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小叶章生态系统根际土壤微生物及 CO_2、CH_4、N_2O 动态

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摘要：研究了培养 45d 的小叶章根际土壤微生物和二氧化碳，甲烷及氧化亚氮产生与消耗之间的关系。结果表明好氧微生物与厌氧微生物的空间分布与甲烷，二氧化碳及氧化亚氮的产生和氧化有着密切的关系。好氧微生物与甲烷产生呈负相关，与二氧化碳和氧化亚氮的产生呈正相关关系。厌氧微生物与甲烷的产生呈正相关，与二氧化碳和氧化亚氮的产生呈负相关关系。

关键词：好氧微生物；厌氧微生物；甲烷；二氧化碳；氧化亚氮

Linking of microorganisms to CO_2, CH_4 and N_2O dynamics in *Calamagrostis angustifolia* rhizosphere soil

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Abstract: To understand the relationship between microbes and greenhouse gases, four soil cores dominated by *Calamagrostis angustifolia* were investigated to determine bacterial community as well as the production and consumption of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) after 45 days incubation in an open area. Aerobic and anaerobic microorganisms were counted to evaluate the bacterial community, and the results suggested a significantly negative correlation between the aerobic and anaerobic. Meanwhile, there appeared to be obvious relationship between the bacterial community and CH_4, CO_2 and N_2O dynamics. The aerobic microbes were negatively related to CH_4 production, and positively related to CO_2 and N_2O production. The inverse relationship was observed between anaerobic microbe and the dynamics of these greenhouse gases.

Key words: aerobic microbe; anaerobic microbe; methane; carbon dioxide; nitrous oxide

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Methane, carbon dioxide and nitrous oxide are three major greenhouse gases in atmosphere, accounting for more than 80% of global warming[1,2]. This has highlighted the importance of the dynamics of greenhouse gases in recent years. While there are many studies on greenhouse gas dynamics in terrestrial ecosystem[3~7], however there are few reports in wetland ecosystems[8]. Wetlands ecosystems play an important role in controlling the terrestrial carbon cycle because of their ability to sequester atmospheric carbon in peat[9]. Northern peatlands, for example, contain approximately 500×10^{15} g of C[10] and thus are a major global carbon sink. Though wetlands have acted as carbon sinks since the last glaciation by incorporation of C into accumulating peat and organic matter, they also release C back into the atmosphere over time. The main sources of loss of C are: mineralization to CO_2 through plant respiration and aerobic peat decomposition; CH_4 production from the lea anaerobic

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decomposition of peat; and as dissolved organic carbon (DOC) from the leaching of soil organic matter in water[11], with the former two accounting for most of the C efflux from wetland and thus the greatest impact on the greenhouse effect. While there is extensive on nitrous oxide[12~14], there are only a few studies in wetland ecosystems, and none for rhizosphere soil in wetland.

The *Calamagrostis angustifolia* ecosystem is one of the main wetland types in Sanjiang Plain, the most extensive natural wetland in China. So the spatial dynamic of greenhouse gases in *Calamagrostis angustifolia* rhizosphere soil that contributed to global warming is a very important area for understanding of carbon cycling in China.

The aims for this research are: (1) determine the bacterial community, (2) measure the dynamics of three major greenhouse gases, and (3) estimate the spatial linkages between bacterial community and greenhouse gas dynamics in *Calamagrostis angustifolia* rhizosphere. To achieve these objectives, an experiment was carried out to simulate the effect of wetland plants on rhizosphere soil. Comparisons of bacterial community, evaluated based on the abundance of aerobic and anaerobic micro biota, was used to reflect oxidation-reduction environment and its link to CH_4, CO_2 and N_2O dynamics in rhizosphere soil of *Calamagrostis angustifolia*.

1 Materials and methods

1.1 Experiment scheme

Soil used in this experiment was taken from 0～15cm layer in agricultural tillage plot in the Sanjiang Experimental Station, CAS. The natural condition was showed as Cheng G[15]. The altitude is 55.4～57.9m, annual mean temperature 1.9℃, non-frost period of 125 d and seasonal freezing of the soil. The annual precipitation is 550mm～600mm, concentrated at July and August, which accounts more than 65% of annual precipitation. Bulk density, organic matter, Kjeldahl N and available P (dilute HCl-H_2SO_4 extractable) of the experiment soil are 1.98g/cm^3, 4.40%, 0.81% and 157.3 μg/g, respectively. Its water holding capacity (WHC) is 35% w/w. The soil was sieved (< 3mm), homogenized, and air-dried before use. Then the soil was placed into the polystyrene containers (I. D. 25cm × H30cm) with ca 10 Kg dry soil in each container. The nylon gauze bags (400 mesh, I. D. 5cm)[16] were set in the center of soil core, and five clusters of grass were planted individually around nylon gauze bag (Figure 1). Water was added to keep the soil at the field WHC. The containers were incubated in the open area located approximately 100 m away from natural *Calamagrostis angustifolia* wetland. Weeds were removed weekly.

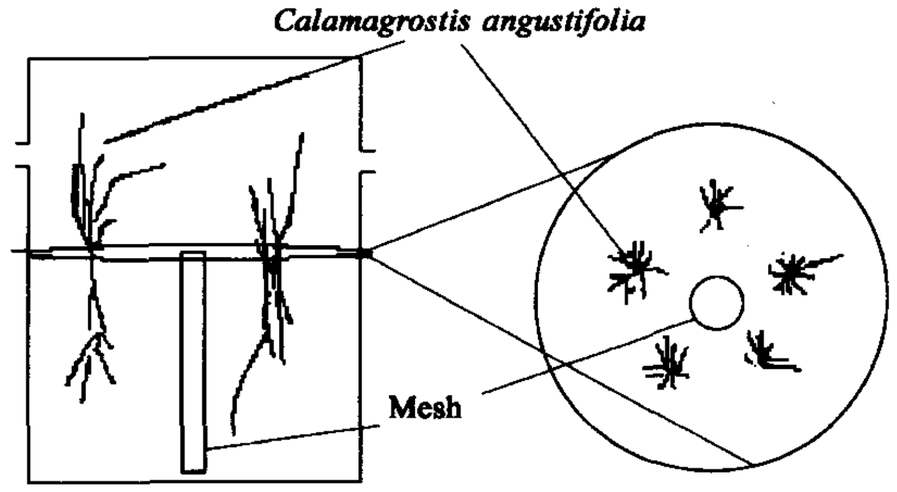

Fig. 1　Experiment scheme
Left graph is horizon view, right is vertical view from up

1.2 Sampling

Soil samples collected during the experiment were divided into three layers, topsoil (0～4cm), root layer soil (5～16cm), and under-layer soil (17～20cm), based on visual inspection of the cores. The topsoil was divided into two categories, rhizosphere and non-rhizosphere soil, as was the under-layer soil. The root layer soil samples included four divisions: root-free soil, bulk soil, rhizosphere soil, and rhizoplane soil. The rhizoplane, defined as the external surface of roots together with closely adhering soil particles and debris, is nearly 2mm away from the roots. Rhizosphere soil, defined as soil that surrounds and is influenced by the roots of a plant, is about 10mm away from root. The soil samples including root-free soil, bulk soil, rhizosphere soil and rhizoplane soil were obtained followed the methods described by Cheng[17]. After 45 days of plant growth, four replicate pots were destructively sampled for rhizoplane soil, rhizosphere soil, and bulk soil as well as root-free soil. Root-free soil was taken from the center of the pot where roots were excluded by the nylon bag (400 mesh) membrane column. Bulk soil was taken from the homogenized soil not attached to roots but within the rooting zone. Rhizosphere soil was the soil loosely attached to the root system, removed by hand shaking. The soil firmly attached to the root system and did not fall off after hand shaking was the rhizoplane soil, which was washed off in deionized water in a beaker. Each sample was taken from four containers and mixed together. Then they were stored at room temperature (ca. 15±3℃) for one day before been sieved and assayed. The soil sample names were abbreviated as RT for rhizoplane soil in top layer, NT for non-rhizosphere soil in top

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layer, PR for rhizoplane soil in root layer, SR for rhizosphere soil in root layer, BR for bulk soil in root layer, RR for root-free soil in root layer, RU for rhizosphere soil in under layer, and NU for non-rhizopshere soil in under layer.

1.3 Assay in laboratory

Total organic carbon (OC) of the soil sub-sample was determined by potassium dichromate oxidation[18,19]. Results were expressed as % (OC mass divided by dry soil mass). The pH values were measured using electrical potential method (soil was extracted with water, at a ratio of soil to water of 1 : 5). The physical-chemical characteristics of the soils are showed in Table 1.

Table 1 Soil properties

	RT	NT	PR	SR	BR	RR	RU	NU
pH	5.67	5.56	5.9	5.65	5.54	5.21	5.44	5.19
SOM(%)	5.84±0.21	5.52±0.08	8.92±0.12	7.27±0.29	6.03±0.23	4.41±0.21	5.51±0.22	4.59±0.22

Bacterial community was determined by microbe counting. The number of aerobic bacteria was obtained with a plate count method. The soil suspension was diluted 106 fold, and 0.5ml of the diluted suspension was added to four replicate Petri dish with beef broth medium. The number of anaerobic bacteria was obtained with plate counting followed the pyro-gallic acid method described by Li Z Y[20]. The Petri dish was kept at room temperature, 10±5℃, representative of field conditions.

1.4 Gas analyses

Methane, carbon dioxide and nitrous oxide were monitored to determine changes in their concentration over time during incubation in sealed mason jars at room temperature (22±5℃). Accumulating CH_4, CO_2 and N_2O were measured with gas chromatography (Agilent 4890) equipped with FID and ECD. CH_4 was separated with 2m stainless steel column with inner diameter of 2mm 13XMS column (60/80 mesh), with FID at 200℃, carrier gas was high-pure nitrogen with flow speed of 30ml/min. CO_2 was separated with 2m stainless steel column with inner diameter 2mm Porapak Q (60/80 mesh), FID works at 200℃ using high-pure nitrogen as a carrier gas at a flow rate of 30ml/min. N_2O was separated using a 1m stainless steel column with inner diameter 2mm Porapak Q (80/100 mesh). The ECD was set at 330℃ using high-pure nitrogen as a carrier gas at a flow rate of 35ml/min. The column temperatures were maintained at 55℃ for the separation. For each sample, four 3 g (dry weight) replicates were incubated in four 300 ml jars. CH_4, CO_2 and N_2O dynamics were quantified as their accumulation in the headspace of jars of soil with water hold capacity about 40%.

1.5 Statistics analysis

Origin 7.0 for Windows, SPSS 10.0 and Excel 2000 for Windows were used to deal with the data of the experiment.

2 Results and discussion

2.1 Bacterial community

There was a significant negative correlation between the numbers of aerobic microbe, compared to the abundance of anaerobic microbes at a given location. For topsoil or sub-topsoil, diffusion of oxygen through aerenchyma, the conducts of gas-filled intercellular spaces in plant roots[21~23], created oxidized conditions, and hence lead to greater abundance of aerobic microbes, compared to anaerobic microbes. The rhizoplane soil and rhizosphere soil have a similar bacterial community as the topsoil with its oxidized condition. For the deep soil and the soil far away from root, reducing conditions lead to a greater abundance of anaerobic microbe, compared to the aerobic.

Figure 2 shows the serrated curves of microbe numbers, which is attributed to the effect of the distribution of soil carbonic because that is the main energy source for microbial

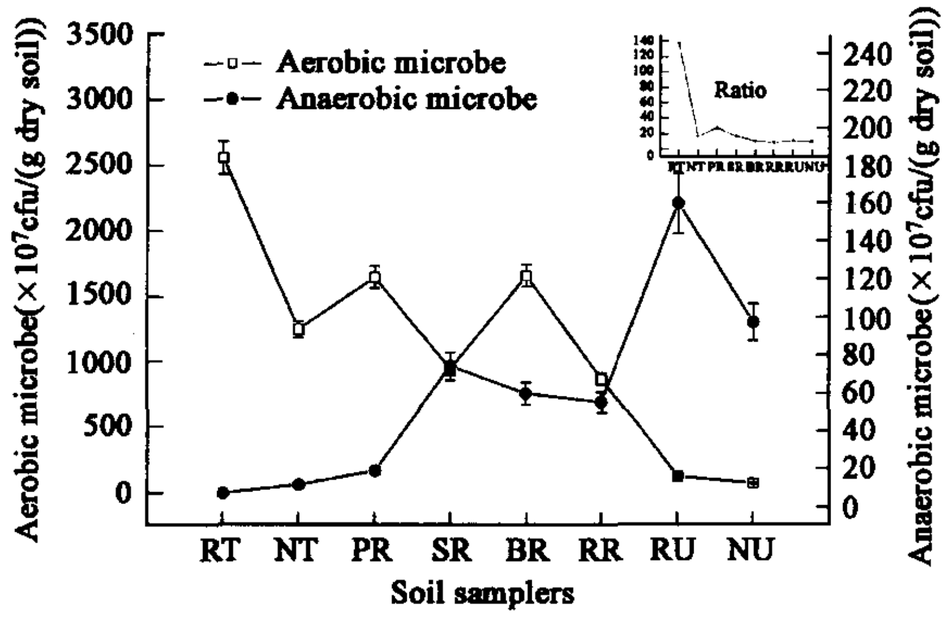

Fig. 2 Spatial distribution of soil microbe

The values are shown as the mean of four replicates, with error bars representing the standard error; The curve in top right corner is the ratio of aerobic to anaerobic microbial abundance; The samples was named as the description in sampling part; the same below

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activity. Although the absolute number of microbe number is variable, the ratio of aerobic-to anaerobic microbe varied regularly. The ratio of rhizosphere soil in the top was near 136, higher than those of other soils whose ratio were around 10. In the root layer soil, outward from rhizoplane soil, the ratio gradually decreased, suggesting the increasing reducing environment. The investigation from Chen[24] was also consistent with our results. Generally, aerobic- and anaerobic microorganism were negatively correlated each other; however, the effect from soil OC could not been ignored[25~27]. Further research is needed to clarify the role of the decomposability and availability of soil OC on these processes.

Table 2　Methane dynamics

t	0.5h	1h	1.5h	2.5h	4.5h	8h
RT	−144.92±−10.14	37.49±2.62	−18.83±−1.32	18.34±1.28	1452.58±101.68	−827.47±−57.92
NT	−144.51±−14.45	49.44±4.94	−39.39±−3.94	11.48±1.15	717.03±71.70	−402.20±−40.22
PR	−120.65±−12.06	5.71±0.57	−20.67±−2.07	22.42±2.24	208.30±20.83	−115.86±−11.59
SR	−93.40±−11.21	28.91±3.47	−27.52±−3.30	26.75±3.21	207.50±24.90	−114.50±−13.74
BR	−90.28±−7.22	51.21±4.10	5.02±0.40	17.13±1.37	237.90±19.03	−124.53±−9.96
RR	−79.28±−10.31	−9.19±−1.19	22.27±2.90	30.49±3.96	201.31±26.17	−107.32±−13.95
RU	−87.49±−17.50	27.62±5.52	22.60±4.52	−19.40±−3.88	207.81±41.56	−104.02±−20.80
NU	−60.72±−3.04	−12.38±−0.62	64.40±3.22	−12.63±−0.63	224.40±11.22	−118.43±−5.92

2.2 Methane dynamic

During the first 2.5h, methane dynamics in all soil layers are similar to each other, which may result from disturbance from field conditions[17]. However, after 2.5h of incubation, the soil began producing methane, which was then oxidized. Overall, the process appeared result in methane oxidation[26], but the methane production from topsoil after 5h needs to be further investigated. We postulate that these observations reflect the effect of higher OC content leading to rapid growth of anaerobic microbe, and hence to methane production.

2.3 Carbon dioxide dynamic

The basal respiration during incubation was largest in topsoil, likely due to its high levels of soil organic matter, and the abundance of aerobic microbes. Higher levels of microbial respiration thereby produced higher levels of CO_2. Figure 4 shows the varied trends of C mineralization for the different soil samples. Initially C was mineralized rapidly, decreased in a short term, and stabilized after 2.5h. We attribute disturbance during sampling to rapid decrease in C mineralization within the first 2.5h. During incubation period, the carbon mineralization was greatest for the rhizoplane soil in the root layer. Levels were, initially 455.99mg C/kg dry soil/h, significantly higher than normal mineralization, but then decreased to 9.37mg C kg^{-1} dry soil h^{-1} after 2.5h of incubation. The topsoil with highest soil OC content had a relative low C mineralizing rate, after 2.5h of incubation, but they all reached a stable level. So the variation of initial 2.5h is attributed to soil disturbance during sampling[17]. The C mineralization during incubation period was lower in subsoil, which may be related to the low OC content or low microbial activity. The mineralization rate after 2.5h, was positively correlated with soil OC content, with the topsoil and rhizosplane soil having more carbon, therefore, more rapid mineralization.

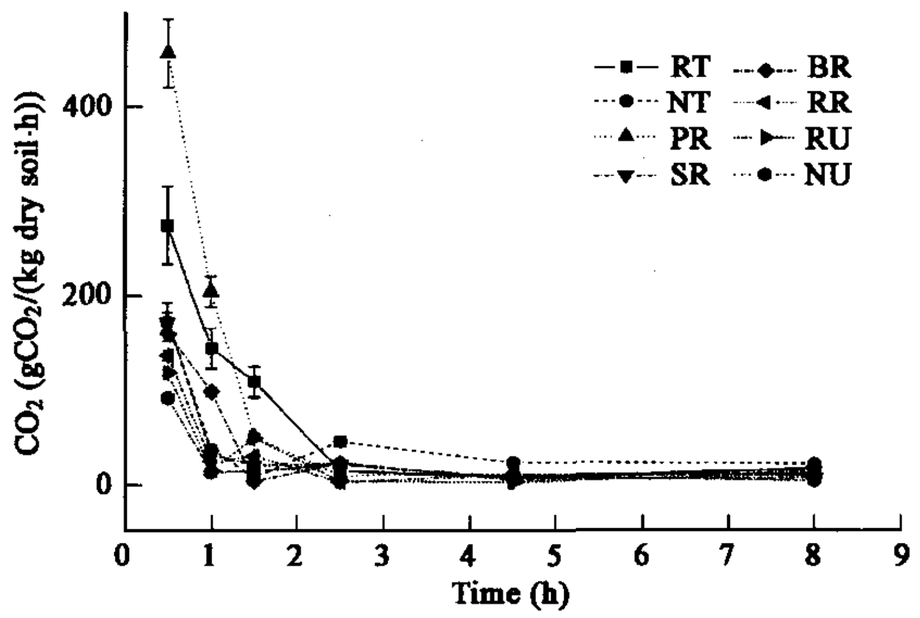

Fig. 3　Carbon dioxide dynamics

2.4 Nitrous oxide dynamic

Nitrous oxide levels varied easily due to its instability. But the general pattern suggests that the topsoil produced nitrous oxide, while the subsoil consumed nitrous oxide. Patric studied nitrous oxide dynamics of rice paddy soil and found that the nitrous oxide dynamics was related with Eh[28], which could explain the result obtained in this experiment. The topsoil produced nitrous oxide because of its highly oxidizing conditions, and subsoil consumed nitrous oxide under reducing conditions. Finally, nitrous oxide was produced when the soils all became oxidized after 8 h of incubation.

2.5 Correlations among the items of the rhizosphere soil

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The correlation coefficient matrix is showed in Table 3. The pH value was significantly and positively correlated to soil OC and CO_2 production. The explanation is that soils used in this experiment are all acidic, pH<7, resulting in more active of aerobic microbes, and thus greater production of CO_2. Soil organic matter was significantly positively correlated to CO_2 production at $p=0.05$ level, presumably because soil OC is the main substrates for CO_2 production[25]. Also the aerobic- and anaerobic microbes are negatively correlated ($R^2=-0.818$; $p=0.05$) for reasons discussed previously. The significantly negative correlation between CH_4 and N_2O was related to differences in the microenvironment, although this phenomenon merits further investigation. The spatial distribution of gases also provides some important information suggesting that the soil closed to root has more active OC transformation and transportation.

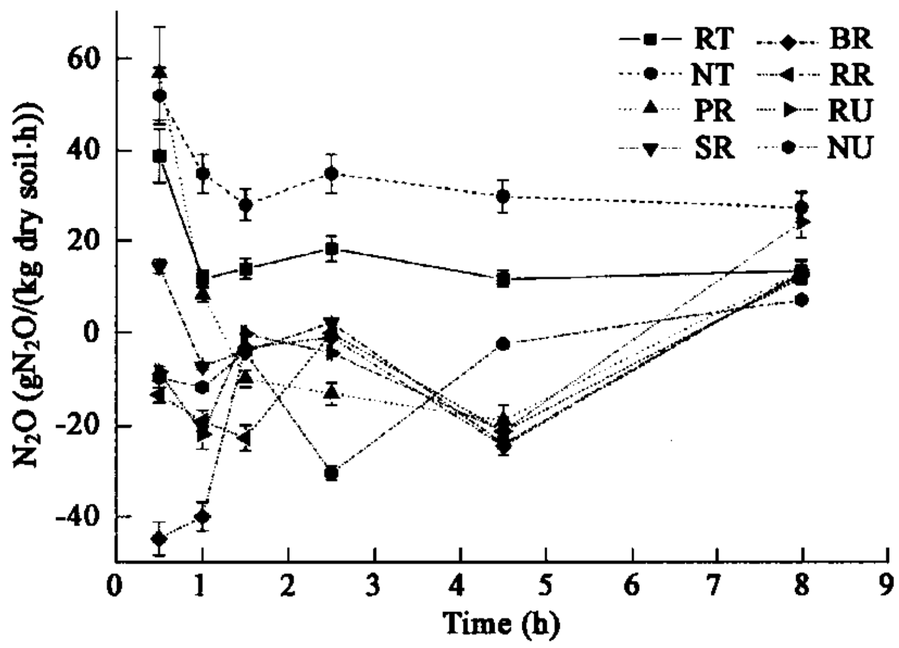

Fig. 4 Nitrous oxide dynamics

Table 3 Correlation coefficient matrix

	pH	SOM	Aerobic microbe	Anaerobic microbe	CH_4	CO_2	N_2O
PH	1						
SOM	0.909**	1					
Aerobic microbe	0.658	0.397	1				
Anaerobic microbe	−0.497	−0.312	−0.818*	1			
CH_4	−0.383	−0.285	−0.437	0.664	1		
CO_2	0.837**	0.804*	0.697	−0.619	−0.638	1	
N_2O	0.562	0.406	0.387	−0.626	−0.837**	0.523	1

* Correlation is significant at the 0.05 level; $N=32$ ** Correlation is significant at the 0.01 level

3 Conclusion

The abundance of aerobic- and anaerobic microbes varied spatially, and exhibited a significant negative correlation to each other ($R^2=-0.818$, $p=0.05$). Meanwhile, there appeared to be an obvious relationship with CH_4, CO_2 and N_2O dynamics. The aerobic microbe was negatively related to CH_4 production, and positively related to CO_2 and N_2O production. However, anaerobic microorganisms was positively related to CH_4 production, and negatively related to CO_2 and N_2O production. At the same time, CH_4 production appeared significantly negatively related to N_2O production ($R^2=-0.837$, $p=0.05$).

The disturbance of fresh samples required more than 3h of incubation to stabilize. Bacterial community varied significantly with the distance from root. The methane and carbon dioxide and nitrous oxygen dynamics were strongly correlated to the distance from root.

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