Nitrogen removal and the fate of Nitrate in Riparian buffer zones

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NITROGEN REMOVAL AND THE FATE OF NITRATE IN RIPARIAN BUFFER ZONES

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Abstract

Riparian buffer zones, adjacent to waterways, may protect water quality by intercepting and removing nitrogen in runoff from agricultural land. This research comprised four parts: (1) a field study of nitrogen buffering by differently vegetated riparian zones in a United Kingdom (UK) sheep-grazed pastoral catchment; (2) a field study of surface and subsurface runoff hydrology, and nitrogen flux, in a UK riparian wetland; (3) a laboratory study (^{15}N tracer-isotope dilution) of microbial inorganic nitrogen production and removal processes in the UK riparian wetland soil; and (4) a laboratory microcosm study (^{15}N tracer) of nitrate removal processes in bare and plant-inhabited (*Glyceria declinata*) New Zealand (NZ) riparian wetland soil.

Dissolved organic nitrogen and ammonium were generally more important components of subsurface runoff than nitrate in the three UK riparian zones. All riparian zones were poor buffers having minimal effect on the nitrogen concentration of subsurface runoff. In the UK riparian wetland site subsurface (saturated zone) and surface 'preferential flow paths' typically conveyed large quantities of catchment runoff rapidly into, and across the site, and hindered nitrogen buffering. However, under low flow conditions, runoff-riparian soil contact increased and the wetland decreased the catchment nitrogen flux by 27%. In the UK riparian wetland soil most nitrate removal was attributable to denitrification (87-100%) as opposed to dissimilatory nitrate reduction to ammonium (DNRA) (0-13%) and immobilisation (0-10%). Total ($^{14}N+^{15}N$) transformation rates for these processes were 1.3-47, 0.5-1.5 and 0.6-2.5 $\mu g\ N\ g\ soil^{-1}\ hr^{-1}$, respectively. In the NZ riparian wetland soil *Glyceria declinata* assimilated 11-15% of nitrate but, more importantly, increased soil oxidation and altered the proportions of nitrate removal attributable to denitrification (from 29% to 61-63%) and DNRA (from 49 to <1%), but not immobilisation (22-26%). Denitrification and, thus, nitrogen buffering might be enhanced, in some riparian zones by increasing the extent of moderately anoxic soil with plants that release oxygen from their roots or with water table management.

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Chapter 1

Nitrate removal in riparian buffer zones: an introduction and review

1.1. The nitrate problem

Nitrate concentrations in surface and ground waters of developed countries steadily increased in the latter half of the 20th century (Burden 1982, Heathwaite *et al.* 1993, Johnes and Burt 1993, Powlson 2000). This trend is illustrated for four United Kingdom rivers where records of nitrate concentration have been kept for several decades or more (Figure 1.1). In rural catchments, excessive application of nitrogenous fertilisers and the increasing intensification of farming practices have been identified as the major cause (Burt 1993, Ledgard *et al.* 1998, Davies 2000). The link between agricultural land use and nitrate enrichment of waterways is illustrated for two United Kingdom rivers (Figure 1.2). Ammonium and organic nitrogen in fertiliser, crop residue and livestock excrement are readily transformed to nitrate in the soil by microorganisms. Nitrate is highly water-soluble and is easily leached from fields to waterways with surface and subsurface runoff, especially in autumn and winter when vegetation uptake is reduced and precipitation exceeds evapo-transpiration (Davies 2000).

Nitrate enrichment of waterways is of considerable concern to government authorities (local and national), and to other agencies involved in water resource management, due to the serious environmental and public health implications. Increased inputs of nitrate to natural waters contribute to eutrophication, a process typically synonymous with reduced water clarity and excessive growth of weeds and algae (Vollenweider 1968, OECD 1982). In drinking water, elevated concentrations of nitrate have been linked to the occurrence of gastric cancer and to methaemoglobinaemia in infants (Winton 1971, Shuval and Gruener 1977).

World-wide concern about the negative effects of nitrate enrichment has resulted in the establishment of recommended maximum limits for nitrate in waterways, principally those used for drinking water extraction, by various national governments and international agencies. The World Health Organisation set a limit of 11.3 mg l^{-1} NO_3-N (World Health Organisation 1970), which has been adopted by the European Union (European Community 1991 – Nitrate Directive 91/676). This limit also applies in some countries outside of the European Union, e.g. New Zealand (Ledgard *et al.* 1998).

For agricultural catchments, the onus is now on water resource managers to identify nitrate-enriched waterways, and implement appropriate strategies to maintain, or lower,

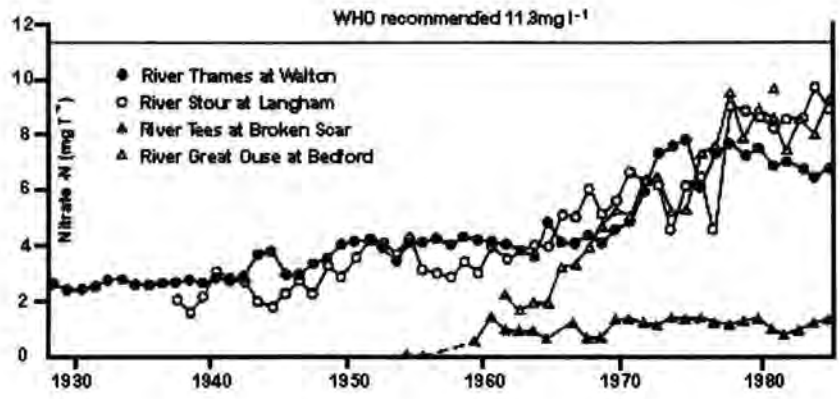


Figure 1.1. Long term trends in nitrate concentration in the River Thames, River Stour, River Tees and River Great Ouse, United Kingdom (Roberts and Marsh 1987).

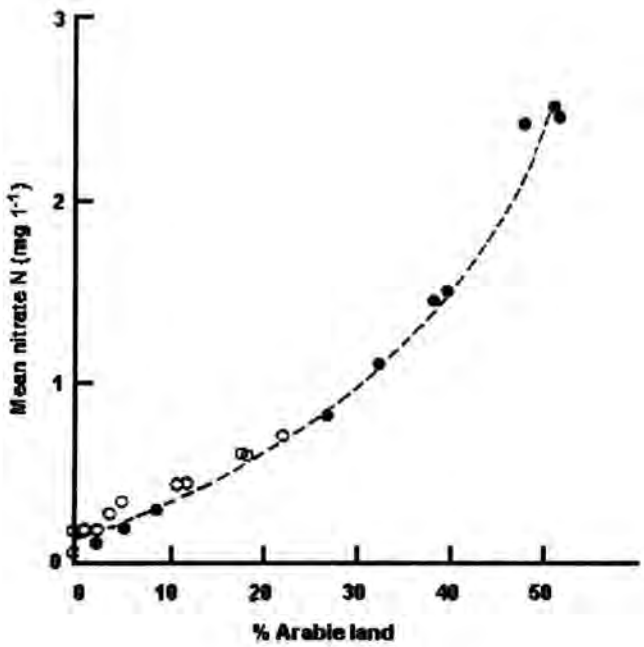


Figure 1.2. The effect of the amount of arable land on the nitrate concentration of river water for the River Dee (O) and River Don (●) in Scotland (Edwards *et al.* 1990).

nitrate concentrations below the required limit. These strategies may be classified into three broad categories (Table 1.1):

(1) control of nitrate leaching at the source;
(2) treatment prior to discharge into waterways; and
(3) treatment of nitrate pollution in-stream or after abstraction

Table 1.1
Strategies to maintain or lower nitrate concentrations in waters derived from agricultural catchments.

Control at source	Treatment prior to discharge	In stream treatment
- Changes in land management practice (more efficient use of fertiliser, catch crops, minimising ploughing) - Changes in land use (e.g. to forestry, parkland etc)	- Riparian buffer zones - Other buffering landscape features (e.g. hedgerows, mid-field forests, ponds etc).	- Chemical treatment -Blending with low nitrate water -Reservoir storage and encouraging biological remediation

These three categories represent a hierarchy of environmental protection, with the least detriment to the environment resulting from the 'control at source' strategies and the greatest from dependence on 'in-stream treatment'. The hierarchical order is obviously reversed when economic costs to the farmer are considered instead. Given the conflict inherent in the adoption of either extreme, nitrate removal utilising riparian buffer zones represents a reasonable compromise to water resource managers.

1.2. Riparian buffer zones: structure and function

In agricultural landscapes, a riparian buffer zone is an area of undrained land directly adjacent to the stream or river channel (Figure 1.3). The water table is in close proximity to the ground surface and it is this feature that characterises the riparian buffer zone. It is an ecotone, which is defined as; "a zone of transition between two adjacent ecological systems having a set of characteristic uniquely defined by space and time scales and by the strength of the interactions between adjacent ecological systems" (Holland 1988). In the case of the riparian buffer zone, the adjacent ecological systems are the 'unsaturated' terrestrial environment and the 'saturated' aquatic system.

The steep gradient from unsaturated to saturated soils in the near-stream zone creates a wide range of macro- and micro- environments, which encourages biological diversity and high productivity. Together with the reduced topographic relief generally encountered in near-stream zones, this creates a huge potential for the interception and regulation of upslope runoff and for the transformation of water-borne nutrients (Risser 1990). Studies in a number of developed countries on the nutrient processing capacity of riparian zones

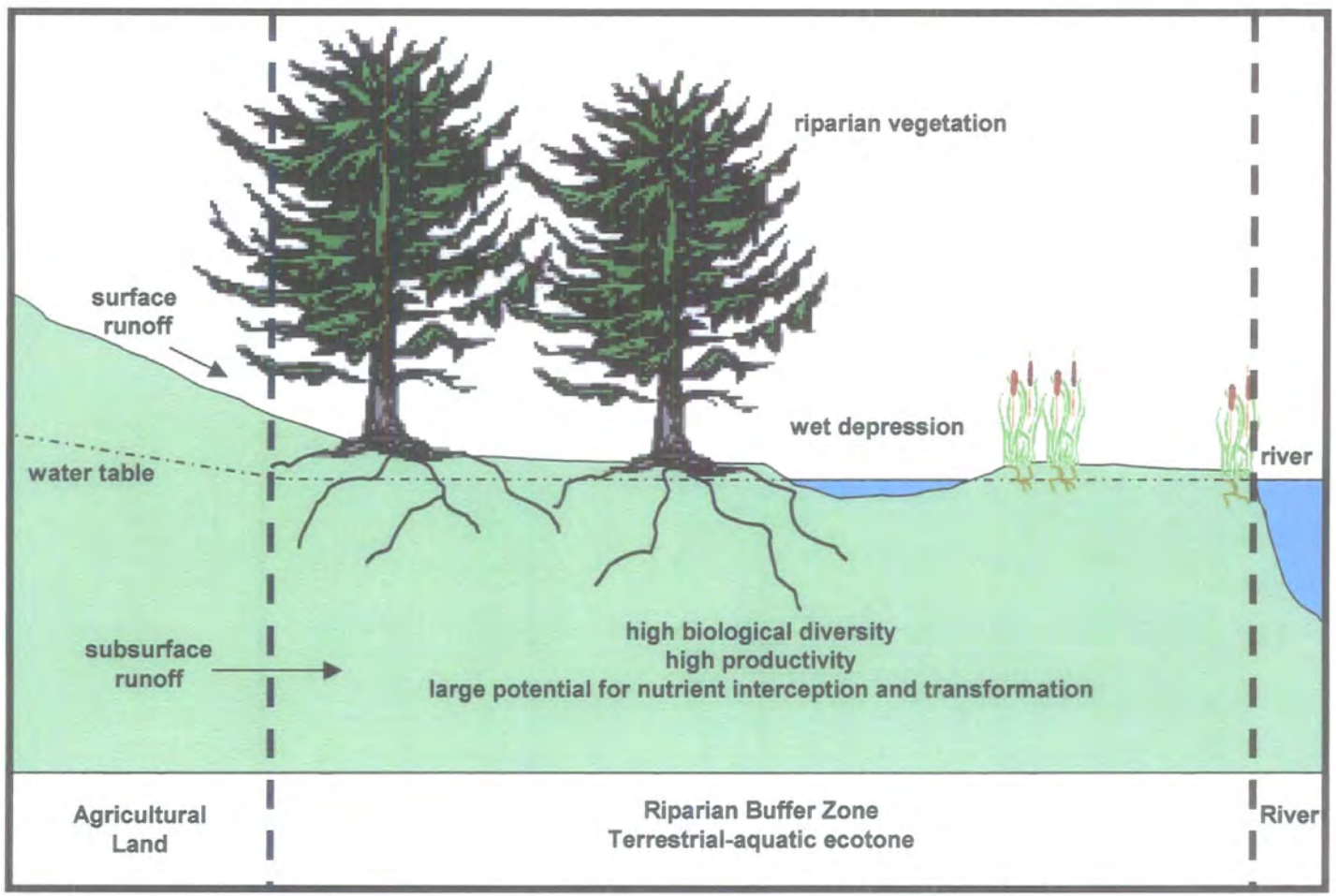


Figure 1.3. Generalised structure of a riparian buffer zone.

support this notion (Table 1.2). All have observed substantial reductions in nitrate and, sometimes, other water-borne nutrients, as runoff passes through riparian buffer zones.

Table 1.2

The nitrate removal capacity of riparian buffer zones observed in studies from a number of developed countries.

Country	RBZ type	Nitrate removal	Reference
England	Woodland	99%	Haycock and Pinay 1993
	Pasture	84%	
France	deciduous forest	100%	Pinay and Decamps 1988
Germany	woodland	61%	Knauer and Mander 1989
New Zealand	pasture (organic soil)	>90%	Cooper 1990
	pine forest	98%	Schipper *et al.* 1993
USA – North Carolina	forest	>90%	Jacobs and Gilliam 1985
- Georgia	deciduous forest	90%	Lowrance *et al.* 1984
- Maryland	forest	90-98%	Peterjohn and Correll 1984
- Rhode Island	deciduous forest	>80%	Simmons *et al.* 1992

Artificial drainage of the near-stream zone destroys the terrestrial-aquatic ecotone, and hence the riparian buffer zone, by intimately linking the terrestrial and aquatic systems. Runoff from the hillslope and near-stream zone is fast-tracked to the stream and the gradient in soil saturation, so critical to the nutrient transforming capacity of the near-stream zone, is diminished. The nitrate concentrations measured in runoff channelled through artificial drains are typically much higher than those reported to drain naturally from riparian buffer zones. In the USA, Neely and Baker (1989) measured 10-20 mg NO_3-N l^{-1} in agricultural drainage while, in the UK, Rose *et al.* (1991) report that maximum concentrations ranging from 30-50 mg NO_3-N l^{-1} are expected during the winter months.

While the presence of non-agricultural vegetation in riparian buffer zones is often desirable (Table 1.3) it does not appear to constitute a necessary component for nitrate removal. Some studies have shown that pasture riparian buffer zones can still function effectively (Cooper 1990, Haycock and Pinay 1993). The main requirement is the presence of a soil saturation gradient. This may exist naturally or may be achieved via the use of controlled drainage structures (Gilliam *et al.* 1979; Gilliam *et al.* 1986, Franklin *et al.* 1992).

Riparian buffer zones exist today for three main reasons:

(1) because land has been too wet to be agriculturally productive and the installation of artificial drainage has not been feasible;

(2) because there has been active protection by the landowner due to some perceived value (such as those outlined in Table 1.3); or

(3) because there has been riparian buffer zone restoration

Table 1.3
Values associated with the presence of non-agricultural vegetation in riparian buffer zones.

Anthropocentric (people) values	Ecological values
- aesthetic or scenic value (e.g. obvious 'greening' of the agricultural landscape)	- habitat for wildlife/river corridor for species migration
- science and educational value (gene pool and ecological processes)	- biological diversity
- amenity and recreational value (bird-watching, shooting etc)	- stream temperature control by shading (warm water detrimental to many aquatic organisms)
- intrinsic value	- source of large woody debris to the stream which creates complexity in channel morphology and habitat diversity for aquatic organisms
- cultural and spiritual value	- bank stability

Already in the United Kingdom (Tytherleigh 1997) and New Zealand (Cooper *et al.* 1997, Downes *et al.* 1997) riparian restoration schemes are being trialled on the basis of studies which have indicated substantial removal of nitrate, and other contaminants, by riparian buffer zones. This is occurring despite the fact that our knowledge of nutrient processing mechanisms in riparian buffer zones still remains far from complete. A very urgent research need, particularly if adoption of riparian restoration schemes is to be encouraged, is determining the sustainability of riparian buffer zones as protectors of water quality. This is inherently linked to the fate of contaminants within the riparian buffer zone soil and is discussed for nitrate below.

1.3. Nitrate removal processes in soils

1.3.1. Overview

There are four biological processes responsible for nitrate removal in riparian buffer zone soils: denitrification, dissimilatory nitrate reduction to ammonium (DNRA), microbial immobilisation and plant uptake (Figure 1.4). The occurrence and regulation of each process are detailed separately below. The implication of each process to nitrogen buffering in riparian zones is then discussed. The final section summarises current understandings of nitrate removal partitioning between the four processes.

1.3.2. Denitrification

Of all the pathways in the nitrogen cycle, denitrification is one of the most well-studied. As a result, this section is somewhat more comprehensive than those that follow on the

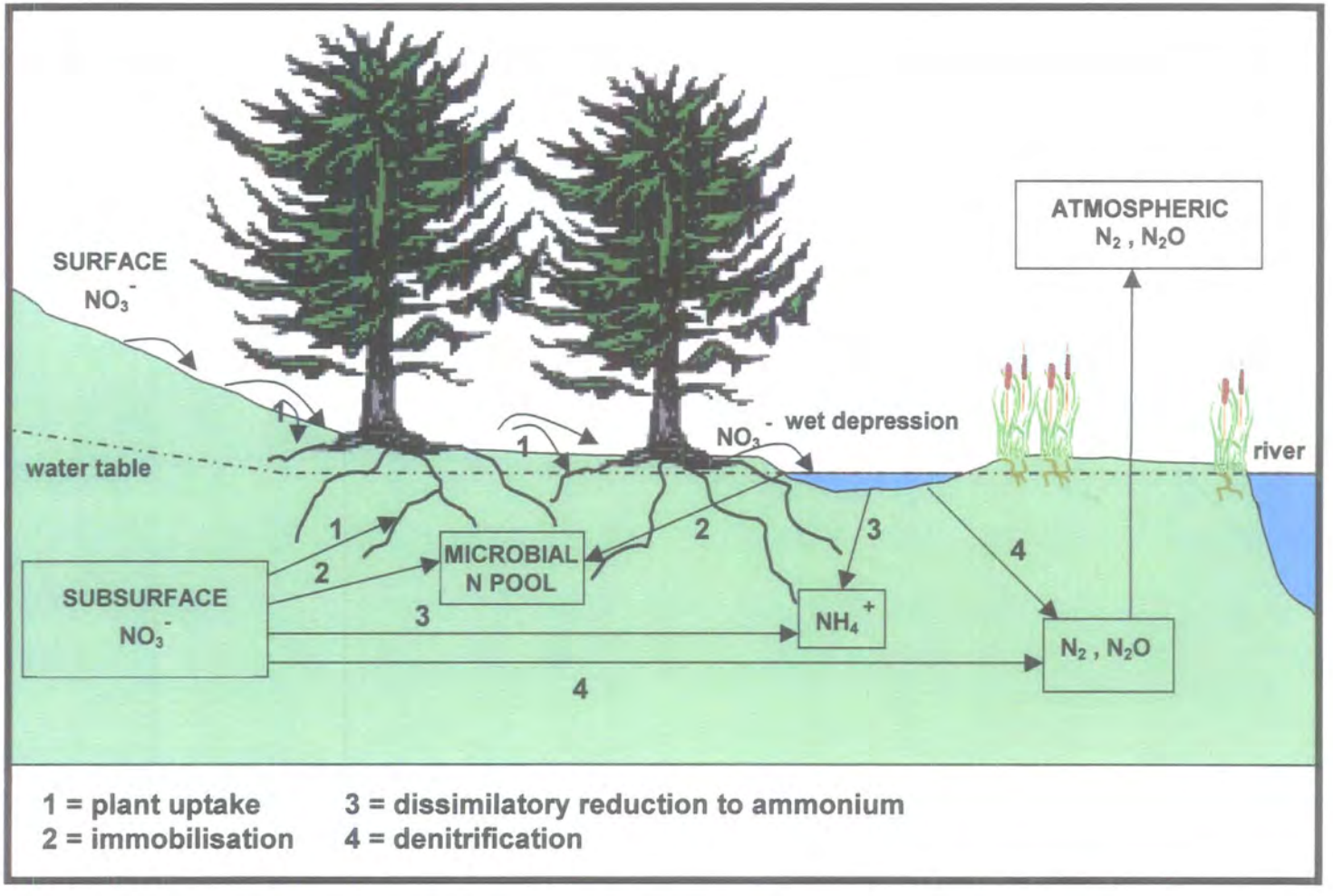


Figure 1.4. The possible fates for nitrate in surface and subsurface runoff entering a riparian buffer zone.

other nitrate removal processes. Denitrification refers to the formation of gaseous nitrogen species, predominantly di-nitrogen (N_2) and nitrous oxide (N_2O), from the reduction of nitrate (Sprent 1987). The occurrence of denitrification is of considerable importance to agriculture as it results in the loss of available nitrogen for plant growth (Vinten and Smith 1993). It is also of interest to those involved in water quality management and research, as it reduces the eutrophication potential of catchment runoff entering downstream lakes and rivers (Heathwaite *et al.* 1993).

Denitrifying soil microorganisms are typically facultatively anaerobic, preferring growth under aerobic conditions, but also able to survive under anaerobic conditions in the presence of nitrate. They utilise nitrate as an alternative electron acceptor to oxygen when the latter becomes limiting (Tiedje 1982). Most denitrifying microorganisms in the soil are heterotrophic (or organotrophic) utilising organic substances such as plant litter and root exudates, as an energy source (Knowles 1981, Tiedje 1982). The wide variety of organic substances utilised, ranging from simple sugars to complex aromatics, reflects the diversity of microorganisms involved in this process (Killham 1994). The most common heterotrophic denitrifiers found in nature are species of *Pseudomonas* and *Alcaligenes* (Tiedje 1988). Other heterotrophic denitrifiers (e.g. species of *Azospirillum*, *Bacillus*, *Halobacterium*, *Rhizobium*) are much less ubiquitous. In addition to the heterotrophs there are some denitrifiers that are chemolithotrophic using H_2 (e.g. *Paracoccus denitrificans*) or S (e.g. *Thiobacillus denitrificans*) for energy. One species, *Rhodopseudomonas sphaeroides* is photolithotrophic (Tiedje 1988, Killham 1994).

There are five proximate regulators of denitrification, which affect the immediate environment of the microbial cell (Schipper 1991, Matchett 1998):
(1) the presence or absence of oxygen;
(2) an available electron acceptor (nitrate);
(3) an available energy source or electron donor (e.g. carbon);
(4) temperature; and
(5) pH.

The presence of **oxygen** represses the microbial enzymes involved in denitrification (Knowles 1981), although complete inhibition of denitrifying activity in soils does not seem to occur. Under aerobic conditions Parkin and Tiedje (1984) found that the rate of denitrification was measurable, but a negligible fraction (0.3 to 3%) of that which occurred under anaerobic conditions. The presence of active denitrifying enzymes has also been reported in well-drained, sandy, and presumably, aerated soil (Smith and Tiedje 1979). The oxygen threshold below which 'significant' denitrification begins to occur is at least as low as 10 μmol O_2 l^{-1} (Tiedje 1988) but may be as low as 6.2 μmol O_2 l^{-1} which is equivalent to 0.2 mg O_2 l^{-1} (Knowles 1982, Seitzinger 1988). In terms of redox potential a

threshold of +420 mV (at pH 7) is proposed (Killham 1994). In soils, the level of oxidation is dependent on the rate at which oxygen can diffuse into, and through the soil, and the strength of the microbial respiratory sink (Tiedje 1988). As the diffusion rate of oxygen through aqueous media is considerably slower than through porous media, increases in soil saturation typically result in lower levels of soil oxidation (Schipper 1991).

Denitrification responds to increasing concentrations of nitrate in a manner described by Michaelis-Menton kinetics (Tiedje 1988). The denitrification rate increases markedly with increasing nitrate at low concentrations. At higher concentrations further additions of nitrate generally have minimal effect on rates of denitrification (Knowles 1981). The nitrate concentration beyond which any further increase does not stimulate the denitrification rate (Km value) is typically much higher in soils than in pure cultures. This is attributed to more restricted nitrate diffusion in soils (Tiedje 1988). The availability of nitrate frequently limits *in situ* denitrification in soils (Pinay and Decamps 1988, Cooper 1990, Pinay *et al.* 1993, Burt *et al.* 1999). This is usually inferred from large discrepancies between potential rates (laboratory assay with excess nitrate) and actual rates of denitrification. *In situ*, the availability of nitrate for denitrification is the sum of: (1) supply from extrinsic sources (groundwater, surface water and diffusion), (2) production via nitrification, (3) utilisation by non-denitrifying microorganisms and by plants, and (4) leaching losses (Tiedje 1988). The generally anaerobic nature of wet soils precludes substantial *in situ* production of nitrate. However, some nitrification may be possible at the interfaces between aerobic and anaerobic conditions (i.e. near the soil surface and near the roots of plants where oxygen is released) (Reddy and Patrick 1984).

Organic carbon has a dual role in the denitrification process: providing energy for microbial respiration and hence, facilitating oxygen consumption in the soil environment, and as an energy source (electron donor) for denitrifiers. The rate of denitrification is often regulated by the availability of the organic carbon source for microbial activity (Burford and Bremner 1975, Kaplan *et al.* 1979). Some studies have found that denitrifying activity is positively correlated with the organic carbon content of the soil incubations, and even more so with the glucose (Stanford *et al.* 1975), water-soluble carbon content (Burford and Bremner 1975) or mineralisable carbon content (Bijay-Singh *et al.* 1988). Presumably, these latter carbon sources represent pools of more 'microbially-available' carbon. Denitrification in mineral soils is usually limited by available carbon and in laboratory studies, an organic carbon source is often added to maximise denitrification rates (Nichols 1983). In wet soils, there are generally larger quantities of organic carbon due to the accumulation and decomposition of plant tissues and/or root exudation (Stefanson 1973, Bailey 1976), but also because fermentative microorganisms

and oxygen-stressed cells provide additional sources of available carbon (Tiedje 1988, Schipper *et al.* 1994).

Denitrification, along with many other biological processes, is regulated by temperature. At low temperatures, between 0 and 5°C, denitrification is thought to be very low but measurable (Knowles 1981, Groffman and Hanson 1997). The actual lower threshold for denitrification may vary depending on the supply of organic substrate and has been found to be low as -2°C with additions of glucose and alfalfa (Dorland and Beauchamp 1991). The optimum temperature for denitrification lies between 60 and 75°C but above this, activity rapidly declines to zero (Bremner and Shaw 1958, Keeney 1973). In between these lower and upper limits, the denitrification rate and temperature should, as proposed for all microbial processes by the Arhennius equation, increase exponentially with increasing temperature (Matchett 1998). In practice, an approximate doubling of denitrification rates for each 10°C increase between 11 and 35°C has been reported (Bailey and Beauchamp 1973). More recently, Garcia-Ruiz *et al.* (1998) found that temperature increased the denitrification rate in river sediments by 1.5 to 3.5 times between 6 and 15°C and by 1-2 times between 15 and 24°C. However, in studies such as these, determining the effect that temperature alone has on denitrification, in isolation from other regulating factors, is difficult to determine precisely. Temperature also affects oxygen availability (by affecting oxygen solubility and rates of oxygen diffusion), nitrate availability (by affecting nitrate diffusion rates and nitrification and mineralisation process rates) and organic substrate availability (by affecting the diffusion rate of soluble carbon) (Schipper 1991).

Denitrification typically increases with increasing pH, reaching an optimum between 6 and 8. The process is, however, able to occur at pH as low as 3.5 and up to 11 (Matchett 1998). The pH also regulates the relative proportion of end-products of the denitrification process. As the pH decreases the mole fraction of nitrous oxide increases relative to di-nitrogen. At pH 4 nitrous oxide is thought to be the major end-product of denitrification. Low pH (<5) may also enable 'abiological denitrification' or 'chemo-denitrification' to occur whereby nitrite is reduced to di-nitrogen and nitrous oxide amongst other products (Knowles 1981).

1.3.3. Dissimilatory nitrate reduction to ammonium (DNRA)

The microorganisms responsible for DNRA reduce nitrate to ammonium with constitutive cell enzymes (Jorgensen 1989), and the end-product is excreted from the cell. This contrasts with microbial immobilisation (assimilatory nitrate reduction), discussed in the following section, which is essentially the same process except the end-product remains in the cell (Tiedje *et al.* 1981).

DNRA is carried out by fermentative microorganisms that have an obligately, or facultatively, anaerobic metabolism. The microorganisms capable of DNRA in soils or sediments include *Escherichia coli*, *Achromobacter fischeri*, *Erwinia carotovora*, *Campylobacter sputorum*, *Wolinella succinogenes*, *Desulfovibrio* spp, *Citrobacter* spp. *Klebsiella* spp, *Clostridium* spp and the *Vibrio/Aeromonas* group (Tiedje 1988). A number of these microorganisms are capable of both denitrification and DNRA. However, there are some species, i.e. *Clostridium tertium* that cannot denitrify and are only capable of DNRA (Caskey and Tiedje 1979). Microorganisms with the capability of DNRA are thought to be widespread in soil, and probably more numerous than those with the denitrifying capability (Tiedje *et al.* 1982).

Comparatively little study of the proximate regulators of DNRA alone has been made. However, a number of studies have measured denitrification and DNRA activity together while varying soil oxidation state, available nitrate and available carbon.

As with denitrification, the oxidation level of the soil appears to regulate DNRA activity. Increased amounts of DNRA in soils have been measured in laboratory incubations when the oxidation level of soil has been artificially reduced;

(1) to a redox potential of –225 mV by the addition of *L*-cysteine (Chen *et al.* 2000); and

(2) to redox potentials of 0 and -200 mV by the use of controlled redox incubators (Buresh and Patrick 1978, Buresh and Patrick 1981).

Higher amounts of DNRA have also been reported in littoral stands of declining, as opposed to healthy, *Phragmites australis*, where accumulating organic matter, lower redox potential and increased methanogenesis, are linked to the reduced capacity of the plant to oxidise rhizosphere sediments (Nijburg and Laanbroek 1997a, Picek *et al.* 2000). Conversely, lower amounts of DNRA have been reported when the oxidation level of the soil has been artificially increased by the addition of high nitrate concentrations (King and Nedwell 1985, Moraghan 1993). As DNRA microorganisms are capable of fermentative growth in the absence of nitrate (Tiedje 1988), but denitrifiers are not, it seems reasonable to expect that they would be most prevalent in long-term, highly anoxic soils, while denitrifiers would prevail in moderately, or temporarily, anoxic soils (Nijburg and Laanbroek 1997a).

As with denitrification, nitrate must be present for DNRA to occur. However, nitrate is an oxidising substance (Buresh and Patrick 1981) and presumably must be present at low concentrations to avoid repressing DNRA activity. Like denitrification, DNRA also requires an electron donor. Along with organic carbon, electron donors for DNRA include H_2, formate and a number of sulphur-reduced compounds including H_2S, FeS and $S_2O_3^{2-}$

(Killham 1994, Brunet and Garcia-Gil 1996). The DNRA process accepts more electrons per molecule of nitrate reduced (eight) than denitrification (five). This makes DNRA a more effective electron sink for the reoxidation of NADH. This is postulated as the main reason why the process might be of benefit to microorganisms, particularly in electron donor-rich, electron acceptor-poor (i.e. low nitrate concentration) environments (Tiedje 1988).

1.3.4. Immobilisation

Immobilisation refers to the incorporation of inorganic nitrogen (nitrate and ammonium) into organic nitrogen within microbial cells. Ammonium is the preferred species of inorganic nitrogen taken up by microorganisms. However, nitrate can also be immobilised if microorganisms have the assimilatory nitrate reductase enzyme (Sprent 1987). Nitrate is first reduced to ammonium by the enzyme and the ammonium is then converted, within the cell, into glutamate and glutamine. These key elements are then used in the synthesis of cellular material (Wood 1989). The immobilisation of nitrate proceeds only at the rate which ammonium is required for cell growth. Hence, this process often occurs at a low rate (Tiedje *et al.* 1981).

In contrast to denitrification and DNRA, the level of soil oxidation has little regulatory effect on the occurrence of nitrate immobilisation (Cole and Brown 1980). Both aerobic and anaerobic microorganisms from various genera are capable of immobilising nitrate including: *Azotobacter*, *Bacillus*, *Clostridium*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Rhizobium* and *Vibrio* (Payne 1973). Note that many of these genera have also been identified as capable of denitrification or DNRA. However, immobilisation of nitrate is considered to be higher under oxidised conditions as aerobic microorganisms have greater nitrogen requirements than anaerobic microorganisms (Reddy and Patrick 1984). Mechanisms that enhance the oxidation level of the soil (e.g. high nitrate additions, oxygen release from plant roots) may increase the overall amount of nitrate immobilisation by stimulating the activity of aerobic, as opposed to anaerobic, microorganisms.

Nitrate immobilisation results from the utilisation of organic substrates by heterotrophic microorganisms for energy and synthesis of new biomass (Rosswall 1982). When the substrate undergoing decomposition contains a high enough carbon to nitrogen ratio that additional nitrogen is needed then immobilisation of inorganic nitrogen (nitrate or ammonium) occurs. There is considerable debate as to the critical carbon to nitrogen ratio above which immobilisation occurs; a ratio of around 20:1 is often cited (Alexander 1977, Killham 1994). When the organic substrate undergoing decomposition (e.g. plant litter or dead cells) contains nitrogen in excess of the requirements of decomposers,

inorganic nitrogen is released into the soil as ammonium. This process is termed mineralisation. Due to the variable distribution and nature of organic substrates in the soil, the processes of immobilisation and mineralisation operate simultaneously.

The presence of nitrate induces nitrate and nitrite reductase enzyme synthesis in microorganisms but the presence of ammonium inhibits enzyme synthesis (Payne 1973, Tiedje *et al.* 1981). This regulation of the nitrate immobilisation process probably occurs so that ammonium is only produced by this mechanism at the rate required for biosynthesis of cell materials (Payne 1973). Rice and Tiedje (1989) found that even low concentrations of ammonium (0.1 μg NH_4-N g $soil^{-1}$) could markedly repress nitrate immobilisation. Despite the occurrence of typically higher ammonium concentrations than this in soils (>5 μg NH_4-N g $soil^{-1}$), especially those that are wet, significant proportions of nitrate removal (5-20%) have been attributed to immobilisation (Ambus *et al.* 1992, D'Angelo and Reddy 1993, Cooke 1994). These observations might be explained by:

(1) nitrate immobilisation occurring in ammonium-free soil microsites (Ambus *et al.* 1992);

(2) the residual rate of nitrate immobilisation in the presence of ammonium being sufficient to account for the accumulation of organic nitrogen (Rice and Tiedje 1989); or

(3) nitrate being first dissimilated to ammonium (extracellularly by DNRA), followed by immobilisation of ammonium, as opposed to nitrate, into the cell.

1.3.5. Plant uptake

Nitrate can be assimilated into plant root cells following the induction of a nitrate-specific permease in the plasma membrane. Within the plant tissues nitrate is first reduced to ammonium via nitrite. Ammonium is then assimilated into glutamate, and this compound is then utilised by the plant for the biosynthesis of amino acids and proteins, principally, but also nucleic acids and other nitrogen-containing compounds. These processes may occur in the root tissues of some plants (e.g. many woody species) or alternatively, in those plants that lack the necessary enzymes in root tissues (e.g. *Xanthium*), nitrate may be transported to the plant shoots (via the xylem) prior to processing (Bray 1983).

In the early stages of plant growth the xylem transports most nitrogenous compounds derived from nitrate assimilation in the roots to the shoots. However, a small fraction is sequestered by the roots for storage as nitrogen reserves or for utilisation in actively growing areas of the root. Nitrogenous compounds transported to shoots in excess of shoot requirements accumulate in soluble nitrogen pools. As the plant matures and shoot senescence and/or seed production begins, nitrogenous compounds (typically amino acids) start to be exported from shoots via the phloem. Exported compounds may be

used in seed development (in annuals), or stored in bark (in woody deciduous species) or perennial roots and rhizomes (in many herbaceous species) as a nitrogen reserve for spring growth (Bray 1983).

Along with nitrate, plants also assimilate ammonium and, to a lesser extent, nitrite and some simple organic compounds (e.g. free amino acids). Preferential uptake of one form may occur under specific environmental conditions. Plants that typically grow in wet soils, where levels of ammonium are normally high and nitrate is virtually non-existent, assimilate ammonium much more efficiently than nitrate. This has been documented for *Typha* species (Reddy 1983), rice, cranberries and blueberries (Nichols 1983, Gunterspergen *et al.* 1991).

Wetland plants, however, grow best with a mixture of ammonium and nitrate. This is because assimilating only ammonium can result in ammonium toxicity. This syndrome develops because ammonium is positively charged, requiring roots to excrete protons to maintain charge balance, and this can result in severe acidification of the rhizosphere (B. Sorrell personal communication). Evidence exists to support the notion that nitrate assimilation by wetland plants does occur. Moraghan (1993) demonstrated nitrate assimilation by *Typha* spp in a freshwater marsh using ^{15}N-labelled nitrate.

In addition to availability of nitrate (for uptake and to induce the nitrate reductase enzyme) the key regulators of nitrate assimilation by plants in wet soils are light, temperature and oxygen. Plants require light for photosynthesis and it is this process which generates energy and carbon for the plant. The availability of this energy source and the soil temperature regulate the uptake of nitrate by the plant. Nitrate uptake is markedly reduced at low temperatures (Lewis 1986). Hence, plant uptake of nitrate occurs principally during the spring and summer months ("the growing season") when light levels, for photosynthesis, and temperatures are higher. The extent to which nitrate uptake occurs outside of the growing season is not well understood (Bowden 1987).

Oxygen is required by plant roots in order to respire. In wet soils, oxygen is generally limiting so plants facilitate the diffusion of oxygen from the atmosphere to the roots via internal, aerenchymatous tissues. Due to the permeability of root surfaces (resulting from the need to enable nutrient uptake), some oxygen is lost to the surrounding soil. Although the loss of oxygen to the surrounding soil may appear wasteful and costly to the plant, there may be a benefit to the plant in this occurring. Provision of oxygen to surrounding soil may stimulate microbial nitrification in the rhizosphere, which, in turn, can supply nitrate back to the wetland plant thereby preventing ammonium toxicity (B. Sorrell personal communication).

1.3.6. Implications of the four processes for nitrogen buffering in riparian zones

Denitrification is the only process that results in permanent removal of nitrogen from soils and is, therefore, beneficial to downstream water quality. Dissimilatory reduction to ammonium merely transforms nitrate to a different nitrogenous water pollutant. The European Union recommended limit for ammonium in drinking water (0.38 mg NH_4-N l^{-1}) is much lower than for nitrate (European Community 1991). Ammonium, however, is much less mobile in soil-water systems than nitrate. It is readily bound to cation exchange sites on clay or organic matter and hence, may be retained in the soil (Lindau *et al.* 1994).

Death of microbial cells will eventually release immobilised nitrate back to the soils' pool of available organic nitrogen. Organic nitrogen may then be transformed to ammonium by the process termed 'mineralisation', and from ammonium back to nitrate by the process called 'nitrification' (Figure 1.5). Similarly, plant uptake generally results in only temporary removal of nitrogen from the soil. Nitrate assimilated into plant tissues will eventually be returned to the soil as organic nitrogen via leaching and decomposition of litter (Lowrance *et al.* 1995). However, both plant and microbial nitrogen pools are potential nitrogen sinks during phases of biomass expansion. While there is some evidence that plant and microbial biomass can be stimulated by nutrient inputs (Ehrenfeld 1987, Smith and Duff 1988), it is unlikely that such growth could continue indefinitely. Plant and microbial pools will probably become nitrogen-saturated at some point with net nitrogen removal declining or stopping entirely thereafter (Aber *et al.* 1989, Groffman *et al.* 1992).

There are two mechanisms by which plant uptake may lead to permanent nitrogen loss. The first occurs naturally, the second occurs via human intervention. The first mechanism begins with the translocation of nitrogen from deeper soil layers to the soil surface. This occurs via uptake of inorganic nitrogen (nitrate, ammonium) by plant roots and the subsequent fall of litter onto the overlying water or surface soil at the end of the growing season. Microbial activity is often much higher in surface soils compared to deeper soils and the soil aerobic-anaerobic interface is typically located close to the soil surface. As a result, this translocation may enhance overall nitrogen loss with sequential mineralisation-nitrification-denitrification reactions occurring in the surface soils in the vicinity of the aerobic-anaerobic interface (Lusby *et al.* 1998). The second mechanism is the harvest of plant shoots. Where the riparian zone is planted with crops this is common practice. However, harvest of non-agricultural vegetation might also be undertaken with economic benefit. Many non-agricultural wetland plants make good livestock fodder, especially grasses like *Glyceria* spp, *Echinochloa* spp and *Vossia* spp (Sculthorpe 1967).

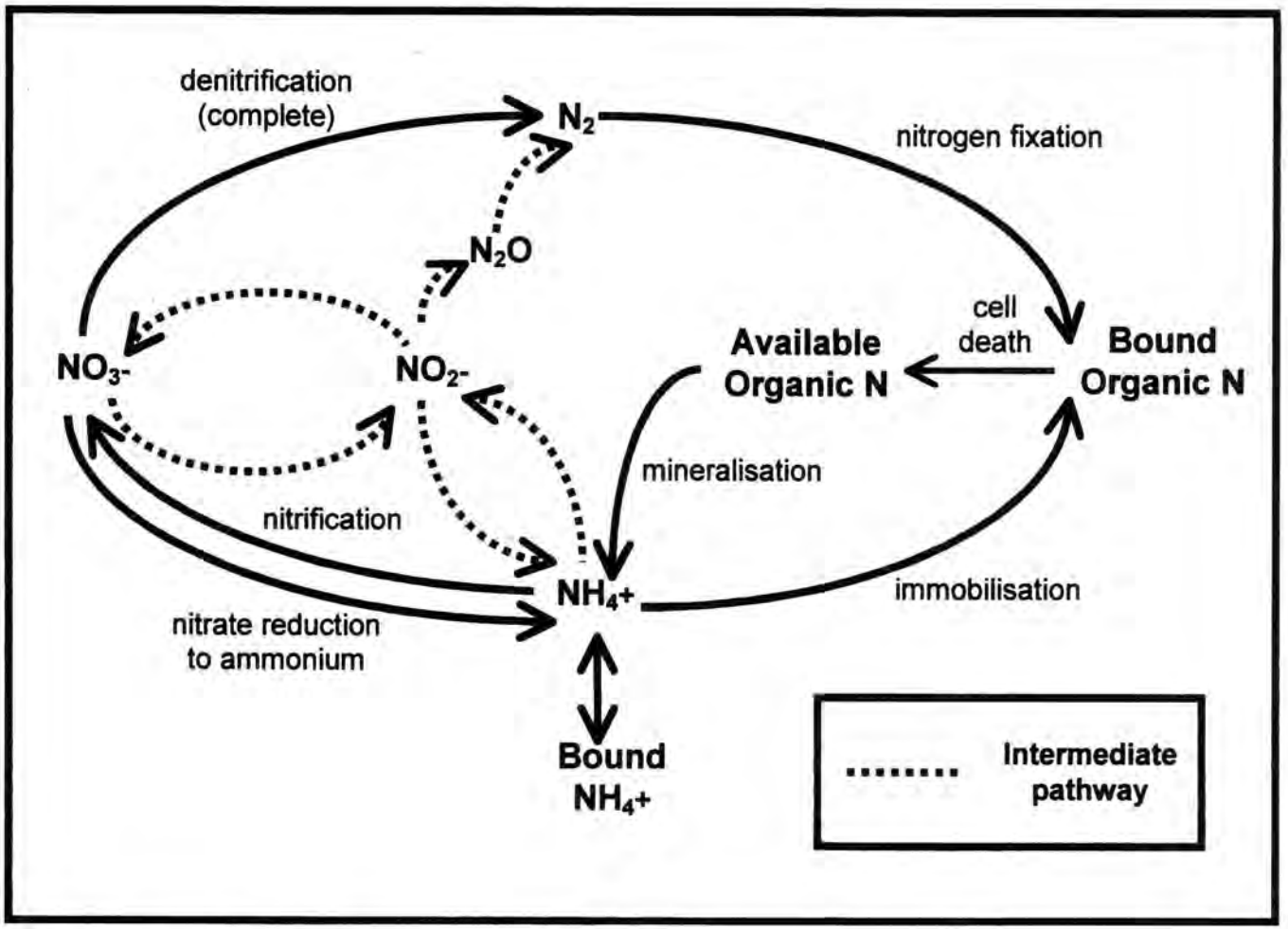


Figure 1.5. The nitrogen cycle.

Provided that all harvested vegetation can regenerate naturally (i.e. no fertilisation), nitrate uptake by these plants will result in permanent nitrogen loss. The true value of both these mechanisms, however, to the nitrate removal capacity of riparian buffer zones, is strongly dependent on plant uptake coinciding with nitrate fluxes from the catchment. If one is to assume that plant uptake occurs only during the 'growing season' (spring and summer) and that nitrate fluxes are greatest outside of this (autumn and winter) these mechanisms probably have minimal value. Further research is required to determine whether these assumptions are generally valid.

1.3.7. Partitioning between nitrate removal processes in wet soils

1.3.7.1. The nature of studies undertaken

The majority of studies on partitioning between nitrate removal processes in wet soils have been performed in the laboratory with sub-samples of mixed, or sometimes intact, soil. Results regarding nitrate partitioning from these studies are shown in Table 1.4, along with relevant information regarding incubation conditions. While studies of this type are relatively quick and easy to perform it is only possible to measure partitioning between denitrification, DNRA and immobilisation; plant uptake is omitted. In some cases, researchers have chosen to omit immobilisation also (Sorensen 1978, Kaspar 1983, King and Nedwell 1985). Nearly all studies have utilised ^{15}N-labelled nitrate (King and Nedwell 1985 is the exception) which enables the researcher to clearly identify the fate of transformed nitrate and, assuming that multiple transformations have not taken place, the process responsible. A number of these studies have also manipulated one or more experimental parameters to investigate the potential factors controlling nitrate partitioning in wet soils. The parameters manipulated include nitrate, carbon substrates, other potential electron donors e.g. sulphide, and redox potential.

Most of the studies have been undertaken with marine or estuarine sediments (Koike and Hattori 1978, Sorensen 1978, Buresh and Patrick 1981, Kaspar 1983, King and Nedwell 1985, Goeyens 1987, Jorgensen 1989). Only five studies utilised soils from freshwater habitats, two were performed with rice paddy soil (Buresh and Patrick 1978, Chen *et al.* 2000), one with lake sediment (D'Angelo and Reddy 1993), one with sediment from a natural wetland receiving sewage effluent (Cooke 1994), and one with riparian fen soil (Ambus *et al.* 1992). The origin of soil in the remaining studies (Reddy *et al.* 1980, Tiedje *et al.* 1981, de Catanzaro *et al.* 1987, Ragab *et al.* 1994) is not stated.

Nitrate partitioning studies undertaken in field plots or in planted laboratory microcosms are detailed in Table 1.5. Only six studies have been identified and only one of these studies has measured partitioning between all (four) nitrate removal processes (i.e.

Nijburg and Laanbroek 1997a). Five of the studies utilised freshwater wet soils (O'Neill and Gordan 1994, Nijburg and Laanbroek 1997a) or sites (Hemond 1983, Peterjohn and Correll 1984, Moraghan 1993). Only one study is estuarine (Lindau *et al.* 1988a). Minimal manipulation of experimental conditions is generally evident in these studies (Moraghan 1993 is the exception) which contrasts with the 'soil only' studies detailed Table 1.4. This presumably results from the increase in experimental scale and associated processing costs.

1.3.7.2. Partitioning between denitrification and DNRA

All of the 'soil only' studies (Table 1.4) have addressed partitioning between these two processes. In the 'plant and soil' studies (Table 1.5) only Nijburg and Laanbroek (1997a) measured partitioning between denitrification and DNRA.

Two key hypotheses have been put forward regarding partitioning between denitrification and DNRA (Tiedje *et al.* 1982, Tiedje 1988, Nijburg and Laanbroek 1997a). They are:

(1) denitrification is favoured by moderately anoxic habitats while DNRA is favoured by highly anoxic habitats
(2) denitrification is favoured by a low electron donor (e.g. organic carbon) to electron acceptor (nitrate) ratio while DNRA is favoured by a high electron donor to electron acceptor ratio

These hypotheses are linked in some respects. Increases in available nitrate will simultaneously increase the oxidation level of soil (as it is an oxidising substance) and lower the electron donor to electron acceptor ratio. This favours denitrification according to both hypotheses. Increases in available organic substrates (e.g. organic matter, organic carbon) may simultaneously lower the oxidation state of the soil by stimulating microbial oxygen demand and raise the electron donor to electron acceptor ratio.

A small number of studies have investigated hypothesis 1 in isolation from hypothesis 2. Buresh and Patrick (1978) used anaerobic pre-incubation of 1 or 20 days, Buresh and Patrick (1981) used controlled redox potential apparatus and Chen *et al.* (2000) used chemical reducing agents, to reduce the oxidation level of the soil without adding microbial carbon substrates. Results from Buresh and Patrick (1978) and Buresh and Patrick (1981) consistently support hypothesis 1. In the study of Chen *et al.* (2000) two rice-paddy soils were tested. Results from one of the soils (Griffiths) are also in support of hypothesis 1 but results from the other soil (Yangzhou) are not.

Table 1.4

Summary of results obtained in laboratory 'soil-only' studies investigating partitioning between nitrate removal processes. Relevant incubation details are included.

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide) Native	Added	Potential oxidising agents (i.e. nitrate) Native	Added	Partitioning between nitrate-reducing processes in the soil (%) Denitrification	DNRA	Immobilisation
Buresh & Patrick 1978 #	rice paddy soil	soil-water slurry	total carbon (8 mg C g^{-1})	none	nitrate (20 µg N g^{-1})	nitrate (100 µg N g^{-1})	76	2	1
	"	"	"	glucose (1 mg C g^{-1})	"	"	63	9	19
	"	"	"	rice straw	"	"	84	3	3
	"	"	"	methanol	"	"	71	1	<0.1
	"	"	"	pre-incubation (1 d)	"	"	92	1	<0.1
	"	"	"	pre-incubation (20 d)	"	"	? (64)	31	5
	"	"	"	glucose (1 mg C g^{-1}) & pre-incub.(1 d)	"	"	40	34	18
	"	"	"	glucose (1.5 mg C g^{-1}) & pre-inc (1 d)	"	"	29	36	34
	"	"	"	rice straw & pre-incubation (1 d)	"	"	89	2	4
	"	"	"	methanol & pre-incubation (1 d)	"	"	83	1	<0.1
	"	"	"	glucose (0.5 mg C g^{-1}) & pre-incub (1 d)	"	"	?	36	34
	"	"	"	glucose (0.5 mg C g^{-1} & pre-incub (1 d) & ammonium (50 µgN g^{-1})	"	"	?	43	18
Koike & Hattori 1978	coastal sediment (Simoda Bay)	soil-water slurry	organic nitrogen (0.56 mg N g^{-1})	-	-	nitrate (15 µg atom N l^{-1})	80	16	4
	coastal sediment (Tokyo Bay)	"	organic nitrogen (3.6 mg N g^{-1})	-	-	"	60	7	33
	coastal sediment (Mangoku-Ura)	"	organic nitrogen (7.8 mg N g^{-1})	-	-	nitrate (30 µg atom N l^{-1})	33	52	15

Table 1.4 continued

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide) Native	Added	Potential oxidising agents (i.e. nitrate) Native	Added	Partitioning between nitrate-reducing processes in the soil (%) Denitrification	DNRA	Immobilisation
Sorensen 1978 #	coastal sediment (0-3 cm depth)	soil-water slurry	-	-	nitrate (<0.1 μmol N cm^{-3})	nitrate (1.5 μmol N cm^{-3})	48	25	-
	coastal sediment (3-6 cm depth)	"	-	-	"	"	34	38	-
	coastal sediment (6-9 cm depth)	"	-	-	"	"	35	38	-
	coastal sediment (9-12 cm depth)	"	-	-	"	"	13	30	-
Reddy *et al.* 1980	organic soil	soil-water slurry (28°C)	total carbon (451 mg C g^{-1})	-	-	nitrate (100 μg N ml^{-1})	97	2.5	0.6
	"	soil-water column (28°C)	"	low BOD floodwater (52 mg l^{-1})	-	nitrate (10-50 μg N ml^{-1}) (~3-15 μg N g^{-1})	96	0.1	4
	"	soil-water column (18°C)	"	"	-	nitrate (10-50 μg N ml^{-1})	93	4	3
	"	soil-water column (8°C)	"	"	-	nitrate (10-50 μg N ml^{-1})	91	5	4
	"	soil-water column (28°C)	"	high BOD floodwater (183 mg l^{-1})	-	nitrate (10-50 μg N ml^{-1})	92	0.4	8
	"	soil-water column (18°C)	"	"	-	nitrate (10-50 μg N ml^{-1})	93	3	4
	"	soil-water column (8°C)	"	"	-	nitrate (10-50 ug N ml^{-1})	80	5	15
Buresh & Patrick 1981	estuarine sediment	soil-water column	total carbon (78 mg C g^{-1})	-	-	nitrate to water (4.5 μg N g^{-1})	85	0.5	15
	"	"	"	-	-	nitrate to soil (4.5 μg N g^{-1})	72	1.6	26
	"	"	"	-	-	nitrate to water (45 μg N g^{-1})	84	0.1	16
	"	"	"	-	-	nitrate to soil (45 μg N g^{-1})	84	0.2	16
	"	soil-water slurry	"	controlled redox apparatus (+300 mV)	-	nitrate (60 μg N g^{-1})	95	m	5
	"	"	"	controlled redox apparatus (+0 mV)	-	nitrate (60 μg N g^{-1})	82	13	5
	"	"	"	controlled redox (-200 mV)	-	nitrate (60 ug N g^{-1})	65	26	9

Table 1.4 continued

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide) Native	Added	Potential oxidising agents (i.e. nitrate) Native	Added	Partitioning between nitrate-reducing processes in the soil (%) Denitrification	DNRA	Immobilisation
Tiedje *et al.* 1981	organic soil (Carlisle muck)	soil-water slurry	-	ammonium (7 μg N g^{-1})	nitrate (100 μg N g^{-1})	nitrate (200 μg N g^{-1})	~81	<5	<3
	"	"	-	ammonium (7 μg N g^{-1}) & glucose (1 mg C g^{-1})	"	"	~89	4	7
Kaspar 1983	inter-tidal sediment	soil-water slurry	volatile solids (32 mg g^{-1})	-	-	nitrate (0.3 ml of 1mM)	79	21	-
	"	"	volatile solids (75 mg g^{-1})	-	-	"	85	15	-
	"	"	volatile solids (20 mg g^{-1})	-	-	"	70	30	-
	"	"	volatile solids (22 mg g^{-1})	-	-	"	82	18	-
King & Nedwell 1985 #	salt marsh sediment	soil-water slurry	-	-	-	nitrate (250 μM)	45	53	-
	"	"	-	-	-	nitrate (500 μM)	54	44	-
	"	"	-	-	-	nitrate (1000 μM)	70	29	-
	"	"	-	-	-	nitrate (1500 μM)	80	16	-
	"	"	-	-	-	nitrate (2000 μM)	83	10	-
deCatanzaro *et al.* 1987 #	unidentified soil	static soil incubation	-	none (1st experiment)	-	nitrate (200 μg N g^{-1})	17	0.5	25
	"		-	alfalfa (46 mg C g^{-1})	-	"	49	38	9
	"	"	-	wheat straw (40 mg C g^{-1})	-	"	83	6	11
	"	"	-	glucose (12 mg C g^{-1})	-	"	71	1	27
	"	"	-	none (2nd experiment)	-	"	48	0.5	52
	"	"	-	alfalfa (2nd exp) (23 mg C g^{-1})	-	"	33	39	28
	"	"	-	glucose (2nd exp) (6 mg C g^{-1})	-	"	79	0.2	21

Table 1.4 continued

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide) Native	Added	Potential oxidising agents (i.e. nitrate) Native	Added	Partitioning between nitrate-reducing processes in the soil (%) Denitrification	DNRA	Immobilisation
deCatanzaro *et al.* 1987 continued	unidentified soil	static soil incubation	-	glucose (6 mg C g^{-1}) & sulphide (0.6 mg g^{-1})	-	"	6	22	36
	"	"	-	glucose (6 mg C g^{-1}) & sulphide (140 mg g^{-1})	-	"	9	2	90
Goeyens *et al.* 1987	coastal sediment	soil-water slurry	-	-	nitrate (35 μM)	nitrate (105 μM)	63	32	4
Jorgensen 1989	estuarine sediment (early autumn	intact soil		ammonium (1000 μM)	nitrate (10-300 μM)	nitrate (700 μM)	11	29	4
	estuarine sediment (later winter)	intact soil		"	"	"	37	8	3
	estuarine sediment (early autumn)	soil-water slurry		"	"	"	30	11	3
	estuarine sediment (late winter)	soil-water slurry		"	"	"	88	4	2
Ambus *et al.* 1992	riparian fen soil	soil core	organic carbon (255 mg C g^{-1})	-	-	nitrate (24.1 μg N g^{-1})	approx. 80	-	approx. 20
	"	"	"	ammonium (50 μg N g^{-1})	-	nitrate (24.1 μg N g^{-1})	approx. 80	-	-
	"	soil-water slurry	"	ammonium (57 μgN g^{-1})	-	nitrate (228 μg N g^{-1})	approx. 80	5-7%	-
	"	"	"	glucose (2.3 mg C g^{-1}) & ammonium (57 μg N g^{-1})	-	nitrate (228 μg N g^{-1})	approx. 80	1-6%	-
	"	"	"	pre-incubation (7 d)	-	nitrate (228 μg N g^{-1})	approx. 80	6-12%	-

Table 1.4 continued

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide)		Potential oxidising agents (i.e. nitrate)		Partitioning between nitrate-reducing processes in the soil (%)		
			Native	Added	Native	Added	Denitrification	DNRA	Immobilisation
D'Angelo & Reddy 1993 #	hypereutrophic lake sediment (bulk)	soil-water slurry	porewater org. C (~32 mg C l^{-1}) (~1400 mg C g^{-1})	-	-	nitrate (1 mg N l^{-1}) (~20-50 μg N g^{-1})	52	34	10
	hypereut. lake sediment (bulk)	"	"	-	-	nitrate (10 mg N l^{-1})	68	12	2
	hypereut.lake sediment (bulk)	"	"	-	-	nitrate (100 mg N l^{-1})	58	4	2
	hypereut. lake sediment (0-2 cm depth)	"	porewater organic carbon (27 mg C l^{-1})	-	-	nitrate (1 mg N ml^{-1}) (~50 mg N g^{-1})	32	13	21
	hypereut. lake sediment (15-20 cm depth)	"	porewater organic carbon (32 mg C l^{-1})	-	-	"	56	29	8
	hypereut. lake sediment (35-40 cm depth)	"	porewater organic carbon (48 mg C l^{-1})	-	-	"	5	6	16
Cooke 1994	sewage wetland surficial soil	soil core	-	-	-	nitrate (10 mg N l^{-1})	60-70	25-35	5-10
Ragab *et al.* 1994 #	Unidentified soil	soil-water column	organic carbon (1.5 mg g^{-1})	none	nitrate (0.3 μg N g^{-1})	nitrate (100 μg N g^{-1})	86	4	4
	"	soil-water column	"	wheat straw (2.3 mg g^{-1})	"	"	93	<0.1	7
	"	soil-water column	"	ammonium (100 μg N g^{-1})	"	"	87	4	4
		soil-water column	"	wheat straw & (2.3 mg g^{-1}) ammonium (100 μg N g^{-1})	"	"	95	<0.1	5
Chen *et al.* 2000	rice paddy soil (Yangshou)	static soil incubation	-	-	-	nitrate (100 μg N g^{-1})	84	13	3
		"	-	reducing agent 1† (redox -100 mV)	-	"	95	4	1
		"	-	reducing agent 2‡ (redox -225 mV)	-	"	95	4	1

Table 1.4 continued

Author	Soil type	Incubation type	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide) Native	Added	Potential oxidising agents (i.e. nitrate) Native	Added	Partitioning between nitrate-reducing processes in the soil (%) Denitrification	DNRA	Immobilisation
Chen *et al.* 2000 continued	rice paddy soil (Griffiths)	"	-	-	-	"	86	12	2
		"	-	reducing agent 1† (redox -100 mV)	-	"	82	15	3
		"	-	reducing agent 2‡ (redox -225 mV)	-	"	61	27	12

† reducing agent 1 = sodium thioglycollate (0.5 g l^{-1})
‡ reducing agent 2 = *L*-cysteine (0.25 g l^{-1})

partitioning reported as a percentage of nitrate added as opposed to a percentage of nitrate removed

Table 1.5

Summary of results obtained from *in situ* and microcosm 'plant and soil' studies investigating partitioning between nitrate removal processes. Relevant incubation details are included.

Author	Soil type	Type of study	Potential reducing agents (i.e. ammonium, carbon, organic matter, pre-incubation, sulphide)		Potential oxidising agents (i.e. nitrate)		Partitioning between nitrate-reducing processes in the soil (%)			
			Native	Added	Native	Added	Denitrification	DNRA	Immobilisation	Plant uptake
Hemond 1983	*Sphagnum* bog soil	*in situ* plot with ^{15}N label	-	-	-	nitrate to water (~8 ng N g^{-1})	? (75)	25	? (75)	? (75)
Peterjohn & Correll 1984	broadleaved, deciduous riparian forest	watershed nitrogen budget					——— 67 ———			33
Lindau *et al.* 1988a	coastal swamp sediment	*in situ* plot with ^{15}N label	-	-	-	nitrate to water (10 g N m^{-2}) (~1 mg N g^{-1})	94	——— 6 ———		
Moraghan 1993	*Typha* marsh soil	*in situ* plot with ^{15}N label	-	-	nitrate in runoff (1-10 mg l^{-1})	nitrate to water (15.4 mg N l^{-1}) (~1.5 mg N g^{-1})	56	——— 12 ———		32
"	"	"	-	-	"	nitrate to water (46 mg N l^{-1}) then (15.4 mg N l^{-1})	82	——— 7 ———		11
O'Neill & Gordon 1994	unidentified loam soil (artificial riparian zone)	bare & planted (Poplar) soil microcosms	-	-	-	nitrate to soil (12-24 mg N l^{-1})	——— 86-89 ———			11-14
Nijburg & Laanbroek 1997a	*Phragmites australis* lake littoral sediment	intact cores (including plant rhizomes) with ^{15}N label	organic carbon (11 mg C g^{-1})	-	nitrate (0 µg N g^{-1})	nitrate to soil (2 µg N g^{-1})	25	4	10	61

Most of the studies undertaken have investigated the importance of the electron donor to electron acceptor ratio in regulating partitioning. They have done this by either manipulating the amount of electron donor (e.g. carbon sources, such as glucose, plant residues) or the amount of electron acceptor (nitrate). Results in support of hypothesis 2 have consistently arisen from studies that have manipulated only the amount of electron acceptor; i.e. nitrate (King and Nedwell 1985, D'Angelo and Reddy 1993); and *not* the amount of electron donor.

Results from studies manipulating the electron donor are generally inconsistent. Some authors have found that additions of electron donor enhance both denitrification and DNRA. With additions of alfalfa and wheat straw, de Catanzaro *et al.* (1987) found that both processes were enhanced, although alfalfa enhanced DNRA more and wheat straw enhanced denitrification more. Ragab *et al.* (1994) also found that wheat straw enhanced denitrification but in their study it did not enhance DNRA at all. Glucose has also been found to enhance denitrification but not DNRA in some studies (Tiedje *et al.* 1981, de Catanzaro *et al.* 1987). However, in another study DNRA was enhanced and denitrification reduced with comparable additions of glucose, more so when combined with a 1 day pre-incubation (Buresh and Patrick 1978). In the same study, additions of rice straw and methanol did not stimulate DNRA at all, or denitrification to any marked extent. Sulphide, also a potential electron donor, was found to enhance DNRA and inhibit denitrification at moderate concentrations but inhibited both DNRA and denitrification at high concentrations (de Catanzaro *et al.* 1987). Ammonium was found to enhance DNRA but not denitrification by Buresh and Patrick (1978).

1.3.7.3. Partitioning between DNRA and immobilisation

DNRA and immobilisation processes both result in nitrate being transformed to ammonium but differ in respect to where that ammonium ends up. With DNRA it is excreted from the cell while with immobilisation it is utilised within the cell. Presumably many microorganisms are capable of both DNRA and immobilisation. So, when is ammonium utilised by the cell and when is it excreted?

Three hypotheses have been put forward regarding the occurrence of immobilisation in soils:

(1) that a high soil, or substrate, carbon to nitrogen ratio (>20) stimulates immobilisation and a low carbon to nitrogen ratio (<20) represses immobilisation

(2) the presence of ammonium represses immobilisation

(3) higher soil oxidation will increase immobilisation as aerobic microorganisms are more efficient than anaerobic microorganisms.

Additions of organic matter or organic carbon to the soil might therefore be expected to stimulate immobilisation of nitrate while additions of nitrate might be expected to reduce immobilisation of nitrate. This is very similar to the scenario discussed previously for DNRA. But, in contrast to DNRA, is immobilisation consistently stimulated by additions of carbon?

The results from previous studies provide little evidence in support of hypothesis 1. Additions of glucose were shown to increase immobilisation in a rice paddy soil (Buresh and Patrick 1981) and in an unidentified soil (Tiedje *et al.* 1981). However, glucose did not increase immobilisation in another unidentified soil (de Catanzaro *et al.* 1987). High BOD floodwater did enhance immobilisation in relation to low BOD floodwater in an organic soil (Reddy *et al.* 1980) and rice straw marginally increased immobilisation in rice paddy soil (Buresh and Patrick 1978). However, additions of methanol (Buresh and Patrick 1978), alfalfa (de Catanzaro *et al.* 1987) and wheat straw (Buresh and Patrick 1978, de Catanzaro *et al.* 1987) did not increase immobilisation. Interestingly, additions of sulphide, especially at high concentrations, stimulated immobilisation considerably in an unidentified soil (de Catanzaro *et al.* 1987). Sulphide might be utilised as an electron donor by some microorganisms and this result supports hypothesis 1.

In support of hypothesis 1, the most consistent results come from studies (or treatments within studies) where additions of nitrate alone have been varied. Lower nitrate additions have typically resulted in higher amounts of immobilisation (Buresh and Patrick 1981, D'Angelo and Reddy 1993).

Hypothesis 2, regarding repression of immobilisation by ammonium has received comparatively less attention in nitrate partitioning studies. Ragab *et al.* (1994) found that the addition of 100 μg NH_4-N g $soil^{-1}$ did not decrease immobilisation. However, the fraction of nitrate removed by immobilisation in this soil was low (4%). Relatively high amounts of immobilisation (20-40% of nitrate removal) have frequently been reported in wet soils (Koike and Hattori 1978, Buresh and Patrick 1981, D'Angelo and Reddy 1993, Ragab *et al.* 1994) where high ammonium concentrations tend to accumulate naturally due to inhibition of nitrification by reducing conditions. Although repression of nitrate assimilation has been demonstrated for some soil microorganisms (Rice and Tiedje 1989), its importance with respect to *in situ* conditions is essentially unknown.

There is little support for hypothesis 3, increased immobilisation under more oxidised conditions, in the studies detailed. Instead, immobilisation was typically lower under more oxidised conditions i.e. no pre-incubation (Buresh and Patrick 1978) and higher soil redox potential (Buresh and Patrick 1981, Chen *et al.* 2000). In relation to this hypothesis it has been proposed that the amount of immobilisation in soil depth profiles will be

highest in the surface soil layer and decline with depth as a result of reduced amounts of oxygen penetration. The only study to investigate immobilisation in different layers of soil was that of D'Angelo and Reddy (1993). Although they found the highest amount of immobilisation in the surface layer (21%), there was no consistent decline with depth. Instead, 8% and 16% were measured at depths of 15-20 cm and 35-40 cm, respectively.

1.3.7.4. Partitioning between plant uptake and microbally-mediated processes

Only a single hypothesis has been put forward regarding nitrate partitioning between plant uptake and microbially-mediated processes. The hypothesis is that plant uptake will only remove nitrate during the 'growing season' while microorganisms may continue to transform or immobilise nitrate outside of the growing season. This hypothesis has not yet been rigorously tested despite its' extreme relevance to nitrate removal in soil environments. Typically, the greatest fluxes of nitrate from the catchment coincide with increased precipitation and lower evapo-transpiration, in the autumn and winter months (Burt and Arkell 1987). If the above hypothesis is correct, then plant uptake is probably a process of little significance to nitrate removal in soils. If the hypothesis is not correct, the importance of plant uptake relative to other nitrate removal processes requires investigation both during, and outside of, the growing season. Nearly all of the studies detailed in Table 1.5 were undertaken *in situ* (Hemond 1983, Peterjohn and Correll 1984, Moraghan 1993), or outdoors (O'Neill and Gordon 1994), during the growing season, or in the laboratory under mimicked 'growing season' conditions (Nijburg and Laanbroek 1997a). Lindau *et al.* (1988a) did not state when their study was undertaken.

The results of these studies show that plant uptake accounted for up to 61% of nitrate removal. O'Neill and Gordon (1994) found that plant uptake by juvenile Carolina poplar removed just over one tenth of nitrate in an artificially created riparian zone. Peterjohn and Correll (1984) estimated that 33% of nitrate removed in a riparian forest was due to assimilation into the broadleaved, deciduous forest. The highest amount of uptake (61%) was measured for reed, *Phragmites australis*, in lake littoral sediments (Nijburg and Laanbroek 1997a). Denitrification, DNRA and immobilisation in this same study accounted for 25, 4 and 10% of nitrate removal, respectively. *Phragmites australis* therefore competed very successfully with soil microorganisms for available nitrate. In contrast, Lindau *et al.* (1988a) measured very high denitrification (94%) and minimal nitrate retention in coastal swamp sediment. They did not isolate the contribution of plant uptake from DNRA or immobilisation in nitrate retention but it could not have exceeded 6%. The vegetation of their coastal swamp forest (cypress, tupelo and gum) therefore competed poorly with denitrifying microorganisms for available nitrate. Hemond (1983) found that at least one quarter of the nitrate removed in a *Sphagnum* bog was retained in

the soil via DNRA but he was unable to determine partitioning between the other three pathways with any certainty.

In the only study where an attempt was made to manipulate experimental conditions *in situ,* Moraghan (1993) found that the amount of nitrate removed by plant uptake decreased from 32 to 11% when the experimental plots were 'primed' with a dose of high nitrate prior to the experiment. Nitrate retention by DNRA and immobilisation (jointly assessed) also decreased from 12 to 7%, while denitrification increased from 56 to 82%. Either oxidation of the marsh soil or a decrease in the soil electron donor to acceptor ratio, resulting from 'priming' the soil with additional nitrate, appeared to increase the competitiveness of denitrifying microorganisms relative to the plant, in particular, but also relative to the DNRA and immobilising microorganisms. The decreased competitiveness of DNRA and immobilising microorganisms with high concentrations of nitrate is consistent with the results from 'soil only' experiments discussed in the previous two sections.

1.3.7.5. Synthesis

In summary, despite a number of studies being undertaken, it is not possible to predict partitioning between nitrate removal processes with any great certainty. However, increased additions of nitrate do appear to make denitrifying microorganisms more competitive in wet soils in relation to plants, DNRA and immobilising microorganisms. Increasing the oxidation level of wet soil also seems to have a similar effect. This suggests that the 'nitrate' effect is essentially one of oxidising the soil rather than altering the electron donor to electron acceptor ratio. The inconsistent results from experiments that have manipulated the ratio by addition of electron donor (e.g. organic matter, organic carbon substrates) support this notion. Future research should therefore concentrate on evaluating the variability in nitrate partitioning both within, and between, habitat types (e.g. riparian buffer zones, lake sediments etc), with additions of nitrate, and levels of soil oxidation, that reflect those encountered *in situ.*

Chapter 2

Research design

2.1. Research aim and objectives

The overall aim of this research was to evaluate the sustainability of the nitrogen removal function in riparian buffer zones.

To achieve this aim it was necessary to satisfy the following objectives:

(1) to measure nitrate and total nitrogen removal in representative sites; and

(2) to determine the fate of removed nitrate.

2.2. Special considerations

2.2.1. Research undertaken in the United Kingdom and New Zealand

This research is sponsored by the National Institute of Water and Atmospheric Research in New Zealand (NIWA) and the European Union-funded NICOLAS (NItrogen COntrol by Landscape structures in Agricultural EnvironmentS) project. NIWA has a large research group studying river ecosystem functioning but many publications relating specifically to riparian buffer zones have been produced. NICOLAS project researchers are studying the nitrate buffering capacity of landscape structures, particularly riparian buffer zones. As a consequence, this study is intended to generate both United Kingdom and New Zealand based data relating to nitrogen removal and the fate of nitrate in riparian buffer zones for the benefit of the NICOLAS project and NIWA, respectively.

While sponsorship has imposed some restriction on the countries and, to a certain extent, the localities where fieldwork must be conducted, there are some major advantages arising from it:

(1) access to specialist ^{15}N analytical facilities at NIWA, which are particularly useful for studying the processes responsible for nitrate removal in riparian buffer zones;

(2) the opportunity to collaborate with other researchers on the NICOLAS project;

(3) the opportunity to undertake riparian buffer zone research in two countries where the context for this research i.e. 'the nitrate problem' is somewhat different (Table 2.1).

Table 2.1

The differing context of the nitrate problem in the United Kingdom and New Zealand.

United Kingdom	New Zealand
- relatively high level of nitrate pollution	- relatively low level of nitrate pollution
- tendency towards phosphorus limitation of algal growth (OECD 1982)	- tendency towards nitrogen limitation of algal growth (White *et al.* 1986)
- focus on public health threat as pollution occasionally exceeds EC acceptable limit in some rivers	- focus on environmental threat (need to maintain low nitrate pollution due to 'ecotourism' industry)
- long history of agriculture (legacy of tile drains and many modified riparian buffer zones)	- short history (150 years) of agriculture (many riparian zones in pristine state)

2.2.2. Focus on autumn and winter seasons

Attempts have been made to focus much of this research on nitrogen removal in riparian buffer zones during the autumn and winter months for the following reasons:

(1) in temperate climates, such as the United Kingdom and New Zealand, the greatest transport of water and nitrate from the catchment typically occurs in autumn and winter (Burt and Arkell 1987, Addiscott *et al.* 1991).

(2) most previous studies of nitrogen and nitrate removal in riparian buffer zones have focussed on the growing season (except Haycock and Burt 1993a) and there is an urgent requirement for further information on the extent of removal, and the processes responsible, outside of the growing season.

2.3. Thesis structure

In addition to this chapter and the preceding introductory one, the thesis consists of four 'experimental' chapters and a final 'synthesis and conclusions' chapter. The four 'experimental' chapters represent separate studies on different aspects of nitrogen removal and the fate of nitrate in riparian buffer zones. Attempts have been made to present each of these chapters in a generalised scientific journal format.

The first experimental chapter (**Chapter 3**) is essentially a comparative study of nitrogen removal from subsurface agricultural runoff in three riparian buffer zone sites (same stretch of river, different vegetation cover): a pastoral site, a woodland site and a wetland site. A key objective of this study was to determine the occurrence and the extent of nitrate removal in the three sites during the autumn-winter period. However, a second complementary objective was to ascertain the importance of other dissolved nitrogen fractions (ammonium and organic nitrogen), relative to nitrate, in agricultural runoff and as

potential end-products of nitrate removal within the riparian buffer zones. This field-based study was undertaken using extensive grid networks of piezometers to monitor changes in the concentrations of dissolved nitrogen forms from agricultural land, through the riparian buffer zones, to the river.

In the second experimental chapter (**Chapter 4**) a more detailed hydrological and hydrochemical investigation of the riparian wetland site is detailed. The riparian wetland site was singled out for further investigation of this nature for the following reasons:

(1) the hydrology of this site appeared to be more complex, principally as a result of inflowing, standing, and outflowing surface water in addition to subsurface runoff;

(2) it seemed that the site intercepted comparatively more catchment runoff due to its location in a hillslope hollow; and

(3) nitrogen inputs to this site in surface runoff, principally nitrate, were high.

The basic subsurface hydrological and hydrochemical data collected for the previous study (Chapter 3) were supplemented with additional measurements, enabling the calculation of water and dissolved nitrogen *fluxes* across the site. Key objectives of this study were:

(1) to validate, or otherwise, the subsurface nitrogen removal efficiency, estimated with nitrogen concentration data, by calculating removal efficiency with nitrogen flux data;

(2) to determine the surface-water nitrogen removal efficiency of the site with nitrogen flux data.

Further investigation of the riparian wetland site, this time in a biological sense, was also undertaken, as described in the third experimental chapter (**Chapter 5**). This chapter outlines a laboratory study of partitioning between nitrate removal processes (denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and immobilisation) in the riparian wetland soil using ^{15}N-labelled nitrate as a tracer. This study was intended to directly complement the autumn-winter field investigation of the site (Chapters 3 and 4); the soil used in this study was collected from the riparian wetland in late winter. In addition, three different nitrate treatments were used in this study, which corresponded to the range of nitrate concentrations encountered *in situ*. Isotope dilution methodology was also jointly employed in this study to enable concurrent measurement of mineralisation, and estimates of total ($^{14}N+^{15}N$) nitrogen transformation rates (principally denitrification, DNRA, immobilisation and mineralisation) as opposed to those based on tracer (^{15}N) alone. The concurrent measurement of mineralisation was considered especially pertinent as observed increases in ammonium in the riparian wetland site may have resulted from this process, instead of, or in addition to, DNRA.

The potential role of the wetland plant in nitrate removal in riparian buffer zones is then addressed in the last experimental chapter (**Chapter 6**). This laboratory microcosm study was undertaken in New Zealand with soil and plants collected from a riparian wetland site there that, like the United Kingdom site, intercepted nitrate-rich runoff from a sheep-grazed pastoral catchment and had comparable vegetation cover (i.e. *Glyceria*). Key objectives of the study were:

(1) to measure nitrate removal partitioning between plant uptake, denitrification, DNRA and immobilisation;

(2) to determine the effect of the wetland plant (in harvested and non-harvested form) on partitioning between nitrate removal processes in the soil. The effect of (shoot) harvest is of interest as it results in permanent loss of nitrate (as opposed to recycling) through plant uptake.

The final chapter of this thesis (**Chapter 7**) brings together the findings of the four experimental chapters and discusses their significance with respect to current understandings of nitrogen removal and the fate of nitrate in riparian buffer zones.

Chapter 3

Dissolved nitrogen removal in subsurface agricultural runoff in pasture, wood and wetland riparian buffer zones

3.1. Abstract

Dissolved nitrogen concentrations (nitrate, ammonium, dissolved organic nitrogen (DON) and total dissolved nitrogen (TDN)) were measured in subsurface runoff moving from sheep-grazed pasture through three riparian zones in a United Kingdom agricultural catchment from late summer to late winter. Water samples were collected on a monthly basis from a grid network of piezometers that extended from the pastoral upland to the river edge across each riparian zone. In inputs to the riparian zones, DON concentrations (<0.1-3.2 mg N l^{-1}; median 0.7) and ammonium concentrations (<0.1-4 mg N l^{-1}; median 0.2) frequently exceeded nitrate concentrations (<0.1-1.7 mg N l^{-1}; median 0.02). In the pastoral upland, the median TDN concentration of subsurface runoff (<2 mg N l^{-1}) was markedly lower than that of hill-side spring water (22 mg N l^{-1}), which suggests that the slowly-permeable catchment soils may buffer >90% of leached nitrogen. Near the middle of the wood and wetland riparian zones high concentrations of inorganic nitrogen (2-20 mg N l^{-1}) were frequently detected. An internal 'vegetative' source and an external 'spring' source were proposed to explain the occurrence of these high concentrations at the wood and wetland sites, respectively. All riparian zones were poor buffers of agriculturally-derived nitrogen in subsurface runoff as total dissolved nitrogen concentrations were rarely altered significantly (wood; 1 of 7 sampling dates, late autumn, 46% decrease) or never altered significantly (wetland and pasture) by transit across them. However, the wood and wetland riparian zones sometimes functioned as transformers of dissolved nitrogen, replacing one form with others. The riparian wood significantly decreased the DON concentration on 3 of 7 sampling dates by 58-92% and the riparian wetland significantly decreased the highest nitrate input concentrations (0.14-1.7 mg N l^{-1}) on 3 of 7 sampling days by 93-99%.

3.2. Introduction

Riparian zones, positioned between agricultural land and rivers, have been recognised since the early 1970s as important interceptors of subsurface runoff from catchments (Waikato Valley Authority 1973). These zones are often synonymous with the river floodplain (Haycock and Burt 1993b, Burt *et al.* 1999). Given their transitional location between terrestrial and aquatic environments, riparian zones are characterised by high physico-chemical and biological diversity. This high diversity denotes a considerable potential for the assimilation and transformation of water-borne solutes (Risser 1990,

Holland and Risser 1991). Of great interest is the potential for removal of nitrogen in runoff from agricultural catchments where the presence of stock and/or the application of nitrogenous fertilisers increases the amount of nitrogen that may be leached from soils. Increasing concentrations of nitrogen in catchment runoff can contribute to the progressive enrichment, or eutrophication, of downstream water bodies.

The nitrogen buffering potential of various types of vegetated riparian zone has been studied in recent years, including grassland or pasture sites (Cooper 1990, Haycock and Burt 1993a, Burt *et al.* 1999), woodland or forested sites (Lowrance *et al.* 1984, Peterjohn and Correll 1984, Pinay and Decamps 1988, Haycock and Pinay 1993, Jordan *et al.* 1993) and wetland sites (Hanson *et al.* 1994). Only a small number of studies have attempted to compare the relative effectiveness of different types of vegetated riparian zone and results are somewhat inconclusive (Haycock and Pinay 1993, Osborne and Kovacic 1993). However, there is general agreement in the literature that the capacity for nitrogen buffering in a riparian zone will be determined, to a large extent, by the hydrological characteristics of the site (Hill 1996, Tabacchi *et al.* 1998, Burt *et al.* 1999). In particular, the extent and duration of runoff contact with organic-rich and biologically-active surface soils, and with the plant root zone, appears to be a critical determinant.

In this study the capacity of three types of vegetated riparian zone (pasture, wood and wetland) to transform and remove nitrogen in subsurface runoff was compared in a single, sheep-grazed pastoral catchment. Specific objectives were: (1) to measure nitrate, ammonium and DON concentrations in subsurface runoff entering, and in transit across, the riparian zones; and (2) to concurrently monitor the position of the water table and the redox potential of runoff in order to gain insights into the likely biological transformations responsible for nitrogen concentration changes.

3.3. Materials and method

3.3.1. Study site description

3.3.1.1. The catchment and study area

The three riparian zone study sites are located in close proximity to one another along a headwater stretch of the River Skerne near Trimdon, County Durham, United Kingdom (latitude 54° 42'N, longitude 1° 23'W, National grid reference NZ397343) (Figure 3.1). Mean daily maximum and minimum temperatures and mean annual rainfall (1961-1990 averages) at nearby Durham Observatory are 4.9 °C, 12.3 °C and 650 mm, respectively. The catchment area for the River Skerne above the study sites is approximately 8 km^2 with 80% agricultural land use, mainly sown grassland and cereal crops. The discharge

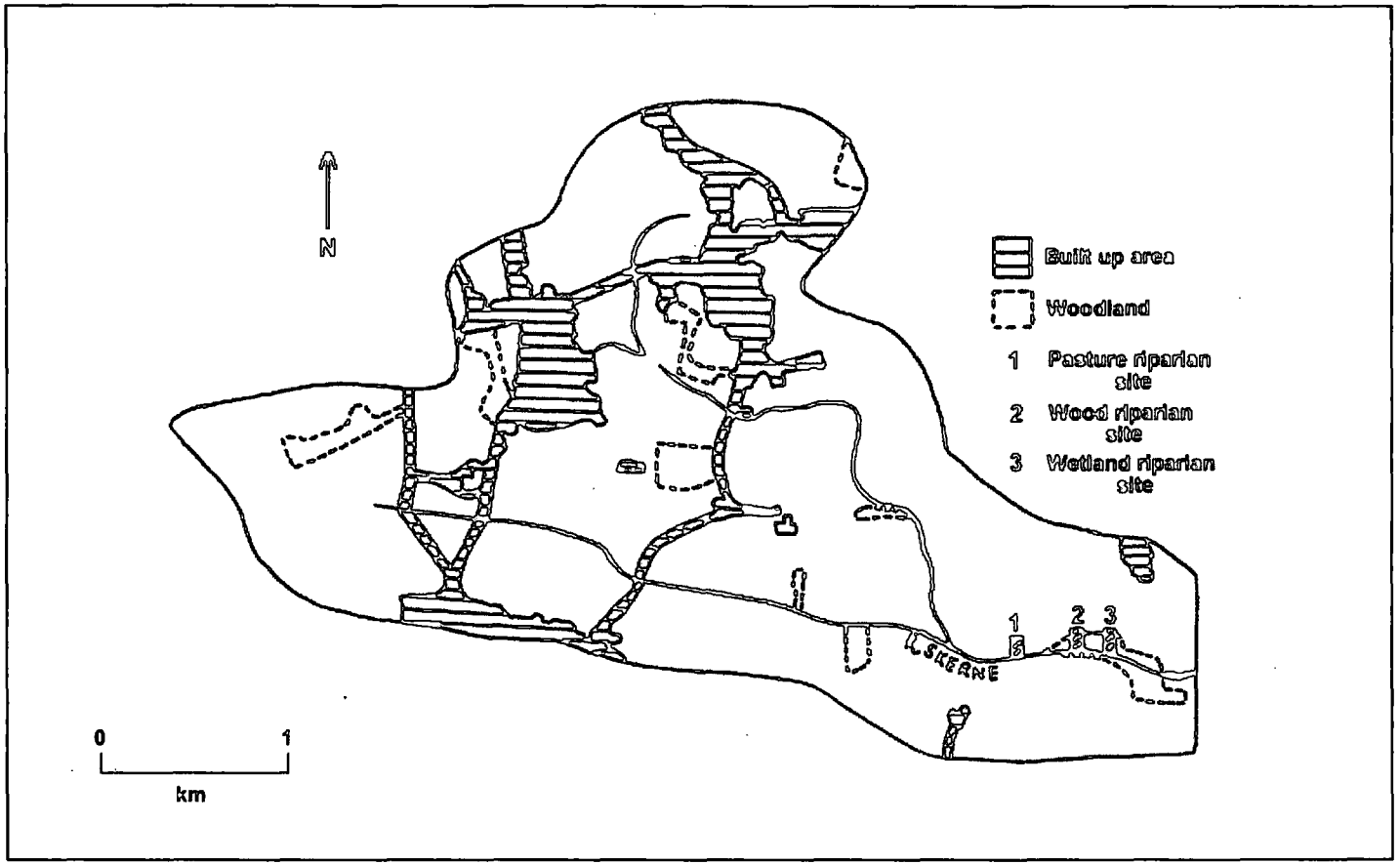


Figure 3.1. The catchment and study area.

and dissolved nitrogen concentration of the River Skerne adjacent to the study sites are around 0.5 m^3 sec^{-1} and 5 mg N l^{-1}, respectively.

All three riparian zones are backed by long (100-200 m), sheep-grazed pastoral hillslopes of moderate (4-5°) angle. During the study period, all areas under pasture were infrequently fertilised with farmyard (cattle, sheep) manure. At all study sites, a 10-20 cm thick organic topsoil overlies an approximately 30 m thick boulder clay deposit of Pleistocene origin. Subsurface runoff travels through the topsoil and more permeable horizons of the boulder clay deposit close to the ground surface. Deeper horizons (2-3 m below the ground surface) are firmly compacted and gleyed, and probably act as an aquiclude to downward percolating runoff. The boulder clay is underlain by Magnesian Limestone (about 130 m thick) and, at even greater depth, by coal measures of the Upper Carboniferous Period. A number of surface springs occur in the area surrounding the study sites. One spring, originating high up on the hillslope above the wetland riparian zone eventually flows into this site. These springs are associated with irregular sand and gravel deposits that occur close to the ground surface in conjunction with the boulder clay (British Geological Survey 1975a, 1975b). They are not considered to be deep groundwater springs associated with the Magnesian Limestone.

3.3.1.2. The pasture riparian site

The pasture site is located furthest upstream. The hillslope above the riparian zone and the riparian zone itself are vegetated entirely by herbaceous, pastoral vegetation; perennial ryegrass (*Lolium perenne*), rough meadowgrass (*Poa trivalis*) and white clover (*Trifolium repens*). The entire site, including river bank and channel, is accessible to grazing stock. The floodplain is elevated considerably above the river channel and the banks are steep and eroding (Figure 3.2 and Plate I).

3.3.1.3. The wood riparian site

The wood site is located approximately 300 m downstream of the pasture site and is vegetated by 50-60 year old sycamore (*Acer pseudoplantanus*), beech (*Fagus sylvatica*) and larch (*Larix decidua*). The area of woodland is ~25 m wide and is located between grazed pasture upslope and a ~15 m width of grassland next to the river (Plate II and Figure 3.3). The grassland consists of perennial ryegrass (*Lolium perenne*), daisy (*Bellis perennis*), white clover (*Trifolium repens*), creeping buttercup (*Ranunculus repens*) and common sorrel (*Rumex acetosa*). The wood and river edge grass areas are separated from the pasture by a fence to prevent stock grazing. However, during the study period a small number of sheep (<5) broke through the fence on occasion.

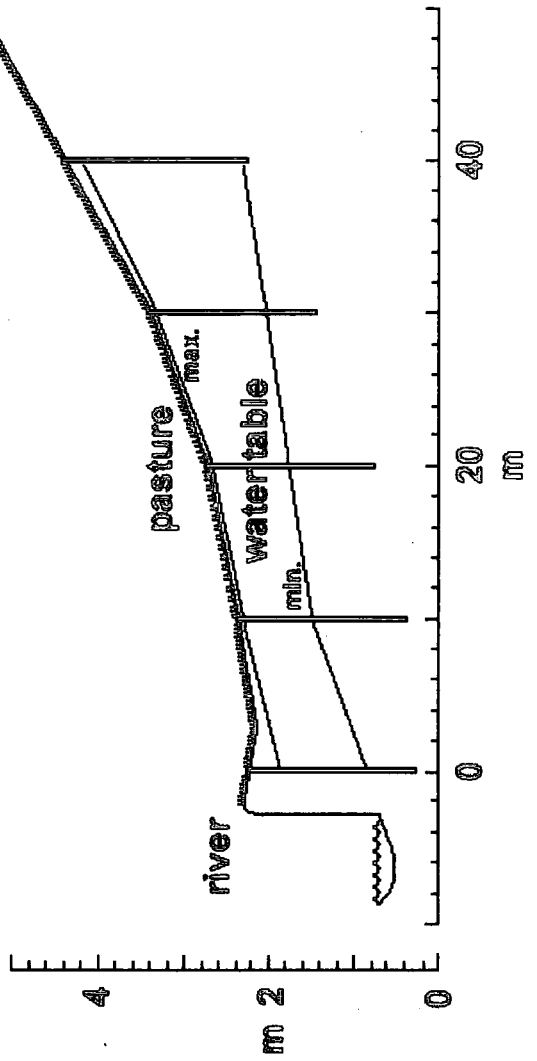


Figure 3.2. Cross-section of the pasture riparian site.

Plate I. The pasture riparian site.

Plate II. The wood riparian site.

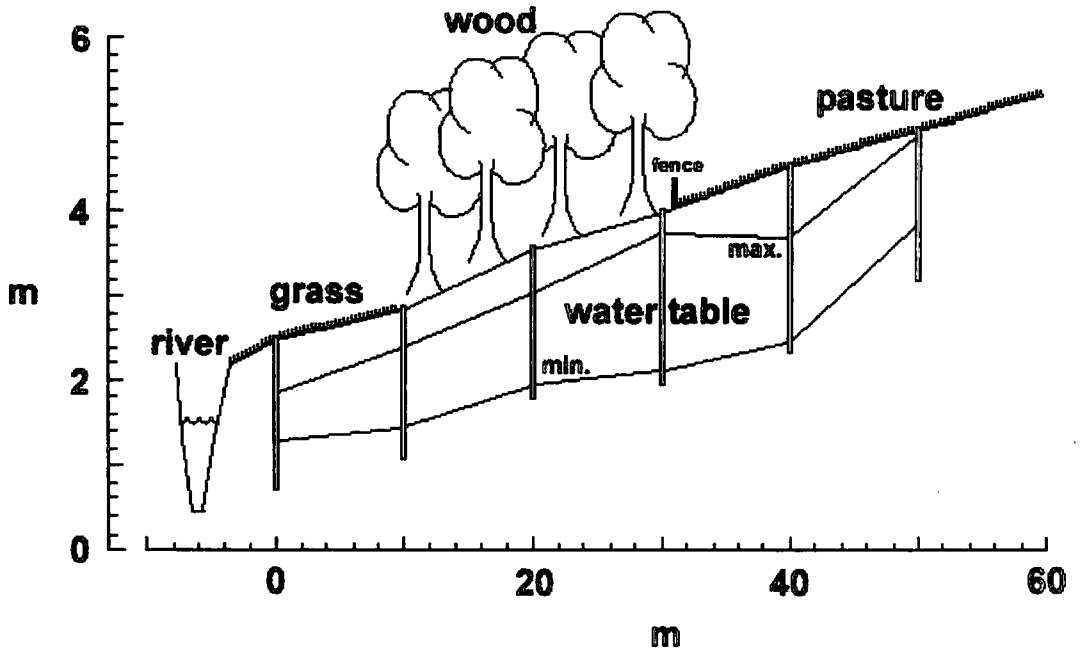


Figure 3.3. Cross-section of the wood riparian site.

The river channel adjacent to this site is much deeper and wider than the channel at the pasture site, and the river flows more slowly. This is due, at least in part, to the presence of a large reservoir (Hurworth Burn) located only a short distance downstream that restricts discharge. The effect of this restriction on river flow is most obvious at, and downstream of, the wood site. The river channel adjacent to this site has been dredged and straightened in the past.

3.3.1.4. The wetland riparian site

The wetland site is located approximately 200 m downstream of the wood site. This site consists of a ~45 m width of variably saturated herbaceous wetland located between grazed pasture upslope and a ~15 m width of artificially elevated grass river edge (Figure 3.4 and Plate III). The wetland is vegetated by floating sweetgrass (*Glyceria fluitans*), water forget-me-not (*Myosotis scorpiodes*), watercress (*Nasturtium officinale*) and soft rush (*Juncus effusus*). The grass river edge is vegetated by the same herbaceous species as the river edge at the wood site. The wetland and grass areas are separated from the pasture hillslope by a fence that prevents stock grazing; however, as noted for the wood site, a small number of sheep sometimes managed to break through the fence. The river channel adjacent to this site is slightly deeper and wider than the channel at the wood site. The channel here has also been dredged in the past and dredge spoil has been placed along the river edge to create an artificially elevated grass berm. This berm appears to act as a barrier to the flow of surface water from this site as water ponds behind it; however a single outflow channel has developed through a low point in the berm. Surface flow is most obviously generated at this site by the inflow of the single, hillslope spring. However, the surface discharge from the site is visually greater than this inflow, which indicates that upwelling of subsurface runoff occurs within the wetland. Surface water flows and chemistry were measured and monitored in a companion study of the interactions between subsurface and surface runoff at this site (Chapter 4).

3.3.2. Piezometer installation

Piezometers to monitor and sample subsurface runoff across the study sites were installed as a 10 x 10 m grid network at the pasture and wood sites, and a 15 x 15 m grid network at the wetland site. The networks consisted of: four upslope-pasture to river-edge transects of five piezometers at the pasture site (Figure 3.5a), four transects of six piezometers at the wood site (Figure 3.5b) and five transects of six piezometers at the wetland site (Figure 3.5c).

Piezometers consisted of an open hole drilled to an approximate depth of 2 m with a hand or power auger. A PVC pipe (5 cm inner diameter) with holes drilled around the

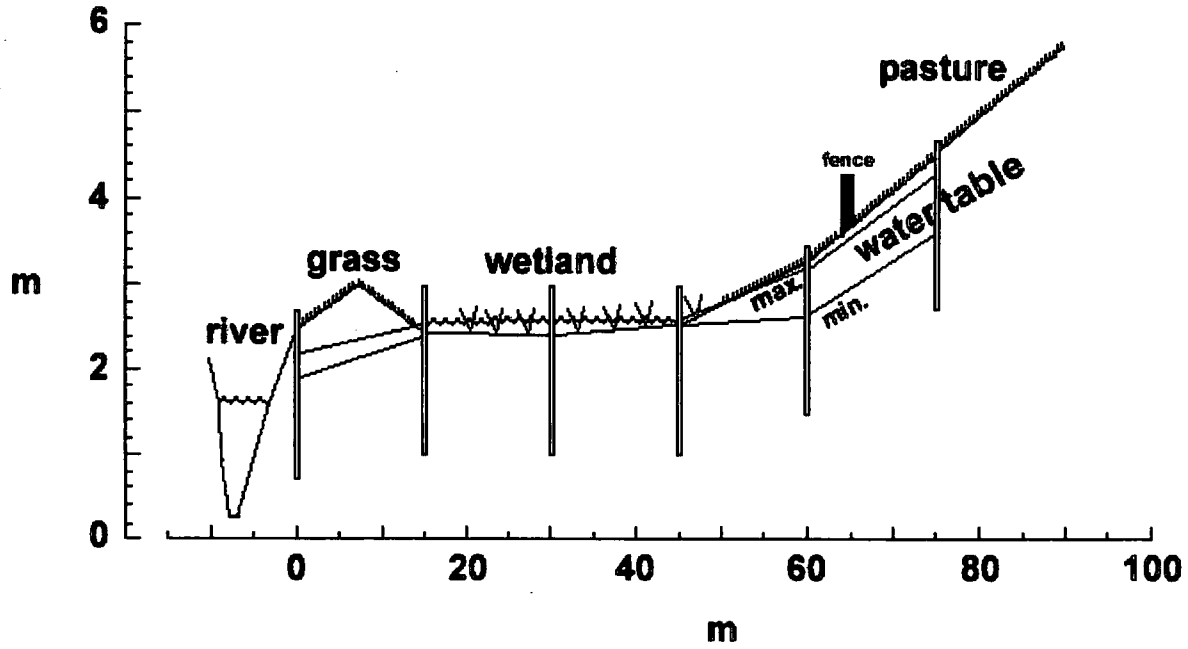


Figure 3.4. Cross-sectiion of the wetland riparian site.

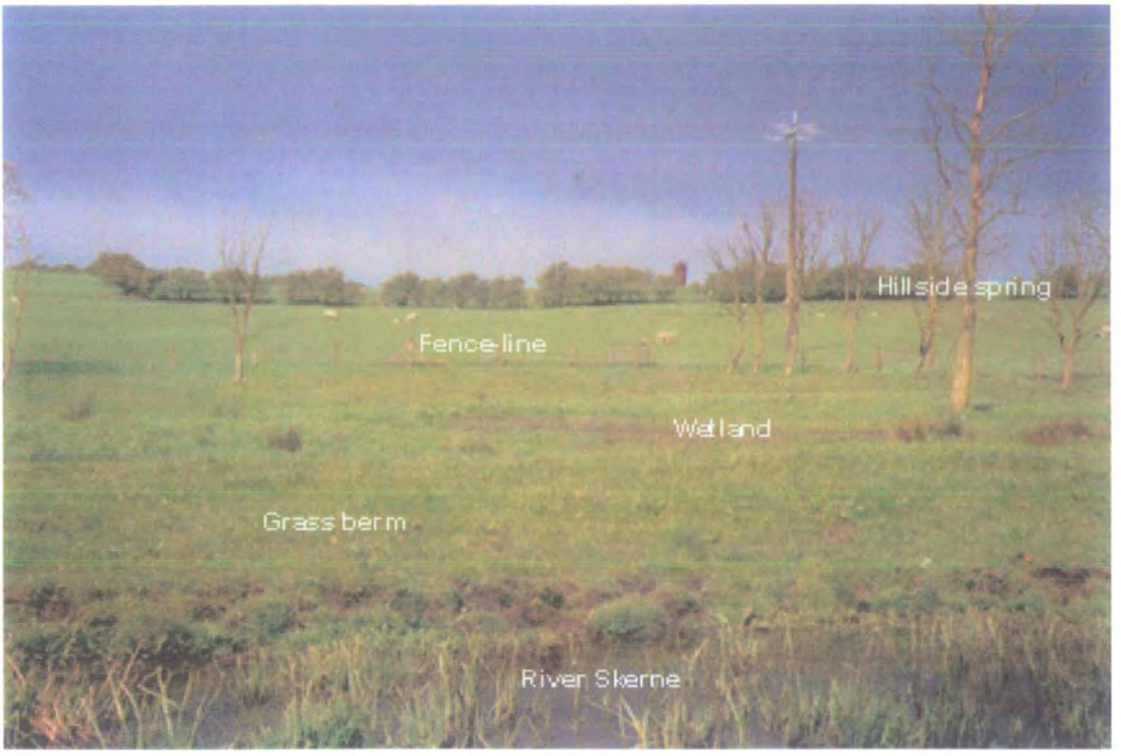


Plate III. The wetland riparian site.

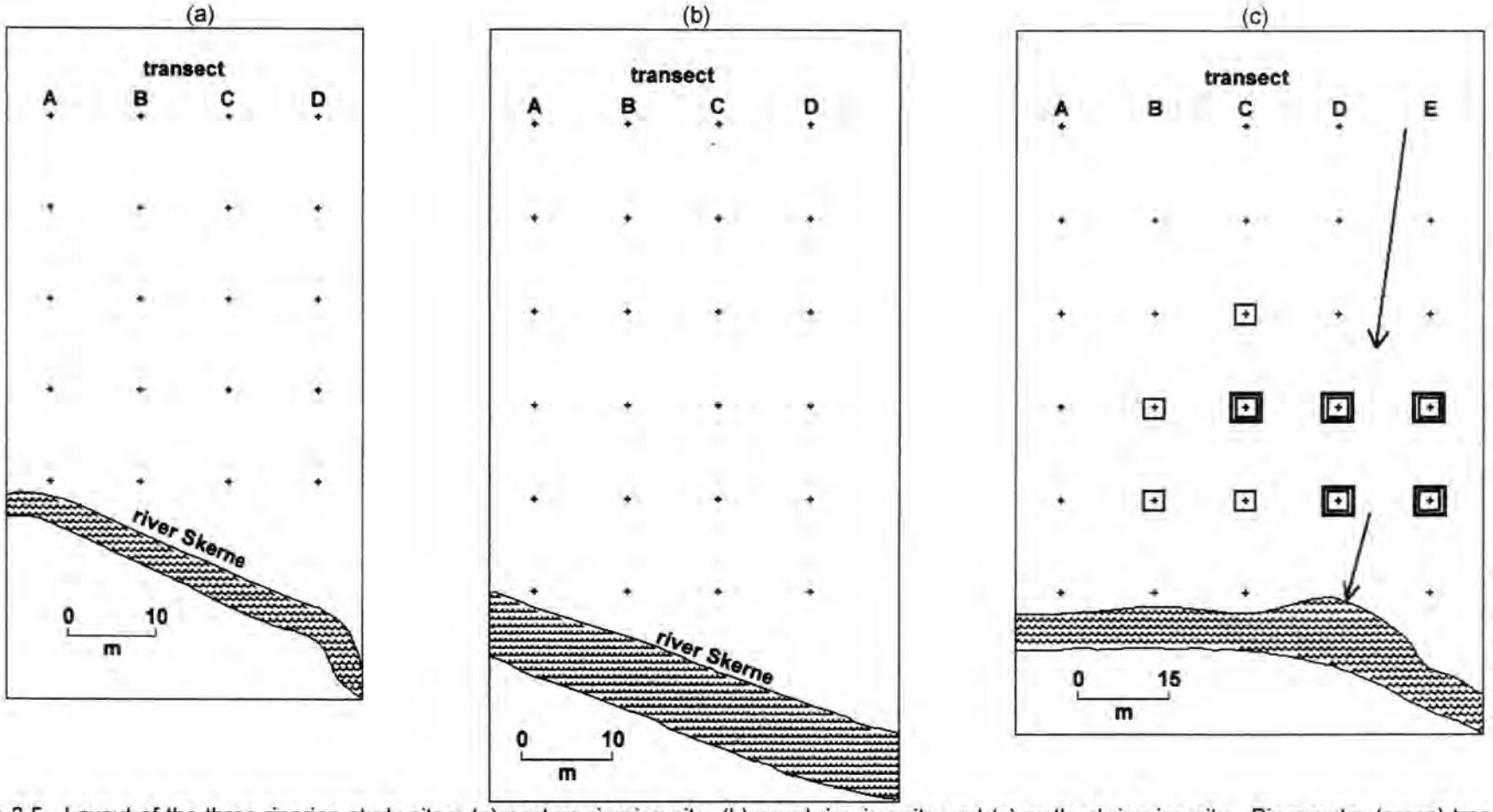


Figure 3.5. Layout of the three riparian study sites; (a) pasture riparian site, (b) wood riparian site and (c) wetland riparian site. Piezometer (cross) transects extending from the pastoral upland to the river edge are shown. At the wetland riparian site, piezometers located in soil that is periodically flooded with surface water are indicated by a single square border, piezometers located in soil that is permanently flooded with surface water are indicated by a double square border. Arrows indicate the approximate position of the surface water inflow and the surface water outflow to the river at the wetland riparian site.

circumference for the length of the pipe and a capped bottom end were fitted snugly into the open holes. The original excavated soil was used as back-fill to close any gaps between the slightly wider auger hole and the pipe. Very occasionally deeper holes and longer tubes had to be prepared where the water table was especially deep. For piezometers at the wetland site, where the water table was often above the ground surface, the top 0.5 m of the each pipe was not drilled with holes. Piezometer holes here were augered to 1.5 m and the pipe inserted. The non-perforated section of the pipe then protruded 0.5 m above the soil surface preventing the inflow of surface water. Some longer piezometer tubes had to be prepared for upslope areas where the water table was sometimes deeper than 1.5 m. In the grazed pasture, piezometer tubes protruding above the ground surface were cut down by approximately 0.4 m shortly after installation as sheep rubbed against the taller tubes. All piezometers tubes were covered: with wooden plates at the pasture and wood sites where tubes did not protrude above the ground surface and with plastic cap covers at the wetland site. The latter were prone to being knocked off by sheep and/or strong winds, and eventually had to be secured to the tubes after every sampling with tape. Three piezometers became redundant shortly after installation at the wetland site; two in the grazed pasture and one at the river edge. Installation of all three had been complicated by the presence of surface water (spring or river) and consequently they were not installed at sufficient depth to intercept subsurface runoff for the duration of the study period.

3.3.3. Topographic survey and water table position

All study sites were topographically surveyed using an EDM laser level (theodolite) to establish the relative surface position and elevation of piezometers and the river channel. Water table depth below the ground surface was measured by inserting an electronic water level probe into piezometer tubes.

3.3.4. Water sampling and analysis

3.3.4.1. Sample collection

Water samples were drawn out of piezometers into high-density polyethylene bottles placed inside a perspex collection chamber. The collection chamber was linked to both a collection tube line and a hand-held vacuum pump line (Figure 3.6). The collection tube was inserted into the piezometer to around 10 cm depth below the water table level and the entire collection apparatus including tube, chamber and bottle was flushed with approximately 200 ml of water sample. The tube was then reinserted into the piezometer for collection of the sample proper. Samples were stored in a cool box for transport back to the laboratory.

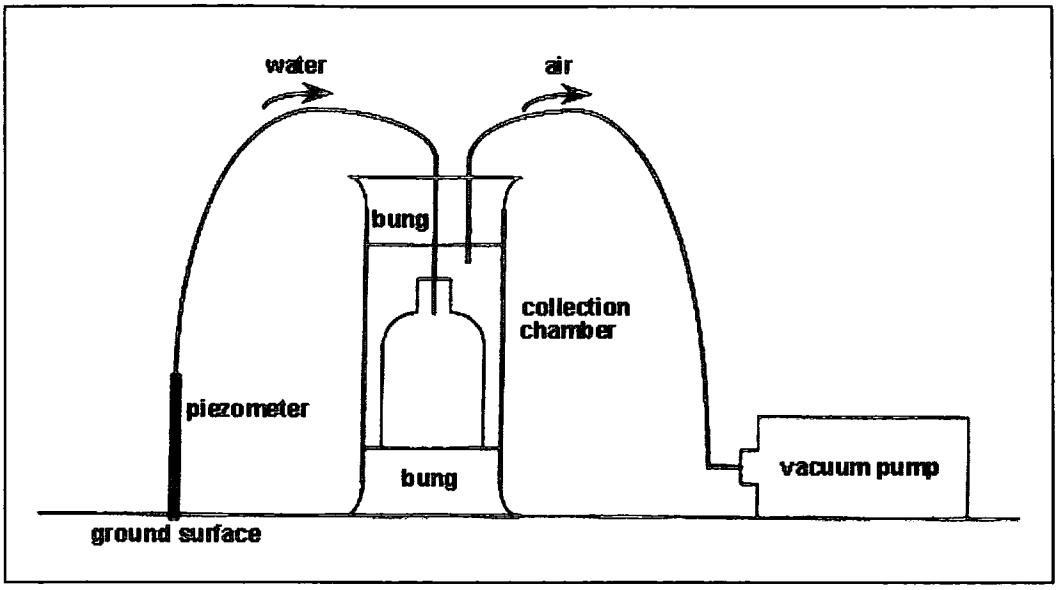


Figure 3.6. Subsurface runoff collection apparatus.

3.3.4.2. Field analyses

The redox potential of water samples was measured immediately after collection with a BDH Gelplas combination redox/ORP probe.

3.3.4.3. Laboratory analyses

On the same day as field collection, samples were filtered through acid-cleaned (1M HCl soak, 3 x deionised water rinse, storage in deionised water) Whatman GF/C glass-fibre filter papers. Samples were stored for a maximum of one week at 4 °C prior to analysis for nitrate, ammonium and total dissolved nitrogen.

Nitrate and ammonium concentrations were determined by ion chromatography with a Dionex DX500 machine. Suppressed conductivity techniques were used with a GP40 tertiary gradient pump and an ED40 electrochemical detector and conductivity cell. For nitrate (anion) measurement an Ionpac ATC trap column, an AG11 guard column and an AS11 analytical column were employed with an ASRS self-regenerating suppressor. For ammonium (cation) measurement a CG12A guard column and a CS12A analytical column were used with a CSRS self-regenerating suppressor.

The total dissolved nitrogen concentration was determined following persulphate digestion and autoclaving that converted all nitrogen to the nitrate form (APHA 1995). The digested sample was then analysed for nitrate as described above. Dissolved organic nitrogen was calculated as the difference between the total nitrogen concentration and the total inorganic nitrogen concentration (nitrate plus ammonium).

3.3.5. Statistics

All statistical analyses were performed using Datadesk 6.0 software. The non-normal distribution of inorganic nitrogen data dictated the use of non-parametric statistical techniques. The Mann-Whitney test was employed to identify significant differences between two samples and Kendalls Tau technique was employed to identify significant correlations between two variables.

3.4. Results

3.4.1. Nitrogen inputs to the riparian zones

3.4.1.1. Nitrate

Nitrate concentrations in subsurface runoff entering the riparian zones were frequently very low, although occasionally concentrations ranging from 0.5 to 2.5 mg N l^{-1} were measured in one or more input piezometers (Figure 3.7). Data from all piezometer transects are shown for nitrate, and other forms, here, and elsewhere, because of the high spatial variability in concentrations. Overall median concentrations for the study period were 0.02. <0.01 and 0.04 mg N l^{-1} at the pasture, wood and wetland sites, respectively. Median monthly concentrations ranged from <0.01 to 0.22 mg N l^{-1} at the pasture site, <0.01 to 0.05 mg N l^{-1} at the wood site and <0.01 to 1.7 mg N l^{-1} at the wetland site. Nitrate concentrations in excess of 0.5 mg N l^{-1} were measured in one or more input piezometers in the following months: late summer and early winter at the pasture site, late autumn and mid-winter at the wood site, and late autumn, mid winter and late winter at the wetland site.

3.4.1.2. Ammonium

Ammonium concentrations were also low for the majority of the study period but, like nitrate, some moderately high concentrations (in the range of 0.5 to 4 mg N l^{-1}) were occasionally measured (Figure 3.8). Overall median concentrations for the study period were 0.17, 0.32 and 0.14 mg N l^{-1} at the pasture, wood and wetland sites, respectively. Median monthly concentrations ranged from <0.01 to 0.64 at the pasture site, <0.01 to 1.5 at the wood site and <0.01 to 0.49 at the wetland site. Ammonium concentrations in excess of 1 mg N l^{-1} were measured in one or more input piezometers in the following months: mid-autumn at the pasture site, early autumn and mid-autumn at the wood site and mid-autumn, late autumn, early winter and late winter at the wetland site.

3.4.1.3. Dissolved organic nitrogen

Dissolved organic nitrogen (DON) concentrations were often higher than nitrate and/or ammonium concentrations and concentrations below 0.2 mg N l^{-1} were infrequently measured (Figure 3.9). Overall median concentrations for the study period were 0.92, 0.59 and 0.65 mg N l^{-1} for the pasture, wood and wetland sites, respectively. Median monthly concentrations ranged from 0.33 to 1.2 mg N l^{-1} at the pasture site, 0.32 to 1.5 mg N l^{-1} at the wood site, and 0.45 to 1.0 mg N l^{-1} at the wetland site. DON concentrations tended to exhibit a less skewed distribution than nitrate and ammonium.

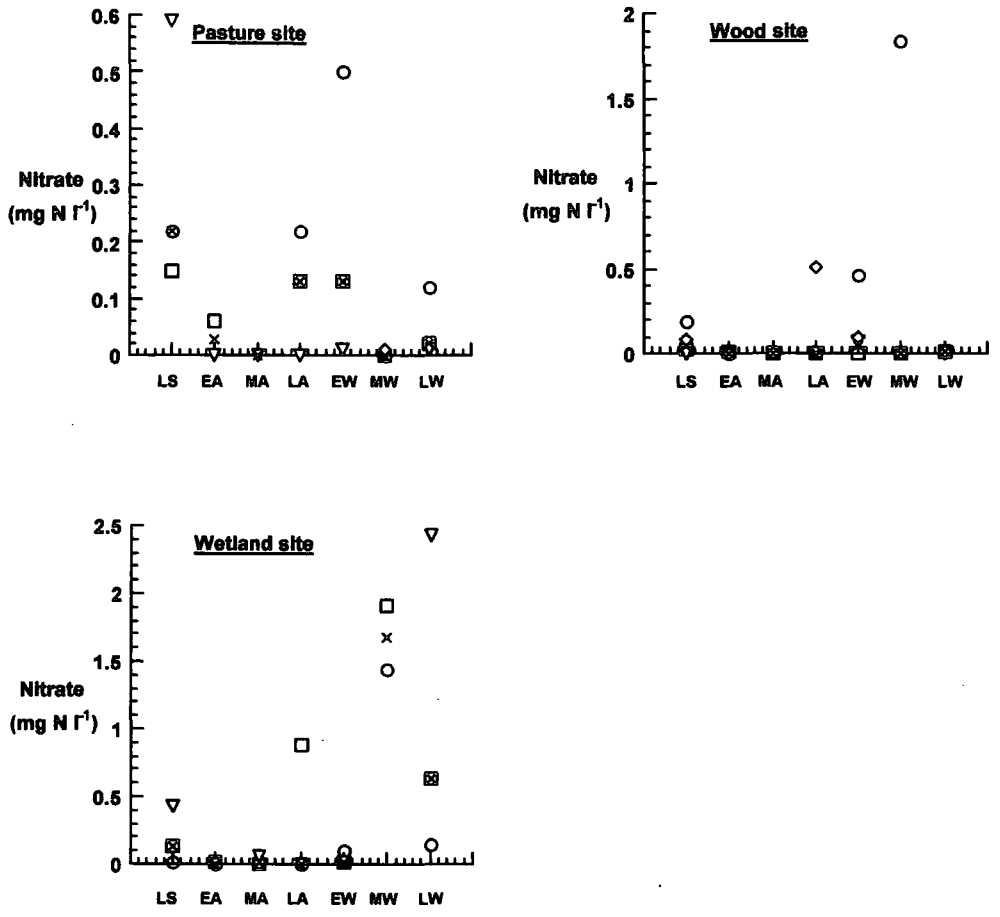


Figure 3.7. Nitrate concentrations in subsurface pastoral runoff entering the three riparian zones on monthly sampling dates. Transect A (O), transect B (□), transect C (∇), transect D (◇) and median (x). LS = late summer, EA = early autumn, MA = mid autumn, LA = late autumn, EW = early winter, MW = mid winter and LW = late winter.

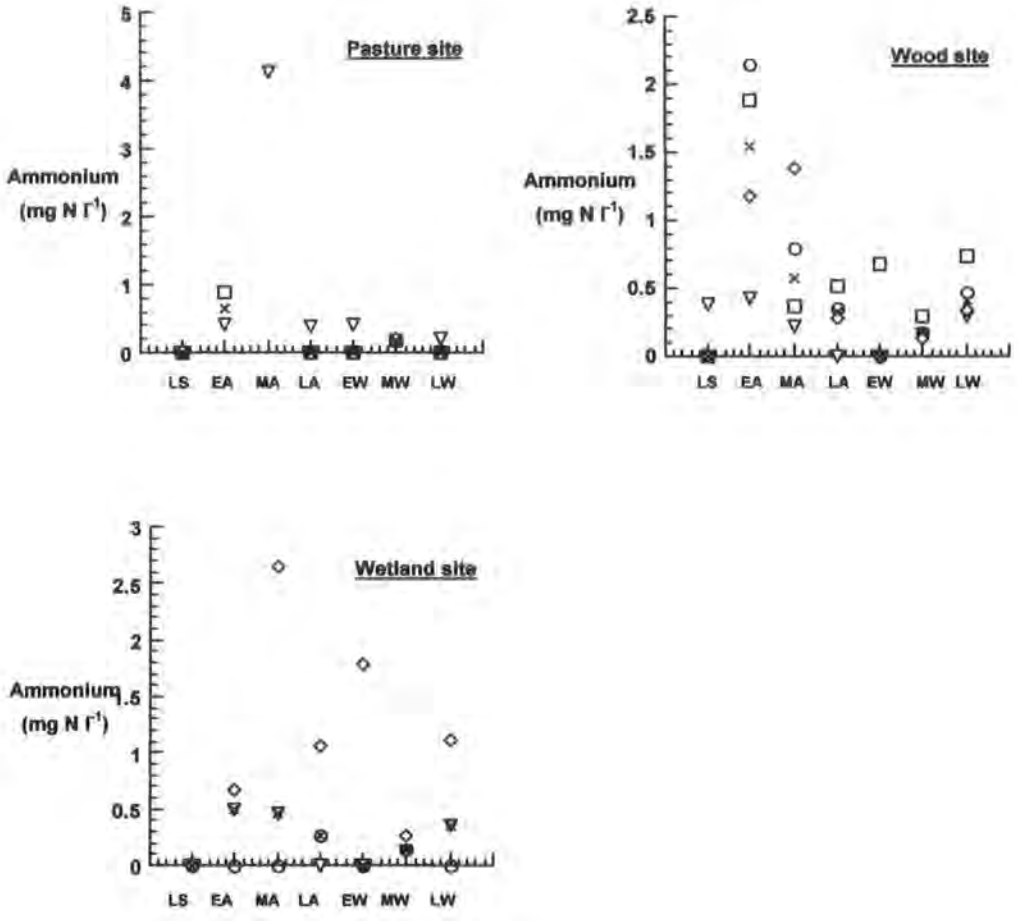


Figure 3.8. Ammonium concentrations in subsurface pastoral runoff entering the three riparian zones on monthly sampling dates. Transect A (O), transect B (□), transect C (∇), transect D (◇) and median (x). LS = late summer, EA = early autumn, MA = mid autumn, LA = late autumn, EW = early winter, MW = mid winter and LW = late winter.

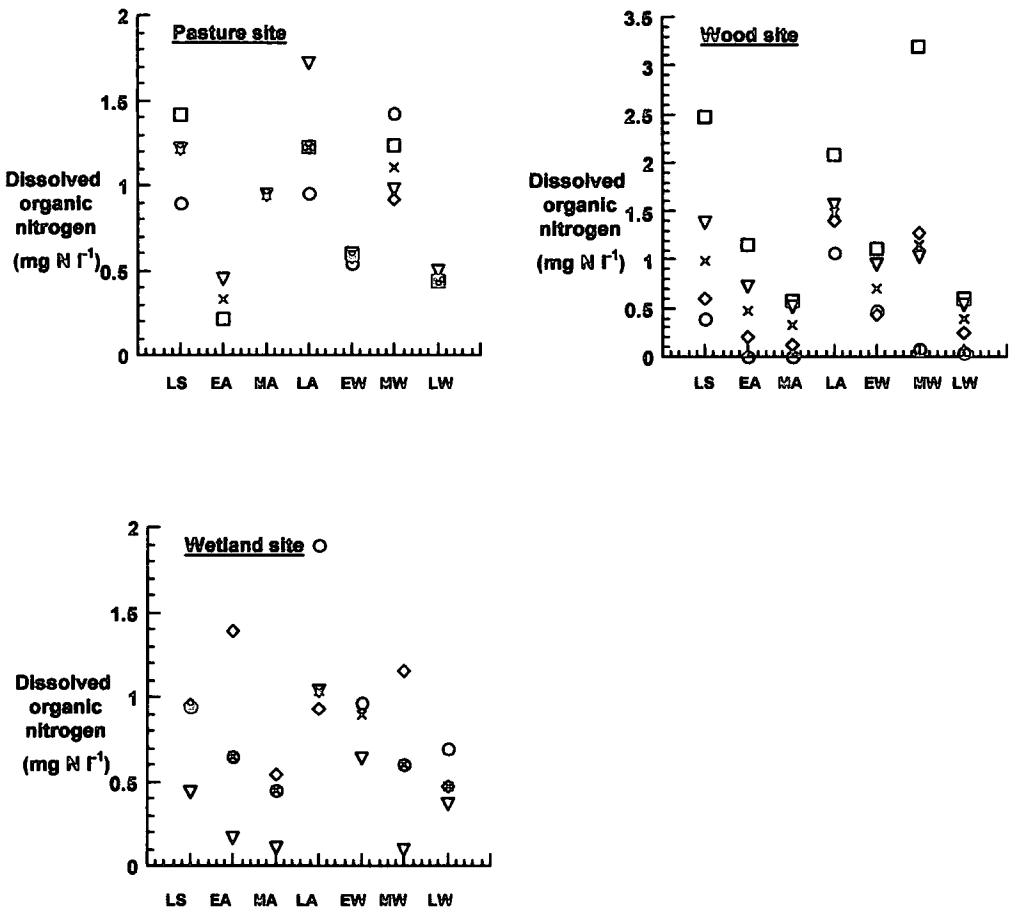


Figure 3.9. Dissolved organic nitrogen concentrations in subsurface pastoral runoff entering the three riparian zones on monthly sampling dates. Transect A (O), transect B (□), transect C (▽), transect D (◇) and median (x). LS = late summer, EA = early autumn, MA = mid autumn, LA = late autumn, EW = early winter, MW = mid winter and LW = late winter.

Concentrations of 0.5 to 1 mg N l^{-1} or higher were measured at all sites in most months. The maximum-recorded concentration was 3.2 mg N l^{-1} at the wood site in mid-winter.

3.4.2. Nitrogen concentrations across the riparian zones

3.4.2.1. Nitrate

At the pasture site, the occurrence of nitrate above negligible concentrations (i.e. >0.1 mg N l^{-1}) was generally restricted to input and river edge piezometers, at 40 m and 0 m distance from the river edge, respectively (Figure 3.10). Even then, the occurrence of measurable nitrate was highly sporadic between transects. The highest nitrate concentrations at this site (up to 2.6 mg N l^{-1}) were associated with a single river edge piezometer. This appeared to be an isolated case, as other river edge piezometers contained little, or no, nitrate; the overall median river edge concentration was 0.02 mg N l^{-1}.

At the wood site, nitrate concentrations were often higher in piezometers located between, and exclusive of, input and river edge piezometers. On most sampling dates concentrations greater than 1 mg N l^{-1} were detected in one or more piezometers located between 10 and 40 m of the river edge (Figure 3.11). The exceptions were early autumn and mid-autumn when nitrate concentrations across the site were ubiquitously low. In mid-winter, a number of piezometers between 10 and 40 m contained nitrate concentrations between 2 and 5 mg N l^{-1}. Nitrate concentrations in river edge (0 m) piezometers were generally low on all sampling dates; the overall median concentration was 0.01 mg N l^{-1}. The only exception was an isolated measurement of 2.2 mg N l^{-1} in one river edge piezometer in late winter.

Similarly to the wood site, higher nitrate concentrations were generally restricted to piezometers located between, and exclusive of, input (75 m) and river edge (0 m) piezometers at the wetland site. Nitrate concentrations measured in one or more piezometers between 15 and 60 m of the river edge were in the range of 2-20 mg N l^{-1} during the study period (Figure 3.12). Concentrations above 5 mg N l^{-1} were measured in two or more piezometers between 15 and 60 m on all sampling dates except late summer. Notably, the high nitrate concentrations were mostly associated with piezometers that were *not* located within the area of permanent standing surface water (i.e. A and B transects). Very low nitrate concentrations were consistently measured in all river edge piezometers; the median concentration was <0.01 mg N l^{-1}.

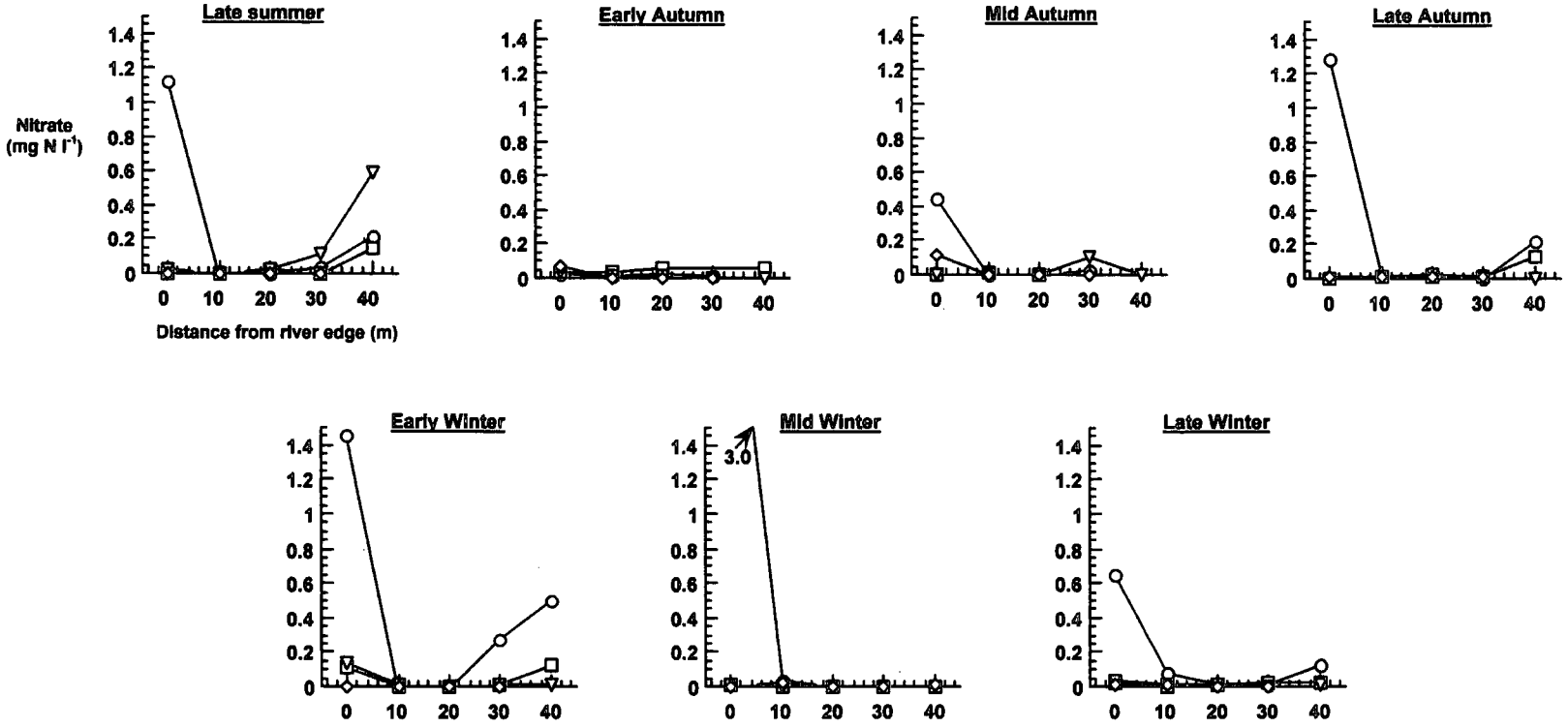


Figure 3.10. Nitrate concentrations in piezometer transects across the pasture site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽) and transect D (◇). Transects extend from the pastoral upland (40 m) to the river edge (0 m).

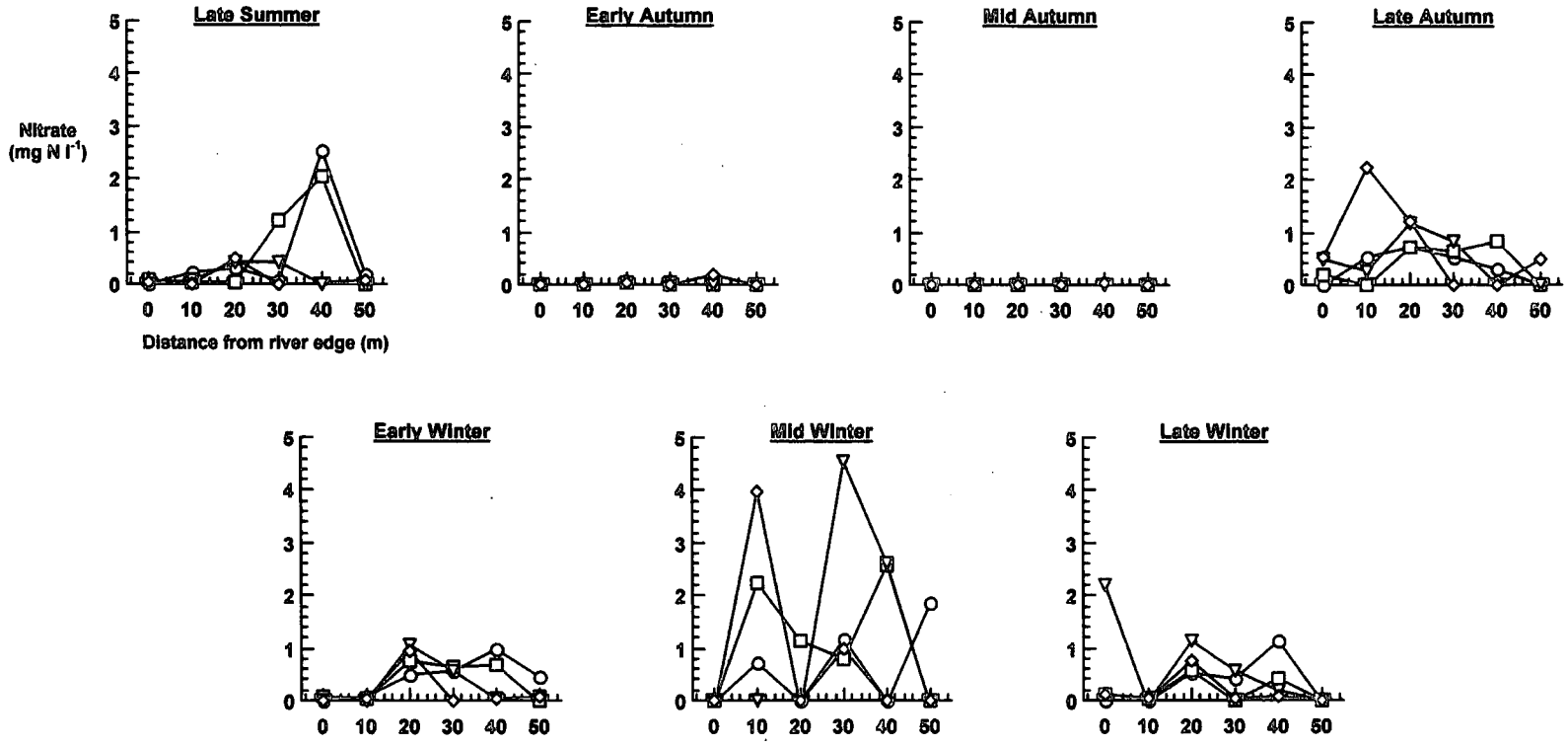


Figure 3.11. Nitrate concentrations in piezometer transects across the wood site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽) and transect D (◇). Transects extend from the pastoral upland (50 m) to the river edge (0 m).

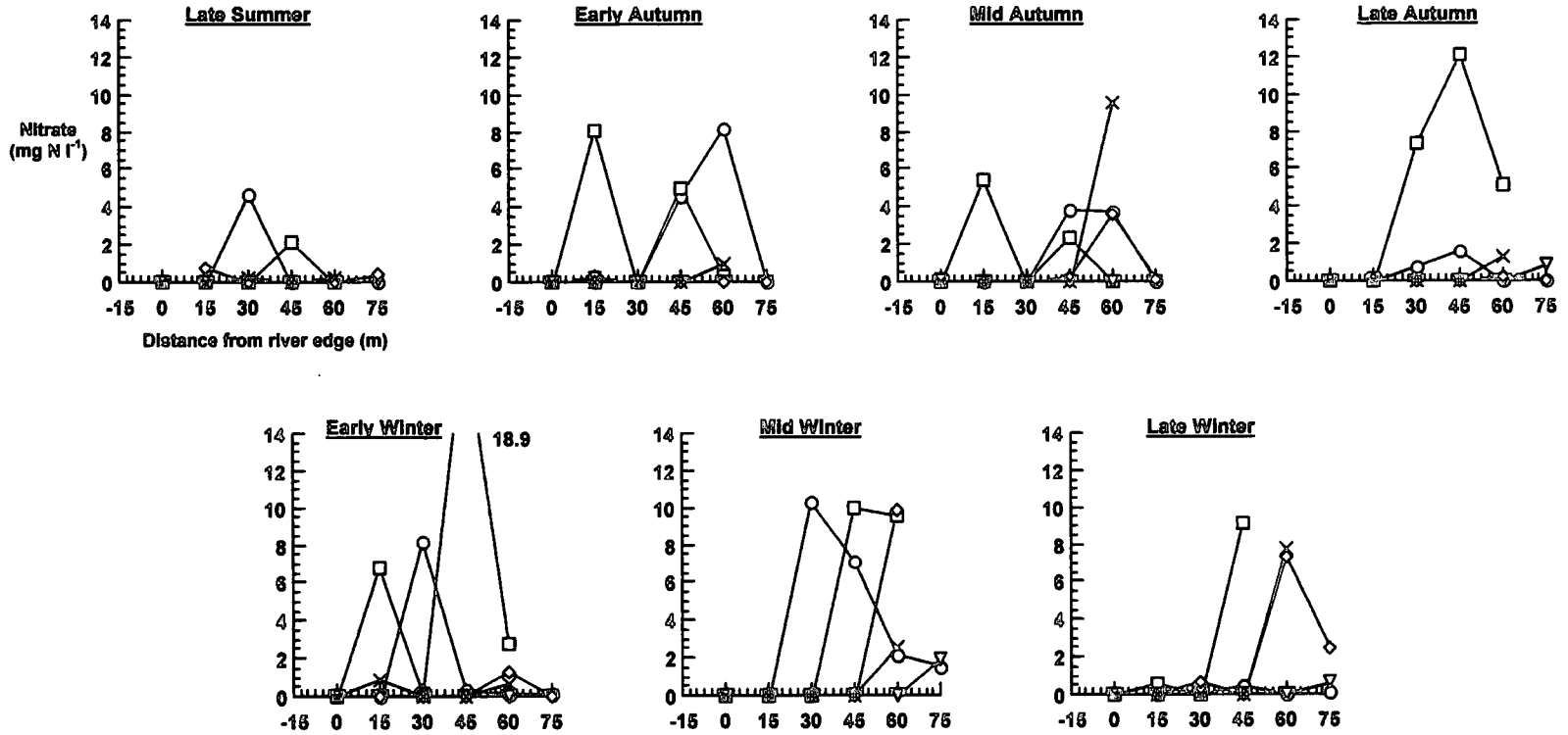


Figure 3.12. Nitrate concentrations in piezometer transects across the wetland site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽), transect D (◇) and transect E (x). Transects extend from the pastoral upland (75 m) to the river edge (0 m).

3.4.2.2. Ammonium

At the pasture site, no clear spatial pattern with respect to ammonium concentrations was evident from piezometer transects (Figure 3.13). Across the site, concentrations were typically very low (the overall median concentration was 0.01 mg N l^{-1}). Higher ammonium concentrations (1-4 mg N l^{-1}) were detected sporadically across the site (0 m, 20 m, 40 m) in the early autumn and mid-autumn.

At the wood site, ammonium concentrations across the site were generally somewhat higher than at the pasture site; the overall median concentration was 0.33 mg N l^{-1}. Moderately high ammonium concentrations (2-5 mg N l^{-1}) were detected in some piezometers at a distance of 10-20 m from the river edge in late summer and early autumn (Figure 3.14). Even higher ammonium concentrations (5-20 mg N l^{-1}) were measured in some piezometers at 10-30 m distance in mid-autumn (note the change in y-axis scale of the mid-autumn plot). Concentrations in river edge piezometers (0 m) were generally much lower and similar to input (60 m) concentrations throughout the study period.

At the wetland site, ammonium concentrations ranging from 2-10 mg N l^{-1} were frequently measured in piezometers located between 15 to 60 m of the river edge (Figure 3.15). The very highest ammonium concentrations were associated with one or more piezometers at 30 m. Notably, these piezometers were located within the area of standing surface water (i.e. transects C and D). In river edge piezometers ammonium concentrations were variable, ranging from <0.1 to 4 mg N l^{-1}, and there was no clear temporal trend. Concentrations in the river edge piezometers (overall median concentration of 0.65 mg N l^{-1}) were often higher than the concentrations measured in input piezometers.

3.4.2.3. Dissolved organic nitrogen

At the pasture site, DON concentrations showed no clear spatial pattern across the site (Figure 3.16). Temporally, concentrations across the entire site increased noticeably between mid-autumn and late autumn; the overall median concentration increased from 0.4 to 1.4 mg N l^{-1}. Some higher concentrations were also sporadically detected across the site in late summer.

At the wood site, DON concentrations also exhibited no consistent spatial pattern across the site (Figure 3.17). A trend of declining concentrations from input piezometers to the river edge was evident in the late summer, late autumn and early winter while concentrations were consistently low across the site in late winter.

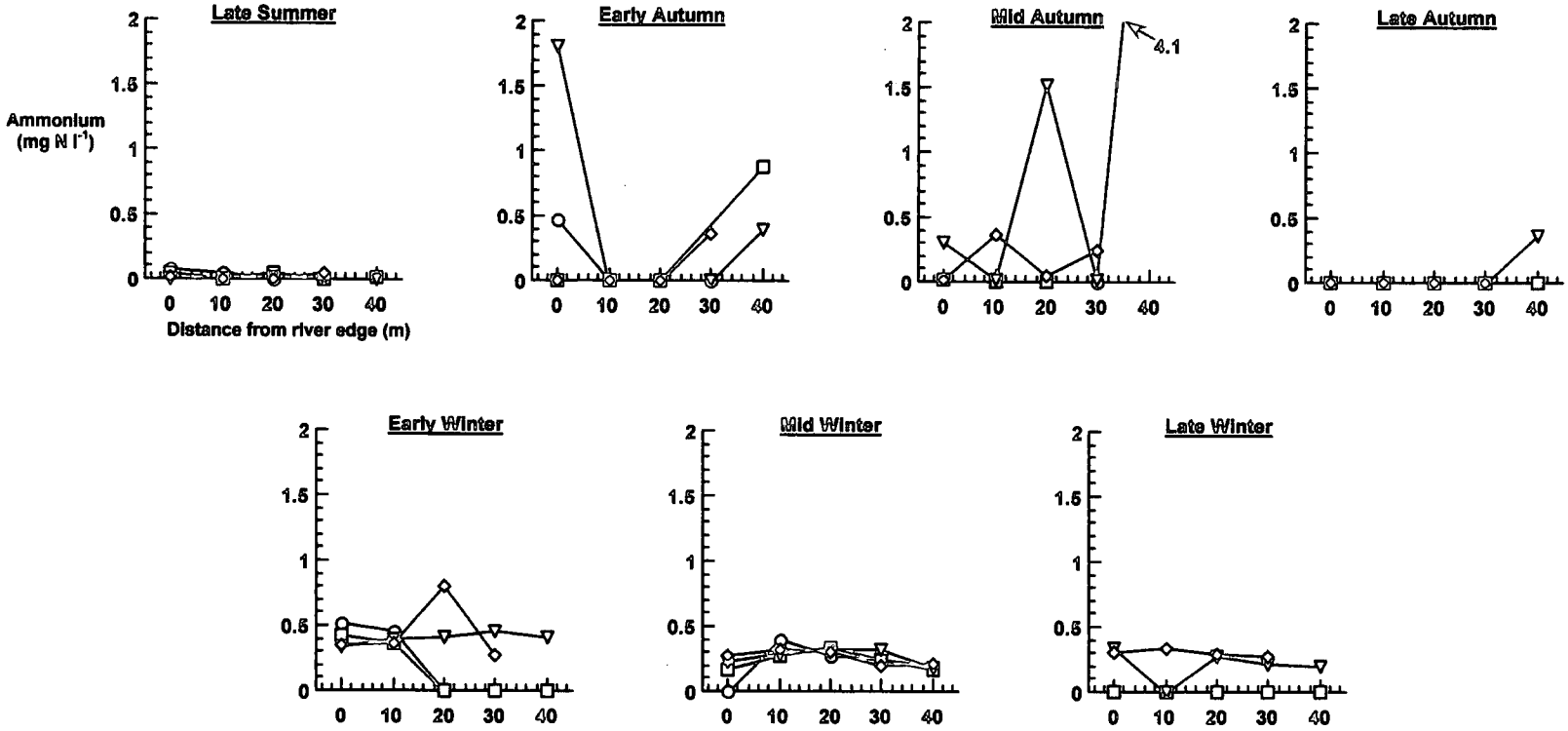


Figure 3.13. Ammonium concentrations in piezometer transects across the pasture site on monthly sampling dates. Transect A (O), transect B (□), transect C (∇) and transect D (◇). Transects extend from the pastoral upland (40 m) to the river edge (0 m).

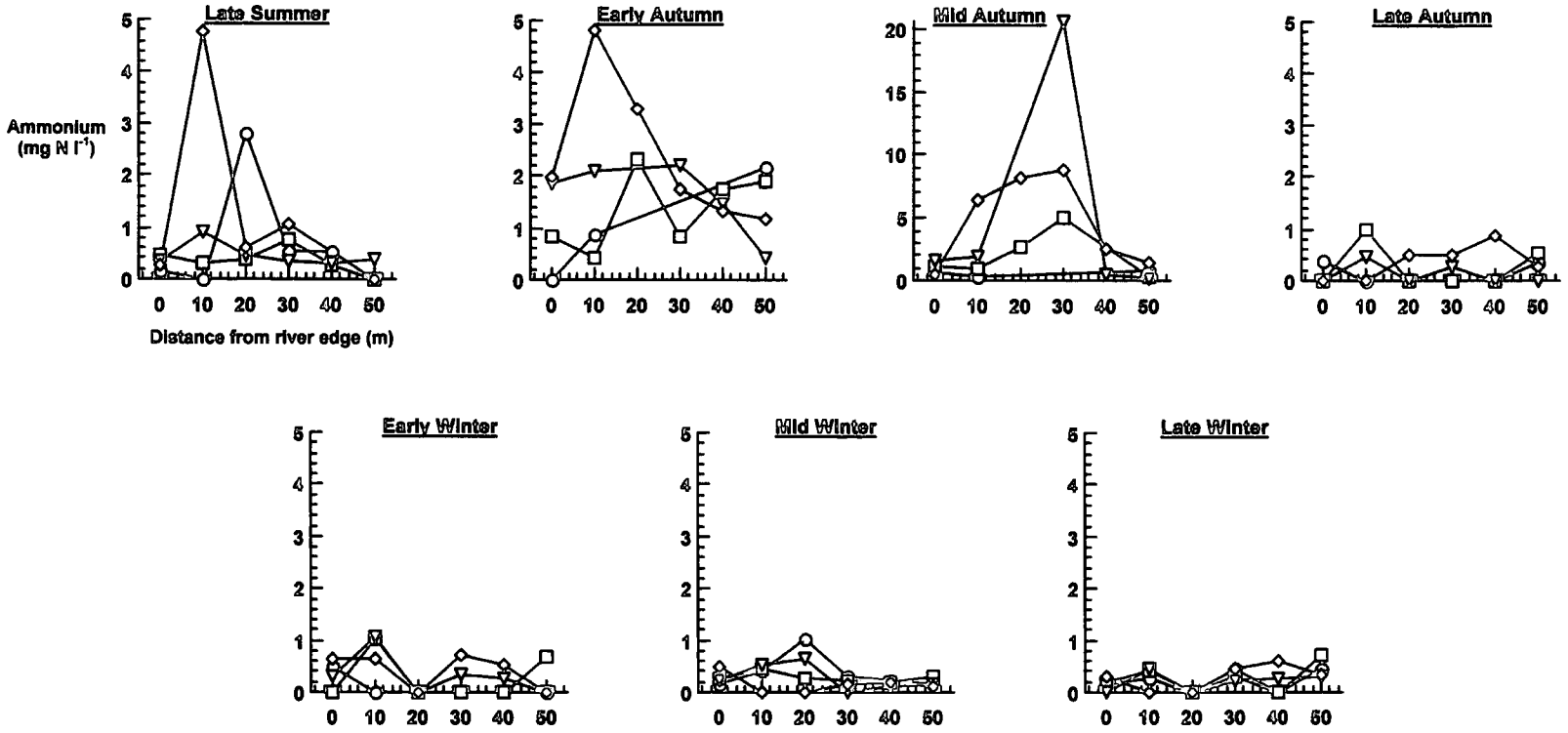


Figure 3.14. Ammonium concentrations in piezometer transects across the wood site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽) and transect D (◇). Transects extend from the pastoral upland (50 m) to the river edge (0 m).

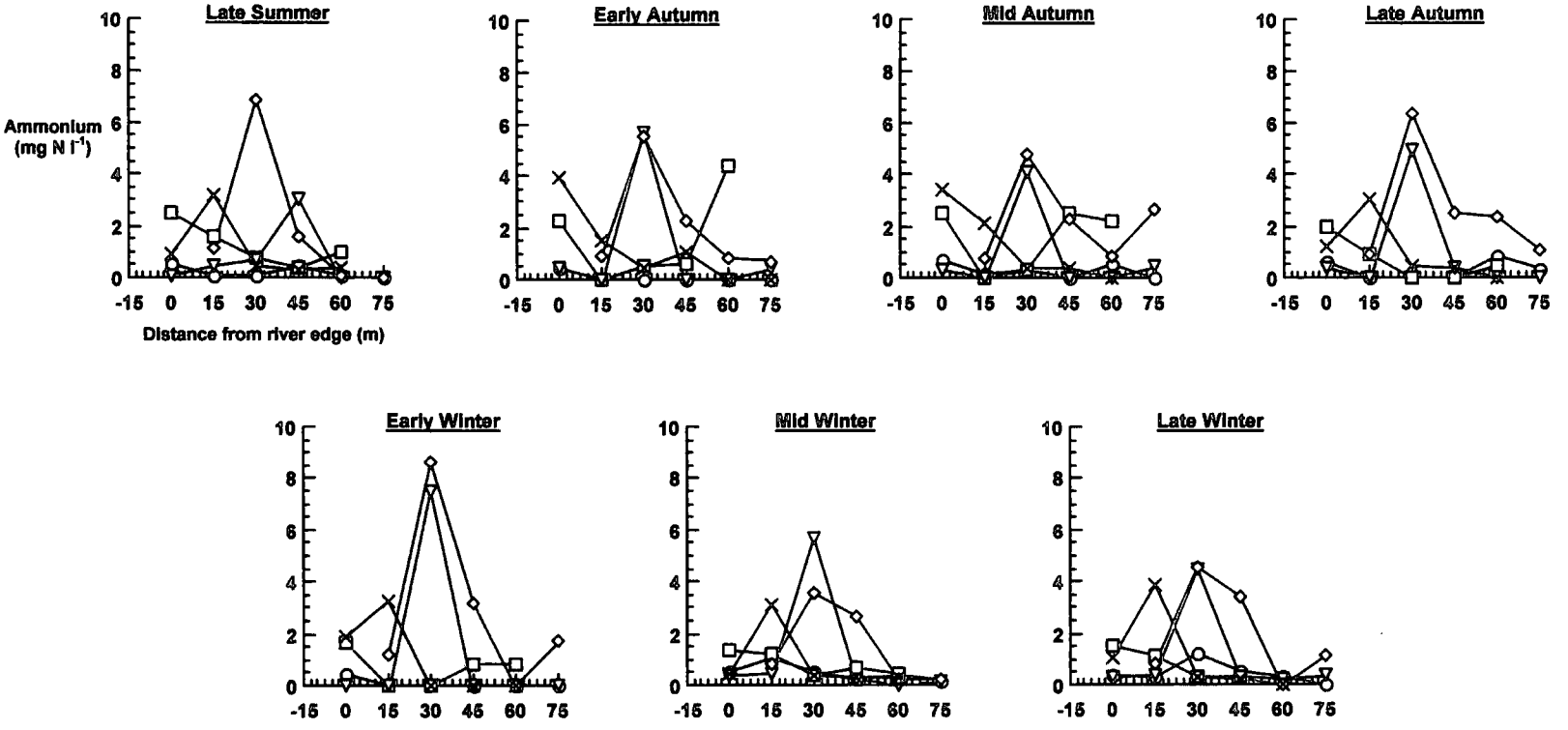


Figure 3.15. Ammonium concentrations in piezometer transects across the wetland site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽), transect D (◇) and transect E (x). Transects extend from the pastoral upland (75 m) to the river edge (0 m).

High DON concentrations (3-11 mg N l^{-1}) were measured in some piezometers at 20-30 m from the river in mid-autumn. This occurrence coincided with several nearby piezometers containing very high ammonium concentrations (as previously discussed).

As for the pasture and wood sites, DON concentrations were highly variable across the wetland riparian zone (Figure 3.18). Slightly higher DON concentrations were detectable in some piezometers between 15 and 60 m of the river. However, no clear spatial or temporal pattern was evident at this site.

3.4.3. Water table features of the riparian zones

3.4.3.1. Temporal trends

Overall, the water table at all sites exhibited a temporal pattern during the study period of minimum level in early autumn and maximum level in mid-winter (Figure 3.19). The pattern is more obvious at the pasture and wood sites than at the wetland site where the position of the water table below the ground surface fluctuated to a lesser extent.

3.4.3.2. Spatial trends

Water table elevation and downslope flow:

Downslope flow of subsurface runoff across each site is inferred from contour plots of water table elevation; water flows from positions of higher to lower elevation and at right angles to contour lines.

At the pasture site there was generally a steady decline in water table elevation across the site indicating constant downslope flow (Figure 3.20). Contour lines indicate the flow direction was almost perpendicular to the river edge with a slight (upstream) skew towards transect A. This skewness is explained by the river edge piezometers in transects A through D being progressively further from the river channel (~2m at A to ~14m at D). A generally consistent pattern of downslope flow across the site is evident even during the extremes of water table level measured in the study period; the very low water table in early autumn and the very high water table in mid-winter.

At the wood site, water table elevation decreased sharply immediately downslope of input piezometers, at 30-50 m from the river edge, indicating rapid downslope flow of subsurface runoff in this part of the site (Figure 3.21). The decrease in water table elevation was less at a distance of 0 to 30 m from the river edge but still more than sufficient to indicate downslope flow as opposed to stagnation. The only exception was

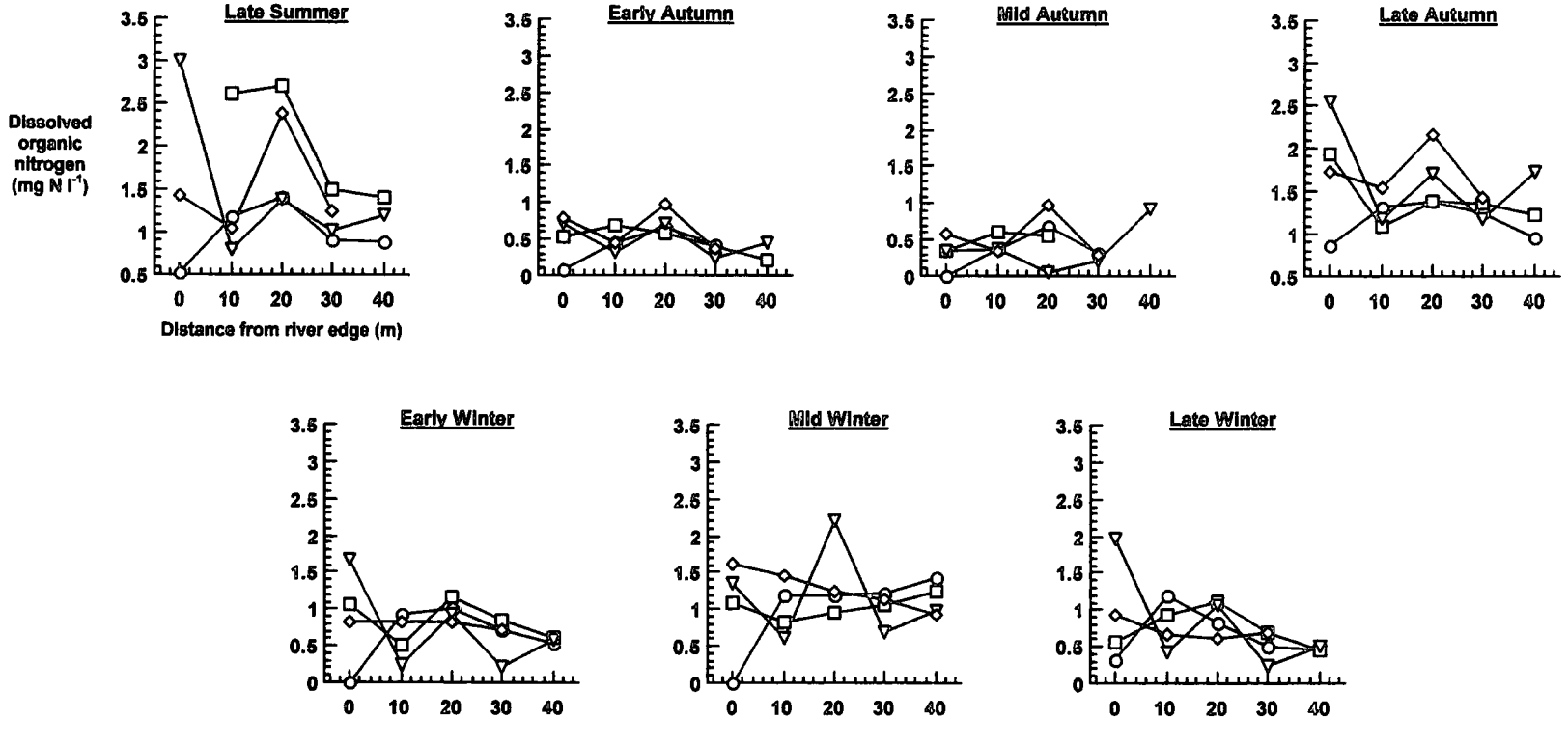


Figure 3.16. Dissolved organic nitrogen concentrations in piezometer transects across the pasture site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽) and transect D (◇). Transects extend from the pastoral upland (40 m) to the river edge (0 m).

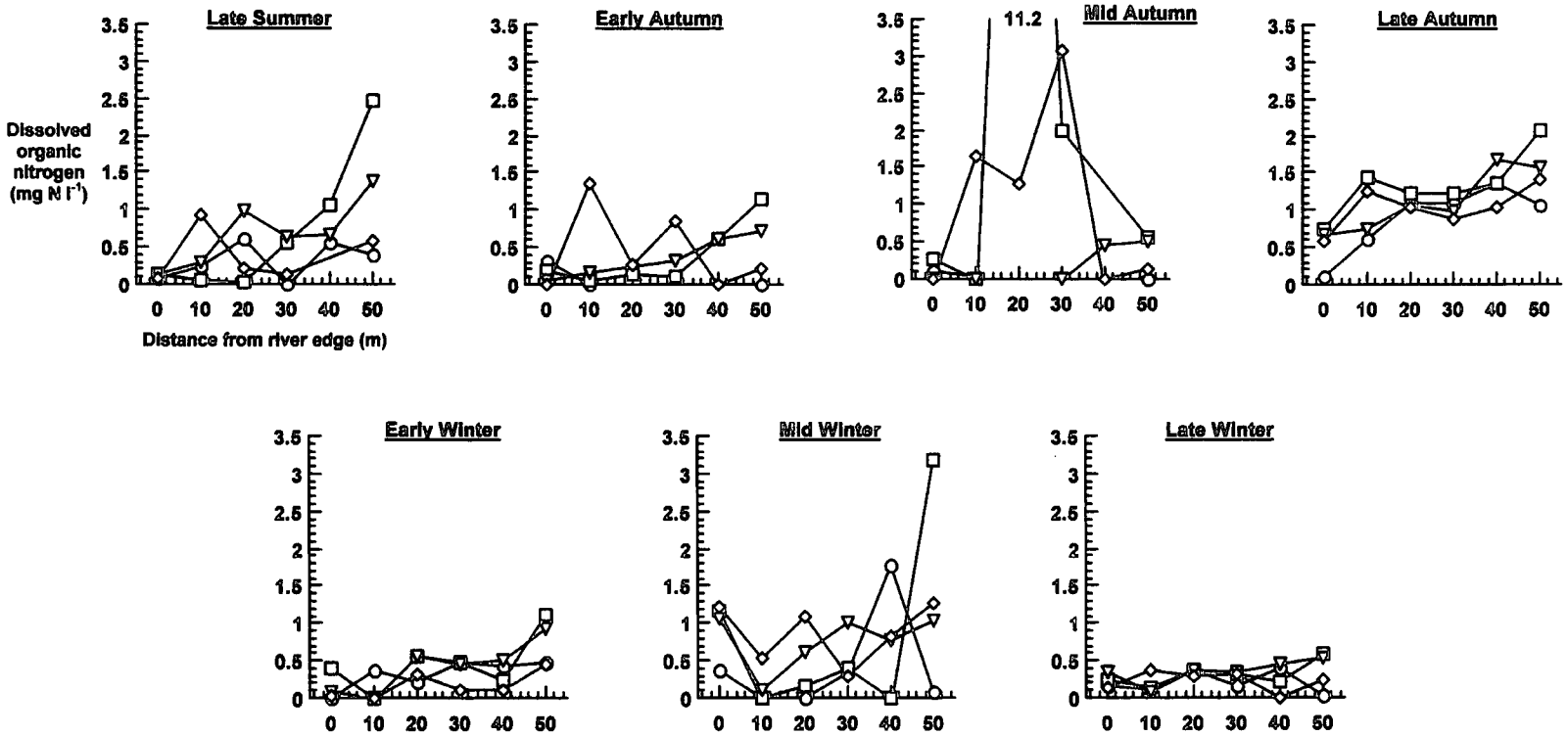


Figure 3.17. Dissolved organic nitrogen concentrations in piezometer transects across the wood site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽) and transect D (◇). Transects extend from the pastoral upland (50 m) to the river edge (0 m).

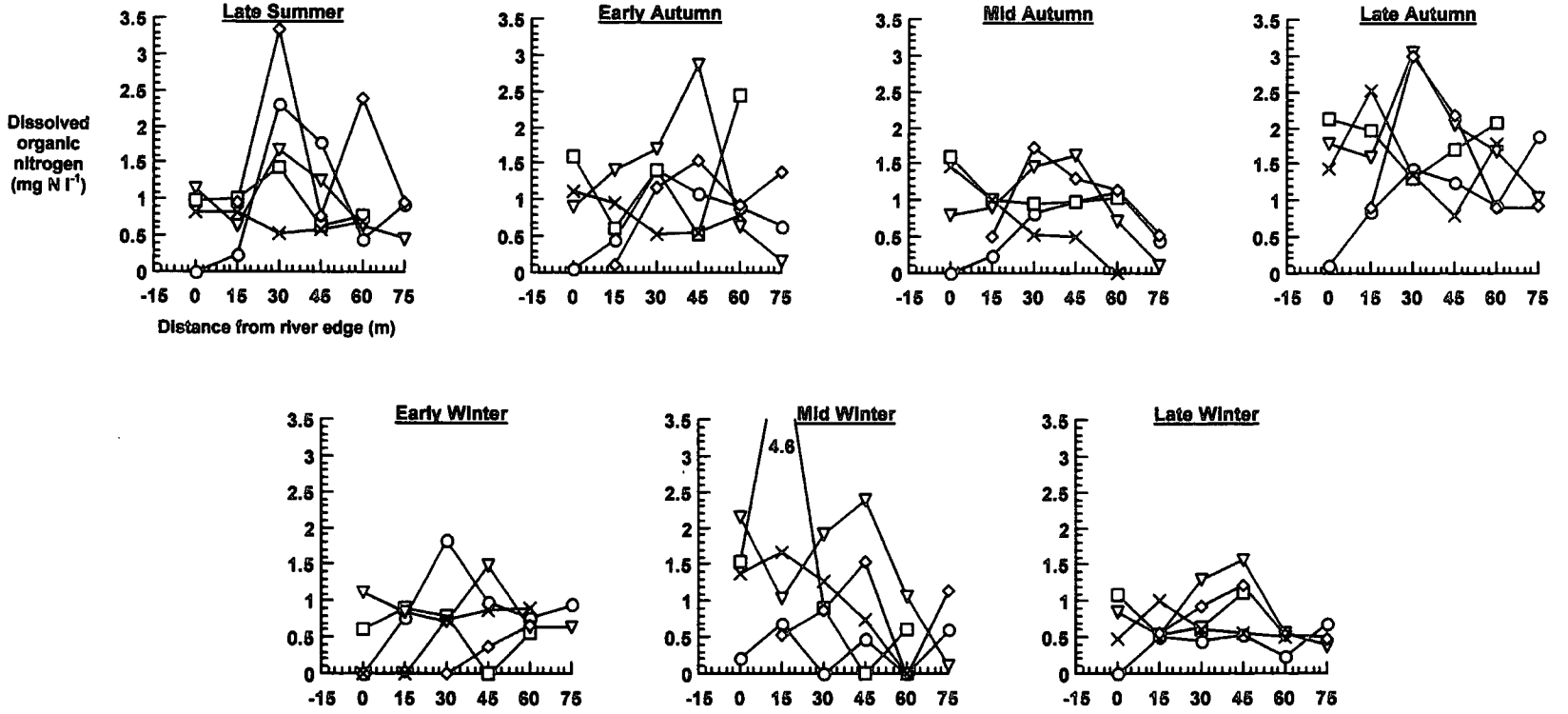


Figure 3.18. Dissolved organic nitrogen concentrations in piezometer transects across the wetland site on monthly sampling dates. Transect A (O), transect B (□), transect C (▽), transect D (◇) and transect E (x). Transects extend from the pastoral upland (75 m) to the river edge (0 m).

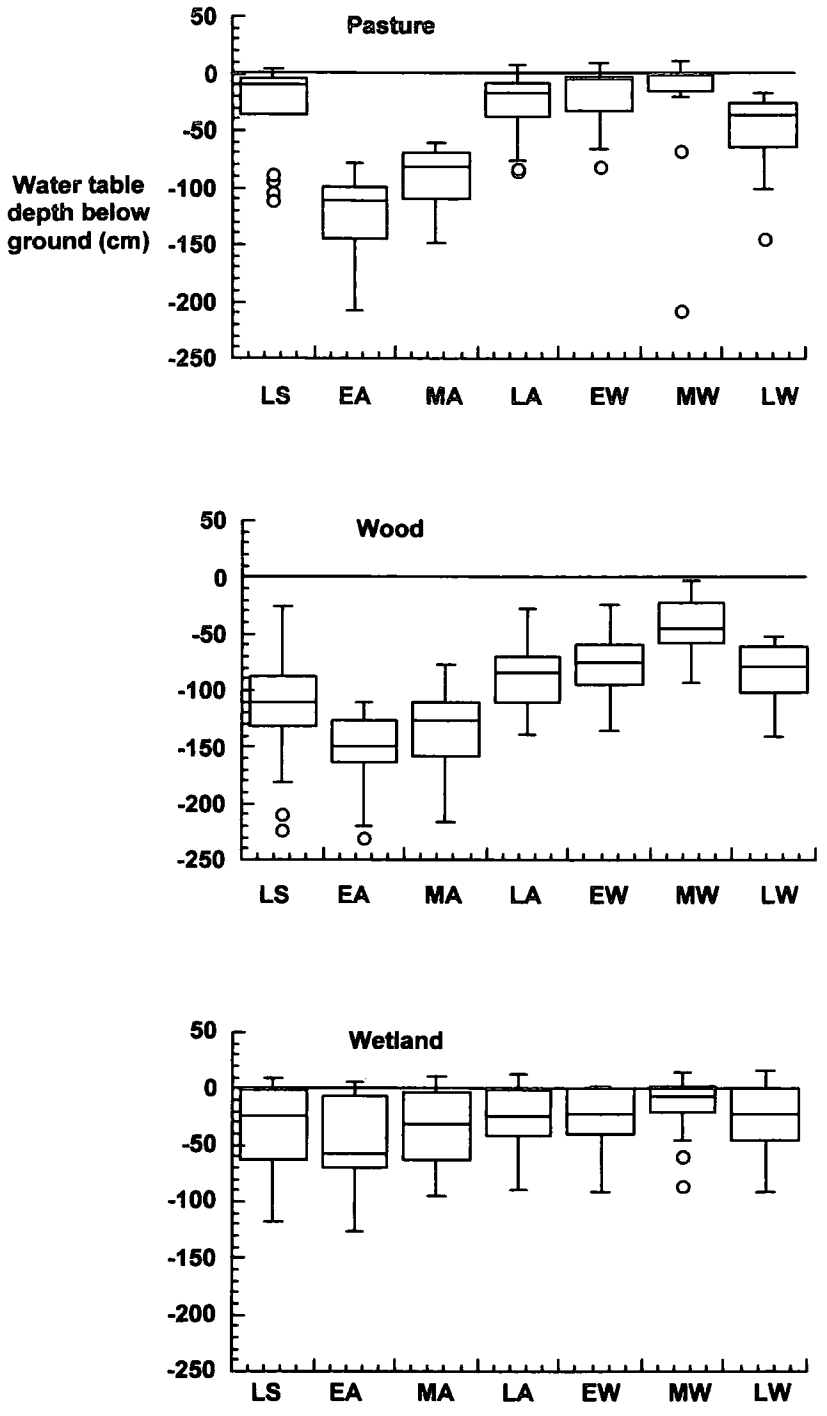


Figure 3.19. Depth of the water table below the ground surface for the three riparian sites on monthly sampling dates. LS = late summer, EA = early autumn, MA = mid autumn, LA = late autumn, EW = early winter, MW = mid winter and LW = late winter. For each box, the line across the box is the median, the box represents data between the 25th and 75th percentiles, the box and whiskers represent all data except outliers. Outliers are those values that exceed the upper quartile + 1.5* interquartile distance or values that are less than the lower quartile – 1.5* interquartile distance.

late summer where some irregular, low water table elevations suggested that water may be ponding in some areas between 0 and 30 m of the river edge. Regarding general flow direction across the wood site, contour lines were again slightly skewed towards Transect A (upstream) but, like the pasture site, river edge piezometers in transects A through D were progressively further from the river channel. The flow direction is therefore virtually perpendicular to the river at this site. Similarly to the pasture site, the pattern of water table elevation was reasonably constant between the extremes of water table position, apart from the somewhat anomalous observation in late summer.

At the wetland site, there was a steady decline in water table elevation between 75 m and 45 m from the river edge indicating constant downslope flow (Figure 3.22). Further downslope, between 45 and 15 m of the river edge, the water table elevation changed very little, which is indicative of water ponding behind the river edge berm deposit. Between 15 and 0 m of the river edge there was evidence of downslope flow through the berm in transects B and C but not A and E. The general flow direction across the site was perpendicular to the river. At this site, river-edge piezometers in transects A-D were similar distances from the river channel (~1 to 2 m) while only the river-edge piezometer at transect E was slightly further away (~6 m). Although the topography of this site was somewhat bowl-shaped, runoff did not appear to flow slightly towards the centre of the site as expected. Water table elevation contours suggest that flow at the outer transects A and E is away from the centre of the site, not towards it. Similarly to the pasture and wood sites, the pattern of water table elevation was reasonably constant between the extremes of water table level evident during this study.

Depth below the ground surface:

At the pasture site, the water table was consistently closest to the ground surface at 10-30 m distance from the river edge, and deeper on the hillslope (40 m) and at the rivers edge (0 m) (Figure 3.23). The water table was very close to, or above, the ground surface in the middle width of the site in some months.

The level of the water table below the ground surface was generally more consistent across the wood site than for the pasture site (Figure 3.24). However, some variability in water table depth between transects was evident at 20-40 m distance from the river edge. In contrast to the pasture site, the water table at the wood site did not, at any time, rise above the ground surface.

At the wetland site, the water table was generally closest to, and in some places above, the ground surface, at distances of 15-45 m from the river edge (Figure 3.25). It was typically deeper at 60-75 m from the river edge, in the upslope part of the riparian zone,

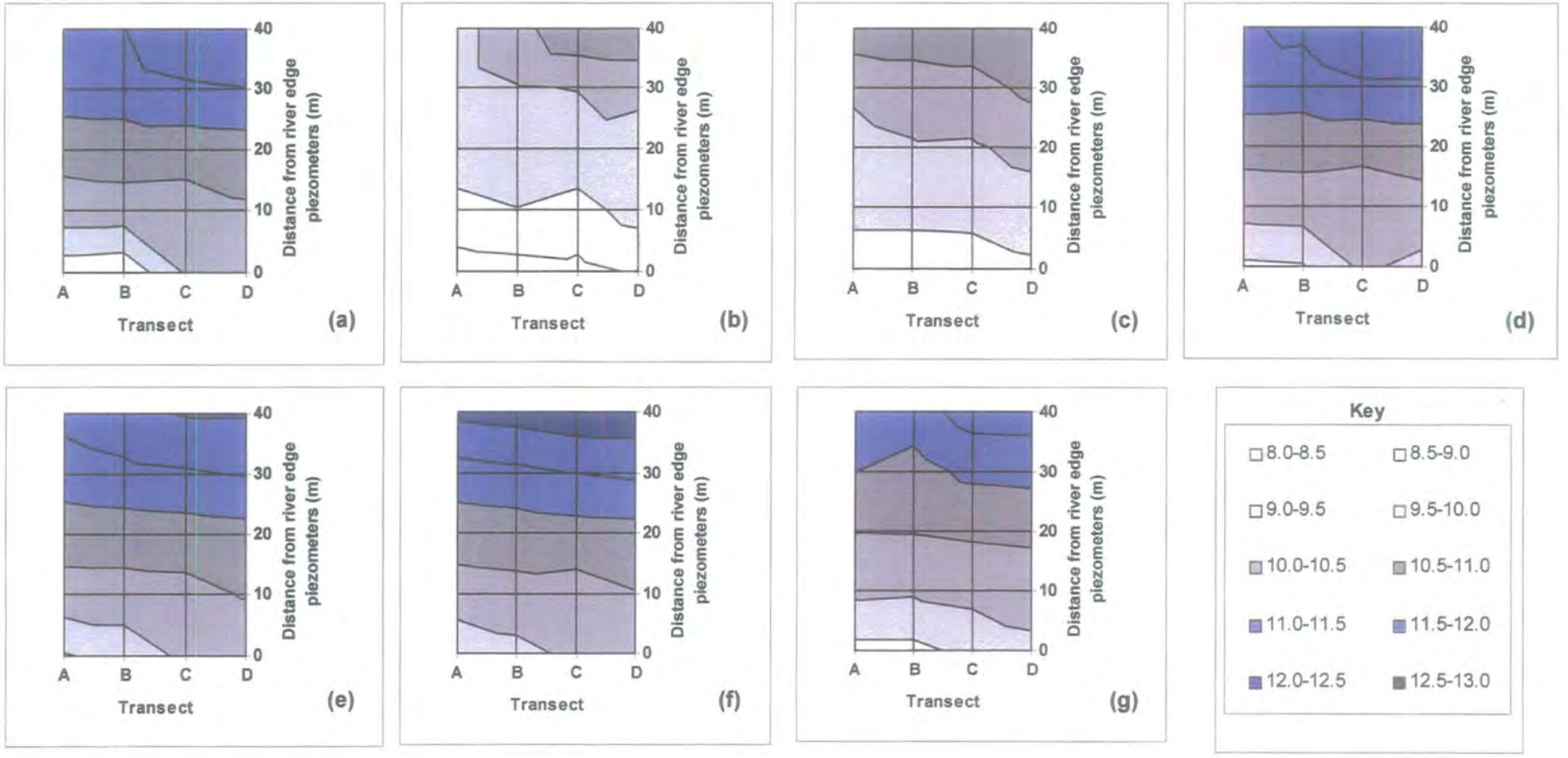


Figure 3.20. Water table elevation contours across the pasture riparian zone on monthly sampling dates. Late summer (a), early autumn (b), mid-autumn (c), late autumn (d), early winter (e), mid-winter (f) and late winter (g). Elevation (m) is above an arbitrary datum.

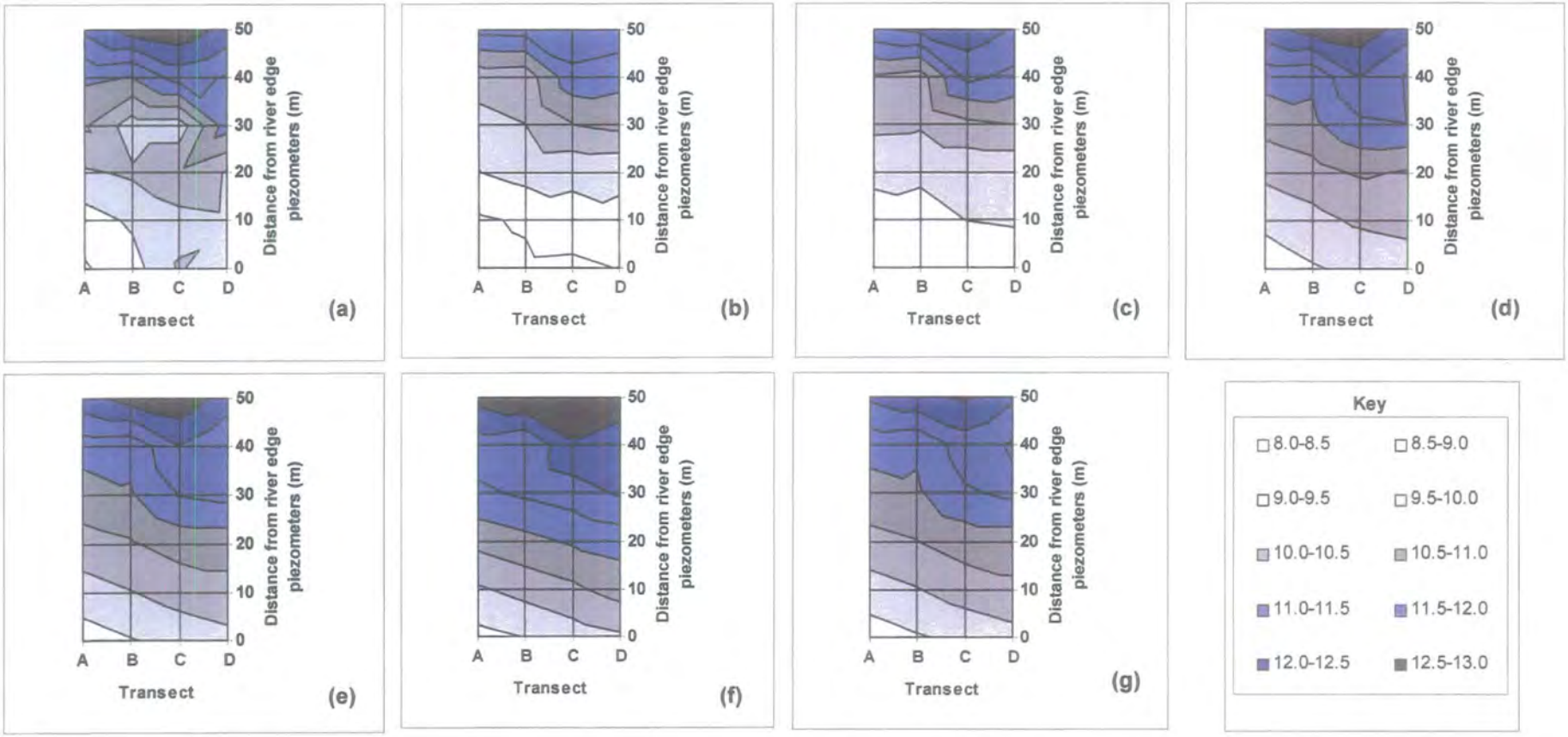


Figure 3.21. Water table elevation contours across the wood riparian zone on monthly sampling dates. Late summer (a), early autumn (b), mid-autumn (c), late autumn (d), early winter (e), mid-winter (f) and late winter (g). Elevation (m) is above an arbitrary datum.

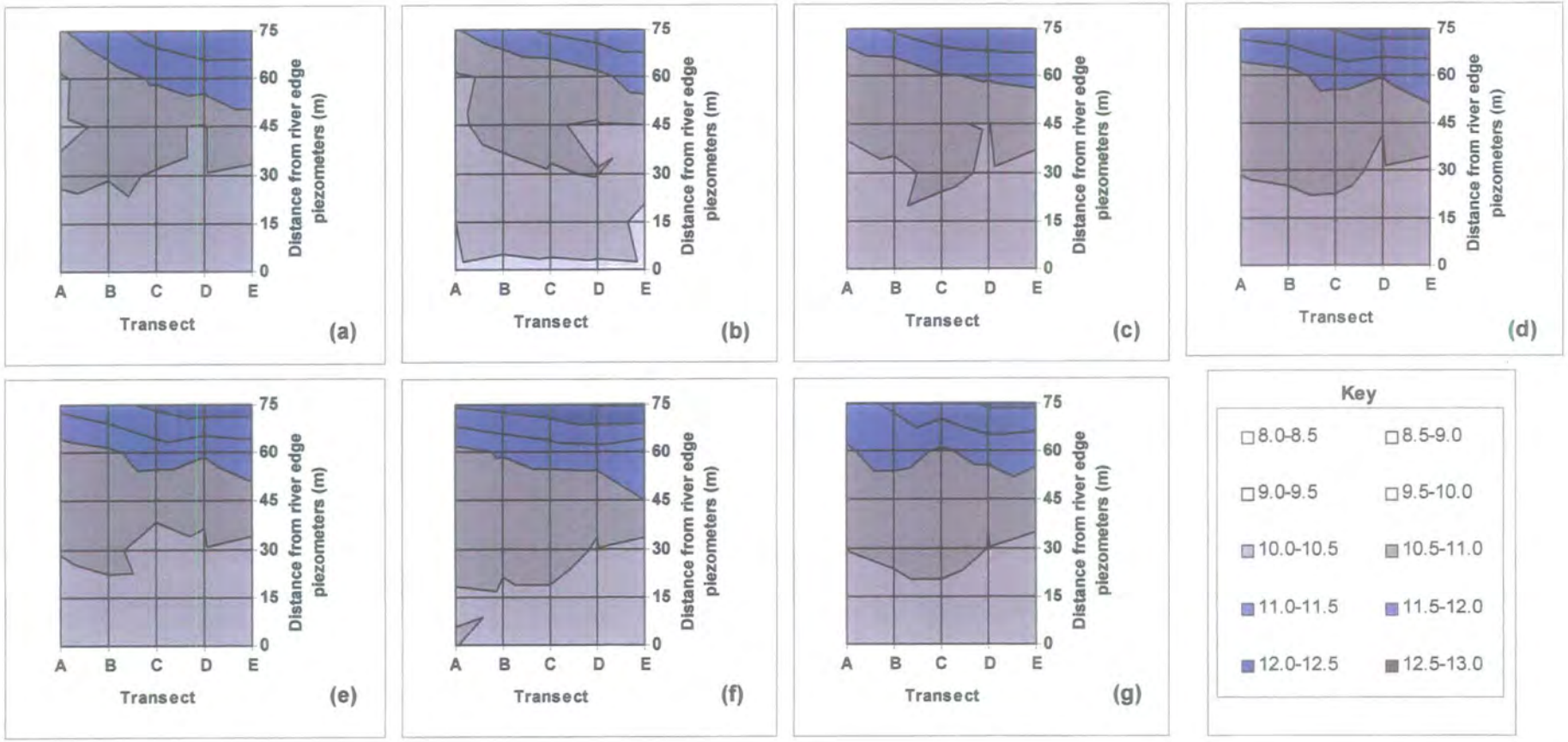


Figure 3.22. Water table elevation contours across the wetland riparian zone on monthly sampling dates. Late summer (a), early autumn (b), mid-autumn (c), late autumn (d), early winter (e), mid-winter (f) and late winter (g). Elevation (m) is above an arbitrary datum.

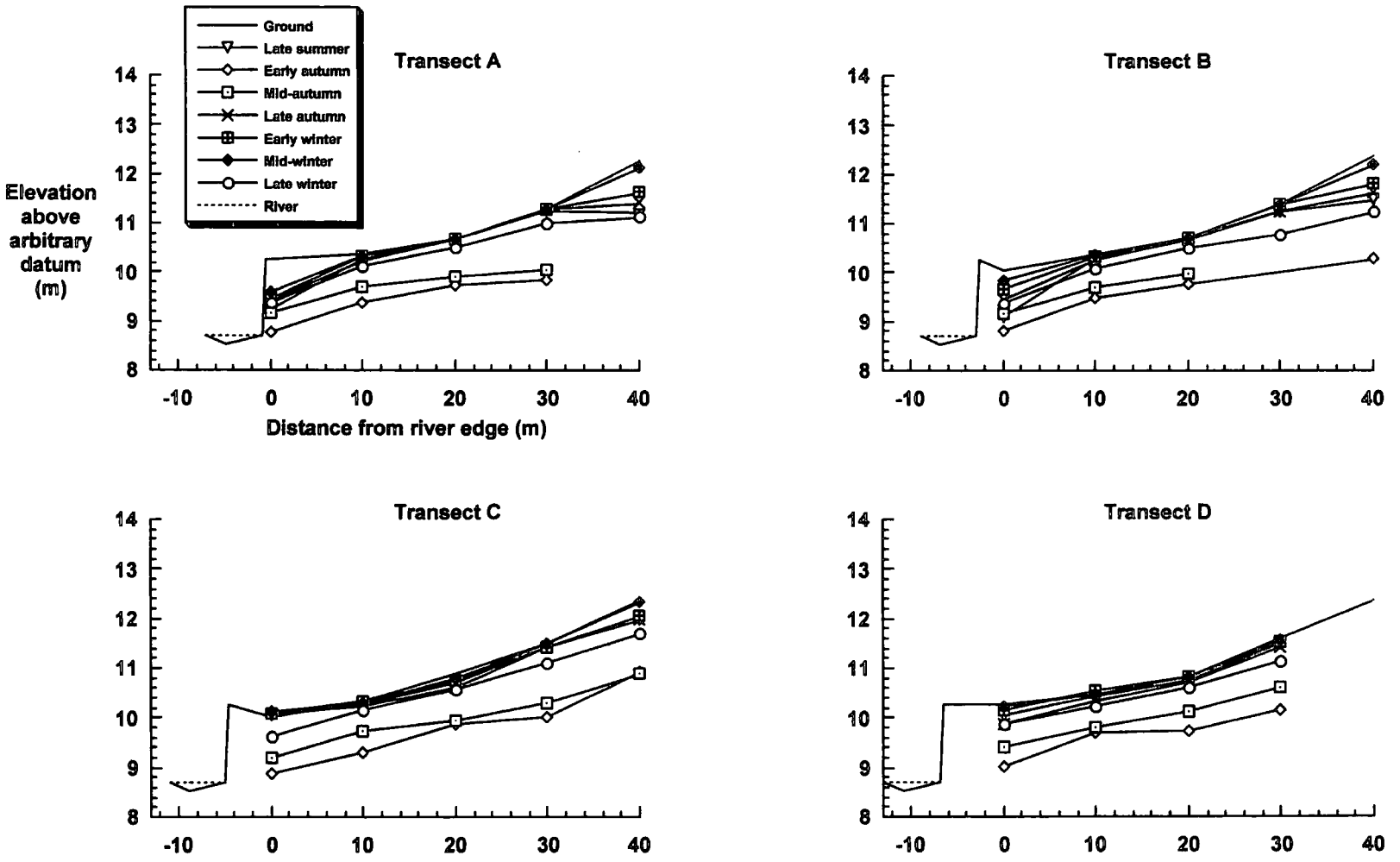


Figure 3.23. The position of the water table in relation to the ground surface for piezometer transects across the pasture riparian zone on monthly sampling dates. Transects extend from the pastoral upland (40 m) to the river edge (0 m).

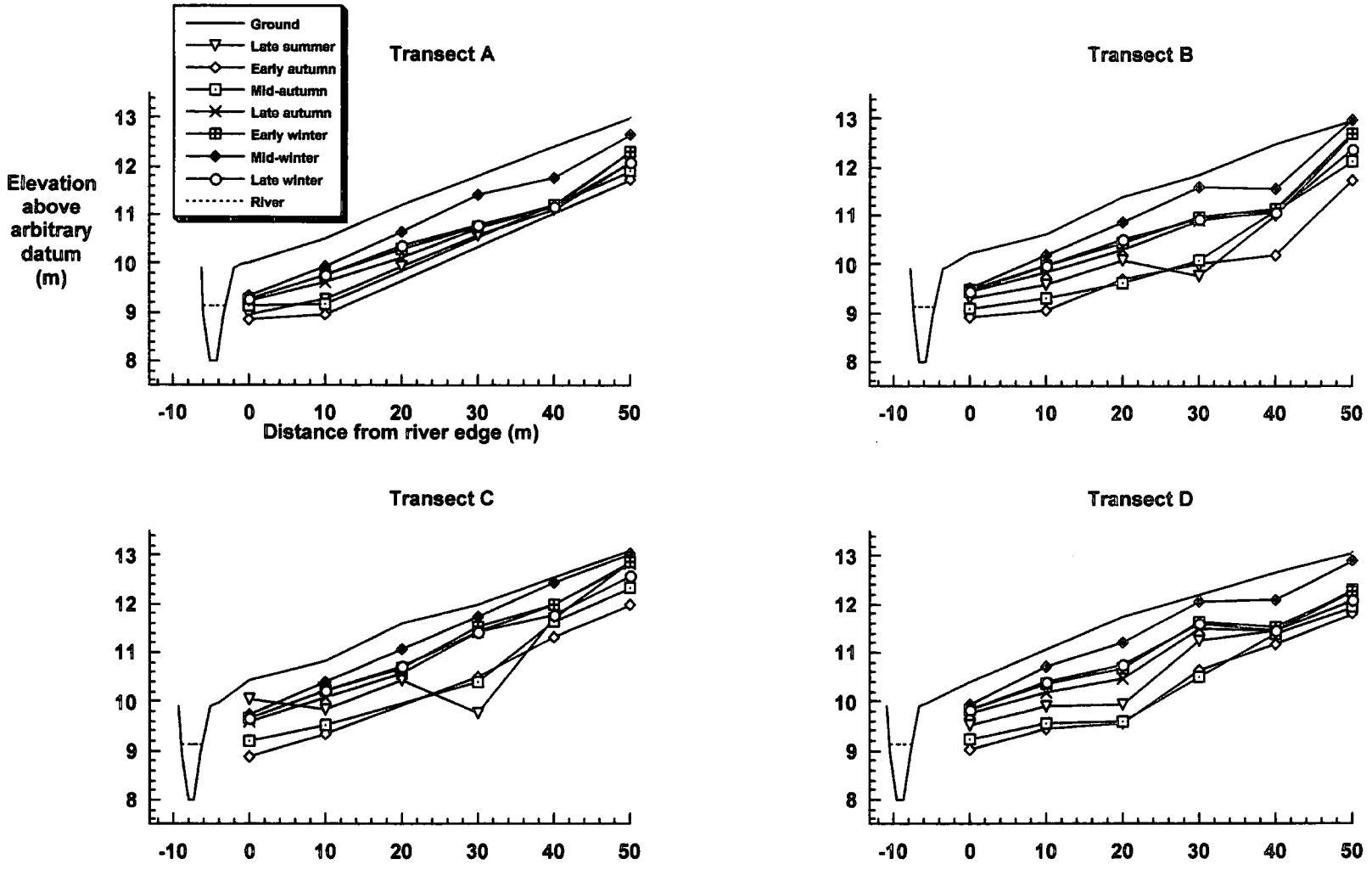


Figure 3.24. The position of the water table in relation to the ground surface for piezometer transects across the wood riparian zone on monthly sampling dates. Transects extend from the pastoral upland (50 m) to the river edge (0 m).

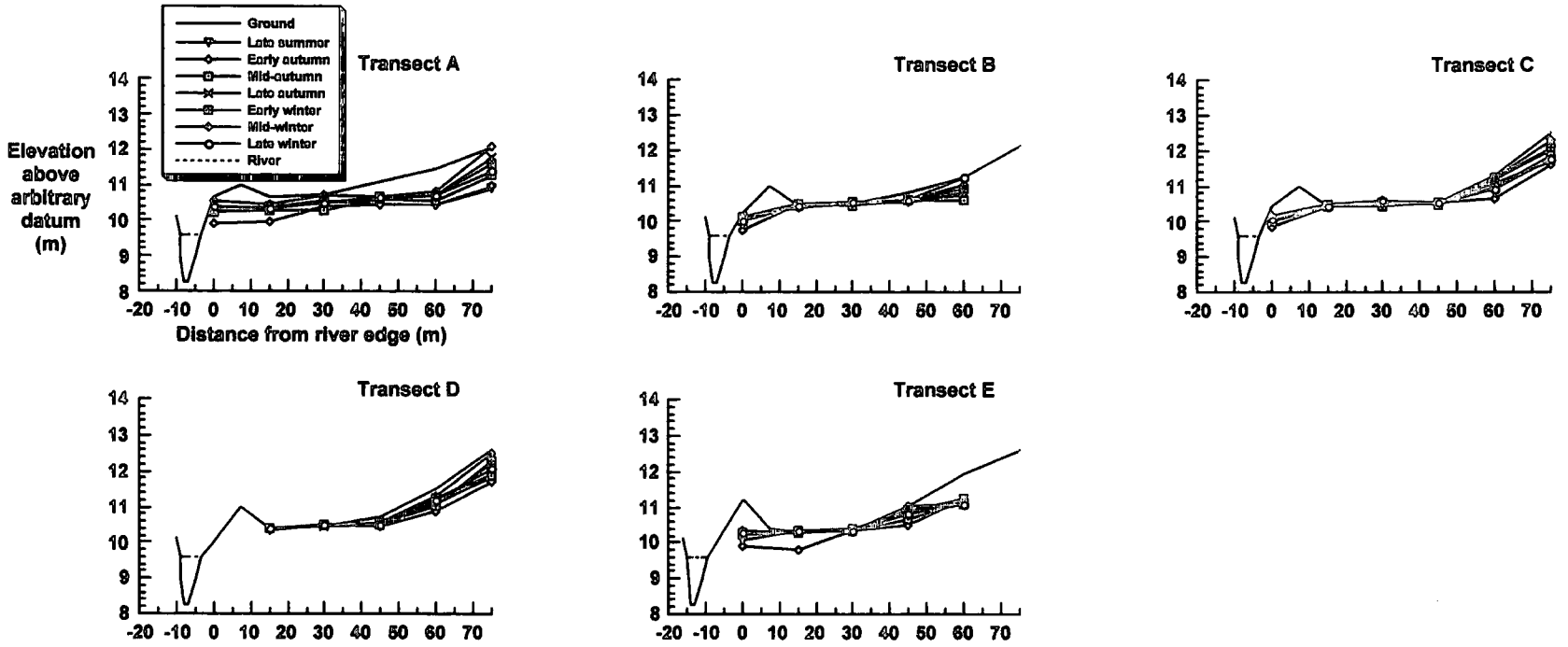


Figure 3.25. The position of the water table in relation to the ground surface for piezometer transects across the wetland riparian zone on monthly sampling dates. Transects extend from the pastoral upland (75 m) to the river edge (0 m).

and at the river edge (0 m). The considerable depth at the river edge is due to the presence of the artificially raised berm.

3.4.4. Redox potentials across the riparian zones

At the pasture site, measurements of redox potential indicated that runoff across the entire site was moderately reducing (100-400 mV) during the early autumn and mid-autumn periods when the water table was low (Figure 3.26). When the site became wetter in the late autumn the redox potential increased across the site to 400-500 mV presumably as a result of 'new', oxidised water inputs. During the winter months, runoff on the pastoral upland and at the river edge remained aerated (500-600 mV) but was moderately reducing in the middle width of the riparian zone (200-350 mV).

At the wood site, few spatial trends were evident with respect to redox potential except for the ubiquitously low values (100-300 mV) across the site in the low water table period as was found for the pasture site (Figure 3.27). At other times the redox potential was variable across the riparian site; in moderately wet periods (late summer and late autumn) and in the wettest period (mid-winter) the range of values was 200-500 mV. The redox potential of the pastoral upland (50 m) was often less variable than within the riparian zone.

At the wetland site the redox potential across the site was less than 350 mV in the early autumn and less than 300 mV in the mid-autumn indicating moderately reducing conditions (Figure 3.28). Higher redox potentials were measured in a variety of areas within the site at other times. Throughout the study period runoff in the river edge zone was moderately reducing with a redox potential of 100-300 mV.

3.4.5. Relationships between nitrogen concentrations and water table depth

Distinct relationships were evident between water table depth and the occurrence of high concentrations of nitrate, ammonium and dissolved organic nitrogen at the three sites (Figure 3.29).

At the pasture site, high nitrate and ammonium concentrations (>1 mg N l^{-1}) occurred where the water table was deep (75-150 cm depth). However a significant arithmetic correlation (Kendalls Tau) with water table (low water table, high nitrogen) was found only for nitrate. In contrast, high DON concentrations (>2 mg N l^{-1}) occurred where the water table was shallow (0-50 cm depth). A highly significant (P<0.001) positive correlation for DON with water table (high water table, high nitrogen) was found.

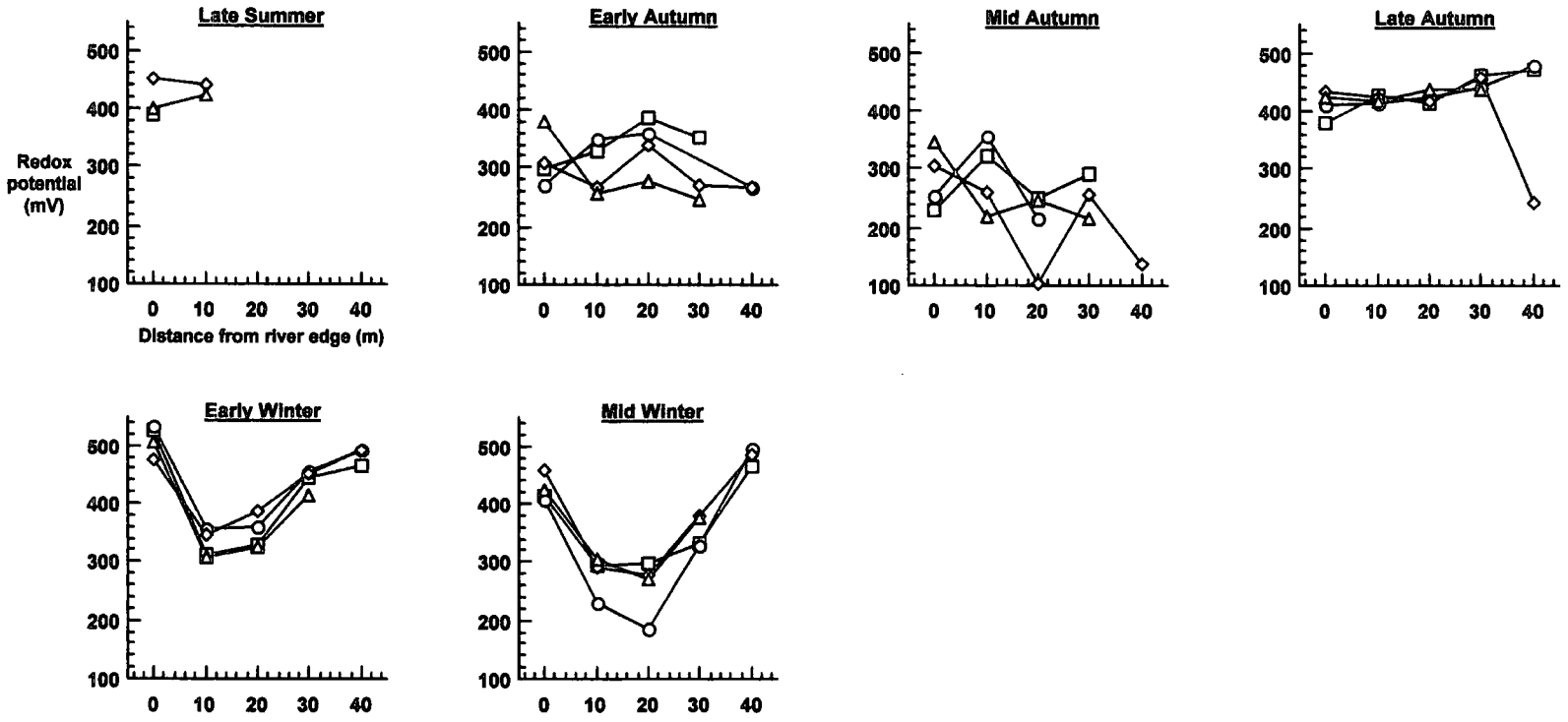


Figure 3.26. Redox potentials in piezometer transects across the pasture riparian zone on monthly sampling dates. Transect A (O), transect B (□), transect C (Δ) and transect D (◇). Transects extend from the pastoral upland (40 m) to the river edge (0 m). Data for all piezometers in late winter and most piezometers in late summer are missing due to equipment (millivolt meter) failure.

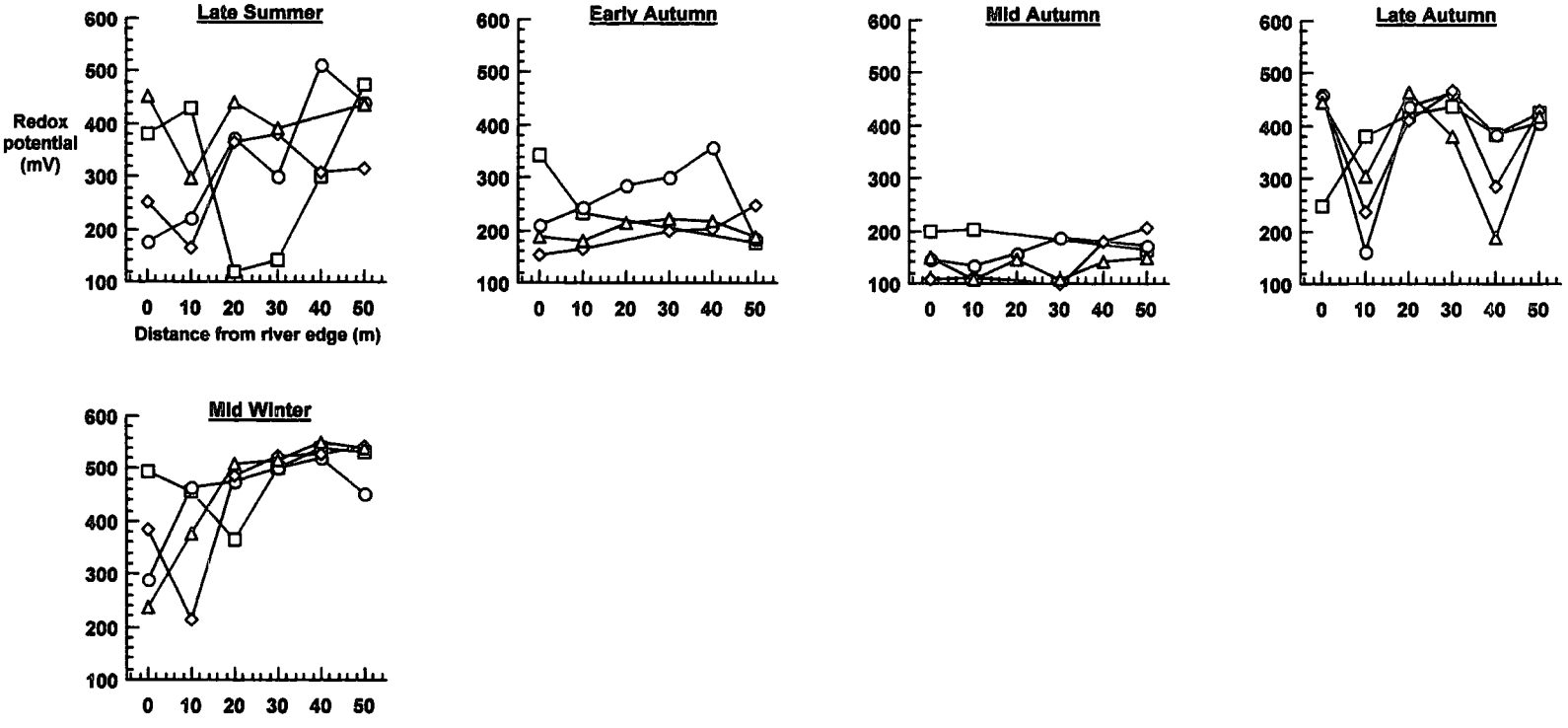


Figure 3.27. Redox potentials in piezometer transects across the wood riparian zone on monthly sampling dates. Transect A (O), transect B (□), transect C (Δ) and transect D (◇). Transects extend from the pastoral upland (50 m) to the river edge (0 m). Data for all piezometers in early winter and late winter are missing due to equipment (millivolt meter) failure.

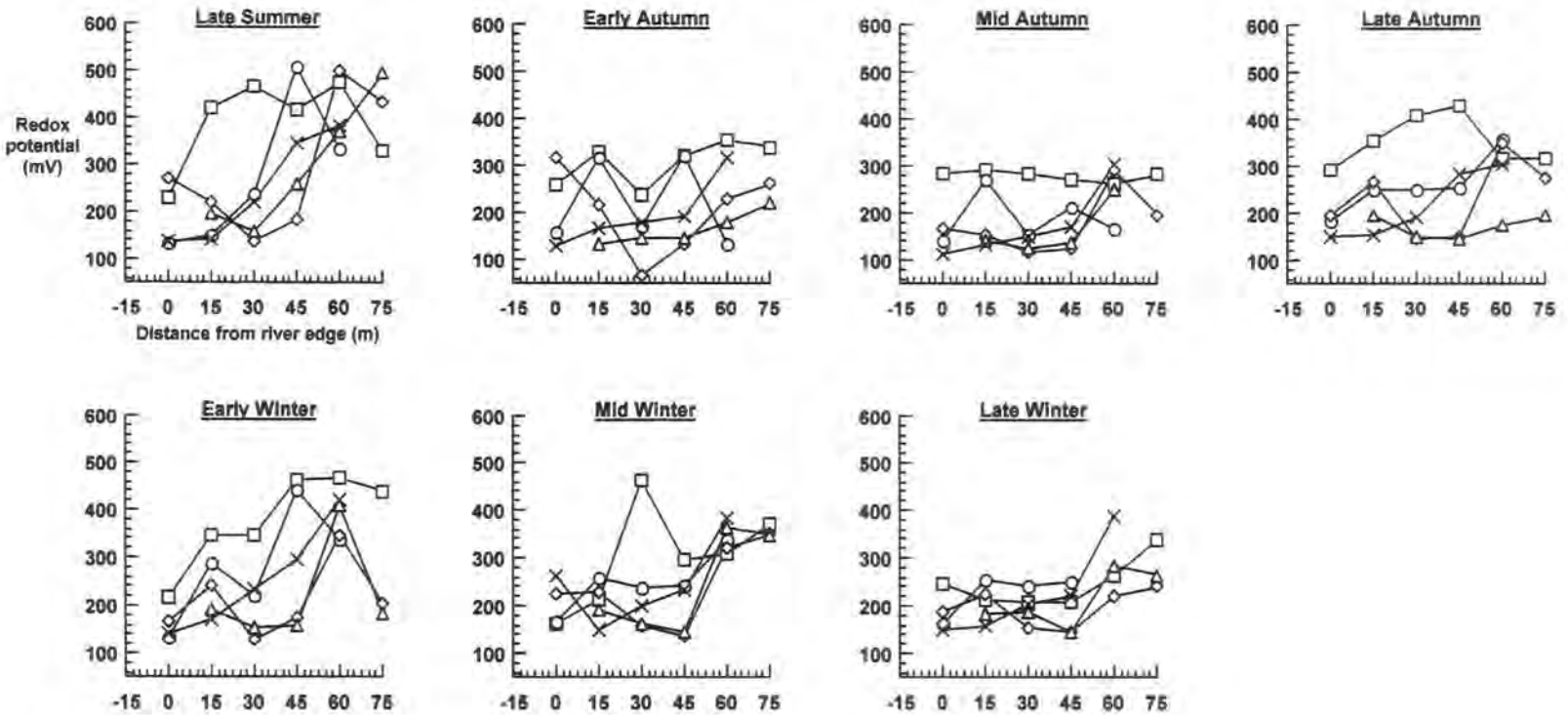


Figure 3.28. Redox potentials in piezometer transects across the wetland riparian zone on monthly sampling dates. Transect A (O), transect B (□), transect C (Δ), transect D (◇) and transect E (x). Transects extend from the pastoral upland (75 m) to the river edge (0 m).

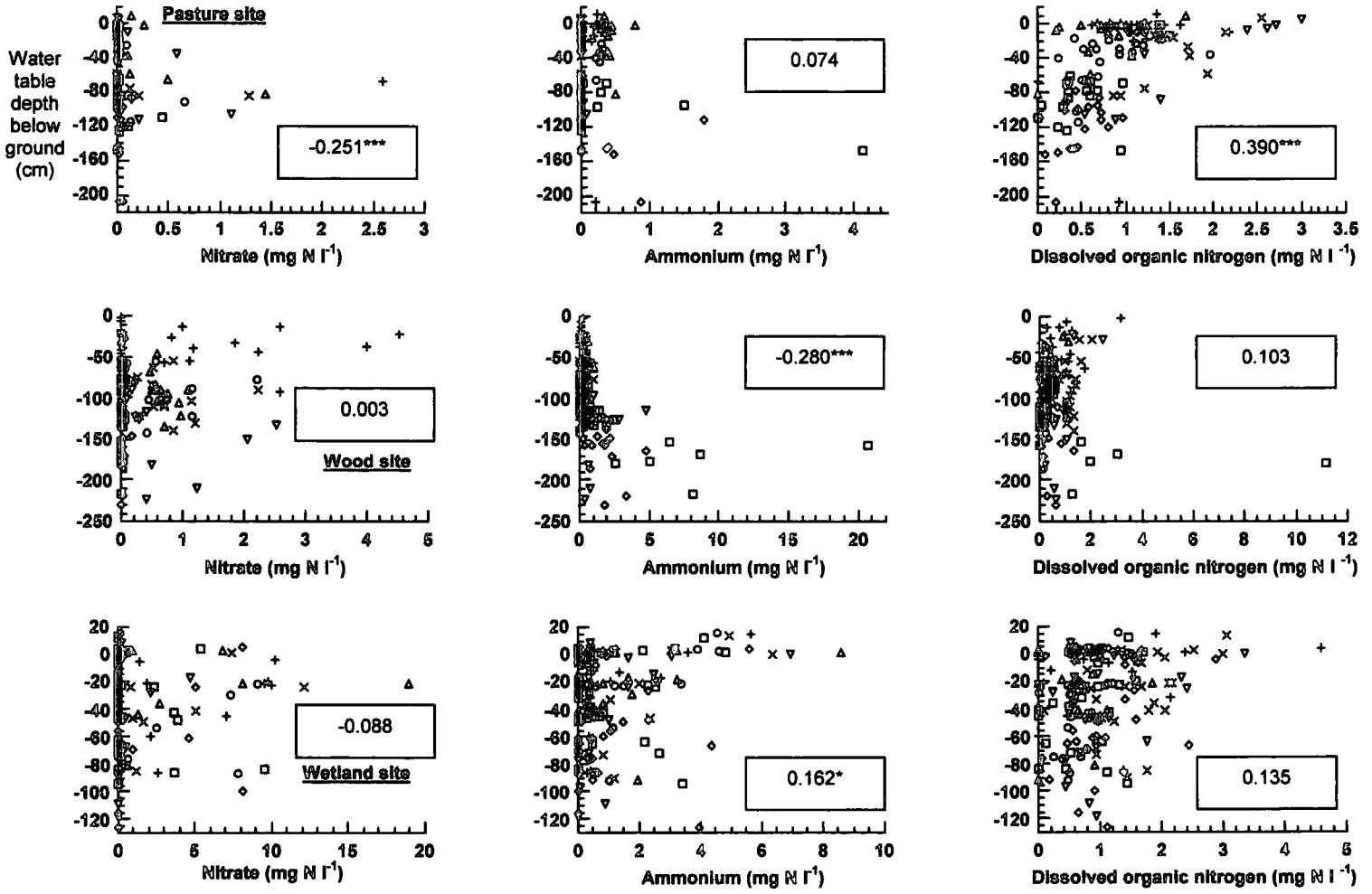


Figure 3.29. Plots of water table depth versus dissolved nitrogen forms (nitrate, ammonium or dissolved organic nitrogen) for the three riparian sites. Data from different monthly sampling dates have different symbols; late summer (▽), early autumn (◇), mid autumn (□), late autumn (×), early winter (Δ), mid winter (+) and late winter (O). The correlation coefficient (Kendall's Tau) for the entire data set is given in each plot. The significance of the correlation is indicated by the number of asterisks; * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Similarly to the pasture site, high ammonium concentrations at the wood site occurred where the water table was deep. However, in this instance, the correlation coefficient was highly significant ($P<0.001$). In contrast to the pasture site, the highest nitrate concentrations at the wood site occurred where the water table was shallow but the correlation coefficient was not significant. At this site, there was no clear relationship (graphical or arithmetic) between DON concentrations and water table depth.

At the wetland site, high nitrate concentrations were detected across the range of water table depths measured and there was no significant correlation. High ammonium and DON concentrations occurred where the water table was shallow but the correlation was only significant ($P<0.05$) for ammonium.

3.4.6. Relationships between nitrogen concentrations and redox potential

At the pasture site, there was a significant ($P<0.05$) positive correlation between nitrate and redox potential (Figure 3.30). Ammonium exhibited no significant correlation with redox potential. DON was positively correlated ($P<0.01$) to redox potential at this site.

Similarly to the pasture site, nitrate and redox potential were positively correlated at the wood site but in this case the relationship was highly significant ($P<0.001$). There was also a highly significant negative correlation between ammonium and redox potential at this site. Regarding DON there was no significant correlation with redox potential.

At the wetland site, nitrate and redox potential exhibited a significant positive correlation as was found at the other sites. There was also a highly significant negative correlation between ammonium and redox potential as was found at the wood site. DON was negatively correlated ($P<0.01$) to redox potential at the wetland site.

3.4.7. Nitrogen concentrations in river and spring water

The river Skerne contained higher nitrate concentrations than subsurface runoff from the catchment (Figure 3.31). Nitrate concentrations in the river water ranged from 3-7 mg N l^{-1} during the study period with the highest concentration evident in the late autumn. Ammonium and DON concentrations in the river water were often low (<0.1 mg N l^{-1}) and rarely above 1 mg N l^{-1}.

The hillside spring contained higher nitrate concentrations than subsurface runoff or river water. Nitrate concentrations ranged from 17-26 mg N l^{-1} during the study period with the highest concentrations in the late summer to mid-autumn period. Ammonium and DON

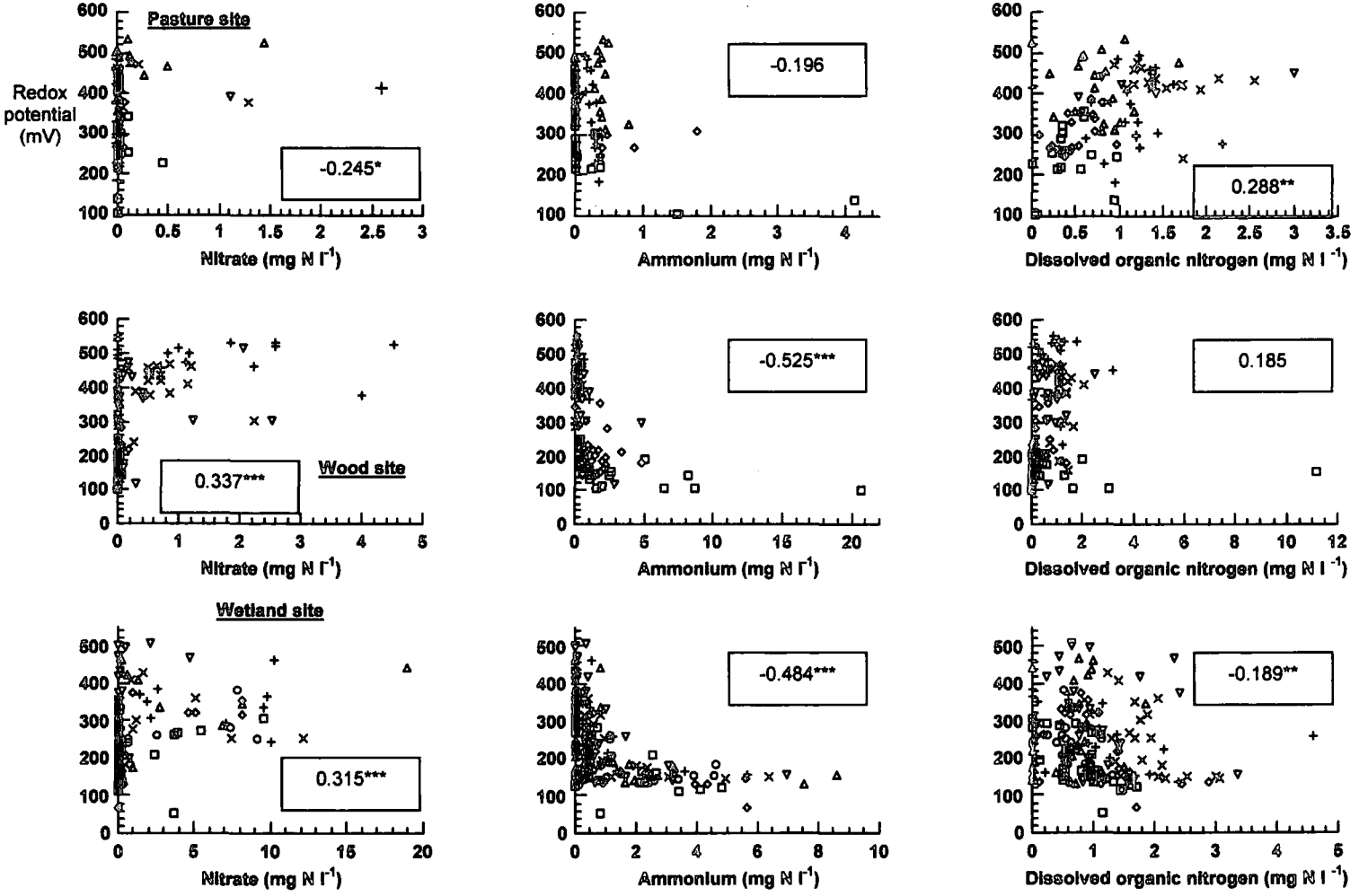


Figure 3.30. Plots of redox potential versus dissolved nitrogen forms (nitrate, ammonium or dissolved organic nitrogen) for the three riparian sites. Data from different monthly sampling dates have different symbols; late summer (∇), early autumn (◇), mid autumn (□), late autumn (x), early winter (Δ), mid winter (+) and late winter (O). The correlation coefficient (Kendall's Tau) for the entire data set is given in each plot. The significance of the correlation is indicated by the number of asterisks; * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

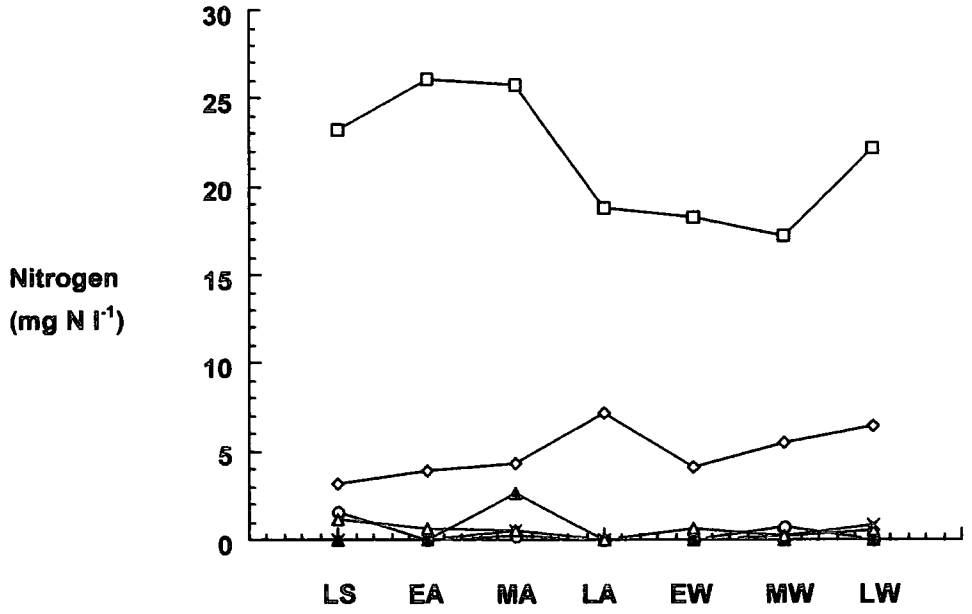


Figure 3.31. Dissolved nitrogen concentrations in the River Skerne and a nearby spring on monthly sampling dates. Spring nitrate (□), river nitrate (◇), spring ammonium (O), river ammonium (x), spring dissolved organic nitrogen (▲) river dissolved organic nitrogen (Δ).

concentrations were often low (<0.1 mg N l^{-1}). The highest concentrations of both were measured in late summer, 1.6 mg N l^{-1} for ammonium and 2.7 mg N l^{-1} for DON.

3.4.8. Nitrogen buffering efficiencies of the riparian zones

The nitrate, ammonium, DON and total dissolved nitrogen (TDN) removal efficiencies of the three riparian zones were evaluated for each monthly sampling date, with statistical analysis (Mann-Whitney test) of median input versus median output concentrations (Tables 3.1-3.3). TDN is the sum of nitrate, ammonium and DON.

The pasture riparian zone did not significantly alter the nitrate, ammonium, DON or TDN concentrations of subsurface pastoral runoff at any time.

The wood riparian zone did not significantly alter nitrate or ammonium concentrations at any time. For DON, the wood riparian zone significantly decreased concentrations in the late summer (by 89%), late autumn (by 58%) and early winter (92%). Regarding TDN, the wood riparian zone significantly decreased the concentration by 46% in the late autumn only.

In contrast to the pasture and wood riparian zones, the wetland, on occasion, significantly altered nitrate and ammonium concentrations. When nitrate input concentrations were highest in the late summer (0.14 mg N l^{-1}), mid-winter (1.68 mg N l^{-1}) and late winter (0.64 mg N l^{-1}), the wetland significantly decreased concentrations by 93, 99 and 97%, respectively. In the late summer, the wetland significantly increased the ammonium concentration. However, like the pasture riparian zone, the wetland did not significantly alter the concentrations of DON or TDN at any time.

3.5. Discussion

3.5.1. Nitrogen inputs to the riparian zones

3.5.1.1. Nitrate

Nitrate concentrations measured in subsurface pastoral runoff entering the three riparian zones were variable and often very low. Concentrations were ubiquitously low (<0.1 mg N l^{-1}) in input piezometers when the water table was very low in early autumn and mid-autumn. At other times, when the water table was higher, concentrations in some piezometers tended to be higher, especially at the wetland site. This is consistent with other observations of nitrate leaching and transport in subsurface runoff being highest in wetter periods (Burt and Arkell 1987, Burt and Trudgill 1993, Armstrong and Burt 1993).

		Late summer	Early autumn	Mid autumn	Late autumn	Early winter	Mid winter	Late winter
Nitrate	Input	0.22	0.03	<0.01 (n=1)	0.13	0.13	<0.01	0.02
	Output	0.01	0.03	0.06	0.01	0.13	0.01	0.03
	Source, sink or no change?	**no change**	**no change**	insufficient data	**no change**	**no change**	**no change**	**no change**
Ammonium	Input	0.01	0.64	4.12 (n=1)	<0.01	<0.01	0.19	<0.01
	Output	0.03	0.24	0.01	<0.01	0.39	0.20	0.15
	Source, sink or no change?	**no change**	**no change**	insufficient data	**no change**	**no change**	**no change**	**no change**
DON	Input	1.21	0.33	0.94 (n=1)	1.22	0.58	1.11	0.45
	Output	1.43	0.63	0.35	1.83	0.94	1.22	0.74
	Source, sink or no change?	**no change**	**no change**	insufficient data	**no change**	**no change**	**no change**	**no change**
TDN	Input	1.57	1.00	5.06 (n=1)	1.35	1.00	1.29	0.57
	Output	1.73	0.73	0.55	2.05	1.79	1.74	1.10
	Source, sink or no change?	**no change**	**no change**	insufficient data	**no change**	**no change**	**no change**	**no change**

Table 3.1. Nitrogen buffering efficiency of the pasture riparian zone as determined by statistical evaluation (Mann-Whitney test) of input and output concentrations of nitrate, ammonium, dissolved organic nitrogen (DON) and total dissolved nitrogen (TDN) on monthly sampling dates. Values are input/output median concentrations (mg N l^{-1}). A significant increase in nitrogen concentration between input and outputs is termed 'source', a significant decrease is termed 'sink' and no significant change is termed 'no change'. All significant differences are reported at the 95% confidence interval (P<0.05).

		Late summer	Early autumn	Mid autumn	Late autumn	Early winter	Mid winter	Late winter
Nitrate	Input	0.05	<0.01	<0.01	<0.01	0.05	<0.01	0.01
	Output	0.03	0.01	<0.01	0.35	0.01	<0.01	0.10
	Source, sink or no change?	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**
Ammonium	Input	<0.01	1.54	0.58	0.32	<0.01	0.17	0.41
	Output	0.29	1.33	0.91	<0.01	0.41	0.25	0.17
	Source, sink or no change?	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**
DON	Input	0.98	0.47	0.32	1.49	0.71	1.16	0.39
	Output	0.11	0.12	0.07	0.63	0.06	1.11	0.20
	Source, sink or no change?	**SINK**	**no change**	**no change**	**SINK**	**SINK**	**no change**	**no change**
TDN	Input	1.21	1.77	0.87	1.89	0.95	1.76	0.71
	Output	0.43	1.46	1.11	1.02	0.49	1.36	0.54
	Source, sink or no change?	**no change**	**no change**	**no change**	**SINK**	**no change**	**no change**	**no change**

Table 3.2. Nitrogen buffering efficiency of the wood riparian zone as determined by statistical comparison (Mann-Whitney test) of input and output concentrations of nitrate, ammonium, dissolved organic nitrogen (DON) and total dissolved nitrogen (TDN) on monthly sampling dates. Values shown are median concentrations (mg N l^{-1}). A significant increase in nitrogen concentration between input and outputs is termed 'source', a significant decrease is termed 'sink' and no significant change is termed 'no change'. All significant differences are reported at the 95% confidence interval (P<0.05).

		Late summer	Early autumn	Mid autumn	Late autumn	Early winter	Mid winter	Late winter
Nitrate	Input	0.14	<0.01	0.02	<0.01	0.02	1.68	0.64
	Output	0.01	<0.01	0.03	<0.01	0.01	<0.01	0.02
	Source, sink or no change?	**SINK**	**no change**	**no change**	**no change**	**no change**	**SINK**	**SINK**
Ammonium	Input	<0.01	0.49	0.46	0.27	<0.01	0.14	0.36
	Output	0.50	1.39	1.62	0.88	1.07	0.48	0.70
	Source, sink or no change?	**SOURCE**	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**
DON	Input	0.94	0.65	0.45	1.03	0.90	0.60	0.47
	Output	0.91	1.01	1.13	1.62	0.31	1.46	0.67
	Source, sink or no change?	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**
TDN	Input	0.96	0.67	0.57	1.99	1.00	2.16	1.37
	Output	1.49	2.62	2.69	2.40	1.53	2.17	1.37
	Source, sink or no change?	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**	**no change**

Table 3.3. Nitrogen buffering efficiency of the wetland riparian zone as determined by statistical comparison (Mann-Whitney test) of input and output concentrations of nitrate, ammonium, dissolved organic nitrogen (DON) and total dissolved nitrogen (TDN) on monthly sampling dates. Values shown are median concentrations (mg N l^{-1}). A significant increase in nitrogen concentration between input and outputs is termed 'source', a significant decrease is termed 'sink' and no significant change is termed 'no change'. All significant differences are reported at the 95% confidence interval (P<0.05).

The slightly higher concentrations at the wetland site during these wetter periods may result from inputs of high-nitrate water from the nearby hillside spring.

Although considerable variability was evident between input piezometers at all sites, nitrate concentrations infrequently exceeded 0.5 mg N l^{-1} and never exceeded 2.4 mg N l^{-1} during the study period. Cooper (1990) similarly reported variable nitrate concentrations in subsurface pastoral runoff entering a streamside riparian zone in New Zealand. However, nitrate concentrations tended to be higher in his study: the 5th and 95th percentiles for upslope concentrations were around 0.05 and 2.5 mg N l^{-1}, respectively, and the median concentration was 0.36 mg N l^{-1}. The median concentration in the present study was very low (0.02 mg N l^{-1}).

It seems unlikely that the generally low concentrations of nitrate in runoff in the present study are the result of low nitrogen availability in the soil. Soil organic matter typically accumulates under pasture or grassland via rhizodeposition and turnover of the large root biomass (Clement and Williams 1967, DeLuca and Keeney 1993). The pasture soil in the present study contained a high percentage (~12%) of organic matter (see Appendix A, soil pit 6 data). Above-ground inputs including stock excreta and leaf litter also contribute to the accumulation of soil organic matter (Williams and Haynes 1997) and, thus, the pool of available nitrogen. The pastoral uplands of the present study area were intensively grazed by stock (predominantly sheep) in the months preceding, and throughout most of the investigation period, so nitrogen inputs in the form of stock excreta would have been considerable, although probably very unevenly distributed (Jarvis *et al.* 1997). The most likely explanation for the generally low nitrate concentration of subsurface pastoral runoff is that very little nitrate accumulated in the soil profile to be leached. In undrained clay soils, such as those in the present study, their inherently low permeability slows the rate of water movement and increases anoxia (Burt and Haycock 1992, Burt 1993). This, in turn, is likely to discourage the accumulation of nitrate in the soil in two ways; (1) by inhibiting nitrification which produces nitrate from ammonium in the presence of oxygen and; (2) by encouraging denitrification which transforms nitrate to gaseous nitrogen in the absence of oxygen. The redox potential of the subsurface pastoral runoff during the study period seemed sufficiently low (median +337 mV, range +138 mV to +543 mV), especially during the drier periods (early autumn and mid-autumn), to support this notion.

3.5.1.2. Other nitrogen forms

Concentrations of other nitrogen forms in pastoral runoff, especially dissolved organic nitrogen (DON), were frequently much higher than nitrate. The median ammonium and DON concentrations in runoff exceeded that of nitrate by 0.2 mg N and 0.7 mg N l^{-1}, respectively. Few previous studies of agricultural nitrogen buffering in riparian zones

have reported ammonium and DON concentrations in subsurface runoff inputs to, or across, the sites. Any that have (Peterjohn and Correll 1984, Jordan *et al.* 1993, Correll *et al.* 1997) were undertaken in arable catchments, as opposed to pastoral catchments. In all of these studies ammonium and DON each comprised less than 2% of the total dissolved nitrogen concentration. However, soils at all of these sites were sandy, which presumably facilitates drainage and aeration, and inhibits the accumulation of reduced nitrogen forms (i.e. ammonium and DON).

The results of the present study suggest that most of the dissolved nitrogen in subsurface runoff in some pastoral catchments may be DON and/or ammonium rather than nitrate. The landscape settings where higher concentrations of reduced nitrogen forms in subsurface runoff might be expected, relative to nitrate, probably include: (1) where soils are slowly permeable and prone to anoxia preventing the accumulation of nitrate (e.g. clay soils); and/or (2) where there is organic fertilisation of soil via intensive livestock grazing or mechanical application of farm-yard manure, urea-based fertilisers or dairy shed effluent.

3.5.2. Nitrogen buffering in pastoral upland soils?

The generally low concentrations of total dissolved nitrogen (nitrate plus ammonium plus DON) in pastoral runoff could be indicative of a natural nitrogen buffering mechanism inherent in the slowly, permeable upland-pastoral soils of this catchment. If, (1) the upland spring water is assumed to represent catchment runoff that has had minimal contact with upland soil; and (2) the subsurface runoff sampled in riparian zone input piezometers is assumed to represent catchment runoff that has had maximum contact with upland soil; then the difference in the total nitrogen concentration of the two is indicative of the nitrogen buffering 'potential' of these soils. The difference in nitrogen enrichment is large; the median TDN concentration of the spring water was 22 mg N l^{-1} whereas the median TDN concentration of subsurface runoff was <2 mg N l^{-1}. Based on this analysis, the nitrogen buffering potential of the upland soils appears to be very high; the TDN concentration is reduced by >90%.

The extent to which runoff moves through slow-flowing subsurface pathways as opposed to fast-flowing 'spring' routes in this catchment is essentially unknown. The approximate contribution might be derived from a simple comparison with the river water concentration (5 mg N l^{-1}). Assuming that the spring and subsurface runoff were the principal contributors to the river discharge, relative contributions would appear to be around 20% and 80%, respectively. This result suggests that nitrogen removal within the upland soils may be a very important mechanism of water quality protection in this catchment.

3.5.3. Nitrogen concentration changes within the riparian zones

In contrast to many previous studies that have reported substantial decreases in nitrogen concentration for subsurface runoff moving through riparian zones (e.g. Peterjohn and Correll 1984, Haycock and Pinay 1993, Jordan *et al.* 1993, Schipper *et al.* 1993), the present study found evidence for minimal change and, in some instances, concentration increases instead.

In the pasture riparian zone concentrations of all nitrogen forms exhibited little change across the site. This presumably results from most nitrogen in pastoral inputs being DON and ammonium rather than nitrate. In contrast to nitrate, transformation of these nitrogen forms by soil microorganisms, particularly ammonium, is stimulated by aerobic conditions. Measurements of water table position and redox potential suggest that the clay soils of the pasture riparian zone were at least as, and often more, waterlogged and anaerobic than soils of pastoral upland. Minimal transformation of DON and ammonium under these conditions is, therefore, not unexpected.

Within the wood and wetland riparian zones, concentrations of nitrate and ammonium were often substantially higher than in input or river edge (output) piezometers. Very different spatial and temporal patterns were evident for these high inorganic nitrogen concentrations at each of the sites suggesting that different biological and/or physical processes were responsible. At both sites the high inorganic nitrogen concentrations cannot be accounted for by the transformation of DON in pastoral inputs; input DON concentrations were generally much too low. A possible 'internal' source of additional nitrogen at both sites is the riparian vegetation, via the decomposition of litter and root exudates. Another possible 'external' source is 'spring' water, routed into the riparian zone from the upland via irregular gravel or sand deposits.

The hillside spring that was sampled in the catchment throughout the study period contained consistently high nitrate concentrations (17-26 mg N l^{-1}) but low ammonium and DON concentrations. However, as nitrate can be reduced to ammonium by microbially-mediated dissimilatory nitrate reduction to ammonium (DNRA) under anaerobic conditions (Tiedje 1988), the high concentrations of both inorganic nitrogen forms within the wood and wetland riparian zones could be attributable to spring water inflows. The nitrate concentration of the sampled spring water was sufficiently high to account for the highest concentrations of both nitrate and ammonium measured in the two riparian zones on all sampling dates. However, at the wood site, the observation that high inorganic nitrogen concentrations were not consistently measured in the same piezometers or in the same areas of the site makes a spring source seem more unlikely at this site and an internal vegetative source more probable. At the wetland site, the

consistent association of high inorganic nitrogen concentrations with certain piezometers and areas of the site is more consistent with the notion of spring inflows.

In downslope areas of the wood and wetland site, inorganic nitrogen concentrations (and therefore, TDN concentrations) decreased back to levels that were generally comparable to pastoral input concentrations (see below). This result indicates that the downslope, near-river areas of the two riparian zones provide conditions conducive to removal of the high inorganic nitrogen concentrations that developed immediately upslope.

3.5.4. 'Net' nitrogen transformation and buffering by the riparian zones

Statistical comparison of nitrogen concentrations in inputs to, and outputs from, the riparian zones indicated that all riparian zones differed in the 'net' effect they had on the subsurface pastoral runoff. However, none of the riparian zones were found to consistently alter the concentrations of nitrogen forms in runoff during the period of investigation. The pasture riparian zone had no 'net' effect on the nitrate, ammonium, DON or TDN concentration of pastoral runoff and therefore did not function as a nitrogen buffer or nitrogen transformer at any time.

The wood riparian zone had no 'net' effect on the nitrate and ammonium concentration of pastoral runoff at any time but on 3 of 7 sampling dates the DON concentration was significantly decreased and on 1 or 7 sampling dates (late autumn) the TDN concentration was also significantly decreased. This result demonstrates that the wood riparian zone sometimes functioned as a nitrogen transformer and occasionally functioned as a nitrogen buffer. The decrease in TDN and DON in the late autumn and early winter may have been due to plant uptake, a microbially-mediated mechanism, or both. Some plants can assimilate simple organic nitrogen compounds such as free amino acids (Scarsbrook 1965). Sequential mineralisation-nitrification-denitrification transformations carried out by soil microorganisms might also be responsible for DON removal, but it is surprising that nitrate and ammonium concentrations were not also lowered significantly if nitrification and denitrification processes were occurring. For sequential mineralisation-nitrification-denitrification to account for the nitrogen removal, runoff must have come into contact with areas of differing aeration in the subsurface of the wood riparian zone. Redox potential measurements suggest that there was indeed considerable variation in aeration within the riparian zone in the late autumn, especially in the downslope area, that could account for this phenomenon.

The wetland riparian zone had no 'net' effect on the DON or TDN concentrations of pastoral runoff at any time during the study but on 3 of 7 sampling dates the nitrate concentration decreased significantly (by 93-99%). On one occasion the decrease in

nitrate concentration was accompanied by a significant increase in ammonium concentration. The wetland riparian zone does not therefore function as a nitrogen buffer, but sometimes functions as a nitrogen transformer, replacing nitrate with other nitrogen forms, most obviously ammonium. Processes that could be responsible for nitrate removal within the wetland riparian zone include plant uptake, denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and microbial immobilisation. Although concurrent production of ammonium is suggestive of DNRA, and the highly waterlogged condition of some of the site is favourable for this process (Tiedje 1988), it is not possible to conclusively rule out the operation of other processes. Ammonium might also be produced via mineralisation of organic nitrogen contained in the wetland plant litter.

3.5.5. Riparian zone nitrogen outputs to the river

Similarly to the pastoral inputs to the riparian zones, the principal nitrogen forms in subsurface runoff outputs from the riparian zones to the river were DON and ammonium. Nitrate concentrations were consistently very low (<0.1 mg N l^{-1}) from the wetland site. At the other two sites occasional, moderately high concentrations (1-2.6 mg N l^{-1}) were detected in one or more piezometers at the river edge. However, these concentrations are still well below the European Union recommended limit for nitrate in drinking water of 11.3 mg N l^{-1} (European Community 1991).

Like nitrate, DON and ammonium are biologically available nitrogen forms and may similarly contribute to the eutrophication of downstream waterbodies. The European Union also have guidelines for ammonium in potable waters (European Community 1991) and waters supporting fish (European Community 1978); 0.38 mg N l^{-1} for potable waters, 0.031 mg N l^{-1} for salmonid fish and 0.156 mg N l^{-1} for cyprinid fish. The concentration of ammonium in subsurface runoff leaving the riparian zones sometimes exceeded 0.4 mg N l^{-1} at the pasture and wood sites. At the wetland site, concentrations were often in the range of 1 to 3 mg N l^{-1}.

The European Union Directive for drinking water quality limits the organic nitrogen concentration to 1.0 mg Kjeldahl N l^{-1} (European Community 1980). Although DON was not measured by the Kjeldahl method, and a persulphate digestion was used instead, comparable and consistent recovery (~90%) of organic nitrogen standards was obtained in preliminary tests of the two methods. DON concentrations in subsurface runoff leaving the riparian zones often exceeded 1 mg N l^{-1} at the pasture and wetland sites but infrequently exceeded 1 mg N l^{-1} at the wood site.

Although subsurface runoff discharging into the river contained mainly DON and ammonium, and often at water quality threatening levels, the river water generally

contained very low concentrations of these nitrogen forms. Most of the nitrogen in the river water was nitrate, typically >99%. Presumably, movement of runoff into the faster-flowing, and more oxidised body of river water facilitates the transformation of the reduced nitrogen forms to nitrate. River water nitrate concentrations ranged from 3-7 mg N l^{-1} during this study, well below the European Union recommended limit.

3.6. Conclusions

This study has shown:

(1) that reduced nitrogen forms, especially DON, were typically more important components of subsurface runoff entering the riparian zones from the pastoral upland, than nitrate, during the late summer to late winter period of investigation.

(2) that the markedly lower total dissolved nitrogen concentration of subsurface runoff relative to 'spring' water in the pastoral upland is indicative of a high nitrogen buffering capacity associated with the slowly-permeable upland soils of this catchment.

(3) that inorganic nitrogen concentrations in runoff increased, sometimes substantially, within central areas of the wood and wetland riparian zones but decreased again downslope, close to the river.

(4) that all three riparian zones studied were generally poor buffers of agriculturally-derived nitrogen in subsurface runoff with total dissolved nitrogen concentrations typically remaining unchanged with transit from the pastoral upland to the river.

(5) the wood and wetland riparian zones sometimes functioned as transformers of dissolved nitrogen, replacing one form (i.e. DON and nitrate, respectively) with others.

(6) that subsurface runoff emitted to the river from the riparian zones often had DON and/or ammonium concentrations at water quality threatening levels. However river water contained mostly nitrate, and little DON or ammonium, and complies with European Union water quality guidelines.

Chapter 4

Dissolved nitrogen buffering by a riparian wetland intercepting surface and subsurface runoff from an agricultural catchment

4.1. Abstract

An 8-month study was conducted to determine surface and subsurface discharges and dissolved nitrogen fluxes across a riparian wetland site in a sheep-grazed pastoral catchment. The study was conducted principally during the autumn and winter months when nitrogen losses from the catchment in runoff were expected to be highest. Discharges from the site were found to comprise mostly surface water (>99%) with volumes ranging from 171 to 1367 m^3 d^{-1}. Surface water inflows (28-3385 m^3 d^{-1}) were always lower than surface water outflows and presumably subsurface water upwelling within the wetland made up the difference (8-1229 m^3 d^{-1}). The subsurface discharge entering the wetland was seriously underestimated by 'spot' measurements made through the network of piezometers (0.2-0.6 m^3 d^{-1}). A large quantity of runoff from the upland is probably conveyed to the wetland via a subsurface 'preferential flow path' associated with very irregular, but highly permeable (gravel or sand) soil deposits. Using the surface water inflow as a baseline, this flow path appears to decrease the nitrogen concentration of runoff by 27-56%. The generally low permeability of soils in the wetland forces most subsurface runoff to upwell and move across the surface of the wetland to the river. This constituted a second type of 'preferential flow path' for catchment runoff. The subsurface nitrogen flux was of little importance to the overall buffering efficiency of the wetland as it contributed less than 0.1% to the overall nitrogen flux from the wetland. Due to upwelling subsurface water, the surface water nitrogen flux often increased substantially (by 3-4 times) with transit across the wetland. However, under low flow conditions (8 m^3 d^{-1} upwelling and 171 m^3 d^{-1} outflow) when runoff-soil contact was higher, microbial transformation processes in the wetland soil clearly decreased the nitrogen flux from the catchment by 27%. Engineering structures that increase runoff-soil contact might improve the nitrogen buffering efficiency of the riparian wetland.

4.2. Introduction

Riparian wetlands have been found to act as important buffers of nitrogen in runoff from agricultural land (Pinay and Decamps 1988, Cooper 1990, Hill 1990, Simmons *et al.* 1992, Haycock and Burt 1993a, Schipper *et al.* 1993). These sites, positioned between agricultural uplands and receiving waters, are characterised by a low topographic gradient and surface expression of the water table for much of the year (Cirmo and

McDonnell 1997). Both features are indicative of their function as principal convergence zones for catchment runoff.

Riparian wetlands may, in some cases, intercept surface water flows and/or groundwater upwelling from deep aquifers, in addition to shallow subsurface runoff (Warwick and Hill 1988, Hill 1990). The occurrence of waters of different origin and the mixing of these waters within the riparian zone can complicate the study of nitrogen buffering. A good understanding of the hydrology of the riparian zone under investigation is recognised as an essential component of riparian zone nitrogen buffering studies (Hill 1996, Cirmo and McDonnell 1997, Burt *et al.* 1999, Hill *et al.* 2000).

The previous chapter investigated the nitrogen buffering efficiency of a riparian wetland, and two adjacent riparian zones, based on nitrogen concentration changes in subsurface runoff flowing from the pastoral upland to the river. A constant subsurface discharge of water across the site and minimal interaction between the surface water inflow and the subsurface runoff was assumed. In this chapter, the interaction between the surface and subsurface flow hydrology and the consequent effect on the nitrogen buffering efficiency of the riparian wetland site were investigated. The specific objectives were; (1) to measure and compare surface and subsurface water discharges and nitrogen fluxes across the riparian wetland site; (2) to determine the degree of interaction between surface and subsurface waters; and (3) to gain insights into the structure of the subsurface environment by assessment of selected soil physical and chemical properties.

4.3. Materials and method

4.3.1. Study site description

The riparian wetland study site is described in Chapter 3. However, as that chapter focused on subsurface runoff, the surface water flows were only briefly described. Further relevant information is as follows:

Within the studied area (that which lies within the piezometer grid) there is a single surface water inflow and two surface water outflow points. The inflow and first outflow points were previously described (Chapter 3). The second outflow point is located in the area between piezometers E2 and E3 and represents a downvalley flow of surface water. The inflow and the first (berm) outflow were confined to small channels of 0.5-2 m depending on the volume of flow. The second (downvalley) outflow was more diffuse and the discharge was frequently spread across a distance of 20-25 m. However, during low water table periods flows tended to converge and form one or two distinct channels of 2-3 m width.

4.3.2. Rainfall data

Rainfall data for the period of investigation were obtained from nearby Durham Observatory.

4.3.3. Methods pertaining to surface runoff

4.3.3.1. Flow velocity and discharge

Surface water discharges into and out of the wetland were gauged just prior to water sampling by the velocity-area method (Mosley and McKerchar 1992). Flow velocities were measured using a Sensa-RC2 ADS digital flow velocity meter set to record 30-second average readings in m s^{-1}.

4.3.4. Methods pertaining to subsurface runoff

4.3.4.1. Piezometer installation

The procedure for piezometer installation is described in Chapter 3.

4.3.4.2. Water table height

The procedure for measuring water table height is described in Chapter 3.

4.3.4.3. Saturated hydraulic conductivity

The saturated hydraulic conductivity (Ksat) of subsurface runoff was estimated at individual piezometers by the method of Freeze and Cherry (1979) which involves instantaneously lowering the water level and monitoring the rate of recovery. The unrecovered head difference ($H-H_t/H_t-H_0$) is calculated where H = height of original water level above an arbitrary datum, H_0 = height of water level after piezometer is emptied, and H_t = height of water level during recovery at time t. A plot is made of the normalised unrecovered head difference versus time using a log scale for the head difference. The time (T_0) where an unrecovered head difference of 0.37 occurs is used in the following equation to calculate saturated hydraulic conductivity:

$$Ksat = (rw^2 . \ln(L/R))/(2LT_0)$$

where rw = radius of perforated portion of piezometer

R = radius of unperforated portion of piezometer

L = length of unperforated portion of piezometer

4.3.4.4. Subsurface discharge and nitrogen flux

Subsurface discharge was calculated for 15 m (cross-slope) x 15 m (downslope) sections of the wetland site from measurements of water table slope, cross-sectional area and saturated hydraulic conductivity using Darcy's formula (Figure 4.1). Calculations were made for subsurface runoff moving between upslope-downslope pairs of piezometers (e.g. A6-A5, A5-A4, A4-A3 etc for each transect). Subsurface nitrogen flux was calculated by multiplying the discharge for the section and the total dissolved nitrogen concentration of runoff sampled in the downslope piezometer.

The subsurface discharge and nitrogen flux for cross-slope rows (e.g. row 5-4) were calculated by summing section measurements (e.g. A5-A4, B5-B4, C5-C4, D5-D4 and E5-E4). Where any section measurements were missing the average value of other sections in the row was substituted and utilised in the sum for the row. Where negative discharges or nitrogen flux values occurred these were added to the upslope row and excluded from the sum for the row they occurred in.

4.3.5. Water chemistry

4.3.5.1. Sample collection

The collection of subsurface water samples from piezometers is described in Chapter 2. Surface water samples were collected from inflow and outflow channels by the procedure described for river water in Chapter 3. A set of additional surface water samples (~20) was collected on Day 89 at a range of pre-determined points within the area of standing surface water.

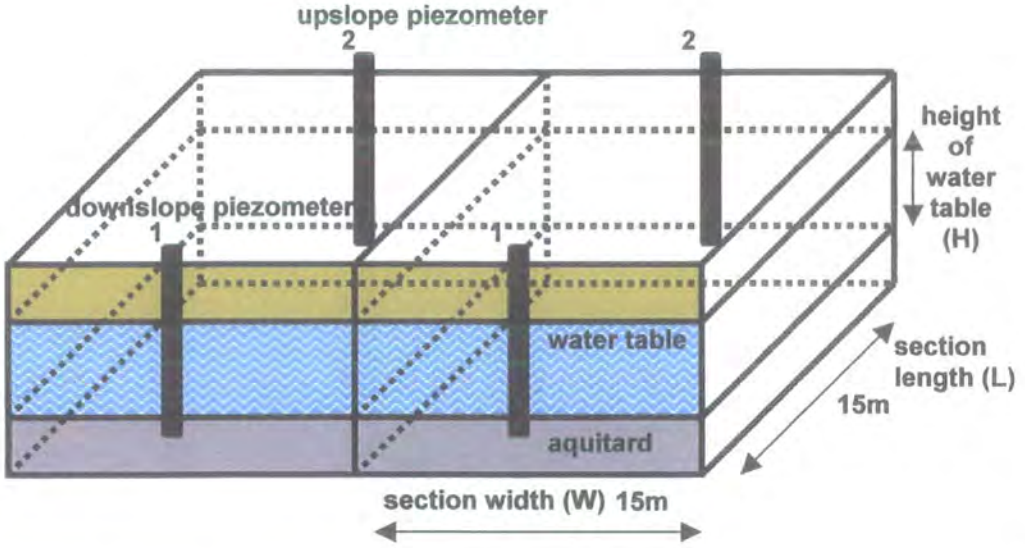
4.3.5.2. Chemical analyses

Dissolved nitrogen forms (nitrate, ammonium, DON and TDN) in water samples were analysed by the methods described in Chapter 3.

4.3.6. Soil physical and chemical properties

4.3.6.1. Field evaluation and soil sampling

In addition to notes taken on soil structure during installation of the piezometers (Chapter 3) four soil pits were constructed at strategic locations across the site; halfway between each of the following piezometer pairs A5-A4, C5-D5, A3-A2 and B1-C1 (see Figure 3.5c, Chapter 3). Soil cores (3 cm inner diameter, 10 cm length) were collected from various horizons in these pits.



Water discharge (Q) per section ($m^3\ d^{-1}$)= A x V
where A = Area of water table (m^2);
$A = H_1 \times W$
V = Subsurface flow velocity ($m\ d^{-1}$);
$V = [(X_2 - X_1)/L] \times K_1$
where X = water table altitude (m)
K = hydraulic conductivity ($m\ d^{-1}$)

Flux of total dissolved nitrogen per section ($g\ N\ d^{-1}$) = Q x C_1
where C = Concentration of total dissolved nitrogen ($mg\ N\ l^{-1}$)

NB: subscripts $_1$ & $_2$ refer to measurements made at downslope and upslope piezometers respectively.

Figure 4.1. The procedure used for calculating subsurface runoff discharge and total dissolved nitrogen flux for 15 m (cross-slope) x 15 m (downslope) sections of the riparian wetland.

Additional surface soil cores (taken at depths of 0-15 cm) were collected from two other locations across the site; the pastoral upland and the area of standing surface water.

4.3.6.2. Laboratory analyses

Measurements of soil bulk density were attained from the weight of fresh soil in a known core volume. The moisture content of soil was measured gravimetrically after drying at 105°C for 24 hours. The organic matter content of soil was determined by loss on ignition at 500°C for 4 hours. The particle size distribution of the <2mm fraction was measured on a Coulter LS Particle Size Analyzer.

4.4. Results

4.4.1. Rainfall

Rainfall at Durham Observatory, approximately 20 km from the study site, was very unevenly distributed during the study period. Rainfall occurred on 58.3% of days (Figure 4.2a). The maximum daily rainfall of 32 mm was recorded in November (late autumn) which had a high proportion of rain days (21 – 70.0%). August had the least number of rain days (12 – 38.7%) and January had the most raindays (23 – 74.2%). As expected, monthly rainfall frequently differed from the 1961-90 average (Figure 4.2b). Rainfall in February was only 37.5% of average and rainfall in October and March was nearly twice the average. Rainfall in August and December was also much lower than average. Overall, however, total rainfall for the August to March period of this investigation was higher than average, 460.2 mm compared to 390.5 mm.

4.4.2. Surface runoff

4.4.2.1. Flow velocity, direction and discharge

Flow velocities across the wetland tended to be highest at the inflow and outflow channels and much lower in between (Figure 4.3). Over some of the site surface waters did not flow perpendicular to the river. Inflow water tended to flow in a slightly upvalley direction. Surface water in the vicinity of piezometers E3, D3, E2, C2 and B2 flowed in a downvalley direction, almost parallel to river flow.

Measured surface inflows into the study site ranged from 28 to 385 m^3 d^{-1} while the discharges from the berm and downvalley outflows were 81 to 1108 m^3 d^{-1} and 82 to 782 m^3 d^{-1}, respectively (Table 4.1). Outflows were always larger than inflows indicating that another water source was contributing to surface water outflows. This additional water presumably upwelled from the subsurface as no other surface inflows were evident. The

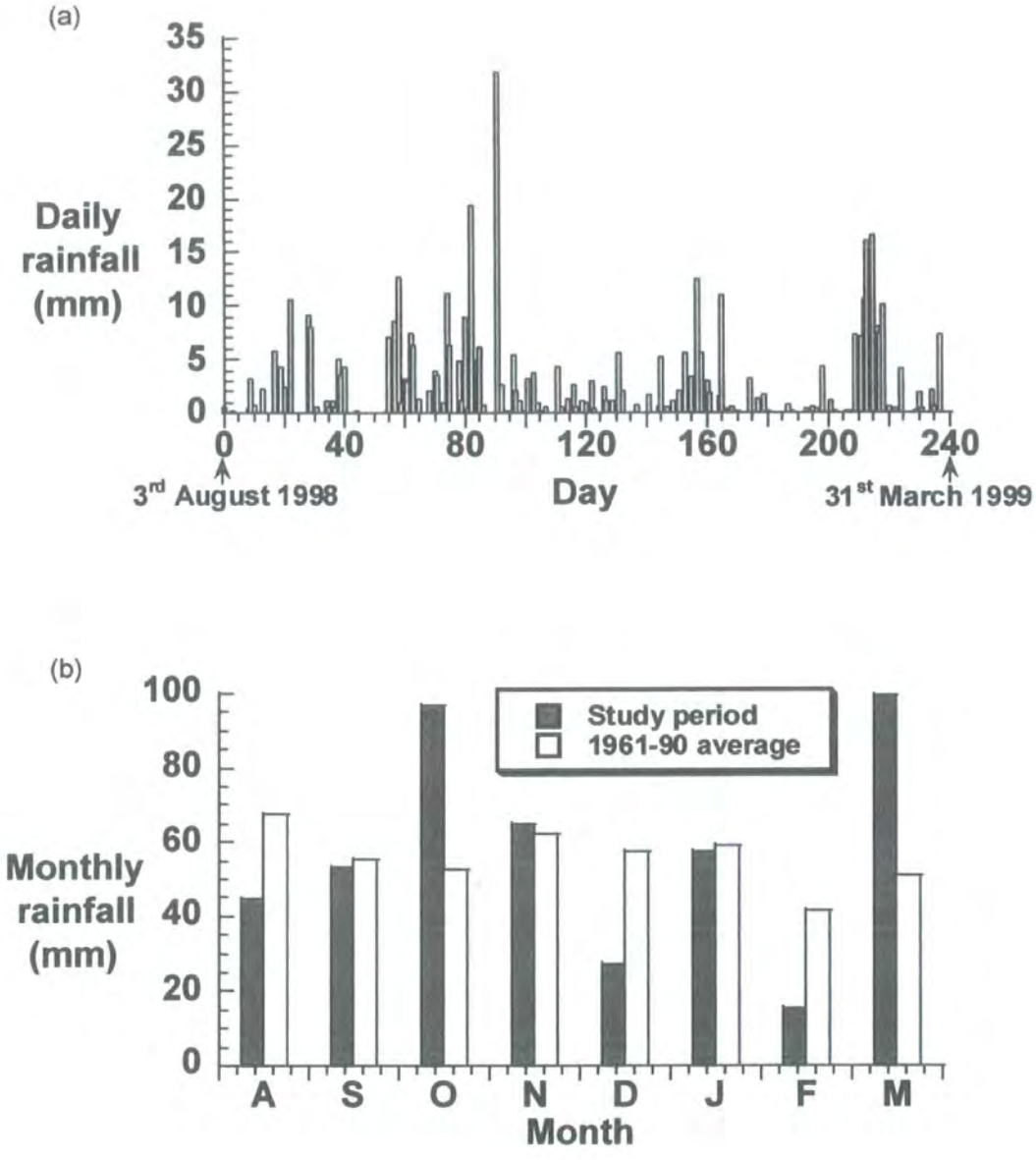


Figure 4.2. Rainfall at nearby Durham Observatory during the study period; (a) daily rainfall and (b) monthly rainfall.

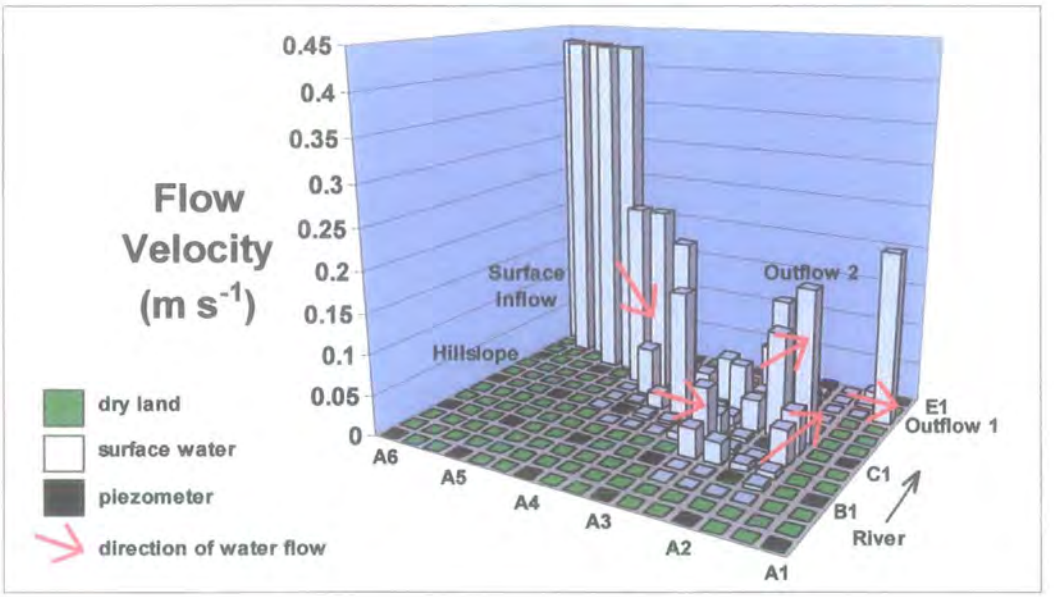


Figure 4.3. Occurrence, velocity and direction of surface flow across the riparian wetland site on Day 227 (early spring).

Table 4.1.

Riparian wetland surface water inflow and outflow discharges and the probable contribution of upwelling subsurface water on various sampling dates.

Days since 3rd August (Late summer)	Discharge (m^3 d^{-1})				
	Inflow	Outflow 1 (Berm)	Outflow 2 (Down-valley)	Total Outflow	Upwelling Water #
54	113	165	729	894	781
76	137	585	782	1367	1229
89	67	198	82	280	213
116	98	315	n	n	n
159	168	448	575	1023	855
187	163	81	90	171	8
216	385	1108	n	n	n
227	28	200	180	380	352

n = no data

calculated from total outflow – inflow

amount of this upwelling water, calculated as the difference between surface inflow and outflow discharges, was highly variable (8-1229 m^3 d^{-1}). The percentage contribution of upwelling water to the total surface outflows was very high (>75%) for upwelling discharges greater than 200 m^3 d^{-1}. On the one occasion (Day 187) when the upwelling discharge was very low (8 m^3 d^{-1}) the contribution it made to surface outflows was only 5% (Figure 4.4).

Visual evidence of upwelling water was apparent only in early spring (Day 227) when a point of upwelling was detected between piezometers B3 and C3 at the very edge of the area of standing surface water. On this date, the wetland was devoid of the partially-floating, herbaceous vegetation that had covered the site for much of the study period. Vegetation cover had persisted through much of the early cold season but eventually harsh frosts and snow-fall in mid-winter (~Day 159) had led to widespread die-off. By early spring much of the decaying litter had also disappeared, presumably swept away during storms or sinking below the water surface. With the vegetation cover and litter gone, perturbations of the water surface caused by an upwelling water plume, were then detectable.

From the point of upwelling, the B3-C3 discharge formed two fairly distinct channels that flowed towards the deeper, main body of surface water. It was therefore possible to gauge the discharge at 104 m^3 d^{-1}. The amount of upwelling water calculated by subtracting the surface inflow from the surface outflows at this time was 352 m^3 d^{-1} (Table 4.1), so 30% of the upwelling water discharge was attributable to this single point.

4.4.2.2. Total dissolved nitrogen concentration and flux

Surface water inflows to the wetland contained higher concentrations of total dissolved nitrogen (TDN) (17.5-27.6 mg N l^{-1}) than surface water outflows (8.6-19.8 mg N l^{-1}) (Table 4.2). Virtually all dissolved nitrogen in surface inflows and outflows was in the nitrate form. Decreases in TDN concentration between the inflow and the outflows ranged from 14 to 61%.

Additional 'across-site' surface water samples collected on Day 89 showed a sharp decrease in the TDN concentration of surface runoff as it became less channelised and entered the larger area of standing surface water (Figure 4.5). This occurred between piezometer rows 4 and 3. Concentrations of 18-20 mg N l^{-1} decreased to 10-16 mg N l^{-1} at this point. However, TDN concentrations were then somewhat variable downslope and downvalley and no clear pattern of further decrease was evident.

Surface water nitrogen influxes (1224-3560 g N d^{-1}) to the wetland were generally lower than nitrogen effluxes (2597-10381 g N d^{-1}), except on one occasion (Day 187) (Table 4.3).

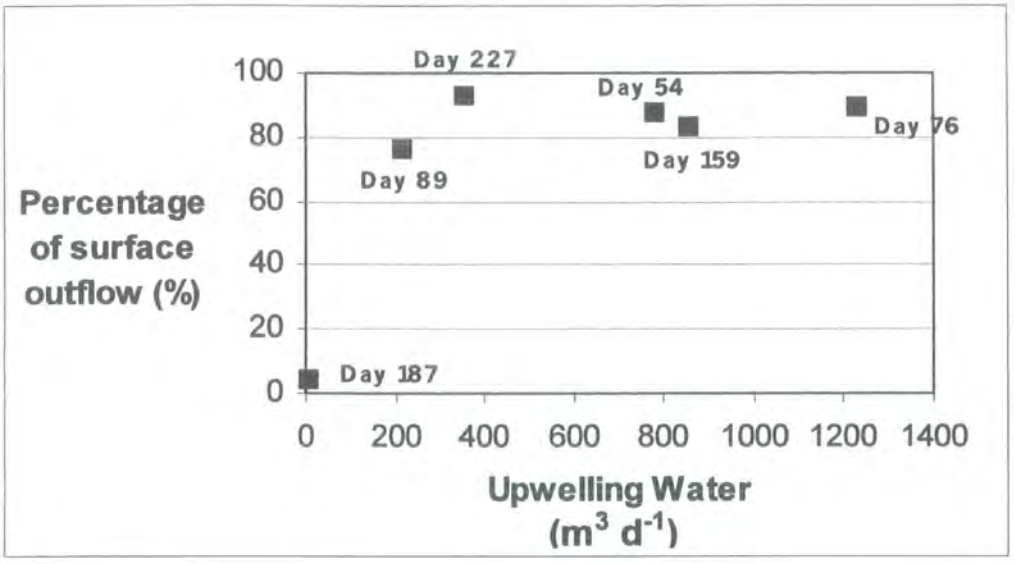


Figure 4.4. Relationship between estimated discharge and percentage contribution to surface water outflows for upwelling water in the riparian wetland during the study period.

Table 4.2

Concentrations of total dissolved nitrogen (TDN) in surface water inflow and outflow discharges from the riparian wetland on various sampling dates.

Days since 3rd August (Late summer)	TDN (mg N l-1) #					
	Inflow		Outflow 1		Outflow 2	
0	27.6	[84.3]	11.2	[76.6]	n	n
29	26.4	[98.7]	10.2	[87.0]	19.1	[92.1]
54	22.9	[>99.9]	19.8	[89.5]	9.8	[>99.9]
89	18.2	[>99.9]	14.5	[>99.5]	10.1	[90.8]
116	17.6	[>99.9]	15.2	[94.7]	9.4	[97.6]
159	17.5	[98.3]	9.5	[>99.9]	8.6	[86.9]
187	21.8	[>99.9]	14.5	[94.4]	15.8	[99.2]

n= no data

the percentage of total dissolved nitrogen that is nitrate is shown in square brackets.

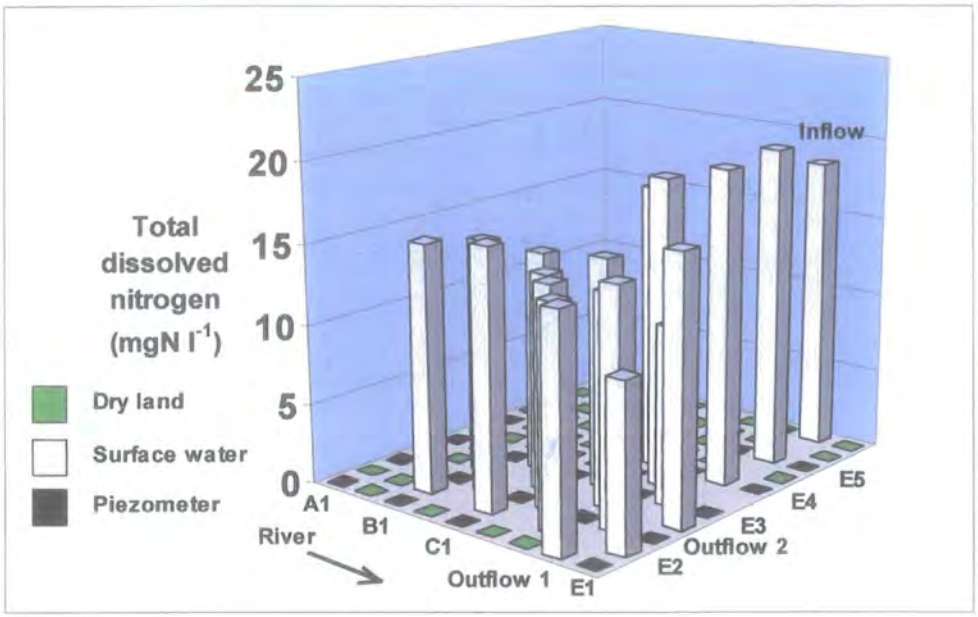


Figure 4.5. Occurrence of surface flow and concentrations of total dissolved nitrogen in surface water samples taken across the riparian wetland site on Day 89 (late autumn).

Table 4.3

Dissolved nitrogen fluxes in surface water inflow and outflows and that added to, or removed from, surface waters in transit across the riparian wetland on various sampling dates.

Days since 3rd August (Late summer)	Dissolved nitrogen flux (g N d^{-1})		
	Inflow	Outflows	Added (+) or removed (-) in transit #
54	2590	10381	+7791
89	1224	3697	+2473
159	2940	9187	+6247
187	3560	2597	-963

calculated as outflows-inflow

The flux of surface water nitrogen increased approximately 3-4 fold with passage across the riparian wetland on the first three sampling dates. On the last sampling date (Day 187) the surface water nitrogen flux decreased by 27% with passage across the riparian wetland.

4.4.3. Subsurface runoff

4.4.3.1. Flow velocity and discharge

The saturated hydraulic conductivity of soil was highly variable across the wetland and no clear spatial pattern was evident (Figure 4.6a). Measurements ranged from 0.4-235 cm d^{-1}. The subsurface flow velocity, which was derived from measurements of saturated hydraulic conductivity and water table slope (see Figure 4.1), was also highly variable (Figure 4.6b). Measurements ranged from <1-21 mm d^{-1}.

Subsurface discharges from 15 m cross-slope x 15 m down-slope sections of the wetland site were highly variable and ranged from –0.172 to 0.649 m^3 d^{-1} during the course of this study (Figure 4.7). The highest discharges (~0.300 m^3 d^{-1} or higher) occurred in section B5-B4 on Days 0, 89, 116 and 159, in section A5-A4 on Day 159 and in section E2-E1 on Day 0 only. At other times discharges from these sections were low or negative. A number of negative discharges were measured on most sampling days with the exception of Days 89 and 116. These negative discharges were the product of negative water table slopes and indicated that water in affected sections was flowing towards the pastoral upland rather than the river.

The subsurface discharge from cross-slope rows across the wetland (i.e. the sum of cross-slope sections) ranged from 0.053 to 1.057 m^3 d^{-1} during the study period (Table 4.4). The discharge at the river edge (row 2-1) was generally lower than the discharge from the pastoral upland (row 6-5) with the exception of Day 0 when the discharge increased approximately 2-fold after transit across the wetland. On other days the discharge decreased from the upland to river edge by varying amounts (64-90%). However, there was no consistent pattern of discharge decrease across the wetland from the upland to the river edge and discharges for rows 5-4 to 3-2 were highly variable. The decrease in discharge from upland to river edge is assumed to represent subsurface water upwelling to the surface. The quantity of upwelling subsurface water was 0.179 to 0.563 m^3 d^{-1} during the study period. On Day 0 the increase in discharge between the upland and river edge indicates surface water downwelling into the subsurface. The magnitude of this downwelling discharge was 0.303 m^3 d^{-1}.

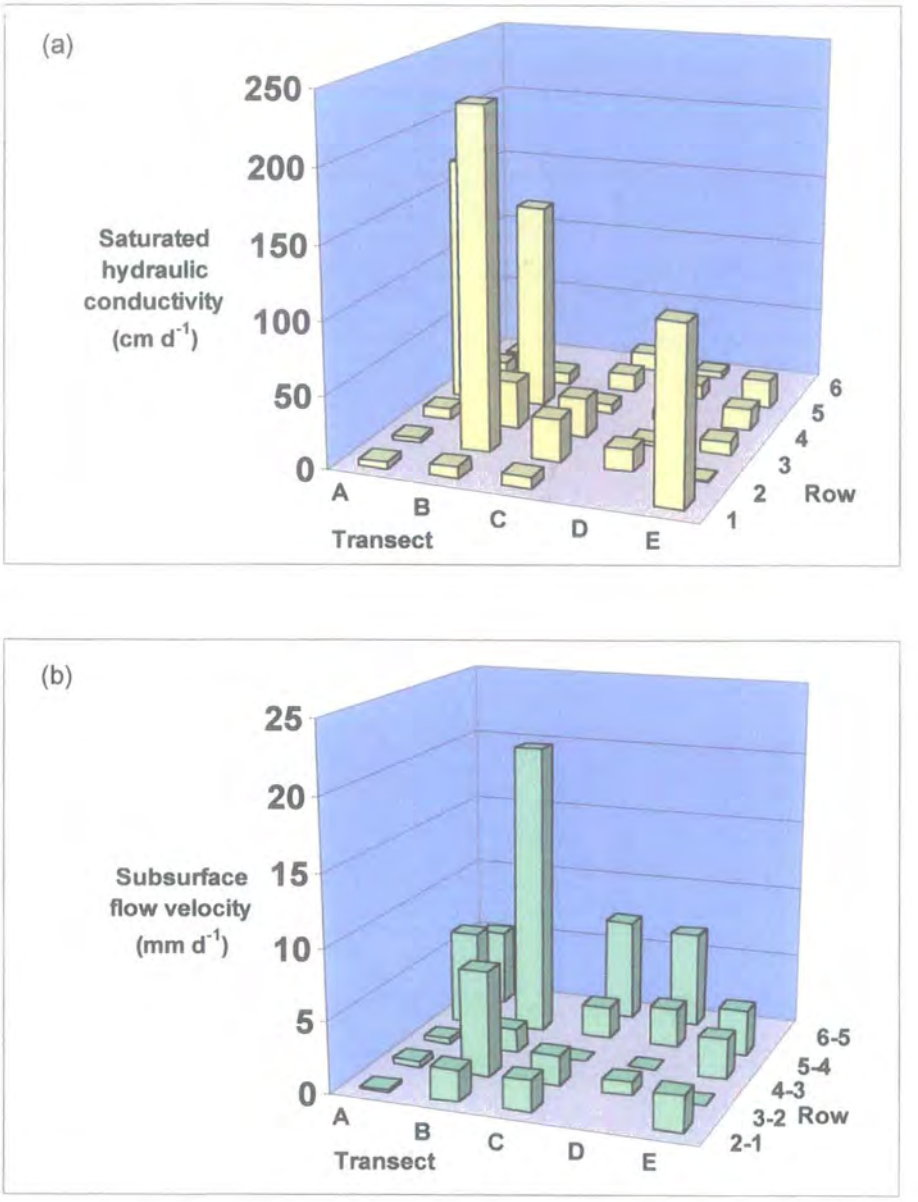


Figure 4.6. Measurements of subsurface water movement across the riparian wetland; (a) saturated hydraulic conductivity and (b) subsurface (Darcy) flow velocity. The manner of calculation for both parameters is detailed in Figure 4.1.

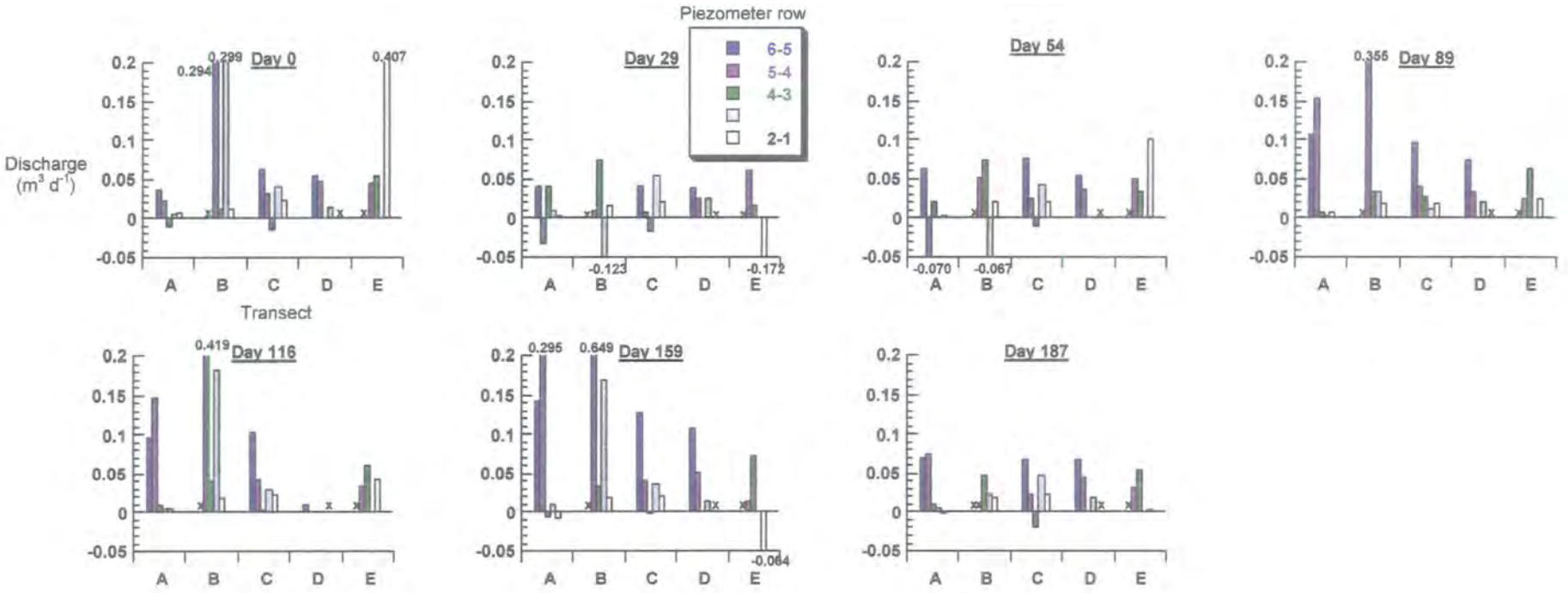


Figure 4.7. Subsurface discharges from sections of the riparian wetland site on various sampling dates. The manner of calculation is detailed in Figure 1. **x** = no data.

Table 4.4

Subsurface discharges across the riparian wetland site and the net change in discharge from the pastoral upland to the river edge on various sampling dates.

	Subsurface water discharge ($m^3 d^{-1}$)						
	Day 0 Late summer	Day 29 Early autumn	Day 54 Mid-autumn	Day 89 Late autumn	Day 116 Early winter	Day 159 Mid-winter	Day 187 Late winter
Row 6-5	0.260	0.232	0.390	0.465	0.344	0.628	0.340
Row 5-4	0.465	0.120	0.174	0.608	0.645	1.057	0.239
Row 4-3	0.066	0.254	0.196	0.136	0.114	0.109	0.110
Row 3-2	0.360	0.262	0.043	0.072	0.218	0.302	0.096
Row 2-1	0.563	0.053	0.145	0.088	0.114	0.065	0.074
Net change	+0.303 2-fold increase	-0.179 77% decrease	-0.245 64% decrease	-0.377 81% decrease	-0.230 66% decrease	-0.563 90% decrease	-0.266 78% decrease

Discharges from adjacent cross-slope sections have been summed. The pastoral upland is represented by row 6-5. The river edge is represented by row 2-1. Regarding the net changes, negative values indicate subsurface water upwelling to the surface, positive values indicate surface water infiltrating into the subsurface.

4.4.3.2. Total dissolved nitrogen flux

Like the discharges, subsurface nitrogen fluxes from 15 m x 15 m sections of the wetland site were highly variable and ranged from –0.871 to 7.867 g N d^{-1} (Figure 4.8). The highest nitrogen fluxes (2-8 g N d^{-1}) were associated with two of the three sections (B5-B4 and A5-A4) where high discharges were simultaneously recorded.

The subsurface nitrogen flux from cross-slope rows ranged from 0.099-8.273 g N d^{-1} during the study period (Table 4.5). The highest nitrogen fluxes (3-8 g N d^{-1}) were found for row 5-4 on sampling days between and inclusive of 89-159 (late autumn to mid-winter). The nitrogen flux generally decreased from the pastoral upland to the river edge except on Day 0 when it increased approximately 3-fold. On other days, the decrease was greater than 50% (51-95%). The largest decrease in nitrogen flux in terms of percent removal (95%), but also in terms of quantity (2.4 g N d^{-1}), occurred on Day 159 (mid-winter).

4.4.4. Soil physical and chemical properties

4.4.4.1. Aquitard position

During piezometer installation an aquitard layer of dense blue-gray clay was found at depths below the ground surface ranging from 0.9m at piezometers B1 and C4 to around 2.2m at piezometer A6. At piezometer E1, the depth to this clay layer was even greater (2.5m) but this was due to the artificial, river-edge berm being especially high at this point. Unfortunately this clay layer was generally located below the maximum depth to which the soil pits could be safely constructed, and hence it was excluded from this additional analysis.

4.4.4.2. Soil analyses

Full details of the soil pit results are provided in Appendix A. Only the particle size data are presented here.

The sand, silt and clay fractions in soil samples, taken at depths below the ground surface ranging from 4-86 cm, were variable (Figure 4.9). The sand fraction was most variable and measurements ranged from 10.8-73.6%. The silt fraction was slightly less variable and ranged from 20.9-68.6%. The clay fraction was least variable and ranged from 5.5-20.5%. According to the United States Department of Agriculture soil textural triangle the soils represented across the wetland site are silts, silt loams, loams and sandy loams (Rawls *et al.* 1992). Soil at intermediate depths (30-80 cm) tended to have more variable percentages of sand, silt and clay than shallower or deeper soil.

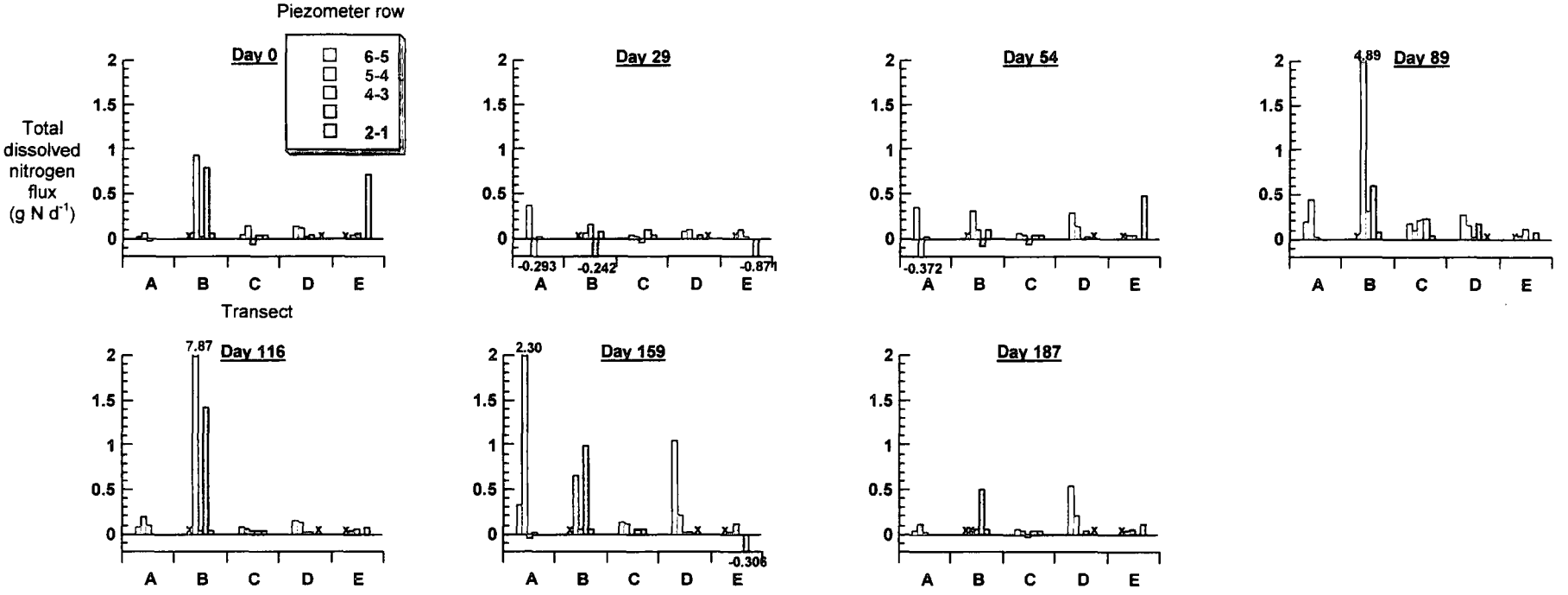


Figure 4.8. Subsurface nitrogen fluxes from sections of the riparian wetland site on various sampling dates. The manner of calculation is detailed in Figure 1. x = no data.

Table 4.5

Subsurface nitrogen fluxes across the riparian wetland site and the net change in nitrogen flux from the pastoral upland to the river edge on various sampling dates.

	Subsurface nitrogen flux (g N d^{-1})						
	Day 0 Late summer	Day 29 Early autumn	Day 54 Mid-autumn	Day 89 Late autumn	Day 116 Early winter	Day 159 Mid-winter	Day 187 Late winter
Row 6-5	0.333	1.050	1.519	1.030	0.508	2.530	1.048
Row 5-4	1.358	0.329	0.589	5.620	8.273	3.338	0.514
Row 4-3	0.099	0.410	0.235	0.671	0.222	0.173	0.114
Row 3-2	0.874	0.995	0.060	0.841	1.459	1.392	0.573
Row 2-1	0.988	0.119	0.746	0.239	0.178	0.130	0.236
Net change	+0.655 3-fold increase	-0.931 89% decrease	-0.773 51% decrease	-0.791 77% decrease	-0.330 65% decrease	-2.400 95% decrease	-0.812 77% decrease

Discharges from adjacent cross-slope sections have been summed. The pastoral upland is represented by row 6-5. The river edge is represented by row 2-1.

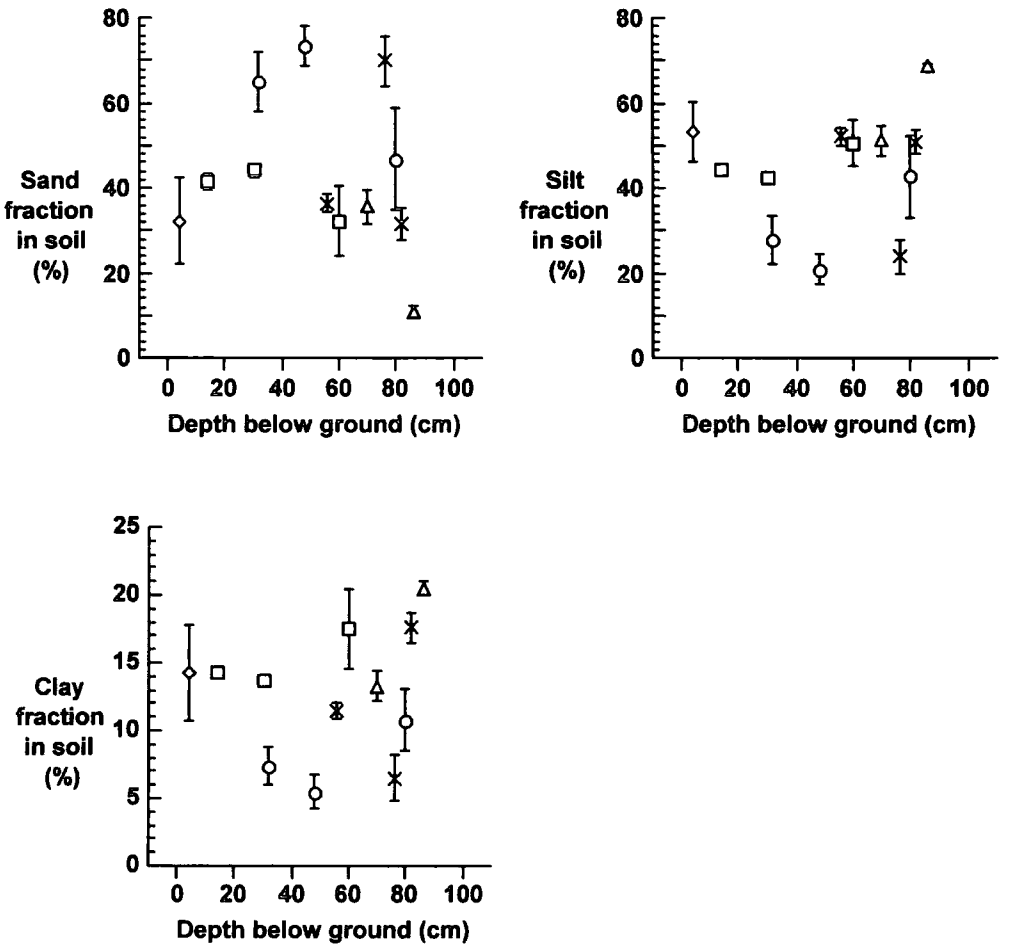


Figure 4.9. Sand, silt and clay fractions in soil versus depth below the ground surface at the riparian wetland site. A4-A5 soil pit (O), C5-D5 soil pit (□), A2-A3 soil pit (Δ), B1-C1 soil pit (x) and surface soil beneath standing surface water (◇). Values are means (± standard deviation, n=5 except for surface soil where n=11).

At these intermediate depths some samples contained higher amounts of sand and lesser amounts of silt and clay than shallower or deeper soil. The deepest soil samples collected (at ~86 cm depth) contained the highest amount of silt and clay and the lowest amount of sand.

4.5. Discussion

4.5.1. Water flows

Two types of 'preferential flow path' are illustrated in this study; 1) a surface flow path from the wetland to the river and, 2) a subsurface flow path from the pastoral upland to the wetland.

Most water discharged from the wetland as surface flow as opposed to subsurface flow. The surface and subsurface components of the total water discharge from the wetland were >99% (171-1023 m^3 d^{-1}) and <1% (0.05 to 0.56 m^3 d^{-1}), respectively. The dominance of surface water flows suggests that the soils of the wetland are generally too impermeable to convey the bulk of the catchment runoff to the river. Measurements of saturated hydraulic conductivity in the wetland soil support this notion. Although there was considerable variability across the site, as is characteristic of riparian sediments (Hill 1996), values were typically low. The range of values in the present study (0.4-235 cm d^{-1}) are comparable to measurements made in other glacial deposits. Hinton *et al.* (1993) reported values of 0.9-225 cm d^{-1} for glacial till and 2.6-259 cm d^{-1} for associated podzolic soils, and Roulet (1990) found values of 0.1-234 cm d^{-1} for glacial deposits in a headwater basin wetland. Regarding riparian zone deposits specifically, the range of values is slightly lower than that found by Burt *et al.* (1999) for clayey floodplain alluvium in a riparian zone in southern England (50-530 cm d^{-1}). However, in one of the most comprehensive studies of hydrological phenomenon in riparian zones to date, Devito *et al.* (2000), reported saturated hydraulic conductivities of 0.1-10 cm d^{-1} for surficial peat deposits and 10-100 cm d^{-1} for underlying sand in a riparian zone in Canada. They also measured higher values (200->750 cm d^{-1}) in a coarser sand and fine gravel layer of limited extent. The values reported in the present study are most similar to their measurements for the less permeable peat and fine sand deposits.

'Bypass' flow via preferential paths may be a common feature of riparian wetlands where impermeable surficial soils and alluvium deflect runoff to flow either: (1) across the soil surface as 'return flow', or (2) at depth if the site is underlain by more permeable deposits. In the present study, deposits at greater depth (i.e. <0.9-2.5 m) were more impermeable so flow was deflected to the surface. Brusch and Nilsson (1993) reported a similar occurrence for a riparian wetland in Denmark. Surficial peat and gyttja in the wetland

were significantly less permeable than upland Quaternary sands so subsurface runoff from the catchment was forced to flow across the wetland surface. Burt *et al.* (1999) reported an instance of 'bypass' flow occurring at depth in a riparian zone in southern England, with runoff being deflected through permeable gravel lenses underlying the relatively impermeable floodplain alluvium at 2.5-3 m. Devito *et al.* (2000) also found that most runoff moving through a riparian zone in Canada bypassed the low conductivity, surficial peat matrix and flowed through underlying permeable sands. However, they also reported that some runoff upwelled through irregular open cavities, or 'pipes', in the peat to emerge at the surface as springs.

Measured discharges leaving the wetland were always higher than measured discharges entering the wetland. With no other surface water inflows evident, the extra water is assumed to upwell from the subsurface. Since the upwelling discharge was highly variable during the study period (8-1229 $m^3 d^{-1}$), a local source, as opposed to a regional one, seems most likely. Discharges associated with a regional groundwater aquifer are expected to be reasonably constant with little seasonal fluctuation (Hill 1996) and this was not evident in the present study. In any case, the regional aquifer is many metres below the impermeable boulder clay overburden. Moreover, overall discharges from the wetland appear to be comparable to estimates of runoff from the local catchment. The catchment area for the riparian wetland is 0.05 km^2 and monthly rainfall averaged 57.4 mm (range 15.6-99.7 mm) during the study period. Assuming evapo-transpiration losses of 35%, as are reportedly typical for this region of the United Kingdom (Petts and Foster 1985), runoff is estimated to be 613 $m^3 d^{-1}$ (monthly range 181-1045 $m^3 d^{-1}$) for the study period. This value is not too dissimilar to the measured discharge from the riparian wetland that averaged 686 $m^3 d^{-1}$ (sampling range 171-1367 $m^3 d^{-1}$).

The upwelling discharge was generally very large in comparison to subsurface discharges estimated from the piezometer network. The most likely explanation for this discrepancy is that catchment runoff is conveyed through highly permeable (i.e. gravel or sand), but very irregular, deposits in the boulder clay from the pastoral upland to the wetland. Irregular lithological features of this type are not uncommon, especially in glaciated terrain where the geology is inherently complex (Smith and Wheatcraft 1992, Cirmo and McDonnell 1997). In the present study, the extent of this type of 'preferential flow path' was seriously underestimated with 'spot' piezometer measurements of saturated hydraulic conductivity. As a heavy clay aquitard was consistently identified at relatively shallow depth (0.9 to 2.5 m) below the ground surface across the study site, it seems most likely that the permeable deposits conveying catchment runoff from the upland to the wetland were located above this. In some upslope parts of the wetland site, surficial deposits (0-70 cm depth especially) contained large amounts of gravel (i.e. C5-B5 pit) or sand (i.e. A4-A5 pit). Permeable soil deposits of this type could be expected to occur at similarly shallow depths in the adjacent pastoral upland. The hill-side spring

above the study site that provided the surface inflow to the wetland, occurred at the downslope edge of a large, surficial gravel deposit (see Chapter 3). It is possible that smaller, unmapped, surficial deposits of this kind occur at other locations within the catchment.

4.5.2. Nitrogen buffering

Despite substantial 'net' changes to the subsurface nitrogen flux with transit across the riparian wetland (decreases of >50% on 6 of 7 sampling dates and a 2-fold increase on 1 of 7 sampling days), this was of little consequence to the overall nitrogen buffering efficiency of the riparian wetland. Subsurface discharge from the wetland contributed a negligible amount of nitrogen to the river (0.1-1.0 g N d^{-1}) relative to surface water (2500-10400 g N d^{-1}). The relative contribution of surface water and subsurface water to nitrogen efflux from the wetland was >99.9% and <0.1%, respectively. Regarding the surface water nitrogen flux, clear evidence of nitrogen buffering was evident on only one of four sampling dates. On this sampling date, Day 187, the nitrogen flux decreased by 27%. On the other sampling dates (Days 54, 89 and 159) the surface water nitrogen flux increased 3-4 fold with transit across the riparian wetland.

It seems likely that nitrogen contained in the upwelling water is responsible for increases in surface water nitrogen flux across the wetland. If the upwelling discharge is the sole nitrogen contributor, then the nitrogen concentration of this water can be calculated as 10.0, 11.6 and 7.3 mg N l^{-1} on Days 54, 89 and 159, respectively. These derived concentrations are considerably lower than those measured in the surface inflow to the wetland (22.9, 18.8 and 17.5 mg N l^{-1}). If the surface inflow can be assumed to be represent 'minimally altered' catchment runoff, then some nitrogen removal must occur as runoff moves from the upland to the surface of the wetland via the subsurface 'preferential flow path'. The percentage decrease in nitrogen concentration is 56.3, 27.1 and 42.3% on Days 54, 89 and 159, respectively. The spatial pattern of surface water nitrogen concentrations across the wetland measured on Day 89 suggests that lower nitrogen upwelling water dilutes higher nitrogen surface inflow water. A sharp decrease in nitrogen concentration between rows 3 and 4 piezometers coincides with the observed upwelling point at row 3 (B3-C3). and the lack of a further nitrogen concentration decrease as the surface water flows towards the outflow points is indicative of the decrease occurring prior to upwelling. Removal of nitrogen from runoff that is conveyed through the subsurface preferential flow path from the upland to the wetland is most likely to occur as water moves through more biologically-active, organic surface soils (i.e. when percolating through the organic topsoil in the upland or when upwelling through the organic-rich surface sediments in the wetland).

The decrease in surface water nitrogen flux with transit across the wetland on Day 187 is clearly due to biological removal processes in the riparian zone. The decrease in nitrogen flux did not occur as a result of the wetland 'trapping' discharge as the outflow discharge was still higher than the inflow discharge. The decrease in nitrogen flux from the catchment by the riparian wetland is presumably linked to the low flow conditions in the wetland. The upwelling and outflow discharges were the lowest recorded at this time. An approximate positive relationship is also evident between surface outflow volumes and the change in nitrogen flux if data from all four sampling days are considered.

The decrease in nitrogen flux associated with the lowest flow conditions presumably occurs as a result of increased runoff-soil contact that, in turn, facilitates biological transformation of nitrogen in the wetland soil. Any biological transformation must be microbially-mediated as wetland vegetation was clearly dormant at this time. As most of the total dissolved nitrogen in surface water discharges was nitrate, the microbial processes that could be responsible for the nitrogen removal include denitrification, immobilisation and dissimilatory nitrate reduction to ammonium (DNRA). The result clearly demonstrates that the riparian wetland soil has the 'potential' to buffer nitrogen in catchment runoff that passes through it. However, this 'potential' is probably not often fulfilled because runoff moves too quickly across the soil surface and there is insufficient contact between runoff and soil for biological remediation to occur.

The nitrogen buffering efficiency of this wetland site might be improved if engineering structures such as diffuser canals (Chescheir *et al.* 1987) or level spreaders (Franklin *et al.* 1992) were utilised to laterally disperse the runoff across a wider area of the riparian zone and increase the degree of runoff-soil contact. However, hydrological engineering of this riparian wetland site, or others, should only be undertaken after carefully considering the following: (1) the potential impacts that re-routing of runoff may have on the structure and function of the wetland; and (2) the necessity, appropriateness and cost of utilising artificial structures in the wetland in the context of management of the wider catchment and possible alternative strategies to enhance the removal of nitrogen from catchment runoff (Pinay and Burt 2001).

4.6. Conclusions

Two types of 'preferential flow path', a subsurface one operating from the pastoral upland to the wetland and a surface one operating from the wetland to the river, were identified in this study. The subsurface flow path conveyed most catchment runoff through irregular, permeable deposits to the wetland. Evidence for this water upwelling and diluting surface water in the wetland suggests that some nitrogen is removed from this runoff in transit (27-56%). The relatively impermeable soils of the riparian wetland forced

subsurface runoff from the upland to upwell in this area and flow mostly across the wetland soil surface to the river. This surface flow path presumably decreases runoff-wetland soil contact and, consequently, the potential for further biological removal of nitrogen as catchment runoff flows to the river. One set of measurements taken during a low flow period clearly show that the riparian wetland is able to lower the nitrogen flux from the catchment by at least 27% when lesser amounts of runoff move through the subsurface and surface preferential flow paths. Engineering structures that increase runoff-soil contact might increase the nitrogen buffering efficiency of this riparian wetland.

Chapter 5

Inorganic nitrogen transformation processes in a riparian wetland soil measured by a ^{15}N tracer and isotope dilution technique

5.1. Abstract

A short-term (250 minute) anaerobic incubation experiment using a joint ^{15}N tracer-isotope dilution technique was undertaken to simultaneously determine potential rates of denitrification, dissimilatory nitrate reduction to ammonium (DNRA), nitrate immobilisation, mineralisation, ammonium immobilisation, anammox-coupled nitrification-denitrification and nitrification in a riparian wetland soil. The technique utilised 'paired' incubations of labelled (^{15}N) nitrate-unlabelled ammonium and unlabelled nitrate-labelled (^{15}N) ammonium and three different input concentrations (0.4, 4 and 24 μg N g soil^{-1}). Determinations of transformation activities were made after 10, 70 and 250 minutes of incubation. Soil 'disturbance' and 'priming' effects are proposed to account for high transformation activities in phase 1 (0-10 minutes). An apparent 'lull' in transformation activities in phase 2 (10-70 minutes) is attributed to a microbial population switch brought about by oxygen depletion, and phase 3 (70-250 minutes) was, thus, dominated by 'anaerobic' nitrate removal. Most (87-100%) nitrate removal in the riparian wetland soil is attributed to denitrification, which ranged from 1.3-47 μg N g soil^{-1} hr^{-1} with the highest rate in phase 1. DNRA (0.5-1.5 μg N g soil^{-1} hr^{-1}) increased relative to denitrification as the nitrate input level decreased and as the carbon to nitrate ratio of the soil increased. DNRA occurred concurrently with high rates of heterotrophic nitrification (11-35 μg N g soil^{-1} hr^{-1}) suggesting that DNRA can occur under more oxidised conditions than previously thought. Mineralisation activity (0.49 μg N g soil^{-1} hr^{-1}) did not occur concurrently with DNRA and was only detected when the inorganic nitrogen input was low. It seems likely that both processes contribute to ammonium production in the riparian wetland soil but under different physico-chemical conditions. Most ammonium removal in the riparian wetland soil was due to an unaccounted mechanism, possibly ammonia volatilisation, but ammonium immobilisation (2.7-8.5 μg N g soil^{-1} hr^{-1}) was also important. Finally, some methodological issues are discussed.

5.2. Introduction

Riparian wetlands are recognised as potentially important interceptors and transformers of agriculturally-derived nitrate in catchment runoff (Hill 1996, Cirmo and McDonnell 1997). Of the possible nitrate transformation processes operating in riparian soils, denitrification has received the most attention in past research (Warwick and Hill 1988, Cooper 1990, Groffman *et al.* 1992, Pinay *et al.* 1993, Schipper *et al.* 1993, Hanson *et al.*

1994, Burt *et al.* 1999, Nguyen *et al.* 1999b). However, dissimilatory nitrate reduction to ammonium (DNRA) and immobilisation might also be involved (Hill 1996). In some riparian wetlands studied, DNRA and/or immobilisation were thought to be of little importance because decreases in the nitrate concentration of runoff were not accompanied by concurrent increases in ammonium and/or organic nitrogen concentration (Haycock and Burt 1993a, Jordan *et al.* 1993, Burt *et al.* 1999). However, few studies have directly measured denitrification, DNRA and immobilisation activities in riparian soils in order to evaluate their relative importance (Ambus *et al.* 1992).

Previous research at a riparian wetland site in the United Kingdom (Chapter 3) showed that decreases in the nitrate concentration of subsurface runoff flowing from the pastoral upland, through the riparian zone, to the river, were sometimes accompanied by increases in ammonium. A similar phenomenon was reported by Nguyen *et al.* (1999a) for a riparian wetland in New Zealand. It is unclear whether nitrate removal and ammonium production occurs: (1) directly by the DNRA process, or (2) indirectly by denitrification and mineralisation (of ammonium from organic matter) processes. To distinguish between these possibilities a joint ^{15}N-tracer and isotope dilution technique needs to be employed. With this technique, soil is incubated with 'paired' inorganic nitrogen (nitrate and ammonium) treatment solutions of equivalent concentration. The first treatment solution contains labelled (^{15}N) nitrate and unlabelled ammonium and the second treatment solution contains unlabelled nitrate and labelled (^{15}N) ammonium. Such a technique has previously been utilised to concurrently measure denitrification, DNRA, ammonium immobilisation and mineralisation activities in riparian fen soil (Ambus *et al.* 1992) and in anaerobic muck soil (Tiedje *et al.* 1981). The technique also offers the opportunity to concurrently measure nitrate immobilisation, nitrification (autotrophic and heterotrophic) and the evolution of nitrogen gas from ammonium. The latter process might occur by coupled nitrification-denitrification reactions (Reddy *et al.* 1989) or by the process of anaerobic ammonium oxidation (Anammox) (van de Graaf *et al.* 1995). Tiedje *et al.* (1981) did not report activities for any of these latter processes. Ambus *et al.* (1992) additionally reported nitrate immobilisation and nitrification activities only. However, the simultaneous measurement of other processes may yield valuable additional information that can aid in the evaluation of nitrogen transformation mechanisms in riparian wetland soils.

The concentrations of inorganic nitrogen in runoff entering riparian wetlands are rarely constant and the relative importance of inorganic nitrogen transformation processes may vary with different input concentrations. As an example, partitioning between denitrification and DNRA is apparently regulated by the magnitude of nitrate input to the soil (King and Nedwell 1985). Higher additions of nitrate oxidise the soil (Buresh and

Patrick 1981) and decrease the carbon to nitrate ratio, both of which favour denitrification over DNRA (Tiedje *et al.* 1982, Moraghan 1993).

The overall objective of the present study was to determine the relative importance of inorganic nitrogen transformation processes in riparian wetland soil. This was achieved by simultaneously measuring denitrification, DNRA, nitrate immobilisation, mineralisation, ammonium immobilisation, anammox-coupled nitrification-denitrification and nitrification activities with a range of inorganic nitrogen inputs representative of those encountered by the soil *in situ*.

5.3. Materials and method

5.3.1. Study site description

The riparian wetland site from which soil for this experiment was collected is described in Chapters 3 and 4.

5.3.2. Soil sampling, storage and preparation for the experiment

Ten cores (10-cm length, 5-cm internal diameter) of near-surface (10-20 cm depth) soil from the riparian wetland were collected in late winter (February 1999) from the area of standing surface water. The ten soil cores were bulked together in the laboratory and thoroughly mixed. A 1 litre plastic pot was filled completely with the soil, to exclude as much air as possible from the sample, sealed and stored at 2°C. The sample pot was air-freighted (on dry ice) to the National Institute of Water and Atmospheric Research in Hamilton, New Zealand where it was stored at 2°C. Prior to use, the soil was wet sieved (2 mm) and some initial chemical and microbiological properties were determined (Table 5.1).

A preliminary experiment was undertaken in June 1999 to determine the optimum time for incubation of the soil with three additions of nitrate (0.2, 2 and 12 μg N g soil^{-1}) which covered the approximate range of concentrations encountered by the soil *in situ*. The disappearance of nitrate was monitored in soil slurries at regular intervals over a 48-hour incubation period. The overall incubation period and sampling intervals chosen for the experiment proper were based on these results. The experiment proper was undertaken in August 1999.

5.3.3. Incubation procedure

Subsamples of soil (5 g wet weight) were weighed into plastic Monovette® vials (37 ml). The sample was then made into slurry by the addition of 10 ml of a treatment solution. Treatment solutions were designed to provide 3 levels of inorganic nitrogen input: low (0.4 μg N g $soil^{-1}$), intermediate (4 μg N g $soil^{-1}$) and high (24 μg N g $soil^{-1}$). At each nitrogen level, ammonium and nitrate were added in equal proportions. In treatments 1, 2 and 3, nitrate was added as ^{15}N (99 atom % enrichment) while ammonium was added as unlabelled ^{14}N (Table 5.2). In treatments 4, 5 and 6 nitrate was added as unlabelled ^{14}N while ammonium was added as ^{15}N (99% atom enrichment). The ^{15}N background (natural abundance) in unlabelled nitrate and ammonium solutions was assumed to be 0.3663 atom %.

After the addition of treatment solution, each vial was sealed with a plastic cap fitted with a rubber gas sampling septum, vigorously shaken (20 sec), and flushed with helium (2 min) to render it anoxic. Helium (He) was chosen as the inert gas rather than nitrogen since He had the advantage of reducing background ^{14}N nitrogen in the headspace of the vials, aiding detection of the comparatively tiny quantities of gaseous ^{15}N evolved during the experiment. Following the flushing procedure, three replicate vials of each treatment were immediately destructively sampled. The remaining six replicate vials of each treatment were incubated for 60 minutes (3), or 240 minutes (3), in the dark at 20°C on a rotary shaker (60 rpm).

The destructive sampling procedure consisted of the following steps:
(1) duplicate 3.5ml gas samples were removed from each vial and stored in separate 3 ml draw vacutainers pending analysis of $^{15}N_2$+$^{15}N_2O$;
(2) microbial activity in vials was stopped by the addition of 210 μl of 40 mg l^{-1} mercuric chloride ($HgCl_2$), vigorously shaken to evenly distribute the preservative through the slurry, and immediately refrigerated (1°C) pending further processing.

The time that elapsed between addition of the treatment solution to the soil and complete preservation of initial samples was estimated to be 10 minutes. This took into account the slight time delay between addition of treatment solution and collection of gas samples (2 min) and the probable time delay between addition of the $HgCl_2$, refrigeration and complete preservation of the sample (8 min). The three sampling times were therefore adjusted to 10 minutes, 70 minutes and 250 minutes.

The following day preserved samples were extracted with 15 ml of 2M potassium chloride (KCl). After 1 hour on a rotary shaker (90 rpm) and centrifuging (3500 rpm for 10 minutes) the extract solution was filtered through acid-cleaned (1M HCl), Whatman GF/C

Table 5.1

Some chemical and microbiological properties of the riparian wetland soil.

Property	Unit	Mean
Organic matter (loss on ignition)	%	31.6
Moisture content	%	49.4
Total carbon	mg C g soil^{-1}	63
Total nitrogen	mg C g soil^{-1}	5.3
pH (1:2 H_2O)		7.14
Redox potential	MV	170
KCl-extractable ammonium	μg N g soil^{-1}	8.6
KCl-extractable nitrate	μg N g soil^{-1}	0.31
Denitrifying enzyme activity	μg N g soil^{-1} hr^{-1}	1.0
Microbial biomass carbon	mg C g soil^{-1}	4.5

Table 5.2

Experimental treatment solutions.

Treatment solution	Inorganic nitrogen input level	μg N g soil^{-1}			
		^{15}N-nitrate	^{14}N-nitrate	^{15}N-ammonium	^{14}N-ammonium
1	Low	0.2	-	-	0.2
2	Intermediate	2	-	-	2
3	High	12	-	-	12
4	Low	-	0.2	0.2	-
5	Intermediate	-	2	2	-
6	High	-	12	12	-

filters and stored at 1°C until nitrogen isotope analysis. A 2 ml aliquot of this filtered extract was diluted 1/20 with deionised water and analysed for nitrate and ammonium by automated hydrazine reduction-sulphanilimide/NEDD diazotization and phenol/hypochlorite colorimetry, respectively (APHA 1995). The extracted soil pellet was air-dried, finely ground and stored at room temperature (23°C) pending nitrogen isotope analysis.

KCl extracts were prepared for analysis of ^{15}N-ammonium and ^{15}N-nitrate by sequential diffusion onto filters (Sorensen and Jensen 1991). Inorganic nitrogen forms were diffused onto duplicate 6 mm disks of acid-cleaned Whatman GF/C filter paper impregnated with 10 µl of 2.5M $KHSO_4$. Diffusion flasks were incubated on a laboratory bench at room temperature (23°C) for two (sequential) 6 day periods and the contents of each flask was carefully mixed (swirling contents by hand) once a day. Diffused extracts and ground soils were analysed for percent nitrogen and atom % ^{15}N enrichment on an isotope-ratio mass spectrometer (Delta Plus, Finnigan MAT). Trapped gases were analysed for percent nitrogen and atom % ^{15}N enrichment on an isotope analyser (Europa Scientific 20/20). The atom % enrichment was converted to atom % excess after taking into account the background natural abundance level (0.3663 atom %). The concentration of excess ^{15}N for a particular nitrogen pool was calculated as the product of the total concentration of the pool with atom % excess.

The probable pH of soil slurries during the experimental period was determined by preparation and incubation of a set of vials in an identical manner to the experimental proper, with the only exception being that ^{14}N-labelled solutions replaced those that were ^{15}N-labelled. Vial caps had to be removed to make the pH measurements at 10, 70 and 250 minute intervals. After measurement, the vial in question was discarded.

5.3.4. Isotope dilution assumptions and equations

Use of the isotope dilution technique in this study is based on the following assumptions: (1) the added ^{15}N mixes uniformly with the native soil inorganic N pool, (2) transformation processes do not discriminate between ^{14}N and ^{15}N isotopes, (3) transformation process rates remain constant over the time interval assessed, and (4) immobilised ^{15}N is not remineralised (Kirkham and Bartholomew 1954). Incubation of the riparian wetland soil as well-mixed slurry satisfies the first assumption. Application of N isotope to generate a high atom % ^{15}N enrichment, and thereby cover small differences in abundance due to isotopic fractionation, satisfies the second assumption. To satisfy the third and fourth assumptions as short an incubation period as possible is utilised (Davidson *et al.* 1991, Tietema and Wessel 1992, Barraclough 1995).

The rates of removal and production of nitrate and ammonium were determined from the changes with time in the concentration and isotopic ratio ($^{15}N/^{14}N$) of nitrate and ammonium pools. Equations used to calculate production and removal rates are based on changes to the total ($^{14}N+^{15}N$) concentration and/or atom % excess of the inorganic nitrogen pool and are as follows:

(1) when both the total concentration and atom % excess of the inorganic N pool changed significantly, the following pair of equations were used (Kirkham and Bartholomew 1954, Di *et al.* 2000).

$$F_I = [(Q1 - Q2).\ln(A1/A2)]/[(t2-t1).\ln(Q1/Q2)] \quad \text{[Equation 5.1]}$$

$$F_O = F_I - [(Q2-Q1)/(t2-t1)] \quad \text{[Equation 5.2]}$$

where F_I is the production rate, F_O is the removal rate, t1 is the first point in time and t2 is the second point in time, Q1 and Q2 is the total ($^{14}N+^{15}N$) concentration of the inorganic nitrogen pool in question at t1 and t2, respectively, and A1 and A2 are the atom % excess values at t1 and t2, respectively.

(2) when the atom % excess of the inorganic nitrogen pool changed significantly but the total ($^{14}N+^{15}N$) concentration did not, the following equation was used (Kirkham and Bartholomew 1954, Di *et al.* 2000)

$$F_I = F_O = F = [\ln(A1/A2)].Q/(t2-t1) \quad \text{[Equation 5.3]}$$

As the total concentration during the incubation period did not change the production and removal rates must be equal.

(3) when the total concentration changed but the atom percent % excess did not, the following equations were used:

$$F_O = (Q1-Q2)/(t2-t1) \quad \text{[Equation 5.4]}$$

$$\text{and } F_I = 0 \quad \text{[Equation 5.5]}$$

This scenario indicates that only removal from the inorganic nitrogen pool occurred.

5.3.5. Nitrogen transformation rates derived from combined isotope pool dilution and ^{15}N tracer methodology

Nitrogen ($^{14}N+^{15}N$) transformation rates for specific processes were calculated from the nitrate and ammonium removal rates derived from isotope dilution, and the percentage contribution that processes were found to make to overall removal by ^{15}N tracer

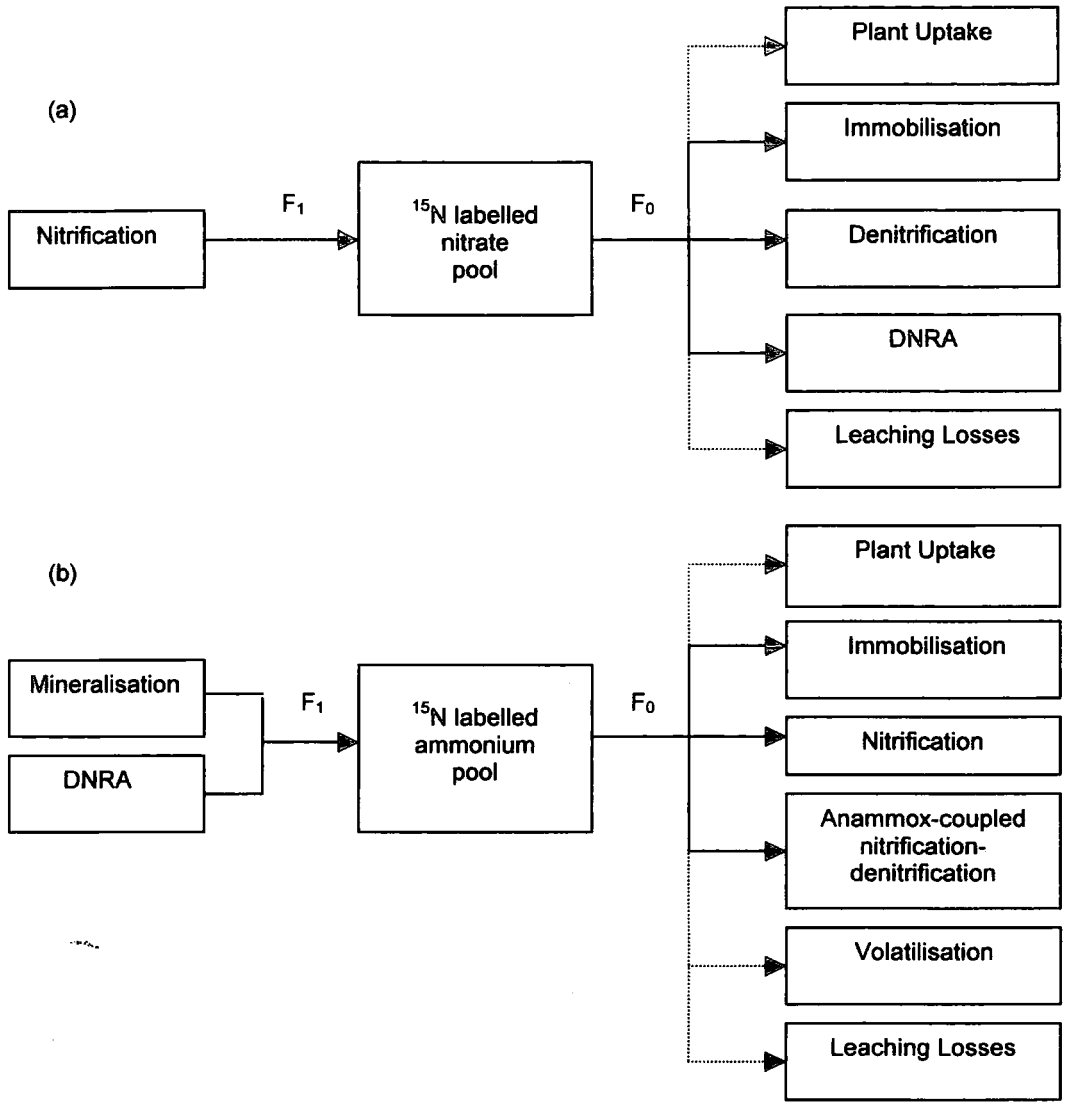


Figure 5.1. Conceptual model (adapted from Di *et al* 2000) of inorganic nitrogen production (F_1) and removal (F_0) processes assessed by the isotope dilution technique with a ^{15}N-labelled nitrate pool (a) or a ^{15}N-labelled ammonium pool (b). Where F_1 or F_0 are represented by more than one process the ^{15}N tracer technique must be used in conjunction with isotope dilution to determine the relative contribution of individual processes to the overall production or removal rate. Solid lines indicate processes applicable to the laboratory incubation procedure adopted in this study. Dashed lines indicate processes that are not applicable in this study but might be in other laboratory or field studies.

methodology. Nitrate removal includes denitrification, DNRA and immobilisation, and ammonium removal includes immobilisation, autotrophic nitrification and anammox-coupled nitrification-denitrification (Figure 5.1). Mineralisation rates were determined by subtracting the rate of DNRA (determined as a proportion of nitrate removal above) from the rate of ammonium production. Heterotrophic nitrification was determined by subtracting the rate of autotrophic nitrification (determined as a percentage of ammonium removal above) from the rate of nitrate production (total nitrification).

5.3.6. Statistics

The two-sample t-test (Datadesk 6.0) was used for the determination of significant differences ($P<0.05$) between measured time-series means (10, 70 and 250 minutes) for atom % excess values and excess ^{15}N, ^{14}N and total ($^{14}N+^{15}N$) concentrations. The t-test of individual means (Datadesk 6.0) was used to determine whether each time-series mean differed significantly ($P<0.05$) from the value calculated for time zero (0 minutes – addition of treatment solution).

5.4. Results

5.4.1. Inorganic nitrogen transformation processes following the addition of ^{15}N-labelled nitrate

5.4.1.1. KCl-extractable soil nitrate pool

With all inorganic nitrogen inputs (low, intermediate and high), decreases in the concentration of excess ^{15}N in the soil nitrate pool were greatest in phase 1 of the experiment (0-10 minutes) and less in later phases, 2 (10-70 minutes) and 3 (70-250 minutes) (Figure 5.2a). These decreases indicate nitrate removal by denitrification, DNRA and/or immobilisation. The concentration of excess ^{15}N in phase 1 decreased by 98, 87 and 52% with low, intermediate and high nitrogen inputs, respectively. In phase 2 there was no further decrease in the concentration of excess ^{15}N with any input but in phase 3 further decreases of 11 and 30% were evident with intermediate and high inputs, respectively.

The concentration of ^{14}N in the soil nitrate pool increased significantly with intermediate and high nitrogen input in phase 1 but did not change significantly with the low input (Figure 5.2b). The increases indicate 'net' nitrate production via nitrification from unlabelled nitrogen pools i.e. ammonium and organic nitrogen. No changes in the concentration of ^{14}N were evident in phase 2 but significant decreases occurred with all inputs in phase 3 indicating 'net' nitrate removal.

5.4.1.2. KCl-extractable soil ammonium pool

With all nitrogen inputs the concentration of excess ^{15}N in the ammonium pool increased significantly in phase 1 (Figure 5.3a). These increases indicate DNRA. The only further significant increase in later phases was with the low input in phase 3. With all inputs the concentration of ^{14}N decreased significantly in phase 1 indicating 'net' ammonium removal (Figure 5.3b). In phase 2, the concentration of ^{14}N decreased significantly further only with low input. In phase 3 there were no further significant changes with any input.

5.4.1.3. Headspace gaseous nitrogen pool

With all nitrogen inputs the concentration of excess ^{15}N in the gaseous nitrogen pool did not increase significantly until phase 3 (Figure 5.4a). As expected, given the large gaseous ^{14}N pool, no significant changes were detected in the concentration of ^{14}N in any phase with all inputs (Figure 5.4b).

5.4.1.4. Soil total nitrogen pool

The excess ^{15}N in the soil total nitrogen pool with low input did not change significantly during any phase (Figure 5.5a). With intermediate and high inputs the excess ^{15}N increased significantly only in phase 1, indicating nitrate immobilisation. The concentration of ^{14}N was constant during all phases with high input (Figure 5.5b). With low and intermediate inputs significant increases were detected in phase 2 and phase 1, respectively.

All data utilised in Figures 5.2 to 5.5 can be found in Appendix B, Tables B1-B3.

5.4.1.5. The relative importance of nitrate removal processes

The proportion of removed excess ^{15}N-nitrate attributable to denitrification, DNRA and immobilisation processes differed with low, intermediate and high nitrogen inputs (Table 5.3). The values from which these relative proportions are derived are given in Appendix B – tables B7-B9. Values were only derived where statistically significant changes were observed. Denitrification was represented by directly measured excess ^{15}N evolution in the gaseous headspace but also by the excess ^{15}N-nitrate unaccounted for in other nitrogen pools. The observation that most excess ^{15}N-nitrate removal occurred in phase 1 but significant increases in gaseous excess ^{15}N did not occur until phase 3 suggests a lag period prior to emergence in the headspace of vials. Gaseous nitrogen produced by denitrification was probably entrapped in the waterlogged soil and only slowly diffused out (Lindau *et al.* 1988b).

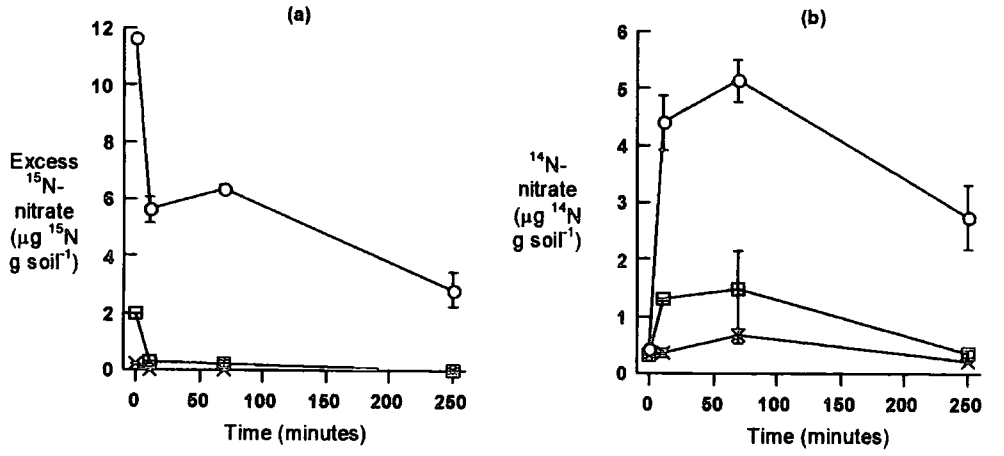


Figure 5.2. Labelled (a) and unlabelled (b) components of the KCl-extractable soil nitrate pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled nitrate during the experimental period. Values are means (± standard deviation, n=3).

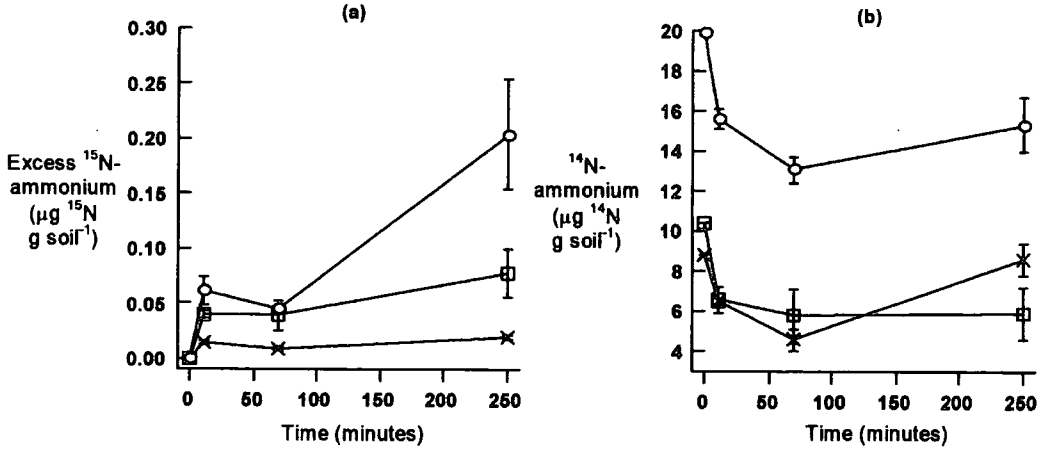


Figure 5.3. Labelled (a) and unlabelled (b) components of the KCl-extractable soil ammonium pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled nitrate during the experimental period. Values are means (± standard deviation, n=3).

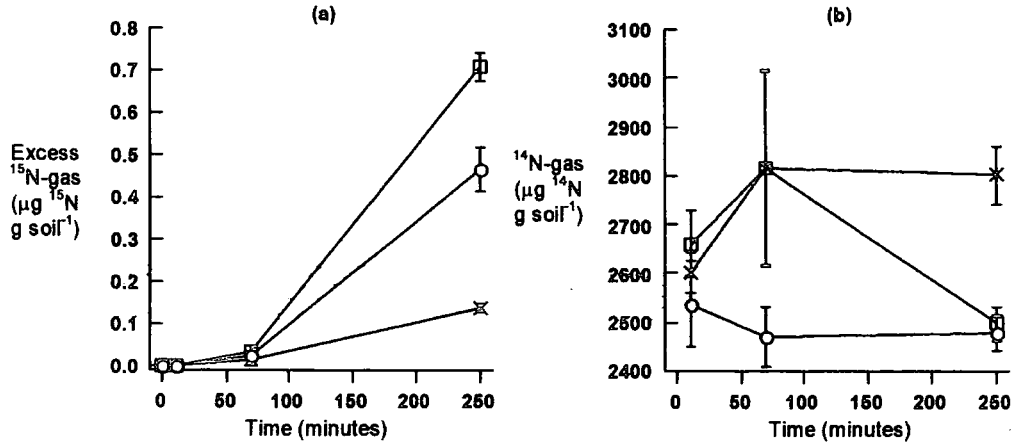


Figure 5.4. Labelled (a) and unlabelled (b) components of the headspace gaseous nitrogen pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled nitrate during the experimental period. Values are means (± standard deviation, n=3).

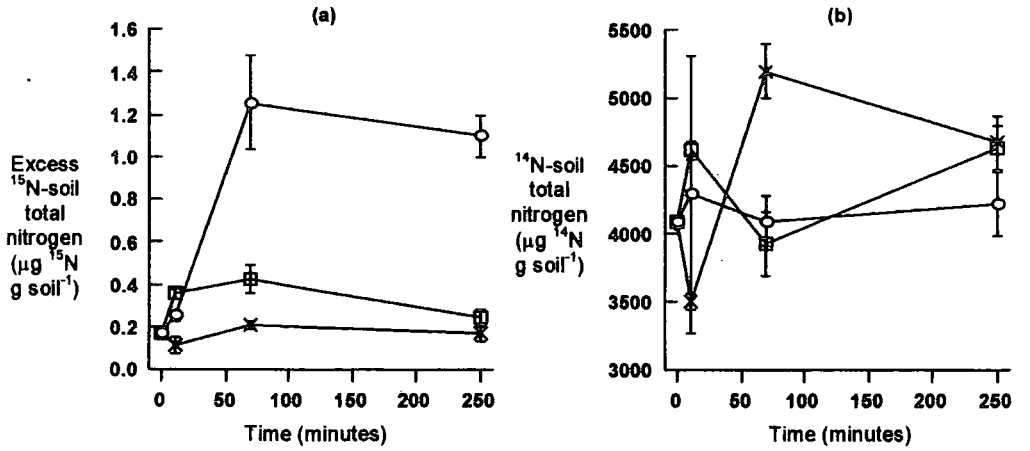


Figure 5.5. Labelled (a) and unlabelled (b) components of the soil total nitrogen pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled nitrate during the experimental period. Values are means (± standard deviation, n=3).

Table 5.3

Proportions of removed ^{15}N-nitrate attributable to various nitrogen transformation processes with different levels of inorganic nitrogen input during the experimental period. Data are given for phases 1, 2 and 3 of the experiment and overall.

Inorganic nitrogen input level		Denitrification (%)	DNRA (%)	Immobilisation (%)
Low	Phase 1	87.0	13.0	0
	Phase 2	-	-	-
	Phase 3	-	-	-
	Overall	**87.0**	**13.0**	**0**
Intermediate	Phase 1	87.4	2.2	10.4
	Phase 2	-	-	-
	Phase 3	100	0	0
	Overall	**88.8**	**2.0**	**9.2**
High	Phase 1	97.6	1.0	1.3
	Phase 2	-	-	-
	Phase 3	100	0	0
	Overall	**98.5**	**0.6**	**0.8**

'-' = no ^{15}N-nitrate removal detected

Denitrification accounted for most ^{15}N-nitrate removal (87-100%) with all nitrogen inputs and proportions increased with increasing input. The proportion attributable to DNRA (0-13%) exhibited the opposite trend to denitrification, and decreased as the nitrogen input increased. The proportion of nitrate removal attributable to immobilisation (0-10%) was highest with intermediate input. DNRA and immobilisation activities were only detected in phase 1 but denitrification activity was detected in phases 1 and 3.

5.4.2. Inorganic nitrogen transformation processes following the addition of ^{15}N-labelled ammonium

5.4.2.1. KCl-extractable soil nitrate pool

With low input no significant changes to the concentration of excess ^{15}N nitrate were evident during any phase (Figure 5.6a). With intermediate and high inputs, significant increases were evident in phase 1 and phase 2, respectively, indicating autotrophic nitrification from ^{15}N-labelled ammonium. The concentration of ^{14}N changed with all inputs but the pattern of change was variable (Figure 5.6b). With low input the concentration of ^{14}N increased significantly in phase 2 indicating 'net' nitrate production from unlabelled sources. With intermediate and high input, decreases were evident in phase 3 and in phases 1 and 3, respectively, indicating 'net' nitrate removal.

5.4.2.2. KCl-extractable soil ammonium pool

No change in the concentration of excess ^{15}N was evident with low input (Figure 5.7a). With intermediate and high inputs the concentration decreased significantly in phase 1 indicating ammonium removal by autotrophic nitrification, anammox-coupled nitrification-denitrification and/or immobilisation. A further significant decrease was evident in phase 2 with intermediate input only. The proportion of ^{15}N-labelled ammonium that was removed in phase 1 was 0, 26 and 30% with low, intermediate and high inputs, respectively. In phase 2 a further 14% was removed with intermediate input.

The concentration of ^{14}N decreased significantly with all inputs in phase 1 indicating 'net' ammonium removal (Figure 5.7b). No further change was evident with low and intermediate inputs, but with high input a significant increase occurred in phase 2 indicating 'net' ammonium production.

5.4.2.3. Headspace gaseous nitrogen pool

The concentration of excess ^{15}N in the gaseous nitrogen pool with low input did not change significantly in any phase of the experiment (Figure 5.8a). With intermediate and

high inputs the concentration increased significantly in phase 2 only, indicating anammox-coupled nitrification-denitrification activity. The concentration of ^{14}N was constant in phases 2 and 3 with all inputs (Figure 5.8b). It was not possible to determine the change in concentration in phase 1.

5.4.2.4. Soil total nitrogen pool

The concentration of excess ^{15}N with low input did not change significantly in any phase of the experiment (Figure 5.9a). With intermediate and high inputs the concentration of excess ^{15}N increased significantly in phase 2 and in phases 1 and 2, respectively, indicating immobilisation. The concentration of ^{14}N was constant in all phases with all inputs (Figure 5.9b).

All data utilised in Figures 5.6 to 5.9 can be found in Appendix B, Tables B4-B6.

5.4.2.5. The relative importance of ammonium removal processes

The proportion of ^{15}N-ammonium removal attributable to anammox-coupled nitrification-denitrification, autotrophic nitrification, immobilisation and unaccounted processes differed with intermediate and high inputs (Table 5.4). The values from which these relative proportions are derived are given in Appendix B – tables B10-B12. No ammonium removal activity was detected with low input.

Ammonium removal activity with high input occurred only in phase 1 but with intermediate input activity was also detected in phase 2. The largest proportions of ^{15}N-ammonium removal with intermediate and high inputs were attributable to immobilisation (19-98% and 22% respectively) or unaccounted processes (0-81% and 75% respectively). With intermediate input unaccounted processes prevailed in phase 1 but immobilisation was the predominant process operating in phase 2. Anammox-coupled nitrification-denitrification activity was detected in different phases of the experiment with intermediate and high inputs, phase 2 and phase 1, respectively. In both cases the proportion of ammonium attributable to this process was small (<3%). Autotrophic nitrification activity was only detected in phase 1 and represented a larger fraction of ammonium removal with high input (2.9%) than with intermediate input (0.8%).

5.4.3. Soil slurry pH

The pH of a set of replicate incubation vials ranged from 7.14-7.37 during the 250-minute incubation period. The pH did not differ markedly with treatment type or with time.

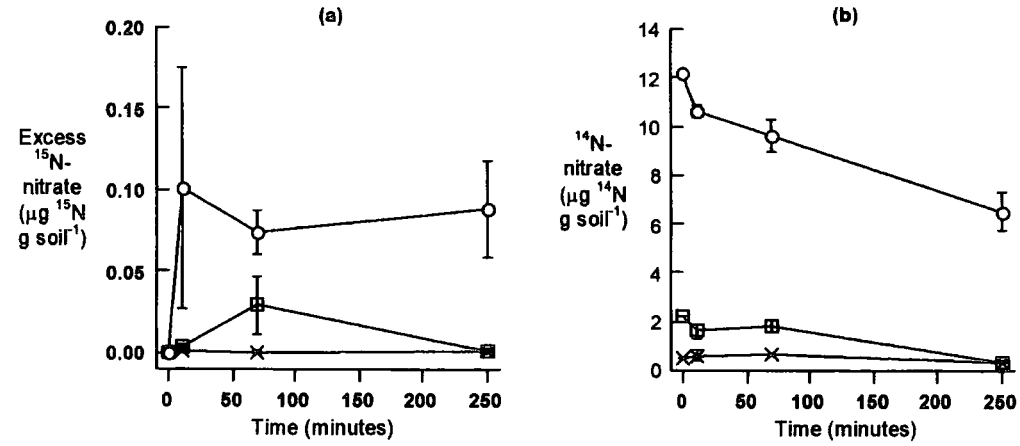


Figure 5.6. Labelled (a) and unlabelled (b) components of the KCl-extractable soil nitrate pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled ammonium during the experimental period. Values are means (± standard deviation, n=3).

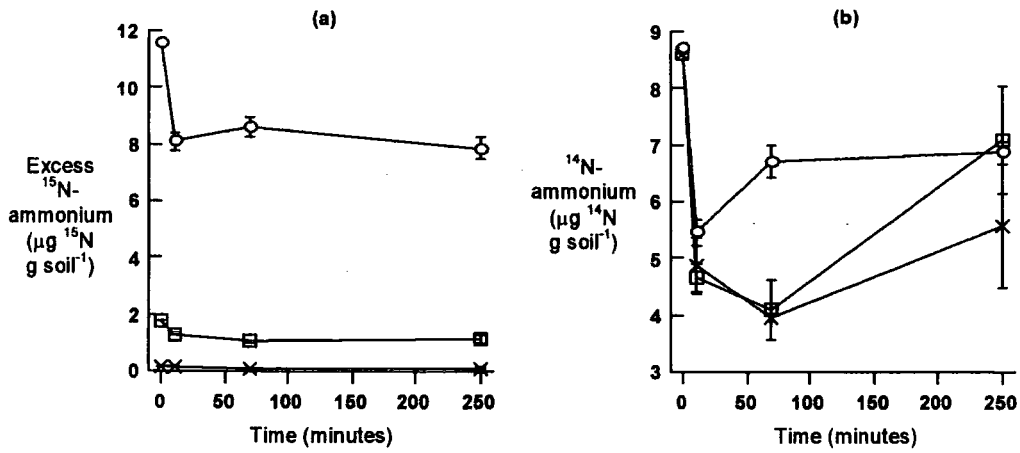


Figure 5.7. Labelled (a) and unlabelled (b) components of the KCl-extractable soil ammonium pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled ammonium during the experimental period. Values are means (± standard deviation, n=3).

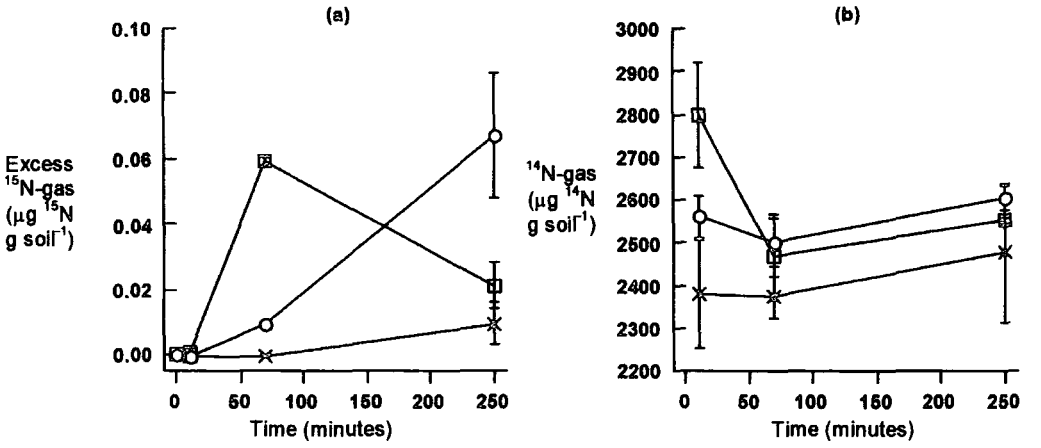


Figure 5.8. Labelled (a) and unlabelled (b) components of the headspace gaseous nitrogen pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled ammonium during the experimental period. Values are means (± standard deviation, n=3).

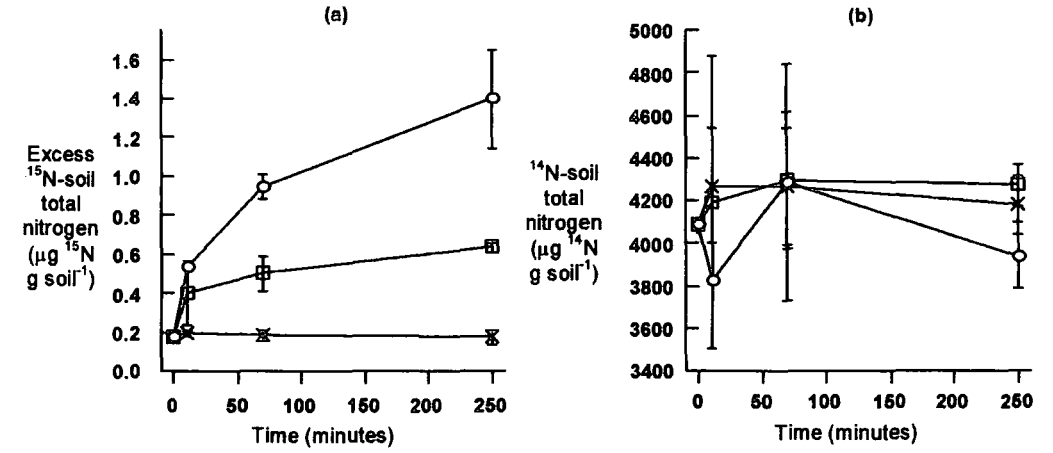


Figure 5.9. Labelled (a) and unlabelled (b) components of the soil total nitrogen pool with low (x), intermediate (□) and high inputs (O) of treatment solution containing ^{15}N-labelled ammonium during the experimental period. Values are means (± standard deviation, n=3).

Table 5.4

Proportions of removed ^{15}N-ammonium attributable to various nitrogen transformation processes with different levels of inorganic nitrogen input during the experimental period. Data are given for phases 1, 2 and 3 of the experiment and overall.

Inorganic nitrogen input level		(%) Autotrophic nitrification	Anammox-coupled nitrification-denitrification	Immobilisation	Unaccounted
Low	Phase 1	-	-	-	-
	Phase 2	-	-	-	-
	Phase 3	-	-	-	-
	Overall	**-**	**-**	**-**	**-**
Intermediate	Phase 1	0.8	0	18.6	80.6
	Phase 2	0	2.3	97.7	0
	Phase 3	-	-	-	-
	Overall	**0.5**	**0.8**	**46.3**	**52.4**
High	Phase 1	2.9	0.3	22.3	74.6
	Phase 2	-	-	-	-
	Phase 3	-	-	-	-
	Overall	**2.9**	**0.3**	**22.3**	**74.6**

'-' = no ^{15}N-ammonium removal detected

5.4.4. Nitrate and ammonium production and removal rates estimated by the isotope dilution technique

Nitrate and ammonium production and removal rates during the experimental period are presented in Table 5.5. The data utilised in the calculation of these rates can be found in Appendix B, tables B13-B15.

All nitrate-transforming activity (removal or production) essentially occurred in phases 1 and 3 with all inputs. Zero nitrate production and negative nitrate removal values were derived in phase 2 as a result of the total nitrate pool increasing significantly with no concurrent change in the atom % excess. Generation of these values is indicative of experimental error as production of nitrate from unlabelled sources should result in dilution of the labelled nitrate pool (i.e. the atom % excess should decrease). Nitrate production and removal rates were much higher in phase 1 than in phase 3. Nitrate removal was equivalent to, or higher than, nitrate production; the latter scenario was more common. Nitrate removal rates in phase 1 and phase 3 increased with increasing input. Nitrate production also increased with increasing input in phase 1. In phase 3 nitrate production was negligible with low and high inputs.

No ammonium removal or production activity was detectable in phase 2. The highest rates of ammonium removal occurred in phase 1 and these increased with increasing input. A low rate of ammonium removal also occurred in phase 3 with low input only. Ammonium removal rates were less than nitrate removal rates with intermediate and high input. With low input ammonium removal was higher, although only slightly in phase 1. Ammonium production was low (low input phase 3), zero (other inputs phase 3, high input phase 1) or negative (low and intermediate inputs phase 1). The negative values occur because decreases in total ammonium ($^{14}N+^{15}N$) accompanied increases in the atom % excess of the pool. The result may be indicative of experimental error or isotope discrimination in favour of the lighter (^{14}N) isotope by processes removing ammonium (autotrophic nitrification, anammox-coupled nitrification-denitrification, immobilisation, and unaccounted processes). If no isotopic discrimination occurs during ammonium removal then no change in the atom percent enrichment/excess of the ammonium pool is expected.

5.4.5. Nitrogen transformation rates estimated by the combined ^{15}N tracer and isotope dilution technique

Denitrification rates in phase 1 ranged from 9.7-47 μg N g soil^{-1} hr^{-1} and increased with increasing inorganic nitrogen input (Table 5.6). Denitrification rates in phase 3 were much lower (1.3-2.0 μg N g soil^{-1} hr^{-1}) where detected (intermediate and high inputs only),

Table 5.5

Total ($^{14}N+^{15}N$) nitrate and ammonium production and removal rates estimated by isotope dilution with different levels of inorganic nitrogen input during the experimental period. Data are given for the three phases of the experiment.

Inorganic nitrogen input level	Process	Transformation rate (μg N g soil^{-1} hr^{-1}) Phase 1 (0-10 mins)	Phase 2 (10-70 mins)	Phase 3 (70-250 mins)
Low	Nitrate production	11.12	-	-
	Nitrate removal	11.12	-	-
	Ammonium production	-11.58	-	0.49
	Ammonium removal	11.15	-	0.49
Intermediate	Nitrate production	19.10	-	1.26
	Nitrate removal	23.89	-	1.26
	Ammonium production	-12.17	-	-
	Ammonium removal	14.51	-	-
High	Nitrate production	35.91	0.00	0.00
	Nitrate removal	48.28	-1.48	1.97
	Ammonium production	0.00	-	-
	Ammonium removal	38.12	-	-

Table 5.6

Nitrogen transformation rates with different levels of inorganic nitrogen input estimated by a combined ^{15}N tracer and isotope dilution technique (ID + T) and by the ^{15}N tracer approach alone (T only) during the experimental period. Data are given for the three phases of the experiment.

		Transformation rate (µg N g soil^{-1} hr^{-1})					
		Phase 1		Phase 2		Phase 3	
Input level	Process	ID + T	T only	ID + T	T only	ID + T	T only
Low	Denitrification	9.67	0.95	-	-	-	-
	DNRA	1.45	0.14	-	-	-	-
	Nitrate immobilisation	0.00	0.00	-	-	-	-
	Autotrophic nitrification	0.00	0.00	-	-	-	-
	Heterotrophic nitrification	11.2	-	-	-	-	-
	Anammox-CND	0.00	0.00	-	-	-	-
	Ammonium immobilisation	0.00	0.00	-	-	-	-
	Unaccounted ammonium	0.00	0.00	-	-	-	-
	Mineralisation	-13.0	-	-	-	0.49	-
Intermediate	Denitrification	20.8	9.22	-	-	1.26	0.07
	DNRA	0.53	0.23	-	-	0.00	0.00
	Nitrate immobilisation	2.48	1.10	-	-	0.00	0.00
	Autotrophic nitrification	0.12	0.02	-	-	0.00	0.00
	Heterotrophic nitrification	19.0	-	-	-	1.26	-
	Anammox-CND	0.00	-	-	0.01	-	-
	Ammonium immobilisation	2.70	0.52	-	0.25	-	-
	Unaccounted ammonium	11.7	2.25	-	-	-	-
	Mineralisation	-12.7	-	-	-	-	-
High	Denitrification	47.1	35.3	-	-	1.97	1.17
	DNRA	0.48	0.37	-	-	0.00	0.00
	Nitrate immobilisation	0.63	0.49	-	-	0.00	0.00
	Autotrophic nitrification	1.11	0.61	-	-	-	-
	Heterotrophic nitrification	34.8	-	-	-	0.00	-
	Anammox-CND	0.11	0.06	-	-	-	-
	Ammonium immobilisation	8.50	4.65	-	-	-	-
	Unaccounted ammonium	28.4	15.6	-	-	-	-
	Mineralisation	-0.48	-	-	-	-	-

but similarly increased with increasing input. DNRA activity was only detected in phase 1 and rates, which decreased with increasing input, ranged from 0.48-1.45 μg N g soil^{-1} hr^{-1}. Where detectable (intermediate and high inputs phase 1 only) nitrate immobilisation rates ranged from 0.63-2.48 μg N g soil^{-1} hr^{-1} and were highest with intermediate input. For all three nitrate removal processes, the proportions of the total ($^{14}N+^{15}N$) transformation rate that could have been determined by ^{15}N tracer methodology alone were approximately 10, 43 and 75% with low, intermediate and high inputs, respectively.

Autotrophic nitrification was only detected with intermediate and high inorganic nitrogen inputs and only in phase 1. The rates (0.12 and 1.11 μg N g soil^{-1} hr^{-1}) increased with increasing inputs. Heterotrophic nitrification rates were considerably higher and ranged from 11.2-34.8 μg N g soil^{-1} hr^{-1} in phase 1. Activity was detected with all inputs and increased with increasing input. It was also possible to measure heterotrophic nitrification in phase 3 with intermediate input but the rate was much lower (1.3 μg N g soil^{-1} hr^{-1}) than in phase 1. Where detectable (high input, phase 1 only) the anammox-coupled nitrification-denitrification rate was low (0.11 μg N g soil^{-1} hr^{-1}). Ammonium immobilisation rates were only measurable with intermediate and high inputs and solely in phase 1. Rates ranged from 2.7-8.5 μg N g soil^{-1} hr^{-1} and increased with increasing input. Rates of ammonium removal associated with unaccounted processes (intermediate and high inputs, phase 1 only) were high and ranged from 11.7-28.4 μg N g soil^{-1} hr^{-1}. For all ammonium removal processes (autotrophic nitrification, anammox-coupled nitrification-denitrification, immobilisation and unaccounted processes) the proportions of the total transformation rate that could have been determined by ^{15}N tracer methodology alone were approximately 19 and 55% with intermediate and high inputs, respectively.

Finally, rates of mineralisation were negative with all inputs in phase 1. These negative values are derived from negative or zero measurements of ammonium production, as outlined in the previous section, minus DNRA. These negative values indicate that mineralisation was essentially zero in early phases of the experiment. In phase 3 of the experiment, mineralisation activity was detected only with low input where the rate was 0.49 μg N g soil^{-1} hr^{-1}.

5.5. Discussion

5.5.1. Phases of microbial activity

Most microbial activity occurred in phase 1 of the experiment. This initial phase of activity probably results from 'disturbance' and/or 'priming' effects associated with the set-up of the experimental vials and/or additions of inorganic nitrogen to the soil. Initial effects have rarely been documented (Davidson *et al.* 1991), and an 'equilibration' period of 0.25

to 1 hour is often applied in soil nitrogen transformation studies prior to making 'initial' measurements. However, initial effects such as those observed in the present study may be worthy of further consideration. After all, soils *in situ*, especially those in riparian zones, are likely to be subjected to natural 'disturbance' or 'priming' effects associated with water table fluctuations. The initial activity measured in the present study could be likened in some ways to the *in situ* response of riparian soil to autumnal re-wetting following dry-out in the summer period.

Davidson *et al.* (1991) similarly reported considerable removal of ^{15}N-nitrate and ^{15}N-ammonium in grassland and forest organic soils in an initial 15-minute period, which was then followed by a 24-hour incubation. For their grassland soil, 32% of added ^{15}N-ammonium was removed in the initial period. The removal of ^{15}N-nitrate was not determined. For their forest organic soil, 18% of added ^{15}N-nitrate and 12% of ^{15}N-ammonium were removed in the initial period. The proportions removed in the first ten minutes of the present study are higher for added ^{15}N-nitrate (52-98%) but overlap for added ^{15}N-ammonium (0-30%). Davidson *et al.* (1991) found that the removal of ammonium in their study was due to abiological immobilisation (clay or organic matter fixation), as opposed to biological immobilisation, as equivalent proportions were removed with sterilised and non-sterilised soil. In the present study it was not possible to distinguish between ^{15}N-ammonium that was abiologically or biologically immobilised into the soil nitrogen pool. However, immobilisation by either means does not account for all ^{15}N-ammonium initially removed and another unidentified mechanism must be operating. This mechanism may be ammonia volatilisation. Although ammonia volatilisation is generally thought to contribute significantly to nitrogen loss in saturated soils at pH of 7.5 or higher (Reddy and Patrick 1984), Blasco and Cornfield (1966) demonstrated that ammonia volatilisation accounted for a large proportion (39-59%) of ammonium sulphate added to a clay soil with a pH of 7.2 under saturated conditions (150% moisture). The pH of the soil during incubation in the present study ranged from 7.14-7.37.

In the present study, Phase 2 of the experimental period (10-70 minutes) was characterised by a general lack of microbial activity in the soil, apart from a small amount of ammonium removal (by immobilisation and anammox-coupled nitrification-denitrification) with intermediate input. This phase may also be part of the soil equilibration process following experimental set-up and additions of inorganic nitrogen. As microbial activity, notably nitrate removal, resumed in phase 3 (70-250 minutes), the 'lull' in activity in phase 2 is probably indicative of a shift in the microbial population to those species more tolerant of anaerobic conditions and lower inorganic nitrogen concentrations. Low or negligible rates of nitrate production measured in phase 3 support the notion of more reducing conditions. Presumably the high transformation activity in phase 1 consumed any residual oxygen in the anaerobically incubated vials.

The nature of microbial activity in phase 3 of the experiment may be representative of the riparian wetland soil response following more prolonged anoxia during wetter periods of the annual cycle.

5.5.2. Denitrification and mineralisation versus DNRA

The results of the present study show that most nitrate removal in the riparian wetland soil is due to denitrification rather than DNRA. Denitrification and DNRA accounted for 87-100% and 0-13% of nitrate removal respectively, with nitrate inputs that ranged from 0.2 to 12 μg N g soil^{-1}. Comparable proportions of nitrate removal have previously been attributed to denitrification and DNRA in rice paddy soil (96 and 2%) by Buresh and Patrick (1978) and (84-85 and 12-13%) by Chen *et al.* (2000), in organic soil (97 and 2.5%) by Reddy *et al.* (1980), in anaerobic muck soil (~81 and <5%) by Tiedje *et al.* (1981) and in riparian fen soil (~80% and 3-6%) by Ambus *et al.* 1992.

Most denitrification activity and all DNRA activity was detected in phase 1 of the experiment. Denitrification rates were initially very high in phase 1 but were lower in phase 3. Measurements made in phase 3 are within the range of potential denitrification rates previously reported for riparian wetland soils, but phase 1 rates are higher (Table 5.7). DNRA rates in phase 1 were 0.5-1.5 μg N g soil^{-1} hr^{-1}. These rates of DNRA are considerably higher than that previously reported by Ambus *et al.* (1992) for riparian fen soil (0.02-0.27 μg N g soil^{-1} hr^{-1}) and by Tiedje *et al.* (1981) for anaerobic muck soil (0.01-0.03 μg N g soil^{-1} hr^{-1}).

Table 5.7
Potential denitrification rates measured in riparian soils.

Author	Method used	Measurement period (mins)	Potential denitrification rate (μg N g soil^{-1} hr^{-1})
This study – Phase 1	^{15}N tracer – isotope dilution	0-10	10-47
This study – Phase 3	^{15}N tracer – isotope dilution	70-250	1.3-2.0
Nguyen *et al.* 1999b	Acetylene inhibition	30-180	4-6
Ambus *et al.* 1992	^{15}N tracer – isotope dilution	0-1360	2-6
Schipper *et al.* 1993	Acetylene inhibition	15-90	0.8
Ambus and Lowrance 1991	Acetylene inhibition	60-360	<0.1-0.7
Warwick and Hill 1988	Acetylene inhibition	0-180	0.13

The results of the present study support previous work showing that the proportion of nitrate removal attributable to DNRA relative to denitrification increases with lower nitrate

inputs (King and Nedwell 1985) and with a higher soil carbon:nitrate ratio (Tiedje *et al.* 1982). In the present study, additions of a range of nitrate inputs but no carbon amendment, simultaneously produced a range of soil carbon:nitrate ratios. The ratio of DNRA to denitrification increased from 1:7 to 1:39 to 1:98 as nitrate inputs increased and the soil carbon:nitrate ratio decreased.

Surprisingly, nitrate removal by DNRA was measured concurrently (in phase 1) with high rates of nitrate production by heterotrophic nitrification. Without measurements of oxygen status in the vials it is unclear whether this result indicates that nitrifying microorganisms are capable of operating under anaerobic conditions or that DNRA microorganisms can function under more oxidising conditions. However, the latter explanation seems more likely as additions of nitrate are known to increase the redox potential of the soil (Buresh and Patrick 1981) and, because the measured rates of nitrate production were very high relative to DNRA.

It seems likely that both DNRA and mineralisation are jointly responsible for ammonium production in the riparian wetland soil but that the two processes occur under different physico-chemical conditions. DNRA and mineralisation activities were temporally separated during the experimental period with the latter only being detectable in phase 3 and with low inorganic nitrogen input. The mineralisation rate of 0.49 μg N g $soil^{-1}$ hr^{-1} is higher than rates previously reported for riparian fen soil by Ambus *et al.* (1992) (0.33 μg N g $soil^{-1}$ hr^{-1}) and for anaerobic muck soil by Tiedje *et al.* (1981) (0.04-0.13 μg N g $soil^{-1}$ hr^{-1}).

5.5.3. Nitrate and ammonium immobilisation

Immobilisation activity (with nitrate or ammonium) was only detectable in the early phases of the experiment and only with the two higher inorganic nitrogen inputs. Immobilisation accounted for 0-10% of nitrate removal and 19-98% of ammonium removal. These proportions overlap with those previously found for other freshwater, saturated soils. Nitrate immobilisation accounted for 2-3% in rice paddy soil (Buresh and Patrick 1978, Chen *et al.* 2000), 0.6% in organic soil (Reddy *et al.* 1980), 2-10% in hypereutrophic lake sediment (D'Angelo and Reddy 1993), and ~20% in riparian fen soil (Ambus *et al.* 1992). Ammonium immobilisation was found to account for 31% of ^{15}N-ammonium removal in an unidentified, but anaerobically incubated, soil (Ragab *et al.* 1994). In a 4-month field plot study of cattail marsh soil, Dean and Biesboer (1985) reported that 22-33% of added ^{15}N-ammonium was immobilised into the soil nitrogen pool.

To the author's knowledge, no previous study has reported rates of total (^{14}N+^{15}N) nitrate immobilisation in freshwater saturated soils, which in the present study were

0.6-2.5 μg N g soil^{-1} hr^{-1}. Rates of ammonium immobilisation in the present study (2.7-8.5 μg N g soil^{-1} hr^{-1}) overlap with those reported by Ambus *et al.* (1992) for riparian fen soil (2.1-3.1 μg N g soil^{-1} hr^{-1}). They are higher, however, than those found by Tiedje *et al.* (1981) for anaerobic muck soil (0-0.03 μg N g soil^{-1} hr^{-1}).

5.5.4. Methodological issues

5.5.4.1. Negative transformation rates

The isotope dilution equations applied in this experiment occasionally yielded negative transformation rates. Theoretically, it is not possible to derive negative values. The most likely explanation is that there was experimental error in determining some 'significant' time-series changes in atom % excess and/or total ($^{14}N+^{15}N$) concentration values. Although isotope discrimination in favour of the lighter ^{14}N isotope might explain the significant increase in atom % excess of the ammonium pool, that caused negative values to be generated for ammonium production, this explanation seems unlikely. Isotope discrimination is not thought to be a significant source of error in ^{15}N labelling experiments (Myrold 1990, Barraclough 1995). Future work with this methodology would be more statistically robust if larger numbers of experimental replicates were utilised.

5.5.4.2. Underestimated transformation rates by ^{15}N tracer methodology

Soil nitrogen transformation rates derived from ^{15}N tracer experiments (e.g. Ragab *et al.* 1994, Nijburg and Laanbroek 1997a) underestimate overall ($^{14}N+^{15}N$) rates, especially when the unlabelled (^{14}N) soil pool is large relative to the addition of labelled (^{15}N) nitrogen. This point is highlighted by the results of the present study. The contribution of ^{15}N-label to overall transformation rates increased as the amount of ^{15}N-label added increased, from 10 to 77% for nitrate transformations and from 19 to 55% for ammonium transformations. The additions of ^{15}N label utilised in the present study (0.2, 2 and 12 μg N g soil^{-1}) increased ambient inorganic nitrogen concentrations by factors of 1.6, 7.5 and 40 times, respectively, for nitrate and by factors of 1 (no effective increase), 1.2 and 2.4 times, respectively, for ammonium. Methodologies that establish overall transformation rates are clearly advantageous where the objective of the research is to determine activities whilst minimising additions of inorganic nitrogen to the soil.

5.5.4.3. Nitrate production in anaerobic slurries

High rates of nitrate production, presumably due principally to heterotrophic nitrification, were evident in the present study despite anaerobic incubation. Like most other microbial activities measured, nitrate production occurred principally in phase 1 (0-10 minutes) of

the experimental period. Nitrate production occurred concurrently with, but was less than, nitrate removal. Ambus *et al.* (1992) similarly reported nitrate production in anaerobic slurry incubations of riparian fen soil. The rate of nitrate production in their study was 0.43 μg N g $soil^{-1}$ hr^{-1}, which is much lower than the rates reported in the present study (11-35 μg N g $soil^{-1}$ hr^{-1}). Ambus *et al.* (1992) proposed that nitrate was released following anaerobic breakdown of soil organics such as oximes, nitroso- and nitro compounds. A similar phenomenon might explain nitrate production in the present study. However, a more likely explanation is that some oxygen was introduced into the soil matrix in the set-up of experimental vials enabling nitrification to occur for a short period after the onset of incubation. Presumably microbial activity rapidly consumed residual oxygen and nitrifying activity was repressed to a greater extent in later phases of the experiment.

5.6. Conclusions

This study has demonstrated the following:

(1) Denitrification is the principal mechanism of nitrate removal operating in the riparian wetland soil with the range of nitrate inputs encountered *in situ*.

(2) DNRA activity increased relative to denitrification as nitrate inputs decreased and the carbon to nitrate ratio of the soil increased.

(3) DNRA activity was detected concurrently with high heterotrophic nitrification activity, which suggests that DNRA might occur under more oxidised conditions than previously thought.

(4) DNRA and mineralisation processes are probably both responsible for ammonium production in the riparian wetland soil but under different physico-chemical conditions.

(5) Ammonium removal was mostly due to an unaccounted mechanism (possibly ammonia volatilisation) and immobilisation into the soil nitrogen pool.

(6) Distinct phases of nitrogen transforming activity were evident during the experimental period. Very high initial activities (0-10 minutes) were attributed to 'disturbance' and 'priming' of the soil associated with the set-up of vials and additions of inorganic nitrogen. The 'lull' in activities that followed (10-70 minutes) was attributed to a switch in the microbial population, following consumption of residual oxygen, to species more tolerant of anaerobic conditions. Anaerobic microorganisms were then mostly responsible for nitrogen transforming activities in the final phase of the experiment (70-250 minutes).

Chapter 6

The effect of the wetland plant, *Glyceria declinata*, with and without shoot harvest, on nitrate removal processes in a riparian wetland soil

6.1. Abstract

Nitrate removal in riparian wetlands depends on the transformation and cycling of nitrogen (N) in the soil-plant system. This study aimed to investigate nitrate removal processes in a riparian wetland soil that intercepted surface and subsurface runoff from a sheep-grazed pastoral catchment. The fate of ^{15}N-nitrate in 42 soil microcosms that were: (1) bare, (2) inhabited by shoot-harvested *Glyceria declinata,* and (3) inhabited by non-harvested *Glyceria declinata,* was studied over a 32-day period. ^{15}N-nitrate (0.5 μg N g soil^{-1}, 99 atom % ^{15}N) was added to each microcosm every 2 days by injecting 20 μl of ^{15}N-nitrate solution (7.9 mg N l^{-1}) at 1-cm intervals from 0-14 cm depth at 4 randomly-selected points. The presence of the wetland plant had a marked effect on the fate of ^{15}N-nitrate. In both types of *Glyceria*-inhabited microcosm, similar proportions were denitrified (61-63%), soil-immobilised (24-26%), plant-assimilated (11-15%) and reduced to ammonium (<1%). However, in bare soil microcosms, 49% was reduced to ammonium, 29% denitrified and 22% immobilised. Dissimilatory reduction of nitrate to ammonium (DNRA) was the most predominant nitrate removal process in bare soils and occurred under conditions of higher redox potential (102-259 mV) and lower pH (5.6-5.8) than previously thought possible. In *Glyceria*-inhabited microcosms, predominance of the denitrification process was attributed to the higher level of soil oxidation, which is considered to be the principal regulator of nitrate removal partitioning between denitrification and DNRA. The single shoot harvest at the beginning of the experiment did not affect the fate of ^{15}N-nitrate, but it decreased new shoot production (by 75%), inhibited new root production and increased the nitrate assimilation capacity of shoots (5 fold).

6.2. Introduction

Riparian wetlands play an important role in the protection of river water quality by removing excess nitrate from agricultural drainage water (Hill 1996). The wetland plant can be a key component of the nitrate-buffering system as it directly assimilates nitrate from drainage water. Two important processes of nitrate removal in the wetland soil are denitrification and dissimilatory nitrate reduction to ammonium (DNRA), both of which are performed by microorganisms in a dissimilatory manner, with end-products (di-nitrogen and nitrous oxide for denitrification and ammonium for DNRA) not being utilised by the cell (Tiedje 1988). A third process, microbial immobilisation (or assimilatory nitrate

reduction), where nitrate is incorporated into the cell, is of less importance as it is restricted by the rate of cell growth (Tiedje *et al.* 1981).

In addition to direct uptake of nitrate, wetland plants in saturated soils have been shown to stimulate nitrification (Reddy *et al.* 1989), alter the composition of the microbial nitrate-reducing community (Nijburg and Laanbroek 1997b, Nijburg *et al.* 1997) and release organic substances into the rhizosphere (Scaglia *et al.* 1985). It is hypothesised that wetland plants change the oxidation level of the soil by oxygen release from roots (Armstrong 1979) and, thereby, alter the relative importance of denitrification and DNRA (Nijburg and Laanbroek 1997a). Denitrification is performed by aerobic microorganisms that have the ability to utilise nitrate when oxygen is not available, while DNRA is carried out by obligately anaerobic, fermentative microorganisms (Tiedje 1988). Denitrification is, therefore, likely to be more important in soil that is moderately anoxic (with, for example, some oxidation from wetland plants), and DNRA should be more prevalent when the soil is highly anoxic. The ratio of electron donor (carbon) to electron acceptor (nitrate) is also proposed to regulate the relative proportions of nitrate removed by denitrification and DNRA; denitrification being more important when the ratio is low, and DNRA when the ratio is high (Tiedje *et al.* 1982). The release and decomposition of organic substances in the plant rhizosphere may increase the availability of carbon and the electron donor to acceptor ratio.

From a water quality perspective, permanent loss of nitrogen (via denitrification) from the wetland system is preferable to nitrogen retention (via DNRA, and also nitrate immobilisation). Plant uptake of nitrate does not result in permanent loss as plant nitrogen is eventually returned to the soil upon the plants' demise. However, plant shoots (leaves and stems) could potentially be harvested, mechanically or by livestock grazing, and this would enable some plant-assimilated nitrate to be permanently removed from the wetland system.

This study was undertaken to determine what effect, if any, the wetland plant, *Glyceria declinata*, in non-harvested (un-cut) and harvested (cut) form, has on nitrate removal processes, and, thus, on the overall balance of nitrogen loss versus retention, in riparian wetland soil. The specific objectives were: (1) to measure the relative proportions of ^{15}N-labelled nitrate subjected to denitrification, DNRA, immobilisation and plant uptake in soil microcosms with un-cut *Glyceria*, cut *Glyceria* and bare soil; and (2) to measure soil oxidation state, microbial biomass and activity, and the amount of carbon in the soil profile of un-cut *Glyceria*, cut *Glyceria* and bare soil, and relate these results to those obtained in (1).

6.3. Materials and method

6.3.1. Study site

The study site is located in steep (17-20°) hill country at Whatawhata Research Station approximately 30 km west of Hamilton, New Zealand (latitude 37° 48' S; longitude 175° 5' E). The riparian wetland (around 400 m^2) intercepts nitrate-rich natural drainage (groundwater seepage and occasional surface runoff) from the surrounding sheep-grazed pastoral catchment.

The wetland vegetation is predominantly reed sweetgrass, *Glyceria declinata*, with some rush, *Juncus effusus,* around the margins. In early winter (June) *Glyceria declinata* shoot biomass (oven dry 60°C) was 1.8 kg m^{-2}. Root biomass (volume basis) was 19.7 kg m^{-3} but, as the rhizosphere extended to only around 25 cm depth, root biomass (areal basis) was 4.9 kg m^{-2}. Organic matter-rich surface sediment (to 20-30 cm depth) is underlain by clay. This organic soil, through which the majority of drainage is expected to pass, was collected from 0-20 cm depth for the laboratory microcosm study. Some of its chemical properties are presented in Table 6.1.

6.3.2. Laboratory microcosm construction

Fifteen blocks of organic surface soil (approximately 20 cm square cubes) randomly collected from the riparian wetland were sealed immediately in individual plastic bags. Around 200 individual *Glyceria declinata* plants were collected from the wetland 9 days later and stored together in a large container with a shallow overlying layer of wetland water from the site. An additional 100 litres of water draining from the wetland at a constructed weir was collected for use in the experiment.

In the laboratory, the soil blocks were sieved through a 2 mm mesh to remove stones, woody debris and plant roots. The bulked and homogenised soil sample was stored in a sealed plastic container at 1°C for 14 days prior to the construction of the experimental microcosms. The shoots of individual *Glyceria declinata* plants of similar size were cut back to 2 cm which stimulated the growth of new root and shoot tissues. The container of plants was kept in the laboratory at room temperature, and under natural light, for 5 days prior to planting in microcosms. Wetland water was stored at 1°C prior to use.

Soil was placed in 42 plastic, cylindrical pots (11.2 cm diameter x 18.2 cm deep), that were wrapped in aluminium foil and tape (the latter on top), to generate a 15 cm deep soil profile. The foil wrap kept soil and plant roots in the dark and restricted oxygen diffusion between soil microcosms and the atmosphere (except via surface water overlying soil

microcosms). Approximately 1500 g of wet (fresh) soil (~190 g oven dry weight) was used in each microcosm. For 28 microcosms, three *Glyceria declinata* plants were carefully planted in evenly-spaced, pre-determined positions. Above-ground shoots were again cut back to 2 cm to stimulate new root and shoot development. The remaining 14 microcosms remained unplanted.

All microcosms were incubated in a constant temperature room (20°C) with a 12-hour light (0300-1500),12-hour dark (1500-0300) cycle. Plant growth lighting was used giving a mean PAR (photosynthetically available radiation) reading of ~460 μmol m^{-1} s^{-1} at plant height. Plants were initially given 24 hours to stabilise in the soil before wetland water (10 ml) from the site was added to each microcosm to create a 0.1 cm surface water layer. The overlying water layer level was then monitored daily (0900) and additions were made that maintained a minimum 0.2 cm level, to mimic the field situation, for the remainder of the experiment. The volume of water added each day to *Glyceria*-inhabited microcosms increased steadily over time relative to that added to the bare soil microcosms which remained constant. This was due to transpiration, which increased as the plants grew, in addition to evaporative losses. All microcosms were left to stabilise for 32 days prior to the start of the 32-day experiment using ^{15}N-labelled nitrate.

6.3.3. ^{15}N-labelled nitrate experiment

On completion of the 32-day stabilisation period, and at Day 0 (0900) of the experiment proper, the shoots of plants in half (14) of the *Glyceria*-inhabited microcosms were harvested to 2 cm. Twelve millilitres (ml) of ^{15}N-labelled potassium nitrate (7.9 mg N l^{-1} as nitrate with 99 atom % ^{15}N) were injected into each microcosm, which added 0.5 μg ^{15}N g $soil^{-1}$ to the soil. The solution was injected at four randomly selected points; the surface area of each microcosm having been divided into a (4x4) grid, each square numbered and four grid squares randomly chosen. Twenty microlitres (μl) of nitrate were dispensed at 1 cm intervals from 0 to 14 cm depth at each of these points. ^{15}N-nitrate was injected into all microcosms in this manner every 2 days up until, and including, Day 30 of the experiment. During the experimental period, the 0.2 cm overlying water layer was maintained by additions of distilled-deionised water.

6.3.4. Destructive sampling procedure

6.3.4.1. Timing

Randomly selected microcosms from each treatment were destructively sampled on Days 0, 8, 16, 24 and 32. Four replicate microcosms from each treatment were taken on Days 0 and 32 while only two were taken on Days 8, 16 and 24.

6.3.4.2. Soil pH, oxygen saturation and redox potential

Immediately prior to sectioning of each microcosm, soil pH, oxygen saturation and redox potential in soil profiles were measured. The soil pH was measured at a depth of 5 cm in the soil profile using a combination pH electrode. Oxygen saturation was measured at 0.1 cm intervals through the soil profile by linear sweep voltammetry (DLK-100 electrochemical analyser, AIS Inc., Flemington, NJ, USA) at a gold amalgam microelectrode, ϕ (phi) = 100 μm. Redox potential was measured at 0.5 or 1 cm intervals using a glass-encased, 45 cm length, platinum electrode and a silver/silver chloride reference electrode. Redox potential readings were corrected for the potential of the reference electrode (+199 mV).

6.3.4.3. Microcosm sectioning

Following oxygen and redox profiling, the plant shoots of *Glyceria*-inhabited microcosms were harvested and sealed in plastic bags. Each soil microcosm was sectioned into three depth layers (0-5cm, 5-10cm, and 10-15cm). The sectioning procedure was as follows: beginning with the uppermost layer, replicate intact mini-cores (4-5) of soil were collected using a 3 cm^3 plastic syringe (with cropped nozzle). Mini-cores were dispensed into duplicate 50 ml centrifuge tubes, until 10 g of soil had been placed in each tube, amended with 15 ml 2M potassium chloride (KCl), and stored (1°C) pending extraction. Remaining soil in the layer, and plant roots where present, were carefully removed (plant roots had to be cut) and placed in a large plastic, aluminium foil-wrapped container. The process was then repeated for each of the underlying layers in turn. Plant roots were later extracted from the bulked soil-plant root sample for each depth layer using forceps. The roots were carefully washed and placed in sealed plastic bags. All plant and soil samples were stored at 1°C for 24 hours after which they were oven-dried at 60°C for 24 hours, ground and stored at room temperature pending nitrogen isotope analysis.

6.3.5. Soil extraction procedure

Soil samples in 50 ml centrifuge tubes (previously amended with 2M KCl) were extracted for 1 hour on a rotary shaker (100 rpm) and centrifuged at 3500 rpm for 10 min. Supernatants in duplicate vials were decanted and pooled together, filtered through Whatman GF/C glass-fibre filters, and stored (1°C) pending dissolved organic carbon, dissolved inorganic nitrogen and nitrogen isotope analysis. Extracted soil was removed from duplicate centrifuge tubes, pooled, air-dried, ground and stored at room temperature prior to total nitrogen and nitrogen isotope analysis.

6.3.6. Chemical and microbiological analyses

Dissolved organic carbon in soil extracts was determined on a Total Organic Carbon analyser (Shimadzu TOC-5000A, Japan). Nitrate and ammonium in soil extracts were determined by automated hydrazine reduction-sulphanilimide/NEDD diazotization and phenol/hypochlorite colorimetry, respectively (APHA 1995).

The moisture content of the bulked soil sample from each layer was determined by gravimetric loss at 105°C for 24 hours. Microbial biomass carbon content was determined by the chloroform fumigation extraction method (Vance *et al.* 1987). The determination of aerobic and anaerobic readily mineralisable carbon content involved incubating 5 g of soil in a 22 ml vacutainer and measuring the evolution of carbon dioxide and methane after 7 days incubation in the dark at 20°C. For the anaerobic assay, vacutainers were flushed with helium (2 mins) to render them anoxic. The total carbon content of the soil, following air-drying and grinding, was determined on a CN analyser (Elementar VarioMAX, Germany).

6.3.7. Nitrogen isotope analyses

To measure the atom % ^{15}N of the nitrate and ammonium pools in soil KCl extracts, samples were first diffused sequentially on to acidified filters (Sorensen and Jensen 1991). Ammonium and nitrate were diffused in turn (with 0.2 g magnesium oxide (MgO) and 0.2 g MgO + 0.4 g Devarda's alloy, respectively) on to duplicate 6 mm diameter circles of acid-cleaned Whatman GF/C filter paper impregnated with 10 µl of 2.5M potassium hydrogen sulphate ($KHSO_4$) and enclosed within polytetrafluoroethylene (PTFE) tape, which floated on the extract. An aliquot (10 ml) of soil KCl extract was added to each diffusion flask (250 ml polyethylene bottle) into which an additional 10 ml of 2M KCl and 2 small glass beads (to aid mixing) were added. Diffusion flasks were then incubated on a bench in a positive-pressure laboratory, at 23°C, for two (sequential) 6-day periods with the flasks standing open for 24 hours in-between to remove residual ammonium. Flasks were carefully hand-swirled on a daily basis. Duplicate filters, removed from the PTFE tape, were dried together in a plastic multiple-well tray in ammonia-free air (dessicator with concentrated sulphuric acid (H_2SO_4) as the dessicant). The atom % ^{15}N of these filters, the percent nitrogen and atom % ^{15}N of KCl-extracted soil, and the percent nitrogen and atom % ^{15}N of plant roots and shoots were determined on a Finnigan MAT Delta-Plus mass spectrometer. Corrections were applied for isotopic dilution of the atom % ^{15}N values for nitrate and ammonium, caused by small amounts of nitrate and ammonium in diffusion reagents and in the acidified filters.

6.4. Results

6.4.1. Natural abundance values for nitrogen pools

The natural abundance atom percent (%) ^{15}N in all nitrogen pools was measured in Day 0 samples (Table 6.2). Day 0 samples had not received any ^{15}N-nitrate solution. The soil nitrogen and plant nitrogen pools had natural abundance atom % ^{15}N values close to those of atmospheric nitrogen (0.3663) and there was little difference between samples from un-cut *Glyceria*, cut *Glyceria* and bare soil microcosms. The natural abundance atom % ^{15}N of soil nitrate and ammonium pools was more variable especially in *Glyceria*-inhabited microcosms. The soil nitrate pool in the uppermost soil layer (0-5 cm depth) of *Glyceria*-inhabited microcosms had high natural abundances of ^{15}N (~1.0-1.1 atom % ^{15}N), as did the soil ammonium pool in the deeper (5-10 cm and 10-15 cm depth) soil layers (~0.7-0.8 atom % ^{15}N). These high values are indicative of nitrogen transformation processes in the soil discriminating in favour of the lighter (^{14}N) nitrogen isotope. As these high values are associated only with the *Glyceria*-inhabited microcosms the isotopic discrimination may be associated with the plant uptake process or with soil nitrogen transformations stimulated by the presence of the plant, such as nitrification and denitrification (Reddy *et al.* 1989). The excess ^{15}N values shown in later sections were calculated using the above, measured, natural abundance atom % ^{15}N values.

6.4.2. KCl-extractable soil nitrate pool

Nearly all of the ^{15}N-nitrate added to all microcosms (98-100%) was transformed during the experimental period. The amount added to the soil was 0.5 μg ^{15}N-nitrate g $soil^{-1}$ every two days. The non-transformed excess ^{15}N-nitrate measured in all soil layers of all microcosms was always less than 0.12 μg ^{15}N g $soil^{-1}$ and typically less than 0.03 μg ^{15}N g $soil^{-1}$.

The concentration of KCl-extractable total nitrate ($^{14}N+^{15}N$) in all soil microcosms ranged from 0.3-3.5 μg N g $soil^{-1}$ during the experimental period. Concentrations generally increased (by 70-240%) during the experimental period (data not shown). The exceptions were the surface soil layers (0-5 cm depth) of the un-cut *Glyceria* and cut *Glyceria* treatments where concentrations after 32 days of incubation were slightly less, or the same, respectively. Increases in KCl-extractable total nitrate concentration cannot be attributed to additions of ^{15}N-nitrate to the soil microcosms as non-transformed ^{15}N concentrations were negligible. Production of ^{14}N-nitrate from unlabelled soil nitrogen pools (i.e. ammonium and organic nitrogen) must be mostly responsible.

Table 6.1

Some chemical properties of the riparian wetland soil (0-20 cm depth)

Property	Unit	Mean
pH (1:2H_2O)		5.6
Moisture content	%	87.3
Organic matter content (loss on ignition)	%	42.5
Total carbon	%	11.2
Total nitrogen	%	0.8
KCl-extractable ammonium	μg NH_4-N g $soil^{-1}$	18.7
KCl-extractable nitrate	μg NO_3-N g $soil^{-1}$	1.1

Table 6.2

Natural abundance atom % ^{15}N values for nitrogen pools in soil microcosms (Day 0)

Nitrogen pool	Soil depth (cm)	Atom % ^{15}N Un-cut *Glyceria*	Cut *Glyceria*	Bare soil
KCl-extractable soil nitrate	0-5	0.9914	1.1184	0.3748
	5-10	0.2476	0.5093	0.3483
	10-15	0.4371	0.3561	0.2977
KCl-extractable soil ammonium	0-5	0.4798	0.4300	0.3975
	5-10	0.7506	0.7181	0.3773
	10-15	0.8071	0.7136	0.3798
Soil total nitrogen	0-5	0.3687	0.3671	0.3671
	5-10	0.3685	-	-
	10-15	-	-	0.3683
Plant shoots	-	0.3687	-	-
Plant roots	0-5	0.3692	0.3630	-
	5-10	0.3674	0.3668	-
	10-15	0.3686	0.3673	-

'-' not applicable or not determined

6.4.3. KCl-extractable soil ammonium pool

In bare soil microcosms a considerable amount of excess ^{15}N-ammonium (1.5-3.7 μg ^{15}N g soil^{-1}) accumulated during the experimental period (Figure 6.1). More ^{15}N-ammonium accumulated in deeper soil layers, especially 5-10 cm depth, relative to the surface soil layer. In contrast to the significant accumulation of ^{15}N-ammonium in the bare soil microcosms, virtually none was found in *Glyceria*-inhabited microcosms.

Bare soil microcosms had significantly higher concentrations of KCl-extractable total ammonium ($^{14}N+^{15}N$) than *Glyceria*-inhabited microcosms throughout the experiment. Concentrations in *Glyceria*-inhabited microcosms ranged from 6.9-17.2 μg N g soil^{-1} and no marked differences were evident between soil layers. Concentrations in bare soil microcosms ranged from 173-216 μg N g soil^{-1} for the uppermost soil layer and from 326-503 μg N g soil^{-1} in deeper soil layers. Ammonium concentrations in all microcosms generally increased (by 1-50%) during the experimental period (data not shown). The magnitude of the increase was not influenced by microcosm type or soil depth. The increase in ammonium was presumably due principally to mineralisation of unlabelled (^{14}N) organic nitrogen as production of ^{15}N-ammonium from ^{15}N-nitrate was negligible by comparison.

6.4.4. Soil total nitrogen pool

In all microcosms, excess soil ^{15}N accumulated during the experimental period (Figure 6.2). After 32 days, similar amounts of excess soil ^{15}N were found in all microcosms, despite occasional marked differences at interim sampling intervals (e.g. Day 16). In all microcosms, the accumulation of excess soil ^{15}N was two to three times higher in the uppermost soil layer relative to deeper soil layers. Regarding total ($^{14}N+^{15}N$) soil nitrogen there were no marked differences in concentrations with time, depth or between treatments (data not shown). Concentrations ranged from 5.3-7.8 mg N g soil^{-1} during the experimental period.

6.4.5. Plant nitrogen pool

Shoot dry matter in un-cut *Glyceria* and cut *Glyceria* microcosms increased, from 4.2 to 10.4 mg dry matter g soil^{-1} and from 0 to 1.6 mg dry matter g soil^{-1}, respectively, in the 32-day experimental period (Figure 6.3). The shoot dry matter that developed during the experimental period was higher in un-cut *Glyceria* (by 48%) and lower in cut *Glyceria* (by 62%) than that (~4.2 mg dry matter g soil^{-1}) formed during the equivalent pre-experiment stabilisation period.

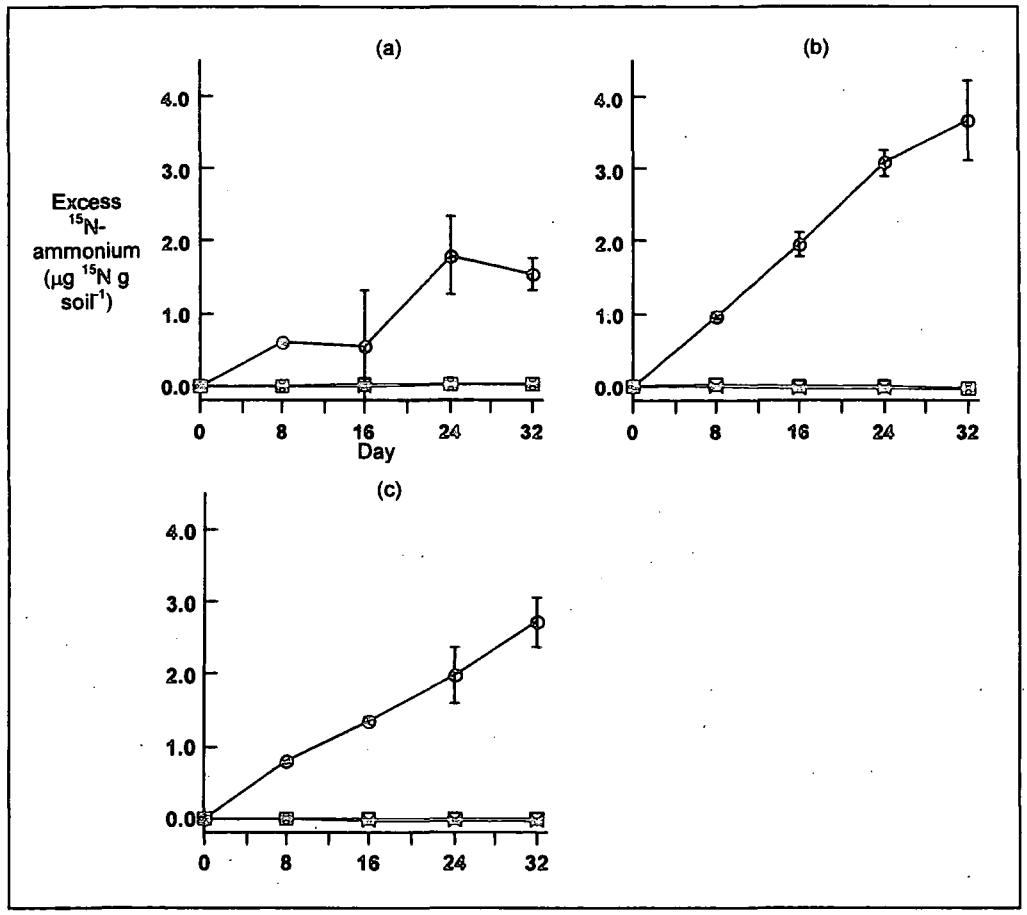


Figure 6.1. Excess ^{15}N-ammonium concentrations in soil depth layers of un-cut *Glyceria* (x), cut *Glyceria* (□) and bare (O) soil microcosms during the experiment; (a) 0-5 cm depth, (b) 5-10 cm depth and (c) 10-15 cm depth. Values are means (± standard deviation, n=4 Days 0 and 32, n=2 Days 8,16 and 24).

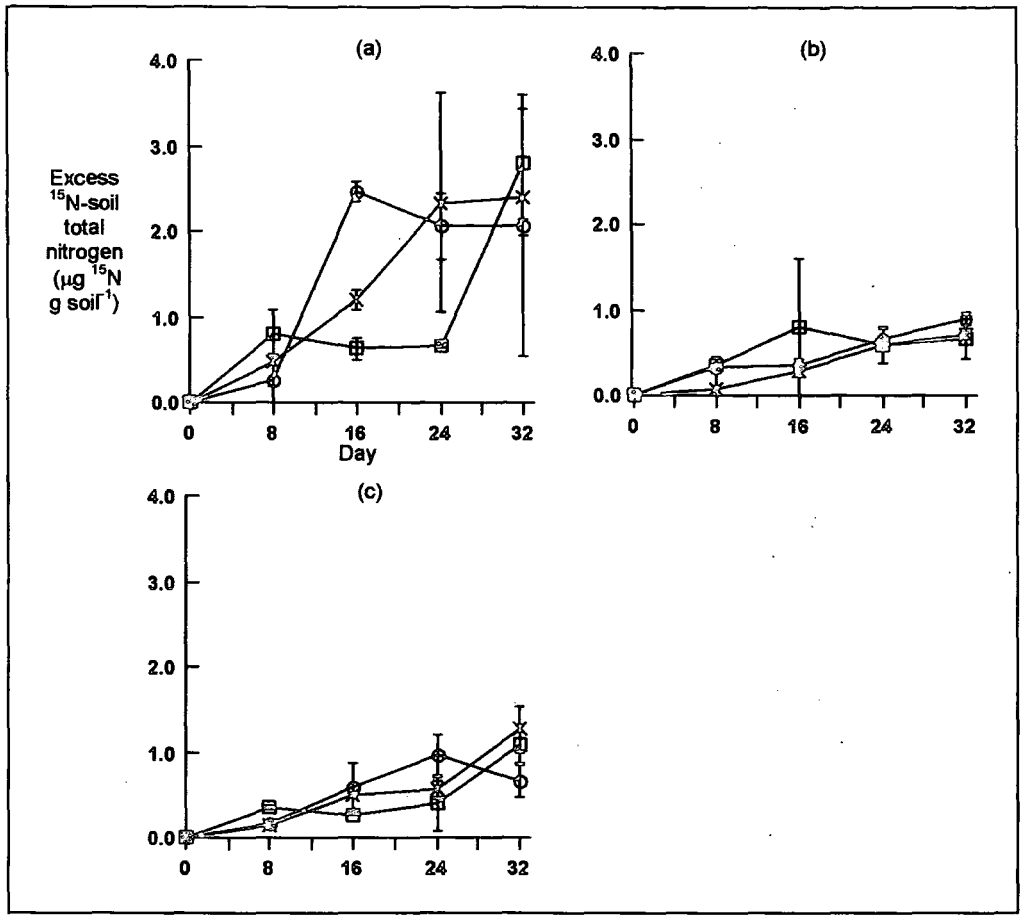


Figure 6.2. Excess ^{15}N-soil total nitrogen concentrations in soil depth layers of un-cut *Glyceria* (x), cut *Glyceria* (□) and bare (O) microcosms during the experiment; (a) 0-5 cm depth, (b) 5-10 cm depth and (c) 10-15 cm depth. Values are means (± standard deviation, n=4 Days 0 and 32, n=2 Days 8, 16 and 24).

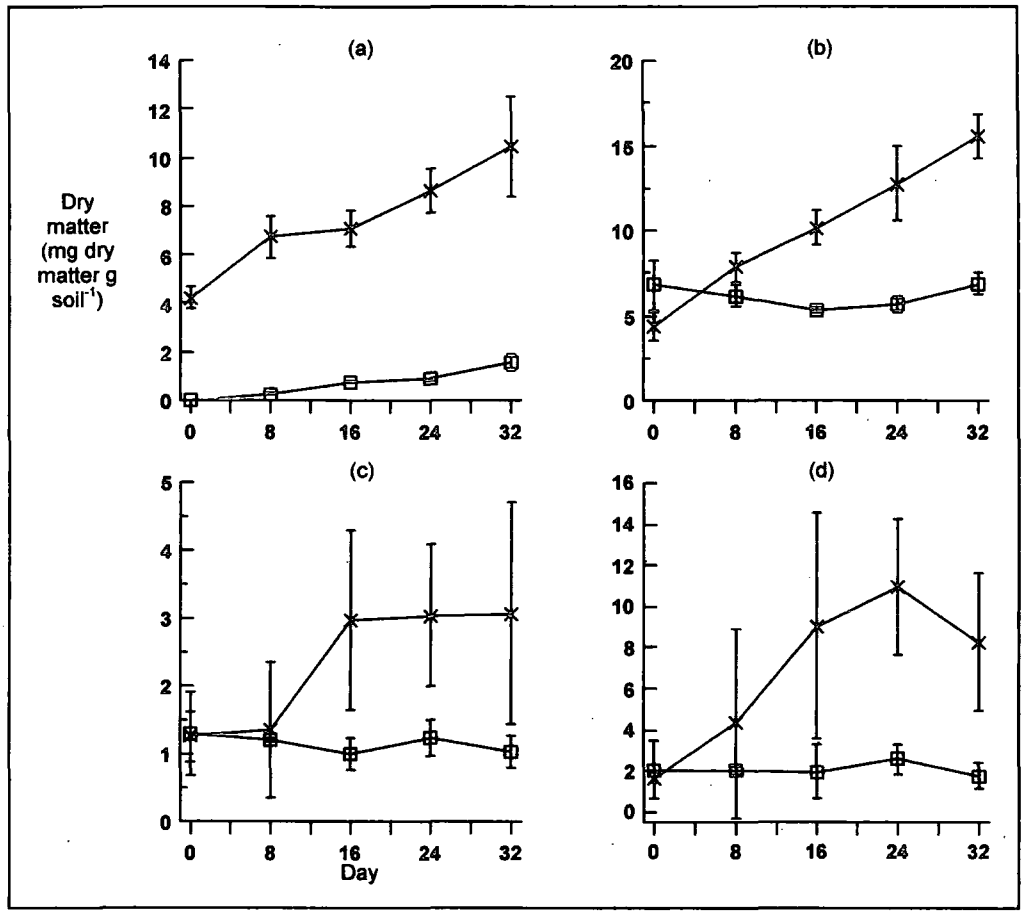


Figure 6.3. Dry matter of plant (a) shoots, (b) roots 0-5 cm soil depth layer, (c) roots 5-10 cm soil depth layer, and (d) roots 10-15 cm soil depth layer relative to dry soil mass in uncut *Glyceria* (x) and cut *Glyceria* (□) microcosms. For shoots, the dry soil mass of the entire microcosm was used. For roots, the dry soil mass of the depth layer in question was used. Values are means (± standard deviation, n=4 Days 0 and 32, n=2 Days 8,16 and 24).

The shoot dry matter that accumulated in un-cut *Glyceria* microcosms (0.27 kg m^{-2}) and cut *Glyceria* microcosms (0.04 kg m^{-2}) was 15 and 2%, respectively, of the *in situ* biomass level.

In un-cut *Glyceria* and cut *Glyceria* microcosms, the main bulk of root dry matter that developed in the 32-day pre-experiment stabilisation period occurred in the 0-5 cm soil layer (Figure 6.3). Lesser amounts of root dry matter occurred in deeper layers, especially at 5-10 cm depth. Root dry matter in un-cut *Glyceria* microcosms continued to increase in the uppermost soil layer throughout the experiment and in deeper soil layers up until Day 16. In contrast, root dry matter in cut *Glyceria* microcosms did not increase following the Day 0 shoot harvest. The root dry matter that accumulated in un-cut *Glyceria* microcosms (1.49 kg m^{-3}) and cut *Glyceria* microcosms (0.54 kg m^{-3}) was 8 and 3%, respectively, of the *in situ* biomass level. The above results on shoot and root dry matter suggest that the shoot harvest substantially reduced the subsequent production of new shoot and root tissues.

The excess ^{15}N per gram of dry shoot matter was considerably higher in cut *Glyceria* than un-cut *Glyceria* microcosms (Figure 6.4). The rate of excess ^{15}N uptake into shoots in the cut *Glyceria* microcosms was highest between Days 0 and 8 (~22 μg ^{15}N g dry $matter^{-1}$ d^{-1} compared to 2.7 μg ^{15}N g dry $matter^{-1}$ d^{-1} between days 8 and 32). The rate of excess ^{15}N uptake by un-cut *Glyceria* shoots was constant at around 1.5 μg ^{15}N g dry $matter^{-1}$ d^{-1} during the experimental period.

The mean excess ^{15}N per gram of dry root matter was often higher in cut *Glyceria* than un-cut *Glyceria* microcosms in all soil layers (Figure 6.4). However, standard deviations of mean values for one or both treatments were frequently large and overlapped. For both treatments, the excess ^{15}N per gram of dry root matter was around two to three times higher in the uppermost soil layer relative to deeper soil layers.

Mean values of excess ^{15}N in plant root and shoot dry matter relative to soil mass were often higher for un-cut *Glyceria* than cut *Glyceria* microcosms (Figure 6.5). However, large and overlapping standard deviations for most mean values suggest that there were few marked differences between the two treatments. For both treatments, the excess ^{15}N in plant root dry matter per gram of soil was higher in the uppermost soil layer relative to deeper soil layers (by 3-15 times).

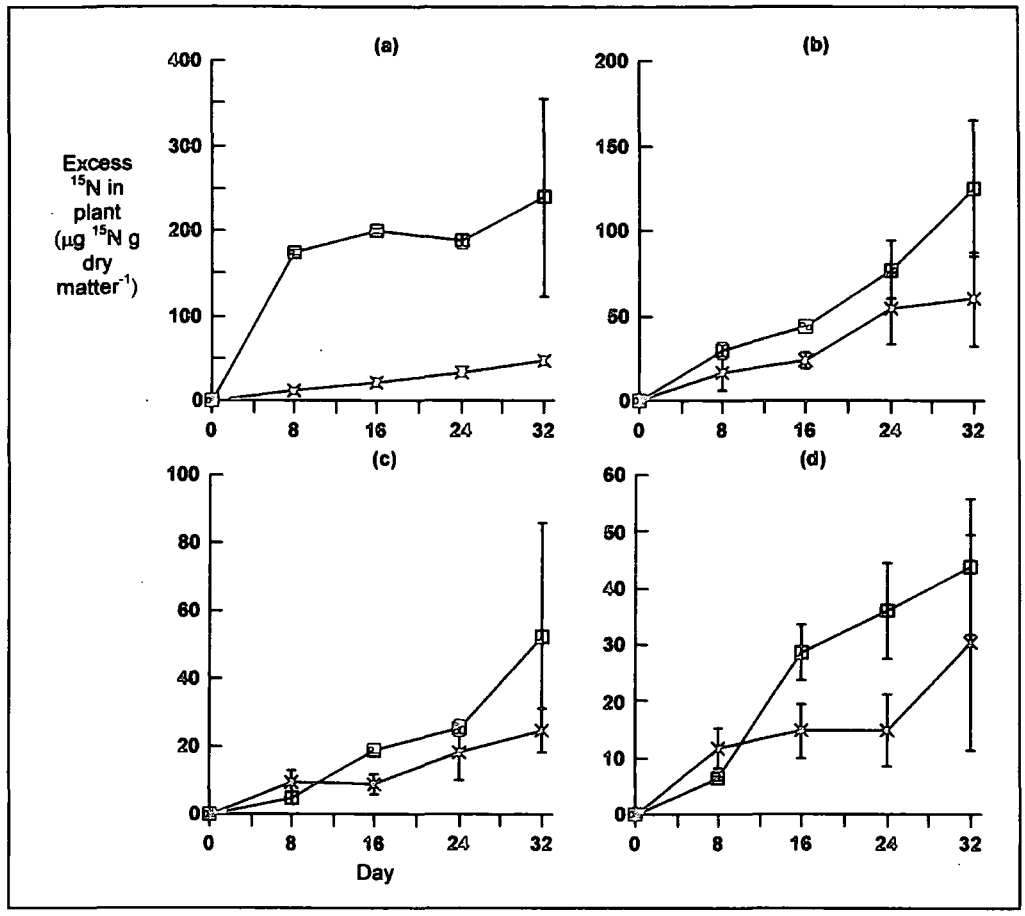


Figure 6.4. Excess ^{15}N concentrations in plant (a) shoots, (b) roots 0-5 cm soil depth layer, (c) roots 5-10 cm soil depth layer, and (d) roots 10-15 cm soil depth layer in un-cut *Glyceria* (x) and cut *Glyceria* (□) microcosms. Values are means (± standard deviation, n=4 Days 0 and 32, n=2 Days 8,16 and 24).

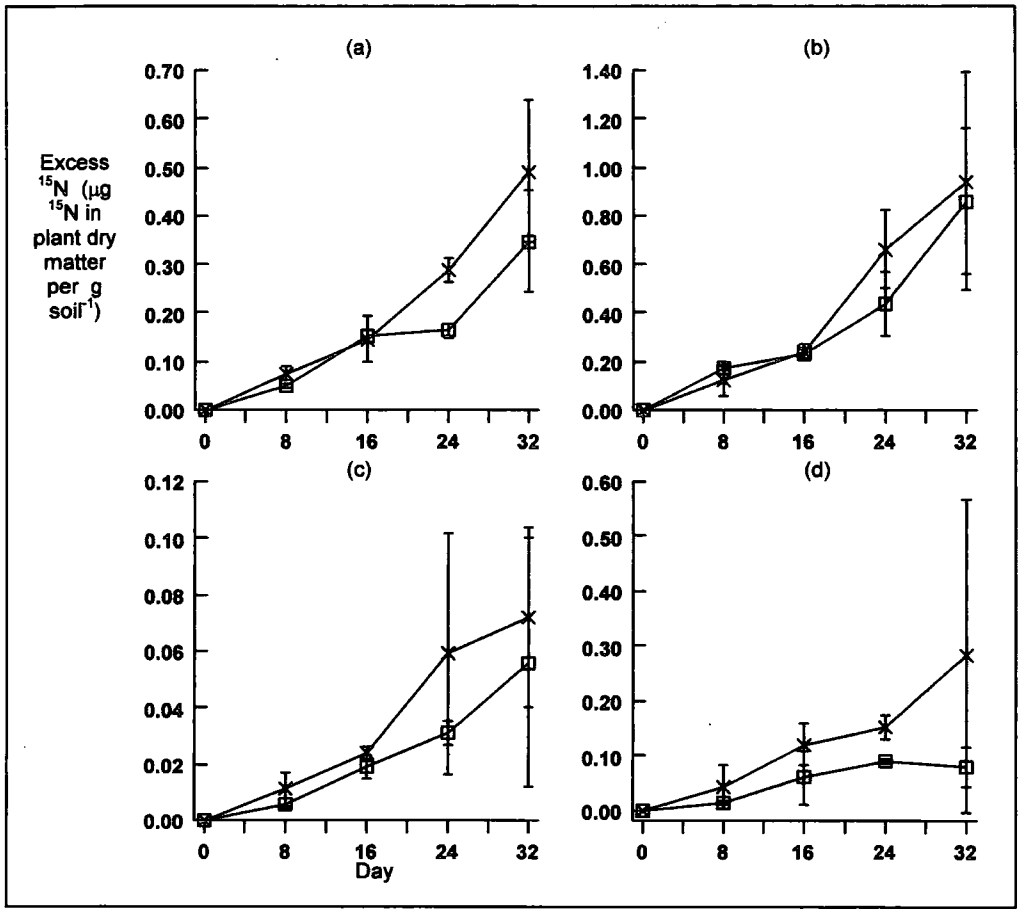


Figure 6.5. Excess ^{15}N in plant (a) shoots, (b) roots 0-5 cm soil depth layer, (c) roots 5-10 cm soil depth layer, and (d) roots 10-15 cm soil depth layer in soil relative to dry soil mass in un-cut *Glyceria* (x) and cut *Glyceria* (□) microcosms. For shoots, the dry soil mass of the entire microcosm was used. For roots, the dry soil mass of the depth layer in question was used. Values are means (± standard deviation, n=4 Days 0 and 32, n=2 Days 8,16 and 24).

6.4.6. Overall fate of ^{15}N-nitrate

After 32 days the fate of added ^{15}N-nitrate in un-cut *Glyceria* microcosms did not differ markedly from that in cut *Glyceria* microcosms (Table 6.3). While a considerable proportion (24-26%) of the added ^{15}N-nitrate was immobilised into the soil nitrogen pool, or assimilated into the plant N pools (11-15%), most (61-63%) was unaccounted for. This unaccounted fraction probably represents gaseous loss by denitrification. In bare soil microcosms almost 50% of added ^{15}N-nitrate was reduced to ammonium while only 29% was denitrified. Immobilisation of nitrate in bare soil microcosms accounted for a similar proportion (22%) to *Glyceria*-inhabited microcosms.

6.4.7. Soil pH and moisture content

Soil pH in the 0-5 cm layer decreased by approximately 0.5 and 0.2 units in *Glyceria*-inhabited and bare microcosms respectively, between Days 0 and 32 of the experimental period, and the pH was higher in bare soil than in *Glyceria*-inhabited soil. The mean pH values (± standard deviation) measured on Days 0 and 32 were 5.19 (± 0.18), 5.22 (± 0.14) and 5.80 (± 0.23) and 4.73 (± 0.08), 4.68 (± 0.16) and 5.60 (± 0.23) for un-cut *Glyceria*, cut *Glyceria* and bare soil microcosms respectively.

The moisture content of the soil at depths of 0-5 cm, 5-10 cm and 10-15 cm did not differ markedly between un-cut *Glyceria*, cut *Glyceria* and bare soil microcosms during the experiment. Over the 32-day period, the moisture content of the uppermost soil layer (81-85%) tended to be slightly higher than that at greater depth (78-82%), which may be due to the addition of water to the soil surface or to slight consolidation of the soil at depth. The range of soil moisture contents measured is slightly lower than that measured for the soil immediately after field collection (87%), suggesting that the watering regime was approximate to *in situ* conditions.

6.4.8. Oxygen saturation and redox potential profiles

Oxygen saturation profiles revealed substantial differences in the degree of oxygen penetration into the soil between *Glyceria*-inhabited and bare soil microcosms (Figure 6.6). Oxygen penetrated to around 0.3-0.4 cm in bare soil microcosms but to 0.7-2 cm in *Glyceria*-inhabited microcosms. Oxygen penetration in all microcosms appeared to increase between Day 0 and Day 32, most distinctly in those inhabited by *Glyceria*.

Redox profiles of the soil were more variable in *Glyceria*-inhabited microcosms compared to bare soil microcosms (Figure 6.7). Below a depth of 2 cm, where diffusion of atmospheric oxygen into the soil was negligible, measured redox potentials ranged from

Table 6.3

The fate of added ^{15}N-nitrate in soil microcosms (Day 32)

Microcosm type	Proportion (%) of added ^{15}N-nitrate found in various nitrogen pools – mean (± S.D.)				
	Soil nitrate pool (No transformation)	Soil ammonium pool (DNRA)	Soil total nitrogen pool (Immobilisation)	Plant nitrogen pool (Plant Uptake)	Unaccounted (Denitrification)
Un-cut *Glyceria*	0.04 (± 0.02)	0.18 (± 0.12)	23.9 (± 3.06)	15.2 (± 3.68)	61.1 (± 2.76)
Cut *Glyceria*	-0.02 (± 0.02)	-0.05 (± 0.02)	25.6 (± 4.24)	11.4 (± 3.16)	63.1 (± 2.38)
Bare soil	-0.01 (± 0.02)	48.6 (± 2.66)	21.9 (± 9.86)	-	29.5 (± 3.63)

S.D., standard deviation (n=4)

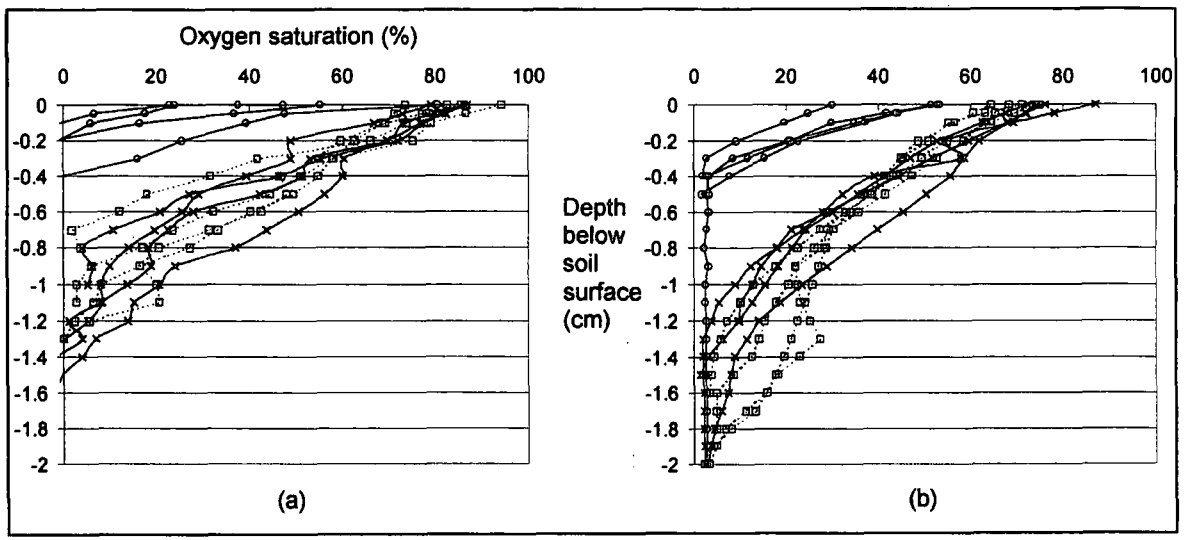


Figure 6.6. Oxygen saturation profiles in soils on (a) Day 0 and (b) Day 32 in un-cut *Glyceria* (x), cut *Glyceria* (□) and bare (O) soil microcosms.

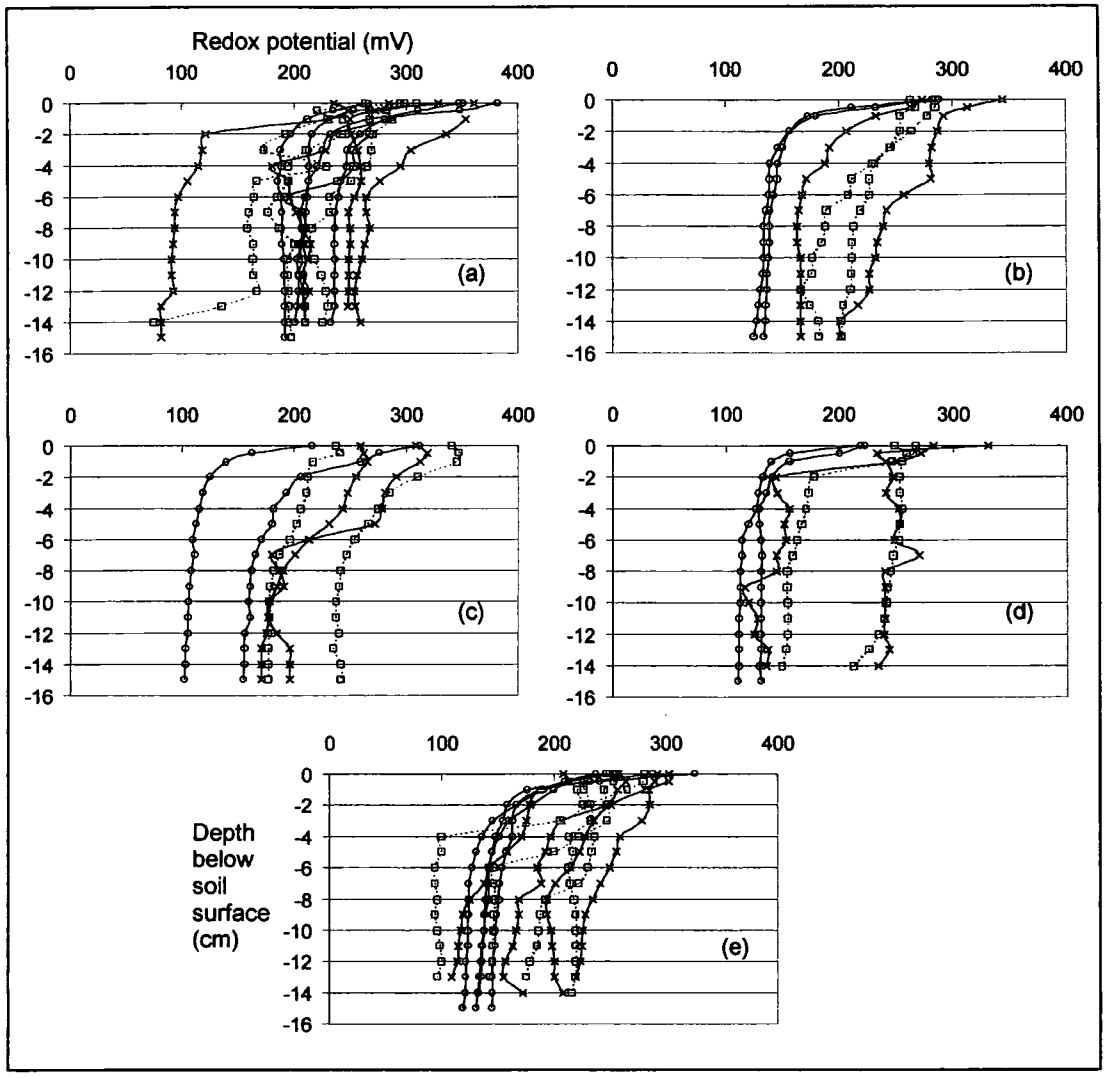


Figure 6.7. Redox potential profiles in soils on (a) Day 0, (b) Day 8, (c) Day 16, (d) Day 24, and (e) Day 32 in un-cut *Glyceria* (x), cut *Glyceria* (□) and bare (O) soil microcosms.

+336 to +82 mV in un-cut *Glyceria*, +271 to + 75 mV in cut *Glyceria*, and +259 to +102 mV in bare soil microcosms. However, throughout the experiment, soils that exhibited higher oxidised redox profiles were always associated with *Glyceria*-inhabited microcosms, reflecting the consistently higher oxygen saturation in these microcosms (probably resulting from the release of oxygen from *Glyceria* roots).

6.4.9. Soil carbon

Few consistent differences were evident in pore-water dissolved organic carbon, microbial biomass carbon, and aerobic and anaerobic readily mineralisable carbon between treatments (Table 6.4). Anaerobic readily mineralisable carbon was higher in bare soil microcosms than in *Glyceria*-inhabited microcosms but only in the uppermost soil layer. This result indicates that more carbon is available to anaerobic microorganisms in near-surface wetland soil in the absence of the wetland plant but the reason for this is unknown. Pore-water dissolved organic carbon increased markedly in all soil layers of all microcosm treatments between Day 0 and Day 32 but no time-series differences were evident for microbial biomass carbon and aerobic and anaerobic readily mineralisable carbon.

The similarity in microbial biomass carbon between treatments, over time, and as a proportion (4-10%) of total soil carbon (112 mg C g soil^{-1}), indicates that the wetland soil is likely to be sustainable in terms of soil microbial activity and carbon substrate for soil microorganisms. The microbial biomass carbon content of the soil (4-11 mg C g soil^{-1}), and the proportion of soil total carbon as microbial biomass carbon, are comparable to those reported in other wetland soils (Duncan and Groffman 1994, Nguyen 2000). This is probably due to high plant productivity and, hence, an abundant supply of labile organic carbon for microbial activity in the wetland where soil was collected. The studied wetland also receives farm nutrients and animal excreta in surface runoff and subsurface flow from the upland sheep-grazed pasture. These inputs would potentially be a significant source of nutrients and carbon for microbial activity.

6.5. Discussion

This study of a riparian wetland soil has shown that the relative importance of the two dissimilatory nitrate-reducing pathways, denitrification and DNRA, is strongly influenced by the presence of the wetland plant, *Glyceria declinata*. In bare riparian wetland soil DNRA was the principal mechanism of nitrate removal, accounting for 49% of added ^{15}N-nitrate, whereas in the presence of *Glyceria*, denitrification was the principal mechanism of nitrate removal (~60%) and DNRA was insignificant (<1%).

Table 6.4

Types of carbon in soil depth layers of microcosms at the beginning and end of the experiment

Soil layer depth (cm)	Microcosm Type	mg C g soil^{-1} – mean (± S.D.) Porewater Dissolved Organic Carbon		Microbial Biomass Carbon		Aerobic Readily Mineralisable Carbon		Anaerobic Readily Mineralisable Carbon	
		Day 0	Day 32	Day 0	Day 32	Day 0	Day 32	Day 0	Day 32
0-5	un-cut	0.89 (± 0.01)	1.47 (± 0.18)	8.08 (± 3.74)	8.42 (± 1.34)	0.99 (± 0.13)	1.02 (± 0.03)	1.25 (± 0.76)	1.74 (± 1.02)
	cut	0.82 (± 0.12)	1.39 (± 0.13)	9.72 (± 2.22)	6.71 (± 1.82)	1.34 (± 0.10)	1.03 (± 0.07)	1.49 (± 0.61)	1.47 (± 0.89)
	bare	0.90 (± 0.14)	1.60 (± 0.18)	7.84 (± 2.86)	7.50 (± 1.60)	1.14 (± 0.21)	0.87 (± 0.11)	2.91 (± 0.19)	2.30 (± 1.33)
5-10	un-cut	0.77 (± 0.09)	1.44 (± 0.11)	7.39 (± 2.72)	6.40 (± 0.70)	0.89 (± 0.14)	0.89 (± 0.06)	1.16 (± 0.75)	1.22 (± 0.51)
	cut	0.73 (± 0.15)	1.32 (± 0.24)	11.0 (± 4.22)	6.06 (± 1.42)	0.88 (± 0.04)	0.78 (± 0.04)	1.37 (± 0.87)	0.37 (± 0.07)
	bare	0.68 (± 0.04)	1.17 (± 0.16)	7.89 (± 3.30)	6.38 (± 1.86)	0.85 (± 0.15)	0.71 (± 0.09)	1.10 (± 0.49)	0.77 (± 0.62)
10-15	un-cut	1.02 (± 0.11)	1.46 (± 0.17)	7.07 (± 3.78)	6.23 (± 1.16)	0.80 (± 0.06)	0.91 (± 0.05)	0.91 (± 0.62)	1.00 (± 0.82)
	cut	0.84 (± 0.17)	1.32 (± 0.15)	9.38 (± 2.96)	6.70 (± 1.28)	0.82 (± 0.14)	0.72 (± 0.05)	0.34 (± 0.14)	0.44 (± 0.07)
	bare	0.72 (± 0.04)	1.20 (± 0.06)	4.44 (± 0.84)	7.57 (± 1.46)	0.77 (± 0.07)	0.63 (± 0.05)	0.78 (± 0.38)	0.76 (± 0.59)

S.D., standard deviation (n=4)

This striking difference in nitrate fate can be attributed to differences in redox conditions induced by the presence of the plant. *Glyceria* induced a less reducing environment, more favourable for denitrification, which is conducted by facultative anaerobes, than DNRA, which is conducted by obligate anaerobes. Soil inhabited by the wetland plant was considerably more oxidised than bare soil. Oxygen penetrated to a greater depth, and redox potential profiles were frequently higher, in *Glyceria*-inhabited soil. This difference in soil oxidation was not a function of soil saturation level as the moisture contents of *Glyceria*-inhabited and bare soils were very similar.

This study shows that the carbon:nitrate ratio *per se* is not important to partitioning between denitrification and DNRA. The high proportion of nitrate removal attributable to DNRA in the bare soil microcosms was not linked to higher soil carbon or, conversely, to lower soil nitrate. Anaerobic readily mineralisable carbon was higher in bare soil microcosms relative to *Glyceria*-inhabited microcosms but only in the uppermost soil layer. Moreover, production of ^{15}N-ammonium by DNRA was greater in the deeper soil layers where the anaerobic readily mineralisable carbon was comparable to that in *Glyceria*-inhabited microcosms.

Evidence for the importance of the carbon to nitrate ratio in denitrification/DNRA partitioning comes principally from experiments where the ratio has been artificially manipulated by the addition of a carbon source (Tiedje *et al.* 1981, Tiedje *et al.* 1982), or variable additions of nitrate (King and Nedwell 1985). Additions of glucose and other carbon sources to the soil stimulate microbial activity, increase biological oxygen demand and result in more reducing conditions, as well as increasing the soil carbon to nitrate ratio. Conversely, additions of nitrate oxidise the soil (Buresh and Patrick 1981, Moraghan 1993), in addition to lowering the carbon to nitrate ratio. The key regulator of DNRA/denitrification partitioning in these studies may, therefore, be the level of soil oxidation. Buresh and Patrick (1981) attempted to demonstrate the effect that manipulating soil oxidation alone could have on the relative importance of DNRA and denitrification for an estuarine sediment. Under (semi-) controlled redox potentials of +300 mV, 0 mV and –200 mV they found that proportion of nitrate removal attributable to DNRA increased from 0.4-1.8% to 3.7-15% to 18-35%, respectively. It is worth noting that they added a considerable quantity of nitrate (60 μg $^{15}NO_3$-N g soil^{-1}) to all treatments which temporarily raised the redox potential up to +100 mV and +150 mV in the 0 mV and –200 mV treatments, respectively. As previously discussed, this would have favoured denitrification.

The proportion of nitrate removal attributable to DNRA in bare soil in the present study is larger than has previously been reported for a range of saturated, freshwater soils. Ambus *et al.* (1992) reported only 3 to 9% DNRA in riparian fen soil and Buresh and

Patrick (1978) measured 1 to 21% in rice paddy soil. Nijburg and Laanbroek (1997a) measured 9% DNRA in lake littoral soil and D'Angelo and Reddy (1993) detected 13 to 32% DNRA in hyper-eutrophic lake sediments. The higher proportion of DNRA found in the present study may be due to the experimental technique utilised. The present study attempted to mimic *in situ* conditions as closely as possible by: (1) making minimal nitrate additions (0.5 μg ^{15}N g $soil^{-1}$), that barely raised the concentration above ambient levels (~1.1 μg N g $soil^{-1}$) and (2) having 'non-stirred' incubation of soil. Previous studies have typically used higher nitrate additions (2-50 μg $^{15}NO_3$-N g^{-1}) and/or soil slurry incubations. Both of these factors can enhance denitrification at the expense of DNRA by oxidising the soil. The inhibitory effect of soil 'stirring' on DNRA has been demonstrated by Jorgensen (1989), who measured lower activity when incubating the same anaerobic estuarine sediment as a slurry, compared to as an intact core.

Although the proportion of nitrate removal attributable to DNRA in bare soil in this study is high relative to others, the rate of ^{15}N-ammonium production by DNRA in bare soil microcosms (0.003 μg^{15}N g $soil^{-1}$ hr^{-1}) is less. This is presumably due to the higher additions of substrate (nitrate) utilised in other studies. Nijburg and Laanbroek (1997a) measured DNRA rates of 0.005-0.184 μg ^{15}N g $soil^{-1}$ hr^{-1} for cores of bare and *Phragmites*-inhabited lake littoral sediment incubated for 1 hr with a single addition of 2-3 μg ^{15}N g $soil^{-1}$. Similarly, Ambus *et al.* (1992) reported DNRA rates of 0.015-0.270 μg ^{15}N g $soil^{-1}$ hr^{-1} for cores and slurries of riparian fen soil, incubated for 24-26 hrs with 24.1 and 228 μg ^{15}N g $soil^{-1}$ (10.3 and 25.4 atom % ^{15}N), respectively.

Results of the present study suggest that DNRA microorganisms are more widespread in nature, and/or more versatile, than previously thought. It has often been stated that: (1) DNRA is important only under intensely reducing conditions (redox potential below –200 mV) and, (2) DNRA occurs principally in high pH soils, (Nommik 1956, Buresh and Patrick 1978, Tiedje *et al.* 1982, Tiedje 1988). In the present study, considerable DNRA was measured in the bare riparian soil, especially below 5-cm soil depth. Redox potentials ranged from +259 mV to +102 mV (below 2-cm depth) and the pH was moderately acidic (5.6-5.8).

The relatively small fraction of nitrate removal in both *Glyceria*-inhabited treatments attributable to plant uptake (11-15%) contrasts with the findings of Nijburg and Laanbroek (1997a). They found that *Phragmites australis* roots and rhizomes (leaves had been cut) assimilated 61% of available nitrate while denitrification, DNRA and immobilisation removed only 25%, 4% and 10%, respectively, in an 8-hr incubation of plant-inhabited lake littoral sediment with ~2-3 μg ^{15}N-nitrate g $soil^{-1}$. The results suggest that wetland plant species differ in their influence on nitrate transformation processes, and hence overall nitrate removal, in saturated soils. Indications are, from the present study, that

Glyceria declinata may be a good plant species to use in constructed or restored riparian wetlands since 61-63% of added nitrate was removed by denitrification in the presence of this plant. However, the effect of this plant, and others (e.g. *Phragmites australis*), on nitrate transformation processes in saturated soils may vary under different environmental conditions (e.g. soil physical and chemical characteristics, nitrate inputs, and soil temperatures). Moreover, the plant shoot and root biomass attained in the present experiment was considerably less than the *in situ* level. A higher ratio of plant biomass to soil could alter the effect of the plant on soil nitrate removal processes.

In the relatively short time period (~1 month) within which this study was conducted shoot harvest had no significant effect on the fate of nitrate in *Glyceria*-inhabited soil. Comparable percentages of added ^{15}N-nitrate were dissimilated to ammonium, denitrified, immobilised into the soil nitrogen pool and assimilated by the plant. Shoot harvest also seemed to have no significant impact on soil oxidation and carbon to nitrate ratio. However, shoot harvest reduced shoot growth by around 75%, inhibited further root growth, and increased the nitrate assimilation capacity of shoots 5 fold. The marked increase in the nitrate assimilation capacity of shoots did not translate into enhanced plant uptake of nitrate due to the reduced rates of new tissue production. It is unclear whether the increased nitrate assimilation capacity of shoots following a single harvest might translate into enhanced plant uptake in the longer term, or with repeated harvesting, given the lower rates of new tissue production. This aspect needs further investigation since there is considerable interest in the effect of periodic shoot removal (by livestock grazing or mechanical harvest) on the transformation and removal of nitrate in riparian wetlands.

6.6. Conclusions

This study has demonstrated that:

(1) DNRA is responsible for a substantial proportion (49%) of nitrate removal in bare riparian wetland soil;

(2) DNRA can occur under less intensely reducing conditions and lower pH than previously thought;

(3) the plant, *Glyceria declinata*, oxidises the riparian wetland soil, markedly repressing DNRA and increasing denitrification;

(4) the level of soil oxidation is probably the principal regulator of the relative importance of dissimilatory nitrate reducing processes, and not the carbon to nitrate ratio of the soil;

(5) a single harvest of *Glyceria declinata* shoots did not alter the relative importance of nitrate removal processes or soil oxidation and carbon levels in this 32-day study, but markedly decreased new shoot production, inhibited new root production and markedly increased the nitrate assimilation capacity of shoots.

Chapter 7

Nitrogen removal and the fate of nitrate in riparian buffer zones: synthesis and conclusions

7.1. Nitrate versus reduced nitrogen forms in riparian zone studies

Reduced forms of nitrogen (ammonium and dissolved organic nitrogen) were measured alongside nitrate (oxidised nitrogen) in a field-based study of agricultural runoff entering, and moving through, three riparian zones (pasture, wood and wetland) in a United Kingdom (UK) grazed pastoral catchment (Chapter 3). Dissolved organic nitrogen (<0.01-3.2 mg N l^{-1}; median 0.7) was frequently the principal nitrogen form in subsurface runoff entering the riparian zones, and ammonium (<0.01-4 mg N l^{-1}; median 0.2) was often higher than nitrate (<0.01-2.4 mg N l^{-1}; median 0.02). Nitrate was the principal form (84->99.9%) of dissolved nitrogen in surface (spring) runoff (18-28 mg N l^{-1}), which flowed into the riparian wetland site only.

Few previous studies of agricultural nitrogen buffering by riparian zones have measured dissolved organic nitrogen, ammonium and nitrate concentrations in subsurface and surface runoff (Peterjohn and Correll 1984, Jordan *et al.* 1993, Correll *et al.* 1997) and none, to the author's knowledge, in grazed pastoral catchments. However, the value of the riparian zone as an agricultural nitrogen buffer can only be accurately evaluated if the concentrations and/or fluxes of all dissolved nitrogen forms are known. In some landscape settings, measurements of reduced nitrogen forms are probably more crucial than in others. These settings include: (1) where agricultural soils are slowly-permeable and prone to widespread anaerobiosis, which discourages the accumulation of nitrate; and/or (2) where there is organic fertilisation of agricultural soils (e.g. the application of farm-yard manure, dairy shed effluent, urea-based fertilisers, excrement of grazing stock).

7.2. Some riparian zones are poor nitrogen buffers

The three UK riparian zones in this study were poor buffers of nitrogen in agricultural runoff during the period of investigation (autumn and winter months). At all sites the nitrogen buffering efficiency was assessed by measuring total dissolved nitrogen (dissolved organic nitrogen, ammonium and nitrate) concentration changes in subsurface runoff from the pastoral upland, across the riparian zone, to the river edge (Chapter 3). Additionally, at the riparian wetland site, nitrogen buffering was assessed by the measurement of nitrogen fluxes, in surface and subsurface water flows, across the site (Chapter 4). The result of this latter analysis is discussed in the 'riparian hydrology' section below.

The pasture and wetland riparian zones did not significantly alter the TDN concentration of runoff at any time and the wood riparian zone effected a net decrease on only one of seven sampling occasions. Poor nitrogen buffering efficiency in these riparian zones highlights the likely inefficiency of 'blanket' strategies of riparian zone restoration and/or preservation for catchments. The set-aside or protection of riparian land to buffer agricultural nitrogen will clearly only be useful where the riparian zone in question can significantly decrease the nitrogen content of agricultural runoff.

7.3. Riparian zone hydrology

One of the key reasons for the failure of some riparian zones to buffer nitrogen in catchment runoff is that there is inadequate contact between runoff and riparian soil. In the present study of water and nitrogen fluxes across a UK riparian wetland (Chapter 4), nitrogen removal was clearly hindered by the occurrence of a 'preferential flow path' that moved surface 'spring' runoff, and upwelling subsurface runoff, rapidly across the riparian soil surface to the river. When flow via this preferential route was lessened, under low flow conditions, runoff-riparian soil contact increased and a 27% reduction in nitrogen flux from the catchment was evident. Other studies have also attributed poor, or reduced, nitrogen buffering efficiencies in some riparian zones to the existence of surface or subsurface (saturated zone) 'preferential flow paths' (Brusch and Nilsson 1993, Burt *et al.* 1999, Devito *et al.* 2000). Where these hydrological features exist, runoff-riparian soil contact will probably only be enhanced by undertaking substantial engineering work in the affected riparian zone (Burt *et al.* 1999).

Riparian zones are, inherently, complex and heterogeneous lithological structures, as they are subjected to the landscape-forming processes of both terrestrial and fluvial systems (Vanek 1997, Matchett 1998). It is not surprising therefore, that in many cases, runoff does not move uniformly across these zones. However, riparian zones in some sedimentary or geomorphological settings will probably be more prone to 'preferential' or 'bypass' flow than others. Highly complex and variable stratigraphy (e.g. in formerly glaciated terrain) is most likely to harbour 'preferential flow paths' for catchment runoff.

Determining the nature and course of hydrological flow paths across riparian zones is clearly essential to accurately evaluate catchment runoff interaction with riparian zone soil. However, the subsurface complexity of these sites means that this is rarely a straightforward task, and detailed study is generally required. The need for comprehensive hydrological study of riparian zones, in conjunction with hydrochemical (nitrogen) measurement, has been recognised for some time (Cooper 1990, Hill 1996), but, to date, few such studies have been undertaken (Devito *et al.* 2000). In the present study, hydrological measurements made via a seemingly 'extensive' grid network of

piezometers across a riparian wetland (Chapter 4) failed to accurately estimate the subsurface discharge entering, and then upwelling within, the riparian zone. Most subsurface runoff was apparently channelled into the riparian wetland through preferential flow paths associated with irregular, high permeable deposits in the saturated zone, which clearly rendered the 'spot' analyses imprecise. Future hydrological research in this riparian zone, and others, may benefit from ground penetrating radar techniques that can help to locate and map irregular subsurface structures (Poole *et al.* 1997). This technology has recently been used to confirm the presence of natural subsurface 'pipe' features in upland peat (Holden 2000).

7.4. Nitrogen transformation and removal processes in riparian zones

7.4.1. The two methodologies employed

Separate investigations of nitrogen transformation and removal processes in two riparian wetland soils (Chapters 5 and 6) utilised the ^{15}N isotope of nitrogen. In the study of a New Zealand (NZ) riparian wetland soil (Chapter 6) the isotope was employed solely as a tracer to determine the relative importance of nitrate removal processes (denitrification, DNRA, immobilisation and plant uptake) in bare and plant-inhabited (*Glyceria declinata*) soil microcosms over a 32-day period. In the study of a UK riparian wetland soil a joint ^{15}N tracer and isotope dilution technique was utilised to determine the relative importance of a broader range of processes associated with the transformation of both inorganic nitrogen forms (ammonium and nitrate) in short-term (<5 hr) soil slurry incubations.

The joint ^{15}N tracer-isotope dilution methodology employed in the present study has the following advantages over the more traditional ^{15}N tracer methodology: (1) it is possible to measure inorganic nitrogen production processes (i.e. mineralisation and nitrification) alongside inorganic nitrogen removal processes; and (2) it is possible to determine total (^{14}N+^{15}N) nitrogen transformation rates, as opposed to rates based solely on the transformation of ^{15}N. The main disadvantages of the technique are: (1) it is only applicable for short-term (<1 week) incubation studies; and 2) to study all nitrate and ammonium removal and production processes simultaneously, as undertaken in the present study, ^{15}N-labelled nitrate and ^{15}N-labelled ammonium must be utilised at the same concentrations due to the paired experimental design. It was also apparent from the present study that the statistical robustness of the technique would probably be improved by using more than the minimum triplicate replication of treatment vials, making it comparatively more labour-intensive than the ^{15}N tracer methodology.

7.4.2. The relative importance of nitrogen transformation and removal processes

7.4.2.1. Denitrification: the key nitrogen buffering process

Efficient nitrogen buffering in riparian zones is due to the denitrification process, which results in permanent loss of nitrogen from the soil-water system. Although ammonia volatilisation and anaerobic ammonium oxidation may also transform inorganic nitrogen to gaseous forms, there is little evidence currently available to suggest that these processes are important transformers of nitrogen in riparian zone soils. In the present study, anaerobic ammonium oxidation was measured by the ^{15}N tracer-isotope dilution methodology in the UK riparian wetland soil (Chapter 5) and was found to be negligible. In the same study, ammonia volatilisation may have been responsible for the large fraction of removed ^{15}N-ammonium that was unaccounted for, but no direct measurement of ^{15}N-ammonia gas was made that could confirm this. Moreover, this process is generally considered to be significant only in rare instances where the pH of soil is above 8 (Reddy and Patrick 1984). All other nitrogen transformation processes operating in riparian soils (i.e. dissimilatory nitrate reduction to ammonium (DNRA), immobilisation, plant uptake, mineralisation and nitrification) retain nitrogen within the soil-water system and generally do not facilitate long-term nitrogen buffering. The only potential exceptions are (1) nitrification, which transforms reduced nitrogen to nitrate that can then be denitrified (i.e. coupled nitrification-denitrification) and (2) plant shoot harvest, which can permanently remove some plant assimilated nitrogen from the soil-water system. In the present study, denitrification was found to be a key process of nitrate removal in both riparian wetland soils studied. Denitrification accounted for 87-100% and 29-63% of nitrate removal in the UK soil (Chapter 5) and in the NZ soil (Chapter 6) respectively.

7.4.2.2. Denitrification versus DNRA

Of special interest in these soil studies was nitrate removal partitioning between denitrification and the other dissimilatory nitrate reducing pathway, DNRA. These two processes compete directly for nitrate in anaerobic soils. As the former process removes nitrogen, while the latter process conserves it, the outcome of this competition is clearly critical to the nitrogen buffering efficiency of riparian zones.

In the study of the UK riparian wetland soil, the potential for DNRA was low by comparison with the denitrification potential. DNRA accounted for only 0-13% of nitrate removal, and total ($^{14}N+^{15}N$) DNRA rates (0.5-1.5 μg N g soil^{-1} hr^{-1}) were much lower than total denitrification rates (1.3 to 47 μg N g soil^{-1} hr^{-1}). It was concluded from this study that most nitrate removal in the UK riparian wetland was probably attributable to denitrification. In the microcosm study of the NZ riparian wetland soil, the potential for

DNRA was higher and the potential for denitrification was lower than found for the UK soil, despite comparable nitrate inputs to the soils in the two experiments. In bare soil microcosms of NZ soil DNRA accounted for 49% of nitrate removal while denitrification accounted for only 29%. In the UK soil slurry experiment, a nitrate input of 0.2 μg N g^{-1} $soil^{-1}$ (low input) was added to an ambient concentration of 0.3 μg N g^{-1} $soil^{-1}$. In the NZ soil microcosm experiment, a nitrate input of 0.5 μg N g^{-1} $soil^{-1}$ was added to an ambient concentration of 1.1 μg N g^{-1} $soil^{-1}$. The marked difference in DNRA and denitrification potentials between the UK and NZ (bare) soils may result from (1) differences in inherent soil properties or (2) differences in experimental technique. Should the differences be due solely to the experimental technique employed, the microcosm study presumably yielded the most realistic estimate of DNRA and denitrification potentials in riparian wetland soils, because *in situ* conditions were more closely replicated. Regardless of these uncertainties, the result clearly shows that DNRA may be an important mechanism of nitrate removal in some riparian wetlands.

The joint ^{15}N tracer-isotope dilution study of the UK riparian wetland soil utilised a range of nitrate input concentrations comparable to those encountered *in situ*, and results showed that partitioning between DNRA and denitrification was a function of the level of nitrate input to the soil. As the nitrate input level increased, the proportion of nitrate removal attributable to denitrification increased and the proportion attributable to DNRA decreased. This result is consistent with the findings of other previous studies (King and Nedwell 1985, Moraghan 1993). The two hypotheses put forward to explain the 'nitrate' effect on partitioning are that (1) higher additions of nitrate lower the carbon:nitrate ratio of the soil, which favours denitrification over DNRA (Tiedje 1988); and (2) higher additions of nitrate oxidise the soil to a greater extent, which favours denitrification over DNRA (Moraghan 1993).

The soil oxidation and carbon:nitrate ratio hypotheses relating to denitrification/DNRA partitioning were examined in the microcosm study of a New Zealand riparian wetland soil. In this study, soil oxidation and soil carbon levels were measured concurrently with nitrate removal partitioning between denitrification and DNRA in microcosms of bare and plant-inhabited riparian wetland soil. This study showed that soil oxidation level was probably the critical determinant of nitrate removal partitioning between denitrification and DNRA and not the carbon to nitrate ratio of the soil. The wetland plant, *Glyceria declinata*, oxidised the riparian wetland soil, as indicated by soil oxygen saturation and redox potential measurements, and markedly increased the proportion of nitrate removal attributable to denitrification from 29% (bare soil) to 63%. In contrast, the proportion of nitrate removal attributable to DNRA decreased from 49% (bare soil) to <1%. The study concluded that nitrate removal by denitrification is probably favoured by moderately

anoxic conditions, while nitrate removal by DNRA is probably favoured by highly anoxic conditions. This finding has important implications for riparian zone management:

(1) the water table in some riparian zones could be artificially manipulated to maximise the extent of moderately anoxic soil, as opposed to highly anoxic or oxic soil, to enhance nitrogen loss by denitrification. Drainage structures that disperse excess runoff into less saturated soils within the riparian zone might achieve this.

(2) chemical enhancement of denitrification in riparian zones with artificial amendments of carbon may lower soil oxidation levels (by stimulating microbial activity, and subsequently oxygen consumption), which, in some cases, may increase the proportion of nitrate removal that is attributable to DNRA relative to denitrification. Two key pioneering studies with a saw-dust 'denitrification wall' in a grazed pastoral catchment (Schipper and Vojvodic-Vukovic 1998, Schipper and Vojvodic-Vukovic 2000) found no evidence of ammonium accumulation within this structure and concluded that DNRA did not contribute markedly to nitrate removal. However, future research, in other landscape settings, should ensure that this possibility is evaluated.

7.4.2.3. Nitrate immobilisation

Immobilisation of nitrate in the two riparian wetland soils studied was also measured as the third, and final, microbially-mediated process of nitrate removal. This process is generally assumed to occur at lower rates than the two dissimilatory nitrate removal pathways (denitrification and DNRA) because it is linked to cell growth (Tiedje *et al.* 1981). Immobilisation was always less than denitrification in the two soils studied. In the UK soil, immobilisation accounted for up to 10% of nitrate removal. Total (^{14}+^{15}N) nitrate removal rates (0.6-2.5 μg N g soil^{-1} hr^{-1}) were comparable with DNRA rates. In the NZ soil, immobilisation accounted for 22-26% of nitrate removal and did not appear to be affected by differences in soil oxidation resulting from the presence or absence of the wetland plant. Comparatively less is known about the importance of this nitrate removal process, relative to dissimilatory nitrate reducing pathways, especially denitrification. The relatively high proportion of nitrate removal that can clearly be attributable to this process, up to 26% in the present study, suggests that this process should be more closely evaluated in future riparian zone studies.

7.4.2.4. The role of the wetland plant

In the microcosm study of the NZ riparian wetland soil, soil inhabited by the wetland plant *Glyceria declinata* was noticeably less anoxic than bare soil, presumably as a result of oxygen release from roots (Armstrong 1979). This higher soil oxidation level dramatically

enhanced nitrogen removal by denitrification, relative to overall nitrogen retention by other processes, in this riparian wetland soil. The plant itself only assimilated a small proportion of the available nitrate (11-15%). The management implication of this finding is that certain plants, like *Glyceria declinata*, that oxidise the soil and assimilate a small proportion of available nitrate, may enhance the nitrogen buffering potential of some riparian zones. However, this 'enhancement' can only occur when the plant is active. Little is currently known about plant activity outside of the growing season, when leaching and transport of nitrogen in runoff from catchments is probably highest. In the NZ riparian wetland studied, *Glyceria declinata* did not die back during the winter but the level of plant activity at this time is unknown. In the UK riparian wetland studied, complete die-back of wetland vegetation (*Glyceria fluitans, Myosotis scorpiodes, Nasturtium officinale*) occurred in mid-winter (January), but the level of plant activity during the preceding autumn and early winter months is unknown. Plant-induced mechanisms of nitrogen buffering in riparian zones will presumably be most important where (1) plant activity persists outside of the growing season and/or (2) nitrogen losses from the catchment are evenly distributed throughout the annual cycle.

7.5. Conclusions

7.5.1. Experimental findings

(1) Concentrations of reduced nitrogen forms, especially dissolved organic nitrogen, were higher than concentrations of nitrate in subsurface runoff, while nitrate was the principal nitrogen form in surface runoff, from the sheep-grazed pastoral catchment studied in north-east England, UK.

(2) The three UK riparian buffer zones studied were found to be poor buffers of agricultural nitrogen in subsurface runoff based on concentration changes.

(3) A surface 'preferential flow path' through the riparian wetland site appeared to hinder overall nitrogen buffering by decreasing runoff-riparian soil contact.

(4) In the UK riparian wetland soil the potential for denitrification was high, and most nitrate removal occurring at the field site was probably attributable to this process.

(5) The level of nitrate input affected nitrate removal partitioning between denitrification and DNRA in the UK riparian wetland soil, with denitrification favoured by higher amendments.

(6) In the NZ riparian wetland soil DNRA accounted for a markedly higher proportion of nitrate removal than denitrification in the absence of the wetland plant, *Glyceria declinata*.

(7) The presence of *Glyceria declinata* in the NZ soil reversed the relative importance of denitrification and DNRA and increased soil oxidation, presumably by oxygen release from roots.

(8) Nitrate removal partitioning between denitrification and DNRA in the NZ riparian wetland soil appeared to be regulated by the soil oxidation level rather than the soil carbon:nitrate ratio, with denitrification favoured by moderately anoxic conditions (in the presence of the wetland plant) and DNRA favoured by highly anoxic conditions.

(9) Immobilisation was a more important process of nitrate removal in the NZ soil than in the UK soil.

(10) Uptake by the wetland plant, *Glyceria declinata*, accounted for a lower proportion of nitrate removal than denitrification and immobilisation in the NZ soil.

7.5.2. Research design findings and recommendations

(1) Future studies of nitrogen buffering in riparian zones should measure all nitrogen forms in runoff entering, and moving through these sites, not just nitrate.

(2) Hydrological flow path determinations in some riparian zones may be aided by utilising ground penetrating radar, especially where irregular lithological deposits could act as preferential flow conduits for runoff. It is clearly more difficult to locate and map these features with traditional 'spot' sampling techniques.

(3) Joint ^{15}N tracer-isotope dilution methodology, as employed in the present study, offers some advantages over traditional ^{15}N tracer methodology for the measurement of nitrogen transformations in soils (more processes can be measured and transformation rates are not based solely on the added tracer), but the method is more labour-intensive and restrictive with respect to research design.

7.5.3. Management implications

(1) 'Blanket' strategies of riparian zone preservation or restoration in catchments to buffer agricultural nitrogen will probably be inefficient as some riparian zones will be poor nitrogen buffers.

(2) Engineering structures that increase runoff-riparian soil contact may enhance the nitrogen buffering potential of some riparian zones, particularly where 'preferential flow paths' cause a large proportion of catchment runoff to have minimal contact with riparian zone soils.

(3) Riparian zone management practices that increase the proportion of moderately anoxic soil in riparian zones, relative to highly anoxic or oxic soil, may enhance denitrification, and thus, nitrogen buffering. These practices could include water table management or encouraging the growth of plants, like *Glyceria declinata*, which release oxygen from their roots in waterlogged soils.

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Appendix A

Soil pit data
(Chapter 4)

Soil Pit 1

Location: Exactly half way between piezometers A5 and A4 Date of Excavation: 20.1.1999

Horizon	Munsell Colour	Notes	Depth Samples Taken (cm)	% Clay*	% Silt*	% Sand*	% Moisture*	% Organic Matter*	Bulk Density ($g\ cm^{-3}$)*
A (0-24cm)	5YR3/1 very dark gray	many fine medium & coarse roots, occasional medium to large stones	no samples taken						
B1g (24-43cm)	10YR3/3 dark brown; mottles of yellow (10YR 5/8) and orange (7.5YR 6/8)	Slightly stony (gravel)	28-38	7.4 ± 1.4	27.6 ± 5.6	65.0 ± 7.0	23.1 ± 2.5	5.4 ± 2.0	1.59 ± 0.12
B2g (43-69cm)	10YR3/3 dark brown; mottles as for B1	more sandy than overlying layer	43-53	5.5 ± 1.3	20.9 ± 3.5	73.6 ± 4.7	20.8 ± 1.2	3.4 ± 0.2	1.76 ± 0.17
B3G (69cm+)	10YR4/1 dark gray; mottles of yellowish brown (10YR4/6) and strong brown (7.5YR 5/8	clay texture, stoneless	75-85	10.8 ± 2.3	42.6 ± 9.7	46.6 ± 12.0	23.4 ± 1.1	4.8 ± 0.5	1.84 ± 0.09

* values are means ± standard deviation (n=5)

Soil Pit 2

Location: Exactly half way between piezometers C5 and D5 Date of Excavation: 27.1.1999

Horizon	Munsell Colour	Notes	Depth Samples Taken (cm)	% Clay*	% Silt*	% Sand*	% Moisture*	% Organic Matter*	Bulk Density ($g\ cm^{-3}$)*
Ahg (0-18cm)	10YR3/2 very dark grayish brown; mottles of black (Gley N2.5), and along grass roots in particular, mottles of weak red (10R4/4) and red (2.5YR 4/6)	Stony (1-12cm diameter), abundant small roots	10-18	14.3 ± 0.4	44.2 ± 1.4	41.5 ± 1.8	28.8 ± 1.1	10.7 ± 0.5	1.61 ± 0.08
B1G (18-53cm)	10YR3/3 dark brown; mottles of brownish yellow (10YR 6/8)	Very stony (5-20cm diameter)	25-35	13.7 ± 0.4	42.2 ± 1.4	44.1 ± 1.6	27.0 ± 1.1	7.8 ± 0.5	1.74 ± 0.03
B2G (53cm+)	10YR4/1 dark gray	Clay texture, stoneless	55-65	17.5 ± 3.0	50.4 ± 5.4	32.1 ± 8.2	27.3 ± 0.5	7.2 ± 0.2	1.70 ± 0.04

* values are means ± standard deviation (n=5)

Soil Pit 3

Location: Exactly half way between piezometers A3 and A2 Date of Excavation: 28.1.1999

Horizon	Munsell Colour	Notes	Depth Samples Taken (cm)	% Clay*	% Silt*	% Sand*	% Moisture*	% Organic Matter*	Wet Bulk Density ($g\ cm^{-3}$)*
Ah (0-22cm)	7.5YR3/1 very dark gray		no samples taken						
B1g (22-52cm)	10YR4/1 dark gray; mottles of strong brown (7.5YR 5/6) and yellowish brown (10YR 5/8)		no samples taken						
B2G (52-80cm)	10YR5/1 gray	slightly stony (5-8cm diameter), numerous small areas of coal and white, yellow and orange sand	65-75	13.3 ± 1.1	51.1 ± 3.6	35.6 ± 4.0	19.9 ± 1.3	4.7 ± 0.7	1.91 ± 0.04
B3G (80cm+)	Gley N6/ gray	clay texture, stoneless	82-92	20.5 ± 0.5	68.6 ± 0.8	10.8 ± 1.3	24.9 ± 0.4	6.9 ± 0.4	1.82 ± 0.01

* values are means ± standard deviation (n=5)

Soil Pit 4

Location: 5 metres from B1 towards C1 and then 5m directly upslope (on top of berm). Date of Excavation: 28.1.1999

Horizon	Munsell Colour	Notes	Depth Samples Taken (cm)	% Clay*	% Silt*	% Sand*	% Moisture*	% Organic Matter*	Bulk Density ($g\ cm^{-3}$)*
Ah (0-8cm)	10YR3/1 very dark gray	dredged river channel sediments, abundance of fine grass roots	no samples taken						
B1g (8-74cm)	10YR2/1 black; mottles of yellowish brown (10YR5/4)	dredged river channel sediments, occasional small stone and fragment of coal	52-62	11.5 ± 0.6	52.1 ± 2.1	36.4 ± 2.1	36.2 ± 1.2	29.5 ± 2.8	1.43 ± 0.05
B2G (74-78cm)	10YR2/1 black	sandy, gravelly texture, abundant small coal fragments	74-78	6.5 ± 1.7	23.8 ± 4.2	69.9 ± 5.9	33.3 ± 0.8	51.6 ± 4.7	1.10 ± 0.12
B3G (78cm+)	2.5Y 3/2 very dark grayish brown	clay texture, stoneless	78-88	17.6 ± 1.1	51.0 ± 2.8	31.4 ± 3.8	32.1 ± 1.2	12.9 ± 1.7	1.65 ± 0.07

* values are means ± standard deviation (n=5)

Soil Pit 5 (Officially not a soil pit, merely cores taken from sediment surface)

Location: area of standing water at the site. Date of Excavation: 28.1.1999

Horizon	Munsell Colour	Notes	Sample Location	% Clay	% Silt	% Sand	% Moisture	% Organic Matter	Bulk Density ($g\ cm^{-3}$)
0 (0-10cm)	Gley N2.5 black	Saturated sediments directly underlying lush growth of wetland vegetation	B1-B2	14.2	58.7	27.1	60.7	30.5	1.22
			C1-C2	18.0	55.2	26.8	52.5	22.3	1.33
			D1-D2	16.3	53.4	30.3	48.8	16.0	1.39
			E1-E2	10.6	50.2	39.2	55.7	40.0	1.08
			B2-B3	10.8	55.2	34.0	45.2	19.0	1.43
			C2-C3	21.6	70.8	7.6	54.0	15.6	1.40
			D2-D3	13.0	54.0	33.0	44.0	25.0	1.18
			E2-E3	10.2	43.8	46.0	61.6	40.2	1.14
			C3-C4	17.4	57.1	25.5	48.2	14.5	1.38
			D3-D4	10.9	45.1	44.0	51.8	12.6	1.48
			E3-E4	13.7	46.2	40.1	37.5	11.1	1.55
			Mean	14.3 ± 3.5	53.2 ± 7.1	32.2 ± 10.2	50.9 ± 6.9	22.4 ± 9.9	1.30 ± 0.13

Soil Pit 6 (Officially not a soil pit, merely cores taken from topsoil)

Location: Between piezometers in sheep grazed field above riparian wetland. Date of Excavation: 28.1.1999

Horizon	Munsell Colour	Notes	Sample Location	% Clay	% Silt	% Sand	% Moisture	% Organic Matter	Bulk Density ($g\ cm^{-3}$)
Ah (0-20 cm)	5YR3/1 very dark gray	Samples taken from 4-14 cm depth	A6-B6				23.6	9.5	1.78
			B6-C6				34.3	14.3	1.56
			C6-D6				28.4	10.8	1.67
			D6-E6				27.2	9.5	1.67
			A6-A5				29.2	11.3	1.66
			Mean				28.5 ± 3.5	11.6 ± 1.6	1.67 ± 0.07

Appendix B

Additional joint ^{15}N tracer-isotope dilution experiment data (Chapter 5)

Table B1

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with a low level of ^{15}N-labelled nitrate and unlabelled ammonium (Treatment 1) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	37.2352 a	0.8871 *(± 0.0651)* b	0.7764 *(± 0.2628)* b	0.7676 *(± 0.3065)* b
	^{15}N excess (μg g soil^{-1})	0.1854 a	0.0032 *(± 0.0005)* b	0.0050 *(± 0.0020)* b	0.0017 *(± 0.0007)* b
	^{14}N (μg g soil^{-1})	0.3108 a	0.3648 *(± 0.0739)* ab	0.6578 *(± 0.1267)* ab	0.2188 *(± 0.0010)* b
Ammonium	Atom % excess	0.0000 a	0.2006 *(± 0.0107)* b	0.1945 *(± 0.0169)* b	0.2256 *(± 0.0089)* b
	^{15}N excess (μg g soil^{-1})	0.0000 a	0.0133 *(± 0.0014)* b	0.0090 *(± 0.0018)* b	0.0194 *(± 0.0012)* c
	^{14}N (μg g soil^{-1})	8.7937 a	6.5426 *(± 0.3201)* b	4.5608 *(± 0.5143)* c	8.6023 *(± 0.8340)* ab
Gaseous nitrogen	Atom % excess	0.0000 a	0.0000 *(± 0.0000)* a	0.0006 *(± 0.0005)* a	0.0048 *(± 0.0003)* b
	^{15}N excess (μg g soil^{-1})	0.0000 a	-0.0004 *(± 0.0007)* a	0.0150 *(± 0.0135)* a	0.1358 *(± 0.0099)* b
	^{14}N (μg g soil^{-1})	nd	2601 *(± 40.8)* a	2817 *(± 202)* a	2803 *(± 60.0)* a
Soil total nitrogen	Atom % excess	0.0042 a	0.0033 *(± 0.0011)* a	0.0040 *(± 0.0002)* a	0.0035 *(± 0.0007)* a
	^{15}N excess (μg g soil^{-1})	0.1710 a	0.1166 *(± 0.0369)* a	0.2077 *(± 0.0152)* a	0.1652 *(± 0.0332)* a
	^{14}N (μg g soil^{-1})	4085 a	3501 *(± 57.2)* a	5197 *(± 202)* bc	4670 *(± 202)* ac

Table B2

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with an intermediate level of ^{15}N-labelled nitrate and unlabelled ammonium (Treatment 2) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	85.6225 a	16.3657 *(± 1.5661)* b	12.7227 *(± 0.6519)* b	1.3758 *(± 0.3095)* c
	^{15}N excess (μg g soil^{-1})	2.0121 a	0.2540 *(± 0.0243)* b	0.2270 *(± 0.1199)* bc	0.0055 *(± 0.0020)* c
	^{14}N (μg g soil^{-1})	0.3293 a	1.2923 *(± 0.0243)* b	1.4680 *(± 0.6905)* ab	0.3625 *(± 0.0725)* a
Ammonium	Atom % excess	0.0000 a	0.5909 *(± 0.0376)* b	0.6161 *(± 0.1186)* b	1.2670 *(± 0.0761)* c
	^{15}N excess (μg g soil^{-1})	0.0000 a	0.0391 *(± 0.0038)* b	0.0390 *(± 0.0135)* ab	0.0779 *(± 0.0224)* ab
	^{14}N (μg g soil^{-1})	10.3758 a	6.5912 *(± 0.6600)* b	5.7797 *(± 1.3607)* ab	5.8908 *(± 1.3117)* ab
Gaseous nitrogen	Atom % excess	0.0000 a	0.0001 *(± 0.0000)* a	0.0012 *(± 0.0006)* a	0.0282 *(± 0.0011)* b
	^{15}N excess (μg g soil^{-1})	0.0000 a	0.0015 *(± 0.0004)* a	0.0320 *(± 0.0146)* a	0.7108 *(± 0.0330)* b
	^{14}N (μg g soil^{-1})	nd	2660 *(± 68.4)* a	2817 *(± 198)* a	2497 *(± 36.8)* a
Soil total nitrogen	Atom % excess	0.0042 a	0.0076 *(± 0.0005)* b	0.0109 *(± 0.0020)* b	0.0052 *(± 0.0007)* ab
	^{15}N excess (μg g soil^{-1})	0.1710 a	0.3535 *(± 0.0195)* b	0.4238 *(± 0.0673)* b	0.2409 *(± 0.0377)* ab
	^{14}N (μg g soil^{-1})	4085 a	4615 *(± 68.4)* bc	3930 *(± 234)* ac	4630 *(± 167)* a

Table B3

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with a high level of ^{15}N-labelled nitrate and unlabelled ammonium (Treatment 3) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	96.1097 a	55.9180 *(± 4.4217)* b	52.8044 *(± 2.3004)* b	50.6960 *(± 1.2789)* b
	^{15}N excess (μg g soil^{-1})	11.6427 a	5.6198 *(± 0.4522)* b	6.3518 *(± 0.1442)* b	2.8463 *(± 0.5940)* c
	^{14}N (μg g soil^{-1})	0.4269 a	4.3954 *(± 0.4607)* bc	5.1419 *(± 0.3633)* b	2.7571 *(± 0.5678)* c
Ammonium	Atom % excess	0.0000 a	0.3930 *(± 0.0841)* b	0.3447 *(± 0.0369)* b	1.2786 *(± 0.1705)* c
	^{15}N excess (μg g soil^{-1})	0.0000 a	0.0615 *(± 0.0127)* b	0.0449 *(± 0.0003)* b	0.2042*(± 0.0499)* b
	^{14}N (μg g soil^{-1})	19.8750 a	15.6262 *(± 0.5202)* b	13.0669 *(± 0.6312)* b	15.3493 *(± 1.3596)* ab
Gaseous nitrogen	Atom % excess	0.0000 a	0.0000 *(± 0.0000)* a	0.0010 *(± 0.0005)*a	0.0187 *(± 0.0018)* b
	^{15}N excess (μg g soil^{-1})	0.0000 a	-0.0007 *(± 0.0008)* a	0.0234 *(± 0.0110)* a	0.4658 *(± 0.0509)* b
	^{14}N (μg g soil^{-1})	nd	2538 *(± 88.4)* a	2470 *(± 62)* a	2477 *(± 36.4)* a
Soil total nitrogen	Atom % excess	0.0042 a	0.0064 *(± 0.0013)* a	0.0303 *(± 0.0047)* b	0.0258 *(± 0.0014)* b
	^{15}N excess (μg g soil^{-1})	0.1710 a	0.2519 *(± 0.0231)* b	1.2564 *(± 0.2244)* b	1.0968 *(± 0.1032)* b
	^{14}N (μg g soil^{-1})	4085 a	4292 *(± 1024)* a	4091 *(± 182)* a	4226 *(± 246)* a

Table B4

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with a low level of unlabelled nitrate and ^{15}N-labelled ammonium (Treatment 4) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	0.0000 a	0.2807 *(± 0.2832)* a	0.0699 *(± 0.1186)* a	0.2250 *(± 0.0159)* a
	^{15}N excess (μg g soil^{-1})	0.0000 a	0.0006 *(± 0.0006)* a	0.0005 *(± 0.0008)* a	0.0008 *(± 0.0004)* a
	^{14}N (μg g soil^{-1})	0.5061 a	0.5892 *(± 0.1964)* ab	0.6624 *(± 0.0019)* b	0.3673 *(± 0.0743)* ab
Ammonium	Atom % excess	2.0129 a	2.6794 *(± 0.1163)* b	2.4934 *(± 0.1063)* b	1.7318 *(± 0.0853)* a
	^{15}N excess (μg g soil^{-1})	0.1776 a	0.1358 *(± 0.0194)* a	0.1014 *(± 0.0040)* a	0.0967 *(± 0.0139)* a
	^{14}N (μg g soil^{-1})	8.6102 a	4.8778 *(± 0.4945)* b	3.9517 *(± 0.0734)* b	5.5771 *(± 1.0886)* ab
Gaseous nitrogen	Atom % excess	0.0000 a	0.0000 *(± 0.0000)* a	0.0000 *(± 0.0000)* a	0.0004 *(± 0.0003)* a
	^{15}N excess (μg g soil^{-1})	0.0000 a	-0.0008 *(± 0.0002)* a	-0.0004 *(± 0.0002)* a	0.0094 *(± 0.0065)* a
	^{14}N (μg g soil^{-1})	nd	2379 *(± 125)* a	2373 *(± 49.6)* a	2476 *(± 162)* a
Soil total nitrogen	Atom % excess	0.0042 a	0.0045 *(± 0.0006)* a	0.0044 *(± 0.0009)* a	0.0041 *(± 0.0008)* a
	^{15}N excess (μg g soil^{-1})	0.1710a	0.1908 *(± 0.0151)* a	0.1842 *(± 0.0252)* a	0.1739 *(± 0.0368)* a
	^{14}N (μg g soil^{-1})	4085 a	4270 *(± 266)* a	4267 *(± 270)* a	4177 *(± 140)* a

Table B5

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with an intermediate level of unlabelled nitrate and ^{15}N-labelled ammonium (Treatment 5) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	0.0000 **a**	0.2209 *(± 0.0096)* **b**	1.3672 *(± 0.6312)* **ab**	0.4664 *(± 0.1822)* **ab**
	^{15}N excess (μg g soil^{-1})	0.0000 **a**	0.0036 *(± 0.0007)* **b**	0.0290 *(± 0.0175)* **ab**	0.0015 *(± 0.0003)* **b**
	^{14}N (μg g soil^{-1})	2.2218 **a**	1.6171 *(± 0.2653)* **a**	1.8123 *(± 0.2774)* **a**	0.3665 *(± 0.0746)* **b**
Ammonium	Atom % excess	16.9745 **a**	20.7974 *(± 0.8171)* **b**	20.8328*(± 2.2533)* **abc**	13.8772 *(± 0.1922)* **c**
	^{15}N excess (μg g soil^{-1})	1.7715 **a**	1.3064 *(± 0.0371)* **b**	1.0553 *(± 0.0318)* **c**	1.1453*(± 0.1367)* **abc**
	^{14}N (μg g soil^{-1})	8.6263 **a**	4.6597 *(± 0.2386)* **b**	4.1018 *(± 0.5220)* **b**	7.1017 *(± 0.9486)* **ab**
Gaseous nitrogen	Atom % excess	0.0000 **a**	0.0000 *(± 0.0000)* **ac**	0.0002 *(± 0.0001)* **b**	0.0008 *(± 0.0003)* **bc**
	^{15}N excess (μg g soil^{-1})	0.0000 **a**	0.0007 *(± 0.0008)* **a**	0.0592 *(± 0.0013)* **b**	0.0211 *(± 0.0070)* **ab**
	^{14}N (μg g soil^{-1})	nd	2797 *(± 122)* **a**	2467 *(± 102)* **a**	2551 *(± 7.6)* **a**
Soil total nitrogen	Atom % excess	0.0042 **a**	0.0088 *(± 0.0024)* **ab**	0.0120 *(± 0.0028)* **b**	0.0148 *(± 0.0004)* **b**
	^{15}N excess (μg g soil^{-1})	0.1710 **a**	0.4021 *(± 0.1662)* **ab**	0.5027 *(± 0.0886)* **b**	0.6335 *(± 0.0287)* **b**
	^{14}N (μg g soil^{-1})	4085 **a**	4190 *(± 688)* **a**	4295 *(± 320)* **a**	4272 *(± 102)* **a**

Table B6

Atom % excess values and concentrations of excess ^{15}N and ^{14}N in KCl-extractable soil inorganic nitrate and ammonium, gaseous nitrogen and soil total nitrogen in soil slurries amended with a high level of unlabelled nitrate and ^{15}N-labelled ammonium (Treatment 6) during the incubation period. Values are means (± 1 standard error, n=3). The script 'nd' means 'not determined'. Different subscripts (a,b etc) across a row indicate significant differences (P<0.05).

Nitrogen pool	Measurement	After 0 minutes expected	After 10 minutes measured	After 70 minutes measured	After 250 minutes measured
Nitrate	Atom % excess	0.0000 **a**	0.9605 *(± 0.7164)* **ab**	0.7594 *(± 0.1429)* **b**	1.2578 *(± 0.3257)* **b**
	^{15}N excess (μg g soil^{-1})	0.0000 **a**	0.1010 *(± 0.0742)* **ab**	0.0740 *(± 0.0141)* **b**	0.0889 *(± 0.0295)* **ab**
	^{14}N (μg g soil^{-1})	12.2091 **a**	10.6488 *(± 0.2632)* **b**	9.6489 *(± 0.6699)* **b**	6.5427 *(± 0.7714)* **c**
Ammonium	Atom % excess	55.9215 **a**	59.4732 *(± 1.6352)* **a**	56.0568 *(± 1.1896)* **ac**	53.1327 *(± 0.6123)* **c**
	^{15}N excess (μg g soil^{-1})	11.5744 **a**	8.0888 *(± 0.2934)* **b**	8.6201 *(± 0.3522)* **b**	7.8650*(± 0.4074)* **b**
	^{14}N (μg g soil^{-1})	8.7215 **a**	5.4600 *(± 0.2374)* **b**	6.7000 *(± 0.2851)* **c**	6.8701 *(± 0.1853)* **c**
Gaseous nitrogen	Atom % excess	0.0000 **a**	0.0000 *(± 0.0000)* **ac**	0.0004 *(± 0.0000)* **b**	0.0026 *(± 0.0007)* **bc**
	^{15}N excess (μg g soil^{-1})	0.0000 **a**	-0.0008 *(± 0.0007)* **a**	0.0094 *(± 0.0004)* **b**	0.0673 *(± 0.0192)* **ab**
	^{14}N (μg g soil^{-1})	nd	2562 *(± 49.6)* **a**	2500 *(± 57.2)* **a**	2606 *(± 28.4)* **a**
Soil total nitrogen	Atom % excess	0.0042 **a**	0.0140 **b**	0.0224 *(± 0.0014)* **bc**	0.0349 *(± 0.0049)* **c**
	^{15}N excess (μg g soil^{-1})	0.1710 **a**	0.5388 **b**	0.9467 *(± 0.0658)* **c**	1.3953 *(± 0.2508)* **c**
	^{14}N (μg g soil^{-1})	4085 **a**	3828 **a**	4281 *(± 556)* **a**	3938 *(± 154)* **a**

Table B7

The fate of ^{15}N-nitrate with low input in the three phases of the experiment. Only significant (P<0.05) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Nitrate removal	0.1822	-	-
Denitrification	0.1585	-	-
Measured denitrification	*0.1358**	-	-
Probable denitrification (unaccounted ^{15}N)	*0.0227*	-	-
DNRA	0.0133+0.0104*	-	-
Immobilisation	-	-	-

* measured in phase 3 but attributable to transformation in phase 1

Table B8

The fate of ^{15}N-nitrate with intermediate input in the three phases of the experiment. Only significant (P<0.05) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Nitrate removal	1.7581	-	0.2215
Denitrification	1.5365	-	0.2215
Measured denitrification	*0.4893**	-	*0.2215*
Probable denitrification (unaccounted ^{15}N)	*1.0472*	-	-
DNRA	0.0391	-	-
Immobilisation	0.1825	-	-

* measured in phase 3 but attributable to transformation in phase 1

Table B9

The fate of ^{15}N-nitrate with high input in the three phases of the experiment. Only significant (P<0.05) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Nitrate removal	6.0229	-	3.5055
Denitrification	5.8805	-	3.5055
Measured denitrification	-	-	*0.4658*
Probable denitrification (unaccounted ^{15}N)	*5.8805*	-	*3.0397*
DNRA	0.0615	-	-
Immobilisation	0.0809	-	-

Table B10

The fate of ^{15}N-ammonium with low input in the three phases of the experiment. Only significant (P<0.05) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Ammonium removal	-	-	-
Autotrophic nitrification	-	-	-
Anammox-coupled nitrification-denitrification	-	-	-
Immobilisation	-	-	-
Unaccounted	-	-	-

Table B11

The fate of ^{15}N-ammonium with intermediate input in the three phases of the experiment. Only significant ($P<0.05$) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Ammonium removal	0.4651	0.2511	-
Autotrophic nitrification	0.0036	-	-
Anammox-coupled nitrification-denitrification	-	0.0059	-
Immobilisation	0.0865*	0.2452	-
Unaccounted	0.3750	-	-

* measured in phase 2 but attributable to transformation in phase 1

Table B12

The fate of ^{15}N-ammonium with high input in the three phases of the experiment. Only significant ($P<0.05$) changes are shown.

Process	($\mu g^{15}N$ g $soil^{-1}$) Phase 1 (0-10 minutes)	Phase 2 (10-70 minutes)	Phase 3 (70-250 minutes)
Ammonium removal	3.4856	-	-
Autotrophic nitrification	0.1010	-	-
Anammox-coupled nitrification-denitrification	0.0094*	-	-
Immobilisation	0.3678+0.4079*	-	-
Unaccounted	2.5995	-	-

* measured in phase 2 but attributable to transformation in phase 1

Table B13

Isotope dilution calculation for inorganic nitrogen production and removal with low input data. Italics indicate no significant change.

		Total nitrogen (μg N g soil^{-1})		Atom % excess		Time (hrs)				
		Q1	Q2	A1	A2	T1	T2	F1	F0	Equation
Phase 1										
Treatment 1	^{15}N-nitrate	*0.4960*	*0.3680*	37.2353	0.8871	0	0.1667	11.1213	11.1213	5.3
Treatment 4	^{15}N-ammon	8.8200	5.0320	2.0130	2.6794	0	0.1667	-11.5816	11.1464	5.1 & 5.2
Phase 2										
Treatment 2	^{15}N-nitrate	*0.3680*	*0.6640*	*0.8871*	*0.7764*	0.1667	1.1667	-	-	-
Treatment 5	^{15}N-ammon	*5.0230*	*4.0680*	*2.6794*	*2.4934*	0.1667	1.1667	-	-	-
Phase 3										
Treatment 3	^{15}N-nitrate	*0.6640*	*0.2200*	*0.7764*	*0.7676*	1.1667	4.1667	-	-	-
Treatment 6	^{15}N-ammon	*4.0680*	*5.7470*	2.4934	1.7318	1.1667	4.1667	0.4942	0.4942	5.3

Table B14

Isotope dilution calculation for inorganic nitrogen production and removal with intermediate input data. Italics indicate no significant change.

		Total nitrogen (μg N g soil^{-1})		Atom % excess		Time (hrs)				
		Q1	Q2	A1	A2	T1	T2	F1	F0	Equation
Phase 1										
Treatment 1	^{15}N-nitrate	2.3500	1.5520	85.6225	16.3658	0	0.1667	19.0975	23.8855	5.1 & 5.2
Treatment 4	^{15}N-ammon	10.4360	5.9880	16.9746	21.8704	0	0.1667	-12.1749	14.5131	5.1 & 5.2
Phase 2										
Treatment 2	^{15}N-nitrate	*1.5520*	*1.7013*	*16.3658*	*12.7227*	0.1667	1.1667	-	-	-
Treatment 5	^{15}N-ammon	*5.9880*	*5.1760*	*21.8704*	*20.8328*	0.1667	1.1667	-	-	-
Phase 3										
Treatment 3	^{15}N-nitrate	*1.7013*	*0.3693*	12.7227	1.3758	1.1667	4.1667	1.2614	1.2614	5.3
Treatment 6	^{15}N-ammon	*5.1760*	*8.2773*	*20.8328*	*13.8773*	1.1667	4.1667	-	-	-

Table B15

Isotope dilution calculation for inorganic nitrogen production and removal with high input data. Italics indicate no significant change.

		Total nitrogen (μg N g soil^{-1})		Atom % excess		Time (hrs)				
		Q1	Q2	A1	A2	T1	T2	F1	F0	Equation
Phase 1										
Treatment 1	^{15}N-nitrate	12.1140	10.0520	96.1097	55.9180	0	0.1667	35.9114	48.2834	5.1 & 5.2
Treatment 4	^{15}N-ammon	19.9520	13.5987	55.9215	59.4732	0	0.1667	0	38.1198	5.4
Phase 2										
Treatment 2	^{15}N-nitrate	10.0520	11.5360	*55.9180*	*55.1048*	0.1667	1.1667	0	-1.4840	5.4
Treatment 5	^{15}N-ammon	*13.5987*	*15.3760*	*59.4732*	*56.0568*	0.1667	1.1667	-	-	-
Phase 3										
Treatment 3	^{15}N-nitrate	11.5360	5.6240	*55.1048*	*50.6960*	1.1667	4.1667	0	1.9707	5.4
Treatment 6	^{15}N-ammon	*15.3760*	*14.7893*	*56.0568*	*53.1127*	1.1667	4.1667	-	-	-