Changing Phosphorus Availability with Ammonium Sulphate and Carbon Dioxide Enrichments in a Calcareous Soil

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LARGE amounts of ammonium sulphate and carbon dioxide have been released into the atmospheric environment due to intensive industrial and agricultural activities, such as an increased use of fossil fuels and disturbance of forests and soils.¹ These inputs have led researchers to wonder what effects the changing atmospheric environment would have on global ecosystems. In a calcareous soil, addition of ammonium sulphate enhanced the fraction of anion resin exchangeable phosphorus, which represents the most bioavailable phosphorus forms. This finding suggests that deposition of ammonium sulphate may cause transport of phosphate anions, as well as some essential cations,² from terrestrial to aquatic systems, accelerating potential eutrophication of water resources. Under high levels of CO₂, phosphorus availability was decreased probably due to abiotic adsorption of phosphorus onto complexes of soil minerals and CO₂ or microbial products induced by CO₂ enrichment. This result suggests that with increasing atmospheric CO₂, reduced phosphorus availability may limit plant productivity in calcareous soils, and hence CO₂-stimulating photosynthesis would not continue to remove the greenhouse gas.

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As considerable amounts of ammonium sulphate are deposited, attention has been paid to leaching losses of metal ions, such as K⁺, Mg²⁺, and Ca²⁺, from soil systems.²,³ There are, however, few reports about subsequent soil phosphorus transformation. Speculation, as stated below, indicates that ammonium sulphate will solubilise phosphate compounds. Unless biological uptake of dissolved phosphorus is followed, the mobilisation may cause substantial transport of dissolved phosphorus from land to aquatic systems. Consequently, the quality of both land and water resources may degrade due to reduced nutrient retention in the former and eutrophication in the latter.

Following climatological warning that an increasing concentration of atmospheric CO₂ could warm up the globe by preventing solar energy from radiating back into space,⁴ there have been numerous investigations on ecological responses to the changing environment. For example, it may enhance photosynthetic rates in green plants.⁵ In addition, it may lead to shifts in plant communities by influencing the competitive ability of each plant for light and nutrient utilisation.⁶,⁷ It will also alter the plant-herbivore interactions because the plentiful supply of CO₂ contributes to the production of organic matter with high carbon/nutrient ratios.⁸

There is, however, little research on effects of elevated concentration of atmospheric CO₂ on chemical and biological processes of soil, particularly with respect to phosphorus availability. Soil processes are of critical importance to ecosystem structure and function because most nutrient elements are supplied through pathways linked to plant root and soil systems. These pathways play a significant role in plant production, which is stimulated by a plentiful supply of CO₂. Furthermore, since natural soils are composed of aerobic and anaerobic components,⁹ it is necessary to carry out studies which provide information on biogeochemical processes, especially of soil spots or matrices under deficient O₂ or excess CO₂ pressure. The current study addresses a potentially important aspect of soil responses to increased deposition of (NH₄)₂SO₄ and concentration of atmospheric CO₂. We focused on changes in pool size of the most bioavailable soil phosphorus fraction.

Soil was taken from subsurface of a limestone area in Chungnam Province of South Korea in July of 1990. The soil was air-dried, sieved (2 mm), and stored at room temperature. The soil had a pH of 7.2, and contained very little organic
carbon. Approximately 0.1 g C/kg soil was respired when incubated under favorable conditions for 10 days: soil moisture was 60 % field capacity and temperature 20° C.

Soil was incubated as described by Lee and his coworkers. A basic incubation unit was five-gram soil (oven dry basis) placed in an approximately 10 ml glass vial. For each unit, organic carbon and nitrogen sources were supplied as 1.0 g glucose-C/kg soil and 0.2 g (NH₄)₂SO₄-N/kg soil, respectively. Each chemical solution was pipetted to the unit in an aliquot of 0.5 ml after an appropriate amount was dissolved in distilled water. Finally, soil moisture was adjusted to 60 % field capacity with distilled water. Less than 0.02 % water loss was noted during incubation.

There were two experimental sets, each of which consists of six incubation jar systems: one set of 250 ml glass jars, where the CO₂ evolved during incubation was unremoved and the other set of 250 ml nalgene plastic jars connected to soil respirometer, where the CO₂ evolved was absorbed by 10 ml of 0.6M KOH solution. From now on, they are called CO₂-unremoved and CO₂-removed set, respectively. The six experimental systems are diagramatically compared and designated in Figure 1. For example, System A consists of two experimental units treated with distilled water only and two units with nitrogen solution, while System D contains two units with nitrogen and two units with glucose and nitrogen. Hence a series of CO₂ and O₂ concentrations was expected in the soil atmospheric environments during incubation. The respirometer measured the amount of CO₂ evolved in the CO₂-removed set every hour. Variations of cumulative soil respiration measured in the CO₂-removed set were used to estimate relative concentrations of CO₂ in the CO₂-unremoved set (Figure 2). There were three replicates for System A through E of experimental sets, and a duplicate for System F. The soils were incubated at 20° C until respiration rates reach approximately steady state.

The concentrations of the CO₂-unremoved set were calculated using the amounts of CO₂ evolved in the CO₂-removed set and the volumes of jar, soil, and vial glass part. Figure 2 shows the variations in CO₂ levels during the incubation period. It was assumed that there was no interferences between experimental units in the CO₂ unremoved systems. Although the assumption may not be correct because
Changing Phosphorus Availability with Ammonium Sulphate

Nitrogen and glucose represent the addition of nitrogen and glucose as 0.2 g (NH₄)₂SO₄-N/kg soil and 1.0 g glucose-C/kg soil, respectively. Water indicates the addition of distilled water for all units to have 60% field capacity of soil.

Figure 1. Pictorial expression of experimental systems. Nitrogen and glucose represent the addition of nitrogen and glucose as 0.2 g (NH₄)₂SO₄-N/kg soil and 1.0 g glucose-C/kg soil, respectively. Water indicates the addition of distilled water for all units to have 60% field capacity of soil.

there is the inhibitory effect of CO₂ on microbial activity, the order of CO₂ levels in the six systems should be correct. It can also be argued that more than 10% of atmospheric CO₂ level, as generated in System E and F during the experiment, will not be realistic. However, it was demonstrated that the levels of CO₂ must not be such high due to less microbial respiration in CO₂-unremoved set (Table 1). In addition, a level of 10% CO₂ concentration in soil air has been observed under current atmospheric condition, and it will be more frequently occurs if the level of atmospheric CO₂ is doubled in the next century. Therefore the atmospheric CO₂ levels in the CO₂-unremoved set must be comparable to those in certain current and future natural soil air.

Differences of soil respiration rates in Systems B, C, and D, where the same amount of glucose had been contained (see Figure 1), were widened during first week and then became narrow. A similar trend is also found between System E and F (Figure 2). This indicates that the addition of N enhanced soil respiration rate during the initial stage of incubation period, but its effect was diminished as
Figure 2. Variations of CO₂ levels in incubation jar systems A through F for 18 days. It was assumed that soil bulk density was 1.3 and the amount of CO₂ evolved in each system of the CO₂-unremoved set was the same as that in the corresponding system of the CO₂-removed set. The volume of gas phase in the system was approximately 200 ml (250 ml of system volume minus the volumes occupied by soil and vial glass). Standard deviations of three replicates are too small to be seen on the plot, indicating that the CO₂ measurements are very reliable.

It is noted that in the experimental design, respiration rate of each incubation unit can be estimated by comparing symbols in units of experimental systems (Figure 1). If there was no interference between units in a system, and when the amounts of CO₂ evolved from experimental systems and units treated with distilled water only, nitrogen only, glucose only, and nitrogen plus glucose are defined as A, B, ..., F, W<sub>CO₂</sub>, N<sub>CO₂</sub>, G<sub>CO₂</sub>, and NG<sub>CO₂</sub>, respectively, the following relationships will hold true:

\[ F = D + E - C \]
\[ A = B + D - E \]

\[ W_{CO₂} = (A + B - C)/4 \]
\[ = (2A - N_{co₂})/4 \]
Changing Phosphorus Availability with Ammonium Sulphate

Table 1. Soil carbon (mg C/kg soil) respired in the calcareous soil with various experimental treatments.a

<table>
<thead>
<tr>
<th>Treatment name</th>
<th>CO₂-unremoved set</th>
<th>CO₂-removed set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water only</td>
<td>N</td>
</tr>
<tr>
<td>A</td>
<td>134.81 (6.52)</td>
<td>142.89 - -</td>
</tr>
<tr>
<td>B</td>
<td>119.44 (5.58)</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>165.13 (41.28)</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>145.00 (13.38)</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note that the CO₂ contents of System A through F in the CO₂-unremoved set were estimated as 3.37, 7.09, 7.20, 7.86, 11.27, and 12.17 %, respectively, at the end of 18-day-incubation. Symbols N and G indicate the addition of nitrogen and glucose as 0.2 g (NH₄)₂SO₄-N/kg soil and 1.0 g glucose-C/kg soil, respectively. Numbers of parentheses represent standard deviations of three replicates.

\[
N_{CO2} = (A + C - B)/4
\]

\[
G_{CO2} = (B + C - A)/4
\]

\[
NG_{CO2} = F/4
\]

\[
= (D + E - C)/4
\]

Immediately after 18-day-incubation, one of duplicated soil incubation units in each jar was fumigated with ethanol-free chloroform in a desiccator for 24 hours as described by Jenkinson and Powlson. Following fumigant removal, soil was incubated for 10 days with the respirometer at 20 °C, and CO₂ evolved was measured.

bNote that the CO₂ contents of System A through F in the CO₂-unremoved set were estimated as 3.37, 7.09, 7.20, 7.86, 11.27, and 12.17 %, respectively, at the end of 18-day-incubation.

cSymbols N and G indicate the addition of nitrogen and glucose as 0.2 g (NH₄)₂SO₄-N/kg soil and 1.0 g glucose-C/kg soil, respectively.

dNumbers of parentheses represent standard deviations of three replicates.
In Figure 3, the calculated soil respirations were relatively close to observed values. So we can assume that there was no interference between experimental units in a CO$_2$ removed set, suggesting that microbial respiration was not nearly limited until a level of approximately 9 % O$_2$ (21 % of initial atmospheric oxygen level - 12 % of the maximum level corresponding to microbial consumption during incubation) once the evolved CO$_2$ had been removed. Organic carbon respired in CO$_2$-removed set during the period of 18 day incubation was then estimated to be 175, 186, 585, and 639 mg C/kg soil with the treatments of water only, N only, G only, and N + G, respectively.

Immediately after the incubation, one of duplicate soil units in a incubation jar was fumigated with chloroform as described by Jenkinson and Powlson, and then soil respiration was measured for 10 days. This confirmed appropriate sealing of incubation jars. More organic carbon was respired in the chloroform-fumigated soils which were previously contained in the CO$_2$-unremoved set (Table 1), indicating that the evolved CO$_2$ was confined and more organic carbon remained unrespired at least in the jars of the CO$_2$-unremoved set. It is apparent that microbial use of organic carbon was depressed by CO$_2$ stress rather than O$_2$. 

Figure 3. Comparison of the observed and calculated amounts of carbon respired for 18-day incubation.
deficiency. Partitioning of unrespired organic carbon into biotic and abiotic components was unknown, however.

The other units of soil in incubation jars were used for phosphorus analyses. The most biologically available phosphorus in soil is represented by anion resin exchangeable fraction.\textsuperscript{14} Resin-extractable phosphorus of soils in the CO\textsubscript{2}\textsuperscript{-} unremoved set were determined immediately after the jars were open. Two grams (oven-dry basis) of incubated moist soil was placed with 50 ml of distilled water and one bag of resin in the bicarbonate form, and shaken for 24 hours. The resin bag was washed with distilled water and shaken with 50 ml of 0.5 M HCl for four hours. Phosphorus in HCl solution was measured colorimetrically.

Table 2 shows that the resin extractable phosphorus was lowered by glucose addition. In general, the supply of organic carbon activated soil microorganisms and caused uptake of dissolved phosphorus.\textsuperscript{10,15} The addition of ammonium sulphate enlarged pool sizes of resin extractable phosphorus fraction. Phosphate ions might be released due to exchange with sulphate ions and acidification in soil minerals. It is widely accepted that local soil acidification mobilises phosphate compounds.\textsuperscript{16} Solubility of iron increases by a factor of approximately 1,000 as one unit of pH decrease above pH 4.0.\textsuperscript{17} With supply of ammonium sulphate, activated nitrifiers may also contribute to the solubilisation of phosphate compounds as it increases soil acidification. In particular, ammonium sulphate, with increasing levels of atmospheric CO\textsubscript{2}, may have an synergistic influences on the mobilisation of soil phosphate compounds. As a whole, activities of nitrifying

<table>
<thead>
<tr>
<th>Treatment name</th>
<th>Chemical added</th>
<th>Water only</th>
<th>N</th>
<th>G</th>
<th>N + G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original soil</td>
<td></td>
<td>3.53(0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>3.06(0.16)</td>
<td>3.11(0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>3.08(0.22)</td>
<td></td>
<td>2.18(1.31)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td>2.52(0.27)</td>
<td>1.19(0.42)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td>2.53(0.00)</td>
<td></td>
<td>2.54(1.36)</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td>1.12(0.22)</td>
<td>2.42(0.24)</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.67(0.47)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}All symbols are the same as in Table 1.
bacteria are favored by high levels of CO$_2$. For instance, more nitrification occurs with CO$_2$ at the levels of 0.07 to 0.23% than at 0.035%. The synergistic effect was not identified in the present experiment, however, because the bioavailable pool of phosphorus became small with increasing CO$_2$ levels, regardless of ammonium sulphate.

Bioavailable phosphorus pool size was reduced when soils were exposed to high levels of air CO$_2$. The increasing concentration of atmospheric CO$_2$ can influence the reactivity of phosphate and metals in soils in several ways. In soils, most of inorganic phosphorus exists as compounds of Al, Fe, or Ca. The solubility of such phosphate compounds is largely regulated by the redox potential and pH of soil solution. Whereas ferric state of iron Fe$^{3+}$ is hardly soluble, for example, ferrous form Fe$^{2+}$ is easily mobilised, which is obviously dominant under anaerobic conditions. The increased CO$_2$ in soil solution was expected to dissociate iron phosphate compounds because it drives soil solution to reduced and acidic status to a degree. On the other hand, carbonate can prevent or at least retard the crystallisation of aluminum hydroxide in soil, which may be related to phosphate adsorption. Because water near soil surface is exposed to the atmosphere, the equilibrium concentration of dissolved CO$_2$ is a function of the liquid phase CO$_2$ mole fraction and the partial pressure of CO$_2$ in the atmosphere. According to Henry's law, the concentration of carbonate will be enhanced in soil solution as the partial pressure of CO$_2$ increases in the atmosphere.

In addition, bioavailability of phosphorus in soil can vary with the status of atmospheric CO$_2$ since the solubilisation of phosphate compounds is largely associated with microbial activities in soil. As stated before, concentration of CO$_2$ in soil air is directly related to that in the atmosphere. Hence, anaerobic or anoxic zones become enlarged as the concentration of atmospheric CO$_2$ is elevated. This may, in turn, toxicate aerobic microbes, but favor at least some anaerobic organisms in the soil. For example, certain fungal growth can be stimulated by CO$_2$ fertilisation. Becard and Piche observed that the growth of a VAM fungus was promoted in 0.5 percent CO$_2$ than in normal air (0.03 percent) since the fungus could use CO$_2$ as a carbon source. As the composition of aerobic and anaerobic microorganisms is altered, by-products of microbial metabolisms should be changed. Yeasts, fungi, certain Pseudomonas, and wood-decaying
basidiomycetes produce high-affinity iron-chelating compounds, although it is unknown if such microbes favor anaerobic surroundings preferentially. The chelators may be able to mobilise iron phosphates. It is also suggested that oxalate, a metabolic product of certain fungi could contribute to the solubilisation of calcium phosphates. Accordingly, it was anticipated that more phosphate would be mobilised with enhanced levels of atmospheric CO₂, especially in calcareous soils.

However, this study demonstrated that the CO₂ stress suppressed bioavailable phosphorus pool in the soil. There is the possibility that CO₂ stress might stimulate microbial uptake of phosphorus from the bioavailable pool. Future determination of microbial phosphorus with chloroform treatment technique will validate this possibility. In a similar study where a mildly acidic soil (pH 5.1) and soil organic matter mixture (pH 7.7) were incubated under five different levels of CO₂ up to 1.5%, with increasing CO₂ soil microbial P was increased, but phosphomonoesterase activity was depressed (manuscript in preparation).

An alternative hypothesis, that abiotic adsorption might be elevated by the complexes of soil mineral and CO₂ and/or microbial by-products under high levels of CO₂, was tested with the phosphorus adsorption procedure. Briefly, one gram (oven-dry basis) of moist soil was mixed with 50 ml of 1.5 mg P/L solution, which as prepared by dissolving an appropriate amount of KH₂PO₄ in 0.01 M CaCl₂ solution. To inhibit microbial uptake, the phosphorus solution also include 5 ml of chloroform per one liter. The mixture was shaken for 24 hours and filtered through Whatman 42 filter paper. Phosphorus content in the filtrate was immediately determined. Amount of phosphorus adsorbed onto soil must be inversely related to the content in solution.

There was tendency that phosphorus adsorption increased in soils incubated with CO₂ enhancement (Table 3). This tendency is similar to that of Table 2. It is suggested, therefore, that soil phosphorus availability was associated with CO₂ level in air and/or microbially mediated processes of organic carbon, and that abiotic adsorption of phosphorus onto soil was enhanced with increasing CO₂ level. It is noted that calcium carbonate coprecipitates with soluble reactive phosphorus in a naturally eutrophic lake.

In conclusion, the results have many implications with respect to environmental
Table 3. Phosphorus concentration ($\mu$g P/L) of solution 24 hours after 1.0 g of the incubated soil and 50 ml of 1.5 mg P/L solution were mixed$^a$

<table>
<thead>
<tr>
<th>Treatment name</th>
<th>Chemical added</th>
<th>Water only</th>
<th>N</th>
<th>G</th>
<th>N + G</th>
</tr>
</thead>
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<tr>
<td>Original soil</td>
<td></td>
<td>45.33(4.93)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>70.67(4.04)</td>
<td>59.00(5.20)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>67.33(4.73)</td>
<td>-</td>
<td>66.33(12.10)</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>-</td>
<td>59.67(9.07)</td>
<td>59.33( 1.53)</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>-</td>
<td>59.67(5.67)</td>
<td>-</td>
<td>56.33(3.06)</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>-</td>
<td>-</td>
<td>54.67( 2.08)</td>
<td>57.66(1.15)</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>54.33(3.06)</td>
</tr>
</tbody>
</table>

$^a$All symbols are the same as in Table 1.

Concerns. Wet and dry deposition of ammonium sulphate will solubilise phosphate compounds. Unless biotic immobilisation is parallel to the dissolution, an amount of dissolved phosphorus forms will be transported by runoff from land to water systems and cause eutrophication of water resources. Under high levels of CO$_2$, on the other hand, abiotic adsorption of phosphorus was enhanced probably by the complexes of soil minerals. CO$_2$ and/or microbial-products, and hence reduced the availability of phosphorus in the soil. In the above-mentioned similar experiment with a mildly acidic soil and soil organic matter mixture, 2 M KCl-extractable NO$_3$-N and NH$_4$-N in the soil and mixture decreased as CO$_2$ increased. Increased concentration of atmospheric CO$_2$ also contributes to production of organic matter with high ratios of carbon to nutrient. It may eventually decrease nutrient availability for plants as microbes compete for their nutritional balance, while decomposing the organic matter. In the calcareous soil, it can not be accepted that increasing atmospheric CO$_2$ stimulates primary productivity and hence greenhouse effect will then be relieved. Rather degraded availability of nutrients will limit the productivity under enhanced atmospheric CO$_2$. However, we did not consider the effect of global warming-up with increasing CO$_2$, which will elevate soil temperature, speed up the weathering of hardly soluble phosphate compounds and mineralisation of organic phosphorus, and thus release more phosphate ions into soil water. Such a cascade process will generate a positive feedback on immobilisation or solubilisation of phosphate compounds by escalating biological activities with enhanced N-fixation and warming. The
Changing Phosphorus Availability with Ammonium Sulphate

processes also deserve attention in the future. In addition, mineralisation of the organic matter may lead to relatively more production of extracellular organic acids due to a large portion of organic carbon compared to other organic nutrient elements. In particular, the microbial synthesis of organic acids is promoted under anaerobic conditions. Such organic acids may contribute, to some extent, to solubilisation of hardly insoluble phosphate compounds, as well as to adsorption of dissolved soil phosphate.

References


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