CHAPTER V

BACTERIAL COMMUNITY STRUCTURE: CULTURE-DEPENDENT STUDY

5.1 Introduction

Knowledge of diversity and structure of soil bacterial communities enables a better understanding of soil bacterial processes. Land management may lead to changes in soil bacterial populations and, consequently, in soil functions (Diaz-Ravina and Baath, 1996; Smart *et al.*, 1997). Soil can be considered as a heterogeneous environment, in which habitats are determined by various factors, such as soil pH, water content, organic matter under decomposition, the low rate of mixing, and soil bacterial activities.

Soil is inhabited by diverse varieties of bacteria. Bacterial characteristics of soils are being evaluated increasingly as sensitive indicators of soil quality because of clear relationships between bacterial diversity, soil and plant quality, and ecosystem sustainability (Doran *et al.*, 2002). Therefore, quantitative and qualitative changes in the composition of soil bacterial communities may serve as important and sensitive indicators of changes in soil which are caused by different land managements.

Bacterial diversity in soils has been studied traditionally by the cultivation of bacteria on laboratory media. A simple method based on bacterial colony development on agar plates for the physiological characterization of bacterial communities in soil was used to quantify bacterial colony development on agar plates over a number of days and determine ecophysiological differences between different soils by applying *r*- and *K*-concepts to slow and fast growing bacteria from these different soils (De Leij *et al.*, 1993). In this chapter, the results of an investigation

applying the bacterial colony development method to determine the total bacterial counts and ecophysiological differences in bacterial communities inhabiting soils under different land management practices were presented and the impact of land management on soil bacterial counts and communities were also discussed.

5.2 Materials and methods

Bacterial community structure: Cultural-dependent method

Fresh soil samples were dispersed in 0.85% NaCl. The resulting slurry was decimally diluted with 0.85% NaCl. Each dilution was spread on a nutrient agar (NA) plate to which 2.5 µl/ml of amphoterricin B (Fungizone™, Bristol-Myers, USA.) was added as a fungal growth inhibitor. The NA plates were incubated at the temperature equal to the average temperature at the sampling site for up 10 days, and colonies were enumerated on a daily basis. Therefore, 10 classes were generated per plate. Only plates that contained between 30-300 colonies were selected for enumeration. The number of bacteria in each class was expressed as a proportion (%) of the total count. The distribution of *r*- and *K*-strategists in each sample can be characterized according to the fast growth in response to enrichment of *r*-strategists and the slow growth in response to enrichment of *K*-strategists (Andrew and Harris, 1986). Fast growers were defined as bacteria that produced visible colonies on NA within 24-48 hours. The different colony distribution curves were analyzed.

Total bacterial counts obtained after 10 days of incubation were expressed in colony forming units (CFU) per gram soil (fresh weight). CFU counts were used for comparisons between bacterial quantity and physical conditions, by means of analyses of variance.

The distribution of the 10 classes of bacterial colonies in each sample was expressed by an ecophysiological index (EPI) (De Leij *et al.*, 1993), a modification of the Shannon diversity index,

$$EPI = - \Sigma(p_i \log_{10} p_i)$$

where p_i represents each proportion of the ten classes to the total population in that sample. EPI values were compared using analysis of variance.

5.3 Results and discussion

5.3.1 Soil bacterial counts

After 10 days of growth on nutrient agar plates, the average numbers of colony forming units (CFU) were lowest (5.17×10^5 CFU·g^{-1}) in the organic farm soil in wet season, and highest (9.33×10^5 CFU·g^{-1}) in the forest soil in wet season (Figure 5.1). No significant differences (after 10 days growth) were found between sites and seasons (ANOVA, df = 5, $p = 0.204$).

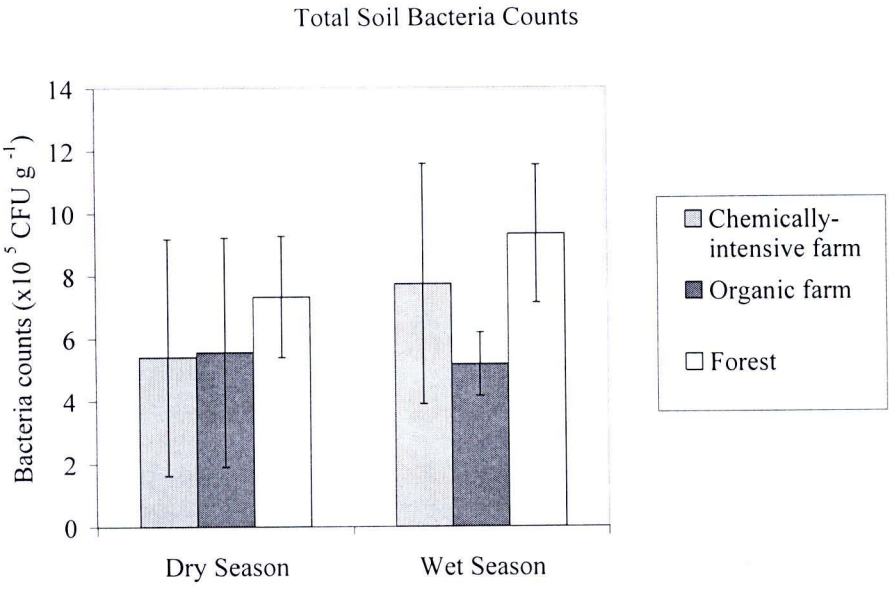

Figure 5.1 Comparison of colony forming unit (CFU) counts of soil bacterial populations from different sites, grown on nutrient agar plate, presented as average ± standard error (n=6).

Correlations between bacterial numbers, soil physical factors, and soil nutrients

Although there was no significant difference of bacterial numbers among sites and between seasons, the CFU values were positively correlated with soil pH and soil nutrients (organic matter, organic carbon and total nitrogen) in all sites (Pearson correlation, $p<0.01$; Appendix 1). The forest soil had relatively high numbers of bacteria in both seasons that were correlated with soil pH and soil nutrient content in the forest, which was higher than other sites (Tables 4.1-4.4). The results were consistent with the findings by Kirschner *et al.* (1999) in which a positive correlation was shown between the numbers of members in the Mycobacterium complex and the content of humic and fulvic acids, two important constituents of organic matter. Previous studies have also indicated that some bacteria benefit from acidity in growth

media (Katila and Mattila, 1991; Portaels and Pattyn, 1992) which was comparable with soil pH values, namely slightly acidic (ranged from 5.54-6.81), in this study.

5.3.2 Soil bacterial community structure

The agar plate method based on *r/K* distribution patterns of populations showed that bacterial community in all soils was dominated by fast-growing bacteria (Figure 5.2). The *r*-strategists were more predominant on the nutrient agar plate of the forest soil compared to the chemically-intensive farm soil and the organic farm soil in dry season (Figure 5.2). Similarly, in wet season, bacteria from the forest soil grew more rapidly than those from the 2 agricultural soils on the nutrient agar plates. On the basis of the ecophysiological index (EPI), physiological diversity was observed to be higher in the bacterial community of the organic farm soil (EPI = 0.669) than those of the chemically-intensive farm soil (EPI = 0.639) and the forest soil (EPI = 0.401) in the dry season. In the wet season, however, the EPI value was much higher in the bacterial community in the organic farm soil (EPI = 0.519) than those of the chemically-intensive farm soil (EPI = 0.450) and the forest soil (EPI = 0.386). Nevertheless, there was no significant difference in EPI values between sites and seasons (Figure 5.3).

Higher values for EPI imply a more even distribution of proportions of bacteria developing on different days (i.e. different classes of bacteria). The mean of EPI (Figure 5.3), ranging from 0.401-0.669, were similar to values previously reported (De Leij *et al.*, 1993), indicating a more even distribution of different classes in this study. However, EPI does not differentiate between *r*-strategist dominance and *K*-strategist dominance in microbial studies of the present nature. For example, if

80% of the colonies appeared on day 1 of one sample (*r*-strategists dominant) and 80% of colonies appeared on day 10 of another sample (*K*-strategists dominant) calculated EPI would be the same provided the development of the other 20% of colonies was spread similarly for the two samples (Sarathchandra *et al.*, 1997).

The *r/K* strategy concept proposes that organisms strive to maximize their survival in either uncrowded (*r*-selection) or crowded (*K*-selection) environments (Sarathchandra *et al.*, 1997). This concept suggests that there are differences between organisms in their ability to exploit and survive in different environments. Generally, *r*-strategists are considered to dominate unstable environments and *K*-strategists are characteristic of stable environments (Kunito *et al.*, 2001). From the results (Figure 5.2), it appears that the forest soil contained a greater proportion of bacteria which are *r*-strategists and are therefore able to respond and grow quickly in response to abundant, easily available nutrients which were of higher values than other sites (Chapter IV). Nutrients from animal wastes, plant litter and fertilizer may have contributed to the *r*-strategist dominance of surface soil (De Leij *et al.*, 1993). Likewise, the chemically-intensive farm and the organic farm bacteria appear to be more adapted to stable environments controlled by land management and a large proportion of them were also *r*-strategists, being able to colonize the uncrowded environments, although slower than in the forest soils (Figure 5.2). In long-term land management, such as the chemically-intensive farm and the organic farm, the bacterial colonies may have adapted to these conditions and survive and compete effectively with forest soil bacteria.

The difference in soils and plant growth patterns in the area may explain these observations. In this study, forest soils are high in organic matter and other nutrients, compared with agricultural soils. The uninterrupted plant growth over long periods in

the forest may also allow the adaptation of bacterial flora to particular plant species. A hypothesis has been proposed that plant and bacterial species may adapt to the presence of each other under natural conditions (Chanway *et al.*, 1990). It is possible that some of the bacterial communities colonizing the chemically-intensive farm are unique and have adapted to their habitat by utilizing specific compounds such as chemical inputs added during agriculture.

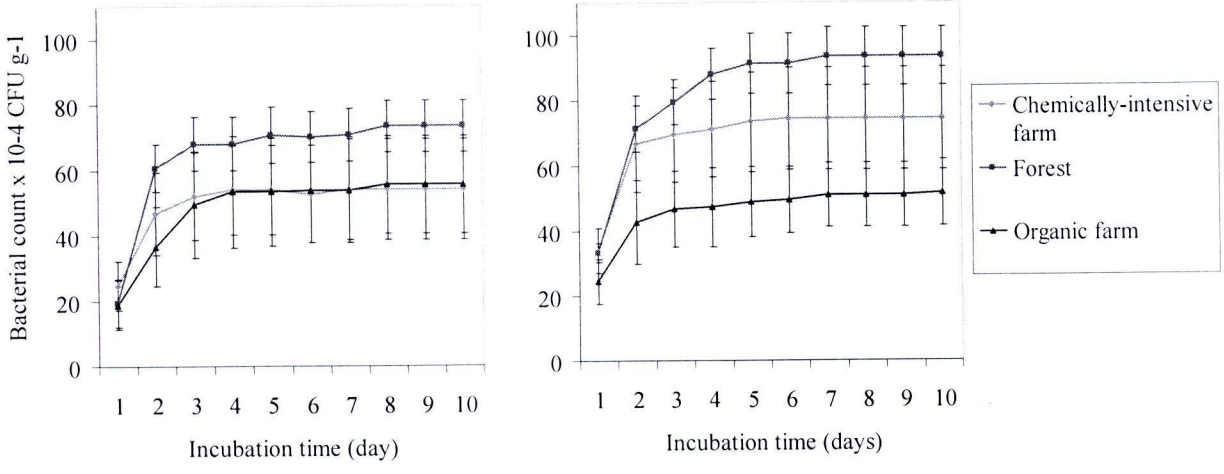

Figure 5.2 Bacterial community structures of soil bacteria from 3 sites in the dry season (A) and wet season (B). Data derived from bacterial colonies appearing on NA over a period of 10 days. (n=6) Each point and error bar represents the averages ± SE.

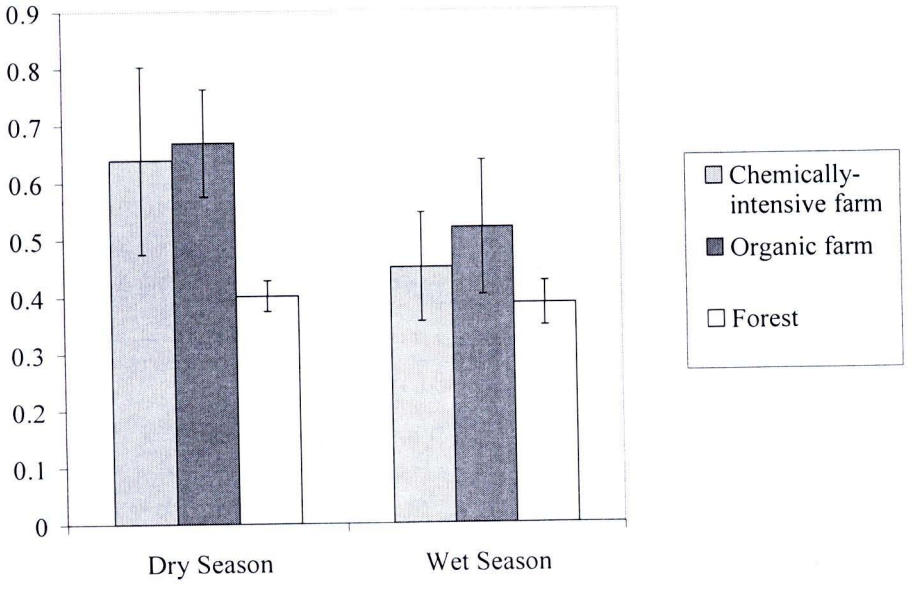

Figure 5.3 Comparison of ecophysiological index (EPI) values of soil bacterial populations from different sites, grown on nutrient agar plate, presented as average ± standard error (n=6).

Only minor differences were observed between different land management practices and seasons. However, the statistical relationships between different soil samples were so weak that it was rather difficult to apply *r-K* concepts to the colony development of these chemically-intensive farm and organic farm bacteria. Even though the bacterial counts and EPI were not significantly different, it did not exclude any change in the bacterial community. Land management may affect the bacterial communities, but the bacterial communities have resilience for disturbances and could adapt to new environment, or the new community could replace the old ones but preserving similar ecological functions, as the results of the bacterial numbers and EPI showed no difference between sites with different land management.

Simple and relatively rapid methods are needed to obtain a better understanding of the functional diversities of the soil bacterial communities. The use

of bacterial colony development rates and the ecophysiological index appears to be sufficiently sensitive to detect highly significant differences in the rate of colony development from different samples collected from a single habitat over a relatively short time span. Such simple methods would warrant further evaluation in different systems, perhaps including the use of simple and recalcitrant substrates.

Total bacterial counts might be useful to quantify nutrient richness of the environment. Also, total bacterial counts in the chemically-intensive farm and the organic farm soil were lower than the forest soil. This can be explained in terms of nutritional status because the organic matter content of the chemically-intensive farm and the organic farm were less than the forest. This means that only quantitative changes in nutritional status of a habitat might be reflected in the total bacterial counts. Qualitative nutritional changes will result in a change of microbial population structure, but not necessarily in a change in total numbers. Since quantitative, as well as qualitative nutritional changes in the environment are important parameters in risk assessment, both population structure assessments and total bacterial population counts can be valuable.

Land management may affect the bacterial communities, but the bacterial communities may be resilient and functions are reserved such that no significant changes were observed in the bacterial numbers and EPI showed no difference between sites with different land management practices. Nonetheless, the results were obtained using a culture-dependent technique, which accounts for only 0.01% of all bacterial communities in soils. Additional information may be gained from other methods available.

5.4 Conclusions

Soil bacterial counts showed that bacterial communities obtained from soils of the forest, the chemically-intensive farm, and the organic farm were dominated by the *r*-strategist bacteria. Although, no significant difference was found in bacterial numbers in both between sites and seasons, the CFU values were positively correlated with soil pH and soil nutrients (organic matter, organic carbon and total nitrogen) in all sites. Ecophysiological index (EPI) values were higher in the bacterial community of the organic farm soil than those of the chemically-intensive farm soil and the forest soil. However, there was also no significant difference in EPI values between sites and seasons.