Nutrient Status in Alpine Soils of the Colorado Front Range Using the Nitrogen/Phosphorus Ratio Index

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The suitability of the foliar N/P ratio was evaluated as a predictor of nutrient limitation in an alpine ecosystem of the Colorado Front Range. We hypothesized that foliar N/P ratios are directly correlated with the alpine soil nutrient status. We used a long-term fertilization experiment conducted in three alpine plant communities, where 48 plots were established consisting of four replicates of control, N, P, and N + P additions. We characterized four extractable P fractions, maximum P sorption capacity, and extractable NO_3–N and NH_4–N, measured N and P adsorption fluxes, determined the soil moisture content, and measured the total N and P concentrations in soils and below- and aboveground plant material. The fertilized plots exhibited significantly higher nutrient concentrations in above- and belowground plant materials and adsorption fluxes of N and P ($P < 0.001$ for all additions) than the control plots. The N-added plots were significantly acidified (<3.8) vs. the control plots (>4.14), which may have partially contributed to increased P flux. A correlation analysis showed that N and P accumulations in aboveground plant material could not be predicted by the traditional extractable N and P tests. Nutrient adsorption flux measurements and especially P showed better correlation with nutrient accumulation in below- and aboveground plant material ($r = 0.53$ and 0.43, respectively, $P < 0.001$). This moderate correlation suggested, however, that the N/P ratio index is somewhat limited in providing a definitive answer for the nutrient limitation status of this alpine ecosystem.

Abbreviations: PSI, phosphate sorption isotherm; WSP, water-soluble phosphorus.

Most terrestrial ecosystems are N and P limited, which directly affects plant community composition and plant growth (Chapin et al., 2002; Aerts and Chapin, 2000). Measurement of foliar N/P ratios has been suggested as a method for estimating which of the nutrients most limits plant growth (Vitousek and Howarth, 1991; Vitousek et al., 1988; Aerts and Chapin, 2000). Considerable attention to the N/P ratio technique was generated by Koerselman and Meuleman (1996), who postulated that at the community level the response to an addition of the limiting nutrient is an increase in total primary production. They have tested this response by using an extensive literature survey and determining whether a given ecosystem was limited by either N or P, or was N + P colimited. A similar approach has been used in other terrestrial ecosystems with various degrees of success (e.g., Corbin et al., 2003; Güsewell et al., 2003; Carreira et al., 1997).

A significant amount of research has been done in recent years in the alpine soil–plant milieu of Niwot Ridge, Colorado, focusing on the influence of nutrient availability on plant primary productivity, abundance, and species diversity. In a series of experiments, Bowman et al. (1993) found that N availability limited primary production in an alpine dry meadow community while N and P availability colimited production in a wet meadow community. In general, there was a greater production response to the N and N + P amendments in the dry meadow than the wet meadow. Seastedt and Vaccaro (2001) studied the influence of N and P limitations across a snowpack gradient and showed that the addition of either N or P enhanced alpine plant foliage productivity ($P = 0.05$ and $P \leq 0.03$, respectively). Phosphorus additions had the strongest single treatment effect on plant productivity in this experiment, but neither P nor N had a significant effect on species richness. More recently, Bowman et al. (2003) investigated the alpine landscape variation in the foliar N/P ratio and found that foliar N and P concentrations of three common species were poor indicators of soil N and P supply, as estimated by ion exchange resin bags. They presented several possible explanations for this lack of correlation, most notably the redistribution of nutrients between foliar tissues and belowground nutrient storage in plants and the use of organic N by plants. Similar results were reported by Soudzilovskaia et al. (2005), who found that N/P ratios were not useful in predicting biomass production and nutrient limitation in a Caucasian alpine tundra plant community. On the other hand, Güsewell and Koerselman (2002) found that although N/P ratios in vegetation were poorly correlated with various measures of nutrient availability in the soil, the effects of fertilization on the biomass production of wetland vegetation were well related to the N/P ratios in the foliar tissues and not to the nutrient concentrations,

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thus supporting the idea that N/P ratios, rather than N or P concentrations in plants, indicate the type of nutrient limitation.

A possible problem with the use of the N/P ratio as an indicator of soil nutrient deficiency is the lack of a clear definition for plant-available soil P. This parameter is fully described by three factors, namely intensity, quantity, and capacity (White and Beckett, 1964). The intensity factor is the concentration of P in the soil solution, the quantity factor is the exchangeable desorbed phosphate following plant uptake, and the capacity factor is a ratio describing the change in the status of P in the soil system. This capacity factor is defined as the ratio of the change in sorbed P to the change in dissolved P and reflects the ability of the soil to buffer changes in solution P concentration following P fertilization or plant uptake (Kuo, 1990). None of the recent studies that examined the relationships between the N/P ratio and available soil P quantified the many possible forms of plant-available P. Hence, the lack of correlation between the foliar N/P ratio and alpine soil nutrient supply calls for more systematic evaluation of the various forms of soil available P and the N/P ratio index in alpine soils. We hypothesized that foliar N/P ratios are directly correlated with the alpine soil nutrient status. To test this hypothesis, we used a long-term fertilization experiment and paid special attention to a wide array of N and P labile pools and adsorption fluxes for the purpose of correlating these soil nutrient indicators with the N/P ratios of the aboveground plant material.

MATERIAL AND METHODS

Field Site

The study was conducted in the Niwot Ridge saddle at an elevation of 3500 m, located in the Front Range of the Colorado Rocky Mountains about 5 km east of the Continental Divide (40°03′ N, 105°35′ W). This site is a UNESCO Biosphere Reserve and a Long-Term Ecological Research Network site. Climate is characterized by long, cool winters and a short growing season (1–3 mo). Since 1951, mean annual temperature is −3.8°C and annual precipitation is 1000 mm, as reported from a site located about 400 m above the current study site (Williams et al., 1996). The research site is referred to as *the saddle*, is located at ~3400 m in elevation, and represents a trough oriented along a north–south axis and becoming progressively steeper to the east and west (Fig. 1). Five alpine plant communities, including fellfield, dry meadow, moist meadow, wet meadow, and snow bed, dominate the snow depth–soil moisture gradient of the saddle (Walker et al., 1993). In the present work, we studied the three alpine plant communities strongly influenced by the snow depth–soil moisture gradient. The dry meadow is characterized by low snow cover and is dominated by *Kobresia myosuroides* (Vill.) Fiori, *Acomastylis rossii* (R. Br.) Greene, *Polygonum viviparum* (L.) Delarbre and various species of *Trifolium*. The moist meadow exhibits a snowpack of between 0.5 and 2 m of winter snow and is dominated by *Deschampsia cespitosa* (L.) P. Beauv. and *A. rossii*. The late-melting snow bed community exhibits variable snow cover, a shorter growing season, and is dominated by *D. cespitosa*, *Caltha leptosepala* DC., *Sibbaldia procumbens* L., and *Trifolium parryi* A. Gray. The soils of the study are Cryochrepts and are approximately 1 to 2.0 m in depth over granitic parent material (Burns and Tonkin, 1982).

Experiment Design

In 1993, 16 plots (2 by 2 m) were established in three of the plant community sites—dry meadow, moist meadow, and snow bed—for a total of 48 plots. The plots in the dry and moist meadows were placed as a completely randomized design but with irregular layout as was dictated by the field conditions (Fig. 1). The snow bed plots were also completely randomized but with a square layout of 2- by 2-m plots with a 1-m buffer strip between plots. The snow bed plots are located northwest of a snow fence, across a shrub field, on a mesic–moist meadow. The 16 plots in each community site consist of four replicates of N, P, and N + P additions as well as four control plots. Plots were initially heavily fertilized to overwhelm any microbial immobilization potentials. Nitrogen at a rate of 20 g N m^{-2} and P at a rate of 2 g m^{-2} were added in 1993, 1994, and 1996. Nitrogen was in the form of NH_4NO_3 (25% of all N added) or $(NH_4)_2SO_4$ (75% of all N added). Phosphorus was in the form of superphosphate (P_2O_5). Beginning in 1997 and thereafter, N additions, all in the form of NH_4NO_3, were reduced to 10 g N m^{-2} and P additions consisted of 1 g P m^{-2} as superphosphate. Fertilizer additions were made in mid-August toward the end of the flowering season (1997, 1998, 1999, 2001, and 2004); hence, the fertilization addition in the summer of 2004 did not affect the results reported here.

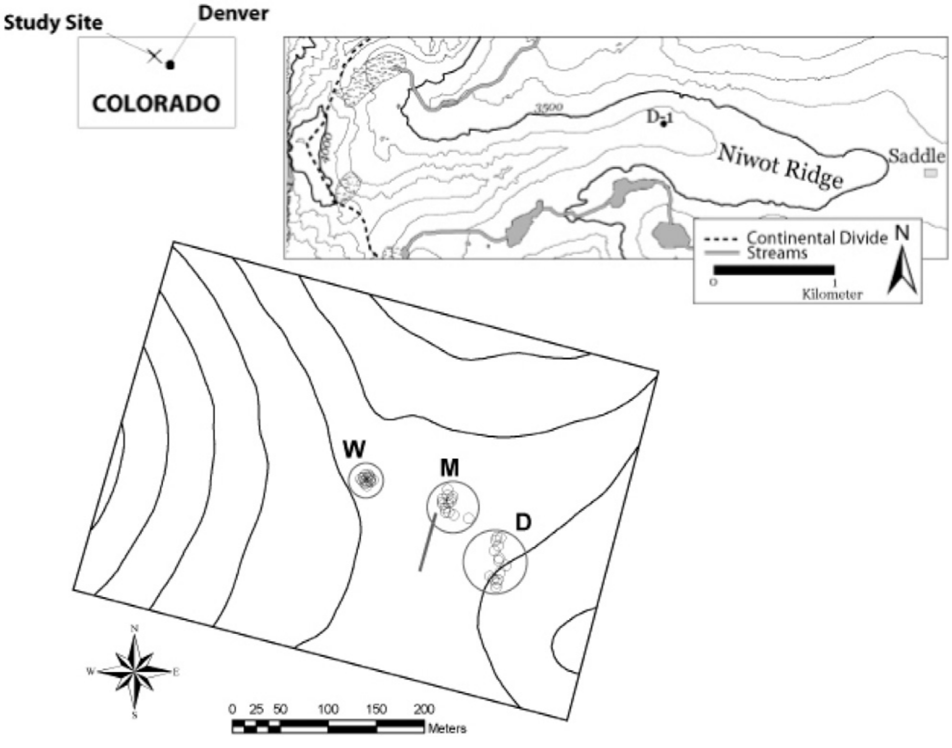

Fig. 1. The saddle study site at Niwot Ridge and the location of the experimental plots in the dry meadow (D), moist meadow (M), and snow bed (W) sites.

Soil and Plant Analyses

Soil samples were taken in October of 2003 after all the aboveground tissues of the alpine plants had senesced to the surface of the ground and the soils were in their driest natural state. Soils were collected at all plots from a depth of 0 to 15 cm, where the bulk of the plant roots extend. Because the soils were extremely dry during sampling and are frozen throughout most of the winter, while plant N uptake has normally ceased by September and does not commence until mid-June the following year (Monson et al., 2001), we assumed that soil N pools measured in October would be similar to the N pools available to the plants in the beginning of the growing season of the following year. All the soil samples were passed through a 2-mm sieve before laboratory analysis was conducted. Soil pH was determined in 1:10 soil/water suspensions using a glass electrode, organic matter content was determined by the dry combustion method (Nelson and Sommers, 1982), and bulk density was determined using intact aggregates by the clod method (Blake and Hartge, 1986). Soil moisture was measured at all plots twice a week during the growing season (June–August 2004), using the time-domain reflectometry (TDR) method (Topp et al., 1980). The TDR probes were inserted vertically through the top 15 cm of the soil, and soil moisture was measured as voltage waveforms using the portable TRASE system 6050X1 (SoilMoisture Equipment Corp., Santa Barbara, CA). Particle size distribution of the soil samples was determined using a low-angle laser diffraction particle-size analyzer (Malvern Instruments, Malvern, UK).

The available soil P was determined using destructive and nondestructive procedures. The destructive procedures included water-soluble P (WSP), readily desorbable P (RDP), and $NaHCO_3$ extractable P (Olsen P). The WSP is the simplest extraction procedure and provides a quick estimate of available P (Zhou et al., 2001; Sims, 2000); however, WSP could be problematic because it may not necessarily extract all forms of available P. The RDP method uses neutral salt solutions (0.01 mol L^{-1} $CaCl_2$) to measure the easily desorbed and released P. Olsen P is a widely used soil P test that uses a 0.5 mol L^{-1} $NaHCO_3$ solution to extract most available P including the fraction that normally is not extracted by the WSP and RDP procedures (McDowell and Sharpley, 2003). The extraction procedures for WSP, RDP, and Olsen P followed Sims (2000) and Self-Davis et al. (2000). Maximum P sorption in the alpine soils was evaluated using the phosphate sorption isotherm (PSI) technique described by Bache and Williams (1971).

A major disadvantage in using the destructive procedures described above is the possibility of extracting P that is otherwise stable and immobile. To circumvent this problem, we used the Fe-impregnated filter paper soil test (FeO P), which is a nondestructive procedure described by Chardon (2000). The Fe filters were prepared by immersing the filter papers (15-cm diam., Whatman no. 541) in an acidified $FeCl_3$ solution, followed by quickly plunging into a 2.7 mol L^{-1} NH_4OH solution to convert the $FeCl_3$ to Fe oxide. The filters were then cut into 2- by 10-cm strips. The filters were installed vertically on a weekly basis throughout the 2004 growing season (mid-June to late August) in each of the 48 plots for 48 h. Following the 48-h equilibration time, the probes were removed, rinsed thoroughly of adhering soil particles, and the adsorbed P was dissolved with 0.1 mol L^{-1} H_2SO_4. The Fe oxide filters were also used in conjunction with the RDP method as an added sink to prevent potential Ca-P precipitation during the extraction experiment.

The available NO_3–N and NH_4–N in the alpine soils were determined using a 2 mol L^{-1} KCl extracting solution (Keeney and Nelson, 1982). To estimate the amount of N available to the plants in a nondestructive fashion, we used plant root simulator probes (PRS, Western Ag Innovations, Saskatoon, SK, Canada), which are ion exchange resin in a membrane form that act more or less analogously to the withdrawing behavior of a plant root (Qian and Schoenau, 2002). These probes ensure a constant surface area and adequate contact with the soil. The PRS probes were converted to a bicarbonate form before installing in the soil. The probes were vertically inserted into the topsoil horizon on a weekly basis throughout the 2004 growing season in all 48 plots for a period of 48 h. Following the 48-h equilibration time, the probes were removed from the soils, washed with deionized water, and the nutrients were eluted from the probes using 0.5 mol L^{-1} HCl. The concentrations of inorganic N (NO_3^- and NO_2^-) eluted from the PRS probes represent the N adsorption flux across the PRS probe area during the 48-h insertion. The term *flux* is a nutrient concentration expressed per unit of contact area of the PRS (and the Fe-impregnated filter paper) per time of burial of the probe, thus representing a flux of the nutrient ion toward the resin (Qian and Schoenau, 2002). The concentrations of N and P in all the extractions were determined using a flow-injection colorimetric autoanalyzer (Lachat Instruments, Loveland, CO).

The accumulation of N and P in the alpine plants was determined from the leaves of at least 50 individual plant species per plant community collected in 1 d in peak season (mid-July to mid-August 2004). Belowground N and P accumulation was estimated by collecting 48 soil cores of 5-cm diam. by 15-cm depth from each plot. Cores were washed over a 0.5-mm sieve to remove the soil. Roots were sorted into live and dead fractions by visible criteria including the light color of young roots, the presence of a light-colored cortex in brown roots, and resistance to breaking following the procedure of Fisk et al. (1998). Sorted roots were washed and oven dried for 48 h at 60°C. Total N and total C in soils and plants were determined using micro-Dumas combustion on a Carlo-Erba (Milan, Italy) CHN analyzer. Total P in the soils and plants was determined using the $NClO_4$ digestion method described by Bender and Wood (2000). We calculated N/P ratios by dividing the N concentration by the P concentration (measured as the percentage by weight of each nutrient in the sample) within each plant tissue sample.

Statistical Analyses

The differences in soil nutrient concentrations were tested by ANOVA using a factorial design of three sites and four fertilization treatments in a randomized complete block design with four replications per site. The differences in nutrient concentrations in aboveground and belowground plant material were tested by comparing the four fertilization treatments with three different alpine plant species. The differences in adsorption fluxes were tested using a split-plot design where the sites and treatments were the main factors while the weekly adsorption flux measurements were considered replications. Where there were no significant interactions, specific differences among the main effects were explored using various multiple comparison tests with $\alpha = 0.05$. All statistical analyses were performed using SPSS Version 11 (SPSS, Inc., Chicago, IL). The evaluation of the source of difference in the interaction effect between the three sites and a treatment with four replicates each was performed by running a deviation contrast analysis. The contrast represents a linear combination of the site and treatment on the distribution of the nutrient availability. We tested the hypothesis that there are no statistical differences between the various site × treatment combinations, and computed the F statistics for each contrast as well as the Boneferroni-type simultaneous confidence intervals, which are

Table 1. Summary statistics (mean ± standard error of estimates) of selected physical and chemical soil properties (0–15 cm) collected in the saddle research site at Niwot Ridge, Colorado Front Range, in October 2003 (n = 16 per site).

Property	Dry meadow	Moist meadow	Snowbed
Sand, %	39.7 ± 3.0	45.5 ± 4.0	45.7 ± 3.0
Silt, %	34.0 ± 0.8	32.5 ± 1.5	34.4 ± 1.6
Clay, %	26.3 ± 2.0	22.0 ± 2.0	19.9 ± 2.0
Bulk density, g cm^{-3}	0.8 ± 0.06	0.9 ± 0.05	1.05 ± 0.06
Total C, g kg^{-1}	159 ± 10	174 ± 20	173 ± 20
Total N, g kg^{-1}	12 ± 0.5	13 ± 0.7	14 ± 0.7
Total P, mg kg^{-1}	121 ± 5.0	135 ± 5.0	131 ± 7.0
C/N ratio	12.9 ± 0.1	13.1 ± 0.13	12.6 ± 0.1
PSI†, mg kg^{-1}	550 ± 35	725 ± 40	715 ± 40

† Phosphate sorption isotherm.

based on Student's *t*-distribution. The correlation between the various N and P availability extractions, N and P accumulation in above- and belowground plant material, and the nutrient adsorption fluxes were conducted using Pearson correlation analysis.

RESULTS

General Attributes

There were no statistical differences in the concentrations of total soil C, particle size distribution, or bulk density among the three sites or the types of fertilizer applied (Table 1). The only general soil attribute that varied significantly between sites and treatments was pH. The soils in the snow bed community were significantly more acidic than the soils in the moist meadow, which in turn were more acidic than the soils of the dry meadow (Fig. 2). The sites that were fertilized with N and N + P were significantly more acidic (mean pH of 3.8 and 3.73, respectively, $P < 0.001$) than the control and P-added plots, which averaged 4.14 and 4.3, respectively.

The temporal distribution of the soil moisture content exhibited a clear drying pattern as the summer progressed, coupled with short intermittent episodes of rewetting following rainstorms (Fig. 3). All three alpine sites exhibited more than 30% moisture content in the beginning of the summer due to snowmelt. The moist meadow was significantly wetter in the early season because it receives additional snowmelt from a snowdrift created by a large snow fence experiment located just south of the moist meadow plots. As was expected, the *Kobresia*-dominated meadow was the driest locale among the three sites during the early season because of lack of snow. As the summer progressed and the relative contribution of snowmelt dwindled, the differences in soil moisture among the three sites correspondingly diminished (Fig. 3). By the end of the summer, the soil moisture content in all three sites was the same.

Nutrient Pattern

The concentrations of total N and total P in the soils did not vary significantly among sites or treatments (Tables 1 and 2). On the other hand, total P in the belowground plant material was significantly higher in the P and N + P plots ($F = 14$, $P < 0.001$), than in control and N plots. Similarly, the total N in the belowground plant material was significantly higher in the N and N + P plots ($F = 2.9$, $P < 0.05$) compared with the P and control plots. The common available P indices such as Olsen P

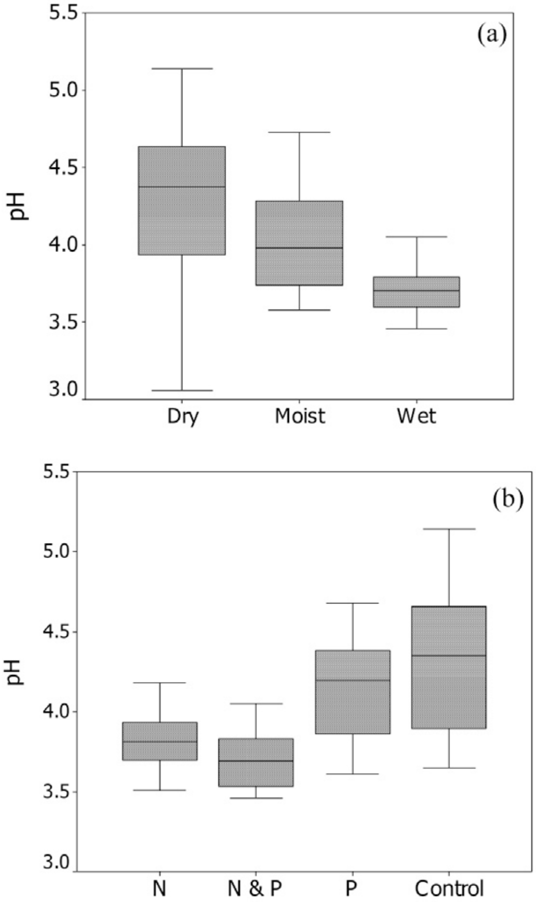

Fig. 2. Distribution of pH in the top soil horizons (upper 15 cm) across the saddle site at Niwot Ridge (a) according to site and (b) by treatments measured in October 2003. The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median.

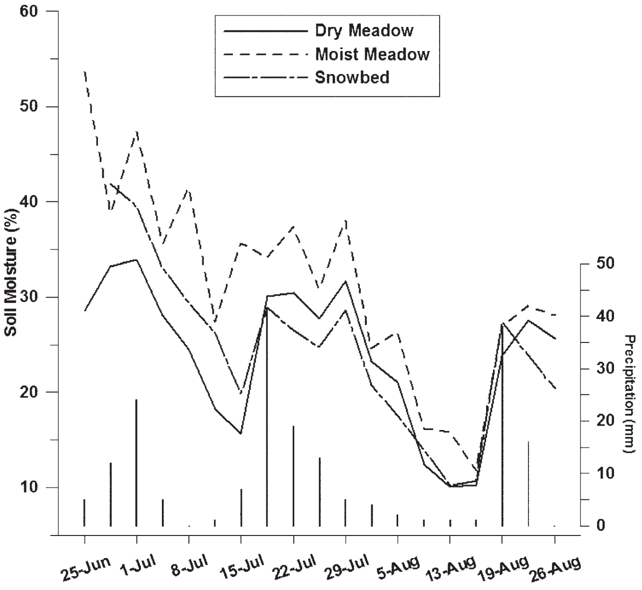

Fig. 3. The distribution of soil moisture content in the top soil horizons (upper 15 cm) across the saddle site at Niwot Ridge during the growing season, summer 2004. The vertical bars represent the daily accumulation of rain.

Table 2. Summary statistics (mean ± standard error of estimates) of nutrient concentrations in plant material and soils (0–15 cm) within a long-term fertilization experiment ($n = 16$) at Niwot Ridge, Colorado Front Range.

Nutrient	N treatment	N + P treatment	P treatment	Control
	g kg^{-1}			
Belowground plant†				
Total N	17 ± 0.8	17 ± 0.9	14 ± 1.4	13 ± 1
Total P	0.6 ± 0.04	0.8 ± 0.05	1.1 ± 0.09	0.7 ± 0.05
Aboveground plant				
Total N	34.6 ± 1.7	36.2 ± 2.0	27.4 ± 1.0	27.9 ± 1.1
Total P	1.9 ± 0.09	2.5 ± 0.14	2.5 ± 0.13	1.9 ± 0.07
Soils‡				
Total N	12 ± 0.6	13 ± 0.8	14 ± 0.9	13 ± 0.6
Total P	0.12 ± 0.006	0.13 ± 0.009	0.14 ± 0.009	0.12 ± 0.006

† Sampled during summer 2004.

‡ Sampled in October 2003.

Table 3. Summary statistics (mean ± standard error of estimates) of the concentrations of selected available P and N extracts in the various treated soils (0–15 cm) collected in October 2003 ($n = 16$) at Niwot Ridge, Colorado Front Range.

Extract†	N treatment	N + P treatment	P treatment	Control
	g kg^{-1}			
WSP	4.1 ± 0.1	3.7 ± 0.2	3.7 ± 0.3	4.5 ± 0.2
RDP	1.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	2.3 ± 1.1
Olsen P	16 ± 1.3	17 ± 2	23 ± 2	22 ± 3
FeO P	26 ± 2	24 ± 2	36 ± 3	31 ± 4
NH_4–N	20 ± 6	19 ± 3	26 ± 9	27 ± 11
NO_3–N	7 ± 1	8 ± 3	15 ± 5	19 ± 5

† WSP, water-soluble P; RDP, readily desorbable P extracted with 0.01 mol L^{-1} $CaCl_2$ solution; FeO P, P extracted with 0.01 mol L^{-1} $CaCl_2$ solution coupled with FeO-impregnated filter paper.

Table 4. Summary statistics (mean ± standard error of estimates) of nutrient adsorption fluxes measured by resin membranes during summer 2004. The number of adsorption flux measurements per each cell (treatment per site) varied from 41 to 44. Some probes failed or were destroyed by the weather or animal activity.

Treatment	Dry meadow	Moist meadow	Snowbed
		μg m^{-2}	
		N adsorption flux	
N	236 ± 30	260 ± 30	220 ± 30
N & P	50 ± 30	265 ± 20	100 ± 30
P	25 ± 30	12 ± 20	20 ± 30
Control	17 ± 20	20 ± 30	14 ± 30
		P adsorption flux	
N	7 ± 2	11 ± 2	9 ± 2
N & P	11 ± 2	16 ± 2	24 ± 2
P	16 ± 2	15 ± 2	16 ± 2
Control	5 ± 2	8 ± 2	7 ± 2

and FeO P were higher in the P-fertilized plots (Table 3) but no significant differences in available P extracts existed among the three sites in spite of large differences in sorption capacities as measured by the PSI (550 mg kg^{-1} in the dry meadow vs. >700 mg kg^{-1} in the wetter sites, $F = 5.6$, $P < 0.001$). The differences in sorption capacity among the sites were attributed to higher sesquioxide concentrations in the wetter sites (Litaor et al., 2005).

Somewhat surprisingly, the N and N + P plots exhibited significantly lower concentrations of extractable NH_4–N and NO_3–N compared with the P and control sites ($F = 4.1$, $P < 0.01$). This may result, however, from the higher levels of total N found in the above- and belowground plant material of these plots depleting soil N pools (Table 2). These findings indicate that alpine plants are efficiently utilizing the added nutrients, thus the level of inorganic nutrient accumulation in the soils due to long-term additions of N + P was negligible.

The magnitude of the nutrient adsorption flux was more related to the type of treatment than the site (Table 4). The results of the ANOVA indicated that the N adsorption fluxes were significantly affected by the interactions between site and treatment (Table 5). The N adsorption fluxes were always higher at the N plots followed by the N + P plots in the wetter sites throughout the entire sampling period. The P adsorption fluxes were significantly higher at the P and N + P plots regardless of site during almost the entire sampling campaign.

Nitrogen/Phosphorus Ratio Index

The evaluation of the N/P ratio to assess nutrient availability and limitation was mainly derived from the aboveground plant analysis. The results of the ANOVA showed that the N concentrations in the aboveground plant material did not change significantly among the three alpine sites but there was significant change with treatment and plant species (Table 5). In addition, the ANOVA showed significant interactions between treatment and plant species. A closer examination of this interaction showed that the mean N concentrations in *Polygonum* collected from the N and N + P plots was above 4% and was significantly higher than N concentrations in *Kobresia* and *Accomastylis* in all treatment types (Fig. 4). These results were in accordance with our N adsorption flux measurements, which showed that the N adsorption flux in the N-fertilized plots was significantly higher than in P and control plots (Table 4).

Similarly, the P concentrations in the aboveground material did not vary significantly with site but was greatly affected by treatment and plant species (Table 5). The mean P concentrations in *Polygonum* sampled in the N + P and P treatments exceeded 0.3% and were significantly higher than P concentrations in *Kobresia* and *Accomastylis* (Fig. 5).

The N and P concentrations in the aboveground plant material were used to compute the N/P ratio index for the three alpine sites (Fig. 6). The N/P ratios did not vary significantly between sites but differed considerably by treatment ($F = 65$, $P < 0.001$). The highest N/P ratios were found in the N-added plots (17.3–19) while the P-added plots exhibited the lowest N/P ratio excepting the dry meadow site (Fig. 6). This pattern agrees rather well with our basic assumptions of the research hypothesis, which stated that N/P ratios would increase if N addition rates were experimentally increased and conversely to experimental additions of P. The control and N + P sites showed fairly similar N/P ratios.

A correlation matrix between the various extractable P and N attributes exhibited complex relationships (Table 6). Total

Table 5. Results (*F* values and significance) of the ANOVA testing the effects of site and treatment for N and P adsorption fluxes and the effects of treatment and plant species for nutrient concentrations in the aboveground plant material at a long-term fertilization experiment at Niwot Ridge, Colorado Front Range, 2003.

Source	df	*F* N	*F* P
Nutrient adsorption fluxes			
Site	2	4.8**	2.3*
Treatment	3	42.2***	9.4***
Site × treatment	6	3.8***	1.25
Error	475		
Nutrient concentrations in aboveground plant material			
Treatment	3	25.1***	24.9***
Plant species	2	139.3***	59.3***
Treatment × plant species	6	5.0**	4.1**
Error	84		

* Significant at $P \le 0.05$.

** Significant at $P \le 0.01$.

*** Significant at $P \le 0.001$.

P in the soils was only moderately correlated with total soil C and N ($r = 0.57$ and 0.58, respectively, $P < 0.01$), indicating that, unlike N, some of the added P is accumulating in the soils in the inorganic phase. This finding is in accordance with an earlier study that found that the inorganic P fraction varied between 10 and 45% of total P across the alpine topographic–snow gradient of the saddle (Litaor et al., 2005). There was a high negative correlation ($r = -0.98$, $P < 0.001$) between the PSI and WSP fraction, while the correlation between the PSI and other P extractants decreased (Table 6). As was expected, the correlation analysis showed that total soil C and total soil N were highly correlated ($r = 0.98$, $P < 0.001$) while no correlation at all was found between these variables and extractable NH_4–N and NO_3–N. The total N in the belowground plant material was only moderately correlated with the N adsorption flux ($r = 0.36$, $P < 0.01$) but showed no correlations with the other extractable N variables.

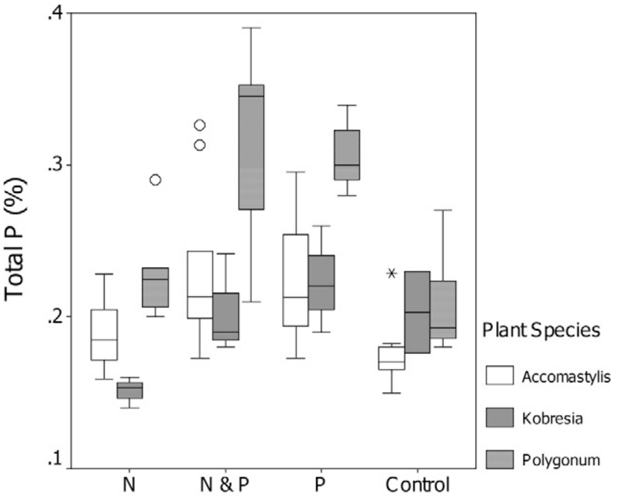

Fig. 5. The distribution of P concentrations in aboveground plant material, according to treatment and plant species, measured in the summer 2004. The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median, and the circles and asterisks are outliers and extreme values, respectively.

DISCUSSION

Koerselman and Meuleman (1996) suggested that N/P ratios below 14 indicate N limitation, ratios higher than 16 indicate P limitation, and ratios between 14 and 16 indicate N and P colimitation. According to the N/P ratio index depicted in Fig. 6, all the N-added plots are P limited, all the P-added plots are N limited, and the N + P plots are mostly colimited. The N/P ratio index indicated that the control plots in the dry and

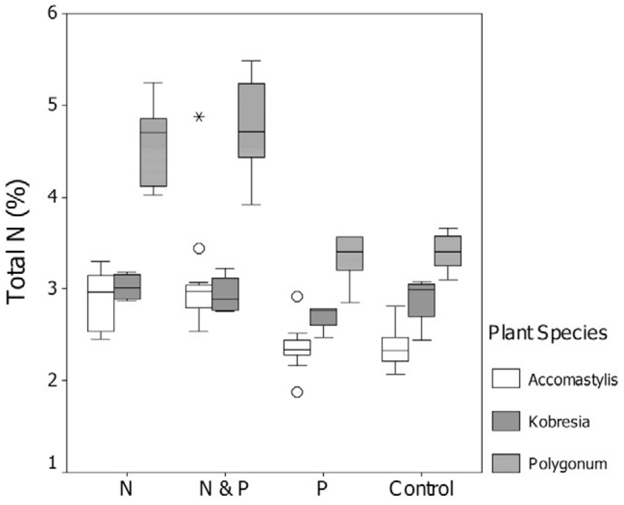

Fig. 4. The distribution of N concentrations in aboveground plant material, according to treatment and plant species, measured in summer 2004. The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median, and the circles and asterisks are outliers and extreme values, respectively.

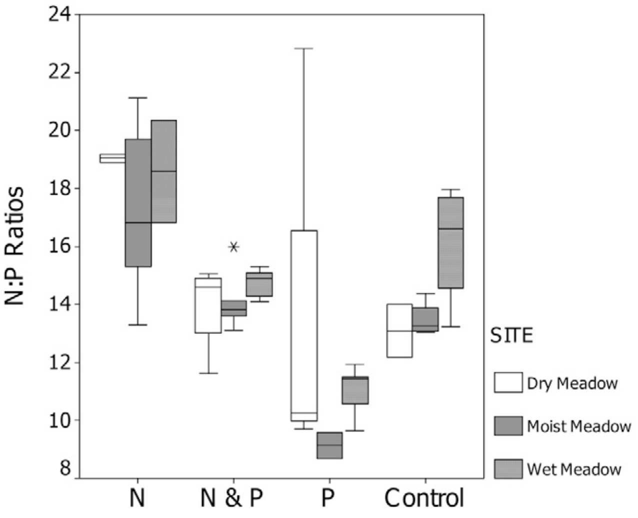

Fig. 6. The N/P ratio distribution using pooled plant species (*Acomastylis rossii*, *Kobresia*, and *Polygonum*) sampled by site and treatment (*n* = 48) during summer 2004. The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median, and the circles and asterisks are outliers and extreme values, respectively.

Table 6. Correlation matrix of selected soil (0–15 cm) P extractants, fluxes, and above- and belowground plant material from a long-term fertilizer experiment at Niwot Ridge, Colorado Front Range.

Parameter†	WSP	RDP	Olsen P	FeO P	PSI	N_{APM}	P_{APM}	P flux	N flux	N_{BPM}	P_{BPM}
WSP	1	0.62**	0.46**	0.53**	−0.98**	0.03	−0.03	−0.36*	−0.28*	−0.20	−0.21
RDP		1	0.71**	0.61**	−0.60**	0.11	−0.12	−0.27	−0.18	−0.19	−0.23
Olsen P			1	0.88**	−0.46**	0.15	0.13	−0.19	−0.25	−0.24	0.17
FeO P				1	−0.52**	0.04	0.07	−0.29*	−0.24	−0.37	0.18
PSI					1	0.01	0.05	0.37*	0.32*	0.23	0.25
N_{APM}						1	0.08	0.24	0.36	0.84**	0.13
P_{APM}							1	0.43**	−0.34	0.13	0.80**
P flux								1	0.11	0.42*	0.53**
N flux									1	0.28*	−0.03
N_{BPM}										1	0.26
P_{BPM}											1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.001 level (2-tailed).

† WSP, water-soluble P; RDP, readily desorbable P extracted with 0.01 mol L^{-1} $CaCl_2$ solution; FeO P, P extracted with 0.01 mol L^{-1} $CaCl_2$ solution coupled with FeO-impregnated filter paper; PSI, phosphate sorption isotherm; APM, aboveground plant material; BPM, belowground plant material.

moist meadows are N limited while the control plots in the snow bed community are N and P colimited. The results for the control plots are somewhat surprising since the entire area has been considered N enriched due to atmospheric N deposition, which should push the alpine ecosystem toward P-limited conditions. In an earlier investigation using exactly the same plots of the current study, Seastedt and Vaccaro (2001) found that the N + P treatment plots in both dry and moist meadows exhibited significantly higher foliage production than the other plots but no increase in foliage production was observed in the snow bed community. When they pooled their data together across all sites, however, N additions increased the plant productivity to 335 g m^{-2} in N-added plots vs. 269 g m^{-2} in plots not receiving N, while P additions increased the alpine plant productivity to 341 g m^{-2} vs. 263 g m^{-2} in plots not receiving P. Other alpine plant studies have demonstrated a similar pattern; for example, Bowman et al. (2006) found a very modest aboveground biomass gain occurring mostly in subdominant plant species during 8 yr of a N fertilization study of a dry meadow located in the saddle area. Soudzilovskaia et al. (2005) reported no correlation between total alpine plant community biomass and N additions in the Caucasus Mountains. The results of Seastedt and Vaccaro (2001) may have been obtained before the current ratios emerged, but in any event these are not in accordance with the N/P ratio if one accepts the hypothesis that this ratio is a reliable index of nutrient availability and limitation.

The complete lack of correlation between total N in below- and aboveground plant material and the inorganic available N fractions in soils suggests that most of the N in this alpine soil system is bound to the organic fraction, as reported by Bowman et al. (2003), or that the system is saturated with respect to inorganic N and its excess is being transported to streams, as suggested by Williams et al. (1996). The high correlation between N and P concentrations in below- and aboveground plant material (Table 6) suggests that the N/P ratio index commonly determined from aboveground plant material also represents the belowground nutrient status of the alpine plants. Moreover, the overall distribution of the nutrient concentrations in soils and belowground plant material and the magnitude and distribution of the nutrient fluxes observed in this experiment suggest that most of the added N and P are accumulating in the belowground plant material as live and dead organic matter rather than in the soil itself, supporting the luxury consumption mechanism used by Bowman (1994) and Bowman et al. (1995). Luxury consumption refers to the ability of the alpine plants to take up nutrients in excess of the amount needed for growth. This process occurs only at relatively high rates of nutrient supply, while foliar nutrient concentrations would increase only after belowground storage pools became saturated. For example, Lipson et al. (1996) found that rhizome N concentrations accounted for all of the luxury consumption in fertilized relative to control *Polygonum* plants, and that after fertilization, 100% of the plant demand was met using belowground storage reserves. Similar results were found in the current work with higher uptake of nutrients by *Polygonum* in the N and especially in the N + P plots that was transformed into large leaves and a luxuriant appearance that was quite evident in the field.

The high negative correlation between the PSI and the WSP fraction is explained by the high dependency of WSP on the sorption–desorption mechanism. On the other hand, the correlation between the PSI and other P extractants decreased with increasing extractant strength. The stronger extracts released less easily labile P fractions (Tiessen and Moir, 1993), which are unaffected by the sorption capacity of the soil. The high correlation between vastly different P extraction methods (i.e., FeO P and Olsen P, see Table 6) suggests that both techniques extract a similar fraction from the labile P pool of the soils. None of the different P extractions, however, were correlated with P concentrations in below- and aboveground plant material. On the other hand, we found a moderate correlation between the P adsorption flux and P accumulation in below- and aboveground plant material and low to moderate correlation between N adsorption flux and N accumulation in below- and aboveground plant material (Table 6). The lack of a high correlation between extractable nutrient tests and nutrient accumulation in plants was previously attributed to the fact that these tests only measure instantaneous nutrient availability, which fluctuates strongly in space and time and may or may not correspond with the chemical fractions actually available to plants (Verhoeven et al., 1988; Perez-Corona et al., 1996).

Bowman et al. (2003) estimated the rates of soil N and P supply using ion exchange resin bags deployed during the first half of the growing season, when the majority of plant nutrient uptake occurs. They found no correlation between foliar N and P concentrations with rates of soil N and P supply. These were the reasons we performed extractable nutrient tests and measured nutrient fluxes on a weekly basis to capture the available nutrient pools and the temporal variation in nutrient concentrations during the short growing season of this alpine ecosystem. The nutrient fluxes, however, were only moderately correlated with the nutrient concentrations in below- and aboveground plant material, suggesting that the N/P ratio index is somewhat limited in providing a definitive answer for the nutrient limitation status of this alpine ecosystem.

CONCLUSIONS

The hypothesis that foliar N/P ratios are directly correlated with the alpine soil nutrient status was tested using a long-term fertilization experiment and experimental testing of a wide array of N and P labile pools and adsorption fluxes. We found that the highest N/P ratios were observed in the N-added plots, while the P-added plots exhibited the lowest N/P ratios. The N and P adsorption fluxes were significantly higher throughout the growing season at the N- and P-added plots, but no accumulation of N and P was observed in the soils, indicating that alpine plants are efficiently utilizing the added nutrients. Support for this conclusion was found in the high correlation between N and P concentrations in the below- and aboveground plant material. The nutrient adsorption fluxes, however, were only moderately correlated with the nutrient concentrations in below- and aboveground plant material, suggesting that the N/P ratio index is somewhat limited in providing a definitive answer for the nutrient limitation status of this alpine ecosystem.

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