CHAPTER II

LITERATURE REVIEW

2.1 Soil

Soil is home to a large proportion of the world's genetic diversity. The linkages between soil organisms and soil functions are observed to be exceptionally complex. The interconnectedness and complexity of the soil food web means any appraisal of soil function must necessarily take into account interactions among the living communities that exist within the soil. An incredible diversity of organisms makes up the soil food web. They range in size from the tiniest unicellular bacteria, algae, fungi, and protozoa, to the more complex nematodes and micro-arthropods, to the visible earthworms, insects, small vertebrates, and plants. As these organisms eat, grow, and move through the soil, they make it possible to have clean water, clean air, healthy plants, and moderated water flow. Therefore, the soil food web is evidently an integral part of landscape processes.

Soil is made up of a multitude of physical, chemical, and biological entities, with many interactions occurring among them. It is a variable mixture of broken and weathered minerals and decaying organic matter. Together with the proper amounts of air and water, it supplies, in part, sustenance for plants as well as mechanical support. The diversity and abundance of soil life exceeds that of any other ecosystem. Plant establishment, competitiveness, and growth are governed largely by the below-ground ecology, so understanding this system is an essential component of plant sciences and terrestrial ecology.

Soil factors, such as organic matter content, soil structure, nutrient content, nutrient cycling, nutrient availability, and water holding capacity, are all influenced

by, or dependent upon, soil bacteria (Torsvik and Øvreås, 2002). Bacteria can be further subdivided based on genetic relationships, specific functions or habitats in which they survive. Important soil bacterial functions such as organic matter breakdown and nitrogen fixation are fairly well understood, but this is only a small fraction of bacterial capabilities.

Intensive studies have described impacts of different land use on soil physical factors. These include effects of land use on soil moisture variation (Fu *et al.*, 2003; Mehta *et al.*, 2005), on soil water holding capacity (Agele *et al.*, 2005), on soil texture and soil characteristics (Graham *et al.*, 2000; Emadi *et al.*, 2008), and on soil nutrients, such as distribution of soil organic matter, organic carbon, total nitrogen and available phosphorus (Hofmann *et al.*, 2003; Zhang *et al.*, 2005; Gong *et al.*, 2005). These studies suggested that the use and management of land significantly altered soil physical factors.

2.2 Soil bacteria

Soil is a complex environment colonized by an immense diversity of microorganisms. Soil microbiology focuses on the soil viruses, bacteria, actinomycetes, fungi, and protozoa, but it also has traditionally included investigations of the soil animals such as the nematodes, mites, and other microarthropods. These organisms, collectively referred to as the soil biota, function in a below-ground ecosystem utilizing plant roots and litter as food sources. The surface layers of soil contain the highest numbers and variety of microorganisms, because these layers receive the largest amounts of potential food sources from plants and animals (Liu *et al.*, 2006). The soil biota form a below-ground system based on the energy and nutrients that they receive from the decomposition of plant and animal

tissues. The primary decomposers are bacteria and fungi. Modern soil microbiology therefore represents an integration of microbiology with the concepts of soil science, chemistry, and ecology to understand the functions of microorganisms in the soil environment.

Bacteria are the most numerous denizens of soil, with populations ranging from 100 millions to 3 billions in a gram (Madigan *et al.*, 2006). They are capable of very rapid reproduction by binary fission in favorable conditions. Most soil bacteria live in close proximity to plant roots, and are often referred to as rhizobacteria. Bacteria live in the soil solution, including the film of moisture surrounding soil particles, and some are able to swim by means of flagella. The majority of beneficial soil-dwelling bacteria need oxygen, and are thus termed aerobic bacteria. On the contrary, those that do not require oxygen are referred to as anaerobic bacteria, and tend to cause putrefaction of dead organic matter. Aerobic bacteria are most active in a soil that is moist and pH-neutral, and where there is plenty of food available such as carbohydrates and micronutrients from organic matter. Hostile conditions will not completely kill bacteria; rather, bacteria will stop growing and get into a dormant stage, and those individuals with pro-adaptive mutations may compete better in the new conditions. Gram-positive bacteria produce spores in order to wait for more favorable circumstances, and Gram-negative bacteria get into a non-culturable stage. Microbiologists traditionally isolate pure strains of microorganisms by using culture techniques. However, methods that do not rely on culturing bacteria have been developed, including microscopic observation and biochemical or genetic analysis of specific cell constituents. The rates or controlling factors for bacterial processes are studied by using various methods from chemistry, biology, and ecology.

What is known about soil bacteria fills many volumes of textbooks, but remains far exceeded by what is unknown. As an example, soil testing using genetic analysis methods has estimated that a single teaspoon of soil may contain as many as 10,000 distinct species of bacteria. Of these only about 1% or less can be cultured, and even fewer of these are known to have specific soil functions (Kirk *et al.*, 2004).

Bacteria have a key role in the processing of materials that maintain life on earth. Transformation of elements between forms can be described conceptually as the elemental cycles (Stark *et al.*, 2007). In the carbon cycle, bacteria transform plant and animal residues into carbon dioxide and the soil organic matter known as humus. Humus improves the water-holding capacity of soil, supplies plant nutrients, and contributes to soil aggregation.

Bacteria may also directly affect soil aggregation, which determines the workability of the soil. Bacteria are important in the formation of soil structure (Lynch and Bragg, 1985; Tisdall, 1991). Actinomycetes produce hyphal threads that bind soil particles together. Extracellular polysaccharides produced by bacteria assist in building soil structure. Humic materials from bacterial action form organic matter/clay complexes and help aggregate the soil. This action reduces erosion, allows good water infiltration, and maintains adequate aeration of the soil. Soil aggregation can be increased by the addition of residues, resulting in additional bacterial activity (Charman and Murphy, 1998). The ability of bacteria to influence aggregation varies with species and is substrate-dependent (Leeper and Uren, 1993).

Soil bacteria are also important constituents in the nitrogen cycle (Stark *et al.*, 2007). The atmosphere is approximately 80% nitrogen gas (N_2), a form of nitrogen that is available to plants only when it is transformed to ammonia (NH_3) by either soil bacteria (N_2 fixation) or by humans (manufacture of fertilizers). Soil bacteria also

mediate denitrification, which returns nitrogen to the atmosphere by transforming NO_3^- to N_2 or nitrous oxide (N_2O) gases. In addition, bacteria are crucial to the cycling of sulfur, phosphorus, iron, and many micronutrient trace elements.

The immense varieties of natural substances that are used by bacteria indicate that soil bacteria possess diverse mechanisms for degrading various compounds. Human activity has polluted the environment with a wide selection of synthetic or processed substances. Many of these hazardous or toxic substances can be degraded by soil bacteria. This is the basis for the treatment of contaminated soils by bioremediation, or the use of bacteria or bacterial processes to detoxify and degrade environmental contaminants. Soil microbiologists therefore study these bacteria, their metabolic pathways, and relevant environmental conditions that can be used to eliminate pollutants from the soil environment.

2.3 Soil bacteria in relation to the soil properties

In addition to provision of physical habitats for soil bacteria, soils provide sustenance for the bacteria, both as energy sources and required nutrients. Nutrients for bacterial growth are divided into three categories: primary, secondary, and micronutrients. Nitrogen, phosphorus, and potassium are primary nutrients, which are needed in fairly large quantities compared to the other nutrients. Carbon and nitrogen pools delimit bacterial biomass and decomposition rates (Knapp *et al.*, 1983). Calcium, magnesium, and sulfur are secondary nutrients which are required in lesser quantities but are no less essential than the primary nutrients. Zinc and manganese are micronutrients which are required in very small amounts. Most secondary and micronutrient deficiencies are easily corrected by keeping the soil at the optimum pH value. As primary decomposers, bacteria occupy a critical position in the soil food

web and 90–95% of all nutrient cycling passes through this group of organisms to higher trophic levels. Soil bacteria constitute a large dynamic source and sink of nutrients in all ecosystems and play a major role in plant litter decomposition and nutrient cycling (Smith and Paul, 1990; Cambardella and Elliott, 1992; Collins *et al.*, 1992). Thus, function, and possibly diversity, of bacteria will be a large factor in determining the functioning of an ecosystem (Lynch, 1983).

Bacteria are also important for the breakdown of organic material. Most natural and xenobiotic compounds can be broken down by soil bacteria with few compounds becoming recalcitrant (Alexander, 1977). Organic matter improves soil physical properties, increases water holding capacity and nutrient availability, and acts as a cementing agent for holding soil particles together. Bacteria thrive best in different soil pH ranges, most do best in slightly acidic soils (pH 5.8 to 6.5) (Pansu and Gautheyrou, 2006). Soil pH values above or below these ranges may result in less vigorous growth or symptoms of nutrient deficiencies.

Bacteria are sensitive to disturbances, such as those induced by agriculture, pollution, and other stress (Elliott and Lynch, 1994). Understanding the effects of disturbance on soil bacterial diversity and functioning may contribute greatly to understanding of soil quality and development of sustainable agroecosystems (Thomas and Kaven, 1993). Different land use and management practices significantly modify soil properties (Islam and Weil, 2000; Braimoh and Vlek, 2004; Shepherd *et al.*, 2000, Chen *et al.*, 2001). For example, conversion of natural vegetation to other land uses caused decreases in nutrient concentrations (Lichon, 1993). Consequently, qualitative and quantitative changes in soil properties may result in changes in soil bacteria community (Braimoh and Vlek 2004; Vitousek *et al.* 1997).

2.4 Assessment of soil bacterial diversity

Knowledge of bacterial diversity is important in understanding of the bacterial ecology in soil and other ecosystems (Atlas, 1984). Soil bacterial communities remain some of the most difficult communities to characterize, because of their immense phenotypic and genotypic diversity. A representative estimate of bacterial diversity is a prerequisite for understanding the functional activity of microorganisms in such ecosystems (Garland and Mills, 1994; Zak *et al.*, 1994)

Several methods have been developed for analyzing the structure and diversity of bacterial communities (Amann *et al.*, 1995; Emerson *et al.*, 1993; Holben *et al.*, 1988; Liesack and Stackebrandt, 1992; Navarro *et al.*, 1992; Sørheim *et al.*, 1989; Hill *et al.*, 2000). Plate counts of bacterial populations provide quantitative yields and specific information on biodiversity (Torsvik *et al.*, 1990). On the other hand, plating studies are inadequate methods for estimating biodiversity, since less than 1% of the total bacterial population has proven to be culturable on standard media (Fægri *et al.*, 1977; Ward *et al.*, 1990; Kirk *et al.*, 2004). To get a better insight into microbial processes within an ecosystem, it is important to study functional diversity in combination with taxonomic diversity. Current knowledge regarding genetic diversity is deficient; even less is known regarding the way genetic and taxonomic diversities affect functional diversity and ecosystem properties (Kennedy and Smith, 1995). Currently, understanding bacterial community composition and structure is restricted because of methodological limitations and the lack of basic taxonomic information. Because taxonomic analysis of bacteria on a large scale is practically impossible; therefore, the focus is shifted to microorganisms of primary importance for the function of an ecosystem.

Comparisons of phenotypic and DNA-based analyses of bacteria diversity provide insights into understanding of bacterial populations in soil. Substrate utilization or production profiles may also provide useful information on functional biodiversity (Zak *et al.*, 1994; Haack *et al.*, 1995; Garland, 1996). Recently, molecular techniques have also been frequently applied to biodiversity assessment. These include measurements of nucleic acids in the environment (Giovannoni *et al.*, 1990; Holben and Harris, 1995; Holben *et al.*, 1988; Torsvik *et al.*, 1990, Stahl and Amann, 1991; Liesack and Stackebrandt, 1992; Navarro *et al.*, 1992), 16S rRNA fingerprinting (ARDRA) (Martinez *et al.*, 1995; Massol *et al.*, 1995) and denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.*, 1995; Wawer and Muyzer, 1995; Ferris *et al.*, 1996; Øvreås *et al.*, 1997; Jensen *et al.*, 1998), DNA sequencing (Lane, 1991; Amann *et al.*, 1995), and repeated extragenic palindromic sequence PCR (rep-PCR) genomic fingerprinting (Versalovic *et al.*,1991; Bruijn, 1992). The combination of direct retrieval of rRNA sequences and *in situ* hybridization also promises to help identifying microbial phylogeny, and to detect and identify individual microbial cells without cultivation (Amann *et al.*, 1995). At present, the lack of rDNA sequence data for many described species is probably the major cause of failure in molecular identification (Borneman *et al.*, 1996; Borneman and Triplett, 1997). Available databases include only a few sequences of organisms isolated from the environment for which an extended phenotypic description has been accomplished.

Methods for assessment of bacterial diversity can be grouped largely into culture-dependent and culture-independent approaches. Different methods explore and reflect different aspects of bacterial diversity and community structure. Advantages and limitation of various methods are detailed below.

2.4.1 Culture-dependent methods: Dilution plating and culturing methods

Traditionally, the analysis of soil bacterial communities has relied on culture techniques using a variety of culture media designed to maximize the recovery of different bacterial species. This is particularly the case for soil health studies, in which numerous examples showed that these techniques have revealed bacterial diversity associated with various soil quality parameters, such as disease suppression and organic matter decomposition (Tunlid *et al.*, 1989; Boehm *et al.*, 1993, 1997; de Leij *et al.*, 1993; Workneh *et al.*, 1993; Alvarez *et al.*, 1995; Hu and van Bruggen, 1997; Maloney *et al.*, 1997). Although there have been recent attempts to devise suites of culture media to maximize the recovery of diverse bacterial groups from soils (Balestra and Misaghi, 1997; Mitsui *et al.*, 1997), it has been estimated that less than 0.1% of the bacteria found in typical agricultural soils are culturable using current culture media formulations (Torsvik *et al.*, 1990a; Atlas and Bartha, 1998; Kirk *et al.*, 2004). This is based on comparisons between direct microscopic counts of bacteria in soil samples and recoverable colony forming units.

Generally, bacterial populations have been used as indicators of ecosystem change. It is rarely possible to count soil bacteria, even on selective media, to give an error less than 0.5 log units (a 300% change), which many would regard as a catastrophic change on an ecosystem (Lynch, 2002). An improvement is to measure colony development on isolation plates with time (De Leij *et al.*, 1993). Counts are made for each of incubation day and the number of colonies in each daily group assessed as a population. To express the distribution of the bacterial population in each sample in a single number, the Shannon diversity index was calculated.

Since the Shannon diversity index is usually used as a measure for the number of species and the abundance of each species in a population, this diversity index is

strictly not appropriate for the quantification of classes of bacteria with similar development characteristics on agar. Therefore, the ecophysiological index (EPI) is used (De Leij *et al.*, 1993). EPI is a more sensitive indicator of perturbation and has more ecological significance than routine colony counting at one moment in time. However, the problem with all counting techniques is that probably no more than 1% of species are culturable and so a totally false impression might be gained.

The primary perceived advantage is that the direct culturing of the sample produces a recognizable number, typically colony forming units (CFU). Therefore, the culturing methods may provide definitive information about the identity and physiological or functional properties of the bacteria and/or the enumeration of specific bacteria when selective medium is employed. However, because of the incredible complexity of the bacterial populations, unless a number of different tests are run, the use of only a few specific bacteria as indicator organisms may provide an inadequate estimation of the true levels of diversity.

2.4.2 Culture-independent methods: Molecular techniques

Because of the inherent limitations of culture-based methods, soil microbial ecologists are turning increasingly to culture-independent methods of community analysis. Using culture-independent methods, the composition of communities can be inferred based on the extraction, quantification, and identification of molecules from soil that are specific to certain bacteria or bacterial groups; or advanced fluorescence microscopic techniques. Useful molecules for such studies include phospholipid fatty acids and nucleic acids (Morgan and Winstanley, 1997)

Among macromolecules tested to date, nucleic acids have been the most useful in providing a new understanding of the structure of bacterial communities.

For example, in recent studies of soil bacterial diversity, re-association kinetics of DNA isolated from soil was compared with that of pure cultures of bacteria. Using the assumption that the greater the sequence diversity of the DNA, the greater the DNA reannealing time, it was estimated that the genetic diversity of soil was 200 times greater than the diversity among bacteria cultured from the same soil (Torsvik *et al.*, 1990a,b, 1996; Ovreas and Torsvik, 1998). This shows that soil bacterial communities are much more complex than they have been previously recognized and that the analysis of DNA sequences may provide a greater understanding of the bacterial diversity that exists in soil than could be gained from culture-dependent methods.

Of the various nucleic acid techniques used to estimate bacterial community composition and diversity in complex habitats, the most useful is the determination of the sequences of 16S ribosomal RNA (rRNA) genes in prokaryotes and 5S or 18S rRNA genes in eukaryotes (Ward *et al.*, 1992). These small subunit (SSU) rDNA molecules are particularly suited for such studies for a number of reasons. First, they are found universally in all three domains of life: Bacteria, Archaea, and Eukarya (Woese *et al.*, 1990). Second, these molecules are composed of both highly conserved regions and regions with considerable sequence variation (Woese, 1987). Because of differential rates of sequence evolution, phylogenetic relationships at several hierarchical levels can be measured from comparative sequence analyses. Third, phylogenetic information held in the SSU rDNA molecule is further enhanced by its relatively large size (e.g. ~1.5 Kb for the 16S rDNA molecule) and the presence of many secondary structural domains (Ludwig and Schleifer, 1999). Consequently, evolutionary changes in one domain do not affect the rate of change in

other domains. Finally, 16S rDNA can be easily amplified using polymerase chain reaction (PCR) and rapidly sequenced.

Perhaps the greatest advantage of the analysis of 16S rDNA is that bacteria from natural habitats can be studied and characterized without culturing. Various studies have shown that rDNA from over 90% of the bacteria that can be observed microscopically *in situ* can be extracted and analyzed (Steffan and Atlas, 1988; Steffan *et al.*, 1988; Tsai and Olsen, 1992; More *et al.*, 1994; Zhou *et al.*, 1996; Porteous *et al.*, 1997; Kirk *et al.*, 2004) as compared with less than 0.1% of the bacteria observed in soil that can be recovered on culture media.

Numerous studies have applied molecular techniques to study soil bacterial diversity (e.g. Felske *et al.*, 1997; Miskin *et al.*, 1998; Henne *et al.*, 1999; Miller *et al.*, 1999; Brady and Clardy, 2003; Kauffmann *et al.*, 2004; Venter, 2004; Bertand, 2005; Lim, 2005; Wexler *et al.*, 2005; Voget *et al.*, 2006). In nearly all of these studies, novel microbial lineages have been discovered, confirming a lack of understanding of the bacterial species that inhabit soils and their potentially important roles in ecosystem function. For example, studies have shown that agricultural soils contain a diversity of Archaea, organisms previously thought to exist only in extreme environments (Ueda *et al.*, 1995; Bintrim *et al.*, 1997; Buckley *et al.*, 1998). Other studies have shown that some soil microbes, which have previously not been cultured and described, are global in their distribution and may play important roles in soils worldwide (Liesack and Stackenbrandt, 1992; Felske *et al.*, 1997; Kuske *et al.*, 1997).

Recently, the use of denaturing gradient and temperature gradient gel electrophoresis (DGGE/TGGE) for separating individual amplicons has been described (Muyzer *et al.*, 1993; Ferris and Ward, 1997; Heuer *et al.*, 1997; Muyzer

and Smalla, 1998). This technique allows one to separate mixtures of PCR products that are of the same length but differ in sequence. Theoretically, any 16S rRNA gene found in the mixed template DNA extracted from soils could be specifically amplified and resolved on a DGGE gel. Once rDNA amplicons have been cloned or separated by DGGE or TGGE, they can be sequenced and analyzed for similarity to other known sequences in public-domain databases (e.g. the NCBI GeneBank database [http://www.ncbi.nlm.nih.gov], and the Ribosomal Database Project [http://www.cme.msu.edu/RDP/] (Maidak *et al.*, 1997). By estimating phylogenetic relatedness to other sequences in the databases, the identity of the bacteria from which the 16S rRNA gene was derived can be determined. It is hoped that the potentially close phylogenetic relationships of non-culturable bacteria with known species can be utilized to devise culturing techniques for many of these bacteria.

One of the limitations of DGGE is the separation of only relatively small fragments, up to 500 base pairs (Myers *et al.*, 1985). This limits the amount of sequence information for phylogenetic inference as well as for probe design. It has been demonstrated that it is not always possible to separate DNA fragments which have a certain amount of sequence variation. Vallaeys *et al.* (1997) found that 16S rDNA fragments obtained from different methane-oxidizing bacteria could not be resolved by DGGE although they had substantial sequence variation. Similarly, Buchholzcleven *et al.* (1997) demonstrated that it was not possible to separate rDNA fragments differing in two to three nucleotides under the electrophoretic conditions they used. Furthermore, Kowalchuk *et al.* (1997) showed that double bands in the DGGE patterns were a result of the presence a so-called *wobble base* (either a C or a T) in the reverse primer. When a mixture of the reverse primers was used, two bands

were visible in the DGGE pattern, while when the two primers were used in separate PCR reactions only one band per reaction was found (Muyzer and Smalla, 1997).

Another limitation of DGGE involves its resolution or the maximum number of different DNA fragments which can be separated by the technique. For instance, by using DNA-DNA reannealing experiments, Torsvik *et al.* (1990) found that there were as many as 104 different genomes present in soil samples. It was evident that DGGE could not separate all of the 16S rDNA fragments obtained from such a variety of bacteria. In general, these electrophoretic techniques will only display the rDNA fragments obtained from the predominant species present in the community. Several different studies revealed that bacterial populations that make up 1% or more of the total community can be detected by PCR-DGGE (Muyzer *et al.*, 1993; Murray *et al.*, 1996). A similar value has been found by Lee *et al.* (1996) using PCR SSCP to characterize bacterial community structures.

Furthermore, co-migration of DNA fragments can be a problem for retrieving clean sequences from individual bands. Another problem in the study of community diversity on the basis of 16S rRNA genes, using DGGE, TGGE or cloning strategies is the presence in some bacteria of multiple *rrN* operons with sequence microheterogeneity. DGGE and TGGE can visualize this sequence heterogeneity (Nubel *et al.*, 1996), which might lead to an overestimation of the number of bacteria within natural communities. The same is true for the double bands in the DGGE or TGGE patterns which were produced by the use of degenerate primers in the PCR reactions (Kowalchuk *et al.*, 1997).

2.5 The use of 16S rDNA to estimate bacterial diversity

Ribosomal DNA (rDNA) are sequences encoding ribosomal RNA, and are located within nucleolus. These rDNA regions, also referred to as nucleolus organizer regions, are parts of expanded chromosomal loops that carry the transcriptional units encoding ribosomal RNA, clustered in tandem repeats. These sequences regulate amplification and transcription initiation and contain transcribed and nontranscribed spacer segments.

The low rate of polymorphism in the rDNA transcription unit allows characterization of the rDNA of each species using only a few specimens, and makes this DNA useful for interspecific comparisons. In addition, different coding regions of the rDNA repeats usually show distinct evolution rates. As a result, this DNA can provide information about almost any systematic level (Hillis *et al.*, 1991)

16S ribosomal RNA (rRNA), when translated, is the small subunit of the ribosome in prokaryotes. The majority of research on the bacterial diversity and/or population has focused on analysis of the 16S rRNA gene. Determining the 16S rRNA gene of bacteria has become a routine part of phenotypic characterization and has replaced many culture-based techniques. As the ribosome is essential to cellular function the gene, it remains highly conserved and can be used to determine the bacterial evolutionary relatedness. The vast range of 16S rRNA gene sequences in online databases also makes comparative analysis viable.

The gene that codes for 16S rRNA is approximately 1.5 Kb, containing highly conserved regions interspersed with variable regions. The length of the gene allows for meaningful analysis on variation within the gene sequence, while being short enough to amplify the whole gene by PCR with ease. Conserved regions of the gene can be used to design universal primers for the amplification of a wide range of

bacteria and variable regions can be used to distinguish various species from one another.

2.6 Phylogenetic analysis of soil bacterial diversity

The success of any of the preceding methods for community characterization relies on a suitable phylogenetic analysis because many of the organisms that are likely to be described from soil communities have not been studied previously. A number of phylogenetic methods have been utilized in studies of microbial ecology (Woese, 1987). While rDNA and rRNA are commonly used as characters in phylogenetic analysis, the list of characters is extensive and can range from molecular to morphological traits (Olsen and Woese, 1993). For microorganisms, molecular data often provide the greatest wealth of information because microorganisms such as bacteria simply do not have the diversity of form to make morphological characteristics useful in establishing phylogenies.

Aside from the derivation of taxonomies, phylogenetic analyses are important in identifying similarities between organisms, leading to the ability to understand the physiology and ecology of non-culturable species. Unfortunately for taxonomists, phylogenetic analyses have at least one major drawback. The fact that an analysis based on a single type of molecule results in a close relationship between taxa does not necessarily mean that another equally suitable molecule will support these results, although this often occurs (Olsen and Woese, 1993). When based on a limited set of taxonomic criteria, it is difficult to say with certainty whether or not those criteria can resolve an unknown microorganism from other known microorganisms. Therefore, bacterial phylogenies should be interpreted with caution when used in soil bacterial community analyses.

2.7 Molecular bacterial diversity of soil samples

Marilley and Aragno (1999) studied bacterial diversity in the rhizosphere of *Trifolium repens* and *Lolium perenne* in Eschikon, Zurich. After isolation and purification of DNA from soil, 16S rDNA was amplified by PCR and cloned to obtain a collection of 16S rRNA genes representative of the bacterial diversity. The cloned 16S rRNA genes were then partially sequenced and analyzed by a molecular phylogenetic approach. The data showed a high diversity in the bulk soil, which was dominated by clones related to Gram-positive, *Cytophaga-Flexibacter-Bacteroides, Proteobacteria*, and *Holophaga-Acidobacterium* groups. The ubiquity of members of the *Holophaga-Acidobacterium* group was composed of sequences of yet-uncultivated microorganisms. The plant roots have a selective effect towards the γ-*Proteobacteria*, to the detriment of the Gram-positive and the *Holophaga-Acidobacterium* bacteria, leading to a dominance of *Pseudomonas*. This work also showed that the phylogenetic diversity decreases in the proximity of plant roots.

In 2003, Zhao *et al.* studied bacterial diversity in two similar sandy surface soils from Virginia and Delaware (USA) using a small subunit ribosomal RNA (SSU rRNA) gene-based cloning approach with about 400-700 SSU rDNA clones obtained from each sample. The clones showing less than 85% similarity to the sequences in the current databases were fully sequenced. Phylogenetic analysis indicated that these sequences fell into 10 of the 35-40 known phylogenetic divisions. Many of the clones were affiliated with *Acidobacterium*, while a substantial portion of the clones belong to *Proteobacteria*, *Planctomycetes*, and Gram-positive bacteria. About 4% of clones were related to other bacterial divisions, including *Cytophaga*, green sulfur bacteria, *Nitrospira*, OP10, and *Verrucomicrobia*. Eight sequences had no specific association with any of the known divisions or candidate divisions and were phylogenetically

divided into three novel division level groups, named AD1, AD2 and AD3. The community structures were similar between these two widely separated, sandy, oligotrophic, surface soils under grass vegetation in a temperate, humid climate but somewhat dissimilar to community structures revealed in similar studies in other types of soil habitats.

16S rRNA gene sequences were used to investigate the bacterial diversity under *Acacia tortilis raddiana* in the dry land part of Senegal (West Africa). Bacterial DNA was purified directly from soil samples and subjected to PCR with primers specific for bacterial 16S rRNA gene sequences. 16S rDNA clone libraries were constructed from two soil samples taken at dry season and wet season. The 16S rDNA of was partially sequenced, and phylogenetic analysis of these sequences revealed extensive diversity. Comparative sequence analysis of these clones identified members of the *Gammaproteobacteria* as the most important group, followed by the *Firmicutes* division. *Alphaproteobacteria*, *Betaproteobacteria*, *Acidobacteria* and *Actinobacteria* were found to be less represented. The results suggested that bacterial communities under *Acacia tortilis raddiana* might differ according to the season. The relative composition of the populations was different in both samples: *Acidobacteria* were present in a much higher percentage in the dry season than in the wet season while the inverse effect was observed for the members of the other groups. Within the *Gammaproteobacteria*, a shift was found between the dry season and the wet season from pseudomonads to *Acinetobacter* and *Escherichia* related organisms. (Diallo *et al.*, 2004)

The SSU rDNA gene library study of the uncultured eubacterial diversity of a soil sample collected below a dead seal, Cape Evans, McMurdo, Antarctica by an SSU rDNA gene library approach revealed a high diversity in the soil sample from

Antarctica. More than 50% of clones showed homology to *Cytophaga-Flavobacterium-Bacteroides* group; sequences also belonged to *α, β, γ Proteobacteria, Thermus-Deinococcus* and high GC Gram-positive group; Phylogenetic analysis of the SSU rDNA clones showed the presence of species belonging to *Cytophaga* spp., *Vitellibacter vladivostokensis*, *Aequorivita lipolytica*, *Aequorivita crocea*, *Flavobacterium* spp., *Flexibacter* sp., *Subsaxibacter broadyi*, *Bacteroidetes*, *Roseobacter* sp., *Sphingomonas baekryungensis*, *Nitrosospira* sp., *Nitrosomonas cryotolerans*, *Psychrobacter* spp., *Chromohalobacter* sp., *Psychrobacter okhotskensis*, *Psychrobacter fozii*, *Psychrobacter urativorans*, *Rubrobacter radiotolerans*, *Marinobacter* sp., *Rubrobacteridae*, *Desulfotomaculum aeronauticum* and *Deinococcus* sp. (Shravage *et al.*, 2007).

Moreover, a 16S rDNA-based approach was taken to study the bacterial community associated with the soil of a native cerrado area and an area that has been converted to pasture in Brazil. The bacterial group most abundantly identified in the cerrado soil was the α-*Proteobacteria* while in the pasture area the *Actinobacteria* were the most abundant (Quirino *et al.*, 2007). Kim *et al.* (2007) studied bacterial diversity and community structure of a pristine forest soil and an anthropogenic terra preta from the Western Amazon forest using molecular methods to identify the predominant phylogenetic groups. Bacterial community similarities and species diversity in the two soils were compared using oligonucleotide fingerprint grouping of 16S rRNA gene sequences and by DNA sequencing. The results showed that both soils had similar bacterial community composition over a range of phylogenetic distances, among which *Acidobacteria* were predominant, but that terra preta supported approximately 25% greater species richness.

Bacterial diversity at different habitats in a Japanese paddy field ecosystem was studied by Asakawa and Kimura (2007). Bacterial diversity was compared by an analysis of the denaturing gradient gel electrophoresis (DGGE) band patterns and the sequenced DGGE bands. The samples from plow layer soil and rice straw compost buried in soil showed considerably higher index values for diversity while those from rice straw placed on soil surface had lower values of the indices than other habitats. Sequences of DGGE bands were assigned to phyla or classes. Distribution of bacterial members to phylogenetic taxa was different among the respective habitats. Inhabitants in plow layer soil were most widely distributed among the groups (nine phyla: *Proteobacteria, Chloroflexi, Chlorobi, Verrucomicrobia, Acidobacteria, Nitrospira*, candidate division OP10, *Cyanobacteria*, and *Actinobacteria*), while those in floodwater were restricted to only three phyla (*Proteobacteria, Bacteroidetes*, and *Actinobacteria*). *Proteobacteria* and *Bacteroidetes* were found at all the habitats and the habitats except for plow layer soil, respectively, whereas abundant members belonged to *Chloroflexi* and *Actinobacteria* in plow layer soil.

2.8 Bacterial diversity in agroecosystems and land management

Bacterial diversity is critical to ecosystem functioning because of the diversity of processes for which bacteria are responsible, such as decomposition and nutrient cycling, soil aggregation and pathogenicity. However, knowledge may be limited to only a small percentage of the characteristics of cultured bacteria (Hawksworth, 1991), which is an even smaller percentage of the total population. Molecular investigations suggest that the diversity in the soil is greater than what can be understood with cultural techniques (Holben and Tiedje, 1988; Torsvik *et al.*, 1990b). It is necessary to increase knowledge of biotic and functional diversity to better

understand the desired level of bacterial diversity and the optimum management practices for the living system.

Bacterial redundancy is thought to be a factor in bacterial functioning. It has been proposed that loss of species may not change the function of the ecosystem, including biologically mediated processes or biochemical transformations (Walker, 1992). Redundancy in bacterial function may not be an important issue because a high level of specialization may exist in bacterial systems. Functionally similar organisms may exhibit various survival and growth requirements and tolerate different environments or habitats (Perry *et al.*, 1989).

Temporal and spatial distribution of bacteria further influences the immensity of bacterial diversity. The diversity of soil bacteria is much greater than the diversity of above-ground species (Kennedy, 1999), yet because of the small physical size, it is much more difficult to assess. The microsite environment is also a key factor defining the functioning of bacterial processes. Conditions of a microsite in one soil pore may be quite different from an adjacent microsite, leading to the development of microsite-specific communities, and thus increasing the diversity of a given soil microsite. Soil texture and pore space can directly affect community composition. Macropores are the site of high bacterial activity (Lee and Foster, 1991), possibly the result of the greater root and micro- and macrofaunal activities in these areas. In the smaller pores, water-saturated microsites may enhance anaerobic activity, thus increasing the variability of nutrient cycling processes (Tiedje *et al.*, 1984). Landscape position also influences the distribution and diversity of selected bacteria. Bacterial populations and activity levels decline with depth in the soil profile (Turco and Bezdicek, 1987). Although metabolically active and diverse populations have been found at great depths (Fredrickson *et al.*, 1991), the lack of carbon limits proliferation and activity.

Crop rotation is a key component in sustainable systems because it enhances beneficial species and interactions, interrupts the cycle of pathogens and reduces weed populations. Many studies have shown the positive effects of crop rotation on crop growth, attributing this to changes in the bacterial community composition (Shipton, 1977; Cook, 1981; Johnson *et al.*, 1992). The populations and aggressiveness of pathogens can be altered with crop rotation, illustrating changes in bacterial diversity and function due to land management (El Nashaar and Stack, 1989).

Resiliency of an ecosystem to buffer the effects of extreme disturbances may depend in part on the diversity and interactions within the ecosystem (Perry *et al.*, 1989; Elliott and Lynch, 1994). It may be important to monitor the bacterial diversity or possibility of these interactions as indicators of change or in response to a stress. The extinction rate of species within a system may be an important indicator of the status of the system and may be critical in determining the level of diversity necessary to maintain an agroecosystem. Actual numbers and composition of species may not be as important as the flux of species or functioning and interaction of individuals within a system.

Bacterial diversity can depict the status of bacterial communities and their response to natural or human disturbances. Bacterial communities in ecosystems may change with land use and management history (Kennedy, 1999). For example, the diversity of actinomycetes was greater in organically managed soil when compared with conventionally managed systems and reduction in diversity correlated with the appearance of tomato pathogens (Workneh and van Bruggen, 1995). Bacterial diversity indices can function as bioindicators of the stability of a community and can be used to describe the ecological dynamics and the impact of stress on that community (Mills and Wassel, 1980; Atlas, 1984). The absence of detailed

information on the bacterial species composition of soil environments limits the greater use of these indices (Torsvik *et al.*, 1990).

The enormity of bacterial diversity is incomprehensible and knowledge of the genetic diversity within the bacterial genome is limited. Bacterial diversity influences nutrient cycling and decomposition, soil structure and biological interactions. The identification of obvious bacterial functions is attainable but it is more difficult to further dissect species function and relationships. The challenge ahead is to identify the level of bacterial diversity, species composition and distribution to maintain resiliency and withstand stress. The importance of soil bacterial diversity and its role in ecosystems needs to be realized.