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 CO_2, phosphorus availability was decreased probably due to abiotic adsorption of phosphorus onto complexes of soil minerals and CO_2 or microbial products induced by CO_2 enrichment. This result suggests that with increasing atmospheric CO_2, reduced phosphorus availability may limit plant productivity in calcareous soils, and hence CO_2-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^+, Mg^{+2}, and Ca^{+2}, 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 CO_2 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 CO_2 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 CO_2 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 CO_2. 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 O_2 or excess CO_2 pressure. The current study addresses a potentially important aspect of soil responses to increased deposition of $(NH_4)_2SO_4$ and concentration of atmospheric CO_2. 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.[10] 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 $(NH_4)_2SO_4$-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 CO_2 evolved during incubation was unremoved and the other set of 250ml nalgene plastic jars connected to soil respirometer,[11] where the CO_2 evolved was absorbed by 10 ml of 0.6M KOH solution. From now on, they are called CO_2-unremoved and CO_2-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 CO_2 and O_2 concentrations was expected in the soil atmospheric environments during incubation. The respirometer measured the amount of CO_2 evolved in the CO_2-removed set every hour. Variations of cumulative soil respiration measured in the CO_2-removed set were used to estimate relative concentrations of CO_2 in the CO_2-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 CO_2-unremoved set were calculated using the amounts of CO_2 evolved in the CO_2-removed set and the volumes of jar, soil, and vial glass part. Figure 2 shows the variations in CO_2 levels during the incubation period. It was assumed that there was no interferences between experimental units in the CO_2 unremoved systems. Although the assumption may not be correct because
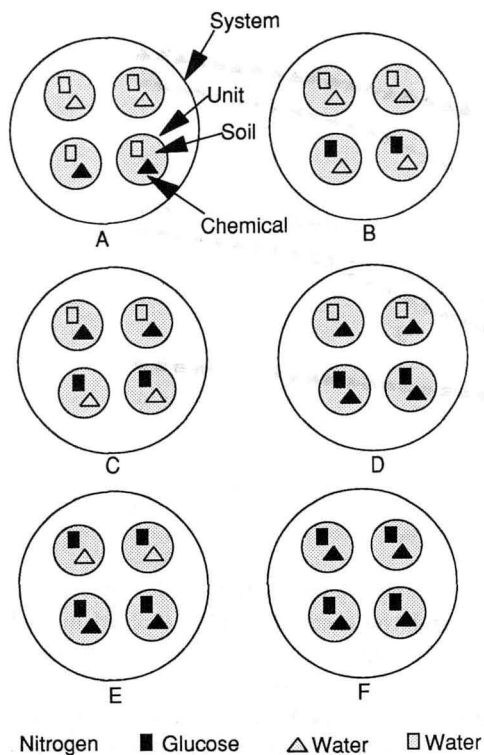
Figure 1. Pictorial expression of experimental systems. Nitrogen and glucose represent the addition of nitrogen and glucose as 0.2 g $(NH_4)_2SO_4$-N/kg soil and 1.0 g glucose-C/kg soil, respectively. Water indicates the addition of distilled water for all units to have 60% field capacity of soil.

there is the inhibitory effect of CO_2 on microbial activity, the order of CO_2 levels in the six systems should be correct. It can also be argued that more than 10 % of atmospheric CO_2 level, as generated in System E and F during the experiment, will not be realistic. However, it was demonstrated that the levels of CO_2 must not be such high due to less microbial respiration in CO_2-unremoved set (Table 1). In addition, a level of 10 % CO_2 concentration in soil air has been observed under current atmospheric condition,[12] and it will be more frequently occurs if the level of atmospheric CO_2 is doubled in the next century. Therefore the atmospheric CO_2 levels in the CO_2-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
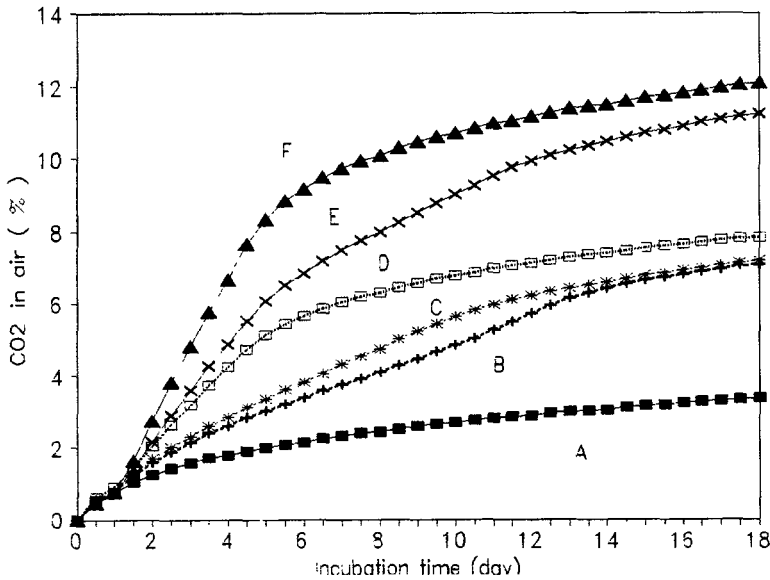
Figure 2. Variations of CO_2 levels in incubation jar systems A through F for 18 days. It was assumed that soil bulk density was 1.3 and the amount of CO_2 evolved in each system of the CO_2-unremoved set was the same as that in the corresponding system of the CO_2-removed set. The volume of gas phase 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 CO_2 measurements are very reliable

time passed. Presumably, units with no N addition compensated N limiting effect with recycling of microbial N and/or N-fixation.

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 CO_2 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_{CO_2}, N_{CO_2}, G_{CO_2}, and NG_{CO_2}, respectively, the following relationships will hold true:

F = D + E - C

A = B + D - E

$$W_{CO_2} = (A + B - C)/4$$
$$= (2A - Nco2)/4$$

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

Treatment name[b]	CO_2-unremoved set				CO_2-removed set			
	Water[c] only	N	G	N + G	Water only	N	G	N + G
A	134.81 (6.52)[d]	142.89 (27.21)	–	–	75.75 (8.52)	60.67 (7.75)	–	–
B	119.44 (5.58)	–	182.49 (79.36)	–	84.20 (5.23)	–	151.45 (21.60)	–
C	–	165.13 (41.28)	214.56 (30.34)	–	–	86.87 (3.14)	163.67 (61.15)	–
D	–	145.00 (13.38)	–	176.44 (32.82)	–	77.24 (20.75)	–	93.27 (11.80)
E	–	–	189.20 (10.06)	170.98 (43.38)	–	–	132.11 (8.30)	123.93 (15.85)
F	–	–	–	154.50 (6.69)	–	–	–	116.29 (6.69)

[a]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.[13] Following fumigant removal, soil was incubated for 10 days with the respirometer at 20 °C, and CO_2 evolved was measured.

[b]Note that the CO_2 contents of System A through F in the CO_2-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.

[c]Symbols N and G indicate the addition of nitrogen and glucose as 0.2 g $(NH_4)_2SO_4$-N/kg soil and 1.0 g glucose-C/kg soil, respectively.

[d]Numbers of parentheses represent standard deviations of three replicates.

$$= (2B - Gco2)/4$$

$$N_{CO_2} = (A + C - B)/4$$

$$= (C + D - E)/4$$

$$G_{CO_2} = (B + C - A)/4$$

$$= (C + E - D)/4$$

$$NG_{CO_2} = F/4$$

$$= (D + E - C)/4$$

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 eestimated 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,[13] 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
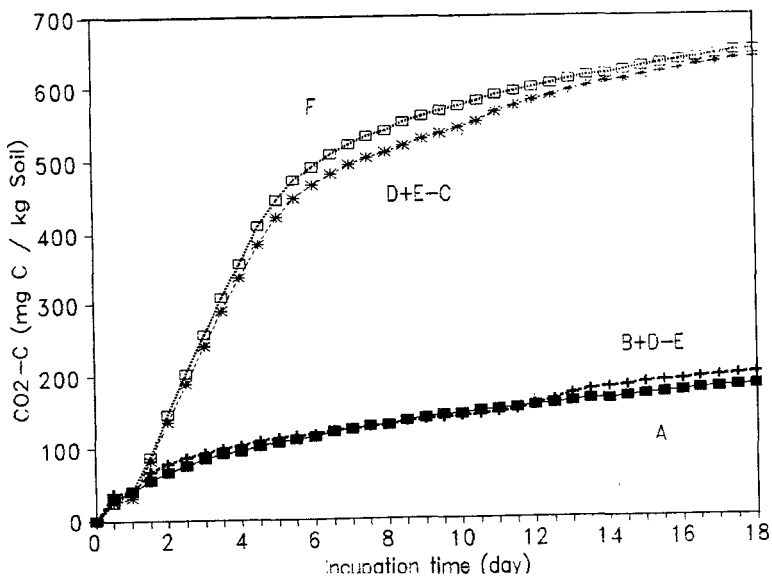

Figure 3. Comparison of the observed and calculated amounts of carbon respired for 18-day incubation.

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.[14] Resin-extractable phosphorus of soils in the CO_2-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.[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.[16] Solubility of iron increases by a factor of approximately 1,000 as one unit of pH decrease above pH 4.0.[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_2, may have an synergistic influences on the mobilisation of soil phosphate compounds. As a whole, activities of nitrifying

Table 2. Anion resin exchangeable phosphorus (mg P/kg soil) of the calcareous soil[a]

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

[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%.[12] 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,[17] 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,[18,19] 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.[20] 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,[21] although it is unknown if such microbes favor anaerobic surroundings preferentially. The chelators may be able to mobilise iron phosphates.[17] It is also suggested that oxalate, a metabolic product of certain fungi could contribute to the solubilisation of calcium phosphates.[22] Accordingly, it was anticipated that more phosphate would be mobilised with enhanced levels of atmospheric CO_2, especially in calcareous soils.

However, this study demonstrated that the CO_2 stress suppressed bioavailable phosphorus pool in the soil. There is the possibility that CO_2 stress might stimulate microbial uptake of phosphorus from the bioavailable pool. Future determination of microbial phosphorus with chloroform treatment technique[15] 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 CO_2 up to 1.5%, with increasing CO_2 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 CO_2 and/or microbial by-products under high levels of CO_2, 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 KH_2PO_4 in 0.01 M $CaCl_2$ 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 CO_2 enhancement (Table 3). This tendency is similar to that of Table 2. It is suggested, therefore, that soil phosphorus availabiiity was associated with CO_2 level in air and/or microbially mediated processes of organic carbon, and that abiotic adsorption of phosphorus onto soil was enhanced with increasing CO_2 level. It is noted that calcium carbonate coprecipitates with soluble reactive phosphorus in a naturally eutrophic lake.[23]

In conclusion, the results have many implications with respect to environmental

Table 3. Phosphorus concentration(μg 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]

Treatment name	Chemical added			
	Water only	N	G	N + G
Original soil	45.33(4.93)	–	–	–
A	70.67(4.04)	59.00(5.20)	–	–
B	67.33(4.73)	–	66.33(12.10)	–
C	–	59.67(9.07)	59.33(1.53)	–
D	–	59.67(5.67)	–	56.33(3.06)
E	–	–	54.67(2.08)	57.66(1.15)
F	–	–	–	54.33(3.06)

[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.[8,24] 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.[25]

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

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.[26] Such organic acids may contribute, to some extent, to solubilisation of hardly insoluble phosphate compounds, as well as to adsorption of dissolved soil phosphate.[10,22,27]

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