Oecologia (2012) 169:85–94
DOI 10.1007/s00442-011-2183-x

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH PAPER

Legumes mitigate ecological consequences of a topographic gradient in a northern Mongolian steppe

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Received: 14 April 2011 / Accepted: 20 October 2011 / Published online: 23 November 2011

Abstract Topography should create spatial variation in water and nutrients and play an especially important role in the ecology of water-limited systems. We use stable isotopes to discern how plants respond both to ecological gradients associated with elevation and to neighboring legumes on a south-facing slope in the semi-arid, historically grazed steppe of northern Mongolia. Out of three target species, *Potentilla acaulis*, *Potentilla sericea*, and *Festuca lenensis*, when >30 cm from a legume, all showed a decrease in leaf δ^{15}N with increasing elevation. This, together with measures of soil δ^{15}N, suggests greater N processing at the moister, more productive, lower elevation, and more N fixation at the upper elevation, where cover of legumes and lichens and plant-available nitrate were greater. Total soil N was greater at the lower elevation, but not lichen biomass or root colonization by AMF. Leaf δ^{13}C values for *P. acaulis* and *F. lenensis* are consistent with increasing water stress with elevation; δ^{13}C values indicated the greatest intrinsic water use efficiency for *P. sericea*, which is more abundant at the upper elevation. Nearby legumes (<10 cm) moderate the effect of elevation on leaf δ^{15}N, confirming legumes' meaningful input of N, and affect leaf δ^{13}C for two species, suggesting an influence on the efficiency of carbon fixation. Variation in leaf %N and %C as a function of elevation and proximity to a legume differs among species. Apparently, most N input is at upper elevations, pointing to the possible importance of grazers, in addition to hydrological processes, as transporters of N throughout this landscape.

Keywords C isotopes · Montane steppe · Moisture gradient · Nitrogen fixation · N isotopes

Introduction

Topography is a major factor underlying local, spatial variation in vegetation structure because its elements (e.g., elevation, slope, and aspect) create spatial patterning in important environmental factors impacting plant performance. Topography results in landscape scale heterogeneity in soil moisture and nutrient availability (Schimel et al. 1991; Knapp et al. 1993; Hook and Burke 2000) affects productivity and biodiversity (Knapp et al. 1993; Fisk et al. 1998; Fu et al. 2004; Nippert et al. 2011), and underlies the distribution of individual species (Barnes and Harrison 1982) and variation in plant functional traits (Choler 2005; Craine and Towne 2010) across the landscape. Topography may hold particular importance for the ecology of semi-arid steppe systems (Barnes and Harrison

Communicated by Russell Monson.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-011-2183-x) contains supplementary material, which is available to authorized users.

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1982; Milchunas et al. 1989; Singh et al. 1998; Hook and Burke 2000), where water plays a large role in limiting productivity (Burke et al. 1998).

Because the stable isotopic ratios of common elements in plant tissues are indicators of how plants perceive or adjust to soil resource variation, they can be especially useful in illuminating responses to topographic gradients. Carbon isotopic ratios (δ^{13}C) offer an integrative record of the ratio of the partial pressures of leaf intercellular CO_2 to ambient CO_2 (c_i/c_a). This metabolic set point represents a balance between the carboxylation of CO_2 by Rubisco and the stomatal conductance (g) of CO_2 into the leaf (Farquhar et al. 1989). The intrinsic water use efficiency of a leaf (photosynthesis, A, divided by g; A/g) is directly related to c_i/c_a, and, thus, δ^{13}C measurements can yield information on true plant water use efficiency (WUE; A divided by transpiration; Farquhar and Richards 1984; Ehleringer 1993). Additionally, any systematic change in carboxylation capacity that can affect A independent of g, such as plant N status, will also affect c_i/c_a and will be manifest in δ^{13}C. Therefore, δ^{13}C offers an integrated view of the optimization of leaf carboxylation capacity and stomatal regulation as plants respond to N availability and soil moisture across topographic gradients.

Plant N isotopic ratios (δ^{15}N) largely track the isotopic composition of soil N, which is influenced by multiple biological processes. Little isotopic fractionation takes place during N fixation (Högberg 1997; Makarov 2009), so recent products of N fixation have N isotopic compositions close to atmospheric ratios. As cycling of N takes place within the system, fractionation occurs during nitrification, denitrification, and ammonification (Högberg 1997; Makarov 2009), resulting in the preferential loss of the lighter isotope (^{14}N) through gaseous or dissolved nitrogenous compounds and generating enrichment in soil δ^{15}N. Plant roots seem not to discriminate against δ^{15}N, but mycorrhizae do (Hawkes 2003; Hobbie and Colpaert 2003), although fractionation by ectomycorrhizae appears far stronger than by arbuscular mycorrhizae (Craine et al. 2009; Hobbie and Ouimette 2009), the predominant type found in herbaceous species.

Here, we used stable isotope analysis of leaf N and C, together with information on spatial variation in soil resources and local aboveground plant productivity, to understand the ecological significance of a topographic gradient in the semi-arid, montane steppe of northern Mongolia. We were especially interested in how plants respond to soil moisture variation and in the importance of legumes as N fixers across the topographic landscape. The steppe holds great importance for the livelihood of nomadic herder populations, as it has for thousands of years (Barfield 1989), but its ecology has not received much attention. We know of no previous work on resource gradients corresponding to topographic relief or associated plant responses in this system.

We analyze leaves from two eudicots, *Potentilla acaulis* and *Potentilla sericea*, and one grass species, *Festuca lenensis*, across a 128-m elevational gradient for N and C concentration and isotopic composition. All three are non-N fixing species. We collected plants both in close proximity to legumes, given their potential for N fixation, and away from legumes, for an integrated picture of variation due to elevation. We also measured a number of abiotic and biotic environmental factors at the lower and upper extremes of the elevational gradient to help us interpret the variation in isotopes we identified: abundance of the three target species, community-level plant productivity, abundance of legumes and lichens, abundance and isotopic composition of soil N and organic C, plant-available soil ammonium and nitrate, soil moisture, and root colonization by arbuscular mycorrhizal fungi (AMF).

We asked three main questions. Do measures of leaf N and C concentration and isotopic composition, as indicators of physiological traits, show intraspecific plant responses to the topographic gradients in soil moisture and N availability? Does proximity to legumes alter the relationships between these physiological trait indicators and the topographic gradient? Are there differences among species in N or C isotopic composition consistent with the distribution of these species with respect to topography?

Materials and methods

Description of site and study species

We worked in montane steppe on a south-facing slope in the Dalbay River valley, on the eastern shore of Lake Hövsgöl (51°01.405′N 100°45.600′E; ranging from 1,660 to 1,800 m in elevation), northern Mongolia. The incline is gentle to flat at the bottom of the slope but becomes much steeper (20° incline) with elevation. The average annual air temperature in this region is −4.5°C, with the coldest average monthly (January) temperature of −21°C and warmest (July) of 12°C (Nandintsetseg et al. 2007). The average annual precipitation measured for the last 40 years, averaged for three weather stations, south (Hatgal), west (Renchinlhumbe) and north (Hanh) of Lake Hövsgöl, is 265 mm (Namkhaijantsan 2006). Most precipitation falls in summer. In 2009, the year the leaf material for this study was collected, our on-site meteorological station recorded summer precipitation, June to August, of 201 mm.

The steppe vegetation is a mixture of sedges (e.g., *Carex pediformis*, *Carex dichroa*), grasses (e.g., *Festuca lenensis*, *Koeleria macrantha*, *Agropyron cristatum*, *Helictotrichon schellianum*, *Stipa krylovii*) and short forbs (e.g., *Aster* *alpinus*, *Potentilla* spp., *Artemisia commutata*, *Thymus gobicus*). The most common legumes are *Oxytropis viridiflava*, *Oxytropis strobilacea*, *Astragalus mongholicus*, and *Vicia multifida*. The soil is of sandy loam texture, of alluvial origin, and classified as a non-carbonated Dark Kastanozem (Aridic Boroll or Typic Ustolls). Bedrock consists of Cenozoic volcanic deposit (Batkhishig 2006). Permafrost is not present on the south-facing slope, but is found in a nearby riparian zone and on north-facing slopes under taiga forest (Sharkhuu et al. 2007), consisting mostly of *Larix sibirica* with *Pinus sibirica* less commonly in the forest interior. The valley is presently grazed mainly in late summer and autumn by yaks, horses, and a mixed herd of sheep and goats.

The three non-N fixing species chosen for analyses of leaf N and C concentration and isotopic (δ^{15}N, δ^{13}C) composition, *Potentilla acaulis* L. and *P. sericea* L. (Rosaceae) and *Festuca lenensis* Drobow (Poaceae), are all distributed across the south-facing slope. The leaves of *P. acaulis* and *P. sericea* are basal, highly pubescent, and compound but more gray-green in *P. acaulis*. Both species, but especially *P. acaulis*, are prostrate in growth form, and both produce nearly erect flowering stems and yellow flowers. *F. lenensis* is a tufted grass, consisting of dense tillers of narrow (<1.0 mm) blue-green leaves that may reach 10–15 cm in height. *P. acaulis* is one of the first species in the valley to flower, in early June, while *P. sericea* flowers in late June to early July concurrent with *F. lenensis*.

In July 2009, leaves of the study species were collected at five locations along three transects running up the south-facing slope. Transects were separated by at least 60 m; sampling locations along each transect were separated by at least 10 m, but the exact elevations differed somewhat among transects. At each location, five leaves from each of five individuals per species were collected, both near (<10 cm) and away from (>30 cm) legumes. Leaves from a single individual plant were combined for N and C analyses. Legumes were not found at the two lowest sampling locations on any transect.

Abundance and distribution of study species

The percentage cover of our study species, in late June–early July 2008, was determined at two elevations on the south-facing slope, roughly corresponding to the lowest and uppermost elevations of our sampling transects. Eight 9 × 9 m blocks, separated by at least 30 m, were laid out at each elevation. One 0.5 × 1.0 m sampling quadrat was placed at an arbitrary location outside each side of the block perimeter. Quadrats were sampled using a grid divided into 50 cells of 10 × 10 cm with taut string, and percentage cover per species per quadrat was compiled from estimates to the nearest 10% in each cell.

Structure of the plant community

The quadrats, described above, were also used to quantify percentage cover of all vascular plants, percentage cover of legumes, vascular plant biomass, and lichen biomass at the lower and upper elevations. Lichen abundance was measured because of their potential role in N fixation (Henriksson and Simu 1971; Englund and Meyerson 1974). Lichen biomass and vascular plant biomass were determined by clipping all vegetation in two 10 × 30 cm areas of fixed location per quadrat and separating lichens from vascular plants before drying and weighing.

Soils

Soils were sampled in the middle of one clipped area per quadrat for measuring the concentration and isotopic composition of surface (0–15 cm) soil organic C and total N. Soil samples were collected with a 5-cm-diameter corer. Large roots and rocks were discarded, and the soil in each core thoroughly mixed before subsamples were removed and transported to the Department of Earth and Environmental Sciences, University of Pennsylvania, where they were air-dried and stored prior to analyses. Data are missing from a single quadrat in one upper elevation block for N and from one quadrat in one lower elevation and two upper elevation blocks for C, resulting in total sample sizes of $n = 62$ and 61 for N and C, respectively.

Plant available NO_3^- and NH_4^+ were measured at the lower and upper elevations in 2009 using plant root simulator (PRS)^{TM} probes (Western Ag Innovations, Saskatoon, SK, Canada; http://www.westernag.ca/innov/prs-probes/), which employ ion-exchange membranes. On 27 June, two anion probes and two cation probes were inserted in each plot of an ongoing climate warming study and left in place for 21 days. Only measurements of NO_3^- and NH_4^+ from the untreated control plots are reported here. Plots were grouped within blocks laid out in 2008, described above, with one control plot per block. In 2009, there was one fewer block at the upper elevation than in 2008 and one block on the lower slope was not sampled with PRS probes, resulting in 7 control plots in each location. The plots were hexagonal in shape, 1.5 m from side to side, and the probes were placed in a row 40–60 cm from one corner. Retrieved PRS probes were cleaned and transported as recommended. Western Ag Innovations analyzed eluate from the probes for NO_3^- and NH_4^+ concentrations. The two probes per plot of the same ionic charge were eluted together to produce a single measurement ($n = 14$, total), which was expressed in μg per 10 cm^2 ion exchange surface per day.

Volumetric soil moisture content of surface soil at a depth of approximately 6 cm was measured in all 15 control plots between 1000 and 1100 hours on almost every rainless day from 26 June until 13 August in 2009 using a calibrated WET-2 sensor (Delta-T Devices). Measurements were taken at arbitrary locations each day but generally <30 cm from the PRS probes ($n = 15$ control plots, total). An average value for the whole sampling period was calculated for each control plot.

N and C analyses

N and C concentration and isotopic signatures (%N, %C, δ^{15}N, δ^{13}C) of collected soil and leaf materials were determined at the Department of Earth and Environmental Sciences, University of Pennsylvania, using an elemental analyzer (Costech Analytical Technologies, CA, USA) coupled to an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus, Bremen, Germany). Dried leaves and soils were ground in liquid N and 3–4 mg subsamples were weighed and analyzed. Isotope ratios were calculated as:

$$\delta\left[{}^{15}\text{N},{}^{13}\text{C}\right]_{\text{sample}} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1,000$$

where, for example, $\delta^{13}\text{C}_{\text{sample}}$ is the isotope ratio in per mil (‰), R_{sample} and $\text{R}_{\text{standard}}$ are the ^{13}C/^{12}C molar abundance ratios of the sample leaf and of the internationally accepted standard (V-PDB), respectively. The internal laboratory standards were IAEA-C3 and NIST Tomato.

Root colonization by AMF

For *P. acaulis* and *F. lenensis*, root colonization by AMF was scored for material collected in late August 2009. One individual of each species was collected, including shoot and roots, from each of five 50 × 50 cm plots, located randomly at the lowest and highest transect elevations, with at least 30 m spacing between plots. The ten individuals of each species were brought back alive to the University of Pennsylvania. Roots used for scoring AMF colonization were cleared in hot 10% KOH, acidified in 5% HCl, and stained with hot 0.1% Trypan blue/lactoglycerol (Phillips and Hayman 1970). Stained roots were stored in tap water at 4°C before mounting on microscope slides in polyvinyl lactic acid glycerol; 100 intersections per sample were scored at ×200 using the modified line-intersect method (McGonigle et al. 1990) for mycorrhizal fungal colonization, vesicles, and arbuscules.

Statistical analyses

For response variables with a single measurement per block: volumetric soil water content, plant-available soil NO_3^-, NH_4^+, and total plant-available soil N, lower and upper elevations were compared using a Student's *t* test. A nested, mixed-model ANOVA was used to compare between elevations for response variables with multiple measurements per block: soil %N and %C, soil δ^{15}N and δ^{13}C, lichen and vascular plant biomass, and percent cover of species or plant groups. Elevation was treated as a fixed effect with quadrat as a random effect nested within block. A mixed ANOVA model was also used to compare AMF percentage root colonization, and the occurrence of arbuscules and vesicles between elevations; elevation and species identity (*P. acaulis* or *F. lenensis*) were treated as fixed effects and block as a random effect. Analyses employing *t* tests and ANOVA were conducted in R version 2.11.1 (R Development Core Team 2010).

Analyses of covariance (ANCOVA) were used to examine changes in leaf δ^{15}N, δ^{13}C, %N, %C across the elevational transects for plants with and without legumes nearby. Response variables were analyzed separately for each species, and in preliminary analyses, proximity to legume was a fixed effect, transect was a random effect, and elevation was the covariate. Elevation was entered as a covariate because elevations of sampling locations differed among transects. Tests for homogeneity of slopes were carried out by including an interaction term of elevation × presence of legumes. The slopes differed in most cases, indicating that plants near legumes and plants away from legumes did not show the same relationship between elevation and a given response variable. Therefore, data for plants near legumes and away from legumes were split and results of 24 separate ANCOVAs reported here (3 species × 4 variables × 2 categories of distance to legume). Models were fitted using REML methods. For each analysis, outliers were detected using studentized residuals; a datum point was dropped if its studentized residual was >2 in absolute value. Between 1 and 13 outliers per analysis were detected (median = 4).

To compare leaf δ^{15}N and δ^{13}C among target species, only plants away from legumes and only at lower (1,662–1,685 m) and upper (1,751–1,790 m) elevations were examined. Species and elevation were treated as fixed effects in a two-way ANOVA with transect included as a random effect but not crossed with species or elevation. Sample sizes of 20 for each species × elevation combination were randomly selected from all data points, excluding outliers, to give a total sample size of 120 plants.

Results

Edaphic factors

Volumetric soil moisture content averaged over the 2009 growing season was greater at the lower elevation ($n = 8$; 17.3 ± 0.4%) than at the upper elevation ($n = 7$; 10.9 ± 0.5%; $df = 13$, t statistic = 9.61, $P \leq 0.001$).

Table 1 Results of separate analyses comparing lower and upper elevation slope locations for a list of response variables

Response variable	$\text{Average}_{\text{lower}}$ ± SE	$\text{Average}_{\text{upper}}$ ± SE	MS_{slope}	$\text{MS}_{\text{within}}$	% $\text{var}_{\text{within}}$	% $\text{var}_{\text{error}}$	*P*
Soil N (%)	0.25 ± 0.02	0.19 ± 0.02	0.055	0.001	41.6	30.8	*
Soil C (%)	2.59 ± 0.21	1.97 ± 0.21	6.20	1.33	42.7	33.8	*
Soil δ^{15}N (‰)	6.70 ± 0.26	4.74 ± 0.26	60.29	2.08	12.8	22.6	***
Soil δ^{13}C (‰)	−26.5 ± 0.25	−25.9 ± 0.25	4.56	1.04	0	95.4	ns
Vascular plant cover (%)	77.2 ± 4.1	57.4 ± 4.1	250.27	21.04	0	13.8	**
Legume cover (%)	0.19 ± 0.49	3.36 ± 0.49	160.66	5.84	0	63.5	***
Vascular plant biomass (g per 0.01 m^2)	1.28 ± 0.11	0.91 ± 0.11	2.16	0.36	3.9	81.1	*
Lichen biomass (g per 0.01 m^2)	0.09 ± 0.07	0.25 ± 0.07	0.44	0.16	35.0	53.8	ns
Potentilla acaulis cover (%)	7.94 ± 1.84	8.49 ± 1.84	4.84	107.90	38.8	61.2	ns
Potentilla sericea cover (%)	0.68 ± 0.32	1.76 ± 0.32	18.71	3.31	20.6	58.9	*
Festuca lenensis cover (%)	5.75 ± 0.81	4.14 ± 0.81	41.28	20.78	24.5	70.3	ns
AMF root colonization (%)	69.1 ± 6.3	79.5 ± 6.3	540.80	250.28	0	90.6	ns
AMF vesicles (%)	5.4 ± 4.3	12.2 ± 4.3	231.2	139.75	15.6	76.2	ns
AMF arbuscules (%)	1.25 ± 2.4	3.75 ± 2.4	31.35	51.88	66.3	33.7	ns

All results are based on ANOVA with multiple measurements nested within spatial or experimental blocks. For vegetation cover, vegetation biomass, and soil variables, *df* = 1.14, *n* = 8; for AMF variables, *df* = 1.8, *n* = 5

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$

Soils at the lower elevation showed significantly greater N and C concentrations (%N, %C) compared to soils at the upper elevation (Table 1). Soils at the lower elevation also showed greater enrichment in δ^{15}N (Table 1) and a non-significant trend for more negative values of δ^{13}C (Table 1; $P = 0.056$).

Plant available NO_3^-, measured over 21 days in 2009, was greater at the upper elevation ($n = 7$; 15.6 ± 2.0 µg per 10 cm^2 ion exchange surface per day) than at the lower elevation ($n = 7$; 8.5 ± 2.1; $df = 12$, t statistic = 2.42, $P \leq 0.05$). Plant available NH_4^+ and total plant available N did not differ with elevation.

Plant community

Total percentage cover and total biomass of the vascular plant community were greater at the lower elevation than at the upper elevation (Table 1). Legumes made up a greater percentage cover at the upper elevation (Fig. 1; Table 1); the cover of lichens, but not their biomass, was also greater at the upper elevation (Table 1).

As measured by percentage cover, *P. acaulis*, the most common of the three studied species, and *F. lenensis* were equally distributed between the lower and upper elevations. The least common, *P. sericea*, was relatively more abundant at upper elevations (Fig. 1; Table 1).

Colonization by AMF

For *P. acaulis* and *F. lenensis*, the two species for which AMF colonization was scored, there was no difference between the lower and upper elevations in total AMF colonization or in the abundance of vesicles or arbuscules (Table 1). We conducted an additional analysis in which we examined these two species together with two others for which we had the same data, *Aster alpinus* and *Koeleria macrantha*, and likewise found no difference in AMF colonization between the lower and upper elevations (results not shown).

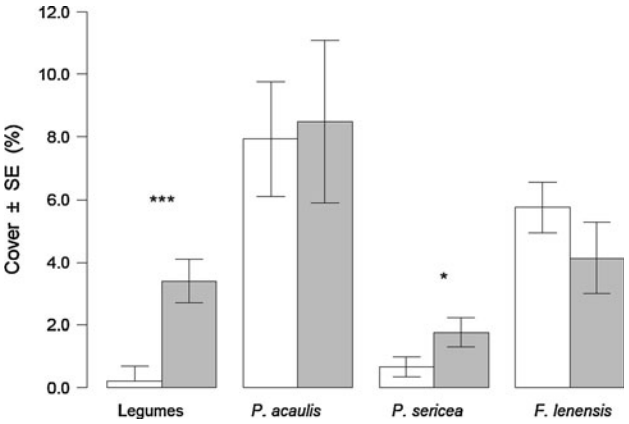

Fig. 1 Percent cover (±SE) for all leguminous species and each of our study species at the lower (*open bars*) and upper elevation (*filled bars*) locations. Data from 32 quadrats of 0.5 × 1.0 m in each location. Significance levels indicate differences between elevations: *$P \leq 0.05$, ***$P \leq 0.001$

Leaf N and C

For leaf N and C concentration and isotopic composition, we were interested both in variation across elevational

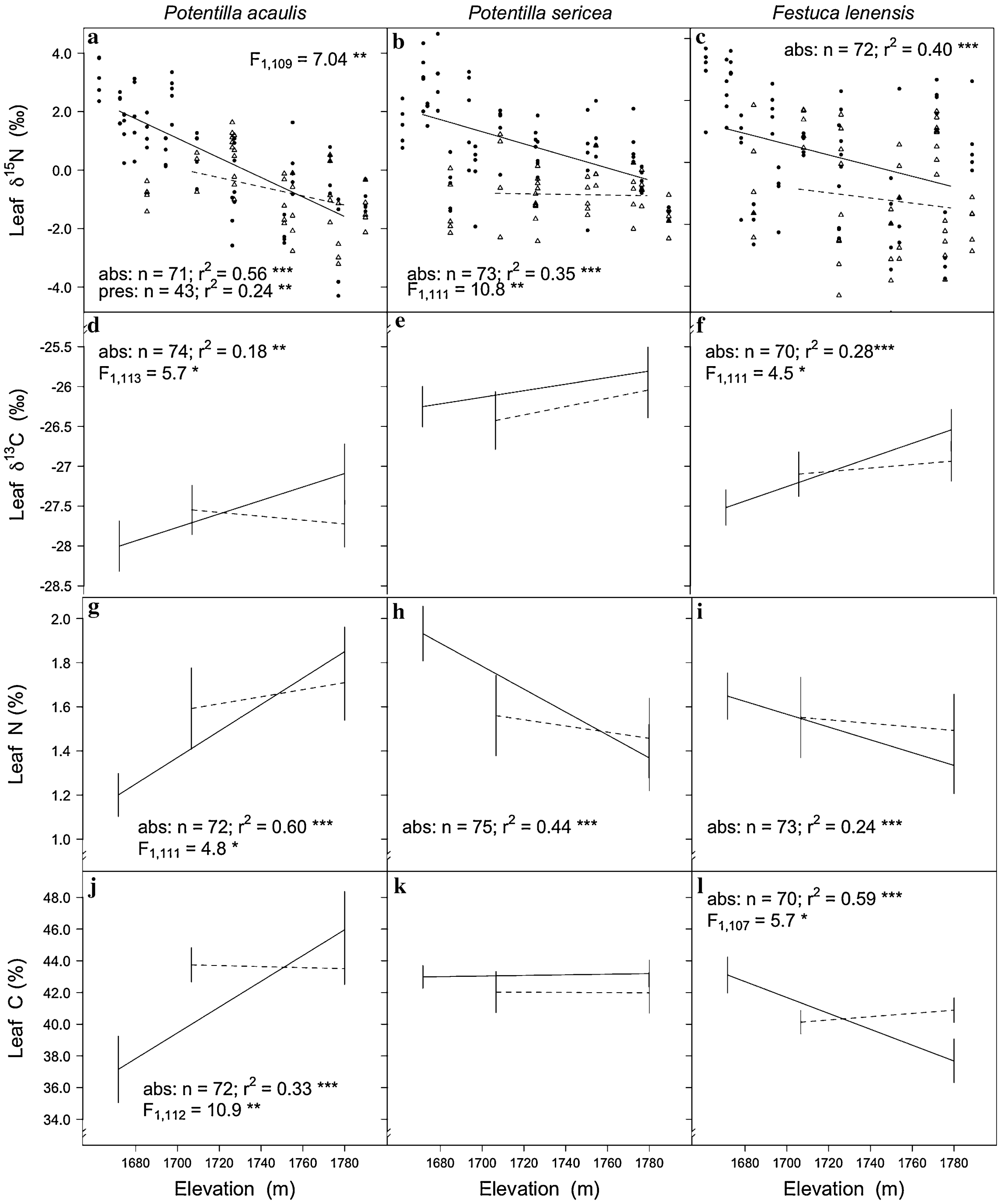

Fig. 2 Regression relationships for leaf δ^{15}N, δ^{13}C, %N and %C as a function of elevation for each study species. All data points and regression lines are shown for δ^{15}N with values for plants >30 cm from a legume (*filled circles*, *solid lines*) and plants <10 cm from a legume (*open triangles*, *broken lines*). Outliers, omitted from the analyses, are not graphed. For simplicity, the other variables are shown only as averages for the lowest and highest elevations (±SE). The r^2 values are given for significant regression relationships with elevation for plants <10 cm from a legume (*pres.*) and plants >30 cm from a legume (*abs.*): *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. *F*-statistics represent a significant difference in slopes between the two groups of plants. There were no legumes at the two lowest elevations. Regression equations presented as an online resource (ESM 1)

transects and how any relationship with elevation might change with proximity to legumes. Leaf δ^{15}N decreased significantly with elevation for plants away from legumes (>30 cm) for all three species, but most strongly for *P. acaulis* (closed circles, solid lines in Fig. 2a, b, c). For plants near legumes (<10 cm), the relationship between leaf δ^{15}N and elevation varied among species (open triangles, broken lines in Fig. 2a, b, c). For plants of

P. acaulis near legumes, leaf δ^{15}N showed a significant decrease with increasing elevation, but with a different slope compared to plants away from legumes ($F_{1,109} = 7.04$; $P \leq 0.01$), suggesting that proximity to a legume influences the relationship between leaf δ^{15}N and elevation. For *P. sericea*, δ^{15}N in plants near legumes did not vary significantly with elevation, and the slope of the relationship between δ^{15}N and elevation differed between plants near and away from legumes ($F_{1,111} = 10.75$; $P \leq 0.01$). For *F. lenensis* near legumes, leaf δ^{15}N did not vary significantly with elevation, but the absence of plants near legumes at the two lowest sampling elevations, instead of a real effect of legumes, may have contributed to this result. This interpretation is supported by *F. lenensis* showing no significant difference in the slopes of the relationship between leaf δ^{15}N and elevation for plants near legumes versus away from legumes.

For plants away from legumes, leaf %N increased in *P. acaulis* with increasing elevation, but decreased in *P. sericea* and *F. lenensis* (solid lines, Fig. 2g, h, i). For plants near legumes, %N did not vary significantly with elevation for any species (broken lines, Fig. 2g, h, i), and only for *P. acaulis* did the slope of the relationship between elevation and %N differ for plants near and away from legumes ($F_{1,111} = 4.840$, $P \leq 0.05$). Thus, for *P. acaulis*, the lack of a relationship between leaf %N and elevation for plants near legumes was probably not simply due to smaller sample sizes at the lower elevations as it may have been for the other two species.

Leaf δ^{13}C increased significantly with elevation in plants away from legumes for *P. acaulis* and *F. lenensis* (solid lines, Fig. 2d, f), but increased only marginally with elevation for *P. sericea* ($P = 0.052$; Fig. 2e). For plants near legumes, leaf δ^{13}C did not show a relationship with elevation for any species (broken lines, Fig. 2d, e, f). The slopes of the relationship between elevation and leaf δ^{13}C differed with proximity to legumes for both *P. acaulis* ($F_{1,113} = 5.746$, $P \leq 0.05$) and for *F. lenensis* ($F_{1,111} = 4.581$, $P \leq 0.05$). These differences in slope confirm that, in fact, plants near legumes did not show as much increase in δ^{13}C with elevation as did plants away from legumes (Fig. 2d, f).

Leaf %C increased with elevation in plants away from legumes for *P. acaulis*, showed no relationship for *P. sericea*, and decreased for *F. lenensis* (solid lines, Fig. 2j, k, l). For plants near legumes, no species showed a relationship between leaf %C and elevation. For *P. acaulis* ($F_{1,\ 112} = 10.941$, $P < 0.01$) and for *F. lenensis* ($F_{1,107} = 5.673$, $P < 0.05$), slopes of the relationship between leaf %C and elevation differed between plants near and away from legumes, suggesting real differences between these two groups.

Using data only for plants away from legumes and only at the lowest and highest elevations in the sampling transects, a two-way ANOVA revealed that leaf δ^{15}N did not differ among species (*P. acaulis* $= 0.55 \pm 0.44$; *P. sericea* $= 1.19 \pm 0.44$; *F. lenensis* $= 0.43 \pm 0.44$; $n =$ 40 for each). A power analysis using GPOWER (Faul and Erdfelder 1992) determined that our total sample size ($n = 120$) was greater than the sample size necessary ($n = 108$) to detect a difference with power set at 0.8. All three species differed from each other in leaf δ^{13}C ($F_{2,114} =$ 48.08, $P \leq 0.001$), with *P. sericea* showing the greatest average value and *P. acaulis* the lowest (*P. sericea* $=$ -26.17 ± 0.12; *P. acaulis* $= -27.77 \pm 0.12$; *F. lenensis* $= -27.03 \pm 0.12$; $n = 40$ for each). The two elevations used in this ANOVA differed both in leaf δ^{15}N ($F_{1,114} =$ 61.72; $P \leq 0.001$) and in leaf δ^{13}C ($F_{1,114} = 23.39$; $P \leq 0.001$), consistent with how these values changed across elevations in the sampled transects (Fig. 2).

Discussion

The decrease in leaf and soil δ^{15}N with increasing elevation is consistent with enrichment of δ^{15}N caused by greater soil microbial activity at lower elevations, where soils are wetter and plant productivity is greater. Other studies have shown δ^{15}N to vary systematically along topographic gradients concurrent with variation in soil moisture, but the relationship between soil water and δ^{15}N may vary with scale. Similar to our results, foliar δ^{15}N in two non-N-fixing evergreen shrubs, soil δ^{15}N, and soil moisture declined with only a 2-m elevation gain in a subtropical savanna (Bai et al. 2008), and depressions within an irrigated Saskatchewan field showed more δ^{15}N enrichment (Sutherland et al. 1993). In contrast, a survey of 250 plant species over a transect spanning 3,300 m in altitude, from a subtropical arid system to snowline, revealed the opposite pattern, with more δ^{15}N enrichment in the drier, lower elevations (Liu and Wang 2010). The results of that study are more aligned with global patterns showing more enrichment of δ^{15}N, on average, with increasing mean annual temperature and decreasing mean annual precipitation (Amundson et al. 2003; Craine et al. 2009). Here, we have the additional factor of legumes being more abundant at upper elevations, so greater N fixation there is likely to contribute to the topographic gradient in δ^{15}N we identified. Discrimination against the heavy isotope via mycorrhizae (Craine et al. 2009; Hobbie and Ouimette 2009) could explain why some leaf values were below atmospheric isotopic levels ($\delta^{15}\mathrm{N} = 0$) at upper elevations, but because mycorrhizae do not change in abundance with elevation, we have no indication that their effects on δ^{15}N are more important at upper elevations than elsewhere.

The moderating effect of legumes on leaf δ^{15}N in *P. acaulis* and *P. sericea* provides additional evidence that

N fixation by legumes is an important N source in this system. Plants in close proximity to legumes, regardless of elevation, have δ^{15}N values more similar to those found higher on the slope. Proximity to a legume also increases leaf %N concentration at lower elevations for *P. acaulis* (but not for the other two species) suggesting an effect of legumes on overall soil N availability. Bai et al. (2008) similarly found that local density of, and proximity to, a woody leguminous species affects δ^{15}N in two non-N-fixing shrubs.

In general, greater leaf δ^{13}C values higher on the slope suggest plants experience lower soil water availability there, consistent with our direct measures of soil moisture. While *P. sericea* is the only species not to show a strong increase in δ^{13}C in drier soils, its δ^{13}C values were also greater overall than those of either *P. acaulis* or *F. lenensis*. Even though *P. sericea* shows a significant decrease in leaf N with elevation and the moderating effects of nearby legumes, there is no elevational trend for δ^{13}C in *P. sericea*, suggesting this species operates at a relatively constant setpoint for c_i/c_a. Considering the greater abundance of *P. sericea* on the drier upper slope, its high and constant value of δ^{13}C likely reflects stomatal control and higher WUE. Thus, *P. sericea* seems to exhibit greater WUE or tolerance to dry soil (Midgley et al. 2004; Diefendorf et al. 2010) and, consequently, may be less competitive in the wetter, more productive lower elevations (Grime 1974).

In contrast, our results also provide evidence that interactions between carboxylation capacity and stomatal conductance can affect δ^{13}C. This is based on leaf %N and water availability holding a relationship with leaf δ^{13}C in *P. acaulis* and *F. lenensis*. For *P. acaulis* plants located away from legumes, both leaf δ^{13}C and leaf %N increases as elevation and soil dryness increase. From these data alone, it is not possible to determine if c_i/c_a decreases due to increased stomatal closure (and hence WUE). On the other hand, individuals of *F. lenensis* located away from legumes showed decreasing leaf %N and increasing δ^{13}C with elevation. Since it is unlikely that carboxylation capacity increases as %N decreases, the observed δ^{13}C response, in this case, is probably due to enhanced WUE along the slope. Variable N and water availability can often force contrasting response patterns on δ^{13}C (Toft et al. 1989; White et al. 1990; Sparks and Ehleringer 1997; Prentice et al. 2011). Perhaps the most interesting observation comes for plants growing near legumes where, for both *P. acaulis* and *F. lenensis*, leaf δ^{13}C shows no pattern with elevation. Regardless of the mechanism (variation in stomatal conductance vs. carboxylation capacity), it appears that growing near legumes allows both *P. acaulis* and *F. lenensis* to maintain a relatively constant c_i/c_a in a manner similar to *P. sericea*.

We cannot be certain why leaf %N and %C increases with elevation in *P. acaulis* while the other two species show a decrease in %N with elevation and either a decrease in %C with elevation (*F. lenensis*) or no relationship between %C and elevation (*P. sericea*). We consider the fact that *P. acaulis* starts growth and flowers earlier than the other two species. Its earlier phenology may mean it has access to more N than the others, but by the time leaves were collected in late July, this very prostrate species is mostly overtopped by surrounding vegetation at lower elevations, and mobile forms of N and C might have already been extracted from the leaves.

When comparisons are made across species representing a diversity of leaf lifespans, leaf N concentration is inversely correlated with structural traits that maximize leaf longevity (Reich et al. 1997, 1999; Chapin et al. 2002), which may explain negative correlations between leaf N and C concentration in some interspecific comparisons (Albert et al. 2010; Cerabolini et al. 2010). However, positive correlations between leaf N and C concentration sometimes occur for species of similar growth form (Golodets et al. 2009) or in intraspecific comparisons (Maron et al. 2007; Albert et al. 2010), just as we found with *P. acaulis* and *F. lenensis*. A positive correlation might arise if leaf C concentration largely tracks the storage products of photosynthesis, since leaf N concentration is a good predictor of photosynthetic capability (Field and Mooney 1986; Reich et al. 1997). That proximity to legumes erases the elevational trend in leaf %C is also perplexing, but since we measured bulk leaf organic C, we cannot say whether variation is primarily due to structural or storage-based components.

This study demonstrates a clear topographic gradient in soil moisture and a reverse gradient in recently fixed N versus N that has undergone more biological transformations, but is there a similar gradient in N availability? Our measurements using ion exchange membranes as root analogs suggest there is, with more plant-available NO_3^- on the upper slope. It is tempting to conclude that water is more limiting than N on the upper slope while N availability is more limiting on the wetter, lower slope, but we are cautious with this interpretation. First, both *P. sericea* and *F. lenensis* show a decline in leaf %N with elevation despite greater plant-available N at the uppermost elevation. Second, analysis of surface soils showed more total N (and more total organic C) on the lower slope. Third, the N available to our root analog probes is a function of mineralized N and N uptake by surrounding vegetation, and we would expect uptake by vegetation to be greater on the more productive lower slope. It is also possible that plants have more N available to them than we are able to measure in inorganic form because we now know that arbuscular mycorrhizae can directly access organic forms of N (Hodge and Fitter 2010), and there is greater total soil N on the lower slope. While it is true that we did not find a difference in root colonization by AM fungi between the lower and upper slope, we did not measure soil hyphal abundance or N uptake rates via mycorrhizae in the different locations.

Since N inputs, via N fixation, increase with elevation, the question arises as to whether the less productive, upper slope serves as the main source of N for this steppe as a whole. Some movement of N down slope is likely to occur through hydrological processes, but since this is a semi-arid system, transport via water could be limited. Just as wild grazers play a crucial role as transporters of organic matter and nutrients in many systems (Frank et al. 1994; Walker et al. 2003; Schoenecker et al. 2004; Holdo et al. 2007), we suggest that domestic herds must have played a similar role in the Mongolian steppe for millennia. Currently, the livestock herds largely sort themselves across the topographic gradient, with sheep and goats foraging more often on steeper, upper slopes. This may be explained, in part, because these animals prefer plants with lower silica content (Massey et al. 2009) than the sedges (Hodson et al. 2005) that dominate at lower elevations.

In summary, our study reveals much about the ecological consequences of topography in a montane steppe system for which there was little prior ecological information. Results suggest that both elevation and proximity to a legume affect plant N and C economies. The potential for N inputs to be highly localized leads us to be very interested in the importance of the various domestic animals as N transporters and their overall impacts on nutrient dynamics and the ecological health of this ecosystem.

Acknowledgments We are indebted to a number of undergraduates from the National University of Mongolia and the University of Pennsylvania for assistance in the field, processing samples, and data entry and to our support staff at our ger camp in the Dalbay Valley. The work was funded by the U.S. National Science Foundation grant number OISE-0729786.

Conflict of interest The authors declare that the research reported here complies with current laws of Mongolia. The authors have no conflicts of interest. No data/results used in this manuscript have been published elsewhere.

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