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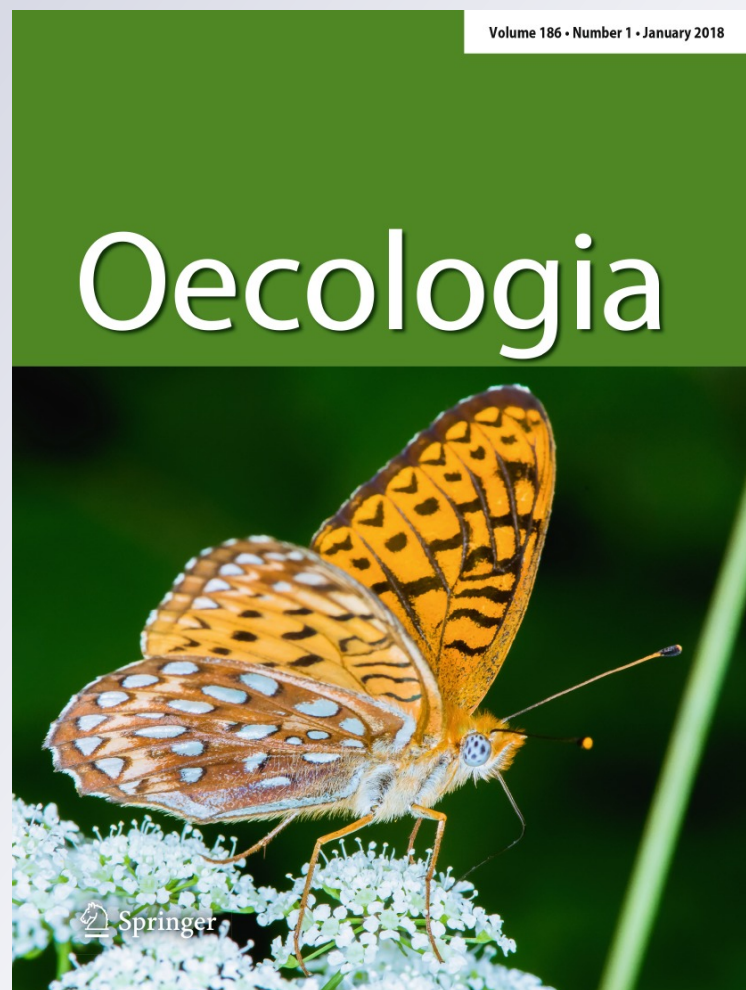
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Growth responses of the common arctic graminoid *Eriophorum vaginatum* to simulated grazing are independent of soil nitrogen availability

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Abstract Plant compensatory growth responses to herbivory are mediated by soil fertility and can have significant feedbacks that affect overall ecosystem nutrient cycling. The sedge *Eriophorum vaginatum* is the dominant graminoid in arctic mesic tundra, and is heavily consumed by caribou. Here, we compare the principal compensatory growth models in explaining the impact of a single episode of simulated caribou grazing at two clipping intensities on *E. vaginatum* total growing season shoot production, nitrogen concentrations, and nitrogen pools, over two successive years across a soil nitrogen fertilisation gradient. The clipping treatments had no effect on shoot production in the growing season when they were applied, but substantially reduced growth in the following year. Surprisingly, these reductions were consistent across all levels of soil nitrogen availability. The Limiting Resource Model can best explain this legacy effect on production because it predicts alternate compensatory growth responses depending on whether or not the herbivory affects availability of the resource that most limits plant growth. Accordingly, our results suggest that shoot compensatory growth in the year after the clipping was limited by some resource other than nitrogen—probably internal

carbohydrate reserves or soil phosphorus. The clipping treatments initially enhanced shoot nitrogen concentrations and pools, but shoot nitrogen pools had decreased by the end of the second year due to the legacy effect of reduced shoot production. Finally, inflorescence removal substantially stimulated new shoot production in both years. Together, our results suggest that herbivory can significantly enhance temporal and local spatial heterogeneity in graminoid growth and nitrogen cycling.

Keywords Apical inflorescence meristem · Caribou · Climate change · Compensatory growth · Herbivory

Introduction

Plant growth in the Arctic is generally nutrient limited (Chapin et al. 1995; Jonasson et al. 1999) and, therefore, the increases in soil fertility predicted with climate change (Nadelhoffer et al. 1991; Shaver et al. 2006) are expected to stimulate primary production. However, herbivory by small and large mammals may be particularly important in constraining plant growth responses to warming (Olofsson et al. 2009; Post and Pedersen 2008; Ravolainen et al. 2014; Zamin and Grogan 2013). These herbivore impacts may be due to direct tissue removal as well as distinct herbivore nutrient-cycling feedbacks that can significantly modify soil nutrient availability (Hobbs 1996; Olff and Ritchie 1998; Pastor et al. 2006).

Current theory of herbivore impacts on plant–soil nutrient cycling suggests that there are two categories of opposing feedbacks, whose relative influence depends on the initial level of soil fertility (Pastor et al. 2006; see Fig. 1). For example, herbivory in tundra can decelerate nutrient cycling through selective browsing (McInnes et al. 1992; Stark and

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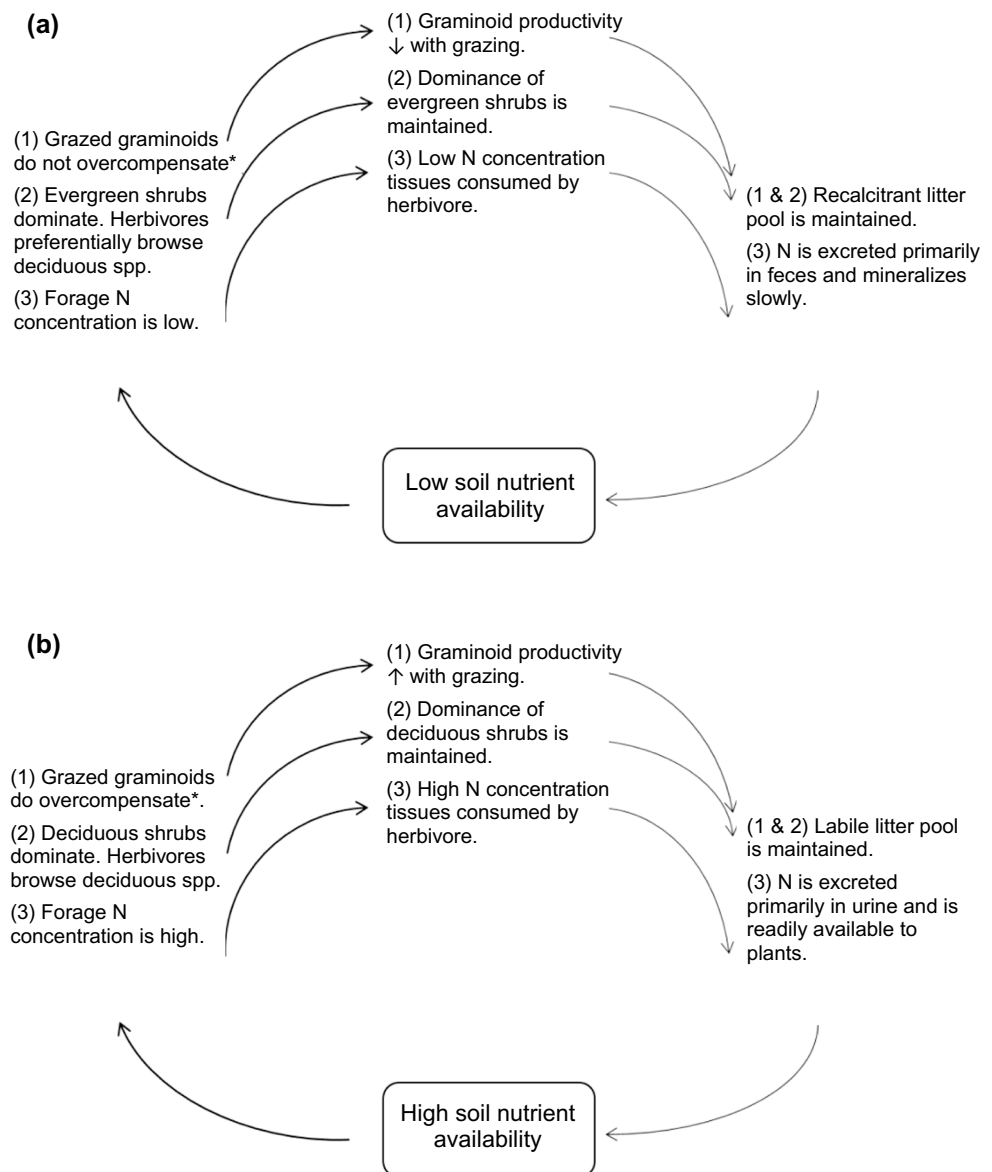
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Fig. 1 A synthesis of conceptual model predictions of the role of soil fertility in herbivore–nutrient cycling feedbacks that is specifically adapted to determine the ultimate impact of herbivores on production and relative abundance responses of the principal plant growth forms in low arctic tundra vegetation. Asterisk indicates that the predictions vary among models; numbers in parentheses indicate citations for the predictions: (1) Pastor et al. (1993); Jefferies et al. (1994), Frank and Groffman (1998), (2) Hilbert et al. (1981), McNaughton (1983), Maschinski and Whitham (1989), Hawkes and Sullivan (2001), Wise and Abrahamson (2005), (3) Hobbs (1996), Pastor et al. (2006)



Grellmann 2002) of graminoids and deciduous shrubs as compared to evergreen shrubs, resulting in feedbacks that promote low levels of soil fertility (Fig. 1a). By contrast, under relatively fertile conditions, plant leaf nutrient concentrations are enhanced, and tundra herbivores can promote high soil nutrient levels by consuming those tissues and short-cutting the decomposition process by rapidly returning the plant nutrients to the soil via urine (Frank and Groffman 1998; Fig. 1b; Hobbs 1996). Soil fertility is, therefore, a key determinant of herbivore impacts on ecosystem function, because it significantly influences vegetation community structure (Chapin 1980), constitutive plant tissue chemistry (Bryant et al. 1983; Jones and Hartley 1999), regrowth following browsing (Hawkes and Sullivan 2001; McNaughton 1983), and herbivore nutrient return (Pastor et al. 2006; Fig. 1). As the climate warms in the Arctic and caribou

populations change (ACIA 2005; Epstein et al. 2012; Zamin and Grogan 2013), understanding the potential influences of these herbivore–nutrient cycling feedbacks is critical to predicting shifts in tundra vegetation community structure.

Three main mechanisms maintain these opposing herbivore–nutrient cycling feedbacks: (i) plant compensatory growth responses (Hawkes and Sullivan 2001; Hilbert et al. 1981; Maschinski and Whitham 1989); (ii) the influence of herbivores on vegetation community structure (Frank and Groffman 1998; Jefferies et al. 1994; Pastor et al. 1993); and (iii) consumed plant tissue nutrient concentrations because they influence herbivore-mediated nutrient return to the soil (Hobbs 1996; Pastor et al. 2006). Our study focuses on the first mechanism, compensatory growth, which is generally defined as a positive growth response of plants to injury (Belsky 1986). Compensatory

growth may range from under-compensation, or partial replacement of lost tissue, to equal compensation, or exact replacement of lost biomass, to over-compensation, or net productivity greater than that of undamaged control plants (Belsky 1986). Meta-analyses have indicated that compensatory growth responses may be non-linear and highly variable across resource levels and between functional groups (Georgiadis et al. 1989; Hawkes and Sullivan 2001), due to the complexity of intrinsic (e.g. plant physiology and development) and extrinsic (e.g. soil moisture or nutrients) drivers (McNaughton 1983).

Several models have been developed to predict the impact of variation in soil fertility on plant compensatory growth responses to herbivory (Hilbert et al. 1981; Maschinski and Whitham 1989), and their conclusions are well supported in a meta-analysis of the research literature (Hawkes and Sullivan 2001). For example, the compensatory continuum hypothesis predicts that tolerance to herbivory should be greater in high resource, low competition or otherwise benign environments (Maschinski and Whitham 1989). By contrast, the growth rate model predicts that tolerance to herbivory will be greater at low resource levels since the targeted plants will not be growing at the maximum possible relative growth rate (RGR) and, therefore, have the potential to increase their RGR (Hilbert et al. 1981). The more recent Limiting Resource Model (LRM) of compensatory growth in response to herbivory is distinctive in that it incorporates the developmental plasticity of plants in balancing acquisition of multiple resources (Wise and Abrahamson 2005, 2007). This model concludes that herbivore damage can either exacerbate an already existing growth limitation or cause an alternative resource to become limiting, and predicts that whether plants under-, exact-, or over-compensate for lost tissue depends on how herbivory affects both the original growth-limiting resource and the alternative (originally non-limiting) resources (Wise and Abrahamson 2005, 2007). Since the extent of graminoid compensatory growth is a critical component of the overall impact of herbivores on production and species relative abundances in tundra vegetation at differing levels of fertility (Fig. 1), characterising the relative effectiveness of these three models in a low arctic context is important.

Perennial plants may be affected by herbivory not only immediately, but also in subsequent years. In the Arctic and other highly seasonal environments in particular, the inter-annual pattern of plant compensatory growth responses to herbivory are particularly relevant because plant growth in a given growing season is strongly influenced by the previous growing season's nutrient availability and weather conditions (Jonasson and Chapin 1985; Shaver et al. 1986). Therefore, our study was conducted over two successive growing seasons to test for legacy effects of single-episode clipping treatments.

Compared to the impacts of only leaf tissue removal, herbivory that damages or removes a plant's apical meristems results in distinctive growth responses (Wise and Abrahamson 2008). Previous comparative studies have focused on dicots and generally found positive growth responses to meristem damage (see list in Wise and Abrahamson 2008), which may be non-linear across soil fertility gradients (Irwin and Aarssen 1996). In the Arctic, graminoid inflorescences are heavily consumed by caribou (White and Trudell 1980) and, therefore, it is particularly important to include graminoid reproductive shoot apical meristem damage when considering herbivory impacts on compensatory growth. Accordingly, our study compares growth responses to simulated herbivory between graminoid plants in which some of the inflorescence apical meristems were removed during clipping, with plants in which only leaf material was removed.

In summary, here we evaluate the impact of a single episode of moderate or intense simulated grazing (i.e. clipping) on the shoot productivity and nutrient pools of the widespread arctic graminoid, tussock cottongrass (*Eriophorum vaginatum* L.), over two growing seasons in plots of varying experimental N addition. We focus on *E. vaginatum* since it is the dominant graminoid in mesic tundra (CAVM 2003; Miller 1982), is highly responsive to increased soil fertility (Chapin 1980; Shaver et al. 1986), and is heavily consumed by caribou (White and Trudell 1980). We address the following research questions:

1. Which conceptual model of compensatory growth best describes *E. vaginatum* total annual shoot growth responses to clipping along a gradient of increasing soil nitrogen availability?
2. Are there significant legacy effects of clipping on *E. vaginatum* total annual shoot production and nitrogen accumulation over the following two growing seasons?
3. Does removal of some *E. vaginatum* inflorescence shoot apical meristems during intense clipping have significant impacts on subsequent shoot production over the following two growing seasons?

Materials and methods

Study site and treatments

We conducted a clipping experiment on tussock cottongrass (*E. vaginatum* L.) in which we manipulated both clipping intensity and soil N availability at three levels of each treatment. This study was located in mesic birch hummock tundra vegetation near the Daring Lake Research Station, Northwest Territories (64° 52' N, 111° 33' W) in the central Canadian low Arctic (see Zamin et al. 2014 for more

information). At the site, mean diel temperatures (\pm SEs) range from an annual minimum of -38 ± 0.7 °C in January to a maximum of 20 ± 0.4 °C in July, and mean annual rainfall is about 138 ± 13 mm (Bob Reid, Indian and Northern Affairs Canada, unpublished data 1996–2011; range bound at 2011 to match sampling in this study).

Birch hummock tundra vegetation is dominated by evergreen shrubs and lichens, with sedges constituting about 6% of aboveground vascular plant biomass (Zamin et al. 2014), almost all of which is *E. vaginatum* (unpublished data). *E. vaginatum* ranges across the circum-Arctic (Miller 1982; Porsild and Cody 1980), is a dominant graminoid in the vegetation types of tussock-sedge/dwarf-shrub/moss tundra (G4; CAVM 2003) and sedge/moss/low-shrub wetland (W4; CAVM 2003), and constitutes about 21% of total vascular plant biomass in the acidic tussock tundra vegetation type at Toolik Lake, Alaska (Chapin et al. 1995).

Three soil fertility treatments were used: control, low-N addition ($1 \text{ g N m}^{-2} \text{ yr}^{-1}$) and high-N addition ($10 \text{ g N m}^{-2} \text{ year}^{-1}$; $n = 5$ per treatment; plot size $5 \times 7 \text{ m}$). These plots were established in flat patches of similar birch hummock tundra vegetation within a $\sim 200 \times \sim 300 \text{ m}$ area of gently sloping valley, and randomly allocated to N addition treatment in July 2004 (see map, Figure S1).

Since then, granular ammonium nitrate (NH_4NO_3 —agricultural grade) has been applied yearly, generally in late summer of all years and specifically on August 28th in 2009, on August 13th in 2010, and on September 4th in 2011 (i.e. always after the plant harvest sampling at the end of each growing season to determine the impact of the clipping treatments in this study). The N addition treatment levels were chosen to match similar experiments in mesic low Arctic tundra at Toolik Lake, Alaska (Chapin et al. 1995) and Abisko, Sweden (Jonasson et al. 1999), with the high N level intended to be well in excess of growth requirements and, therefore, to remove N limitation (Chapin et al. 1995). However, in the context of future climate, the extent to which warming may enhance nutrient availability remains largely unknown (Giesler et al. 2012; Hobbie et al. 2002), but is unlikely to reach the high-N addition level used in this study (but see Mack et al. 2004).

Twelve *E. vaginatum* patches were located within each of the five replicate control, low-N and high-N addition plots (each 35 m^2), and randomly assigned to one of three levels of clipping treatment: control (i.e. no clipping), simulated moderate graze, and simulated intense graze. Biomass responses to the clipping treatments were measured at the individual patch level, but then averaged across the four patches of each clipping level within each plot for the statistical analysis (i.e. $n = 5$ per clipping treatment per soil N addition level). The patches consisted of individual clumps or tussocks of *E. vaginatum* that ranged in area from ~ 0.0025 to 0.01 m^2 , and were deliberately chosen so as to be at least 1 m apart

to minimise likelihood of rhizome connections, except that we had to include two (out of 180 total) that were no more than 45 cm apart because of low *E. vaginatum* density in several plots. The clipping treatment was performed on a $5 \text{ cm} \times 5 \text{ cm}$ area within each tussock or clump patch, as this was the approximate area of sedge removed in a typical caribou grazing event (pers. obs.). Since *E. vaginatum* is a clonal species with carbon and nutrients being absorbed and shared among the clonal parts, the larger sized patches where some of the plant shoots would have been left unclipped could compensate for the clipped parts, whereas the relatively small patches where clipping was applied to the entire plant might have responded differently. To overcome this potential impact of variation in patch size on our results, we allocated patches of similar size equally across the clipping treatments, i.e. if there were three large tussocks in a plot, one would be assigned to each level of clipping treatment.

To ensure the designated sampling areas could be relocated, we created a map of their locations in each plot, and marked them by inserting pairs of small bamboo barbecue sticks or 10-cm nails into two diametrically opposite corners so that they protruded $\sim 4 \text{ cm}$ above the soil surface. For large patches, this clipped area was located in the centre and constituted only a portion of the tussock, whereas for small patches this area may have included the entire patch. Patches included both whole tussocks and smaller clumps, and probably contained single genotypes, although this may not have always been the case given clonal growth in *E. vaginatum* (Callaghan et al. 1992). Although all plots were freely accessible to caribou (i.e. unfenced) throughout the study period, very few animals were observed near the plots during the two summers of the study (pers. obs.). Furthermore, the Bathurst caribou herd that migrates through the study region has been in continual decline since mid-1980s, with the population less than 7% of its previously recorded maximum at the time of this study (see Zamin et al. 2014 for details).

The clipping treatments to simulate caribou grazing were applied only once in the entire study—on July 17, 2010. For the simulated ‘moderate’ graze plants, all leaf blades (and any inflorescence stems—see below) emanating from within the designated 25 cm^2 sampling area were gathered together and held vertically prior to clipping all but the basal 5 cm of leaf/tiller length above the soil surface (measured from the green–brown transition in the moss), which equated to removing $\sim 50\%$ of the control (i.e. not fertilised) plot graminoid aboveground biomass in this vegetation type. For the simulated ‘intense’ graze plants, all blades within the 25 cm^2 area were clipped to the soil surface, removing 100% of aboveground biomass. The same methods and treatment levels were executed across all control soil, low-N and high-N addition plots. The control-grazing plant patches in each of these plots were marked but not disturbed. Following

the clipping treatments, all experimental plants were left to grow/re-grow, and then all of the aboveground biomass within the previously delineated 25 cm² patches from control, moderate, and intense simulated graze plants was harvested on August 11, 2010 (i.e. 25 days after clipping). Peak biomass at this site is around August 1st, and so we assume that our harvests represent total *E. vaginatum* shoot production in that growing season. In the following year, these same experimental patches were harvested on August 19 or 27, 2011 by clipping all aboveground biomass within the designated 25 cm² sample areas.

Biomass and chemical analyses

Harvested tissue from each patch was sorted in the lab into live blade and live inflorescence categories (the sum of which is referred to as ‘total shoot production’ for here on), and dried separately at 40 °C for 2 days to constant mass, and weighed. To achieve a sufficient sample mass for chemical analyses, tissue from the four patches of each clipping treatment within each plot was combined (i.e. $n = 5$ datum points per clipping treatment per soil treatment). This tissue was then ground to a 0.5 mm particle size (IKA MF 10 Microfine grinder, Staufen, Germany) and analyzed for total C and N via dry combustion on an Elemental Analyzer (Elementar, Hanau, Germany).

For the year of the simulated grazing treatments (2010), total season’s production for the clipped plants was calculated as the sum of the biomass removed during the clipping treatments on July 17th and the biomass harvested on August 11th. Likewise, to calculate the total growing season shoot N pools for 2010, we summed the N pools of the tissue removed during clipping on July 17th and the tissue harvested on August 11th. To be able to test for the impact of inflorescent apical meristem removal during the clipping treatments on subsequent shoot production, we examined the July 17th biomass harvest from each patch sampling area and determined whether any emerged (i.e. fully formed) *E. vaginatum* inflorescences were present in the clippings. 15–30% of those patch sampling areas contained inflorescences that were removed during clipping. By contrast, the majority of *E. vaginatum* plant clump or tussock patches contained inflorescences outside, but adjacent to, the designated sampling areas and, therefore, their apical meristems remained intact after clipping (Table S1). These proportional distributions were very similar across the N addition treatments (Table S1). The exact amount of plant shoot regrowth following clipping was calculated for the intensely grazed individuals, but this variable could not be determined for the moderately grazed patches since their final harvest included the basal 5 cm of tissue that was not removed on July 17th. Therefore, our analysis of the impact of inflorescence apical meristem removal during clipping on the subsequent

growing season’s new shoot production is confined to the intensely clipped plant patches only, and for 2010 included apices removed on July 17th, but for 2011 included apices removed either on July 17, 2010 or August 11, 2010. Note that since arctic *E. vaginatum* inflorescence abundance is highest in early summer (White and Trudell 1980), the likelihood of new inflorescences emerging after mid-July is small, and in fact only 6% of the patches that had been intensely clipped in July had inflorescences removed in the August harvest. However, although we think it is unlikely, we acknowledge that the difference in timing of potential inflorescence removal between the intensely clipped plots (July) and the moderately clipped plots that could possibly have had newly emerged reproductive shoots between the clipping treatment in July and the subsequent harvest in August might affect our inflorescence production data for both 2010 and 2011.

Statistical analyses

The shoot production, N concentrations and pools, and inflorescence data in this nested experimental design (clipping treatment nested within soil N treatment) were analysed using a general linear mixed effects model in which the N addition and clipping intensity treatments, and year were fixed effects, and plot was included as a random effect. To more clearly interpret the shorter term impacts of the clipping and N addition treatments on shoot production, these data were then analysed separately for each year using the same linear mixed effects model approach. Note that for all the above statistical analyses, the four patch sampling areas for each clipping treatment within each plot were averaged to make a single experimental replicate unit, and so there were a total of five replicate (i.e. plot) datum points for each of the three clipping treatments nested within each of the three N addition treatments. We analysed the impact of inflorescence apical meristem removal on subsequent shoot production (i.e. regrowth) in the high-intensity clipping treatment using a similar mixed effects model as described above. While inflorescences were present in a minority of subplots (Table S1), their aggregation up to the plot level ensured equal sample sizes in the analysis of the impact of apex removal. Data transformations are indicated in Table 1, and all statistical analyses were performed in R 3.1.2 (R Core Team 2015).

Results

The impacts of the clipping intensity and N addition treatments on total *E. vaginatum* shoot production over the full growing season (i.e. the sum of shoot growth pre- plus post-clipping) had completely opposing effects between the two

Table 1 Statistical results of linear mixed model analyses of the N addition and clipping intensity treatments on total growing season shoot production of *E. vaginatum* combined across the two years (a),

and on total shoot production, shoot N concentration, N pools and inflorescence production in each growing season separately (i.e. in 2010—when the clipping treatments were applied, and in 2011) (b)

(a) Multi-year analysis		Both years					
		<i>df</i>	<i>F</i>	<i>P</i>			
Total production ^a							
N addition		2, 12	3.8	0.053 [†]			
Clipping		2, 60	5.6	0.006*			
Year		1, 60	136.0	< 0.001*			
N addition × clipping		4, 60	2.0	0.106			
N addition × year		2, 60	3.3	0.042*			
Clipping × year		2, 60	9.3	< 0.001*			
N add. × clipping × year		4, 60	0.2	0.913			
(b) Single-year analyses		Year of simulated graze (2010)			Year following simulated graze (2011)		
		<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Total production ^{b,a}							
N addition	2, 12	7.5	0.008*	2, 12	1.8	0.214	
Clipping	2, 24	0.5	0.639	2, 24	9.0	0.001*	
N addition × clipping	4, 24	0.7	0.579	4, 24	1.0	0.447	
N concentration ^{c, c}							
N addition	2, 12	34.9	< 0.001*	2, 12	16.1	< 0.001*	
Clipping	2, 24	164.5	< 0.001*	2, 24	11.1	< 0.001*	
N addition × clipping	4, 24	1.2	0.332	4, 24	1.2	0.344	
N pools ^{b, a}							
N addition	2, 12	10.5	0.002*	2, 12	3.6	0.061 [†]	
Clipping	2, 24	5.8	0.009*	2, 24	5.2	0.014*	
N addition × clipping	4, 24	0.3	0.877	4, 24	1.1	0.375	
Inflorescence production ^{b, b}							
N addition	2, 12	1.0	0.411	2, 6	1.0	0.437	
Clipping	2, 24	1.6	0.216	2, 6	1.0	0.422	
N addition × clipping	4, 24	0.6	0.681	4, 6	1.1	0.433	

Data transformations are indicated following each test: ^alog, ^bsquare root, ^carcsine square root*P* < 0.05 and < 0.10 are indicated by * and †, respectively

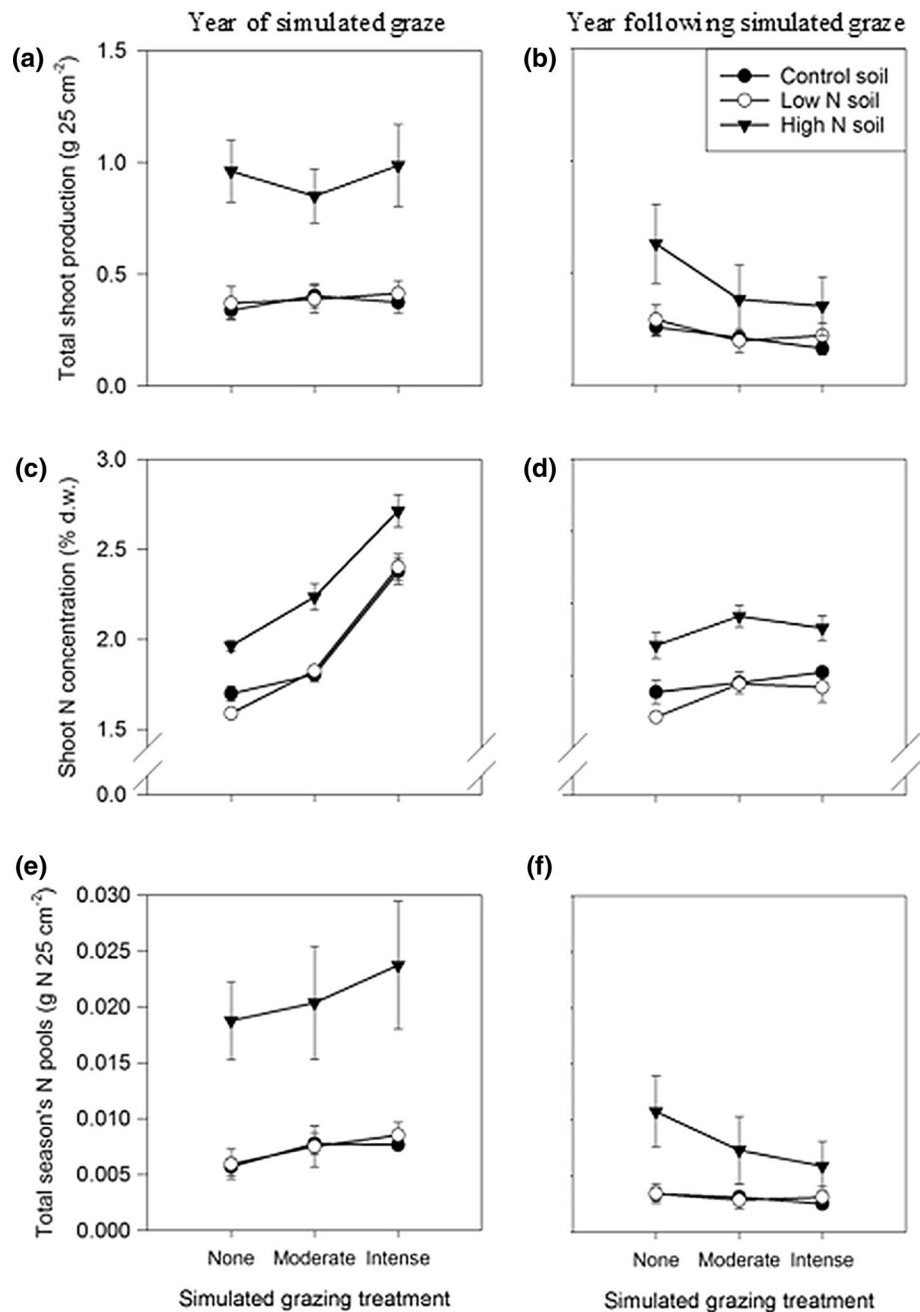
successive years (Table 1a; Fig. 2a, b). In the summer that the clipping treatments were applied, plants at all levels of soil fertility demonstrated equal compensation, i.e. total shoot production within each of the fertility treatments was unaffected by these simulated grazing events (Table 1a, b; Fig. 2a). Meanwhile, the N addition treatment significantly enhanced total shoot production in the first year (Table 1a, b; Fig. 2a), and this effect was driven by the response to the high-N addition treatment level only (control-high N, Tukey HSD: *P* = 0.001; low N-high N, Tukey HSD: *P* = 0.011; Fig. 2a).

By contrast, this pattern of treatment effects was entirely reversed in the year following the simulated grazing. Shoot production was substantially reduced in the plants that had been clipped in the previous year (Table 1a, b; Fig. 2b), and there was no significant effect of N addition (Table 1a, b).

Although this legacy grazing effect seemed particularly strong for plants in the high-N addition plots (Fig. 2b), high variability among replicates precluded a statistically significant interaction between the two treatments (Table 1b), leading to the conclusion that soil N availability did not alter the second-year plant regrowth responses.

Each of the clipping treatments significantly increased N concentrations in new shoot tissue produced in the month after the clipping event (Table 1b, Fig. 2c). This clipping effect was maintained in the following year, although to a much smaller magnitude (Table 1b, Fig. 2d). As might be expected, the high-N addition treatment increased shoot N concentrations in both years (Fig. 2c, d; 2010 control-high N, Tukey HSD *P* < 0.001, low N-high N, Tukey HSD *P* < 0.001; 2011 control-high N, Tukey HSD *P* = 0.012, low N-high N, Tukey HSD *P* < 0.001), but there were no

Fig. 2 Effect of clipping intensity and soil N addition treatments on *Eriophorum vaginatum* total growing season shoot production (**a, b**), N concentrations (**c, d**), and N pools (**e, f**) in mesic birch hummock tundra in the same year as the simulated graze, and in the following year. Error bars are standard errors ($n = 5$)



significant effects of the low-N addition. As a result, clipping increased aboveground *E. vaginatum* total shoot N pools in the year of the clipping event (Table 1b; Fig. 2e), even though it did not alter shoot biomass in that year. By contrast, clipping decreased aboveground N pools in the following year, in accordance with the patterns of shoot production (Fig. 2f). Finally, total shoot aboveground N pools were significantly increased by N addition in the first year (Table 1b; Fig. 2e) and tended to increase in the second year (Table 1b; Fig. 2f), but these effects occurred in the high-N addition treatment only (2010: control–high N, Tukey HSD $P < 0.001$, low N–high N, Tukey HSD $P < 0.001$).

New shoot production was significantly enhanced in both years in those patches where the intense clipping treatment had removed shoot apical inflorescence meristems (Table 2, Fig. 3a, b). This growth stimulation effect of apex removal was greatest in the high-N addition plots resulting in a nearly fivefold larger shoot biomass compared to the unclipped unfertilised plants in both years (Fig. 3a, b), and a statistically significant interaction of apex removal and N addition in the year of the clipping treatment (Table 2; Fig. 3a). Finally, production of

Table 2 Statistical results of linear mixed model analysis of the impacts of N addition and inflorescence (apical meristem) removal during the intense clipping treatment on total *E. vaginatum* new shoot production in each growing season

	Year of simulated graze (2010)			Year following simulated graze (2011)		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Apex removal on shoot production ^{b, a}						
N addition	2, 12	21.5	< 0.001*	2, 12	0.0	0.969
Apex removal	1, 7	15.6	0.006*	1, 7	11.7	0.011*
N addition × apex removal	2, 7	7.0	0.021*	2, 7	1.9	0.223

Superscript letters indicate data transformations as described in Table 1

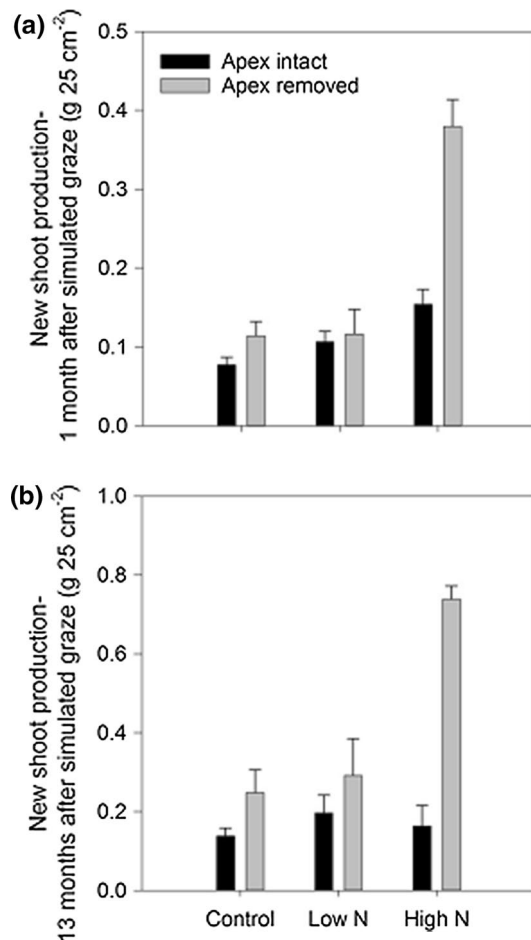


Fig. 3 Effect of inflorescence apical meristem removal during the intense clipping treatment on *Eriophorum vaginatum* new shoot production across the soil N addition treatment plots in mesic birch hummock tundra in the same year as the clipping (a), and in the following year (b). Error bars are standard errors ($n = 5$)

inflorescences (~ 10% of total shoot production) was not significantly impacted by either the clipping or the soil N addition treatments in either year (Table 1b, Fig. S2a, b).

Discussion

Compensatory growth responses to clipping were unaffected by N availability

The relationship between the clipping treatments and total growing season shoot production in *E. vaginatum* was unaltered across a gradient of N availability in both years, with consistent exact compensation across control, moderate, and high-intensity clipping levels in the year of the treatments, and consistent under-compensation in the following year. This result cannot be readily reconciled with either the Compensatory Continuum Hypothesis or the Growth Rate Model Hypothesis which predict that compensatory growth across a fertility gradient would either increase or decrease, respectively (Hilbert et al. 1981; Maschinski and Whitham 1989). The Limiting Resource Model (LRM) is more flexible, and predicts that plant responses to grazing would be consistent across a N fertility gradient if herbivory did not affect N availability or use, but instead resulted in growth limitation by some other resource (Wise and Abrahamson 2005; Fig. 4, upper pathway). By contrast, had herbivory increased N limitation, then plants in the high-N addition plots would have had higher tolerance to herbivory than those in the low-N or control plots because they would be less N-limited (Wise and Abrahamson 2005, 2007; Fig. 4 lower pathway). In our case, shoot growth in the first year was clearly N-limited as indicated by the significant overall effect of N addition (Table 1b, Fig. 2). However, the lack of a statistical interaction between the N addition and clipping treatment factors in that first year (Table 1b) indicates that simulated herbivory did not exacerbate the N limitation on shoot growth. The exact compensatory growth across clipping levels in that year may have been fuelled from belowground tissue carbon (C) reserves that augmented the impoverished leaf photosynthate C supply due to the clipping (Fig. 4, upper pathway). In the second year, the legacy of the clipping treatments restricted shoot growth—presumably as a result of larger depletion of belowground tissue carbon (C) reserves in the more intensely clipped

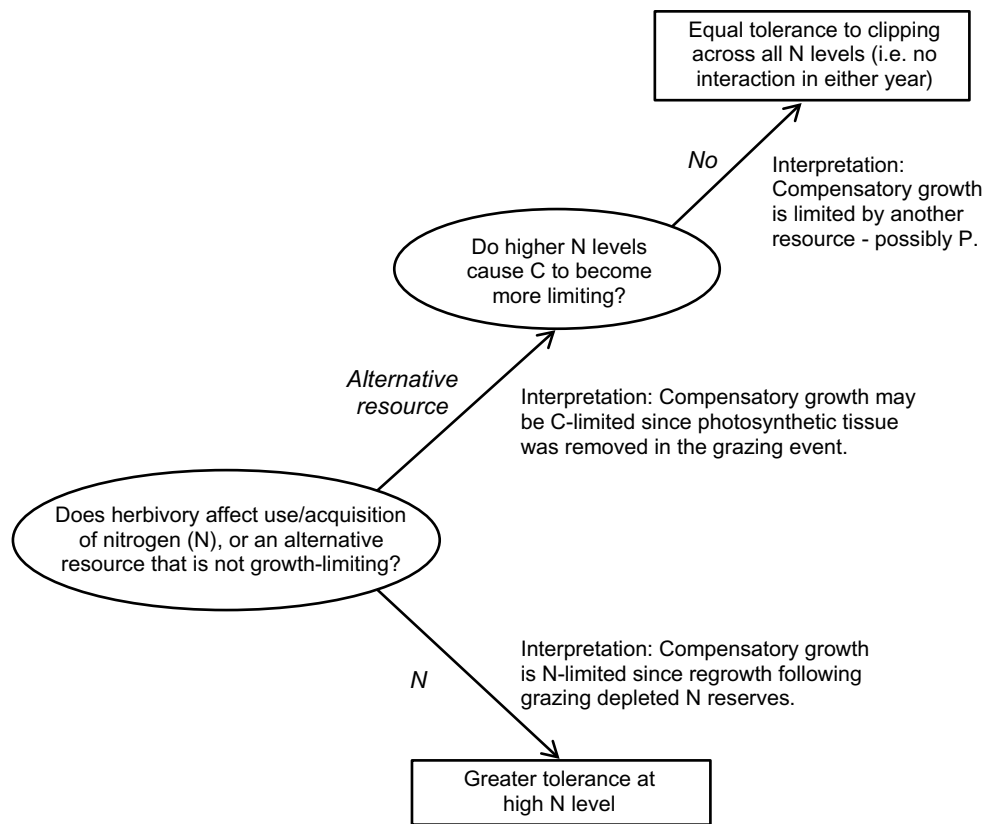


Fig. 4 Conceptual framework for understanding our data on the herbivory tolerance of the common arctic sedge, *Eriophorum vaginatum*, using a decision tree with nitrogen (N) as the focal growth-limiting

resource and carbon as the initial alternative non-limiting resource that is adapted from the Limiting Resource Model of compensatory growth by Wise and Abrahamson (2005)

plants over the previous year. However, once again there was no significant interaction between the two experimental treatments indicating that increasing N availability did not *exacerbate* the potential for C-limited growth. Therefore, according to the LRM conceptual framework, our results together over both years suggest that the clipping treatments may have caused shoot compensatory growth to become ultimately limited by an external resource other than N (Fig. 4 upper pathway).

E. vaginatum growth in mesic tundra has been previously identified as primarily N-limited over 1–2-year study durations (Shaver et al. 1986), but over longer periods, it can clearly be co-limited by nitrogen and phosphorus (P) (e.g. see Zamin et al. 2014 for results after 8 years of factorial fertiliser additions in our research site at least). Scandinavian studies comparing nutrient availability across different tundra vegetation types suggest that mineral phosphorus availability is lower in herb- and graminoid-dominated vegetation than in tundra heath vegetation (Eskelinen et al. 2009; Sundquist et al. 2014). Furthermore, intense reindeer grazing in graminoid-dominated vegetation can lower soil P availability, potentially promoting P-limitation of plant growth (Sitters et al. 2017). Altogether, these study results

and the LRM conceptual framework suggest that our clipping treatments may have increased *E. vaginatum* growth limitation by P and/or some other belowground resource.

Although we have outlined the rationale for why the most likely resources that became limiting to compensatory growth were belowground tissue carbohydrates and/or soil phosphorus, soil moisture could also have contributed. Annual rainfall input in 2010 was equal to the annual average over the period 1996–2011, but relatively low in 2011 (138 and 88 mm, respectively-see “Materials and methods” for more details), and likewise continuous records of soil moisture during the corresponding growing seasons were also relatively low in the later year (Grogan, unpublished data). Therefore, in the context of arctic climate change, we conclude that warming effects on the availabilities of not just N, but also P, other soil nutrients, and soil moisture are important considerations for predicting graminoid plant responses to herbivory. Although growing season temperature increases are projected to generally enhance tundra soil fertility (ACIA 2005; Nadelhoffer et al. 1991; Shaver et al. 2006), the extents to which particular nutrients’ availabilities will be affected depends on the distinctive biogeochemistry of each (Schlesinger 1997). Our results suggest

that graminoid shoot growth compensation in response to herbivory may be unaffected by warming-induced increases in soil N availability unless those increases are matched by proportional increases in the other potentially growth-limiting resources.

The LRM approach has recently been successfully applied in a recent tundra study to explain patterns of woody plant compensatory growth in response to simulated herbivory. Moderate and high leaf stripping treatments on birch shrubs growing at two levels of N addition over two years resulted in full and under-compensation in leaf area responses, respectively, but there was no impact of N fertilisation in either case (Champagne et al. 2012). As the authors acknowledge, the timeframe for the fertilisation to take effect on growth of a woody plant is very short, and may constrain their confidence in using the LRM, but nevertheless the similarity in their results to those from our study (of a very different plant growth form) warrant more focussed tests of the applicability of the LRM using long-term full factorial designs with multiple nutrients on the full range of arctic plant growth forms. More fundamentally, in the context of the overall conceptual framework to understand and predict herbivore impacts on vegetation through nutrient cycling feedbacks (Fig. 1-adapted Pastor et al. 2006), our results suggest that compensatory growth of tundra graminoids at least is largely independent of soil N fertility. Therefore, even if climate warming enhances N availability as widely anticipated, we conclude that the negative feedback loop represented in the upper part of the framework (Fig. 1a) will tend to predominate with caribou/reindeer herbivory maintaining current nutrient cycling rates and tundra vegetation structure.

Legacy effects of the clipping treatments on shoot production in the following growing season

E. vaginatum shoot production and N accumulation responses to clipping differed strongly between the two successive years. Clipping had no effect on growth in the first year, but significantly reduced total shoot production in the second year (Table 1a, b; Fig. 2 a, b). Graminoids such as *E. vaginatum* exhibit sequential leaf development in which one or two leaves are grown at a time throughout the season, with growth fuelled from stored reserves until late June, after which it is then fuelled primarily from nutrient retranslocation from older senescing leaves (Jonasson and Chapin 1985). Nutrient storage pools are replenished by fresh uptake from the soil after late June and by nutrient retranslocation prior to leaf senescence (Jonasson and Chapin 1985). Consequently, we expect that the exact compensation which we observed in the first growing season immediately following clipping in mid-July would have been facilitated by reallocation of recently replenished stored reserves and/or new

nutrient uptake (Chapin and Slack 1979; Stowe et al. 2000). By contrast, the clipping-induced reduction in shoot growth (under-compensation) observed in the second year of our study was likely due to depletion of reserves by the regrowth in the first year and the lack of replenishment via new uptake or retranslocation. Since this latter response was independent of soil N availability, it further supports the conclusion that shoot regrowth in both the first and second years after clipping was limited by a resource other than soil N.

We did not measure belowground biomass and acknowledge that shifting resource allocation is a key mechanism of plant tolerance to herbivore damage because the altered root–shoot ratio following aboveground tissue removal may indirectly drive compensatory growth by changing source–sink relationships (Stowe et al. 2000; Thorne and Frank 2009). In this context, it is noteworthy that although both above- and belowground biomass components of *E. vaginatum* were enhanced by fertilisation in a previous tundra study, root–shoot ratios were not significantly affected (Shaver et al. 1986). Nevertheless, future research on this topic would clearly benefit from investigations that include compensatory growth responses across the whole plant. Note too that the Shaver et al. (1986) study reported the opposing pattern to that found here, in that shoot production was substantially reduced in the year of the clipping and unaffected in the following year. However, the herbivory treatment in that study involved a very high grazing intensity of nine clipping events within a growing season, which probably greatly exceeds the frequency of natural caribou grazing in most tundra locations.

Positive feedback between shoot production and inflorescence removal at high fertility

The strong effect of inflorescence apical meristem removal on *E. vaginatum* shoot production indicates that regrowth following grazing is not only affected by the quantity of biomass but also by the tissue type that is removed. While the effect of disrupted apical dominance has been well documented for dicots (see list in Wise and Abrahamson 2008), corresponding research in monocots of the graminoid growth form is less common (but see Tomlinson and O'Connor 2004). Nevertheless, similar impacts may be expected during flowering because the otherwise basal graminoid shoot apical meristem converts to a reproductive meristem and is extended upwards (Tanaka et al. 2013). Similar to the dicot release from apical dominance, the removal of some of the graminoid inflorescence meristems may alleviate suppression of lateral meristems, and promote increased shoot growth via tillering (Shaver et al. 1986). For dicots, this effect is strongest at intermediate soil fertility levels when competition is removed (Irwin and Aarssen 1996). We did not observe increased growth at our intermediate

soil fertility treatment (i.e. the low-N addition) perhaps because the levels of soil N availability created through our low- and high-N addition treatments (a tenfold difference in fertilisation rates) fell above and below that which would be considered intermediate to *E. vaginatum*. Furthermore, aboveground competition is unlikely to be important because *E. vaginatum* tussocks in this vegetation type do not have significant competitors for light. Since caribou consumption of inflorescences is high in early summer (White and Trudell 1980) and *E. vaginatum* inflorescence production is strongly stimulated by the combination of high N plus P availability (Table S2 and Fig. S2a, b; Shaver et al. 1986), our results indicate the potential for a positive feedback on total shoot production whereby increased soil fertility promotes shoot flowering, which when grazed, promotes tillering and leaf production that should ultimately enhance soil fertility by increasing graminoid litterfall.

Clipping increased spatial and temporal variation in productivity and N pools

Total shoot N pools were significantly enhanced by the clipping treatments at the end of the following month. This effect which was common across all N addition levels may be driven by enhanced N uptake from soil, and/or by retranslocation of N to new growth (Chapin and Slack 1979; Thomas et al. 2008), altogether providing a mechanism for herbivory-enhanced nutrient cycling within the ecosystem. Multiple defoliations within a single growing season can enhance *E. vaginatum* N uptake did not alter root N concentrations (Chapin and Slack 1979). However, that treatment did reduce root carbohydrate and P concentrations, suggesting that allocation of N to new aboveground growth was replenished by soil uptake whereas that of P was not (Chapin and Slack 1979). These results are consistent with our conclusion that herbivory on tundra graminoids may increase growth limitation by nutrient resources other than N. Shoot N pools increased shortly after the clipping, but decreased the following year, likely contributing to increased temporal variation in N cycling in and immediately around the clipped plant patch, but also increasing spatial variation across the ecosystem. Since caribou are highly selective in patch use at the local scale (Skarin et al. 2008), this increased variability in local N cycling may contribute to heterogeneity in plant and soil nutrient pools, with implications for productivity (Hutchings et al. 2003) if not diversity (Laliberte et al. 2014). These short-term, small-scale effects on spatial patterning in nutrient availability are complemented by longer term variation in *Rangifer* population trends and range use. For example, graminoid abundance can significantly decline following 10 years of release from intensive *Rangifer* browsing (Eskelinen and Oksanen 2006). Overall

then, our results suggest that herbivory of *E. vaginatum* likely contributes to substantial local spatial and temporal heterogeneity in graminoid shoot N pools and, therefore, in mesic tundra ecosystem N cycling.

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Author contribution statement TJZ and PG conceived and designed the experiment. TJZ performed the experiment and analyzed the data. PG and TJZ wrote the manuscript.

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