The impact of 36 years of grazing management on soil nitrogen (N) supply rate and *Salix repens* N status and internal cycling in dune slacks.

Millett, J[1]* and Edmondson, S[2]

[1]Loughborough University

[2]Prescot, Merseyside

*Corresponding author: Centre for Hydrological and Ecosystem Sciences, Department of Geography, Loughborough University, Loughborough, LE11 3TU

T: +44 (0)1509222739

Email: j.millett@lboro.ac.uk

Abstract

Aims: To determine the impact of long-term rabbit and sheep grazing on *Salix repens* N status (green and abscised leaf N content and C:N ratio), internal N dynamics and soil N supply rate in dune slacks.

Methods: Herbivore exclosures were erected in dune slacks at Ainsdale Sand Dunes NNR, creating three grazing treatments: rabbit grazing; rabbits excluded for 36 years; rabbit grazing followed by sheep and rabbit grazing for 18 years. Soil N supply rate was analysed using ion exchange membranes; leaf N dynamics of *S. repens* were measured over one summer.

Results: soil N supply rate was higher in ungrazed plots. There was no difference in green leaf N_{MASS} or C:N ratio between treatments, but N dynamics differed. Adding sheep to existing rabbit grazing reduced *S. repens* N resorption efficiency (R_{EFF}) from 67% to 37%; excluding rabbits had no impact. Litter N_{MASS} was lower and C:N ratio higher in ungrazed plots.

Conclusions: Grazing can impact significantly on leaf N resorption, but this impact depends on the grazing regime.

Key words: herbivory; grazing; mammals; wetland; nitrogen; biogeochemical cycling

Introduction

Mammalian herbivores can have significant impacts on plant – soil interactions. These impacts can alter ecosystem functioning and responses to other environmental drivers such as climate change. Herbivory can alter ecosystem nutrient cycling in a number of ways: indirectly through impacts on individual plants (Sirontnak and Huntley 2000) and on species composition (Jaramillo and Detling 1992; Parsons et al 1994), and directly through deposition of faeces and urine, and due to physical impacts on the soil such as compaction (Schrama et al 2013). These impacts can retard (Ritchie et al 1998; Stark and Grellmann 2002) or accelerate (Frank and Evans 1997; Van der Wal et al 2004) the rate of nutrient cycling depending on ecosystem characteristics such as productivity, the identity of the herbivore and the timing of herbivory (Millard et al 2001; Stark and Grellmann 2002). These impacts can also change over the long-term (e.g. Olofsson et al 2007), but the majority of studies are short- to medium-term, which limits our understanding.

Most conceptual models predict that herbivores will speed up nutrient cycling in fertile systems and slow down nutrient cycling in low nutrient systems (Wardle et al 2004). This is because when leaf N concentrations are low herbivores consume a smaller proportion of net primary productivity (NPP). Return of nutrients to the soil is then dominated by less labile litter rather than fecal inputs and community composition becomes dominated by plants with low quality litter. Experimental studies, however, find contrasting results. Bakker et al (2009), for example, found that the opposite is the case for grasslands, but this might be due to the short term nature of experiments. Stark et al. (2014) found that herbivory decreased soil N cycling in more N replete tundra systems but increased cycling in N deficient systems. One hypothesis is that plant nutrient stoichiometry is a more important driver of herbivore impacts (Cherif and Loreau 2013), while Stark et al. (2014) considered that nutrient redistribution by herbivores might be the mechanism. What is clear is that plant – particularly leaf – and litter N concentrations and C:N ratios are key to understanding how grazing affects N cycling. These two traits are directly linked through the process of leaf nutrient resorption.

During leaf senescence plants withdraw N from their leaves, this is known as N resorption. Plants store this N in perennial tissues and use it for growth in the following year (Millard 1996). N not withdrawn remains in the leaf and is returned to the soil as litter. Leaf nutrient resorption is, therefore, a key process that controls nutrient fluxes from plants to the soil and nutrient availability for storage and reuse (Millard 1996). Between-site variability in leaf nutrient resorption appears to be controlled by plant nutrition; more nutrient replete plants are less efficient than more nutrient limited plants (Kobe et al 2005; Vergutz et al 2012). Within-site controls are less clear, but leaf nutrient resorption can be affected by competition (Millett et al 2005) and plant growth stage (Yasumura et al 2005), but not irradiance (Yasumura et al 2005). Herbivory may potentially influence resorption by altering litterfall timing, phenology or leaf chemistry (Chapman et al 2006b). Surprisingly, however, few studies have considered the impact of grazing on leaf nutrient resorption.

In terms of measuring the impact of herbivory a variety of leaf traits are interesting to study because they are strongly correlated with plant performance (e.g. mass, area, mass per unit area, nitrogen concentration), are potentially affected by herbivory and have the potential to impact on rates of herbivory and N cycling. The impact of herbivory is predicted to be strongly related to leaf N concentrations and C:N ratios (Bakker et al 2009). Leaves with more N and a lower C:N ratio tend be more palatable to herbivores; litter with more N and a lower C:N ratio tends to decompose faster (Cornwell et al 2008). Once grazed, plant tissue C:N ratios, leaf area and leaf mass can change, with impacts on plant function, decomposability and the probability of further herbivory (Kielland et al 1997). There are therefore clear links between green leaf chemistry, herbivory and decomposability (Wardle et al 2002).

In this *in-situ* experimental study we measured the impact of different grazing regimes – rabbit grazing (*Oryctolagus cuniculus*) or rabbit plus sheep (*Ovis aries*) grazing – after 36 years of grazing treatments. Rabbits and sheep are important mammalian herbivores, which might be expected to have different impacts on N cycling. Rabbits are small sized, hind gut fermenters; sheep are medium sized ruminants. These differences might have important impacts on ecosystem N cycling. Small animals should be more selective, choosing higher quality plants (Dement and Van Soest 1985), should redistribute N more widely in smaller pellets which release their N more quickly (Pastor et al 1996), and small burrowing animals such as rabbits should increase soil aeration, whereas larger animals should increase soil compaction. We, therefore, measured impacts on two key N cycling processes: soil N supply rate and leaf N cycling in the typical dune slack plant species *Salix repens*.

Herbivory has been a constant component of the ecology and management of dune slack systems, which are of high conservation importance (for example being listed in Annex 1 of the EU Habitats Directive). We have previously reported changes in plant community composition in these communities, in response to different grazing regimes (Millett and Edmondson 2013). We were interested in determining whether there were also changes in nitrogen cycling in these communities. We measured soil nitrogen (N) supply rate and leaf N traits for *S. repens* within dune slacks in Ainsdale Sand Dunes National Nature Reserve in North West England to assess the effects of three different grazing regimes: 1. grazing prevented for 36 years (‘*ungrazed*’), 2. continued rabbit grazing (‘*rabbit*’) and 3. sheep grazing added to existing rabbit grazing for 18 years (‘*rabbit+sheep*’). Specifically, we addressed the following questions: 1. does grazing over the long-term slow down or speed up soil N supply rate and alter *S. repens* leaf N content and autumn leaf N resorption, 2. how does adding sheep to existing rabbit grazing impact soil N supply rate and alter *S. repens* leaf N content and autumn leaf N resorption?

Materials and Methods

The details of the study site and experimental set-up have been described in full in Millett and Edmondson (2013); we will provide only pertinent details here.

Study site

This study was conducted in dune slacks within the Ainsdale Sand Dunes National Nature Reserve, which is part of the Sefton Coast Special Area of Conservation (SAC). Dune slacks are low-lying seasonally flooded areas in coastal dune systems. They have high alpha plant diversity and contribute to beta and gamma diversity due to the high proportion of specialist species. Ainsdale Sand Dunes NNR has received long-term grazing by rabbits (Houston 2004); more recently—in 1991—additional sheep grazing was added. The sheep graze the dune system intermittently between September/October and May/June each year, at a density of approximately 1.65 sheep per hectare. During the study mean±se annual rainfall was: 849±18.4 mm; mean annual maximum and minimum temperatures were respectively 13.6±0.2ºC and 6.1±0.1ºC. Atmospheric N deposition was approximately 1.37±0.03 g N m^{-2} $year^{-1}$ and remained relatively constant throughout the study. This level of N deposition is intermediate for the UK and is at the critical load threshold for this habitat of 1.0-1.5 g N m^{2} $year^{-1}$ (Bobbink and Hettelingh 2011). The water table varies by over 50 cm most years, with 30% of the slacks flooded in winter (10-30 cm) and 10% remaining flooded in the summer (Clarke and Sanitwong Na Ayutthaya 2009).

Experimental design

We erected three 1.5 x 1.5 m and 1 m high rabbit exclosures (30 mm mesh) in each of four dune slacks in 1974. By 2009 one exclosure had failed in one slack and all three had failed in another slack leaving a total of 8 functioning rabbit exclosures. 10 x 10 m and 1 m high sheep exclosures (mesh size 150 mm mesh) were added around the rabbit exclosures in two of the slacks in 1991, at the same time that sheep grazing was introduced. These exclosures prevented sheep access, but still allowed rabbit access. Counts of rabbit faecal pellets confirmed that no rabbits were able to enter the rabbit exclosures and that rabbit abundance was similar inside and outside the sheep exclosures (Millett and Edmondson 2013a). Specifically, in 2009 we found no faecal pellets inside the rabbit exclosures; outside the exclosures numbers were not significantly different inside and outside the sheep exclosure and varied from 0 – 121 per m^2, being on average 22.6 per m^2.

Plant community

The plant community in the dune slacks has been monitored since 1974; changes over time and the impact of grazing treatments are described in detail by (Millett and Edmondson 2013). In 1974 the community was dominated by *S. repens* and *Agrostis stolonifera* with abundant *Carex flacca*, *Lotus corniculatus* and *Hydrocotyle vulgaris*. Over time, this community changed, and was affected by the grazing treatments. In 2009 in the plots with no grazing the vascular plant community were dominated by *Rubus caesius* and *S. repens*, with the ground layer dominated by *Lotus pedunculatus* and *H. vulgaris*. In the grazed plots the plant community was dominated by *S. repens*, *Festuca rubra* and *L. pedunculatus* with *H. vulgaris* also dominating in those grazed by both rabbits and sheep. Also abundant in grazed plots, but not the ungrazed plots, were *Carex nigra*, *C. flacca*, *Anagallis tenella* and *A. stolonifera*. *Equisetum variegatum* and *L. corniculatus* were also abundant in plots grazed by rabbits and sheep but not in ungrazed plots or those grazed by only rabbits. Overall, grazing removal resulted in an increase in woody plant abundance and a decrease in forb and graminoid abundance. Graminoid and forb cover were higher in the plots grazed by rabbits and sheep than those grazed by rabbits alone. The weighted Ellenberg N score of the plant community in the ungrazed plots was higher (4.2±1.6) than in the grazed plots, but did not differ significantly between grazing treatments (Rabbit grazing: 3.8±2.2 Rabbit and sheep grazing: 3.7±1.2).

Measurements

The plots used in this study are identical to those in (Millett and Edmondson 2013), allowing direct comparison between the plant community and N cycling. We established one study plot inside each of the rabbit exclosures (‘ungrazed’: *n*=8 in total), 2 plots inside each of the sheep exclosures (‘rabbit grazed’: *n*=4 in total) and three near to the exclosures but in areas where sheep and rabbits were able to graze (‘Sheep and rabbit grazed’: *n*=11 in total).

In 2009 nitrogen cycling was investigated in the plots using two approaches. Firstly, leaf-N dynamics were measured for *S. repens* in each plot. This species was present in all plots and is characteristic of dune slack vegetation, making it a good indicator species for the impacts of grazing on plant-N cycling. Fully formed leaves (*n*=30 per plot) were removed on 6th July 2009 and 31st August 2009. On 25th October 2009 a sample of abscised leaves was removed from each plot. These were leaves that fell off readily when touched. The leaves from each plot were scanned using a flat-bed scanner. Leaf area was determined from the resulting image using image analysis software (ImageJ, Abramoff et al. 2004). Leaves within each plot were pooled and dried at 70ºC for 72 hours, weighed and milled to a fine powder using a ball mill (Retsch MM200). Carbon (C) and N content was then determined using a Perkin Elmer 2400 Series II CHN Elemental Analyzer. The height of *S. repens* in each plot was also measured at 10 random points in each plot.

Soil N availability was determined using Plant Root Simulator Probes (PRS^{TM}, Western Ag Innovations, Saskatoon, SK, Canada). These use either an anion- or cation-exchange resin membrane (total of 17.5 cm^2 per probe) housed in a plastic casing, the counter ions are Na^+ and HCO_3^-. The absorption of NH^+_4 and NO^-_3 by the resin membrane in the PRS^{TM} probes give an integrative index of the flux of plant available inorganic N over the burial period (Hangs et al 2004). This is a function of the size of N pools and the transformation of N to these NH^+_4 and NO^-_3 pools. Therefore, they are unable to provide any measure of differences in the size of these pools between grazing treatments. Nonetheless, they are a useful measure of differences in terms of rates of N supply rate, particularly the impact of net mineralisation on N supply (because we removed root interactions). They are also broadly comparable to measurements made by (Bakker et al 2009) who measured N supply rate using resin bags.

Three pairs of PRS^{TM} probes (one anion and one cation, 5 cm apart) were buried in each plot on 6th July 2009 with at least 40 cm between pairs. Each probe was inserted into a slit cut into the soil so that the membrane was level with the top of the soil, extending down 5 cm into the soil. To prevent root interaction with the ion exchange membrane all vegetation was removed along with as much of the root system as possible (minimising soil disturbance) from a 10 cm diameter circle around each pair. Roots were severed with a serrated knife around the perimeter of this circle. The probes were removed on 31st August 2009 (a burial period of 60 days). On removal the probes were rinsed in the field with deionised water to remove as much soil as possible, they were later carefully scrubbed with a brush and deionised water to remove all remaining soil residue. The PRS probes were sent to Western Ag Innovations where they were extracted using 0.5 N HCl for 1 h. The extractant was analysed for NH^+_4 and NO_3^-, for the cation and anion exchange membranes respectively, using colourimetry with a flow injection analysis system (FIAlab-2600 automated procedure). The amount of N absorbed by the PRS^{TM} probes was calculated by adding the total amount of N contained in the extractants (so is the sum of NH^+_4-N and NO^-_3-N).

Data analysis

Initial data exploration showed that the pattern of treatment differences in leaf N concentrations differed between July and August for N_{AREA} – lower for leaves in *rabbit+sheep* grazed plots in July but not in August – but did not differ for N_{MASS} – no significant differences between treatments in July or August. Leaves collected in July had consistently higher N concentrations on a mass and area basis than those collected in August. We therefore used the July leaves as our ‘green’ leaves and the leaves collected in October as our ‘abscised’ leaves. Leaf N resorption before autumn leaf abscission (R_{EFF}) is best measured on a leaf area basis because this changes less than leaf mass during senescence (van Heerwaarden et al 2003). Thus, R_{EFF} was calculated as follows (Aerts 1996; Killingbeck 1996):

Equation 1: $$R_{EFF} = \frac{(N_{\mathrm{AREA}}\text{ of green leaf} - N_{\mathrm{AREA}}\text{ of abscised leaf})}{N_{\mathrm{AREA}}\text{ of green leaf}}$$

We used a number of leaf traits. Leaf N content is directly related to plant nutritional status and is a key parameter for understanding ecosystem responses to herbivory (Bakker et al 2009). We therefore calculated leaf N concentration on an area (N_{AREA}) and mass (N_{MASS}) basis. We calculated the C:N ratio for green and abscised leaves because of the hypothesised importance of nutrient stoichiometry for herbivore impacts on N cycling. We also used leaf-mass, leaf-area and leaf mass per unit area (LMA) because woody perennials alter biomass allocation patterns as a result of herbivory (Hester et al 2004) and this may be captured in these traits.

We tested for differences in these leaf traits and R_{EFF}, PRS^{TM} probe N absorption and *S. repens* height between grazing treatments using a Linear Mixed Model (LMM) in SPSS 20.0 (IBM Corp. 2011) with grazing treatment and dune slack as fixed effects. The effect of grazing treatment was also tested using a priori tests of the difference between ungrazed and grazed (the mean of *rabbit* and *rabbit+sheep* grazed plots) plots and the difference between *rabbit* and *rabbit+sheep* grazed plots were tested separately using custom hypothesis tests with the 'TEST' subcommand in SPSS. The relationship between green leaf N content and abscised leaf N content was assessed using linear regression.

We undertook further analysis of plant community data from the plots (presented in (Millett and Edmondson 2013). We calculated an index of impact of grazing removal on the most common vascular plant species found in the plots. The index was calculated as follows:

Equation 2: $$Grazing\ index = \frac{(ungrazed\ abundance - grazed\ abundance)}{(ungrazed\ abundance + grazed\ abundance)}$$

This index yields a number for each species from -1 to +1. A value of +1 indicates that species is only present in ungrazed plots, a value of -1 indicates that species is only present in grazed plots and a value of 0 indicates that abundance is the same in grazed and ungrazed plots.

Results

During the 60 day soil incubation period considerably more inorganic N was absorbed by ion and cation exchange membranes in the PRS^{TM} probes in the *ungrazed* plots than in either of the grazing treatments (Table 1, Fig. 1). *Salix repens* was present in every plot and plants were taller in the *ungrazed* treatments than either of the grazed treatments (Table 1, Fig. 2). Leaf traits in July differed between grazing treatments. Leaves of *S. repens* in grazed plots had, on average, higher mass than those growing in ungrazed plots and those grazed by only rabbits had a higher leaf mass than those grazed by rabbits and sheep (Table 1, Fig. 3a). Leaves of *S. repens* in ungrazed plots also had a smaller area than those in grazed plots, with no significant difference between *rabbit* or *rabbit+sheep* grazed plots (Table 1, Fig. 3b). As a result LMA of *S. repens* in grazed plots was not different on average to that of plants in *ungrazed* plots. LMA of plants in *rabbit* grazed plots was, however, significantly higher than that of plants in *rabbit+sheep* grazed plots (Table 1, Fig. 3c). In terms of leaf nutrients, only leaf N content on an area basis was affected by the grazing treatments (Table 1), those in *rabbit* grazed plots had higher N_{AREA} than those in *rabbit+sheep* grazed plots. There was no impact of the treatments on green leaf C:N ratios or N content on a mass basis.

On average *S. repens* leaf-N decreased by 51.0±5.1% between July and abscission. Leaf N dynamics were also affected by the grazing treatments (Table 1, Fig. 4). Leaves of plants which had higher green-leaf N concentrations withdrew more N from these leaves than those with lower green-leaf N concentrations. The result of this was a weak negative relationship between green-leaf N content and abscised-leaf N content (Linear regression: P=0.053, r^2=0.174), and significantly lower leaf N resorption efficiency (R_{EFF}) of *S. repens* plants in the *rabbit+sheep* grazing treatment than that of plants in the *ungrazed* or *rabbit* grazed plots (Table 1, Fig. 5a). Abscised leaf N_{MASS}, however, was significantly lower in *ungrazed* plots and C:N ratio was significantly higher in ungrazed plots.

Grazing removal had a positive impact on the abundance of only four of the most common species (Fig. 6). There was no discernible impact on the abundance of *S. repens* and a negative impact on the remaining 14 most common species, with six of these species being absent from ungrazed plots.

Discussion

The impact of herbivory on leaf nutrient resorption is relatively poorly studied and the controls over leaf N resorption are not yet fully resolved. Millett et al (2005) found no impact of low levels of simulated herbivory on autumn N resorption by *Betula pubescens* but Silla and Escudero (2003) found that foliar herbivory reduced green-leaf N content and so leaf N resorption efficiency. We found an impact of grazing regime on leaf N resorption efficiency. The leaf N resorption efficiency of *S. repens* growing in plots grazed by *rabbits+sheep* was approximately half that of those in *ungrazed* or *rabbit* grazed plots. This suggests that herbivory might be an important control over within site variability in leaf nutrient resorption, but this effect is dependent on the grazing regime. Green-leaf N_{AREA} was lower in *rabbit+sheep* grazed plots but abscised-leaf N_{AREA} did not differ between treatments. Similarly to Silla and Escudero (2003) these differences in green-leaf N concentrations appear to have resulted in the observed differences in leaf N resorption efficiency. This suggests that herbivore impacts on leaf nutrient resorption may depend on impacts on green-leaf nutrient content. Herbivory might, therefore, reverse the usual between site pattern of positive correlation between green- and abscised-leaf N content and negative correlation between plant N status and resorption efficiency (Vergutz et al 2012).

Green-leaf N_{MASS} and so C:N ratio of *S. repens* growing in grazed plots was unaffected by grazing but abscised-leaf N_{MASS} was increased in grazed plots, resulting in lower C:N ratios. The differences between N_{MASS} and N_{AREA} might be explained by the herbivore induced production of C-based secondary metabolites (Mithöefer and Boland 2012), which might be less mobile within the plant and so less likely to be withdrawn from the leaves. This would alter N_{MASS} by changing the mass of the leaf, but not N_{AREA} because the area of the leaf would remain unchanged. The results here show that adding sheep grazing to existing rabbit grazing reduced green-leaf N content and N resorption efficiency, reducing the leaf N pool available to *S. repens* plants for resorption and reuse. This might be a result of increased herbivory resulting in removal of more N from *S. repens* plants in *rabbit+sheep* grazed plots than in only *rabbit* grazed plots, reducing total plant N content and so leaf N content. This might have consequences for *S. repens* nutrition in following years. Further studies are needed to better understand the impacts and implications of grazing for leaf nutrient resorption.

Long-term exclusion of herbivores resulted in an increase in N ion absorption onto the PRS^{TM} probes. This suggests an increased soil N supply rate due to acceleration of N cycling below-ground, and so increased plant N availability. This effect was consistent for both rabbit grazing and when sheep grazing was added to the rabbit grazing. It is maybe surprising that this increased soil N supply did not result in altered N status of *S. repens*. The reasons cannot be unambiguously determined but might be because the plants were not N limited – as indicated by relatively high leaf N content; or due to an interaction between increased soil N supply and decreased herbivory – both changes are confounded; or maybe the additional N was used by the plants for growth – they were taller in ungrazed plots – rather than to increase tissue N concentration.

Grazing impacts on ecosystem N cycling are often observed (Hobbs 1996), though the impacts are variable (Milchunas and Lauenroth 1993; Olofsson et al 2007). Models predict that herbivores will increase soil N supply in high nutrient systems and decrease soil N supply in low nutrient systems (Bardgett and Wardle 2003). Bakker et al (2009), however, found the opposite in a relatively short term (6 – 8 years) experiment. Our measurements are broadly comparable to those of Bakker et al (2009). We only measured nutrition of leaves in one species, but Jones et al (2004) found tissue N concentrations in entire dune slack plant communities ranged from 0.94-1.67% in the UK, which is in the mid to high part of the range studies by Bakker et al (2009). The *S. repens* plants in our plots had relatively high leaf N concentrations (2.1% in ungrazed plots and 2.0% in grazed plots) and low C:N ratios (24 in ungrazed plots and 26 in grazed plots); grazing had a negative impact on N supply rates (-62% with *rabbit* grazing and -30% with *rabbit+sheep* grazing). These results are consistent with those found by Bakker et al (2009), but after 36 years of grazing exclusion (for *rabbit* grazing), and so provide good support of their findings.

Changes in soil N supply could be due to changes in the soil microbial community, the accumulation of litter due to increased above-ground biomass (e.g. Olofsson et al 2007) or the return of plant-N to the soil in a more readily available form by the herbivores or due to changes in litter chemistry and so decomposability. Our evidence suggests that changes in litter chemistry are not responsible. *Salix repens* was present in all plots, forming a significant proportion of vascular plant cover (19-30%). Therefore it might be expected that this single species would have a disproportionate impact on ecosystem N cycling. *S. repens* leaf litter N concentration (N_{MASS}) decreased and C:N ratios increased when grazing was removed, which would likely decrease litter decomposability and so slow N cycling (Bardgett and Wardle 2003), rather than speed up soil N supply rate as we found.

Grazing removal caused significant changes in the plant community, resulting in an increase in the weighted Ellengberg N value (Millett and Edmondson 2013). These changes could potentially impact on N cycling if the N uptake and cycling characteristics of the species in the respective communities differ (Hobbs 1996; Chapman et al 2006a). We have summarised the impact on individual species in Fig. 6. There is no evidence from these data that changes in the plant community might have caused the grazing removal induced increase in soil N supply. In fact grazing removal resulted in a reduction in the abundance of plant species which might be expected to increase soil N supply (e.g. N-fixing species and herbaceous species). It, therefore, seems unlikely that changes in plant community composition were the reason for the changes in soil N supply.

Stark et al (2014) considered that the movement of N within ecosystems by herbivores might be the mechanism that causes the negative impacts of herbivores on soil N cycling in tundra systems. This could be the case on dune slacks where the N content of vegetation varies between plant communities (Jones et al 2004). Rabbits and sheep may remove nutrients from relatively nutrient replete areas such as dune slacks to relatively nutrient poor areas such as mobile and semi-fixed dunes. Excluding herbivores would prevent this movement. Alternatively, Schrama et al (2013) hypothesise that soil compaction by herbivores reduces oxygen availability in wet soils such as those in the present study, resulting in a reduction in N mineralisation. This warrants further study and maybe sand dune systems are the ideal place to study this because very different hydrological conditions can exist within meters of each other—from very wet dune slacks to very dry dune summits.

In summary, *S. repens* plants in grazed plots were shorter and had bigger (mass and area) leaves than those in ungrazed plots. The leaves of plants in plots grazed by both rabbits and sheep had a lower LMA, which might reflect increased grazing pressure and/or different grazing patterns. These relatively subtle additive impacts of sheep resulted in considerably lower leaf N resorption efficiency. This decreased resorption efficiency will reduce the size of the resorption pool available to the plants, increasing reliance on root N uptake. Soil N supply rate increased significantly when rabbits were excluded, but was not affected by adding sheep. So *S. repens* plants in the *rabbit+sheep* plots are likely to have to compete more – due to reduced leaf N resorption – for a more limited soil N supply. We found, however, no impact of herbivory on *S. repens* N status, but *S. repens* litter N concentrations were higher and C:N ratios lower in grazed plots, which will likely increase rates of litter decomposition. We conclude that grazing can impact significantly on leaf N resorption; sheep grazing seems to be more important than rabbit grazing for autumn leaf N resoprtion in *S. repens*. Soil N supply is also significantly affected by grazing, but seems to be indifferent of herbivore identity and additive effects. Further study of herbivore impacts on leaf nutrient resorption is required to be able to fully understand how herbivores affect ecosystem nutrient cycling.

Acknowledgements

This study was funded by the Botanical Society of the British Isles. The manuscript was greatly improved by the comments of three anonymous reviewers and Harry Olde Venterink.

Conflict of interests

The authors have no conflicts of interest.

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Table 1

Linear Mixed Model (LMM) results for traits of *Salix repens* growing in dune slack plant communities subjected to different grazing regimes. Presented are the *P*–values for the effect of grazing regime on characteristics measured in 2009, changes in characteristics during 2009, and the interaction between the two. LMA is leaf mass per unit area, N_{AREA} and N_{MASS} are N per unit area and mass respectively, R_{EFF} is autumn leaf N resorption efficiency on an area basis.

Fig. 1 Dune slack soil N availability under three different grazing regimes. Presented are the estimated marginal mean+SE inorganic N (NH_4^+ + NO_3^-) uptake by Plant Root Stimulator (PRS^{TM}) probes.

Fig. 2 Height of *Salix repens* plants growing in three different grazing treatments. Presented are mean±SE.

Fig. 3 Leaf traits of *Salix repens* plants growing in three different grazing treatments. Presented are estimated marginal mean±SE of: a. leaf mass b. leaf area; c. LMA.

Fig. 4 Leaf traits of *Salix repens* plants growing in three different grazing treatments. Presented are estimated marginal mean±SE of leaf N content on a. and b. an area basis and c. and d. a mass basis; and e. and f. leaf C:N ratio. Data are presented for green leaves in July (a, c, and e) and for abscised leaves in October (b, d and f).

Fig. 5 Autumn leaf N resorption efficiency (R_{EFF}) of *Salix repens* plants growing in three different grazing treatments. Presented are estimated marginal mean±SE for resorption efficiency calculated on a per unit area basis.

Fig. 6 Index of impact of grazing removal on most common vascular plant species found in dune slacks. A value of +1 indicates that species is only present in ungrazed plots, a value of -1 indicates that species is only present in grazed plots and a value of 0 indicates that abundance is the same in grazed and ungrazed plots..

Table 1

Measure	**d.f.**	**Treatment**	**Grazed vs Ungrazed**	**Rabbit vs Rabbit+sheep**
S. repens height	2, 16	**0.003**	**0.001**	0.974
S. repens summer leaf area	2, 16	**0.013**	**0.005**	0.725
S. repens summer leaf mass	2, 16	**0.001**	**0.001**	**0.005**
S. repens summer LMA	2, 16	**0.049**	0.887	**0.017**
S. repens N_{AREA} (July)	2, 16	**0.004**	0.289	**0.002**
S. repens N_{AREA} (October)	2, 16	0.093	n/a	n/a
S. repens N_{MASS} (July)	2, 16	0.637	n/a	n/a
S. repens N_{MASS} (October)	2, 16	**0.011**	**0.004**	0.980
S. repens leaf C:N ratio (October)	2, 16	**0.005**	**0.001**	0.801
S. repens R_{EFF}	2, 16	**0.018**	0.096	**0.038**
PRS^{TM} probe N absorption	2, 16	**<0.001**	**0.017**	0.958

Fig. 1

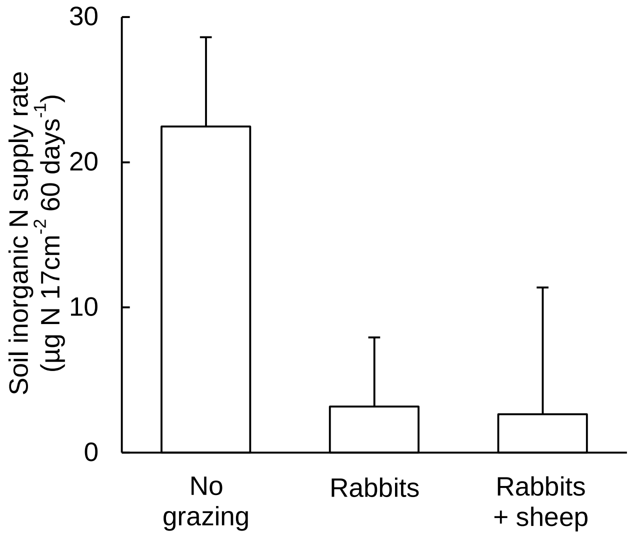

Soil inorganic N supply rate
(µg N 17cm-2 60 days-1)
30
20
10
0
No
grazing
Rabbits
Rabbits
+ sheep

Fig. 2

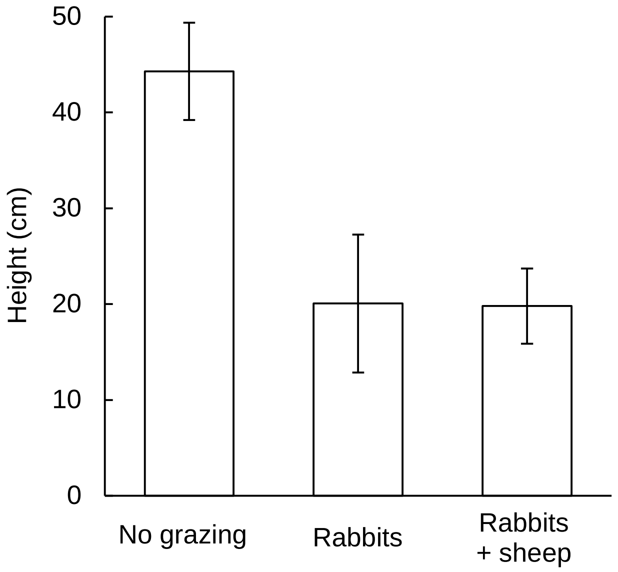


Fig. 3

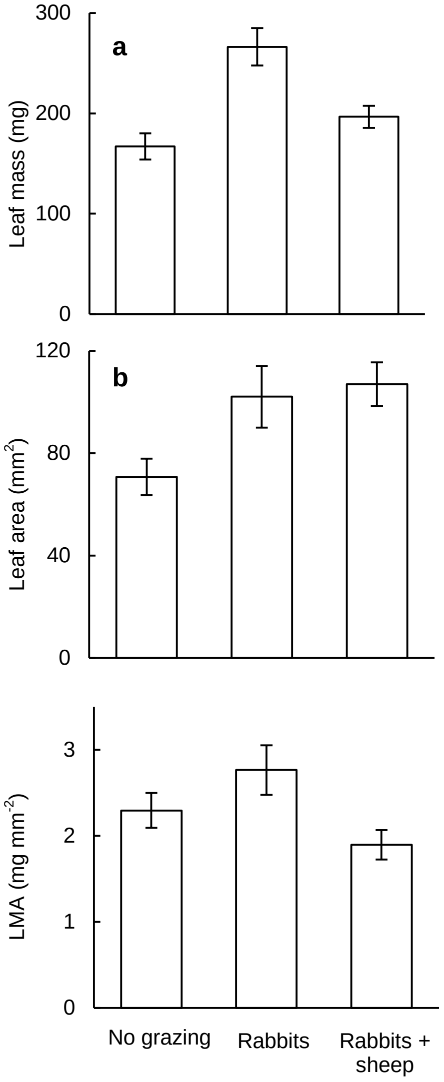

a
Leaf mass (mg)
300
200
100
0
b
Leaf area (mm^2)
120
80
40
0
LMA (mg mm^{-2})
3
2
1
0
No grazing
Rabbits
Rabbits + sheep

Fig. 4

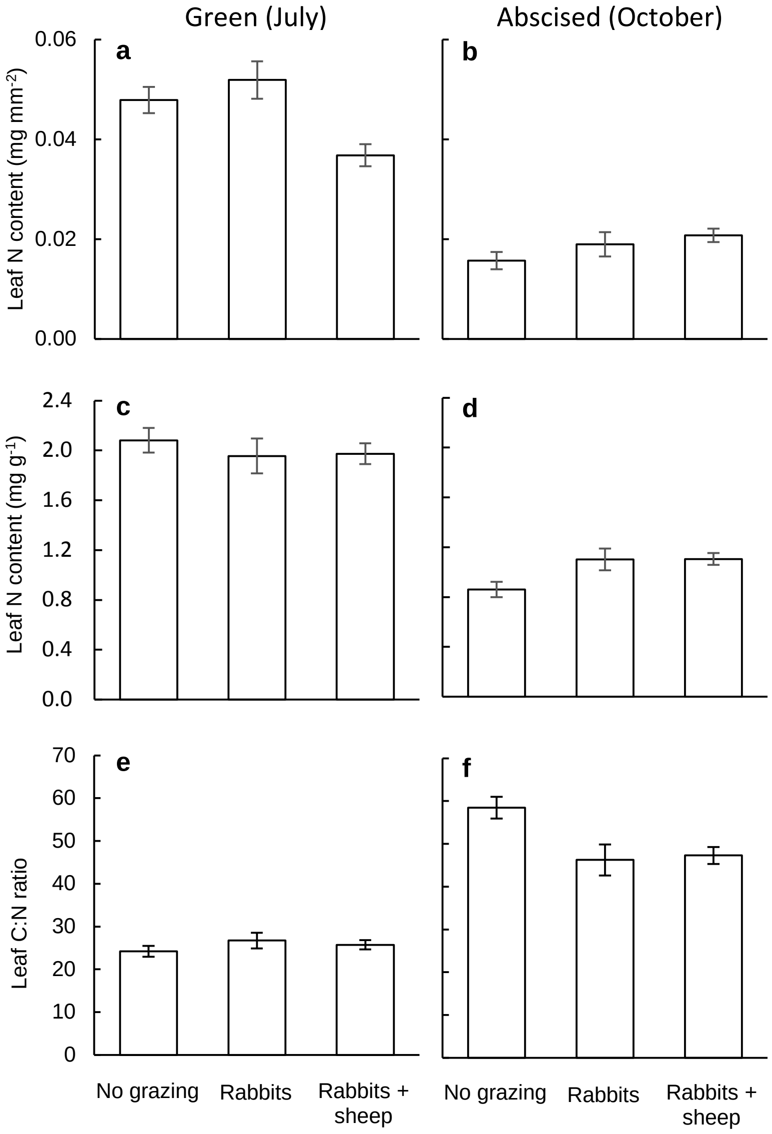

Green (July)
Abscised (October)
a
b
c
d
e
f
Leaf N content (mg mm^{-2})
0.06
0.04
0.02
0.00
Leaf N content (mg g^{-1})
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Leaf C:N ratio
70
60
50
40
30
20
10
0
No grazing
Rabbits
Rabbits + sheep
No grazing
Rabbits
Rabbits + sheep

Figure 5

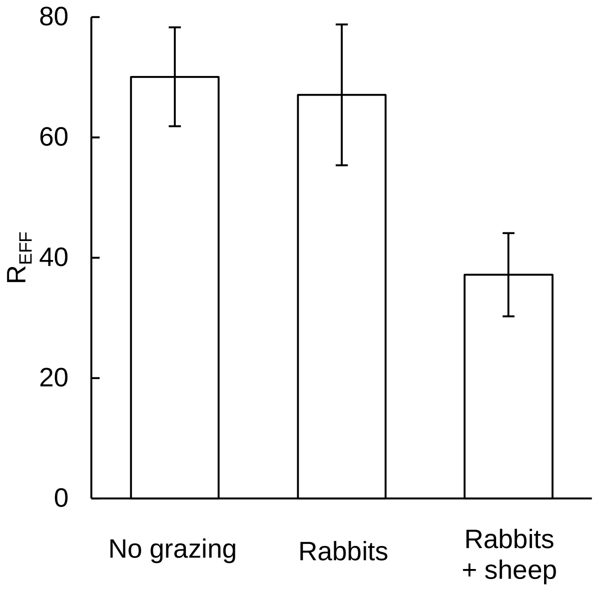
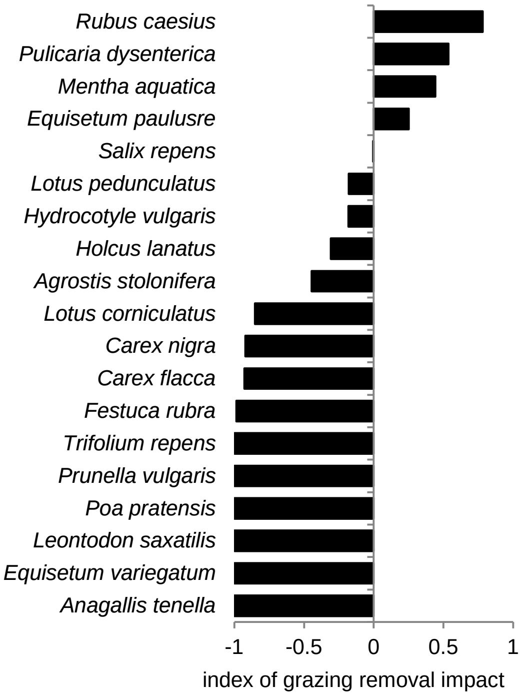


Figure 6


Rubus caesius
Pulicaria dysenterica
Mentha aquatica
Equisetum paulusre
Salix repens
Lotus pedunculatus
Hydrocotyle vulgaris
Holcus lanatus
Agrostis stolonifera
Lotus corniculatus
Carex nigra
Carex flacca
Festuca rubra
Trifolium repens
Prunella vulgaris
Poa pratensis
Leontodon saxatilis
Equisetum variegatum
Anagallis tenella
-1
-0.5
0
0.5
1
index of grazing removal impact