DINITROGEN FIXATION IN ALPINE TUNDRA, NIWOT RIDGE, FRONT RANGE, COLORADO, U.S.A.

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ABSTRACT

The process of dinitrogen fixation was studied in the alpine tundra ecosystem at Niwot Ridge, Colorado. In situ fixation activities were measured by the acetylene reduction method in the soils of the major plant community habitats found on the ridge: dry *Trifolium* fellfield, dry *Kobresia* meadow, moist *Deschampsia* meadow, wet *Caltha* meadow, moist *Sibbaldia* snowbed, and moist *Salix* shrub tundras. The highest overall acetylene reduction rates are found in the *Caltha* meadow and *Salix* shrub tundra sites (5.8 to 7.8 μmol ethylene produced in m^{-2} h^{-1}), while the lowest activities are found in the soils of the xeric sites, *Kobresia* meadow and fellfield tundras (0.13 to 0.33 μmol ethylene m^{-2} h^{-1}). The mean 1980 (summer season) acetylene reduction rate for all sites is 1.4 μmol ethylene m^{-2} h^{-1}. Soil moisture is the primary environmental factor influencing in situ rates of acetylene reduction in this alpine system. Acetylene reduction activity was found associated with the vascular plants *Dryas octopetala* L. ssp. *hookeriana* and *Trifolium dasyphyllum* T. & G. (0.13 to 5.4 μmol ethylene m^{-2} h^{-1}), and the lichens *Peltigera aphthosa* and *Stereocaulon alpinum* (0.05 to 0.42 μmol ethylene g^{-1} h^{-1}). Low activity associated with the moss *Pohlia* is attributed to epiphytic cyanobacteria. The contribution of nitrogen by dinitrogen fixation, estimated to be 5 mg N m^{-2} annually, does not apparently constitute the major source of nitrogen input to this tundra system.

INTRODUCTION

Significant progress has been made in recent years in our knowledge of the cycling of nutrients and their balance in tundra ecosystems, especially the nitrogen cycle in arctic tundra, as a result of the International Biological Programme (IBP) studies. The nitrogen supply and its cyclic transformations in soils are fundamental to the fertility of any environment and have a direct influence on its biological productivity. In cold-dominated tundras, "new" nitrogen input via dinitrogen fixation (the bacterial conversion of atmospheric N_2 to NH_4^+) is perhaps more critical to productivity than in any other terrestrial system.

A low annual net productivity is a definitive charac-

teristic of tundra vegetation (Webber, 1974). Boyd (1958) and Haag (1974) both concluded that the limitation on primary production by the low available nitrogen levels in tundra communities is largely the result of low soil temperatures which act to restrict organic decomposition and microbial transformation of nitrogen; i.e., a low recycling rate rather than the total nitrogen supply itself limits production. In this respect then, input by dinitrogen fixation becomes significant to the available nitrogen supply and the total nitrogen economy of the tundra system.

Results from the IBP studies on the nitrogen cycle have established the importance of the contribution of dinitrogen fixation to the nitrogen economy of tundra ecosystems. In the arctic coastal tundra at Barrow, Alaska (Barsdate and Alexander, 1975; Alexander et al., 1978),

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dinitrogen fixation is an active process and is the major source of annual nitrogen input to that system, representing 75% of the total annual input. Similar conditions are thought to exist in subarctic alpine tundras as well (Alexander and Schell, 1973; Alexander, 1974).

At Barrow and other circumpolar IBP sites, moisture, temperature, and solar radiation (amount and length of time) were found to be the major abiotic factors influencing both the distribution(s) of dinitrogen-fixing organisms and in situ rates of dinitrogen fixation (Alexander, 1974; Alexander et al., 1978). Fixation activities were highest in low wet areas such as polygon troughs and marshes having grasses and mosses as the predominant vegetation. Dry areas, such as high-centered polygons dominated by grasses and lichens, usually had the lowest rates of activity (Alexander et al., 1978).

The importance of cyanobacteria (blue-green algae), and their ability to fix dinitrogen has been demonstrated in the Antarctic (Horne, 1972), and in Arctic tundra regions during the IBP investigations. Included in the IBP surveys were arctic sites in Alaska and Devon Island, Canada, and subarctic Fennoscandian sites. At the majority of these sites the dominant dinitrogen fixers (measured using acetylene reduction method) were cyano-

bacteria, either free-living in surface layers and associated with mosses (e.g., *Anabaena, Nostoc*), or as symbionts in the lichens *Peltigera* and *Stereocaulon* (Alexander, 1974, 1975; Jordan et al., 1978). Similar findings are reported for the muskeg environment of James Bay, subarctic Canada (Blasco and Jordan, 1976). In the alpine tundra regions of Alaska, fixation activity was largely due to free-living and lichen genera, but significant activities were also found associated with vascular plants, the legume *Oxytropis* and the actinomycete-nodulated *Dryas octopetala* L. at these sites (Alexander and Schell, 1973; Alexander, 1974).

The major part of the U.S. IBP Tundra Biome research on dinitrogen fixation was conducted in the arctic tundra at Barrow. Alpine tundra sites in subarctic Alaska were surveyed, but on a less intensive scale. The purpose of this study was to investigate the dinitrogen fixation process in a dry continental or mid-latitudinal alpine tundra ecosystem with the following objectives in mind: (1) to determine in situ rates of fixation in the soils of representative plant community-types; (2) to identify organisms involved in the fixation process; and (3) to compare the results with those of similar studies from other tundra ecosystems.

MATERIALS AND METHODS

Description of the Study Area and Sites

The study area is a wind-swept tundra upland in the Indian Peaks region of the Front Range of the southern Rocky Mountains, Colorado (Figure 1). Niwot Ridge has been a study area of the U.S. IBP Tundra Biome Program: the main site is located in an area called the Saddle (40°3′N, 105°36′W, altitude 3650 m); (Webber and May, 1977; Komárková and Webber, 1978).

Periglacial features commonly occur on the ridge surface, over an igneous and metamorphic bedrock mainly of Precambrian age (Benedict, 1970; Gable and Madole, 1976). Soils are predominantly acidic (pH 4.5 to 6), coarse

textured and well drained, with thin organic-rich surface layers (Marr, 1967; Webber and May, 1977). Permafrost is too deep and sporadic to influence the vegetation (Ives and Fahey, 1971). Average length of the growing season is 90 d (Barry, 1973).

Xeric fellfield communities characterized by *Trifolium dasyphyllum* and *Selaginella densa* account for some 40% of the vegetation on the ridge, while dry meadows dominated by *Kobresia myosuroides* cover about 20%. The remainder of the vegetation is composed of moist to wet meadows, snowbank, and shrub tundra communities (Komárková and Webber, 1978). Lichens and mosses are
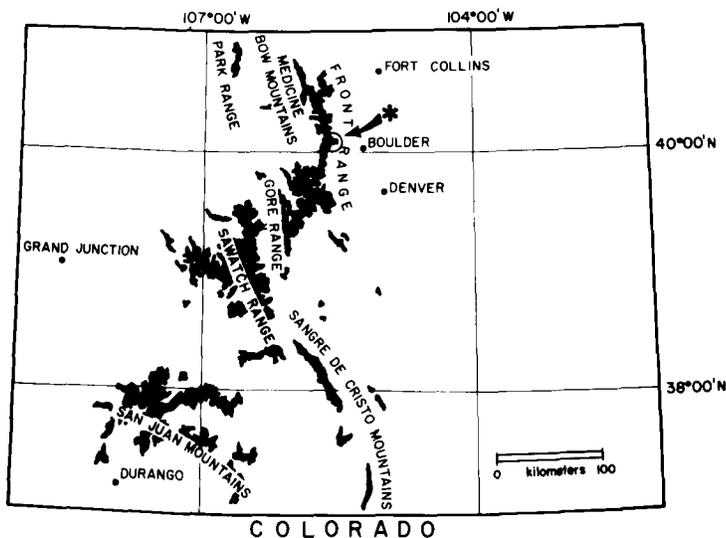

FIGURE 1. The location of Niwot Ridge (*) in the southern Rocky Mountains, Colorado (from Komárková and Webber, 1978).

never abundant in any community (Webber and May, 1977; Flock, 1978). The well-developed community types, largely determined by wind and snow distribution influencing the moisture supply, are typical of the regional alpine tundra environment (Marr, 1967).

Sites for dinitrogen fixation study were chosen in six vegetation community-types or vegetation noda (Table 1) that are representative of the major plant community habitats found at Niwot Ridge (Webber and May, 1977). All noda are found within the Saddle area, and have been described and mapped (Komárková, 1976; Komárková and Webber, 1978).

FIELD METHODS

Dinitrogen fixation activity was measured in situ by the acetylene reduction or acetylene-ethylene technique. This assay for dinitrogen fixation, although indirect, is based upon the ability of the nitrogenase enzyme complex to reduce the alternative substrate acetylene (C_2H_2) to ethylene (C_2H_4) (Stewart et al., 1967; Hardy et al., 1973). Field sampling for acetylene reduction (dinitrogen fixation) activity was conducted in each of the six nodal sites during the summers of 1979 and 1980. At each site soil cores (20 cm^2 × 5 cm) were taken at random from a designated 1-m^2 plot. The undisturbed cores were placed into incubation chambers constructed of modified glass jars (250 ml) with serum septa fixed into the lids to permit gas addition and sampling (Stutz and Bliss, 1973).

Acetylene was generated from calcium carbide (acetylene produced in this manner contained no ethylene detectable by gas chromatography) in the field and injected by syringe into the gas phase of each incubation chamber to adjust the internal atmosphere to 10% acetylene. The incubation jars were inverted (glass bottom up) and then submerged partially in water of ambient temperature (20-30°) at the soil surface to approximate the thermal regime of the surface. Five replicate cores were incubated in this manner to give the closest approximation of in situ fixation rates. One additional soil core per site was incubated as above but without the addition of acetylene as a control to determine the amount of ethylene produced endogenously by the soil microflora. A further control for establishing dinitrogen fixation activity, as discussed by Postgate (1972), utilizing inhibitors of the nitrogenase enzyme such as ammonium (NH_4^+), was not used in this study. In the usual low activity natural samples (in situ conditions), the occurrences of nonnitrogenase catalyzed acetylene reduction by either biological or nonbiological systems is rare if it can be detected at all. Thus, the use of a sample minus acetylene as a control (as we have done) is apparently sufficient to correct for errors introduced in this way (Hardy and Holsten, 1977). Following a 2- to 4-h incubation period, samples of the gas phase were collected into Vacutainer tubes for subsequent gas chromatographic analysis of the ethylene content. Incubation and sampling were conducted on each date between 0900 and 1500 h, when net solar radiation at the soil surface at Niwot Ridge is near maximal and least variable (LeDrew and Weller, 1978). Soil temperatures at the surface and at 10 cm belowground were measured for each nodal site at the time of gas sampling using standard soil thermometers.

Lichens and mosses were tested for acetylene reduction activity by the method of Crittenden (1975). Acetylene was injected to adjust the gas phase to 20%, and following a minimum 1-h incubation, gas samples were collected as above. Lichen and moss samples tested were dried to constant weight at 105°C. Vascular plants were tested for associated activity by the following method: plants were collected as cores (20 cm^2), gently shaken to remove soil, and treated as soil cores as above.

GAS CHROMATOGRAPHIC METHODS

Ethylene production from acetylene was determined using a Tracor 550 gas chromatograph equipped with flame-ionization detectors. Hydrocarbon separation was achieved using a 1.2 m × 3.1 mm (ID) glass column packed with 80/100 mesh Porapak N (Waters Associates).

TABLE 1

Vegetation noda designated as study sites for dinitrogen fixation

Site	Nodum description[a]
I. dry *Trifolium* fellfield	Exposed xeric fellfield communities dominated by *Trifolium dasyphyllum, Silene acaulis,* and *Carex rupestris;* snow-free period more than 200 d.
II. dry *Kobresia* meadow[b]	Moderately dry sedge meadow dominated by *Kobresia myosuroides, Selaginella densa,* and *Acomastylis rossii,* snow-free period between 150 and 200 d.
III. moist *Deschampsia* meadow[b]	Moist meadow dominated by *Acomastylis rossii* and *Deschampsia caespitosa,* snow-free period of 100 to 150 d.
IV. wet *Caltha* meadow	Wet meadow dominated by *Caltha leptosepala* and *Carex scopulorum,* snow-free period about 100 d.
V. moist *Sibbaldia* snowbed	Snowbank community (later-melting) dominated by *Sibbaldia procumbens* and *Carex pyrenaica,* snow-free period is less than 75 d.
VI. moist *Salix* shrub tundra	Moist willow shrub community dominated by *Salix planifolia* and *Salix villosa,* snow-free period between 100 to 150 d.

[a]From Komárková and Webber (1978).
[b]U.S. IBP Tundra Biome study site.

Gas samples were measured for ethylene content by gas chromatography within 48 h of collection in the field. Results are expressed in micromoles (μmol) and nanomoles (nmol) of ethylene produced. For comparison purposes, we have assumed the theoretical ratio of 3:1 for converting moles of acetylene reduced to moles of N_2 fixed, a ratio found valid for most natural soil samples (Hardy and Holsten, 1977; Alexander et al., 1978). Therefore, multiplying ethylene production data (in μmol) by 9.33 (28/3) will convert results reported in μmol ethylene produced to micrograms ammonia-N produced.

SOIL ANALYSES

Standard soil analyses were conducted on composite soil samples from each nodal site. Soil pH, inorganic nitrogen, and organic carbon were determined by the methods of Peech (1965), Bremner (1965a), and Allison (1965), respectively. Soil moisture (percent of dry weight) was measured for each nodal site on each sampling date (gravimetric method). Total nitrogen analyses, determined using the standard Kjeldahl method (Bremner, 1965b), were performed by the Weld County Agricultural Laboratory, Greeley, Colorado. All analyses were done in triplicate and averaged.

Primary isolation of dinitrogen-fixing cyanobacteria was achieved by transferring samples of soil and mosses to BG-11$_0$ liquid medium, a variant of BG-11 medium lacking combined nitrogen (Rippka et al., 1979). Subcultures were maintained under photoautotrophic conditions and supplemented with cycloheximide to eliminate contaminating eukaryotes (Zehnder and Hughes, 1958). Final isolates were identified by light and phase contrast microscopy according to the criteria of Rippka et al. (1979), and later tested for acetylene reduction ability by the method of Rippka and Waterbury (1977).

RESULTS

Our initial survey for dinitrogen fixation activity at Niwot Ridge in 1979 (Table 2) suggested in situ rates of acetylene reduction were higher in the moist to wet sites (III-VI) than in the dry fellfield and sedge meadow communities that are typical of the vegetation on the ridge. Rates ranged from 34.0 nmol to 2.48 μmol of ethylene produced m^{-2} h^{-1}, equivalent to a nitrogen input of 0.32 to 23.1 μg of nitrogen m^{-2} h^{-1}.

The 1980 data are shown (Figure 2) such that sampling date means and seasonal trends are evident. Based on these data, we conclude that acetylene reduction activity is highest (single sampling date and overall) in the moist *Salix* shrub tundra and *Caltha* meadow community-types (Figure 2a), and lowest in the dry *Kobresia* meadow and *Trifolium* fellfield tundra community-types (Figure 2c). Intermediate activities are found in the moist *Deschampsia* meadow and *Sibbaldia* snowbed tundra sites (Figure 2b). Rates for 1980 are generally greater than those from 1979 and ranged from 18.1 nmol to 7.79 μmol ethylene m^{-2} h^{-1}, which is equivalent to a nitrogen input of 0.17 to 72.7 μg nitrogen m^{-2} h^{-1}. Analysis of variance of the 1980 data indicates the rates of acetylene reduction in sites I-VI differed significantly ($p = 0.003$).

Acetylene reduction activities show a distinct maximum in June or early July, and decline through the remainder of the summer season. This pattern is most probably related to the seasonal distribution of soil moisture. In the alpine tundra at Niwot Ridge slowly melting snowbanks provide the initial and major source of soil moisture (LeDrew and Weller, 1978). As the growing season progresses, soil moisture decreases as soil temperatures, at the surface and 10 cm belowground, increase (Table 3). A moisture stress develops through the growing season as convective precipitation is apparently not sufficient to maintain the moisture supply (LeDrew and Weller, 1978). Acetylene reduction activity in most sites parallels this seasonal decline in soil moisture levels, and is positively correlated with soil moisture data (see below), especially during June and July. Only in sites I and IV, fellfield and wet meadow, are there slight increases in activity during August. Acetylene reduction activity was not detected (< 10 nmol ethylene m^{-2} h^{-1}) in any site during two sampling periods in September 1980, the probable result of both low soil temperatures and continuous cloud cover on both dates.

The 1980 data for rates of acetylene reduction in sites I to VI are not correlated (coefficient r) at significant levels with soil temperatures (Table 3), soil pH, organic carbon, total nitrogen, or C/N ratios (Table 4). These rates are positively correlated with soil moisture levels (Table 3), at the 0.01 probability level, especially for June and July ($r > 0.9$), and inversely correlated ($r < -0.7$) with levels of inorganic nitrogen (Table 4).

During the survey of 1980 significant dinitrogen fixation activity (1.2 to 51 μg nitrogen m^{-2} h^{-1}) was found associated with the vascular plants *Dryas octopetala* L. ssp. *hookeriana* (Juz.) Hultén and *Trifolium dasyphyllum* T. & G., as assayed by acetylene reduction (Table 5). The

TABLE 2
Summary of in situ acetylene reduction assays on Niwot Ridge, 1979[a]

Site	June 29	July 12
I	34 (0.32)	184 (1.72)
II	42 (0.39)	180 (1.68)
III	158 (1.47)	117 (1.09)
IV	416 (3.88)	125 (1.17)
V	—[b]	134 (1.25)
VI	2480 (23.14)	205 (1.91)

[a]Data expressed as nmol ethylene produced m^{-2} h^{-1}; the approximate conversions to μg of N m^{-2} h^{-1} are in parentheses.
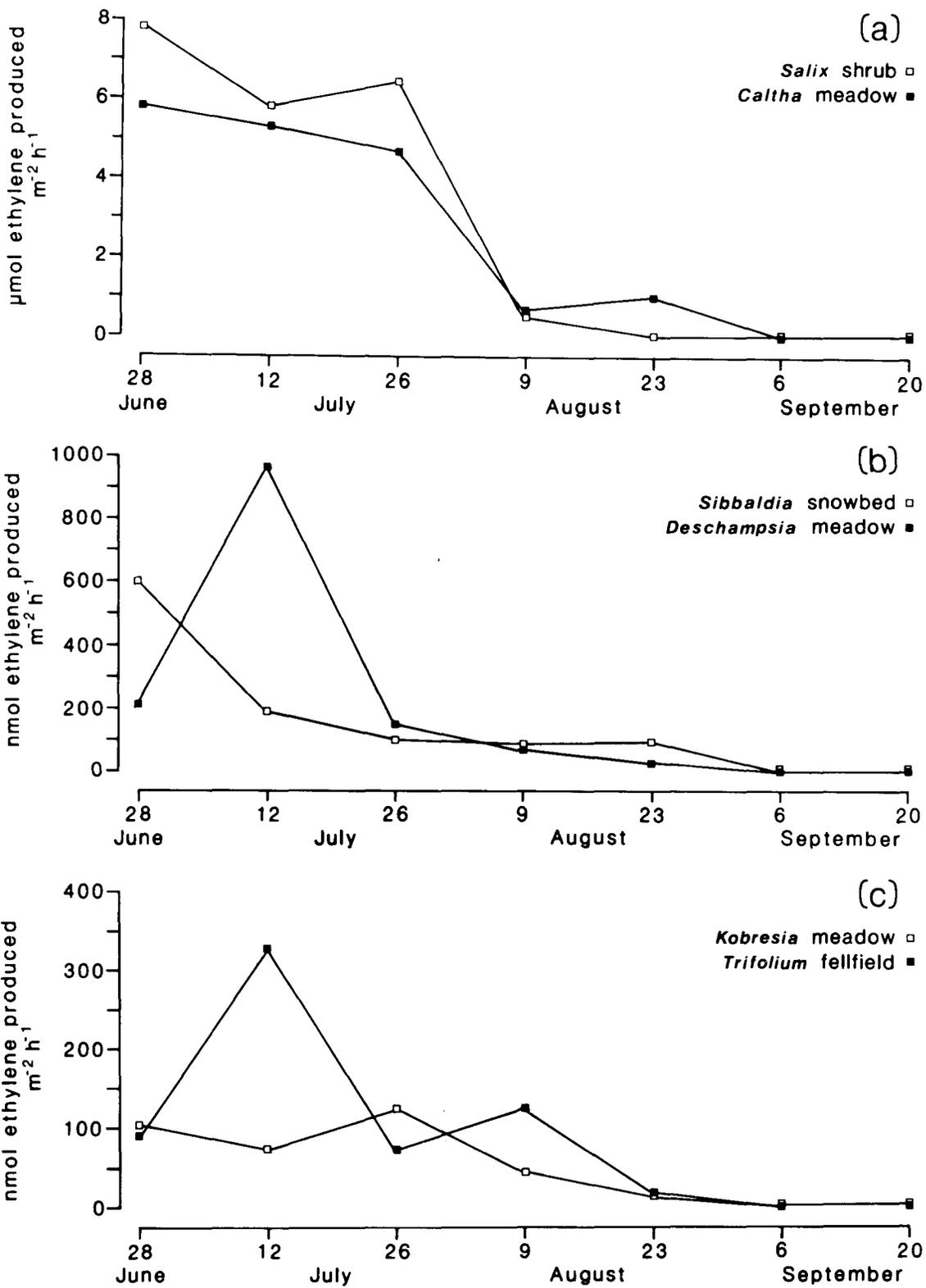[b]Site V inaccessible due to snowpack.

FIGURE 2. Acetylene reduction activities, Niwot Ridge, 1980: (a) *Salix* shrub tundra, site VI and *Caltha* meadow, site IV; (b) *Sibbaldia* snowbed, site V, and *Deschampsia* meadow, site III; (c) *Kobresia* meadow, site II and *Trifolium* fellfield, site I.

TABLE 3
Table 3
Soil moisture levels and soil temperatures, Niwot Ridge, 1980

Site	Measurement	June 28	July 12	July 26	August 9	August 23	September 6	September 20
I	Moisture[a]	2.6	15.0	8.1	16.7	14.9		
	Temperature[b]	38/21	14/12	17/14	17/13	18/13	15/8	
II	Moisture	75.8	19.0	37.2	26.5	33.4		
	Temperature	30/11	18/10	17/10	20/11	17/12	14/9	
III	Moisture	58.3	77.0	69.8	46.7	24.8		
	Temperature	35/8	15/11	17/11	23/12	17/13	15/9	10/8
IV	Moisture	233.0	147.0	132.0	109.0	117.0		
	Temperature	21/5	18/7	17/9	18/10	20/9	13/9	11/8
V	Moisture	176.0	60.3	50.3	25.2	18.9		
	Temperature	18/5	18/10	17/11	20/14	16/13	13/10	
VI	Moisture	144.0	67.1	77.2	41.8	34.9		
	Temperature	21/10	19/10	17/11	20/12	16/11	13/10	10/8

[a]Moisture, in % of dry weight.
[b]Temperature, °C (at surface/10 cm belowground).

Table 4
Soil properties, Niwot Ridge

Site	pH	Organic C (%)	Total N (%)	Inorganic N μg g dry soil^{-1}	C/N Ratio
I	5.4	10.9	0.765	17.5	14.3
II	5.5	13.0	0.967	20.3	13.4
III	4.6	22.4	1.350	18.0	16.6
IV	5.0	16.2	0.759	17.3	21.3
V	4.9	23.7	0.946	17.0	25.1
VI	4.5	12.6	1.010	15.4	12.5

Table 6
Mean rates of dinitrogen fixation and estimated annual nitrogen input in sites I to VI, Niwot Ridge, 1980

Site	Mean fixation rate[a] (μg N m^{-2} h^{-1})	Annual nitrogen input (mg N m^{-2})
I	1.19	1.15
II	0.68	0.66
III	2.40	2.33
IV	32.6	31.6
V	1.99	1.93
VI	38.4	37.2

[a]June 28–August 23.

Table 5
In situ dinitrogen fixation activity in lichens and vascular plants, Niwot Ridge, 1980

	nmol ethylene g^{-1} h^{-1} June 28	August 9
Peltigera aphthosa (VI)[a]	421.0	358.0
Stereocaulon alpinum (III)	1.79	55.6
Cetraria nivalis (III)	0.00	
Pohlia sp. (VI)	0.97	
Dryas octopetala L. ssp. hookeriana (I)	131.0[b]	(7/12)
Trifolium dasyphyllum T. & G. (II)	5410.0	(8/23)

[a]Number in parentheses designates site of collection.
[b]Activities expressed as nmol ethylene produced m^{-2} h^{-1}, date following in parentheses.

lichens *Peltigera aphthosa* and *Stereocaulon* sp. (probably *S. alpinum*) were found to actively reduce acetylene (Table 5), while the common lichen *Cetraria nivalis*, not having a cyanobacterial symbiont, showed no measurable activity.

Low rates of acetylene reduction, ≈1.0 nmol ethylene produced g^{-1} h^{-1}, were associated with samples of the moss *Pohlia* (Table 5), which were collected from the moist depressions beneath the *Salix* canopy in the shrub communities (site VI). This dinitrogen fixation activity is attributed to epiphytic cyanobacteria as later microscopic examination of numerous moss samples revealed the presence of large numbers of cyanobacteria resembling *Nostoc*. *Anabaena* and *Nostoc* were routinely identified in samples of other conspicuous mosses, including *Bryum, Drepanocladus, Mnium,* and *Tortella* sp., collected from this site. *Calothrix* and nonheterocystous filaments resembling *Oscillatoria* and *Pseudanabaena* were also present in some of these moss samples. Only *Nostoc*

was identified in soil samples from sites I, II, and IV, all of which have a pH of 5 or above (Table 4). Subsequently, acetylene reduction activity was demonstrated for purified isolates of *Nostoc* and *Anabaena* grown in liquid culture: activities ranging from 0.15 to 0.75 nmol ethylene $min^{-1} \cdot mg$ protein^{-1}, respectively, were comparable to that of *Anabaena variabilis* Kütz, PCC 6309 (Rippka et al., 1979), 0.46 nmol ethylene $min^{-1} \cdot mg$ protein^{-1}.

An estimate of the annual nitrogen input to the alpine system at Niwot Ridge by dinitrogen fixation for 1980 is calculated by the method of Alexander et al. (1978). The 1980 seasonal averages of nitrogen input based on measured fixation rates (Table 6) range from 0.68 μg nitrogen m^{-2} h^{-1} in *Kobresia* meadow (site II) to 38.4 μg nitrogen m^{-2} h^{-1} in the *Salix* shrub site (VI). The 1980 seasonal mean for all sites is 12.9μg m^{-2} h^{-1} (1.4 μmol ethylene m^{-2} h^{-1}). The annual nitrogen input, assuming a 12-h fixation day (average) over a 90-d growing season (Barry, 1973) with a linear decline in rate to zero during a 10-d period at each end of the season, ranges from 0.66 to 37 mg nitrogen m^{-2} for the *Kobresia* meadow and *Salix* shrub sites, respectively (Table 6). The mean nitrogen input for all sites would then be 12.5 mg nitrogen m^{-2} annually. However, if the areal extents of the six vegetation noda on Niwot Ridge (Komárková and Webber, 1978) are considered, the annual nitrogen input would be less, ca. 5 mg nitrogen m^{-2}. As noted above, much of the ridge (>60%) is covered by dry fellfield and sedge communities, having the lowest rates of acetylene reduction overall.

DISCUSSION

Webber (1974) has suggested, based on similarities in vegetation structure and patterns, that arctic and alpine tundras could be considered part of the same natural biome. Within this concept, it follows that there may be like parallels in physiological processes between the two tundras. The results from this study indicate that the process of dinitrogen fixation in a dry continental alpine ecosystem is similar to the process as it occurs in the arctic tundra of Alaska, despite the considerable differences between the two environments. Although the rate of nitrogen entering the alpine system by fixation is rather low, we have found dinitrogen fixation to be an active process in the soils at Niwot Ridge, and soil moisture to be the primary environmental factor influencing in situ activities. Significant fixation activity was found associated with photoautotrophic organisms, including vascular species, lichens, and cyanobacteria. The principal differences between these two tundras lie in the magnitude of the observed rates of fixation, and the relative importance of the contribution of nitrogen to the nitrogen economy of these environments by the process.

Dinitrogen fixation activity increases along a soil moisture gradient at Niwot Ridge. The highest overall rates of acetylene reduction are found in the moist *Salix* shrub and wet *Caltha* meadow tundra sites while the lowest overall occur in soils of the xeric communities, *Kobresia* meadow and fellfields. Activities parallel the soil moisture regime in the sites showing a general decline through the growing season (Figure 2). Soil temperatures at the surface and at 10 cm belowground (Table 3) apparently have no direct influence (based on correlation analyses) on activities, yet there exists a strong negative correlation ($r < -0.82$) between soil temperature(s) and soil moisture for the 1980 sampling period. This suggests that soil moisture is the more important abiotic factor affecting in situ fixation activities at Niwot Ridge, and that temperature may have a significant influence on activities only when there is an adequate moisture supply available. Such a situation may exist earlier in the season, prior to the period our sampling was conducted. For these same sites at Niwot Ridge monthly temperature means in the plant canopy (5 cm aboveground) are 0°C or above by mid May, while monthly means in the soil (10 cm belowground) lag somewhat, reaching 0°C or above in early June (May and Webber, 1975). The lower temperature limit for significant fixation activity by tundra organisms is in the range 0 to 5°C, and maximum activity occurs in the 15 to 20°C range (Alexander, 1974; Jordan et al., 1978). Thus, temperatures in the soil and at the tundra surface on Niwot Ridge are very likely to be favorable for significant fixation by early June, provided the soil moisture supply is not dissipated. Whether this is the situation could not be determined: the above-normal snowpack from the 1979/80 winter prevented access to the ridge above treeline until late June. A more extensive period of soil measurements and analysis of fixation rates is necessary to determine this with certainty.

The major dinitrogen-fixing components of tundra ecosystems are cyanobacteria, free-living and as symbionts in lichens, although in some alpine regions vascular plants and lichens appear to have a more important role (Alexander, 1975). We found the lichens *Peltigera* and *Stereocaulon*, the plant *Dryas*, and the legume *Trifolium*, to be active dinitrogen fixers in this alpine system.

These lichens, having cyanobacteria as symbionts, are only locally abundant in the cryptogam layer of several communities. *Peltigera aphthosa* was restricted to the moist, mossy depressions beneath the *Salix* canopy in the shrub tundra site (VI), while *Stereocaulon alpinum* was found as scattered clumps in moist *Deschampsia* meadows (site III), and to a lesser extent in snowbed communities (site V).

The contribution of nitrogen to the alpine system by the vascular plants is considerable, as both of these species are abundant and often dominant in the xeric communities that predominate especially on the east half of Niwot Ridge. Vegetation units having *Dryas octopetala* ssp. *hookeriana* as the dominant or abundant species cover more than 10% of the ridge, while *Trifolium dasyphyllum* is dominant in or present in the fellfield and sedge

meadows that cover more than 30% of the ridge (Komár-ková and Webber, 1978). In the alpine tundra of the Alaska Range, significant fixation activity was found with *Dryas octopetala* L. and two *Oxytropis* species. There *D. octopetala* exists as scattered individuals and contributes little to total biomass (Alexander et al., 1978). In contrast, *D. octopetala* ssp. *hookeriana* often forms dense, soil-stabilizing mats on exposed, gravelly slopes in the alpine tundra of Colorado (unpublished observations; Marr, 1967).

Other lichen and vascular species presumably having associated dinitrogen-fixing activity (but not tested in this study) are common components of the vegetation on Niwot Ridge, especially the legumes *Trifolum nanum* Torr. and *Trifolium parryi* Gray ssp. *parryi*. *Trifolium parryi* is an abundant species in the moist grass meadows that predominate on the western end of the ridge. Several *Peltigera* spp. are found on Niwot Ridge, largely in moist communities (Flock, 1978).

The contribution of free-living cyanobacteria to the nitrogen regime of this alpine system is probably minor. *Anabaena* and *Nostoc* are prevalent in the moist depressions under the willow canopy (site VI) where they are associated with mosses, and to a lesser extent in the soils from three sites. However, the overall dryness of the ridge would tend to severely restrict their occurrence in soils and thus preclude widespread, significant activity by these organisms. The low pH (5.5) of all sites is probably not favorable for maximal activity, as acidic conditions (pH < 5) are known to effectively limit both the distribution and fixation activities of cyanobacteria in tundra soils (Alexander, 1974). Thus, significant fixation by these organisms may occur solely in the moss layer of shrub and wet meadow communities where optimum conditions of soil moisture, pH, and reduced oxygen tension (Alexander et al., 1978) are usually encountered. Such habitat on Niwot Ridge is limited, restricted to a few communities with very low (7%) areal extent (Komárková and Webber, 1978).

For the purpose of comparison with data from Barrow and other IBP study sites we have estimated the annual nitrogen input to the alpine system on Niwot Ridge by fixation to be 5 mg N m^{-2} for 1980. This estimate is considerably less than similar input estimates for the arctic system at Barrow, 100 mg N m^{-2} (Alexander et al., 1978), and for other arctic and subarctic sites, which range between 23 and 380 mg N m^{-2} (Alexander, 1974). Thus, nitrogen input to Niwot Ridge annually via fixation is comparable to that of Signy Island, Antarctica, 2 mg nitrogen m^{-2} yr^{-1} (Horne, 1972; Alexander, 1974).

Much of the difference in estimated inputs between the Niwot Ridge and arctic tundra sites such as Barrow could be attributed to differences in their soil moisture regimes. The former is a windswept ridge having considerable topographic and microenvironmental variation. The predominance of fibrous-rooted vegetation is indicative of

its dry character (Webber and May, 1977). Maximum solar radiation intensities at the surface and diurnal ranges are greater, due to more frequent clear skies in the summer over Niwot than at Barrow (LeDrew and Weller, 1978). In contrast, the coastal tundra at Barrow is characterized by extensive wet meadows over perennially frozen ground and a lack of topographic variation (Tieszen, 1978). Consequently, there is generally little or no moisture stress due to a continual standing moisture supply in the soils during the entire growing season (LeDrew and Weller, 1978). Soil moisture levels of 200 to 800% are common at Barrow (Alexander et al., 1978). High moisture levels of this range are required by cyanobacteria and lichens for maximum fixation activity (Alexander, 1974). The levels of soil moisture measured for Niwot Ridge (Table 3) certainly are not adequate for maximum in situ dinitrogen fixation to occur.

The most noteworthy difference between the two tundras concerns the relative importance of dinitrogen fixation to the nitrogen economies of these two systems. Dinitrogen fixation does not apparently constitute the major source or perhaps a significant source of nitrogen input to the alpine system at Niwot Ridge, in marked contrast to the situation at Barrow (cf. Barsdate and Alexander, 1975). For the Indian Peaks region of the Front Range, nitrogen input (mainly inorganic forms) from precipitation alone has been estimated as high as 500 to 600 mg N m^{-2} yr^{-1} (Grant, 1981, pers. comm.; Fehsenfeld, 1981, pers. comm.). Much of this supply of nitrogen, presumably of anthropogenic origin, has been related to the increasing amounts of nitric (HNO$_3$) acid in the precipitation (Lewis and Grant, 1980; Grant and Lewis, 1982). Further, these authors indicate the acidity of the precipitation in the region is unexpectedly high and increasing at a significant rate.

It is interesting to speculate that the high amounts of nitrogen contributed by precipitation to the Indian Peaks region may be exerting a detrimental influence on dinitrogen fixation activity in the soils. Knowles and Denike (1974) have shown nitrogenase activity in soil is suppressed by as little as 5 μg NH$_4^+$-nitrogen $\cdot$ g soil^{-1}, suggesting that fixation activity is very sensitive to even low concentrations of inorganic nitrogen (ammonium and nitrate) in natural soils. Our data suggest an inverse relationship between in situ acetylene reduction rates and soil inorganic nitrogen levels. In a related study at Niwot Ridge, Youmans (1980) found similar levels of nitrate in soils, levels sufficiently high to promote rates of denitrification (loss of nitrogen by microbial reduction of nitrate to N$_2$) that were significantly greater than those estimated for the tundra at Barrow. Thus, the ecologically important amounts of nitrogen entering the system at Niwot Ridge via precipitation play a major role in the nutrient balance and cannot be ignored (Grant and Lewis, 1982). The effect(s) of this source on nitrogen related microbial processes in the soils deserves further study.

ACKNOWLEDGMENTS

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