. This is a very powerful bond that needs a great deal of energy to break it in the industrial Haber–Bosch process (Chapter 4), appreciably more than is available in most biological processes. How do the *Rhizobium* microbes in the nodules manage it? They manufacture the *nitrogenase* enzyme, which is one of the very few enzymes able to catalyse the cleavage of a triple bond with the amount of energy available in soils in the field. This enzyme can also catalyse the cleavage of other triple bonds such as those in acetylene (H–C≡C–H) and cyanide (–C≡N).

Nitrogenase is readily deactivated by oxygen, so the microbe has to maintain anaerobic conditions around the enzyme for it to fix nitrogen from the air. This must be one reason why the higher plants failed to evolve the capacity to fix nitrogen. The root nodule provides an environment in which the microbes can control the oxygen concentration around the enzyme while receiving the energy from the plant to do so. Its position also enables it to feed the fixed nitrogen into the same stream as nutrients taken up by the root.

Could the benefits be spread?

The bacteria, the nodules and their plant hosts still attract interest from researchers. The legumes are currently the only plants capable of forming the associations through which nitrogen is fixed and there are obvious benefits to extending this capacity to other plants. However, the specificity of symbiotic relationships means that, even with genetic modification as a tool, we are still a long way from being able to develop a cereal crop able to form a relationship with a bacterium through which it could obtain fixed nitrogen. A cereal crop with this property would be invaluable in developing countries where nitrogen fertilizer is often too expensive for smallholder farmers (Chapter 11), at least partly because of transport costs. It would also save immense amounts of energy in the developed world at a time when we are becoming increasingly aware of the limits to oil and gas reserves. Such a crop would have some limitations, but they are trivial compared with the potential benefits. The bacteria in the nodules use carbohydrate that the crop has photosynthesized and the crop would therefore not yield as much grain as a crop grown with fertilizer. Also the fact that the crop was grown without fertilizer would not mean that no nitrate was lost to the environment!

Our existing nitrogen-fixing crops make a major contribution to nitrogen supply in the temperate zones, particularly in grassland farming. The Canterbury Plains in the South Island of New Zealand depend almost entirely on nitrogen fixed by clover and are highly productive. And in hilly or other areas where the terrain is difficult, clover is often a more practical proposition than nitrogen fertilizer. In arable systems, peas and beans are important crops in their own right and also provide 'break crops' for limiting the transfer of disease from one cereal crop to another. Also nitrogen residues from legumes become available through mineralization to the following crop or to crops grown with the legumes in inter-cropping systems (systems in which the legume and non-legume crop are grown side-by-side in alternate rows). Organic farming systems depend for their nitrogen supply on legumes and atmospheric deposition. But where the legumes really come into their own is in the developing world. For large areas of sub-Saharan Africa, and in many other places, they are unquestionably the main source of nitrogen. Beans, soya and groundnuts have a vital culinary and economic role in the lives of hundreds of millions of people.

Is Nitrate the Preferred Form of Nitrogen for Plant Uptake?

It has long been assumed that nitrate is the main form of nitrogen taken up by plants but that most plants can also take up ammonium. This was so widely accepted that the present section would not have been added were it not for the topic recently becoming a matter of controversy. Perakis and Hedin (2002) reported that nitrogen was lost as dissolved organic compounds in stream waters flowing from pristine South American forests but mainly as inorganic nitrate in streams flowing from North American forests that suffer deposition of nitrogen oxides. This led van Breeman (2002) to claim that the uptake of dissolved organic nitrogen by plants was the norm in unpolluted ecosystems and that nitrate uptake was an adaptation to pollution. He suggested that the standard thinking about how nature deals with nitrogen needed to be re-evaluated. This in turn led *New Scientist* to publish an article entitled 'Botched Botany' (Pearce, 2002) which berated scientists for getting the nitrate story wrong all along.

Addiscott and Brookes (2002), however, pointed out that the presence of dissolved organic nitrogen in the pristine streams did not prove that nitrogen of this kind was that taken up by plants, particularly as 'dissolved' was defined as passing though a filter of 1 μm pore size and would have included molecules up to 1000 larger than those likely to have been taken up by plants, together with colloidal organic matter and bacteria. They suggested that a forest ecosystem with no nitrogen inputs would evolve to

recycle all usable nitrogen, inorganic or organic, and minimize its loss in streams. And the dissolved organic nitrogen was in the streams not because it was what the plants took up, but because it was what they were unable to use. No re-evaluation of our current thinking about nitrogen uptake seems necessary at the moment. (And was 'Botched Botany' perhaps a case of botched journalism?)

How nature deals with nitrogen depends greatly on temperature. Ryegrass plants given equal concentrations of ammonium-N and nitrate-N took up an increasing proportion of ammonium as the temperature became cooler (Clarkson and Warner, 1979). Recent research in plant physiology, reviewed by Williams and Miller (2001), has shown that plants are equipped with transport mechanisms for a variety of nitrogen-containing organic solutes. And field research in northern forests with cold temperatures (Persson and Nasholm, 2001) has shown that plants can absorb small organic molecules such as amino acids. This flexibility may have evolved simply because cold unfertilized soils do not contain much nitrate. Nitrifying bacteria become less active as the temperature declines and are inhibited at 3–5°C, leaving ammonium and small organic molecules as the main source of nitrogen. The ammonifying bacteria are probably inhibited at around 0°C, depending on the genera involved.

Plants in the South American forests studied by Perakis and Hedin (2001) may well have taken up some small organic nitrogen molecules, because the temperatures were quite low (4–11°C). But this does not require a re-evaluation of the standard thinking about how nature deals with nitrogen. My guess, based on the evidence above, is that plants generally use nitrate but as the temperature declines they increasingly take up first ammonium and then amino acids and other small nitrogen-containing molecules.