Oecologia (1988) 76:298–302

Oecologia

Contribution of nitrogen fixation to nitrogen nutrition in an alpine sedge community (*Caricetum curvulae*)

H.-P. Holzmann and K. Haselwandter
Institut für Mikrobiologie, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria

Summary. *In situ* acetylene reduction assays (ARA) were carried out over two growing seasons at 2550 m in the upper alpine zone of the Tyrolean Central Alps of Austria. For comparative purposes, some Fabaceae species introduced into the upper alpine zone from lower elevation (2000 m) were subjected to ARA. At the end of the growing season the potted plants were transferred to the laboratory where their acetylene reducing activities were measured again. *In situ* nitrogenase activity is very low. The highest values were found in association with *Leucanthemopsis alpina* and *Veronica bellidioides* (150 and 217 nmol ethylene 24 h^{-1} per pot respectively). Higher levels of activity were detected in pots transferred to the laboratory (maximum value 750 nmol ethylene 24 h^{-1} per pot; assay temperature about 12° C higher than in the field) and in the Fabaceae transferred to the upper alpine zone (14×10^3 nmol ethylene 24 h^{-1} per pot of *Trifolium badium* and *T. pallescens*). Maximum nitrogen input in the field is in the range of 8 mg m^{-2} a^{-1}. Therefore, under natural circumstances biological nitrogen fixation contributes only very small amounts of nitrogen to this alpine vegetation system, the process being inhibited by low soil temperatures. Possible alternative sources and patterns of N acquisition are discussed in relation to the overall nitrogen economics of plants of the upper alpine zone.

Key words: Nitrogenase (acetylene reducing) activity – Microbial ecology – Soil – Plant analyses – Nitrogen economy

Biological nitrogen fixation has previously been investigated in several nutritionally and climatically stressed arctic, subarctic, and alpine environments. The results indicate that in most cases biological nitrogen fixation is low, and primarily due to the activity of cyanobacteria associated with mosses or fungi (Blasco and Jordan 1976; Jordan et al. 1978; Henry and Svoboda 1986), or rhizobia in symbiosis with plants (Labroue and Carles 1977; Tosca and Labroue 1981). The estimated N inputs reached up to 460 mg N m^{-2} a^{-1}. Haselwandter et al. (1983) working in the nival zone (3100–3200 m) of the Central Austrian Alps showed that N_2 fixation yielded only small quantities of N compared with those obtained from snow-melt water, and that the latter source was sufficient to meet the requirements of nival zone plants during their short growing season. At lower elevations with longer growing seasons plant communities dominated by grasses and sedges are found and it is possible that there, in the upper alpine zone (2400–2700 m), biological nitrogen fixation may be more important. In studies of a *Carex curvula* community on acid soils of the upper alpine zone Rehder and Schäfer (1978) showed that rates of N mineralization were low. Under these circumstances biological fixation could represent an important additional source of nitrogen for the plants. In this study we aimed to determine the role of nitrogen-fixing microorganisms in the nitrogen budget of the upper alpine zone.

In addition to studies of the natural population of the *Caricetum curvulae*, we also analysed the N_2 fixing activity of some Fabaceae which were transferred into the *Caricetum curvulae* for comparative purposes.

Materials and methods

Study site

The *Caricetum curvulae* studied is located at Obergurgl (46° 52′ N, 11° 2′ E) near to the summit of Hohe Mut (elevation 2550 m) in the Tyrolean Central Alps of Austria. A detailed description of the vegetation is provided by Grabherr et al. (1978). Thirteen plant species (with their surrounding rhizosphere soil) characteristic of this association were selected for the present study: the dominant sedge *Carex curvula*, the subdominant grasses *Festuca halleri, Oreochloa disticha, Avena versicolor, Luzula lutea, Poa alpina*, and the herbs *Veronica bellidioides, Geum montanum, Homogyne alpina, Leucanthe mopsis alpina, Potentilla aurea, Primula glutinosa, Silene acaulis*, and *Minuartia sedoides*.

Soil temperatures were recorded continuously at 5 cm soil depth in summer 1978 and 1979 by means of Pt resistance thermometers. Temperature profiles are presented in Fig. 1. For calculation of polynomial regression lines daily means of maximum or minimum temperature were used. While acetylene reduction assays (ARAs) were carried out soil temperature (5 cm soil depth) was registered under midday radiation conditions inside and outside the reaction chamber using a maximum-minimum thermometer. From these measurements we calculated a mean difference of 1.5° C between undisturbed soil (8.6° C ± 4.1) and soil enclosed in reaction chambers for ARA (10.1° C ± 4.7).

Acetylene reduction assays

Nitrogenase activity in soils associated with plants of the *Caricetum curvulae* was measured *in situ* during the growing

Offprint requests to: H.-P. Holzmann

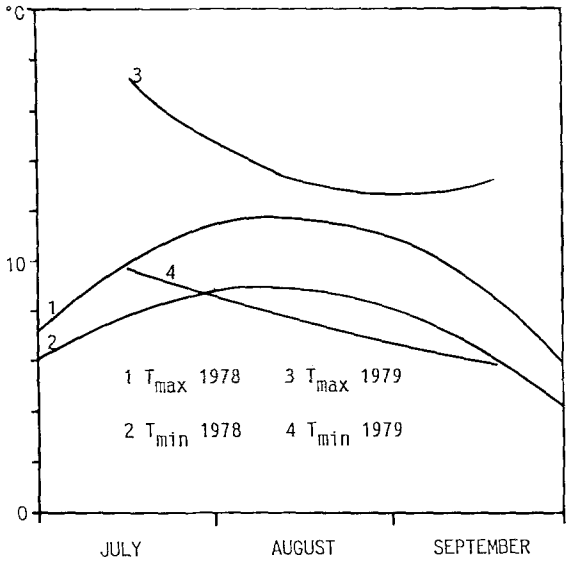

Fig. 1. Soil temperature profile (at 5 cm soil depth) for the growing seasons 1978 and 1979

Table 1. Maximum values for nitrogenase activity (nmol ethylene production per pot 24 h^{-1}) of intact systems under laboratory and field conditions

Plant species[a]	Laboratory	Field
Naturally occurring:		
Carex curvula	525	<1
Festuca halleri	24	<10
Avena versicolor	<1	<10
Veronica bellidioides	135	217
Geum montanum	180	<1
Homogyne alpina	105	<1
Leucanthe mopsis alpina	750	150
Potentilla aurea	150	75
Silene acaulis	<1	<10
Minuartia sedoides	360	<10
Transplanted:		
Trifolium badium	–	14×10^3
T. pallescens	–	14×10^3
Lotus alpinus	–	12×10^3

[a] nomenclature follows Flora Europaea

seasons of 1979 (27–29 August, 18–20 September, 21 October) and 1980 (29–31 July, 14–16 August, 6–7 and 27–28 September) using the acetylene reduction assay. Cores containing soil and plants of various dominant species of the *Caricetum curvulae* were excavated and transferred intact to plastic pots (13 cm diameter, 11 cm high) 4 weeks before the first ARA in 1979. The pots were sunk in the ground and exposed to the natural environment for the duration of experimental period.

For the ARA clear plastic domes (plant propagator No 312, Stewart Plastics, Ltd., Croydon, England) were sealed onto the rims of all pots using adhesive tape. The drainage holes of the pots were plugged with rubber stoppers. A syringe was inserted through the stoppers to withdraw 10% by volume of the air from each pot. This volume was replaced by injecting acetylene into the pots. Immediately after injecting acetylene, gas samples were taken from the sealed chambers using evacuated 5 ml vacutainer tubes (B-D Vacutainer, 4507F, Becton, Dickinson-France S.A. 38043 Grenoble, France) fitted with double needles (B-D Vacutainer needle no 05741-11). Following this initial sampling the sealed systems were incubated in the presence of acetylene for up to 48 h, further sampling of the gas within the pots being carried out at intervals during the incubation. Pots receiving no acetylene were included as controls as recommended by Postgate (1972) in order to determine natural levels of ethylene production. Vacutainers containing the sampled gas were transported to the laboratory where they were stored at 4° C prior to analysis by the standard GC method.

Transfer experiments

The Fabaceae *Trifolium badium, T. pallescens* and *Lotus alpinus*, which occur naturally at altitudes below the *Caricetum curvulae* zone, were carefully excavated from their habitat at 2000 m, potted and transferred to the *Caricetum curvulae*. They were then subjected to the ARA procedure as described above.

When the *in situ* analyses were completed at the end of the 1980 growing season, all pots except those with Fabaceae plants were transferred to the laboratory where further ARAs were carried out at higher temperatures (22°–25° C).

Soil and plant analyses

Soil cores with representative groups of individual plant species were collected at three different locations during the growing season of 1980 and were transported in cool boxes to the laboratory for analysis. Water content, pH, nitrate, and ammonium levels of the soil were determined. Nitrate was extracted with distilled water and determined by the phenol disulfonic acid method. Exchangeable ammonium was extracted with 2N HCl, distilled in the presence of MgO and determined by the Nessler procedure. All these procedures followed Allen et al. (1974). Organic nitrogen of soil and plant materials was determined by a modified semimicro-Kjeldahl method (Bremner 1960). After soil samples had been air-dried, sieved and ground to a fine powder, 200 mg portions of each sample were digested at 350° C on a metal block heater. Prior to analysis plant material was freeze-dried for 24 h and ground to a fine powder in a mortar mill. 25 mg of each sample were digested as described above.

Results

Nitrogenase activity of intact plant-soil systems in situ

A total of about 600 samples were analysed for ethylene content. The results are summarized in Table 1. Only 5% of all samples contained ethylene. The highest values were obtained in pots of *L. alpina* and *V. bellidioides* where levels of ethylene production reached 150 and 217 nmol 24 h^{-1} per pot respectively. In contrast, pots containing *F. halleri, A. versicolor, S. acaulis*, or *M. sedoides* produced only traces of ethylene (<10 nmol 24 h^{-1} per pot). Using the maximum values for *L. alpina* and *V. bellidioides* and extrapolating from the rate per pot to a square meter, and a conver-

Table 2. Chemical characteristics of plants and soil. Values without standard deviation are averages of duplicate determinations of pooled samples from 3 different locations. Samples were taken at time of presumptive biomass peak (30 July) except where otherwise noted

Plant species	Plant Organic N (% dw)		Soil				
	Shoot	Root	Organic N (% dw)	NH_4-N (µg/g dw)	NO_3-N[a] (µg/g dw)	pH[b] (0.01M $CaCl_2$)	Water content[c] (% dw)
Carex curvula	1.98	0.58	0.90	4.8	1.26±0.03	4.2	34± 7.5
Oreochloa disticha	2.14	1.05	0.84	2.4	–	3.6	36±10
Avena versicolor	2.52	0.88	1.42	11.4	–	4.0	30± 8
Luzula lutea	2.93	0.85	–	3.8	0.46±0.08	4.0	27± 5
Veronica bellidioides	1.90	0.99	1.07	2.8	–	3.9	34± 5.8
Geum montanum	3.35	1.62	1.39	–	–	3.6	36± 1.9
Homogyne alpina	2.40	1.69	1.15	2.2	–	3.8	42± 5.9
Leucanthe mopsis alpina	2.68	–	0.99	1.8	0.59±0.04	3.8	38± 3.4
Potentilla aurea	3.86	2.06	1.13	8.2	0.76±0.06	3.7	40± 7
Primula glutinosa	2.76	0.80	0.56	2.2	0.59±0.02	3.9	31± 8
Silene acaulis	1.52	1.19	–	1.5	–	3.9	33± 6
Minuartia sedoides	1.54	1.32	0.82	–	–	4.1	34± 7

[a] 16 August, $n=6$
[b] 7 September, $n=2$
[c] mean (22 and 30 July, 16 August, 7 September) over growing season

sion factor of 3, the possible extent of N input attributable to N_2 fixation was calculated. Assuming a N_2 fixation rate of 60 nmol N 24 h^{-1} per pot, the maximum possible N input is 8 mg N m^{-2} a^{-1}. The Fabaceae species transplanted into the *Caricetum curvulae* showed, in contrast, high levels of acetylene reduction. The highest rates for *T. badium* were as high as 14×10^3 nmol C_2H_4 24 h^{-1} per pot, equivalent to a N input of 790 mg m^{-2} a^{-1}.

Nitrogenase activity in the laboratory

When ARA levels were measured in the laboratory at 22°–25° C, considerable increases in fixation rates were observed (Table 1). About 60% of the gas samples from laboratory assays contained ethylene. For *L. alpina* a maximum N_2 fixation rate of 30 mg N m^{-2} a^{-1} can be calculated. The control chambers, which received no acetylene, showed no evidence of ethylene production.

Soil and plant analysis

Soil pH was in the range of 3.6 to 4.2 within the study site (Table 2). Water content of soil varied from 27% to 42% of dry weight. Concentration of inorganic nitrogen is very low. Amounts of extractable NH_4-N were between 1.5 and 11.4 µg g^{-1} dry weight, while extractable NO_3-N reached levels between 0.5 and 1.3 µg g^{-1} dry weight. Levels of organic nitrogen were between 0.6% and 1.4% of dry weight. This is in contrast to results from a nival ecosystem where about half of the nitrogen sources are present in mineral form (Haselwandter et al. 1983).

All plants showed high tissue nitrogen contents. In roots, nitrogen levels varied from 0.6% to 2.1% of dry weight, whereas shoot nitrogen content was between 1.5% and 3.9% of dry weight. Highest concentrations were found in root as well as shoot tissue of *P. aurea* and *G. montanum*. The level of organic nitrogen content of root and shoot tissue of the dominant species *C. curvula* is lower than in most of the other plant species occurring at the same site.

Discussion

Nitrogen fixation studies

Soil temperature may be one of the main factors limiting asymbiotic nitrogenase activity in the *Caricetum curvulae*, as indicated by the increase in acetylene reduction rate from field to laboratory measurements (Table 1). Similar conclusions have been reported by Basilier et al. (1978) for a subarctic mire, by Alexander et al. (1974) for the arctic tundra, and by Jordan et al. (1978) for arctic areas in general.

Both seasonal fluctuations and annual differences in soil temperature (Fig. 1) may have important effects on nitrogen fixation. In years when soil temperature is higher, the nitrogen input through asymbiotic nitrogen fixation may contribute more to the nitrogen nutrition of plants than in others.

Under field conditions, despite the low soil temperature, the Fabaceae plants transferred to the alpine sedge community showed remarkably high levels of nitrogenase activity. This may be of particular relevance for revegetation programmes at high elevation (Johnson and Rumbaugh 1986), in which seeds of Fabaceae might be included in the seed mixtures applied. Once established, a plant cover with nitrogen-fixing Fabaceae, even growing beyond their natural area of distribution, may become less dependent upon nitrogen fertilization than a community without plants with symbiotic nitrogen fixation.

Soil and plant analyses

Chemical analyses indicate low soil pH and low levels of extractable inorganic nitrogen. In nutrient turnover studies in alpine grass and sedge communities in the Central Alps of Austria it was shown that in the *Caricetum curvulae* the net mineralisation of N was very low (Rehder 1976; Gökçeoğlu and Rehder 1977; Rehder and Schäfer 1978). However, plants of the *Caricetum curvulae* have high levels of organic N in root and shoot tissues (Table 2). It has been observed previously that plants from high latitudes and alti-

tudes exhibit higher tissue nitrogen contents than plants from temperate zones (Chapin et al. 1975; Babb and Whitfield 1977; Körner et al. 1986; Körner and Diemer 1987). A higher nitrogen content in leaves will enhance photosynthetic performance, a direct mechanism to increase annual carbon gain and to compensate for the low temperatures (Chapin 1983).

Nitrogen economy in the Caricetum curvulae

Rehder and Schäfer (1978) reported that the net mineralisation rates in the *Caricetum curvulae* are very low and by far the lowest for alpine grass and sedge communities. Mineralisation yields about 110 mg (ammonium + nitrate)-N m^{-2} a^{-1} (Grabherr and Rehder, pers. communication). Precipitation might provide an additional nitrogen input in the range of 85 mg inorganic N m^{-2} a^{-1}. In arctic and subarctic ecosystems nitrogen input through precipitation reaches 56 mg ammonium-N and 70 mg nitrate-N m^{-2} a^{-1} (Kallio and Veum 1975). Rosswall et al. (1975) give a similar figure for a subarctic mire (about 100 mg N m^{-2} a^{-1}.

The net primary production rate in the *Caricetum curvulae* community at Obergurgl is in the range of 100–160 g m^{-2} GS^{-1} (Grabherr et al. 1978). Assuming that 1.5% of plant dry weight is nitrogen the annual requirement for this element is about 2 g m^{-2}. This is similar to the amount required for annual plant growth in a subarctic mire (Rosswall et al. 1975). All this indicates that the nitrogen budget in the alpine *Caricetum curvulae* is tight. It seems likely that the annual nitrogen supply per unit ground area provided by nitrogen fixation (8 mg m^{-2}), mineralisation (110 mg m^{-2}) and precipitation (85 mg m^{-2}) is not sufficient to support the reported annual plant growth. Nitrogen analyses of plant material, however, show no evidence of nitrogen deficiency (Table 2).

Specific adaptations of alpine and arctic plants to multiple environmental stresses have been reported by Chapin et al. (1975) and Larcher (1983). Towards the end of the growing season N is translocated from the shoot into the root system (Chapin et al. 1975; Holzmann, in preparation) from which it is released at the beginning of the next growing season supporting new plant growth.

An increment in the root/shoot ratio may also be of ecological significance. Körner and Renhard (1987) found a fourfold increase in root length of high as compared to low altitude plants. This may not only provide a bigger absorbing system (Chapin 1980), but also a bigger nutrient reservoir into which nutrients such as N are translocated at the end of the growing season (see above).

Plant nutrition is strongly affected by mycorrhizal infection (Harley and Smith 1983; Moser and Haselwandter 1983). The majority of the plants in the *Caricetum curvulae* is infected with vesicular-arbuscular mycorrhizal fungi (Haselwandter and Read 1980; Hofmann 1983; Haselwandter 1987). It has been demonstrated that vesicular-arbuscular mycorrhizal infection enhances not only P (Sanders 1975) but also N supply to the plant (Ames et al. 1983).

All these various adaptations to cold environments, including seasonal translocation of nutrients within plants and increase in the root/shoot ratio, as well as mycorrhizal infection, are likely to have significant effects on the nutrition of alpine plants. In comparison to the annual N supply provided by mineralization and precipitation the N input through asymbiotic N fixation in the rhizosphere of the *Caricetum curvulae* plants is small. Under conditions of low N availability even small amounts may be valuable for plant nutrition (Giller and Day 1985), although the extent to which asymbiotically fixed N is translocated from the rhizosphere to the plant has yet to be quantified.

Acknowledgements. We wish to thank the Forschungsinstitut für Hochgebirgsforschung, Universität Innsbruck, for financial support, and Dr. Battista of the Institut für Gerichtsmedizin, Universität Innsbruck, for his help with the GC analyses.

References

Alexander V, Billington M, Schell DM (1974) The influence of abiotic factors on nitrogen fixation rates in the Barrow, Alaska, arctic tundra. Rep Kevo Subarctic Res Stat 11:3–11

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1974) In: Allen SE (ed) Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford London Edinburgh Melbourne

Ames RN, Reid CPP, Porter LK, Cambardella C (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytologist 95:381–396

Babb TA, Whitfield DWA (1977) Mineral nutrient cycling and limitation of plant growth in the Truelove Lowland ecosystem. In: Bliss LC (ed) Truelove Lowland, Devon Island, Canada: A high Arctic Ecosystem. The University of Alberta Press, Edmonton-Canada, p 589–606

Basilier K, Granhall U, Stenström T-A (1978) Nitrogen fixation in wet minerotrophic moss communities of a subarctic mire. Oikos 31:236–246

Blasco JA, Jordan DC (1976) Nitrogen fixation in the muskeg ecosystem of the James Bay Lowlands, Northern Ontario. Can J Microbiol 22:897–907

Bremner JM (1960) Determination of nitrogen in soil by the Kjeldahl method. J Agr Sci 55:1–23

Chapin FS III (1980) The mineral nutrition of wild plants. Ann Rev Ecol Syst 11:233–260

Chapin FS III (1983) Direct and indirect effects of temperature on arctic plants. Polar Biol 2:47–52

Chapin FS III, Van Cleve K, Tieszen TT (1975) Seasonal nutrient dynamics of tundra vegetation at Barrow, Alaska. Arctic and Alpine Research 73:209–226

Giller KE, Day JM (1985) Nitrogen fixation in the rhizosphere: significance in natural and agricultural systems. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Blackwell Scientific Publications, Oxford London Edinburgh Boston Palo Alto Melbourne, p 127–147

Gökçeoğlu M, Rehder H (1977) Nutrient turnover studies in alpine ecosystems III. Communities of lower altitudes dominated by *Carex sempervirens* Vill. and *Carex ferruginea* Scop. Oecologia (Berlin) 28:317–331

Grabherr G, Mähr E, Reisigl H (1978) Nettoprimärproduktion und Reproduktion in einem Krummseggenrasen (*Caricetum curvulae*) der Ötztaler Alpen, Tirol. Oecol Plant 13 3:227–251

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Haselwandter K (1987) Mycorrhizal infection and its possible ecological significance in climatically and nutritionally stressed alpine plant communities. Angew. Botanik 61:107–114

Haselwandter K, Read DJ (1980) Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. Oecologia (Berlin) 45:57–62

Haselwandter K, Hofmann A, Holzmann H-P, Read DJ (1983) Availability of nitrogen and phosphorus in the nival zone of the Alps. Oecologia (Berlin) 57:266–269

Henry GHR, Svoboda J (1986) Dinitrogen fixation (acetylene re-

duction) in high arctic sedge meadow communities. Arctic and Alpine Research 18 2:181–187

Hofmann A (1983) Die vesikular-arbuskulare Mykorrhiza in einer alpinen Grasheide und ihre Beziehung zum Phosphorhaushalt der Wirtspflanzen. Dissertation, Innsbruck

Johnson DA, Rumbaugh MD (1986) Field nodulation and acetylene reduction activity of high-altitude legumes in the Western United States. Arctic and Alpine Research 18 2:171–179

Jordan DC, McNicol PJ, Marshall MR (1978) Biological nitrogen fixation in the terrestrial environment of a high arctic ecosystem (Truelove Lowland, Devon Island, N.W.T.). Can J Microbiol 24:643–649

Kallio P, Veum AK (1975) Analysis of precipitation at Fennoscandian Tundra sites. In: Wielgolaski FE (ed) Ecological Studies 16. Fennoscandian Tundra Ecosystems. Springer Verlag, Berlin Heidelberg New York, pp 333–338

Körner Ch, Diemer D (1987) In situ photosynthetic responses to light, temperature and carbon dioxide in plants from low and high altitude. Functional Ecology 1:179–194

Körner Ch, Renhardt U (1987) Dry matter partitioning and root length/leaf area ratios in herbaceous perennial plants with diverse altitudinal distribution. Oecologia (Berlin) 74:411–418

Körner Ch, Bannister P, Mark AF (1986) Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. Oecologia (Berlin) 69:577–588

Labroue L, Carles J (1977) Le cycle de l'azote dans les sols alpins du Pic du Midi de Bigorre (Hautes-Pyrénées). Oecol Plant 121:55–77

Larcher W (1983) Ökophysiologische Konstitutionseigenschaften von Gebirgspflanzen. Ber Deutsch Bot Ges 96:73–85

Moser M, Haselwandter K (1983) Ecophysiology of mycorrhizal symbioses. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological Plant Ecology III. Springer Verlag, Berlin Heidelberg New York, p 391–421

Postgate JR (1972) The acetylene reduction test for nitrogen fixation. In: Norris JR, Ribbons DW (eds) Methods in Microbiology 6B Academic Press, London New York, p 343–356

Rehder H (1976) Nutrient turnover studies in alpine ecosystems I. Phytomass and nutrient relations in the *Caricetum firmae*. Oecologia (Berlin) 23:49–62

Rehder H, Schäfer A (1978) Nutrient turnover studies in alpine ecosystems IV. Communities of the Central Alps and comparative survey. Oecologia (Berlin) 34:309–327

Rosswall T, Flower-Ellis JGK, Johansson LG, Jonsson S, Ryden B-E, Sonesson M (1975) Stordalen (Abisko), Sweden. In: Rosswall T, Heal OW (eds) Structure and Function of Tundra Ecosystems. Ecol Bull 20 Stockholm, p 265–294

Sanders FE (1975) The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London New York San Francisco, p 261–276

Tosca C, Labroue L (1981) Le cycle de l'azote dans les milieux supra-forestiers des Pyrénées Centrales: contribution à l'évaluation des gains. Acta Oecologica Vol 2(16)1:41–52

Received July 14, 1987