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

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A B S T R A C T

Dune slacks are biodiverse seasonal wetlands which experience considerable fluctuation in water table depth. They are under threat from eutrophication and lowered water tables due to climate change and water abstraction. 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 CH_4 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. 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 and 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

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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 declines, with N_2 generally replaced by N_2O (Brady and Weil, 2002). N_2O production is favoured at low soil pH and 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 enhance organic matter accumulation (Jones et al., 2008). In systems which are N limited, elevated nitrogen inputs tend to increase decomposition rates. Decomposition is often measured by CO_2 emissions (soil respiration), an indicator of aerobic microbial decomposition. Methane emissions are used as an indicator of anaerobic microbial decomposition of soil organic matter (Whalen, 2005), however CO_2 can also be produced in anaerobic conditions (Ferry, 1993).

The measurement of extracellular enzyme activities within soils can further quantify biogeochemical processes linked to nutrient and carbon cycling 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 and Popova, 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 elevated nitrogen inputs 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 organic matter decomposition? Does groundwater nitrogen contamination increase dune slack soil denitrification? And does groundwater nitrogen contamination increase soil BG enzyme activity?

2. 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. A mesocosm experiment 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.

3. Experimental designs

3.1. 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 recreated 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, as the mesocosms were located outside and exposed to natural levels of rainfall and sunlight.

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 × 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 −20 cm 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 20 L 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

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

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