Phosphorus and nitrogen limitation to photosynthetic microbes in high-elevation soils

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Abstract

Environmental change in the form of increased nitrogen (N) deposition is occurring at high elevation sites across the Rockies at sites such as Niwot Ridge. It is unknown if, or how, this N input affects soil microbes, specifically those in talus sites where microbes are ecologically significant. Microbes are abundant and ubiquitous across the planet, especially in soils. Understanding whether nutrient inputs are affecting the growth strategies of talus microbes is important, as demonstrated by (i) the influence of microbes on biogeochemical cycling of nutrients, (ii) the impact of microbes on plant production through symbioses and nutrient pulses caused by microbial turnover and (iii) the role of microbes as "filters" of groundwater to downhill systems, such as watersheds with economic relevance. Our results indicate that nutrient inputs can cause shifts in the growth strategies of microbes, and this could influence the kinetics of microbial growth and the subsequent downhill transport of nutrients and materials from these sites. An environment dominated by copiotrophs (microbes dependent on high nutrient levels in soil) as was found in N amendments, could lead to a rapid depletion of resources, which could negatively influence the growth and development (succession) of other microbes at talus sites as well as potentially influencing the transport to downhill systems highlighted in the Landscape Continuum Model (Seastedt et al., 2004 BioSci, 54(2):111). Future experiments focusing on how various types of N (NH_4^+ vs. NO_3^-) impact microbial communities, studies measuring the growth and development of other ecologically significant talus soil microbes, as well as incubation experiments with more replicates will all be valuable to better understand how talus soil

microbial communities will respond to ongoing environmental changes such as N input from various sources.

Background and Introduction

Mountains are unique ecological areas characterized by extreme environmental conditions, such as high solar radiation, extreme temperature fluctuations (Freeman et al., 2009), and high wind disturbance (Williams et al. 1996). These environmental conditions all affect the life strategies of organisms living in these landscapes (Seastedt et al., 2004). The Landscape Continuum Model (LCM), developed based on work at Niwot Ridge, Colorado, conceptualizes the transport of nutrients from a mountain downhill across terrestrial ecosystems via redistribution mechanisms such as wind (Seastedt et al, 2004). The model highlights the importance of abiotic processes, such as wind, in the redistribution and transport of endogenous or exogenous (originating from the alpine versus from outside ecosystems) from alpine ecosystems to downhill ecosystems (Seastedt et al., 2004). The LCM provides a framework to understand the importance of high-elevation ecosystems because the latter are the sources of many inputs to downhill systems via transport of both endogenous and exogenous sources of nutrients to ecosystems at lower elevation (Seastedt et al., 2004). Furthermore, high-elevation alpine ecosystems are valuable due to impact of these soils on economically important goods, such as drinking water and water quality. Microbes act as filters of groundwater by removing nutrients and other chemical compounds unwanted in drinking water, by assimilating them into biomass (Williams et al., 1996). This

"purified" water travels downhill and eventually becomes the drinking water for the large downhill populations. Generally, the economic effect of microbes on drinking water is related to the costs of processing of water for drinking. Studying microbial growth response to nutrient inputs could help better understand how water quality may change in response to shifts in microbial community composition and growth strategies of the microbes occurring there.

Recent studies have identified changes, e.g. increased nitrogen (N) deposition at high elevation sites such as Niwot Ridge Long-Term Ecological Research (LTER) site in the Front Range of Colorado (Williams et al., 1996 and Seastedt et al., 2004). Increased N deposition is caused by (i) increased precipitation at higher elevation and (ii) an increase in the N concentration in precipitation (Williams et al., 1996). Increased N inputs have not had dramatic effects on high elevation plant communities (Bowman et al., 2002); however, in freshwater systems lake algae have experienced an increased rate of growth (McKnight et al., 1988 and Williams et al., 1996). While these latter studies support the view that N inputs affect certain biota of the region, it has yet to be described how these inputs impact microbial species, specifically in unvegetated (no vascular plants) talus ("barren," unvegetated) soils where microbes are the primary source of photosynthetic primary production (Freeman et al. 2009) and biogeochemical cycling of nutrients such as N (Ley et al. 2004, Schmidt et al. 2009).

Microbes consist of organisms from all three domains of life: Archaea, Eukarya and Bacteria (Woese and Fox, 1977). Collectively, these organisms possess a vast metabolic diversity (Pace, 1997) that enables them to inhabit numerous

varied ecosystems across the planet. In terrestrial ecosystems microbes are ubiquitous and it is estimated that up to 10^6 different species can inhabit a single gram of soil (Fierer et al., 2007). Soil microbial species have important roles in ecologically important processes, such as plant primary (photosynthetic) production (van der Heijden et al., 2008) and biogeochemical cycling of nutrients like nitrogen (N) (Schmidt et al., 2007, King et al., 2010 and Nemergut et al., 2008). Rhizobia and cyanobacteria are examples of bacteria that contribute to the vast amounts of atmospheric N_2 entering ecosystems via N_2 reduction into biologically available forms (e.g., ammonium) in specialized structures (heterocysts for cyanobacteria or in root nodules for rhizobia). The symbiotic relationship between plants and rhizobia exemplifies how microbes contribute to both the biogeochemical cycling of nutrients by fixing N, and primary plant production by supplying plants with limiting nutrients for growth (N). Microbes further influence plant production via other roles, e.g. providing nutrients to plants in soil through processes such as microbial turnover or succession, large N inputs into ecosystems caused by microbial community shifts (Schmidt et al., 2007). These shifts are an important source of N for plant growth (Schmidt et al., 2007), especially in areas where the growing season is short such as Niwot Ridge. The various roles of microbes demonstrate (i) the influence of these microscopic organisms on large-scale, ecosystem stability and (ii) the importance of studying how soil microbial communities respond to on-going climate and other anthropogenic changes.

Microbial growth is characterized by four stages: lag, exponential, stationary, and death phases (Figure 1). In the lag phase of growth, population growth is slow

or delayed while important enzymes and growth factors are being synthesized or while population sizes reach a quorum to coordinate growth. In exponential phase, the microbe is growing at the highest rate corresponding to the high availability of limiting nutrients, enzymes and cofactors. The exponential phase is a valuable because it used to infer the growth (r) of a microbial population (phototrophs in this case) in response to a nutrient. Once resources have begun to be depleted, individuals in a population begin to die at an equal rate as they divide and the population growth plateaus. This plateau is the maximum amount of individuals/species an environment can support with its given nutrients, thus it can be inferred to be the carrying capacity (K) of the environment. K is also an important number for microbial growth because it tells us how many microbes can grow given these conditions. Lastly, resources will become so depleted that growth stops and microbes enter the death phase of growth when the population numbers decline due to a stop in growth and an increase in death (Figure 1).

Recent work by Fierer and colleagues (2007) has demonstrated that the growth strategies of certain phyla (groups) of bacteria can be differentiated based on the availability of nutrients in an environment. These groups of bacteria are applied to a growth strategy continuum similar to the r and K selection continuum described by MacArthur and Wilson (1967) to determine the life history traits of microbes. In this continuum, an oligotroph (K-selected species) would be more adapted to lower nutrient availability, have higher enzyme affinities (able to capture nutrients for growth when they are less concentrated in the environment), slower rates of growth and a larger carrying capacity while a copiotroph (r-selected

species) would be adapted to higher nutrient availability, have a higher rate of growth, lower enzyme affinities, and a smaller carrying capacity (Fierer et al. 2007). This "ecological framework" provided by Fierer et al. (2007) allows one to predict which types of species- copiotroph or oligotroph -would be more abundant in an environment given the characteristics of the area.

Talus soils offer an interesting environment to study microbial growth response to nutrients or succession (development and change in abundance/composition of the microbial community over time) because of a historically low availability of nutrients (Ley et al., 2004 and King et al., 2008), such as N, P, calcium (Ca), and potassium (K). Equally interesting is the high rate of microbial turnover at talus sites (Schmidt et al., 2007). The rapid succession of microbes can be explained by their small size, high surface area : volume ratios and how they respond to the environment. Microbes are able to grow and reproduce on much shorter time scales, and therefore are able to rapidly respond to fluxes in nutrients that result in a seasonal turnover of species (succession) (Schmidt et al., 2007). As previously mentioned, it is unknown how the growth of talus soil microbes from the Rocky Mountains will respond to the increased N input in a controlled laboratory setting however, previous studies can provide valuable insight for making predictions regarding the Rocky Mountain microbial phototroph community and its growth response to nutrients.

Preliminary studies have estimated that a P-limitation exists in early successional talus soils located in the Peruvian Andes, likely due to a low availability of inorganic P from rock on newly exposed areas (Schmidt et al., 2011). Previous

work by King et al. (2010) has demonstrated soil microbial communities at high elevation sites, such as Peru and Colorado, have similar spatial patterns based on soil chemistry. By recognizing that microbial communities are similar across high elevation sites (King et al., 2010), the Rocky Mountain soils can be hypothesized to be similar to the Andean sites, thus a P-limitation existing at Niwot Ridge is not out of the question.

I hypothesized that the microbial phototrophs abundant in talus sites at Niwot Ridge (Freeman et al., 2009) are limited by P. This is because, when supplied with ample amounts of C and N, phototrophs will quickly deplete the available P, as a result of high enzyme affinities and high surface area to volume ratios (Schmidt et al., 2011), and be limited for subsequent growth after the original P is assimilated. This hypothesis can be tested by measuring phototrophic growth (response) to nutrient additions in a controlled laboratory setting (incubation) for a sufficient period of time to allow for microbial growth (several weeks). I predict that soils with P additions will have a higher carrying capacity and lower growth rate than N additions because oligotrophs will be dominant in P soils. I further hypothesize that copiotrophs will be favored when N is added to soils because there will be a higher availability of nutrients (N), therefore these treatments will favor copiotroph growth, thus I predict they will have lower carrying capacity and higher rates of growth.

Materials and Methods

Samples were collected from the top-5-cm of soils located in a talus field above the high-elevation D1 site near Green Lake Five at the CU Mountain Research Station (40 3'24"N 105 37'30"W). The sampling site is a talus field located within sites previously described by Ley et al. (2004), Freeman et al. (2009) and King et al. (2008,2010). Roughly 200 g of soil were collected from 3 different sites within a 200-m^2 area. Samples were sieved using a 2.46-mm sieve, homogenized and stored in a -20˚C freezer until they were prepared for incubation. Prior to setting up the experiment, 3 sub-samples of the homogenized soil were placed in metal tins and dried overnight in an oven at 60˚C to determine percent moisture. Soil pH was measured at the beginning of the experiment by mixing a 5:1 ratio of distilled water to soil, shaking samples for 60 minutes and measuring with a OakTron pH probe (OakTron, Vernon Hills, IL, USA). Percent moisture [g/g] was calculated for both unprocessed and the sieved/homogenized soils, while pH was calculated using four replicates of the homogenized soils.

For the incubation experiment, an average of 14.3g (dry) of the homogenized and sieved soil was added to 24 sterile mini petri dishes (20x60mm) and placed under a full spectrum 60W light bulb. Four treatments, each with a different nutrient addition (+N, +P and +N+P) were assembled; six replicates were set up for all treatments and control samples. Soils were sampled from two time points– at day 35 and day 55. Soils were incubated for a total of 55 days at room temperature (~21˚C) under a photoperiod of 12 h light/12 h dark, watering was performed two to three times per week to maintain samples around 60% of the field water holding

capacity (WHC), the ability of soil to hold water against the force of gravity, as determined gravimetrically similar to King et al. (2008).

Nutrient amendments for incubated soils consisted of N, (+N), P, (+P) and N and P (+N+P) respectively. Fertilizer solutions of 0.016M and 0.017M were prepared from 0.2135 and 0.0142% mono and dibasic sodium phosphate (Na_2HPO_4 and NaH_2PO_4 respectively) and ammonium nitrate (NH_4NO_3) respectively. Fertilizer solution was added during the first watering of the incubation at day 0, by adding 2.22mL of solution to each treatment plate with 75µg of nutrient (e.g. N, P or N and P) per gram of dry soil (14.3g per dish). Control plates received equal volumes of sterilized distilled water. After the first addition of either fertilizer solution or water, all plates were watered with sterilized distilled water two to three times per week in an attempt to maintain water availability for each plate at 60% WHC. This was done by recording the mass of each plate at 60% WHC, then weighing plates every two or three days and adding an appropriate volume of water to reach the desired mass for each plate.

During the incubation period of 55 days, visual observations were performed on plates using a low-power microscope (Bausch & Lomb, New York, USA) at 45X. Plates were assessed for the presence of ecologically significant groups, microbial photoautotrophs (photosynthetic organisms that rely on the energy from the sun [photons] to fix carbon from the atmosphere into sugars) such as cyanobacteria and micro-algae. These groups were categorized as "green" for observational purposes. Fields of view (what one could see in the microscope) were identified as either containing "green," cyanobacterium or algae, a "plant," either a liverwort or a moss,

or “blank,” suggesting barren soil. Observations were made on each Petri dish along transect lines that ran up and down the plate along the length of the plate, transects were not drawn on Petri dishes but rather lines created by moving the microscope in a straight line from one edge to the next. Transects were sampled in a random manner in order to perform statistics on the data points collected during observations twice a week. If a member of the aforementioned groups, such as a cyanobacterium for green, was present and observed in the 45X field of view (FOV), a tally was recorded. If no species were observed in a given FOV then a “blank” was marked indicating the soil was barren of any visible microbes or bryophytes. A total of 50 FOV for each plate at each time point were recorded to determine the relative abundance of microbial groups over time. Observations were made on 12 sub-samples, beginning on day 7 of the incubation with observations continuing for twice a week for a total of 55 days.

Observation data obtained during the incubation period were plotted and statistically analyzed using Kaleidagraph (Synergy Software, Reading, PA, USA). The population growth of phototrophs in each replicate plate for 12 treatments was plotted and calculated using a logistic equation proposed by Schmidt et al. 2011:

$$C(t) = K / (1 + e^{-r(t-i)}) \qquad (1)$$

where C(t) is the % ground cover at time t, r is the intrinsic rate of increase (with units of $days^{-1}$), i represents inflection point of the sigmoidal curve (with units of days) and K is the carrying capacity in the same units as C(t). The average value of relevant variables was recorded for each plate and analyzed with analysis of variance (ANOVA) and Tukey (T) tests.

After 55 days, percent cover was estimated for each plate using a dot that was drawn on the ocular of the microscope as a reference point. 100 total points were measured following vertical transects along plates for each replicate plate as described above. The previously mentioned key with the "green," "bryophyte" and "blank" was used. This method is similar to plant community surveys in that it allows us to infer the percent of ground cover of a particular group of microbes by measuring the total amount of times an individual was present in a field and dividing it by the total number of points. Percent cover was estimated by dividing the total number of occurrences for each category by the number of points (100) and multiplying by 100 to give a percent.

Additional soil biogeochemical properties such as microbial biomass carbon (the amount of C stored in soil microbial cells) were determined using chloroform fumigation experiments as done by previously by Weintraub and colleagues (2007) and Nemergut et al. (2010). 5g of each soil was combined in a 50mL conical test tube with 20mL of 0.5M K_2SO_4 and shaken at 300 rpm for 1 hour. Next, soil slurries were filtered through Whatman #1 paper (Whatman Ltd, USA). This first step allowed us to infer the amount of non-biomass organic C present in soil samples because C assimilated into microbial biomass would not be present in solution without fumigation with chloroform. An additional 5g of soil was placed in a falcon tube with 20mL 0.5M K_2SO_4 then placed in a desiccator with 30mL chloroform, sealed air tight with a vacuum pump and covered with a black bag for 72 hours. After 72 hours samples were filtered and extracted as described above. The microbial C in samples was estimated by subtracting the organic C present in the

unfumigated samples (first extraction) from the total C of the fumigated samples (second, fumigated extraction). Extractions were measured with a high temperature combustion total CN analyzer (Shimadzu TOCvcpn, Kyoto, Japan) as done previously by Nemergut et al. (2010). The elemental characteristics of each treatment (C and N) were measured and averaged to determine if and how soil properties shifted over time in response to nutrient amendments. Data were compared across treatments using ANOVA and t-tests to determine differences among the means of treatments.

Results/Discussion

Applying equation 1 (see above), the count data indicates that P is a limiting nutrient for growth of high alpine microbial phototrophs. This is supported by the P, (+P), and the N and P, (+N+P), plates having significantly higher carrying capacities than the N (+N) plates ($p<0.05$). Microbial growth curves (Figures 2 and 3) suggest that the (+P) and (+N+P) treatments reached the stationary phase (Figure 1) with the highest carrying capacity relative to the other plates. This result supports the hypothesis that P is limiting phototrophic growth in high alpine soils because, when P was added to soils, microbial communities increased in abundance (% cover). Had another nutrient been limiting, P would have had a curve similar to N where a "weedy" copiotroph species takes over and carrying capacity is decreased (Figures 2 and 3).

The K values for carrying capacity were significantly higher in plates to which P had been added (+P) and plates which N had been added (+N: Table 1 and Figure 2 and 4), indicating P was likely limiting to growth and thus carrying capacity increased when there was a higher resource availability. P-limitation relates to a previously mentioned study by Schmidt et al. (2011) that found a P-limitation in high elevation talus sites in the Andes.

The similar, slow growth rate observed between P addition and the control plates, as well as the significantly lower growth rate of the control and P compared to N addition may suggest that a different type of microbe was favored when N was added than those naturally favored in talus soils in the Rockies. When applied to the continuum proposed by Pianka (1970) and Fierer et al. (2007), oligotrophs were naturally abundant in talus soils in the Rockies. This is supported by lower rates of growth and higher carrying capacity of the control plates. Talus soils favoring slow growing oligotrophs is supported by previous findings by Freeman and colleagues (2009).

The data indicate that P is limiting the growth of microbial phototrophs because, when P is available in high concentrations, microbes are able to grow and reach a higher carrying capacity than the N and control treatments. Equally interesting is the impact of N indicated by the data. N seems to increase the growth rate of phototrophs, but lowers the carrying capacity in both the (+N) and (+N+P) as compared to the control and the P (+P) treatments (Figures 2 and 5). This response suggests that N is in some way negatively impacting the carrying capacity of the

talus soil phototroph community, likely by selecting for copiotrophs over oligotrophs due to high resource availability of N.

Insignificant results were obtained in microbial biomass and percent cover assays. Percent cover survey methods indicated no significant difference in percent cover of phototrophs between the control and the nutrient inputs. Microbial biomass fumigation assays likewise revealed no significant differences between the amount of C (C) in the cells of microbes across the different treatments ($P>0.05$), as determined by ANOVA tests and Tukey's T-test. Although microbial biomass assays did not yield to significant results, the value of the chloroform fumigation technique to detect C and N biomass levels in soils is still recognized for use for future research regarding soil nutrient levels and microbial influence. Treatments receiving P (+P and +N+P treatments) had the highest biomass, as demonstrated by a higher microbial C content than either the control or (+N) treatments. Although not statistically significant, these results agree with carrying capacity values previously discussed, indicating that P was a limiting nutrient because biomass increased when P was added. Future studies with more replicates could potentially demonstrate a statistically significant difference between the microbial biomass C in response to various fertilizer treatments therefore future research is warranted.

Further research is necessary to elucidate the specific impacts of N on soil microbial phototrophs (Figure 3) due to multiple types of N (NH_4^+ and NO_3^-) used in our N treatments. Further research with various nitrates or ammonium additions could lead to insight about if and how specific types of compounds affect microbial communities differently. It could be hypothesized that NH_4^+ (ammonium) affects

soil microbial communities differently than NO_3^- (nitrates) because ammonium would likely lower the pH of the soil due to excess H+ ions entering the system. Soil microbes are greatly affected by soil pH. Fierer and Jackson (2006) demonstrated that soil microbial communities are the most diverse and species rich in neutral pH (~7) soils with both richness and diversity decreasing with increasing or decreasing pH. The results of N addition decreasing carrying capacity could be due to a potential decrease in the soil pH over the course of the experiment caused by ammonium use. This decrease in pH would not have supported as many phototrophs as other treatments and explained the lower carrying capacity of N treatments. Determining what different forms of N mean for microbial communities in terms of growth and function may help better predict how microbial communities in land and ocean environments will respond to changing climatic variables such as N deposition occurring at high alpine sites or increased N concentrations in run-off water into rivers, lakes and oceans.

These results suggest that increased N deposition at Niwot Ridge is causing shifts in microbial growth strategies of phototrophs that could further impact rates of biogeochemical cycling of N and C by changing the abundance and distribution of oligotrophic as well as copiotrophic bacteria. Copiotroph or "weedy" (fast growing, resource depleting) species of microbes will utilize nutrients quicker (Fierer et al., 2007) and could potentially create nutrient fluxes through metabolic processes or successional turnover (Schmidt et al., 2007) that are different due to differences in growth requirements for certain species. At high-elevation sites, changes in nutrient dynamics would subsequently affect downhill ecosystems (Seastedt et al.,

2004) and their nutrient inputs. Shifts in nutrient fluxes could be a concern to citizens whose water originates from watersheds downhill of these areas. Shifts in the availability of nutrients in downhill ecosystems will impact production and input of nutrients such as C and N

Figures/Tables

Equation 1: $C(t) = K / (1 + e^{-r(t-i)})$

Figure 1:

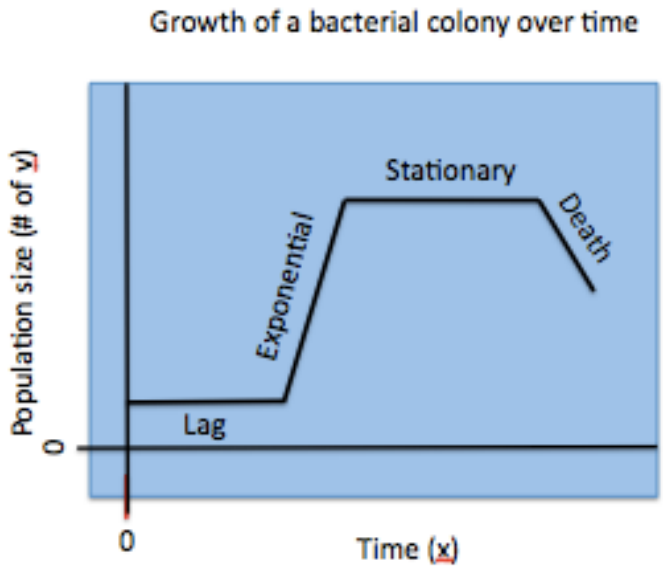

Figure 1: A basic graph depicting the four basic stages of microbial growth. The exponential phase has importance because it is the stage that the growth rate is inferred. Stationary phase is important because it is the stage where the colony reaches its carrying capacity.

Table 1:

Treatment	K	r
control	39.36	0.077
N	27.002	0.246
P	50.44	0.156
NP	44.74	0.248

Table indicating the average carrying capacity (K) in units of % cover and the growth rate (r) in units of % per day in response to various treatments.

Figure 2:

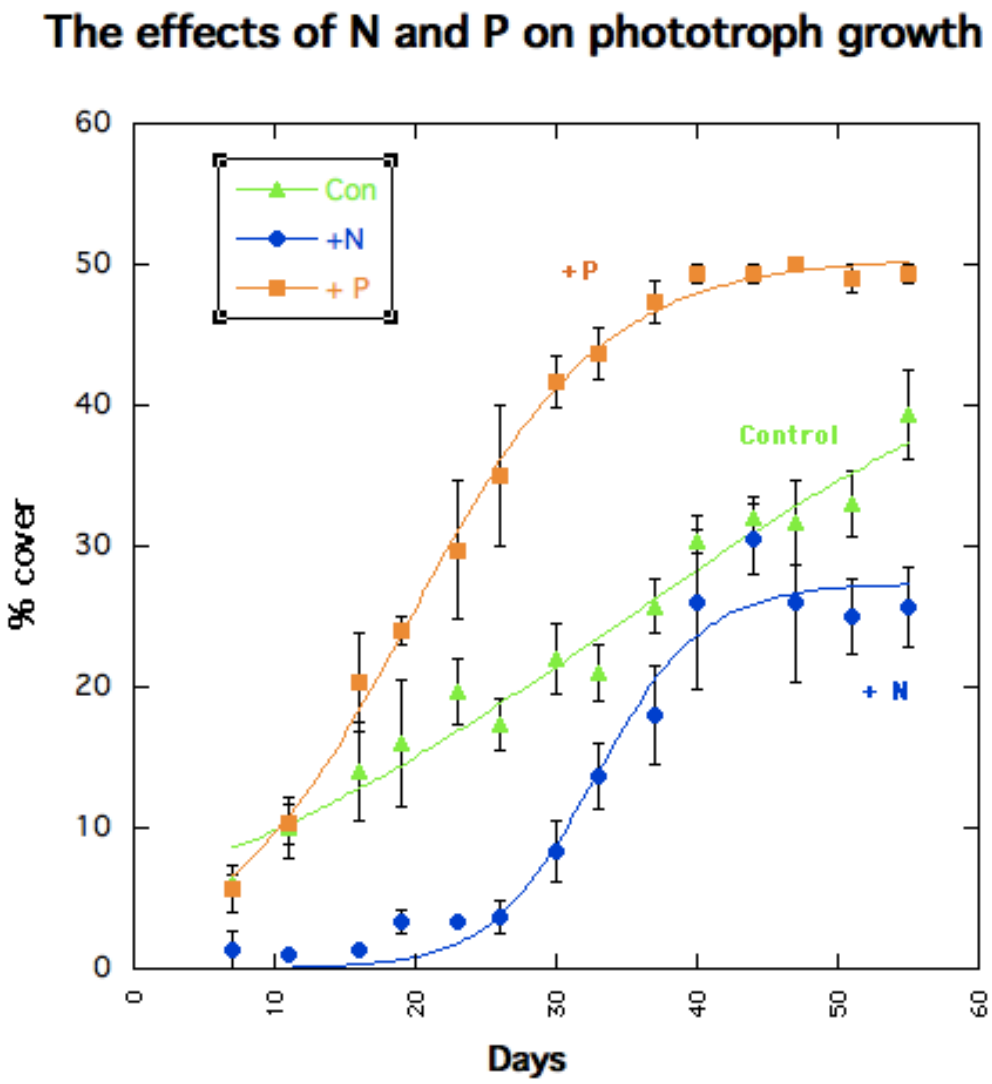

Figure 2: Growth response of talus soil phototrophs during incubation with various nutrient inputs of N and P. The graph displays the relative effects of N and P to the control soils. The slope of the lines as well as the height at which the curve flattens out are important indicators of the growth rate and carrying capacity during each nutrient regime.

Figure 3:

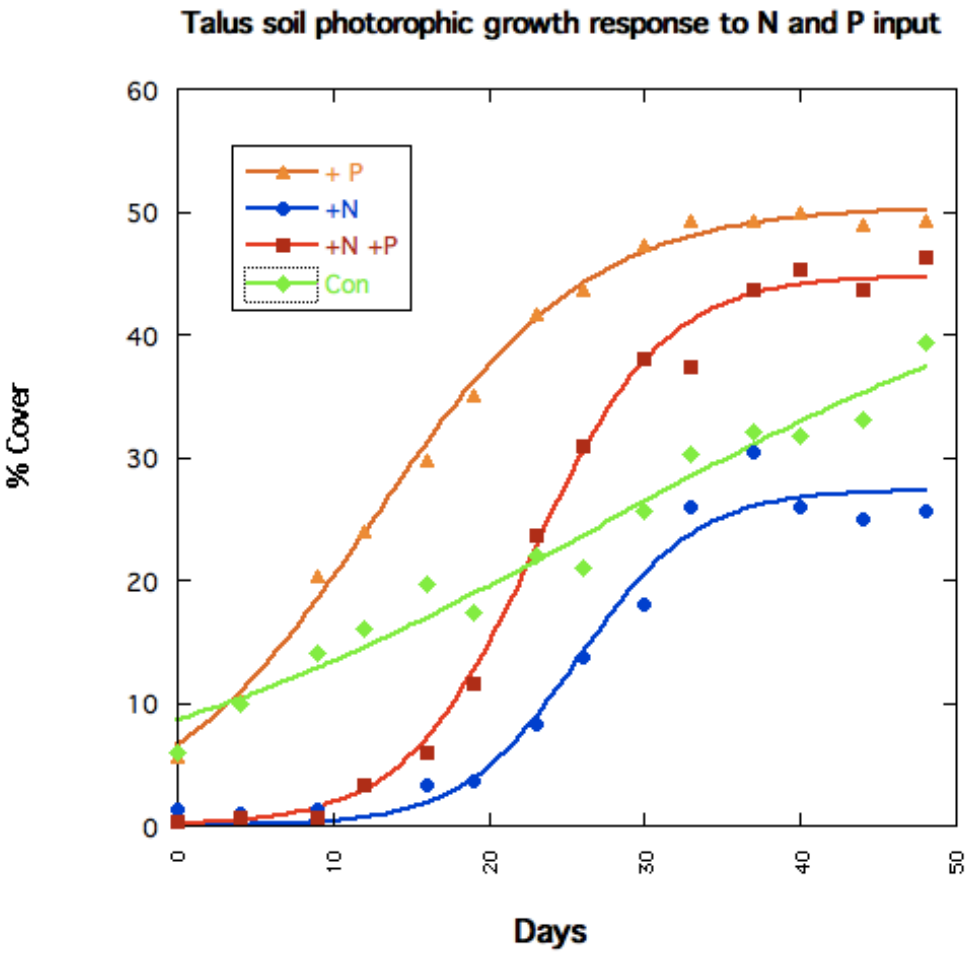

Figure 3: Average growth response of phototrophs to N and P inputs. The control soils had the slowest average rates of growth and had a lower carrying capacity than P and NP treatments. N input had a lower carrying capacity a high rate of growth (0.246 % per day) compared to other treatments. P inputs had a higher average carrying capacity and a slower average rate of growth.

Figure 4:

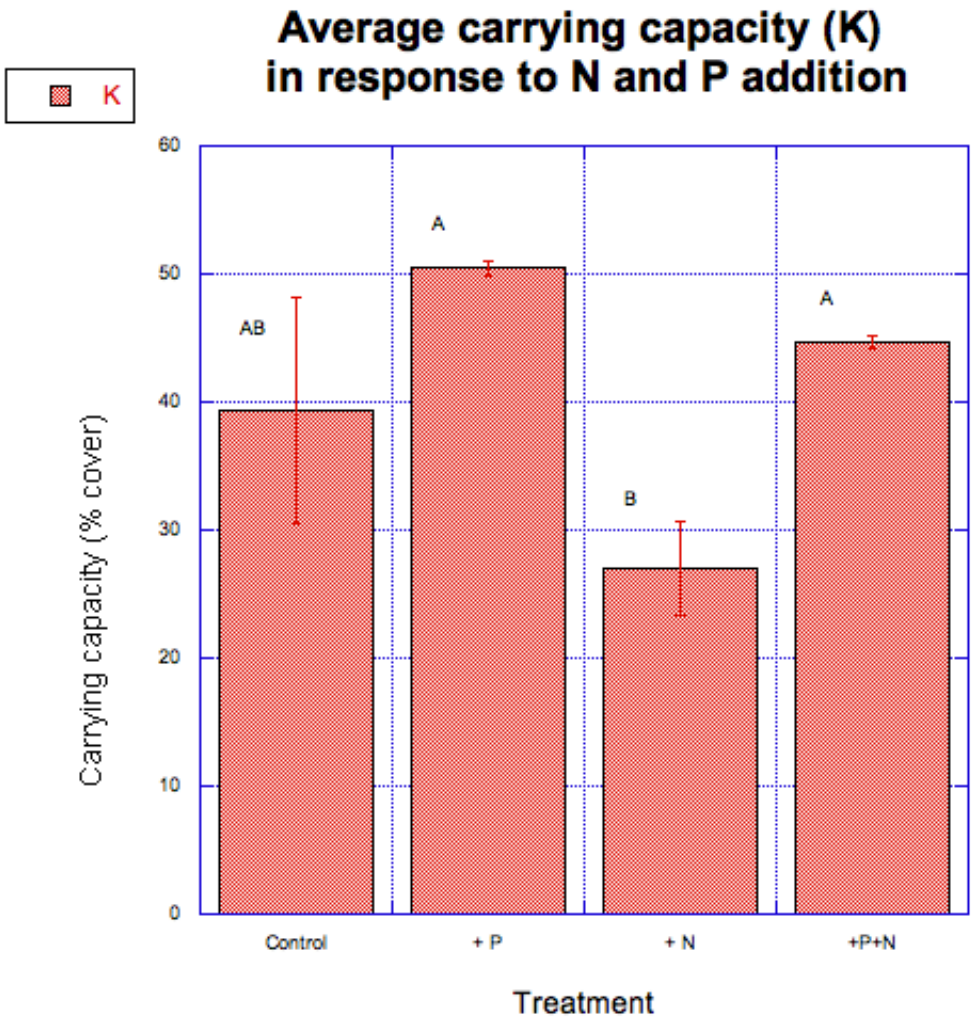

Figure 4: Visualizing the impact of N and P on carrying capacity. Treatments with P had the highest carrying capacity values while N plates had a significantly lower carrying capacity than P (p=0.007) and NP (p=0.03).

Figure 5:

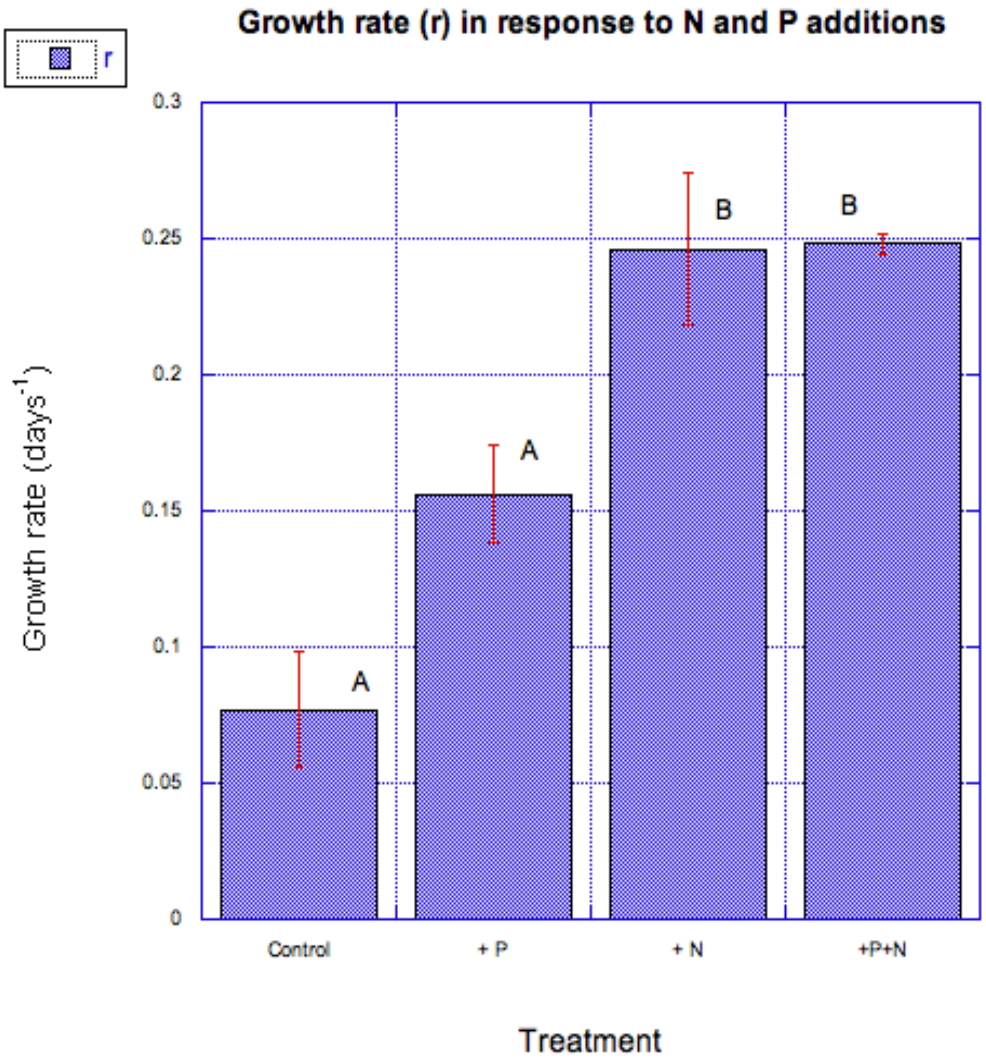

Figure 5: A representation of the average growth rates of talus soil phototrophs in response to nutrient inputs with N and P. N and NP treatments had significantly higher rates of growth than the control and P treatments ($p=0.04$).

References

Bowman, W.D., D.M. Cairns, J.S. Baron and T.R. Seastedt. 2002. Islands in the sky: Alpine and treeline ecosystems of the Rockies. 183-202 in Baron JS, ed. Rocky mountain futures: An ecological perspective. Washington DC: Island Press.

Fierer, N. and R.B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, 103: 626-631.

Fierer, N., M.A. Bradford, and R.B. Jackson. 2007. Toward an ecological classification of soil bacteria. *Ecology,* 88(6): 1354-1364.

Freeman, K.R., M.Y. Pescador, S.C. Reed, E.K. Costello, M.S. Robeson and S.K. Schmidt. 2009. Soil CO_2 flux and photoautotrophic community composition in high-elevation, "barren" soils. *Environmental Microbiology* 11: 674-686.

King, A.J., A.F. Meyer, and S.K. Schmidt. 2008. High levels of microbial biomass and activity in unvegetated tropical and temperate alpine soils. *Soil Biology and Biochemistry*, 40(10): 2605-2610.

King, A.J., K.R. Freeman, K.F. McCormick, R.C. Lynch, C.A. Lozupone, R. Knight, and S.K. Schmidt. 2010. Biogeography and habitat modeling of high-alpine bacteria. *Nature Communications,* 1:53. DOI: 10.1038/ncomms1055.

Ley, R.E., M.W. Williams, and S.K. Schmidt. 2004. Microbial population dynamics in an extreme environment: Controlling factors in talus soils at 3750 m in the Colorado Rocky Mountains. *Biogeochemistry*, 68: 313–335.

MacArthur, R. and E.O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA.

McKnight, D.M., C. Miller, R.L. Smith, J.A. Baron and S.A. Spaulding. 1988. Phytoplankton populations in lakes in Loch Vale, Rocky Mountain National Park, Colorado; Sensitivity to acidic condition and nutrient enrichment. Denver (CO): US Geological Survey. Water-Resources Investigations Report 88-4115.

Nemergut, D. R., A.R. Townsend, S.R. Sattin, K.R. Freeman, N. Fierer, J.C. Neff, W. D. Bowman, C.W. Schadt, M.N. Weintraub and S.K. Schmidt. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: Implications for carbon and nitrogen cycling. *Environmental Microbiology,* 10(11): 3093-3105.

Nemergut, D.R., C.C. Cleveland, W.R. Wieder, C.L. Washenberger and A.R. Townsend. 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biology and Biochemistry*, 42(12): 2153-2160.

Pianka, E. 1970. On r- and K-selection. *American Naturalist*, 104: 592-597.

Pace, N.R. 1997. A molecular view of microbial diversity and the biosphere. *Science,* 276: 734-739.

Ramirez, K.S., C.L. Lauber, R. Knight, M.A. Bradford, and N. Fierer. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology,* 91: 3463-3470.

Seastedt, T.R., W.D. Bowman, T.N. Caine, D. McKnight, A. Townsend, and M.W. Williams. 2004. The landscape continuum: A model for high-elevation ecosystems. *Bioscience,* 54(2): 111-121.

Schmidt, S.K., E.K. Costello, D.R. Nemergut, C.C. Cleveland, S.C. Reed, M.N. Weintraub, A.F. Meyer and A.M. Martin. 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology*, 88(6): 1379-1385.

Schmidt S.K., D.R. Nemergut, A.E. Miller, K.R. Freeman, A.J. King, A. Seimon. 2009. Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. *Extremophiles* 13: 807-816.

Schmidt, S.K., C.C. Cleveland, D.R. Nemergut, S.C. Reed, A.J. King and P. Sowell. 2011. Estimating phosphorus availability for microbial growth in an emerging landscape. *Geoderma* 163: 135-140.

Townsend, A.R. and R.W. Howarth. 2010. Fixing the Nitrogen Problem. *Scientific American*, Feb 2010, pp 64-71.

Van der Heijden, M.G.A., R.D. Bardgett and N.M. van Straalen. 2008. *Ecology Letters* 11: 296-310.

Williams, M.W., P.D. Brooks, A. Mosier and K.A. Tonnessen. 1996. Mineral nitrogen transformations in and under seasonal snow in a high-elevation catchment in the Rocky Mountains, United States. *Water Resources Research*, 32(10): 3161-3171.

Weintraub, M.N., L.E. Scott-Denton, S.K. Schmidt, and R.K. Monson. 2007. The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon and nutrient availability in a subalpine forest ecosystem. *Oceologia*, 154: 327-338.

Woese, C.R. and G.E. Fox. 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *PNAS,* 74: 5088-5090.