Nitrogen processes in terrestrial ecosystems

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Executive summary

Nature of the problem

- Nitrogen cycling in terrestrial ecosystems is complex and includes microbial processes such as mineralization, nitrification and denitrification, plant physiological processes (e.g. nitrogen uptake and assimilation) and physicochemical processes (leaching, volatilization). In order to understand the challenges nitrogen puts to the environment, a thorough understanding of all these processes is needed.

Approaches

- This chapter provides an overview about processes relating to ecosystem nitrogen input and output and turnover. On the basis of examples and literature reviews, current knowledge on the effects of nitrogen on ecosystem functions is summarized, including plant and microbial processes, nitrate leaching and trace gas emissions.

Key findings/state of knowledge

- Nitrogen cycling and nitrogen stocks in terrestrial ecosystems significantly differ between different ecosystem types (arable, grassland, shrubland, forests).
- Nitrogen stocks of managed systems are increased by fertilization and N retention processes are negatively affected.
- It is also obvious that nitrogen processes in natural and semi-natural ecosystems have already been affected by atmospheric N_r input.
- Following perturbations of the N cycle, terrestrial ecosystems are increasingly losing N via nitrate leaching and gaseous losses (N_2O, NO, N_2 and in agricultural systems also NH_3) to the environment.

Major uncertainties/challenges

- Due to their complexity, ecosystem nitrogen stocks and nitrogen cycling processes are not well studied, as compared to those of carbon. However, strong ecosystem feedbacks to global changes have to be expected, especially with regard to nitrate leaching, C sequestration and emissions of the primary and secondary greenhouse gases N_2O and NO.

Recommendations

- In view of the still limited knowledge on nitrogen and carbon interactions at ecosystem and landscape scales and effects of global changes (climate, N deposition, landuse, land management) on C and N cycling, multi-disciplinary research needs to be initialized, encouraged and supported. Interdisciplinary and multi-scale studies should focus on simultaneous and comprehensive measurements of all major N_r fluxes at site and landscape scales including plant uptake/release of organic and inorganic N compounds as well as microbial N_r conversion.
- Based on an in-depth understanding of nitrogen cycling processes, best management options need to be developed to minimize negative environmental impacts of global change on N cycling, C/N interactions and biosphere–atmosphere–hydrosphere exchange processes.

6.1 Introduction

Nitrogen (N) is a key nutritional element for any life form on earth. Living organisms use N to form a number of complex organic compounds such as amino and nucleic acids, chitin and proteins. In unperturbed terrestrial systems N is a factor which limits net primary ecosystem production (Vitousek and Howarth, 1991). However, this situation has changed dramatically during the last decades. In most regions of Europe there is little limitation of biomass production by N, due to intensive use of fertilizers in agricultural systems and increasing N deposition to natural and semi-natural systems. The effects of increased N availability include changes in biosphere–atmosphere exchange (such as increased aerosol formation and emissions of the greenhouse gas N_2O from terrestrial systems), eutrophication of terrestrial and aquatic systems (with consequences for species composition and richness, carbon sequestration, surface and drinking water quality), or acidification of soils and water bodies following the deposition of reactive nitrogen (N_r). Nitrogen cycling in terrestrial ecosystems and landscapes is mainly driven by microbiological and plant processes, with physico-chemical processes such as diffusion, emission, volatilization, leaching or erosion leading to displacement of N on site, regional and global scales (Galloway et al., 2003; Erisman et al., 2008). To better understand how N is affecting ecosystem functioning and to predict future ecosystem responses to increased N availability, it is necessary to have detailed knowledge of the processes involved in N cycling in terrestrial systems. This chapter provides an overview of current knowledge on N stocks in terrestrial ecosystems (Section 6.2), sources of N inputs (Section 6.3), N cycling at the ecosystem scale (Section 6.4), N loss pathways (Section 6.5) and N effects at the ecosystem scale (Section 6.6). The chapter has a particular focus on forest ecosystems, while agricultural systems are more thoroughly considered in Jarvis et al., 2011 (Chapter 10 this volume).

6.2 Nitrogen in terrestrial ecosystems

6.2.1 Nitrogen as a key element in biogeochemistry

Nitrogen is a key element for global biogeochemistry and its cycling is closely linked to the carbon cycle. Nitrogen availability often limits net primary production in agricultural as well as natural and semi-natural ecosystems (Vitousek and Howarth, 1991; De Vries et al., 2006). Nitrogen bound in organic compounds is an essential part of all proteins and enzymes and thus, N is driving the key metabolic processes involved in growth and energy transfer. Furthermore, N is a part of chlorophyll, the green pigment of the plant that is responsible for photosynthesis. A large fraction of N in primary producers is utilized directly in capturing energy in photosynthesis (Evans, 1989). Nitrogen has different oxidation states from −3 in NH_3 to +5 in NO_3^- and a series of microbial processes such as nitrification (autotrophic, heterotrophic), denitrification or anaerobic ammonium oxidation (anammox) have evolved to either gain energy from the oxidation process or to use oxidized N compounds as an alternative electron acceptor when growing anaerobically.

In contrast to global C cycling, where the largest fluxes are associated with the net primary production of terrestrial and marine systems, the global biogeochemical cycle of N is dominated by microbial processes in soils, sediments and water bodies (Seitzinger et al., 2006). This is also a major difference from the global cycling of phosphorus, which becomes available to the biosphere mostly through mineral weathering.

Depending on ecosystem type and land use, N cycling and N storage in soils and vegetation varies considerably. In agricultural systems, N cycling is dominated by N fertilization and crop removal, while in natural and semi-natural systems N cycling is largely affected by climatic, edaphic and landscape conditions and the sum of N inputs via N deposition and biological N fixation. Across differing climates, shrublands in Europe have been predominantly N-limited systems, i.e. plant production was suboptimal due to shortages in available N. In such systems N availability is mostly hampered by poor soil properties, e.g. high sand content and thus reduced ion exchange capacities and low organic matter content, which negatively affects retention of reactive nitrogen (N_r; here and in the following: all organic N forms plus inorganic N forms except N_2) in the system. Also the human use of these systems (e.g. grazing, fodder collection) over centuries has depleted the nutrient reservoirs. At the present time, shrublands and heathlands exposed to high N deposition are often showing indications of N saturation, such as changes in species composition or nitrate leaching (Schmidt et al., 2004). Wetlands are also mostly N-limited systems, due to accelerated losses of N_r via the denitrification pathway. Forests are naturally N-limited systems, at least for European climatic conditions, whereas tropical rainforests are often N-rich systems. This situation has changed markedly during the last decades due to atmospheric N deposition (De Vries et al., 2007). Forest foliage is more effective at receiving N deposition compared to other vegetation types, resulting in an increased N deposition. Signs of N saturation of forests have been widely reported and include accelerated growth and significant N_r losses via nitrate leaching and N trace gas emissions (Dise et al., 2009; Pilegaard et al., 2006).

6.2.2 Distribution of nitrogen stocks in the soil and plant system

Based on a detailed database of soil properties, Batjes (1996) estimated global amounts of soil N to be 133–140 Pg N for the upper 100 cm of the soil profile. By comparison, about 10 Pg N is held in the plant biomass and about 2 Pg N in the microbial biomass (Davidson, 1994). This shows that on an ecosystem scale, soils are the main reservoir for N.

We have compiled data for representative European terrestrial ecosystems on N pools and fluxes in representative
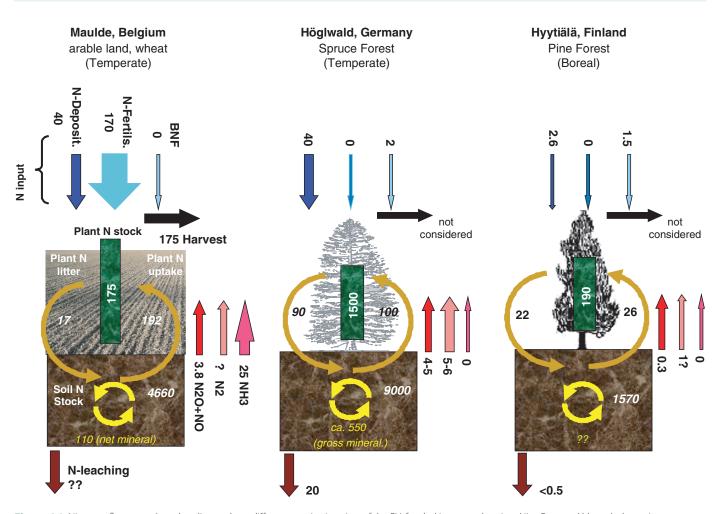
Figure 6.1 Nitrogen fluxes, pools and cycling at three different monitoring sites of the EU-funded integrated project NitroEurope. Although these sites are currently among the best investigated, uncertainties remain high and fluxes of, e.g. denitrification N_2 losses or gross (net) rates of microbial turnover are scarcely measured if at all. BNF: biological N_2 fixation.

ecosystem types (supplementary material, Chapter 6). Using three well-studied sites selected from those listed in supplementary material (Chapter 6), the major differences in pools and fluxes between agricultural and forest systems as well as between N saturated and N limited forests can be seen (Figure 6.1). Our data (supplementary material, Chapter 6) show that the dominance of soil N as a reservoir (1350–9000 kg N ha^{-1} on mineral soil) is more pronounced for agricultural systems, with greater than 90%–95% of N_r being stored in the soil as compared to forest systems, where N storage in biomass can be up to 30%–50% (Figure 6.1). Most of the N in soil, is bound to organic material (soil organic matter) and in natural systems only a very tiny portion is available as inorganic N in the form of NH_4 or NO_3: 1–10 kg N ha^{-1} in natural systems and 10–200 kg N ha^{-1} in fertilized systems (supplementary material, Section 6.6 and Figure 6.1). Even though N cycling is largely dependent on microbial processes (Section 6.4) the microbes only hold 40–1300 kg N ha^{-1}, with the largest in fertilized arable systems (supplementary material, Chapter 6).

6.3 Inputs of nitrogen into ecosystems

6.3.1 Input by biological nitrogen fixation

In preindustrial times biological N_2 fixation was the major pathway of N_r creation in terrestrial ecosystems and in more pristine regions this may still be the case (Cleveland *et al.*, 1999). Even though dinitrogen (N_2) is the major component of the earth's atmosphere and nitrogen is essential for all forms of life, N_2 cannot be used directly by biological systems to synthesize the chemicals required for growth, maintenance and reproduction. The general chemical reaction for the fixation of N ($N_2 + 3H_2 \rightarrow 2NH_3$) is identical for both the chemical (Haber–Bosch) and the biological processes. The triple bond of N must be broken – which requires substantial amounts of energy – and three atoms of hydrogen must be added to each of the N atoms. Living organisms use energy derived from the oxidation ('burning') of carbohydrates to reduce molecular nitrogen (N_2) to ammonia (NH_3).

The capacity of biological N fixers to convert N_2 to organic N is substantial, more than enough to maintain N pools in

Table 6.1 Reported ranges for biological N_2 fixation in natural and managed ecosystems.

Ecosystem type	Crop	N fixation rate kg N ha⁻¹ yr⁻¹	Source
Boreal forests and boreal woodland		1.5–2	Cleveland *et al.* (1999)
Temperate forests and forested floodplains		6.5–26.6	Cleveland *et al.* (1999)
Natural grasslands		2.3–3.1	Cleveland *et al.* (1999)
Mediterranean shrublands		1.5–3.5	Cleveland *et al.* (1999)
Agricultural ecosystems	Common bean	30–50	Smil (1999)
	Faba bean	80–120	Smil (1999)
	Soya bean	60–100	Smil (1999)
Managed grasslands	*Trifolium pratense* and *T. repens* mixture	31–171	Carlsson and Huss-Danell (2003)
	Medicago sativa with different grasses	65–319	

ecosystems and to replenish N losses. In many systems, N fixers drive the accumulation of fixed N on long time scales, bringing N supply close to equilibrium with other potentially limiting resources (Vitousek *et al.*, 2002). Significant biological N_2 fixation on ecosystem scale is most often associated with symbiotic N_2 fixation, with the classic example of the bacterium *Rhizobium* infecting the roots of leguminous plants (e.g. peas and beans, soya bean, clover, peanut). Leguminous symbiotic N_2 fixation can be up to approximately 200 kg N ha⁻¹ yr⁻¹ (Table 6.1). Therefore, legumes are often used in agricultural systems, representing a major direct source of food and forage for livestock. In low-input and organic farming systems, leguminous crops are included in rotational designs in order to provide N for other crops within the rotation (Ball *et al.*, 2005). Total global biological N fixation by agricultural crops is most likely in the range of 40–60 Tg N yr⁻¹ (Herridge *et al.*, 2008). Also in natural systems numerous genera of non-leguminous angiosperms, such as *Alnus*, *Casuarina*, *Coriaria*, *Myrica*, etc., exist that are capable of supporting symbiotic biological N_2 fixation. These associations may achieve fixation rates as high as 100 kg N ha⁻¹ y⁻¹ and may occur as climax vegetation or as pioneer species in adverse soil environments.
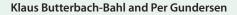
Another major pathway of biological N_2 fixation is by heterotrophic bacteria in soils and sediments. Heterotrophic fixation during the decomposition of plant litter is thought to be important in terrestrial ecosystems and may account for 1–5 kg N ha⁻¹ yr⁻¹. The net contribution of heterotrophic N fixation to ecosystem N budgets may be greater in wetland soils. N budgets of flooded rice suggest that 50–100 kg N ha⁻¹ yr⁻¹ may be added by N fixation (Cassman and Harwood, 1995), and heterotrophic fixers contribute a substantial proportion of this total (Eskew *et al.*, 1981). Similarly, high rates of heterotrophic fixation may support plant production in some natural wetlands.

While understanding of the symbiotic system in a few legume crop plants is relatively advanced, much less is known about N fixation in non-agricultural legumes or in other N-fixing organisms, such as symbiotic cyanobacteria or free-living heterotrophic bacteria. In many ecosystems, the contribution of such organisms is assumed to be significant and needs to be accounted for. However, extreme difficulties in measuring rates of N_2 fixation accurately at ecosystem scale have so far hampered a better understanding of the importance of biological N_2 fixation for most terrestrial ecosystems.

6.3.2 Nitrogen input via atmospheric deposition

Nitrogen is deposited from the atmosphere in both 'wet' (in precipitation) and 'dry' (as gases processes; and particles) for further information see Hertel *et al.* (2011) (Sections 9.4–9.6, this volume). In short vegetation, wet deposition constitutes the largest part of the atmospheric N input. Whereas forest canopies are efficient sinks for atmospheric gases and particles. Hence, N deposition on forests is up to a factor of 2–3 times larger than on open land (Fowler *et al.*, 2004). As forests are the most exposed system, this chapter focuses on issues related to N input to forests.

Deposition to forests ranges from less than 5 kg N ha⁻¹ yr⁻¹ in Northern Europe to greater than 60 kg N ha⁻¹ yr⁻¹ in Central and Western Europe (Dise *et al.*, 2009) and the range for short vegetation is about half, i.e. 3–30 kg N ha⁻¹ yr⁻¹. At remote sites in the southern hemisphere wet deposition is less than 1 kg N ha⁻¹ yr⁻¹ (Vitousek *et al.*, 1997), hence total pre-industrial inputs to forests were probably below 2 kg N ha⁻¹ yr⁻¹, assuming a small contribution from dry deposition. In large parts of Europe ammonium is the dominant form of atmospheric N input. Ammonium originates from ammonia emitted primarily from animal husbandry. Nitrate originates from N oxides emitted by fossil fuel burning, including automotive exhaust. The nitrate fraction of the atmospheric input is mainly below 10–15 kg N ha⁻¹ y⁻¹ and is relatively uniform across sites (Faegerli and Aas, 2008). Also there may be an additional wet deposition input of dissolved organic nitrogen (DON) with a magnitude of approximately 1 kg N ha⁻¹ yr⁻¹ (e.g. 0.5–1.1 kg N ha⁻¹ yr⁻¹ at the
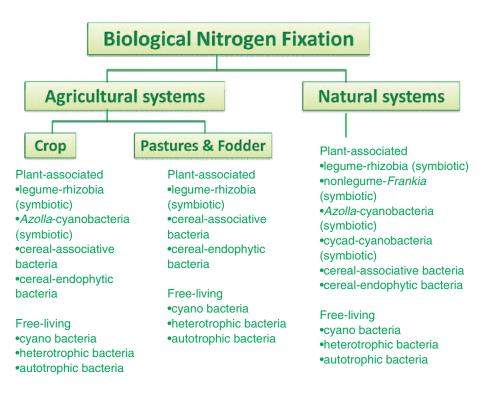
Biological Nitrogen Fixation

Agricultural systems

Crop

Plant-associated
•legume-rhizobia (symbiotic)
•*Azolla*-cyanobacteria (symbiotic)
•cereal-associative bacteria
•cereal-endophytic bacteria

Free-living
•cyano bacteria
•heterotrophic bacteria
•autotrophic bacteria

Pastures & Fodder

Plant-associated
•legume-rhizobia (symbiotic)
•cereal-associative bacteria
•cereal-endophytic bacteria

Free-living
•cyano bacteria
•heterotrophic bacteria
•autotrophic bacteria

Natural systems

Plant-associated
•legume-rhizobia (symbiotic)
•nonlegume-*Frankia* (symbiotic)
•*Azolla*-cyanobacteria (symbiotic)
•cycad-cyanobacteria (symbiotic)
•cereal-associative bacteria
•cereal-endophytic bacteria

Free-living
•cyano bacteria
•heterotrophic bacteria
•autotrophic bacteria

Figure 6.2 Biological N_2-fixing agents in agricultural and terrestrial natural systems (Herridge *et al.*, 2008).

Höglwald Forest, Germany (Rothe *et al.*, 2002), which is not yet accounted for in current inventories (Cape *et al.*, 2004)).

Although European models of total N deposition may reproduce the general continental pattern in deposition and produce reliable regional total N deposition estimates (Faegerli and Aas, 2008), local variability in N deposition can be large and is not accounted for in these models (Dragosits *et al.*, 2002). Particularly in fragmented and complex forest land with many edges towards open land the N deposition at the edges is several times higher than in the interior of the forest (Spangenberg and Kölling, 2004). This 'edge effect' is mainly caused by the increased turbulence created by the edge, and may extend 50–100 m into the forest. Larger 'edge effects' (extending up to 200 m into the forest) may be found close to local ammonia sources from animal farms (Spangenberg and Kölling, 2004).

The sum of throughfall and stemflow N is often used as a proxy for total deposition of N to forests although this is most probably an underestimation due to retention by leaf and bark processes (Lovett and Lindberg 1993; Kreutzer *et al.*, 2009) and retention by microorganisms on canopy surfaces. In forest, most of the evaluations of N budgets in Europe are based on N fluxes in throughfall. Thus much of our knowledge on N deposition and its effects in forests relies on the assumption that throughfall (+ stemflow) represents the total N input. Often only the inorganic N components of the throughfall are considered since the sources of organic N are not well known. In many cases the contribution from stemflow is also ignored even in broadleaf stands where the contribution may be significant (Rothe *et al.*, 2002).

When inorganic N in wet deposition is compared to canopy throughfall at the same site, a net uptake in the canopy is indicated at wet deposition rates below 5–10 kg N ha^{-1} yr^{-1} (Kristensen *et al.*, 2004). This may be due to ion exchange reactions in the canopy and uptake by epiphytes in the canopy. These uptakes may also occur at higher deposition rates, but only a few investigations have quantified the uptake. Kreutzer *et al.* (2009) estimated an uptake of 2–3 kg N ha^{-1} yr^{-1} at the Höglwald Forest site, Germany, which included also the potential uptake of N gasses directly through stomata (Figure 6.1). Assessments based on canopy budget models give somewhat higher uptake rates (Staelens *et al.*, 2008), but these models do not include potential transformations to organic N and do rely on assumptions that need further testing. It is however, uncertain how much of the uptake actually ends up inside the leaf. In the canopy, microbes, lichens and other epiphytes may sequester N from wet and dry deposition. Also decomposing leaves, twigs and branches with high C:N ratio may sequester N. In a large-scale 3-yr experiment adding 18 kg N ha^{-1} yr^{-1} to the canopy in mist using a helicopter, Gaige *et al.* (2007) found up to 70% retention in the canopy, but part of it may have been assimilated by epiphytes or microorganisms on the canopy surface. A detailed analysis of the fate of the N addition (including a ^{15}N trace) in this N-deficient forest showed that only 1%–3% of the added N was recovered in the bole wood and a similar amount in the foliage (Dail *et al.*, 2009). Conversely, 25%–56% of the N addition was recovered in bark and branches, but unfortunately it was not possible, with the methods used, to separate out how much of this was localized in tree tissue or in mosses, lichens and bacteria living on the bark (Dail *et al.*, 2009). Thus bark and epiphytes may play an important role in canopy N retention. Besides retaining deposition N, the microbes and lichens may take up ammonium and nitrate and release organic N. Furthermore, nitrification of ammonium to nitrate has been observed in the canopy (Papen *et al.*, 2002).

The N retained in epiphytes may be counted in the litterfall N flux as part of the foliage, bark and other organic

material. However at the Solling site, a forest in Northern Germany, throughfall was collected, stored and filtered to measure the flux of elements from suspended matter in the throughfall. It appeared that this material contributed a considerable fraction of the nutrient input to the soil: for N, 10–14 kg ha^{-1} yr^{-1} (Gundersen et al., 1998c). The nutrient composition of the material was comparable to that found in the very fine litter particles with C:N ratios of 11–14 (g g^{-1}) but differed from that in needle litter (C:N 38). This suspended matter input to the soil is not included in throughfall sampling (that is usually filtered) nor in conventional litterfall sampling that collects material above a certain mesh-size, typically 0.5 mm. To get a better quantification of the total deposition to forest and to more quantitatively know the potential underestimation of deposition N by the throughfall methods more studies (including ^{15}N labelling) are needed.

6.3.3 Fertilizer and manure input into agricultural systems

The agricultural use of N in the form of synthetic fertilizers has increased dramatically over the past 50 years (Erisman et al., 2008; Galloway et al., 2004, see also Erisman et al., 2011, (Chapter 2 this volume) and Jensen et al., 2011 (Chapter 3 this volume)), in Europe and across the world. In 1950, global annual consumption of fertilizer N was less than 4 Mt, but had increased to 32 Mt by 1970 and to greater than 80 Mt by the 1990s (Roy and Hammond, 2004). Over the EU-27 as a whole, the main source of N input to agricultural land is now mineral fertilizer, with livestock manure a close second. Mineral fertilizer N use in Europe increased from 4.6 Mt in 1960 to 11.8 Mt by 1995 (van Egmond et al., 2002), when it accounted for 50% or more of total N input in Denmark, Germany, Greece, France, Luxembourg, Finland and Sweden. However, in Belgium and the Netherlands livestock manure was still responsible for more than 50% of N inputs (Vall and Vidal, 1999). In addition to fertilizer and manure N, a further 2.2 Mt is introduced annually into agroecosystems through biological N fixation, by forage and grain legume crops (van Egmond et al., 2002). The average N application rate to European agricultural land (fertilizer plus manure) increased in parallel with the increased use of fertilizers, reaching an average of 123 kg N ha^{-1} by the 1990s (van Egmond et al., 2002; note that this study excluded the area of former Soviet Union). Since then, modest decreases have occurred in some countries, driven mainly by regulations to limit water pollution by leached nitrate; recent steep fertilizer price increases may well further reduce N use. Further information on fertilizer and manure use in agricultural systems and shifts in the use of different types of mineral fertilizers (e.g. increased use of urea) is provided in Jarvis et al. (2011) (Chapter 10, this volume).

6.4 Internal nitrogen cycling in terrestrial ecosystems

6.4.1 Nitrogen cycling processes

Nitrogen cycling in terrestrial ecosystems is characterized by a variety of N transformations involving both organic and inorganic (ammonium and nitrate) N species and uptake/immobilization of N by microbes and plants, as shown in Figure 6.3.

A growing temperate forest has a net N demand of approximately 5–10 kg N ha^{-1} yr^{-1} (Scarascia-Mugnozza et al., 2000), while N cycling due to fine root and leaf production and turnover varies mostly from 60–100 kg N ha^{-1} yr^{-1} (Kreutzer et al., 2009). Compared to this, microbial N turnover via mineralization, nitrification and immobilization may be up to a magnitude higher (Corré et al., 2003; Kreutzer et al., 2009). Losses to the environment in form of leaching or gaseous volatilization are – under N mass balance considerations – mostly small and in a range of a few kg per hectare.

Litter production and decomposition (and the balance between these two processes) are the major drivers of storage and release of N$_r$ from the soil organic matter pool. During the decomposition process soil organic matter is cleaved from large polymers to largely bio-available monomers which are accessible to both plants and microbes. Microbes can further degrade these organic monomers to form ammonium (ammonification or N mineralization). Ammonium as well as organic N can also be oxidized to nitrate (nitrification). Both ammonium and nitrate can either be taken up by plants or immobilized by microorganisms. Thus, plants and microorganisms might compete for both bio-available organic N compounds (e. g. amino acids) and mineral N under N-limited conditions. Furthermore, N cycling in terrestrial ecosystems is characterized by internal loops, which occur at extremely variable timescales, from seconds to decades and can be mediated by both microorganisms, soil fauna (grazing of microorganisms) or plants (e.g. uptake of N and subsequent death of plants or root exudation). All mechanisms of internal N cycling favour ecosystem N retention, and thus are of crucial ecological significance (Corré et al., 2003).

The common view of the terrestrial N cycle has undergone a considerable change in the past two decades (for detailed reviews see Schimel and Bennett, 2004; Chapman et al., 2006: Jackson et al., 2008; Rennenberg et al., 2009). Until the 1990s the perception and understanding of N cycling in terrestrial ecosystems was dominated by the paradigms that (1) N mineralization is the limiting step in N cycling, (2) plants take up only inorganic N, (3) plants poorly compete for N against microbes and therefore use only the N which is 'left over' by microbes (Schimel and Bennett, 2004). This perception led to the definition of net N mineralization as the sum of N which is exceeding the microbial demand and, thus, is available for plant uptake and pathways of N losses from the ecosystem. Consequently, net rate assays like the buried bag technique (Eno et al., 1960) have been widely used in order to measure plant available N.

Since the late 1980s, researchers have increasingly become aware that net rates may be a poor approximation of the real status of N cycling in soils (Davidson et al., 1991; Hart et al., 1994), especially with regard to losses such as N trace gas emissions. Abundant published studies utilizing the ^{15}N pool dilution technique for the determination of gross rates of ammonification, nitrification and inorganic N immobilization highlighted the limitations of net rate assays and provided a new and more advanced insight into actual N turnover. The ^{15}N pool dilution studies mostly revealed significantly higher rates of gross ammonification and nitrification while little or no ammonium and nitrate accumulated (Davidson et al., 1992; Hart et al., 1994; Neill et al., 1999; Verchot et al., 2001; Ross
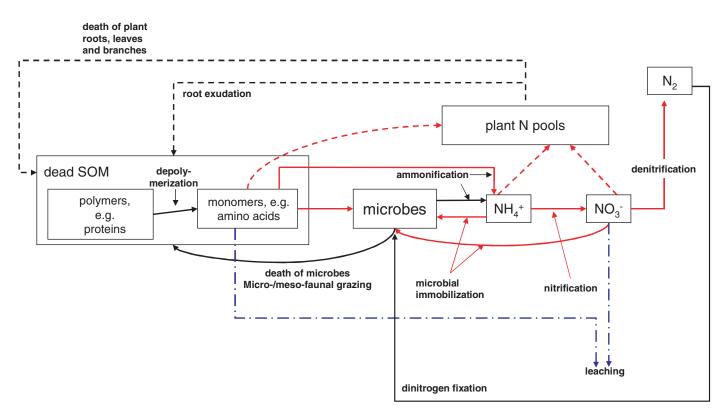
Figure 6.3 Scheme of major ecosystems processes of N cycling, including internal N retention pathways. Dashed lines: plant processes; solid lines: microbial processes; red dashed and solid lines: competitive processes between plants and microorganisms. Blue lines: hydrological transport pathways. SOM: soil organic matter. Taken from Rennenberg *et al.* (2009).

et al., 2004). This illustrated the complex dynamics of microbial production and consumption of inorganic N which cannot be evaluated by net rate assays.

Since the 1990s, many studies have shown that in quite a broad range of ecosystems, including arctic tundra, boreal and temperate forests, desert ecosystems and low productivity grasslands, plants are able to take up organic N, e.g. amino acids (Näsholm *et al.*, 2009; Rennenberg *et al.*, 2009). Further studies suggested that mycorrhyzal fungi can claim an important role in the acquisition of organic N by mycorrhyzal plants (see review by Meyer *et al.*, 2009), with approximately 90% of all plants supporting mycorrhyzation.

The increasing awareness of the role of DON in plant nutrition in natural ecosystems such as heathlands or forests and the surprisingly competitive strength of plants also led to a shift in paradigms concerning N mineralization. Compared to the old paradigm, which was centred around a simple N_r mineralization term (ammonification of organic N) as the rate-limiting step, the new, more holistic definition of N mineralization takes into account two steps: (1) the depolymerization of organic macromolecules to bioavailable DON and (2) mineralization/ammonification of bioavailable DON to ammonium. The new paradigm of terrestrial N cycling leaves the core concept unchallenged, i.e. that microorganisms are responsible for breaking down complex organic material into plant-available form. However, it widens the definition of N_r mineralization into the steps of depolymerization into monomers and subsequent ammonification to ammonium. The new view on N_r cycling is centred around depolymerization as the rate-limiting step (Figure 6.3).

A summary of the synthetic analysis of ^{15}N pool dilution studies of Booth *et al.* (2005) showed that soil organic matter quality – and here the C:N ratio – is a major tool for understanding differences between ecosystem types (e.g. at the same soil organic matter concentrations, grassland soils with relatively low C:N ratios show high N mineralization rates, while forest soils with relatively high C:N ratios showed lower N mineralization rates) and that N mineralization in agricultural or other soils is not necessarily stimulated by fertilization. The study also showed that soil C losses due to agricultural practices, changes in vegetation, or climatic factors, may increase the ability of nitrifiers to compete with heterotrophic microorganisms for NH_4, which in consequence will lead to higher N losses due to leaching or volatilization. Thus, a key role in potential N retention and N loss from terrestrial ecosystems has been attributed to the balance of inorganic N immobilization by microbes and (autotrophic) nitrification (Tietema and Wessel, 1992; Stockdale *et al.*, 2002). Subsequently the ratio between nitrification and immobilization and the ratios of nitrification to N mineralization (relative nitrification) and ammonium immobilization to N mineralization (relative ammonium immobilization) have recently been used as indices for potential N loss or the N retention capacity of ecosystems (Stockdale *et al.*, 2002; Accoe *et al.*, 2004; Dannenmann *et al.*, 2006). However, experimental assays for the determination of gross rates of N turnover are mostly based on root-free soil and thus ignore plant–microbe interactions. Therefore, it remains unclear to which extent the understanding of major controls of the terrestrial N cycling is transferable to actual N cycling in ecosystems. It was, for instance, indicated that dissimilatory

nitrate reduction to ammonium (DNRA, see later in this section) may also be an important process involved in terrestrial ecosystem N cycling (Silver *et al.*, 2001). Please note that in the context of ecosystem N_r cycling denitrification is not considered, but that it is discussed later as a pathway of N_r loss.

Decomposition and mineralization

Litter production and decomposition are the major drivers of terrestrial N turnover. Nitrogen enters the soil organic matter pool after internal N cycling via the plants (Figure 6.3). Depolymerization of organic matter is carried out by extracellular enzymes of fungi and bacteria (Jackson *et al.*, 2008). The bioavailable N resulting from the cleavage of macromolecules can be used either by plants or microorganisms. Microorganisms can further convert bioavailable DON into ammonium (N mineralization or ammonification). Ammonification is performed by unspecific heterotrophic microorganisms both under aerobic and anaerobic conditions (Jarvis *et al.*, 1996).

Depolymerizing and ammonifying heterotrophic microbes are supposed to be C-limited, and thus should be negatively affected by very low C:N ratios. However, this might be compensated by root exudation, root turnover and mycorrhyzal turnover adding both labile N and C sources to the soil (Bais *et al.*, 2006; Hogberg and Read, 2006). These root-derived C sources are supposed to claim a key role in C substrate supply to N mineralizing microbes. The strong dependence of depolymerizing and ammonifying heterotrophic microorganisms on labile C sources illustrates the tight coupling of the C and N cycles.

Nitrification

Nitrification is the biological oxidation of ammonium (NH_4^+) or ammonia (NH_3) via hydroxylamine to nitrite (NO_2^-) or nitrate (NO_3^-). Ecologists have identified nitrification to be a key process in ecosystem N cycling (Figure 6.4) in view of its relevance for ecosystem nutrient loss, atmospheric chemistry (with N_2O losses during nitrification being in the range of 0.1–10‰; Breuer *et al.*, 2002; Ingwersen *et al.*, 1999), streamwater quality and soil acidification. Nitrification of ammonium increases the probability that the converted N_r is lost from the ecosystems, since the end-products nitrite/nitrate are susceptible to losses by leaching along hydrological pathways or further reduction to gaseous NO, N_2O and N_2 via denitrification (Figure 6.4). On the other hand the likelihood that nitrified N_r will be immobilized by microbes or taken up by plants is somewhat lower in comparison to that of ammonium, since N can be only incorporated into the cell walls in reduced form (Glass *et al.*, 2002). Furthermore, nitrate is more susceptible to leaching, since the anion exchange capacity of soils is markedly lower than that for cations, thus, promoting retention of ammonium rather than of nitrate in the soil matrix. Both the high probability of loss along hydrological as well as gaseous pathways and the unfavourable energetics of consumption by plants and microbes make nitrate a critical N species with regard to N_r loss from terrestrial ecosystems. Increasing nitrification has opened the N cycle of many terrestrial ecosystems towards increased N losses. Nitrification can be performed both along heterotrophic and autotrophic pathways. Autotrophic nitrification is performed in two steps by different groups of microorganisms (Costa *et al.*, 2006; Wrage *et al.*, 2001). In a first step, an ammonia oxidizer oxidizes ammonium or ammonia to hydroxylamine (NH_2OH) and further to nitrite (NO_2^-). *Nitrosomonas europaea* is the best known autotrophic ammonia oxidizer. In a second step a nitrite-oxidizer (e.g. *Nitrobacter*) convert nitrite to nitrate.

While autotrophic nitrifiers use the oxidation of NH_4^+ or NO_2^- as an energy source for carbon dioxide (CO_2) fixation, heterotrophic nitrifiers use organic N_r both as an energy source and a C-source. Heterotrophic nitrifiers may be able to oxidize both ammonium and organic N compounds. The enzymatics
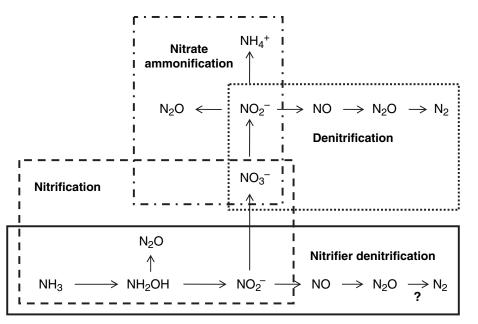

Figure 6.4 Nitrification is a central process for the formation of several N forms in the soil. Microbial sources of N_2O in soils are highlighted (Baggs, 2008).

of heterotrophic nitrification may – despite the intermediates and products being the same – differ from autotrophic nitrification (Wrage *et al.*, 2001; Conrad, 2002). Detailed reviews of nitrification pathways involving enzymes and their regulation were provided by Wrage *et al.* (2001) and Conrad (2002).

Nitrification is in general an aerobic process. Consequently, the oxygen availability in the soil exerts a major influence (Conrad, 2002). The optimum soil water content for nitrification is – depending on the soil texture – in the range of approximately 30%–60% water-filled pore space (Bouwman, 1998). However, autotrophic nitrification is affected by soil pH, the optimum pH for nitrification is thought to be at pH 5.5–6.5 (Machefert *et al.*, 2002). Another potential environmental control on nitrification is soil temperature (Machefert *et al.*, 2002), although nitrification can also take place in frozen soil (Freppaz *et al.*, 2007).

Plants may influence nitrification via consumption of ammonium, thus removing the substrate for nitrification. However, there might also be a direct effect of plants on nitrification activity via toxic compounds in root exudates (Castaldi *et al.*, 2009). Root exudates of some tropical pasture grasses contain nitrification inhibitors that block both the monooxygenase and the hydroxylamine-oxidoreductase pathways in *Nitrosomonas* (Subbarao *et al.*, 2006).

Microbial immobilization of N in the soil

Nitrogen is crucial for the growth and activity of heterotrophic soil microorganisms. Various organic and inorganic (ammonium, nitrate) N species can be taken up by both bacteria and fungi. Ammonium immobilization appears to be largely related to and controlled by gross ammonification and available C. Heterotrophic microorganisms in the soil prefer ammonium over nitrate (Rice and Tiedje, 1989; Recous *et al.*, 1990; Jansson *et al.*, 1955) due to energy costs (Tiedje *et al.*, 1982). Consequently a meta-analysis of available literature revealed a distinct, negative relationship between ammonification and the ratio of nitrate assimilation to total assimilation (Booth *et al.*, 2005). Nevertheless, microbial nitrate immobilization is a significant process in a wide range of terrestrial ecosystems (Booth *et al.*, 2005) especially in relatively undisturbed soils of semi-natural ecosystems (Dannenmann *et al.*, 2006; Myrold and Posavatz, 2007), but also exceptionally in agricultural soils (Burger and Jackson, 2003).

Nitrate assimilation has been shown to be dependent on available C (Azam *et al.*, 1988; Trinsoutrot *et al.*, 2000; Nishio *et al.*, 2001; Booth *et al.*, 2005), since C fuels the growth and activity of heterotrophic microorganisms. Additionally, a meta-analysis of ^{15}N-pool-dilution studies also revealed a dependence of nitrate immobilization on microbial biomass N and gross nitrification as the process supplying the substrate (Booth *et al.*, 2005). This might be explained by microsite variability (Sexstone *et al.*, 1985; Parkin, 1987; Schimel and Bennett, 2004) in the heterogeneous soil system which is likely to include microsites with high available C and low NH_4^+, where NO_3^- will readily be assimilated by microorganisms (Schimel and Bennett, 2004; Booth *et al.*, 2005). This

'microsite hypothesis' is confirmed by the observation that nitrate immobilization is larger in undisturbed soils than in physically disrupted soils (Booth *et al.*, 2005). Furthermore, nitrate immobilization has been shown to be especially large close to decomposing residues high in C (Cliff *et al.*, 2002; Moritsuka *et al.*, 2004).

Plant uptake

The acquisition of resources depends both on the phenology of the plants and on the supply of nutrients. Mobile resources including water and N may have a strong seasonal pattern of availability in many ecosystems. Plants tend to take up most of their N during vegetative growth (until flowering). However, distinct uptake patterns may be observed according to plant functional groups (annuals versus perennials) or even according to species. Co-existing plant species tend to decrease their competition for a limiting resource by taking up N at distinct times or soil depths, or using distinct N sources in a way that the composition of the plant community may be related to partitioning of differentially available forms of a single limiting resource.

Plants predominantly take up inorganic N forms and here mostly NH_4^+ in presence to NO_3^- to cover their N demand for growth. However, it was shown recently that in N-poor and cold ecosystems amino acids and other organic monomers are readily taken up by plants as well, thus, plant N uptake may by-pass microbial N mineralization. However, when N is not strongly limiting as in agricultural systems or N-deposition-affected forests, inorganic N remains the major nutrient for plants (Harrison *et al.*, 2007; Jackson *et al.*, 2008). Plants take up N via transport systems in the plasma membrane of root cells. According to the growth requirements, N uptake is regulated by root system architecture and mechanisms that regulate the activity of the transport systems. High-affinity transport systems regulate N uptake at soil DIN concentrations between 1 µmol l^{-1} and 1 mmol l^{-1}, while low affinity transport systems become significantly active above concentrations of approx. 0.5 mmol (Jackson *et al.*, 2008). Plant N uptake can be mediated by mycorrhyzal symbiosis even though the magnitude and ecological significance of this is still a matter of debate (Meyer *et al.*, 2009).

Nitrogen acquisition by plants depends much on the plant species and on the respective growth potential but also on abiotic factors such as temperature (which affects root activity as well as N availability), soil pH and competition by microbes (Jackson *et al.*, 2008). Soil pH is one of the factors that can influence the relative proportion of nitrate and ammonium in the soil. When both inorganic forms are present at similar concentrations most plant species take up ammonium preferentially. However, many plants are sensitive to high ammonium concentrations, showing 'ammonium syndrome'. The toxicity of ammonium nutrition is universal, although the threshold at which it is observed is species-dependent. As a general rule fast growing species are more ammonium-sensitive than slow-growing species. Ammonium toxicity has been associated with

ionic unbalance, cytoplasmatic pH changes and futile cycles (Britto and Kronzucker, 2002).

For N-saturated spruce forests, it has been shown that increased concentrations of NH_4^+ can inhibit NO_3^- uptake, which is most likely due to the energy dependence of nitrate uptake (Jackson *et al.*, 2008). Thus, plant nitrate immobilization ceases, followed by high rates of nitrate leaching. The actual uptake of N by plants is regulated by internal factors such as C and N metabolites and external factors like ammonium, nitrate and organic N compound concentrations in soil, light, temperature and soil pH.

Dissimilatory nitrate reduction to ammonium

In addition to nitrate assimilation, dissimilatory nitrate reduction to ammonium (DNRA) represents a second microbial pathway of ecosystem nitrate retention. Dissimilatory nitrate reduction to ammonium is an anaerobic process, catalyzed by fermentative bacteria and reducing NO_3^- via nitrite (NO_2^-) to ammonium (NH_4^+). DNRA has been shown to occur predominantly in anaerobic sludge and sediments (Tiedje *et al.*, 1982; Ambus *et al.*, 1992; Bonin, 1996; Nijburg *et al.*, 1997). By providing NH_4^+ for plant uptake and microbial immobilization and by reducing the size of the NO_3^- pool available for denitrification and nitrate leaching, DNRA has the potential to play an important role in ecosystem N retention (Silver *et al.*, 2001). Both DNRA and denitrification appear to be favoured by similar soil conditions (low redox potential, high NO_3^- and labile C availability). The ratio of C to electron acceptors seems to control the partitioning of nitrate to denitrification and DNRA in such a way that DNRA is favoured when this ratio is high (Tiedje, 1988). DNRA has been recognized as a significant process mainly in wetland ecosystems. Silver *et al.* (2001) found DNRA to be an important process in moist tropical forest soils with high clay content. DNRA might also be a significant process in temperate grassland soils (Müller *et al.*, 2004, 2007). The significance of DNRA in aerobic upland soils however, remains largely unclear and urgently needs to be investigated.

6.4.2 Controls on nitrogen cycling

Factors affecting nitrogen cycling

Nitrogen cycling in ecosystems is strongly affected by environmental conditions such as climate, soil properties, vegetation type and management activities.

Temperature and moisture are major controllers on temporal and spatial scales and microbial N cycling is high if neither temperature nor moisture are limiting. As an example, it has been shown that mineralization as well as nitrification respond positively to increasing soil moisture as long as the soil is not saturated and, thus becomes anaerobic (Stark and Firestone, 1995; Breuer *et al.*, 2002, Corré *et al.*, 2003). The N_r mineralization has been shown to increase with rising temperature up to approximately 30 °C (Shaw and Harte, 2001), this also applies for nitrification. However, the temperature

response of nitrification will depend upon NH_4 availability. As assimilation of NH_4 also increases with temperature and even faster than mineralization (Binkley *et al.*, 1994), nitrification can be substrate-limited at higher temperatures (Binkley *et al.*, 1994). Soil properties such as texture and clay content can affect N turnover in soils in several ways. On the one hand fine textured soils have a higher water holding capacity than coarse (sandy) textured soils and tend to have higher soil organic carbon concentrations. At the same precipitation level fine textured soils will show higher soil moisture values and become more often and more intensively anaerobic after intensive rainfall events. In consequence, gaseous N losses and (in this case especially important from an environmental point of view), N_2O and NO emissions are higher from fine-textured soils due to the more frequent stimulation of the predominantly anaerobic process of denitrification. On the other hand, the reduced ion exchange capacity and the improved soil drainage of sandy soils promotes nitrate leaching. Other important factors affecting N cycling are soil organic carbon content (SOC), C:N ratio and soil pH. The pace of N cycling has been found to be closely linked to increasing SOC (Li *et al.*, 2005). Moreover, the soil C:N ratio has been identified in several studies as a major indicator for leaching and gaseous N losses on the ecosystem scale. For example studies by Gundersen *et al.* (1998a) and Klemedtsson *et al.* (2005) show that at C:N ratios of less than 20–25 N_2O losses and nitrate leaching increase dramatically from the forest ecosystem whereas losses are usually negligible from soils above this C:N (see later). It is widely accepted that soil pH has a significant effect on soil N_r cycling and the associated production and emission of N trace gases from soils, as it influences the three most important processes that generate N_2O and NO: nitrification, denitrification and DNRA (Stevens *et al.*, 1998; Kesik *et al.*, 2006). N_2O as well as NO emissions have shown the tendency to increase under low soil pH conditions. This is most likely due to a combination of factors including increased N trace gas 'leakage' rates during nitrification and denitrification, shifts in microbial communities or contribution of chemo-denitrification to N trace gas production for ecosystems with soil pH values of less than 4.0 (Yamulki *et al.*, 1997; Ormecci *et al.*, 1999; Kesik *et al.*, 2006).

Vegetation type also exerts a major effect on ecosystem N cycling. For example litter quality is a major controlling factor for mineralization. Furthermore, other vegetation parameters such as canopy structure, leaf geometry or root distribution have been reported to affect N trace gas exchange, soil nitrate leaching and rates of microbial N turnover via effects on soil aeration, soil moisture distribution or substrate availability (Brumme *et al.*, 1999; Butterbach-Bahl *et al.*, 2002; Rothe *et al.*, 2002).

Bearing in mind the still incomplete list of factors affecting ecosystem N cycling, it becomes obvious that human management is of outstanding importance and for many ecosystems the crucial factor. Drainage, fertilization, irrigation, tillage practices, amendment of soils with manure or lime, crop rotations, soil compaction, grazing or human-induced N deposition

are altering ecosystem state variables at a multitude of temporal and spatial scales via effects on substrate availability, litter quality, aeration, microbial community composition, soil moisture and even temperature profiles.

Effects of land use and management on nitrogen cycling

Land-use changes have a tremendous effect on N cycling. Conversion of natural land into arable land is not only characterized by losses of ecosystem C stocks, but is mostly also accompanied by significant losses of ecosystem N stocks along hydrological pathways, gaseous volatilization or through erosion (Tiessen *et al.*, 1982; McLauchlan, 2006). Based on a review of reported data, Murty *et al.* (2002) concluded that the conversion of forest soils to agricultural use resulted in an average loss of 15% N. This is less than the average loss of SOC, indicating that the C:N ratio narrows when agriculture is introduced (Murty *et al.*, 2002; McLauchlan, 2006). However, intensification of land use results not only in a loss of N stocks and a narrowing of C:N ratios, but mostly goes along with an increase in soil pH due to liming, compaction of the soil and an increase in soil temperature and moisture amplitudes due to the reduction of the aboveground biomass cover. Not taking into account other management strategies such as fertilization, irrigation or drainage, this already has major effects on ecosystem N cycling. Mineralization rates are mostly slowed down in temperate agricultural systems as compared to native grasslands or forests (Booth *et al.*, 2005). However, the likeliness of nitrate leaching losses increases, since nitrification is stimulated by external N inputs (Watson and Mills, 1998) and denitrification is often C-limited. Recent studies also show that soil microbial communities involved in N cycling are adapting to different land uses, which is accompanied by changes in the composition of species and functional groups (Coloff *et al.*, 2008). However, it remains mostly unclear if these changes are accompanied by losses in functionality. Agricultural management can also support an accumulation of N and recovery of N cycling. Introduction of leguminous plants in the crop rotation (Tiessen *et al.*, 1982) or application of organic fertilizers (Griffin *et al.*, 2005) can support an accretion of N stocks.

In summary, there are several studies available which show that land use and land management are of utmost importance for N cycling and biosphere–hydrosphere–atmosphere exchange at site and regional scales and that for example, intensification of agriculture has resulted in dramatic changes in regional and global N cycling (Galloway *et al.*, 2003; Gundersen *et al.*, 2006; Coloff *et al.*, 2008).

Effects of climate change on nitrogen cycling

Predicted climate changes are likely to feedback on ecosystem N cycling and biosphere–hydrosphere–atmosphere exchange processes. Based on field manipulation experiments at four different shrubland sites in Denmark, UK, the Netherlands and Spain, Beier *et al.* (2008) found that N mineralization was relatively insensitive to temperature increases (approximately 1 K). The lack of direct connection between temperature and N mineralization in this study suggests that changes in the N cycle appear to occur more slowly and to have little direct influence on the C cycle (Beier *et al.*, 2008). Contrary results were reported by Rustad *et al.* (2006) on the basis of a meta-data analysis for 32 research sites in North America, representing four broadly defined biomes, including high (latitude or altitude) tundra, low tundra, grassland, and forest. They found that experimental warming in the range of 0.3–6.0 K significantly stimulated rates of soil respiration (+20%) and N mineralization (+46%) as well as plant productivity (+19%). The differences between the analyses could be due to the confounding effect of soil water availability. Temperature stimulation effects can only be expected if moisture is not limiting. The study by French *et al.* (2009) on climate change effects on agricultural soils provides some indication which microbial-mediated processes that play an important role in the N cycle may also be influenced as a result of climate change. Modelling studies dealing with climate change effects on N trace gas exchange or nitrate leaching of forest ecosystems in North America and Europe indicate that the stimulation of microbial N turnover in soils with climate change may be accompanied by increased losses of nitrate and increased emissions of NO rather than of N_2O (Kesik *et al.*, 2006a, b; Campbell *et al.*, 2009), mostly due to large increases in N mineralization and nitrification.

6.4.3 Spatial and temporal variability of nitrogen cycling

Describing N cycling on spatial and temporal scales remains a challenge, much of which is associated with the fact that small areas (hotspots) and brief periods (hot moments) often account for a high percentage of N turnover and in these cases especially denitrification activity and N losses (Groffman *et al.*, 2009). While the knowledge on factors affecting N cycling such as substrate availability, temperature, oxygen or moisture is pretty well established (see above), the understanding of how these factors are interacting on spatial and temporal scales remains low. The best known are, for example, emission pulses of N trace gases following re-wetting of dry soil in e.g., Mediterranean and subtropical climates (Davidson *et al.*, 1993), fertilization or irrigation/drainage events (Dobbie *et al.*, 1999) or freeze-thaw-related N_2O emissions from agricultural or natural soils (Christensen and Tiedje, 1990; Wolf *et al.*, 2010). Such pulse emissions, lasting often only for a few days, may contribute up to 80% of total annual emissions. Also the understanding that certain components of landscapes, for example riparian zones, could be viewed as hotspots of denitrification and zones of intensive N exchange between biosphere–hydrosphere and atmosphere (McClain *et al.*, 2003; Groffman *et al.*, 2009) is still increasing. A better characterization of N cycling processes and quantification of N exchange across spheres therefore requires research at landscape scales and a better understanding of how biological, climatic, soil and hydrological factors converge to create hotspots and hot moments at these scales (Groffman *et al.*, 2009).

6.5 Outputs of nitrogen from ecosystems

Several natural processes remove N from terrestrial ecosystem, which together with low inputs is the reason why N limitation may persist in many natural ecosystems (Vitousek and Howarth, 1991). The most important pathways are volatilization in fires, denitrification and N leaching. Moreover, erosion processes can contribute to N limitations and redistribution of nutrients. These N outputs may be modified by exploitation and management of the ecosystem. In the following we discuss the natural removal processes and how these are changed by human activities.

6.5.1 Fire

Wildfires have coexisted with human activities and have shaped the landscapes of the Mediterranean but also those of temperate climates. Although fire has always existed as a natural phenomenon, the use of fire for activities such as grazing, agriculture, and hunting has significantly modified fire regimes, primarily in the Mediterranean region. More recently, the increase in population density in Europe, and the extensive use of natural and forest regions for recreation has increased the number of man-made fires (Barbosa et al., 2009). According to the EU Fire Database, almost 600 000 ha of forest land burn every year (2000–2005); of these c. 500 000 ha yr⁻¹ occurred in Mediterranean countries (Miranda et al., 2009). Although the number of fires has steadily increased in the last decades, the total burned area has not increased in Europe. This is mostly due to a fire suppression policy and the efficiency of (and the large investment in) fire-fighting equipment (Miranda et al., 2009).

Burning is a major pathway of N loss for ecosystems exposed to high fire frequencies. Depending on fire intensity (heat), 50%–99% of aboveground biomass N is lost as N_2 (pyrodenitrification) or as NO_x, N_2O and NH_3. Part of the litter layer N may also be lost, whereas the N pool in the mineral soil usually is unaffected (Alexis et al., 2007). Several uncertainties exist with regard to regional N mass balances but the largest are related to the characteristics of the fuel and fuel consumption. According to the CORINAIR emission inventory, forest fires cause 0.2% of NO_2, and 1.3% of NH_3 emissions, in Europe.

Fire has significant short-term effects on soil N availability and therefore on water quality through soil heating effects and mid-term effects (1–3 years) on decomposition rates. The N deposition may also enhance nitrate runoff to stream waters one to three years after fire events (Johnson et al., 2007; Gimeno et al., 2009). Fire can have substantial long-term effects (decades to a century) on ecosystem C and N by causing changes in vegetation, often by facilitating the occupancy of the burned site with N-fixing vegetation (Wan et al., 2001).

6.5.2 Gaseous losses

Total N losses by denitrification

Denitrification is defined as the dissimilatory reduction of nitrate, nitrite, nitric oxide (NO) or nitrous oxide (N_2O) to N_2O

and N_2 by microbes. The ability to carry out denitrification is not only found in bacteria, but also in some fungi and archaea (see review by Hayatsu et al., 2008; Zumft, 1997). In general, denitrifying enzyme activity occurs and is expressed under oxygen-limiting conditions, i.e. (if available) nitrogen oxides are used as an alternative electron acceptor. However, several bacteria have been shown to reduce nitrite or nitrate to gaseous nitrogen and a combined inhibitor and isotope tracer study by Bateman and Baggs (2005) suggests that aerobic denitrification occurred at 20% water-filled pore space.

In addition to microbial denitrification, chemo-denitrification may also occur, i.e. in the presence of Fe^{2+} (formed through weathering of minerals) and an alkaline pH, nitrate can be chemically reduced (Van Cleemput, 1998; Samarkin et al., 2010).

N_2 can also be produced via the anammox process (anaerobic ammonium oxidation). This process, discovered in 1995 combines (under strict anaerobic conditions) ammonium and nitrite directly into N_2 (Hayatsu et al., 2008). So far it has not been possible to show that this process is of significance in soils (instead only in bioreactors, wastewater plant and landfills). This is despite the fact that anammox sequences of the relevant bacteria (e.g. *Candidatus Brocadia anammoxidans*) have been detected in permafrost soil, agricultural soil and in samples associated with nitrophilic or nitrogen-fixing plants production (Humbert et al., 2010). It seems that the diversity of anammox bacteria is higher in terrestrial systems in comparison to marine systems, which is possibly a consequence of the larger variability of soil habitats and specific ecological requirements (Humbert et al., 2010).

Microbial denitrification is the main pathway of N_r loss in terrestrial ecosystems. It is remarkable that the importance of denitrification is more constrained at regional and global scales as compared to the site scale. In an analysis involving major watersheds in the USA and Europe, Van Breemen et al. (2002) concluded that only 23% of N_r applied to the landscapes was found in riverine exports. Even if one accounts for changes in N storage due to land use change and biomass increment or export of N_r with food, feed and wood products this still leaves 37% of N_r left to be denitrified within the landscape. This estimate of the magnitude of landscape denitrification is well in-line with estimates on a global scale by Seitzinger et al. (2006), who estimated that approximately 40% of the global addition of 270 Tg N_r yr⁻¹ to terrestrial ecosystems is removed via soil denitrification. Based on a calculation of $^{15}N{:}^{14}N$ ratios, Houlton and Bai (2009) concluded that approx. 28 Tg of N_2 are lost annually via denitrification to N_2 in the terrestrial soil beneath natural vegetation, with an $N_2{:}N_2O$ ratio ranging from 2.2 to 4.6 at the global scale.

Estimates of denitrification rates for different ecosystem types vary largely in dependence of ecosystems type, soil properties and management (Barton et al., 1999; Hofstra and Bouwman, 2005), but also with regard to the method applied for the quantification of N losses via denitrification (Groffman et al., 2006; Hofstra and Bouwman, 2005). Based on a detailed literature review on denitrification in soils, Barton et al. (1999) showed that, on average, denitrification rates in agricultural
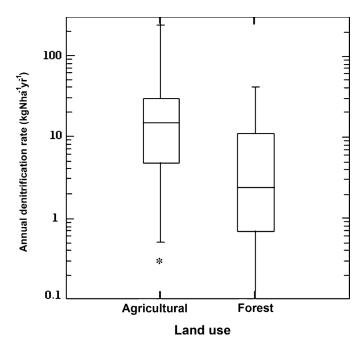
Figure 6.5 Box-plots comparing annual denitrification rates in agricultural and forest soils. The centre vertical line marks the median, the edges of the box (hinges) mark first and third quartiles, and the central 50% of annual rates are within the range of the box. Vertical lines show the range of values that fall within 1.5 (midrange) of the hinges. Asterisks are values outside inner margins (1.5 (|median-hinge|)). (Figure taken from Barton *et al.*, 1999.)

soils are one order of magnitude higher than in natural soils (Figure 6.5). They concluded that 'most annual denitrification rates reported in the literature appear to be fairly low, with over half of the rates in forest soils being less than 1 kg N ha^{-1}.yr^{-1} (with a mean of 1.9 kg N ha^{-1}.yr^{-1}). Rates of denitrification in agricultural soils tend to be higher than in forest soils, with 85% of rates reported being greater than 1 kg N ha^{-1}.yr^{-1}, and a mean rate of 13 kg N ha^{-1}.yr^{-1}.' Numerous soil, site, and management factors have been reported (Barton *et al.*, 1999; Groffman *et al.*, 2009) to affect the denitrification process *in situ*. The literature indicates that the highest rates of denitrification can be expected in N-fertilized soils, or where site management increases soil nitrate availability. Where nitrate is non-limiting, denitrification rates appear to be highest in irrigated loam soils. The review suggests that it is 'difficult to predict denitrification rates based on our current understanding, and that plot studies should still be conducted if soil N balances are required'.

Hofstra and Bouwman (2005) showed that soil-core-based estimates are a factor of two lower than those based on mass balance approaches. The situation becomes even more complex if the huge variability of denitrification across temporal and spatial scales is taken into account (Groffman *et al.*, 2009). At the present site scale, estimates of denitrification are highly uncertain, despite more than eight decades of research on the process. It depicts partly our current lack of understanding of denitrification in soils and partly the problem of variability that will remain and will always induce large uncertainty at field scale.

The challenge to estimate denitrification losses at the site scale is closely linked with the difficulties in measuring denitrification rates in soils at a background N_2 concentration of 78%. The commonly used C_2H_2 blockage technique, i.e. using C_2H_2 to block the further reduction of N_2O to N_2 during denitrification, has been shown to fail in the presence of O_2 (Bollmann and Conrad, 1997), since C_2H_2 catalyses the conversion of NO (another intermediate of denitrification) to nitrite/nitrate which is then metabolized by soil microbial processes. Therefore, this method can lead to an underestimation of N losses. The most reliable methods for quantifying denitrification in soils are the use of stable isotope techniques or the soil core gas flow technique (Butterbach-Bahl *et al.*, 2002). However, these methods also have severe drawbacks with regard to the experimental complexity, representativeness of soil cores, sensitivity of measurements and cost of experiments (Groffman *et al.*, 2006).

Nitrous oxide

From a mass balance perspective, global N_2O losses from terrestrial ecosystems are small and in the magnitude of 10 Tg N yr^{-1}. However, N_2O is important from the perspective of stratospheric ozone destruction and climate protection (Butterbach-Bahl *et al.*, 2011; Chapter 19, this volume). On a global scale, the main sources of N_2O are associated with soil emissions (e.g. Smith, 1997) and, more specifically, mainly with emissions from agricultural soils and tropical rainforest soils. Even though it is known that on a global scale soils are a major source for N_2O (approximately 60% of all sources), there is still an ongoing debate regarding the source strength of individual ecosystems for N_2O, the potential of soils to function also as sinks for atmospheric N_2O (Chapuis-Lardy *et al.*, 2007) and about losses of N_2O following N_r application/deposition. This is due to the variability of N_2O emissions from soils on temporal and spatial scales, our shortcomings with measuring techniques (mainly chamber-based, with significant drawbacks with regard to spatial representation), the lack of continuous year-round measurements and the still limited understanding of microbial processes driving soil N_2O formation. It is remarkable that, based on a thorough literature review, IPCC (2007) assumes that 1.0% of fertilizer N applied to fields is directly emitted in the form of N_2O, while the top-down approach by inverting the global atmospheric N_2O budget yielded loss rates of N_2O from newly created N_r that are in the range of 3.5%–4.5% (Crutzen *et al.*, 2008). A re-evaluation of the top-down approach of Crutzen *et al.* (2008) by Davidson (2009) indicates that 2.5% of fertilizer N-production has been converted to N_2O, either directly following fertilizer application or indirectly following Nr cascading through downwind/downstream ecosystems. This means that research is still needed to get a better understanding of direct and indirect N_2O emissions following N_r input to terrestrial systems. Major uncertainties here are specifically the ratio of N_2 to N_2O emissions and how this ratio is affected by environmental conditions and involved microbial processes. Based on the work of Seitzinger *et al.* (2006), approximately 40% of the 270 Tg N_r that is brought annualy into terrestrial ecosystems is

denitrified in soils (100 Tg). Since N_2O emissions from terrestrial ecosystems are in the magnitude of 10 Tg yr^{-1}, the N_2:N_2O ratio should at least be on average 10 (assuming that all terrestrial emissions are emissions from soils). Good experimental background data exists which shows that the N_2:N_2O ratio is highly variable (Simek et al., 2002).

Nitric oxide

Soil emissions of NO from heavily fertilized areas can reach the same magnitude as the anthropogenic NO release in urban areas (on a per area and time basis) (Ludwig et al., 2001) and annual loss rates have been reported to be as high as 12–52 kg N ha^{-1} yr^{-1} for fertilized (280 kg N ha^{-1} yr^{-1}) and irrigated maize fields in southwestern France (see also Figure 6.6). Estimates of NO emissions from agricultural soils for EU-15 states are vary within a range of 49 to 190 kt NO-N for the year 2000, depending on the approach used (Butterbach-Bahl et al., 2009). For forests in the EU-15, soil NO emissions have been estimated to be 75 kt NO-N yr^{-1} (Kesik et al., 2006a, b). In total, NO emissions from soils in EU-15 have been calculated to be 4%–6% of annual anthropogenic NO/NO_2 emissions (Butterbach-Bahl et al., 2009). The drivers of soil NO emissions are in principle the same as for N_2O, i.e. soil moisture and temperature, and N availability, but also soil properties (texture, soil organic carbon, pH, etc.) and management practices such as tillage and irrigation have been shown to affect the timing and magnitude of soil NO emissions (Ludwig et al., 2001). In contrast to N_2O, the main microbial process responsible for NO production in soils seems to be nitrification rather than denitrification (Conrad, 2002). Thus, optimum moisture conditions in NO emissions from soils have often been found to be in the range of 50%–70% water-filled pore space. Since soil–atmosphere NO exchange is the product of simultaneously occurring production and consumption processes the role of denitrification for NO exchange is often associated with the uptake of NO produced from nitrification which is already within the soil matrix, i.e. NO consumption (Conrad, 2002). The close relationship between N availability and NO emissions is demonstrated by the close

positive correlation between N deposition and the magnitude of NO emissions from coniferous forest soils, e.g. as shown by Pilegaard et al. (2006). NO is a highly reactive N trace gas and reacts quickly – already partly within the canopy – with O_3 to form NO_2. The NO_2 can be readily taken up and metabolized by plants. Hence high soil NO emissions are often correlated with substantial NO_2 re-deposition to plant and soil surfaces (Sparks, 2009). Soil NO emissions and re-deposition on and uptake of NO_2 by plant leaves can be regarded as a potentially important process of nutrient dispersal (Butterbach-Bahl et al., 2004; Sparks, 2009). In summary, little is known about the regulation of NO turnover in soils and ecosystems, and what is known is based on empirical observations but is not understood theoretically on the basis of microbial and plant metabolism (Conrad, 2002). To improve understanding there is a need for (a) continuous measurements (year-round) for understanding temporal variability and to improve loss estimates and (b) the study of microbial and plant processes involved in production and consumption of N trace gases in soils and ecosystems.

Ammonia

For agricultural systems and under certain environmental conditions (high pH environments, application of organic and inorganic fertilizers) NH_3 losses are also very important from a mass balance point of view (Jarvis et al., 2011, Chapter 10 this volume). In the case of synthetic fertilizers, urea has the greatest potential for loss of NH_3 to the atmosphere by volatilization, especially from alkaline soils. Major losses of NH_3 to the atmosphere also arise from animal manures, with estimates of the fraction of their N content lost in this way ranging from 20% to 33% (Bouwman et al., 1997; IPCC, 1997). The calculated median NH_3 loss from global application of synthetic N fertilizers (78 million tons N per year) and animal manure (33 million tons N per year) amount to 14% (10%–19%) and 23% (19%–29%), respectively (Bouwman et al., 2002). Across the EU-27, Oenema et al. (2009) estimated NH_3 losses from agriculture in the year 2000 of 2873 Gg N. The principal measures to reduce these NH_3 emissions include avoidance of excessive
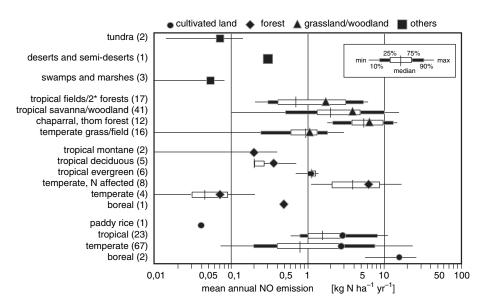

Figure 6.6 Published data on NO emissions from soils from various ecosystem and land use types. (Figure taken from Ludwig et al., 2001). With kind permission from Springer Science+Business Media.

Table 6.2 Characteristics of coniferous forest ecosystems with low, intermediate and high N status (Gundersen *et al.*, 2006). Nitrogen input is not a good indicator of N status, but the ranges given are typical for low, intermediate and high N status ecosystems

Nitrogen status	Low N status (N-limited)	Intermediate	High N status (N-saturated)
Input (kg N ha^{-1} yr^{-1})	0–15	15–40	40–100
Needle N% (in spruce)	< 1.4	1.4–1.7	1.7–2.5
C:N ratio (g C g N^{-1})	> 30	25–30	< 25
Soil N flux density proxy (litterfall + throughfall) (kg N ha^{-1} yr^{-1})	< 60	60–80	>80
Proportion of input leached (%)	<10	0–60	30–100

N in agricultural production systems, i.e. feeding animals and fertilizing fields and pastures according to requirements, covering manure storage and use of low emission application systems rather than surface spreading. The use of N fertilizers with high losses (such as ammonium bicarbonate and urea) is to be avoided.

6.5.3 Leaching

Ammonium and dissolved organic nitrogen

The mobility of N in soils largely depends on the form of dissolved N (NH_4^+, NO_3^- or DON). Ammonium is absorbed on the soil cation exchange complex and is thus quite immobile in the soil profile. As a result, NH_4^+ concentrations are generally low in seepage water and very low in streams. Ammonium usually contributes less than 5% to the total dissolved N concentration in soil water except in extremely NH_4^+-loaded soils (Dise *et al.*, 2009; De Vries *et al.*, 2007). Concentrations of DON are below 0.6 mg N l^{-1} and often even below 0.1 mg N l^{-1} in both seepage water from well-aerated soils (Michalzik *et al.*, 2001) and streams (Campbell *et al.*, 2000; Perakis and Hedin, 2002). No relation to concentrations of dissolved inorganic N was found in these studies (Michalzik *et al.*, 2001). In pristine forest streams, DON constitutes the dominant N leaching loss, in the order of 1–3 kg N ha^{-1}y^{-1}, since nitrate concentrations were very low in those streams (Campbell *et al.*, 2000; Perakis and Hedin, 2002).

A recent analysis by Brookshire and others (2007) showed that elevated atmospheric N deposition (spanning an input gradient of 5–45 kg N ha^{-1} yr^{-1}) increased DON output from temperate forested watersheds in the Appalachian Mountains of the USA. However, over a similar gradient across 12 European sites no significant relation between throughfall N and forest floor DON leaching was found (Park and Matzner, 2006). Nitrogen addition experiments in the USA showed a consistent increase in DON effluxes following the increase of N input (Pregitzer *et al.*, 2004; McDowell *et al.*, 2004) whereas no such effects were shown in European N addition experiments (Gundersen *et al.*, 1998b; Raastad and Mulder, 1999; Sjöberg *et al.*, 2003).

Nitrate leaching and N status indicators

Nitrate is the constituent in seepage and stream water that responds most to input changes or disturbances of the plant cover. Nitrate is highly mobile in soils and input and release in excess of plant and microbial uptake requirements will be transported through the soil profile and leached. In consecutive compilations of input–output budgets for N in European forests nitrate leaching increased with increasing N input (MacDonald *et al.*, 2002; De Vries *et al.*, 2007; Dise *et al.*, 2009). However, elevated nitrate leaching hardly ever occurs below a throughfall N deposition of 8–10 kg N ha^{-1} yr^{-1} and always occurs above 25 kg N ha^{-1} yr^{-1}. Within this N input interval both full retention and no retention in forests can be found. This variability in the response to N deposition is to a large extent determined by the 'N status' or N availability of the system; N, poor systems have a high retention and N-rich systems have a low retention (Table 6.2). The type of N input (ammonium or nitrate) also influences retention. Those sites dominated by ammonium retain a higher proportion of the input (Emmett *et al.*, 1998) probably due to absorption of ammonium on the soil cation exchange complex and subsequent biological processing.

In agricultural soils, leaching losses can increase rapidly when rates of fertilizer N exceed plant N demand (Vinten *et al.*, 1994), but losses can also be influenced by rainfall, cultivation and soil management (Shepherd *et al.*, 2001; Misselbrook *et al.*, 1996).

From N input manipulation experiments and analyses of available European datasets from coniferous forests, three classes of forest N status can be distinguished, based on N concentrations and fluxes (Table 6.2). The forest floor C:N ratio is a good indicator of N status, at least for coniferous forests, but a more broadly applicable indicator is the C:N ratio of the top mineral soil (Gundersen *et al.*, 2009). A negative relationship between C:N ratio and nitrate leaching has been found and below a C:N ratio threshold of 25 all sites had elevated nitrate leaching (Gundersen *et al.*, 1998a), even though other studies have found a less clear relationship (De Vries *et al.*, 2007). MacDonald *et al.* (2002) found an average retention of 68% and 35% of the input at sites above and below a forest floor C:N ratio of 25, respectively. An important mechanism behind the shift of the balance between retention and leaching seems to be the onset of net nitrification at forest floor C:N ratios around 24–27 (or 1.4%–1.6 N in organic matter) (Aber *et al.*, 2003; Kriebitzsch, 1978). This emphasizes the importance of soil C content on N retention, where C-rich soils may have large N retention potentials, especially if the C:N ratio is greater than 25. This threshold for N retention compares well with the

threshold (C:N >25) for growth response to N fertilization in conifers (Hyvönen *et al.*, 2008).

N content in needles correlates with the forest floor C:N ratio and is also a good indicator of N status (Kristensen *et al.*, 2004). At N contents in needles below 1.4%, no nitrate leaching occurs and the system appears N-limited (Table 6.2), whereas above 1.4% N in needles leaching often occurs. This level corresponds surprisingly well with the threshold of 1.3%–1.4% N above which conifer stands show no growth response to N fertilizer additions (Sikström *et al.*, 1998).

In accordance with the definition of N saturation, nitrate leaching occurs if the soil flux density of mineral N (defined as N deposition plus net mineralization) exceeds the capacity of N uptake by plants. Net mineralization, which is a measurable parameter, includes (by definition) the microbial demand, i.e. the microbial immobilization. Datasets combining N deposition and net mineralization suggest a threshold in N flux density of 100 kg N ha^{-1} yr^{-1} for elevated nitrate leaching (Andersson *et al.*, 2002; Fisk *et al.*, 2002). Net mineralization is not routinely measured, but may be strongly correlated to the N flux with litterfall. Hence, total aboveground N input to the soil (throughfall N plus litterfall N) excluding belowground root litter input can be taken as a proxy for N flux density. Using this parameter the threshold is around 60 kg N ha^{-1} yr^{-1} for conifers (Table 6.2).

Retention of deposited N in soils mainly occurs in the forest floor (Nadelhoffer *et al.*, 1999). This retention may slowly change the organic matter quality and decrease the C:N ratio of forest floors with time. There is no documentation whether this has occurred, but an indication is given by decreasing C:N (increasing forest floor per cent N) with increasing N deposition (Aber *et al.*, 2003; Kristensen *et al.*, 2004). On the other hand, Moldan *et al.* (2006) found no change in C:N ratio after a decade of chronic N addition to a coniferous forest, but an increased accumulation of both C and N in the organic mor layer. The European soil inventory of Vanmechelen *et al.* (1997) showed that currently approximately 40% of the *c.* 4000 sites had forest floor C:N ratios below the threshold of 25, below which elevated nitrate leaching often occurs.

With current N loads, many forest sites may move towards N saturation. At this condition, the ecosystem is very responsive to changes in N deposition (Gundersen *et al.*, 1998b). Reductions in N deposition will, with only a short time delay, translate into a proportional decrease in nitrate leaching. This was shown in experiments where N inputs in throughfall water were removed by roofs. In these experiments, nitrate leaching was reduced and N content in older needles decreased, indicating reversibility of the N-saturated condition (Boxman *et al.*, 1998a; Bredemeier *et al.*, 1998).

6.6 Effects of nitrogen

6.6.1 Nitrogen enrichment and saturation

Since N is considered the major limiting element in terrestrial ecosystems, increased N deposition would be expected to be retained in the ecosystem and stimulate growth. However, N deposition in large areas in Europe exceeds the normal growth requirement of forests and other (semi-natural) ecosystems and they may thus over time become enriched in N. Central to our understanding of N enrichment and its effects is the concept of 'nitrogen saturation', that describes a series of temporal changes in ecosystem functioning in response to increased N input.

Nitrogen saturation can be defined in several ways (Ågren and Bosatta, 1988; Aber *et al.*, 1989). The most widely used definition is in conditions where 'availability of mineral N may exceed the combined nutritional demands of plants and microbes' (Aber *et al.*, 1989) which then can be determined as elevated nitrate leaching from the rooting zone. In this situation, what also occurs is that N (derived from deposition) leaves terrestrial ecosystems and may potentially affect downstream ecosystems through the 'N cascade' (Galloway *et al.*, 2003). Ågren and Bosatta (1988) have defined an N-saturated system as 'an ecosystem where N losses approximate or exceed the inputs of N' which implies an accumulation in the system close to zero. From a theoretical point of view, this may be the most proper use of the term 'saturation'. However, this type of 'true saturation' may be seen as the end point of the N-saturation continuum where there is practically no biological control over N retention. Agricultural systems are often N-saturated according to the first definition, since it is usually economical to add more fertilizer N than can be taken up by the crop. On the other hand, they will never reach true saturation since a major fraction of the N input will be removed in harvested crops.

The development of N saturation by increased N inputs involves a complex interaction of the processes in the N cycle (Aber *et al.*, 1989, 1998). The progression from N limitation to N excess and the potential effects of N deposition may be explained as follows. In the first phase primary production increases, plants and microbes effectively absorb added N and the N content of plants increases. Retranslocation of N from senescent foliage (and roots) may decrease leading to higher N contents in litter materials and thus increased litterfall N flux. The internal cycling of N is accelerated through increases in litterfall N, net mineralization and tree N uptake. As N availability is increased, the composition of the forest floor vegetation may gradually change towards more nitrophilic species and other essential resources (P, K, Ca, Mg, or water) may at least periodically limit growth. In the accelerated N cycle, net nitrification becomes important and nitrate starts to appear in soil water. When elevated nitrate leaching becomes chronic, soil acidification resulting from N transformations becomes significant. Destabilization and potential forest decline from excess N deposition have been shown in case studies where the nutritional imbalance was important (Roelofs *et al.*, 1985). Recent synthesis efforts support this general scheme although the understanding of processes and interactions has become more complex and detailed (Aber *et al.*, 1998, 2003; Emmett *et al.*, 1998; Gundersen *et al.*, 1998b). The timing of the changes in ecosystem N function at a certain N deposition load is still not well understood, nor is the cumulative load required to change the N status of a low-N ecosystem known.

6.6.2 Biodiversity and vegetation change

Nitrogen deposition has the potential to impact on biodiversity in a wide range of ways (see also Dise *et al.*, 2011, Chapter 20, this volume). Aboveground impacts on vegetation include direct toxicity to sensitive species (Britto and Kronzucker, 2002), reduced resistance to environmental stresses such as frost (Caporn *et al.*, 2000), increased susceptibility to pests and disease (Brunstig and Heil, 1985) and soil-mediated effects of acidification and eutrophication (Stevens *et al.*, 2006).

The degree to which N deposition has either a positive or negative impact on an individual species depends on the tolerances and requirements of the respective species. At a community level, the impact is usually negative, resulting in a change in the species composition from that typically found at a given location. Additionally, declines in species richness have been widely reported in a number of vegetation communities, both from experimental studies (Bobbink *et al.*, 1998; Suding *et al.*, 2005), studies using natural deposition gradients (Stevens *et al.*, 2004; Maskell *et al.*, 2009) and studies of species change over time (Dupré *et al.*, 2010).

Changes in species composition of the vegetation are likely to have impacts on N cycling. Species may have differential abilities to use N or a preference for either nitrate or ammonium. Consequently, changing species composition may impact on the relative rates or form of N uptake (Britto and Kronzucker, 2002). Other feedbacks between plant community change and the N cycle may result from different concentrations of N stored in plant tissues, plant growth rates and life-span and ease of decomposition of dead materials, all resulting in changes in the availability of N or residence times in N pools.

6.6.3 Carbon cycle

Effects on plant growth and aboveground C sequestration

In agricultural systems, N additions from fertilizer usually lead to increased plant growth even though the system is N-saturated as defined above, but in this case other nutrients (P, K, and micronutrients) are added as well. When NPK fertilizers are used in forest ecosystems strong growth responses are usually observed there as well (Jarvis and Linder, 2000), but when N alone is added growth response is more modest and dependent on N status prior to fertilization (Hyvönen *et al.*, 2008). Growth responses to repeated annual N fertilization in northern Europe decrease with soil C:N ratio, with an indicated threshold for a growth response at C:N ratio 25 (Hyvönen *et al.*, 2008). Under high loads of N fertilization the growth response can be reversed, as other nutrients become limiting (Högberg *et al.*, 2006).

Experiments more closely simulating chronic N inputs from deposition (lower doses split in several additions over the growing season or the whole year) show quite variable growth responses: the European NITREX sites showed no growth response to N addition (Emmett *et al.*, 1998), whereas a long-term experiment at four sites in Michigan, USA, did show an increase in woody biomass (500 kg C ha^{-1} yr^{-1}) after adding 30 kg N ha^{-1} yr^{-1} over 10 years (Pregitzer *et al.*, 2008). In an

N$_r$-enriched site, a growth increase after trees were relieved from excess N deposition has also been observed (Boxman *et al.*, 1998a). The experiments simulating chronic N inputs were criticized for adding N to the soil, rather than to the canopy as happens with N deposition (Sievering, 1999). Recently it was shown that canopy uptake of N stimulated plant growth at a low-deposition (3 kg N ha^{-1} yr^{-1} total) site in the USA (Sievering *et al.*, 2007) and a modelling analysis indicate that direct canopy N uptake, by-passing the competition for nutrients exerted by soil microbes, could have a significant effect on the sensitivity of tree growth (Dezi *et al.*, 2009). However, a study by Dail *et al.* (2009) revealed a limited canopy N uptake (see Section 6.3.2). Thus the experimental evidence is somewhat inconclusive. Recent multi-factorial analysis of forest growth at nearly 400 plots across Europe showed significant responses to N deposition at 1%–2% growth increase per kg N ha^{-1} yr^{-1} when analysed at stand level (Solberg *et al.*, 2009) as well as at individual tree level (Laubhann *et al.*, 2009). A similar analysis of deposition effects on forest C storage, using, North American data showed comparable results (Thomas *et al.*, 2010). Stronger responses were seen for sites with high C:N ratios (Solberg *et al.*, 2009) as also observed in the fertilizer experiments (Hyvönen *et al.*, 2008).

Forest growth has increased in Europe by more than 60% over the past 50 years (Ciais *et al.*, 2008), resulting in a strong increase in C sequestration. N deposition has been suggested as the main driving factor for this increase in forest growth (Karjalainen *et al.*, 2008). On the one hand, it has been argued that N only makes a minor contribution to this sink since most of the N is retained in soils at a C:N ratio of approx. 30 (Nadelhoffer *et al.*, 1999). Recently, Magnani *et al.* (2007) suggested that a large fraction of ecosystem C sequestration potential in temperate and boreal forests could be attributed to the effects of atmospheric N deposition. This study has fuelled a new debate on the effect of elevated N deposition on C sequestration (de Vries *et al.*, 2008, 2009; Sutton *et al.*, 2008). Sutton *et al.* (2008) suggested that the proposed sensitivity of about 200 kg C per kg N (Magnani *et al.*, 2008) could be an artefact resulting from the parallel effects of other environmental factors and should be reduced to 50–75:1. De Vries *et al.* (2009) compiled data from a range of different approaches (observational, experimental and modelling) to analyse the impact of N deposition on C sequestration. The results of the various studies were in close agreement with regard to the effect of N addition for aboveground C sequestration (i.e. 15–30 kg C:1 kg N), but more variable for soil values (i.e. 5–35 kg C:1 kg N). All together these data indicate a total C sequestration range of 30–70 kg C per kg N deposition.

The discrepancy in estimated C–N sensitivity of forest ecosystems between different studies could stem from several sources of error. On the one hand, studies based on regional N deposition datasets (Magnani *et al.*, 2007) ignore the fact that higher dry N deposition rates are generally observed over forest canopies, as a result of their greater roughness. By underestimating N deposition, they would overestimate the response to a unit dose of added N. On the other hand, ecosystem manipulation studies could also be affected by artefacts, as they neglect the potentially important role of canopy N uptake and often apply

doses (up to 100 kg N ha^{-1} yr^{-1}) well in excess of natural N deposition over most forest ecosystems (Dise *et al.*, 2009).

Whatever the sensitivity, the question is whether this apparent C sequestration from N deposition will be sustained in the long-term if forest ecosystems become N-saturated. At true N saturation (i.e. no or very low N accumulation) the ecosystem may also be C-saturated (no C sequestration) at least in the soil compartment (trees in managed systems may still grow and sequester N, but with no further response to N). The long-term fertilizer experiments in Sweden (Hyvönen *et al.*, 2008) show that C sequestration per unit of added N decreases with dose (i.e. the response levels of above *c.* 30 kg N ha^{-1} yr^{-1}). It remains to be seen if the same is true for increasing N deposition or for cumulative loads. An important question could be if there is an optimum level of N deposition for C sequestration or if a critical threshold exists for the effect of N on C sequestration.

Effect on soil C processes and C sequestration

Although there is observational as well as experimental evidence for increased wood production, there is no evidence for an increase in leaf litter production due to elevated N deposition. Despite the relatively unsophisticated sampling of this ecosystem flux, it has not been part of the European forest monitoring programme and no data compilation seems to be available which could allow an analysis of the effect of N on leaf litter fluxes. N addition experiments show no response in leaf litter mass but instant increases in N concentrations of litter and thereby in the aboveground litterfall N flux (Gundersen *et al.*, 1998b; Pregitzer *et al.*, 2008). As the litterfall N flux increases, N mineralization also increases (Gundersen *et al.*, 1998b; Nave *et al.*, 2009). The N content in all compartments increases and major internal N fluxes increase. The responses of soil C processes are more complex and less well understood.

Early stages of litter decomposition may also respond positively to elevated N deposition, since microorganisms on high C:N ratio litter material need to immobilize N for the decomposition (Berg, 2000). At later stages, on the other hand, when the easily decomposable organic matter has been processed, decomposition may actually decrease with N availability (Berg and Matzner, 1997). Thus with a constant leaf litter C input, SOM is expected to accumulate due to reduced decomposition at elevated N deposition. However, the mechanism whereby N addition is accompanied by a decrease in decomposition and increase in soil C stocks is still unclear (Janssens *et al.*, 2010). A prevailing hypothesis is the lower production of lignolytic enzymes and phenol oxidases (see review in Janssens *et al.*, 2010). There is growing evidence of reduced soil respiration from chronic N addition experiments (Burton *et al.*, 2004; Hagedorn *et al.*, 2003; Janssens *et al.*, 2010) in parallel with a decline in soil microbial biomass (Treseder, 2008). Since soil respiration, however, includes not only a component from decomposition but also respiration components from roots and mycorrhizae, we cannot conclude that reduced soil respiration indicates increased SOM-C accumulation (C sequestration), as it could also indicate reduced root respiration.

A critical gap in our knowledge is the contribution of belowground litter input (roots, mycorrhyzae and exudates) to SOM formation versus that from aboveground plant litter (leaves, etc.) (Rasse *et al.*, 2005). Likewise the responses of these belowground components to elevated N deposition are not well known, but total belowground C allocation is known to decline with increasing productivity and N availability (Palmroth *et al.*, 2006). Root biomass (Boxman *et al.*,1998b; Nadelhoffer, 2000) and turnover (Majdi, 2004) may decrease with N availability. Likewise ectomycorrhyzal mycelial growth was also negatively affected by N additions after N fertilization (Nilsson and Wallander, 2003; Parrent and Vilgalys, 2007), as an effect of N deposition (Nilsson *et al.*, 2000) and with increasing N availability in natural nutrient fertility gradients (Nilsson *et al.*, 2005). Alternatively, accumulation of SOM by reduced decomposition could be outweighed by reduced contributions from other sources of SOM-C (roots, mycorrhyzae and exudates).

The integrated response of these processes to elevated N in the form of increased SOM accumulation may be difficult to measure due to the size and the variability in this pool (Yanai *et al.*, 2003). However, long-term forest N fertilization experiments from Sweden and Finland did reveal an increase in SOM-C from N addition (Hyvönen *et al.*, 2008) and for the first time a significant response of SOM-C was shown for a chronic N addition experiment at four forest sites in Michigan, USA (Pregitzer *et al.*, 2008). A long-term N addition experiment on heathland also revealed increased SOM-C accumulation (Evans *et al.*, 2006). Further measurements of increased organic layer thickness over 40 years in an intensive network of sites across Sweden (Berg *et al.*, 2009) indicate that C sequestration in organic layers could be a widespread phenomenon. A metadata analysis of studies on the response of CO_2 flux from N additions in multiple terrestrial and wetland ecosystem types by Liu and Graever (2009) showed a large variation of net ecosystem CO_2 exchange (NEE) for non-forest ecosystems (grassland, wetland and tundras), thus leading to a statistically insignificant effect.

6.6.4 N leaching associated effects

Nitrate leaching is an acidifying process in soils and an important process in acidification of lower soil horizons (Velthof *et al.* 2011, Section 21.4 this volume). The input may be as nitric acid or as ammonia/ammonium that can be nitrified in the soil and release protons. Soils have an ability to neutralize acids through the supply of base cations from weathering and cation exchange reactions. Increased acidification of forest soils has been observed during recent decades (Falkengren-Grerup *et al.*, 1987; Wesselink *et al.*, 1995) with pH declining by up to 1 unit, which in part may be caused by air pollution including deposition of N compounds. Both proton (H^+)-producing and proton-consuming processes including N species occur in soils, but a net acidification only occurs when nitrate is leached from the system (Gundersen and Rasmussen, 1990). Each 14 kg N ha^{-1} yr^{-1} of nitrate leached is equivalent to the production of 1 kmol H^+ ha^{-1} yr^{-1}. Depending on the acid status of the soil, base cations and/or Al will be leached

with the nitrate. An increasing fraction of the acidity in acid-sensitive surface waters is related to nitrate (Stoddard *et al.*, 1999; Durand *et al.*, 2011, Chapter 7, this volume). In Europe, approximately 30% of monitored forest sites leach between 7 and 50 kg N ha^{-1} yr^{-1} (De Vries *et al.*, 2007; MacDonald *et al.*, 2002), equivalent to an acid production of 0.5–3.5 kmol H$^+$ ha^{-1} yr^{-1}. At the majority of these sites this was buffered by Al release (Dise *et al.*, 2001; De Vries *et al.*, 2007) and nitrate and aluminium are usually positively correlated in acid soil and surface waters. In the long-term this may lead to significant nutrient loss, impairment of base cation uptake by Al toxicity and potentially reduced forest production (Velthof *et al.*, 2011, Chapter 21, this volume) as well as Al toxicity in surface water (Havas and Rossland, 1995).

6.6.5 N-driven vulnerability to disturbances

Nitrogen deposition may play a significant role in increasing forest susceptibility to wildfires, as documented in mixed coniferous forests of southern California (USA). High levels of ozone and nitrogenous compounds derived from regional urbanization and industrialization cause specific changes in forest tree C, N, and water balances that enhance individual tree susceptibility to drought, bark beetle attack, and disease, and when combined contribute to the whole ecosystem susceptibility to wildfire (Grulke *et al.*, 2009). Similar findings have been documented for deserts and coastal sage scrub formations in southern California where N deposition and frequent fire promotes increased grass biomass from invasive species and increases the risk of fire further as it provides the fuel for subsequent fires (Brooks *et al.*, 2004; Rao *et al.*, 2009).

6.7 Summary

This section summarizes the main conclusions on the terrestrial N cycling processes and their importance. Major uncertainties and gaps in knowledge are also highlighted.

Nitrogen pools and N availability

(1) On the ecosystem scale, soils are the main reservoir for N. This is more pronounced for agricultural systems, with more than 90%–95% of N$_r$ being stored in the soil as compared to forest systems, where N storage in soil is 50–70%.

(2) N availability varies with temperature and humidity gradients in Europe.

(3) The contemporary global biogeochemical cycle of N in terrestrial ecosystems is dominated by microbial processes in soils.

Nitrogen inputs in non-agricultural systems

(4) An understanding of biological N$_2$ fixation in a few legume crop plants is relatively advanced, but much less is known about biological N$_2$ fixation in non-agricultural legumes or in other N$_2$-fixing organisms. The difficulties of measuring rates of biological N$_2$ fixation accurately at the ecosystem scale have so far hampered a better understanding of the importance of biological N$_2$ fixation for most terrestrial ecosystems.

(5) The estimation of N deposition inputs at the site scale is affected by neglecting the input of dissolved organic nitrogen (DON) and by the uncertainty in atmosphere canopy interaction of N species.

Nitrogen cycling

(6) The understanding of N cycling in terrestrial ecosystems has undergone a paradigm shift since 1990. Until then, the perception was that (i) N mineralization is the limiting step in N cycling, (ii) plants take up inorganic N, and (iii) plants poorly compete for N against microbes and use only the N which is 'left over' by microbes. Consequently, net N mineralization has been assessed to measure plant-available N. Since then studies have shown that plants effectively compete for N with microorganisms and take up organic N, in a broad range of ecosystems.

(7) Nitrogen mineralization/ammonification is the dominant control of gross nitrification.

(8) Microbial nitrate immobilization is a significant process of N$_r$-retention in a wide range of terrestrial ecosystems that depend largely on gross nitrification.

(9) The importance of other recently recognized processes (DNRA, anammox, nitrifier denitrification) for N$_r$-cycling in ecosystems is not well developed.

(10) Denitrification rates in soils are highly uncertain, despite more than eight decades of research, partly because of our lack of understanding and partly due to the large spatial and temporal variability.

(11) Factors controlling N$_r$ fluxes include moisture content (water-filled pore space) and soil temperature, soil properties, such as clay content, carbon content, C:N ratio and pH and vegetation factors, which are all affected by land use change and climatic change.

Nitrogen outputs

(12) Burning is a major pathway of N$_r$ loss for ecosystems exposed to high fire frequencies. The influence of N$_r$ deposition on fuel N build-up should be considered in order to estimate wildfire NO$_x$ emissions. This effect is neglected at present.

(13) Major outputs in ecosystems not exposed to fires are nitrate leaching and gaseous losses (N$_2$O, NO, N$_2$ and in agricultural systems also NH$_3$) to the environment. N$_2$ emissions are most uncertain due to the difficulties to quantify N$_2$ emissions and to constrain what is driving denitrification on site and regional scales.

(14) The C:N ratio of the forest floor or the top mineral soil is a good indicator of N status related to NO$_3^-$ leaching. At C:N above 25, mineral N is usually retained, whereas below 25, NO$_3^-$ leaching often occurs and increases with increasing N deposition.

Nitrogen effects

(15) Atmospheric N$_r$ input has caused N saturation in terms of a decline in the soil C:N ratio in the forest floor, associated

with an increase in (i) N_r (nitrate) leaching and in gaseous N losses (N_2O, NO, N_2), (ii) N availability and related biodiversity change, and (iii) the occurrence of pests and diseases.

(16) Atmospheric N_r input has led to an increase in aboveground C sequestration but the impact on soil C sequestration is less clear. A critical gap in our knowledge is the impact of N_r deposition on belowground litter input (roots, mycorrhyzae and exudates) and through that on soil C sequestration.

Future studies on terrestrial N cycling

(17) Interdisciplinary and multi-scale studies should focus on simultaneous and comprehensive measurements of all major N_r fluxes at site and landscape scale, including plant uptake/release of organic and inorganic N compounds as well as microbial N_r conversion.

(18) Linking plant physiological and soil microbial N_r cycling, as well as soil hydrological N_r transport, to more reliable estimates of ecosystem N fluxes will be a major research challenge for coming years. In particular, this will include further development of methodological approaches and experimental assays that allow direct assessment of N turnover processes in the larger context of intact plant–soil-systems, where competitive mechanisms between microorganisms and plants persist. This will require an intensified interdisciplinary cooperation between plant scientists and soil ecologists/soil microbiologists.

Acknowledgements

Supplementary materials

Supplementary materials (as referenced in the chapter) are available online through both Cambridge University Press: www.cambridge.org/ena and the Nitrogen in Europe website: www.nine-esf.org/ena.

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