Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors

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\textbf{A R T I C L E   I N F O}

\textbf{Article history:}
Received 20 May 2011
Received in revised form 18 October 2011
Accepted 10 December 2011

\textbf{Keywords:}
Denitrification
Groundwater nitrate
Denitrification bioreactor

\textbf{A B S T R A C T}

Laboratory denitrifying bioreactors, which use an organic carbon (C) rich media to enhance microbial reduction of nitrate (NO\textsubscript{3}\textsuperscript{−}) to nitrogen (N) gases, are used worldwide to protect surface and groundwater. To highlight potential adverse effects of denitrifying bioreactors, NO\textsubscript{3}\textsuperscript{−} removal rates (g NO\textsubscript{3}\textsuperscript{−}-N m\textsuperscript{−2} d\textsuperscript{−1} removed), NO\textsubscript{3}\textsuperscript{−} removal efficiencies (% removed minus production of other N species) and release of greenhouse gases and solutes (ammonium (NH\textsubscript{4}\textsuperscript{+}), phosphorus (P) and organic carbon (C)) were compared in this study using different media: lodgepole pine woodchips (LPW), cardboard, lodgepole pine needles (LPN), barley straw (BBS) and a soil control. Results showed that NO\textsubscript{3}\textsuperscript{−} removals were consistently >99% for all media for initial leaching and steady-state periods. When pollution swapping was considered, this ranged from 67% for LPW to 95% for cardboard. Sustained P releases over the threshold for the occurrence of eutrophication were measured in all media. Greenhouse gas emissions were dominated by carbon dioxide (CO\textsubscript{2}) and methane (CH\textsubscript{4}) fluxes with little nitrous oxide (N\textsubscript{2}O) release due to the anaerobic conditions prevalent within the bioreactors. Comparisons of different media, under steady-state conditions, showed that C fluxes were highest for cardboard and BBS bioreactors. Carbon fluxes from cardboard bioreactors ranged from 11.6 g C m\textsuperscript{−2} d\textsuperscript{−1} to 13.9 g C m\textsuperscript{−2} d\textsuperscript{−1}, whilst BBS emissions ranged from 3.9 g C m\textsuperscript{−2} d\textsuperscript{−1} to 4.4 g C m\textsuperscript{−2} d\textsuperscript{−1}. These C emissions were correlated with the total surface area exposed within the media. Such investigations highlight the need to consider pollution swapping during the initial leaching period and should improve design criteria for field-scale bioreactors used to mitigate shallow groundwater NO\textsubscript{3}\textsuperscript{−}.

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1. Introduction

Excess reactive nitrogen (N) may occur in soil, aquatic and atmospheric environments (Stark and Richards, 2008). Legislative instruments such as the European Union (EU) Water Framework Directive (WFD; 2000/60/EC, \textit{Council of the European Union}, 2000) and basic programmes of measures such as the Nitrates Directive (91/676/EEC, \textit{Council of the European Union}, 1991) aim to reduce N losses to sensitive receptors by removing pollution sources and accounting for the connectivity between waterbodies. Even after the removal of the pollution source, flushing of nitrate (NO\textsubscript{3}\textsuperscript{−}) to deeper groundwater or towards a surface waterbody may take a long time (Fenton \textit{et al.}, 2011a). In Ireland, NO\textsubscript{3}\textsuperscript{−} varies spatially and temporarily in shallow groundwater (<30 m) due to variable denitrification potential of glaciated subsoils, recharge variation and soil physical characteristics (Fenton \textit{et al.}, 2011b). In such settings, supplementary measures may be required in low denitrification potential areas to remediate NO\textsubscript{3}\textsuperscript{−} already migrating along subsurface pathways.

In situ denitrification bioreactors are engineered structures, which intercept contaminated water (e.g. shallow groundwater, or outlets of natural or artificial drainage systems). Denitrification, or reduction of NO\textsubscript{3}\textsuperscript{−} to N\textsubscript{2} gas by microbial degradation of organic carbon (C), occurs naturally in soils and aquifers. Natural conditions, such as high dissolved oxygen (DO) concentrations, low organic C bioavailability or low transit times, can limit natural attenuation. Denitrifying bioreactors use a variety of C-rich reactive media (Table 1), creating ideal conditions for high rates of denitrification (Schipper \textit{et al.}, 2010).

Of the various media used, woodchip-based materials are the most popular (Schipper and Vojodic-Vukovic, 2001; Robertson and Merkley, 2009) due to their low cost and high C/N ratio (Gibert \textit{et al.}, 2008). In addition, they do not require replenishment as C is not rapidly depleted from them, although the duration of their effectiveness will be affected by the longevity of the C supply to the denitrifying microorganisms (Moorman \textit{et al.}, 2010). For
a comprehensive review of the performance of various materials used in denitrifying bioreactors, the reader is referred to Schipper et al. (2010). NO$_3^-$ removal rates – expressed in terms of reactor volume – from these systems range from 0.62 (Jaynes et al., 2008) to 203 g NO$_3^-$ N m$^{-3}$ d$^{-1}$ (Xu et al., 2009). They are affected by operation temperature (Cameron and Schipper, 2010), influent DO concentration (Robertson, 2010), hydraulic loading rate (HLR) (Xu et al., 2009), NO$_3^-$ loading rates, and C concentrations and bioavailability (Schipper et al., 2010).

In this paper, laboratory bioreactors were used to reproduce NO$_3^-$ bioremediation in shallow groundwater in heterogeneous glacial tills. Nutrients lost from agricultural systems originate from organic and inorganic fertilizer sources. In such subsols, NO$_3^-$ occurrence in shallow groundwater varies spatially and temporally, and has been correlated with saturated hydraulic conductivity ($k_s$) and denitrification parameters such as nitrogen gas (N$_2$)/argon (Ar) ratios (Fenton et al., 2011a). The $k_s$ of glacial tills can vary considerably e.g. sandy, silty tills in Scandinavia range from $5 \times 10^{-3}$ m s$^{-1}$ to $5 \times 10^{-4}$ m s$^{-1}$ (Lind and Lundin, 1990). The scenarios covered in this paper represented $k_s$ of moderate permeability tills ranging from $5 \times 10^{-3}$ m s$^{-1}$ to $5 \times 10^{-4}$ m s$^{-1}$ (Fenton et al., 2011b).

### 1.1. Potential adverse effects of denitrification bioreactors

In general terms, ‘pollution swapping’ may be defined as ‘the increase in one pollutant as a result of a measure introduced to reduce a different pollutant’ (Stevens and Quinton, 2009). Such a definition must include: (1) greenhouse gases (GHG) and ammonia (NH$_3$) (which may be lost vertically above a bioreactor, as well as down-gradient as de-gassing/diffusion occurs from a surface and/or subsurface waterbody) and (2) dissolved contaminants such as NH$_3$, phosphorus (P), dissolved organic carbon (DOC) and metals, which can adversely affect aquatic ecosystems (Fig. 1). In the present study, consideration of pollution swapping goes beyond N transformations.

In order to assess total pollution swapping and the associated risk in terms of GHG emissions and release of dissolved contaminants, the following parameters need to be quantified: (1) losses of dissolved and gaseous N species (2) leaching of non-nitrogen species from the soil and carbon media (e.g. DOC and P); and (3) production of gases (e.g. CH$_4$) or solutes resulting directly (e.g. manganese (Mn) or iron (Fe)) or indirectly (e.g. metals or P) from microbiologically mediated reactions occurring at low redox potential in bioreactors (Gibert et al., 2008). Researchers evaluate the performance of treatment systems, but infrequently include this factor (Gibert et al., 2008). High N inputs into bioreactors may result in gaseous N losses via either NH$_3$ volatilisation or nitrous oxide (N$_2$O) emission in the absence of complete denitrification to N$_2$. Whilst no previous studies have examined NH$_3$ emissions from bioreactors directly, there is ample evidence of NH$_3$ measurement from other sources (e.g. directly from slurry tanks and waste stabilization ponds) in the literature. The principle determinants of NH$_3$ volatilisation are: (1) an ammonium (NH$_4^+$) source (2) temperature (3) pH > 7 and (4) a concentration gradient between the source and the atmosphere (Ni, 1999). Other dissolved N species, such as NH$_4^+$, can be lost. In addition, microbial decomposition and/or anaerobic digestion of the organic C media has the potential to lead to both gaseous losses as carbon dioxide (CO$_2$) and methane (CH$_4$), as well as DOC losses, or other solutes.

Initial leaching of the media in denitrification bioreactors has been shown to favour the release of large concentrations of dissolved C, N or P (Gibert et al., 2008; Cameron and Schipper, 2010; Schipper et al., 2010). This initial period contrasts with steady-state conditions when pollution swapping due to leaching from the media is assumed to be negligible in comparison to the release of gases and solutes linked to microbial–mediated reactions. The characterisation of solute release in the initial leaching period allows for the establishment of design criteria to attenuate high pollution loads to receptors in the early stages of the experiment.

### Table 1

A selection of laboratory bioreactor studies.

<table>
<thead>
<tr>
<th>Media used</th>
<th>Influent concentration</th>
<th>Loading rate</th>
<th>NO$_3^-$ removal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>1.13 kg NO$_3^-$ N m$^{-3}$ d$^{-1}$</td>
<td>3.0 m h$^{-1}$</td>
<td>&gt;99%</td>
<td>Phillips and Love (2002)</td>
</tr>
<tr>
<td></td>
<td>2.52 kg NO$_3$ N m$^{-3}$ d$^{-1}$</td>
<td>3.0 m h$^{-1}$</td>
<td>&gt;99%</td>
<td>Bedessem et al. (2005)</td>
</tr>
<tr>
<td>Sawdust and native soil</td>
<td>n.a.</td>
<td>2.8 m h$^{-1}$</td>
<td>98%</td>
<td>Vrtovsek and Rol (2006)</td>
</tr>
<tr>
<td>Soil</td>
<td>67% (TN)</td>
<td>31% (TN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC plastic and powdered activated carbon (PAC)</td>
<td>45 mg NO$_3^-$ N L$^{-1}$</td>
<td>1.9 g NO$_3^-$ N m$^{-2}$ d$^{-1}$</td>
<td>&gt;90%</td>
<td>英文 text here</td>
</tr>
<tr>
<td>Woodchip and sand</td>
<td>200 mg L$^{-1}$</td>
<td>2.9 mg NO$_3$ N kg$^{-1}$ d$^{-1}$</td>
<td>97%</td>
<td>Healy; (2006)</td>
</tr>
<tr>
<td>Woodchips and wheat straw</td>
<td>200 mg NO$_3$ N L$^{-1}$</td>
<td>99%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Softwood and sand</td>
<td>50 mg NO$_3$ N dm$^{-3}$</td>
<td>0.3 cm$^3$ min$^{-1}$</td>
<td>&gt;96%</td>
<td>Gilbert et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>1.1 cm$^3$ min$^{-1}$</td>
<td>66%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodchip and soil</td>
<td>50 mg L$^{-1}$</td>
<td>2.9 cm d$^{-1}$</td>
<td>100%</td>
<td>Greenan et al. (2009)</td>
</tr>
<tr>
<td>Maize cobs</td>
<td>n.a.</td>
<td>11.6 cm d$^{-1}$</td>
<td>29%</td>
<td>Cameron and Schipper (2010)</td>
</tr>
<tr>
<td>Green waste</td>
<td>n.a.</td>
<td>15–19.8 g N m$^{-3}$ d$^{-1}$</td>
<td></td>
<td>Cameron and Schipper (2010)</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>n.a.</td>
<td>7.8–10.5 g N m$^{-3}$ d$^{-1}$</td>
<td></td>
<td>Cameron and Schipper (2010)</td>
</tr>
<tr>
<td>Softwood</td>
<td>n.a.</td>
<td>5.8–7.8 g N m$^{-3}$ d$^{-1}$</td>
<td></td>
<td>Cameron and Schipper (2010)</td>
</tr>
<tr>
<td>Hardwood</td>
<td>n.a.</td>
<td>3.0–4.9 g N m$^{-3}$ d$^{-1}$</td>
<td></td>
<td>Cameron and Schipper (2010)</td>
</tr>
<tr>
<td></td>
<td>n.a.</td>
<td>3.3–4.4 g N m$^{-3}$ d$^{-1}$</td>
<td></td>
<td>Cameron and Schipper (2010)</td>
</tr>
</tbody>
</table>

* Considers pollution swapping, n.a. not available.

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**Fig. 1.** Diagram of a laboratory scale bioreactor. "Not measured in current study."
The objectives of the current laboratory study were to: (1) determine the effectiveness of different media – lodgepole pine woodchips (LPW), cardboard, lodgepole pine needles (LPN), barley straw (BBS) and a soil control – in reducing water loaded at a HLR of 3 cm d⁻¹ from influent to leachate and quantify pollution swapping from the initial leaching of nutrients and subsequent losses through transformational processes and gaseous losses.

2. Methods

2.1. Construction of bioreactors

0.1 m-diameter x 1 m-deep acrylic columns, comprising a 0.015 m long ‘water tank’ (built using a fine metal mesh) at the base to allow uniform distribution of influent water into the column (Fig. 1), were constructed and operated in a temperature-controlled room at 10°C. 0.8 m-deep reactive media rested on top of the metal mesh. Influent water was applied at the base of each column at a HLR of 3 cm d⁻¹ using a peristaltic pump (operated continuously) and the water exited the column via a 0.01 m-diameter tube positioned just above the reactive media surface. This mode of operation was after Della Rocca et al. (2007), Salling et al. (2007), Moon et al. (2008) and Hunter and Shaner (2010), and prevented the occurrence of preferential flow paths that may occur if the system was loaded from the surface. Water sampling ports (rubber septum stoppers) were positioned at depths of 0.2, 0.4, 0.6 and 0.8 m along the side of the columns (Fig. 1). The C source-to-soil volume ratio was 1 and the C-rich media were placed in the bioreactors in alternating 0.03 m-deep layers with soil. All bioreactors were covered with black plastic to prevent photosynthesis. Prior to operation, each bioreactor was seeded with approximately 1 L of bulk fluid containing heterotrophic bacteria from a wastewater treatment plant and was then loaded with NO₃-N solution varying from 19.5 to 32.5 mg L⁻¹. The DO in the influent water was kept low (<2 mg L⁻¹) by bubbling Ar gas through the water daily. This was to replicate DO conditions in shallow groundwater.

2.2. Analysis of water, media and gases

Water samples from the inlet, outlet and at the 3 sampling ports (Fig. 1) along each column were tested in accordance with the standard methods (APHA, 1995) for the following parameters: pH, DO, chemical oxygen demand (COD), NH₄⁺-N, NO₃⁻-N, nitrite-N (NO₂⁻-N) and orthophosphorus (PO₄³⁻-P). The C, N and P of each media (including soil) are presented in Table 2. The C and N content were determined using a thermal conductivity detector, following combustion and separation in a chromatographic column, and the P content of the media was determined by inductively coupled plasma emission spectroscopy (ICP-ES) after aqua regia digestion. The soil used in the bioreactors were air dried at 40°C for 72 h, crushed to pass a 2 mm sieve and analysed for Morgan’s P (the national test used for the determination of plant available P in Ireland) using Morgan’s extracting solution (Morgan, 1941).

The emission of GHG, comprising CO₂, CH₄ and N₂O, were measured from each column at specific times over their operation period using the static chamber technique (Hutchinson and Mosier, 1981). The headspace above each column was flushed with Ar gas for 5 min at a flow rate of 3 L min⁻¹. The headspace chamber was then sealed and connected in series to an INNOVA 1412 photoacoustic gas analyser (Lumasense Inc., Copenhagen, Denmark) for 12 min with measurements performed at a rate of one per min. In addition, gas samples were withdrawn at 0, 15 and 30 min, and samples were analysed using a gas chromatograph (GC) (Vario GC 450; The Netherlands) and automatic sampler (Combi-PAL
2.3. Nitrate removal

In this study, NO$_3^-$ removal rates considering bed (NR$_{BV}$) or media volume (Eq. (1), Schipper and Vojodic-Vukovic, 2000) and effective porosity (NR$_{EP}$) or fluid volume (Eq. (2), Schipper et al., 2010) were calculated using:

$$NR_{BV} = \frac{q \times A \times \Delta [NO_3^- - N]}{\text{media volume}} \left( g \, N \, m^{-3} \, d^{-1}, \text{units of media volume} \right)$$ (1)

$$NR_{EP} = \frac{NR_{BV}}{n_e} \left( g \, N \, m^{-3} \, d^{-1}, \text{units of fluid volume} \right)$$ (2)

where $q$ is Darcy velocity (m d$^{-1}$), $A$ is cross-sectional area of the bioreactors (m$^2$) and $n_e$ is effective porosity. Effective porosity was calculated using hydraulic retention time (HRT), the length of the bioreactor and Darcian velocity. A conservative tracer (NaBr, 10 g L$^{-1}$) was used to estimate the average HRT using methods detailed in Levenspiel (1999). The tracer was applied as a pulse in one constant hydraulic loading interval to each bioreactor using a peristaltic pump. A fraction collector (REDIFRAC, Amersham Pharmacia Biotech, Bucks, UK), positioned at the outlet of each bioreactor, collected effluent samples in timed increments. The sample volumes were subsequently measured and tested for bromide (Br$^-$) concentration using a Konelab 20 Analyser (Konelab Ltd., Finland). Bioreactor HRTs and $n_e$ are presented in Table 2.

NO$_3^-$ removal efficiencies of the reactive media were defined as the % of NO$_3^-$ converted to di-nitrogen (N$_2$) gas in the column by accounting for the HRT of each bioreactor. Any measured concentrations of intermediary products of denitrification, such as N$_2$O or NO$_2^-$, as well as other N species produced by other NO$_3^-$ reduction processes (e.g. dissimilatory NO$_3^-$ reduction to ammonia (DNRA) leading to NH$_4^+$) or leaching of the media, were subtracted from the measured NO$_3^-$-N concentrations at the effluent port. Instead of estimating average removal using the total running period of the bioreactors, the bioreactor data were separated into leaching and steady-state periods. Such periods were defined as occurring when COD in the effluent reached equilibrium. NO$_3^-$ concentrations were also taken into account for the soil control (e.g. soil N and release of NO$_3^-$ from the soil due to mineralisation). For equivalent initial leaching and steady-state periods, the NO$_3^-$ removal ($\%$) was also calculated not including pollution swapping.

3. Results

3.1. Column media and influent/effluent parameters

Initial leaching and steady-state period boundaries are presented in Table 2. Discrimination between the initial leaching and steady-state periods was possible for all media except BBS, as these bioreactors did not reach steady-state as defined by effluent COD concentration. Soil had a much earlier boundary between initial leaching and steady-state periods (26, 29 d), followed by LPN (168 d), whilst LPW (300, 288 d) and cardboard (327, 305 d) were similar. Temporal variation of NO$_3^-$-N concentration in influent and effluent solutions is presented in Fig. 2. For all media, effluent NO$_3^-$-N concentrations were much lower than influent concentrations. Maximum NO$_3^-$-N concentration in effluent water occurred in the early stage of the experiment for LPN (4.5 mg L$^{-1}$) and BBS (0.9 mg L$^{-1}$). In contrast, high concentrations occurred later in the experiment for LPW (1.8 mg L$^{-1}$) and for cardboard (0.2 mg L$^{-1}$). Soil effluent NO$_3^-$-N concentrations were generally much higher than in the other media (maximum of 82.2 mg L$^{-1}$ at the beginning of the experiment), and after 107 d operation, influent and effluent NO$_3^-$-N concentrations were similar. 

Barley (14.0–21.8 d) and LPW (13.0–17.5 d) had the highest HRT (meaning the $n_e$ was therefore highest), whilst cardboard (8.5–11.0 d) and LPN (9.9–11.7 d) had the shortest ($n_e$ therefore lowest) (Table 2). Soil HRT was between these two ranges (11.8–15.5 d). Carbon content of media was similar and ranged from 41.6% for cardboard and 51.2% for LPN. N content was variable and ranged from 0.1% for LPW to 1.1% for LPN. For soil, both C and N contents were 0.1% and under detection limits, respectively.

NO$_3^-$ removals and efficiencies (taking average NO$_3^-$-N and NH$_4^+$-N effluent concentrations into account) for the assigned periods are denoted in Table 2. In the soil only bioreactors, NR$_{BV}$ ranged from 0.00 to 0.28 g NO$_3^-$-N m$^{-3}$ d$^{-1}$ in the initial and steady-state periods, respectively (Fig. 3a). In all other media, this varied from 0.81 (LPW, initial leaching period) to 1.06 g NO$_3^-$-N m$^{-3}$ d$^{-1}$ (cardboard, steady-state period). NO$_3^-$ removals per unit of media volume were smaller in the initial period for all media except for LPN (Fig. 3a). No correlation with HRT was observed.

The NO$_3^-$ removal rate (NR$_{BV}$) (Table 2), measured in all bioreactors (except the soil control), ranged between approximately 1.12 and 2.67 g NO$_3^-$-N m$^{-3}$ d$^{-1}$ in the BBS and LPN bioreactors during the initial leaching period and 1.51 and 3.03 g NO$_3^-$-N m$^{-3}$ d$^{-1}$ in the LPW and cardboard bioreactors during the steady-state period. From Eq. (2) (as $n_e$ is calculated from HRT), decreasing NR$_{BV}$ is expected for increasing HRT between bioreactors for the same media (Fig. 3b).

NO$_3^-$ removal (%) in all media showed comparable values of >99.72% (Fig. 3c). When pollution swapping was considered, NO$_3^-$ removal efficiency (Fig. 3d) in the initial period ranged from 66.83% (LPN, column 2) to 86.78% (LPN, column 1). In the steady-state period, removal efficiencies varied from 86.61% (LPW, column 3) to 95.38% (LPN, column 1). Pollution swapping decreased between initial leaching and steady-state periods for LPW, cardboard and LPN. For both initial leaching and steady-state periods, cardboard and LPN NO$_3^-$ removal efficiencies tended to increase when HRT significantly decreased. For LPW, NO$_3^-$ removal efficiency in the initial leaching and steady-state periods tended to increase with increasing HRT. For all other media, the highest NO$_3^-$ removal efficiencies were often observed at the lowest HRT. The soil media exhibited smaller NO$_3^-$ removal efficiencies in both periods, with increasing removal for shorter HRTs.

The range of pH in the effluent water was similar for soil, barley and LPW treatments (from 7.3 to 8.5). Cardboard and LPN media had generally lower pH in the initial period (as low as 5.1 for LPN and 6.6 for cardboard) and higher pH in the steady-state period (up to 7.2 for LPN and 8.6 for cardboard). General COD and NH$_4^+$-N patterns within the initial leaching and steady-state periods were comparable for LPW and cardboard media, whilst other patterns were more variable (Fig. 4a and b). In the initial leaching phase, COD concentrations were higher in the LPN and BBS media (Fig. 4c and d), and smaller in the soil media (Fig. 4e). LPN had the highest initial leaching and steady-state COD concentration (over 10,000 mg L$^{-1}$ and 1000 mg L$^{-1}$, respectively). This media also had the strongest red pigmentation in the effluent, which was indicative of COD release (results not illustrated). The range of NH$_4^+$-N concentrations was similar for all media, except for a minority of samples.
which displayed high concentrations in the LPN (Fig. 4c) and BBS (Fig. 4d) media. Such columns had the highest HRTs. In both periods, except for the soil media, NH$_4^+$-N and COD concentrations did not show correlated trends. Ortho-phosphate concentrations in the initial leaching period were generally greater than in the steady-state period (max concentration up to 1.1 mg L$^{-1}$ for LPW). These differences were more significant for both LPW and the cardboard media (Fig. 5a and b) than for the LPN media (Fig. 5c). The BBS media (Fig. 5d) displayed similar PO$_4$-P concentrations to the LPN media. In the steady-state period, LPN displayed the lowest PO$_4$-P concentrations. In the soil media (Fig. 5e), PO$_4$-P concentrations were similar to LPN. Generally, variations of concentrations between media were higher than between columns of the same media. Phosphorus concentrations in the different media ranged from 41.9 mg kg$^{-1}$ for LPW to 832.0 mg kg$^{-1}$ for LPN (Table 2). The soil used in the columns had a low soil test phosphorus (STP) concentration of 4.95 ± 1.75 mg PL$^{-1}$, expressed as Morgan’s plant available P.

3.2. Longitudinal patterns of NO$_3$-N and NH$_4$-N in steady state period

NO$_3^-$ reduction to near or below detection limits was observed for LPW, cardboard and LPN bioreactors at a maximum distance of 0.4 m from the inlet (Fig. 6a–i). For all media, no significant differences in NO$_3^-$ removal patterns were observed. The bioreactors showing the smaller HRT (Fig. 6b and e) generally showed higher NO$_3^-$-N concentrations at a distance of 0.2 m from the inlet than in all other bioreactors. NH$_4^+$-N generally increased in concentration along the column. The LPW bioreactor displayed smaller concentrations of NH$_4^+$-N at the shortest HRT (Fig. 6a–c), as well as significant increases and decreases in concentration between adjacent ports. In contrast, NH$_4^+$-N patterns in the cardboard
bioreactors were more similar between bioreactors, except for
the third sampling interval (interval C, Fig. 6d–f) where NH₄⁺−N
remained constant or decreased along the bioreactors.

3.3. Greenhouse gas column emissions

Greenhouse gas emissions were dominated by CO₂ and CH₄
fluxes with little N₂O release. Nitrous oxide emissions were
extremely low, with the highest values observed during the ini-
tial loading phase for LPN bioreactors (Table 2). Once steady-state
was achieved, the values for all amendments were lower than
0.6 mg N₂O-N m⁻² d⁻¹, with the exception of LPW 1 and 2, where
N₂O emissions were 1.45 mg N₂O-N m⁻² d⁻¹ and 2.15 mg N₂O-
N m⁻² d⁻¹, respectively (Table 1). Emissions varied both in terms
of total C lost and the proportions of CO₂ and CH₄ comprising the
total emissions (Fig. 7). During the initial phase, there was a large
increase in CO₂ efflux from the LPN and BBS bioreactors relative
to the soil control, with these emissions comprising the entire C lost
from the system (Fig. 7). Initial phase CO₂ fluxes were 12.5 g CO₂-
C m⁻² d⁻¹ for LPN and 5.7 g CO₂-C m⁻² d⁻¹ for BBS, compared to the
baseline flux of 0.43 g CO₂-C m⁻² d⁻¹. Once steady-state conditions
were achieved, CO₂ fluxes decreased substantially to 1.2 g CO₂-
C m⁻² d⁻¹ for LPN (Fig. 7). Barley did not reach steady-state during
the experiment. However, the total C flux from LPN was not signifi-
cantly higher than the soil control at steady-state.

In contrast, carbon fluxes from the cardboard bioreactors were
11.6 g C m⁻² d⁻¹ and 13.9 g C m⁻² d⁻¹ for sampling times 1 and 2,
respectively. Whilst there were no significant differences of total
C loss between the two sampling dates for cardboard, there was a
trend towards increasing CH₄ over time. The proportion of CH₄
comprising the total C flux ranged from 31 to 47% for the card-
board bioreactors. The C flux from the LPW bioreactors, whilst
significantly higher than both LPN and the soil control, was much
lower (1.8 g C m⁻² d⁻¹) than the above C-amendments, with CO₂
comprising over 80% of total C flux.

These C emissions were correlated with the total surface area
exposed within the media (Fig. 8, R² = 0.637). Both the BBS and
cardboard had similar average surface areas of media at 4.7 m²
per bioreactor. As a result, there was a greater area of C substrate
available for microbial degradation.

When all GHG emissions were expressed in terms of global
warming potentials and cumulated to annual fluxes (i.e. CO₂-
equivalents per unit area), there was a similar trend in that the
highest emissions were recorded for the cardboard-amended
bioreactors, followed by BBS and then LPW (Fig. 9). Total GHG
emissions were dominated by CH₄, which comprised 91%, 86% and
54% of BBS, cardboard and LPW emissions, respectively. By
contrast, N₂O emissions were highest for the soil control (0.8 t CO₂-
equiv. ha⁻¹ yr⁻¹) and zero for the LPW.

4. Discussion

The HLR on the columns represented a Darcy flux (q) of
3.47 x 10⁻⁷ m s⁻¹. For a typical groundwater hydraulic gradient
of 1%, such a Darcy flux implies a kₜ value of 3.47 x 10⁻⁵ m s⁻¹.
Effective porosity in glacial tills can vary from 2.5 to 40%. These
values are generally smaller than those observed in the present study (Table 2). Nevertheless, present values imply groundwater velocities typical of a high permeability zone in glaciated tills where low denitrification potential is expected. Higher denitrification potential zones, or hotspots, occur in areas of lower $k_s$ and natural attenuation is likely to protect waterbodies in such zones. In situ bioreactors in glaciated tills should nevertheless be installed in more permeable zones where NO$_3^-$ fluxes are higher and denitrification potentials are lower.

Dissolved oxygen concentrations in the columns were below the threshold value for denitrification in groundwater of <2 mg L$^{-1}$ as presented by Rivett et al. (2008). However, some studies show that concentrations of up to 4 mg L$^{-1}$ can facilitate denitrification (Rivett et al., 2008). Circumneutral pH was observed in this study and present favourable conditions for denitrification. The temperature inside the bioreactors was kept constant at 10 °C, which is close to the mean annual groundwater temperature of 11.6 °C measured at the field site from which the soil was extracted. Influent NO$_3^-$ concentrations used in the study are at least twice that of the EU maximum admissible concentration for groundwater bodies (11.3 NO$_3^-$-N mg L$^{-1}$).

4.1. Bioreactor media and influent/effluent parameters

As mentioned by Schipper et al. (2010), mitigation measures are needed (e.g. pre-washing of organic carbon media) to limit initial leaching of COD, NH$_4^+$-N and P to groundwater. In the current study, such leaching occurred for a significant time due to very high residence times in the bioreactors, which reflect glaciated subsoil permeability.

For all carbon media, Fig. 2 illustrates almost complete NO$_3^-$ removal. For the present study, low residence times, high C availabilities and low DO concentrations were responsible for such removals. Hydraulic residence times appear to be a key control on NO$_3^-$ removal e.g. using softwood in 0.9 m-deep columns, Gibert et al. (2008) found >96% NO$_3^-$ removal (removal from
48 mg NO$_3^{-}$-N L$^{-1}$ to <2 mg NO$_3^{-}$-N L$^{-1}$) for a HRT of 6.6 d. In the same experiment, a shorter HRT of 1.7 d achieved 66% NO$_3^{-}$ removal. In a laboratory-scale bioreactor filled with woodchip (6.1 m length) and with influent NO$_3^{-}$ concentrations of 25.7 mg NO$_3^{-}$-N L$^{-1}$, Chun et al. (2009) observed complete NO$_3^{-}$ reduction with a much shorter HRT of 19.2 h. Contrastingly, NO$_3^{-}$ removal dropped to a minimum of 10% for a HRT of approximately 2 h.

At a maximum NREP of 3.03 g NO$_3^{-}$-N m$^{-3}$ d$^{-1}$ calculated for the cardboard bioreactors, the NO$_3^{-}$ removal rates measured in this study were comparable to other studies (Xu et al., 2009 and others). Greenan et al. (2009) found that when the HLR on a laboratory denitrifying bioreactor filled with a mixture of woodchip and soil was increased from 2.9 to 13.6 cm d$^{-1}$, the NO$_3^{-}$-N removal rates increased from 11 to 15 mg NO$_3^{-}$-N kg$^{-1}$ wood d$^{-1}$. Gibert et al. (2008) found similar results. In the current study, complete denitrification was observed at a distance of 0.4 m from the base of the reactors (Fig. 6). In this study, for all treatments almost full NO$_3^{-}$ removal was achieved (Fig. 3c). Therefore, differences in NREP between bioreactors or media, as outlined in Eq. (2), relate mostly to differences in $n_e$ (and therefore HRT) rather than actual differences in NO$_3^{-}$ removal (Fig. 3b). In the present study, NREP incorporates the total length of the column in calculations rather than merely sections where denitrification is maximal. Therefore, the calculated NREP are likely to underestimate the actual NO$_3^{-}$ removal in first sections of the column (where highest denitrification occurs).

In contrast, NR$_{BV}$ were very similar between bioreactors and across treatments. Slightly lower values in the initial leaching period were probably due to the high initial release of NO$_3^{-}$-N from the soil (Fig. 2e). An increase in HLR would likely result in
a decrease in NO$_3^-$ reduction (i.e. an increase in the length of column needed to achieve nearly complete denitrification). As a consequence, NR$_{WW}$ would increase with increasing HLR until a threshold could be reached wherein a decrease in NO$_3^-$ reduction would not compensate for an increase in HLR. Schipper et al. (2010) recommended the use of NR$_{WW}$ instead of NR$_{WP}$ to allow a direct comparison of removal rates across bioreactor studies. Such an approach, as confirmed by the present study, enables one to investigate the efficacy of different media to remove NO$_3^-$ at laboratory-scale. Some authors have reported that denitrification can follow zero order (Robertson and Cherry, 1995; Greenan et al., 2006; Gibert et al., 2008) or first order Monod kinetics (Chun et al., 2009). In this study, it is likely that denitrification followed different kinetics before and after the 0.2 or 0.4 m sampling ports. Even if the HRTs reported in this study are longer than those from the literature (mentioned earlier), they appear to have a significant impact on denitrification transformational processes along the columns: typically, higher HRT leads to complete denitrification at shorter distances from the influent port of the columns (Fig. 6).

Even if these low residence times allow for nearly complete NO$_3^-$ removal, they are also responsible for the production of unwanted solutes and gases. This is illustrated in Fig. 3c and d when comparing NO$_3^-$ removal and NO$_3^-$ removal efficiency (accounting for NH$_4^+$-N production). In the current study, high C release and low NO$_3^-$-N concentrations were observed in the two first ports of the bioreactors (Fig. 6). These conditions and the subsequent increase of NH$_4^+$ along the bioreactors suggest that DNRA may have occurred. In this process, under anaerobic conditions, NO$_3^-$ is reduced to NH$_4^+$ according to:

\[
2\text{CH}_2\text{O} + \text{NO}_3^- + 2\text{H}^+ \rightarrow \text{NH}_4^+ + 2\text{CO}_2 + \text{H}_2\text{O}
\] (3)
Dissimilatory NO$_3^-$ reduction to NH$_4^+$ is a counter-productive process that has been identified as a potential fate of NO$_3^-$ by Gibert et al. (2008) and Greenan et al. (2009), and results in sustainable NH$_4^+$ increases in the effluent rather than removal of N as N$_2$ gas. In a 135-d batch study examining the denitrification potential of various organic substrates, Gibert et al. (2008) found that DNRA contributed up to 9% of the NO$_3^-$ removal in some substrates, but varied with media used. Greenan et al. (2009) also found similar results, and speculated that NH$_4^+$-N release was independent of the NO$_3^-$ loading rate and may have been related to the media within the bioreactors. Wildman (2002) showed in woodchip and woodchip/gravel bioreactors that NO$_3^-$ removal improved from low (3–11%) to high (95%) in the first few months and subsequent operation periods. Accounting for NH$_4^+$-N production is particularly valid for the initial leaching period, where NO$_3^-$ removal efficiencies (Fig. 3d) are significantly lower than NO$_3^-$ removal (Fig. 3c). This approach also enables us to differentiate the performances of different media or differences in HRT between columns with regard to pollution swapping. Typically, a higher HRT will favour higher NH$_4^+$-N production, as observed for several media in this study. This implies that a HRT that maximises NO$_3^-$ reduction and minimises NH$_4^+$-N release is critical design criterion for bioreactors.

Under field conditions, variations of water temperature, influent NO$_3^-$-N or DO concentrations may further complicate such criteria. On the site where the soil of this study was extracted, shallow groundwater NO$_3^-$-N and NH$_4^+$-N concentrations were less than 16.9 and less than 2.8 mg L$^{-1}$, respectively. On this site, a point-source from an up-gradient dairy soil water irrigation system was identified (Fenton et al., 2009). If a bioreactor was installed in this glacial setting, it would result in a great increase in denitrification potential and in the generation of additional NH$_4^+$. Depending on the proximity of surface water bodies and on the adsorption capacity of the intermittent aquifer, this could result in increasing risk to aquatic ecosystems.

The release of organic C, as expressed in this study by COD concentrations, strongly decreased in the steady-state period to reach values comparable to those of the soil media, except for the LPN media. This is possibly due to the less resistant structure of this media. Similar COD release for all other media and soil, but with different denitrification rates in the steady-state period, implies that a greater proportion of bioavailable carbon was released from the carbon media. Long-term studies have showed that woodchip can sustain NO$_3^-$ removals over long periods (Robertson et al., 2000; Moorman et al., 2010; Long et al., 2011). Very little data exist for the bioavailability of carbon across media types.

Besides N and organic C pollution swapping, other losses involve P in the effluent. Degradation and anaerobic conditions triggered the release of P from the reactors (Fig. 5). As P movement through soil is a function of soil type and structure, sediment and water temperature, number of flow paths, soil P and organic matter content (Sallade and Sims, 1997; Algoazany et al., 2007), special attention needs to be paid to the positioning of denitrifying bioreactors. Lodgepole pine needles and BBS phosphorus release were similar to the soil control, indicating no further inputs from the media. For LPW and cardboard, P losses were higher, indicating losses from both the soil and the media. The soil used in the columns had a low STP concentration of 4.95 ± 1.75 mg P L$^{-1}$, expressed as Morgan’s plant available P, which minimised losses of nutrients to the environment. Such soils may achieve sufficient dry matter yields, but the herbage P concentration would not meet the dietary requirements of grazing animals. To minimise P losses in the initial leaching period, a soil with not only the required hydraulic conductivity but also a very low STP of 0–3 mg L$^{-1}$, should be chosen. Such soils are found in areas that have been out of production for some time and therefore ideal for excavation and transport to the bioreactor site. Schulte et al. (2010) showed that it may take many years for elevated STP concentrations to be reduced to optimum agronomic levels to reduce risk to water quality. Therefore, sustained P release in shallow groundwater could be expected where high P index soils are used.
4.2. Greenhouse gas column emissions

Anthropogenic GHG emissions are dominated by CO₂ release, which comprises 72% of global emissions and arise primarily from fossil fuel burning and land-use change (Hofmann et al., 2006). The predominant non-CO₂ GHGs are CH₄ and N₂O, which comprise 18% and 5% of global emissions, respectively. Both arise primarily from the agriculture, land-use and waste sectors, and also contribute to stratospheric ozone depletion (IPCC, 2007). In addition, atmospheric deposition of volatilised NH₃ can indirectly contribute to both increased eutrophication and N₂O emissions (Asman et al., 1998). Increases in these gaseous losses may offset some or all of the remediation benefit of particular abatement techniques. For example, in a study on constructed wetlands whilst there was a decreased eutrophication, gaseous losses of N₂O and CH₄ increased by 72 t CO₂-equiv. ha⁻¹ (Alford et al., 1997).

Ideally, a denitrifying bioreactor should force an endpoint to the N cascade (Galloway et al., 2002) by denitrifying all N₂O⁻ back to N₂ without N₂O production. Generally, N₂O emissions are lowest in a fully saturated bioreactor. In this experiment, N₂O emissions were indeed very low, ranging from 0.11 to 2.15 mg N₂O-N m⁻² d⁻¹ once steady-state conditions had been achieved. Similar results were found by Woli et al. (2010), who measured N₂O emissions of 0.24–3.12 mg Nm⁻² d⁻¹ from a bioreactor bed. In a laboratory column experiment, Greenan et al. (2009) found that N₂O emissions accounted for 0.003–0.028% of the N₂O⁻ denitrified. In a nine-year study, Moorman et al. (2010) investigated the denitrification potential of woodchip bioreactors and found that there was no significant difference in N₂O emissions between a control (soil only) and the bioreactor. Moorman et al. (2010) found that N₂O losses exported in drainage water exiting the bioreactor accounted for 0.0062 kg N₂O-N kg⁻¹ N₂O-N, or 0.62% of N₂O⁻ removed. Emissions from a large denitrification bed were on average 78.58 µg m⁻² min⁻¹ N₂O-N (reflecting 1% of the removed N₂O⁻), 0.238 µg m⁻² min⁻¹ CH₄ and 12.6 µg m⁻² min⁻¹ CO₂. Dissolved N₂O-N increased along the length of the bed. The bed released on average 362 g dissolved N₂O-N per day and, coupled with N₂O emission at the surface, about 4.3% of the removed N₂O⁻ as N₂O. Dissolved CH₄ concentrations showed no trends along the length of the bed, ranging from 5.28 µg L⁻¹ to 34.24 µg L⁻¹ (Warneke et al., 2011).

Whilst N₂O emissions are low, NH₃ may represent a major loss pathway for reactive N in bioreactors. Within the columns, pH conditions were generally alkaline, apart from the LPN media in the initial leaching period. Typically, such conditions will favour NH₃ volatilisation with a correlated decrease in NH₄⁺ in solution. Within waste stabilization ponds, high rates of ammonia removal (99%), principally attributed to volatilisation, have been shown to result from high pH and high (>22 °C) water temperatures (Leite et al., 2011).

Methane and CO₂ efflux, resulting from acetate fermentation, also occurs from denitrifying bioreactors, but will reduce as C leaches from the system and the reactive media decays (Jaynes et al., 2008). This rate of reduction will depend on the media, temperature and HRT. Elgood et al. (2010) measured N₂O and CH₄ emissions of 2.4 mg N m⁻² d⁻¹ and 297 mg C m⁻² d⁻¹, respectively, from a stream bed denitrifying bioreactor containing woodchips. A closer look at these average figures presents a very high CO₂ equivalent of CH₄ (30.6 t CO₂ ha⁻¹ yr⁻¹). Comparable CH₄ emissions for the LPW bioreactors were observed in this experiment. However, these emissions were dwarfed by those from the BBS and cardboard bioreactors. Indeed, the rate of CH₄ release from these treatments was more comparable to those from landfills, where CH₄ emissions can range from 9 to 1800 g C m⁻² d⁻¹ (Borjesson and Svensson, 1997; Chanton and Liptay, 2000). The high initial levels of CO₂ and subsequent shift to CH₄ production under steady-state conditions were most likely due to a shift from aerobic respiration as the bioreactors saturated to acetate fermentation which produces both CH₄ and CO₂. Subsequent reduction of the produced CO₂ can generate more CH₄ (Bogner et al., 1997). Therefore, C loss, particularly CH₄ emissions – and not N₂O – from these denitrifying bioreactors appear to be most pressing issue in terms of pollution swapping. This may be partially ameliorated by the soil capping with soils of low STP of denitrifying bioreactors, as similar capping of landfill systems can oxidise up to one-third of the generated methane (Stern et al., 2007). Another way to limit methane production is to optimise the residence time within the bioreactor to ensure that N₂O⁻ is only just removed as it exits the bioreactor. This ensures that most of the bioreactor is not methanogenic.

5. Conclusions

1. In the initial leaching period, highest NO₃⁻ removal efficiencies were recorded for cardboard (~94%), followed by LPN (~75%), BBS (~74%) and LPW (~70%). Effluent COD release was one order of magnitude higher during this period for LPN. PO₄-P was highest for LPW, followed by cardboard, LPN and BBS.

2. In the steady-state period, the NO₃⁻ removal efficiency order did not change, but the efficiency increased. Effluent COD release in this period remained highest for LPN. PO₄-P for all media decreased, but remained above environmental thresholds.

3. Highest GHG emissions (CO₂-equivalents per unit area) were recorded for cardboard, followed by BBS, LPW and LPN. Greenhouse gas emissions were dominated by CH₄ and N₂O emissions were highest for the soil control.

4. Recognising the transitional risk of solute and gaseous losses between initial leaching and steady-state periods is important for the future design of denitrifying bioreactors. For all media, NO₃⁻ removal efficiencies (with pollution swapping) improved from the initial leaching to the steady-state periods.

5. NO₃⁻ removal efficiency (with pollution swapping) and rate (without pollution swapping) should be used to select a carbon media, which maximises denitrification and minimises adverse environmental consequences.

Acknowledgements

This research was funded by the Department of Agriculture Fisheries and Food under the Research Stimulus Fund (RSF 07 525). The authors would also like to thank Cathal Somers, Dr. Karl Richards & M.M.R. Jahangir.

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