Chapter 7

Nitrogen processes in aquatic ecosystems

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Executive summary

Nature of the problem

- Freshwater ecosystems play a key role in the European nitrogen (N) cycle, both as a reactive agent that transfers, stores and processes N loadings from the atmosphere and terrestrial ecosystems, and as a natural environment severely impacted by the increase of these loadings.

Approaches

- This chapter is a review of major processes and factors controlling N transport and transformations for running waters, standing waters, groundwaters and riparian wetlands.

Key findings/state of knowledge

- The major factor controlling N processes in freshwater ecosystems is the residence time of water, which varies widely both in space and in time, and which is sensitive to changes in climate, land use and management.
- The effects of increased N loadings to European freshwaters include acidification in semi-natural environments, and eutrophication in more disturbed ecosystems, with associated loss of biodiversity in both cases.
- An important part of the nitrogen transferred by surface waters is in the form of organic N, as dissolved organic N (DON) and particulate organic N (PON). This part is dominant in semi-natural catchments throughout Europe and remains a significant component of the total N load even in nitrate enriched rivers.
- In eutrophicated standing freshwaters N can be a factor limiting or co-limiting biological production, and control of both N and phosphorus (P) loading is often needed in impacted areas, if ecological quality is to be restored.

Major uncertainties/challenges

- The importance of storage and denitrification in aquifers is a major uncertainty in the global N cycle, and controls in part the response of catchments to land use or management changes. In some aquifers, the increase of N concentrations will continue for decades even if efficient mitigation measures are implemented now.
- Nitrate retention by riparian wetlands has often been highlighted. However, their use for mitigation must be treated with caution, since their effectiveness is difficult to predict, and side effects include increased DON emissions to adjacent open waters, N_2O emissions to the atmosphere, and loss of biodiversity.
- In fact, the character and specific spatial origins of DON are not fully understood, and similarly the quantitative importance of indirect N_2O emissions from freshwater ecosystems as a result of N leaching losses from agricultural soils is still poorly known at the regional scale.
- These major uncertainties remain due to the lack of adequate monitoring (all forms of N at a relevant frequency), especially – but not only – in the southern and eastern EU countries.

Recommendations

- The great variability of transfer pathways, buffering capacity and sensitivity of the catchments and of the freshwater ecosystems calls for site specific mitigation measures rather than standard ones applied at regional to national scale.
- The spatial and temporal variations of the N forms, the processes controlling the transport and transformation of N within freshwaters, require further investigation if the role of N in influencing freshwater ecosystem health is to be better understood, underpinning the implementation of the EU Water Framework Directive for European freshwaters.

7.1 Introduction

The scope of this chapter is to document the extent of knowledge regarding the fate of nitrogen (N) in European freshwater systems (wetlands, standing and running waters, the hyporheic zone and groundwaters). Another aim is to highlight those areas where knowledge is currently sparse and future research is required to underpin the development of sound evidence-based environmental policy for the wide range of nutrient enriched waters across Europe. The key processes and controls of nitrogen turnover in freshwaters are discussed to understand the observed trends and the impacts of these processes on the ecological status and societal value of European freshwaters.

The definition of groundwater used in this chapter includes the vadose zone beyond the reach of the root system of terrestrial vegetation, running waters are considered down to the limit of their tidal influence, wetlands are restricted to riparian areas receiving surface and groundwater but including the hyporheic zone, and standing waters include all freshwater lakes, ponds, pools and reservoirs.

This chapter first describes the main factors controlling nitrogen cycling in freshwater systems, the distribution of N forms in waters and their origin, and the role of N in the ecology of those systems. The specific characteristics of N cycling in different types of freshwater systems are then highlighted.

## 7.2 Factors controlling N cycling in freshwaters

7.2.1 The water cycle

Fresh water is defined as water with less than 0.5 g/l of dissolved salts. Some 3% of the water on Earth is freshwater. About two thirds of it is frozen in polar caps and glaciers, most of the remainder is present as groundwater, and only 0.3% is surface water. Freshwater ecosystems occupy over 3% of the Earth's surface. In Europe, freshwaters cover 1% of the surface and wetlands 0.8% (European Environment Agency, 2005).

The classical figure of the water cycle (Figure 7.1) illustrates that one main feature of aquatic ecosystems is their interconnectivity. Water infiltrating from terrestrial ecosystems recharges groundwater. In flat, low lying areas the groundwater table reaches the surface, determining the extent of wetlands. These wetlands are often located close to the streams or lakes (riparian wetlands). Streams and lakes are fed by groundwater discharge, but also by surface overland flow and subsurface interflow in variable proportions. The direction of fluxes between groundwater, wetlands, streams and lakes varies according to hydrological conditions.

Owing to natural or artificial obstacles to flow, surface waters create standing water bodies that are generally connected to the hydrological network. They can be located at the source of the streams or along the main course, and vary widely in extension and depth. Standing waters also occur in topographic depressions in the landscape including, for example, kettle holes in some post-glacial landscapes which are often less well connected to the running water network. The main hydrological driver is the excess rainfall, defined here as the amount of water available for groundwater recharge or runoff after interception and evapotranspiration. In Europe, this excess rainfall varies from a few mm/yr in the driest Mediterranean zones to more than 1000 mm in North-Western coasts. In most of Europe, it is between 150 mm and 500 mm (Figure 7.2).

The seasonality of lotic (running water) ecosystems is largely determined by the hydrological regime. In Europe, three major hydrological regimes exists (Figure 7.3): (1) the oceanic temperate regime, with moderate variations in mean rainfall distribution over the year, and increased evapotranspiration during summer, leading to low summer discharge and winter flooding; (2) the Mediterranean regime with low rainfall and high evapotranspiration in summer leading to extremely low summer discharge and flooding in spring and autumn; and (3) the snowmelt controlled regime (mountainous and Nordic regions) with high discharge during spring or early summer. The first two regimes are characterized by low discharge during the periods with the highest temperature and light intensity, while in the snowmelt regime, the most productive period occurs before the summer flood, under sub-optimal light and temperature conditions.
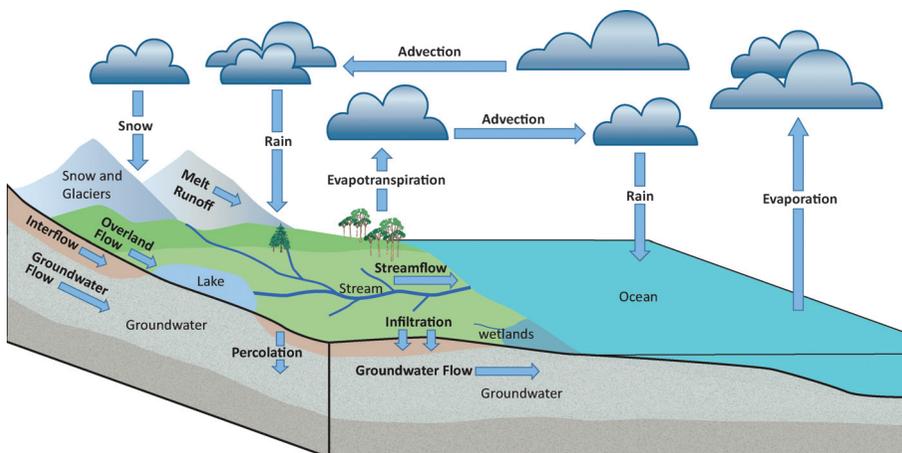
Figure 7.1 The global water cycle (courtesy of Sandra Süß).
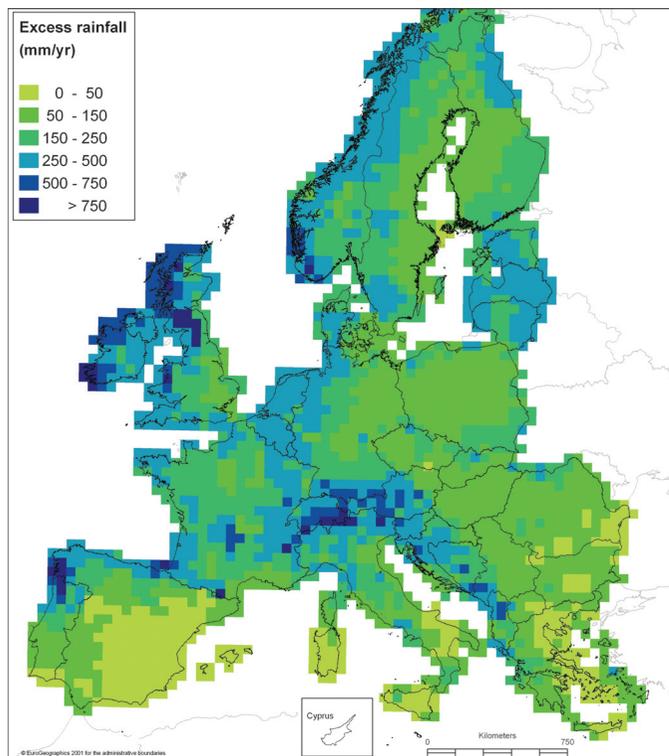
Figure 7.2 Map of excess rainfall in Europe, based on long term rainfall data and computed actual evapotranspiration (Mulligan *et al.*, 2006) (© 2006, JRC, European Commission).

7.2.2 Stream order

The fate of nitrogen in freshwater depends strongly on the stream network geometry, which can be described in a synthetic and meaningful way by the stream order concept.

The Strahler stream order system (Strahler, 1952) was proposed to define stream sizes based on a hierarchy of tributaries. Headwaters are first-order streams. When two first-order streams connect they form a second-order stream and so forth. The ecological functioning of water bodies, and therefore the N cycling, varies according to Strahler's order as follows.

Streams of Strahler's order 1 to 3 are characterized by shallow depth and narrow width, steep slope and a relatively high contribution of lateral inputs of water with respect to the volume of the reach. Most inputs of energy are in the form of coarse organic material from riparian vegetation. Shading by riparian trees is common in these reaches, limiting light availability for stream flora. The overall metabolism of the system is typically heterotrophic, dominated by fungi and shredder invertebrates.

Mid-reach river systems are wider, deeper and less strongly influenced by dilution. They receive more light so that autochthonous primary production can occur either through macrophyte (typically stream order 4–5) or planktonic (typically stream order 5–7) development. The overall metabolism of the system becomes autotrophic. Organic matter, either of autochthonous origin or transferred from upstream systems, is dominated by fine particulate organic material and supports a community of collector or grazer invertebrates.

Lower reach systems (stream order >7) are still deeper and wider. They are no longer influenced by substantial lateral hydraulic dilution and show a decline of autochthonous primary production because of increased depth and turbidity which limits primary production capacity. They are again often heterotrophic, with stream metabolism strongly dependent on organic material brought in from upstream reaches. Sedimentation of fine material is possible and sediments are often rich in organic material.

7.2.3 Residence times

In all aquatic ecosystems, N cycling is controlled by the energy sources (light, organic matter and reduced inorganic compounds such as sulphur (S) or ferrous minerals), redox conditions (oxygen availability) and the nutrient loads. The main factor differentiating N turnover rates in the different types of freshwater systems, however, is the residence time of the water. The mean residence time of a well mixed reservoir is defined as the ratio between the volume of water (V) and the flux that goes through it (Q). Depending on the lines of flux, the actual residence time may vary considerably within a given water body: for example, in groundwater and lakes the residence time increases with depth, and in streams it is higher near the banks and the bed than in the middle of the stream. For running waters residence times are often defined for reaches (the portion of the stream between two confluences). For lakes and wetlands, residence time may be very short along the primary flow channels, but very long in areas less well connected to the primary flow channels. This is particularly evident in lakes formed over flooded river valleys, and in wetlands with a clear point of inflow and outflow.

Typical residence times increase from order 1 streams (minutes to hours) to larger streams and wetlands (weeks), standing waters (weeks to decades) and groundwaters (decades to centuries). The highest variability in residence time is found in standing waters (from days to decades) and for groundwater (from months to thousands of years). Residence time also varies over time within a system. Bearing in mind the general definition of residence time as V/Q, since the flux is far more variable than the volume, the variability of residence time is controlled by the variations in discharge from the catchment. Therefore, rivers with low flows in summer have residence times similar to riverine lakes, and numerous lakes and wetlands are subjected to flushing episodes during high flow events.

7.2.4 Nitrogen delivery to freshwaters

Nitrogen can reach freshwaters through a number of pathways: by atmospheric deposition on the catchment or directly on the water body; by leaching from diffuse sources within the catchment, such as those resulting from fertilizer and manure application; by sediment erosion of N rich soils and surface applications of manure in catchments; and by direct input from point sources such as sewage treatment works. A further source of reactive N (N_r) is nitrogen fixation. Some prokaryotes, such as some cyanobacteria, also possess the nitrogenase enzyme that allows atmospheric nitrogen to be converted into ammonia
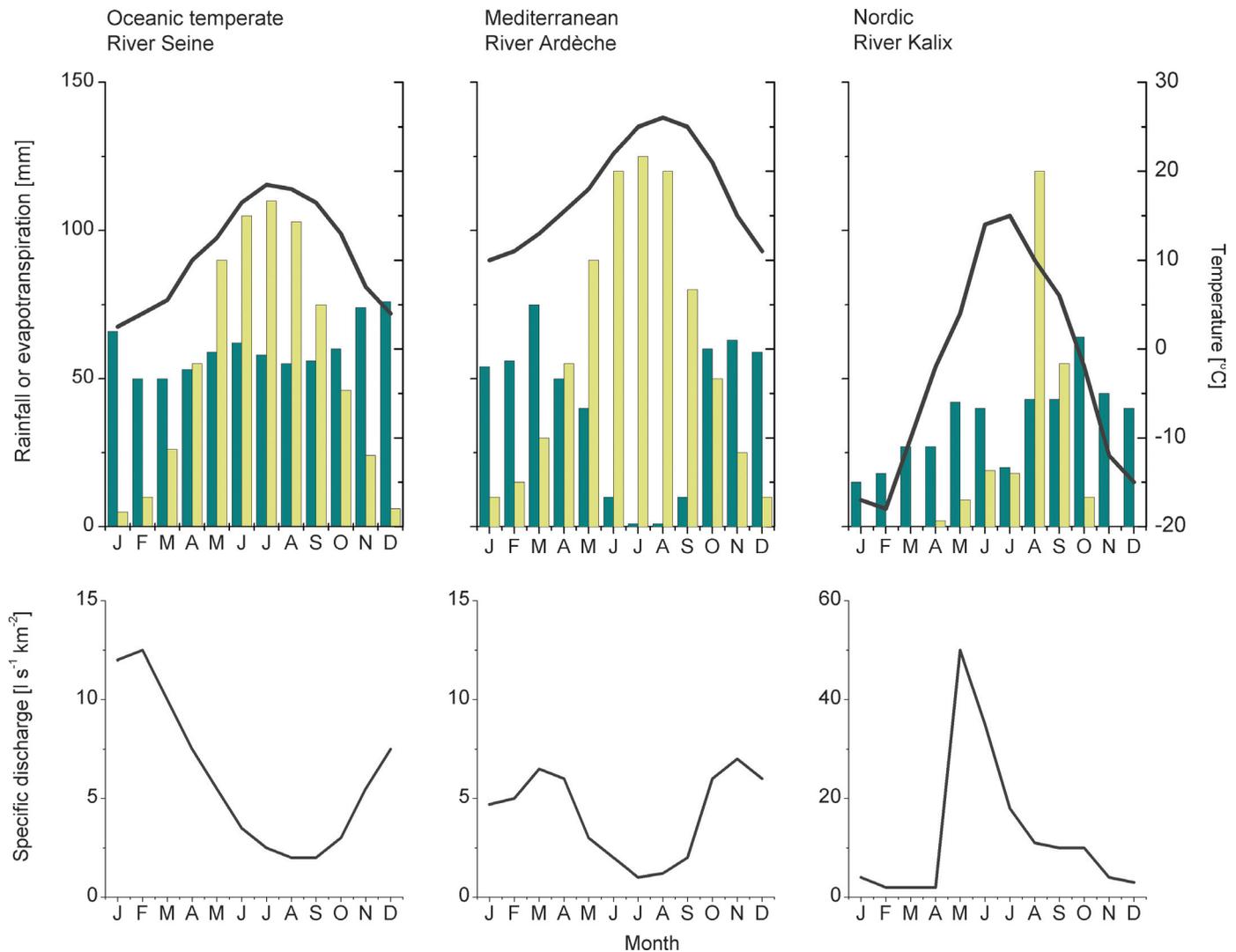
Figure 7.3 Examples of the three major hydrological regimes of European rivers. Blue bars: rainfall; yellow bars: evapotranspiration; brown lines: temperature; dark lines: specific discharge.

and thus can exploit dissolved nitrogen gas in freshwaters. A few freshwater diatoms such as *Epithemia* and *Rhapalodia* also possess this ability via endosymbiotic inclusions believed to be derived from a cyanobacterium related to *Cyanothece* (Prechtl *et al.*, 2004), although this mechanism is not widely found in European freshwaters.

The nutrient load delivered to aquatic ecosystems from diffuse catchment and atmospheric sources depends strongly on the hydrological processes, particularly the relative importance of different water pathways in the transfer of the various N forms from terrestrial to aquatic systems (Figure 7.4).

Overland flow is responsible for the transport of particulate forms of N and, to a lesser extent, of dissolved organic N (DON) and ammonium. In agricultural areas, dissolved inorganic N (DIN) concentrations in overland flow are usually low compared to those of subsurface water, causing dilution of DIN in streamwater during flood events (Durand and Juan Torres, 1996; Durand *et al.*, 1999; Kemp and Dodds, 2001). This is not always true in situations where subsurface waters are

low in DIN and where surface accumulation of N occurs, for example: during snowmelt events when atmospheric N deposition has accumulated in the snowpack; shortly after fertilizer applications; in intensively farmed outdoor stock enterprises; in Mediterranean forested zones during floods (Bernal *et al.*, 2006; Johnes, 2007a).

Most N leaving the soil in the form of nitrate will be transported to adjacent water bodies by water that has infiltrated in the soil, but the conditions controlling this transfer vary depending on the relative importance of shallow pathways (interflow, return flow, shallow groundwater seepage) and of deep infiltration (Creed *et al.*, 1996; Molenat *et al.*, 2002). DON is mostly delivered during storm events, with N-rich soil porewater flushed to the hyporheic zone and adjacent surface waters. Less DON is transported to groundwater stores in permeable areas with deep aquifers, where the pathway is dominated by nitrate flux. In these areas unsaturated vertical flow can be very long and lead to significant accumulation of nitrate in the vadose zone. The temporal variation of nitrate fluxes
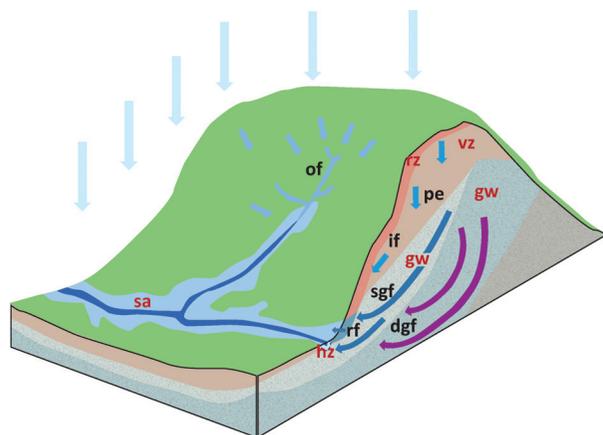
legend:

rz: root zone
vz: vadose zone
hz: hyporheic zone
gw: groundwater
sa: saturated area

of: overland flow
pe: percolation
if: interflow
rf: return flow (exfiltration)
sgf: shallow groundwater flow
dgf: deep groundwater flow

Figure 7.4 Schematic of the different flows in a headwater catchment. The colours of the arrows symbolize the age of water from the most recent (light blue) to the oldest (purple) (courtesy of Sandra Süß).

from groundwater to rivers is damped, and the mean residence time of water and solutes in these areas can be in the order of several decades (Wade *et al.*, 2006).

7.3 Nitrogen forms and sources in freshwater

7.3.1 Particulate and dissolved N components

Nitrogen cycling in aquatic ecosystems is complex and involves a variety of N forms and associated oxidation states. Both the oxidized and reduced inorganic N species (NO_2^-, NO_3^-, NH_4^+, NH_3) and organic N fractions (DON, PON) are commonly found in all freshwater, estuarine and coastal waters across Europe. Nitrate, nitrite, ammonium and DON are directly available for plant uptake, supporting production in both the algal and higher plant communities. In addition the gaseous forms (N_2, N_2O, NO) are exchanged with the atmosphere.

N speciation in streams varies along a gradient of N enrichment. Nitrate is the dominant form in highly enriched rivers, whereas DON is the dominant form in less enriched rivers. DON can also be an important secondary constituent of the TN load even in the most enriched rivers.

Figure 7.5 presents nitrogen species concentrations in different European streams ranging from oligotrophic to hypertrophic. Data were collated for the present work from all available national databases and both published and unpublished research on the relative proportion of total N present in the various N species forms in European rivers. Data were only included where at least the inorganic N species and total N had been determined at high sampling frequency (typically weekly to daily sampling frequency). For some sites only total organic N (TON) concentrations were reported (shown yellow on Figure 7.5) alongside inorganic N species concentrations. For the majority of sites, however, the full species range had been determined, including both DON and PON fractions. A total of 84 separate annual records for 57 streams were found which fulfilled these criteria. Data have been plotted along a gradient of increasing total N concentrations, from the lowest concentrations typically found in boreal headwater streams to the highest concentrations found in rivers draining the intensively farmed lowland regions of Europe (Figure 7.5).
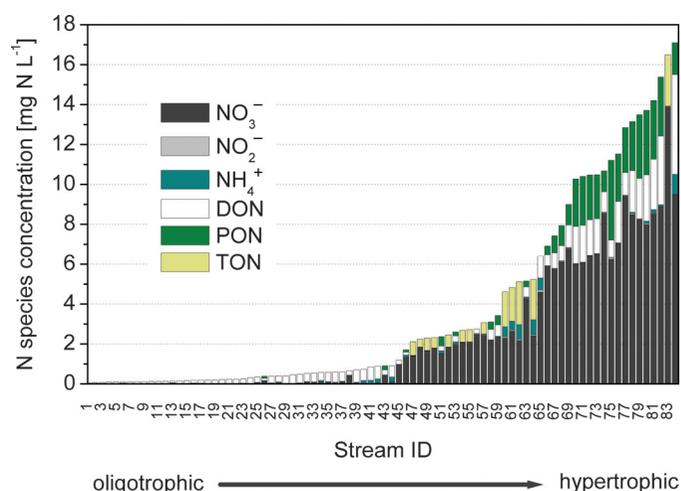
Figure 7.5 Concentrations of N species in 57 European streams and rivers (an explanation of data sources is given in the text. Stream ID, sampling locations and observation periods are provided in the Supplementary Material of Chapter 7).

Two clear patterns emerge from Figure 7.5: nitrate concentrations increase in absolute terms and also as a proportion of total N along a gradient from ultra-oligotrophic to hyper-trophic waters, and mean annual DON concentrations also increase along this gradient but as a decreasing proportion of total N. Concentrations of nitrate range from <0.002 mg NO_3^--N/l in the least disturbed catchments to over 14 mg NO_3^--N/l in the most intensively farmed catchments. DON concentrations are initially substantially higher than nitrate concentrations in undisturbed sites, with mean annual DON concentrations ranging from <0.15 mg N/l in low nutrient status upland waters (Willetts *et al.*, 2004; Kortelainen *et al.*, 2006; Skoulikidis and Amaxidis, 2009) to over 3 mg N/l in the highly enriched rivers of lowland Britain and the Netherlands, and a maximum of 5 mg N/l in the River Ter in Spain, with maximum daily concentrations in excess of 9 mg N/l (Johnes and Burt, 1991). This pattern, with nitrate concentrations increasing at a proportionally higher rate than DON concentrations as nutrient enrichment occurs is highlighted in Figure 7.6.

Ultra-oligotrophic systems, where total N concentrations are less than 1 mg N/l, are highly N limited, and inorganic N is taken up rapidly by the biota to sustain production. DON,

(a)

$$y = 14.921 * \ln(x) + 36.731$$
$$R^2 = 0.655$$

(b)

$$y = 39.287 * x^{-0.453}$$
$$R^2 = 0.735$$
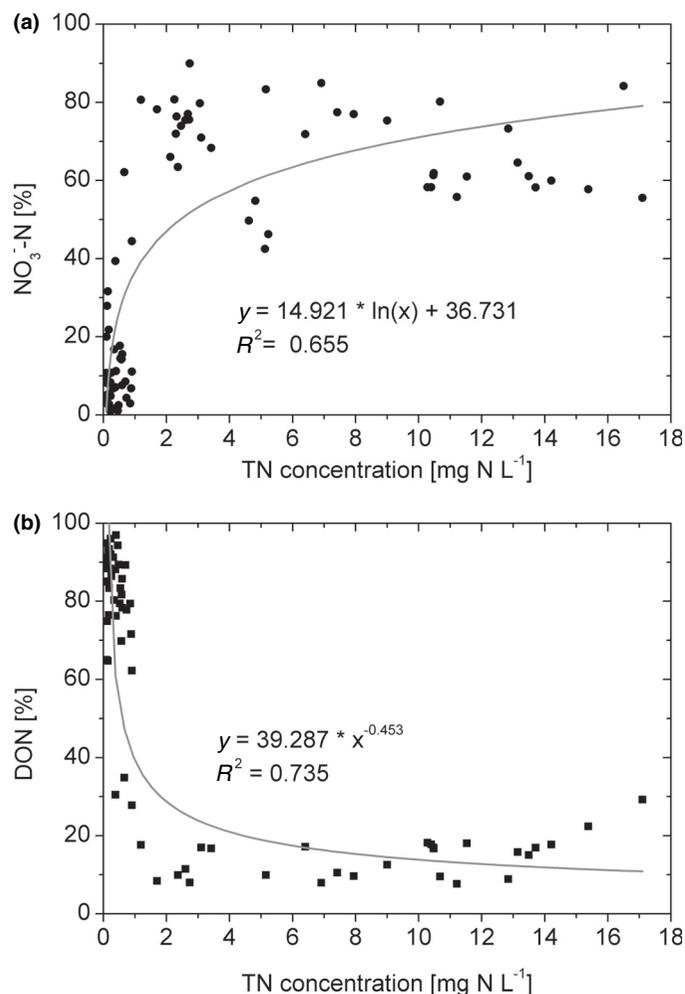
Figure 7.6 Proportion of total N present in the form of (a) nitrate-N and (b) DON in European rivers along a gradient of N enrichment (an explanation of data sources is given in the text).

as a by-product of microbial breakdown of organic matter, is released to adjacent waters through flushing of soil porewaters during and after rainfall, providing a substrate for microbial metabolism in situ and a mechanism for N transport downstream. In these systems, DON typically constitutes over 60% of total N, and nitrate concentrations are typically less than 40% of total N. Above 1 mg N/l there is surplus inorganic N being introduced to the system as a result of increasing anthropogenic disturbance of the catchment and/or increasing atmospheric N deposition rates. These increasingly productive systems often generate more DON which, together with background DON, is flushed downstream together with surplus inorganic N leached from N enriched agricultural and forest soils, or delivered from point source sewage discharges and intensive livestock enterprises. This leads to a rising trend in DON concentrations, but a decrease in the proportion of total N in the form of DON; and a rising trend in nitrate concentrations with an associated increase in the proportion of total N in the form of nitrate. Thus, as catchments become enriched through anthropogenic enhancement of N inputs, the system becomes less efficient at processing these inputs, and as system inefficiency rises, inorganic N concentrations rise disproportionately.

In lowland, intensively farmed agricultural catchments DON contributes up to 30% of the total N. In upland waters, with lower TN loading rates, and a higher proportion of histosol soils in their catchments, the DON fraction can constitute up to 90% of the TN load (Kortelainen *et al.*, 1997; Willetts *et al.*, 2004). Overall in European streams, between 10% and 80% of the total N load, and 11% to 100% of the total dissolved N load may be in the form of DON (Mattsson *et al.*, 2009), for which no specific European legislation exists. DON is also not determined in most European routine water quality monitoring programmes. Evidence from a range of sources indicating a rising trend in dissolved organic carbon (DOC) concentrations in many upland waters (Evans *et al.*, 2005), possibly due to climate change, raises additional concerns since a rise in DON concentrations could also be expected.

There is evidence from experimental and field monitoring studies that low molecular weight DON (amino acids, polyamines) is directly available for plant and algal uptake, and is a key resource in N limited oligo- to mesotrophic estuaries and freshwaters (Maberly *et al.*, 2002; Fong *et al.*, 2004). Further experimental data show that both PON and high molecular weight DON are available for microbial assimilation in both terrestrial and aquatic environments (Antia *et al.*, 1991; Chapin *et al.*, 1993; Seitzinger and Sanders, 1997; Lipson and Näsholm, 2001; Jones *et al.*, 2005). Further, PON can act as a food resource for aquatic filter-feeding organisms, and as an important component of C metabolism in aquatic ecosystems. Both DON and PON provide a key pathway for nitrogen transfer downstream, playing an important role in sustaining the nutrient spiral within rivers, lakes, estuaries and wetlands.

Point sources of nitrogen, including discharges from industrial sources, sewage treatment works and overflow of septic tank systems can be locally significant as sources of N loading to adjacent waters, but are rarely significant contributors to the total N loading delivered to waters from their catchments where atmospheric deposition and agriculture are fully taken into account. These are the primary sources of N enrichment of European waters (Moss *et al.*, 1996; Johnes and Butterfield, 2002). For EU 27, total N excretion by livestock in 2000 was about the same as total fertilizer use (Oenema *et al.*, 2007). Particular problems exist with intensive agricultural regions where heavily fertilized grassland and high stocking densities have led to excessive N loading to these components of the terrestrial ecosystem. It is also a problem in many upland areas of Europe where stocking densities have risen on marginal land with steep slopes, thin soils and high rates of rainfall and snowmelt where the intrinsic nutrient export potential is high. The apportionment of N sources of nitrogen in the major European catchments is described in more detail in Billen *et al.*, 2011 (Chapter 13 this volume).

7.3.2 Gaseous N emissions from freshwater ecosystems

Through the processes of nitrification and denitrification, N_r input may enhance the biogenic production of the greenhouse

gas nitrous oxide (N_2O) in standing waters like lakes and ponds (Mengis *et al.*, 1997), in wetlands (Johansson *et al.*, 2003) and in groundwaters, streams, and rivers (Seitzinger and Kroeze, 1998; Hasegawa *et al.*, 2000). N_2O can also be leached directly into these systems, particularly from agricultural soils, producing super-saturation of N_2O in the soil leachate (Dowdell *et al.*, 1979). Natural aquatic systems are generally considered relatively minor sources of N_2O, e.g. 3%–6% of the direct agricultural emissions in the Seine basin (Garnier *et al.*, 2009). In fact, aquatic systems with low N availability may act as a sink for N_2O (Mengis *et al.*, 1997; Blicher-Mathiesen and Hoffmann, 1999; Dhondt *et al.*, 2004). Whether a system acts as a source or a sink depends on local circumstances including the availability of N_r, temperature and pH.

Natural wetlands are likely to be relatively minor sources of N_2O, due to the low availability of N_r common to these systems (Regina *et al.*, 1996). Relatively high nitrous oxide fluxes have been quantified for constructed and/or riparian wetlands receiving high N_r inputs. However, the evidence base is incomplete and other authors claim that N_2O emissions from constructed wetlands are relatively insignificant at the landscape scale (Søvik *et al.*, 2006). There is clearly a need for further research on the role of wetlands at landscape scale, whether natural or constructed, on N_2O emission rates. Based on measurement and budgeting approaches in the Seine basin, the indirect N_2O emissions (including the ones from wastewater treatment plants, water mirror, and riparian zones) have been evaluated as 13%–17% of the direct emissions (Garnier *et al.*, 2009). Lakes can be sources of sinks of N_2O, but the importance of N_2O emissions from lakes also remains poorly quantified. Many lakes have deep anoxic zones that are undersaturated with N_2O and this is generally considered to be the result of N_2O consumption by denitrifying bacteria (Mengis *et al.*, 1997). In the oxic zone of lakes, incomplete denitrification can result in super-saturations of N_2O and subsequent outgasing to the atmosphere. This source may be especially significant in the littoral zone of eutrophic and hyper-eutrophic lakes where variable redox conditions and plentiful supplies of organic carbon (C) lead to increased N_2O production from nitrification and/or incomplete denitrification (Huttunen *et al.*, 2003).

In headwater streams, emissions are predominantly a result of the physical outgasing of N_2O-supersaturated water entering them via leaching and surface run-off. Such allochthonous N_2O inputs are generally lost through outgasing within a few hundred metres of entry to open water courses (Garnier *et al.*, 2009). However, emissions of N_2O from acid upland soils and peats were found to be a negligible proportion of deposition inputs of N in an intensive study of four headwater moorland catchments in the UK (Curtis *et al.*, 2006). In lowland streams and rivers, in situ production of N_2O may become more important, with this autochthonous N_2O being produced via nitrification and denitrification in aquatic sediments and occasionally in the water column (Dong *et al.*, 2004).

Globally, most of the N_2O emitted from aquatic systems is associated with agriculture (Kroeze *et al.*, 1999; Bouwman *et al.*, 2005). Numerous studies have now reported super-saturation of dissolved N_2O in agricultural drains, aquifers, streams and rivers (MacMahon and Dennehy, 1999; Hasegawa *et al.*, 2000; Dong *et al.*, 2004; Clough *et al.*, 2006; Beaulieu *et al.*, 2008), but the true importance of N_2O losses from this indirect pathway compared to direct emissions from fertilized fields remains an area of considerable uncertainty (Haag and Kaupenjohann, 2001). Clearly more experimental and process-based modelling studies may be needed to better understand the processes underlying N_2O formation in fresh water systems.

7.4 The role of N in freshwater ecosystems

7.4.1 Role as a nutrient

The primary role of N in freshwater ecosystems is as one of the key nutrients, along with carbon, phosphorus (P) and silica (Si), required to support primary production by higher plants and algae. This process of photoautotrophy requires energy from sunlight and C, N, Si and P. Nitrogen also plays a role in determining the food web structure and relative productivity of any water body through microbial, algal and plant uptake of N in the form of both inorganic N species and DON. Any change in the rate of supply of nitrogen to a water body, or the relative abundance of C, N, Si and P, will lead to changes in the productivity of the water body and its microbial metabolism, with associated secondary effects in terms of microbial, plant and animal community species composition and relative abundance, and the structure and balance of the aquatic food web.

The role of N in freshwater ecosystems is clearly illustrated through examination of the changes to ecosystem structure and function in response to nutrient enrichment. This process is termed eutrophication, defined under the EU Urban Wastewaters Treatment Directive (91/271/EEC) as *the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned.* Typical responses to the eutrophication process can range from losses in biodiversity resulting from shifts in higher plant community species composition and relative abundance; increases in phytoplankton productivity leading, in extreme examples, to dominance of N fixing cyanobacteria in the primary producer community; and shifts in food web structure. See Grizzetti *et al.*, 2011 (Chapter 17, this volume) for more detail.

In the phytoplankton (algal) community, because of the relative abundance of C and N relative to P in natural surface waters, productivity is most commonly assumed to be limited by the availability of P rather than N or C (Hecky and Kilham, 1988; Huszar *et al.*, 2006). This is based on the application of Liebig's Law of the Minimum which proposes that the biological response to resource abundance is controlled by the single most limiting resource. However, this is complicated by the interacting effects of ecological factors (Talling, 1979) and because within the phytoplankton community, different species may be limited by different factors (Hecky and Kilham, 1988; Maberly *et al.*, 2002). Consequently, it is likely that more than one nutrient can control the yield or rate of growth even in the phytoplankton community.

In the higher plant community, rooted macrophytes obtain a large part of their nutrients from the sediment (Barko *et al.*, 1991), and N and P are required in a ratio of around 30:1 (Duarte, 1992; Verhoeven *et al.*, 1996). Higher plants require almost twice as much N as phytoplankton, relative to P. In the sediment and the sediment porewater the availability of P is much higher than in the surface water, particularly in nutrient enriched systems, and in contrast to N. Accordingly, rooted and benthic species experience a higher P availability than free floating plant species such as *Lemna* spp., and the free-floating phytoplankton community that derive their nutrient resources from the water column and, in the case of the cyanobacteria, through N fixation from atmospheric sources. For higher plants, therefore, N is more likely to be limiting to productivity than P, and this is confirmed by a number of research studies (Anderson and Kalff, 1986; Barko *et al.*, 1988) including recent work which has shown that high N concentrations seem to be correlated with a low species diversity in the macrophyte community (James *et al.*, 2005) and a low macrophyte abundance in lakes (Gonzalez Sagrario *et al.*, 2005), see also Chapter 17 (Grizzetti *et al.*, 2011, this volume) for more details.

Thus the role of N and P in limiting production from oligotrophic to eutrophic status is complex: different elements of the biota respond to P and N loads delivered to waters in different ways, mediated by the access of each biotic group to each nutrient store within the water body. Submerged aquatic plants, and benthic algal communities in clear water lakes and streams growing on P-enriched sediments are likely to be N-limited. Emergent plant production along lake and river margins will reflect the relative abundance of N and P in the bed and bank sediments. The free-floating phytoplankton community in lakes and slow-flowing lowland river reaches will depend on the relative availability of nutrients in the water column. In all cases, the role of nutrients in limiting biological production may be over-ridden by other factors such as reduced light penetration in turbid and highly coloured waters, or shear stress and abrasion associated with high flow environments. The impact of nitrogen enrichment in European freshwaters is likely, therefore, to lead to changes in community structure and composition, as well as an increase in gross productivity in the system.

7.4.2 Role in water acidification

In undisturbed lake and stream catchments, most N export is in the form of organic N. Spatially representative long term data bases from 21 managed and 42 unmanaged headwater boreal catchments covered by peatlands and forests across Europe, demonstrate that the long term N load is dominated by organic N in pristine and managed catchments (Kortelainen *et al.*, 1997; Mattsson *et al.*, 2003; Kortelainen *et al.*, 2006). In northern European lakes and streams, concentrations of inorganic N are generally very low compared with organic N. The presence of elevated nitrate in undisturbed lake and stream catchments can indicate anthropogenic N deposition where direct catchment sources are absent (intensive agriculture, industrial or urban areas, point sources). Nitrate may originate as NO_x or reduced N (through nitrification) but in either case, leached nitrate in these catchments indicates a net input of acidity. Data from various studies suggest that NO_3^- concentrations in acid-sensitive lakes increased from their very low levels during the 1970s and 1980s (e.g. Grennfelt and Hultberg, 1986; Brown, 1988; Henriksen and Brakke, 1988). Later studies confirmed that nitric acid is making a major contribution to acidification in many parts of Europe and North America (Murdoch and Stoddard, 1992; Allott *et al.*, 1995; Wright *et al.*, 2001).

The resulting decrease of acid neutralizing capacity and pH can significantly affect the productivity and biodiversity of aquatic ecosystems. Even where acidification is not, or no longer, chronic, episodic acidification, e.g. during snowmelt can threaten sensitive biota. Impacts have been reported on a wide range of species, including algae, macrophytes, micro- and macroinvertebrates, batrachians, salmonids and riverine birds (Ormerod and Durance, 2009). Acidification can also affect functions such as organic matter decomposition (Merrix *et al.*, 2006).

While total N deposition is now declining in many parts of Europe, nitrate leaching continues to slow the chemical recovery of freshwaters from acidification, due to N saturation of the terrestrial ecosystems (Butterbach-Bahl *et al.*, 2011, Chapter 6 this volume). Long-term trends are notoriously difficult to detect for nitrate because nitrate concentrations vary strongly over different timescales, ranging from diurnal and seasonal (Reynolds and Edwards, 1995) to climatically driven variations linked to 5–10 yearly fluctuations in the North Atlantic Oscillation index (Monteith *et al.*, 2000). Furthermore, this overall lack of general trend at the European scale was suggested to be the result of opposing factors: the increasing N saturation status of catchments in the context of declining N deposition in some parts of Europe (Wright *et al.*, 2001).

Data from 54 European lake and stream sites included in the ICP Waters Programme (UNECE International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes) show that N concentrations in runoff increase as N deposition levels rise above certain thresholds (Figure 7.7). At sites with inorganic N (TIN) concentrations in precipitation below 0.25 mg N/l, runoff TIN concentrations did not exceed 0.1 mg N/l. This was typically observed at remote sites in Scandinavia and Scotland with natural or semi-natural vegetation cover. In areas with precipitation concentrations in the range of 0.25–0.7 mg N/l, TIN in runoff reached 0.4 mg N/l. This included the more polluted locations in Scandinavia together with sites in the Czech Republic, UK, and Italy. Above this deposition level, runoff concentrations spanned a wide range from below 0.3 mg N/l to more than 4 mg N/l. The latter illustrates that catchment TIN losses are highly variable among sites, depending on landscape characteristics, site history and different types of disturbance. Sites included in this group were located in Germany, the Czech Republic and in Italy.

It is clear that reductions in total N deposition will have to be achieved to further reduce nitrate concentrations in semi-natural catchments in Europe. What is not yet known is the degree to which nitrate leaching could increase if deposition levels are held constant. Recent evidence from an analysis of 20 years of data in the UK Acid Waters Monitoring Network shows
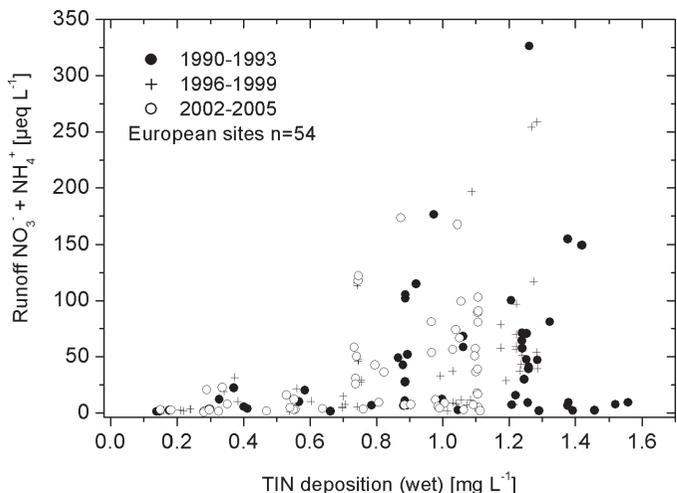
Figure 7.7 Nitrogen (NO_3^- + NH_4^+) concentration in runoff and total inorganic N concentrations in deposition for 54 European ICP Waters sites in 1990–93, 1996–99, and 2002–05. Deposition data are interpolated from adjacent EMEP stations. The sites include lake and stream sites from 8 European countries ranging from the Mediterranean to Scandinavia (1 mg N/l is equivalent to about 70 µeq/l) (from Kaste *et al.*, 2007).

a number of sites with significant increasing trends in nitrate leaching discernible above the 'noise' of temporal variations at various timescales (Figure 7.8), despite static or declining levels of N deposition. While other drivers (climate, de-acidification) may explain some of these increases, the possibility of a nitrogen saturation signal cannot be ruled out (Butterbach-Bahl *et al.*, 2011, Chapter 6 this volume). As excess sulphate concentrations have fallen in response to declining S emissions, the importance of NO_3^- and NH_4^+ in acidification and preventing recovery has increased in relative terms (N vs. S) and at some sites may continue to increase in absolute terms (Curtis *et al.*, 2005; Rogora and Mosello, 2007).

7.5 Nitrogen processing in freshwater systems

Surface freshwaters, including streams, rivers, lakes and other standing waters receive nitrogen both as non-point sources from surface and groundwater runoff from their watershed, and from point sources from direct discharge of treated or untreated urban wastewater. As a rule, nitrate concentrations and fluxes
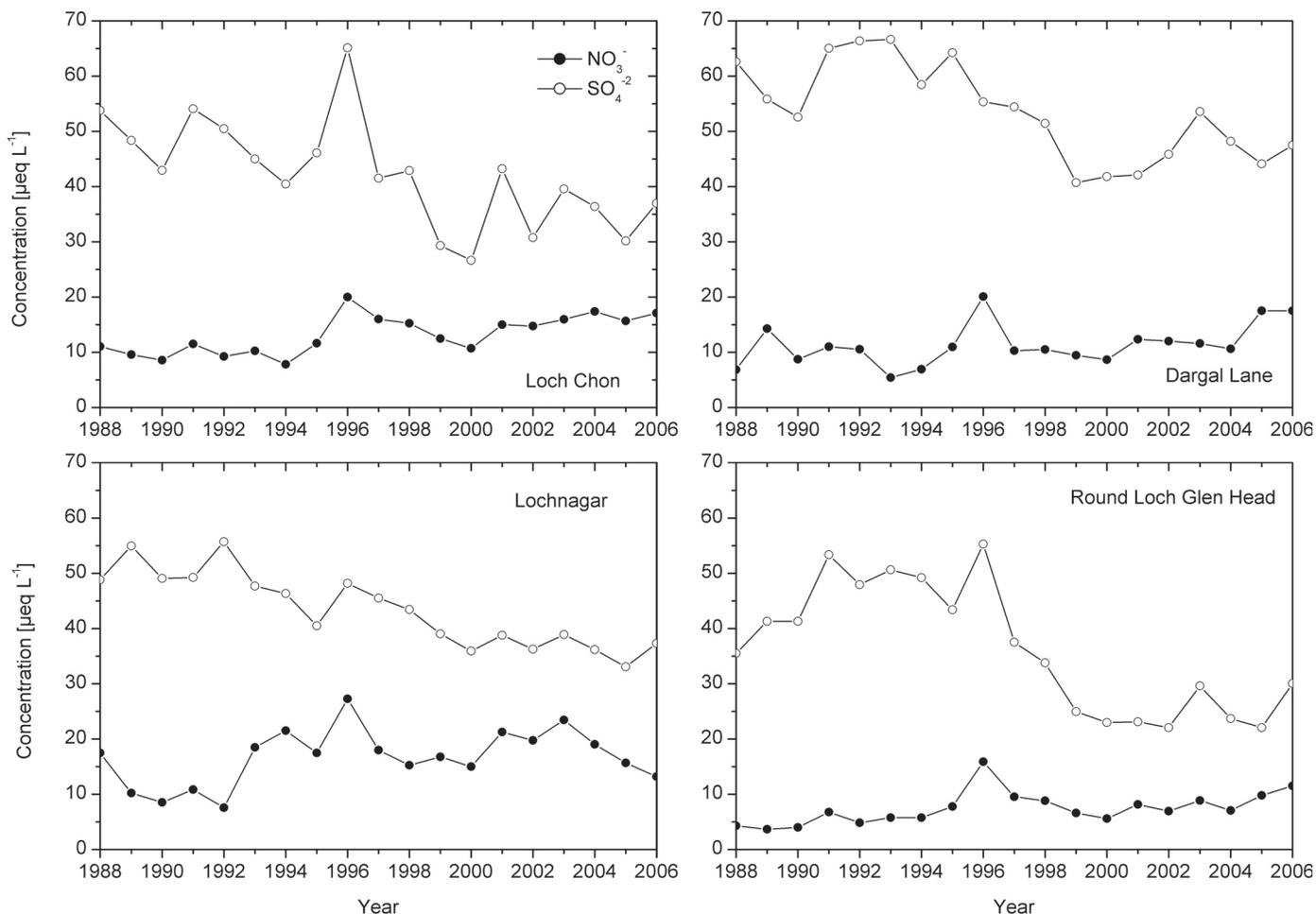
Figure 7.8 Annual mean concentrations of excess (non-marine) sulphate and nitrate in four sites from the UK Acid Waters Monitoring Network. Data for lakes (Lochnagar, Round Loch of Glen Head, Loch Chon) based on quarterly samples, stream (Dargal Lane) monthly samples.

in rivers are significantly lower than in the soil sources in their catchments. A part of this abatement is explained by the role of riparian wetlands in transforming nitrate to gaseous forms and to organic N stored in wetland soil porewaters and biomass, en route from catchment to stream. Another part results from in-stream processes leading to transformation of the N load in transit from headwater streams to estuarine and coastal waters as well as from groundwater to surface water. The following section will discuss the dominant N-relevant processes of the different freshwater systems.

7.5.1 Groundwater

Groundwater (saturated) and vadose (unsaturated) zones have four major roles in the N cycle:

- infiltration and groundwater flow serve to *transfer*, often with significant time lags, nitrogen from soils to groundwater bodies (aquifers) and in turn to surface-water bodies via groundwater baseflow;
- *exchange* of water between surface waters and groundwaters takes place through the biogeochemically active hyporheic zone where rapid cycling of N_r may take place;
- low flow-rates and long travel distances allow these zones to *store* large quantities of nitrogen, principally as nitrate;
- long residence times and specific biogeochemical conditions may allow nitrogen *processing*, especially through biotic reduction processes, particularly denitrification.

Groundwater is hence both a receptor of pollution and a pathway to surface water receptors. Although elevated nitrate has been recognized in groundwater since the 1870s (Addiscott, 1996), the seriousness of this occurrence was not fully appreciated until a century later (Foster *et al.*, 1986; Rivett *et al.*, 2007).

Residence times and storage capacity

Transfer of nitrogen to the subsurface zone (Figure 7.4) may occur via leaching of soils, point-source discharges, particularly via septic tank systems, or river seepage through the hyporheic zone (Dages *et al.*, 2008). Although some discharges, e.g. point sources, may occur in the form of ammonium or DON, nitrification to nitrate is typical in aerated unsaturated zone and shallow groundwater. Nitrate migrates down through the unsaturated zone at a slow rate with nitrate fronts discernible in many systems (Kinniburgh *et al.*, 1999). In many porous or dual-porosity porous-fissured aquifers such as sandstones or chalk-limestones, rates of downward nitrate movement are typically 0.2–1 m/yr (Rivett *et al.*, 2007). Hence it may take years to decades for the main nitrate front to reach deep water tables as 10–50 m thick vadose zones are not uncommon. Even where units are fissured, nitrate diffusion to a porous rock matrix will cause limited flow rates. Below the water table, lateral groundwater flow causes nitrate to spread throughout the aquifer with some flow lines ultimately discharging to receiving surface water bodies and others migrating deeper to aquifer units potentially confined by overlying low permeability units.

Velocities in groundwater are governed by the hydraulic gradient, effective porosity and hydraulic conductivity of units that may vary over orders of magnitude (Martin *et al.*, 2006). Flow rates are typically in the range of 10–100 m/yr. However, in highly fissured karst systems, rates may reach kilometres per day, with significantly contrasting water ages occurring in the subsurface zone (Boehlke and Denver, 1995).

Storage of nitrate in the subsurface-groundwater zone, thought to be relatively small at the global scale, can locally be a significant part of a catchment nitrogen budget, even in areas underlain with crystalline bedrocks (Durand, 2004; Basset-Mens *et al.*, 2006). Nitrate fronts slowly migrating down through unsaturated zones tens of metres thick and of high porosity (and hence large water storage capacity) is of particular concern. The principal impact on the underlying groundwater and in turn receiving surface waters may occur decades after N application at surface. Many aquifers used for water supply or replenishment of surface waters are relatively porous (10%–40%) and where such aquifer thicknesses are large (+50 m) storage of nitrate may be substantial (Jackson *et al.*, 2007). This is evident for example in aquifers of consolidated sedimentary sandstone or chalk/limestone deposits in some lowland parts of Europe. Water quality improvements arising from any protection zone initiatives taken to reduce nitrogen inputs at surface (Johnson *et al.*, 2007) may hence become apparent years to decades after their implementation (Beaudoin *et al.*, 2005; Jackson *et al.*, 2008).

Transformation processes

Microbial transformation of nitrate in the subsurface-groundwater zone is hence critical to the reduction of its impacts. Heterotrophic denitrification, with organic C as an electron donor ultimately forming nitrogen gas, is generally thought to be the dominant attenuation process amongst several other processes (Rivett *et al.*, 2008). The latter include autotrophic denitrification by reduced iron or sulphur, dissimilatory nitrate reduction to ammonium, anoxic oxidation of ammonium (anammox) and abiotic nitrate reduction (Korom, 1992; Burgin and Hamilton, 2007). Biogeochemical conditions, for example redox and the presence and type of electron donors, are key to establishing their relative importance. Rivett *et al.* (2008) conclude that as denitrifying bacteria are essentially ubiquitous in the subsurface zone, even to hundreds of metres depth (Neilsen *et al.*, 2006), the critical limiting factors are electron donor concentration and availability. Variability of other environmental conditions (nitrate concentration, nutrient availability, microbial acclimation, pH, temperature, presence of toxins and other co-contaminants such as pesticides) appear to exert secondary influences on denitrification rates in groundwaters.

Although the number of case studies assessing nitrate migration and attenuation steadily increases (Einsiedl *et al.*, 2005; Barkle *et al.*, 2007; Burgin and Hamilton, 2007; Ruckart *et al.*, 2007; Thayalakumaran *et al.*, 2007; Rivett *et al.*, 2007) the frequency of occurrence of transformation types and their rates remain largely unknown (Korom, 1992; Seitzinger *et al.*,

2006). These studies illustrate that it is technically challenging to investigate the various denitrification/transformation processes in the field with several lines of supporting evidence or methods typically required to characterize bacterial presence, their activity and specificity, nitrogen species present, electron donors, reaction. Often rates estimated are typically low; however, long residence times in the subsurface may result in even low rates of transformation proving important (Rivett *et al.*, 2007). There is hence a crucial need to further develop methods that reliably quantify aquifer denitrification capacities to allow improved prediction of nitrate fluxes (Green *et al.*, 2008).

The ready availability of oxygen in the unsaturated zone and to a lesser extent in unconfined, outcrop aquifer units may cause denitrification to be effectively absent or present at only low rates. For example, studies of the dual-porosity Chalk of south-east England (summarized in Rivett *et al.*, 2007) demonstrated only low rates of denitrification. This was ascribed to relatively good contact with the atmosphere via its fissure network and the low level of in-situ electron donors. The potential for aerobic denitrification was, however, recognized with the existence of anaerobic micro-sites occurring within generally aerobic environments. Although low oxygen conditions may be present in the chalk matrix, the fine pore matrix may exclude bacteria (~1 μm), causing bacterial activity to be largely restricted to fracture sites where it is difficult to establish anaerobic conditions necessary for denitrification without substantial labile C inputs, typically from a pollution source (Gooddy *et al.*, 2002) or perhaps river infiltration.

Importance of DOC

The presence of DOC in groundwater, often thought to be the primary electron donor in denitrification, is typically low. For example, a >11 000 sample dataset covering nine different aquifer types in England and Wales indicated aquifer mean DOC varying from 0.7 to 1.8 mg/l with DOC rarely exceeding 5 mg/l. Such a concentration would drive little denitrification. The stochiometry of heterotrophic denitrification indicates that 1 mg C/l of DOC may convert 0.93 mg N/l of nitrate to N_2. The DOC is, however, preferentially oxidized by dissolved oxygen that requires 1 mg DOC-C/l to convert 2.7 mg O_2/l. In air-saturated groundwater (10 mg O_2/l), up to about 3.8 mg DOC-C/L must therefore be oxidized before denitrification can commence (Rivett *et al.*, 2008). For a fully oxygenated groundwater, this represents a DOC level below which anaerobic conditions may not develop and denitrification does not occur. The greatest concentrations of DOC occur near surface, either where groundwaters are being recharged or water is discharged through riparian zones, organic-rich wetlands or hyporheic zone riverbed sediments (Smith and Lerner, 2008).

The relevance of DOC inputs into aquifers for sustaining heterotrophic denitrification has recently been challenged. Siemens (2003) and Green *et al.* (2008) report DOC fluxes into a sandy aquifer in northern Germany and a set of four sandy aquifers across the USA, and suggest that these are insufficient to account for oxygen and nitrate consumption. In addition, Green *et al.* (2008) suggest that literature denitrification rates are biased towards high rates due to (1) a selection bias to aquifers with high denitrification rates, and (2) solely attributing temporal nitrate concentration gradients to denitrification and ignoring the history of N inputs. Regardless of the actual denitrification rate, the above studies indicate that denitrification in those aquifers relied mainly on the presence of fossil stocks of organic C and reduced sulphur (Kölle *et al.*, 1985). Consumption of organic C, sulphur or ferrous minerals without replenishment increases the risk of a downward oxidation of electron donors and progression of nitrate-rich water further into aquifer units.

In summary, although denitrification rates in groundwater may often be low and difficult to estimate, they are nevertheless important as they may lead to some mitigation of nitrogen pollution delivery to surface waters. The latest global assessment of groundwater denitrification (Seitzinger *et al.*, 2006) gives values ranging from 0 to 7020 kg N/km per yr, and suggests that European aquifers account for 30% of the global groundwater denitrification, which is estimated to be 44 Tg N/yr. This estimate is based on the assumption that heterotrophic denitrification is dominant, and since it is limited by DOC supply from recharge, it is presumed to occur principally in shallower groundwater systems. At present, however, there is insufficient evidence available to support this contention, and this remains an area requiring elucidation through further research.

7.5.2 Riparian wetlands

Status, evolution and diversity of freshwater wetlands

It is believed that at least 50% of the original area of wetlands has been lost in Europe due to drainage for agriculture and urbanization. This destruction is still in progress, since almost 4% of the remaining area has been lost between 1990 and 2000 (Figure 7.9).

Freshwater wetlands comprise a range of very diverse ecosystems. Wetlands dominantly fed by rain water are highly sensitive to atmospheric N deposition. An associated loss of biodiversity occurs where the rate of N deposition increases over time, as is the case in many areas of Europe (Dise *et al.*, 2011, Chapter 20 this volume). The other types can, under certain conditions, contribute to removal of inorganic nitrogen from water (see below).

The conversion of riparian wetlands to urban or agricultural use has resulted not only in an increase in size of the potential source of nutrients subject to transfer to the wider environment, but also in the degradation and loss of ecosystems that are capable of reducing or 'buffering' the flux of inorganic N species from terrestrial to aquatic environments. This nitrate buffering potential of wetlands was first reported in detail in the early 1970s. Subsequently, there have been many publications produced describing the numerous benefits that can be gained from wetlands, often supported by data demonstrating
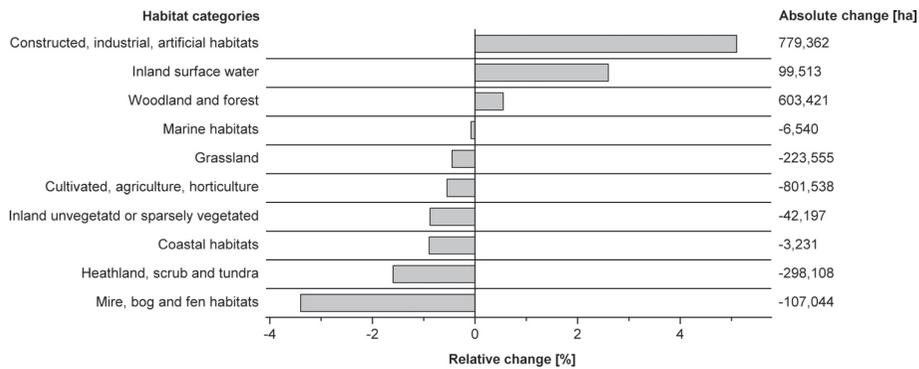
Figure 7.9 Changes in coverage of EUNIS 10 main habitat types from 1990 to 2000 (European Environment Agency, 2005).

their high efficiency in reducing nitrate flux to adjacent surface waters (see Haycock *et al.*, 1997, for a review). This section discusses the potential of freshwater wetlands for regulating water quality by storing and transforming nitrogen through the performance of a variety of physical, chemical and biological functions, and the role of hydrological residence time in controlling this behaviour.

Hydrological linkage between wetlands and the catchment

There are three main aspects to the hydrology of a wetland that control its ability to transform and store nutrients: (i) the hydrological linkage between the wetland and the wider catchment, (ii) the internal hydrological regime of the wetland and (iii) hydrological pathways within a wetland (McClain *et al.*, 2003).

The location of a wetland in the catchment often is the crucial factor in determining its effectiveness as a zone for nitrogen transformation (Johnston *et al.*, 1990). Historically, the assessment of the impact of riparian wetlands on N_r flux at the catchment scale has focused on the total area of wetlands present. However, in wetlands where the main source of water is via diffuse shallow groundwater, it is often the wetland/terrestrial interface that is the active zone in terms of the reduction of nitrate concentrations in inflowing water. The location of nitrate removal at the upslope edge of a riparian buffer zone receiving water from the hillslopes is explained by the combination of high nitrate concentrations in the runoff, high carbon concentrations in the soil (essential as a respiratory substrate for the denitrifying bacteria), and anaerobic conditions resulting from elevated water table. This combination provides optimal conditions for denitrification to occur (Pinay *et al.*, 1989). Nitrate concentrations are sometimes depleted over a few metres within this zone, and the rest of the wetland often shows very low denitrification rates (Sabater *et al.*, 2003). As a consequence, the length of interface between the wetland and upslope sources of nutrient rich runoff is often an important factor determining the impact of the wetland on nitrate reduction.

While the hydrological connectivity between wetlands and sources of nutrients in the catchment controls the degree of contact between inflowing nutrients and wetland biogeochemical cycling processes, the effectiveness of that processing depends on the internal hydrological regime and hydrological pathways of the wetland (Pinay *et al.*, 2002). Wetlands have a wide range of hydrological regimes, and these are influenced by many factors, including soil type, location in the catchment and geomorphology. In soils with low permeability, if the inflow is moderate (upstream locations, baseflow conditions), the residence time of water may be long enough for anoxic conditions to develop, and denitrification may be the dominant process influencing N concentrations in wetland soil porewaters. If the rate of inflow is higher (e.g. during storm events), this function may be bypassed through flushing of soil porewater stores and generation of preferential and/or overland flow to adjacent surface waters. In this circumstance, N retention rates and/or denitrification rates will be substantially reduced. In permeable soils, if the inflow is low, oxic conditions may dominate. This is also the case if the rate of inflow is very high because residence time is not long enough for anoxic conditions to develop. In both cases, denitrification is less significant, and plant uptake with subsequent microbial decomposition of plant material leads to the conversion of nitrate to PON and DON. In high flow events, when the water table rises in these systems, lateral flushing of DON-rich water can occur, so that these wetlands do not reduce total N flux to surface waters, they merely attenuate the rate and N form of the delivery (Prior and Johnes, 2002). It follows that, in general, the actual efficiency of riparian wetlands to denitrify inflow is much less than has been reported in studies where nitrate concentrations only are considered.

Side effects of nitrate removal by wetlands

Although the beneficial effects of buffer zones on nitrate flux to surface waters have been widely documented, the complete environmental assessment of increased N loads in wetlands must include the potential limitations and adverse effects (Haag and Kaupenjohann, 2001). For example, increased N_2O emission rates due to higher N availability and denitrification favouring conditions are likely. Factors such as acidic conditions and low temperatures further alter the N_2O/N_2 ratio in the denitrification process. Increased CO_2 emissions are likely as well, due to increased organic matter oxidation in these systems. Another side effect is the release of DOC and DON into the streams, as mentioned above. Finally, increased N load can damage the ecological status of the wetland. This is obvious in the case of oligotrophic wetlands, but it is also the case for

mesotrophic wetland ecosystems, leading to reduced biodiversity and altered structure and function in the wetland ecosystem. In addition, as the wetland nutrient store reaches capacity, the nitrogen storage and attenuation function of these wetlands will be compromised.

7.5.3 Running waters

N cycling and transformation

N cycling and transformations in river systems occur in the sediment and in the water column and are mainly controlled by the hydrological conditions (Figure 7.10).

Organic matter decomposition and ammonium release by heterotrophic activity in rivers is mainly controlled by the input of organic material either from primary production or from allochthonous origins (litter fall, wastewater release and transfer from upstream). Typical values of bacterial production rate measured in the water column of European rivers ranges between 0.005–0.05 mg C/l per day for uncontaminated forest streams, between 0.01–0.5 mg C/l per day for large rivers, and can reach 2.5 mg C/l per day in rivers heavily contaminated with urban sewage (Billen *et al.*, 1995). Taking into account a C/N ratio of 6–7 for the organic material serving as substrate, and a growth yield of 0.5, these production rates correspond roughly to an in-stream release of ammonium of 0.0008–0.008 mg N/l per day, 0.0015–0.08 mg N/l per day and 0.4 mg N/l per day respectively.

Nitrification occurs in rivers where large amounts of ammonium are present, most often because of untreated urban wastewater release, and can lead to severe oxygen depletion (Chestérikoff *et al.*, 1992). Nitrification rates as high as 0.1 mg N/l per day have been recorded in European rivers (Brion and Billen, 2000). Seeding of river water by nitrifying bacteria

occurs through soil erosion but also by sewage release (Brion and Billen, 2000; Cebron *et al.*, 2004; Cebron *et al.*, 2005). Under low oxygen concentration (<2 mg O_2/l), ammonium oxidizing bacteria are able to make use of nitrite as an alternative electron acceptor. The process, called nitrifying denitrification, leads to the production of large amounts of N_2O (Tallec *et al.*, 2006; Garnier *et al.*, 2007).

Denitrification occurs in the water column of river systems only when the oxygen concentration has dropped below a threshold of about 1 mg O_2/l. There exist a number of well documented situations where this happened and resulted in the complete removal of the river nitrate load (Chestérikoff *et al.*, 1992). Fortunately, the progress of wastewater treatment which was primarily aimed at reducing the organic matter loading and improving the oxygen status of surface water has now resulted in the disappearance of these situations of severe oxygen depletion in most major European rivers. However, denitrification, which occurs in sediments, can be responsible for an important flux of nitrate from the water column (Figure 7.11). The role of periphytonic biofilms in denitrification has been highlighted in many studies, particularly in rivers without any significant deposit of fine sediments, and where significant hyporheic flow occurs through pebble, gravel or sand beds (Boulton *et al.*, 1998; Claret *et al.*, 1998; Mermillod-Blondin *et al.*, 2005; Revsbech *et al.*, 2005).

River primary production and inorganic nitrogen uptake. In streams, effects of eutrophication on primary producers are less visible than in standing waters. Hydraulic flushing prevents algal blooms in these systems and hydraulic drag restricts vertical growth of macrophytes (Biggs, 1996; Hilton *et al.*, 2006). Additionally, limitation by light can be important if the sediment load of a stream is high or if the stream is shaded by riparian forest. Nevertheless, there is much evidence
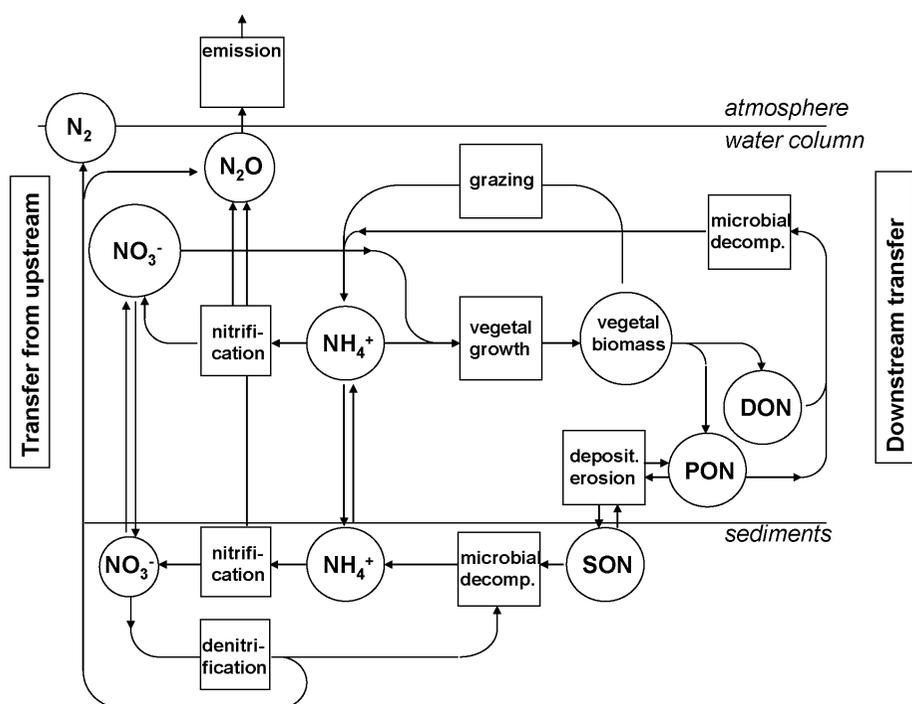
Figure 7.10 The nitrogen spiralling in river systems: a general scheme of the processes affecting nitrogen species during their downstream transfer in rivers.
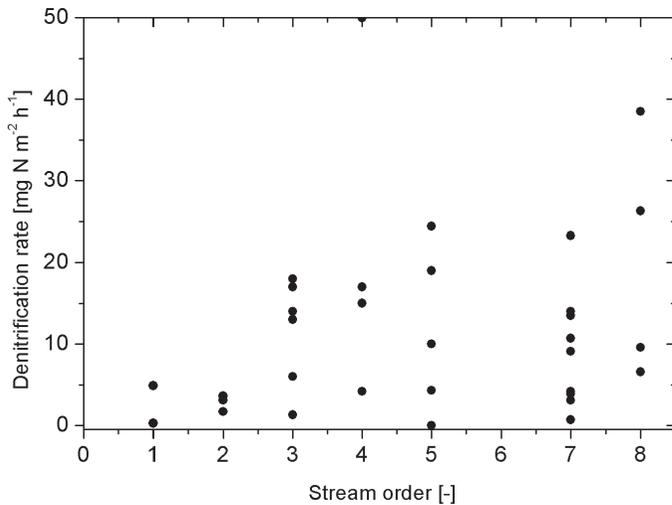
Figure 7.11 Benthic denitrification rates measured with belljars or benthic chambers in European rivers of different stream order (data from Billen *et al.*, 1985; Chestérikoff *et al.*, 1992; Garban *et al.*, 1995; Sanchez, 1996; Thouvenot *et al.,* 2007; Billen *et al.*, 2007; de Klein, 2008).

Figure 7.12 Percent N removal (100*(1 − total N_{out}/N_{in}) vs. water column depth to residence time ratio in a number of European lakes and rivers stretches. Data include Italian lakes (◆); Danish lakes (●) (Andersen, 1977), Seine Reservoirs (■) (Garnier *et al.*, 1999) and a number of river stretches (□) (Cooke and White, 1987; Christensen and Sorensen, 1988; Chestérikoff *et al.*, 1992; de Klein, 2008). Solid lines result from the application of Kelly's model (with Sn respectively 100, 20 and 5 m/yr). The dashed line is the empirical formula proposed by Seitzinger *et al.* (2002), the dotted line is the formula proposed by de Klein (2008).
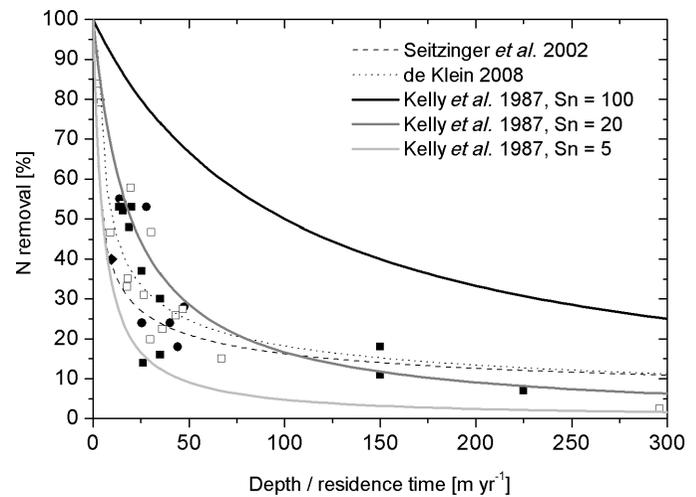
showing that both N and P affect periphyton and phytobenthos growth in streams (Elser *et al.*, 2007) with dense development of filamentous and/or epiphytic algae characteristic of highly eutrophicated rapidly flowing rivers. Uptake by algae or macrophytes is the main mechanism by which N_r can be transformed into organic particulate (or dissolved) form. Low molecular weight DON can also be taken up directly by plants, and can represent an important pathway to support production in higher plant and algal communities in N limited waters from lakes to estuaries and coastal waters (Antia *et al.*, 1991; Seitzinger and Sanders, 1997).

In-stream nitrogen retention

Empirical measurement of nitrogen in-stream retention capacity has been carried out in small experimental streams under steady discharge conditions. In brief, the experiments increase the background concentration of a nutrient in stream water and monitor its downstream spatial and temporal decay (Ensign and Doyle, 2006). The results show a high variability of retention capacity, the broad picture being that this is higher for ammonium than for nitrate and much higher in pristine sites than in N-enriched sites (Marti *et al.*, 2004).

On the scale of catchments and individual waterbodies nitrogen removal can be estimated from balancing incoming (N_{in}) and outflowing (N_{out}) fluxes. The retention fraction (%) is thus defined as *100*(1 − $N_{out}t/N_{in}$)*. For whole system N retention the average hydraulic residence time is a major controlling factor (Nixon *et al.*, 1996; Seitzinger *et al.*, 2002).

Kelly *et al.* (1987) proposed a model of nitrogen removal through benthic denitrification in lakes and Howarth *et al.* (1996) extended this approach to river reaches. The model relates nitrogen loss through denitrification to water residence time (τ), mean water column depth (z), and an average mass transfer coefficient Sn (m/yr) which can be approximated by the ratio between mean areal denitrification rate and mean nitrate

concentration in the water column, if first-order denitrification kinetics is assumed

$$\%N_{removed} = 100^* Sn/(z/\tau + Sn). \tag{7.1}$$

The ratio z/τ is referred to as water displacement (Seitzinger *et al.*, 2002); Sn is typically in the range of 5–100 m/yr in Western European rivers. Figure 7.12 shows that this model fits reasonably well the empirical estimates obtained for a number of European lakes and river reaches. Seitzinger *et al.* (2002) proposed an alternate empirical expression of relation (Eq. (7.1)) which fits both river and lake observations:

$$\%N_{removed} = 88.45 \, (z/\tau)^{-0.37}. \tag{7.2}$$

De Klein (2008) obtained a very similar relationship based on the nitrogen budget of a number of lowland ditches and river systems:

$$\%N_{removed} = 138 \, (z/\tau)^{-0.44}. \tag{7.3}$$

They also stressed that, although the proportion of N inputs that is removed in an individual river stretch is generally low (<20%, see Figure 7.11), the cumulative effect of continued N removal along the entire flow path of a drainage network can result in much higher overall N removal.

7.5.4 Standing waters

N processing in standing waters and major controlling factors

In lakes, N_r can come from direct atmospheric inputs in the form of wet and dry deposition, and N_2 fixed by cyanobacteria. These inputs may be more significant in lakes, compared to rivers, given the greater surface area for exchange to take place. In lakes, N is also derived from influx of both inorganic and organic N from catchment sources delivered by surface and subsurface flow pathways and in some geological environments,

predominantly from groundwater stores. Internal loading derives from the microbial decomposition of N stored in aquatic plant and algal biomass and in lake sediment stores.

Turnover of N in standing waters is controlled by the population dynamics of pelagic (open water), littoral (shoreline) and benthic (bottom dwelling) organisms, with the relative significance of these different groups varying among lake types. For example, in shallow lakes with a high littoral (shallow water) to profundal (deep water) ratio, N turnover is dominated by inorganic and organic N uptake by aquatic plants and organic N decomposition by the microbial community. In deeper lakes, with a low littoral to profundal ratio the free-floating planktonic community will be dominant and exert substantial control over N turnover in the lake. The balance of control is also influenced by water transparency (controlling the depth of light penetration), macro-nutrient availability (N, P and C, derived from external sources, internal stores and sediment-water column interactions), the vertical mixing regime (depending on currents, depth and climate), and the residence time for water entering the lake which determines the length of contact time between N entering the lake and the lake biota. The mixing regime in particular is a specific feature of standing waters as well as large, slow rivers: while the typical pattern of temperate lakes is a dimictic regime with a winter stratification (cooler water at surface) and a summer one (cooler water at the bottom), some lakes are monomictic (stratified only in summer), polymictic (many mixes, e.g. shallow lakes and ponds), or meromictic (never fully mixed because of a stagnant bottom layer). These regimes affect the sensitivity of water bodies to eutrophication and the nitrogen cycling dynamics: for example, in poorly mixed hypertrophic lakes, the hypolimnion (bottom layer including the sediment-water interface) may become anoxic, so denitrification and ammonium accumulation are promoted.

Nitrogen loadings in European lakes and the question of N versus P limitation

The N concentrations in lakes vary widely, depending on the relative rates of atmospheric N deposition, and the intensity of human perturbation of land in the surrounding catchment; N relative to P loading is also highly variable, depending on the relative contribution of atmospheric and diffuse nutrient delivery from atmospheric and terrestrial sources (N rich) and point source (particularly sewage treatment works, P rich) (Johnes, 2007b). Data collated by the International Lake Environment Committee contains information on 40 European lakes (Figure 7.13), including records for varying time periods for 16 large European lakes for which total N and P loading rates are reported (Table 7.1). Annual loads to the catchment vary from <0.02 kg N/ha for Bodensee to 29 kg N/ha for Lake Paajarvi in Finland. Of these, 25% of lakes were reported as N limited or N and P co-limited, compared to over 75% P limited lakes.

The question of N vs. P limitation in lakes has been largely debated in the literature, and what emerges from this debate is a more complex picture than the common idea that P is the cause of eutrophication in freshwaters. Although it is true that P limitation is most commonly reported, N and P co-limitation and N limitation are not rare, at least periodically, and may well occur in many European standing water bodies (Moss *et al.*, 1996; Maberly *et al.*, 2002; James *et al.*, 2003). In cases of N and P enrichment, silica may be also limiting after diatom growth, giving way to possibly undesirable non-diatoms (Schelske and Stoermer, 1971). Note that N limitation or co-limitation can occur in different contexts.

- Exceptionally P rich lakes, and lakes with very low N loads. In both cases, this may result in production by both the phytoplankton community in the open water areas, and the macrophyte community rooted in P rich sediment being limited by N rather than P availability.
- Shallow, well mixed water bodies: since the main store of P is in sediments, it is more readily available in shallow lakes and internal loading of P from these stores makes a substantial contribution to P availability in the open water areas. The diffusion to upper layers in deep, stratified lakes is much slower, typically resulting in P limitation of primarily phytoplankton production in the euphotic zone (where primary production is not limited by light availability).
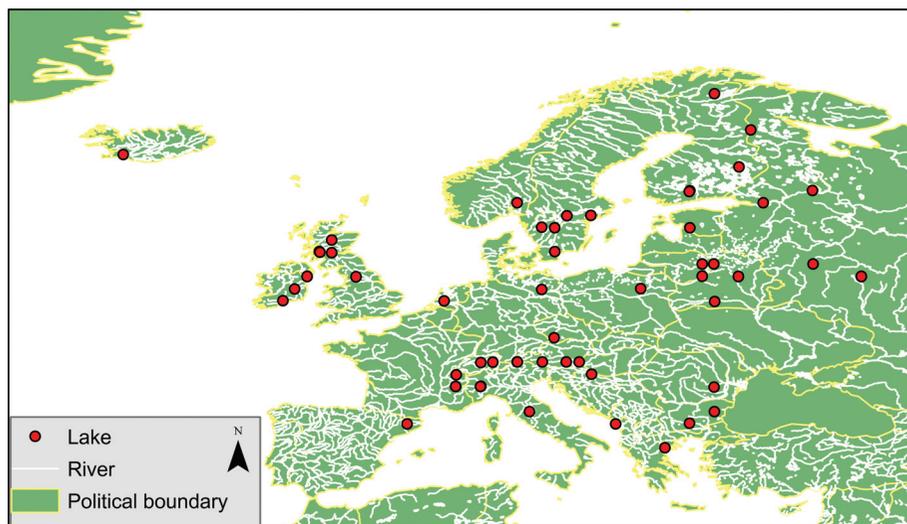
Figure 7.13 European lakes included in the World Lakes Database (International Committee Lake Foundation, 2010).

Table 7.1 Reported TN and TP loadings for large European lakes (International Committee Lake Foundation, 2010)

Lake	Country	Catchment area (km²)	TN loading (t/yr)	TP loading (t/yr)	N:P ratio	N or P limited
Lake Constanz	Germany	10 900	21.0	2.3	9.13	co-limited
Lake Slapy	Czech	12 900	18 200	247	73.7	P
Lac d'Annecy	France	278	550	39.3	14.0	P
Lough Derg	Ireland	10 280	6245	215	29.1	P
Lac Leman	France	7975	1300	1350	0.96	N
Lake Balaton	Hungary	5181	3484	314	10.0	co-limited
Lago Maggiore	Italy	6387	11 000	550	20.0	P
Lago Trasimeno	Italy	396	433	422	10.3	co-limited
Lake Tyrifjordan	Norway	9808	1600	70.0	22.9	co-limited
Lake Mjosa	Norway	16 567	4430	257	17.2	P
Lake Hjalmaren	Sweden	3575	2750	65.0	42.3	P
Lake Malaren	Sweden	21 640	13 400	590	22.7	P
Lake Vanem	Sweden	41 182	17 380	880	19.8	P
Lake Vattern	Sweden	4530	3370	65.0	51.9	P
Lake Paajarvi	Finland	2550	74.3	22.5	30.3	P
Lake Paijanne	Finland	25 400	5475	183	30.0	P
Lake Pielinen	Finland	12 823	2600	114	22.8	P

- Summer N limitation in eutrophic lakes where the earlier algal blooms have exhausted the dissolved N in the water column, and delivery from diffuse catchment sources is at its annual minimum.
- Lakes in either Mediterranean or Nordic environments, where both N and P loadings (mainly atmospheric) are very low (Camacho *et al.*, 2003; Rekolainen *et al.*, 2004; Bergstroem and Jansson, 2006).

While most of the studies have focused on limitation of phytoplankton production, similar results have been found for periphyton (Maberly *et al.*, 2002) and for the macrophyte community (James *et al.*, 2005).

A representative example of these findings is the recent paper by Phillips *et al.* (2008) who studied the relationship between growing season chlorophyll *a* (as a function of phytoplankton production), total P and total N concentrations in over 1000 European lakes under the EU REBECCA programme (Figure 7.14). The database was dominated by lakes from north and central Europe, but a number of Mediterranean sites were also included. Phillips and colleagues concluded that growing season chlorophyll *a* concentrations were significantly related to both TN and TP and that although TP was the best descriptor of growing season chlorophyll *a* for the whole lake database, TN was a better predictor than TP for humic high alkalinity and very shallow polyhumic lakes, suggesting N limitation of phytoplankton productivity in the growing season in these lake types.

High N loads have also been reported to be responsible for loss of biodiversity, especially for submerged plants. In a range of UK and Polish lakes the richest submerged plant communities were associated with winter nitrate concentrations not exceeding 2 mg N/l (James *et al.*, 2005) and the authors propose this as an appropriate target concentration for enriched shallow European lakes to reach 'good ecological status' under the EU Water Framework Directive. Many shallow north temperate European lakes have concentrations well in excess of this limit. Van der Molen *et al.* (1998) for example, reported that 65% of over 200 Dutch lakes had a summer mean total N concentration higher than 2.2 mg N/l. They argue that an appropriate target to protect against ecological deterioration and support recovery of eutrophic lakes is a summer mean concentration of 1.35 mg N/l. In only 30% of lakes studied by Moss *et al.* (1996) was mean summer total N concentration below this target figure.

It is clear that reduction of both N and P is required in shallow eutrophicated waters if both gross productivity is to be reduced and a stable, diverse submerged plant community is to be restored (James *et al.*, 2005). Jeppesen *et al.* (2005) concluded that to improve the ecological status of shallow lakes it may be necessary to control both P and N loading rates. They reported that where external N loading has been reduced to the lakes in their study, the response time in terms of lake total N concentrations is relatively short (<5 years) compared to the response time for lake total P concentrations in relation to external P loading (10–15 years). Jeppesen and colleagues attributed these differences to the relative importance of the sedimentary P store to the lake P budget. These results also suggest that management of freshwaters needs to take a more holistic view of nutrient control than just relying on controlling the supply of P.

(a)

(b)
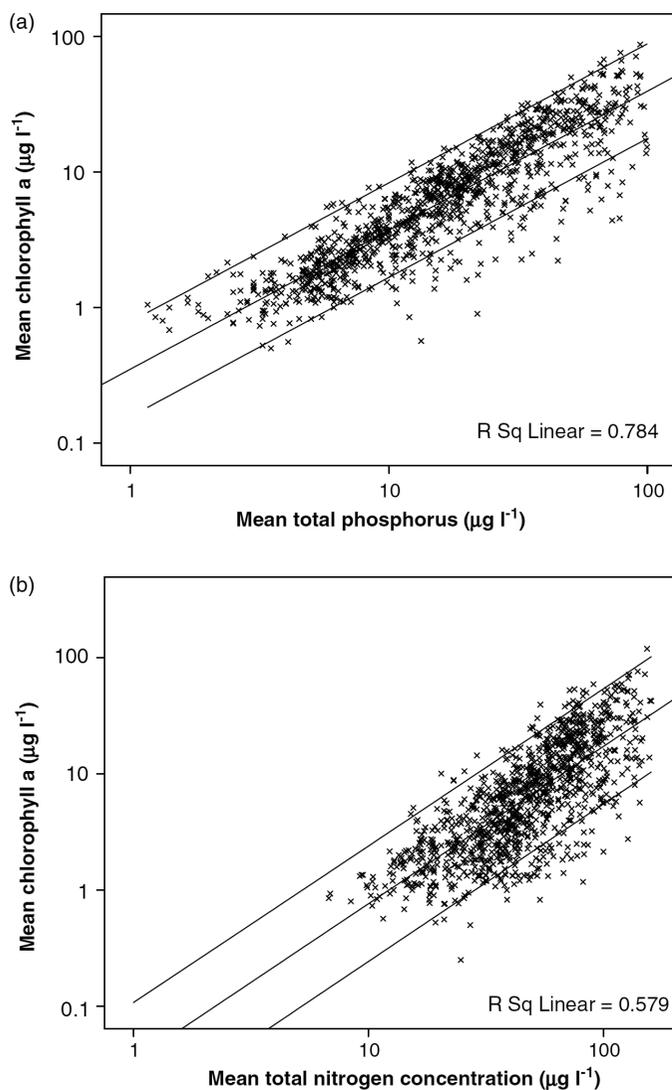
Figure 7.14 Relationship between growing season chlorophyll *a*, total P and total N concentrations in over 1000 European lakes (Phillips *et al.*, 2008).

7.6 Conclusions

Nitrogen enrichment of freshwaters has been taking place throughout Europe, from the advent of modern society, but particularly in the latter half of the twentieth century, where the increasing intensity of agricultural production together with increased industrial and traffic-associated emissions as well as changes in societal management of water and waste-water have led to increased inputs of all plant nutrients to freshwater systems. This enrichment has led to alteration of a wide range of aquatic ecosystem functions including the productivity of water bodies and their microbial metabolism, the microbial, plant and animal community species composition and their relative abundance, and the structure and balance of the aquatic food web.

In terms of ecosystem function, there are relatively few European freshwaters where this has not been altered as a result of N enrichment, either from land-based or atmospheric sources, posing a problem in terms of achieving compliance with the EU Water Framework Directive (2000/60/EC)

as it applies to surface freshwaters, and the EU Groundwater Directive (2006/118/EC) designed to ensure protection of groundwater and groundwater-dependent wetlands against pollution and deterioration in terms of both quantity and chemical quality. The enrichment of European freshwater systems through increases in nitrogen export has compromised a wide range of ecosystem structures, functioning and services and will need to be brought under control through coordinated measures. Consideration will need to be given to a much wider range of sources, practices, and pathways for N delivery to waters and of the total N load delivered and transported to waters not merely the inorganic N fraction if the problem is to be fully addressed. Investigating all effects and managing the cascade of aquatic nitrogen on ecosystem services has not been conducted yet in a comprehensive way and there is a clear need for further research in this respect.

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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