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Evidence for sensitivity of dune wetlands to groundwater nutrients

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Highlights

- We studied a dune system impacted by groundwater nutrients.
- Groundwater nutrients affected vegetation and soils in dune slack wetlands.
- Change in vegetation and soil were observed at 0.2mg/L of DIN within groundwater.

Abstract

Dune slacks are seasonal wetlands, high in biodiversity, which experience considerable within-year and between-year variations in water-table. They are subject to many pressures including climate change, land use change and eutrophication. Despite their biological importance and the threats facing them, the hydrological and nutrient parameters that influence their soil properties and biodiversity are poorly understood and there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations. In this study we examined the impact of groundwater nutrients on water chemistry, soil chemistry and vegetation composition of dune slacks at three distance classes (0-150 m, 150–300 m, 300–450 m) away from known (off-site) nutrient sources at Aberffraw dunes in North Wales, whilst controlling for differences in water-table regime. Groundwater nitrate and dissolved inorganic nitrogen (DIN) and soil nitrate and nitrite all had significantly higher concentrations closest to the nutrient source. Multivariate analysis showed that although plant species composition within this site was primarily controlled by water table depth and water table fluctuation, nitrogen from groundwater also influenced species composition, independently of water table and soil development. A model containing all hydrological parameters explained 17% of the total species variance; an additional 7% was explained following the addition of NO_3 to this model. Areas exposed to elevated, but still relatively low, groundwater nutrient concentrations (mean 0.204 mg/L +/- 0.091 of DIN) had greater abundance of nitrophilous species and fewer basipholous species. This shows clear biological impact below previously suggested DIN thresholds of 0.20 – 0.40 (mg/L).

Keywords:

Dune slacks; Nitrogen; Groundwater; Contamination; Sand dunes; Ecohydrology

1.Introduction

Sand dune systems have a global distribution (Martinez et al. 2004) and support a high biodiversity, including many threatened plant, insect and animal species (Rhind and Jones, 2009; Howe et al. 2010). They contain seasonal wetlands, known as dune slacks, which support a particularly diverse flora in Europe (Grootjans, 2004), including red list species such as the fen orchid *Liparis loeselii* and the liverwort *Petalophyllum ralfsii*.

Sand dune systems have undergone considerable change globally in the last Century (Martinez et al. 2004). In temperate European dune systems these drivers include: changes in land use, crashing rabbit populations, climate change and eutrophication (Provoost et al., 2011; Jones et al. 2011; Beaumont et al. 2014). With regard to the latter; nutrients from atmospheric deposition have increased dramatically from their pre-industrial levels of 2 – 6 kg N ha^{-1} yr^{-1} (Fowler, 2004). As a consequence, the critical load defined for dune slacks, 10-15kg N ha^{-1} yr^{-1} (Bobbink and Hettelingh, 2011), is exceeded across much of Europe. While the effects of atmospheric deposition have received recent attention in dry dune habitats (Plassmann et al., 2009; Remke et al. 2009; Jones et al. 2013), relatively little attention has been given to the impact of other sources of nutrients in dune wetlands, indeed in wetlands in general, and the issue of groundwater or surface water-derived nutrients is not explicitly considered within atmospheric critical loads frameworks. In dune systems that are not isolated hydrologically from surrounding groundwater, there is the potential for nutrient inputs to these habitats from agricultural and other sources via groundwater to add to the nutrient load already received from atmospheric deposition. A collation of dune groundwater chemistry data (Davy et al., 2010) suggested that values > 1 mg/L dissolved inorganic nitrogen (DIN) in dune groundwater indicated probable nutrient contamination of the groundwater within a site, while concentrations above 0.2 mg/L may also signify contamination. A global assessment of aquatic ecosystems concluded that concentrations above 0.5 – 1.0 mg/L of total nitrogen could lead to eutrophication (Camargo and Alonso, 2006). There have been studies in the Netherlands on impacts of highly eutrophic river water around drinking water infiltration ponds (Meltzer and van Dijk, 1986). However, there have been no empirical studies to date testing for biological effects in dune systems resulting from groundwater nutrients at low concentrations.

Species distribution within these ecosystems is governed primarily by water table depth, seasonal water table fluctuations and water chemistry (Curreli et al., 2012; Grootjans et al., 1996; Lammerts et al., 2001; Lammerts et al., 1992; Willis et al., 1959). Yet, there remains a major knowledge gap as to how groundwater nutrients may affect dune slack vegetation and at what concentrations (Jones et al. 2006). Studies of atmospheric nitrogen deposition impacts have been made in many habitats (e.g. Phoenix et al. 2012), with the potential for community shift in extreme cases such as conversion of heathlands into grasslands (Heil and Diemont, 1983). However, in dune slacks there is still relatively little empirical evidence of nutrient impacts either from atmospheric deposition or from other sources, especially at realistic N loads. One of the few studies, using high nutrient loads on dune vegetation at Braunton Burrows demonstrated that *Agrostis stolonifera* dominated a dune slack following surface additions of N and P (Willis, 1963).

Dune slack water tables tend to be at their highest in winter and fall in the summer months (Van Der Laan, 1979) as the water table is highly dependent on precipitation and evaporation. Water tables can also vary substantially from year to year (Ranwell, 1959; Stratford et al. 2013), causing periods of drought and flooding which affect the period in which the rooting zone is in contact with the water table.

These fluctuations also play an important role in controlling nutrient composition within the soils. During periods of high water level, mineralisation of organic matter is reduced thus conserving the low nutrient status favoured by dune slack species (Berendse et al., 1998). Soil processes are important in regulating the impacts of N. Soil exchange sites may actively bind ammonium from the groundwater during periods of inundation, while denitrifying bacteria may release nitrogen back into the atmosphere (Myrold, 1998).

The aim of this investigation was to examine the impact of nutrients on dune groundwater chemistry, soil chemistry and botanical composition along gradients of nutrient input from known sources, and controlling for differences in water-table regime. We tested the following hypotheses: Does nutrient contamination from off-site sources extend into the groundwater under the dune system? If nutrients are present in the groundwater, is there any evidence in the plant assemblages and soils of dune slacks that these nutrients are accessible to the vegetation in the dune slacks, and do they have an adverse ecological impact on the plant community composition?

2.Methods

2.1 Site Description

Aberffraw dunes are located on the south west corner of the island of Anglesey in North Wales, UK (53°11’N, 4°27’W). The site extends for 1km in width and 3 km inland (Fig 1). A small lake, Llyn Coron bounds the north east edge of the system and feeds the river Afon Fraw, which flows along the north-west edge of the dunes down to the sea. The site is in a low valley surrounded on all 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 boundary. A number of streams and ditches draining this heavily fertilised agricultural area lead on to the site.

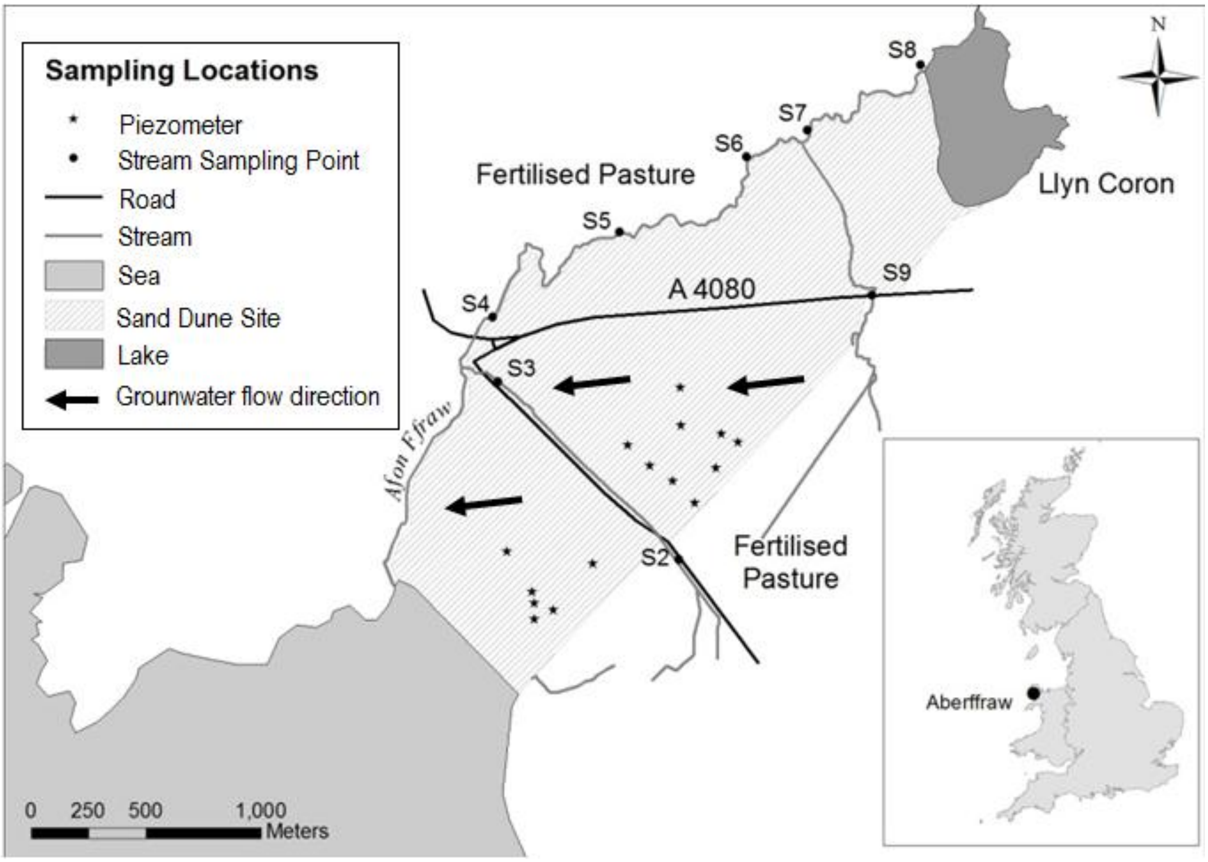

Fig 1- Map of Aberffraw dune system, showing all piezometers and stream (S2 - S9) sampling points. S1 (not shown) was an episodic stream and data were only collected from this sampling point for one month. Cross-hatched area represents designated site. Redrawn from Ordnance Survey.

2.2 Groundwater flow direction

In a preliminary survey, elevation of the water table at each piezometer and at additional locations around the site – measured by auguring down to the water table and then referred to ground surface elevation measured using a Leica 1200 RTKGPS, with a vertical accuracy of ±1 cm, and correcting for water table depth. Groundwater flow direction was estimated by contour analysis in ArcGIS v10.1.

2.3 Sampling design

A preliminary survey was carried out whereby water samples were collected by drilling down to the water table with an augur and sampling the groundwater with a hand pump. This established that there was a possible nitrate contamination gradient that extended into the site from the fertilised pastureland on the south-east site boundary. In order to quantify the possible effects of this contamination 15 piezometers, 2 meters in depth with full-length slotted screens of 0.3mm slots covered by mesh were installed. Installation was restricted to dune slack areas as this is where vegetation and rooting zone are in contact with the groundwater and where impacts are most likely to occur. The sampling strategy aimed at evaluating gradients in water chemistry within three distance classes from the south-east site boundary (0 – 150 m, 150 – 300 m and 300 – 450 m).

2.4 Hydrological monitoring and water chemistry sampling

Monthly manual measurements of groundwater levels were taken from 15 piezometers using a water level meter (Boart longyear), starting in March 2012 for a period of 12 months. Water samples were collected monthly from the top 10 cm of the water table at each piezometer. During periods of inundation, when water table was above ground level in certain slacks, samples of the standing water above the piezometer were collected. Water samples were also collected from streams entering or nearby the site (Figure 1), which could potentially contribute to groundwater nutrients via seepage from the stream bed. Stream water samples were collected at the same time as groundwater, by dipping a clean collecting container into the surface flow. Samples were stored in darkness at 5°C prior to chemical analysis. pH was recorded for each sample which was then filtered through 0.45 µm nylon syringe filter (Avonchem™). Dissolved inorganic anions (chloride, nitrite, nitrate, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on an ion chromatograph (Metrohm, UK Ltd.). Detection limits for all anions and cations were 0.005 mg/L apart from nitrite (0.003 mg/L), nitrate (0.002 mg/L) and ammonium (0.001 mg/L). Dissolved Inorganic Nitrogen was calculated as the sum of NO_3-N, NO_2-N and NH_4-N.

2.5 Botanical Survey

At each of the 15 piezometers vegetation was surveyed in three 1m x 1m quadrats. The quadrats were placed at a 3m distance from the piezometer and arranged on cardinal bearings (North, West and East). Species occurrence was recorded using visual estimates of % cover for all species of vascular plants, bryophytes and lichens. Nomenclature follows Stace (2010) for vascular plants and Hill et al. (1994) for bryophytes. Cover of bare ground and litter were also recorded. The location of each quadrat was recorded at its centre using a Leica 1200 RTKGPS. Mean UK-modified Ellenberg indicator values (Hill et al., 1999, Hill et al., 2007) were then calculated for each quadrat using species presence data.

2.6 Topographical resolution

Elevation of the ground surface at each piezometer and quadrat was measured using the Leica 1200 RTKGPS to 1 cm vertical resolution, which allowed groundwater levels for each quadrat to be calculated using their relative elevation difference from the nearest piezometer.

2.7 Soil Sampling

At each quadrat a soil core (5cm diameter, 15cm depth) was collected and stored in darkness at 5°C, prior to analysis. The thickness of the organic horizon was recorded and any vegetation and large roots were removed. The soil was then homogenised by hand and a sub sample (10-15g field moist soil) was weighed and dried at 105°C and reweighed to measure moisture content. The samples were then re-heated in a furnace at 375°C for 16hrs and re-weighed to determine organic matter content through Loss on Ignition (Ball et al. 1964).

A sub-sample was prepared for chemical analysis using a water extraction of 10g homogenised sample of fresh soil, mixed with 10ml of ultra-high purity water (1:10wt/vol) on a laboratory blender (Stomacher 80, Seward UK). pH was recorded using a calibrated pH electrode and electrical conductivity was measured using a conductivity meter (Primo 5, Hanna Instruments Ltd UK).The remaining solution was centrifuged for 15mins at 5000rpm and filtered through 0.45 µm nylon syringe filter (Avonchem™). Organic anions (chloride, nitrite, phosphate and sulphate) and cations (sodium, ammonium, potassium, calcium and magnesium) were then measured on the Metrohm ion chromatograph, detection limits described above.

2.8 *Rooting depth*

Soil pits > 30cm wide and 1 m deep were dug at 5m distance from six of the piezometers in order to measure rooting depth. Three of these were dug in slacks with a hydrological regime supporting wet slack vegetation communities and three in dry slack communities. On one clean vertical face in each soil pit, the number of visible roots in a 30 cm wide x 20 cm deep section were recorded at 4 depth bands below the surface (-20 to -40 cm, -40 to -60 cm, -60 to -80 cm and -80 to -100 cm). It was not possible to count visible roots in the main rooting zone (top layer 0 to -20 cm) due to the high abundance of roots.

2.9 Statistical Analysis

Quadrats and piezometers were grouped into three classes based on their distance from the south-east site boundary (See Fig. 1) (0-150 m N=15, 150-300 m N=18, 300-450 m N=12). Monthly groundwater (including inundation samples) and streamwater (N=8) chemistry values and pH for each sampling point were averaged to give an annual mean, as preliminary analysis showed no seasonal differences in groundwater chemistry. Data from the three soil samples around each piezometer were also used to test for statistical differences among distance classes using analysis of variance (Minitab v16). Analysis of soil chemistry variables included annual maximum water table elevation as a co-variable. Data that proved not normally distributed (Kolmogorov-Smirnov Test) were transformed using a Johnson's transformation. Where transformation was not sufficient to achieve assumptions of normality- (Soils: phosphate, sodium and ammonium. Groundwater: sulphate and potassium) a non-parametric Kruskal-Wallis Test was carried out. Differences in root abundance between wet and dry slack community soil pits were assessed using analysis of variance using Minitab v16.

Relationships between vegetation and measured soil and water variables were sought using multivariate analyses. An initial DCA of the 45 vegetation quadrats tested the length and strength of the first gradient whilst relationships between vegetation and environmental variables were explored through indirect gradient analysis using PCA. The significance of the relationships with environmental variables was tested singly and within models using Redundancy Analysis (RDA) Monte Carlo methods within CANOCO.

3. Results

3.1 Groundwater direction, groundwater and stream chemistry

The preliminary topographical and water level survey showed that the direction of groundwater flow is approximately westerly (Fig.1). The summary data of annual piezometer water chemistry (Table 1) showed significant differences in annual mean groundwater nitrate concentrations of the piezometers in the three classes with those in the 0-150 m class (0.885 +/- 0.283 mg/L) being significantly greater than those in the 150-300 m (0.360 +/- 0.147 mg/L) or 300-450 m classes (0.092 +/- 0.046 mg/L). Significant difference was also found in annual mean groundwater dissolved inorganic nitrogen concentrations, with those in the 0-150 m class (0.204 +/- 0.091 mg/L) again being significantly greater than those in the 150-300 m (0.084 +/- 0.034 mg/L) or the 300-450 m classes (0.0224 +/- 0.011 mg/L). All other piezometer water chemistry variables showed no significant difference among classes. Nitrate and phosphate concentrations were an order of magnitude higher in the streams running through the site than in the dune groundwater, even in the class of piezometers nearest the south-east site boundary.

Chemistry and pH	Groundwater	Streams
Chloride	68.717 ± 2.249	44.141 ± 2.421
(mg/L)	(16.113, 190.945)	(22.707, 211.436)
Nitrite	0.008 ± 0.001	0.042 ± 0.004
(mg/L)	(0.003, 0.185)	(0.005, 0.211)
Nitrate	**0.468 ± 0.112**	10.945 ± 1.438
(mg/L**)**	**(0.002, 16.706)**	(0.003, 86.833)
Phosphate	0.006 ± 0.000	0.058 ± 0.010
(mg/L)	(0.005, 0.041)	(0.005, 0.520)
Sulphate	16.887 ± 0.872	14.376 ± 0.699
(mg/L)	(1.088, 77.230)	(1.550, 41.391)
Sodium	35.072 ± 1.093	25.806 ± 0.997
(mg/L)	(13.130, 92.294)	(0.005, 123.956)
Ammonium	0.036 ± 0.005	0.031 ± 0.005
(mg/L)	(0.001, 0.585)	(0.003, 0.221)
Potassium	1.857 ± 0.079	4.398 ± 0.330
(mg/L)	(0.005, 6.223)	(0.005, 15.310)
Calcium	83.723 ± 1.336	48.486 ± 1.657
(mg/L)	(40.847, 187.050)	(0.020, 94.846)
Magnesium	7.086 ± 0.167	8.655 ± 0.184
(mg/L)	(0.005, 14.720)	(0.005, 21.234)
Dissolved inorganic N	**0.108 ± 0.002**	2.485 ± 0.030
(mg/L)	**(0.001, 3.829)**	(0.002, 19.609)
pH	7.511 ± 0.021	7.715 ± 0.027
	(6.804, 8.473)	(7.194, 8.620)

Table 1

Summary of annual mean water chemistry from piezometers and streams; values for each variable are expressed as mean ± standard error and brackets show minimum and maximum values. Values in bold show significant differences in groundwater chemistry among distance classes (see text).

Nitrate concentrations at all stream sampling points (Fig.2) do not exceed 20 mg/L apart from S2 which considerably exceeds this concentration. They show a slight seasonal trend with concentrations lower in summer than in winter. By contrast, the stream S2 which drains from the south-east boundary into the site shows a steep and rapid increase in nitrate concentration from mid-April that exceeds 50 mg/L for four months, peaking at 87 mg/L in June before rapidly declining from June to September to concentrations similar to other stream sampling points.

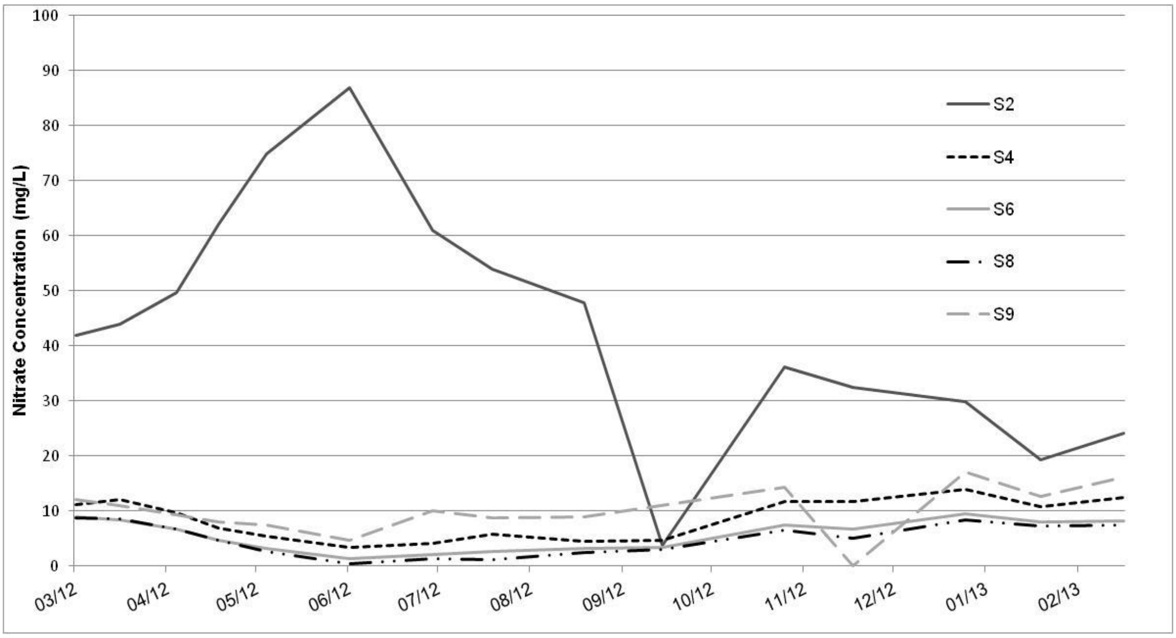

Fig 2- Monthly nitrate concentrations (mg/L) from four representative stream sampling points and from divergent S2 (see Fig 1) over a 12--month period. Samples from streams S3, S5, S7 not shown for clarity, but were not significantly different from S4-S9 shown here.

3.2 Soils

A summary of soil physico-chemistry parameters for the three classes of quadrats are shown in Table 2. Significantly higher soil nitrite concentrations occurred in the 0-150 m class (0.090 +/- 0.033 µg g $^{-1}$ dry soil) compared with that of the 150-300 m class (0.034 +/-0.010 µg g $^{-1}$ dry soil) and the 300-450 m class (0.046 +/- 0.031 µg g $^{-1}$ dry soil). Soil nitrate concentrations were also significantly greater within the 0-150m class (1.967+/- 0.515 µg g $^{-1}$ dry soil) compared with that of both the 150-300 m (0.612 +/-

0.155 µg g^{-1} dry soil) and 300-450 m class (1.087+/- 0.730 µg g^{-1} dry soil). No significance among classes was found for soil dissolved inorganic nitrogen and for all other variables.

Table 2

Summary of soil physico-chemistry parameters for categorised quadrats located 0-150 m (n=15), 150-300 m (n=18) and 300-450 m away from the south-east site boundary (n=12). Values for each variable are expressed as mean ± standard error, brackets show minimum and maximum values for each class. Significant differences among distance classes shown in bold; values denoted by the same letter not significantly different from each other.

Variable		Distance from Fence		
		0-150	150-300	300-450
		n=15	n=18	n=12
Soil Chemistry (µg g^{-1} dry soil)	Chloride	4.105 ± 0.243	3.742 ± 0.334	4.532 ± 0.573
		(2.307, 5.597)	(2.318, 7.950)	(2.337, 10.006)
	Nitrite	**0.090 ± 0.033**^{A}	**0.034 ± 0.010**^{B}	**0.046 ± 0.031**^{B}
		(0.020, 0.519)	**(0.005, 0.159)**	**(0.005, 0.390)**
	Nitrate	**1.967 ± 0.515**^{A}	**0.612 ± 0.155**^{B}	**1.087 ± 0.730**^{B}
		(0.397, 7.838)	**(0.013, 2.485)**	**(0.018, 9.051)**
	Phosphate	0.018 ± 0.005	0.013 ± 0.001	0.013 ± 0.000
		(0.011, 0.081)	(0.012, 0.025)	(0.011, 0.014)
	Sulphate	3.630 ± 0.229	3.713 ± 0.158	3.822 ± 0.441
		(2.176, 5.420)	(2.671, 4.868)	(2.100, 7.302)
	Sodium	2.846 ± 0.660	3.419 ± 0.733	3.279 ± 0.792
		(0.011, 6.886)	(0.012, 7.910)	(0.012, 7.295)
	Ammonium	0.350 ± 0.061	0.367 ± 0.057	0.622 ± 0.210
		(0.012, 0.490)	(0.012, 2.423)	(0.013, 0.849)
	Potassium	3.813 ± 0.610	4.414 ± 0.479	6.174 ± 2.460
		(1.927, 8.228)	(1.214, 35.954)	(0.631, 10.213)
	Calcium	29.244 ± 3.754	28.636 ± 3.185	28.868 ± 2.396
		(10.790, 41.567)	(0.045, 39.389)	(0.034, 52.584)
	Magnesium	1.709 ± 0.639	2.165 ± 0.162	2.169 ± 0.198
		(0.639, 2.339)	(0.412, 3.604)	(1.669, 3.481)
	Dissolved Inorganic N	0.744 ± 0.152	0.434 ± 0.067	0.743 ± 0.321
		(0.041, 0.753)	(0.060, 4.047)	(0.324, 2.588)
Soil Characteristics	pH	7.690 ± 0.147	7.990 ± 0.104	8.054 ± 0.144
		(6.480, 8.370)	(6.952, 8.556)	(6.703, 8.640)
	Electrical conductivity (mS/cm)	28.867 ± 3.216	31.167 ± 2.472	30.667 ± 3.438
		(12.000, 50.000)	(13.000, 48.000)	(11.000, 57.000)
	LOI (%)	5.020 ± 0.421	4.674 ± 0.496	4.728 ± 0.597
		(1.760, 7.618)	(0.464, 9.189)	(2.588, 8.870)
	Observed organic matter	8.286 ± 0.947	9.941 ± 0.860	10.250 ± 1.008

	(cm)	(2.000, 15.000)	(3.000, 15.000)	(5.000, 15.000)

3.3 Vegetation

Figure 3 shows water table variation in relation to rooting depth for two hydrographs typical of a wet and a dry slack community, corresponding to UK National Vegetation Classification SD14d (*Salix repens-Campyllium stellatum* dune slack, *Festuca rubra* subcommunity) and SD15b (*Salix repens-Calliergon cuspidatum* dune slack, *Equisetum variegatum* subcommunity) respectively (Rodwell 2000). Both wet and dry slack communities reveal an asymmetric seasonal pattern whereby the water depth drops steadily over the summer period and increases rapidly in early winter (Fig. 3a). The summary data for hydrological parameters (Fig. 3b) shows the range of fluctuation in water depth with the average minimum at -105 cm and maximum at 48 cm (i.e. above ground surface), with one piezometer as high as 193 cm. The dry slack community has a lower water depth than that of a wet slack all year around, with the greatest differentiation occurring in the drier months of summer (Fig. 3a). Roots were significantly more abundant at depths -40 to -60 cm in dry communities than in wet communities (Fig. 3c), there was no significant difference at all other depths. In the wet slack vegetation community, the main rooting zone (0 to -40 cm) is in contact with the water table for approximately 8 months. In the dry slack vegetation community, the main rooting zone (0 to –60 cm) is also in contact with the water table for a similar duration, suggesting rooting depth is constrained by water levels.

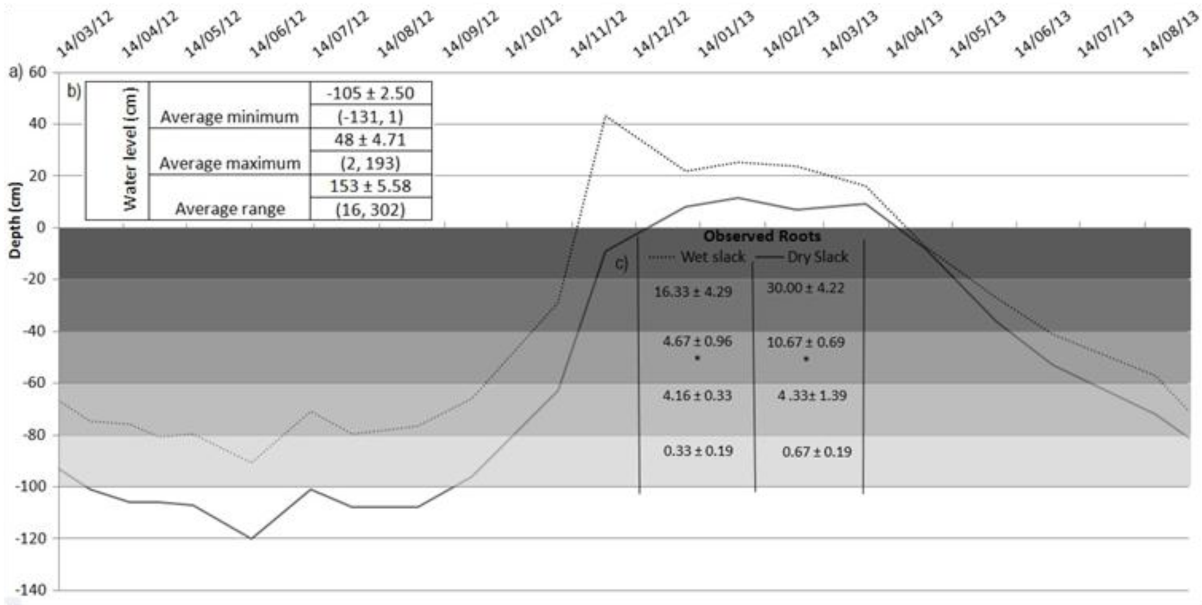

Fig 3- a) Annual hydrographs of two piezometers from a wet and a dry slack. b) Annual average minimum, maximum and range water level data from 13 piezometers expressed as mean ± SE, brackets show minimum and maximum values. c) Visible roots observed at 4 rooting depth zones (-20 to -40 cm, -40 to -60 cm, -60 to -80 cm and -80 to -100 cm) expressed as mean ± SE. Asterisks denote significant difference between root abundance in dry slacks SD 14d and wet slacks SD 15b.

The PCA plot shows the distribution of the 45 quadrats, coded by their distance to the south-east site boundary, with environmental variables overlain to aid interpretation (Figure 4). The overlain environmental variables suggest that axis 1 (Fig.4 a) relates to a hydrological gradient in which annual maximum water level, water level range and Ellenberg F were negatively associated with the axis. i.e. high water tables were found to the left of the diagram, corresponding to low scores on axis 1. The low axis 1 scores (Fig.4 b) were occupied by species tolerant of wet soils *Galium palustre*, *Hydrocotyle vulgaris* and *Carex nigra* whereas the highest axis 1 scores were occupied by drier species such as *Lotus corniculatus* and *Trifolium repens*. Axis 2 (Fig.4 a) related to a combined soil development/nutrient axis where groundwater NO_2 concentrations, soil NO_3 concentrations and Ellenberg N were positively linked with axis 2, and soil pH and Ellenberg R were negatively associated with the axis. The overlain species data reinforce this pattern, with low axis 2 scores (Fig.4 b) occupied by species with higher base status demand such as *Campyllium stellatum* and *Equisetum variegatum* whereas high scores were revealed by higher fertility species e.g. *Rubus caesius* and *Potentilla reptans.* There was no clear separation of quadrats relative to distance to south-east site boundary on Axis 1, however axis 2 (Fig.4 b) segregated the 0-150 m class from the 300-450 m class, with quadrats in the 0-150 m class located higher on axis 2.

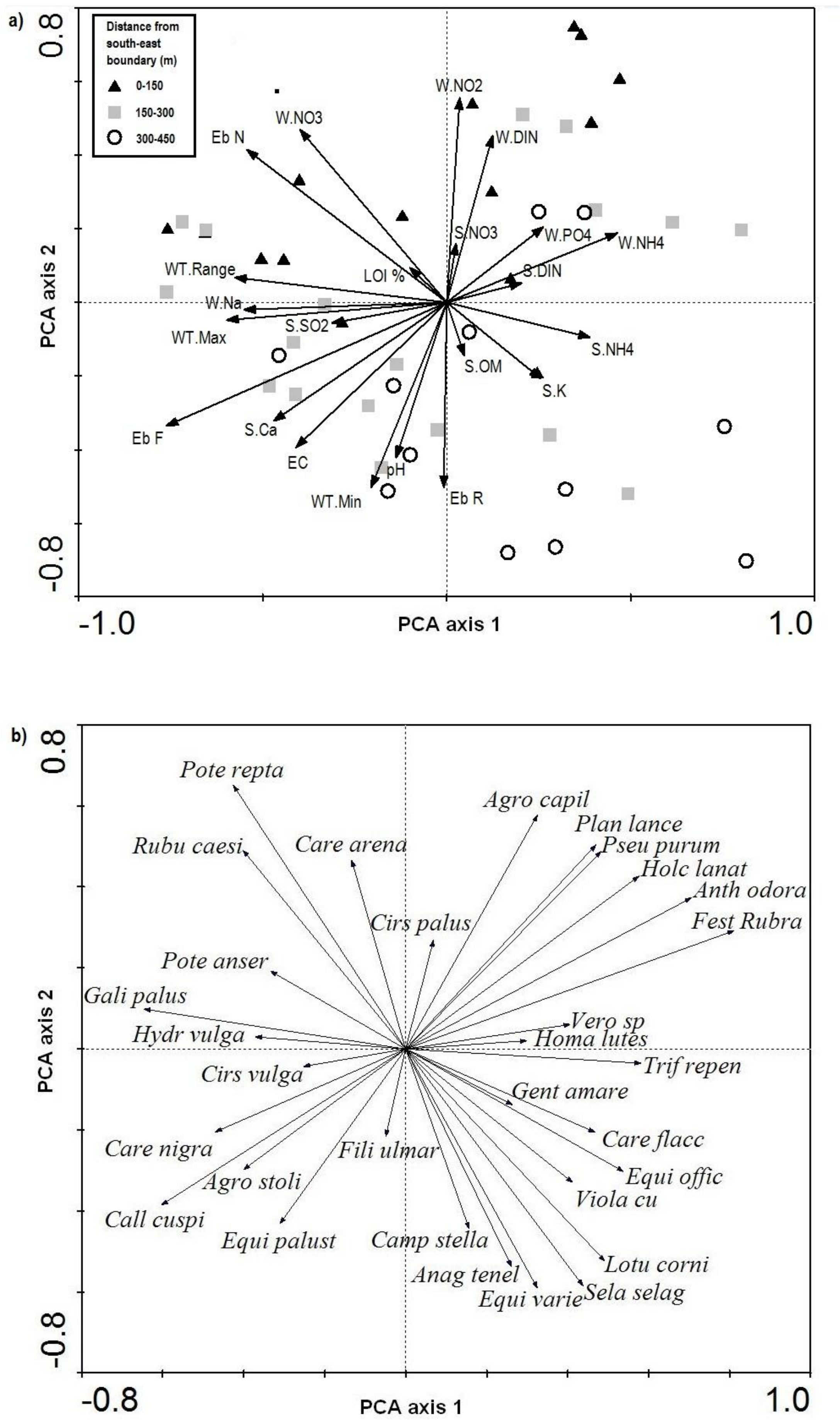
a)
0.8
-0.8
-1.0
1.0
PCA axis 1
PCA axis 2
Distance from south-east boundary (m)
0-150
150-300
300-450
W.NO2
W.NO3
Eb N
W.DIN
S.NO3
W.PO4
W.NH4
S.DIN
LOI %
WT.Range
W.Na
WT.Max
S.SO2
S.NH4
S.OM
S.K
Eb F
S.Ca
EC
pH
WT.Min
Eb R
b)
0.8
-0.8
-0.8
1.0
PCA axis 1
PCA axis 2
Pote repta
Agro capil
Plan lance
Rubu caesi
Care arena
Pseu purum
Holc lanat
Anth odora
Cirs palus
Fest Rubra
Pote anser
Gali palus
Vero sp
Hydr vulga
Homa lutes
Trif repen
Cirs vulga
Gent amare
Care flacc
Care nigra
Fili ulmar
Equi offic
Agro stoli
Viola cu
Call cuspi
Equi palust
Camp stella
Lotu corni
Anag tenel
Sela selag
Equi varie

Fig 4- PCA analysis: (a) the distribution of environmental variables with PCA scores, quadrats coded by distance to south-east site boundary. Only environmental variables and species with axis scores >0.2 are shown for clarity (except LOI). See Tables 2 and 3 for full list of variables. Prefixes denote the following S- Soil; W-groundwater and WT- water table and pH -soil pH.

Using a Monte Carlo permutation test the model containing all variables was highly significant ($p<0.001$), where the first four axes explained 51.8% of the species-environment relationships and explained 45.9% of the total species variance. Most variables tested singly were significant at 0.001 level (Table 3). A model containing all hydrological parameters explained 17% of the total species variance, showing as expected a degree of co-correlation between hydrological variables. When tested singly, NO_3 explained more of the total species variance than any of the individual hydrological variables. When the influence of all hydrological variables was accounted for in a combined model, adding NO_3 explained an additional 7% of species variance. This shows that species variation due to groundwater NO_3 was largely independent of that due to hydrology, and that NO_3 was significantly affecting plant community composition.

Table 3

Environmental variables illustrating percentage of total species variation explained within RDA and significance, when tested singly.

	Variables	Variance (%)	significance
Hydrological variables	Annual maximum water level (m)	8.80%	***
	Annual minimum water level (m)	7.30%	***
	Annual Range (m)	8.40%	***
Soil variables	S.Ca (µg g^{-1} dry soil)	7.30%	***
	S.NH_4 (µg g^{-1} dry soil)	5.90%	***
	S.Mg (µg g^{-1} dry soil)	5.80%	**
	S.pH	5.30%	**
	S.DIN (µg g^{-1} dry soil)	4.00%	*
	EC (mS/cm^{-1})	7.60%	***

	Soil moisture (%)	4.00%	*
	LOI (%)	2.20%	*
Water chemistry	W.NO_3 (mg/L)	9.00%	***
	W.Na (mg/L)	8.20%	***
	W.NO_2 (mg/L)	7.90%	***
	W.NH_4 (mg/L)	7.20%	***
	W.Cl (mg/L)	6.80%	***
	W.DIN (mg/l)	6.10%	***
	W.Br (mg/L)	5.10%	**
	W.PO_4 (mg/L)	4.80%	**
	W.K (mg/L)	4.60%	*
	W.SO_2 (mg/L)	4.10%	*
	W.Ca (mg/L)	4.00%	*
	W.Mg (mg/L)	4.00%	*
Ellenberg indicators	Eb F	13.60%	***
	Eb N	9.80%	***
	Eb R	6.20%	***
Combined models	Hydrological parameters (min, max + range)	17.0%	***
	Hydrological parameters and groundwater nitrate (min, max, range, W.NO_3)	24.0%	***

* significant at 0.05 level

** significant at 0.01 level

*** significant at 0.001 level

4. Discussion

This study has shown that there is a nutrient contamination gradient that extends from the south-east site boundary into the site which is significantly affecting groundwater nitrate and dissolved inorganic N concentrations, soil nitrate and nitrite concentrations and vegetation composition.

Results suggest that the contamination is sourced from the south-east fertilised pasture land and is likely to be due to fertiliser application. Concentrations of nitrate samples from stream S2, which flows onto the site, exceed the 50 mg/L nitrate vulnerable zones designation threshold (Environment agency, 2012) and the 50 mg/L World Health Organisation's guideline value for drinking water. Contamination is not likely to be due to manure within the site, or from the adjoining pasture land as ammonium and phosphate concentrations are relatively low within the streams, groundwater and soils. In sandy soils with low water holding capacity it is probable that nitrate is rapidly leached post fertiliser application (Skiba and Wainright, 1984), particularly after heavy periods of rainfall and in turn is contaminating the groundwater. The sandy nature of the pasture land at Aberffraw allows groundwater to carry pollutants in a westerly direction into the site but the flow rate is unknown, although a study carried out at a nearby site, Newborough Warren, determined that groundwater flows at a speed of 39.6 m/year (Betson et al., 2002). This suggests at least 3 years of contamination as NO_3 concentrations are elevated at up to 150 m into the site. Although knowledge of the local land management history suggests that the adjacent farmland has been intensively managed for several decades and such nutrient concentrations are unlikely to be a recent phenomenon. Since nitrate concentrations determined from stream S2 are much greater than those determined in the groundwater, this suggests that the spatial extent of contamination could represent a number of possibilities: 1) An equilibrium caused by physical dilution and mixing with uncontaminated rainwater that infiltrates through the sand or 2) A result of processing and denitrifying N within the sandy body, or 3) A combination of both processes. Further work is required to assess to what extent dilution and denitrification play a significant role in reducing NO_3 concentrations in this aquifer.

The observed nitrate and nitrite soil gradient is likely to be due to uptake from the groundwater during the winter and spring months, when water tables are at their highest and plant roots are in direct contact with the groundwater or capillary fringe. This allows for possible nutrient uptake by the plants and subsequently the nutrients return to the soil surface via litter fall (Berendse et al., 1998), and direct binding of ammonium by the soil. As farming on this pastureland has been carried out for decades it is likely that the nutrient gradient has accumulated over time, but has not yet lead to significantly increased organic matter accumulation. This could be due to microbial processes maintaining low nutrient levels, as denitrification has been found to significantly increase with NO_3 availability (Merrill and Zak, 1992). It is also likely that in areas of contamination a shift in the microbial community composition has occurred, which supports higher levels of microbial activity (Peacock et al., 2001) and therefore maintaining low organic matter build up. Although denitrification rates can also be limited by other nutrients such as available carbon (Weier et al., 1993).

Assessment of the rooting zones has determined that the water table is likely to be a major factor controlling the rooting depth and as a result the main rooting zones within wet slacks are found in the shallower 0 to -40 cm zone compared with those within dry slacks in the deeper -0 to -60 cm zone. With differing water table regimes in both communities and the effects of capillary reaction, which carries substantial amounts of water 45 cm above it (Ranwell, 1959), both wet and dry slacks main rooting zones are exposed to groundwater for similar periods of the year and therefore are equally vulnerable to groundwater contamination.

Although the main determinant of species composition was water table depth and water table fluctuation, in broad agreement with the literature (e.g. Lammerts et al. 2001), RDA analysis showed that nitrogen was strongly influencing species composition independently of water table and soil development. The results suggest that with increasing availability of N basiphilous species have decreased, while species with higher nutrient status have increased.

If nitrogen pollution within this system continues it is likely that over time the slacks will become more eutrophic, resulting in greater productivity, more rapid soil development, increase in succession rate and loss of species (Jones et al., 2008). Other issues of concern are the projected changes in hydrological regimes, due to climate change, from wet dune slack regimes to dry grassland regimes (Curreli et al., 2012). This is likely to increase the mineralisation of organic matter, such that the desired low nitrogen and phosphorus conditions are not preserved (Lammerts and Grootjans, 1997), which will further exacerbate the eutrophication issue. This study is the first evidence that shows biological impact caused by DIN groundwater concentrations below 0.2mg N/L within dune wetlands, which is below threshold concentrations described by Davy et al. (2010) and Camargo and Alonso (2006).

5. Implications for management

Sandy soils contain very little organic matter or cation exchange sites and therefore have low potential to store nitrogen in the soil, and leach nitrate readily (Rowell, 1994). As a result, it is more cost effective for farmers operating on sandy soils to only apply enough nitrogen that can be directly utilised by the crop. Site specific measures to reduce excess N leaving the site could include a new fertiliser application regime whereby less fertiliser is applied in more frequent doses which will reduce the loss of nitrate through leaching, and installation of fenced buffer zones along pastureland edges and ditches to enhance filtration of nutrients and decrease the rates of runoff (Patty et al., 1997).

6. Conclusions

Aberffraw dune system is exposed to a nutrient gradient in groundwater which is likely to be caused by farming practices on surrounding pastureland. Plant species composition of dune slack wetlands within this site is primarily controlled by water table depth and water table fluctuation. However nitrogen from groundwater is influencing species composition independently of water table and soil development, with evidence of an increase in more eutrophic species and a decrease in basiphilous species in affected areas. While there is increasing evidence of N impacts in dry dune habitats (e.g. Jones et al. 2004; van den Berg et al. 2005; Kooijman 2004; Jones et al. 2013), this is the first field-based evidence for impacts of N in dune slacks at relatively low groundwater nutrient concentrations. This study highlights two key findings: Impacts have been observed at very low nutrient concentrations of around 0.2 mg/L DIN, reinforcing potential impacts on aquatic systems at low levels of N (Camargo and Alonso, 2006). Further, it shows that groundwater nutrient inputs need to be considered in addition to atmospheric N inputs in wetland systems. However, additional work is needed to determine the fluxes of N entering the site, in order to match the critical load approach. Experimental approaches to investigate groundwater nutrient impacts would also be useful, but technically difficult to implement.

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