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Microbial production of CO2 in red soil in Stone Forest National Park

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Lunan stone forest is a kind of typical karst in China, which is mainly developed under red soil. In the winter of 1999, three study sites were chosen in stone forest national park according to vegetation cover, geomorphologic location and soil types. CO2 concentration was measured with Gastec pump at different depths of soil (20, 40, 60 cm) and at the same time soil samples were gathered and soil properties such as soil moisture, pH, soil organic content were analyzed and the total number of viable microbes were counted in laboratory. In the study, dependent variable was chosen as the mean soil log (PCO2), and soil properties were chosen as the independent variables. Multiple stepwise regression analysis showed that the total amount of microbes and soil moisture are the best indicators of the CO2 production, with the equation LOG(PCO2) = - 0.039(TNM) - 0.056(Mo) + 1.215 accounting for 86% of the variation of the soil CO2 concentration, where TNM is the total number of microbes in the soil and Mo is the moisture of soil sample.

Microbial production of CO2 in red soil in Stone Forest National Park LIANG Fuyuan1, SONG Linhua1, TANG Tao2 (1. Inst. of Geographic Sciences and Natural Resources Research, CAS, Beijing 100101, China; 2. State University of New York, College Buffalo, USA) 1 Introduction Soil CO2 is mainly produced in the soil zone (A and B horizons) by both root respiration and microbial decomposition of organism in the soil(e.g, Witamp and Frank, 1969; Lerman, 1972; Fritz et al., 1978). The CO2 produced in the soil undergoes redistribution via gas transport and geochemical reaction with water and various mineral phases. Part of CO2 produced in the soil will diffuse upward to the atmosphere(Dong et al., 1996) and the fluxes of CO2 are significantly positively correlated with the content of the soil organic carbon(Geng et al., 2001). Dissolved CO2 is a key factor controlling the hardness of spring waters (Jiang, 1999) and the denudation of limestone terrains (Kiefer, 1991). The partial pressure of CO2 gas in the unsaturated zone of soil is an important parameter in the chemistry of recharging groundwater and primary weathering reaction. In addition to acceleration of the weathering reaction, increased partial pressure of soil CO2 is important in the leaching of exchangeable cations (Johnson et al., 1977; Drake, 1980; Solomon, 1987). So far it is difficult to determine the ratio of the root respiration, microbial decomposition of organism and the diffusion to the atmosphere in the soil CO2 production, because CO2 concentrations in soil vary temporally and spatially. Many researchers are devoted to predict CO2 in soil with different factors which influence the production of CO2. Working with carbonate spring chemistry data and using temperature as the independent variable, Drake and Wigley (1975) found that there is a relationship between the soil temperature and partial pressure of CO2 in the soil: Log(PCO2) = -1.97 + 0.04T (1) where T is the mean annual temperature of soil in degree; and PCO2 is the partial pressure of CO2. Brook et al. (1983) developed a world model of average soil CO2 concentration during the growing season and found that the best predictor of CO2 concentration at a site is yearly actual temperature. The model was defined by the equation: Log(PCO2) = -3.47 + 2.09 (1-e-0.00172AET) (2) where Log(PCO2) is the base 10 logarithm of the partial pressure of CO2 in soil atmosphere; and AET is the actual evaportranspiration temperature. The regression model relating Log(PCO2) and log(AET) had the R2 value explaining 66% of the variation in PCO2. Soil CO2 data acquired by Kiefer (1990) in Pigeon Mountain, GA, are in good agreement with Brook´s world model. Dyer (1991) developed a model of average CO2 concentration during non-growing season with soil depth in White forest. The equation is: MEANCO2 = 0.035 + 0.0015(DEPTH) (3) where MEANCO2 is the average of CO2 concentration in soil; and DEPTH is the soil depth. The model shows MEANCO2 is highly correlated with soil DEPTH with correlation coefficient R2 = 0.99. Based on the CO2 concentration over a one-year period in six karst soils in the Malay Peninsula at depths of 15, 30 and 50 cm, Crowther (1984) thought that soil depth and bulk density are the best predictors of MAX

CO2 (the highest single CO2 value), with equation (4), which can account for 86% of the variation of the soil CO2 concentration: Log(MAXCO2) = 1.146 (BDEN) + 0.00698 (DEPTH) - 1.227 (4) As the models mentioned above, in the regions with different temperature, precipitation, the best predicators of CO2 concentration are different. Production of soil CO2 is determined by the root respiration and microbial decomposition of organism. So it is unwise not to consider the function of microbes in soil when developing a model to predict the variation of CO2 concentration. Many papers mainly dealt with the seasonal variation of soil CO2 indicated that the highest CO2 concentration occurs during the summer and the lowest concentrations during the winter (Froment, 1972; deJong and Schappert, 1972; Garrett and Cox, 1973; Edwards, 1975; Rightmire, 1978; Reardon et al., 1979; Gunn and Trudgill, 1982; He, 1997). Little is to do with the biogenic CO2 except the experiment of Hendry (1993), which showed that the microbial decomposition is an important source of soil CO2. In southern China, with the most precipitation and the higher temperature at the same period, most of the CO2 is produced by microbial decomposition of organism (He, 1997). This paper aims not to deal with the seasonal variation of soil CO2, but mainly study the relationship between soil CO2 concentration and the amount of microorganisms in soil and tries to develop a preliminary model to predict the soil CO2 concentration. 2 Study area Stone Forest National Park of China is located at 24o49´N and 103o19´E, 85 km southeast of Kunming, the provincial capital of Yunnan, Southwest China. Lunan stone forest (or Shilin literally in Chinese) covers a total area of some 350 km2. It is positioned in the subtropical, monsoon climate, plateau lake area of eastern Yunnan. Climax vegetation is climatic climax-subhumid broad-leaved evergreen with deciduous forest. But the existing vegetation is mostly secondary forest consisting of limestone bearing broad-leaved evergreen coppice shrubwood, Yunnan pine, limestone juniper brushwood and water vegetation. The climate belongs to humid subtropical. Mean annual temperature is 15.6oC, the highest mean monthly temperature is 25.3oC and the lowest is 8.2oC. Average annual precipitation is 967.9 mm, 80-88% of the total annual precipitation is in summer and autumn (May to October), and precipitation in arid seasons (November to April next year) occupies only 12-20%. The annual humidity is 75%. Stone forest and clint mainly developed in Qixia group and Maokou group of Permian limestone, where the calcium carbonate content is about 78-95%. There are two main fractures developed in the Qixia and Maokou strata, one is in the direction of 300-340o, the other is 40-70o. Dominant soil types are red layer and weathering materials of basalt in the study area. 3 Methods, samples collection and analysis Three study sites were chosen in Lunan Stone Forest National Park according to vegetation cover, soil type and geomorphological location. Site S was chosen at the Shizi hill in the west of major stone forest, where soil type is red layer and vegetation cover is man-made lawn. Site D1 is at bottom of a doline mainly developed in the basalt weathering material, D2 is in the rim of the same doline. There is exuberant lawn at site D1 but little at D2. CO2 concentration: The concentration of soil CO2 was measured by GASTEC pump with tubes made in Japan. A steel stick was used to drill a hole into the soil to a certain depth, then the tube was put into the bottom of the hole immediately and the orifice of the hole was covered with soil. The tube was pumped, often 100 ml each time. After 2 or 3 minutes, the tube was taken out from the hole and the number of the tube was read. The number is looked on as in situ concentration at this particular depth, further details are provided by Song (2001). Soil moisture, temperature, organic carbon content, pH and total number of microorganisms: At the same time, sections at the three sites were made and soil samples were collected and sent to the lab for analysis of the moisture, pH, and organic carbon content. Soil temperature was measured in situ by digital thermometer. In each section, soil was sampled every 20 cm vertically. Soil sample was divided into two parts. One was put into airproof aluminum box and was sent to the lab to test the moisture, organism carbon content, pH and so on. The other part was put into an airproof plastic bag and sent to the lab to count the number of viable microorganisms. Aerobic hererotrophic microorganism were extracted from soil with a sodium pyrophosphate buffer solution and cultured on 1% PTYG (peptpne-tryptone-yeast extract-glucose) media; enumerations were then made after incubation at 26oC for 14 days (Balkwill and Ghiorse, 1985; Severson et al., 1992). Further details are provided by Severson et al. (1992). 4 Results and discussion (1) Soil pH The pH of red earth and basalt weathered materials in Stone Forest National Park ranges from 6.37 to 7.68, which is higher than that of other red layer in southern China, which is between 4.5-5.5 (Wen et al., 1983). (2) Soil temperature The relationship between soil temperature and soil depth is shown in Table 1. Generally soil temperature increases with increasing depth in soil with a gradient of 1.81oC per meter. Because soil temperature was measured in December of 1999, it is lower than the annual mean in Stone Forest National Park (15.6oC). At different soil depths, soil temperature at site D2 is the highest, and that at site S1 is the lowest. (3) Soil moisture Volumetric moisture contents versus depth are shown in Table 1. In general, soil moisture increases with increasing depth. The moisture difference is smaller between site D2 and S1 than that between site D1 and D2, and between S1 and D1. (4) Organic carbon Organic carbon is the main source of microorganism activity in soil. The organic carbon analyzed in the study includes two parts: one is the litter organic parts of p lants, the other is soil humus which is combined substantially with soil mineral grain. The average value of red layer in southern China ranges from 4.04% to 6.69% (Wen et al., 1983), but the content of organic carbon in Stone Forest National Park (0.099-0.260%) is much lower than the average, which shows that decomposition of organic materials is rapid. At site S1, organic carbon content is much higher than the other two sites at depths of 20 and 40 cm underground. It must be resulted from the fertilization to the man-made lawn. Table 1 showed organic carbon content decreases with increasing of soil depth. Regression analysis shows that soil CO_2 concentration has no direct relation with soil organic carbon content at certain depth. (5) Total number of soil viable microorganism Direct examination of soil sample confirmed the presence of viable bacteria, actinomycete and fungi population in soil. The population levels were 104 to 108 cells/g of dry weight which were consistent with those reported for an unsaturated geologic media in a mesoscale model (Hendry, 1993). Numbers of bacterial and actinomyces are generally several orders of magnitude greater than that of fungi in soil. Table 1 shows the relationship between the total number of microorganisms and depth. The total number of microorganisms (TNM) varies greatly at different sites. In general, TNM at site D1 is greater than that of site S1, and TNM at site D2 is the least. But at different depths of soil at the same site, TNM varies greatly also. At site D1, TNM at a depth of 60 cm is 28.5×104 cells/g of dry soil, but in deeper soil (100 cm), TNM is only 7.55×104 cells/g of dry soil. At site D2, TNM in deeper soil (60 cm) is more than 4-8 times that at depths of 20 cm and 40 cm underground. Distribution pattern of the number of microorganism in soil is different from large scale unsaturated media. In large scale unsaturated media, microbial abundance decreases with increasing of soil depth (Wood et al., 1984; Bone, 1988; Beloin et al., 1988). But in soil near surface, TNM of microorganism varies greatly. (6) CO_2 concentration Results of measured partial pressure of CO_2 at the three sites at different depths of soil are shown in Table 1. All of the sites did not exhibit a general increase in CO_2 concentration with depth. At sites D1 and D2, CO_2 concentration at a depth of 40 cm is smaller than that of 20 and 60 cm, but at site S1, it is versus. In general, CO_2 concentration of site D1 is the highest of the three sites, site D2 exhibits the lowest CO_2 concentration.

(7) Multiple regression analysis In the study, dependent variable was chosen as the mean soil $\log(PCO_2)$, and the soil depth, soil temperature, soil inorganic carbon, organic carbon, soil pH and total microorganism number were chosen as the independent variables. Stepwise multiple linear regression analysis was conducted to determine if two or more of the variables could provide in one model a better explanation of variation in PCO_2 (Table 2). Model 1, which relates $\log(PCO_2)$ to TNM had a lower adjusted R square value, can be determined by the following equation: $\mathrm{Log}(PCO_2) = -0.054\,(TNM) - 1.218$ (5) $R^2 = 0.620$ $F = 14.074$ Sig. = 0.007 which can account for 62% of the variation of the soil CO_2 concentration. When variable Mo (moisture) was taken into account, model 2 had a higher adjusted R square value and can be determined by the following equation: $\mathrm{Log}(PCO_2) = -0.039\,(TNM) - 0.056\,(Mo) + 1.215$ (6) $R^2 = 0.895$ $F = 25.578$ Sig. = 0.001 where $\mathrm{Log}(PCO_2)$ is the base 10 logarithm of the partial pressure of CO_2 in soil atmosphere. TNM is the total number of the microorganism at certain depth of soil, Mo is the moisture of the soil sample. The model had the R^2 value of 0.895, which can account for 89.5% of the variation of the soil CO_2 concentration. With such a high level of explanation from two readily measured variables of soil, equation 2 is a satisfactory model of CO_2 variations from 20 to 60 cm. The direct relationships between PCO_2 and the total number of microorganism and moisture prove that microbial decomposition of organic matter is an important source of soil CO_2. Brain (1993) studied two field sites and found that at Washington site, subsurface CO_2 production could be expressed as a function of microbe abundance and subsurface temperature except near the end of the growing season. The same direct relationship was shown in the paper of Lin (1997) about the same study area (Table 3). Oxidation of organic carbon, which is carried out by microbes, can be represented by organic $C + O_2 = CO_2$(7) For the production of CO_2 as described in equation (7), metabolically active microorganisms must be present in the soil with a labile organic source, and O_2 must be consumed (Hendry, 1993). But in the study, there is no direction relationship between soil CO_2 and the total organic carbon content, just as proved by Li (1993) on Guizhou plateau in Southwest China (Table 4). It may be because the total organic carbon accounted in this study includes labile and inertia organic source. However only the labile organic carbon was consumed to produce CO_2 in the soil. So how to identify labile organic carbon from total organic carbon is an interesting question and it is important to study the CO_2 production in unsaturated media. Moreover, equation (6) also shows that the environmental variable, such as moisture of soil, may influence the productivity of soil CO_2. Equation (6) shows that moisture exerted greater influence upon soil carbon dioxide evolution than soil temperature did, which is different from the opinion of Froment (1972). He thinks that soil temperature is more important than soil moisture, the former having a correlation coefficient of $r = 0.54$ at a depth of 1 cm and 0.58 at 10 cm while for the latter, $r = 0.39$. But this is not a contradiction because the important influence on CO_2 production of temperature will be shown when CO_2 productivity in summer is contrasted with that of in winter. Many studies have shown that higher concentration of

CO2 in summer while temperature is higher than that of in winter with lower temperature (Forment, 1972; deJong and Schappert, 1972; Garrett and Cox, 1973; Edwards, 1975; Rightmire, 1978; Reardon et al., 1979; Gunn and Trudgill, 1982; He, 1997), and mean soil CO2 correlated strongly with mean soil temperature (Kiefer, 1990). Since carbon dioxide is produced by microbial decomposition of organic matters, the rate of production is higher at higher temperatures under moist condition (Trudgill, 1985). According to the data of Kononova (1966), the rate of CO2 production when the soil was gradually heated and dried in the laboratory was at a maximum at temperature of about 25 to 35oC and moisture contents of 30-40%. Those at lower moisture have been attributed to reduced diffusion of soluble substrates (Griffin, 1981a) and to reduce microbial mobility and consequence access to substrate (Griffin, 1981b; Killham et al., 1993). Higher moisture cause declination in microbial activity, which is attributed to O2 deprivation caused by reduced O2 diffusion (Bridge and Rixon, 1976; Griffin, 1968). Obviously, the result of experiment of Kononova gives the reason that why at site D1 there is much higher CO2 concentration in soil than at sites S1 and D2. 5 Summary The relationships between CO2 concentration with soil properties (sampling depth, organic matter content, inorganic content, soil pH, soil moisture, soil microorganism total number) were studied in Stone Forest National Park, China. Metabolically active microorganisms were presented in soil samples at different sampling depths. Multiple regression analysis results showed that the total number of microorganism and soil moisture is the best predicator of soil CO2 concentration. They can account for 89.5% variation of CO2 in soil. Acknowledgements The authors thank Mr. Wang Fuchang, Li Zhongde, Zheng Binyuan and Miss Zhang Liping of Administration of Stone Forest National Park for their help in the field survey. Thanks are also due to Prof. Yu Jingbao, Nanjing University, Prof. Lin Junshu, the Institute of Geographic Sciences and Natural Resources Research, CAS for their collaboration work.

关键词： soil CO2; microbial decomposition; stone forest