NITROGEN CYCLING IN AN ANTARCTIC ECOSYSTEM
1. BIOLOGICAL NITROGEN FIXATION IN THE VICINITY OF SYOWA STATION

Takayuki NAKATSUBO and Yoshio INO

Department of Biology, School of Education, Waseda University, 6–1, Nishi-Waseda 1-chome, Shinjuku-ku, Tokyo 160

Abstract: As part of the studies of the nitrogen flows in the Antarctic terrestrial ecosystems, nitrogen-fixing activities of moss communities, algae and lichens, collected in various ice-free areas near Syowa Station, East Antarctica, were measured by the acetylene reduction method. Moss communities which grew on the sand at dry habitats had dense cover of cyanobacteria and showed high nitrogen-fixing activities, while those at wet habitats near streams showed weak or no activity. Folious colonies of *Nostoc* sp. showed significant activities. Some colonies of Chlorophyceae *Prasiola crispa* had weak activities which were probably due to associating cyanobacteria. No activity was detected for lichen species tested. The results suggest that nitrogen fixation by cyanobacteria, especially those epiphytic on mosses, plays an important role in the nitrogen budgets of terrestrial ecosystems developed in dry ice-free areas near Syowa Station.

1. Introduction

The Antarctic terrestrial ecosystems are characterized by extremely harsh conditions such as severe cold, drought and nutrient-poor substrates. The organisms that make up the living component of such ecosystems are ecologically interesting since they often have developed rather unique adaptations which enable them to survive under these conditions. Furthermore, because the structure of ecosystems in severe climates tends to be simple, the studies of them may offer some important information about fundamental functions of ecosystems that are difficult to be recognized in complex ecosystems.

Although the constituents of the Antarctic terrestrial ecosystems have been clarified by taxonomic and phytosociological researches, little is known about the function such as interactions among organisms and environment except for maritime Antarctic and sub-Antarctic islands under milder climate conditions. To understand ecological function, it is important to make clear the matter and energy flows. A series of studies about carbon flows in continental Antarctica have been done by INO *et al.* (1980, 1981) and INO (1983a, b). However, it is also important to study the flows of nutrients, especially those of nitrogen, because it is required by organisms in a large amount and nitrogen deficiency sometimes restricts the biological activities and the distribution of organisms in some ecosystems. In continental Antarctica, the soil nitrogen content is very low (YAMANAKA and SATO, 1977; INO *et al.*, 1980), and

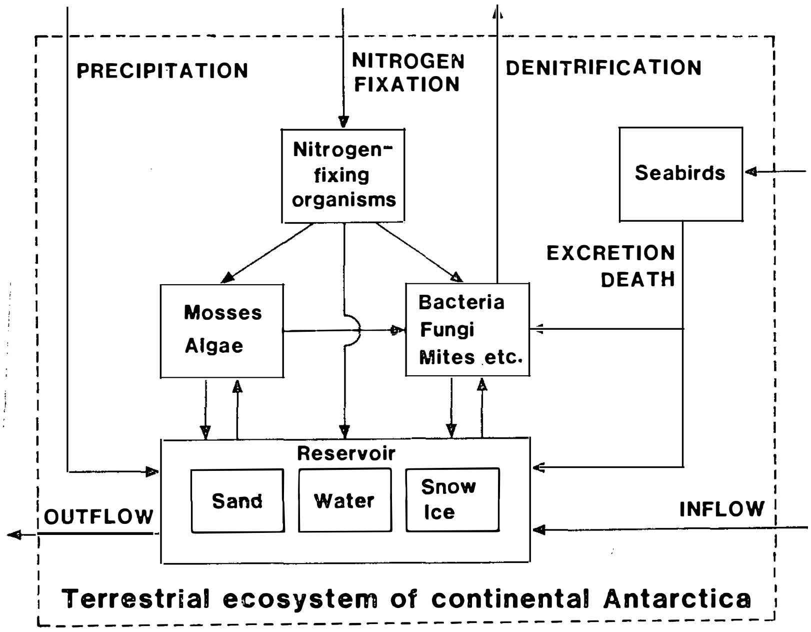

Fig. 1. A compartment model showing possible nitrogen flows in a terrestrial ecosystem of continental Antarctica.

in many cases the nitrogen supply to organisms seems to be restricted.

Figure 1 presents a compartment model showing possible nitrogen flows in a terrestrial ecosystem of continental Antarctica. To represent many interactions among organisms and environment, the compartment models are useful means. As in this model, nitrogen compounds are supplied into the system by precipitation, seabirds and biological nitrogen fixation. MATSUDA (1968) pointed out the importance of seabird excrement as a nutrient resource to the ecosystem. Some portion of the nitrogen compounds may be brought by water flow. Then, available nitrogen is absorbed by mosses and algae directly or after being incorporated in sand or water. Nitrogen compounds are accumulated in organisms and sand, and mineralized by various microorganisms. Outflow of nitrogen compounds from the system is attributed to leaching, run-off, sweeping by wind and denitrification, but no information is available to date. This is a generalized model and it can be modified for each ecosystem investigated.

Biological nitrogen fixation is known to play an important role in the nitrogen budgets of some ecosystems in the polar region. It has been reported that biological nitrogen fixation supplies more nitrogen to arctic ecosystems than precipitation does (BARSDATE and ALEXANDER, 1975).

Nitrogenase activities have been reported on sub-Antarctic Marion Island for free-living cyanobacteria (CROOME, 1973) and bryophyte-cyanobacteria associations (SMITH and ASHTON, 1981; SMITH and RUSSELL, 1982). The amounts of nitrogen fixed by *Nostoc commune* and lichens were measured on Signy Island in maritime Antarctic (FOGG and STEWART, 1968; HORNE, 1972). For continental Antarctica, detailed studies of nitrogen fixation of *Nostoc* sp. were carried out in the Vestfold Hills (DAVEY, 1982, 1983; DAVEY and MARCHANT, 1983).

In the ice-free areas of the Sôya Coast and the Prince Olav Coast, moss communities are often covered with cyanobacteria (FUKUSHIMA, 1959; MATSUDA, 1968; NAKANISHI, 1977; KANDA, 1981), but the measurement of their nitrogen-fixing activities has not been carried out. Thus, as the first step of the studies of the nitrogen flows in the ecosystem of continental Antarctica, we studied biological nitrogen fixation in the vicinity of Syowa Station.

This paper presents the results of the laboratory study of nitrogen-fixing activity, measured by the acetylene reduction method, of moss communities, algae and lichens collected in various ice-free areas near Syowa Station.

2. Materials and Methods

The samples for the measurements of nitrogen-fixing activity were collected by one of the authors (Y.I.) and Dr. H. KANDA (National Institute of Polar Research) from various ice-free areas along the coast in East Antarctica during the austral summers of 1982, 1983 and 1984.

Sampling sites included East Ongul Island (69°00′S, 39°35′E) where Syowa Station is situated, West Ongul Island (69°01′S, 39°34′E), Einstöingen (69°39′S, 38°50′E), Rundvågskollane (69°50′S, 39°09′E), Yukidori Valley in Langhovde (69°14′S, 39°46′E) and a moraine neighboring Richardson Lake (66°46′S, 50°40′E) (Fig. 2).

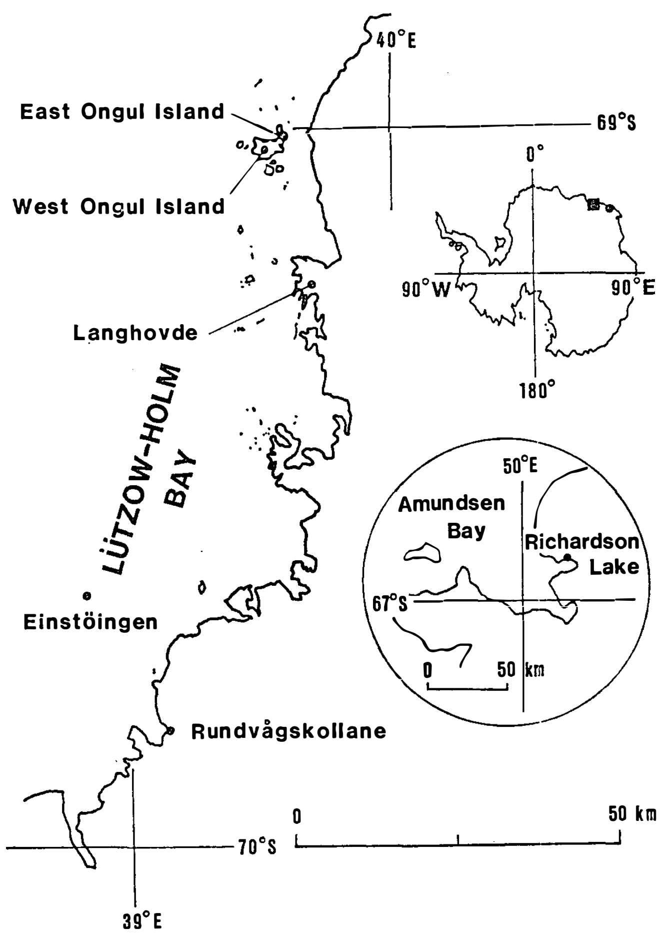

Fig. 2. Map showing the localities where the samples were collected.

Species and sampling sites of the moss communities, algae and lichens used for the measurements are as follows:

1) Mixed community of *Ceratodon purpureus* (HEDW.) BRID. and *Bryum pseudotriquetrum* (HEDW.) GAERTN., MEYER et SCHERB. of East Ongul Island (Fig. 3).

This community grew on the sand near Syowa Station and was dry when it was collected. Its surface was heavily colonized by cyanobacteria and lichens, and was almost black in color. The green part of the community could not be seen in appearance.

2) Mixed community of *Grimmia lawiana* J. H. WILLIS, *C. purpureus* and *B. pseudotriquetrum* of Rundvågskollane (Fig. 4).

It was found on the sand near a small snowdrift. Its growing condition and appearance were similar to those in East Ongul Island.

3) Mixed community of *Bryum argenteum* HEDW. and *C. purpureus* of the Yukidori Valley in Langhovde.

This community grew along a small stream. Its surface was partly covered with cyanobacteria including folious colonies of *Nostoc* sp. The rest part was green.

4) Community of *G. lawiana* of the Yukidori Valley in Langhovde.

This community was found on a dry rock and was dry when it was collected. Its surface was black in color.

5) Community of *C. purpureus* grown in a stream near Richardson Lake (Fig. 5).

No cyanobacterium was observed on the surface. The mean length of the green (living) parts of the shoots was *ca.* 8 mm. The lower part of the community was brown and seemed to be dead. The length of the brown part was more than 10 cm.

6) Folious colonies of cyanobacterium *Nostoc* sp. of West Ongul Island.

They were dry and brittle when they were collected.

7) Colonies of Chlorophyceae *Prasiola crispa* (LIGHTF.) MENEGH. ssp. *antarctica* (KÜTZ.) KNEBEL of Einstöingen.

They were found on the soil near a pond. There were many nests of snow petrel (*Pagodroma nivea*).

8) Fruticose lichen *Usnea sulphurea* (KÖN.) TH. FR. grown on a dry rock in Rundvågskollane.

9) Folious lichen *Umbilicaria decussata* (VILL.) ZAHLBR. grown on the wet sand by a stream near Richardson Lake.

10) Folious lichen *Umbilicaria aprina* NYL. grown on a dry rock near Richardson Lake.

These samples were sent to Japan in a frozen state at −20°C and kept in a freezer at −20°C until the measurements of nitrogen-fixing activity were made.

Each sample was wetted with distilled water and was put in a growth box at *ca.* 10°C, 200 μE m^{-2} s^{-1} photosynthetically active radiation (PAR) for one day before the measurement. During this pretreatment, the samples were kept in a water-saturated condition.

Nitrogen-fixing activities were tested by the acetylene reduction method (STEWART *et al.*, 1967; STUTZ and BLISS, 1973).

Each sample was placed in a 23 m*l* Erlenmeyer flask which was capped with a rubber stopper. The amount of sample in a flask varied depending on its shape but

Fig. 3. Moss community of East Ongul Island.

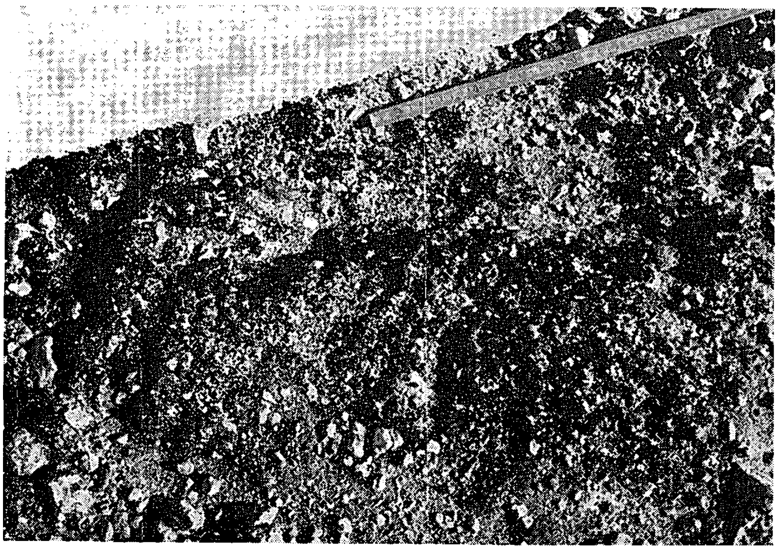

Fig. 4. Moss community of Rundvågskollane.

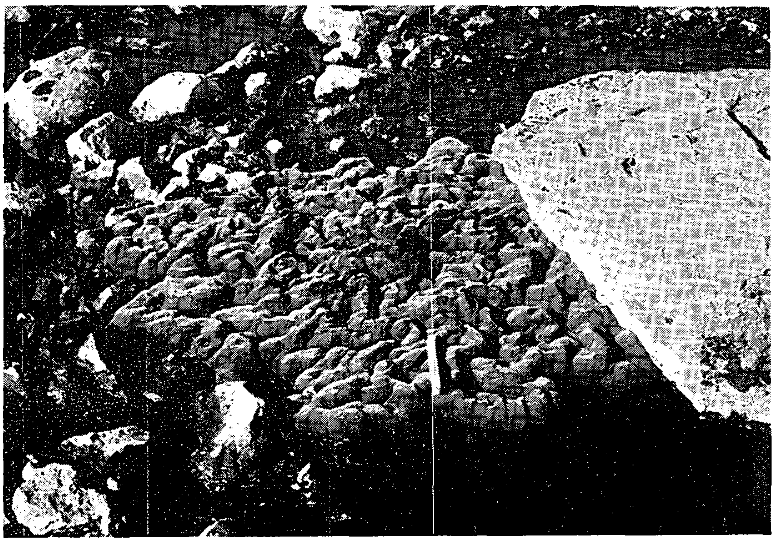

Fig. 5. Moss community in a stream near Richardson Lake.

was less than 5 g in fresh weight. For moss communities, 1 or 2 cm^2 cores taken from them with cork borers were used. When the sample was thicker than 1 cm, the part lower than 1 cm was cut away.

Part of the air within a flask was replaced with acetylene gas generated from calcium carbide to make 0.1 atm C_2H_2. Control flasks, in which only samples were contained and acetylene gas was not added, were used to monitor endogenous ethylene production. All flasks were incubated in a water bath at 10°C and 200 $\mu E\ m^{-2}\ s^{-1}$ PAR for 24 h. This incubation temperature was selected taking into account the fact that the moss surface temperature was often higher than 10°C in summer in the field (MATSUDA, 1968). A long time of incubation was required to detect the low activity. Because reduction rates may not have been constant through experimental time, we have expressed acetylene-reducing activity as nanomoles of ethylene produced per square centimeter of moss community per 24 h. A flask with the sample core and a YSI thermistor probe was placed under the same condition and the surface temperature of the sample was checked. A photo-transistor was used to monitor the light intensities in these flasks. At the end of the incubation period, air samples were withdrawn from the flasks with a syringe for gas chromatographic analysis. The production of ethylene was determined using a Simazu GC-8A gas chromatograph equipped with a flame ionization detector. Hydrocarbon separation was achieved using a 2.0 m×2.6 mm glass column packed with 80/100 mesh Porapak R. Acetylene gas samples were also checked for potential ethylene contamination.

After the measurement of acetylene-reducing activity, the fresh weight and volume of the sample were measured, and the sample was dried to constant weight at 80°C.

To eliminate ethylene contamination, used stoppers were discarded.

3. Results and Discussion

Since all samples used in the present study were stored in a freezer at −20°C,

Table 1. Nitrogen-fixing (acetylene-reducing) activities of moss communities.

No.	Moss species	Cyano-bacteria*	Sampling sites	nmol $C_2H_4\ cm^{-2}\ 24\ h^{-1}$	
				Mean±S.E. (n)	Range
1	*Ceratodon purpureus* *Bryum pseudotriquetrum*	++	East Ongul Island	151±46 (8)	92–240
2	*Ceratodon purpureus* *Bryum pseudotriquetrum* *Grimmia lawiana*	++	Rundvågskollane	31±26 (12)	3– 91
3	*Ceratodon purpureus* *Bryum argenteum*	+	Langhovde	5± 7 (7)	0– 20
4	*Grimmia lawiana*	±	Langhovde	0± 0 (7)	
5	*Ceratodon purpureus*	−	Richardson Lake	0± 0 (3)	

* Degree of colonization of cyanobacteria
++: Surface was heavily colonized by cyanobacteria and was black in color.
+: Surface was partly colonized by cyanobacteria.
−: Surface was not colonized by cyanobacteria and was green in color.
±: Surface was black but no cyanobacterium was detected.

the effect of freezing on the acetylene-reducing activity must be taken into consideration. To examine the effect of the freezing storage on the acetylene-reducing activity, some samples were experimentally frozen at −20°C for 108 days. Acetylene reduction rates after the freezing were not significantly different from those before the freezing. From this result, it was assumed that the decrease in the acetylene-reducing activity by the freezing storage was negligible.

The results of acetylene reduction tests on the moss communities are presented in Table 1. Endogenous ethylene production was not detected.

Among five moss communities tested, the mixed community of *Ceratodon purpureus* and *Bryum pseudotriquetrum* from East Ongul Island showed the highest activity. The community of Rundvågskollane had the next highest mean value, although the activities varied widely among twelve replicates. These communities had dense cover of epiphytic cyanobacteria, and microscopic observation showed that several species of cyanobacteria, probably some species of genera *Nostoc*, *Gloeocapsa* and *Stigonema*, were included. Because some species of these genera are known to be potential nitrogen fixers, the acetylene-reducing activities detected can be attributed to some of these cyanobacteria.

For the mixed community of *C. purpureus* and *B. argenteum* from the Yukidori Valley in Langhovde, some replicates showed relatively high activities but most of them had weak or no activity. Since there were many nests of snow petrel near the habitat of this sample, it is suspected that this community was supplied with nutrients from these nests.

The community of *Grimmia lawiana* was also collected at the Yukidori Valley, but it had grown on a dry rock apart from any water resource and its water and nu-

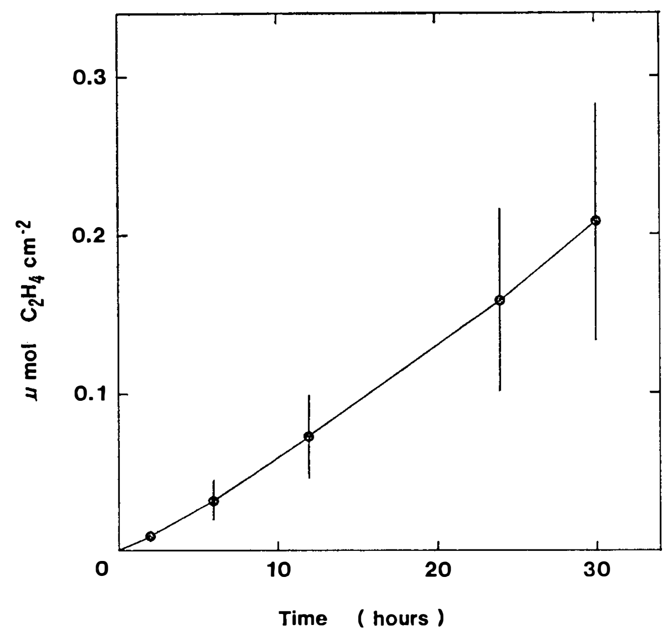

Fig. 6. Cumulative acetylene reduction by the moss community of East Ongul Island as a function of incubation time. Incubation was performed at 10°C and 200 μE m^{-2} s^{-1} PAR. Each value is the mean of five replicates ±1 standard error.

trient supply seemed poor. Its surface was black like that of East Ongul Island, but no cyanobacterium was observed by microscopic observation. No acetylene-reducing activity was detected.

For the community of *Ceratodon purpureus* in a stream near Richardson Lake, no cyanobacterium was observed on the surface and no acetylene-reducing activity was detected. In this community, the nutrients used for its growth seemed to be supplied by the flowing water.

A time course study of acetylene reduction was carried out with the community of East Ongul Island (Fig. 6). The relation between the cumulative acetylene reduction and the incubation time was almost linear. The average acetylene reduction rate was 6.29 nmol C_2H_4 cm^{-2} h^{-1}. DAVEY and MARCHANT (1983) presented year-round *in situ* studies of nitrogen fixation of *Nostoc commune* in the Vestfold Hills in continental Antarctica and reported that the damp moss-*Nostoc* cushions reduced acetylene at an average rate of 3.56 nmol C_2H_4 cm^{-2} h^{-1} during January to mid February 1980, when the soil surface temperature fluctuated between 1.4 and 4°C. The acetylene reduction rates of the community of East Ongul Island in the present study seem to be comparable to those of the Vestfold Hills because our experiments showed that the temperature coefficient (Q_{10}) of this reaction was 2.8 in the range from 1 to 10°C at 200 μE m^{-2} s^{-1} PAR. In the Vestfold Hills, the damp moss-*Nostoc* cushions contribute 119 mg N m^{-2} y^{-1} to the terrestrial ecosystem (DAVEY and MARCHANT, 1983) and it is deduced that cyanobacteria epiphytic on mosses fix a considerable amount of nitrogen in the ice-free areas near Syowa Station as well. Nitrogen input via this pathway appears to be important for the moss communities of relatively dry habitats like those of East Ongul Island and Rundvågskollane because the soil nitrogen content is very low at these habitats. For example, the nitrogen content of the soil beside the moss community of Rundvågskollane was less than 0.01% (INO and NAKATSUBO, 1986). However, to make clear the role of nitrogen fixation in the Antarctic ecosystem, it is necessary to estimate the amount of nitrogen fixed annually.

Table 2. Nitrogen-fixing (acetylene-reducing) activities of algae and lichens.

No.	Species	Sampling sites	nmol C_2H_4 g^{-1} dw 24 h^{-1}	
			Mean±S.E. (n)	Range
6	*Nostoc* sp.	West Ongul Island	5.18±71.1 (7)	0–173
7	*Prasiola crispa*	Einstöingen	0.6± 1.0 (7)	0–2.2
8	*Usnea sulphurea*	Rundvågskollane	0 ± 0 (3)	
9	*Umbilicaria decussata*	Richardson Lake	0 ± 0 (3)	
10	*Umbilicaria aprina*	Richardson Lake	0 ± 0 (3)	

Table 2 shows the results of acetylene reduction tests on algal colonies and lichens. Acetylene-reducing activities of these samples are expressed on a gram dry weight basis because of the difficulty in expressing on a unit area basis. No endogenous ethylene production was detected for these samples.

Acetylene-reducing activities of the folious colonies of *Nostoc* sp. varied from 0 to 173 nmol C_2H_4 g^{-1} dw 24 h^{-1}. The colonies that showed no activity may be dead, because these samples were severely desiccated when they were collected. However, it was difficult to separate the dead one from the living one. Nitrogen fixation by free-living cyanobacteria has been reported for the Vestfold Hills in continental Antarctica (DAVEY, 1983; DAVEY and MARCHANT, 1983), Signy Island in maritime Antarctic (FOGG and STEWART, 1968; HORNE, 1972) and sub-Antarctic Marion Island (CROOME, 1973). Since free-living nitrogen-fixing cyanobacteria are widely distributed in the ice-free areas near Lützow-Holm Bay (AKIYAMA, 1974), the amount of nitrogen fixed by these cyanobacteria may be considerable.

Chlorophyceae *Prasiola crispa* ssp. *antarctica*, which itself has no nitrogen-fixing activity, often grows with cyanobacteria such as the genus *Nostoc* (AKIYAMA, 1974). Among seven replicates tested, two samples showed low but significant acetylene-reducing activities which can be attributed to these cyanobacteria. The habitat of these samples was thought to be eutrophic because there were many nests of snow petrel near the habitat, and the role of nitrogen fixation as a nitrogen resource seemed to be negligible in this site.

Some lichens containing green algal phycobionts were tested for their acetylene-reducing activities to ascertain whether any nitrogen-fixing organisms associated with them. No acetylene reduction was detected. FOGG and STEWART (1968) and HORNE (1972) demonstrated nitrogen fixation by two lichen species on Signy Island, but these species have not been reported in the vicinity of Syowa Station (KASHIWADANI, 1982).

Some soil samples from the Yukidori Valley in Langhovde were also tested for acetylene-reducing activity, but no activity was detected. However, since potential nitrogen-fixing cyanobacteria such as the genera *Nostoc*, *Anabaena* and *Stigonema* have been reported for the soil from the coastal region of Lützow-Holm Bay (AKIYAMA, 1974), it is important to study the nitrogen fixation by soil cyanobacteria in this region.

The results obtained in the present study suggest that nitrogen fixation by cyanobacteria, especially those epiphytic on mosses, plays an important role in the nitrogen budgets of some terrestrial ecosystems in the ice-free areas near Syowa Station. In order to make clear its contribution, annual nitrogen input via nitrogen fixation to the moss community in East Ongul Island will be estimated in the subsequent paper.

Acknowledgments

The authors wish to thank Dr. H. KANDA of National Institute of Polar Research (NIPR) for many helpful discussions and suggestions during this work. We are indebted to Prof. T. MATSUDA, director of NIPR, and Prof. T. HOSHIAI and Dr. Y. OHYAMA of NIPR, and Prof. Y. OSHIMA of Waseda University for their kind advice and encouragement. Our thanks are also due to Prof. Y. MARUYAMA and Mr. H. KAWAKUBO of the University of Tokyo for their helpful advice on the acetylene reduction method.

References

AKIYAMA, M. (1974): Nankyoku Ryutsuo-Horumu Wan engan rogantai no sôrui shokusei (A preliminary note on some algae found in the ice-free area of the coastal region of Lützow-

Holm Bay, Antarctica). Shimane Daigaku Kyôiku Gakubu Kiyô (Shizen Kagaku) (Mem. Fac. Educ. Shimane Univ.), **8**, 37–50.

BARSDATE, R. J. and ALEXANDER, V. (1975): The nitrogen balance of arctic tundra; Pathways, rates and environmental implications. J. Environ. Qual., **4**, 111–117.

CROOME, R. L. (1973): Nitrogen fixation in the algal mats on Marion Island. S. Afr. J. Antarct. Res., **3**, 64–67.

DAVEY, A. (1982): *In situ* determination of nitrogen fixation in Antarctica using a high sensitivity portable gas chromatograph. Aust. J. Ecol., **7**, 395–402.

DAVEY, A. (1983): Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. Polar Biol., **2**, 95–100.

DAVEY, A. and MARCHANT, H. J. (1983): Seasonal variation in nitrogen fixation by *Nostoc commune* VAUCHER at the Vestfold Hills, Antarctica. Phycologia, **22**, 377–385.

FOGG, G. E. and STEWART, W. D. P. (1968): *In situ* determinations of biological nitrogen fixation in Antarctica. Br. Antarct. Surv. Bull., **15**, 39–46.

FUKUSHIMA, H. (1959): Onguru Tô no seibutsu gaihô (General report on fauna and flora of the Ongul Islands, Antarctica). Yokohama Shiritsu Daigaku Kiyô (J. Yokohama Munic. Univ.), Ser. C, **112**, 1–10.

HORNE, A. J. (1972): The ecology of nitrogen fixation on Signy Island, South Orkney Islands. Br. Antarct. Surv. Bull., **27**, 1–18.

INO, Y. (1983a): Photosynthesis and primary production in moss community at Syowa Station, Antarctica. Jpn. J. Ecol., **33**, 427–433.

INO, Y. (1983b): Estimation of primary production in moss community on East Ongul Island, Antarctica. Nankyoku Shiryô (Antarct. Rec.), **80**, 30–38.

INO, Y. and NAKATSUBO, T. (1986): Distribution of carbon, nitrogen and phosphorus in a moss community—soil system developed on a cold desert in Antarctica. Ecol. Res., **1**, 59–69.

INO, Y., OSHIMA, Y., KANDA, H. and MATSUDA, T. (1980): Soil respiration in the vicinity of Syowa Station, Antarctica. 1. Relationships between soil respiration rate and water content or nitrogen content. Nankyoku Shiryô (Antarct. Rec.), **70**, 31–39.

INO, Y., OSHIMA, Y., OHYAMA, Y., KANDA, H. and MATSUDA, T. (1981): Soil respiration in the vicinity of Syowa Station, Antarctica. 2. Estimation of carbon dioxide amount evolved from the naked part of West Ongul Island. Nankyoku Shiryô (Antarct. Rec.), **73**, 124–133.

KANDA, H. (1981): Flora and vegetation of mosses in ice-free areas of Sôya Coast and Prince Olav Coast, East Antarctica. Hikobia Suppl., **1**, 91–100.

KASHIWADANI, H. (1982): Chiirui (Lichens). Nankyoku no Kagaku, 7. Seibutsu (Science in Antarctica, 7. Biology), ed. by Natl Inst. Polar Res. Tokyo, Kokon Shoin, 196–206.

MATSUDA, T. (1968): Ecological study of the moss community and microorganisms in the vicinity of Syowa Station, Antarctica. JARE Sci. Rep., Ser. E (Biol.), **29**, 58 p.

NAKANISHI, S. (1977): Ecological studies of the moss and lichen communities in the ice-free area near Syowa Station, Antarctica. Nankyoku Shiryô (Antarct. Rec.), **59**, 68–96.

SMITH, V. R. and ASHTON, P. J. (1981): Bryophyte-cyanobacteria associations on sub-Antarctic Marion Island; Are they important in nitrogen fixation? S. Afr. J. Antarct. Res., **10/11**, 24–26.

SMITH, V. R. and RUSSELL, S. (1982): Acetylene reduction by bryophyte-cyanobacteria associations on a subantarctic island. Polar Biol., **1**, 153–157.

STEWART, W. D. P., FITZGERALD, G. P. and BURRIS, R. H. (1967): *In situ* studies on N_2 fixation using the acetylene reduction technique. Proc. Natl. Acad. Sci. USA, **58**, 2071–2078.

STUTZ, R. C. and BLISS, L. C. (1973): Acetylene reduction assay for nitrogen fixation under field conditions in remote areas. Plant Soil, **38**, 209–213.

YAMANAKA, M. and SATO, K. (1977): Syowa Kiti fukin no rikujô shokubutsu gunraku no bunpu to suibun oyobi yôbun tono kankei (Distribution of terrestrial plant communities near Syowa Station in Antarctica, with special reference to water supply and soil property). Nankyoku Shiryô (Antarct. Rec.), **59**, 54–67.

(*Received January 31, 1986; Revised manuscript received April 30, 1986*)