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Availability of Biologically Fixed Atmospheric Nitrogen-15 to Higher Plants

THE direct utilization by higher plants of nitrogen fixed by blue-green algae could play a substantial part in the Earth's nitrogen cycle. That certain species of blue-green algae can fix nitrogen when grown in solution culture is well established[1]. The maintenance of soil nitrogen supplies in rice paddy has been attributed to the growth and fixation of nitrogen by blue-green algae. Recently it was postulated that the blue-green algae may be important in the nitrogen economy of grazing lands[2–4].

The blue-green algae are known actively to excrete nitrogenous materials in solution culture[5]. However, the fate of nitrogenous products fixed by the blue-green algae and/or their associated organisms *in situ* has not been completely delineated. In rice culture it was suggested that algal-N became available to the rice plant following decomposition of algal tissue[6].

Surface soil encrustations of blue-green algae and associated organisms are found on semi-arid grassland soils[1]. Grass seedlings may be found rooted in the algal crusts where moisture and fertility conditions may be more favourable than on non-encrusted soils. Several grass seeds (*Artemesia* sp.) unexpectedly germinated in crusts under experimentation to assess the magnitude of nitrogen fixation. The use of atmospheric nitrogen-15 provided a means of tracing the products of nitrogen fixation and the possible ecological contribution of nitrogen to higher plants.

Native algal crusts were removed intact from the surface of desert-grassland soils. Excessive adhering soil was removed from the underside and 1 cm diam. cores were cut from the crust. Composite samples consisting of 60 random cores were placed in a desiccator containing 74·8 per cent nitrogen; enriched with 0·53 per cent atm. ^{15}N; 4·9 per cent carbon dioxide, 20 per cent oxygen and 0·3 per cent argon. The samples were moistened with de-ionized water by means of a cotton wick, and incubated in a plant growth chamber with 12 h days of 35° C with light intensity of 2,000 ft.-candles and 12 h nights at 18° C.

After incubation the seedlings were cut 1 cm above the algal crust and successively rinsed with 1 N HCl and de-ionized water. Nitrogen was determined by the Kjeldahl method using 1 : 3 : 1 sample : acid : salt ratio and a salt composition of K_2SO_4 : $CuSO_4$: Se in a 100 : 10 : 1 ratio on a weight basis. Digestion was continued for 6 h after clearing as recommended by Bremner[7]. Ammonia was recovered by alkaline distillation and prepared for isotopic nitrogen analysis by the hypobromite method[9]. We thank Dr. Arthur Edwards, Agronomy Department, Iowa State University, for the determination of $^{29}N/^{28}N$ ratios on the Consolidated Electrodynamics model 21–620 mass spectrometer.

Grass seeds (*Artemesia* sp.) germinated and grew for some time on an algal crust sample incubated for 160 days. At harvest the grass tops contained 1·22 mg nitrogen which contained 0·3733 per cent ^{15}N while the host crust nitrogen was enriched with 0·3813 per cent ^{15}N. Both enrichments were significantly greater than either the control crust sample (0·3648 per cent ^{15}N) or a commercial ammonium sulphate standard (0·3663 per cent ^{15}N).

The algal crust materials were lyophilized and ground with mortar and pestle to pass an 80-mesh sieve. Approximately 3·4 per cent of the total algal crust nitrogen was found to be water soluble, which included 1 per cent as water soluble ammonium–nitrogen. Both forms of soluble nitrogen contained significant isotopic enrichments of ^{15}N and may have been the form of labelled nitrogen taken up by the higher plant.

Thus, it appears that nitrogen taken up by the grass originated as atmospheric nitrogen, which was fixed by organisms in the algal crust and subsequently released in a form suitable for utilization by higher plants. Whether release of fixed nitrogen occurred via active excretion of nitrogenous products or followed decomposition of the biological nitrogen fixing organisms or both was not resolved. However, active nitrogen fixation was occurring in the algal crust at the 160 day harvest at the same rate as found on previous harvest dates (3, 6, 12, 24 and 52 days). The accumulation of fixed nitrogen was found[9] to fit a linear regression line with a correlation of $R = 0{\cdot}98$.

Soil nitrogen in grassland areas is depleted by removal by grazing animals and may be lost by soil erosion and denitrification processes. These losses gradually lead to an impoverishment of soil nitrogen except where it may be replenished by biological fixation of atmospheric nitrogen or by artificial means. The evidence provided here indicated that the algal crust organisms fix atmospheric nitrogen and release it in forms available for uptake by higher plants growing in association with the crusts. The crust organisms appear to be an important source of nitrogen on semi-arid grassland.

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