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Original article

Geographical structure of soil microbial communities in northern Japan: Effects of distance, land use type and soil properties

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ABSTRACT

Geographical assemblages of biological community are explained by natural selection and/or by stochastic processes of death and recruitment of individuals. We evaluated geographical pattern of the structure of soil bacterial and fungal communities at 32 sites distributed over 8000 km^2 in northern Japan, to examine (1) relative contribution of environmental factors and geographical distance (2) roles of land use types and soil properties, and (3) differences in spatial pattern between bacterial and fungal communities. The abundance and composition of soil bacterial and fungal communities were evaluated by phospholipid fatty acid (PLFA) profiling and denaturing gradient gel electrophoresis (DGGE) method with 16S rDNA and 18S rDNA. PLFA profiling and DNA fingerprinting revealed that geographical distance did not significantly affect geographical patterns of microbial communities, while land use and soil chemical properties had large significant effects. The four land use types (bare ground, agricultural land, grassland, and forest) explained about 30.0% of the total variation in bacterial PLFA abundance, mostly because of their differing soil properties. Correlations with soil properties suggest that litter input from aboveground vegetation determines the bacterial abundance in bare ground and agricultural land, whereas soil pH regulates the bacterial abundance in grassland and forests. DGGE results suggest that fungal community structure is sensitive to human disturbance because agricultural land showed significantly different fungal community structure from other types.

1. Introduction

Pattern of soil decomposer communities plays key roles in primary production and nutrient cycling in terrestrial ecosystems [29]. Reportedly, soil microbes are not randomly distributed, but instead exhibit spatial aggregation [3,22,25]. Therefore, geographic assemblages of soil microbial communities should reflect spatial pattern of decomposition activity of organic matter and nutrient mineralization rate at regional scale [14].

Natural selection and dispersal are frameworks that explain generation of geographical pattern of biological communities [5,21,23]. Geographic distribution and spatial abundance patterns are generated through natural selection in spatial heterogeneity of environments [8] or through stochastic processes of death and dispersal of new individuals from regional species pool [8,21].

The relative importance of natural selection and stochastic events have been tested by comparing spatial variation in community structure with local ecological conditions or with distances between sites [10,16,18,35]. If spatial pattern shows clear correlations with local environmental variables, natural selection should be responsible for generation of spatial pattern [16]; if it shows clear relationships with distance between sites, stochastic dispersal and recruitment processes should be a main cause for spatial pattern [35]. However, the relative importance of environmental factors and geographic distance on biogeographic pattern of soil microbial communities has not been extensively studied compared with plants and animals. We attempted to clarify which mechanism, between natural selection and stochastic process, is involved into spatial pattern of soil microbial communities by relating community data with ecological condition and with geographical distance.

It is known that the aboveground vegetation strongly influences diversity and abundance of decomposer communities through input of different quality of litter into soil [4,22,27]. On the other hand, many studies have reported that soil pH explains most variation in geographic pattern of soil microbial communities [1,13]. We chose vegetation and soil, physical and chemical

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properties as potential environmental factors responsible for spatial pattern of soil microbial communities, and examined which factor was more influential on spatial assemblages of soil microbes.

Bacteria and fungi occupy 80% of the total biomass of soil organisms and account for a large share of soil decomposition activity [11]. We examined the relative importance of geographical distance and local environmental factors on spatial patterns of community assemblages of the two microbial groups. To decouple environments and distance, four land use types were sampled from different geographical area in northern Japan. Phospholipid fatty acid (PLFA) profiling was used as a measure of abundance in major microbial groups [43] and fingerprinting pattern of denaturing gradient gel electrophoresis (DGGE) using 16S rDNA and 18S rDNA fragments for bacteria and fungi, respectively, was used as a measure of microbial community composition [30]. In the present study, we addressed the following three questions: (1) are abundance and composition of soil microbial communities determined by environments or by geographic distance? (2) what is the relative importance of the two factors on assemblages of soil microbial communities? and (3) are geographical patterns of bacteria and fungi communities determined by similar mechanisms?

2. Materials and methods

2.1. Site description

The study was conducted in Aomori prefecture (139°55′9″E –141°31′3″E and 40°19′4″N–°41°31′6″N) located in northern Honshu, Japan (Fig. 1). In Aomori prefecture, the monthly average temperature ranges from −4 °C in January to 25.7 °C in August and that of precipitation ranges from 38.9 mm in January to 170.8 mm in September (Source: Average 1999–2000, Japan Meteorology Agency). We collected soil samples at 32 sites distributed throughout about 8000 km^2 (Fig. 1). The sampling sites extend from the plains along riverbanks and the seashore to mountain areas higher than 1000 m a.s.l. (Table 1). Among the 32 sites, 8 sites each were designated as having one of four land use types: bare ground (less than 50% of vegetation coverage), agricultural land, grassland, or forest. Soil types were volcanic at 29 sites, alluvial at two sites and dune at one site (Table 1).

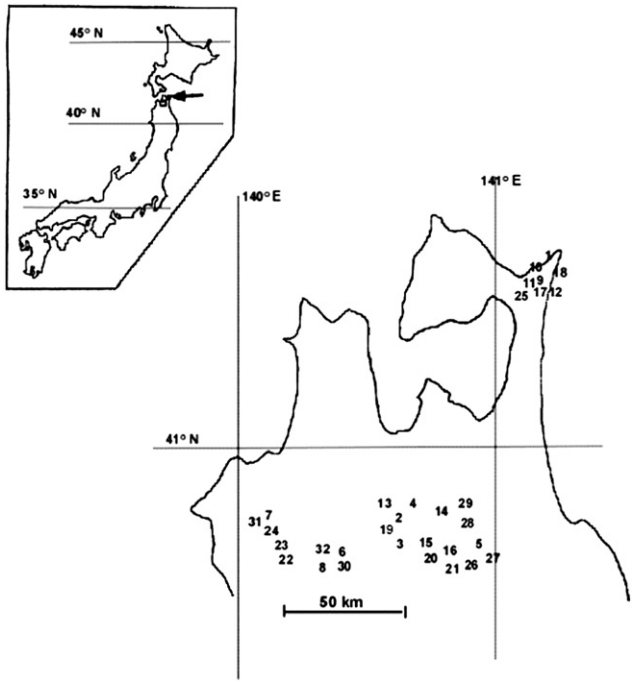

Fig. 1. Map of the study area showing the 32 sampling sites (1–32) selected from bare ground, agricultural land, grassland, and forest areas in Aomori prefecture, northern Honshu Island, Japan. Sites are described in Table 1.

2.2. Sampling and characterization of soils

A plot was randomly selected from sites representing each land use type and soil sample was collected from 0 to 10 cm depth during 13–20 September 2005. For grassland and forest, soils were sampled after cover vegetation or litter layer was removed. The collected soils were sieved through a 2 mm-mesh screen to remove plant roots, rocks, and macrofauna in the laboratory. Immediately after sieving, soil samples were analyzed to characterize their physical and chemical properties. Soil pH was determined with distilled water (50 ml) dissolving 25 g soil. Soil moisture contents were determined by weight loss after drying 10 g fresh soil at 60 °C for 24 h. These properties were measured using three replicates. Soil total N (%) and total C (%) were analyzed using an automatic N–C analyzer (NC-90A; Sumika Chemical Analysis Service Ltd., Tokyo, Japan) for a single sample of each site.

A separate soil sample was collected from each site using a soil core sampler (100 ml volume, ø 50 mm × 50 mm depth) for the analyses of solid, gaseous, and liquid fractions of soil. Bulk density was determined from the dry mass (at 105 °C) of the soil per volume collected in the sampler. Water holding capacity was determined as the content of water at 100% saturation for 24 h per g soil. Soil particle density was determined using 10.0 g dry soil with a 100 ml pycnometer and expressed as g cm^{-3}. Then, the proportions of solid, gaseous, and liquid fractions of soils were determined. These properties were determined using single sample from each of the 32 sites.

2.3. PLFA profiles

PLFA analysis was performed following by a previously described method [20] which is a modified procedure of that explained by other study [15]. Lipids were extracted from fresh soils equivalent to 7.0 g dry weight using triplicate replications for each of the 32 sites. PLFA was analyzed using a gas chromatograph (GC-14B; Shimadzu Corp., Kyoto, Japan) that was equipped with a capillary column of 30 m with 0.25 m inner diameter (SPB-1; Supelco, Bellefonte, USA). In addition, PLFA mixtures extracted from representative soils were analyzed using GC–MS (PQ2010; Shimadzu Corp., Kyoto, Japan) to identify each PLFA peak. We also used a commercial bacterial FAME mixture for peak identification (BAME; Supelco, Bellefonte, USA). The PLFAs of each soil sample were identified from the chromatographic retention time through comparison with nonadecanoic acid (19:0; Supelco, Bellefonte, USA) used as an internal standard. Total PLFA (μg PLFA g^{-1}°soil) was determined as the total microbial biomass using both identified and unidentified PLFAs in each sample; the identified individual PLFA was expressed as nmol PLFA g^{-1} dry soil. Fatty acid nomenclature followed that described by other study [15]. As shown in Table 2, we used 20 selected PLFAs as biomarkers for six microbial groups (general bacteria, Gram-positive bacteria, Gram-negative bacteria, actinomycetes, arbuscular mycorrhizae, and ectomycorrhizal and saprotrophic fungi) following procedures described in previous studies [7,15,38].

2.4. DNA extraction, PCR, and DGGE analysis

DNA was extracted from 1 g fresh soil using a kit (Ultra Clean Soil DNA kit; Mo Bio Laboratories Inc., Solana Beach, USA). Because of