SPATIAL VARIATION OF STABLE CARBON AND NITROGEN ISOTOPE RATIOS AND C:N OF PERENNIAL PLANT SPECIES IN THE STEPPE GRASSLAND OF NORTHERN MONGOLIA

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ABSTRACT

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Stable carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N) and C:N are evaluated in individuals of three species (*Festuca lenensis, Potentilla acaulis, and Pontentilla sericea*) occurring across an elevation gradient along a south-facing slope. δ^{13}C is a common proxy for water use efficiency (WUE) in plants, C:N is a proxy for nitrogen use efficiency (NUE), and δ^{15}N provides insight into N-cycling at the site. Results indicate variation in δ^{13}C values (and hence WUE) that is significantly correlated with elevation (and thus water availability) in *F. lenensis* and *P. acaulis*, but show no such correlation in *P.* sericea. Variation in C:N (and thus NUE) is significantly correlated with elevation (and total soil nitrogen) in *P. sericea* only. That no species simultaneously increased both WUE and NUE suggests a trade-off between the two. The apparent plasticity in WUE seen in *F. lenensis* and *P. acaulis* may explain their abundance along the slope, whereas *P. sericea*—which had the highest WUE—is significantly more abundant where soil moisture levels are low. δ^{15}N results indicate variation in the isotope ratio that is significantly correlated with elevation in all three species. Locations higher on the slope, at the drier end of the aridity gradient, show higher levels of soil nitrate, a greater abundance of lichens and legumes, and decreased plant δ^{15}N. These results are consistent with previous work showing strong topographic effects on local N-cycles, and also the potential that the upper slope is playing a crucial role in bringing nitrogen into the system.

A better understanding of these results, and how legume and lichen abundance will be affected by future increases in temperature and increases in grazing pressure, will help us predict the future plant community composition in the region. The future distribution of these important grazing species will be impacted by their physiological response to different soil moisture levels and the availability of soil nitrogen.

Introduction:

Human induced changes to the major biogeochemical cycles have focused

attention on the ability of terrestrial ecosystems to adapt accordingly. In the case of

the carbon cycle, climate models predict new precipitation patterns that will bring

increased drought to many areas. A better understanding of drought tolerance and

how plants respond to natural moisture gradients at the landscape scale will

improve our interpretation and prediction of future responses on a global scale.

Likewise, the increasing rate of anthropogenic nitrogen deposition worldwide will

also impact plant growth. A better understanding of local N-cycling and its effect on

plant community composition will prove important as we assess these future

changes.

Both water and nitrogen are critical elements limiting plant growth in

natural ecosystems. Plant physiological performance across moisture gradients can

be evaluated using a simple ratio: that of carbon gained to water lost. This ratio is

referred to as the water use efficiency (WUE) of a plant. When soil moisture levels

decrease, plants commonly respond by decreasing photosynthesis, decreasing

transpiration, and decreasing leaf conductance. The magnitude of these responses

differs among photosynthetic pathways (i.e. C_3, C_4, and CAM) and also among

species that share the same pathway. Regardless of the pathway, the response

impacts the isotopic signature of plant leaf tissue, and as a result, stable carbon

isotope discrimination ($\delta^{13}C$) is a common proxy used to estimate WUE in plants.

There are two stable isotopes of carbon: ^{12}C and ^{13}C. ^{12}C is most abundant,

constituting approximately 98.93% of the Earth's carbon. ^{13}C forms the remaining

1.07% (although trace amounts of the radioisotope ^{14}C do exist). The results of past studies indicate substantial variation in plant $\delta^{13}C$ values at both the inter- and intra-specific levels in response to different environmental conditions, such as water availability (O'Leary, 1981; O'Leary, 1988; Osmond et al. 1982).

In addition to water, nitrogen is also a limiting factor for plant growth. In fact, nitrogen is the nutrient that most often limits plant growth in terrestrial ecosystems. This is because most organisms cannot use atmospheric nitrogen directly, which is the largest reservoir. In order for plants to use nitrogen, it must be converted from atmospheric N_2 gas into more chemically available forms. The conversion of atmospheric N_2 to NH_4^+ is known as nitrogen fixation and is done by bacteria that often form symbiotic relationships with plants, known as legumes. The subsequent conversion of NH_4^+ to NO_3^- is known as nitrification and is also done by bacteria. Plants use both NH_4^+ and NO_3^-, but uptake patterns may differ among co-occurring species (Evans, 2001).

Just as $\delta^{13}C$ is used as a proxy for WUE, so too is C:N (carbon: nitrogen ratio) used as a proxy for nitrogen use efficiency (NUE). The limited number of studies involving C:N along an elevation gradient have revealed inconsistent results (Mooney et al. 1978; Korner and Cochrane, 1985; Wegner et al. 2003; Tsialtas and Veresoglou, 2007).

Nitrogen exists as two stable isotopes ^{14}N (~99.63%) and ^{15}N (~0.37%). Each step of the nitrogen cycle differentially discriminates against ^{15}N, the heavy isotope. Recently fixed nitrogen has an isotopic composition similar to that of atmospheric N since no fractionation occurs during N-fixation. On the other hand, nitrification,

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denitrification, mineralization, and leaching discriminate against ^{15}N, leaving the remaining soil N pool enriched in ^{15}N (Handely and Raven, 1992). Because no discrimination during plant uptake occurs under most field conditions, plant δ^{15}N is usually a good approximation of δ^{15}N of the available N source (Marshall et. al 2008). As a result, variation in plant δ ^{15}N across a landscape can be used as an integrative proxy to characterize local nitrogen cycling processes (Handley and Raven, 1992; Amundson, 2003; Bai et. al 2009).

While on a global scale, plant δ^{15}N values have been found to systematically decrease with increasing mean annual precipitation and decreasing mean annual temperature (Amundson, 2003), at the landscape level, topography and soil nutrient availability (Schmidt and Stewart, 2003; Bai, et al. 2009) appear to have strong effects on these values due to their influence on soil moisture (Garten and van Miegroet, 1994), soil flux (Amundson, 2003), and microbial activity (Booth et al. 2005). Differential denitrification, mineralization (Garten and van Miegroet, 1994), and leaching (Kahmen et al. 2008) along these environmental gradients are believed to cause the observed isotopic changes.

This study uses carbon and nitrogen stable isotope analysis to study the effects of landscape scale heterogeneity on plant community composition in the steppe grasslands of Mongolia. The steppe grassland and larch forests surrounding the Lake Hovsgol region of Northern Mongolia are currently experiencing two strong ecological pressures: (1) increases in mean surface temperature as a result of climate change effects in high latitude regions, and (2) increases in grazing as the traditional nomadic culture becomes progressively more sedentary. Over the last

40 years, Northern Mongolia has warmed almost $2^{O}C$, there has been an increase in ecosystem water loss with no increase in precipitation, and the growing season now begins almost one month earlier than it did 10 years ago (Goulden et al. 2000; Nandintsetsg and Goulden, 2003). Permafrost has subsequently begun to thaw, leading to drastic changes in soil moisture levels (Goulden et al. 2003a,b; Sharkhuu, 2004; Sharkhuu et al. 2006). Additionally, excessive land use by nomadic herders has caused changes in plant distribution, community composition, and biomass.

Within the framework of the observed changes in Northern Mongolia, we explored how three important grazing plants (*Festuca lenensis, Potentilla acaulis,* and *Pontentilla sericea*) respond to the natural moisture and nitrogen gradients along a slope at the field site, and also how nitrogen enters the system and whether or not its input is localized. We evaluate the stable carbon and nitrogen isotope compositions of individuals collected along an elevation gradient to answer the following research questions. (1) What level of inter- and intra-specific variation in $\delta^{13}C$ (and thus WUE) and C:N (and thus NUE) exists and do these values change in predictable ways along the slope? (2) Does a species' ability (or lack of ability) to adjust its resource use efficiency in response to environmental gradients correspond to its distribution in space? (3) What level of inter- and intra-specific variation in plant $\delta^{15}N$ exists and do these values change in predictable ways along the slope? (4) Do plant $\delta^{15}N$ values suggest a higher level of N-fixation at the top of the slope (consistent with our observation of increased legume and lichen abundance with elevation)? (5) How important are legumes and lichens in providing newly fixed nitrogen to the surrounding ecosystem?

The answers to these questions will provide us with useful data as we attempt to predict the future effects of drought, changes to the N-cycle, and increased grazing on plant community composition in the region. The future distribution of these important species will be impacted by their physiological response to different moisture levels and the availability of soil nitrogen.

Materials and Methods:

Sample Species:

Leaf samples from three important perennial grazing species, *F. lenesis, P. acaulis,* and *P. sericea,* were collected at the International Long-Term Ecological Research site, located in the Lake Hövsgöl region of Northern Mongolia. *P. acaulis* and *P. sericea* are forbs, and *F. lenesis* is a grass. While *P. acaulis* and *F. lenesis* show no significant difference in percent cover from the bottom to the top of the slope, *P. sericea* is significantly more abundant at the top of the slope (unpublished data).

Field Site:

The sampling site was the south-facing slope of the Dalbay Valley, which drains into Lake Hövsgöl, one of the world's largest lakes, located at 50°N latitude and at 5000 ft. elevation. Elevation gain from the bottom to the top of the slope is approximately 120m. The slope is semi-arid steppe grassland and heavily grazed by yaks, sheep and goats. Vegetation consists predominantly of forbs, legumes, grasses and sedges. Currently, the plant community is more diverse higher on the slope

where it is also drier, but productivity is greater lower on the slope. This difference is mainly due to the overwhelming abundance of two particular sedge species at the bottom of the slope.

As mentioned above, a natural moisture gradient exists on the slope. Relatively high soil moisture levels exist at the bottom of the slope and these levels decrease with elevation. Additionally, available soil nitrate is greater higher on the slope, at the direr end of the aridity gradient, but total soil nitrogen is greater lower on the slope. Locations higher on the slope also show a greater abundance of lichens and legumes.

Sampling Method:

Plant leaves (five per individual) from *F. lenesis*, *P. acaulis* , and *P. sericea* were sampled at five locations (elevations) along three transects running up the south-facing slope. The elevations of the sampling locations differed slightly among transects. At each location, leaves from five individuals of each species were collected both close to (<10cm) and far from (>30cm) legumes. Legumes were not found at the two lowest sampling locations on each transect (locations 1 and 2). Although lichens (also N_2-fixers) are more abundant at the top of the slope, proximity to lichens was not accounted for in the sampling of individuals. See table 1 for an additional representation of the sampling method.

	Legumes Absent	*Legumes Present*
5 (1730-1777m)	5 individuals from each species (>30cm from legume)	5 individuals from each species (<10cm from legume)
4 (1707-1755m)	5 individuals from each species (>30cm from legume)	5 individuals from each species (<10cm from legume)
3 (1685-1726m)	5 individuals from each species (>30cm from legume)	5 individuals from each species (<10cm from legume)
2 (1672-1697m)	5 individuals from each species (>30cm from legume)	None sampled (<10cm from legume)
1 (1662-1682 m)	5 individuals from each species (>30cm from legume)	None sampled (<10cm from legume)

Elevation ↑ (vertical axis label on left)

Table. 1. Sampling method for a representative transect. In the "legumes absent" treatment, 5 individuals (located >30cm from the closest legume) from each species were sampled at 5 different elevations along a vertical transect. In the "legumes present" treatment, 5 individuals from each species (located <10cm from the closest legume) were sampled at the top 3 elevations along a vertical transect. This sampling method was repeated for 3 separate transects.

Data Analysis:

All 225 samples were dried, ground, and weighed and sent to the University of Pennsylvania Earth and Environmental Science Stable Isotope Laboratory to be analyzed for %C, %N, and C and N isotopic composition by mass spectrometry. Outliers were identified using studentized residuals. Data were excluded from statistical analyses if the studentized residuals were greater than 2 in absolute value. Between 0 and 8 outliers were detected per analysis (median = 2 outliers).

Stable carbon isotope discrimination ($\delta^{13}C$) is a common proxy used to estimate WUE in plants. Fractionation of carbon isotopes during photosynthesis results from the differential diffusivities of CO_2 containing ^{12}C and ^{13}C across the stomatal pathway and the preferential discrimination against ^{13}C by Rubisco and

PEP carboxylase (Farquhar, 1989). Isotopic discrimination against $^{13}CO_2$ relative to $^{12}CO_2$ is enhanced as the CO_2 partial pressure inside the leaf (c_i) increases relative to that in the surrounding air (c_{air}). In the case where water is not limiting, stomatal conductance (g) can be high, which causes increased water loss but also increased c_i. This increase in c_i allows for a high level of discrimination against ^{13}C, yielding more negative $\delta^{13}C$ values. This is an example of low WUE. On the other hand, when water is limiting, g is lowered, which reduces both water loss and c_i. In this case, discrimination against ^{13}C is decreased and less negative $\delta^{13}C$ values are observed—an example of high WUE.

$$\delta^{13}C_{plant} = \delta^{13}C_{air} - a \ - \ (b - a)\bullet \ c_i/c_{air}$$

The isotope ratio of air ($\delta^{13}C_{air}$) is constant within a site and the isotopic fractionation associated with diffusion (a) and enzyme discrimination (b) are constant, therefore $\delta^{13}C_{plant}$ is controlled by c_i/c_{air}. Carbon isotope ratios are reported relative to the Pee Dee Belemnite (PDB) standard.

C:N ratio is used to estimate nitrogen use efficiency (NUE) in plants. It can be thought of as the amount of carbon assimilated into plant tissue during photosynthesis per unit of nitrogen.

Fractionation of nitrogen isotopes occurs differentially for each step of the nitrogen cycle. Recently fixed nitrogen has an isotopic composition similar to that of atmospheric N since no fractionation occurs during N-fixation. Nitrification, denitrification, and leaching on the other hand, discriminate against ^{15}N, leaving the

remaining soil N pool enriched in ^{15}N. Because the rate of these N-transforming processes differs spatially as a result of different environmental conditions, the isotopic composition of soil nitrogen also differs spatially. Additionally, because discrimination during plant uptake is only observed when plant N demand is low compared with N supply (Högberg et al. 1999), plant δ^{15}N is a good approximation of δ^{15}N of the available N source under most field conditions (Marshall et. al 2008). Other factors such as rooting depth, timing, preference of NO_3^- vs. NH_4^+, and mycorrhizal status can influence plant δ^{15}N; however, because we are comparing the same species over a small geographic range, it is unlikely that these factors would significantly influence trends within a species. δ^{15}N is calculated as follows:

$\delta^{15}N_{plant}$ = [(R_{sample}/ R_{air})-1] x 1000, where R = the ^{15}N/^{14}N of a sample or standard. The standard here is atmospheric N_2, which is equal to 0.3663 atom% ^{15}N, and is constant over a broad geographic range (Mariotti, 1983).

Comparisons were made among species for δ^{13}C and δ^{15}N values (for plants not near legumes only) based on the overlap of 95% confidence intervals. Data were pooled for all locations.

   To test the effect of elevation on δ^{13}C, %C, δ^{15}N, and %N, separate regressions were performed for each species and legume treatment. Transect was included as a random effect. Models were fitted using REML methods.

   To test the effect of elevation on C:N ratio, both Pearson correlation coefficients and Spearman rank correlation coefficients were calculated. The

Spearman's method was used in addition to the Pearson's method because the data are not normally distributed.

Results:

δ13C and C:N

The mean $\delta^{13}C$ value differed among species (see figure 1). The mean for *P. acaulis* was the lowest (-27.65 ± 0.22‰) and that for *P. sericea* the highest (-26.14 ± 0.17‰) with *F. lenesis* intermediate (-27.01 ± 0.17). Each mean value is significantly different from the others (95% confidence intervals for the means are non-overlapping).
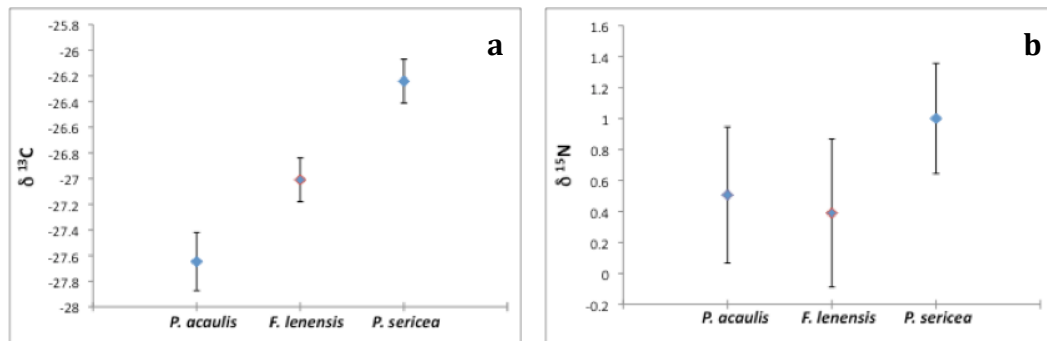

Figure 1. Mean $\delta^{13}C$, $\delta^{15}N$, and their associated 95% confidence intervals for individuals in the absence of legumes. A) shows mean $\delta^{13}C$ values that are significantly different among species. B) shows mean $\delta^{15}N$ values that are not significantly different.

Figure 2 shows $\delta^{13}C$ and C:N as a function of elevation. For individuals with legumes absent, $\delta^{13}C$ values increase with elevation in *F. lenensis* (R^2= 0.108, p<0.0001), and *P. acaulis* (R^2=0.279, p<0.01) but not for *P. sericea*. With legumes present, $\delta^{13}C$ values show no significant response to elevation in any species.

C:N ratios increase with elevation in *P. sericea* in the absence of legumes (R^2=0.246, p<0.0001). The Pearson's test shows a positive relationship between C:N and elevation in *P. sericea* (legumes present) as well (R^2=0.101 , p=0.038), but the significant relationship is not present when the Spearman's rank test is used. For the other species, both the Pearson's and Spearman's test are consistent and show no significant relationship between C:N and elevation. Only individuals in the absence of legumes are shown.
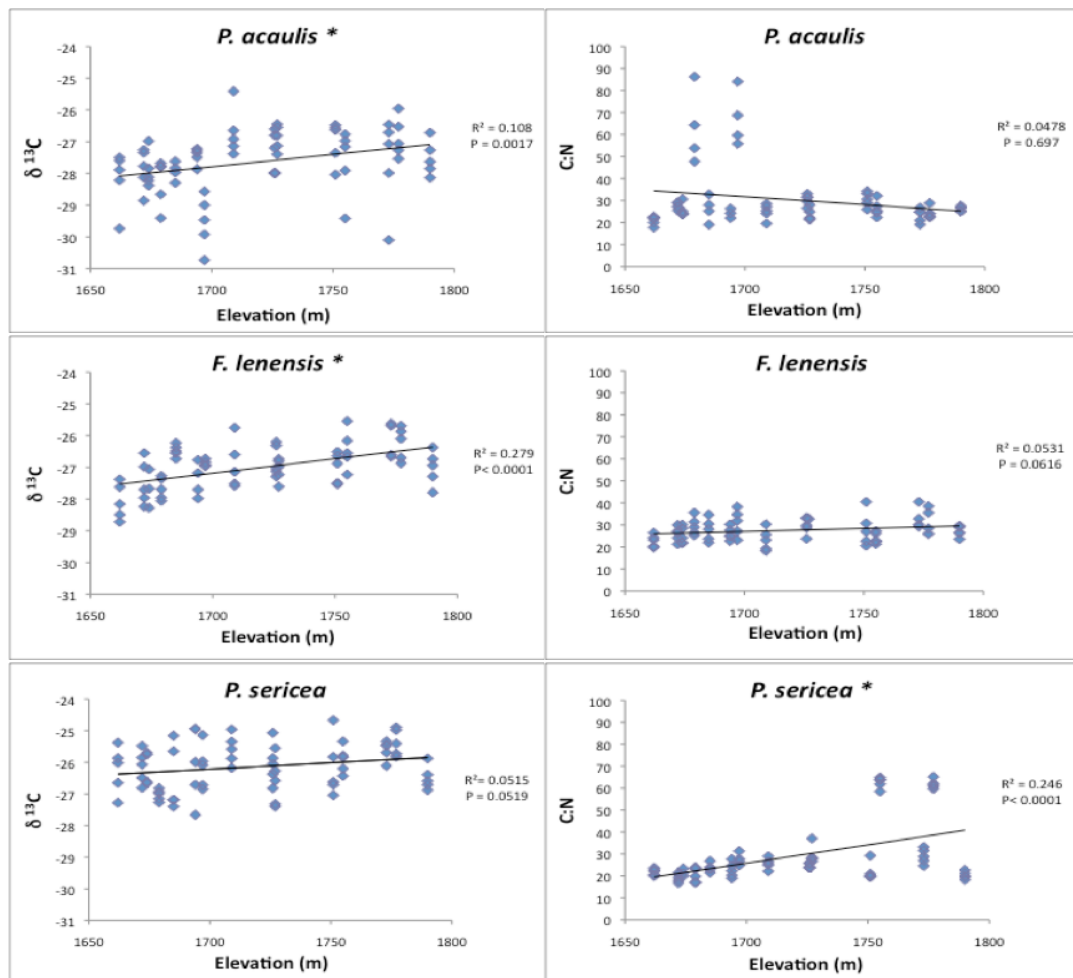

Figure 2. $\delta^{13}C$ and C:N values taken from all transects as a function of elevation. Slopes that are significant are marked with an asterisk. For a given species we see significant relationships in either $\delta^{13}C$ or C:N, but never in both (R^2 and p-values are given above).

%C and %N:

Figure 3 shows %C as a function of elevation. For individuals with legumes absent, %C values increase with elevation in *P. acaulis* (R^2=0.251, p<0.0001), decrease with elevation in *F. lenensis* (R^2= 0.129, p<0.0001), and show no significant response in *P. sericea*. With legumes present, %C values show no significant response to elevation in any species.

Figure 4 shows %N as a function of elevation. For individuals with legumes absent, %N values increase with elevation in *P. acaulis* (R^2=0.286, p<0.0001), and decrease with elevation in both *F. lenensis* (R^2= 0.114, p=0.0015) and *P. sericea* (R^2=0.263, p<0.001). With legumes present, %N values show no significant response to elevation in any species.

$\delta^{15}N$:

Mean $\delta^{15}N$ showed no significant difference among species (see figure 1). Figure 5 shows that $\delta^{15}N$ values where legumes were absent decrease with elevation in all three species, but most strongly in *P. acaulis* (R^2=0.657, p<0.0001), *F. lenesis* (R^2=0.151, p<0.001) and *P. sericea* (R^2=0.302, p<0.0001).

With legumes present, $\delta^{15}N$ values decrease significantly with elevation in *P. acaulis* only (R^2=0.182, p<0.01). No significant response was observed in either *F. lenensis* or *P. Sericea* (in fact, the regression line for *P. sericea* is almost perfectly flat).
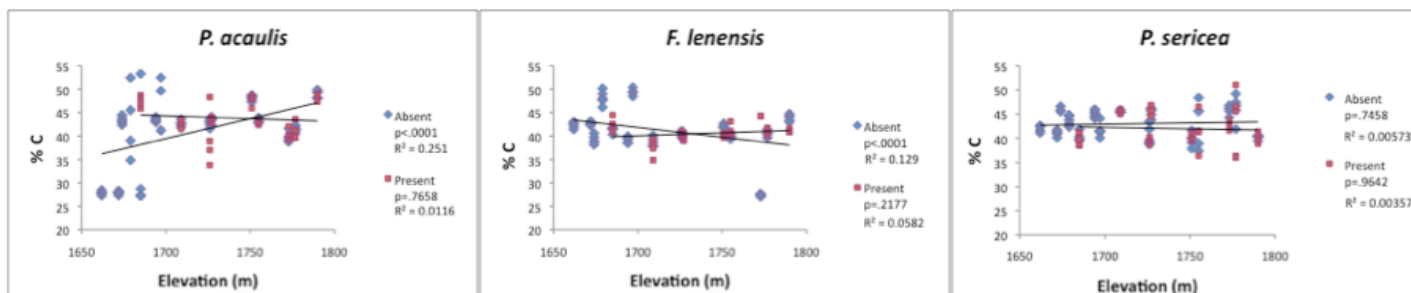
Fig 3. %C values take from all transects as a function of elevation. For individuals with legumes absent, %C values increase with elevation in *P. acaulis* (R^2=0.251, p<0.0001), decrease with elevation in *F. lenensis* (R^2= 0.129, p<0.0001), and show no significant response in *P. sericea*. With legumes present, %C values show no significant response to elevation in any species.
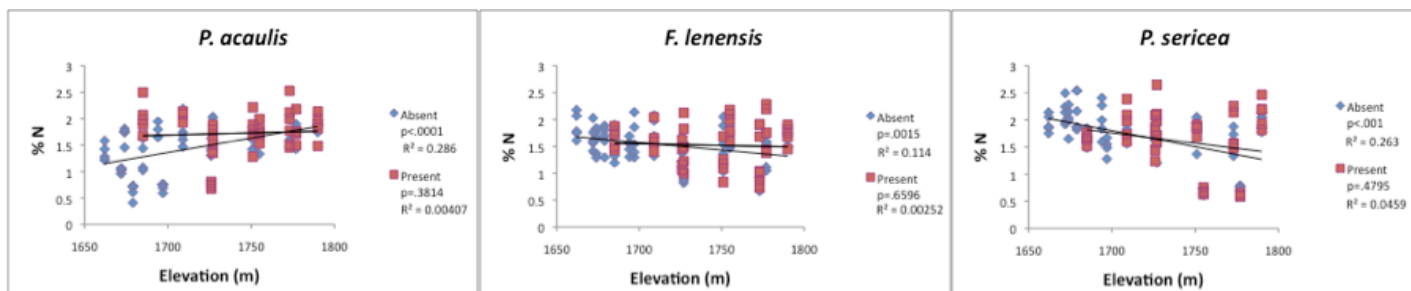
Figure 4. %N values taken from all transects as a function of elevation. In the absence of legumes, %N values increase with elevation in *P. acaulis* (R^2=0.286, p<0.0001), and decrease with elevation in both *F. lenensis* (R^2= 0.114, p=0.0015) and *P. sericea* (R^2=0.263, p<0.001). With legumes present, %N values show no significant response to elevation in any species.

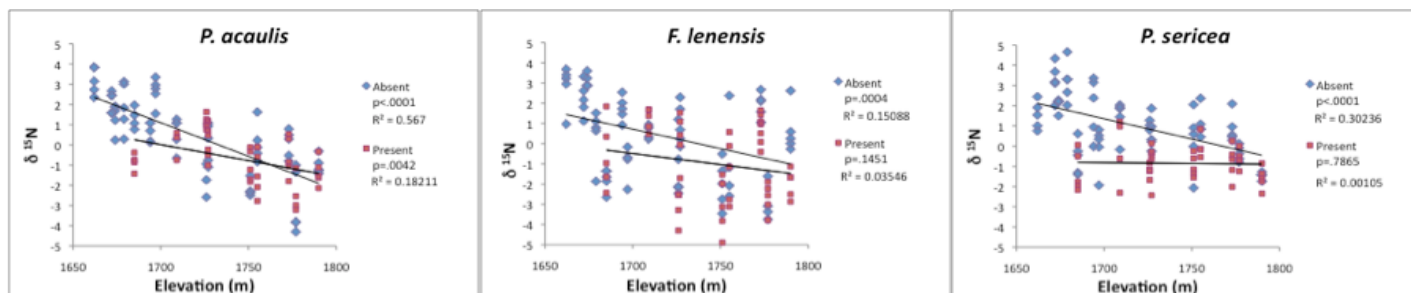**Fig 5.** $\delta^{15}N$ values taken from all transects as a function of elevation. In the absence of legumes, the slopes are significant for all species. In the presence of legumes, the slope is significant in *P. acaulis* only (R^2 and p-values are given above).

Discussion:

Variation in plant $\delta^{13}C$:

The literature supports that, for many plants, the availability of water can affect short term WUE if g (stomatal conductance) and A (assimilation) are differentially affected (Toft and Anderson, 1989). Differential responses of g and A to water availability will lead to differences in c_i and thus differences in WUE. However, if g and A are tightly coupled and change in concert in response to water availability, no change in either c_i or WUE will occur.

The results obtained for *F. lenesis* and *P. acaulis* are what we would expect to see in species in which g and A are differentially affected and readily demonstrate a change in WUE in response to surrounding environmental conditions. Individuals at the bottom of the slope (lower elevation), where soil moisture levels are high, show significantly more negative $\delta^{13}C$ values than do individuals at the top of the slope (higher elevation), where soil moisture levels are low. This suggests that for *F. lenesis* and *P. acaulis*, when water is readily available at lower elevations, g remains high (relative to A) and c_i remains high. This allows for a high level of discrimination against ^{13}C, yielding more negative $\delta^{13}C$ values (lower WUE). Conversely, as water becomes scarce at higher elevations, g is reduced (relative to A) and c_i drops. Discrimination against ^{13}C is decreased and less negative $\delta^{13}C$ values are observed (greater WUE).

P. sericea on the other hand, does not exhibit the same response. Individuals do not show a significant change in isotope composition that is correlated with elevation. Unlike *F. lenesis* and *P. acaulis*, *P. sericea* does not demonstrate a change

in WUE in response to the different soil moisture levels observed on the slope. Similar results have been observed by Wong et al. (1979), who observed no change in WUE with variation in water because A and g changed in concert and c_i was unaffected. Perhaps this can explain the results observed in *P. sericea.* In order to demonstrate this, instantaneous measurements of assimilation and transpiration would need to be taken and compared.

When we combine the isotope data discussed above with the spatial distribution of these species, the results provide us with insight into the determinants of community structure as well as the future distribution of these species as soil moisture levels in the region change with climate.

A species that responds well to changes in environmental conditions would be expected to exist at similar densities across a wide range of habitats. Whereas, a species that does not respond well to such changes would be expected to exist at significantly lower densities where conditions deviate from that species' optimum.

Our $\delta^{13}C$ isotope and species distribution data support this hypothesis. Both *F. lenesis* and *P. acaulis* are continuously distributed on the slope. Each species shows no significant difference in abundance when comparing the top and the bottom of the slope. *P. sericea* is also continuously distributed along the slope, but its abundance is significantly lower at the bottom of the slope than at the top. *P. sericea* has an average $\delta^{13}C$ value that is greater than *P. acaulis* (higher WUE) and exhibits no change in $\delta^{13}C$ with elevation. Thus we would expect *P. sericea* to be more abundant in areas with lower water availability, which is exactly what we

observe. Perhaps in areas of high soil moisture *P. sericea* is outcompeted by species that respond with lower WUE and higher rates of photosynthesis.

Based on these results, we expect *P. sericea* to increase in abundance with future soil moisture declines, while the ability of *F. lenesis* and *P. acaulis* to tolerate a decrease will depend on the full extent of their physiological plasticity and their interactions with other species.

Variation in %C, %N, and C:N:

Studies involving C:N across an elevation gradient have yielded inconsistent results. Previous work has shown increases in %N, and subsequent decreases in C:N with increasing elevation (Korner and Cochrane, 1985; Mooney et al. 1978). This is thought to be the result of decreasing specific leaf area with increasing elevation, and the negative relationship between nitrogen content per unit leaf area and specific leaf area. On the other hand, Tsialtas and Veresoglou (2007) found negative relationships between C:N and total soil N, and between C:N and soil water content, which is consistent with our results for *P. sericea*. Yet, Wegner et al. (2003) observed relative constancy of the C:N ratio within two fern species along an Andean elevation gradient, but were not able to explain the results.

In the context of these inconsistent patterns, here we observe relative constancy of the C:N ratio in *P. acaulis* and *F. lenesis*, and an increase in C:N with elevation in *P. sericea*. These differential trends in C:N with elevation can perhaps be explained by trade-offs between WUE and NUE, and might serve as indicators of the limiting resources for each species.

P. acaulis and *F. lenesis* show no change in C:N (and thus NUE) with elevation, but as discussed earlier, do show an increase in WUE with elevation. Alternatively, *P. sericea* shows an increase in C:N (and thus NUE) with elevation, but shows no change in WUE. These results suggest that plants are facing a trade-off between WUE and NUE, as no species simultaneously increased both values. Additionally, it appears that water availability is having the strongest physiological effect on *P. acaulis* and *F. lenesis*, whereas in *P. sericea*, nitrogen availability might instead exert a stronger control. The fact that *P. sericea* shows an increase in NUE as total soil N decreases, but as soil nitrate increases, suggests that *P. sericea* might prefer NH_4^+ to NO_3^- (i.e. perhaps NH_4^+ declines with elevation). The fact that we observed the greatest mean $\delta^{15}N$ value in *P. sericea* (although not significant) supports the preferential uptake of NH_4^+, which is enriched in ^{15}N compared to NO_3^-.

Based on these results and those discussed above regarding WUE, we expect that *P. sericea* will increase in abundance with future soil moisture declines, and will show a physiological response to future changes in soil N levels. The ability of *P. acaulis* and *F. lenesis* to tolerate changes in future N levels is unknown, but they appear to be unaffected across the range of N levels observed at the site.

Variation in plant $\delta^{15}N$:

Our $\delta^{15}N$ results fit nicely into the context of previous studies that have examined variability in plant $\delta^{15}N$ and landscape level N-cycling processes. For reasons discussed above, we assume here that variation in plant $\delta^{15}N$ reflects variation in $\delta^{15}N$ of the soil pool, which on a landscape scale is influenced by a

variety of biotic and abiotic factors involving the movement of nitrogen throughout the ecosystem. This assumption is strengthened by the fact that no significant difference in $\delta^{15}N$ among species was observed.

At the landscape scale, residual soil is typically enriched in ^{15}N by the preferential loss of depleted N compounds due to nitrification, denitrification, mineralization, and leaching. Spatial variability in soil (and thus plant) $\delta^{15}N$ is influenced by topography because of its control over soil moisture, nutrient availability, and subsequently the rates of N-cycling processes. Consistent with these findings, we observed that both soil water and total soil N decreased with elevation and that plant $\delta^{15}N$ was negatively correlated with elevation in the three species sampled.

While on a global scale, plant $\delta^{15}N$ values have been found to systematically decrease with increasing mean annual precipitation (Amundson, 2003), at the landscape level, positive correlations between water availability and plant $\delta^{15}N$ have been observed (Garten and van Miegroet, 1994; Bai, et al. 2009). This is thought to be the result of elevated microbial activity in wetter areas, resulting in increased losses of ^{15}N-depleted N by nitrification, denitrification, mineralization, and leaching (Garten and van Miegroet, 1994).

Many studies have also shown a positive association between total soil N and plant $\delta^{15}N$. Garten and van Miegroet (1994), Schmidt and Stewart (2003), and Bai (2008) observed positive correlations between total soil nitrogen content and soil/ leaf $\delta^{15}N$ since higher soil N results in greater microbial activity, which leads to preferential losses of ^{14}N (Booth et al. 2005). While these observations are

consistent with our results, measurements of N-processing rates at the top and bottom of the slope are needed to confirm this.

At the field site, we also observed that low-lying portions of the landscape show lower levels of soil nitrate compared to upper portions. Previous studies have shown that ^{15}N depleted nitrate in grasslands is prone to losses by leaching, which consequently increases soil δ^{15}N of the remaining pool (Amundson 2003). Perhaps lower nitrate levels at the bottom of the slope are the result of leaching, which also contributes to the elevated δ^{15}N values observed.

At smaller scales, variation in plant δ^{15}N can reflect the influence of N-fixers on neighboring plants. While most soils are enriched in ^{15}N, soils under N_2-fixing legumes, for example, can be depleted in ^{15}N, which is attributed to the dilution effect of leaf litter (Shearer and Kohl, 1989; Peoples et al. 1991). Furthermore, it is possible for N previously fixed by adjacent fixers to be transferred to neighboring plants (Handley and Raven, 1992; Bai et al. 2009). Looking at our results in the context of these findings, it is possible that in areas of high lichen and legume abundance (the upper slope), these N_2-fixing plants are providing a "shadow" of recently fixed nitrogen that is being used by the surrounding upper slope community (as indicated by low δ^{15}N values). On the other hand, at lower elevations (where lichens and legumes are sparse), plants are utilizing nitrogen derived from decomposition that, after being recycled, is relatively enriched in the heavy isotope.

We don't however see a difference in δ^{15}N values between individuals collected in the absence of legumes and individuals collected in the presence of legumes that is consistently in the direction that the literature would suggest (i.e.

lower $\delta^{15}N$ values for individuals close to legumes). Potentially, this could be the result of our legume absent/present sampling method not accurately reflecting "far from" or "close to" a source of recently fixed nitrogen as it was designed. This could either be because the shadow of recently fixed nitrogen extends beyond 30 cm from the nearest legume or because lichen proximity was not taken into account. For a better understanding of these results, future work that traces the movement of nitrogen through the field site is needed.

More broadly, our results indicate that the pool of available nitrogen varies along the slope, showing ^{15}N enrichment at the bottom, which decreases with elevation. Plant $\delta^{15}N$ values are consistent with previous work showing strong topographic effects on local N-cycles—influencing the relative sizes of the N pools and N-processing rates between upper and lower portions of the landscape. Our results are also consistent with the hypothesis that higher levels of N-fixation at the top of the slope exist and that legumes and lichens might play a critical role in providing newly fixed nitrogen to the surrounding ecosystem. The degree to which each factor is influencing our observations is unknown, but should be the focus of future work.

Conclusion:

While this study provides us with a starting point for understanding the physiological mechanisms that dictate the distribution of these species in space, further investigation is needed to make more certain predictions about the future.

Increased grazing pressure in the region by nomadic herders has already placed stress on these plant communities. How these species respond to the additional stresses of climate change and changes to the N-cycle could have potentially large impacts on nomadic culture in Northern Mongolia. The answers to the posed questions will provide us with useful data as we attempt to predict these future effects.

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