Heterotrophic nitrogen fixation (C₂H₂ reduction) as influenced by phosphorus application in paddy soils

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Summary The influence of phosphorus application on soil nitrogenase and N₂-fixers in tropical paddy soils differing in their properties was investigated in a laboratory incubation study. Application of P stimulated the soil N₂-ase in an alluvial soil and in a P-deficient soil under both flooded and nonflooded conditions. The stimulation of N₂-ase by P was more pronounced under nonflooded conditions. A corresponding increase in N₂-ase occurred with an increase in the P level at least up to 80 ppm level. A depressive effect of P on N₂-ase occurred after 16 days under nonflooded conditions when the level of P was increased to 100 ppm. But under flooded conditions the stimulation was almost continuous. Results indicate that the effect of P on N₂-ase depended on the water regime, level of P and soil type. Addition of P had a little effect on the population of N₂-fixing micro-organisms in alluvial soil. On the contrary, addition of P stimulated the population of Azospirillum and Azotobacter in a P-deficient soil. Data suggested that the alteration in the N₂-fixing microbial populations and the levels of available P might be responsible for changes in the N₂-ase activity in these soils. Application of superphosphate and dicalcium phosphate stimulated N₂-ase activity; while the rock phosphate exhibited an innocuous effect in alluvial and P deficient soils. In Sukinda soil, however, superphosphate slightly stimulated N₂-ase at early stages, while other P sources had innocuous effect. Results indicate that the level and source of applied P exhibited differential influence on N₂-ase and N₂-fixers in tropical paddy soils.

Introduction

Nitrogen fixation is of considerable significance for lowland rice and several factors determine the extent of the contribution and participation of free-living and associative micro-organisms. With the introduction of high yielding rice cultivars, the fertilizer management practices have substantially altered. The high fertilizer input for achieving desired yields and the altered management practices necessitate investigation on their impact on processes of concern to soil fertility.

Phosphorus application to rice soils has often led to the increased crop yields. It has been established that addition of P influenced the N availability and even counteracted the toxic effects of high levels of N. Nitrogen combined with P promoted rhizosphere N₂-ase activity. The P availability enhanced with an increase in the moisture content of the soil in presence of organic matter. P application
favoured the N$_2$-ase activity in the rice rhizosphere under intermediate deep water situations$^{18}$. Application of naturally occurring P sources like rock phosphate, though slow in availability in comparison to synthetic chemical fertilizers, can supplement the P needs of crop over long periods after application$^{6,11}$. Information on the effect of P on soil N$_2$-ase in different soil types under tropical conditions is not available. We investigated the effect of different levels and sources of P on soil N$_2$-ase activity and nitrogen fixers in three rice soils differing in their properties.

Materials and methods

Alluvial, P-deficient and Sukinda soils were collected from the rice growing tracts of India. Some of the properties of these soils are indicated in Table 1. The soils were air dried, screened (<2 mm), amended uniformly with 0.5% cellulose and were placed in B-D vacutainer (New Jersey) tubes in 5-g amounts. One set of the soils were flooded (1.5 cm standing water) and the other was held nonflooded (50% water holding capacity). All the treatments were replicated three times. P was applied as K$_2$HPO$_4$ in solution to provide 0, 10, 20, 40, 80, and 100 ppm P levels.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>Organic carbon (%)</th>
<th>Total nitrogen (%)</th>
<th>Cation exchange capacity (meq/100 g soil)</th>
<th>Soil separates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alluvial</td>
<td>6.3</td>
<td>0.62</td>
<td>0.07</td>
<td>15.5</td>
<td>25.9</td>
</tr>
<tr>
<td>Sukinda (laterite)</td>
<td>7.2</td>
<td>0.63</td>
<td>0.041</td>
<td>6.0</td>
<td>14.6</td>
</tr>
<tr>
<td>P-deficient (alkaline)</td>
<td>8.4</td>
<td>0.60</td>
<td>0.07</td>
<td>51.5</td>
<td>32.6</td>
</tr>
</tbody>
</table>

The influence of different sources of P on N$_2$-ase activity was studied in another experiment. Rock phosphate, dicalcium phosphate and superphosphate were applied to the respective soils to yield the final concentration of 0, 20, 40 and 60 ppm P. The remaining P (Olsen's P) in soils was also determined by colorimetric assay$^8$.

The N$_2$-ase activity of the soil was determined periodically by stoppering the tubes and replacing the gas phase by high purity C$_2$H$_2$ (10% by volume) through a gas-tight hypodermic syringe. Tubes without C$_2$H$_2$ were also included for the assay of indigenous C$_2$H$_4$. The tubes were then kept at 28°C for 24 h in the dark and shaken intermittently to facilitate C$_2$H$_2$ diffusion. A 0.5 ml sample of the gas phase from each tube was analysed for evolved C$_2$H$_4$ on a gas chromatograph fitted with a hydrogen flame ionization detector and 1500 x 3 mm column filled with 100–120 mesh Porapak-R at a column temperature of 60°C$^{12,15}$. High purity nitrogen at a flow rate of 30 ml/min served as the carrier gas. The N$_2$-ase activity (means of three replicates) was expressed as n mole of C$_2$H$_4$ formed for g soil/day. The indigenous production of C$_2$H$_4$ was negligible. The results were subjected to statistical analysis (LSD).

The populations of different groups of N$_2$-fixing micro-organisms in P-amended soils were counted by conventional serial 10-fold dilution technique in N-free media. Azospirillum was counted following the method suggested by Okon et al.$^{16}$ and populations of anaerobic N$_2$ fixers and Azotobacter as per Rao et al.$^{17}$. Results presented are the means of five replicates for Azospirillum and anaerobic N$_2$ fixers and three replicates for Azotobacter populations.