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Small changes in water levels and groundwater nutrients alter nitrogen and carbon processing in dune slack soils

J. Rhymes [1*], L. Jones [2], H. Wallace [3], T.G. Jones [1], C. Dunn [1], N. Fenner [1].

[1]Bangor University, UK; [2]Centre for ecology and hydrology, UK; [3] Ecological surveys (Bangor), UK

*Corresponding author: jenny_rhymes@outlook.com

1.1 Abstract

Dune slacks are biodiverse seasonal wetlands which experience considerable fluctuation in water table depth. They are under threat from lowered water tables due to climate change and water abstraction and from eutrophication. The biological effects caused by the interactions of these pressures are poorly understood, particularly on soil processes. We used a mesocosm experiment and laboratory assays to study the impact of lowered water tables, groundwater nitrogen contamination, and their synergistic effects on soil microbial processes and greenhouse gas emissions. This study showed that just a 10 cm decrease in water table depth led to a reduction in denitrification and to a corresponding increase in soil nitrogen content. Meanwhile N_2O emissions occurred for longer durations within dune slack soils subject to higher concentrations of groundwater nitrogen contamination. The results from extracellular enzyme assays suggest that decomposition rates increase within drier soils shown by the increase in β-glucosidase activity, with further sensitivity to groundwater nitrogen contamination shown by the increase in phenol oxidase activity. Dune slack soils with a 10 cm lower water table had significantly lower methane emissions,

nearly 5 times lower in the drier soils. Our findings demonstrate that dune slacks are sensitive to both small changes in groundwater levels and to groundwater nitrogen contamination. The biological impacts from lowered water tables are likely to be intensified where there is also groundwater nitrogen contamination.

1.2 Introduction

Wet dune slacks are seasonal wetlands occupying low-lying areas within a sand dune system which support a diverse flora of high conservation value (Grootjans et al., 2004). They are subject to seasonal variations in water tables, with water tables highest during the winter and falling during the summer (van der Laan, 1979; Stratford et al. 2013). These fluctuations play a key role in controlling nutrient and carbon processes within dune slack soils, conserving the low nutrient status required by dune slack species (Berendse et al., 1998). These habitats are however, at threat from eutrophication and lowered water tables from climate change and/or water abstraction. It is therefore of importance to identify the effect of predicted water table lowering (Clarke and Ayutthaya, 2010) and increases in nitrogen availability (Galloway and Cowling, 2002) on dune slack soil biogeochemistry and nutrient cycling.

The impacts of atmospheric nitrogen deposition on dry dune habitats have been investigated in a number of studies (e.g. Plassmann et al., 2009, Remke et al., 2009, Jones et al., 2013). Far fewer studies have investigated the impacts of nitrogen inputs on dune slack ecology (e.g. Willis et al., 1959, Plassmann et al., 2010). In particular, the role of groundwater rather than atmospheric nutrient inputs is little studied. In The Netherlands, studies have showed the effects of high nitrogen and phosphorus concentrations in groundwater on the botanical composition of dune wetlands (Meltzer & van Dijk 1986), while a recent UK study focusing on nitrates provides evidence of impacts from dissolved inorganic nitrogen (DIN) contamination at concentrations as low as 0.2 mg/L (Rhymes et al., 2014).

Denitrification is important in regulating nitrogen concentrations within wetland ecosystems (Camargo and Alonso, 2006), including dune slack habitats that are vulnerable to nitrogen contamination (Seitzinger et al., 2006). Denitrification rates are controlled by multiple factors including soil moisture content (Hefting et al., 2004), nitrate concentrations (Merrill and Zak, 1992) and soil O_2 levels (Burgin et al., 2010). During periods when dune slack soils are waterlogged, the anaerobic conditions for denitrification are met (Berendse et al., 1998) and soil nitrate is reduced to gaseous nitrogen products (N_2, N_2O and NO) by microbial processes (Knowles, 1982). Under

complete anaerobic conditions N_2 is the end product, at higher oxygen levels denitrification stops with the formation of NO_x (Brady and Weil, 2002). N_2O production however, tends to occur at low soil pH or high nitrate concentrations. The measurement of N_2O within wetland studies is therefore often used as an indicator of soil denitrification (Bernot et al., 2003, DeLaune and Jugsujinda, 2003) as it is difficult to measure N_2 production against high atmospheric N_2 background concentrations (Groffman et al., 2006).

Decomposition rates are controlled by temperature and soil moisture content, where cooler and wetter soils reduce soil decomposition and subsequently increase soil development (Jones et al., 2008). In systems which are N limited, elevated nitrogen inputs tend to increase decomposition rates. Decomposition can be measured by soil respiration, an indicator of aerobic microbial decomposition, and by methane emissions, an indicator of anaerobic microbial decomposition of soil organic matter (Whalen, 2005).

The measurement of extracellular enzyme activities within soils can further quantify biogeochemical processes linked to nutrient and carbon cycling, allowing an understanding of microbial ecology under different environmental conditions. Extracellular enzyme activities and their response to environmental change have been investigated in multiple soil types (Henry, 2012), however, to our knowledge these measurements have not been carried out within dune slack soils. The hydrolase enzyme N-acetyl-β-glucosaminidase (NAG) is responsible for the breakdown of chitin, an essential process in nitrogen cycling (Kang et al., 2005) and β-glucosidase (BG) for the degradation of cellulose to glucose, providing one of the most important sources of labile carbon for soil microbes (Deng, 2011). Phenol oxidase enzyme (POX) degrades phenolic material (McLatchey and Reddy, 1998). Even though this is not involved with nitrogen cycling directly, the build-up of phenolics from low POX activity can affect the activity of hydrolase enzymes, such as NAG (Freeman et al., 2001). The measurement of POX therefore helps the interpretation of NAG and BG responses to nitrogen contamination and climate change.

This study aimed to investigate the impacts of lowered water tables (Clarke and Ayutthaya, 2010), predicted increases in nitrogen availability (Camargo and Alonso, 2006) and their interaction on dune slack biogeochemistry. We tested the following research questions using analysis of soil chemistry, extracellular enzyme activities and greenhouse gas measurements: Do lowered water tables decrease denitrification? Do lowered water tables increase soil decomposition? Does groundwater nitrogen contamination increase dune slack soil denitrification? and does groundwater nitrogen contamination increase soil carbon processes?

1.3 Methods

Dune slack soil was collected from a previously uncontaminated *Salix repens-Calliergon cuspidatum - Campylium stellatum* dune slack community at Aberffraw (Anglesey, North Wales, UK, 53°11'N, 4°27'W), identified by the presence of pristine vegetation communities and very low groundwater NO_3 concentration (Rhymes et al., 2014). Soil was separated into two horizons; an organic top 10 cm layer and mineral sand from depth range -10 to -50 cm. Roots were removed by hand and soil was homogenised with a clean cement mixer and used for two complementary experiments.

Mesocosms of soil and vegetation representing more natural conditions run for a period of nine months and microcosms for laboratory assays to allow close control of potentially confounding factors and to investigate the effect of nitrogen contamination further.

1.3.1 Experimental designs

Mesocosm experiment

The mesocosm experiment investigated lowered water levels, N loading, and their interactions under controlled water level conditions using reconstructed dune slack soils, planted with four representative dune slack plant species. Each mesocosm was constructed with plastic pipe (50 cm height and 16 cm diameter) with a mesh-lined perforated plastic base attached to the bottom for drainage. The first 42 cm was filled with mineral sand with no organic matter (described above), whilst the top 8 cm was filled with homogenised organic matter to replicate a mature slack soil. Each mesocosm was planted with four typical dune slack species (2 sedge and 2 forb species): one specimen each of *Carex arenaria*, *Carex flacca*, *Leontodon autumnalis* and *Prunella vulgaris*. The mesocosms were then placed into individual buckets filled with a re-created groundwater composition and the nutrient treatments (see details below). Holes within the side of the buckets were used to control water table regimes and were attached to plastic tubing to collect any overflow. Black plastic was used to cover the opening of the bucket to exclude light, to prevent rainfall mixing directly into the groundwater and to avoid water loss through evaporation. The outer part of the mesocosms, buckets and outlet bottles were wrapped in foil to minimise absorption of the sun's heat.

This experiment ran from October 2013 to July 2014 in Bangor, North Wales, UK (53°13'32.0"N, 4°07'55.1"W) and involved three groundwater DIN treatments; control (0.0 mg/L of DIN), low (0.2 mg/L of DIN) and high (10 mg/L of DIN) in factorial combination with a wet or dry hydrological regime, each with eight replicates of each water level x nitrogen combination, giving 48 mesocosms

overall. The hydrological regimes followed a three-stage seasonal pattern. Wet hydrological regimes were altered from -10 cm water table depth in the winter months to -20cm in spring, to -30 cm in the summer months, whilst the dry hydrological treatments were altered to consistently be 10 cm lower than the wet treatment. The artificial groundwater was synthesised by adding the listed compounds (Table 1) to 20L of de-ionised water, to reproduce concentrations of cations, anions and the groundwater pH measured at Aberffraw (Rhymes et al., 2014). Due to rainfall and evaporation the water tables fluctuated in line with typical hydrological regimes in the field, although were unable to flood. On the 1st of July 2014 two litres of artificial groundwater (Table 1) was added to each mesocosm due to a long period without rainfall. Nitrogen treatments were maintained by monitoring the groundwater chemistry monthly and calculating the amounts of ammonium nitrate required to meet the targeted DIN treatment concentrations.

Table 1 Artificial groundwater recipe; compound weights added to 20L de-ionised water

Compound	**Weight (g)**
$CaCO_3$	0.941
$CaCl_2.6H_2O$	7.541
$MgSO_4.7H_2O$	0.370
$MgCl_2.6H_2O$	0.996
KCl	0.089
$NaHCO_3$	6.082

Microcosm experiment

The microcosm experiment investigated N loading only, under controlled conditions, using just the homogenised organic soil. Microcosms were prepared in 50 ml falcon tubes (Corning inc.) wrapped in foil with 15 g of the organic homogenised dune slack soil. Once arranged the microcosms were left to equilibrate for 24 hours and kept in complete darkness at 18 °C for the duration of the experiment. Groundwater treatments with a wider range of N concentrations were produced by adding calculated volumes of ammonium nitrate to groundwater collected from a dune slack with low nitrogen background concentrations (0.075mg/L of DIN), to produce concentrations of 0, 1, 3, and 10 mg/l of DIN. 5 ml of treatment was then added to 15 replicate microcosms for each treatment (10 replicates for enzyme sampling and 5 replicates for gas sampling). Microcosms were sampled for enzyme activity 24 and 74 hours after treatment addition and gas samples were collected 1, 4, 8, 24, 48 and 72 hours after treatment was added.

1.3.1 Gas sampling

Both mesocosm and microcosm gas samples were taken using a 20 cm^3 syringe fitted with a two-way valve (Sigma, Aldrich Ltd.) and a short bevel hypodermic needle then injected into 12 ml evacuated exetainers (Labco Ltd., Lampeter, UK). Mesocosm gas samples were taken 30 min and 1 hour after attaching an air-tight transparent chamber fitted with a Suba-Seal® rubber septa (Sigma Aldrich Ltd., Dorset, UK) (N_2O gas concentrations measured 30 min after incubation and 1 hour for CO_2 and CH_4, ensuring gas linearity). Samples were taken from four randomised replicates of each treatment on a winter day (29/01/14) and summer night (21/07/14) and 6 replicates of each treatment for summer days, over 3 days (22nd to 24thof July, 2 replicates per treatment per day), which coincided with the mesocosm soil sampling (See below). Three ambient samples were taken prior to attaching the chamber. Microcosm lids were fitted with a Suba-Seal® rubber septa and placed onto individual microcosms, with gas samples being collected after an hour. This procedure was carried out 1, 4, 8, 24, 48 and 72 hours after the addition of DIN groundwater treatment. Three ambient gas samples were taken prior to enclosing each microcosm with a lid.

1.3.2 Soil sampling

Mesocosm soil samples were collected over a 3 day period from 22nd to the 24th July 2014, from six replicates of each treatment (2 replicates per treatment per day), where 4 cm length x 6 cm width x 8 cm height soil samples were taken from the middle of the mesocosm and placed into a sealable plastic bag. Samples were then de-rooted, homogenised by hand and weighed (see analysis below). Both mesocosm enzyme activity and soil chemistry were analysed immediately after soil collection and preparation. Ten replicate microcosms were sampled for determining enzyme activity, 5 randomly selected replicates were utilised at 24 and another 5 (Total= 10) at 74 hours after treatment for each treatment. Soil samples from each microcosm were homogenised with a spatula and weighed out.

1.3.3 Laboratory analysis

Gas analysis

Gas samples were analysed by gas chromatography using a Varian model 450 gas chromatograph (GC) instrument, equipped with a flame ionisation detector (FID) with a CO_2 to CH_4 catalytic converter (methaniser), to measure concentrations of CO_2 and CH_4 and an electron capture detector (ECD) for N_2O. Two mL of sample gas was injected via a 1041 on-column injector system onto a PoroPak QS (1.83m x 3.18mm) 80/100 column. Methane, CO_2, and N_2O (retention times 1.08, 1.87

and 2.25 minutes respectively) were quantified by comparison of peak area with that of the standards of known concentration used in the preparation of a standard curve.

Calculating the gaseous fluxes concentrations, from set time periods was achieved by the following equation (adapted from Levy et al. 2011):

$$\text{Flux}\ (\mu g\ m\text{-}2\ h\text{-}1) = \frac{\delta C}{\delta t} \times \left(\frac{V \times M}{a \times V_{mol}} \right)$$

Where δC is rate of change in the gas concentration over the time period; δt is the change in time from the background reading to the final measurement in hours; V is volume of the headspace of the chamber (m^3); M is the molecular weight of the gas; a is the area of the surface of the mesocsom, this is substituted for mass (g) of the soil/water sample in the microcosms and the units changed accordingly; V_{mol} is the volume of a mole of gas (air) at a given temperature ($m^3\ mol^{-1}$) calculated by:

$$p \times (R \times K)$$

Where p is pressure (kPa); R is equal to 8.314 (the ideal gas constant) and K is temperature (Kelvin). We used global warming potential carbon dioxide equivalents of 34 for CH_4 and 298 for N_2O (Myhre et al., 2013) on all measurements.

Soil moisture content and LOI

In both mesocosm and microcosm experiments a sub sample (6-8 g of fresh soil) was weighed, dried at 105 °C and re-weighed to measure moisture content within 24 hours of collection. The samples were then heated in a furnace at 375 °C for 16 h and re-weighed to calculate organic matter content through loss on ignition (Ball, 1963).

Soil chemistry

A sub sample of soil from the mesocosm experiment was prepared for chemical analysis using a water extraction of 5 g of homogenised soil, mixed with 40 ml ultra-high purity water (1:10 wt/vol) for 24 hours on an orbital shaker (Chantigny, 2003). The solution was then centrifuged for 15 min at 5000 rpm and filtered through 0.45 µm nylon syringe filter (Avonchem, Maccelsfield, UK). Nitrate, nitrite and ammonium were quantified on an ion chromatograph (Metrohm, UK Ltd., Runcorn, UK). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3-N, NO_2-N and NH_4-N. Total nitrogen (TN) and total carbon (TC) were analysed by thermal oxidation on a Thermalox TOC/TN

analyser (Analytical Sciences, Cambridge, UK), whilst total inorganic carbon (TIC) was measured using a TIC-reactor on the same instrument. Dissolved organic nitrogen (DON) was calculated by the difference between TN and calculated DIN.

Enzyme analysis

In both experiments soil samples were assayed with fluorogenic-4-methylumbelliferone (MUF) labelled substrates for the activity of three extracellular enzymes, the names and functions for each enzyme are listed in table 2. Hydrolase enzyme activity (N-acetyl-β-glucosaminidase and β-glucosidase) was measured using 1 g of soil and a modified method from Freeman et al. (1995). Phenol oxidase activity was measured using 1 g of soil and a modified method from Pind et al. (1994). Modifications for both methods are described by Dunn et al. (2014). (It should be noted that complete saturation curves were not explored for dune slack soils and must therefore not be compared with the wider literature however, the technique allows for the comparison of potential enzyme activity across treatments rather than absolute activity). Substrates and soils were incubated at 16 °C for mesocosm soils and 18 °C for microcosm soils (16 °C was the recorded temperature during mesocosm soil sample collection, whilst 18 °C was the optimal soil temperature recorded within the field).

Table 2 MUF-labelled substrates required to measure specified extracellular enzyme activity.

Substrate	**Enzyme**	**Abbreviation**	**Enzyme commission number**	**Function**
4-MUF N-acetyl-β-glucosaminide	N-acetyl-β-glucosaminidase	NAG	3.2.1.96	Breaks down chitin
4-MUF β-glucopyranoside	β-glucosidase	BG	3.2.1.21	Hydrolyses carbohydrate molecule
L-Dopa	Phenol oxidase	POX	1.10.3.2	Oxidises phenolic compounds

1.3.4 Statistical analysis

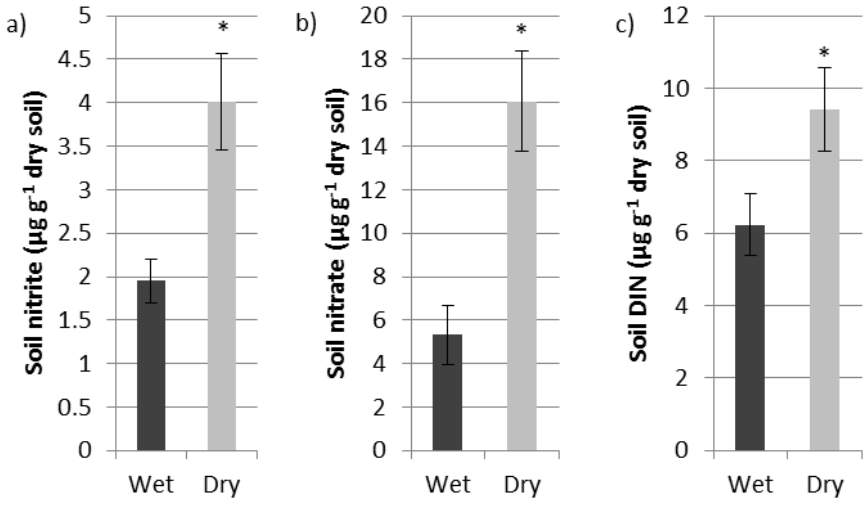
All statistical analyses were performed using Minitab v.16. The normality of data were tested using the Kolmogorov-Smirnov test; data that proved not normally distributed were transformed using a Johnson's transformation, which selects the optimum transformation for the data to achieve a normal distribution.

Mesocosm enzyme activity and gas data were averaged for each treatment over the three day sampling period. Differences in the mesocosm enzyme activity, soil chemistry and greenhouse gas production were tested separately for each time point using general linear models ("water table" "nitrogen" "water table * nitrogen"). The model tested for the individual differences between wet and dry treatments, nitrogen treatments and their interactions. Statistical differences in microcosm enzyme activity and N_2O production were also tested between treatments separately for each time point using ANOVA with Tukey HSD *post hoc* tests.

1.4 Results

1.4.1 Mesocosm

The hydrological regimes (wet or dry treatment), within the mesocosm study showed significant effects on soil chemical parameters, enzyme activity and greenhouse gas fluxes (Fig 1). Soil moisture content at the end of July was 31.83 ± 0.37 % within the wet treatment and 28.07 ± 0.47 % in the dry. Soil nitrite, nitrate and DIN concentrations were significantly higher within the dry treatment than those exposed to the wet treatment (Fig 1 a,b & c), whilst TC and DIC concentrations were significantly lower within the dry treatments than within the wet treatments (Fig 1 d & e). Soil DOC: DON ratios (Fig 1 f) were unaffected by hydrological regimes.



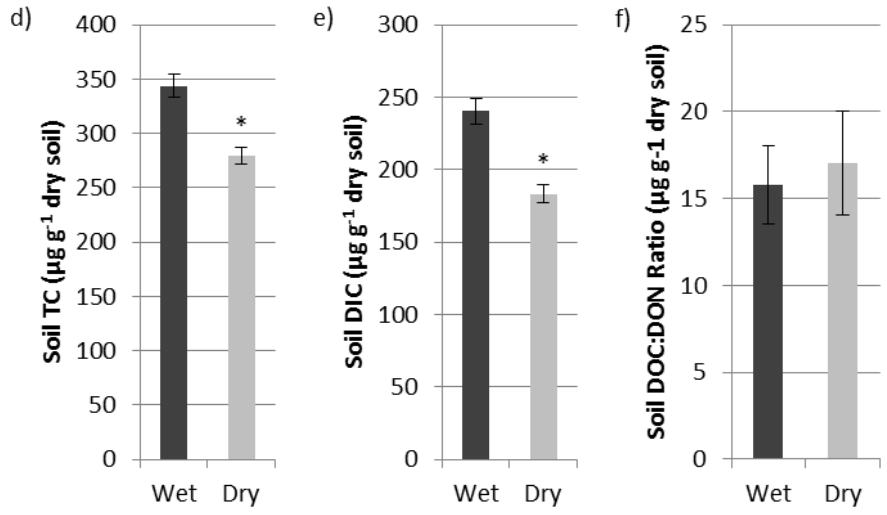


Fig 1 Mean water extractable soil a) nitrite b) nitrate c) DIN d) TC e) DIC and f) DOC: DON ratio. Asterisks denote significance between wet and dry hydrological treatments.

Hydrolase enzyme activities NAG and BG were significantly affected by the hydrological treatment, where soil NAG activity was significantly higher within the wet treatment than within the dry treatment (Fig 2 a) and BG activity was significantly lower within the wet treatment than within the dry treatment (Fig 2 b). POX enzyme activity showed no significant differences between wet and dry treatments (Fig 2 c).

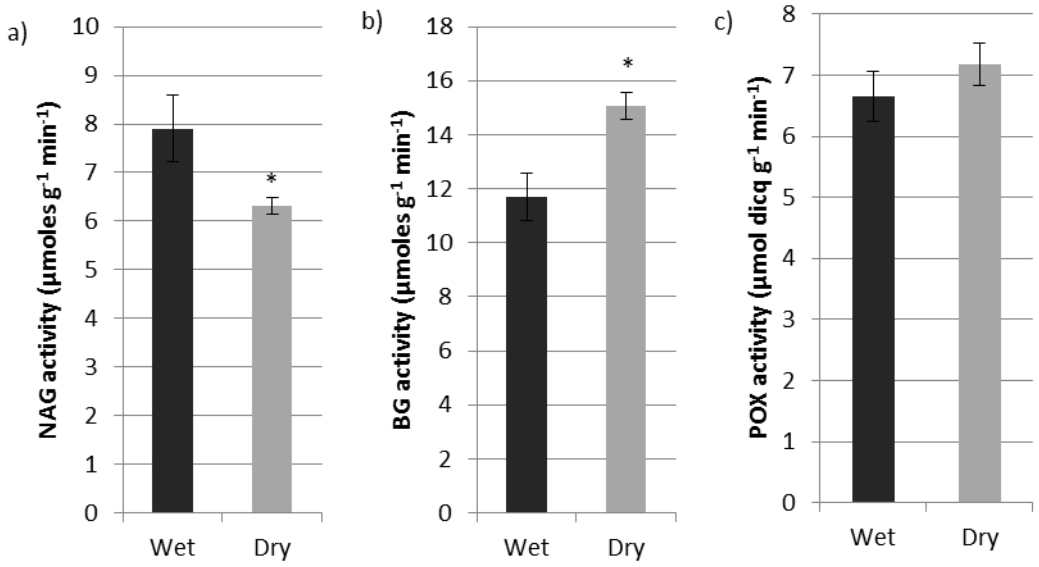


Fig 2 The activity of enzyme a) N-acetyl-β-glucosaminidase (NAG) b) β-glucosidase (BG) and c) Phenol oxidase (POX) within mesocosm soils. Asterisks denote significance between wet and dry hydrological treatments.

Hydrological treatment had no effect on CO_2 uptake (i.e. through photosynthesis) measured on winter (29/01/14) and summer days (22nd to 24th of July), however CO_2 emissions measured on a summer night (21/07/14) were significantly higher ($F= 15.07$ $df= 1$ $p= 0.001$) within wet treatments compared to dry treatments (Fig 3 a). Methane emissions measured on a winter day ($F= 5.82$ $df= 1$ $p= 0.024$), summer day ($F= 39.84$ $df= 1$ $p= 0.000$) and summer night ($F= 38.80$ $df= 1$ $p=0.000$) were all significantly greater within mesocosms subject to the wet treatment than the dry treatment; with greater methane emissions within the summer than in the winter (Fig 3 b). While there was no effect of water table on N_20 fluxes (Fig 3 c), there was a consistent, but non-significant, trend of increased N_2O emissions with increasing groundwater nitrogen treatment (Fig 4).

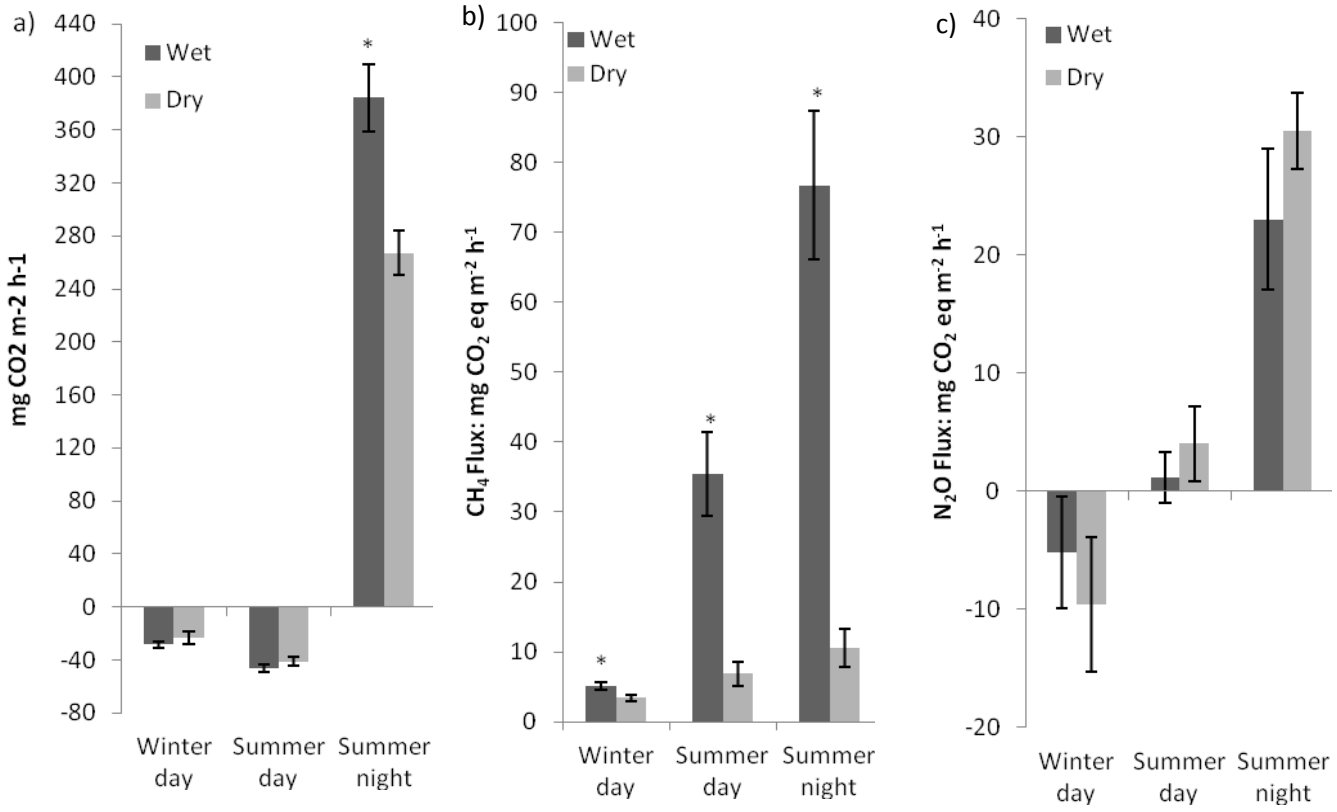


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Fig 3 Wet and dry hydrological treatment effects on the emissions of a) CO_2 and b) CH_4 and c) N_2O. Gas samples collected on a winters day (29/01/14), summer day (coincides with soil sampling for enzyme activity, 22nd to 24th of July) and summers night (21/07/14). Comparisons were only made between treatments for a winter day, summer day and summer night. An asterisk denotes significance between wet and dry hydrological treatments at a single time point (i.e. winter day).

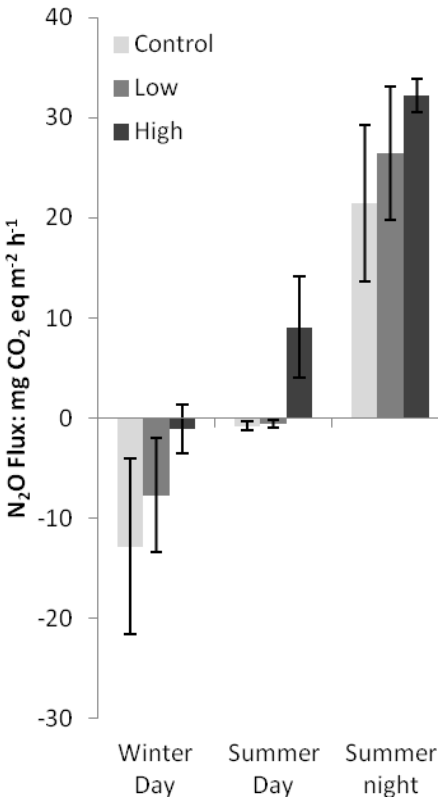

Fig 4 The effects of groundwater nitrogen treatment (Control, low and high) on N_2O emissions. Gas samples collected on a winters day (29/01/14), summer day (coincides with soil sampling for enzyme activity, 22nd to 24th of July) and summers night (21/07/14). Comparisons were only made between treatments for a winter day, summer day and summer night.

1.4.2 Microcosm

In the microcosm experiment, the enzyme assays showed that POX activity was significantly higher in the 10 mg/L DIN groundwater treatment than the 0 mg/L at 72 hours post treatment addition (Fig 5 c), yet was not significantly different at 24 hours. NAG and BG were also not significantly different between groundwater DIN treatments (at both 24 and 72 hours after groundwater nitrogen treatment addition, Fig 5 a & b). NAG and BG hydrolase enzyme activities within all treatments, however decreased from the 24 hour sampling point to the 72 hour sampling point (Fig 5 a & b). Soil moisture percentage decreased from 36.10 ± 0.45 at the 24 hour sampling point to 35.31 ± 0.33 at the 72 hour sampling point, although this data is not shown.

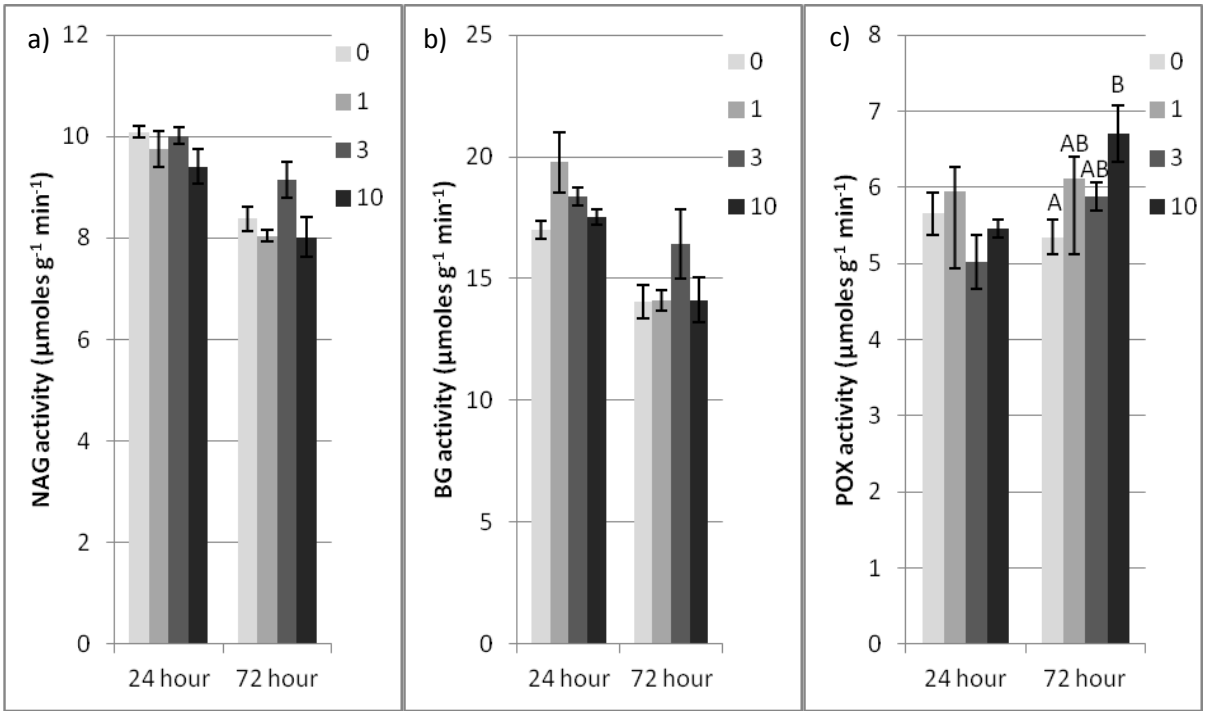

Fig 5 The activity of enzyme a) N-acetyl-β-glucosaminidase (NAG), b) β-glucosidase (BG) and c) Phenol oxidase (POX) in microcosm soils 24 hours and 72 hours after the addition of different groundwater DIN treatments (0, 1, 3 and 10 mg/L of DIN). Enzyme activity is reported as a mean ± one standard error. Letters denote significance between treatments at the 24 hour and 72 hour time points respectively.

N_2O gases were measured at intervals to help identify the time course of denitrification activity within the soils following the addition of DIN groundwater treatments. Negligible amounts of N_2O gas were produced within all DIN treatments for the first 8 hours post treatment addition (Fig 6). At 24 hours post DIN treatment addition a peak in N_2O production was measured for all treatment concentrations, with significantly higher production ($F= 3.86$ $df= 3$ $p= 0.025$) in the 10mg l^{-1} nitrogen treatment than the 3 mg l^{-1} treatment (Fig 6). At 48 hours N_2O gas production had decreased within all treatments (Fig 6), but remained elevated ($F= 2.37$ $df=3$ $p= 0.000$) in the 3 mg l^{-1} and 10 mg l^{-1} nitrogen treatments compared with the 0 mg l^{-1} and 1 mg $^{-1}$ nitrogen treatments, and was substantially higher in the 10 mg l^{-1} nitrogen treatment than in the 3 mg l^{-1}.

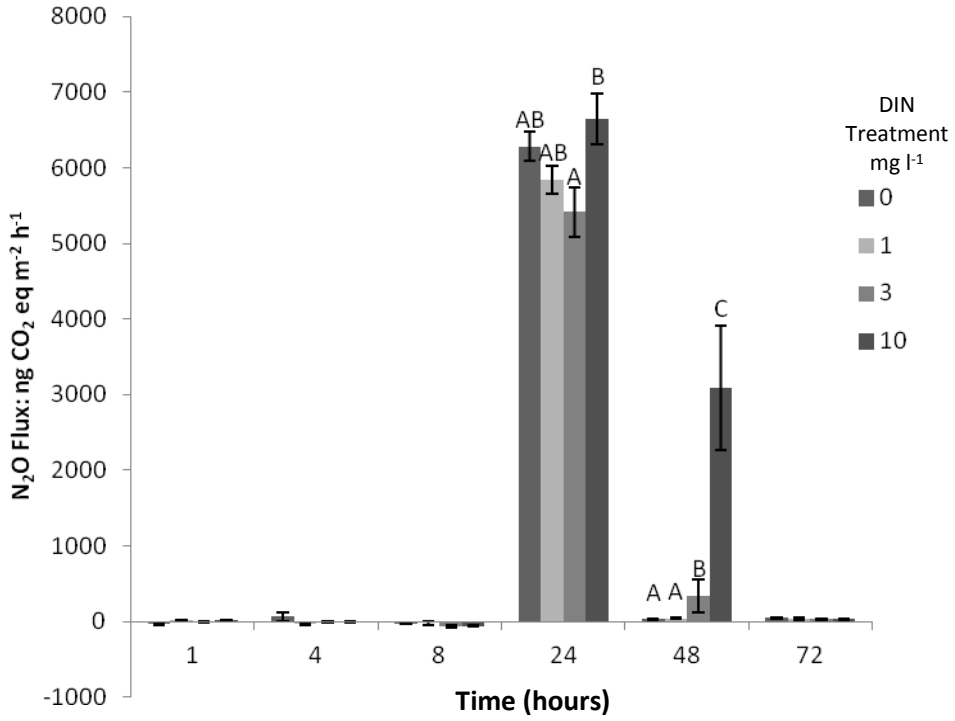

Fig 6 Time series of N_2O production following the addition of groundwater DIN treatments. N_2O production is reported as mean ± one standard error. Letters denote significance within individual time points.

1.5 Discussion

This study investigated the potential impacts of climate change and eutrophication on dune slack soil nitrogen and carbon cycling and specifically, the effects on biogeochemistry, soil processes and greenhouse gas emissions.

Just a 10 cm lowering in water table was sufficient to affect soil chemistry, enzyme activity and greenhouse gas emissions. For nitrogen cycling, the 2-3 fold increase in soil nitrite, nitrate and DIN concentrations in the dry treatment is likely to be as a result of the lowered water table increasing the aerobic zone, resulting in less favourable conditions for denitrification. This is in accordance with the decreased NAG activities also found within the dry treatment.

Soil-water TC concentrations were lower in the dry treatment, possibly due to decreased contributions of soil carbon from DIC (dissolved CO_2), rather than DOC. The smaller contributions of DIC observed can be explained by the increase in BG activity together with decreased anaerobic conditions in the dry treatment, which are known to increase the rates of decomposition and methanogenisis. In agreement, the night-time CO_2 fluxes, representative of both plant and microbial

respiration, were lower in the dry treatment as a result of either; 1. decreased root respiration, 2. a decrease in incomplete decomposition of soil organic matter under more aerobic conditions (Whalen, 2005), 3. decreased methanogenesis, where CO_2 can also result as a by-product of methane production, dependant on the nature of the terminal electron acceptor or by anaerobic methane oxidation (Ferry, 1993) and/or 4. a combination of these processes. Indeed, here, methane production was lower in the dry treatment, which is in line with the consensus (Segers, 1998, Whalen, 2005). Limited data also suggests that uptake of CO_2 within the winter and summer months indicate that CO_2 intake from photosynthetic processes is greater than CO_2 emissions from soil respiration.

Conversely, the wet treatment conserved soil carbon as soil respiration and decomposion rates were reduced by the anaerobic environment (Kang and Freeman, 1999, Flanagan and Syed, 2011). However, in broad agreement with the literature (e.g. Whalen, 2005), methane production was greater in the wet treatment at each season measured. This was expected due to methane production primarily being an anaerobic process (Segers, 1998, Whalen, 2005); where the anarobic conditions reduce the consumption of methane by methanotrophic bacteria, resulting in an increase in methane released to the atmosphere (Pearce and Clymo, 2001). As methane emissions are sensitive to soil conditions and temperature (Whalen, 2005), methane emissions here show temporal and seasonal variation.

The greater BG activity in the dry treatment might suggest increased degradation of cellulose within these soil conditions as a result of increasing microbial biomass (Turner et al., 2002) resulting in increased enzyme synthesis. This is unlikely however, as if this was the case we would see similar results with POX enzyme activity. A more likely explanation is that the BG enzyme is being synthesised by soil microorganisms in response to the occurrence of appropriate carbon substrates or by the increase of inorganic nutrients (Fenner and Freeman, 2011), in this case, soil nitrate and nitrite. Phenol oxidase was not affected by water table, which could be due to the extremely low availability of substrate for this enzyme is sandy soils, i.e. phenolic concentrations (Freeman et al., 1996), however, this was not measured here. These findings therefore suggest that with lowered water tables, cellulose decomposition rates are likely to increase, through BG activity, without demonstrable increases of soil respiration. The implications discussed, from a mere 10 cm lowered water table depth, suggests that such effects are likely to be intensified by climate change, as water tables are predicted to lower by up to 100 cm by 2080 (Clarke and Ayutthaya, 2010).

Previous studies demonstrate the temporal variability of soil denitrification in response to increases in soil water content (Rudaz et al., 1991, Martin et al., 1988), with N_2O production measured within 30 minutes in dry grassland soils (Rudaz et al., 1991) and longer in other studies. In the microcosms, we found that denitrification lasted longer within dune slack soils with higher groundwater nitrogen concentrations. As denitrification has been found to significantly increase with N availability (Merrill and Zak, 1992), it is likely that N stores within the higher DIN treatments are larger than those treated with lower DIN treatments, causing denitrification activity rates to last longer. These findings however were not significant in the mesocosm experiment, where there was a clear increasing N_2O emission trend with increasing nitrogen groundwater treatments, but high variability between replicates. The microcosm experiment illustrates that in these sandy soils, groundwater nitrogen can increase denitrification and therefore N_2O emission, and that this is likely to occur in the field. Indeed denitrification is proposed as a mechanism by which some dune slacks maintain a low nutrient status, despite many years of plant and soil development (Adema et al., 2005)(Adema et al. 2005).

The effect of nitrogen addition on carbon cycling, and POX activity in particular, varies significantly across different studies and the responses are largely explained by the quantity of lignin in plant litter (Waldrop et al., 2004). Peatland soils subject to increased atmospheric nitrogen are seen to have higher POX activity (Bragazza et al., 2006). Our findings are in accordance with those of Bragazza et al. (2006), where POX activity increased within microcosms subject to higher groundwater nitrogen contamination 72 hours after nitrogen treatment addition. In turn, the increase in POX activity reduces polyphenol concentrations and can indirectly stimulate the activity of hydrolase enzymes, such as NAG and BG, by means of the phenol oxidase latch mechanism (Freeman et al., 2001), which ultimately increases decomposition rates. The latch response however was not observed within this study, with NAG and BG activity unaffected by the increased POX activity. Nonetheless, the increase in POX activity suggests that dune slack soil decomposition rates are sensitive to groundwater nitrogen contamination leading to increased decomposition and subsequently the potential for increased carbon losses under elevated groundwater nitrate.

1.6 Conclusions

Our findings suggest that dune slack habitats are highly sensitive to both groundwater nitrogen addition and modest changes in water tables. Should drier conditions prevail, as a result of climate change (Clarke and Ayutthaya, 2010) or water abstraction, dune slack soils are likely to become drier and this, in turn, will reduce denitrification rates, leading to greater nitrogen retention and therefore

a greater eutrophication impact. At the same time, the global availability of nitrogen is increasing (Galloway and Cowling, 2002) and it is therefore likely that DIN availability within dune slack soils will increase, irrespective of water table changes. Subsequently, dune slacks are likely to experience plant community shifts from both a decrease in soil water content (Curreli et al., 2013) and an increase in soil nitrogen availability, both posing a serious threat to endangered dune slack species (Rhymes et al., 2014). With regard to carbon cycling within dune slack soils, BG measurements suggest that decomposition rates are increased with lowered water tables, and with increased groundwater nitrogen concentrations, thereby potentially reducing carbon sequestration in this habitat. Taken together, our findings suggest that there is a hierarchy with hydrology being the dominant factor followed by nitrogen contamination, and so climate change poses more of a threat than eutrophication. The response of dune slack soil processes to such threats are still poorly understood and further research is required to understand the future prospects of dune slack ecology and dune wetlands in a changing environment.

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