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Chapter 18

Nitrogen Removal Processes in Constructed Wetlands

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Abstract. Constructed wetlands can effectively remove nitrogen from waste-waters and diffuse run-off from land. In mature wetlands the dominant N removal process is generally microbial denitrification. N removal from ammonium-rich wastewaters is frequently limited by insufficient oxygen for initial nitrification. Alternative microbial N removal processes, including anaerobic ammonium oxidation, may be important in these situations. Emergent plants enhance N removal mainly via indirect effects on physico-chemical and microbial processes. In particular, they promote settling and retention of suspended solids, transport oxygen into the root-zone, provide surfaces for biofilm growth, and produce organic substrates for denitrifying bacteria.

18.1. Introduction

Constructed wetlands attempt to replicate and optimise treatment processes that occur naturally in swamps, fens and marshes. Efficiency is enhanced by optimising dispersion, flow paths, water depths, residence times, and vegetation characteristics. Constructed wetlands are now widely used to provide "natural" ecotechnological treatment solutions for urban, industrial and agricultural waste-, storm-and drainage-waters (USEPA, 1993, 2000; Kadlec & Knight, 1996; IWA, 2000). Construction and operating costs are low relative to mechanical treatment plants providing suitable land is available, and provision of wildlife habitat and green spaces may provide ancillary benefits. Much of the historical development and application of constructed wetlands has occurred in North America, Europe and Australasia, but interest is now rapidly increasing in Asia, South America and Africa.

Constructed wetland designs can be most simply classified as surface-flow (SF, also known as free-water surface) or subsurface-flow (SSF, also known as vegetated submerged beds, reed-beds and root-zone systems). In SF wetlands,

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the wastewater flows through a shallow "pond" planted with emergent plants such as bulrushes, reeds or sedges, or less commonly, floating or submerged macrophytes. In SSF systems, the wastewaters flow through gravel or similar substrata, and the plants grow rooted in the gravel. SF wetlands have become favoured in many areas of the world because they are cheaper to construct (no gravel media required) and generally have higher wildlife habitat values. SSF wetlands, however, tend to be more effective at suspended solids removal and BOD reduction per unit land area. Because the wastewater remains below the surface in these systems, there is also little possibility for human or wildlife contact with wastewaters and less potential for odours or insect infestation. Intermittently dosed, vertical-flow (VF) constructed wetlands have recently been developed to provide enhanced removal of biochemical oxygen demand (BOD) and nitrogen (IWA, 2000). These wetlands are essentially simple percolating filters with plants and will not be covered further here. The use of hybrid designs incorporating VF, SSF and/or SF sections is becoming increasingly common.

Key features of wetlands that contribute to their nutrient and contaminant removal functions include:

1. Low flow velocities and tortuous pathways through aquatic vegetation, which favour sedimentation and accumulation of particulates. BOD, nutrients and other contaminants associated with settled particulates are thus removed from through-flowing waters and incorporated into the wetland sediments.
2. Intimate contact between water, sediments, plants, detritus, and biofilm, which enhances assimilation of nutrients and substrates, and promotes physical, chemical and biotic interactions. Nutrients taken up by plants are recycled both internally within plants and through leaching and mineralisation of standing and fallen litter. A proportion of nutrients are bound up in detritus and refractory humic compounds which tend to accrete in the wetland. Organic substrates exuded by plants and released from decaying plant tissues can fuel important microbial transformations such as denitrification.
3. A mosaic of aerobic and anaerobic micro-environments, which promotes sequential microbial transformations of a wide range of nutrients, metals, and natural organic and xenobiotic compounds. High loadings of organic substrates and restricted oxygen exchange with the atmosphere create anaerobic conditions, particularly in the sediments of wetlands. Algal and submerged plant photosynthesis during daylight promotes aerobic conditions within the water column and in biofilms. Atmospheric gas exchange across the water surface via diffusion, convection, and release from the internal tissue of plants produce aerobic micro-zones near the water surface, and associated with shoots and the root-zone. In combination these opposing processes of oxygen

consumption and supply create a complex temporal and spatial mosaic of aerobic, anoxic and anaerobic environments in wetlands.

Nitrogen is an important contaminant in many waste, storm, and drainage-waters. Key forms of N in water include the oxidised species nitrate (NO_3) and nitrite (NO_2), and reduced species such as ammoniacal-N (NH_4-N) and N bound in dissolved and particulate organic matter (Org-N). Ammoniacal-N is a major plant nutrient that can promote excessive growth of aquatic plants, leading to eutrophication of water bodies. The un-ionised ammonia (NH_3^+) component (favoured at elevated pH and temperature) is also toxic to aquatic life and may exert a significant nitrogenous oxygen demand (NOD) as a result of bacterial nitrification processes (see below). This chapter introduces the key processes important for nitrogen removal in constructed wetlands, and then uses examples, from work of both the author and others, to illustrate the role of these processes in wetlands constructed for treatment of wastewaters and agricultural drainage waters.

18.2. Microbial Nitrogen Transformation Processes

Cycling of N in wetlands is complex, and includes important microbially mediated transformations (Fig. 1). Biological N-fixation is likely to be negligible compared

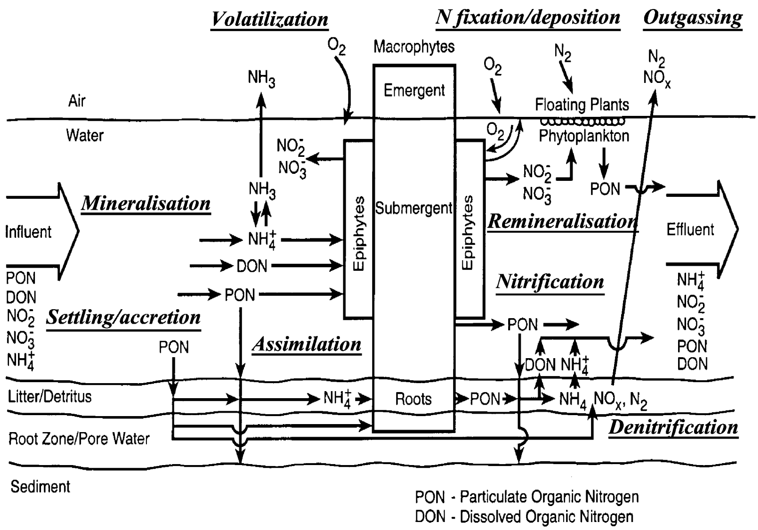

Figure 1: Key nitrogen transformations in SF treatment wetlands (USEPA, 2000). NO_x refers to NO_2 and NO_3-N; PON and DON refer to particulate and dissolved organic forms of N (Org-N).

to N loadings in normal treatment wetlands and the limited measurements of dissimilatory nitrogen reduction to ammonia (DNRA) suggest that it is not likely to be a quantitatively significant process in most treatment wetlands (Bowden, 1986; Tiedje, 1988; van Oostrom & Russell, 1994; Nijburg & Laanbroek, 1997), although it may be under some conditions (Cooke, 1994; Matheson et al., 2002). Reversible adsorption of NH_4-N to sediments, media and biofilms is likely to be a relatively small sink for N under steady state conditions, but may result in rapid removal in systems during start-up and where intermittent loading results in periodic depletion of the sorbed pool (e.g. Tanner et al., 1999; McBride & Tanner, 2000). The dominant transformation processes relevant to constructed wetlands are believed to be:

- *Mineralisation or ammonification* (Org-N $\rightarrow$ NH_4). Anaerobic and aerobic microbial decomposition of organic matter results in the hydrolysis of complex organic forms of N to ammoniacal N.
- *Ammonia volatilization* ($NH_4^+ \rightarrow NH_3$(aqueous) $\rightarrow NH_3$(gas)). As pH climbs above ~ 8 the proportion of un-ionised ammonia rises rapidly, increasing the potential for volatilization and release to the atmosphere (Jayaweera & Mikkelsen, 1991).
- *Plant and microbial Assimilation* ($NH_4^+ \rightarrow$ Org-N). Nitrogen is an important nutrient for plant growth. It is most commonly taken up by plants in the form of NH_4-N but, as in most terrestrial plants, it can also be taken up as NO_3-N and reduced to NH_4-N internally, or sometimes in organic forms (Marschner, 1995). N uptake and storage by plants can be an important removal mechanism, particularly during the establishment phase, however, unless the plants are periodically harvested and tissues removed, or the N is stored in long-lived tissues (e.g. wood), much of the assimilated N will be returned to the wetland when it senesces and decays. A proportion of the N in decomposing tissues is retained in accreted litter and humic compounds in recalcitrant forms. Assimilation by bacteria and fungi will also occur when there is a surplus supply of organic substrates and nutrients, and also when the microbial pool is expanding. Such immobilisation of N is likely to be minimal once the microbial pool has developed and reached a relatively steady state.
- *Nitrification* (NH_4 ($\rightarrow NH_2OH$) $\rightarrow NO_2 \rightarrow NO_3$). Under aerobic conditions and with an adequate supply of alkalinity, chemoautotrophic nitrifying bacteria can convert ammoniacal N to nitrate (NO_3) via hydroxylamine (NH_2OH) and nitrite (NO_2). Commonly, neither NO_3 nor its intermediaries accumulate in constructed wetlands treating organic wastewaters. This is presumed to be due to the presence of carbon-rich, anoxic conditions that limit nitrification but are highly conducive to denitrification (see below), and to close coupling between nitrification and denitrification at aerobic/anaerobic interfaces.

- *Denitrification* ($NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$). This is generally the dominant N removal process in constructed wetlands and involves bacterial conversion of nitrate to N_2O and N_2 gases. Denitrifiers are facultative heterotrophs that use NO_3 and NO_2 as electron accepters in the oxidation of organic matter under anoxic conditions. Although seldom measured directly, available evidence suggests that this pathway may commonly account for 40–90 percent of N removal from constructed wetlands (Tanner et al., 2002). Because it returns N to the vast, relatively inert, atmospheric pool of dinitrogen (N_2) this is generally seen as an ideal, sustainable removal process. However, a proportion of the denitrified (and nitrified) N may be emitted as nitrous oxide (N_2O), which is a potent greenhouse gas in the atmosphere (Houghton et al., 2001).
- *ANAMMOX* ($NH_4 + NO_2 \rightarrow N_2$) *and other alternative pathways*. There is increasing evidence that in oxygen-limited environments nitrification, denitrification and other microbial processes (e.g. methane oxidation) may be much more closely coupled (also described as integrated or simultaneous). They may also include a range of alternative and co-metabolic pathways that overcome oxygen and/or carbon limitations that frequently limit "classical" nitrification and denitrification processes in constructed wetlands.

Anaerobic ammonium oxidation (ANAMMOX) pathways (Fig. 2) have only recently been positively identified in nature (Robertson & Kuenen, 1992; van Loosdrecht & Jetten, 1998; Jetten et al., 1999), despite earlier prediction on thermodynamic grounds. Ammonium oxidising bacteria, which have a higher affinity for oxygen than nitrite oxidisers, are likely to be selectively advantaged under low oxygen conditions. Recent evidence also suggests that "aerobic" ammonium oxidisers have more versatile metabolism than previously assumed, being able to autotrophically denitrify with ammonia as electron donor under oxygen-limited conditions (63% reduction in NOD) or with hydrogen or organic compounds under anoxic conditions, and to use N_2O_4 for ammonium oxidation under both oxic and anoxic conditions (Kuai & Verstraete, 1998; Schmidt et al.,

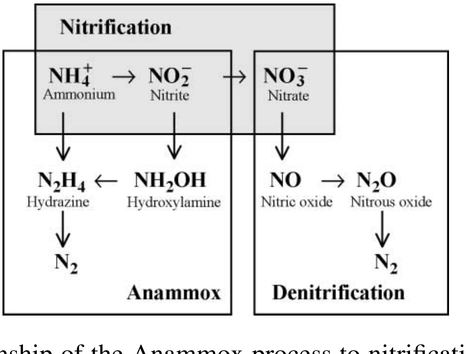

Figure 2: Basic relationship of the Anammox process to nitrification and denitrification.

2002; Sliekers et al., 2002). It has been suggested that "aerobic" nitrifier and anamox bacteria may be natural partners in many oxygen-limited situations (Schmidt et al., 2002), such as those found in many treatment wetlands, and in the root zone of wetland plants generally. Heterotrophic nitrification has been identified, and is sometimes linked directly with denitrification within the same organism (Robertson & Kuenen, 1992). Another option is to "short-cut" the full nitrification–denitrification process and denitrify from nitrite rather than nitrate, thus reducing the oxygen requirement by 25% (Kuai & Verstraete, 1998; Bernet et al., 2001). These alternative pathways need to be investigated further in both natural and constructed wetlands to develop an understanding of their role in wetland N removal.

18.3. N Removal Performance of Constructed Wetlands

General responses of effluent total nitrogen (TN) concentration to N loading are shown in Fig. 3 for North American (USEPA, 1998) and New Zealand (Tanner & Sukias, 2003) SF constructed wetlands treating effluents from domestic and agricultural waste stabilisation ponds. The NZ sewage wetlands for which data was available tended to be relatively highly N loaded compared to those in the North American Wetlands Treatment Database (NADB). Regression equations

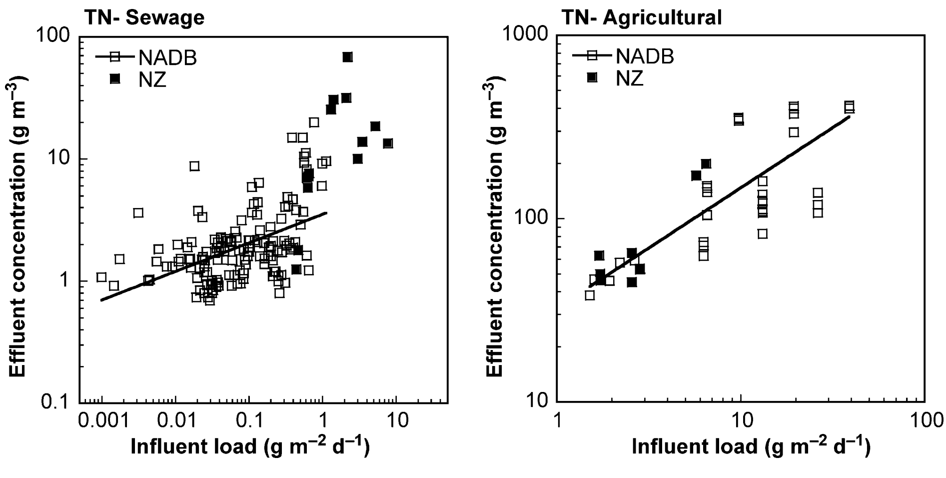

Figure 3: Comparison of mean annual outflow concentration of TN relative to mass loading for SF wetlands treating waste stabilisation pond effluents in NZ and for SF systems reported in the NADB. Each data point is the reported annual average for a specific wetland system or component, and the trend line is a power fit to the NADB data (Tanner and Sukias, 2003).

and rate constants for N removal, derived from North American and European wetland treatment systems are summarised in IWA (2000). These are based mainly on data for systems treating municipal sewage and care should be taken when extrapolating these to other wastewater types where the balance of N forms and/or associated organic loadings (BOD or COD) are different. Considerably higher N removal efficiencies are generally recorded for constructed wetlands treating waste, storm and drainage-waters where N is predominantly present as NO_3-N (van Oostrom & Russell, 1994; Xue et al., 1999; Bachand & Horne, 2000). Ammoniacal N removal can be promoted in wetland systems that incorporate aerobic open-water zones (Hammer & Knight, 1994) or include aerobic phases; e.g. intermittent vertical flow wetlands (IWA, 2000) or fluctuating water levels (Tanner et al., 1999).

18.4. Dominant Mechanisms of N Removal in Constructed Wetlands

18.4.1. Role of plants

TN removal performance for side-by-side studies of planted and unplanted SSF constructed wetlands is compared in Fig. 4 (Tanner, 2001b). Here, despite considerable data scatter, the planted wetlands show a clear trend of improved TN removal.

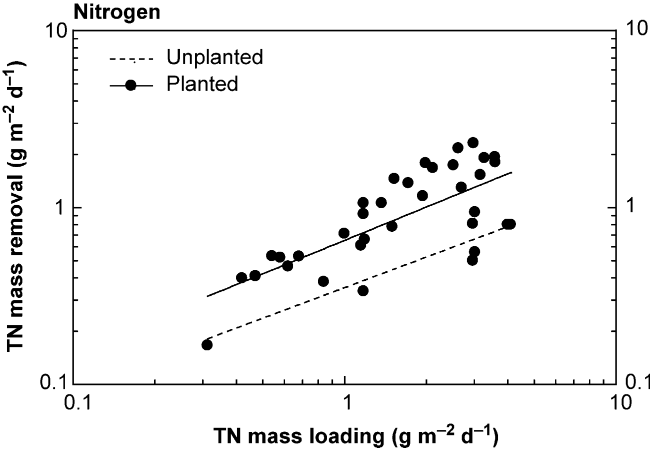

Figure 4: Comparison of mass loading and removal rates of TN for planted and unplanted wetlands. Trend lines shown are power fits (Tanner, 2001b).

The quantity of nutrients that can be taken up and accumulated by live plant biomass per unit of wetland surface area is finite for a given plant species, nutrient regime and set of environmental conditions. Once live plant storage pools approach this limit, little further net annual uptake is possible (Howard-Williams, 1985). In pilot-scale trials where plant storage pools were still actively filling, Gersberg et al. (1986) estimated potential plant uptake could only account for 12–16% of the N removal recorded in SSF wetlands planted with bulrushes. This was 5–7 times less than the additional removal recorded for the planted systems (over that of an unplanted system). In higher loaded SSF systems achieving relatively low N removal, van Oostrom & Cooper (1990) estimated net N uptake by bulrush over an annual period accounted for 25% of wetland TN removal, representing 66% of the additional removal recorded for the planted systems.

Detailed measurements of seasonal uptake by bulrush during the second growth season in four equivalent SSF systems operated over a range of loading rates (Tanner, 2001a) showed that, even in immature systems where plant nutrient pools are actively building, net storage in live plant tissues accounted for only 2–8% of TN removal over an annual period. Net annual plant uptake was responsible for only 3–19% of the additional TN removal recorded for the planted systems. This suggests that plants primarily facilitate improved N removal indirectly via their effects on other removal processes. Plants may enhance N transformation processes (e.g. nitrification and denitrification) through root-zone oxygen release and supply of organic matter. Cycling and accumulation of plant-derived organic matter provides a sustained supply of organic C for microbes (including denitrifiers), sequesters organically bound nutrients, and buffers nutrient release.

18.4.2. Gaseous Emission

Although rarely measured directly in treatment wetlands, microbial denitrification to dinitrogen and nitrous oxide gases is considered to be the primary sustainable nitrogen removal mechanism in wetlands treating wastewaters (IWA, 2000). Ammonia volatilization may also be important in SF wetland systems where the photosynthesis of algae and submerged macrophytes depletes dissolved carbon dioxide in the water column, causing diurnal pH elevation (Jayaweera & Mikkelsen, 1991). In wetlands receiving very high NH_4-N loadings, Poach et al. (2002) found volatilization could account for 12–28% of measured N removal.

Ammonium-Rich Waters. The assumed microbial pathway for NH_4-N removal via denitrification involves initial oxidation to NO_3-N (nitrification). In the predominantly anaerobic waters of treatment wetlands, oxygen availability via atmospheric diffusion and transport through emergent macrophytes is considered to be the main rate-limiting factor for microbial nitrifiers (Gersberg et al., 1986;

IWA, 2000). Because of the presence of abundant organic carbon and reduced compounds, competition for available oxygen is likely to be intense from heterotrophs and other bacteria using alternative electron donors and chemical reductants (Laanbroek, 1990; Adams et al., 1996).

Tanner et al. (2002) attempted to determine the relative importance of different N removal processes along SSF constructed wetlands using experimental cascade mesocosms (wetland tanks in series). Measurements of flow and concentrations of different N species were used, along with a simplified model of sequential N transformations and sinks to infer rates of key N transformation processes down the cascades. When the mesocosms were supplied with four different organic wastewaters, each with contrasting ratios of COD: N and forms of N, it was found that TN and COD mass removal rates varied markedly for the different wastewaters (Fig. 5).

Based on the model, Tanner et al. (2002) found N losses via denitrification accounted for between 60 and 84% of overall TN losses in the cascades, and 0–89% of TN removal in different stages of the cascades. Mean denitrification rates ranged from 0.47–2.0 g N m^{-2} day^{-1} in the different cascades and from 0.0–3.17 g N m^{-2} day^{-1} in individual stages (Fig. 6). Net plant uptake (plant assimilation into above- and below-ground tissues less regeneration from below-ground biomass)

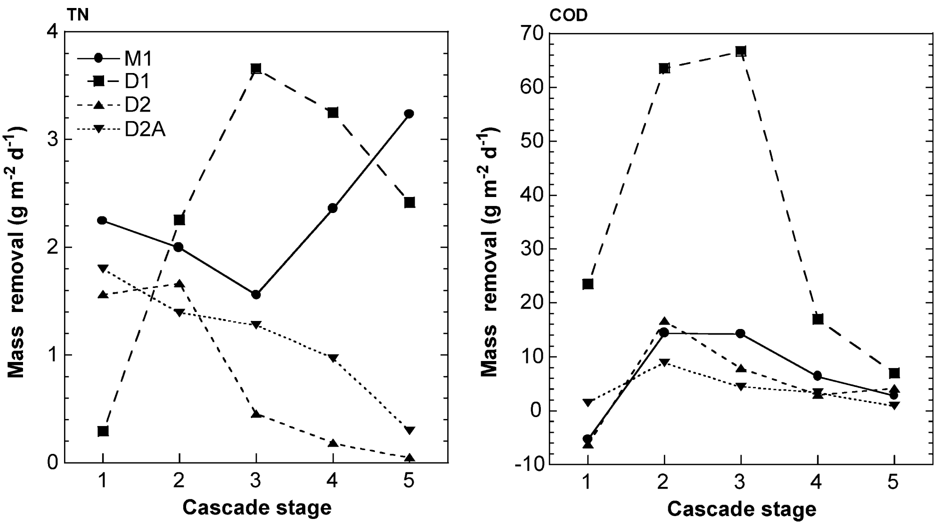

Figure 5: Gradients of TN and COD mass removal during passage through cascade mesocosms simulating horizontal-flow SSF constructed wetlands for four agricultural wastewaters with differing N species balances (Tanner and Kadlec, 2003). The wastewaters had been pretreated in waste stabilisation ponds; M1 = anaerobic-treated meat processing, D1, D2, D2A = anaerobic, facultative and aerated pond treated farm dairy, respectively. See Tanner et al. (2002) for gradients of different N forms.

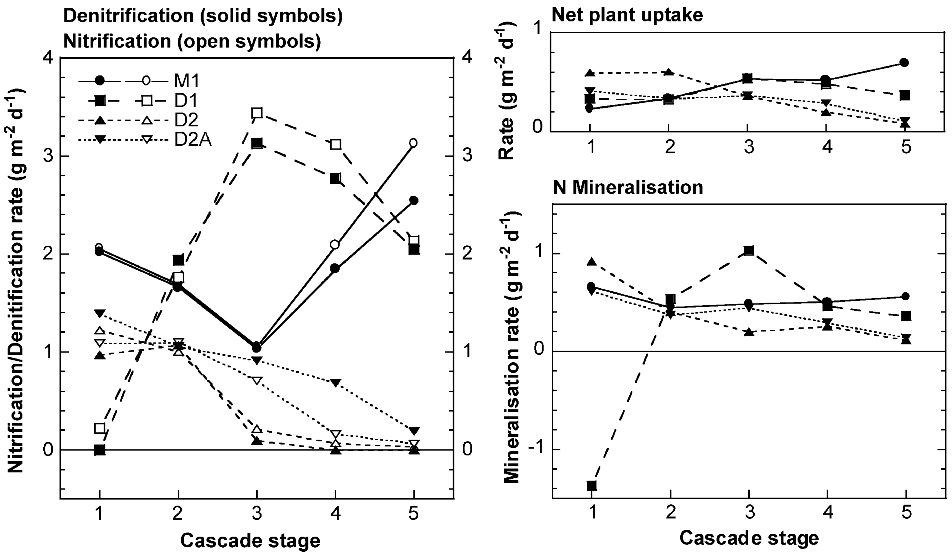

Figure 6: Gradients of key N transformation and removal processes along wetland cascade mesocosms supplied with four different agricultural wastewaters with differing N species balances and simulating horizontal-flow SSF constructed wetlands (Tanner and Kadlec, 2003).

was similar along all the cascades, accounting for $\sim$0.1–0.3 g N m^{-2} day^{-1} (16–40% of overall cascade TN losses). Mineralisation of organic N along the cascades accounted for 0.1–1 g N m^{-2} day^{-1}, increasing the realised NH_4-N loading to the wetlands. Apparent negative mineralisation in the D1 cascades occurred, presumably due to net Org-N generation from accumulated organic matter, which was substantial with this wastewater.

In situations where TN mass removal rates were low (less than $\sim$1 g N m^{-2} day^{-1}) plant N uptake was an important removal mechanism in the cascades. Apparent N removal via nitrification–denitrification became progressively more important as removal rates increased. This increased the corresponding theoretical NOD required to drive nitrification up to 15 g N m^{-2} day^{-1} in the stages of the cascades where the highest N removal rates were recorded (Tanner & Kadlec, 2003).

Cascades receiving wastewaters with differing characteristics showed contrasting nitrogen process gradients. Overall net plant N uptake, which was likely to have been elevated in the small-scale, harvested mesocosms, represented less than 24% of TN removal in the M1, D1 and D2A, and 40% in D2 cascades. Denitrification accounted for 60–84% of overall TN removal in the cascades, but contrary to commonly accepted paradigms, nitrification was apparently occurring

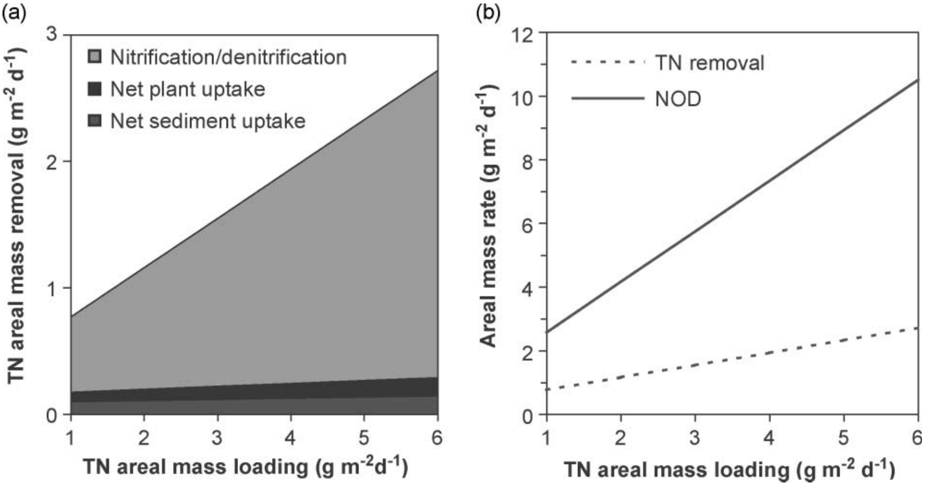

Figure 7: Typical relationships between (a) component TN removal process rates and (b) predicted NOD to wetland TN loading for SSF treatment wetlands (Tanner and Kadlec, 2003).

concurrently with COD removal. General data for N removal in SSF wetlands suggested denitrification is of similar importance in full-scale systems (Fig. 7).

The calculated NOD required to support full nitrification of ammonia and mineralised organic N was in the upper range of that normally able to be supplied by plant root-zone oxygen release. In the organic-rich, predominantly anaerobic environment of SSF wetland beds it is highly unlikely that nitrifiers would be able to compete successfully for more than a small proportion of this oxygen flux (Tanner & Kadlec, 2003). This suggests that oxygen transfer through the wetland surface and via emergent plants is insufficient to support the current paradigm of coupled nitrification–denitrification in SSF treatment wetlands. Better estimates of plant oxygen transport and root-zone release are needed, and the potential importance of recently discovered alternative pathways for nitrogen removal with lower oxygen requirements (e.g. Anammox) need to be explored to improve our understanding of wetland treatment processes.

Nitrate-Rich Waters. Much of the N in mechanically aerated wastewaters, and urban and agricultural drainage waters is commonly converted to the NO_3-N form. Because the process of ammonium oxidation, which is normally rate-limiting, has already occurred, constructed wetlands treating these nitrified waters can generally achieve high removals of N via microbial denitrification (van Oostrom & Russell, 1994; Bachand & Horne, 2000).

Constructed, restored and natural wetlands are increasingly seen as a key tool in the management of diffuse N export from agricultural lands (USEPA, 1993; Mitsch

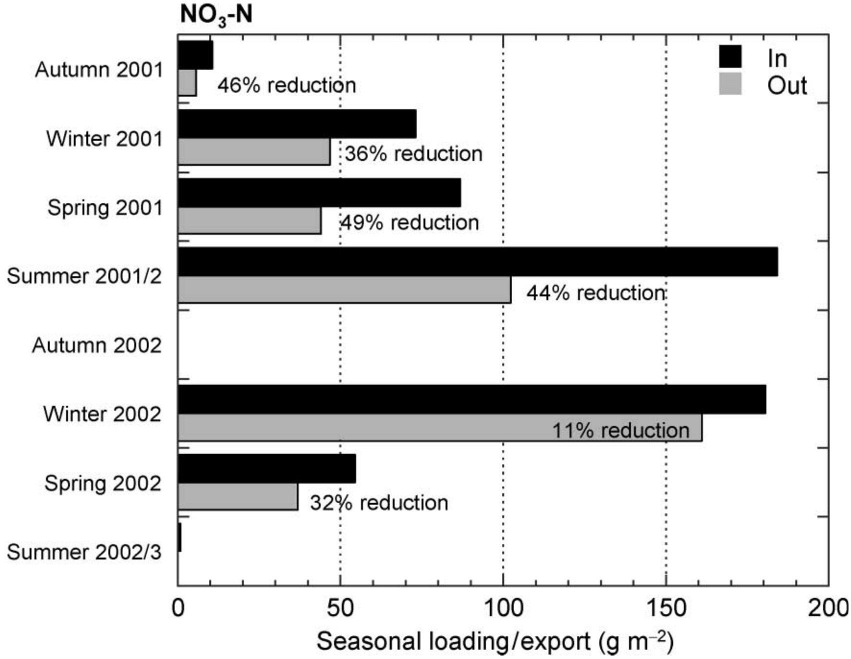

Figure 8: Summary of seasonal mass loads, export and percentage reduction of NO_3-N over 2 years for a constructed wetland treating subsurface agricultural drainage (Tanner et al., 2004).

et al., 2001). Data summarised for a range of North American experimental and field-scale studies (Mitsch et al., 2000) shows NO_3-N removal rates of 95–1,022 g N m^{-2} yr^{-1} for warm climate wetlands and 11–132 g N m^{-2} yr^{-1} for cold climate wetlands. Overall TN removal efficiencies of ~37% TN were reported for an in-stream wetland occupying ~0.8% of an agricultural watershed in North Carolina (Hunt et al., 1999), and also for three wetlands (each ~3% of contributing catchment area) treating cropland tile drainage in Illinois (Kovaic et al., 2000). In a semi-natural wetland (<0.2% of catchment area) treating predominantly subsurface drainage in east–central Illinois, Miller et al. (2002) reported 33% reduction in NO_3-N loads over a 4 year period. Tanner et al. (2004) studying constructed wetland treatment (~1% of catchment) of subsurface drainage from grazed dairy pastures in New Zealand over 2 years found seasonal NO_3-N removal ranging from 11–49%, with overall annual removals of 44% (156 g m^{-2} yr^{-1}) and 16% (52 g m^{-2} yr^{-1}). Variations in the seasonal pattern of N delivery to the wetlands appeared to markedly influence treatment performance (Fig. 8).

Mitsch et al. (2001), summarising data on wetland NO_3^- removal from river waters for multi-year studies carried out in six off-stream wetlands at two sites

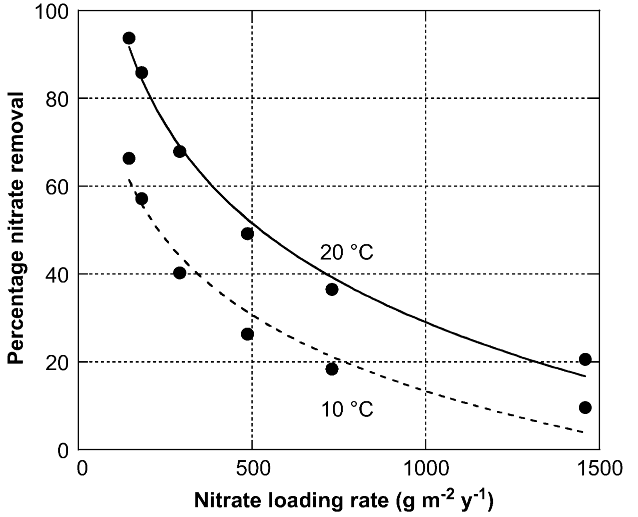

Figure 9: Theoretical relationship between wetland nitrate removal efficiency and loading at 10 and 20°C, based on k-C $*$ tanks-in-series kinetic model (IWA, 2000; Kadlec, 2004) with first order areal annual rate constant $K_{20} = 34$, temperature factor $\theta = 1.09$, hydraulic efficiency parameter $N = 3$, influent NO_3-N = 10 g m^{-3}, and areal hydraulic loading rate 40–400 mm day^{-1}.

in mid-western USA, reported NO_3-N removal rising as a power function from ~12–45 g m^{-2} yr^{-1} as loading increased from 20–200 g m^{-2} yr^{-1}. Using data from 65 SF wetlands, including all those noted above, Kadlec (2004) derived a mean first order areal removal rate constant (k) of 34 ± 3 m yr^{-1} for NO_3^- removal in surface-flow wetlands, and a mean Arrhenius temperature coefficient of 1.09. Theoretical wetland nitrate removal based on this relationship is summarised in Fig. 9. However the dataset, which included wetlands treating a wide range of NO_3^- concentrations and loadings, water types (wastewaters, stormwaters, agricultural drainage and river water) and flow regimes, showed a wide range of mean k values (<10 to >60 m yr^{-1} for wetlands not receiving carbon supplements). Stormwater flows are characteristically highly variable and pulsed. Further studies are required to better understand constructed wetland treatment responses to such fluctuations in annual, seasonal and day-to-day loads and to develop improved design and performance models.

18.5. Conclusions

- Constructed wetlands can provide effective, low-cost N removal from waste, storm and drainage-waters. N mass removal rates typically rise with increasing loading up to at least 6 g N m^{-2} day^{-1} (>2 kg N m^{-2} yr^{-1}) while efficiencies typically decrease from >80 to <20% removal.

- In SF wetlands, plants form the main physical structure in the water column, moderating water flow, stabilising sediments, shading and sheltering the water column, and providing surfaces for biofilm growth and organic substrates for denitrifying bacteria.
- In SSF wetlands, plants enhance TN removal rates predominantly through root-zone oxygen release and supply of organic substrates for denitrifying bacteria.
- Direct plant uptake of N is generally of secondary importance for N removal in constructed wetlands, except during initial establishment and prime growth seasons, or at very low N loadings.
- Microbial transformation to gaseous forms is generally the dominant N removal process, except at very low loadings or where elevated pH promotes ammonia volatilization. Estimated oxygen fluxes into SSF wetlands appear to be insufficient to support apparent rates of nitrification (and subsequent denitrification) seen in some studies of wetland wastewater treatment. Emerging information on alternative microbial pathways that operate under low oxygen conditions may help explain these discrepancies.

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