



## Using chemical, microbial and fluorescence techniques to understand contaminant sources and pathways to wetlands in a conservation site



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### HIGHLIGHTS

- We used chemistry, fluorescence and bacterial counts to study multiple pathways of contamination to a wetland site.
- Contamination sources are primarily fertilizers, causing exceedance of nutrient thresholds in groundwater within the site.
- Contamination pathways into the site include groundwater, surface runoff, and streams, with minor input from on-site grazers.
- The site attenuates nutrient and bacterial concentrations providing an ecosystem service, but with adverse biological effects.

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### ABSTRACT

Nutrients and faecal contaminants can enter wetland systems in a number of ways, with both biological and potentially human-health implications. In this study we used a combination of inorganic chemistry, dissolved organic matter (DOM) fluorescence and *Escherichia coli* and total coliform (TC) count techniques to study the sources and multiple pathways of contamination affecting a designated sand dune site of international conservation importance, surrounded by agricultural land. Analysis of stream samples, groundwater and dune slack wetlands revealed multiple input pathways. These included riverbank seepage, runoff events and percolation of nutrients from adjacent pasture into the groundwater, as well as some on-site sources. The combined techniques showed that off-site nutrient inputs into the sand dune system were primarily from fertilisers, revealed by high nitrate concentrations, and relatively low tryptophan-like fulvic-like ratios <0.4 Raman units (R.U.). The *E. coli* and TC counts recorded across the site confirm a relatively minor source of bacterial and nutrient inputs from on-site grazers. Attenuation of the nutrient concentrations in streams, in groundwater and in run-off inputs occurs within the site, restoring healthier groundwater nutrient concentrations showing that contaminant filtration by the sand dunes provides a valuable ecosystem service. However, previous studies show that this input of nutrients has a clear adverse ecological impact.

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### 1. Introduction

The global availability and mobility of nitrogen have increased rapidly over the past five decades (Galloway and Cowling, 2002) and the damaging impacts it has on freshwater ecosystems are widely documented (Camargo and Alonso, 2006). Aquatic systems are extremely sensitive to nitrogen and are threatened by atmospheric deposition inputs (Fowler et al., 2005) as well as point sources and diffuse sources which can enter aquatic systems via numerous pathways such as through runoff, streams and groundwater. Tracing sources of aquatic pollution is therefore often problematic (Withers et al., 2009).

Within rural areas river water quality (Hooda et al., 2000) and groundwater quality (Oakes et al., 1981) are primarily impacted by agricultural diffuse pollution (Novotny, 1999). Atmospheric nutrients have been demonstrated to have adverse impacts on the ecology of protected dune habitats (Jones et al., 2013; Plassmann et al., 2008; Field et al., 2014). However, the specific impacts of relatively low levels of nutrients from groundwater on aquatic habitats in dune systems have only recently been documented (Rhymes et al., 2014). As well as nutrients, diffuse inputs of dissolved organic matter (DOM) and micro-organisms into groundwater and surface waters also occur via runoff, field drainage and leaching, as a result of agricultural practices such as slurry and fertiliser application. Previously, these diffuse inputs have largely been characterised by using nutrients as a proxy (e.g. Vadas et al., 2007), although more recent studies are now examining diffuse sources and pathways by investigating pathogenic micro-organisms (Kay et al., 2008) and

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characterising DOM by natural fluorescence (Hudson et al., 2007). To date there have been no studies combining chemical, fluorescent and microbial techniques to help decipher multiple diffuse sources and pathways. Excitation emission matrix fluorescence spectroscopy (EEMS) can be used to trace DOM from agricultural sources (Baker, 2002; Old et al., 2012). EEMS is sensitive enough to characterise fulvic-like, humic-like and protein-like substances (Tryptophan-like and tyrosine-like) within the DOM to help characterise and quantify the extent of contamination by effluents from different sources (Hudson et al., 2007). Fulvic-like and humic-like substances are derived from the breakdown of plant material (Stedmon et al., 2003), whereas large inputs of tryptophan-like substances are associated with readily biodegradable material from sewage and farm waste slurry (e.g. Baker, 2001). Agricultural diffuse sources such as animal waste are characterised by high protein-like fluorescence with very high ratios of tryptophan-like to fulvic/humic-like fluorescence compared to stream waters (Baker, 2002); these ratios are sensitive enough to characterise inputs from different livestock animals such as pigs and sheep (Baker, 2002).

Currently, the WHO Guidelines for Drinking Water Quality, adopted as standard in many countries, use total coliforms (TC), or specifically *Escherichia coli* (*E. coli*) a sub-group of faecal coliforms, as faecal indicators for the safety of water supplies. In some countries, such as The Netherlands and Denmark, groundwater is abstracted from sand dune systems to supply drinking water, indicating the importance of understanding the fate and occurrence of TC and *E. coli* within these systems (Smeets et al., 2009). The enumeration of TC and *E. coli* is also used as an indicator of water quality within the revised bathing water directive; *E. coli* counts greater than 10,000 counts per 100 mL and TC counts greater than 2000 counts per 100 mL would fail to meet the required standards in the directive (European Community, 2006). Although the TC group includes the species *E. coli*, which is generally considered to be specific for faecal contamination, it also includes other genera such as *Klebsiella* and *Citrobacter* which are not necessarily of faecal origin and can emanate from alternative organic sources such as decaying plant materials and soils (WHO, 2006).

While some *E. coli* represent enteric pathogens (Savageau, 1983), other strains of this species can grow and maintain populations in the environment if the conditions are suitable (Byappanahalli and Fujioka, 2004). Sources of *E. coli* include septic tanks, sewer lines, wastewater treatment plants, manure spreading on land, livestock and wildlife. These sources also contribute DOM and nutrient inputs. Despite advanced wastewater treatment efforts by water treatment companies, some UK bathing sites do not always produce full compliance with microbial standards (Crowther et al., 2002) due to other diffuse sources within catchments, resulting in a greater proportion of nonconformity due to agriculture. More than 150 different pathogens, associated with both environmental and human health risks can be found in livestock manure which can significantly increase bacterial loading to the subsurface, causing contamination within soils, groundwater and stream water (Gerba and Smith, 2005). The transport time and distance travelled by bacteria reaching the groundwater or streams depend on the rate at which bacteria are released from manure, the presence of preferential pathway networks within soil and the depth to the groundwater (Abuashour et al., 1994; Unc and Goss, 2003). The presence of TCs in surface or groundwater is usually considered evidence of recent faecal contamination, with *E. coli* remaining active for 16–45 days in the subsurface (Taylor et al., 2004).

This study aims to use a combination of inorganic chemistry, DOM fluorescence and culturable *E. coli* and TC counts to evaluate the potential sources and pathways of nutrients and contamination to a sand dune site designated for its international nature conservation importance, known to be affected by nutrients from the surrounding agricultural land (Rhymes et al., 2014). Building on the previous study, a further year of bi-monthly sampling was carried out to separately assess the degree of potential contamination and

likely sources of contaminants from a) off-site sources entering the site from streams, b) off-site sources entering the site via runoff/overland flow, c) groundwater flowing under the site, and d) on-site sources.

## 2. Materials and methods

### 2.1. Field monitoring strategy

Aberffraw sand dune system is part of an internationally designated conservation site in the European Union Natura network, located on the southwest corner of the island of Anglesey in North Wales, UK (53°11'N, 4°27'W). It is designated for its dune habitats, in particular its dune slack wetlands and the rare plant and invertebrate species they support (Curreli et al., 2013). The site is in a low valley surrounded on three sides by agricultural land. The agricultural land is reseeded and fertilised pasture used for sheep and cattle grazing, with feed stations on land immediately adjacent to the south-east dune site edge (Fig. 1). Streams A and B (Fig. 1) drain this heavily fertilised agricultural and both lead onto the site. Flow in stream A is episodic and flows primarily in winter, compared with the permanent and faster flowing stream B. Annual long term average rainfall at the site is 847 mm (Stratford et al., 2013). There are a number of potential pathways by which nutrients and coliforms can enter the site; these include streams and ditches, surface runoff draining agricultural land and flowing onto the site, seepage of nutrients into the groundwater flowing under the site, and on-site sources such as grazing cattle and rabbits. Previous work has previously shown a nitrogen groundwater contamination gradient that extends into the site from the fertilised pastureland on the south east border with groundwater travelling in a south westerly direction (Fig. 1) (Rhymes et al., 2014). To determine the nature and pathways of the contamination, measurements were made bimonthly for a 12 month period (i.e. 6 sample periods) across streams, ditches, standing surface water and groundwater in dune slacks. Stream samples were collected from two streams (A and B) entering the site (Fig. 1). Samples were collected from upstream sampling points (A1 & B1) and downstream sampling points (A2 & B2) by dipping a clean collecting container into the surface flow. Groundwater samples within dune slacks were measured from fifteen groundwater monitoring piezometers across the site, installed to 2 m depth. Four piezometers (Fig. 1, triangles) aimed at looking at impacts from surface runoff. The remaining eleven aimed at evaluating potential gradients in the groundwater of water chemistry, natural fluorescence and TC and *E. coli* abundance with distance from the contamination sources on the south-east edge of the site (Fig. 1, squares). Samples were collected from the top 10 cm of the water table at each well using a sterilised pump and tubing, which was disinfected with Trigene and flushed three times with deionised water between samples into 250 mL sterile plastic bottles. During periods of inundation, for up to 4 months between November and February (Rhymes et al., 2014), when water tables were above ground level in certain slacks, samples of the standing water above the piezometer were taken. Groundwater depth was measured monthly at each piezometer. For fluorescence and *E. coli* measurements, sampling was conducted for 4 of the 6 sampling periods.

### 2.2. Water chemistry analysis

Samples from piezometers and streams were analysed within 24 h of collection and stored in darkness at 5 °C prior to chemical analysis. In the laboratory groundwater pH was recorded for each sample which was then filtered through a 0.45 µm nylon syringe filter (Avonchem™). Dissolved inorganic anions (fluoride, chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then quantified on an ion chromatograph (Metrohm, UK Ltd.). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO<sub>3</sub>-N, NO<sub>2</sub>-N and NH<sub>4</sub>-N. Total nitrogen (TN) and total carbon were analysed by thermal oxidation on a thermalox TOC/TN

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