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Biogeochemical cycling bacterial activity in response to lime and fertilizer applications in pond systems

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Abstract. Lime-induced changes of biogeochemical cycling bacteria were examined using doses of 25, 50, 100, 200, 500, 1000 and 2000 kg ha^{-1} in the first part, and post-lime inorganic and organic fertilization in the second part of the experiment. Heterotrophic, ammonifying, denitrifying, cellulose decomposing and phosphate solublizing bacteria were drastically reduced by 30–90% in 1000 kg ha^{-1} and 51–91% in 2000 kg ha^{-1}. Density differences were minor in the remaining test doses for all groups of bacteria except for the dinitrifiers which were reduced in 500 kg ha^{-1} by 90% over the control. Similarly, reduced rates of ammonification, nitrification and denitrification were dose-dependent. Inorganic fertilization following lime application of 2000 kg ha^{-1} resulted in no marked increment of the counts of bacteria and activity processes, whereas organic manuring increased the density (50–200%) and the activities (212–292%) as well. Lime-induced changes of bacterial population and activities were explained as a result of high pH stress as well as a major shift in the CO_2-HCO_3-CO_3 equilibrium system. Increase of major nutrients in the post-lime organic treatment was significantly higher than that of inorganic fertilization resulting in favourable N/P ratio and water quality conducive to fish farming after 45 days of liming.

Key words: bacterial activities, biogeochemical cycling bacteria, inorganic fertilizer, liming, organic manure

Introduction

Lime is often used in aquaculture ponds to raise total alkalinity in the water column to a level at which carbon is not limiting for phytoplankton growth, whereas it is used in agriculture to raise soil pH to around 6.5 or 7.0 because availability of nutrients is greatest at these pH levels in most soils. In aquaculture ponds, lime is used to correct the acidity of soil, promote bacterial decomposition of organic matter (Alexander, 1961); establish a strong pH buffer system (Wolny, l967); counteract the poisonous effects of excess magnesium, potassium and sodium ions (Jhingran, 1995); reduce the toxic effects of certain heavy metals like copper, nickel, zinc and aluminium

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(Yan *et al*., 1977); fix harmful acids and inorganic acids (Hansell and Boyd, 1980); displace certain other fertilizers from organic colloidal systems and thus make available greater amounts of potash and phosphate (Hora and Pillay, l962) urea and ammonium nitrogen but has no effect on nitrate nitrogen (Chatterjee and Saha, 1986; Broberg, 1978; Soechtig, 1989; Smayda, 1990; Babin *et al*., 1994). However, Masuda and Boys (1994) found that agricultural limestone applied at 0.2 kg m^{-2} did not affect soluble reactive phosphate and total plosphorus concentrations of ponds. Moreover, liming materials release ions that contribute equally to total alkalinity and hardness and are thus useful as fertilizers. Boyd (1982) observed a close relationship between the base saturation of bottom mud and total hardness of water.

Lime, by its toxic and caustic action, kills pathogens and fish parasites as well as other undesirable organisms and helps to prevent diseases like acanthocephaliasis (Huang *et al*., l989), epizootic ulcerative syndrome (Das and Das, 1993) and other diseases of cultured fish. Lime, or alum-treated manganese ores, are known to effectively remove heterotrophic bacteria and *E. coli* from polluted canal water (Prasad and Chaudhuri, 1995), but Russell *et al*. (1993) found substantial increases of bacterial biomass even exceeding that of phytoplankton during the summer because bacteria are less limited to carbon after lime application. However, Bell and Tranvik (1993) found no significant difference in bacterial activity between acidified and limed lakes.

In India, lime treatment comprises an important component of pond management. In grow out ponds, liming is followed by successive fertilizer applications using inorganic and organic materials after two weeks. Information about the consequences of lime application and subsequent pond fertilization on the microbial biogeochemical cycling bacteria are not available for fish ponds in India.

Because of quantitatively different chemical ingredients in inorganic and organic materials, they are likely to exert different physico-chemical *milieu* in ponds primarily by pH shift. Such a shift in pH might influence the functional responses and stability of pond metabolism through microbial pathways. The purpose of the present study was to determine the microbial activities and density differences of some selected nutritional groups of bacteria in relation to physico-chemical characteristics of aquatic systems that differed in subsequent fertilization schedule.

Materials and methods

Two experiments were performed. In the first phase, 24 outdoor tanks (300 l) provided with 5 cm dry soil were filled with dechlorinated ground water and treated with cattle dung (200 g) and poultry droppings (200 g) two weeks before lime application. Commercial grade slaked lime [$Ca(OH)_2$] was applied to 2l tanks, allotted to seven treatments in triplicate, in a two-fold increase in doses starting from

0.75 g to 60 g per tank, corresponding to 25, 50, 100, 200, 500, 1000 and 2000 kg ha^{-1}. Three tanks were not treated with lime and served as controls (Figure 1).

The fertilization protocols followed in the second phase were (a) inorganic, (b) organic and (c) inorganic followed by organic. Fifteen outdoor tanks were initially treated with mixed manure following the same dose as used earlier. After two weeks, 65 g of lime (corresponding to @ 2000 kg ha^{-1}) was applied to 12 tanks, and three were used as controls. After the next two weeks, each of the six, lime-treated tanks were fertilized with urea (4 g) and single superphosphate (16 g), three tanks with organic manure (150 g cattle dung + 100 g poultry droppings) and the remaining three tanks served as controIs. After 15 days, three inorganic fertilized tanks received the earlier dose of organic manure, whereas a second installment of inorganic and organic fertilizers was applied to the respective fertilized groups (Figure 1).

Samples of water and surface sediments were collected every other day or weekly from each tank and were monitored for heterotrophic (HB), ammonifying (AB), ammonia oxidizing (AOB), denitrifying (DNB), phosphate solubizing (PSB) and cellulose decomposing (CDB) bacteria as well as for natural and potential bacterial activity rates of ammonification, nitrification and denitrification. The natural ammonification and nitrification rates were defined as net increase in the concentrations of ammonium-N, nitrite-N and nitrate-N in water and sediment samples afier four and eight days of incubation, respectively, at *in situ* temperature. The decrease in concentrations of three forms of nitrogen after eight days of incubation gave the natural denitrification rates. The bacterial activities in presence of energetic substrates peptone, ammonium sulphate and potassium nitrate were considered as potential ammonification, nitrification and denitrification rates, respectively (Rodina, l972; Antipchuk, 1979). Routine water quality parameters were determined every week following the standard methods described in APHA (1995).

All the results were statistically interpreted. Treatment means were compared by means of one way analysis of variance (ANOVA) performed separately on each date of sampling. Duncan's Multiple Range test was applied to find the treatment differences. The level of significance was accepted at $P < 0.05$.

Results

Bacterial population

There was a dose-dependent response of different groups of bacteria to lime application. The low dose treatments (25–200 kg h^{-1}) showed either unaltered responses in AB, DNB and CDB or marginal declines (2.3–12%) in others (HB, PSB and AOB). Treatment-wise, decline for all groups of bacteria was maximum (51–92%) in the 2000 kg ha^{-1} followed by 1000 kg ha^{-1} (28–58%). While in 500 kg

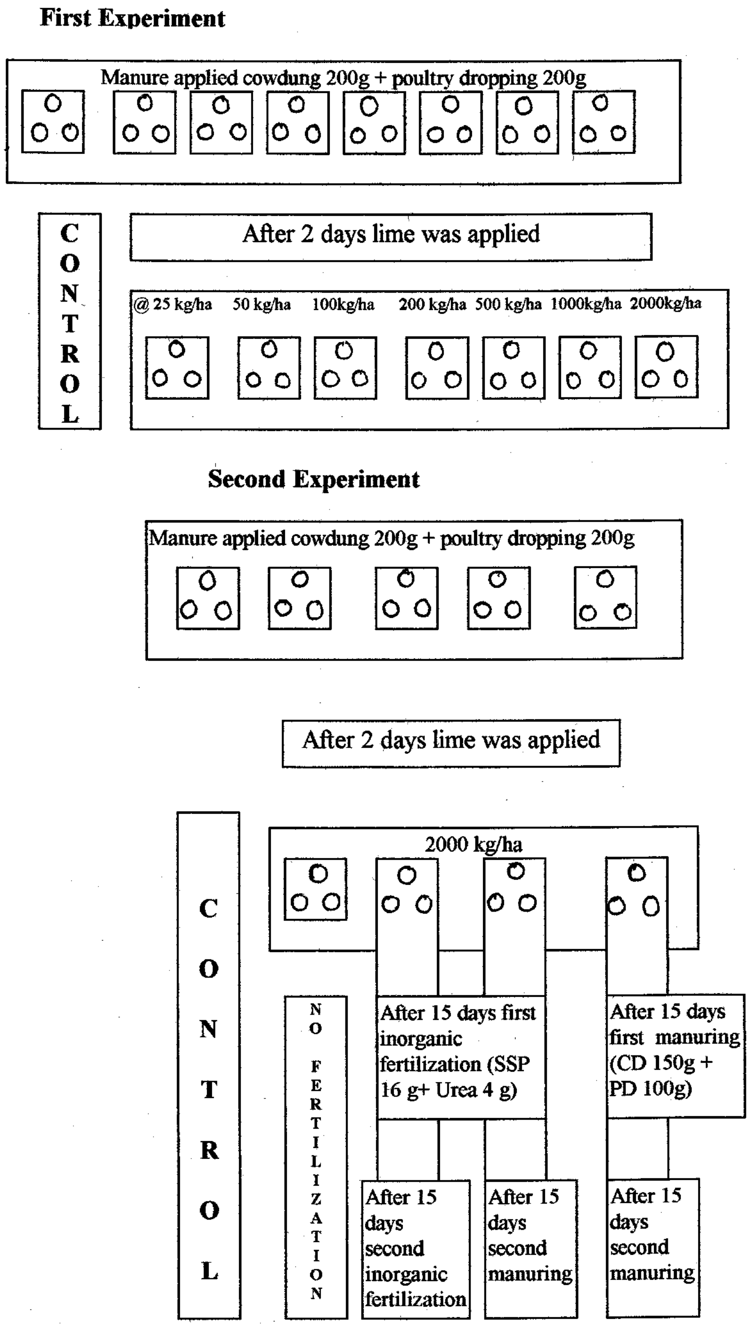


Figure 1. Experimental design and steps followed in the first and second liming trials.

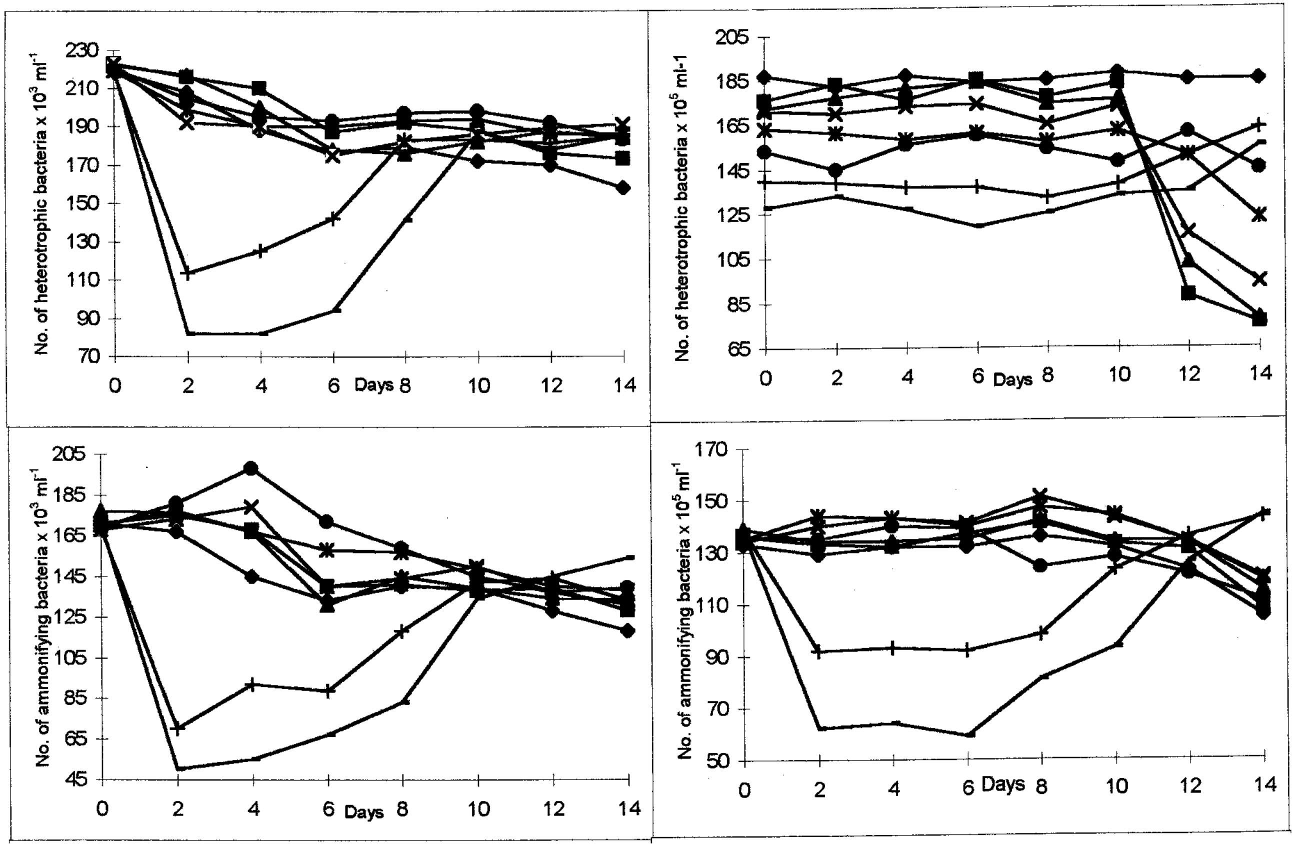
ha^{-1}, there was no marked decline of all groups of bacteria, DNB population showed a decline of 56 and 90% in sediment and water respectively. The recovery of HB (Figure 2) was earlier (8 to 10 days) followed by AB and DNB (12 days) and CDB populations (14 days). Treatment differences were significant (DMR test; $P < 0.05$) for all the groups of bacteria during most of the period of investigation.

The growth curve of all the groups of bacteria in the second experiment consisted of a primary declining phase, a stationary phase, a recovery phase and a secondary declining phase. The primary declining phase was observed two days after lime application, which was more severe (83–89%) in the case of PSB, CDB, DNB and AOB, and relatively less (54–67.4%) in AB and HB. The stationary phase lasted for two days in the case of HB and AB, but extended up to six (PSB and CDB), or eight days (DNB and AOB). As a result, recovery started much earlier in the former than in the latter groups. The peak period ranged from day 14 in PSB, AB and HB to day 22 in CDB, AOB and DNB. Again, the CDB population did not exhibit a peak as conspicuous as the other groups. Thereafter, no marked change was registered in the population counts of CDB, AB and HB, whereas a definite decline was observed in the counts of the AOB population.

The responses of sediment bacteria to lime application were basically similar to that of water, showing a population decline within two days of lime application. The decline of AOB was markedly less (22%) than for the remaining groups of bacteria (55–70%). The stationary phase in DNB was as long as 10 days, whereas it was a mere two days for the rest of the groups. The recovery phase in DNB resulted in the development of peaks about one or two weeks later than for the other groups. The secondary declining phase was observed at the end of the recovery phase. The decline was as high as 80–90% in the case of CDB, DNB and AOB.

Subsequent fertilization by inorganic materials resulted in a substantial rise of all the bacterial groups seven days after application. Increase was more intense (148.7–148.8%), in the case of DNB and AOB, less (66%) in CDB and least (13.8–19%) in others (PSB, AB and HB). Inorganic fertilizer application in the lime-treated tanks induced the growth of sediment bacteria ranging from 41 to 119%. The increase was, however, 7.5–19.8% in the case of AB and HB. Organic manure application either after lime or lime plus IF treatment induced the growth of CDB (63.3–285%) and AOB populations (63–92.2%) over lime plus IF treatment (Figure 3).

In sediment, a further rise in the counts of all the groups of bacteria was observed following application of organic manure either in lime or in lime plus IF treated tanks. The rate of increase was greater in the former than in the latter. Among all groups of bacteria, the AOB and DNB populations increased much less (5–12%) than PSB, CDB, AB, and populations (27–88%) (Figure 3). Treatment differences were significant (DMR test; $P < 0.05$) for all the groups of bacteria during most of the period of investigation.


No. of heterotrophic bacteria x 10³ ml⁻¹
230
210
190
170
150
130
110
90
70
0 2 4 6 Days 8 10 12 14
No. of heterotrophic bacteria x 10⁵ ml-1
205
185
165
145
125
105
85
65
0 2 4 6 Days 8 10 12 14
No. of ammonifying bacteria x 10³ ml⁻¹
205
185
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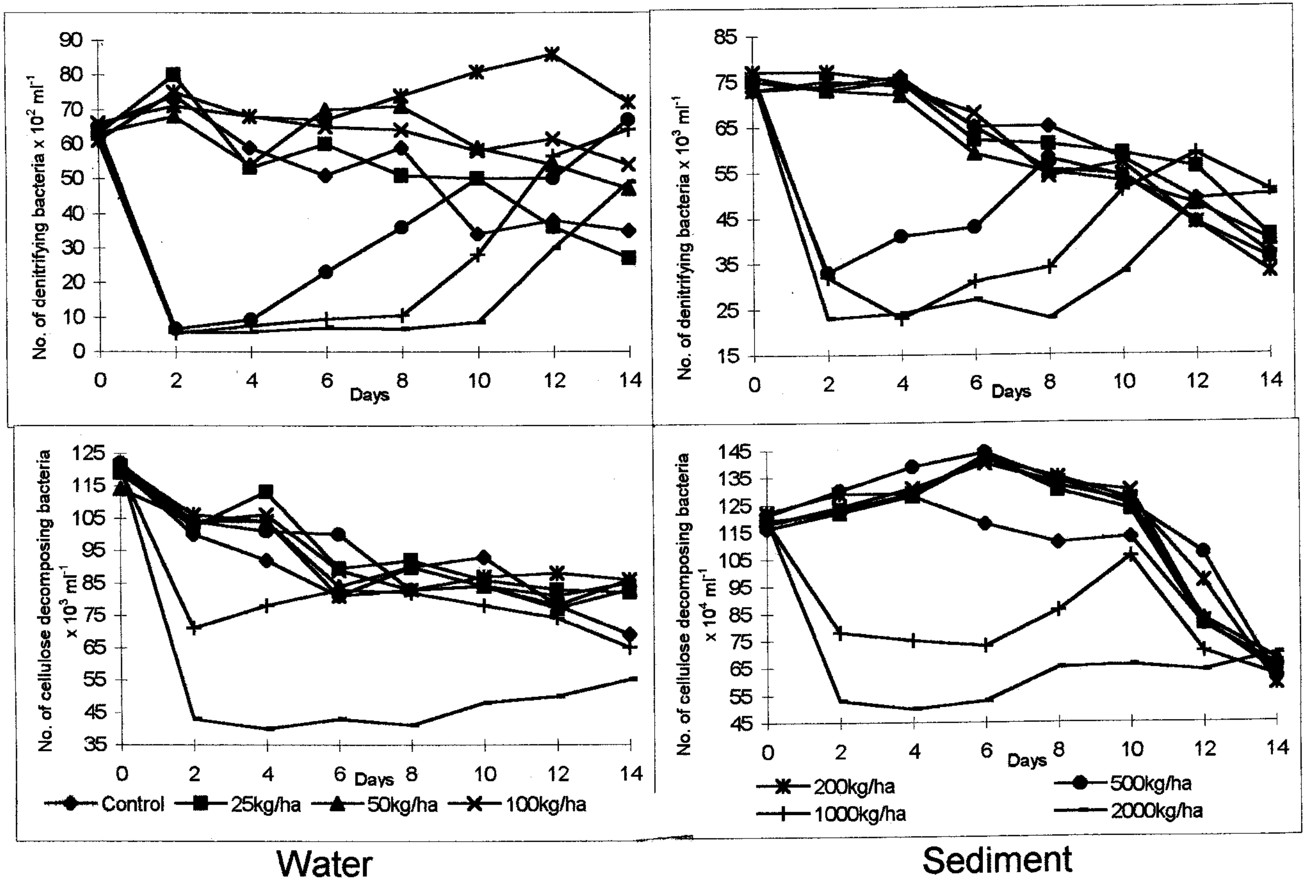

Figure 2. Temporal responses of the counts of heterotrophic (HB), ammonifying (AB), phosphate solubilizing (PSB) and cellulose decomposing bacteria (CDB) to different doses of lime treatment in water and sediment.

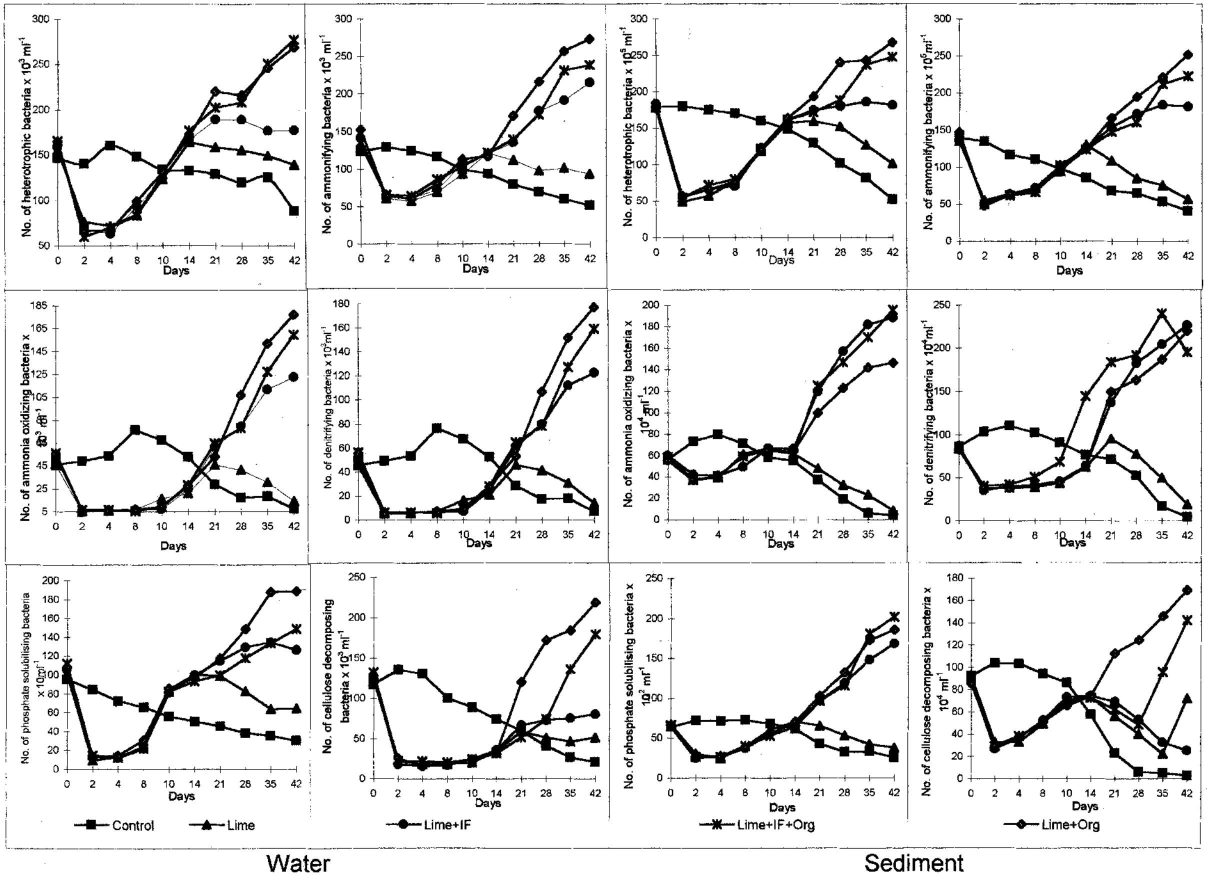

Figure 3. Temporal responses of the counts of heterotrophic (HB), ammonifying (AB), ammonia oxidizing (AOB), denitrifying (DNB), phosphate solubilizing (PSB) and cellulose decomposing bacteria (CDB) to lime and post lime fertilization in water and sediment.

Bacterial activity

Ammonification

Ammonification showed a dose-dependent reduction in both water and sediment. There was complete inhibition of both natural and and potential ammonification on day 4 after lime application at the rate of 1000 and 2000 kg ha^{-1}, which did not achieve pretreatment values, although showed some recovery towards the end of the experiment. The degree of inhibition ranged from 123–164% in the case of 1000 kg ha^{-1} and 140–200% in 2000 kg ha^{-1} in water and sediment. For ammonification potential, inhibition was 42 and 35% in the case of 1000 kg ha^{-1} and 52% and 53% in the case of 2000 kg ha^{-1} in water and sediment respectively (Figure 4).

Responses to qualitatively different fertilization were also similar in the second phase experiment. There were significant treatment differences (ANOVA, $P < 0.05$) due to inorganic and organic fertilization. While there was no marked change in the trend of variation due to inorganic fertilization, organic manure, on the other hand, exerted a strong positive response (57–100%) 14 days after application (Figure 6).

Nitrification

Lime application resulted in inhibition of nitrification at NO_2-N and at NO_3-N as high as 72–210% in the case of 1000 kg ha^{-1} and 81–305% in the case of 2000 kg ha^{-1}. The degree of inhibition was 30–42% higher in water samples than in sediment (Figure 4).

There was complete cessation of both the natural and potential nitrification either at NO_2-N or NO_3-N following four days of lime application. Again, the inhibition was greater in the case of water (250% at NO_2-N and 179% at NO_3-N), than in sediment (l55% and 112%). However, there was slight recovery of inhibition after day 14, but no natural synthesis occurred until day 45 of lime treatment. Organic manure caused the restoration of pretreatment values much earlier (ANOVA, $P < 0.05$) than inorganic fertilization (Figure 6). The pattern of variation was almost identical in sediments.

Denitrification

Similarly, the denitrification response to lime application showed full inhibition in water and sediment samples four days after the application of 1000 and 2000 kg ha^{-1}. Natural denitrification was reduced by 170, 328 and 166% at NO_3-N, NO_2-N and NH_4-N respectively in the case of 1000 kg ha^{-1}, whereas it was 216, 290 and 200% in the case of 2000 kg ha^{-1}. Likewise, potential denitrification at different levels was reduced by 120–146% (Figure 5). Further, the rates of natural and potential denitrification at NO_3-N, NO_2-N and NH_4-N tended to decrease gradually

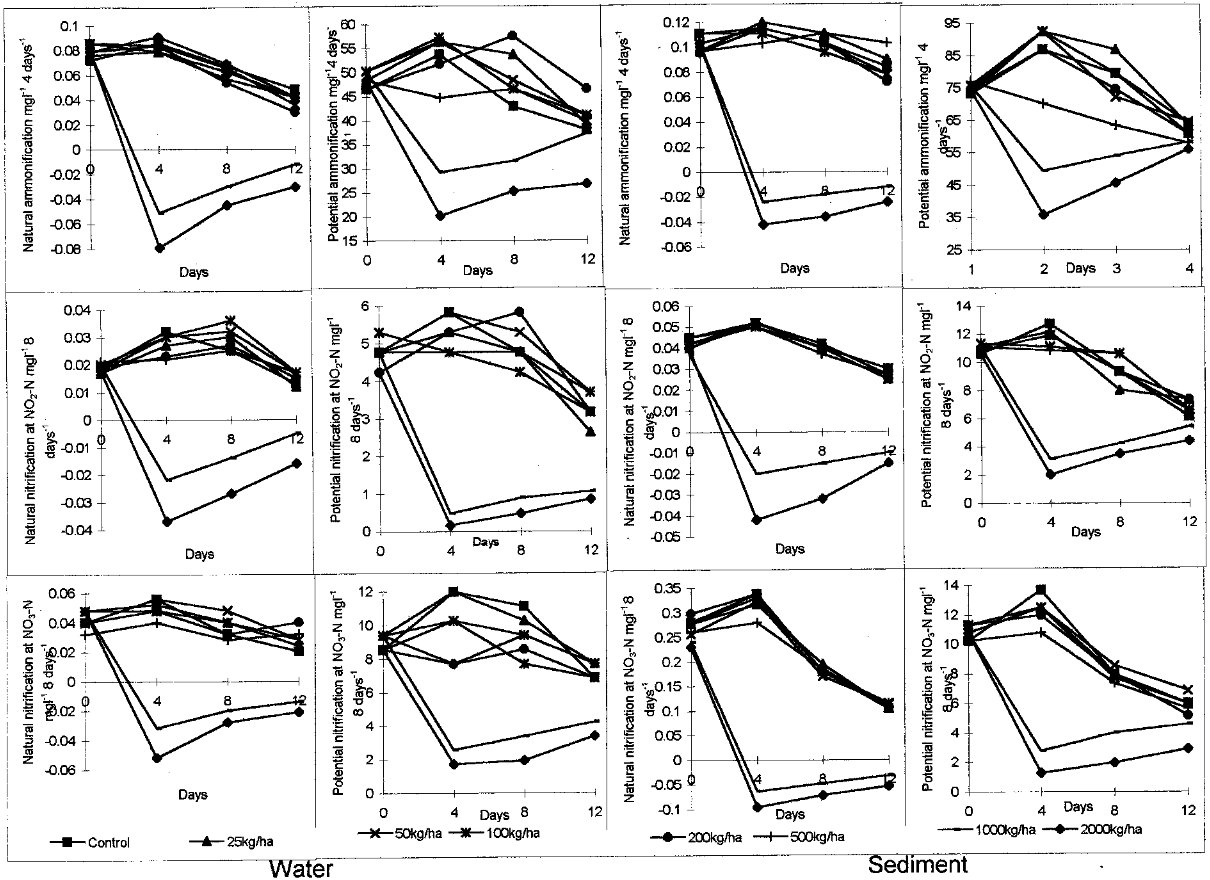

Figure 4. Temporal responses of rate of ammonification and nitrification (natural and potential) to different doses of lime treatment in water and sediment.

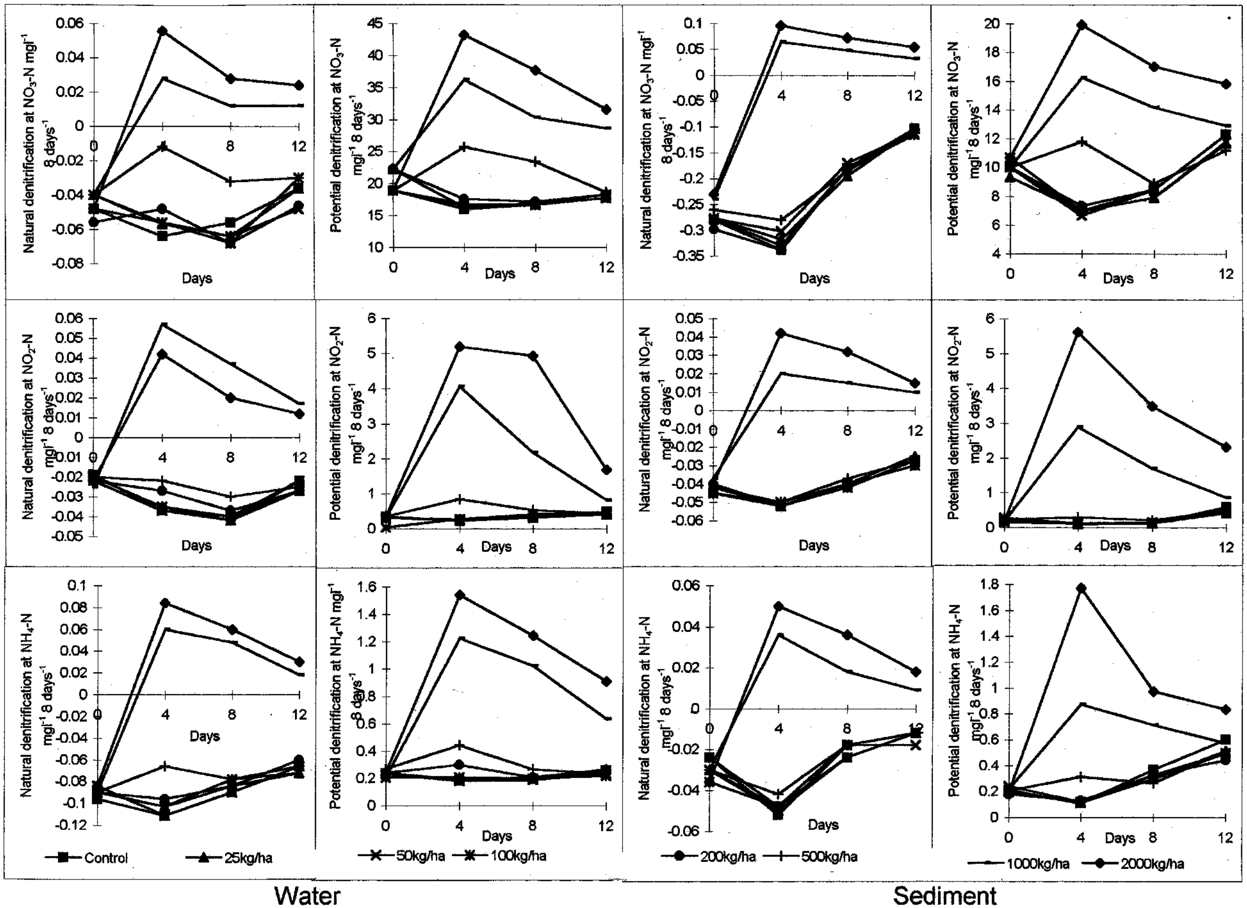

Figure 5. Temporal responses of denitrification (natural and potential) to different doses of lime treatment in water and sediment.

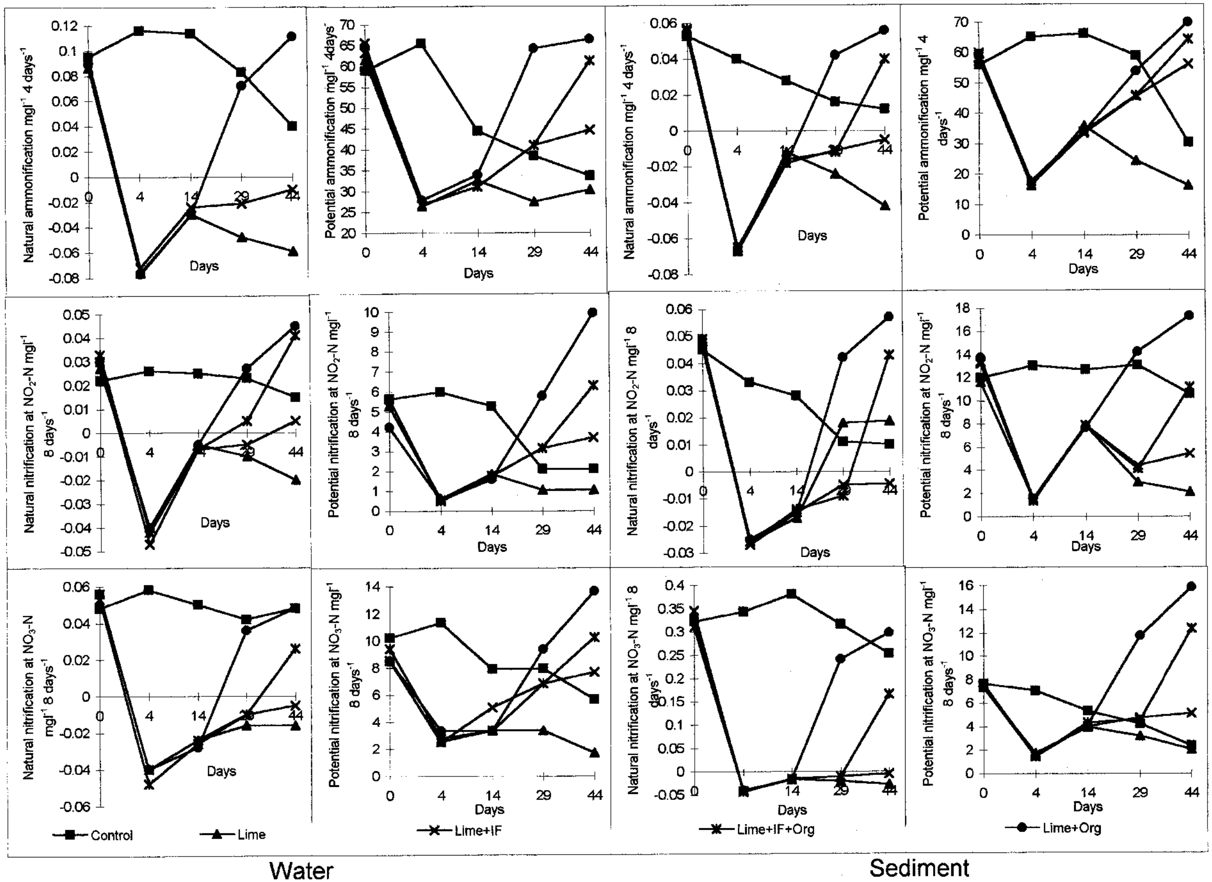

Figure 6. Temporal response of ammonification and nitrification (natural and potential) to lime and post lime inorganic and organic treatment in water and sediment.

over time implying the influence of lime application on nitrate loss in the system. Subsequent organic fertilization caused denitrification rates to increase (ANOVA, $P < 0.05$) over the inorganic fertilization (Figure 7).

Physico-chemical parameters

Water temperature ranged from 27.6–31.3 °C in different treatments during both the experiments. There was considerable increase of pH within two days of lime application and the increase was dose-dependent. Responses of carbonate and bicarbonate alkalinity of water to lime application were similar to pH. The carbonate alkalinity increased to a maximum level of 26 mg l^{-1} in the lime treated system against 5 mg l^{-1} in the control. Bicarbonate alkalinity ranged from 131–159 mg l^{-1} in different doses of lime application compared to 130 mg l^{-1} in controls (Figure 8). After 45 days of lime application, the total alkalinity declined from 151 to 118 mg l^{-1}.

After 14 days of lime application, there was an increase of dissolved oxygen of water to maximum values of 11.9–12.5 mg l^{-1} against 6.7–6.8 mg l^{-1} in the controls. Subsequent organic fertilizer treatment resulted in a decline of dissolved oxygen (8.5 mg l^{-1}) but tended to rise thereafter. The decline of COD ranged from 34–47% in high dose treatments (in 92 mg l^{-1} in 1000 kg ha^{-1}; 73 mg l^{-1} in 2000 kg ha^{-1}) treatments compared to 7–9% decline in the remaining doses (Figure 8). Organic manuring caused COD values to rise by 14% compared to inorganic fertilization (140 mg l^{-1}) (Figure 9).

There was no change in the concentration of dissolved organic carbon in response to lime treatments. Post lime fertilization resulted in 8% and 15% increases in inorganic and organic treatments, respectively (Figure 9).

Application of lime resulted in a considerable decline of ammonia and nitrite concentrations, more pronounced in the highest dose. The nitrate level, on the other hand, showed an increasing trend over time. Post-lime pond fertilization caused the values of nitrogen to rise considerably. The amounts of three species of nitrogen increased much higher in the case of inorganic rather than organic fertilization.

Soluble reactive phosphate increased in the range of 22–38% over control (0.026 mg l^{-1}), after four days of lime application in higher doses (1000 and 2000 kg ha^{-1}), but remained unaltered in the low dose treatments (Figure 8). Inorganic fertilization caused SRP values to rise up to 0.064 mg l^{-1} after two weeks. Organic manuring, on the other hand, did not alter the values during the first week, but showed a rise after the second week.

Application of lime caused substantial decrease (40–50%) in conductivity after two days although there was gradual recovery in the values registering control value after three weeks. Post-lime fertilization resulted in a downward trend in both inorganic and organic treatments showing the lowest in the former than in the latter.

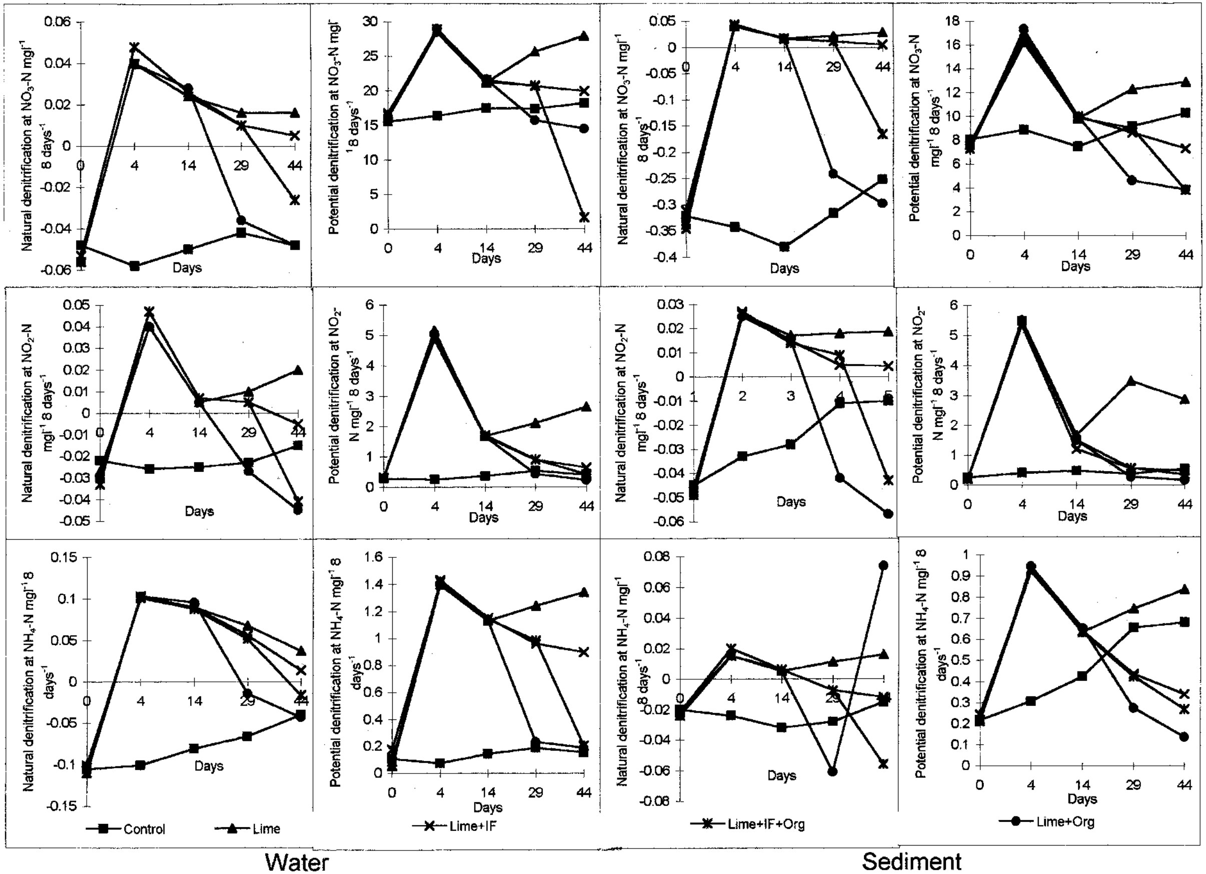

Figure 7. Temporal response of denitrification (natural and potential) to lime and post lime inorganic and organic treatment in water and sediment.

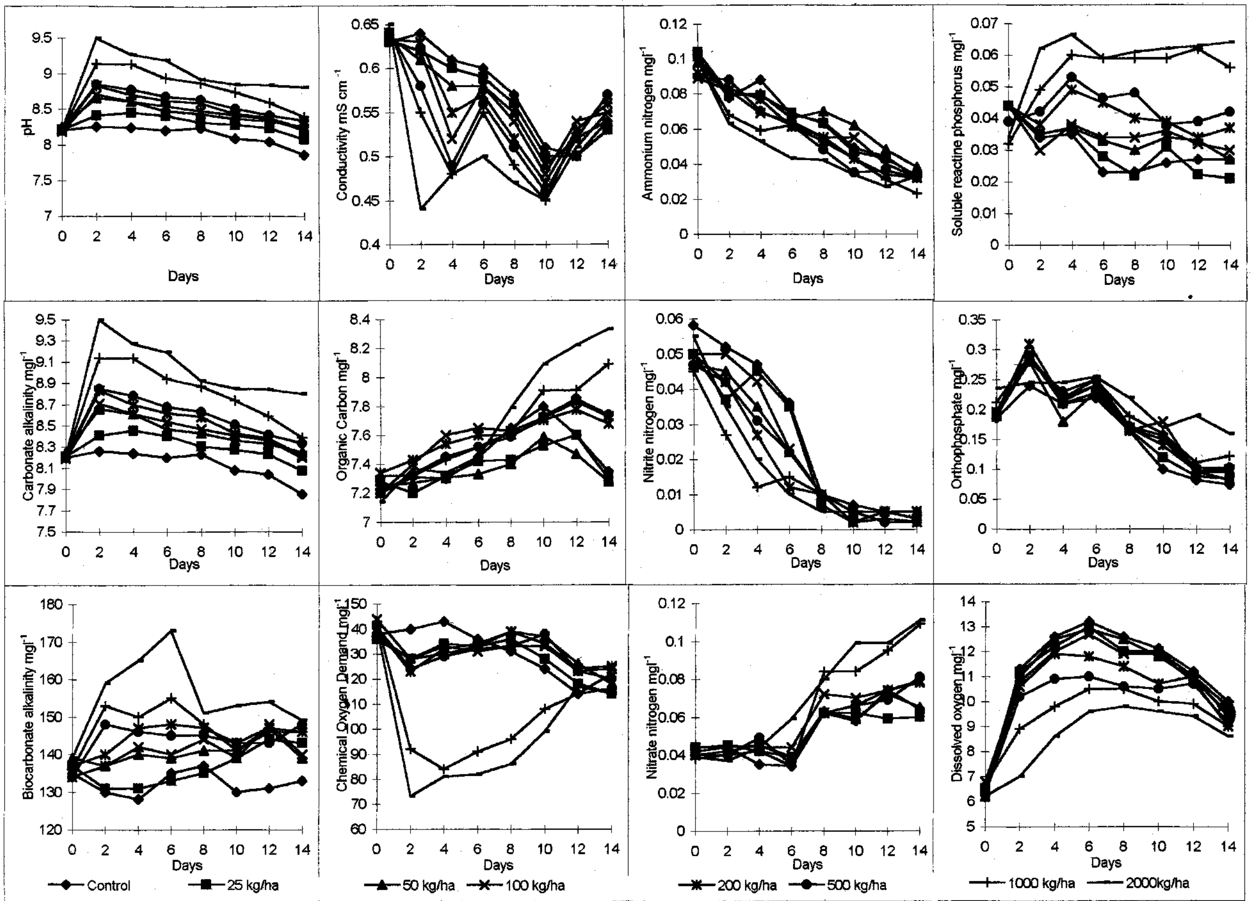

Figure 8. Temporal responses of physico-chemical parameters of water to different doses of lime treatment.

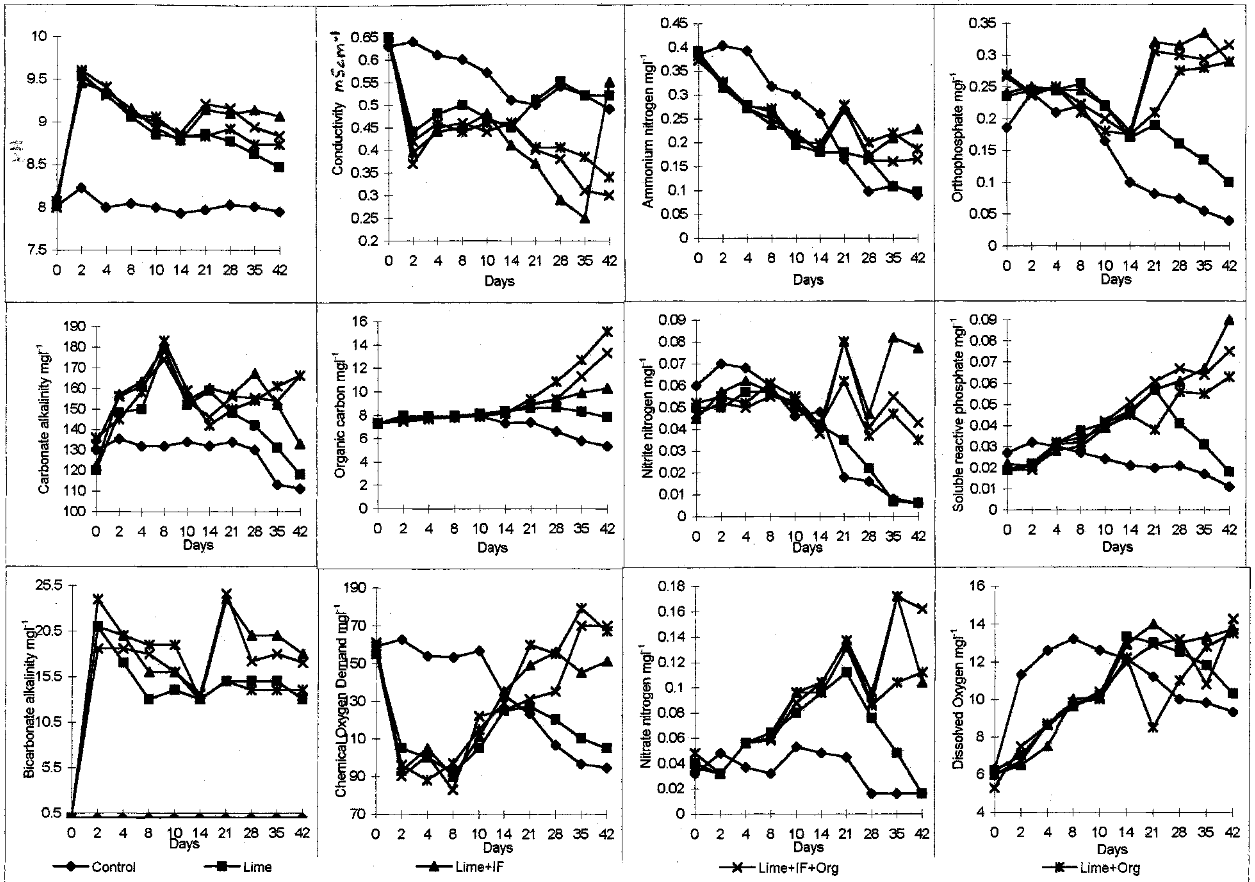

Figure 9. Temporal responses of physico-chemical parameters of water to lime and post lime inorganic and organic treatment.

Discussion

Different groups of microbial populations were found to be strongly affected by lime application > 500 kg ha^{-1}, but remained almost unaffected at low dose treatments (< 500 kg ha^{-1}) which are normally the recommended doses for stabilizing pH conditions in fish growing ponds. Depending upon the soil pH of the pond, lime doses may vary from 200 to 500 kg ha^{-1} (Boyd, 1990). Moreover, liming was of little or no value unless the total alkalinity and total hardness of pond water was below 50 mg l^{-1} as equivalent $CaCO_3$ or the pond soils are acidic (pH < 7.0) (Boyd,1993).

Lime application at 1000–2000 kg ha^{-1}, as employed in the present investigation, was highly bactericidal in the beginning, mediated through high pH stress and major shift in the CO_2–HCO_3–CO_3, equilibrium system. The inverse relationship between the pH and different groups of bacteria ($r > 0.66$; $P < 0.05$) suggests that pH shift towards higher scale was responsible for the growth inhibition of bacteria. Recovery to pretreatment values after 6 to 14 days of lime application was attributed to the reduced stress effect of lime through substantial decline in total alkalinity of water. The same explanation was found to be true for the microbial biogeochemical activities examined. This implied that lime application in shrimp farming ponds in the range of 1000–2000 kg ha^{-1} might be justified from the pond health and microbiological standpoint. The negative effects of acidification on microbial communities are common in disturbed systems, (laboratory system, sediment water slurry), but rare in relatively undisturbed systems (intact sediment core, large *in situ* enclosures, whole lake manipulation and follow-up) (Bell and Tranvik, 1993).

It is further evident from this study that immediate post-lime pond fertilization, regardless of quality, induced all groups of bacteria because of greater amount of nutrient availability in terms of carbon, nitrogen and phosphorus which perhaps compensated the lime-induced pH stress on the biogeochemical cycling bacteria examined.

According to Wolny (1967), the N/P ratio favouring high productions in fish ponds varied between 4 and 8. Since there was sharp reduction of N/P ratio from its initial value of 25 to 6.5 after 45 days of lime application (Figure 10), it is apparent that availability of phosphate increased in greater proportions than nitrogen over time. In the present study, post-lime fertilization with either both inorganic followed by organic or sole organic in two installments, resulted in better N/P ratios (5.28–5.7) compared to two inorganic fertilizations. This suggests fertilizer quality dependent quantitative selection of bacterial population, resulting in favourable N/P ratio in the post-lime organic manured treatment, exhibiting its efficacy over the inorganic treatment for fish husbandry. Lime is known to displace certain other fertilizers from organic colloidal system thus making greater amounts of phosphate available in fish ponds (Hora and Pillay, 1962).

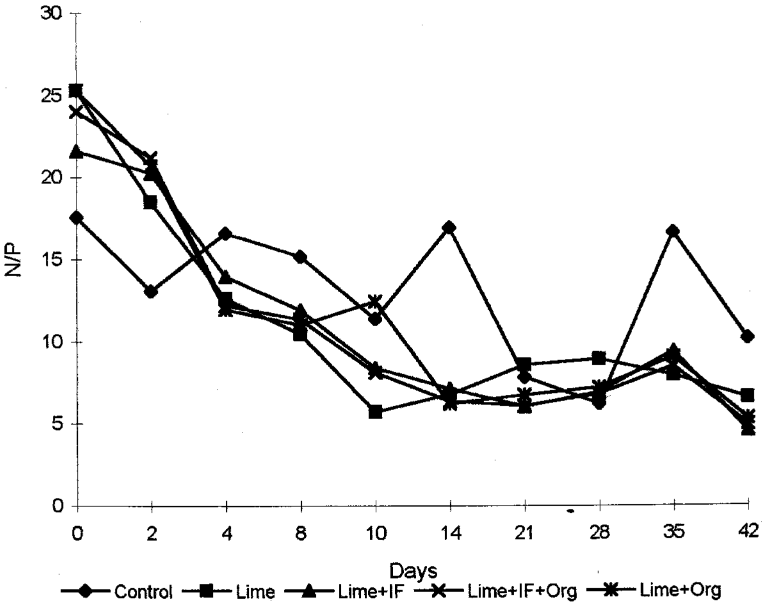

Figure 10. Temporal response of N/P to lime and post lime inorganic and organic treatment.

Conclusions and recommendations

1. Pond liming up to 500 kg ha^{-1}, depending upon the sediment pH, is justified due to least detrimental effect on biogeochemical cycling bacterial populations and their activities, culminating into the physico-chemical *milieu* suitable for fish farming. However, drastic bactericidal effects were manifested at higher doses (> 500 kg ha^{-1}) and are therefore, not recommended.
2. Post-lime organic manuring was desirable in the fish farming system as it induced recovery of microbial activity at a faster rate compared to inorganic fertilization.
3. Reduction of N/P ratio from 25 to 6.5 in the post-lime inorganic and organic treatment suggests that liming may be suitable for fish farming.

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