C-2. 2. 2 Studies on the impact of environmental acidification on soil microorganisms

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Abstract Effect of exposure of artificial acid rain (mixture of 0.25mM solution of HCl, HNO3, and H2SO4;(pH 3.5)) on model soils was determined in terms of diversity and ability of carbon source utilization of soil bacteria. Artificial acid rain reduced remarkably both of diversity (56% of control) and number of carbon source utilized (30% of control) of soil bacterial community at 1 day after the exposure. The diversity level was recovered up to 85% of the control in the long term incubation (1year) but the level of carbon source utilization ability was remained at 44% of the control. It is considered that the bacterial community is shifted to that dominated by strains having simple carbon source utilization ability.

Effects of environmental acidification on soil microbial community both of bacteria and fungi were evaluated in terms of quantity and diversity. Diversities of bacteria and fungi were determined by complexities of carbon source utilization pattern and morphological characteristics pattern, respectively. In quantities measured with the plate counting method, fungi increased but, in contrast, bacteria decreased in some soils acidified compared to a soil unacidified. On the contrary, diversities of both of bacterial and fungal communities remarkably decreased with decrease of pHs of the soils. It is considered that these results strongly suggest that acidification of environment will shift microbial communities to those which are more uniform and are dominated by fungi even in natural soils. Then it may make shrink of material flow systems in environment.

1. Introduction

Climate changes, especially, environmental warming due to green house effects, change of rain precipitation, and environmental acidification may strike severely ecosystem of soil microorganisms which is considered to maintain circulation of materials in our environment. It may result in unpredictable problems in the circulation of materials and flow of energy in this world. However, detecting change in the soil microbial community and evaluating damage in the ecosystem are so difficult because of low sensitivity of methods which are regularly used and instability of the communities in soils.

In this study, new methods to detect structural change in the soil microbial community were developed and used for evaluating the changes under conditions acidified both experimentally and naturally.

2. Research Objective

Objectives of this research are to develop new methods to detect change in soil microbial community and to evaluate impact of environmental acidification on the soil microorganisms.

3. Research Method

(1)Soil used
A soil (pH. 6.1) in a green house (Ecotron) which is located at Institute of Agro-Environmental Sciences is used as a model soil for experiments of artificial acidification.
Five soils (Table 1) acidified naturally and a soil unacidified at Aomori Pref. were used to evaluate impact of environmental acidification under natural condition.

Table 1. Naturally acidified soils tested

Soil	Plant on the ground	pH
Birin	Japanese cedar	5.4
Entsuji	Japanese cedar	3.6
Ryukiko1	Japanese beech	3.4
Oniishi	Bamboo grass	3.2
Ryukiko	none	3.1

(2) Filamentous fungi used as a model community
Twenty five isolates of filamentous fungi classified into 22 in species 7 genus which are isolated regularly by using the plate method were selected as a model community to test the new method for evaluating diversity of soil fungal community.

(3) Artificial acidification of the model soil
The model soil packed into sterilized plastic syringe was treated by an artificial acid rain solution (pH. 3.5, mixture of 0.25 mM of hydrochloric acid, sulfuric acid and nitric acid). Excess solution was removed immediately after treatment and the treated soil was incubated at 25 C under darkness until isolation step of soil bacteria. Sterilized distilled water (pH 6.5) was used as a control.

(4) Evaluation of soil microbial community
Numericalization of traits of bacteria and fungal strains
Fifty strains of bacteria and fungi were isolated randomly from the colonies formed on the PTYG agar (for bacteria) and the Rose bengal agar (for fungi) media after 4 days incubation at 25 °C under darkness.
For bacteria, after purifying each strain twice, a bacterial cell suspension (10^8 cells/ml 0.85% NaCl solution) was tested for use of 95 carbon sources using the BIOLOG bacterial identification panel GN or GP according to the Gram reaction. Plates were incubated for 24 h at 25 °C under darkness.
For fungi, each strain was transferred to PDA media to check their morphological characteristics and incubated for 7 days at 25 C under darkness.
The abilities to utilize the 61 different carbon sources of bacterial strain and the 24

morphological characteristics were converted into 61 (for bacteria) and 24 (for fungi) columns of binary numbers to represent numericalized traits of each strain.

The numericalized traits of each strain were clusterized using cluster procedure in the SAS/STAT and distances between each cluster were calculated.

Diversity index

The diversity index that represents level of diversity of the community tested was calculated as follows,

Diversity index= sum of distances between each cluster x average distance between the clusters.

= sum of distances x sum of distances/number of components

=(sum of distances)^2/number of components

4. Result and Discussion

Morphological diversities of twenty model communities composed of 10 isolates of model filamentous fungi which selected randomly were determined and were compared with each number of genus and species. Remarkable high positive correlations between the morphological and taxonomical diversity were observed (Fig. 1 and 2).

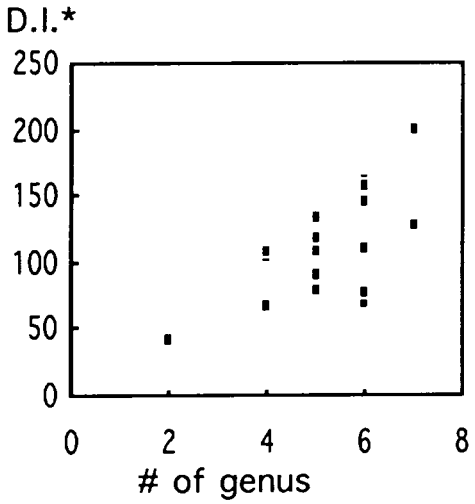

Fig. 1. Relationship between morphological diversity and numbers of genus.

*: Diversity index

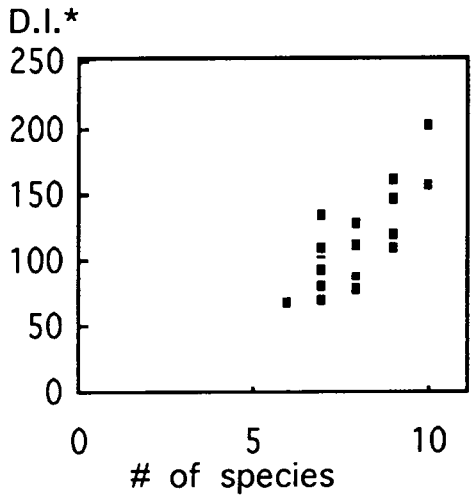

Fig. 2. Relationship between morphological diversity and numbers of species.

*: Diversity index

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Table2. Short term effect of artificial acid rain on soil microbial communtiy

	Treatment	
	Control	Artificial acid rain
Bacteria(cfu/g)	4.3×10^8	1.8×10^8
Actinomycete(cfu/g)	2.6×10^6	3.9×10^6
Diversity of Bacterialcommunity	1353.5	755.8

Table 3. Long term effect of the artificial acid rain on diversity of soil bacterial community

Yeartested	Treatment	
	Artificialacidrain	control
1994	755.8	1353.5
1995	1153.0	1358.4

Table4. Long term effect of the artificial acid rain on carbon source utilization ability of soil
bacterial community

Yeartested	Treatment	
	Artificialacidrain	control
1994	4.2	14.0
1995	6.6	15.2

Effects of environmental acidification on soil microbial community both of bacteria and fungi were evaluated in terms of quantity and diversity. Diversities of bacteria and fungi were determined by complexities of carbon source utilization pattern and morphological characteristics pattern, respectively. In quantities measured with the plate counting method, fungi increased (Fig. 3) but, in contrast, bacteria decreased in some soils acidified compared to a soil unacidified (Fig. 4). On the contrary, diversities of both of bacterial and fungal communities remarkably decreased with decrease of pHs of the soils (Fig.3, 4). It is considered that these results strongly suggest that acidification of environment will shift microbial communities to those which are more uniform and are dominated by fungi even in natural soils. Then it may make

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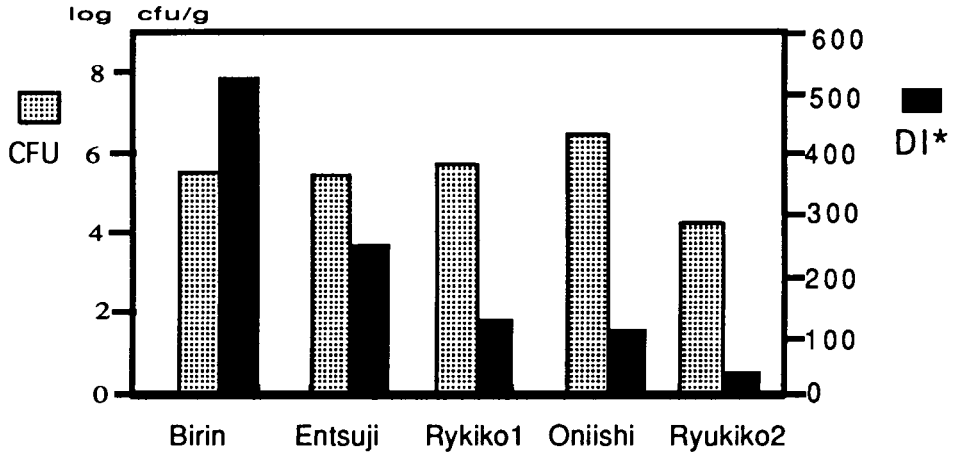

Fig. 3. Diversities and viable counts of fungi in naturally acidified soils
DI*: Diversity index

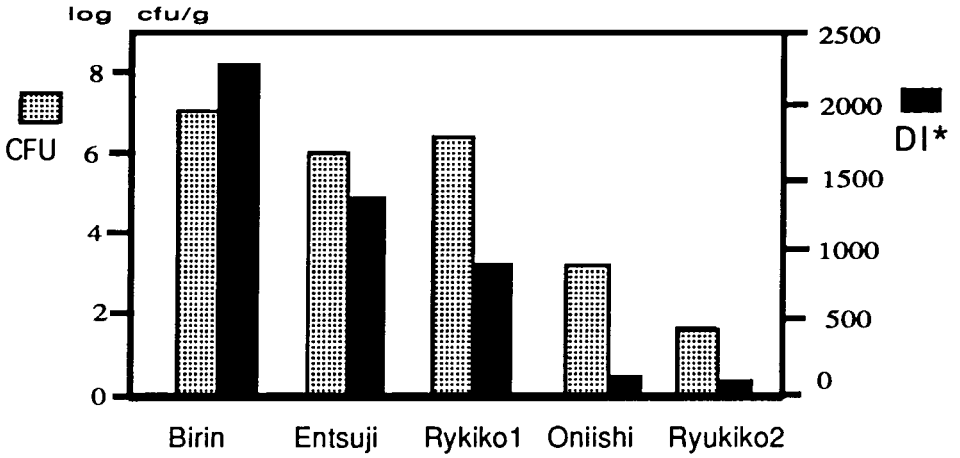

Fig. 4. Diversities and viable counts of bacteria in naturally acidified soils
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