Rhymes, J.; Wallace, H.; Tang, S.Y.; Jones, T.; Fenner, N.; Jones, L. 2018.
Substantial uptake of atmospheric and groundwater nitrogen by dune slacks under different water table regimes.

Abstract

Dune slacks are biodiverse seasonal wetlands which experience considerable fluctuations in water table depths. They are subject to multiple threats such as eutrophication and climate change, and the interactions of both of these pressures are poorly understood. In this study we measured the impact of groundwater nitrogen contamination, as ammonium nitrate (0, 0.2, 10 mg/L of DIN, dissolved inorganic nitrogen), lowered water table depth (lowered by 10 cm) and the interactions of these factors, in a mesocosm study. We measured gross nutrient budgets, evapotranspiration rates, the growth of individual species and plant tissue chemistry. This study found that nitrogen uptake within dune slack habitats is substantial. Atmospheric inputs of 23 kg N ha^{-1} $yr.^{-1}$ were retained by the mesocosms, with no increase of nutrient levels in the groundwater, i.e. there was no leaching of excess N. When N was added to the groundwater (in addition to atmospheric N), total uptake was equivalent to 116 kg N ha^{-1} $yr.^{-1}$, at a groundwater DIN concentration of 10 mg/L. This resulted in increased plant tissue N concentrations showing uptake by the vegetation. The effect of lowering water tables did not influence N uptake, but did alter vegetation composition. This suggests that groundwater can be a substantial input of N to these habitats and should be considered in combination with atmospheric inputs, when assessing potential ecosystem damage.

Keywords

Dune slack, Ecology, Soil, Groundwater, Eutrophication

Introduction

Dune slacks are seasonal wetlands with an annually fluctuating water table (Stratford et al., 2013; van der Laan, 1979). They are highly biodiverse and the vegetation communities they support are adapted to low levels of nutrient input (Grootjans et al., 2004). As a result, these wetland communities are sensitive to multiple threats including eutrophication and lowered water tables, e.g. from climate change or water abstraction (Clarke and Ayutthaya, 2010; Provoost et al., 2011). Many semi-natural habitats are sensitive to excess nutrients (Field et al., 2014), and critical loads were designed as a policy tool to protect plant communities from atmospheric nitrogen deposition. Critical loads are defined as "exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson, 1988). However, in addition to atmospheric inputs, wetlands may receive nutrients from sources including groundwater and overland flow as shown by Rhymes et al. (2014). It is not known how much of that

N is retained, denitrified or otherwise removed by soil and groundwater processes before it reaches a receptor wetland. This is a key knowledge gap in relating non-atmospheric and atmospheric nitrogen sources to biological impacts. The magnitude of N contributions from these other sources is recognised as a gap in knowledge (Achermann and Bobbink, 2003) and there is no framework currently able to account for these combined impacts. Recent studies have shown ecological impacts on dune slack vegetation resulting from low concentrations of nutrients in groundwater from a variety of non-atmospheric sources (Rhymes et al., 2015; Rhymes et al., 2014). Those impacts included changes in plant species composition, with a shift towards nitrophilic species in the more nutrient-enriched areas. Crucially, those shifts were observed at very low nutrient concentrations in the groundwater, at a concentration of 0.2 mg L^{-1} of dissolved inorganic nitrogen (DIN).

In addition to nutrient impacts, hydrology plays a key role in determining composition of wetland communities (Curreli et al., 2013; van der Laan, 1979; Willis et al., 1959), with 40 cm in hydrological regime separating wet from dry communities (Curreli et al., 2013). Hydrological fluctuations may also play a role in conserving the low nutrient status required by dune slack species (Berendse et al., 1998), by moderating rates of nutrient uptake or of denitrification (Adema et al., 2005), but the conditions governing these mechanisms are poorly understood.

However, in the field it is very difficult to manipulate water levels under controlled conditions, and to assess the relative importance of groundwater vs atmospheric inputs. Therefore, we designed an experiment to manipulate three levels of nitrogen concentration in groundwater supply to dune slack mesocosms, to determine whether that N was taken up by dune slack vegetation & soils. In factorial combination with the N treatments, we varied water levels seasonally using two treatments to mimic wetter and drier hydrological regimes, to see whether this affected N uptake.

We tested the following hypotheses: 1) Higher groundwater nitrogen contamination concentrations will increase the quantity of nitrogen taken up by dune slack ecosystems, which will affect growth of dune slack vegetation. 2) Lowered water tables will decrease evapotranspiration rates and reduce exposure to nutrients, both of which will result in lower uptake of N in the drier community.

Materials and methods

Dune slack soil (from a 6 m X 6 m area to a depth of 50 cm) was collected from an uncontaminated *Salix repens-Calliergon cuspidatum stellatum* community dune slack 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 concentrations. These were separated into two soil types: an organic 0 to 10 cm layer and mineral sand from depth range -10 to -50 cm. Roots were removed by hand from both soil types and the soils were separately homogenised with a clean cement mixer.

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 from plastic UPVC 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 the mineral sand collected from Aberffraw (see above) and the top 8 cm was filled with the organic matter collected from Aberffraw (see above) to replicate a mature slack. 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 10 L buckets filled with a synthesised and re-created groundwater (See Appendix I for chemical composition) and the additional nutrient treatments. Holes (1.5 cm diameter) in the side of the buckets were used to control the desired water table depth and were attached to plastic tubing and a collecting vessel to collect any overflow due to rainfall (See Fig. 1). The bucket rim was covered with plastic joined to the side of the mesocosm, thus evapotranspiration was limited to the mesocosm surface. This experiment ran from October 2013 to July 2014 in Bangor, North Wales, UK (53°13'N, 4°07'W). The mesocosms were setup in October 2013 to allow for a seven month equilibration period, i.e. for mineralisation due to soil disturbance to diminish and for the plants to establish before the groundwater N treatments commenced in May.

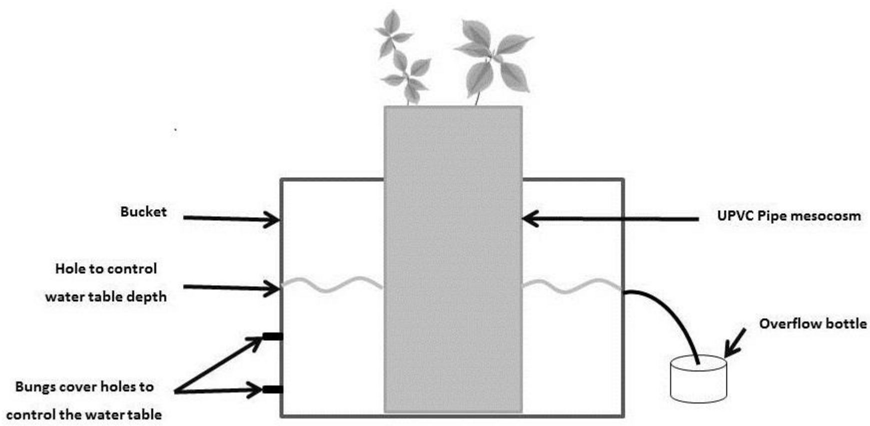


Fig 1: Diagram of constructed mesocosm

There were three groundwater ammonium nitrate dissolved inorganic nitrogen (DIN) treatments; control (0.0 mg L^{-1} of DIN), low (0.2 mg L^{-1} of DIN) and high (10 mg L^{-1} of DIN) in factorial combination with a wet or dry hydrological regime. The hydrological regimes followed a three-stage seasonal pattern. Wet hydrological regimes were altered from -10 cm water table depth in the winter months (1st of October 2013 to 15th of March) to -20 cm in spring (16th March to 31st April), to -30 cm in the summer months (1st of May onwards), whilst the dry hydrological treatments were altered to consistently be 10 cm lower than the wet treatment. There were eight replicates of each nitrogen X hydrological regime combination, giving 48 mesocosms overall. The low N treatment concentration of 0.2 mg L^{-1} of DIN was chosen due to evidence that shows biological impacts on dune slack habitats at these low concentrations (Rhymes et al., 2014). The DIN treatments were maintained monthly and therefore fluctuated throughout the experiment. Mesocosms were located outside and exposed to natural levels of rainfall and sunlight, which allowed for water table fluctuations (below the maximum level controlled by the overflow tubes).

Water table depth, water chemistry sampling and maintenance of treatments

Water table depth was measured once a week. Volume of water within each mesocosm was calculated by the volume of water within the bucket and the water held within the mesocosm sand based on a water-holding capacity of 30 % (Ranwell, 1959). The groundwater chemical composition was measured on a monthly basis by taking a water sample from each bucket and filtering through 0.45um nylon syringe filter (Avonchem™) prior to chemical analysis. NO_3 and NH_4 concentrations were quantified by ion chromatography (Metrohm, UK Ltd.), whist total nitrogen (TN) concentrations were analysed by thermal oxidation on a thermalox TOC/TN analyser. DIN concentrations and water volume were then used to calculate the quantity of ammonium nitrate required to return DIN concentrations to the target DIN treatments on a monthly basis.

Nitrogen and water budget experiment

From the 1st of May to the 22nd of July 2014 a simplified water and nitrogen budget was calculated for each individual mesocosm. Inputs of water were rainfall and added groundwater stock, rainfall volume was measured weekly from a manual rain gauge. Measured outputs of water were water collected in the overflow bottles, measured every two weeks. Water loss from evapotranspiration was calculated on a monthly basis, and combined to give evapotranspiration losses over 84 days.

Nitrogen inputs measured included monthly DIN inputs from the ammonium nitrate treatments added (see above) and monthly atmospheric deposition concentrations and fluxes (described below), DIN and TN concentrations from the overflow bottles were measured bi-weekly. Budget calculations estimated total uptake of N (assumed to include plant and soil uptake and denitrification losses) on a monthly basis, to give total N losses over an 84 day period. Denitrification fluxes were not separately measured in this study.

Atmospheric nitrogen deposition measurements

A monitoring station located 3 metres away from the mesocosms, measured dry and wet N deposition over the three months. Gaseous nitrogen was measured using triplicate nitrogen dioxide diffusion tubes (Gradko International Ltd, Winchester, UK) and triplicate ammonia ALPHA badge samplers (Centre for Ecology and Hydrology, Edinburgh), (Tang et al., 2001), exposed monthly for a three-month period from May to July. Wet deposited nitrogen was sampled weekly for the three month period; rainfall volume was obtained from a rain gauge and NO_3, NH_4, and TN were measured for each weekly rainfall sample (methods described earlier). Dry gaseous NO_2-N and NH_3-N concentrations were converted to N fluxes using a deposition velocity of 1.13 mm s^{-1} for NO_2-N (Jones et al., 2004) and 22 mm s^{-1} for NH_3-N (Jones et al., 2013). Total nitrogen concentrations from weekly rainfall samples were converted to fluxes using rainfall volumes and bulked to a monthly wet deposition flux.

Plant responses

At the end of July species cover was recorded using visual estimates of percentage cover with aid of a custom-built 5 cm X 5 cm grid for each species in each mesocosm. In order to measure plant tissue chemistry four randomly chosen leaves from each *Carex flacca* specimen were harvested, dried for 38 hours at 30 ºC and ground using a ball mill. The samples were then analysed for Total C and total N by dry combustion using Leco Truspec CN analyser (Leco corp., St Joseph, MI, USA).

Statistical analysis

All statistical analysis was performed using Minitab v.16. Data were tested for assumptions of normality. Where transformation was not sufficient to achieve assumptions of normality a non-parametric Kruskal-Wallis test was carried out. Differences in nitrogen uptake (N mg L^{-1}), mean species percentage cover, and *Carex flacca* tissue chemistry were analysed by a general linear model to test for the individual differences caused by the water level and nitrogen treatments and the interaction between the two. Differences in water losses between the wet and dry mesocosms from the 1st of May to the 22nd of July were analysed by analysis of covariance.

Results

Maintenance of treatments

The target treatment DIN concentrations for the whole experimental period were maintained, with values of (average ± standard error): control 0.151 ± 0.170 mg L^{-1}, low 0.218 ± 0.018 mg L^{-1} and high 9.486 ± 0.370 mg L^{-1}. The average monthly DIN treatment concentrations for all nitrogen treatment measurements are shown within a time series (Fig.2 a), whilst the dry hydrological regime treatment was successfully maintained at approximately 10 cm lower than that of the wet hydrological regime treatment (average difference between the water tables was 9.2 cm). Total water losses from evapotranspiration over this period within mesocosms subject to the wet hydrological regime were 403.31 ± 6.88 mm, compared with only 334.04 ± 5.86 mm water losses within mesocosms subject to the dry hydrological regime. Overall, and for many of the individual time points (Fig. 2 c), water losses were significantly greater ($F= 297.85$ $df= 1$ $p= 0.000$) in mesocosms subject to a wet water regime compared to those subject to a dry water regime. The exception was for the first two weeks in July. This was an artefact caused by the supplementary addition of the synthesised artificial groundwater (Fig. 2 c) to all treatments following a dry spell (Fig. 2 b) to reach the desired water table depth, with greater uptake of water to replenish the soil moisture deficit in the drier mesocosm treatment.

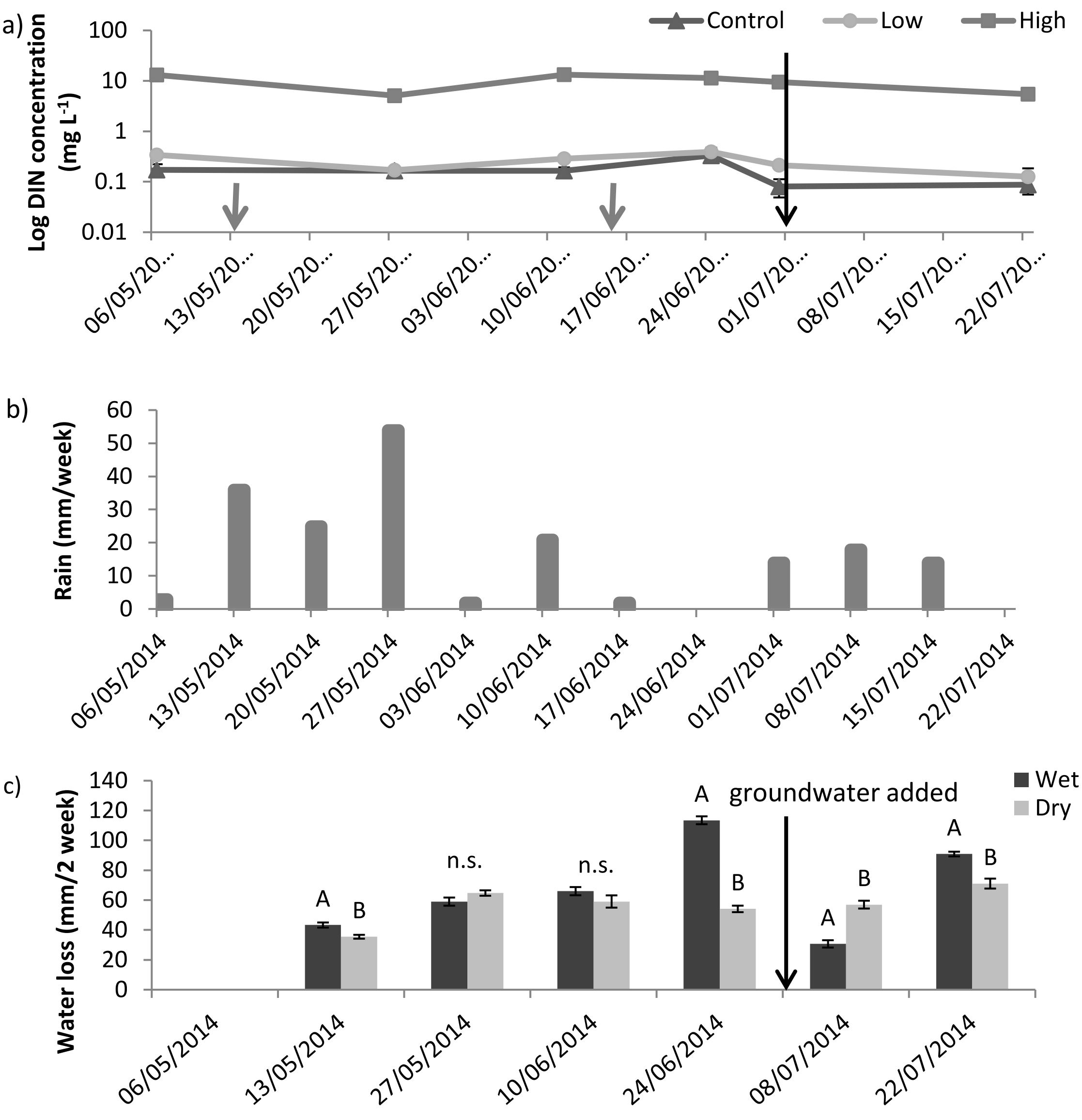
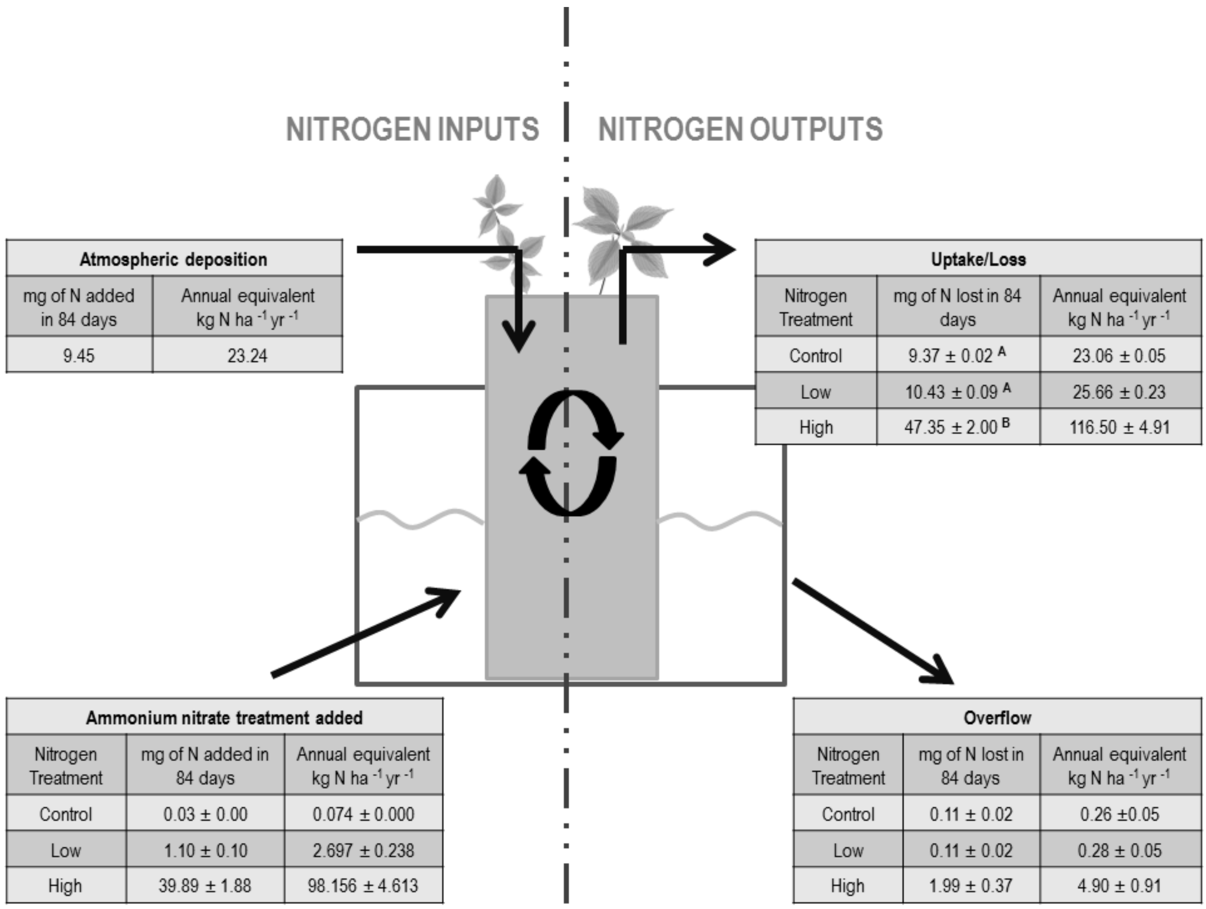


Fig 2: Eighty-four day time series for; a) log DIN concentrations for all nitrogen treatments for the three month period. b) weekly rainfall and, c) fortnightly water loss where different letters denote significance between treatments; n.s = no significance. In Fig. 1a, the short arrows represent when ammonium nitrate treatment was added, grey arrows represent ammonium nitrate only, the black arrow represents when both groundwater stock and ammonium nitrate treatment was added. In Fig. 2c, the long black arrow indicates when 2 litres of water was added to both treatments.

1.1 Nutrient uptake

The sum of nitrogen inputs and outputs for the three months is presented in Fig. 3 and shows that in the highest N treatment, an annual equivalent of 98 kg N ha^{-1} yr^{-1} had to be added to the groundwater, in addition to the 23 kg N ha^{-1} yr^{-1} coming from atmospheric deposition, in order to maintain a concentration of 10 mg L^{-1} DIN in the groundwater. Comparing nitrogen outputs among the three DIN treatments (Fig. 3), the total N uptake by the mesocosms from the 1st of May to the 22nd of July was significantly higher in the high nitrogen treatment than the control and low nitrogen treatments. No significant difference was found between the control and low nitrogen treatments.



Atmospheric deposition	
mg of N added in 84 days	Annual equivalent kg N ha^{-1} yr^{-1}
9.45	23.24

Uptake/Loss		
Nitrogen Treatment	mg of N lost in 84 days	Annual equivalent kg N ha^{-1} yr^{-1}
Control	9.37 ± 0.02 ^{A}	23.06 ± 0.05
Low	10.43 ± 0.09 ^{A}	25.66 ± 0.23
High	47.35 ± 2.00 ^{B}	116.50 ± 4.91

Ammonium nitrate treatment added		
Nitrogen Treatment	mg of N added in 84 days	Annual equivalent kg N ha^{-1} yr^{-1}
Control	0.03 ± 0.00	0.074 ± 0.000
Low	1.10 ± 0.10	2.697 ± 0.238
High	39.89 ± 1.88	98.156 ± 4.613

Overflow		
Nitrogen Treatment	mg of N lost in 84 days	Annual equivalent kg N ha^{-1} yr^{-1}
Control	0.11 ± 0.02	0.26 ±0.05
Low	0.11 ± 0.02	0.28 ± 0.05
High	1.99 ± 0.37	4.90 ± 0.91

Fig 3: Diagram summarising total nitrogen inputs and outputs from the 84 days from the 1st of May to the 22nd of July and calculated annual equivalent kg N ha^{-1} yr^{-1} from the 84 day period measurements. Values are expressed as mean ± standard error and values denoted with the same letter are not significantly different.

Separating atmospheric deposition into wet and dry classes (Table 1) shows that rainfall contributes double the amount of atmospheric nitrogen inputs compared to the total dry gaseous nitrogen inputs. Very high rainfall volumes in May compared with previous months accounted for most of the wet deposition measured, and lead to a relatively high annual equivalent. The highest proportion of gaseous nitrogen inputs is from gaseous ammonia with a small amount contributed by nitrous oxide deposition.

Table 1: Total wet and dry measured atmospheric deposition inputs into individual mesocosms from the 1st of May to the 22nd of July and calculated annual deposition from the 84 day period measurements.

Atmospheric deposition		mg of N deposited in 84 days, per mesocosm	Annual equivalent (kg N ha^{-1} yr^{-1})
Wet	NO_3-N	2.87	7.07
	NH_4-N	3.06	7.52
Dry	NO_2-N	0.67	1.64
	NH_3-N	2.79	6.87

In order to compare the effects of the experimental treatments on nutrient uptake in plants, the nitrogen and carbon content was measured within the leaves of the dominant species within the experiment, *Carex flacca*. The comparison of nitrogen treatments showed that plant tissue nitrogen of *C. flacca* was elevated in the high nitrogen treatment, with values significantly greater (F= 3.87 df= 2 p= 0.029) than the low nitrogen treatment (Fig. 4 a), although the high and low nitrogen treatment were not significantly different from the control. The C:N ratio was not significantly different (p= 0.084) between the nitrogen treatments (Fig. 4 b), although the C:N ratio is nonetheless noticeably lower within the high nitrogen treatment than the control and low nitrogen treatments. No difference was found when comparing the effects of the wet and dry hydrological regime or the interaction between hydrological regime and nitrogen treatment on either nitrogen content or the C:N ratio.

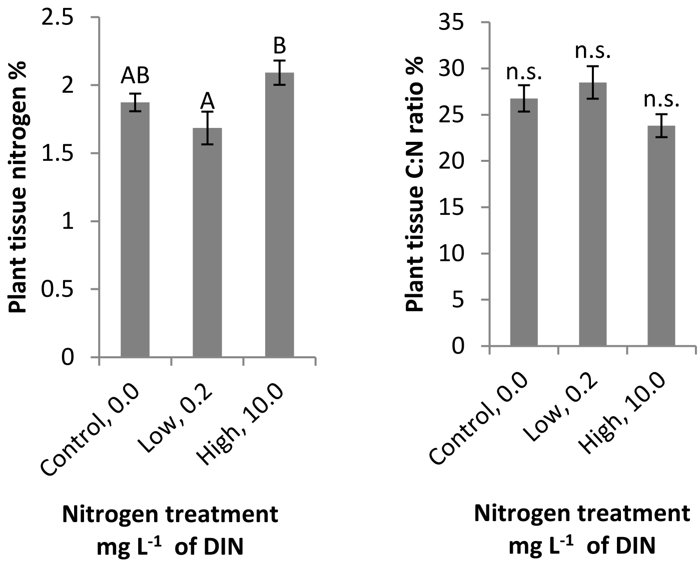


Fig 4: *Carex flacca* tissue composition of a) nitrogen and, b) C:N ratio for all nitrogen treatments. Different letters denote significance between treatments; n.s = not significant.

1.2 Effects of water tables

The plant responses to the wet and dry treatment, the nitrogen treatment and the interaction between the two were analysed. The percentage cover of the forb *Prunella vulgaris* (Fig.5 a) was significantly greater ($F= 19.15$ $df= 1$ $p<0.001$) within the dry treatment than the wet, whereas the sedge *Carex flacca* (Fig.5 c) showed significantly greater ($F= 6.81$ $df= 1$ $p=0.013$) percentage cover in the wet treatment compared to the dry. There were no significant differences between the wet and dry treatments for *Leontodon autumnalis* (Fig.5 b) or *Carex arenaria* (Fig.5 d), and no influence on overall species percentage cover from the nitrogen treatment or the interaction between the wet and dry treatments and nitrogen treatments (Fig.5). *Carex flacca* had the greatest percentage cover within all mesoscosms compared with all other species.

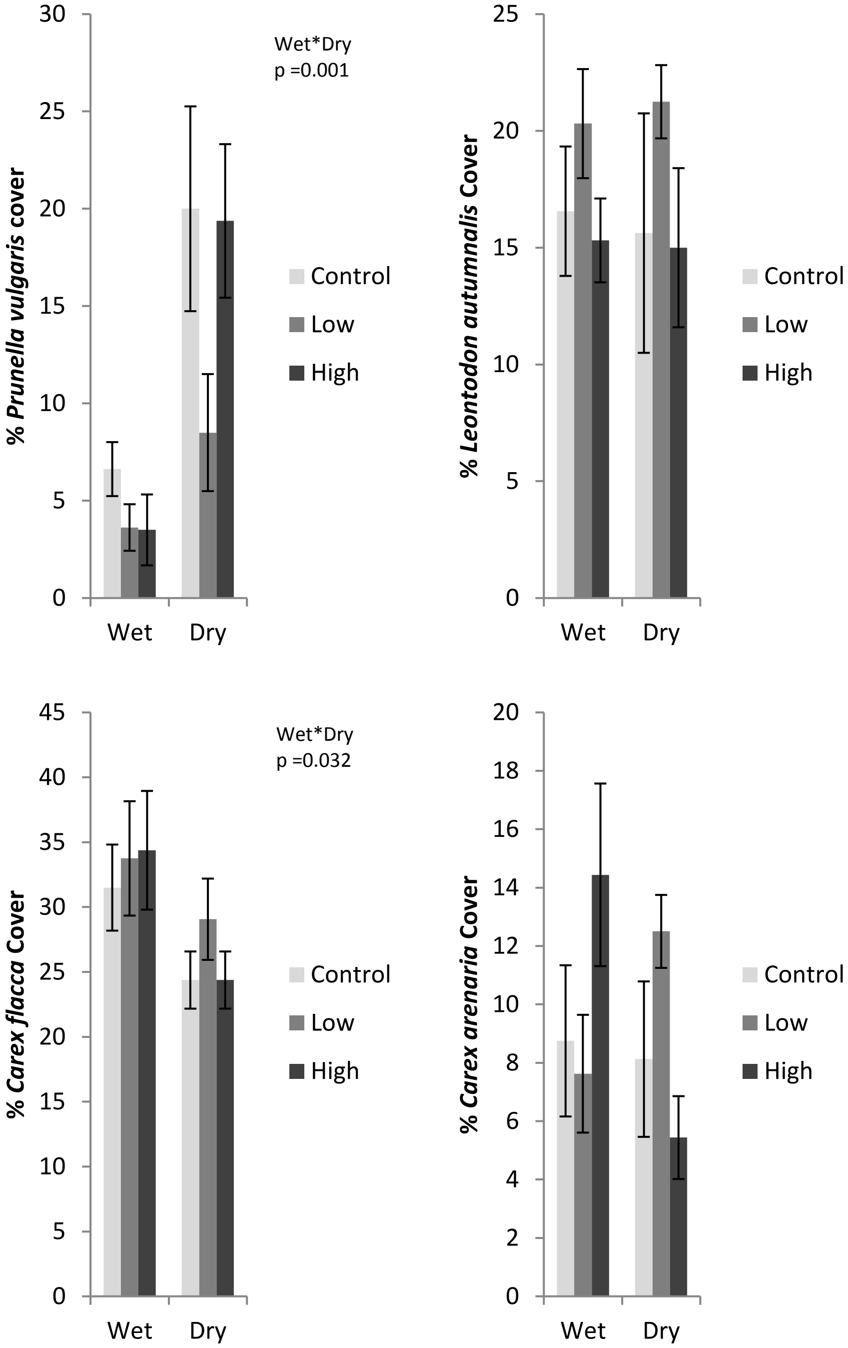


Fig 5: Species percentage cover in wet/dry and nitrogen treatments for a) *Prunella vulgaris,* b) *Leontodon autumalis,* c) *Carex flacca* and, d) *Carex arenaria.* No difference was found between nitrogen treatments; significant differences between wet and dry treatments are indicated. Error bars show +/- 1 s.e.

1.3 Discussion

Results of this study show that high DIN groundwater concentrations increase nitrogen uptake by dune slack mesocosms, however groundwater DIN concentrations ≤ 0.2 mg/Lhad no effect on nitrogen uptake. A water table lowered by only 10 cm resulted in lower water losses in the drier dune slack mesocosms and altered percentage plant cover in a forb and sedge species.

Very high levels of nutrient uptake were revealed in the experiment. The quantity of N which had to be added to the groundwater in order to maintain a concentration of 10 mg L^{-1}DIN suggests that, for sites where groundwater N concentrations are elevated, the input fluxes from groundwater are likely to be substantial, and are larger than the inputs from atmospheric deposition. The annual equivalent atmospheric inputs were also high, due partly to an extremely wet month and to the study being located in an urban area where dry atmospheric loads are typically 47 % higher than non-urban areas (Bettez and Groffman, 2013). The atmospheric deposition contributions already exceed the critical load of 10-20 kg N ha^{-1} yr^{-1} for wet dune slack habitats (Bobbink and Hettelingh, 2010), yet this did not cause groundwater DIN concentrations in the control to rise above those observed in dune groundwater un-impacted by groundwater nitrogen contamination (Rhymes et al., 2014), and additional N had to be added to maintain the 'low' treatment concentration of 0.2 mg L^{-1} of DIN. The implication here is that dune slack soils are able to retain relatively high levels of atmospheric inputs without excess N leaching into groundwater. Taken together, and since N deposition in most dune areas of the UK is < 20 kg N (Field et al., 2014), these findings suggest that, where groundwater concentrations are elevated, the most likely source is terrestrial rather than atmospheric and that the input fluxes, from either source, are high.

However, although dune slack soils appear to be able to process relatively high rates of atmospheric inputs, that does not mean there are no ecological effects. Ecological damage such as altered plant community composition can still occur at very low groundwater N concentrations (Rhymes et al., 2014), even at the concentrations of the low N treatment (0.2 mg L^{-1}) in this study. At higher concentrations, ecological changes can be profound. At a site in South Wales, UK, Jones et al. (2006) discuss the effects of a seasonal limestone spring with high levels of nitrate, equivalent to 8.7 mg L^{-1}DIN. The outflow area of the spring supports eutrophic flood meadow vegetation rather than the typical dune slack vegetation found elsewhere on the site. In The Netherlands, nitrate (and phosphate) concentrations were negatively correlated with dune slack species richness at groundwater nitrate-N concentrations ranging from 0.01 – 2.44 mg L^{-1} of N (Meltzer and Van Dijk, 1986). However, to date there are still relatively few studies that have studied plant community responses to elevated groundwater N in dune systems, and this remains a knowledge gap.

In all three treatments, including the control, the average plant tissue nitrogen content for *Carex flacca* was greater than 1.5 %. By comparing with data from other studies (Jones et al., 2013), these tissue concentrations are broadly comparable with exposure to 22 kg N ha $^{-1}$ yr $^{-1}$ of ammonia fumigation, or >40 kg N ha $^{-1}$ yr $^{-1}$ of bulk deposition in the field, i.e. well above the critical load (Bobbink & Hettelingh 2010). At these loads, both the atmospheric and groundwater inputs of N in this study are likely to cause ecological damage. The overall uptake of N was greater than 116 kg N ha^{-1} yr^{-1} annual equivalent in the high N treatment. Some of this uptake was due to the incorporation into plant tissue by *C. flacca* in the high N treatment, but not all of the potential loss pathways were separately quantified in this experiment. Uptake may consist of a combination of nitrogen incorporation into plant tissues, binding and uptake by the soil and microbes, and losses through denitrification. Denitrification has been found to significantly increase with N availability (Adema et al., 2005; Rhymes et al., 2016). The use of ^{15}N labelling to trace the fate of N would help quantify the relative magnitude of these pathways in future studies. While the experimental nutrient additions ran for only three months, after a seven-month settling period for the mesocosms, this was sufficient to demonstrate substantial uptake of N. However, running the experiment for a full year would have allowed a more accurate annual budget to be calculated.

It is well documented that the species composition and distribution within dune slack habitats are primarily influenced by water table depth (Curelli et al., 2013; Willis et al.,1959). Here we found that a small (10 cm) difference in water level treatments had an effect on both plant growth and water losses. The responses of *Prunella vulgaris* and *Carex flacca* were consistent with the UK National vegetation classification (Rodwell et al., 2000); where the SD16 drier slack communitycontains relatively lower cover of *C. flacca* and higher cover of *P. vulgaris* and the wettest subtype slack community of SD14, SD14b, is characterised by higher *C. flacca* and lower *P. vulgaris* cover. This indicates the sensitivity of individual dune slack species to changes in water tables as small as 10 cm. Similar sensitivity to elevation above the water table in dune slacks has been shown in the field by Hope-Simpson et al. (1979). With only 40 cm differences in water table depth separating the drier from the wetter dune slack communities (Curreli et al., 2013), and the increasing threat of dropping water table depths due to climate change, dune slack communities are likely to change from wetter SD15/14 to drier SD16 communities.

Previous studies show that annual water losses through evapotranspiration are greater in wet dune slacks than dry slacks (Stratford et al., 2007). Overall, this study found that the water losses due to evapotranspiration were significantly greater in the wet hydrological regime than the dry. These findings are comparable to those of Stratford et al. (2007), which suggests that water losses within natural dune slack systems are likely to decrease with lowered water tables from climate change (Clarke and Ayutthaya, 2010), thus providing a degree of negative feedback on hydrological change. However, the response under further lowering of the groundwater level may not be linear since evaporation and transpiration can decouple. In drier conditions, surface soils may be dry but deep-rooted plants still have access to groundwater and can continue transpiring. Further research to assess likely impacts of lowering water tables on evapotranspiration would be useful under both controlled experimental conditions, and in the field.

Despite altered plant growth and water fluxes, nitrogen uptake was not affected by the differing water table regimes. This may be due to the soils and plants within both treatments having equal accessibility to groundwater nitrogen due to capillary processes, which can carry water 45cm above the water table (Ranwell, 1959) and due to deeper rooting depths observed within drier slack communities (Rhymes et al., 2014), i.e. the plant rooting depth is constrained by high water levels, meaning that for the hydrological regimes and species used in this study, the roots maintained similar contact with the water table.

1.4 Conclusions

These results suggest that for sites where nutrient concentrations in dune groundwater are elevated, there is a nutrient source in addition to atmospheric deposition. This highlights the necessity to develop a mechanism to include the contribution of groundwater nitrogen loads when assessing critical nitrogen loads for dune slack and other wetland habitats (Bobbink and Hettelingh, 2010).

This study demonstrates loss of DIN in groundwater suggesting N uptake and processing in dune slacks however, additional work is required to investigate the fate of this N, whether it is stored in soil and plant N pools and microbial biomass, or whether it is denitrified and emitted as N2 or the greenhouse gas N2O.

This study also highlights the vulnerability of dune slack communities to hydrological change. Changes in plant species cover due to a 10 cm change in water table depth emphasises the necessity to consider the potential impacts of climate change and groundwater abstraction on water tables and therefore on botanical composition of dune slacks, and to implement conservation management plans to respond to these combined threats.

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Appendix I

Table 1 – Artificial groundwater recipe compound weights added to 20L of deionised water. Table extracted from Rhymes et al. (2016).

Compound	Weight
$CaCO_3$	0.941
$CaCl_2$	7.541
$MgSO_4$	0.370
$MgCL_2$	0.996
KCl	0.089
$NaHCO_3$	5.082