

The effect of glucose, P, Co and Mo on heterotrophic acetylene reduction in a sub-Antarctic peat

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Addition of P, Co or Mo solutions, separately or together, to peat samples from an oligotrophic mire at Marion Island (46°54'S, 37°45'E) did not significantly increase acetylene reduction rates over those exhibited by samples to which an equal amount of distilled water was added. Addition of glucose, with or without P, Co and/or Mo, markedly enhanced acetylene reduction rates. It is concluded that readily utilizable energy sources limit N fixation in the mire peat but the possibility that inorganic nutrients might be secondarily limiting could not be discounted.

Byvoeging van P, Co of Mo-oplossings, afsonderlik of gesamentlik, tot veenmonsters vanaf 'n oligotrofiese moeras van Marion-eiland (46°54'S, 37°45'O) het nie die asetileen-reduksietempo's betekenisvol teenoor dié van monsters waartoe 'n ekwivalente hoeveelheid gedistilleerde water gevoeg is, verhoog nie. Byvoeging van glukose, met of sonder P, Co en/of Mo, het asetileenreduksietempo's aansienlik gestimuleer. Beskikbare energiebronne beperk N-fiksering in die moerasveen waarskynlik en die moontlikheid dat anorganiese voedingstowwe sekondêr beperkend is, kan nie uitgesluit word nie.

Introduction

Peat samples from an oligotrophic mire at Marion Island (46°54'S, 37°45'E) did not reduce acetylene in the dark unless fortified with glucose and phosphate and incubated anaerobically at 20°C (Smith 1985). A preliminary, qualitative study suggested that the stimulation of nitrogenase activity, measured by the acetylene reduction (AR) assay, was due to glucose rather than phosphate, although possible synergistic effects of adding the two together were not assessed. Molybdenum and iron are important constituents of the enzyme and its activity has been shown to depend on the presence of Mg (Postgate 1978). Sesquioxides are important weathering products of the volcanic parent material at Marion Island (Smith 1979a) and the peats contain substantial concentrations of Fe. Mg is also unlikely to be limiting since, due to the influence of wind-blown sea-spray, it is the most abundant divalent cation in many of the island peats. Alexander *et al.* (1978) demonstrated that Mo and Co stimulated nitrogenase activity in *Peltigera aphthosa* from an Arctic tundra but Smith (1984) found that autotrophic (cyanobacterial) acetylene reduction at Marion Island is depressed by the addition of either or both of these elements.

This paper describes the effects of glucose, phosphate, Co and Mo, added separately or together in various combinations, on heterotrophic acetylene reduction by peat samples from an oligotrophic mire at Marion Island.

Materials and Methods

500 g freshly-collected peat (top 10 cm layer) was slurried with

1000 ml filtered (0.2 µm) distilled water. 50 ml subsamples of the slurry were enriched by adding 1 ml of solution of the particular nutrient/s and mixing thoroughly. Final concentrations (per g wet slurry) of nutrient/s added were: glucose, 1 000 µg g⁻¹; P (as KH₂PO₄), 50 µg g⁻¹; Co (as CoCl₂·6H₂O), 0.01 µg g⁻¹; Mo (as NaMoO₄·2H₂O), 0.15 µg g⁻¹. Filtered distilled water (1 ml) was added to a further 50 ml subsample of the slurry to serve as a control. Aliquots (3-5 g, accurately weighed) of each slurry were placed in six, 10 cm long 9 mm i.d. cylindrical glass incubation tubes, open at the bottom and possessing a short, open side-arm near the top. The remainder of each slurry was used for pH determination (glass combination electrode) and moisture content (oven-drying at 105°C to constant weight).

All of the incubation tubes were stoppered at the bottom and the side-arms fitted with new serum stoppers, through which the tubes were evacuated to 0.01 atm. before flushing with argon. The evacuation-flushing cycle was repeated three times to establish anaerobic conditions. The tubes were placed in the dark at 20°C for 24 h before being placed on a manifold, connected at one end to a supply of Ar containing 10% C₂H₂ which had been scrubbed twice through conc. H₂SO₄. The other end of the manifold was connected via a manometer to a vacuum pump. The flasks were slowly evacuated to 0.01 atm. before relieving the vacuum with Ar/C₂H₂. This evacuation-flushing procedure was repeated four times at intervals of one minute. To two tubes of each fortification series pure Ar was added instead of Ar/C₂H₂, serving as controls for assessing endogenous C₂H₄ production. Subsamples of the scrubbed C₂H₂ were taken to determine levels of C₂H₄ contamination.

Following the gas introduction procedure all the tubes were incubated in the dark at 20°C for 12 h. The tubes were then vibrated strongly on a vortex stirrer for 30 s and the headspace sampled through the serum stopper using double-sided needles into 3 ml Venoject® (Terumo Corp., Japan) tubes which had been re-evacuated to a consistent vacuum. The AR assay procedure described has been shown to maximise AR estimates on waterlogged samples, in that the evacuation-flushing cycles ensure saturation of the peat with the assay gas and the vigorous agitation before headspace sampling facilitates C₂H₄ diffusion out of the peat solution (Matsuguchi *et al.* 1979).

C₂H₄ in the gas samples was determined within 6 h by gas chromatography on Poropak N. N₂ was the carrier gas and detection was by flame ionization. C₂H₄ concentrations were reported directly by a Hewlett-Packard 3390A Reporting Integrator connected to the GC. Observed C₂H₄ concentrations were corrected for pressure/volume changes associated with the transfer of the gas subsample between the incubation vessel and Venoject® tubes and for the amounts of C₂H₄ dissolved in the peat slurry, assuming a solubility product equal to that of pure water. AR rates are expressed per g dry weight of slurry.

Results and Discussion

Levels of "available" P in the island peats are low, generally < $3 \mu\text{g g}^{-1}$ wet weight (Smith & Steyn 1982) and the concentration added here represents a considerable fortification, up to levels found at areas manured by animals and showing enhanced plant vitality (Smith 1979b) and soil bacterial counts (Steyn & Smith 1981). Endogenous concentrations of Mo and Co are unknown; the amounts added in the investigation were within the range of those found by Alexander *et al.* (1978) to stimulate N fixation in *Peltigera aphthosa*.

None of the nutrient additions altered the pH (4.9-5.0) of the peat slurry. Endogenous C_2H_4 production did not occur for any of the peat subsamples. Fortification with P, Co or Mo, either separately or together, did not significantly ($P \leq 0.05$) enhance AR rates above those exhibited by the unamended peat subsamples (Fig. 1). Addition of glucose markedly stimulated AR. The addition of inorganic nutrients in combination with glucose appeared to cause a greater increase in nitrogenase activity than the addition of glucose alone; however, the differences were not significant at $P \leq 0.05$.

A stimulation of AR (at 22°C) by glucose was also found for mesic meadow peats at Devon Island (75°N ; Canadian high-Arctic); in the absence of glucose the peat samples failed to show evidence for N fixation (Jordan *et al.* 1978).

Contrary to the results of previous laboratory incubations (Smith 1984) unamended peat samples from the oligotrophic mire at Marion Island exhibited detectable, although low ($0.5 \text{ nmol C}_2\text{H}_2 \text{ g}^{-1} \text{ h}^{-1}$) AR rates. This may be due to the longer (12 rather than 3 h) incubation times employed in the study reported on here. However, glucose-fortified subsamples exhibited much lower ($20\text{--}27 \text{ nmol C}_2\text{H}_2 \text{ g}^{-1} \text{ h}^{-1}$) reduction rates compared with those ($404\text{--}932 \text{ nmol g}^{-1} \text{ h}^{-1}$) estimated previously on glucose-fortified samples from the same mire (Smith 1984).

The results suggest that heterotrophic bacteria potentially able to fix N are present in the mire peat. However, because of a lack of readily utilisable energy sources, fixation rates are very low. This agrees with the observation that bacterial growth in the island peats is primarily limited by the availability of energy-rich substrates (Lindeboom 1979). Once the energy-substrate limitation to nitrogenase activity has been relieved, inorganic nutrients may have a secondary limiting

role but this could not be unequivocally demonstrated in this study. Low temperatures might be expected to further reduce nitrogenase activity, since field temperatures for most of the year are very much lower than that (20°C) employed in the laboratory incubations.

N fixation by methane-oxidizing bacteria cannot be detected by the AR assay procedure. CH_4 formation may have occurred under the anaerobic conditions imposed in this study but its subsequent utilisation would be strongly inhibited by the same conditions and by the presence of C_2H_2 . Nothing is known regarding the role of methane-oxidizers in the N cycle of sub-Antarctic terrestrial ecosystems; however, it is unlikely that either methane formation or oxidation are important metabolic pathways in the aerobic surface layers of the oligotrophic mire peats at Marion Island.

Photo-autotrophic N fixation (especially by moss-cyanobacteria associations; Smith and Russel 1982) is probably more important than heterotrophic fixation at Marion Island; in this respect the island ecosystem is similar to Arctic and sub-Arctic regions, where heterotrophic fixation is not generally regarded as an important source of reduced N (Alexander 1974; Jordan *et al.* 1978).

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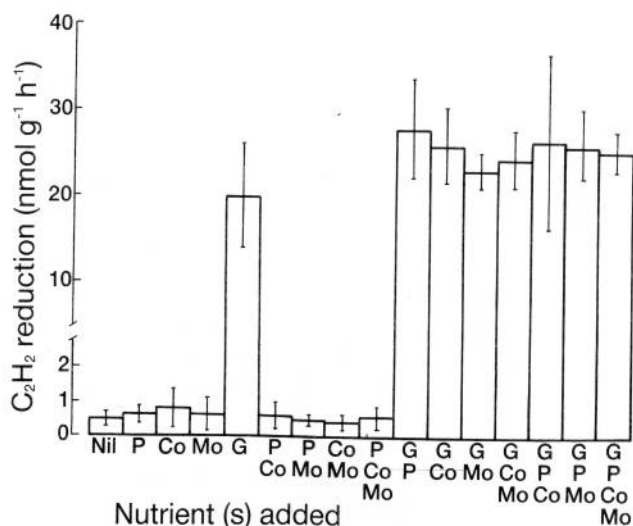


Fig. 1. Acetylene reduction by peat samples with and without added nutrients. Vertical lines in each bar indicate ± 1 standard deviation of the mean ($N=4$). Note change of ordinate scale.