1. Introduction

Nitrate (NO\textsubscript{3}−) contamination of groundwater has become an environmental and public health issue worldwide (Fazal et al., 2003; Rivett et al., 2008). It generally originates from anthropogenic sources, mainly from intensive application of fertilizers and animal manure, from landfill leachates and septic tanks leakages (Della Rocca et al., 2007; Stuart and Lapworth, 2016). NO\textsubscript{3}− easily percolates into groundwater, through which it can be discharged into surface waterbodies (streams, rivers, lakes) adversely affecting ecosystems (e.g. causing eutrophication) (Addy et al., 2016; Rivett et al., 2008). NO\textsubscript{3}− concentration exceeding the nominal limit of 50 mg/L for drinking water set by the World Health Organization have been recorded in numerous aquifers worldwide (Rivett et al., 2008; Huang et al., 2015). Excessive NO\textsubscript{3}− ingestion from polluted drinking waters can induce methemoglobinemia, hypertension, cancers and a number of currently inconclusive health repercussions (Della Rocca et al., 2007).

Remediation of groundwater contaminated by NO\textsubscript{3}− is a matter of active research. Among the available remediation approaches, biological denitrification appears to be the most effective and desirable one (Della Rocca et al.,...
Denitrification is defined as the dissimilatory biological reduction of $\text{NO}_3^-$ to nitrogen gas ($\text{N}_2$) through a multistep process, in which $\text{NO}_3^-$ is sequentially converted into nitrite ($\text{NO}_2^-$), nitric oxide ($\text{NO}$), nitrous oxide ($\text{N}_2\text{O}$) and, finally, harmless nitrogen gas ($\text{N}_2$) as shown in the following equation:

$$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$$  \hspace{1cm} (1)

Microbial nitrate reduction is accomplished by the oxidation under anaerobic conditions of either an organic compound (by heterotrophic bacteria) or an inorganic compound (by autotrophic bacteria). Heterotrophic denitrification, on which most of the denitrification-based treatments rely, can be described by the following overall reaction:

$$5\text{CH}_2\text{O} + 4\text{NO}_3^- \rightarrow 2 \text{N}_2 + 5\text{HCO}_3^- + 2\text{H}_2\text{O} + \text{H}^+$$  \hspace{1cm} (2)

where $\text{CH}_2\text{O}$ represents a generic organic compound. In subsurface environments, the lack of readily available organic carbon has often been reported as the most common hindrance to denitrification (Jahangir et al., 2012). This limitation explains why, until recently, the use of denitrifying bacteria for groundwater treatment has traditionally been reserved to ex-situ treatments in bioreactors. However, latterly attention has focused on the supply of an external organic substrate in the aquifer itself (in-situ treatment).

One option of applying an organic substrate into the aquifer is through a permeable reactive barrier (PRB). A PRB consists in placing a reactive material across the flow path of contaminated groundwater so that the contaminants can be transformed as groundwater flows through the reactive material (Scherer et al., 2000; Kalin, 2004). The selection of the reactive material is crucial: it must be chemically effective to eliminate the target contaminant(s) and must maintain an adequate permeability to ensure flow through the PRB. In a heterotrophic denitrification PRB, the reactive material consists of an organic substrate to stimulate denitrifying activity. PRBs are designed to be passive treatment systems in the sense that, once installed, groundwater typically flows through the reactive zone under its natural hydraulic gradient, and thereby energy input is not needed.

Most of the PRBs currently installed worldwide are based on abiotic processes, and only a small number on biotic processes, mostly for the bioremediation of groundwater contaminated by acid mine drainage (Benner et al., 2002; Gibert et al., 2013), hydrocarbons (McGovern et al., 2002; Gibert et al., 2007) and, as it is the case of the present study, nitrate (Robertson et al., 2008). With regard to the latter case, there is considerable laboratory-based research on promoting denitrification by the addition of organic substrates (Della Rocca et al., 2007; Su and Puls, 2006; Huang et al., 2015; Li et al., 2017). Nevertheless, most of these studies have been performed with configurations (e.g. batch, column and tank tests) and under controlled operational conditions (e.g. constant water composition, constant flow rate, constant temperature, homogeneous reactive materials, biostimulation of denitrifying bacteria) that differ from those in field-scale PRBs, where site weather, geochemistry and hydrology can all fluctuate.

Hence, while lab-studies undoubtedly provide useful information and contribute to a better understanding of denitrification in carbon-amended porous media, their results cannot automatically nor reliably be extrapolated to field-scale PRBs. As highlighted by other researchers (Addy et al., 2016), further research is required, as it is only through accumulated experience at field-scale that such a technology can be successfully applied and tailored to site-specific conditions.

The aim of this study was to evaluate the ultimate performance of a denitrification PRB, the first of its kind in Europe, over a period of 11 months. An aspect that made this PRB unique is that it was equipped with an injection system that allowed controlled concentrations of $\text{NO}_3^-$ to be injected into the aquifer directly upgradient of the PRB, providing an opportunity to evaluate the $\text{NO}_3^-$ removal efficiency of the PRB under increasing $\text{NO}_3^-$ loadings. From a broader perspective, and given the scarce data within this field, the objective of this study was to contribute to a better understanding of these systems and aid design of future PRBs.

### 2.2 Materials and methods

#### 2.1 Site characterization

The site is located on the eastern edge of Ballymena (Northern Ireland), within the relatively flat flood plain of the River Braid. It was used in the past for agricultural purposes until 1998, when the Ballymena Borough Council purchased the site for the Millenium Ecos Centre used to study the local environment.

A total of 21 boreholes, aligned in transects approximately perpendicular to the Braid River, were drilled in the study area to provide the site geologic lithology and to allow the installation of piezometers for monitoring the groundwater hydrology and quality (Figure 1). An additional number of 8 boreholes were drilled beyond the study area to yield information on the lithology of the whole site (data not shown). The intrusive works revealed a shallow aquifer over much of the site, which presented a lithology consisting of an upper layer of silt and clay (generally to 1.5 m below ground level), underlain by a coarse layer (primarily gravel and sand with some silt) ranging in thickness approx. 1.5 to 3 m below ground level, underlain in turn by a stiff boulder clay (subglacial till) that acts as an aquitard beneath the aquifer due to its low permeability. Measurements of groundwater levels showed that groundwater flows in a southwesterly direction, with groundwater flow lines converging to the river (Figure 1). The mean hydraulic conductivity of the aquifer, based on slug tests, is 2 m/d, and the mean hydraulic gradient is 0.53%.
Soil and groundwater analysis demonstrated that there were no early indications of any pollution of the aquifer at the site and that N-species were clearly not of regulatory concern (Table 1).

Table 1: Composition of the groundwater at the Ecos site

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>ORP</td>
<td>mV</td>
<td>21 ± 63</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cond.</td>
<td>μS/cm</td>
<td>270 ± 112</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>mg/L</td>
<td>4.5 ± 3.5</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>mg/L</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>mg/L</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>TOC</td>
<td>mg/L</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Na⁺</td>
<td>mg/L</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>K⁺</td>
<td>mg/L</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>mg/L</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>mg/L</td>
<td>12 ± 4</td>
</tr>
</tbody>
</table>

Figure 1: Map view of the site showing the monitoring well locations and the groundwater flow direction. Dash area corresponds to the denitrification PRB.
<table>
<thead>
<tr>
<th></th>
<th>mg/L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻</td>
<td>14 ± 1</td>
<td></td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>8 ± 7</td>
<td></td>
</tr>
<tr>
<td>Alk (as CaCO₃)</td>
<td>178 ± 62</td>
<td></td>
</tr>
</tbody>
</table>

The site was chosen as there was NO₃⁻ contamination at the site, and therefore the ability for the PRB to naturally exhibit denitrifying potential, and under increasing loads of NO₃⁻ in groundwater was ideal for this study. Therefore the proposed PRB was designed to incorporate a controlled delivery system of NO₃⁻ to test its denitrification performance at different NO₃⁻ loading rates.

### 2.2 Design of the PRB

The design of the PRB was performed according to established guidelines (Gavaskar, 1999; Obiri-Nyarko et al., 2014). Briefly, site characterization allowed setting the location, configuration, orientation and dimensions (width and depth) of the PRB in order to successfully intercept the contaminated plume, while laboratory experiments allowed selecting the most suitable components of the PRB and, with the denitrification kinetics data, determining the required thickness of the PRB to ensure a targeted removal of NO₃⁻. Among the substrates evaluated in this laboratory study, mulch consisting of hardwood with small amounts of leaves supplied by the local company M. Large Tree Services Ltd (Newtownabbey, Northern Ireland) was the top performing substrate, attaining an overall NO₃⁻ removal mostly via denitrification of >96% in both batch and column systems (Gibert et al., 2008).

The PRB was designed as a continuous trench perpendicular to the groundwater flow direction and parallel to the Braid River approximately 13 m from the riverbank (Figure 1). The vertical position of the PRB was selected in order to be keyed into the underlying clay and to intercept the groundwater in the shallow aquifer. The dimensions were 20 m long, 1.7 m deep and 1.8 m thick, and it was positioned 1.5 to 3.2 below the ground level. The excavated trench was backfilled using gabion technology with a mixture of 50% (v/v) mulch and gravel (approx. 5–10 mm size) previously homogenised (porosity of the mixture approx. 0.30) identical to that used in the laboratory experiments (Gibert et al., 2008). Mulch was added as the organic substrate to promote denitrification, while gravel was added to ensure a high permeability within the PRB. Denitrifying bacteria are ubiquitous in groundwater (Rivett et al., 2008), and their proliferation within the PRB was expected under the assumption that mulch would provide a long-term source of carbon and energy.

Two adjacent layers of gravel of 0.75 m thick were placed upgradient and downgradient of the reactive zone to provide a highly permeable zone that facilitated uniform flow of groundwater through the PRB (Figure 2). It is in the upgradient layer of gravel where NO₃⁻ was injected. On completion of backfilling, the top of the PRB was covered with excavated material and compacted to limit oxygen diffusion and flow of infiltrating water into the barrier.
The NO₃⁻ delivery system consisted of two elevated 230 L tanks (with 1.5 m of hydraulic head) connected to a drip irrigation system through which site groundwater with variable concentrations of NO₃⁻ was continuously injected into the upgradient gravel layer, ultimately through the reactive zone. The injection was accomplished by a row of 22 drip emitters situated along the upgradient gravel layer. The close spacing between the drip emitters was to provide a uniform front of NO₃⁻ flowing towards the barrier. This delivery system allowed a precise control of the NO₃⁻ loading rate injected into the aquifer by selecting the NO₃⁻ concentration within the barrels and controlling the injection rate at ca. 0.1 L/min (refilling of barrels approximately every 3 days). A scheme of the drip irrigation system is shown in Figure 2. By using values of groundwater flow rates and injection rates, the expected dilution factor for NO₃⁻ once injected into the upgradient gravel layer was quantified at approx. 10 (this value was later validated by a tracer test).

### 2.3 Monitoring wells network

A monitoring network consisting of 28 wells was installed within and around the PRB to evaluate its performance. Unless otherwise stated the wells were completed in the Gravel Aquifer at 2.5 m below ground level. As shown in Figure 2, two monitoring wells were installed in the upgradient gravel layer (to monitor the groundwater entering the barrier) and two more in the downgradient gravel layer (to monitor treated groundwater). Two additional sets of monitoring wells were emplaced to monitor groundwater inside the barrier. Both consisted of three nested piezometers with a 0.5 m slotted screen each, terminated at depths of 2.0, 2.5 and 3.0 m below ground. Six wells were installed at either side of the PRB and the remaining twelve wells were placed between the PRB and the River Braid (Figure 2b) to assure that water entering the river was satisfying regulatory requirements placed on the research program.

### 2.4 Hydraulic PRB validation

A NaCl tracer test was conducted soon after the installation of the PRB and before any NO₃⁻ delivery in order to validate the flow through the PRB and the dilution factor estimated from the initial hydrogeological survey. For
that purpose, a solution of Cl\(^{-}\) (1621 mg/L as measured) was injected from the barrels into the upgradient gravel layer. Monitoring carried out during the 8 week period of the tracer test showed that: 1) no Cl\(^{-}\) was measured at either side of the PRB (MW11 to MW16), proving that groundwater flow direction was perpendicular to the PRB and that no by-pass flow occurred around the PRB, 2) the maximum measured Cl\(^{-}\) concentration in wells in the upgradient gravel layer and within the PRB was 150 mg/L, indicating a dilution factor of 10.8 (which was in close agreement with the dilution factor of 10 estimated from the design based on hydrogeological modelling), and 3) the time needed for the Cl\(^{-}\) to travel from the upgradient to downgradient layers (residence time within the PRB) was 14 days. All these findings confirmed the design parameters were reflected in the PRB operation as built.

### 2.5.2 NO\(_3\)\(^{-}\) delivery plan

The calculated dilution factor (10.8) was used to select NO\(_3\)\(^{-}\) concentration in the barrels that, after dilution, would provide a desired concentration of NO\(_3\)\(^{-}\) entering the PRB.

A stepwise increase of NO\(_3\)\(^{-}\) concentration in barrels was planned in order to investigate the PRB performance at various NO\(_3\)\(^{-}\) loading rates. Before proceeding with any increment of NO\(_3\)\(^{-}\) loading rate, action and regulatory oversight was agreed with the Environment Agency of Northern Ireland. The delivery plan carried out during the study is presented in Figure 3. NO\(_3\)\(^{-}\) was first spiked into the aquifer with a concentration of NO\(_3\)\(^{-}\) in the barrels of 500 mg/L (before dilution by the groundwater flowing through the PRB) for 157 days. The PRB immediately demonstrated the denitrifying ability was effective at removing all NO\(_3\)\(^{-}\) from groundwater during this first phase, and NO\(_3\)\(^{-}\) concentration in the barrels was increased to 5000 mg/L until day 206, when it was further increased to 10,000 mg/L for the following 49 days. Given that NO\(_3\)\(^{-}\) removal was maintained >97%, it was decided to further increase NO\(_3\)\(^{-}\) concentration in barrels to 20,000 mg/L until day 340.

![Figure 3](image) NO\(_3\)\(^{-}\) delivery plan during the operation of the PRB and dates of sampling events.

### 2.6 Denitrification quantification

We recognized that dilution within the PRB may occur due to changes in flow rates and/or direction of groundwater (caused by e.g. rainfall episodes), and these could bias the interpretation of results. The choice of the nitrate source (KNO\(_3\)) allowed the concentration of NO\(_3\)\(^{-}\) to be normalized with respect to the conservative K\(^+\) ion. K\(^+\) was used for the following reasons: (1) it was found to behave conservatively within the mulch/gravel media (not suffering from lateral mechanisms e.g. adsorption) in lab-tests, (2) it presented very low background concentrations in the site groundwater, and (3) it is easy to detect at low concentration. Hence, NO\(_3\)\(^{-}\) and K\(^+\) were injected together into the PRB in the form of KNO\(_3\) (ratio 1:1). Any observed decrease of NO\(_3\)\(^{-}\) concentration as compared to K\(^+\) concentration would be indicative of NO\(_3\)\(^{-}\) removal due to processes other than simple dilution (e.g. through denitrification).

A denitrification factor (DNF) was thus defined as follows:

\[
\text{DNF} = \frac{\frac{\text{NO}_3^-_{in}}{\text{K}^+_{in}}}{\frac{\text{NO}_3^-_{out}}{\text{K}^+_{out}}}
\]

where subindexes “in” and “out” refer to inlet and outlet of the PRB, respectively. A DNF approaching 0 was indicative of total NO\(_3\)\(^{-}\) removal, whereas a DNF tending to 1 was indicative of no removal at all (and that any observed decrease in NO\(_3\)\(^{-}\) concentration was due to dilution).

### 2.7 Groundwater sampling and chemical analysis

Groundwater was collected from monitoring wells for chemical analysis. Each well was purged using a submersible pump (whales minipurger; flowrate 10 L/min) for about 1 minute prior to sampling (representing over 5
well volumes). River water from upstream and downstream of the PRB was also sampled using the same submersible pump.

Groundwater analysis comprised the determination of pH, nitrate (NO$_3^-$), nitrite (NO$_2^-$), ammonium (NH$_4^+$), dissolved organic carbon (DOC) and major cations and anions (K$^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, Cl$^-$, SO$_4^{2-}$). After collection, samples were immediately stored at 4°C and shipped to an ISO17025 accredited laboratory under the United Kingdom Schemes (UKAS) (Alcontrol Laboratories) for analysis within 48 hours in order to avoid any post sampling microbial degradation. A total of nine campaigns were carried out for groundwater analysis, as shown in Figure 3.

The Kone analyser was used for the analysis of NO$_2^-$ and NO$_3^-$ (AWWA/APHA, 1999). NH$_4^+$ (BS 6068: Part 2.11:1984) (BSI, 1984), SO$_4^{2-}$ (APHA Method 4500E) (AWWA/APHA, 1999) and Cl$^-$ (modified US EPA Method 325.1 & 325.2) (USEPA, 1983). pH was analysed using a GLpH pH meter (BS 1377 Method) (BSI, 1984). DOC was analysed by combustion according to APHA Method 5310 (AWWA/APHA, 1999). Cations Na$^+$ and K$^+$ were analysed by ICP-OES according to APHA Method 3111 (AWWA/APHA, 1999) while Ca$^{2+}$ and Mg$^{2+}$ by ICP-MS according to APHA Method 3125B (AWWA/APHA, 1999). The limits of detection for these analytes (in mg/L) were 0.3 (NO$_3^-$), 0.05 (NO$_2^-$), 0.2 (NH$_4^+$), 3 (SO$_4^{2-}$), 1 (Cl$^-$), 2 (DOC), 0.2 (K$^+$ and Na$^+$), 0.1 (Ca$^{2+}$ and Mg$^{2+}$).

### 2.8 Gases sampling and chemical analysis

In a denitrification system, NO$_3^-$ is ideally converted totally to N$_2$ according to reaction (1). However, incomplete denitrification may result in the accumulation of intermediate species, e.g. NO and N$_2$O. Moreover, degradation of organic matter in anaerobic conditions (either by denitrifying bacteria or other bacteria also present in the system) can lead to the accumulation of carbon dioxide (CO$_2$) and methane (CH$_4$). These gases, together with N$_2$O are known to be potent greenhouse gases of major environmental concern, and in this case by the regulator on site. In fact, N$_2$O and CH$_4$ exhibit a global warming potential for a 100-year time horizon 298 and 25 times greater, respectively, than that of CO$_2$ (IPCC, 2007). In subsurface systems, gases can be emitted via upward diffusion from groundwater through the unsaturated zone to the atmosphere (Jurado et al., 2017). For this reason, emissions of CO$_2$, CH$_4$ and N$_2$O were measured using the closed chamber method (Hensen et al., 2013). The chamber consisted of a plastic box (31 cm x 31 cm) inserted into the soil above the PRB to a depth of 5 cm. The enclosure surface area was 0.0961 m$^2$ and the created headspace volume 0.0115 m$^3$. Gas from the headspace of the chamber was pumped continuously through PTFE tubing connected to the chamber for a 70-min collection period and contents of CO$_2$, CH$_4$ and N$_2$O were analysed using a portable MCERTS accredited FTIR multiparameter gas analyser (Gasmet DX 4030).

To further elucidate the generation of CO$_2$, CH$_4$ and N$_2$O and their potential for upward migration and discharge to the atmosphere, these gases were measured in the headspace of each of the three nested wells at different depths within the PRB. Wells were sealed with gas tight caps prior to and during the measurements to ensure that the headspace of the monitoring wells was not diluted with air. The headspace of the wells was not purged prior to gas analysis in order to measure highest possible concentration. The gas phase of the wells was re-circulated at 1/min for 2 to 3 times during measurements (i.e. approximately 2 to 3 times the volume of the headspace). Spectra were continuously recorded at a rate of 1 measurement per minute.

CO$_2$, CH$_4$ and N$_2$O measurements were performed during the period when the highest nitrate load was spiked in order to assess highest potential concentration of gases (see Figure 3).
However, when NO$_3^-$ concentration in feed barrels was increased to 20,000 mg/L from day 257 onwards, the DNF declined to 0.34 (NO$_3^-$ removal percentage of 66%), making evident that the PRB has an ultimate design limit for NO$_3^-$ load (530 mg/L measured in the inlet gravel layer).

Three observations can be highlighted on the response of the PRB to the increase in the NO$_3^-$ load:

First, the study period saw increasing severity of weather in Northern Ireland, resulting in near monthly flooding of the field by the River Braid and strong perturbations to the groundwater flow regime. This led to measured dilution factors in the upgradient gravel layer (mostly between 10 and 70) higher than that estimated during the tracer test (quantified at 10.8). Moreover, additional dilution (at factors 1-40) was occasionally observed within the PRB, likely due to lateral groundwater inputs during flooding. These dilutions factors resulted in NO$_3^-$ concentrations in the inlet of the PRB (between 0.6 and 530 mg/L) that were up to 100-fold lower than the expected ones without the additional dilution. Although K$^+$-normalized molar ratio [NO$_3^-$]/[K$^+$] accounted for dilution effects and, thus, findings were not compromised by them, for some campaigns, the low measured concentration of K$^+$ prevented reliable calculation of DNF.

Second, neither NO$_2^-$ nor NH$_4^+$ were detected in groundwater samples at relevant concentrations (averaged concentrations of 0.5 mg/L and 0.3 mg/L, respectively) throughout the study, suggesting that most NO$_3^-$ removal was due to denitrification and not through other processes such as dissimilatory nitrate reduction to ammonia (DNRA). A possible NO$_3^-$ removal process that cannot be ruled out from the measurements taken alone is N-immobilization in microbial biomass (Calderer et al., 2016). This process has been found of minor importance in other denitrification PRBs (Schipper and Vojvodic-Vukovic, 1998). However, when NO$_3^-$ concentration in feed barrels was increased to 20,000 mg/L from day 257 onwards, the DNF declined to 0.34 (NO$_3^-$ removal percentage of 66%), making evident that the PRB has an ultimate design limit for NO$_3^-$ load (530 mg/L measured in the inlet gravel layer).

Third, DOC measured in groundwater from within the PRB exhibited a sustained concentration in the range of 4-10 mg/L throughout the study (Figure 5), showing that mulch was providing significantly increased level of DOC to sustain heterotrophic microbial activity. An initial peak of up to 71 mg/L was measured in MW3, likely as a result of washout of organic carbon. Similar high initial releases of DOC have also been observed in other denitrification PRBs filled with natural organic substrates (Robertson and Cherry, 1995; Schipper and Vojvodic-Vukovic, 1998; Schmidt and Clark, 2012).

![Figure 5](alt-text: Fig. 5) Evolution of DOC in groundwater within the PRB at different depths (MW3 to MW5).

The achieved DOC concentration at stabilized conditions was in line with PRB design parameters (gravel to mulch ratio) where calculations showed it should be low enough to avoid both a negative impact on groundwater quality and undesirable processes like DNRA, but high enough to support heterotrophic denitrification activity. We should bear in mind here that other heterotrophic processes consuming DOC may inevitably also take place by other indigenous microorganisms, such as aerobic respiration, sulphate-reduction and methanogenesis, as shown by the following equations, respectively:

$$\text{CH}_3\text{O} + \text{O}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \quad (3)$$

$$2\text{CH}_3\text{O} + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^- + \text{H}^+ \quad (4)$$

$$2\text{CH}_3\text{O} + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^- + \text{H}^+ \quad (5)$$

The observed increases in K$^+$-normalized molar ratio [HCO$_3^-$]/[K$^+$] (which averaged 15-fold) after the passage of groundwater through the PRB suggested that heterotrophic processes (reactions (2) to (5)) were taking place in the PRB. This was further supported by the observed slight decreases of pH from inlet to outlet monitoring wells (averaged decreases of 0.3 units of pH). The occurrence of sulphate-reduction (reaction (4) in the PRB was evidenced by the observed decrease of K$^+$-normalized molar ratio [SO$_4^{2-}$]/[K$^+$], which averaged 30%. Finally, analysis of air in the headspace of the monitoring wells showed an enrichment in CO$_2$, CH$_4$ and N$_2$O (see below), indicating that Eqs. (3), (4) and (5) were taking place.
Competition for CH₂O between denitrifiers, aerobic degraders, methanogens and sulphate-reducers should not be seen necessarily detrimental for denitrification to proceed, as it is known that denitrifiers do not degrade complex polymeric carbon found in organic substrates but depend on a consortium of microorganisms to release more labile, simple organic compounds (Schipper and Vojvodić-Vuković, 1998; Schipper and Vojvodić-Vuković, 2001). The synergies in such a consortium were beyond the scope of this study, but it is likely that the co-occurrence of reactions (2)-(5) helped denitrification to progress.

No change of the level of NO₃⁻ in the river water between upstream and downstream of the PRB was observed throughout the study. At all times, NO₃⁻ in the River Braid water ranged between 11 and 16 mg/L, and these measured concentrations were always higher than concentrations measured in any of the monitoring wells between the PRB and the River Braid.

### 3.3.2 Vertical profile of NO₃⁻ within the PRB

**Fig. 6** illustrates NO₃⁻ vertical profiles inside the PRB at different inlet NO₃⁻ concentrations. Denitrification activity seemed to depend on the vertical location, as DNF generally declined downward from averaged values of 0.51 at depth = 2.0 m to 0.10 at depth = 3.0 m. The greater NO₃⁻ removals at the bottom of the PRB can be justified by the fact that the deeper the location the less atmospheric O₂ intrusion and, therefore, the more reducing conditions, which favours denitrification (Jahangir et al., 2012; Cocco et al., 2018).

![Graph showing DNF ratio vs depth](alt-text: Fig. 6)

It must be underlined that DNF values shown in **Fig. 6** correspond to the monitoring wells in half of the PRB flow-distance. The overall NO₃⁻ removal was higher as groundwater travelled the whole width of the PRB (as shown in **Fig. 4**).

### 3.3.3 Gases analysis

Emissions of generated CO₂(g), CH₄(g) and N₂O(g) from the PRB to the atmosphere can occur vertically via upward diffusion from groundwater through the unsaturated zone. Averaged emissions of CO₂(g), CH₄(g) and N₂O(g) measured from the closed chamber tests were 411.09 mg/(m²·h), 23.05 mg/(m²·h) and 0.403 mg/(m²·h), respectively (Table 2).

<table>
<thead>
<tr>
<th>Flux of emitted gas</th>
</tr>
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<tbody>
<tr>
<td><strong>Table 2: Emissions of generated CO₂, CH₄ and N₂O from the PRB to the atmosphere measured from the closed chamber tests</strong></td>
</tr>
</tbody>
</table>

**Table 2**
The generation of CO$_2$, CH$_4$ and N$_2$O was indicative of heterotrophic processes. CO$_2$ can be formed from HCO$_3^-$ as a product of reactions (2)-(5). CH$_4$ reflected the prevalence of anaerobic conditions (at least in some parts) within the PRB, whereas N$_2$O was indicative of (incomplete) denitrification.

There are no published studies that have examined gases emission from field-scale denitrification PRBs. However, the values obtained in this study compare well with other ecosystems under field conditions. In fact, CO$_2$ emission fluxes were in the same order of magnitude as those measured in restored riparian wetlands (35.1±732 mg/(m$^2$·h)) (Audet et al., 2013), constructed wetlands (32.4±489 mg/(m$^2$·h)) (Savik et al., 2006) and undisturbed natural soil (230±557 mg/(m$^2$·h)) (Yang et al., 2013), but considerably lower than those measured in some Danish agricultural peatlands (403±5500 mg/(m$^2$·h)) (Elsgaard et al., 2012). CH$_4$ emission fluxes were also comparable to those measured in wetlands (4±49 mg/(m$^2$·h)) (Whalen, 2005; Audet et al., 2013), constructed wetlands (1±47 mg/(m$^2$·h)) (Savik et al., 2006) and undisturbed natural soil (1±20 mg/(m$^2$·h)) (Yang et al., 2013), and much lower than those measured in landfills (96±(m$^2$·h)) (Bruun et al., 2017 and references therein). Finally, N$_2$O flux values measured for the PRB fell in the lower end of values reported in literature in riparian soils (0.02±31 mg/(m$^2$·h)) (Audet et al., 2013; Audet et al., 2014; Hinshaw and Dahlgren, 2016; Jurado et al., 2017 and references therein), European agricultural and forested ecosystems (0.4±137 mg/(m$^2$·h)) (Machfert et al., 2002) and constructed wetlands (<0.01±38 mg/(m$^2$·h)) (Savik et al., 2006; Bruun et al., 2017). These values of N$_2$O have been reported to not represent a significant contribution to the atmosphere compared to the direct emissions from agricultural fields (Hinshaw and Dahlgren, 2016; Jurado et al., 2017). Taking into account the small area of the PRB (compared to other systems such as natural or constructed wetlands) it appears that the measured gas emissions in this study represent a minor contribution of total emissions to the atmosphere.

The low CO$_2$, CH$_4$ and N$_2$O emissions do not necessarily imply that the concentrations of these gases in groundwater may not be high. It is well known that gases in groundwater can be over-saturated with respect to the overlying atmosphere (Jurado et al., 2017). CO$_2$, CH$_4$ and N$_2$O concentrations in groundwater were not measured directly in the current study. However, their content was measured in the headspace of the nested monitoring wells within the PRB. Because these wells were filled with groundwater that entered through the screened zone at a different depth, the content of gases in their headspace can be regarded as an indicator for microbial activity being active at different locations in the PRB. It must be bear in mind that these released gases do not diffuse through any soil layer (unlike those analysed in closed chamber tests), and thereby their contents are expected to be higher than those measured in the closed chamber test.

Headspace gas within the three nested wells presented contents notably greater (for CH$_4$), greater (for N$_2$O) and only slightly greater (for CO$_2$) than atmospheric background (Fig. 6). Contents varied depending on the depth of the monitoring well. CO$_2$ contents were higher in the headspace of the shallowest piezometer, likely coming from aerobic respiration, favoured in the upper part where diffusion of atmospheric O$_2$ is not as limited as in deeper parts, and to a lesser extent from denitrification and sulphate-reduction (which require much more reducing conditions) (Stuart and Lapworth, 2016).
The opposite trend was exhibited by N$_2$O. Higher contents were seen in the deepest piezometer while lower contents in the shallowest piezometer. This was consistent with the DNF vertical profile showing that it is in the deepest part where denitrification is favoured. Degassing of N$_2$O generated in the deepest part to the atmosphere is often hindered by 1) the low N$_2$O diffusivity through saturated soil (the diffusion coefficient of N$_2$O in water is around four orders of magnitude smaller than in air), which makes that the larger the water column above a location the lower the amount released to the atmosphere (Heincke and Kaupenjohann, 1999) and 2) the microbial reduction of N$_2$O to N$_2$ during the slow upward diffusion (Jahangir et al., 2012; Jurado et al., 2017; Cocco et al., 2018).

No specific pattern was observed with regard to CH$_4$. More measurements should be done to elucidate whether CH$_4$ differs between monitoring wells at different depths.

### 3.4.3.4 Comparison against other denitrification PRB

To our best knowledge, there are only six other PRBs for NO$_3^-$ remediation worldwide as reported in peer-reviewed journals. Four of these are in Canada and USA and two in Australia and New Zealand. This is, thus, the first PRB of this type in Europe. Table 3 presents an inventory of these denitrification PRBs, together with the one reported here, to facilitate comparisons among them. The principles and design of all them are comparable in general terms. The one reported here is singular in that it is the only one with a NO$_3^-$ delivery system.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dimensions</th>
<th>Composition</th>
<th>t$_R$ (d)</th>
<th>[NO$_3^-$] (mg/L)</th>
<th>[DOC] (mg/L)</th>
<th>Hydraulic performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Up-grad</td>
<td>In the PRB</td>
<td>Down-gradient</td>
<td>Removal up-down</td>
<td>Up-grad</td>
</tr>
</tbody>
</table>
4. Climatic conditions can vary considerably over the year and so do the aquifer hydrologic conditions, in particular following heavy and sustained rainfalls.

2008 rates (with up to 25-fold decreases) or even bypasses around the PRB depending on the season have been reported for four of the six reported denitrifying PRBs (Schipper and Vojvodić-Vuković, 1998, 2000, 2001, Long et al., 2011).

Difficulties in intercepting the plume, "because the majority of the sampled groundwater had flowed beneath the wall".

Difficulties in intercepting the plume, as "most of the groundwater flowed under rather than through the wall".

Difficulties in intercepting the contaminated plume, causing dilution with uncontaminated groundwater within the PRB.

Difficulties in intercepting the contaminated plume, because the groundwater within the PRB flowed under rather than through the wall. Difficulties in intercepting the plume, as "most of the groundwater flowed under rather than through the wall".

Occasional lateral groundwater inputs resulting in dilution within the PRB.

The conclusions of the study can be summarised as follows:

- The denitrification PRB was successful at removing NO₃⁻ from groundwater at inlet concentrations up to 280 mg/L (with NO₃⁻ removal percentages ≥97%). When NO₃⁻ concentration was 530 mg/L the NO₃⁻ removal percentage declined to 66%. To reliably attribute any decrease of NO₃⁻ concentration to NOₓ removal, its concentration was normalized with respect to a conservative ion (K⁺) also spiked with NO₃⁻ (as KNO₃) to account for possible dilution effects.

Successes and failures of these PRBs must be evaluated from both chemical and hydraulic points of view. From a chemical point of view, all reported PRBs demonstrate that denitrification can satisfactorily be promoted in the subsurface by means of a natural solid organic substrate (sawdust, woodchips, mulch). In all cases, NO₃⁻ removal within the PRB was found >70% (with inlet NO₃⁻ inputs ranging between 2 and 438 mg/L) and the main NO₃⁻ removal mechanism was identified to be heterotrophic denitrification, promoted by the release of DOC from the solid natural organic substrate. For two of the PRBs, authors have estimated that, based on the release rate of DOC, the denitrification capacity can be maintained for many decades (Schipper and Vojvodić-Vuković, 2001; Robertson et al., 2008; Long et al., 2011). In some PRBs, denitrification has been confirmed by monitoring denitrification enzyme activity (DEA) (Schipper and Vojvodić-Vuković, 2001; Schipper et al., 2004; Schmidt and Clark, 2012) or by means of microbiological tools (Hiller et al., 2015). Residence time (tᵣ) within these denitrification PRBs ranges from 1.8–13 days (based on the explicitly reported values), which indicates that denitrification is a faster process than sulphate-reduction, for which tᵣ required in a PRB is in the order of (at least) 90 days to satisfactorily take place (Benner et al., 2002; Gibert et al., 2011).

However, it is from hydrogeological perspective that some of these documented PRB show their flaws. In fact, it is not rare that PRBs suffer from unforeseen changes in groundwater directions and/or improper hydraulic characterization of the site prior to PRB installation, which results in limited capture of the NO₃⁻-contaminated plume, diversion and/or partial or total by-pass of the groundwater around the PRB. Fluctuations of groundwater flow rates (with up to 25-fold decreases) or even bypasses around the PRB depending on the season have been reported for four of the six reported denitrifying PRBs (Schipper and Vojvodić-Vuković, 2000; Schipper et al., 2004; Robertson et al., 2008; Schmidt and Clark, 2012). Design flaws on the site characterization with the subsequent loss of hydraulic control have been reported to be the most common cause of PRB failures (Henderson and Demond, 2007), especially when climatic conditions can vary considerably over the year and so do the aquifer hydrologic conditions, in particular following heavy and sustained rainfalls.

4.4 Conclusions

The conclusions of the study can be summarised as follows:

- The denitrification PRB was successful at removing NO₃⁻ from groundwater at inlet concentrations up to 280 mg/L (with NO₃⁻ removal percentages ≥97%). When NO₃⁻ concentration was 530 mg/L the NO₃⁻ removal percentage declined to 66%. To reliably attribute any decrease of NO₃⁻ concentration to NOₓ removal, its concentration was normalized with respect to a conservative ion (K⁺) also spiked with NO₃⁻ (as KNO₃) to account for possible dilution effects.
The marginal levels of NO$_3^{-}$ and NH$_4^{+}$ indicated that NO$_3^{-}$ removal was mainly through denitrification and not through other processes such as DNRA.

Monitoring groundwater at different depths within the PRB provided evidence that NO$_3^{-}$ underwent denitrification preferably at the deepest part of the PRB (removals mostly >60%), where more favourable reducing conditions were achieved.

The higher contents of N$_2$O$_{(g)}$ measured in the headspace of the deeper monitoring well seemed to confirm this trend.

The analysis of other dissolved ion species (SO$_4^{2-}$) and gases (CO$_2$, CH$_4$) revealed that heterotrophic processes other than denitrification were also active within the PRB. These processes included aerobic respiration, sulphate-reduction and methanogenesis.

The intense rainfalls during the operation period of the PRB resulted in greater NO$_3^{-}$ dilutions than expected from the initial site hydrogeological characterization. Although this additional dilution did not pose concern for the denitrification capacity of the PRB, for some campaigns the low concentration of the conservative ion K$^+$ prevented reliable calculation of denitrification removal percentage.

Emission fluxes of CO$_2$, CH$_4$ and N$_2$O were comparable with other ecosystems under field conditions. Because of the small area covered by the PRB, these fluxes do not represent any significant contribution to greenhouse gases to the atmosphere. Further work is required on this field.

## Conflicts of interest
The authors declare no conflict of interests.

## Uncited reference
Capodici et al., 2018

## Acknowledgements
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https://doi.org/10.1016/j.chemosphere.2006.05.015.


**Graphical abstract**

![Graphical abstract](alt-text: Unlabelled Image)

**Highlights**

- The performance of a field-scale PRB for NO₃⁻-contaminated groundwater is reported.
- The design of the PRB allowed operation at different inlet NO₃⁻ concentrations.
- The PRB was successful at removing NO₃⁻ (>97%) at inlet concentrations up to 280 mg/L.
- Denitrification preferentially took place at the deepest part of the PRB.
- Emissions fluxes of greenhouse gases to the atmosphere were not of concern.

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I’ve changed the text: we acknowledge these institutions for their operational support.