Effect of ammonium or nitrate on nitrogen fixation by a terrestrial Blue-green alga

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Summary  The effects of ammonium or nitrate-nitrogen on biological nitrogen fixation by an algal crust are compared. Nitrate-nitrogen up to 3.0 µmoles N g⁻¹ sand/algae crust at 60% water holding capacity did not affect fixation, whereas an ammonium-nitrogen concentration of 0.2 µmoles N g⁻¹ crust markedly depressed fixation. Consequences of these differential effects are considered.

Introduction
Manufactured fertiliser nitrogen is an important source of the nitrogen required for the growth of crops. This nitrogen is usually added to the soil as ammonium and/or nitrate which can be assimilated by plants. In many soils asymbiotic biological nitrogen fixation by prokaryotic organisms may also be, indirectly, an important source of nitrogen for higher plants. Nitrogen fixed by these microorganisms is ultimately made available to higher plants following death and lysis of the microorganisms and subsequent mineralisation of their fixed organic nitrogen to ammonium and nitrate.

Photosynthetic blue-green algae (Cyanobacteria) can grow and form a ‘crust’ on the surface of many soils over a wide climatic range. Many genera are capable of fixing considerable quantities of atmospheric nitrogen in a year. Values range from 1 kg N ha⁻¹ yr⁻¹ in the Antarctic³, to around 30 kg N ha⁻¹ yr⁻¹ in temperate arable soils⁵,¹⁰. However, many investigations using pure cultures of algae grown in liquid media indicate that fixation is suppressed by inorganic nitrogen sources²,⁹. The aim of this investigation is to compare the effects of increasing levels of ammonium or nitrate-nitrogen on the rate of nitrogen fixation by Blue-green algae growing on the soil surface. An understanding of the effects of nitrogen fertilisers on nitrogen fixation by terrestrial Blue-green algae may therefore assist in improving the efficiency of nitrogen fertiliser usage.

Materials and methods
The alga, Nostoc muscorum (No. 1453/12) was obtained from the Culture Centre of Algae and Protozoa, Cambridge, England. It was routinely grown aseptically and maintained as a crust on washed sea sand in glass Petri dishes. The sand was 100% saturated with the medium of Allen and Arnon¹ without inorganic nitrogen at pH 7.5. From these stock cultures small pieces of algal crust ca. 0.5 cm² were placed on the surface of sand in 500 ml beakers 13 cm deep. The sides of the beakers were covered with aluminium foil to prevent algal growth down the inside walls of the beakers. The sand was brought to 60% water holding capacity (to obtain maximum algal growth⁹) with inorganic medium without or with inorganic nitrogen as either NaNO₃ or (NH₄)₂SO₄ and all beakers were incubated at 22°C and 4000 lux for 10 days. To determine the effect of depth of nitrogen fixation, sand cores 3.5 cm deep were taken with a cork borer from beakers without inorganic nitrogen after 10 days incubation and these cores, 8 mm diameter, were divided into 7 0.5-cm portions. For experiments to determine the effect of ammonium or nitrate, cores from beakers amended with ammonium or

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nitrates-nitrogen from 0–0.5 cm depth were used. Each 0.5 cm core was placed in a 7 ml serum bottle and water was added to bring the core to 100% saturation (to obtain maximum nitrogenase activity). The acetylene reduction assay was used to determine comparative nitrogen fixation rates by measuring nitrogenase activity. Briefly, acetylene was injected into each bottle so that the atmosphere was 10% v/v acetylene in air. Ethylene production after 6 h at 22°C and 4000 lux was determined by gas chromatography and standards of ethylene in air were used for calibration. Reduction rates were measured on a per mg chlorophyll basis by extraction of the chlorophyll from each core in 80% acetone and spectrophotometric estimation using a specific extinction coefficient of 84.0 at 663 nm.

Results and discussion

Blue-green algae are photosynthetic organisms and should therefore grow only on the soil surface where the energy and reductant required for growth and nitrogen fixation is provided by photosynthesis. Fig. 1 shows that fixation occurred primarily in the top 0–0.5 cm of the sand core. Some fixation was detected in sand 0.5–1.0 cm below the surface. This may have been due to contamination of the sand during partitioning of the core prior to analysis; or movement of some algal filaments to this depth during growth of the surface crust and initiation of photosynthesis under illumination during the acetylene assay incubation period.

Fig. 2 shows the effects of increasing nitrate or ammonium concentration on fixation by algal crusts at 0–0.5 cm depth. Increasing nitrate concentration had no significant effect whereas a low ammonium concentration markedly depressed fixation. Increasing ammonium concentration gradually depressed fixation further. These findings support earlier work with algae grown in liquid culture.

![Graph](image)

Fig. 1. Nitrogen fixation rates by algae in sand cores from 0–3.5 cm depth. Inorganic medium without combined inorganic nitrogen. Vertical lines represent the standard errors of the means of triplicate samples.