UNIVERSITY OF PADOVA

DOCTORATE SCHOOL OF CROP SCIENCE

CURRICULUM AGROBIOTECHNOLOGY - CYCLE XXIII

Department of Agricultural Biotechnology

Microbial biodiversity in a wooded riparian zone specifically designed for enhancing denitrification process

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THESIS SUBMISSION DATE
January 31st, 2011

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Md. Mizanur Rahman

January 31st, 2011

A copy of the thesis will be available at *http://paduaresearch.cab.unipd.it/*

Dedicated

To

My late Father and family.

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LIST OF ABBREVIATION

AMF: Arbuscular mycorrhizal fungi

ARB: ARBor, Latin: tree;

ARDRA: Amplified rDNA restriction analysis

BOD: Biological oxygen demand

CFU: Colony forming unit

CLPP: Community-level physiological profiling

COD: Chemical oxygen demand

CTC: 5-cyano-2, 3-di-4-tolyl-tetrazolium chloride

D: Simpson dominance index

DGGE: Denaturing gradient gel electrophoresis

FAME: Fatty acid methyl ester

GN: Gram-negative

GP: Gram-positive

$\bar{H}$: Shannon diversity index

HRT: Hydraulic residence time

MAP: Magnesium-ammonium-phosphate

NICOLAS: Nitrogen control by landscape structures

NWWG: North-western working group

OTUs: Operational taxonomic units

PBS: Phosphate buffer solution

PCA: Principal component analysis

PCR: Polymerase chain reaction

PLFA: Phospholipid fatty acid analysis

RISA: Ribosomal intergenic spacer length polymorphism

RMZ: Riparian management zones

SMZ: Streamside management zones

SPZ: Stream protection zones

SSCP: Single-strand conformational polymorphism

SSCU: sole source carbon utilization

TAE: Tris–acetate EDTA

TGGE: Temperature gradient gel electrophoresis

TKN: Total Kjehldahl nitrogen

TN: Total nitrogen

TP: Total phosphate

TRFLP: Terminal restriction fragment length polymorphism

UPGAMA: Unweighted pair group method using arithmetic averages

USDA-SCS: United state department of agriculture-soil conservation service

USEPA: United States environmental protection agency

ABSTRACT

This research is part of a project aimed at verifying the potential of a specifically assessed wooded riparian zone in removing excess of combined nitrogen from the Zero river flow for the reduction of nutrient input into Venice Lagoon. General objectives of this project were to increase knowledge on the processes which allow the riparian strips to act as a buffer and to identify the most appropriate management strategies in order to maximize the efficiency of these systems in supporting the microbial activities involved in the process. For this purpose, specific objectives were pursued to determine seasonal fluctuations of the microbial populations in the soil/water of the wooded riparian strip. The bacterial communities were determined by combined approaches involving cultivation, microscopic approaches and DNA bases techniques to characterize both culturable and total microbial community inside and outside the riparian strip.

ARDRA and DGGE analyses of soil collected at different depths, showed a clear decrease of the microbial diversity in deeper horizons as compared to the medium depth and surface ones. A comparison between this soil and that collected from an undisturbed zone external to the riparian strip, indicated that this effect can be also observed in the external area, although higher microbial diversity was always present in the internal soil. DGGE cluster analysis and PCA of both genetic and chemical properties of water samples indicated that the bacterial populations present at the drainage ditches are rich in denitrifiers as a result of a mixing of bacterial communities carried by the Zero river flux and those already present in the soil of the riparian strip.

Taken together, the overall results confirm what it was demonstrated by other chemical-physical analysis: the wooded riparian buffer zone assessed for water remediation (nitrogen removal from Zero river) is effectively working as a result of the special conditions there produced to support the work of specific microbial populations. The microbiological analysis here accomplished can also contribute to understand the bacterial population dynamic of an agricultural soil when transformed in a wooded strip and to provide key indications for the management of a phytoremediation site.

RIASSUNTO

Questa ricerca fa parte di un più ampio progetto finalizzato a verificare l'attività di zone riparie atte a rimuovere l'eccesso di azoto combinato nel fiume Zero, con il fine ultimo di ridurre l'imput di nutrienti nella Laguna di Venezia. Obiettivo generale di questa ricerca è stato quello di aumentare le conoscenze relative ai processi che consentono alle zone riparie di agire come tamponi e identificare le strategie di gestione più appropriate per massimizzare l'efficienza di questi sistemi nel supportare le attività microbiche coinvolte.

A questo scopo, sono stati perseguiti obiettivi specifici per determinare le fluttuazioni stagionali delle popolazioni microbiche nei suoli/acque della fascia tampone.

Le comunità microbiche sono state determinate tramite un approccio combinato che ha previsto la coltivazione, tecniche microscopiche e tecniche molecolari al fine di caratterizzare sia la comunità microbica coltivabile sia quella totale, all'interno e all'esterno della fascia tampone.

Le analisi ARDRA e DGGE di suoli raccolti a diverse profondità, mostrano una chiara diminuzione della diversità microbica negli orizzonti più profondi rispetto agli strati intermedi e superficiali.

Il confronto tra suoli raccolti nella fascia tampone e suoli raccolti in una zona indisturbata esterna, indicano che questo effetto può essere osservato anche all'esterno sebbene una maggiore diversità microbica sia sempre rilevabile all'interno.

Indagine DGGE e elaborazioni statistiche con PCA, sia delle proprietà genetiche che di quelle chimiche dei campioni d'acqua, hanno indicato che le popolazioni microbiche presenti nelle scoline sono ricche di denitrificanti e sono il frutto del rimescolamento delle comunità microbiche del fiume Zero con quelle già presenti nei suoli della fascia tampone.

Nel complesso, questi risultati confermano quanto dimostrato da altre analisi fisico-chimiche: la fascia tampone messa a punto per il biorimedio delle acque (rimozione dell'azoto dal fiume Zero), sta effettivamente funzionando come risultato delle speciali condizioni che favoriscono specifiche popolazioni microbiche.

Le analisi microbiologiche qui riportate possono inoltre contribuire alla comprensione delle dinamiche di popolazioni in suoli agricoli convertiti in fasce tampone e fornire indicazioni chiave per la gestione di siti di fitorimedio.

1. INTRODUCTION

1.1. The wooded riparian zone

A riparian zone (see an example in Fig 1.1) generally encompasses the vegetated strip of land that extends along streams and rivers and is therefore the interface between terrestrial and aquatic ecosystems (Gregory et al., 1991; Martin et al., 1999). In the literature, in addition to streams and rivers, the definition of riparian zones often includes the banks of lakes, reservoirs and wetlands.

Riparian areas are often included in wetland classifications (Dahl et al., 2007). Wetland definitions commonly include three characteristics: water table near, at or above land surface; these unique soils are typically characterized by reduced conditions, organic matter accumulation and ecological communities adapted to these wet conditions (Mitsch and Gosselink, 1986; Brinson et al., 2002). However, classification systems vary, emphasizing one or the other aspect, depending on purpose (Zoltai et al., 1975; Gosselink and Turner, 1978; Cowardin et al., 1979).

Ingram (1983), Carter (1986) and NWWG (1997) conclude that wetland hydrology is the primary driving force controlling wetland ecology and function. Consequently, the main wetland classification criterion might consider sources of water distinguishing between solely precipitation dependent (bogs), mainly groundwater dependent (fens) or mainly surface water dependent (swamps, marshes and shallow open water). Riparian areas including the latter two types are situated adjacent to both perennial, intermittent, and ephemeral streams, lakes, and estuarine–marine shorelines (Brinson et al., 2002).

Buffer zones or strips have also been variously labelled as Stream Protection Zones (SPZ), Streamside Management Zones (SMZ), or Riparian Management Zones (RMZ). In agricultural landscapes, buffer zones often consist of a fenced area alongside streams and this may be left as a grassy sward, or planted with woody vegetation. In forestry systems, a buffer zone is generally one of production trees left beside the stream when the surrounding area is harvested.

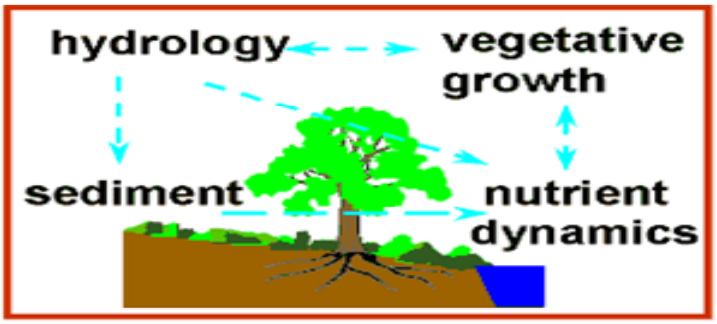


Fig 1.1. A wooded riparian zone

Riparian management can be conducted in different ways, some of which are summarised below:

Grass Filter Strips: Fenced strip of rank paddock grasses to filter nutrients and sediment.

Headwater or riparian wetlands: Fenced wetlands as hotspots for nutrient removal

Rotational Grazing: Filter strips with varied stock grazing practices, such as occasional light grazing by sheep.

Forested or planted native trees: A buffer of native trees to return ecological function to the stream and provide water quality benefits.

Production trees or plants: A buffer of forestry trees left unharvested along stream banks, or production trees that are planted in riparian zones for selective harvesting with minimal disturbance (e.g., Tasmanian blackwoods). Plants such as flax for weaving, or fruit and nut trees, or high value native tree species that can be selectively harvested may also provide ecological function and a mechanism to remove nutrients such as phosphorus from the riparian zone.

Multi-tier system: A combination of buffers where native forest trees may be used beside the stream to enhance ecological function and biodiversity, a buffer of production trees may occur outside of that and at the outer edge beside agricultural land a grass filter strip may be used.

1.1.1. Functions of the riparian buffer zones

Riparian buffer zones are used as a management tool to perform many functions including stabilising channels, preventing stock access to waterways, filtering sediment and other particulates terrestrial and aquatic habitat (Table 1.1). In addition, wet riparian soils, generally rich in carbon and low in oxygen, can promote a significant loss of N through denitrification. Riparian vegetation can also provide corridors for the movement of native fauna and flora between geographically separate areas, although the spread of weed species can also be facilitated in this way (Welsch, 1991).

Riparian zones are commonly areas with heterogeneous vegetation and soils and therefore provide a diverse habitat for terrestrial and semi-aquatic organisms (birds, insects, amphibians and plants) (Boothroyd and Langer,1999). Vegetation in the riparian zone can influence water flow, both in surface and subsurface (through root systems) and has direct effects on stream functioning. Forest vegetation in particular can shade streams and lower stream temperatures. High light levels from deforestation around streams, lead to increase in algae and in-stream primary production, and changes to invertebrate community composition. Stream temperature has a direct impact on aquatic species as most metabolic processes are accelerated with increasing temperature and many fish and invertebrate species have thermal tolerances that can be exceeded in unshaded streams (Quinn et al., 1994; Martin et al., 1999). Trees provide organic matter inputs in the form of leaves and woody debris, creating a diversity of food resources and habitats for in-stream fauna. Terrestrial insects may also be attracted to vegetated riparian zones and become a valuable food source for fish when they fall into the stream (Barling and Moore, 1994).

Key riparian zone functions	Explanatory notes
Stream bank stability	The root systems of trees and grasses strengthen streambanks and groundcover reduces surface erosion – provides habitat stability in the form of refuges during floods.
Filtering overland flow	Surface roughness provided by grassy vegetation, or litter, reduces the velocity of overland flow, enhancing settling of particles. High infiltration of uncompacted soils encourages subsurface flowpaths, with resulting particulate filtering and nutrient uptake by plants and microbes.
Suitable habitat for adult phases of stream insects	Some stream insects spend extended periods (weeks – months) as adults in the terrestrial area. Riparian vegetation may be a key element of these species ability to persist in pastoral streams. (e.g., humidity, temperature, food resources)
Shade for stream temperature	Removal of shade can result in summer temperatures that can be lethal to some invertebrates and fish, or winter temperatures that are too warm for successful trout spawning.
Shade for instream plant control	Shade removal provides light for instream plant growth, sometimes resulting in streams becoming choked and/or variations in dissolved oxygen and pH that stress invertebrates and fish.
Woody debris and leaf litter input	Riparian trees add leaf litter and wood that are an important source of habitat diversity for invertebrates and fish, particularly in silt-bed streams. Leaf litter is also a food resource for stream invertebrates.
Plant nutrient uptake from groundwater	Roots of riparian plants intercept groundwater reducing nutrient input to streams.
Denitrification N Control	Denitrifying bacteria can remove substantial quantities of nitrate from groundwater passing through riparian wetlands, venting this to the atmosphere as nitrogen gases.
Control of direct animal waste input	Preventing direct access of stock to waterways prevents hoof-damage to streambanks and direct input of nutrients, organic matter and pathogens in dung and urine.
Downstream flood control	Well-developed riparian vegetation increases the roughness of stream margins, slowing down flood-flows. This reduces the peak flows downstream but may result in some local flooding. Riparian wetlands provide temporary storage of water during rain events.
Terrestrial biodiversity	Riparian zones contain a high diversity of soil and water conditions, resulting in correspondingly diverse terrestrial plant and animal communities

Table 1.1. Summary of the possible functions of a riparian zone

1.1.2. Modes of particulate and dissolved nutrient transport in the riparian buffer zone

Nitrate and other compounds are gradually transformed through the changing redox zones observed during passage of the aquifer (Dahl, 1995; Hoffmann, 1998; Hoffmann et al., 2006). For this reason, entry point and flow paths to a large extent control where transformation processes take place. The ability of buffer zones to attenuate pollutants will depend upon the mechanisms by which these pollutants reach surface waters. Mainly three transport processes can occur:

- direct pollution (e.g., stock access to streams, bank erosion);
- surface runoff;
- subsurface flow and drainage.

Surface runoff

Surface runoff can occur through several mechanisms. It may result when the surface soil becomes saturated (saturation excess) which is common where flow pathways converge as a result of topography. It may also occur when rainfall intensity exceeds the infiltration capacity of the soil (infiltration-excess) a process that is common in poorly drained clay-rich soils (Muscutt et al., 1993). Surface runoff can be a major transport mechanism for water soluble pollutants, particularly when land beside a stream has been grazed, or fertiliser or livestock waste applied to the land during or prior to rain events. Surface runoff can also be a conduit for sediment and particulate pollutants. Sediment transport can occur through sheet erosion in spatially uniform flows over hillslopes, but is most likely to result from areas where flow is concentrated, and from bare soils (e.g., stock tracks, slips, cultivated soils). Sediment in surface runoff can also carry particulate forms of phosphorus, and a high proportion of total P loss has been found to occur during periods of high flow (Culley & Bolton, 1983; Smith 1987).

Subsurface flow and drainage

Subsurface flow is frequently the major pathway of N transport in catchment runoff and high concentrations commonly occur in artificial subsurface drains (Muscutt et al., 1993). Intensive agriculture is often accompanied by subsurface drainage especially in clay-based soils. These drains provide routes for the rapid transport of water and pollutants from the soil during high water table conditions and, in many cases, can bypass the riparian zone by directly discharging to the stream.

Subsurface flow paths are influenced by the surrounding topography and soil drainage characteristics. For instance, on land that is free draining, water and associated pollutants may bypass the riparian zone, whereas on poorly drained soils or where the water table is high, pollutants in subsurface water may be carried into the soils of the buffer zone. On occasion, subsurface flows may re-emerge, and discharge downslope as surface runoff.

1.2. Nitrogen removal in riparian buffers

The USEPA (United States Environmental Protection Agency) considers nitrogen one of the primary stressors in aquatic ecosystems (USEPA, 2002a). Though nitrogen is an important nutrient for all organisms, excess nitrogen is a pollutant that causes eutrophication in surface water and contaminates groundwater (Carpenter et al., 1998). Streams receive chronic nitrogen inputs in various chemical forms such as nitrate (NO_3^-), ammonia (NH_3), and organic N from upland sources such as fertilizers, animal wastes, leaf litter, leaking sewer lines, atmospheric deposition, and highways (Carpenter et al., 1998; Swackhamer et al., 2004). Subsequent eutrophication leads to environmental impacts such as toxic algal blooms, oxygen depletion, fish kills, and loss of biodiversity (Vitousek et al., 1997). NO_3^- is of particular concern as an environmental stressor because it is biologically reactive, poses a human health risk (i.e., methemoglobinemia; USEPA, 2002b), and often is found in groundwater (Welch, 1991).

Riparian buffers are thought to be an effective, sustainable means of protecting aquatic ecosystems against anthropogenic inputs of nitrogen (Phillips, 1989; Verhoeven et al., 2006), a place where nitrogen species may be transformed by various processes including plant uptake, microbial immobilization or denitrification, soil storage, and groundwater mixing (Lowrance et al., 1984). Especially nitrate removal has been in focus (Hoffmann, et al., 2006; Peterjohn and Correl, 1984; Cooper, 1990; Haycock and Pinay, 1993). In riparian wetlands characterized by shallow lateral groundwater flow originating from upland areas discharging groundwater to a nearby stream, denitrification is believed to be the main process responsible for nitrate removal (Cooper, 1990; Schipper et al., 1993; Hoffmann et al., 2000; Vidon and Hill, 2004a; Gumiero et al., 2011). Denitrification has been determined using different approaches based on stable isotopes (Mariotti et al., 1988; Ostrom et al., 2002), soil sampling and immediate incubation with C_2H_2 (Cooper, 1990; Schipper et al., 1993), conservative tracer experiments with chloride and bromide (Jacobs and Gilliam, 1985; Smith et al., 1996), laboratory microcosmos experiments (Groffman et al., 1996; Hoffmann et al., 2000), and mass balance calculations (Haycock and Pinay, 1993).

1.2.1. Mechanisms of nitrogen removal

Nitrogen removal is achieved by two major processes, physicochemical and biological treatment techniques. Traditional biological nitrogen removal from soil, water and wastewater, primarily composed of a combination of aerobic nitrification and anaerobic denitrification, is usually considered to accomplish optimal and economic nitrogen treatment. The nitrogen removal mechanisms in constructed buffer zones are known to involve ammonification, nitrification-denitrification, plant uptake, and physicochemical methods such as sedimentation, ammonia stripping, breakpoint chlorination, and ion exchange (Kadlec and Knight, 1996; USEPA, 1993; Lee et al., 2009).

1.2.1.1. Ammonification

Ammonification is the process where organic N is biologically converted into ammonia. Pollutants containing nitrogen are readily degraded in both aerobic and anaerobic zones of reed beds, releasing inorganic ammoniacal-nitrogen (NH_4–N). The inorganic NH_4–N is mainly removed by nitrification-denitrification processes in constructed wetlands. Ammonification proceeds more rapidly than nitrification (Lee et al., 2009). The rates of ammonification are fastest in the oxygenated zone and then decrease as the mineralization circuit changes from aerobic to facultative anaerobic and obligate anaerobes. The rates are influenced by temperature, pH, C/N ratio, available nutrients, and soil structure (Reddy and. Patrick, 1984). NH_4–N in subsurface flow systems can be reduced by other processes, which include adsorption, plant uptake and volatilization (Vymazal, 2007). However, it is generally believed that the contribution of these processes to the NH_4–N removal is very limited compared with nitrification-denitrification.

1.2.1.2. Nitrification

Decomposition processes in the wetlands are believed to convert a significant part of the organic nitrogen to ammonia (Mayo and Mutamba, 2004). Biological nitrification, which is performed by nitrifiers such as *Nitrosomonas, Nitropira, Nitrosococcus* and *Nitrobacter*, followed by denitrification is believed to be the major pathway for ammonia removal in both surface flow and subsurface flow constructed wetlands/ buffer zone (Kadlec et al., 2000; Gersberg, et al., 1985).

In traditional nitrogen treatments, the biological nitrogen removal requires a two-step process: nitrification followed by denitrification. Nitrification implies a chemolithoautotrophic oxidation of ammonia to nitrate under strict aerobic conditions and is performed in two sequential oxidative stages: ammonia to nitrite (ammonia oxidation) and nitrite to nitrate (nitrite oxidation). Each stage is performed by different bacterial genera which use ammonia or nitrite as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. The most commonly recognized genus of bacteria is that of *Nitrosomonas* for the ammonia oxidation process and *Nitrobacter* for the nitrite oxidation process. The overall equations for these two reactions can be represented as follows (Metcalf and Eddy, 1991).

$NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + H_2O + 2H^+$ (1)

$NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$ (2)

In the subsurface flow systems, significant nitrification generally does not take place before substantial BOD (Biological Oxygen Demand**)** reduction (Zhao et al., 2004a; Zhao et al., 2004b). The rate of nitrification is influenced by temperature, pH, alkalinity, inorganic carbon source, moisture, microbial population, and concentrations of ammonium–N and dissolved oxygen.

1.2.1.3. Denitrification

In this process, denitrifying bacteria decrease inorganic nitrogen such as nitrate and nitrite into innocuous fundamental nitrogen gas (Prosnansky et al., 2002; Szekeres et al., 2002). From the biochemical point of view, biological denitrification is an oxidation process in which the oxidation of organic substrate differs from respiration with molecular oxygen only in the final step, in which nitrite and/or nitrate-nitrogen serves as an electron acceptor. Many bacteria are capable of growing by reducing ionic nitrogenous oxides to gaseous products and this process requires an organic carbon source. Bacteria belonging to the genera *Rhizobium, Allorhizobium, Azorhizobium, Mesorhizobium, Sinorhizobium* and *Bradyrhizobium* are able to interact symbiotically with legume plants to produce nitrogen-fixing root nodules. While some traits are common among all rhizobia, selected traits such as denitrification and the dissimilatory reduction of nitrate to gaseous nitrogen oxides seem to be randomly distributed (Basaglia et al., 2007). Some species are complete denitrifiers,

for example *Bradyrhizobium japonicum* (Bedmar et al, 2005), while others are only partial nitrogen oxide-reducers, for example, strains of *Rhizobium sullae* (Casella et al., 1986). Denitrifying bacteria (denitrifiers) can be classified into two major groups: heterotrophs and autotrophs. Heterotrophs are microbes that need organic substrates to obtain their carbon source for growth and evolution, and get energy from organic matter. In contrast, autotrophs utilize inorganic substances as an energy source and CO_2 as a carbon source (Rijn et al., 2006). So far, the heterotrophic denitrification process has been mainly engaged in conventional wastewater treatment plants, while autotrophic denitrification has only recently been studied (Kim et al., 2004). The second step of denitrification is conducted by a heterotrophic microorganism (such as *Pseudomonas, Micrococcus, Achromobactor* and *Bacillus*) under anaerobic or anoxic conditions. The proportion of total nitrogen removal by denitrification is typically 60–95%, in comparison to 1–34% assimilated by plants and algae. Heterotrophic microorganisms utilize an oxidized form of nitrogen, NO_3^-, NO_2^-, NO and N_2O as terminal electron acceptors and organic carbon as electron donor under anoxic conditions (Metcalf and Eddy, 1991). Consequently, the denitrification provides energy to denitrifiers and it is also affected by the organic matter as the electron donor. This process is shown in the following Fig 1.2 (Peterjohn and Correll, 1984).

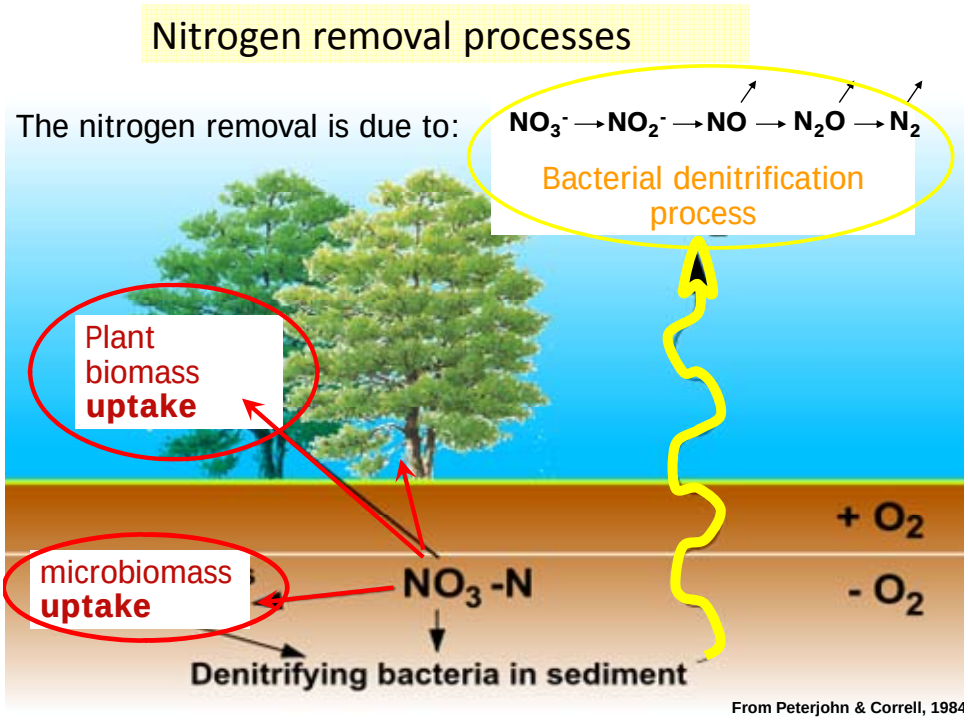


Fig 1.2. Denitrification process (Peterjohn and Correll, 1984)

Denitrification can take place only in the anoxic zones of the systems, as the presence of dissolved oxygen suppresses the enzymatic system required for this process (Metcalf and Eddy, 1991). High concentrations of nitrate in the inlet zones can lead to more vigorous and robust populations of denitrifiers within the inlet sediments (Sirivedhin and Gray, 2006). In constructed wetlands, it is believed that microsites with steep oxygen gradients can be established, which allow nitrification and denitrification to occur in sequence, in very close proximity to each other

The rate of denitrification is influenced by many factors, including nitrate concentration, microbial flora, type and quality of organic carbon source, hydroperiods, different plant species residues, the absence of O_2, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, water level, and the presence of overlying water (Sirivedhin and Gray, 2006; Vymazal, 1995; Bastviken and Eriksson, 2005). Numerous studies have shown that the denitrification rate in organic carbon-restricted water and wastewater can be improved continually by supplementing any carbon sources (Killingstad et al., 2002), even though there are some issues regarding external organic carbon sources in heterotrophic denitrification (Joo et al., 2005).

1.2.1.4. Plant uptake

The uptake of ammonia and nitrate by macrophytes converts inorganic nitrogen forms into organic compounds, as building blocks for cells and tissues (Vymazal, 1995). The capability of rooted plants to use sediment nutrients partly explains their extensive yield compared with planktonic algae in many systems (Wetzel, 2000). Various plant species differ in their favored forms of nitrogen absorbed, depending on the forms available in the wetland. The uptake and storage rate of nutrients by plants depend on the nutrient concentration of their tissues. Thus, desirable features of a plant used for nutrient assimilation and storage include fast growth, high tissue nutrient content, and the ability to obtain a high-standing crop. Conversely, plants that have great biomass accumulation during autumn and winter may release much of their accumulated nitrogen back into the water during the winter season (Vymazal, 2007).

1.2.1.5. Physicochemical processes

The contribution of physicochemical processes to overall nitrogen removal is generally high in newly built wetlands, but decreases with time. Although many physicochemical processes can take place in constructed wetlands, the major mechanisms for nitrogen removal are ammonia adsorption and sedimentation.

1.2.1.5a. Ammonia adsorption

In constructed wetlands, adsorbed ammonia is bound loosely to the substrates and can be released easily when water chemistry conditions change. When the ammonia concentration in the water column is reduced as a result of nitrification, some ammonia will be adsorbed to regain equilibrium with the new concentration. If the ammonia concentration in the water column is increased, the adsorbed ammonia will also increase (Vymazal, 2007). If the wetland substrates are exposed to oxygen, adsorbed ammonium may be oxidized to nitrate by periodic draining (Sun et al., 2005; Sun et al., 2006; Connolly et al., 2004). The ammonium ion is generally adsorbed as an exchangeable ion on clays, and adsorbed by humic substances. The rate and extent of these reactions are reported to be influenced by several factors, such as the type and amount of clay, alternating submergence and drying

patterns, characteristics of soil organic matter, submergence period, and the presence of vegetation.

1.2.1.5b. Sedimentation

Most particulate organic nitrogen in constructed buffer strip is removed by sedimentation (Taylor et al., 2005). Particulates may settle on the wetland floor or may adhere to plant stems. The decomposed materials such as TN, TP, and organics of low molecular weight are used by microorganisms and plants (Kadlec and Knight, 1996). In nitrogen removal in buffer strip, combined physical and chemical processes can be employed. An enhanced sedimentation method using magnesium-ammonium-phosphate (MAP), as added precipitation reagent, has been developed for the removal of nitrogen and phosphorus in wastewater treatment and has the potential to be applied in constructed wetlands.

1.2.2. Environmental factors affecting nitrogen removal efficiency

Considering that pollutants are removed by a variety of physicochemical and biological processes in constructed riparian strip, numerous environmental factors can influence the removal of nitrogen. Major factors include temperature, HRT (Hydraulic residence time), type and density of vegetation, the characteristics of microbial communities, climate, the distribution of wastewater etc. These factors are often related, and a change in one factor can cause a change in the others (Sirivedhin and Gray, 2006). Among these, two of the most significant factors are temperature and HRT (Kuschk et al., 2003).

1.2.2a. Temperature affecting nitrogen removal efficiency

Temperature, as a key environmental factor, is important in relation to the activities of nitrifying bacteria and the denitrification potential in treatment wetlands (Langergraber, 2007). Biological nitrogen removal is most efficient at 20~ 25°C and temperatures affect both microbial activity and oxygen diffusion rates in constructed wetlands (Phipps and Crumpton, 1994). The microbial activities related to nitrification and denitrification can decrease considerably at water temperatures below 15 or above 30°C, and most microbial communities for nitrogen removal function at temperatures greater than 15°C (Kuschk et al., 2003). Several studies have shown that the activity of denitrifying bacteria in

constructed wetland sediments is generally more robust in spring and summer than in autumn and winter (Herkowitz, 1986), and the overall removal rate of nitrate is higher in summer than in winter. While denitrification is commonly believed to cease at temperatures below 5°C, some studies have demonstrated denitrification activity at 4°C or lower, albeit at lower rates (Richardson et al., 2004). Vymazal (2007) reported that the optimum temperature range for nitrification is 30–40°C in soils, and the optimal ammonification temperature is 40~ 60°C, while the optimal pH is between 6.5 and 8.5. At low temperature, nitrification can be insufficient to prevent a net increase in ammonia concentration due to ammonification (Akratos and Tsihrintzis, 2007).

1.2.2b. Hydraulic residence time affecting nitrogen removal efficiency

HRT plays a critical role in nitrogen removal efficiency. Huang et al. (2000) described that ammonium and TKN (Total Kjehldahl nitrogen) concentrations in treated effluent decrease dramatically with increase in wastewater residence time. In most wetland systems, nitrogen removal requires a longer HRT compared with that required for BOD and COD removal. Accordingly, nitrogen removal efficiency varies greatly with flow conditions and residence time (Taylor et al., 2005).

1.2.2c. Types of vegetation affecting nitrogen removal efficiency

The roots of macrophytes provide surface areas for microbial growth and aerobic zones in constructed buffer. The rhizosphere is the most energetic reaction zone in a constructed buffer. Microphytes also play an important role in wastewater treatment through uptake of nutrients, surface bed stabilization, and other mechanisms (Kadlec and Knight, 1996). The type of macrophytes in constructed wetlands has a greater influence on nitrogen removal than the removal of organic matter (Akratos and Tsihrintzis, 2007; He and Mankin, 2002). Common macrophytes used in constructed wetlands are reed *(Phragmites australis*), cattail (*Typha* spp.) and bulrush (*Scirpus* spp.), all characterized as water-tolerant macrophytes that are rooted in the soil but emerge above the water surface (Kadlec et al., 2000). Their growth changes with temperature and dissolved oxygen concentrations in sediment and water.

Buffer systems with vegetation typically remove greater amounts of total nitrogen than non-vegetated systems (Akratos and Tsihrintzis, 2007; Taylor et al., 2006). Nutrient removal by the emergent plants is achieved by two processes: absorption of the plant itself and microorganism activity around the rhizome (Cooper and Boon, 1987). Plants dead matter accumulated in the soils may cause eutrophication, increasing the BOD of fresh water in constructed wetlands for a long time. Thus, the plant harvest should be conducted at an appropriate time. If not, nutrients within the dead plants are re-discharged into the receiving water, so that a variety of adverse effects occur in constructed wetlands, because the ambient CO_2 gas influx is increased in the wetland. In general, the main role of hydrophytes in constructed wetlands is to promote microbial growth within media surfaces, and to assist the permeation velocity of the wastewater for pollutant treatment efficiency. Several operational factors such as water depth, water level, and uneven bed surfaces can be used to control vegetation populations and manage colonization (Bach and Horne, 2000). Hammer (1992) indicates that the optimum plant species in constructed buffers should be autochthonous species suitable for regional climate and soil.

1.3. Biodiversity in soil

Biological diversity (or biodiversity) can be defined as the set of microbial, animal and vegetable species, their genetic material and the ecosystems they belong to, and it includes ecosystem, species and gene diversity (Ohtonen et al. 1997). As the biodiversity of an ecosystem increases, the resilience and stability of the ecosystem should increase (Garbeva et al., 2004; Torsvik and Ovreas, 2002). Conversely, as ecosystems degrade, ecosystem biodiversity decreases (Garbeva et al., 2004; Sun et al., 2004). Loss of biodiversity leads to loss of ecosystem resistance and resilience to anthropogenic as well as natural stresses (van Elsas et al., 2002). Recently, the biodiversity of soil biota is becoming increasingly essential with the requirement of preserving the integrity, function and long-term sustainability of natural and managed terrestrial ecosystems (Chapman et al., 2007; Costa et al., 2007; Lagomarsino et al., 2007; Martins-Loução and Cruz , 2007; Winding and Hendriksen, 2007; Xu et al., 2008a, b).

Soil biodiversity (bacteria, protozoa, fungi, nematodes, springtails, annelids, isopods, and coleopterans) influences a huge range of ecosystem processes that contribute to the

sustainability of life on earth. Soil biodiversity maintains critical and key processes such as carbon storage, nutrient cycling, plant species diversity, soil fertility, soil erosion, nutrient uptake by plants, formation of soil organic matter, nitrogen fixation, biodegradation of dead plant and animal materials, reducing hazardous waste, production of organic acids that weather rocks, and control of plant and insect populations through natural biocontrol (Cragg and Bardgett, 2001; Wolters, 2001; De Deyn et al., 2003). Soil biodiversity is several orders of magnitude higher than that above ground (Heywood, 1995; Swift, 1999) and humans have relied directly on that to provide food and medicinal products (e.g. leeches to antibiotics). The biotic elements (micro and macro-life forms) within soil interact with the soil abiotic elements (chemical and physical properties) to maintain the diverse, multi-functional value of soils (Bater, 1996; Van der Heijden et al., 1998; Hafez and Elbestawy, 2009)

1.3.1. Microbial diversity in soil

Soil microbial diversity is important because it is often regarded as an important index of soil ecosystem health. Soil microbial communities are often difficult to be fully characterized, mainly because of their immense phenotypic and genotypic diversity, heterogeneity, and crypticity. To date, only 1–5% of the world's microorganisms have been identified (Mills et al., 2007; Nannipieri et al., 2003; Hugenholtz et al., 1998). Moreover the fraction of the cells making up the soil microbial biomass that have been cultured and studied in any detail are negligible (Borneman and Triplett, 1997, Torsvik et al., 1990, Garbava et al., 2004): approximately only 1% of the soil bacterial population can be cultured by standard laboratory practices. Few data are available concerning how closely colturable fraction reflects the composition of whole communities.

A hypothesis to explain the large microbial diversity of surface soil is based on the presence of a greater variety and content of organic compounds (Tiedje et al., 2001; Nannipieri et al., 2003). This presence would be responsible for the diverse heterotroph-dominated microbial community in surface soil. However, microbial diversity of soil from preferential flow paths (cracks, fissures, biopores such as earthworm burrows or root channels) was similar to that of the bulk soil (Bundt et al., 2001), inspite of the fact that the

former sample showed a greater concentration of organic C and organic N and greater microbial biomass values than the latter sample.

1.3.1.1. Bacterial diversity in soil

Bacteria are the most abundant and diverse group of organisms in soil, with estimates of 10^4–10^9 distinct genomes per gram of soil (Torsvik and Ovreas, 2002; Garbava et al., 2004; Tiehang et al., 2008; Gans et al., 2005). They are critical to many of the biological, chemical, and physical processes that drive terrestrial ecosystems. Plant growth is affected directly through their activities as plant pathogens or plant growth promoters and indirectly via interactions with other soil microorganisms (Benizri et al., 2001; Compant et al., 2005; Garbaye, 1994; Sturz and Nowak, 2000). Several key inorganic nutrients (C, N S, P, Fe, Ni, Ag, etc.) in soil are transformed and cycled through their metabolic activities (Cole and Brown, 1980, Kandeler et al., 2005, Lovely and Coates, 1997, Tiehang et al., 2008). Soil structure is affected through their production of organic and inorganic acids, facilitating the weathering of soil minerals and formation of soil aggregates (Gorbushina and Krumbein, 2005, Tisdall et al., 1978).

New species and taxa are constantly described, adding to the bacterial list. Consequently, some species or even higher taxa may be transferred to a newly described taxon with more suitable descriptions. Described taxa may be merged together forming a more coherent arrangement and high rank taxa may be sub-divided or even raised to a higher rank. According to the second edition of Bergey's Manual of Systematic Bacteriology (Garrity and Holt, 2001), members of the domain Eubacteria include about 4,000 bacterial species assigned to at least 941 validated genera and organised into 23 phyla.

Comparative phylogenetic analysis of the DNA sequences of cloned 16S rDNA (see below) has shown that members of four major phylogenetic groups are ubiquitous to almost all soil types: class α-proteobacteria and phyla Actinobacteria, Acidobacteria and Verrucomicrobia. These four groups are represented in >75% of 16S rDNA clone library studies of soil bacterial communities (Hugenholtz et al., 1998). Other classes of the phylum Proteobacteria and phyla Firmicutes and Planctomycetes are detected in 25–75% of studies (Hugenholtz et al., 1998). Phyla Proteobacteria, Cytophagales, Actinobacteria and Firmicutes are well represented by culturable organisms and these four phyla account for 90% of all culturable

bacteria characterised by 16S rDNA sequences (result was compiled from 5,224 sequences from cultivated organisms in ARB (ARBor, Latin: tree; a software environment for sequence data) database; Hugenholtz et al., 1998). Some phyla which are revealed by clonal analysis, such as Acidobacteria and Verrucomicrobia, are poorly represented by sequences from cultivated organisms. For example, Acidobacteria appear to be numerically dominant and active members of most soils form up to 52% of 16S rRNA gene sequences in clone libraries (Kuske et al., 1997; Nogales et al., 1999; Felske et al., 2000). However, only few isolates have been obtained from soil (Kishimoto and Tano 1987; Sait et al., 2002). The Proteobacteria not only contain a large number of culturable species but also are well represented by cloned 16S rDNA sequences (Dunbar et al., 1999; Buckley and Schmidt, 2003; Saul et al., 2005). Some brief information on the phyla whose members are related to soil bacterial diversity studies is summarised in the following section.

1.3.1.1a. Proteobacteria

The Proteobacteria are a metabolically diverse group of microorganisms sub-divided into five groups, four of which, α-, β-, γ- and δ-proteobacteria, are commonly detected in soils (Madigan and Martinko, 2005). The α-proteobacteria appears to be one of the most abundant microbial groups in many soils, as assessed by both molecular and cultivation-dependent methods. This diverse microbial group contains many nitrogen-fixing bacteria and certain methylotrophic organisms. Members of β- and γ-proteobacteria, though generally not as abundant as the α-proteobacteria, are also commonly detected in the soils. Microbes known to mediate nitrification are found among the β-proteobacteria, whereas organisms such as the fluorescent pseudomonads, which are well known for their ability to metabolise a diverse array of carbon compounds are in the γ-proteobacteria. The δ-proteobacteria mainly consist of sulphate- and iron-reducing bacteria. These organisms are commonly found in the soils, although, because of their intolerance for atmospheric oxygen concentrations, they are rarely represented in isolate collections grown under aerobic conditions (Gupta, 2000).

1.3.1.1b. Acidobacteria

The acidobacteria have been detected in nearly all soil samples analysed (Dunbar et al., 1999). This bacterial group contains at least six phylogenetic sub-groups (Dunbar et al., 1999). Although acidobacteria are widespread and abundant in soils, little is known about these microbes. Currently, only few strains of acidobacteria have been cultivated under laboratory conditions (Sait et al., 2002; Joseph et al., 2003), providing new insights into the metabolic capabilities of this diverse group of microorganisms.

1.3.1.1c. Verrucomicrobia

The Verrucomicrobia are commonly detected in the soils by molecular techniques, but rarely represented in soil isolate collections. Currently, only a few strains from this group have been characterised. The cultivated strains seem to specialise in the degradation of carbohydrates. It is difficult to speculate on the specific function of Verrucomicrobia in soils, but observation that this group is widespread and abundant in diverse soils indicates that these organisms are important components of soil microbial communities (Janssen et al., 2002).

1.3.1.1d. Cytophagales

Cytophagales are commonly detected in soil clone libraries and frequently isolated from soil samples (Furlong et al., 2002). Many of these organisms are involved in the aerobic degradation of cellulose or chitin and thus suspected to be of great importance in the decomposition of plant materials. Despite their widespread distribution and ease with which many of the members of this group have been obtained in pure culture, there have been few studies addressing the diversity or ecologic significance of these microbes.

1.3.1.1e. Actinobacteria

Members of Actinobacteria are high G + C contents Gram positive microorganisms and tend to be abundant in soil microbial communities. These bacteria are well represented in pure cultures and metabolically diverse. The coryneform bacteria and the filamentous actinomycetes are the Actinobacteria most commonly recovered in soil isolate collections. It is interesting to note that the Actinobacteria are recovered less frequently in clone

libraries collected from soils than in soil isolate collections. This observation may be due to the over-representation of these organisms in culture collections or their under-representation in clone libraries owing to the difficulty in extracting nucleic acid from these resilient Grampositive cells (Janssen et al., 2002).

1.3.1.1f. Firmicutes

The Firmicutes are low G + C Gram-positive bacteria which are well represented in pure cultures and are metabolically diverse. This group contains the endosporeforming bacteria, the lactic acid bacteria and Gram-positive cocci. The over-representation of this group can also be observed in culture collections (Ahmad et al., 2000).

1.3.1.1g. Planctomycetes

Planctomycetes are aerobic organisms that grow best in dilute media. These organisms divide by budding and are one of the few bacterial groups that lack peptidoglycan in their cell walls. Though a number of strains are present in culture collections, few Planctomycetes have been obtained from soil samples. It has been suggested that Planctomycetes are both diverse and abundant members of soil microbial communities. Their display of unusual distinctive features such as compartmentalized cell organization, ability of some species to grow anaerobically and autotrophically via oxidation of ammonium, and the possession of large genomes combined with their wide distribution in a variety of habitats reinforces an increasing interest in them.

1.3.1.2. Effect of different factors on soil microbial diversity

1.3.1.2.1. Soil type as the determinant of the structure of microbial communities in soil.

Soil is fundamental and irreplaceable; it governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles because microorganisms in the soil degrade, sooner or later, virtually all organic compounds including persistent xenobiotics and naturally occurring polyphenolic compounds. Soil problems such as, soil loss, soil degradation, soil contamination are some of the emergencies that mankind must resolve in the third

millennium to safeguard the planet and ensure survival of mankind. Soil is a fascinating biological system with the microorganisms inhabiting soil responsible for much of its broad metabolic capacity (Nannipieri et al., 2003). Therefore, microbiological properties are considered to be more sensitive than chemical and physical properties to changes in management and environmental conditions. Changes in the composition of soil microflora can be crucial for the functional integrity of soil (Insam, 2001).

Soil type likely represents another important factor influencing the structure of microbial communities. Soil, on the basis of different particle size distribution, pH, cation exchange capacity, or organic matter content, thus can affect microbial community structure either directly, i.e., by providing a specific habitat that selects specific microorganisms, or indirectly, i.e., by affecting plant root functioning and exudation in a soil-specific manner. Several relatively recent studies have indeed provided evidence that soil type can be an important determinant of the composition of microbial rhizosphere communities (de Ridder-Duine et al., 2005; Girvan et al., 2003; Nunan et al., 2005). Indeed, bacteria and fungi are highly versatile; they can carry out almost all known biological reactions.

1.3.1.2.2 Plant type as the determinant of the structure of microbial communities in soil

Plant roots release a wide variety of compounds into the surrounding soil, including ethylene, sugars, amino acids, organic acids, vitamins, polysaccharides, and enzymes. These materials create unique environments for the microorganisms living in association with plant roots, in the rhizosphere. Bacteria respond differently to the compounds released by the plant root, and thus different compositions of root exudates are expected to select different rhizosphere communities. The most efficient plant growth– promoting bacteria have been found among-the genera *Pseudomonas, Agrobacterium, Bacillus, Variovorax, Phyllobacterium*, and *Azospirillum,* (Burdman et al., 1997; Molla et al., 2001; Bertrand et al., 2001). Several studies on different plant species in different locations, using a range of cultivation-based and molecular methods, indicated that plant type is indeed a major factor influencing the structure of microbial communities (Table 1.2).

It has been reported that plants have a major influence in shaping rhizosphere microbial communities when they are grown in monoculture (Grayston et al., 1998; Steer and Harris,

2000). Rhizodeposition of carbon from plant roots drives many complex chemical and biological interactions in the soil (Jones et al., 2004; Singh et al., 2004). These include sustaining a complex food web of prokaryotes and eukaryotes in, on or near to the root, the composition of which is thought to be regulated by complex signalling (Phillips et al., 2003). However, it is not conclusively known whether plants actively select beneficial soil microbial communities in their rhizospheres. Some studies have found selection of specific groups of microorganisms in the rhizosphere (Grayston et al., 1998; Smalla et al., 2001; Kowalchuk et al., 2002; Duineveld et al., 2001; Costa et al., 2006). However, other studies have provided evidence of a stronger impact of soil characteristics on the rhizosphere microbial community structure (de Ridder-Duine et al., 2005; Girvan et al., 2003; Nunan et al., 2005), while a few indicated the importance of both plant species and soil types (Alvey et al., 2003; Marschner et al., 2001). There are few reports which studied the impact of plant species and soil type on both microbial (fungal and bacterial) communities (Mougel et al., 2006; Costa et al., 2006).

System and factors studied	Methods used	Results and conclusions	References
Plant species (chickpea, rape, and Sudan grass); soil type (sandy, sandy loam, and clay); root zone location	PCR-DGGE	Bacterial community structure in the rhizosphere is affected by a complex interaction between plant species, and root zone location	Marschner et al.,2001
Soil type, plant type (clover, bean, alfalfa), plant age	PCR-TGGE	The plant species type had the greatest effect in determining microbial community structure	Wieland et al., 2001
Soil type, plant type (wheat, rygrass, bentgrass, and clover)	Biolog CLPP	Plant effect with significant difference in microbial communities from the different plant species	Grayston et al., 1998
Plant cultivar (maize) and soil–effect on the specific bacterial group of *Paenibacillus*	PCR-DGGE	Soil type showed higher effect than plant cultivar type on *Paenibacillus* communities	Da Silva et al., 2003
Plant type (canola, wheat); soil type	FAME	Effect of plant type stronger than that of soil type	Germida et al., 1998
Plant (flax, tomato) and soil type; effect on fluorescent pseudomonads	Cultivation; REP-PCR, RFLP	Soil effect stronger than plant effect	Latour et al., 1996
Plant (maize) development, cultivar, and soil effect	Cultivation–methohd	Between the factors studied, soil had the dominant effect on microbial diversity	Chiarini et al., 1998
Microbial community in the spermosphere as affected by soil type and seed type	Biolog CLPP; FAME	Soil type affected microbial community structure more than seed type	Buyer et al., 1999
Rhizosphere bacterial community composition in natural stands of *Carex* arenaria (sand sedge) is determined by bulk soil community composition	PCR-DGGE	Large differences were observed between the bacterial communities of the different sites for both bulk and rhizosphere soi	De Ridder-Duine et al., 2005
The bacterial community composition in the rhizosphere of canola, clover and two tomato genotypes	PCR-DGGE	Plant growth, mycorrhizal colonization and bacterial community composition of the two tomato genotypes were affected by a complex interaction between tomato genotype	Marschner and Timonen, 2004
Arbuscular mycorrhizal (AM) colonisation influences rhizosphere bacteria differently depending on plant species	PLFA	The plant species had greater effects on the bacterial community in the rhizosphere than AM colonisation and the effect of AM differed between plant species	Soderberg, et al., 2002
The bacterial and fungal rhizosphere communities of strawberry (*Fragaria ananassa* Duch.) and oilseed rape (*Brassica napus* L.)	PCR-DGGE	The structure of different microbial groups in the rhizosphere is influenced by plant species and sampling site.	Costa et al., 2006
Rhizosphere microbial community and its response to plant species and soil history	PCR-DGGE	Plant species and soil type are two important factors affecting the structure of total bacterial, *Pseudomonas* and *Bacillus* community.	Garbeva et al., 2008
Plant growth-promoting rhizobacteria	PCR-DGGE	The bacterial communities analyzed here hardly varied, regardless of different vetiver genotypes	Monteiro et al., 2009
Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils	TRFLP	Growth of plants has a major impact on microbial community structure in soils	Singh et al., 2007

Table 1.2. Effect of plant and soil type on microbial community structure.

1.3.1.2.3. Agricultural management regime as the determinant of the structure of microbial communities in soil.

The effects of alteration in land-use on the physical and chemical properties of soils have been well studied. The land-use change can have significant and long-lasting effects on soil carbon and nutrient contents, soil texture, and pH (Murty et al., 2002); these effects largely arise from changes in plant species composition and associated management practices across land-use types (Christian et al., 2008). Several studies have shown that soil management practices, such as crop rotation, tillage, fertilizer, compost, manure, or pesticide applications and irrigation greatly affect soil microbial parameters (Table 1.3).

Steenwerth et al. (2003) evaluated soil microbial community structure for nine land uses, including irrigated and non-irrigated agricultural sites, non native annual grassland and relict, and never-tilled or old field perennial grassland. The results showed a distinct grouping of microbial communities from different treatments and suggested that non native annual grasses may be associated with a unique microbial community. The impact of long-term grassland management regimes (N-fertilizer application and soil drainage) on microbial community structure was assessed by Clegg et al. (2003) using PCR-DGGE and PLFA profiling. McCaig et al. (2001) compared bacterial communities in grassland under different management regimes by 16S rDNA clone libraries and PCR-DGGE. Palmer & Young (2000) also showed clear effects of soil-management regime on rhizobial diversity in soil, as a higher diversity of *Rhizobium leguminosarum* was measured in arable soil than in grassland soil.

Soil management regimes	Impact	Reference
Organic, low-input, and conventional farming system	Increases in microbial biomass resulting from high organic matter inputs in the organic and low-input systems	Bossio et al., 1998
Application of model herbicide 2,4-D in three different soils	The same population of 2,4-D degraders became dominant in the three soils	Tiedje et al., 1999
Long-term grassland management regimes (N-fertilizer application and soil drainage)	Grassland management practice impacts on Community structure of specific bacterial groups. N fertilizer has significant impact on eubacterial and actinomycete community and soil drainage on actinomycetes and pseudomonad community	Clegg et al., 2003
Unimproved, semi-improved, and improved grassland soil	Clear difference in microbial community between the three differently managed grasslands	McCaig et al., 2001
Plots in sod and cropped to wheat. No-till, subtill, or plow managed	Cropped plots were higher in microbial biomass. Prevalence of mycorrhizal fungi in sod and sensitivity to tillage under wheat-fallow cropping	Drijber et al., 2000
Change from forest to pasture vegetation	Shifts in bacterial community structure. Significantly higher G + C content in the pasture soil	Nusslein and Tiedje, 1999
Improved, unimproved, and semi-improved grassland pastures	Significant effect of soil management on diversity of ammonia oxidizer populations with higher diversity in unimproved soil	Webster et al., 2002
Permanent grassland, arable land under rotation and under monoculture of maize	Significant difference in microbial community structures. Higher diversities in the permanent grassland	van Elsas et al., 2002

Table 1.3. Effects of major soil changes on microbial community structure

1.3.1.2.3a. Cropping effect

Soil microorganisms are critically important in the decomposition of crop residues. The physical and chemical nature of plant materials influences microbial decomposition (Collins et al., 1990). The use of cover crops generally increased soil organic C and stimulated bacterial growth and activity (Bolton et al., 1985; Kirchner et al., 1993; Mullen et al., 1998). Knowledge of the impact of transgenic crop residues on soil microbial ecology is essential for understanding the long-term agronomic and environmental effects of genetically modified crops and for developing appropriate management practices for

minimizing potential negative impacts (Min et al., 2007). Continuous cultivation of the same crop in conducive soil, after some years of severe disease, can eventually reduce disease pressure by stimulating microorganisms antagonistic to the pathogen. Next to cover crops, compost application, and tillage, also crop rotation is important (Abawi and Widmer, 2000), as the densities of both soilborne pathogens and the antagonistic microorganisms are affected.

1.3.1.2.3b. Soil amendment and tillage

Management systems, such as tillage regimens, have also been investigated. Curci et al. (1997) discovered that enzyme activity was higher in the uppermost 20 cm of soil in plots tilled by shallow ploughing and scarification than in those tilled by deep ploughing. Boddington and Dodd (2000) investigated the effect of soil disturbance on the spore density, species richness, and extraradical mycelium lengths of arbuscular mycorrhizal fungi. All factors were reduced in disturbed soil in comparison with undisturbed soil, further indicating that microbial communities are influenced by management practices (Fang et al., 2005).. Although organic farms have been shown to have slightly higher levels of organic matter and carbon than neighboring conventional farms (Lockeretz et al., 1981; Reganold, 1993; Girvan et al., 2003), only limited research has investigated the structures and compositions of microbial communities following a switch to organic farming practices. Soil management including tillage also affects residue decomposition because incorporation in soil often leads to more rapid decomposition than in no-tillage systems in which residues remain on the soil surface (Mungai et al., 2005).

1.3.1.2.4. The soil habitat

Habitat complexity may affect soil biota positively or negatively via changes due to disturbance regimen (Hendrix et al., 1986; Millar and Barbercheck, 2002) and plant identity and diversity (De Deyn et al., 2004; John et al., 2006). Soil represents a highly heterogeneous environment for the microbiota inhabiting it; the different components of the solid fractions in soil (sand, silt, clay, and organic matter) provide myriads of different microhabitats (van Elsas and Trevors, 1997). The organisms resident in soil are exposed to abiotic and nutritional conditions that may vary even over the micrometer scale, i.e., the

scale experienced as their biosphere. In a "stable" system, one can hypothesize that each microhabitat is occupied by organisms that were best able to colonize the niche and become established. These organisms collectively are the underlying catalysts of the biochemical processes in soil.

1.3.1.2.4a. The effect of soil structure and environmental conditions on microbial diversity

Soil is a very complex system that comprises a variety of microhabitats with different physicochemical gradients and discontinuous environmental conditions. Microorganisms adapt to microhabitats (van Elsas and Trevors, 1997) and live together in consortia with more or less sharp boundaries, interacting each other and with other parts of the soil biota. A number of investigations emphasize the impact of soil structure and spatial isolation on microbial diversity and community structure (Tiedje et al., 2001; Sessitsch et al., 2001). Analysis of the spatial distribution of bacteria at microhabitat levels showed that, in soils subjected to different fertilization treatments, more than 80% of the bacteria were located in micropores of stable soil micro-aggregates (2–20 cm) (Tiedje et al., 2001). Such microhabitats offer the most favourable conditions for microbial growth with respect to water and substrate availability, gas diffusion and protection against predation. Particle size had a higher impact on microbial diversity and community structure than did factors like bulk pH and the type and amount of organic compound input. Results showed that the microbial diversity in fractions with small soil particles was higher than that in fractions with large soil particles, and that most of the soil microbial community was particle-specific.

1.3.1.3. Microbial diversity versus community structure

The term biodiversity has been defined in various ways. In microbial terms, it describes the number of different types (species) and their relative abundance in a given community in a given habitat. In molecular-ecological terms, it can be defined as the number and distribution of different sequence types present in the DNA extracted from the community in the habitat. However, the term "community structure" implies that information is included on the numbers of individuals of different recognizable taxa (van Elsas and

Trevors, 1997). These divergent terms are often used interchangeably in publications on soil microbial diversity. With respect to microbial diversity, the number of types present and the evenness of their distribution are important. A habitat with a larger number of species is more diverse, whereas an evenly distributed community is more diverse than an unevenly distributed community with the same number of species (Hedrick et al., 2000).

1.3.1.3.1. Definition and measurement of microbial diversity

Microbial diversity is a general term used to include genetic diversity, that is, the amount and distribution of genetic information, within microbial species; diversity of bacterial and fungal species in microbial communities; ecological diversity, that is, variation in community structure, complexity of interactions, number of trophic levels, and number of guilds. Here we consider microbial diversity simply to include the number of different bacterial species (richness) and their relative abundance (evenness) in soil microflora. Equations used to calculate species richness and evenness and diversity indices, which combine both richness and evenness, have been discussed by Kennedy & Smith (1998). Microbial diversity is measured by various techniques such as traditional plate counting and direct counts as well as the newer molecular-based procedures.

1.3.1.3.1.1 Diversity index

Biological diversity can be quantified in many different ways. The two main factors taken into account when measuring diversity are richness and evenness. A synthetic definition of both these concepts is following:

a) **Richness**

The number of species per sample is a measure of richness. The more species present in a sample, the 'richer' is the sample. Species richness as a measure on its own takes no account of the number of individuals of each species present. It gives as much weight to those species which have very few individuals as to those which have many individuals.

b) **Evenness**

Evenness is a measure of the relative abundance of the different species making up the richness of an area.

1.3.1.4. Methods for the assessment of soil microbial diversity

1.3.1.4.1. Cultivation – based methods

Traditionally, methods to analyze soil microorganisms have been based on cultivation and isolation (van Elsas et al., 1998; Gamble et al., 1977; Ehrenreich et al., 2000; Brettar et al., 2001; Joseph et al., 2003; Burgmann et al., 2004; Heylen et al., 2006). A wide variety of culture media has therefore been designed to maximize the recovery of diverse microbial groups. These methods are fast, inexpensive and can provide information on the active, heterotrophic component of the population.

Limitations include the difficulty in dislodging bacteria or spores from soil particles or biofilms, growth media selection (Tabacchioni et al., 2000), growth conditions (temperature, pH, light), the inability to culture a large number of bacterial and fungal species with current techniques and the potential for colony–colony inhibition or of colony spreading (Trevors, 1998). In addition, plate growth favours those microorganisms with fast growth rates and those fungi that produce large numbers of spores (Dix and Webster, 1995). All of these limitations can influence the apparent diversity of the microbial community

Garland and Mills (1991) developed a technique using a commercially available 96-well microtitre plate to assess the potential functional diversity of the bacterial population through sole source carbon utilization (SSCU) patterns. Gram-negative (GN) and gram-positive (GP) plates are available from Biolog (Hayward, CA, USA, www.biolog.com) and each contains 95 different carbon sources and one control well without a substrate. Inoculated populations are monitored over time for their ability to utilize substrates and the speed at which these substrates are utilized. Multivariate analysis is applied to the data and relative differences between soil functional diversity can be assessed. This method has been used successfully to assess potential metabolic diversity of microbial communities in contaminated sites, plant rhizospheres arctic soils, soil treated with herbicides or inoculation of microorganisms (Kirk et al., 2004).

The Biolog-based method for directly analyzing the potential activity of soil microbial communities, defines a community-level physiological profiling (CLPP) (Garland, 1996). Unfortunately, as a result of biases favouring copiotrophic organisms, the resulting

metabolic fingerprints are unlikely to represent accurately the *in situ* functional diversity in a natural microbial community (Smalla et al., 1998).

In conclusion cultivation-based methods are limited in that only a small fraction of the microbial cells in soil are accessible to study, although a recent study claimed that this percentage can be raised substantially by using special cultivation techniques (Janssen et al., 2002).

1.3.1.4.2. Cultivation- independent methods

1.3.1.4.2.1. Fatty acid methyl ester (FAME) analysis

A biochemical method that does not rely on culturing of microorganisms is fatty acid methyl ester (FAME) analysis. This method provides information on the microbial community composition based on groupings of fatty acids (Ibekwe and Kennedy, 1998). Fatty acids make up a relatively constant proportion of the cell biomass and signature fatty acids exist that can differentiate major taxonomic groups within a community. Therefore, a change in the fatty acid profile would represent a change in the microbial population. It has been used to study microbial community composition and population changes due to chemical contaminants (Siciliano and Germida, 1998; Kelly et al., 1999) and agricultural practices (Bossio et al., 1998; Ibekwe and Kennedy, 1998). For a comprehensive review of the use of fatty acid patterns of phospholipids and lipopolysaccharides to characterize microbial populations see Zelles (1999).

For FAME analysis, fatty acids are extracted directly from soil, methylated and analyzed by gas chromatography (Ibekwe and Kennedy, 1999). FAME profiles of different soils can be compared using multivariate analysis. This method will detect changes in the composition of the bacterial and/or fungal community, as well as enable one to follow signature fatty acids of different groups of microorganisms. Ibekwe and Kennedy (1998) used phospholipid fatty acid analysis (PLFA) and CLPP to study microbial communities in the rhizosphere of plants from the field and from greenhouse pots.

Although FAME analysis is one method to study microbial diversity, if using total organisms, fatty acid analysis is a poor method with limitations. If using fungal spores to study the potential fungal diversity, approximately 130 to 150 spores are needed (Graham et al., 1995) and this may obscure detection of minor species in the population. Cellular

fatty acid composition can be influenced by factors such as temperature and nutrition, and the possibility exists that other organisms can confound the FAME profiles (Graham et al., 1995). In addition, individual fatty acids cannot be used to represent specific species because individuals can have numerous fatty acids and the same fatty acids can occur in more than one species (Bossio et al., 1998).

1.3.1.4.2.2. Metabolically active communities (Direct methods)

Several methods, based on direct microscopy, have been proposed to enumerate metabolically active bacteria, i.e. heterotrophic bacteria found in water samples and soil samples. They include for example CTC (5-cyano-2, 3- ditolyl tetrazolium) , or INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5- phenyl tetrazolium chloride) reduction to CTC- or INT-formazan respectively; the resulting red fluorescent spots or black precipitates inside cells indicate effectively respiring bacteria (Zimmerman et al., 1978; Yu and McFeters, 1994; Pyle et al., 1995; Toffanin et al., 2000; Casella et al., 2001; Lepeuple et al., 2004; Basaglia et al., 2003; Basaglia et al., 2007).

A convincing method, in which microbial activity can be linked to phylogenetic information, is to incorporate 5-bromo-2'-deoxyuridine (BrdU) into DNA, followed by fingerprinting of the active communities (Urbach et al., 1999; Yin et al., 200). In addition, ^{13}C incorporation (stable isotope labeling) followed by separation and fingerprinting has been proposed as a way to assess the metabolically active fractions (Bailey and McGill, 2002).

1.3.1.4.2.3. DNA based methods

In recent years, various culture-independent molecular techniques have been employed; in these nethods the sequence variation in ribosomal RNA (rRNA) genes have been exploited for inferring phylogenetic relationships among microorganisms and used to estimate the genetic diversity of complex microbial communities in natural ecosystems (Hackl et al., 2004; Jin et al., 2006). These molecular techniques are generally based on PCR or RT-PCR of specific or generic targets in soil DNA or RNA. The 16S and 18S ribosomal RNA (rRNA) or their genes (rDNA) represent useful ecological markers for prokaryotes and eukaryotes, respectively. However, the shortcomings of these techniques and their related

problems have also been well documented (Janssen et al., 2002; VonWintzingerode et al., 1997). PCR products generated with primers based on conserved regions of the 16S or 18S rDNA from soil DNA or RNA yield a mixture of DNA fragments representing all PCR-accessible species present in the soil. The mixed PCR products can be used for (a) preparing clone libraries and (b) a range of microbial community fingerprinting techniques.

1.3.1.4.2.3a. Clone libraries

Clone libraries are useful to identify and characterize the dominant bacterial or fungal types in soil and thereby provide a picture of diversity (Axelrood et al., 2002). However, to accurately describe the microbial diversity within a soil sample, clone libraries usually need to be quite large. There are as yet few studies in which the representativeness issue has been satisfactorily resolved, and hence microbial diversity has not been adequately covered in most studies to date. Rarefaction analysis, calculation of coverage values, or other statistical techniques are needed to evaluate whether the number of screened clones is sufficient to realistically estimate the true diversity (Lauber et al., 2008; Lozupone and Knight, 2008). Great progress can be expected in this area, as our capacity for rapid sequencing of large numbers of clones increases and as statistical techniques to determine representativeness improve.

1.3.1.4.2.3b. Microbial community fingerprinting techniques

A range of techniques has been developed to fingerprint soil microbial communities. These include denaturing or temperature gradient gel electrophoresis (DGGE/TGGE) (Heuer et al., 1997; Muyzer et al., 1993; Muyzer and Smalla, 1998; Yu and Morrison, 2004; Yu et al., 2008; Wang et al., 2007; Green et al., 2009), amplified rDNA restriction analysis (ARDRA) (Sharma et al., 2008; Zhang et al., 2008; Zhao et al., 2008; Heylen et al., 2006; Li et al., 2006; James et al., 2006; Kirk et al., 2004)., terminal restriction fragment length polymorphism (T-RFLP) (Osborn et al., 2000; Sakamoto et al., 2003), single-strand conformational polymorphism (SSCP) (Schmalenberger and Tebbe, 2002.), and ribosomal intergenic spacer length polymorphism (RISA) (Ranjard et al., 2001). Although these PCR-based methods are in principle reproducible and robust, they are susceptible to the potential biases described above that are inherent in both nucleic acid extractions and PCR amplifications (Table 1.4).

Advantages
Dependence on efficient cell lysis only and not on the physiological status of cells
Direct picture of the diversity of dominant microbial types, including the unculturables
Direct assessment of shifts in microbial community structure
Ease in handling. Simultaneous analysis of high sample numbers
Reproducible results
Generation of sequences resulting in identification and specific probes to track the specific organism in the ecosystem

Disadvantages
Incomplete lysis of some species, notably gram-positive spore-formers
Possible biases in DNA extraction and PCR amplification, inhibition by soil compounds
Possible presence of one particular sequence or band in different organisms
Heterogeneous bands that may originate from one bacterial strain due to heterogeneity in the rDNA genes
Phylogenetic information only is usually obtained, and the link to functional information is difficult

Table 1.4. Advantages and disadvantages of culture-independent PCR-based microbial community fingerprinting methods.

Denaturing gradient gel electrophoresis (DGGE) / temperature gradient gel electrophoresis (TGGE)

DGGE and TGGE are two similar methods for studying microbial diversity. These techniques were originally developed to detect point mutations in DNA sequences.

Muyzer et al. (1993) expanded the use of DGGE to study microbial genetic diversity. DNA is extracted from soil samples and amplified using PCR with universal primers targeting part of the 16S or 18S rRNA sequences. The 5′ end of the forward primer contains a 35–40 base pair GC clamp to ensure that at least part of the DNA remains double stranded. This is necessary so that separation on a polyacrylamide gel with a gradient of increasing concentration of denaturants (formamide and urea) will occur based on melting behaviour of the double-stranded DNA. If the GC-clamp is absent, the DNA would denature into single strands. On denaturation, DNA melts in domains, which are sequence specific and will migrate differentially through the polyacrylamide gel (Muyzer, 1999). Theoretically, DGGE can separate DNA with one base-pair difference (Miller et al., 1999). TGGE uses

the same principle as DGGE except the gradient is temperature rather than chemical denaturants. DGGE/TGGE have the advantages of being reliable, reproducible, rapid and somewhat inexpensive. Multiple samples can also be analyzed concurrently, making it possible to follow changes in microbial populations (Muyzer, 1999). Limitations of DGGE/TGGE include PCR biases (vonWintzingerode et al., 1997), laborious sample handling, as this could potentially influence the microbial community, (Muyzer, 1999; Theron and Cloete, 2000), and variable DNA extraction efficiency (Theron and Cloete, 2000).

It is estimated that DGGE can detect only 1–2% of the microbial population representing dominant species present in an environmental sample (MacNaughton et al., 1999). In addition, DNA fragments of different sequences may have similar mobility characteristics in the polyacrylamide gel. Therefore, one band may not necessarily represent one species (Gelsomino et al., 1999) and one bacterial species may also give rise to multiple bands because of multiple 16S rRNA genes with slightly different sequences. (Gelsomino et al., 1999; Maarit-Niemi et al., 2001). Maarit-Niemi et al. (2001) used different combinations of DNA extraction and clean-up procedures and reported the method used does influence the banding pattern on DGGE gels. They reported that the Soil DNA Isolation Kit (MO Bio Laboratories Inc., Solana Beach, CA, USA) gave consistent, clear bands with the most extensive banding patterns.

Gelsomino et al. (1999) found that direct and indirect DNA extraction methods yielded DNA fingerprints that were 90% identical, with sample variation for each extraction method being less than 5%. Most of the differences in extraction methods and in reproducibility were between faint bands, presumably representing less dominant species (Gelsomino et al., 1999). Holben et al. (2004) used DGGE in combination with G+C fractionation to assess microbial community diversity and to detect minority populations of bacteria in the digestive tracts of chickens. This approach shows promise in that the fractionation reduces the complexity of the community and allows the detection of species that are present in low abundance. DGGE/TGGE has been used to assess the diversity of bacteria and fungi in the rhizosphere (Duineveld et al., 1998, 2001; Smalla et al., 2001), caused by changes of nutrient addition (Iwamoto et al., 2000) and addition of

anthropogenic chemicals (Torsvik et al., 1998; el Fantroussi et al., 1999; MacNaughton et al., 1999; Whiteley and Bailey, 2000).

The partial community level fingerprints derived from DGGE/TGGE banding patterns have been analyzed for diversity studies based on the number and intensity of the DNA bands as well as similarity between treatments. However, with the limitations of PCR and of banding pattern separation, care must be exercised when interpreting results with respect to microbial diversity. Specific DGGE/TGGE bands can also be excised from gels, re-amplified and sequenced or transferred to membranes and hybridized with specific primers to provide more structural or functional diversity information (Theron and Cloete, 2000). By sequencing bands, one can obtain information about the specific microorganisms in the community.and the taxonomic groups within the community. While the rRNA genes have been the main target of microbial diversity studies using DGGE, some researchers have targeted catabolic genes, such as methane monooxygenase (Knief et al., 2003) or nitrite oxygenase (Nogales et al., 2002; Throbäck et al., 2004) for DGGE analysis. This would provide information on the diversity of specific groups of microorganisms competent in a defined function such as pollutant degradation.

1.3.1.5. Future perspectives

It is important to study microbial diversity not only for basic scientific research, but also to understand the link between diversity and community structure and function. Human influences such as pollution, agriculture and chemical applications could adversely affect microbial diversity, and perhaps also above and below-ground ecosystem functioning. For instance, Buckley and Schmidt (2001) found significantly higher amounts of 16S rRNA for all microbial groups analyzed in fields that have never been cultivated as compared to agricultural fields. This suggests a decrease in bacterial biomass or activity in cultivated fields. Similarly, the diversity of AMF (Arbuscular mycorrhizal fungi) has been shown to increase from arable fields to natural systems (Daniell et al., 2001; Menendez et al., 2001). However, it is not known what these reductions in diversity mean to ecosystem functioning and it is important for sustainability of ecosystems that the link between diversity and function be examined and better understood.

There is disagreement within the scientific community of whether taxonomic or genetic diversity is important as long as functional diversity is maintained. Given the limitations of our ability to study diversity and how diversity relates to function, it would be prudent to assume functional redundancy does not exist and taxonomic diversity is important to maintain. It was once thought that AMF were functionally redundant given a lack of host specificity, but it has since been found that they are not functionally redundant and do provide different benefits to different plant hosts.

Knowledge of microbial diversity and function in soils is limited because of the taxonomic and methodological limitations associated with studying these organisms. Although methods to study diversity (numerical, taxonomic, and structural) are improving for both bacteria and fungi, there is still not a clear association between diversity and function. Even if an organism is functionally redundant in one function, chances are it is not redundant in all functions and will have different susceptibilities and tolerances to abiotic and biotic stresses. It is generally thought that a diverse population of organisms will be more resilient to stress and more capable of adapting with environmental changes.

Bacterial and fungal diversity increases soil quality by affecting soil agglomeration and increasing soil fertility. They are both important in nutrient cycling and in enhancing plant health through direct or indirect means. In addition, a healthy rhizosphere population can

help plants deal with biotic and abiotic stresses such as pathogens, drought and soil contamination.

Our current ability to study and understand soil microbial diversity is wrought with taxonomic and methodological limitations. Soil microbiologists face the difficult task of attempting to define and identify microorganisms and their functions. Although molecular methods have the advantage of obtaining information about non-culturable organisms, they also have limitations that cannot be ignored. It is challenging to soil microbiologists to develop techniques to study soil microbial diversity when it is currently impossible to know how accurate these techniques are. We do not know what is present in a gram of soil, and therefore it is difficult to conclude whether one technique of studying diversity is better than another. Given the current state of knowledge, we feel that the best way to study soil microbial diversity would be to use a variety of tests with different endpoints and degrees of resolution to obtain the broadest picture possible and the most information regarding the microbial community. In addition, methods to understand the link between structural diversity and functioning of below- and above-ground ecosystems need to be developed so that the question of how diversity influences function can be addressed.

Our knowledge of plant–microbe–soil interactions is increasing, but the complexity of interacting biological, chemical and physical factors means that much remains to be understood. As new techniques are developed, our level of understanding will increase and our knowledge expand.

2. EXPERIMENTAL SITE

2.1. Introduction

The experimental site is situated inside the Pilot Demonstrative Farm “Diana”, in the municipal district of Mogliano Veneto (North East part of Italy, Venice Lagoon catchment), managed by Veneto Agricoltura. It was built within the project promoted and carried out by the local drainage authority “Consorzio di Bonifica Dese Sile” (from 2010 renamed “Consorzio di Bonifica Acque Risorgive”) and titled "Environmental restoration actions along the low course of Zero River for the reduction of nutrient input into Venice Lagoon", funded by Veneto Region through the “Plan for pollution prevention in the watershed flowing directly into Venice Lagoon”.

The experimental design was planned according to the protocols and methods of the European project NICOLAS (Nitrogen Control by Landscape Structures in Agricultural Environment - EC DGXII, 1997-2000 ENV4-CT97-0395).

2.2. The study area

North East Italy includes one of the major drained reclamation regions of the country and a considerable portion of the Venice Lagoon watershed area is located within this region (Fig 2.1).

Over the past decades nutrient loads delivered to the Venice Lagoon have attracted considerable concern, so the local government (Regional Authority) in 1995 established a series of targets to reduce eutrophication by reducing the level of nitrogen and phosphorus entering the Lagoon. For Dese and Zero (Fig 2.2) rivers, two of the main rivers managed by the local drainage authority (Consorzio di Bonifica Acque Risorgive), 150 x 10^3 Kg/year of total N and 40 x 10^3 Kg/year of total P loads reduction were established.

In order to reach these objectives several experimental actions were undertaken, one of which was the conversion of a cultivated area of about 30 ha to a forested buffer strip, irrigated with freshwater from the Zero river. Inside this afforested area, a pilot experimental scale system was established in order to find the most suitable conditions for enhancing denitrification activity. The Zero joins the Dese river just before the latter flows into Venice Lagoon; it is a resurgence river 41.5 km long, with a 7283 ha watershed, 94% of which is used for agriculture and 6% as urban areas. The watershed is mostly covered by herbaceous cultivations (corn, soy, wheat) farmed “alla ferrarese”, i.e. in regular plots, longitudinally convex with 1-3% steepness, 30-50 m large and 200-500 m long, bordered

by lateral permanent drainage.The climate is sub continental with temperatures ranging from a daytime average of 1°C in January to 23°C in July and August. The mean value of rainfall is 900 mm per year, with higher peaks in Autumn and Spring and lower values in Winter and Summer.

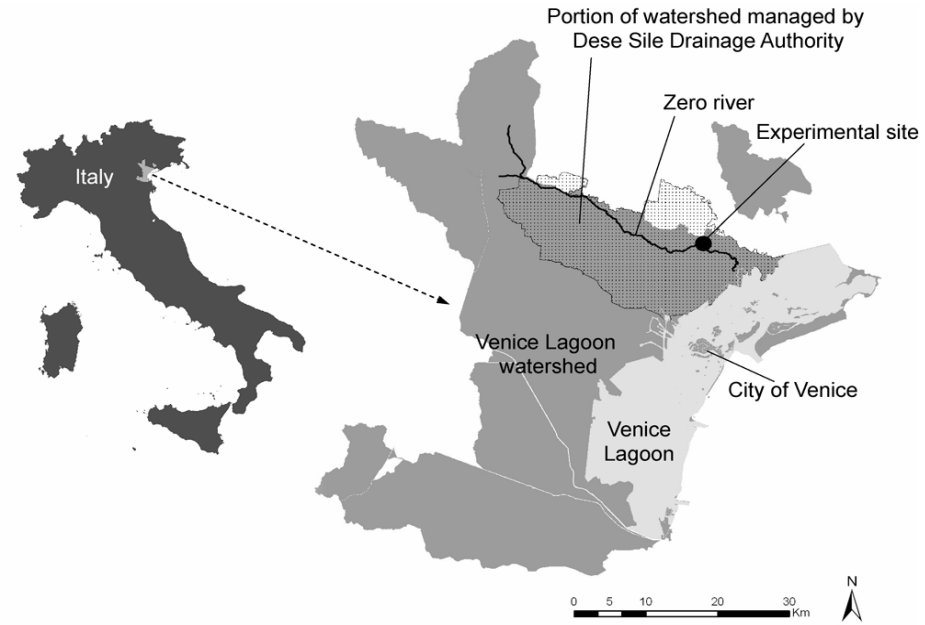


Fig 2.1. The experimental site, situated on the left bank of the terminal reach of Zero river, in the watershed draining into Venice Lagoon. This portion of basin is managed from the drainage authority Consorzio di Bonifica Dese Sile (from 2010 renamed Consorzio di Bonifica Acque Risorgive)

Fig 2.2 The Zero River in the reach adjacent to the experimental site

The soil (texture category “silty clay loam”) belong to the “Zerman soil consociation” according to the “Carta dei suoli del Bacino Scolante in Laguna di Venezia” (Soils map of the watershed draining into Venice Lagoon) (ARPAV 2004).

2.3. Experimental site description

The experimental site is situated within a much wider (about 30 ha) forested buffer zone, developed in lands previously used for arable crops, along the left bank of the lower course of Zero river (locality Bonisiolo) 15 km far from Venice (Fig 2.2).

The afforested area is divided in plots of the same size (0.35 ha each) and structure, but with different forest configuration (Fig 2.3). Each plot is watered through a system of ditches carrying water (through a pumping system), from Zero river.

Fig 2.3. Plan of the 30 ha wide forested buffer zone**.**

The experimental site was built in 1999 in two of these different plots (Fig 2.4). It occupies a total area of about 0.70 ha (227 m long and 30 m wide). It required rebuilding the hydraulic structures (furrows facilitating sub-superficial water flow) and the water pumping plant, upgrading the meteorological station already existing in the Diana farm, installing the piezometric network, preparing the soil, planting the saplings.

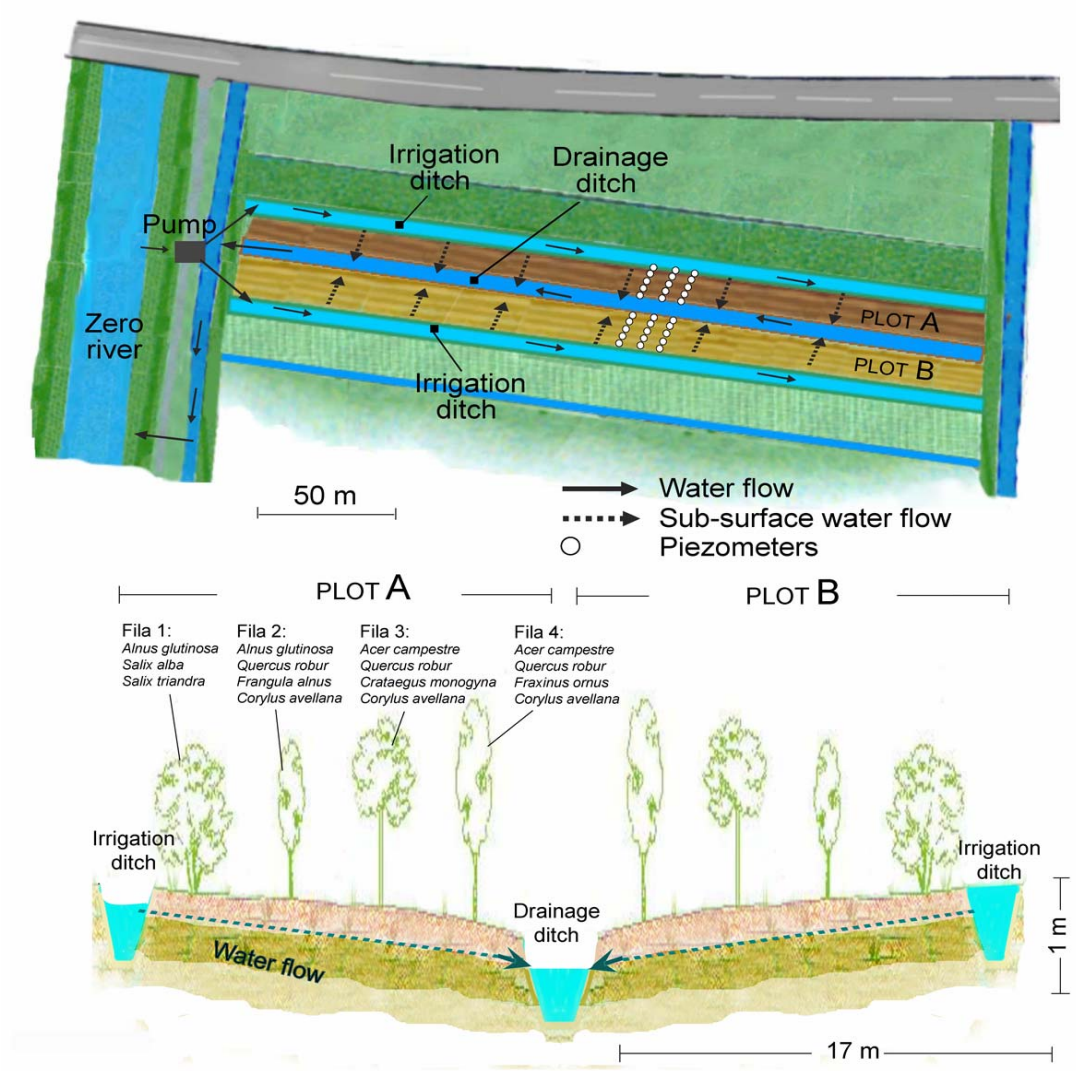


Fig 2.4. Plan (above) and section (below) of the experimental site: each of the 2 plots is watered through an irrigation ditch carrying water from the Zero river. Soil setting allows a difference in elevation among the irrigation ditches and the drainage ditch, resulting in a sub-surface flow of water running through the wooded buffer strips.

2.3.1. Soil characteristics

A soil profile was determined near the experimental site by digging a trench 150 cm deep (ARPAV, 2004) (Table 2.1). Soil is fine textured (according to textural classification USDA-SCS, 1984; Ritchie 1972), with a deep calcic horizon. In particular, the top layer of the soil horizon (0 to a 70 cm depth) is olive-brown, with silty clay loam texture (according to textural classification USDA-SCS, 1984; Ritchie 1972), low limestone content and alkalinity. Underneath the top soil is a weathered subsoil (Bw) 20 cm thick, light olive-brown, with silty-clay texture, lower limestone content.

(Zerman soil – ZMR1 (SINAP 13 profile) –Location: Diana Farm (Bonisiolo)																
Horizion	**Depth**	**pH H_2O**	**Granulometry**			**Textural class**	**Carbonates**	**Limestone**	**Organic carbon**	**Total phosphorous**	**Exchange complex (meq/100g)**					**Ks**
			Sand	**Silt**	**Clay**						**CSC**	**Ca sc.**	**Mg sc.**	**K sc.**	**TSB**	
	cm		%				%			mg/kg	(meq/100g)				%	mm/hr
Ap1	0-40	8.0	12.9	51.4	35.7	FLA	4	1	0.9	22	26.2	21.7	3.4	0.3	100	0.88
Ap2	40-70	8.0	12.2	51.8	36.0	FLA	4	2	0.9	16	20.6	20.9	3.9	0.3	100	
Bw	70-90	8.1	7.4	52.3	40.3	AL	1	1	0.3		19.7	18.9	6.4	0.3	100	1.20
Bk	90-120	8.6	10.5	63.4	26.1	FL	15	13	0.2		14.4	64.2	6.0	0.1	100	0.08
Ckg	120-150	8.4	18.1	64.8	17.1	FL	46	11	0.1		13.6	32.3	5.1	0.1	100	

Table 2.1. Table summarizing the physical and chemical characteristics of the various soil horizons as surveyed in the experimental site in 2001 – Source ©2003-2007 ARPAV.

The following horizon is 30 cm thick, light olive gray with grey and yellow-brown streaks, loamy sand textured, highly calcic and strongly alkaline, characterized by limestone accumulation (calcic horizon Bk) forming irregular concretions or soft masses, of light colour. At 120 cm depth begins the Ckq substratum, with no structure and with colours and texture similar to the previous horizon.

2.3.2. Site hydrology

The experimental site was designed to rigorously monitor the hydrological fluxes and to carefully characterize the hydrology of the buffer system. Ridges and furrows facilitate sub-surface water flow throughout the field from the inlet point, represented by two irrigation ditches where water is pumped through, to the parallel drainage ditches localized at lower

elevation (Fig 2.4). The average slope between irrigation and drainage ditches is 4%. The drainage ditches are connected to a canal which brings back water to Zero river (Fig 2.4).

As a consequence of the irrigation (an average of 55,000 m^3 ha^{-1} $year^{-1}$, about three times rainfall), a perched aquifer was created on the calcic layer located at around 90-150 cm depth.

The water level in the experimental site was always between 25 to 60 cm below the soil surface (Fig 2.5). While the surface soil layer was subjected to the normal seasonal cycle, the medium and depth layers were often saturated.

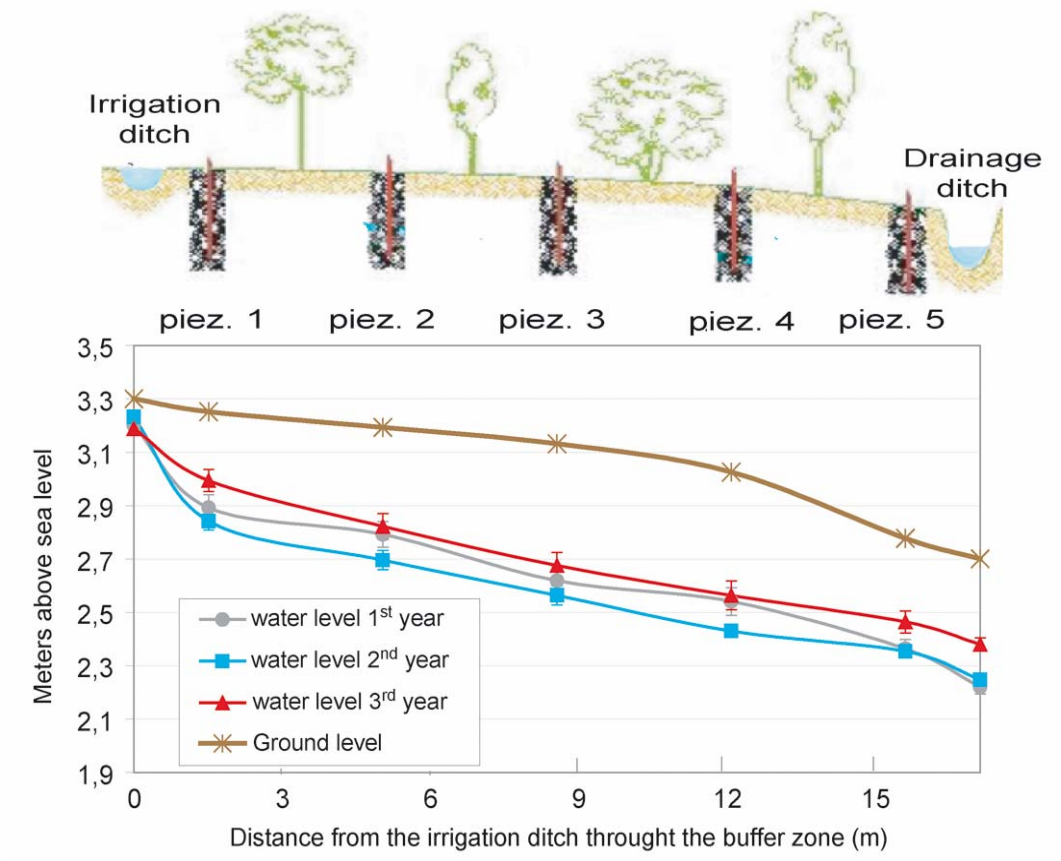


Fig 2.5. Ground level and mean annual water table elevation measured in plot A (monthly measures in ditches and piezometers) during the three monitored years. These values are not significantly different to those from plot B. Bars represent standard error.

2.3.3. Vegetation

Several tree and shrub species white willow (*Salix alba* L.), almond willow (*Salix triandra*), black alder (*Alnus glutinosa* (L.) Gaertner), pedunculate oak (*Quercus robur* L.),

field maple (*Acer campestre* L.), common hazel (*Corylus avellana* L.), common hawthorn (*Crataegus monogyna* Jacq.), manna ash (*Fraxinus ornus* L.), black dogwood (*Frangula alnus* L.) were planted in spring 1999 using 2-3 years old harvested plants and were arranged in four parallel rows for each plot as indicated in Fig 2.4 and Fig 2.6. The chosen forest configuration was: 1.5 m for shrubs and 3.5 m for trees spaced along the row at 3.5 m wide inter-rows, for a total of 4 rows for each plot.

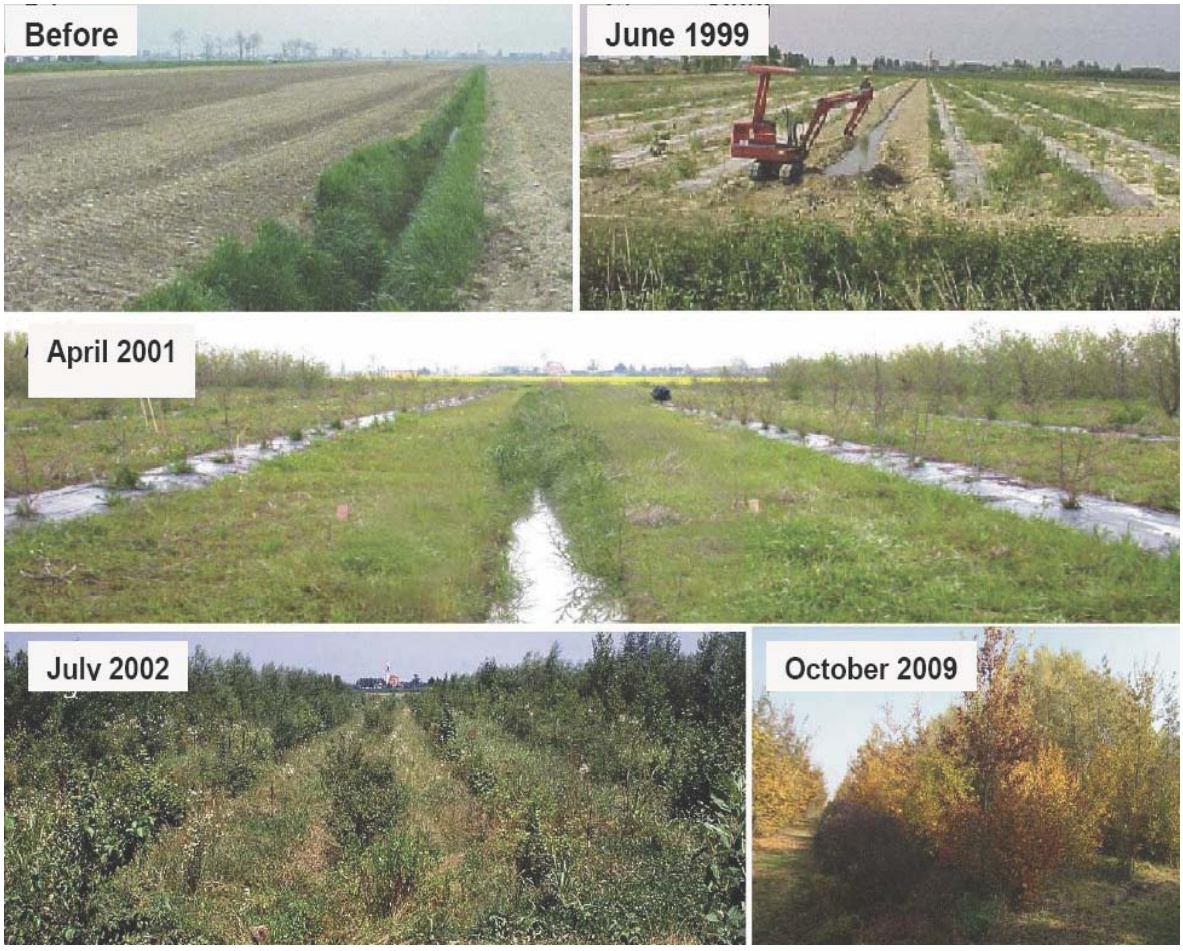


Fig 2.6. Pictures allow to compare the quick transformation occurring in the experimental site from the initial condition of agricultural area with newly-planted saplings to a forested buffer area

3. OBJECTIVES

3.1. General objectives

General objectives of this project are the following

- To quantify the amount of the nitrogen removal due to a 30ha wide wooded buffer zone.
- To increase knowledge on the processes which allow the riparian forest buffer strips to act as buffers.
- To identify the most appropriate management strategies, in order to maximize the efficiency of these systems supporting the microbial processes involved in nitrogen removal.

3.2. Specific objectives

The nitrogen removal mechanisms in constructed buffer zone are known to involve ammonification, nitrification and denitrification processes where microorganisms are directly involved to these processes. So, it is important to identify and characterize types of involved microorganisms, how can they work and how are they distributed.

For this purpose, main and specific objectives were pursued in order to determine spatial and seasonal fluctuations of the microbial communities in the soil/water of the wooded riparian strip.

The following tasks were followed to obtain these main objectives:

a) To determine culturable bacteria of the microbial communities in the soil/water samples.
b) To quantify viable and metabolically active cells of the microbial communities in soil samples.
c) To identify and characterize microorganisms of the microbial communities from soil and water samples.
d) To operate a comparison between the microbial communities in the wooded riparian strip soil (internal) to that outside soil of the experimental site (external).
e) To find out the variations and fluctuations of microbial communities with time by collecting samples at different season in different years.
f) To resolve the distribution of the microbial communities at different soil levels and water sites.

4. Metabolic activity and microbial diversity of the wooded riparian strip soil by using culture dependent and independent methods.

4.1. Introduction

Soil microbial diversity is an important index of agricultural productivity (George et al., 1995, Kent and Triplett, 2002; Tilak et al., 2005). The extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health (Smith and Goodman, 1999) and quality, as a wide range of microorganisms is involved in nutrient cycles (N, C, S, P etc) and, consequently, in soil functions. The nitrogen cycle strongly depends upon microbial activities and it is important to note that combined nitrogen is considered one of the most important limiting factors for plant growth. While nitrogen fixation can be considered as a positive activity for the whole ecosystem, denitrification may play a double role in relation to the context it takes place: while nitrogen removal from a NO_3^--fertilized soil is an absolutely detrimental process, the gasification of nitrate from surface and subsurface water flows of terrestrial and aquatic ecosystems may result as a very useful tool for reducing nitrate and nitrite pollution (Knowles, 1982).

Riparian buffer zones are particularly studied for such a purpose. Nitrogen removal from water fluxes crossing a wooded riparian strip is due to the metabolic activity of soil bacterial communities (in particular denitrification process) (Pinay et al., 1993; Hunter and Faulkner, 2001; Spruill, 2004) and to assimilation and uptake by plants (Hanson et al., 1994; Hefting and Klein, 1998). For this reason, the most important factor to be investigated is the relationship of the soil bacterial communities and the distribution of bacterial communities in different soil layers.

Microbial diversity within the soil is crucial to many functions but it has been difficult to be determined using the traditional plating methods, since only a small fraction of the bacteria known to occur in natural habitats can be cultivated on laboratory media (Colles et al., 2008; Zhang et al., 2008).

Fluorescent stains, such as those contained in the LIVE/DEAD BacLight* Bacterial Viability Kit developed by Molecular Probes Inc, can be used to evaluate microbial viability (Boulos et al., 1999; Lepeuple et al., 2004). Unfortunately direct microscopic methods can provide only an estimated number of viable/dead cells but do not give any information related to biodiversity.

Molecular characterization was developed and adopted almost 20 years ago to detect both culturable and unculturable microbial species (Ranjard et al., 2000; Lynch et al., 2004); it

represents an accurate, effective and fast technology for identification of microbial diversity in different environments (Ogram et al., 1987; Pace, 1997; Hatamoto et al., 2008): individual organisms can be identified from some unique part of their 16S r DNA or RNA, providing definitive information on soil biodiversity (Ward et al., 1990; Zhao et al., 2006; Betancourt et al., 2008; Hatamoto et al., 2008; Mocali et al., 2008; Norton et al., 2008; Sallam and Steinbüchel 2008; Wang et al., 2008). For this purposes, 16S rDNA-based techniques have been widely used to characterize microbial community structure in environmental samples (Sharma et al., 2008; Zhang et al., 2008; Zhao et al., 2008; Heylen et al., 2006; Li et al., 2006; James et al., 2006; Kirk et al., 2004).

In this study, culture and culture independent 16S rDNA-based methods were performed to analyze microbial diversity through: a) quantification of metabolic activity of the soil microbial communities; b) operating a comparison between the microbial communities in the inside (internal) and outside (external) soils of wooded riparian strip; c) find out the fluctuation of microbial communities with time by collecting samples at each season; d) determination of the distribution of the microbial communities at different soil depths.

4.2. Material and Methods

4.2.1. Collection of samples and preparation of serial dilution

Soil samples were collected at four different seasons (March 08, April 08, July 08 and October 08) from the wooded riparian strip at three different depths (surface level: 0-15 cm; medium level: 45-60 cm, and deep level: 80-100 cm). The areas where the soil was sampled are reported in experimental site section (see section 2. Experimental sites).

To get suitable control, in October 08, April 09 and October 09, soil samples were also collected outside of the experimental site (External) (see Fig 4.1) for verification of long term efficiency of the buffer system and compare microbial population dynamics.

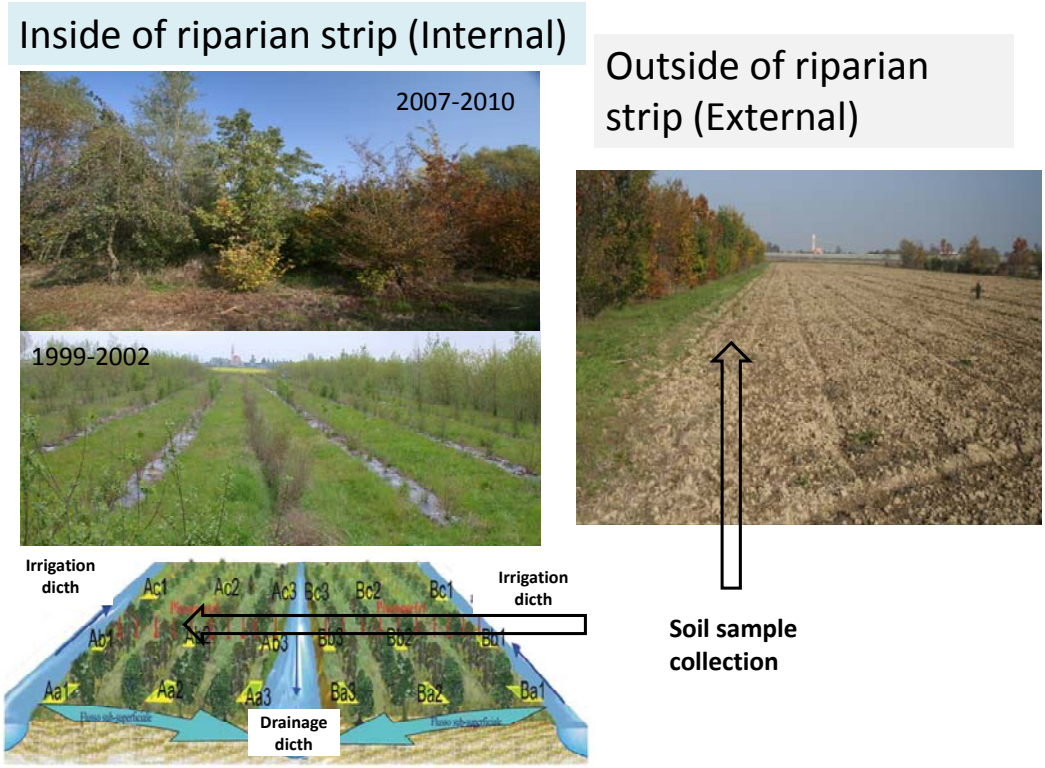


Fig 4.1. Sampling areas

At least 3 independent replicates were sampled for each soil depth. All analyses were performed within 24 h from sampling. 20 g of soil were suspended in sterile NaCl (0.9%) and maintained on a rotary shaker for 45 min at the maximum speed. Soil serial dilutions (1:10) were prepared for the analyses described below. All analyses were performed at least in triplicate.

4.2.2. CTC-assay

CTC (5-cyano-2, 3-di-4-tolyl-tetrazolium chloride) reduction assay indicates, when positive, respiration ability of soil bacteria. In CTC test, the oxidized and soluble tetrazolium chloride enters bacterial cells and it is reduced to the insoluble and fluorescent formazan by the reducing power of metabolically active bacteria (Fig 4.2). The procedure of Gribbon and Barer (1995) was followed: in short, samples of soil serial dilutions were incubated with 4 mM CTC (Polysciences Inc, USA) in phosphate buffer solution (PBS) for 2 h. After staining, aliquots were filtered through a 0.2 μm pore size black polycarbonate membrane filter (Millipore). Filters were air dried and mounted with low-fluorescence

immersion oil (Molecular Probes) on glass microscope slides. CTC-reducing cells were observed by fluorescence microscopy (Olympus fluorescent microscope BX60) equipped with a blue 420-nm exciter filter (Olympus BP 490). At least 20 fields or 300 cells were recorded for each sample.

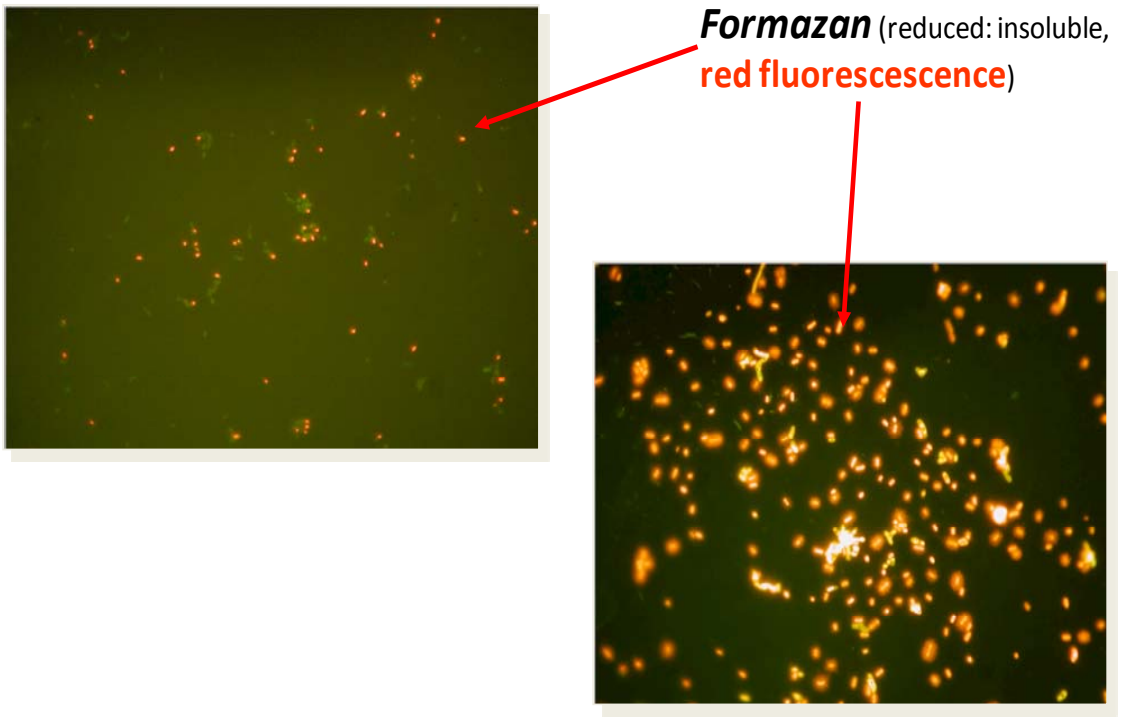


Fig 4.2 Counting metabolically active cells by CTC-assay: red spots correspond to metabolically active bacteria.

4.2.3. Direct Viable Counts

Direct viable and total counts of bacteria were obtained using LIVE/DEAD *Bac*Light* bacterial viability kit provided by Molecular Probes. *Bac*Light contains of two nucleic acid binding stains: SYTO 9 and propidium iodide. SYTO 9 penetrates intact and damaged bacterial membranes staining the cells green, while propidium iodide penetrates only cells with damaged membranes; the combination of the two stains produces red fluorescing cells. Thus bacteria with intact membranes (i.e., viable bacteria) absorb the stain that fluoresces

green while excluding the red fluorescent stain, whereas bacteria with damaged membranes (i.e., dead bacteria) absorb the fluorescent red stain (Fig 4. 3). To perform the test, the two dyes were mixed together (1:1), 3 µL of the mixture were added to1 ml of soil/water suspension. After 15 minutes incubation, the stained sample was filtered through a 0.2-mm black polycarbonate filter (Millipore), and the filter mounted with *Bac*Light mounting oil, as described in the instructions provided by the manufacturer. Fluorescence microscopy conditions were the same as CTC assay.

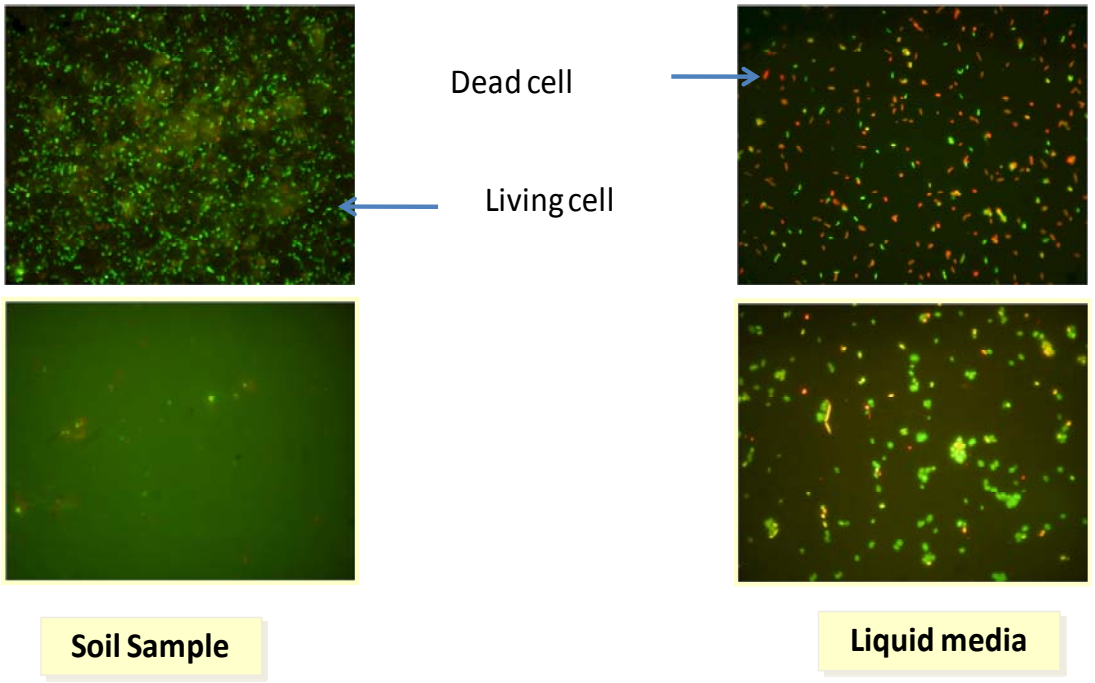


Fig 4.3 Counting of total living cells using LIVE/DEAD *Bac*Light* bacterial viability kit: green cells are living, red cells are dead.

4.2.4. Determination of Culturable Microorganisms

0.1ml aliquots of the soil serial dilution (1:10, 1:100) were dropped and spread onto the solid media plate count agar (PCA) for determinations of total culturable aerobic bacteria. Petri-dishes were incubated aerobically at 30 °C. After 14 days the number and the morphology (shape, size, colour etc) of colonies were recorded. Representative colonies of different morphologies were isolated and stored in glycerol at -20° for molecular analyses.

4.2.5. DNA extraction from colonies

For molecular analysis, DNA was extracted from isolated colonies by alkaline lysis. One colony was suspended in an Eppendorf tube with 50 µL of lysis buffer (2.5 ml 10% sodium dodecyl sulfate, 5 ml 1 M NaOH, 92.5 ml MilliQ water). After 15 min at 95°C and the tube was centrifuged for 5 min at 13,000xg, and the supernatant was transferred to a new tube to add 90 µL MilliQ water. Extracted DNAs were stored at -20°C for further molecular analyses.

4.2.6. Amplified ribosomal DNA restriction analysis (ARDRA)

For amplification of 16SrDNA from cultural bacteria PCR was performed in a final volume of 25 µl containing buffer 10X, 1.0 unit of *Taq* DNA polymerase (Amersham Biosciences), 0.2 mM each of dNTPs, 200 nM of each primer 63F 5'CAGGCCTAACACATGCAAGTC (Marchesi et al., 1998) and 1389R 5'ACGGGCGGTGTGTACAAG (Osborn et al., 2000) and 50 ng template DNA. The thermal cycler (Bio Rad ICycler 170-8740) was programmed for the initial denaturation step (94°C) of 5 min, followed by 44 cycles of 1 min denaturation along with 1 min primer annealing (37°C) and 2 min primer extension (72°C), followed by the 7 min primer extension (72°C) step.

For the ARDRA analysis, 5µl of amplified reaction products were digested using 10U of the *Hinf*1 and *Hpa*II endonucleases (Amersham Biosciences) in final volume of 20 µl for a minimum of 2 h at 37 °C. Fragments were analyzed by 2% agarose gel electrophoresis in 0.5% tris Borate EDTA buffer, at a constant voltage of 100V. Gel images were acquired in digital format using an EDAS 290 Image capturing system (Kodak, Rochester,NY). Profiles were sorted and compared using the BioNumerics software version 4.5 (Applied Maths, Sint-Martens-Latem, Belgium).

4.2.7. The sequence of 16S rDNA culturable bacteria

16S rDNA sequences of culturable bacteria were carried out as described previously (Tan et al., 2007). Operational taxonomic units (OTUs) were defined by a 2% difference and each OTU was represented by a type sequence. The 16S rDNA sequence was analyzed using Chromas LITE (Version 2.01); the most similar bacterial species was found in the GenBank by using BLAST search (http://www.ncbi.nlm.nih.gov). Neighbor-joining phylogenetic

trees were constructed based on 16S rDNA sequences using BioNumerics software version 4.5 (Applied Maths, Sint-Martens-Latem, Belgium).

4.2.8. Diversity index and principal component analysis (PCA)

Based on groupings of unique OTUs (operational taxonomic units) using 16S rDNA sequencing, the diversity indices Shannon's evenness index for general diversity ($H'=-\Sigma Pi \ln Pi$) and Simpson's dominance index ($D = \Sigma ni(ni - 1)/ N(N -1)$ were calculated as described previously (Brandt et al., 2006; Kapley et al., 2007; Wani et al., 2006). In the equations, N is the total number of OTUs in the *i*th (*i* value is 0, 1, 2,…∞) 16S rDNA sequence phylogroup, *Pi* is the proportion of OTUs in the *i*th 16S rDNA sequence phylogroup and n*i* is the number of OTUs in the *i*th 16S rDNA sequence phylogroup. Principal component analysis (PCA) was performed with quantitative data of 16S rDNA sequence phylotype distributions by culturable bacteria using Xlstat 2007 software.

4.3. Results and Discussion

4.3.1. Viable (living) cells, metabolically active cells (CTC+) and culturable bacteria (CFU)

Internal soil

Viable**,** metabolically active cells and culturable bacteria were determined at different soil depths in different seasons of 2008 and 2009. Results are reported in Fig 4.4, 4.5 and 4.6.

Living, metabolically active and culturable cells showed higher values in surface soil and decreased according to depth on both 2008 (Fig 4.4A, Fig 4.5A and Fig 4.6A) and 2009 (Fig 4.4B and Fig 4.5B Fig 4.6B)

In all samples, at any depth and at any time, the culturable bacteria are only a small percentage of CTC+ cells that are themselves a percentage of the total living fraction. These results confirm the well known evidence that in natural environments, such as soil, culturable bacteria are only a small percentage of the totals number of living cells.

External soil

Living, metabolically active and culturable bacteria were also enumerated in a soil from an external area, and results compared with those obtained from the internal one (Fig 4.7 and Fig 4.8). In October 08, living and culturable bacteria showed comparable values, while metabolically active cells showed significantly lower numbers in the external soil (Fig 4.7). Similar results were obtained when internal and external samples of October 09 were compared (Fig 4.8). In both internal and external soil samples the numbers of living, metabolically active and culturable bacteria were higher in surface soil levels and decreased their number with the soil depth.

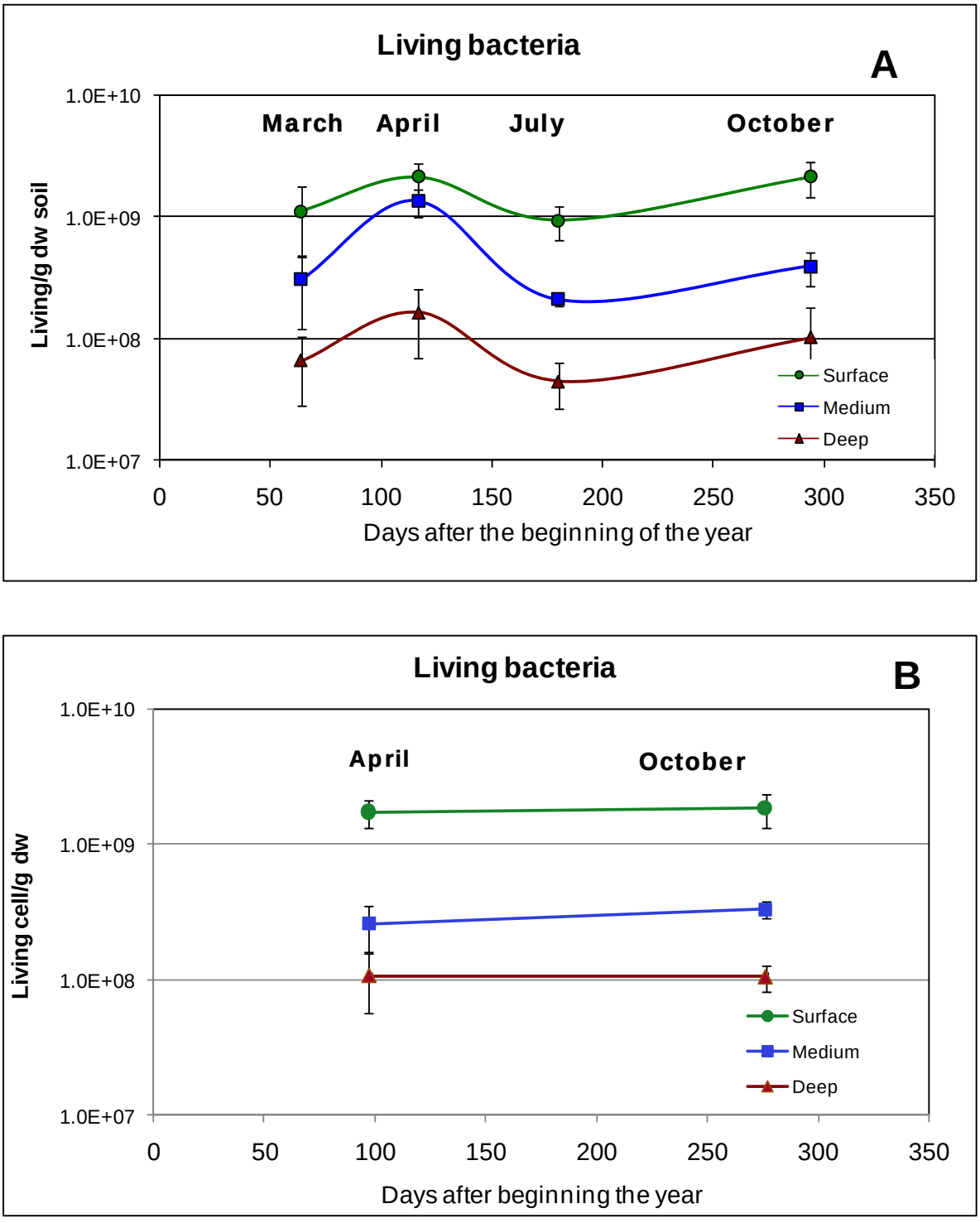
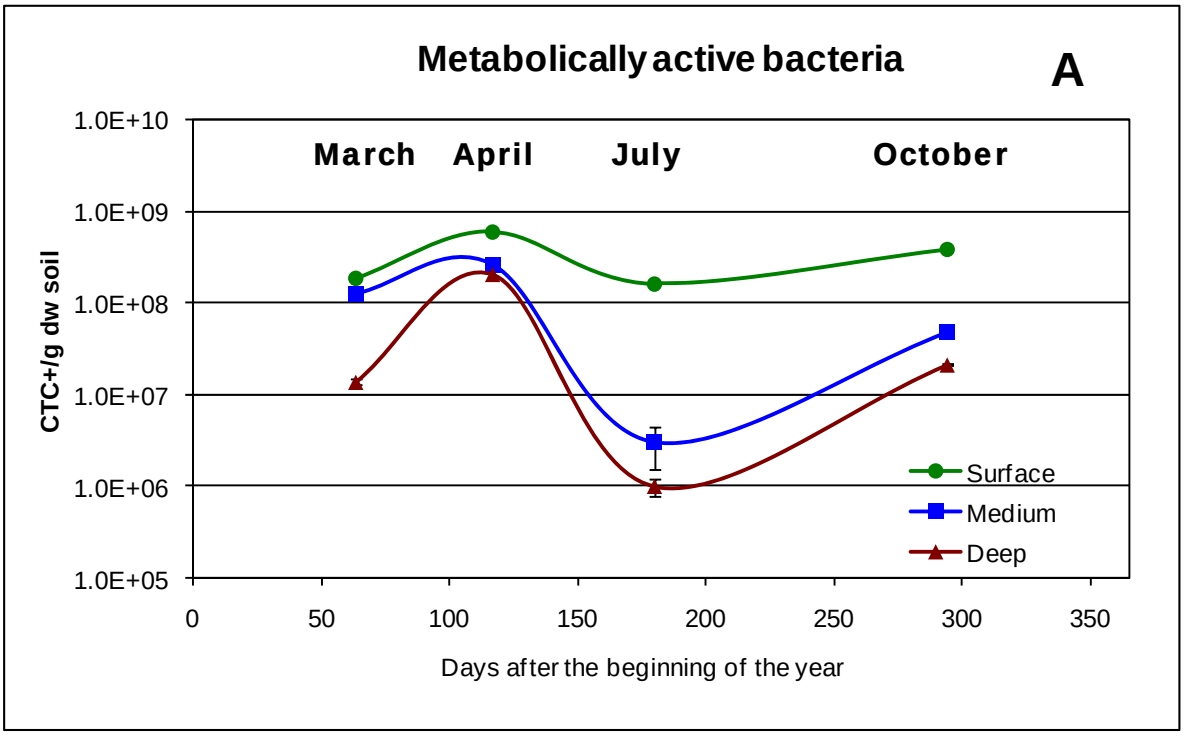


Fig 4.4. Living cells/g dry soil at different soil depths in different seasons. A: results of year 2008; B: results of year 2009.



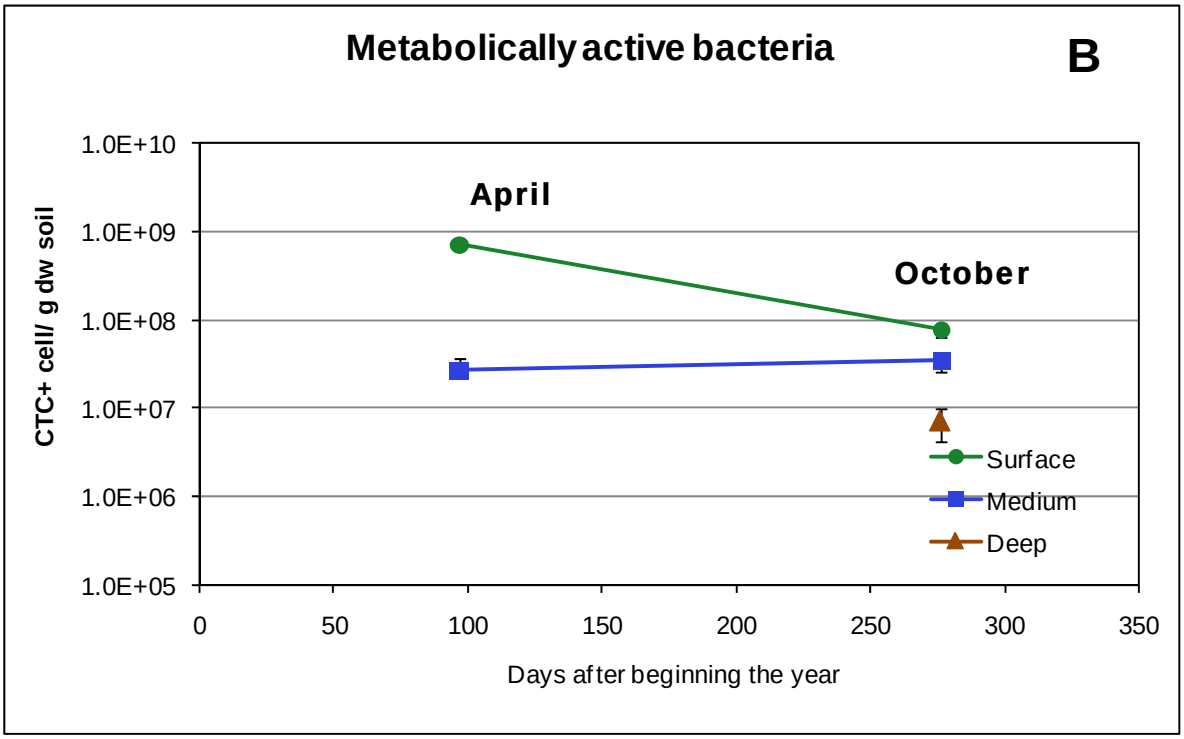


Fig 4.5. Metabolically active cells/g dry soil (CTC+) at different soil depths in different seasons. A: results of year 2008; B: results of year 2009.

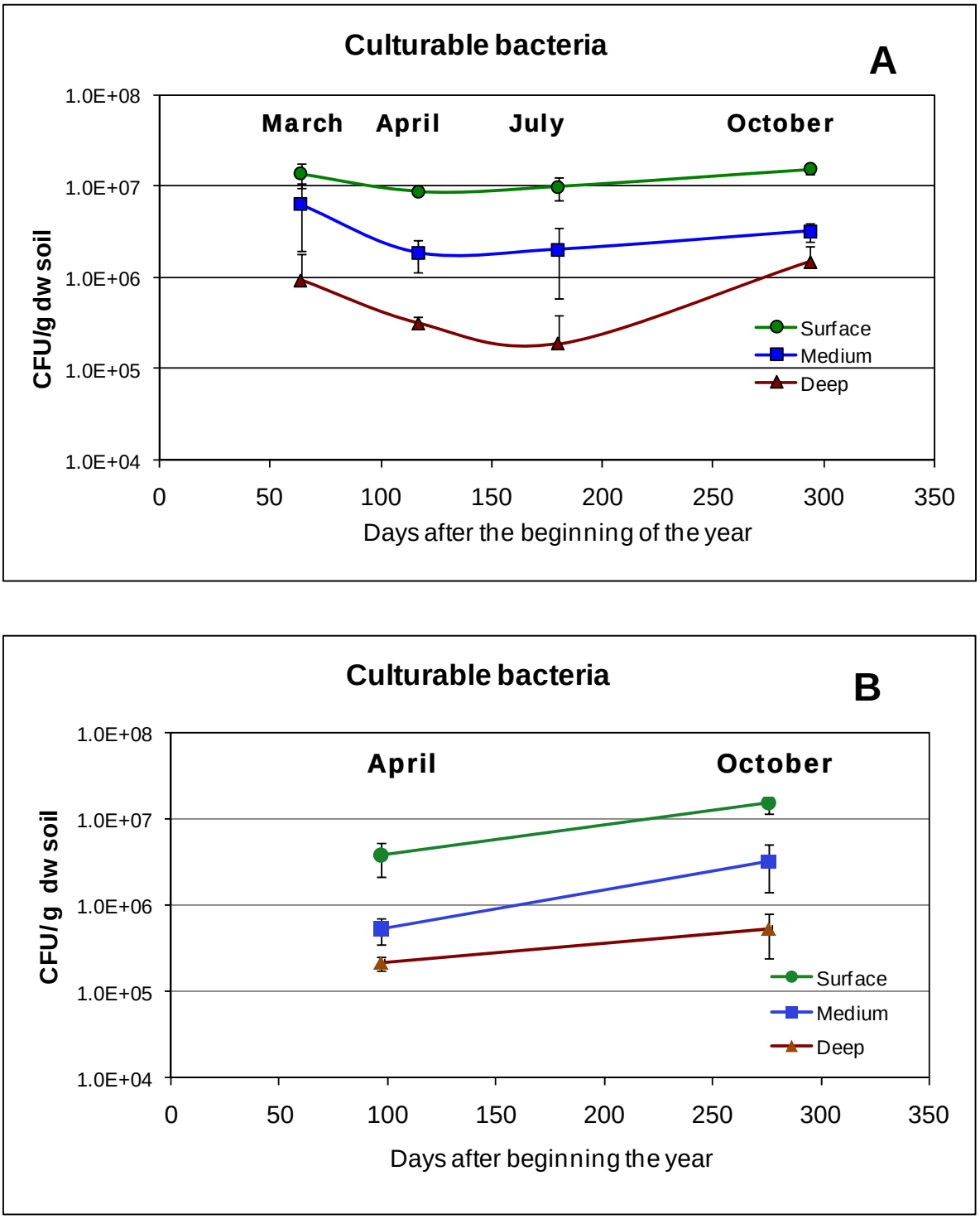
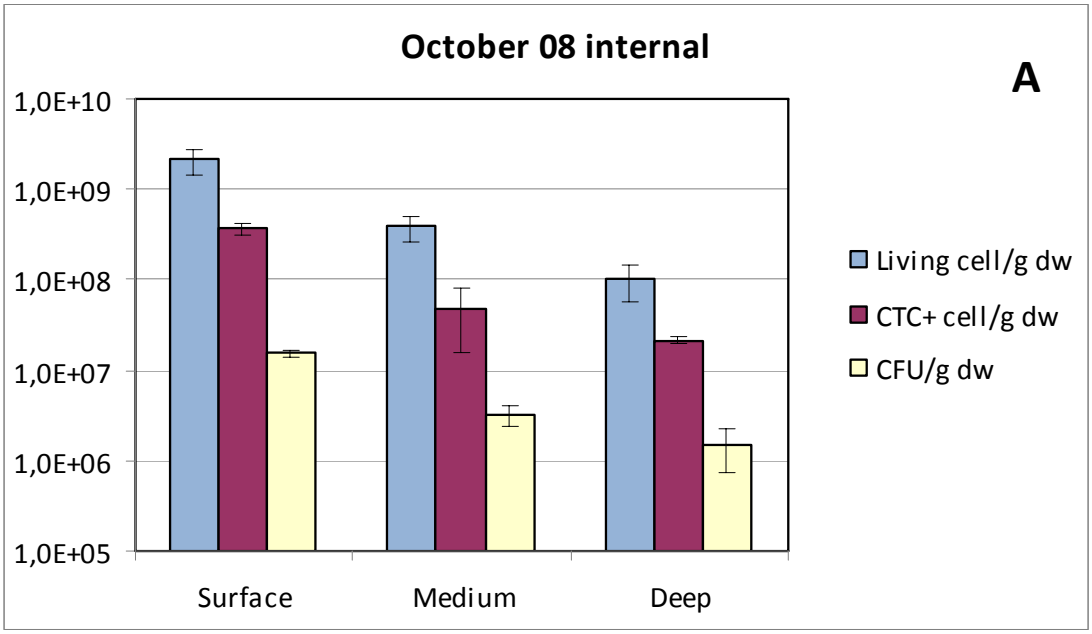


Fig 4.6- Colony forming units/g dry soil at different soil depths in different seasons. A: results of year 2008; B: results of year 2009.



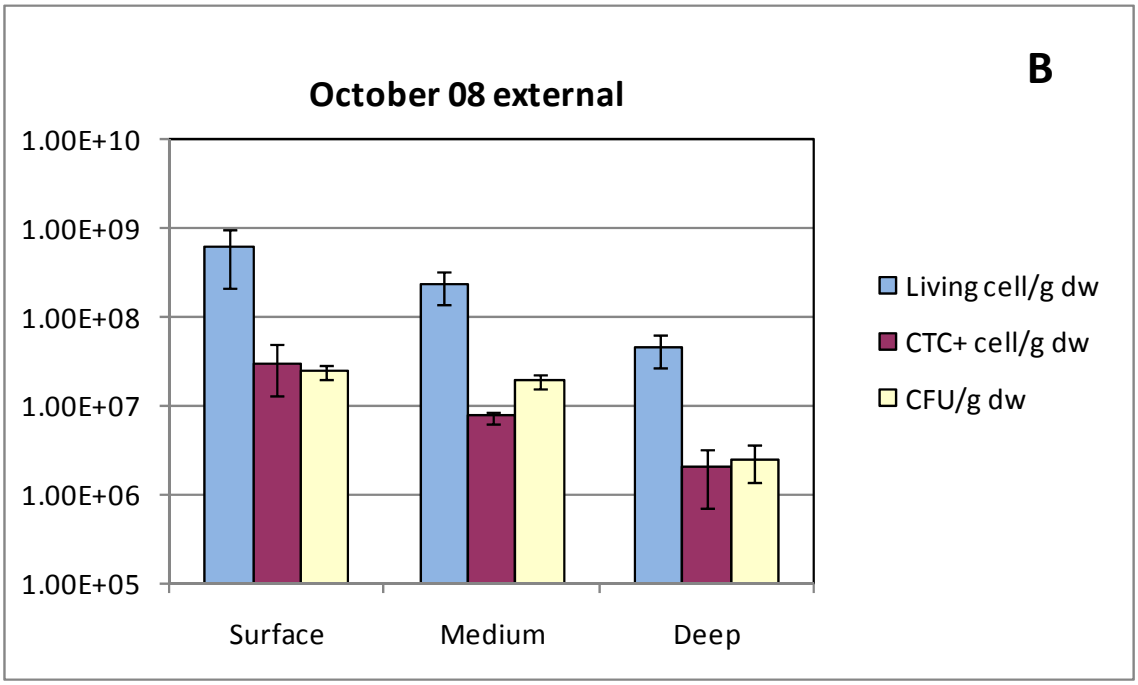
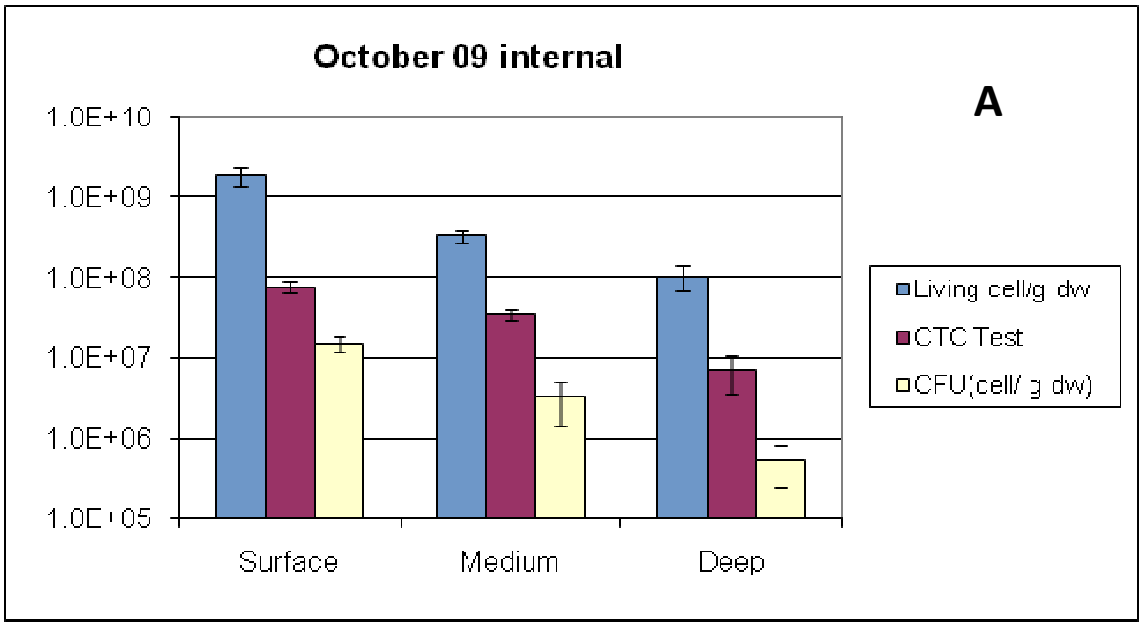


Fig 4.7. Living, metabolically active and culturable cells per g of dry soil inside (Internal, A) and outside (External, B) the buffer strips, at three different soil depths in October 2008.



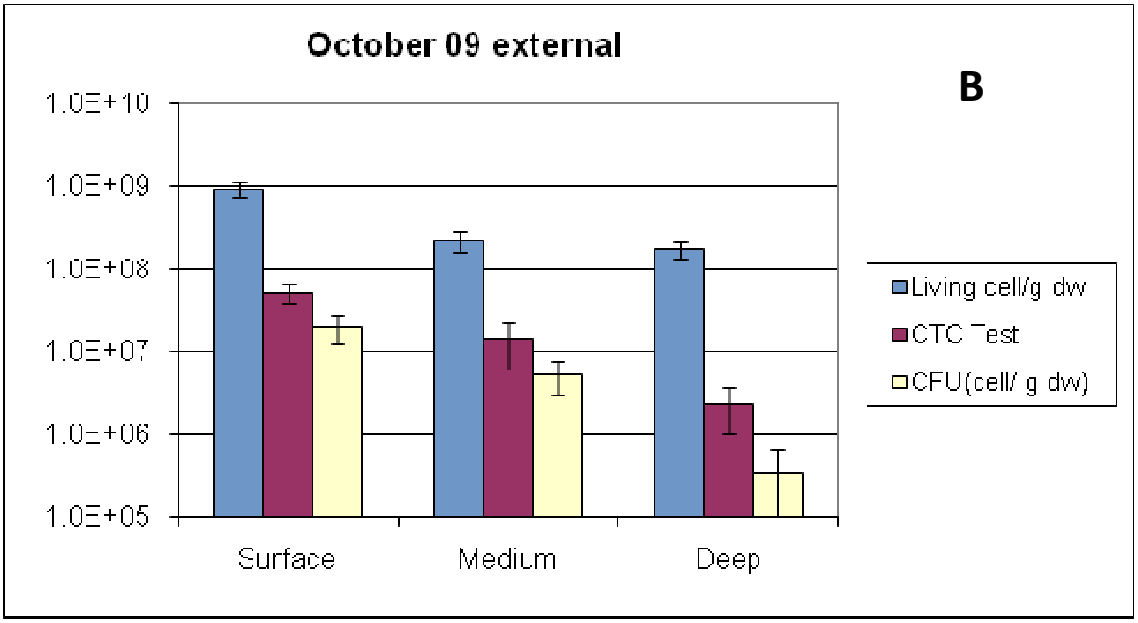


Fig 4.8. Living, metabolically active and culturable cells per g of dry soil inside (Internal, A) and outside (External, B) the buffer strips, at three different soil depths in October 2009.

4.3.2. Biodiversity of culturable bacteria

With the aim to analyze the microbial diversity of the culturable fraction of microbial soil community, a total of 1500 colonies were isolated from the wooded riparian area and from an external soil.

4.3.2a. Morphological observations of colonies

Different morphologies of colonies (colors, margins and shapes) were observed among isolates from inside (Internal) and outside (External) the wooded riparian strip; the related results are reported in Fig 4.9 and Fig 4.10.

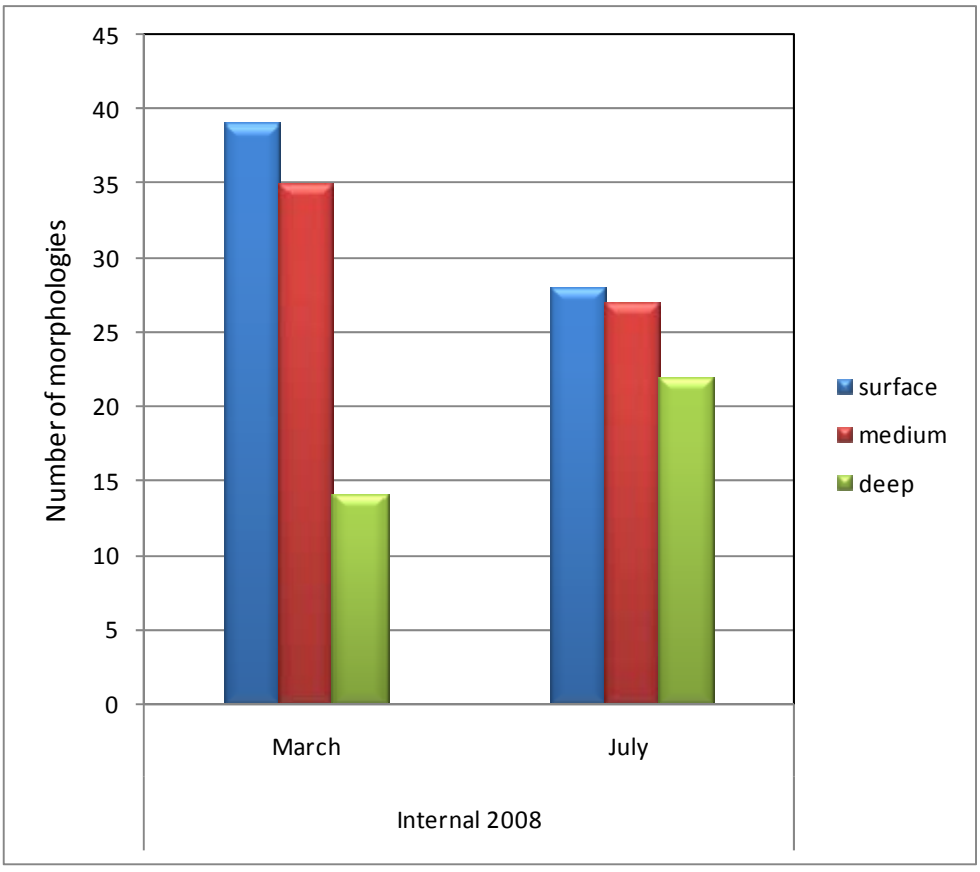


Fig 4.9. Number of morphologies inside the buffer strips (Internal) at three soil depths in 2008 samples.

Taken together, these preliminary results indicate that the culturable fraction of the soil bacterial population shows more biodiversity in March than in July, although the differences among the soil layers lean to be lower in the hot season. In 2009, the differences between internal and external soils were evident only in October.

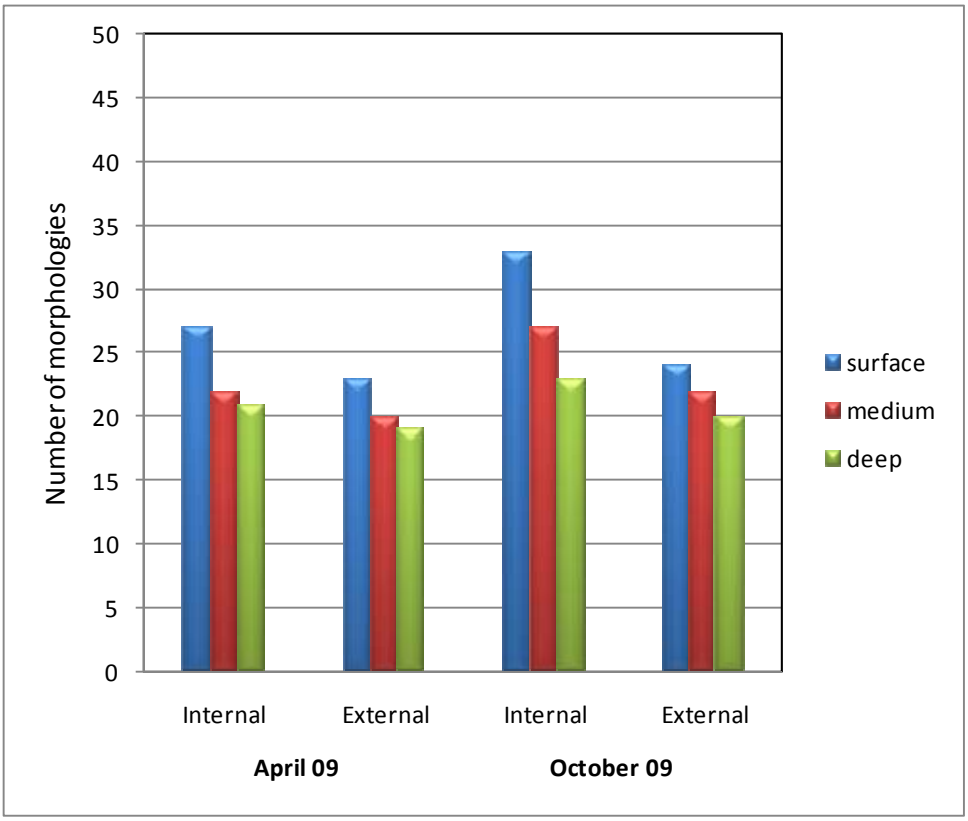


Fig 4.10. Number of morphologies inside (Internal) and outside the buffer strip (External) at three different soil levels for 2009 samples.

Taken together, these preliminary results indicate that the culturable fraction of the soil bacterial population shows more biodiversity in March than in July, although the differences among the soil layers lean to be lower in the hot season. In 2009, the differences between internal and external soils were evident only in October.

4.3.3. Amplification of 16 S rDNA and ARDRA analysis

16S rDNA was amplified from the isolated colonies by PCR. The majority of isolates did produce amplification (examples of amplification of culturable bacteria are shown in Fig 4.11).

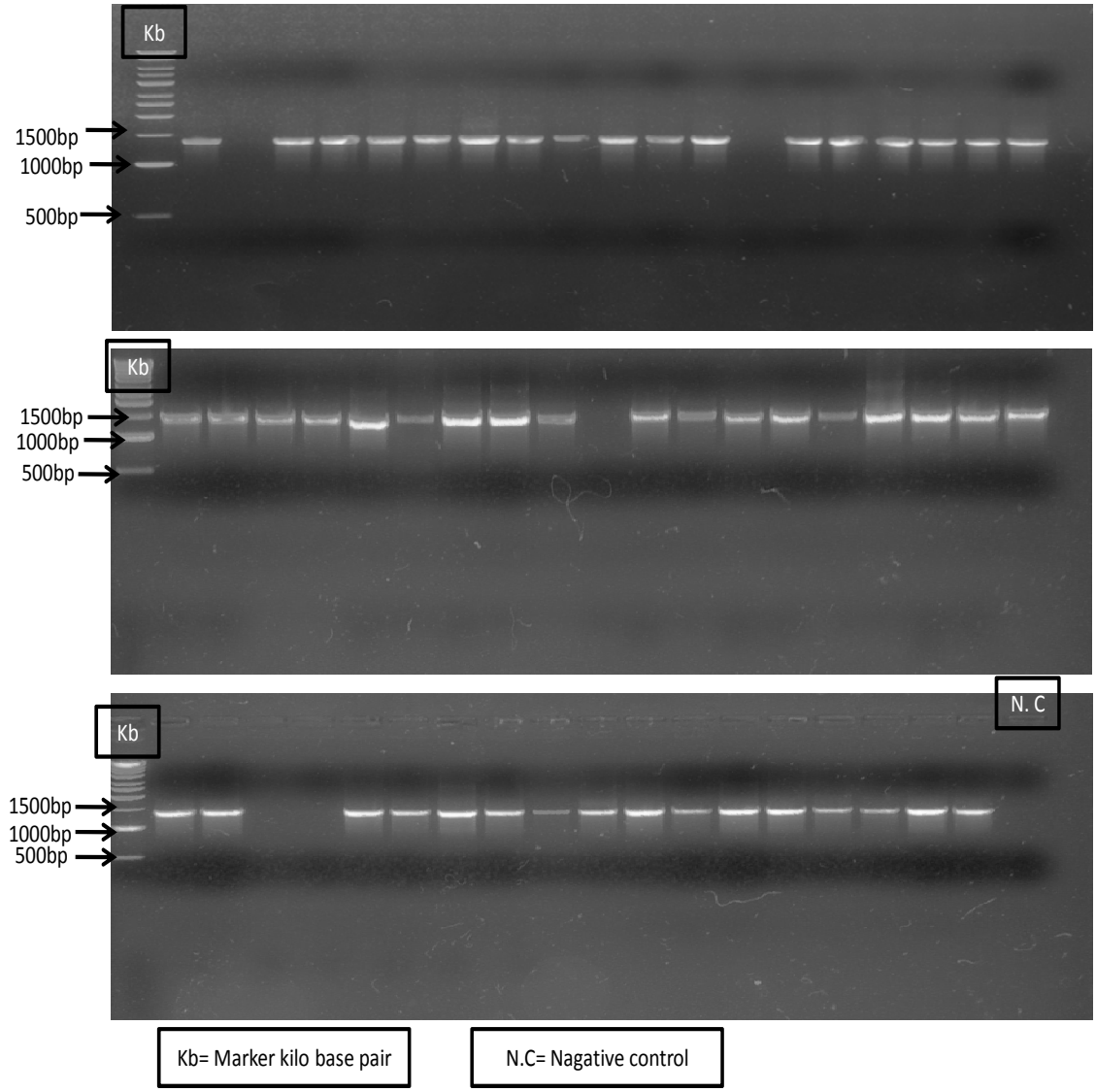


Fig 4.11. Examples of electrophoretograms of 16S r DNA amplicons from culturable bacteria.

Amplified 16S rDNA was digested by two restriction enzymes, *Hin*fI and *Hpa*II. A variety of different DNA fragment patterns was revealed; in total 2420 ARDRA profiles were obtained (examples of restriction patterns of culturable bacteria are shown in Fig 4.12).

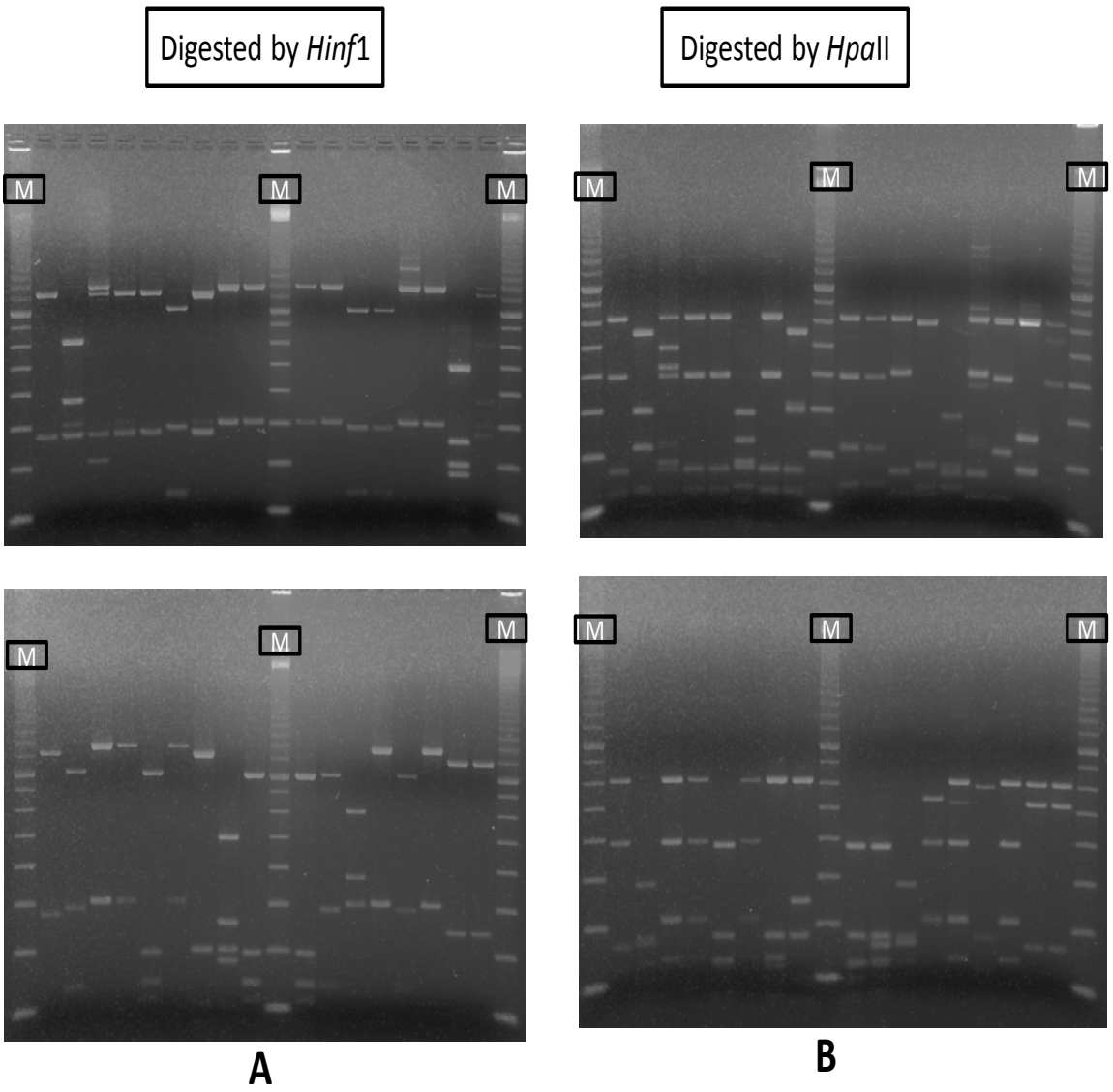


Fig 4.12. Restriction patterns of PCR-amplified 16S rDNA region digested with restriction enzymes: A) *Hinf*1 and B) *Hpa*II.

ARDRA profiles were clustered by computer assistant program BioNumerics, version 4.5 (see Appendix 1) and their analysis discerned high numbers of OTUs (Operational Taxonomic Units) for all the seasons tested in 2008. The 16S rDNA clustered with 151 and 177 colonies and comprised 75 and 103 different ARDRA patterns in March and July, respectively, with higher percentage of OTUs and single OTUs obtained in July (Table 4.1A).

These results confirm the consideration made on the culturable fraction of the soil microbial population by observing the morphology of the colonies, but giving further details on the microbial diversity distribution occurring in the wooded riparian soil.

The same analysis was performed on the samples collected during the year 2009, including the external soil, and the related results are reported in Table 4.1B and Table 4.1C.

Percentage of total OTUs and single OTUs were higher in the internal soil samples when compared with the external. This confirms again that the artificial riparian system seems to sustain higher microbial diversity among the culturable fraction of the microbial population.

Considering the different percentages of common OTUs recovered from the internal and external soils, in different seasons and at different depths, a low level of similarity can be observed between treated and untreated soils. Moreover, it seems to be clear that the soil depth clearly affects the composition of the culturable fraction of the bacterial community.

Sample names	Colony number	OTUs number	OTUs (%)	OTUs% (common)	OTUs (single profile)	OTUs% (single profile)	OTUs (similar profiles)	OTUs% (similar profiles)
March 08 Internal								
Surface	75	45	60.0		28	62.2	17	37.8
Medium	46	24	66.0		13	54.2	11	45.8
Deep	30	18	60.0		13	72.2	5	27.8
Total	**151**	**75**	**49.7**	**13.9**	**47**	**62.7**	**28**	**37.3**
July 08 Internal								
Surface	58	48	82.8		40	83.3	8	13.7
Medium	61	44	72.1		35	79.6	9	20.5
Deep	58	41	70.7		30	73.2	11	26.8
Total	**177**	**103**	**58.2**	**22.6**	**73**	**70.9**	**30**	**29.1**
March and July 08 Internal								
Surface	133	76	57.1	18.3	49	64.5	27	35.5
Medium	107	62	57.9	8.3	36	58.1	26	41.9
Deep	88	48	54.6	18.6	30	62.5	18	37.5
Total	**328**	**155**	**47.3**	**16.7**	**92**	**59.4**	**63**	**40.6**

Table 4.1A. ARDRA analysis of the colonies isolated from the wooded riparian strip in March and July 08 (Internal soil).

Sample names	Colony number	OTUs number	OTUs (%)	OTUs% (common)	OTUs (single profile)	OTUs% (single profile)	OTUs (similar profiles)	OTUs% (similar profiles)
April 09 Internal								
Surface	48	33	68.8		26	78.8	7	21.2
Medium	46	28	60.9		21	75.0	6	21.5
Deep	45	27	60		19	70.4	8	29.6
Total	**139**	**76**	**54.7**	**13.7**	**53**	**69.7**	**23**	**30.3**
April 09 External								
Surface	49	30	61.2		18	60.0	12	40.0
Medium	46	26	56.5		16	61.5	10	38.5
Deep	47	22	46.1		9	40.9	14	63.6
Total	**142**	**63**	**44.4**	**19.2**	**40**	**63.5**	**23**	**36.5**
April 09 Internal and External								
Surface	97	59	60.8	6.35	42	71.2	17	28.81
Medium	92	50	54.4	7.04	36	72.0	14	28.00
Deep	92	46	50.0	6.15	26	56.5	20	43.48
Total	**281**	**120**	**42.7**	**13.7**	**78**	**65.0**	**42**	**35.0**

Table 4.1B. ARDRA analysis of the colonies isolated from the wooded riparian strip and from the external soil in April 2009.

Sample names	Colony number	OTUs number	OTUs (%)	OTUs% (common)	OTUs (single profile)	OTUs% (single profile)	OTUs (similar profiles)	OTUs% (similar profiles)
October 09 Internal								
Surface	56	41	73.2		34	82.9	7	17.0
Medium	58	33	56.9		23	69.7	10	30.3
Deep	54	30	55.6		20	66.7	10	33.3
Total	**168**	**94**	**56.0**	**9.6**	**66**	**70.2**	**28**	**29.8**
October 09 External								
Surface	57	34	59.7		24	70.6	10	29.4
Medium	51	28	54.9		19	67.9	9	32.1
Deep	52	24	46.2		12	50.0	12	50.0
Total	**160**	**73**	**45.6**	**15.1**	**43**	**58.9**	**30**	**41.1**
October 09 Internal and External								
Surface	113	65	57.5	13.3	45	69.2	20	30.8
Medium	109	54	49.5	11.5	32	59.3	22	40.7
Deep	106	46	43.4	14.8	28	60.9	18	39.1
Total	**328**	**130**	**39.6**	**21.2**	**77**	**59.2**	**53**	**40.8**

Table 4.1C. ARDRA analysis of the colonies isolated from the wooded riparian strip and from the external soil in October 2009.

Interestingly, by analyzing the ARDRA profiles of the external soil in the same season, but after one year (October 08 and October 09). The observed differences resulted as not significant (Table 4.1D). This may suggest that the level of biodiversity in the external soil, not conditioned by the treatments applied with the artificial system, could follow well established oscillations that replicate every years.

Sample names	Colony number	OTUs number	OTUs (%)	OTUs% (common)	OTUs (single profile)	OTUs% (single profile)	OTUs (similar profiles)	OTUs% (similar profiles)
October 08 External								
Surface	114	59	51.8	32	54.2	27	45.8	
Medium	76	47	61.8	32	68.1	15	31.9	
Deep	81	50	61.7	31	62.0	19	38.0	
Total	**271**	**137**	**50.6**	**84**	**61.4**	**53**	**38.7**	**12.2**
October 09 External								
Surface	57	34	59.7		24	70.6	10	29.4
Medium	51	28	54.9		19	67.9	9	32.1
Deep	52	24	46.2		12	50.0	12	50.0
Total	**160**	**73**	**45.6**	**15.1**	**43**	**58.9**	**30**	**41.1**

Table 4.1D. Comparison between ARDRA analysis of the colonies isolated from the external soil in October 2008 and after one year, in October 2009.

4.3.4. Sequence analysis of 16S rDNA of culturable bacteria

Before sequencing 16S rDNA of the culturable fraction of bacteria, all ARDRA profiles, obtained from the total 1385 isolates, were clustered together. 508 (OTUs: Operational taxonomic units) unique ARDRA patterns were obtained. Representative bacteria of each unique ARDRA pattern were sequenced, while unsequenced bacteria with identical ARDRA patterns were assigned to the same Phylotypes. The majorities (93.36%) of the sequences were obtained less than 5% different from those present in the current databases; 5.08% of the sequences differed 5 to 10% from those in the databases, 1.17% of the sequences differed 10 to 15%, and only 0.39% of the culturable bacteria differed more than 15%. Detailed results of 16S rDNA sequencing are reported in Table 4.2.

Isolate and Isolation sources	**Closest relative**	**% Similarity**	**Accession number**	**Phylogenetic group (division,subdivision**
A51, surface, March 08	*Micrococcus sp.* AKB-2008-HE95	100	AM988871	Actinobacteria
D27, medium, March 08	*Bacillus muralis*	100	FJ609713	Firmicutes
B23, medium, March 08	*Bacillus arsenicus*	97	AJ606700	Firmicutes
B10, medium, March 08	*Bacillus flexus*	99	EU869200	Firmicutes
D14, medium, March 08	*Brevundimonas kwangchunensis*	97	AY971369	Alphaproteobacteria
F27, deep, March 08	*Enterobacter aerogenes*	97	FJ811873	Gammaproteobacteria
F4, deep, March 08	*Bacillus sp.* BAM565	100	AB330414	Firmicutes
F8, deep, March 08	*Bacillus sp.* BAM565	100	AB330414	Firmicutes
A11, surface, March 08	*Bacillus sp.* A1	100	FJ535468	Firmicutes
C5, surface, March 08	*Bacillus weihenstephanensis*	100	FJ390462	Firmicutes
B67, medium, March 08	*Arthrobacter sp.* 1b-1	99	AY561524	Actinobacteria
A36, surface, March 08	*Actinomycetales bacterium* TLI002	99	EU699479	Actinobacteria
G23, surface, March 08	*Nocardia cummidelens*	91	EU593591	Actinobacteria
A7, surface, March 08	*Janthinobacterium sp.*	100	FJ006906	Betaproteobacteria
D35, medium, March 08	*Acetobacter pasteurianus*	100	FM179769	Alphaproteobacteria
G24, surface, March 08	*Bosea sp.* AKB-2008-KK9	99	AM988982	Alphaproteobacteria
J1, deep, March 08	*Sphingomonas sp.* Tan25	98	FJ459996	Alphaproteobacteria
F16, deep, March 08	*Xanthomonadaceae bacterium*	100	AB461065	Gammaproteobacteria
A50, surface, March 08	*Xanthomonas sp.* L60	98	DQ196469	Gammaproteobacteria
A40, surface, March 08	*Lysobacter sp.* GH41-7	99	DQ462462	Gammaproteobacteria
B68, medium, March 08	*Pseudomonas mandelii*	99	FM955880	Gammaproteobacteria
B32, medium, March 08	*Pseudomonas jessenii*	99	AM707022	Gammaproteobacteria
G12, surface, March 08	*Pseudomonas sp.* TAD001	100	FJ225153	Gammaproteobacteria

A15, surface, March 08	*Flavobacterium sp.* Tan16	98	FJ459992	Flavobacteria
B75, medium, March 08	*Pseudomonas plecoglossicida*	98	AY972231	Gammaproteobacteria
G4, surface, March 08	*Microbacterium sp.* RI48	98	DQ530139	Actinobacteria
G20, surface, March 08	*Arthrobacter sulfonivorans*	93	FM955888	Actinobacteria
G14, surface, March 08	*Bacillus bataviensis*	97	EU334358	Firmicutes
G3, surface, March 08	*Bacillus bataviensis*	100	EU334358	Firmicutes
F6, deep, March 08	*Bacillus boroniphilus*	94	EU620409	Firmicutes
A39, surface, March 08	*Bacillus sp.* GB02-14B	99	DQ078995	Firmicutes
C24, surface, March 08	*Bacillus sp.* CL1.120	99	AM934695	Firmicutes
D48, medium, March 08	*Kribbella karoonensis*	98	AY995146	Actinobacteria
D7, medium, March 08	beta proteobacterium	98	AY162061	Betaproteobacteria
D33, medium, March 08	*Bacillus silvestris*	99	AJ550464	Firmicutes
A24, surface, March 08	*Variovorax sp.* SRS16	99	AY621157	Betaproteobacteria
A48, surface, March 08	*Lysobacter niastensis*	99	DQ462462	Gammaproteobacteria
J26, deep, March 08	*Variovorax sp.* Is-BDOE5	96	EF435021	Betaproteobacteria
C1, surface, March 08	*Microbacterium sp.* Y19	98	FJ654469	Actinobacteria
B76, medium, March 08	*Pseudomonas gingeri*	98	EU196770	Gammaproteobacteria
G16, surface, March 08	*Agromyces sp.*	98	DQ497242	Actinobacteria
G17, surface, March 08	*Bacillus sp.* BC-1	100	FJ584315	Firmicutes
Da31, medium, July 08	*Micrococcus lylae*	99	AF057290	Actinobacteria
Aa10, surface, July 08	*Micromonospora coxensis*	99	FJ532383	Actinobacteria
Fa2, deep, July 08	*Saccharopolyspora sp*	92	EF104116	Actinobacteria
Da29, medium, July 08	*Streptomyces sp.* AR2	99	EF491601	Actinobacteria
Ba16, medium, July 08	*Kocuria sp.*	100	DQ107400	Actinobacteria
Da25, medium, July 08	*Streptomyces exfoliatus*	99	FJ532461	Actinobacteria
Fa10, deep, July 08	*Streptomyces rubrolavendulae*	96	FJ441643	Actinobacteria
Ha4, medium, July 08	*Streptomyces pseudovenezuelae*	99	FJ796459	Actinobacteria
Aa7, surface, July 08	*Bacillus licheniformis*	98	AM910586	Firmicutes
Ha3, medium, July 08	*Thermocrinis sp.* P2L2B	88	AJ320219	Aquificae
Aa20, surface, July 08	*Isoptericola dokdonensis*	96	DQ387860	Actinobacteria
X14, deep, July 08	*Paenibacillus sp.* N8-3	99	EF690420	Firmicutes
Ja3, deep, July 08	*Oceanobacillus profundus*	99	DQ386635	Firmicutes
X22, deep, July 08	*Paenibacillus polymyxa*	100	FJ468005	Firmicutes
Ca4, surface, July 08	*Paenibacillus pabuli*	99	FJ189794	Firmicutes

Da33, medium, July 08	*Paenibacillus sp.* EK-10	94	EU910230	Firmicutes
Ga36, surface, July 08	*Bacillus sp.* DBTMGS2	100	FJ842658	Firmicutes
Ja12, deep, July 08	*Bacillus pumilus*	100	EU647705	Firmicutes
Ba13, medium, July 08	*Bacillus licheniformis*	100	FJ808719	Firmicutes
Ba10, medium, July 08	*Paenibacillus sp.* B22a	100	EU558281	Firmicutes
X16, deep, July 08	*Paenibacillus anaericanus*	99	AM745262	Firmicutes
X26, deep, July 08	*Brevibacillus sp.*	100	AY372923	Firmicutes
Aa17, surface, July 08	*rhizosphere soil bacterium*	95	AJ252586	Unclassified bacteria
Ha18, medium, July 08	*Lysobacter sp.* KNUC361	98	EU239150	Gammaproteobacteria
Ba20, medium, July 08	*Lysobacter sp.* MH24	100	EU182852	Gammaproteobacteria
X3, deep, July 08	*Stenotrophomonas sp.* M445	100	AB461783	Gammaproteobacteria
Da14, medium, July 08	*Pseudomonas sp.* S15	100	FM163469	Gammaproteobacteria
Ha30, medium, July 08	*Pseudomonas plecoglossicida*	99	AY972231	Gammaproteobacteria
Ga2, surface, July 08	*Variovorax sp.* T71	99	FJ719347	Betaproteobacteria
Ga 24, surface, July 08	*Variovorax paradoxus*	99	AJ969086	Betaproteobacteria
Aa28, surface, July 08	actinobacterium CH12i	94	FJ164055	Actinobacteria
Ca8, surface, July 08	*Microbacterium sp. ORS 1418*	98	AJ968704	Actinobacteria
X25, deep, July 08	*Rhizobium leguminosarum*	100	AY505131	Alphaproteobacteria
Ja28, deep, July 08	*Microbacterium foliorum*	100	EU834263	Actinobacteria
X10, deep, July 08	*Microbacterium maritypicum*	100	AJ853910	Actinobacteria
Ca12, surface, July 08	*Ensifer adhaerens*	100	FJ609719	Alphaproteobacteria
Ca7, surface, July 08	*Bacillus pumilus*	100	EU647705	Firmicutes
Ga12, surface, July 08	*Arthrobacter sp.* SMCC G970	100	EU446197	Actinobacteria
Ha8, medium, July 08	*Actinomycetales bacterium*	94	EU699478	Actinobacteria
Ba14, medium, July 08	*Arthrobacter sp. AM55T*	99	AM983491	Actinobacteria
Aa26, surface, July 08	*Frigoribacterium sp.* OS-12A	100	EF612311	Actinobacteria
Ja17, deep, July 08	*Bacillus jeotgali*	100	FJ609706	Firmicutes
Aa22, surface, July 08	*Bacillus sp.* SeaH-As9s	98	FJ607361	Firmicutes
Da17, medium, July 08	*Bacillus sp.* BWDY-19	99	DQ314538	Firmicutes
X4, deep, July 08	*Bacillus senegalensis*	96	EF690434	Firmicutes
Aa9, surface, July 08	*Paenibacillus castaneae*	97	EU099594	Firmicutes
Ga1, surface, July 08	*Curtobacterium flaccumfaciens*	99	DQ512787	Actinobacteria
Ga14, surface, July 08	*Agromyces ramosus*	99	X77447	Actinobacteria
Ga3, surface, July 08	*Pseudomonas brassicacearum*	100	DQ377746	Gammaproteobacteria

Ga31, surface, July 08	*Streptomyces macrosporeus*	98	EF371436	Actinobacteria
Fa3, deep, July 08	*Bacillus sp.* K5T	100	AM983517	Firmicutes
Ba18, medium, July 08	*Microbacteriaceae bacterium*	99	EF540491	Actinobacteria
Ga29, surface, July 08	*Pseudomonas sp.* EC-V20-9	100	AB379687	Gammaproteobacteria
Ga19, surface, July 08	*Stenotrophomonas maltophilia*	100	EF620444	Gammaproteobacteria
Aa5, surface, July 08	*Microbacterium sp.* K6-01	98	EF612295	Actinobacteria
Ja26, deep, July 08	*Brevibacillus ginsengisoli*	95	AB245376	Firmicutes
Ha15, medium, July 08	*Agromyces bracchium*	97	AB023359	Actinobacteria
Ha12, medium, July 08	mercury-resistant bacterium	98	DQ401833	Unclassified bacteria
Ba3, medium, July 08	*Arthrobacter sp.* J3.37	99	DQ157997	Actinobacteria
Ja4, deep, July 08	*Paenibacillus sp.* FYD11	100	EU833937	Firmicutes
Fa14, deep, July 08	*Stenotrophomonas sp.* IK1_83	99	AB461056	Gammaproteobacteria
Ca3, surface, July 08	*Ensifer sp.* MH23	99	EU182851	Alphaproteobacteria
X5, deep, July 08	*Microbacterium sp.* ORS 1417	98	AJ968703	Actinobacteria
Fa16, deep, July 08	*Frigoribacterium sp.* GIC6	99	AY439262	Actinobacteria
Ga34, surface, July 08	*Agromyces sp.* VS2	96	AM039785	Actinobacteria
Jb33, deep,October 08 ex	*Paenibacillus castaneae*	98	EU099594	Firmicutes
Hb44, medium, October 08 ex	*Agromyces italicus*	99	AY618215	Actinobacteria
Hb4, medium, October 08 ex	*Agromyces cerinus*	99	AM410681	Actinobacteria
Jb17, deep, October 08 ex	*Agromyces allium*	100	DQ673874	Actinobacteria
Jb23, deep, October 08 ex	*Actinomycetales bacterium* Tpl	100	EU375385	Actinobacteria
Fb2, deep, October 08 ex	*Lysinibacillus sphaericus*	100	EU741101	Firmicutes
Hb5, medium, October 08 ex	*Bacillus sp.*	86	FJ601631	Firmicutes
Bb33, medium, October 08 ex	*Ochrobactrum anthropi*	92	FJ873801	Alphaproteobacteria
Hb42, medium, October 08 ex	*Bacillus sp.* CS8	100	FM202726	Firmicutes
Hb21, medium, October 08 ex	*Bacillus thuringiensis*	100	FJ772071	Firmicutes
Jb36, deep, October 08 ex	*Arthrobacter polychromogenes*	99	AB167181	Actinobacteria
Xb7 , deep, October 08 ex	*Bacillus sp.* JY01	99	EU798946	Firmicutes
Bb30, medium, October 08 ex	*Bacillus sp.* MHS037	100	DQ993294	Firmicutes
Hb1, medium, October 08 ex	*Arthrobacter humicola*	98	AB279890	Actinobacteria
Xb2, deep, October 08 ex	*Rhodococcus sp.* DM5	99	FJ447540	Actinobacteria
Hb34, medium, October 08 ex	*Arthrobacter scleromae*	100	FM955866	Actinobacteria
Bb29, medium, October 08 ex	*Arthrobacter sulfonivorans*	100	FM955860	Actinobacteria
Bb21, medium, October 08 ex	*Arthrobacter sp*	99	EF451631	Actinobacteria

Hb47, medium, October 08 ex	*Arthrobacter humicola*	100	AB279890	Actinobacteria
Xb17, deep, October 08 ex	*Arthrobacter nitroguajacolicus*	99	AJ512504	Actinobacteria
Xb33, deep, October 08 ex	*Streptacidiphilus sp.* SB-B35	98	DQ904528	Actinobacteria
Hb31, medium, October 08 ex	*Pantoea sp.*	98	EU216737	Gammaproteobacteria
Ab 12, surface, October 08 ex	*Variovorax soli*	98	DQ432053	Betaproteobacteria
Hb20, medium, October 08 ex	*Sphingobacterium sp.*	93	DQ530064	Sphingobacteria
Jb29, deep, October 08 ex	*Achromobacter denitrificans*	96	EU869274	Betaproteobacteria
Ab32, surface, October 08 ex	*Rhodovulum sp.* SMB1	97	DQ868668	Alphaproteobacteria
Bb6, medium, October 08 ex	*Rhodococcus sp.*	93	AY429711	Actinobacteria
Jb4a, deep, October 08 ex	*Mycobacterium sp.* WPCB170	100	FJ006915	Actinobacteria
Ab33, surface, October 08 ex	*Streptomyces tanashiensis*	99	EU841673	Actinobacteria
Bb26, medium, October 08 ex	*Bacillus altitudinis*	100	FM955870	Firmicutes
Db22, medium, October 08 ex	*Bacillus licheniformis*	100	FJ641027	Firmicutes
Xb6, deep, October 08 ex	*Microbacterium sp.*	98	FJ405359	Actinobacteria
Hb38, medium, October 08 ex	*Stenotrophomonas maltophilia*	99	EU931549	Gammaproteobacteria
Bb36, medium, October 08 ex	soil bacterium SI01	99	DQ518548	Unclassified bacteria
Jb31, deep, October 08 ex	*Sphingomonas sp.* BBCT69	99	DQ337553	Alphaproteobacteria
Jb26, deep, October 08 ex	*Sphingomonadaceae bacterium*	93	AB377219	Alphaproteobacteria
Gb6, surface, October 08 ex	*Lysobacter enzymogenes*	96	EU668316	Gammaproteobacteria
Db26,medium, October 08 ex	*soil bacterium* S65D1	98	AY039429	Unclassified bacteria
Fb46, deep, October 08 ex	*Agromyces fucosus*	97	AY158025	Actinobacteria
Cb33, surface, October 08 ex	*Agromyces allium*	98	DQ673874	Actinobacteria
Cb44, surface, October 08 ex	*Stenotrophomonas sp.* DCY38	98	EU873315	Actinobacteria
Hb28,medium, October 08 ex	*Xanthomonas retroflexus*	100	AM495257	Gammaproteobacteria
Jb30, deep, October 08 ex	*Xanthomonas sp.* BBCT38	98	EF471219	Gammaproteobacteria
Xb43, deep, October 08 ex	*Sinorhizobium sp.* T10	93	FJ687972	Alphaproteobacteria
Xb19, deep, October 08 ex	*Serratia marcescens*	97	FJ789679	Gammaproteobacteria
Jb41, deep, October 08 ex	*Pseudomonas chlororaphis*	100	EF620458	Gammaproteobacteria
Hb37,medium, October 08 ex	*Pseudomonas kilonensis*	100	AJ292426	Gammaproteobacteria
Jb34, deep, October 08 ex	*Microbacterium imperiale*	99	AF526906	Alphaproteobacteria
Fb11, deep, October 08 ex	*Pseudomonas sp.* S15	100	FM163469	Gammaproteobacteria
Gb45, surface, October 08 ex	bacterium TLCL5	100	EU086572	Unclassified bacteria
Ab36, surface, October 08 ex	*Pseudomonas plecoglossicida*	99	AY972231	Gammaproteobacteria
Fb6, deep, October 08 ex	*Variovorax sp.* SRS16	98	AY621157	Betaproteobacteria

Ab28, surface, October 08 ex	*Variovorax soli*	99	DQ432053	Betaproteobacteria
Cb6, surface, October 08 ex	*Microbacterium testaceum*	97	EF602568	Actinobacteria
Bb9, medium, October 08 ex	*Microbacterium natoriense*	98	AY566291	Actinobacteria
Db37,medium, October 08 ex	*Microbacterium sp.* ASD	100	AY040877	Actinobacteria
Hb6, medium, October 08 ex	*Microbacterium foliorum*	100	EU834263	Actinobacteria
Jb13, deep, October 08 ex	*Microbacterium arborescens*	99	AM711565	Actinobacteria
Cb17, surface, October 08 ex	*Microbacterium sp.* M412	98	AB461754	Actinobacteria
Hb7, medium, October 08 ex	*Microbacterium resistens*	99	AY277553	Actinobacteria
Jb11, deep, October 08 ex	*Microbacterium sp.* CQ0110Y	100	DQ852355	Actinobacteria
Hb30,medium, October 08 ex	*Microbacterium sp.* d9829	99	FJ595885	Actinobacteria
Fb7, deep, October 08 ex	*Microbacterium sp.* 46	98	AF388031	Actinobacteria
Cb8, surface, October 08 ex	*Sphingobacteriaceae bacterium*	99	EU723088	Sphingobacteria
Cb34, surface, October 08 ex	*Sphingobacterium siyangense*	99	EU046272	Sphingobacteria
Cb13, surface, October 08 ex	*Pantoea agglomerans*	83	FM202485	Gammaproteobacteria
Cb27, surface, October 08 ex	*Enterobacteriaceae bacterium*	99	AB461749	Gammaproteobacteria
Xb10, deep, October 08 ex	*Terribacillus saccharophilus*	99	AB243847	Firmicutes
Cb9, surface, October 08 ex	*Cellulosimicrobium cellulans*	99	DQ359937	Actinobacteria
Jb4b, deep, October 08 ex	*Isoptericola variabilis*	100	FJ502236	Actinobacteria
Cb19, surface, October 08 ex	*Nocardioides panaciterrae*	99	AB257719	Actinobacteria
Jb16, deep, October 08 ex	*Curtobacterium flaccumfaciens*	100	DQ512787	Actinobacteria
Bb2, medium, October 08 ex	*Brevibacillus laterosporus*	99	EU159585	Firmicutes
Hb46,medium, October 08 ex	*Bacillus sp.* SH5	100	EU374137	Firmicutes
Bb28,medium, October 08 ex	*Bacillus sp.* SH60	100	EU374154	Firmicutes
Db8, medium, October 08 ex	*Paenibacillus sp.* L32	99	DQ196465	Firmicutes
Hb36,medium, October 08 ex	*Bacillus niacini*	100	EU221360	Firmicutes
Hb13,medium, October 08 ex	*Bacillus sp.* JSM 081037	99	FJ527422	Firmicutes
Ab21, surface, October 08 ex	*Achromobacter sp.* AO22	99	EU696789	Betaproteobacteria
Fb8, deep, October 08 ex	actinobacterium '#33 white'	96	AF423074	Actinobacteria
Gb20, surface, October 08 ex	*Agromyces sp.* SC19T	99	AM983476	Actinobacteria
Hb40,medium, October 08 ex	*Elizabethkingia miricola*	98	FJ938215	Flavobacteria
Ab30, surface, October 08 ex	*Chryseobacterium indologenes*	97	AY050493	Flavobacteria
Gb22, surface, October 08 ex	*Flavobacterium sp.* AKB-2008-	96	AM988915	Flavobacteria
Gb25, surface, October 08 ex	*Flavobacterium johnsoniae*	98	DQ256490	Flavobacteria
Ab42, surface, October 08 ex	*Agromyces allii*	99	DQ673874	Actinobacteria

Hb1, medium, October 08 ex	*Arthrobacter sp.* S21011	100	D84563	Actinobacteria
Gb55, surface, October 08 ex	*Variovorax paradoxus*	99	AY512828	Betaproteobacteria
Ab40, surface, October 08 ex	*Lysobacter niastensis*	97	DQ462462	Gammaproteobacteria
Ab1, surface, October 08 ex	*Microbacterium oleivorans*	98	EU164543	Actinobacteria
Fb31, deep, October 08 ex	*Microbacterium sp.* AGL 12	100	EU118778	Actinobacteria
Fb24, deep, October 08 ex	*Chryseobacterium sp.* CI27	99	DQ530090	Flavobacteria
Hb26,medium, October 08 ex	*Microbacterium sp.* SMT-5	99	AM689980	Actinobacteria
Xb41, deep, October 08 ex	*Pantoea sp.* GJT-8	87	FJ426593	Gammaproteobacteria
Hb39,medium, October 08 ex	*Lysobacter sp.* G6	98	AB429529	Gammaproteobacteria
Db27,medium, October 08 ex	*Agromyces sp.* VS2	98	AM039785	Actinobacteria
1F3, deep, April 09 in	*Arthrobacter sp.* SMCC G992	100	EU446218	Actinobacteria
1C1, surface, April 09 in	*Isoptericola variabilis*	99	AB167235	Actinobacteria
1J7b, deep, April 09 in	actinobacterium kmd_043	99	EU723094	Actinobacteria
1D33, medium, april 09 in	*Aerococcus sp.* LV65.5:W1	100	AF076639	Firmicutes
1C15, surface, April 09 in	*Bosea sp.* AKB-2008-KK9	98	AM988982	Alphaproteobacteria
1G14, surface, April 09 in	*Bosea sp.* AKB-2008-KK10	99	AM988983	Alphaproteobacteria
1X4, deep, April 09 in	*Bacillus arbutinivorans*	93	AB508884	Firmicutes
1F2, deep, April 09 in	*Frigoribacterium sp.* VC	100	EU734598	Actinobacteria
1X14, deep, April 09 in	*Paenibacillus telluris*	99	AM745265	Firmicutes
1X20, deep, April 09 in	*Arthrobacter sp.* SK1.18	100	AY436810	Actinobacteria
1C7, surface, April 09 in	*Bacillus sp.* PL-26	98	AF326369	Firmicutes
1A16, surface, April 09 in	*Variovorax sp.* MG56	100	AY621157	Betaproteobacteria
1D24, medium, April 09 in	*Paenibacillus panacisoli*	99	AB245385	Firmicutes
1J17, deep, April 09 in	*Bacillus benzoevorans*	100	EU833938	Firmicutes
1X13, deep, April 09 in	*Bacillus sp.* MM4	100	FJ228147	Firmicutes
1X17, deep, April 09 in	*Agromyces cerinus*	100	AY277619	Actinobacteria
1X12, deep, April 09 in	*Kitasatospora kepongensis*	98	AY858888	Actinobacteria
2A3, surface, April 09 ex	*Arthrobacter sp.* SMCC G992	100	EU446218	Actinobacteria
2A10, surface, April 09 ex	*Arthrobacter sp.* S11	99	EU747692	Actinobacteria
2C15, surface, April 09 ex	*Arthrobacter sp.* SMCC G992	99	EU446218	Actinobacteria
2H2, medium, April 09 ex	*Arthrobacter sp.* SMCC G970	99	EU446197	Actinobacteria
2H19, medium, April 09 ex	*Arthrobacter sp.* SMCC G970	100	EU446218	Actinobacteria
2B3, medium, April 09 ex	*Cellulosimicrobium sp.*	99	EU438938	Actinobacteria
2D1, medium, April 09 ex	*Streptomyces sp.* SU1-1/28	99	AM397444	Actinobacteria

2G7, surface, April 09 ex	*Chryseobacterium sp.* AKB-2008	99	AM988902	Flavobacteria
2G20, surface, April 09 ex	*Chryseobacterium taichungense*	98	AJ843132	Flavobacteria
2D24, medium, April 09 ex	*Chryseobacterium taichungense*	99	AJ843132	Flavobacteria
2D2, medium, April 09 ex	*Bacillus sp.* T83	100	FJ719320	Firmicutes
2H9, medium, April 09 ex	*Staphylococcus sp.* AKB-2008	100	AM988975	Firmicutes
2H20a, medium, April 09 ex	*Leifsonia kafniensis*	99	AM889135	Actinobacteria
2D11, medium, April 09 ex	*Paenibacillus pabuli*	99	DQ288948	Firmicutes
2A6, surface, April 09 ex	*Variovorax soli*	99	DQ432053	Betaproteobacteria
2D5, medium, April 09 ex	*Paenibacillus sp.* Gi-662	95	DQ462448	Firmicutes
2A16, surface, April 09 ex	*Microbacterium sp.* JDM308	98	EU260088	Actinobacteria
2C8, surface, April 09 ex	*Pseudomonas sp.* SY7	99	EU073118	Gammaproteobacteria

Table I.2. Analysis of the bacterial isolates on the basis of partial 16S rDNA sequences, including the closest relatives as identified by BLAST program in the GenBank database (**in:** Internal, **ex:** External)

4.3.5. Phylogenetic analysis of culturable bacteria

Fig 4.13, 4.14 and 4.15 show the dendrograms of the phylogenetic analysis of internal soil samples of March and July 08 in surface, medium and deep soil, respectively. The dendrograms indicate that some groups of species seem to be typical for a specific season and soil layer. For example, in the surface soil layer some group of species were found only on March and some only in July, while some groups were common in both March and July (Fig 4.13). Similar results were obtained with the soil coming from the medium depth (Fig 4.14) and deep soil (Fig 4.15). It is interesting to note that in the deeper soil the common groups are no longer present.

Taking into account a comparison between internal and external soils, the phylogenetic analysis also indicates that some groups of species seem to be representative for a specific site. For instance, some groups were found only in the surface internal soil while some ones only in the surface external soil and some groups resulted as common in both internal and external surface soil (Fig 4.16 and Fig 4.19). Similar results were obtained with the soil coming from the medium depth (Fig 4.17 and Fig 4.20) and deep soil (Fig 4.18 and Fig 4.21).

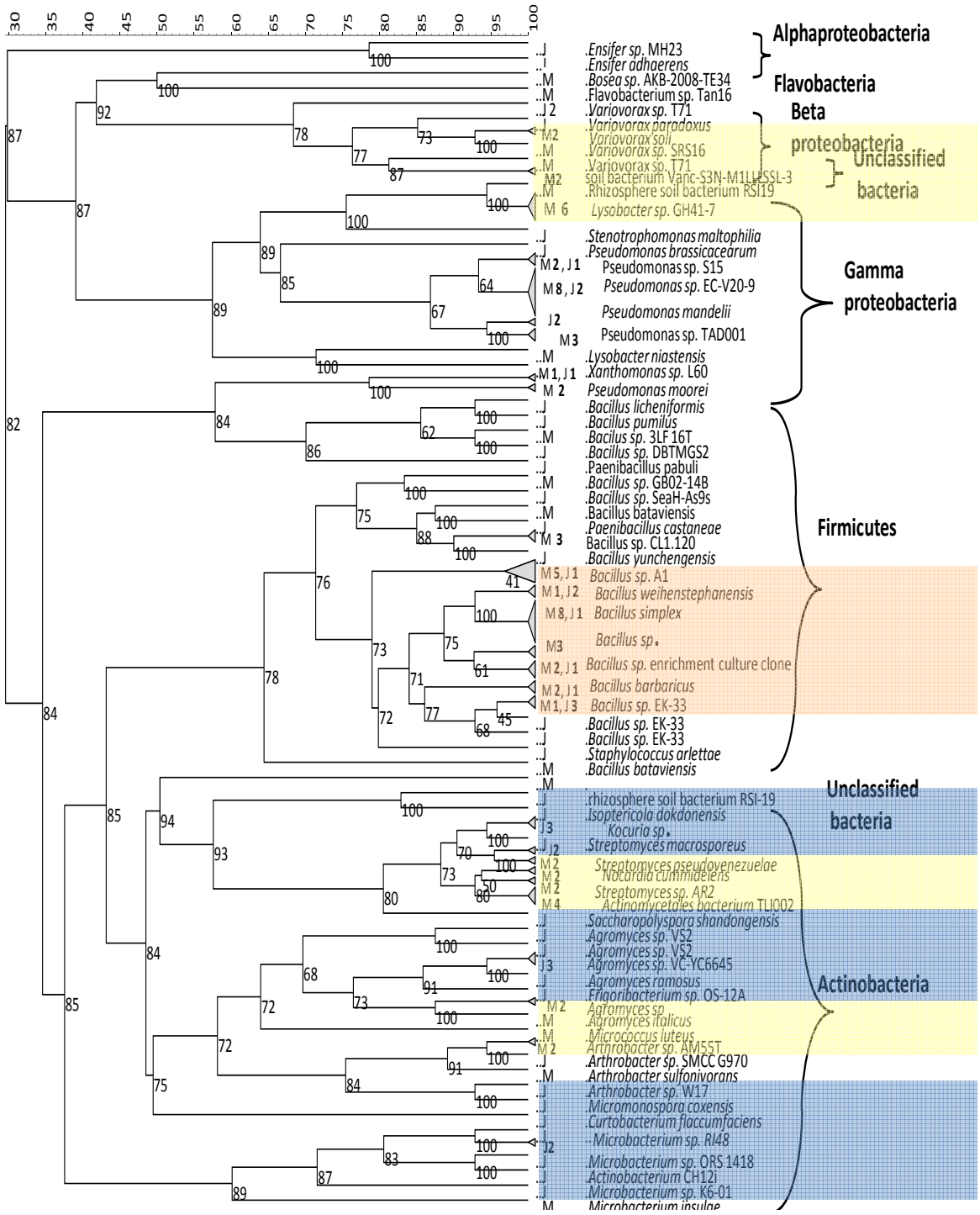


Fig 4.13. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from surface soil levels of March and July. Sequences marked M are those from March, and those marked J are from July. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow, blue and pink colors indicate the groups recovered in March, in July and in both the seasons, respectively.

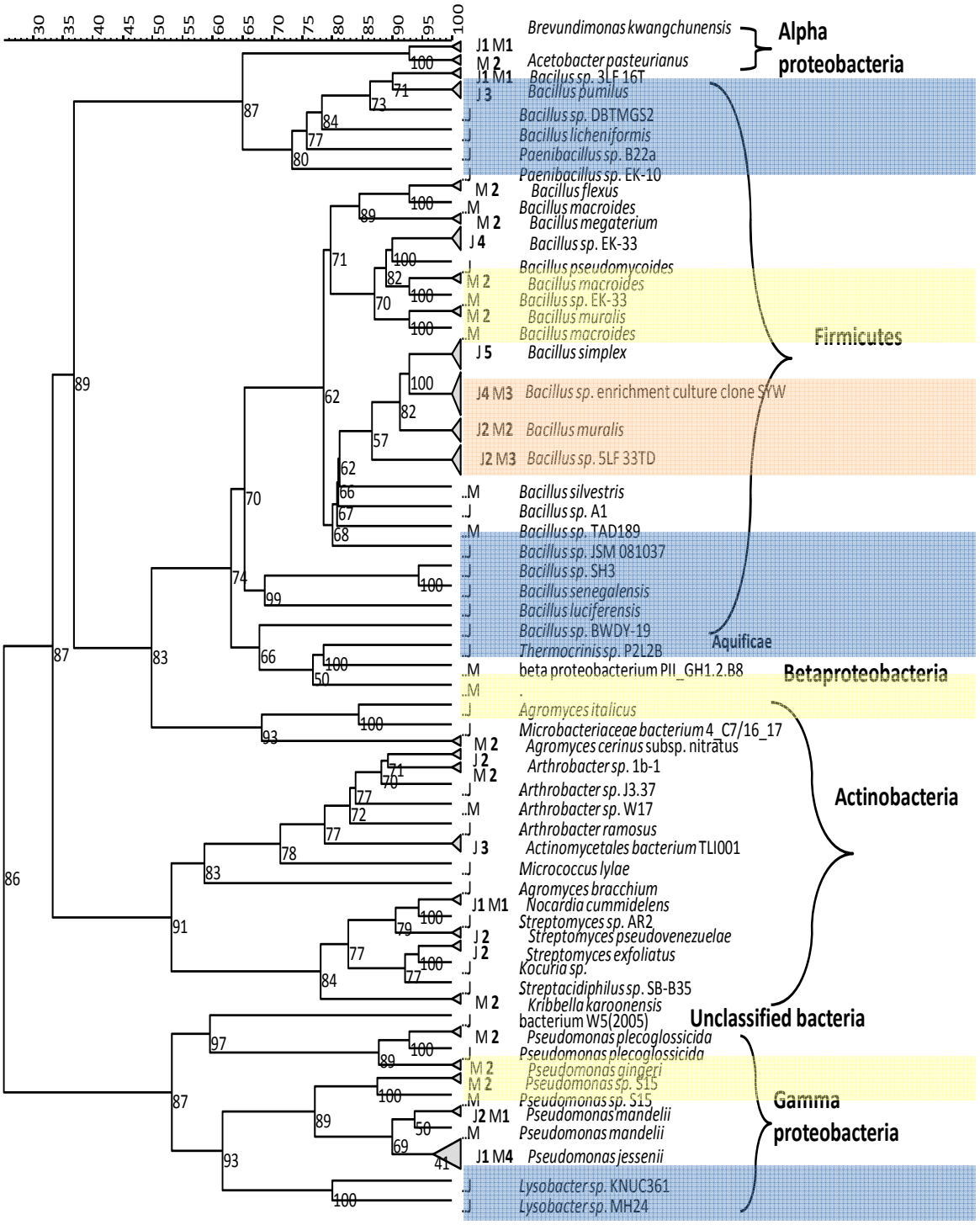


Fig 4.14. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from medium soil levels of March and July. Sequences marked M are those from March, and those marked J are from July. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow, blue and pink colors indicate the groups recovered in March, in July and in both the seasons, respectively.

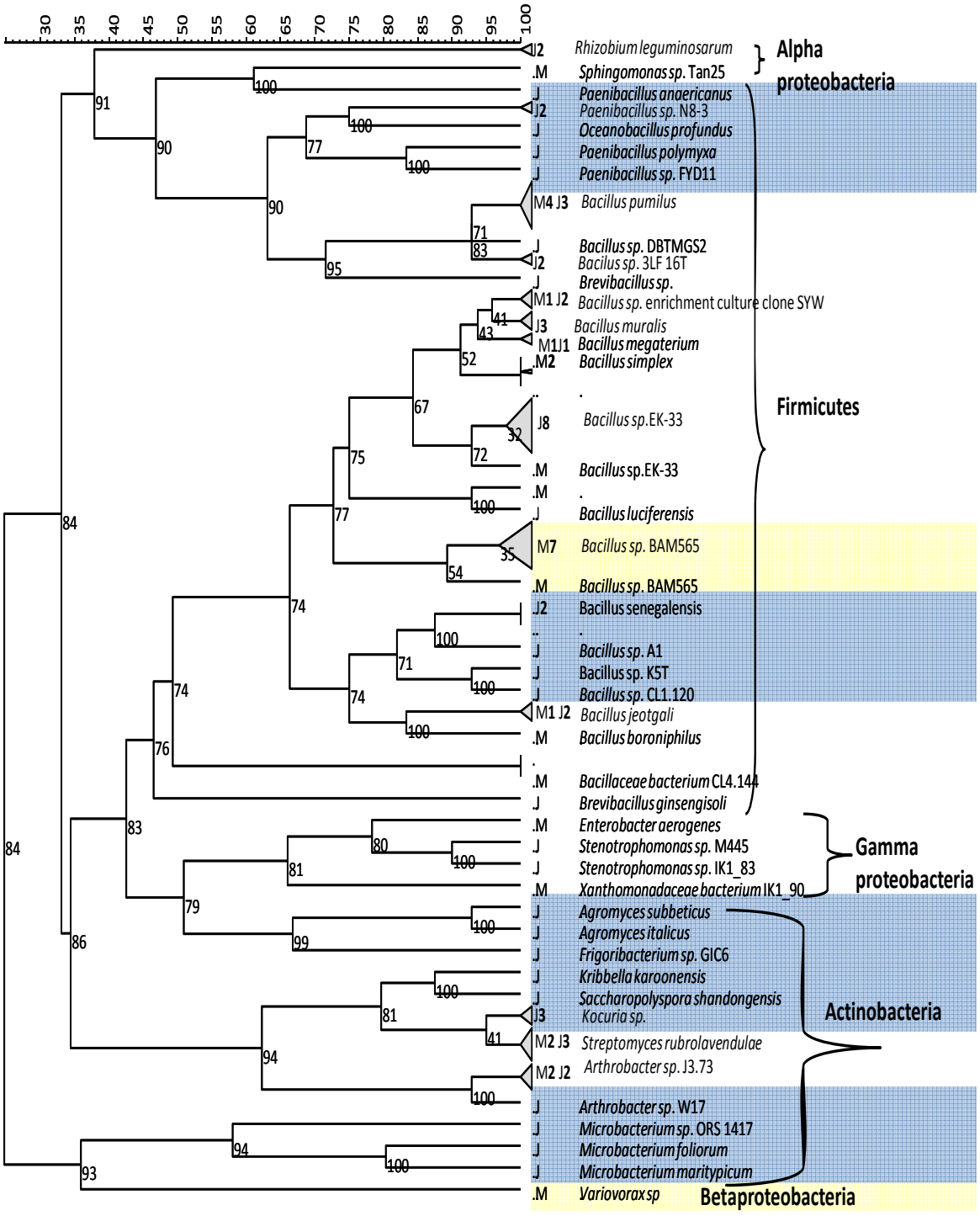


Fig 4.15. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from deep soil levels of March and July. Sequences marked M are those from March, and those marked J are from July. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate the groups recovered in March and in July, respectively.

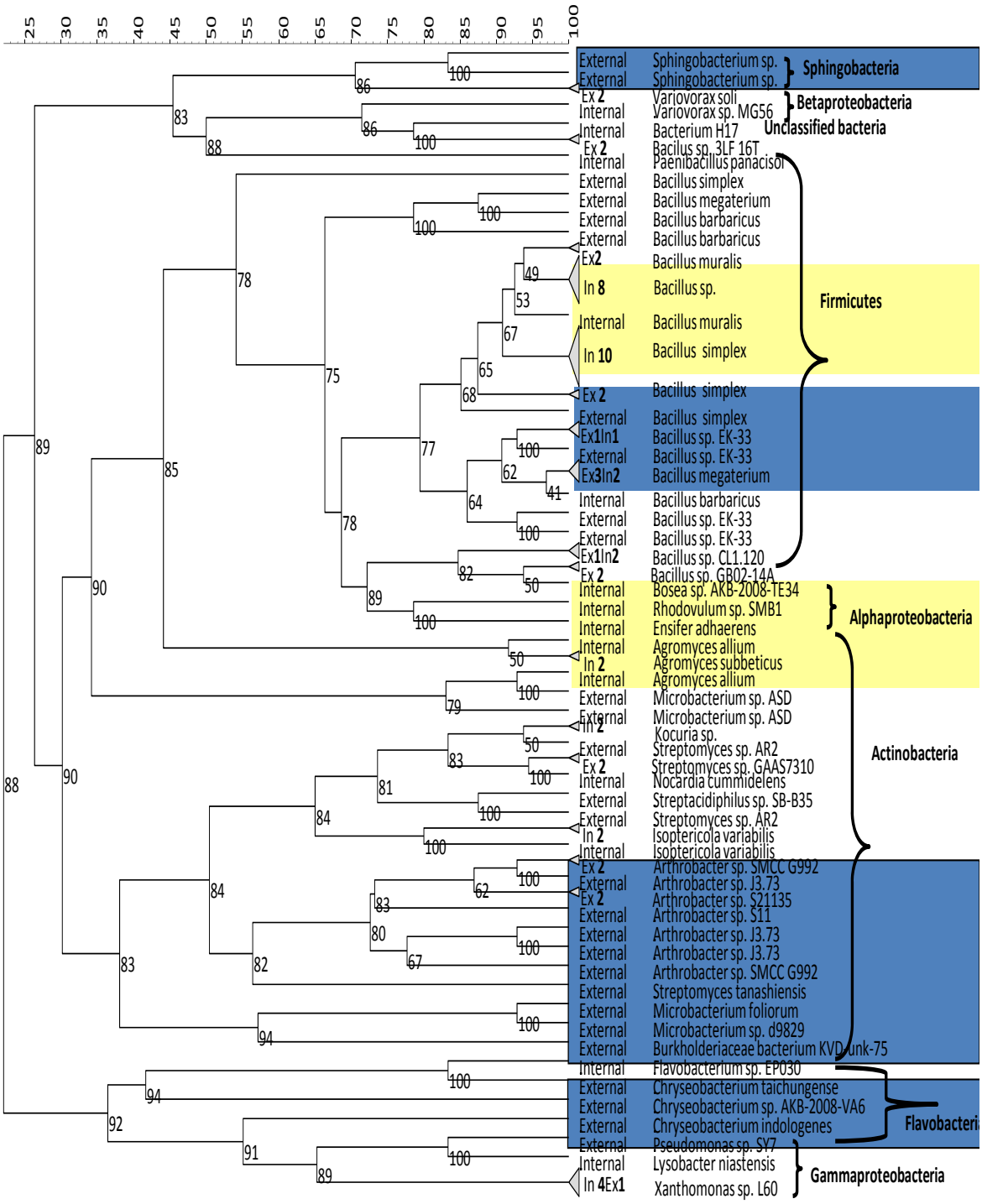


Fig 4.16. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external surface soil of April 09. Sequences marked **In** are from internal and those marked **Ex** from external soil. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

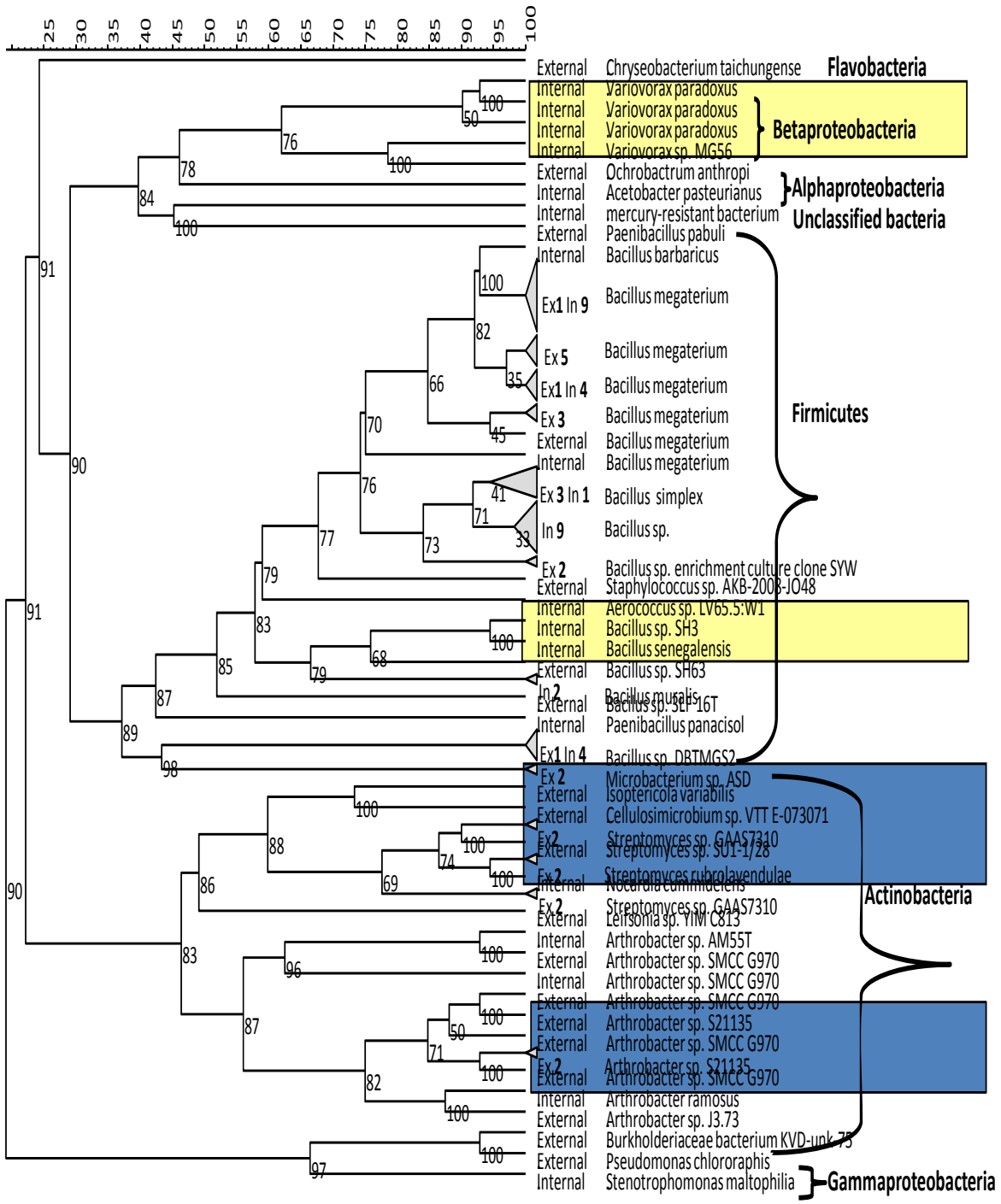


Fig 4.17. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external medium depth soil of April 09. Sequences marked **In** are those from internal and those marked **Ex** from external. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

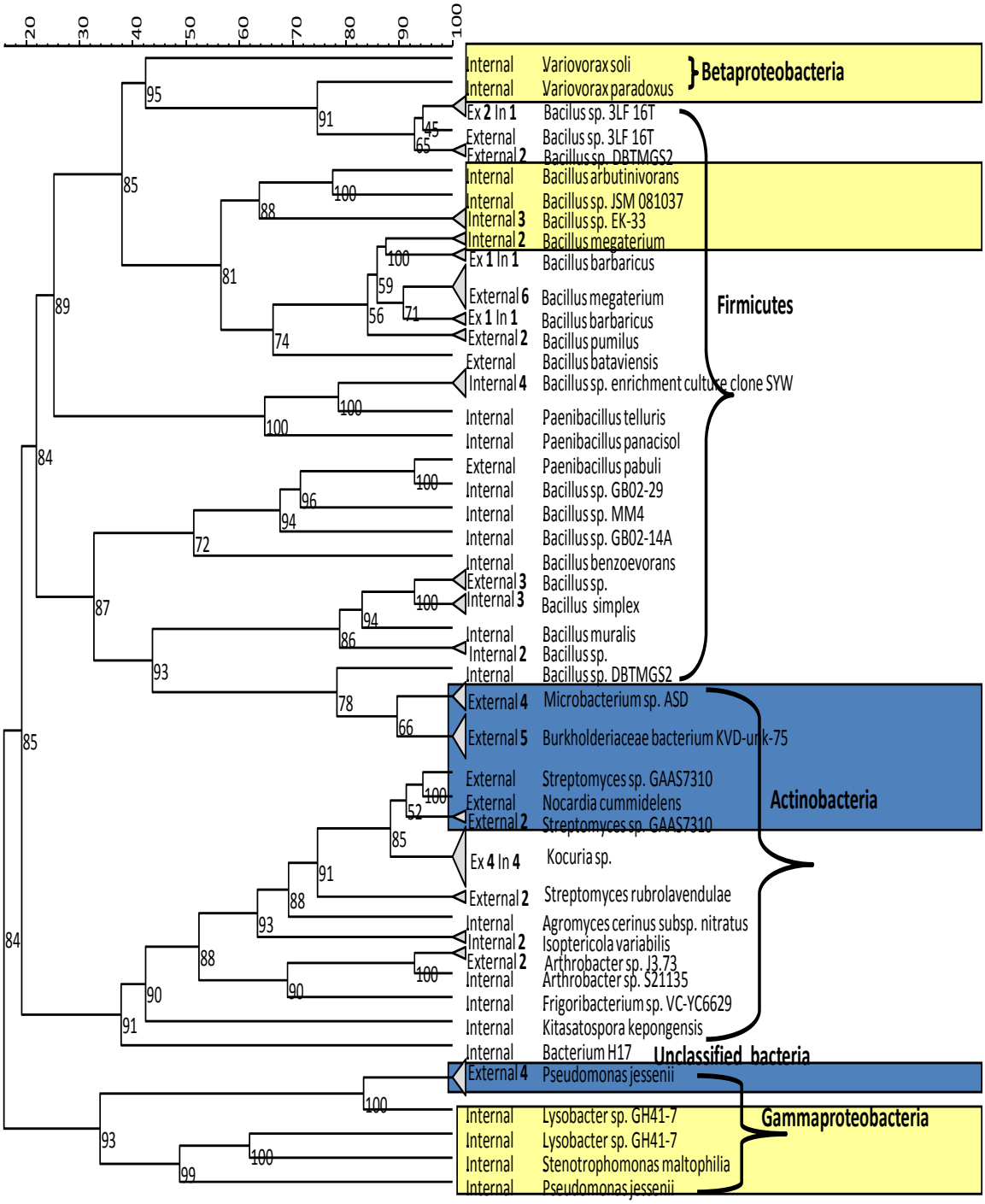


Fig 4.18. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external deep soil of April 09. Sequences marked **In** are those from internal soil and those marked **Ex** are from the external. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

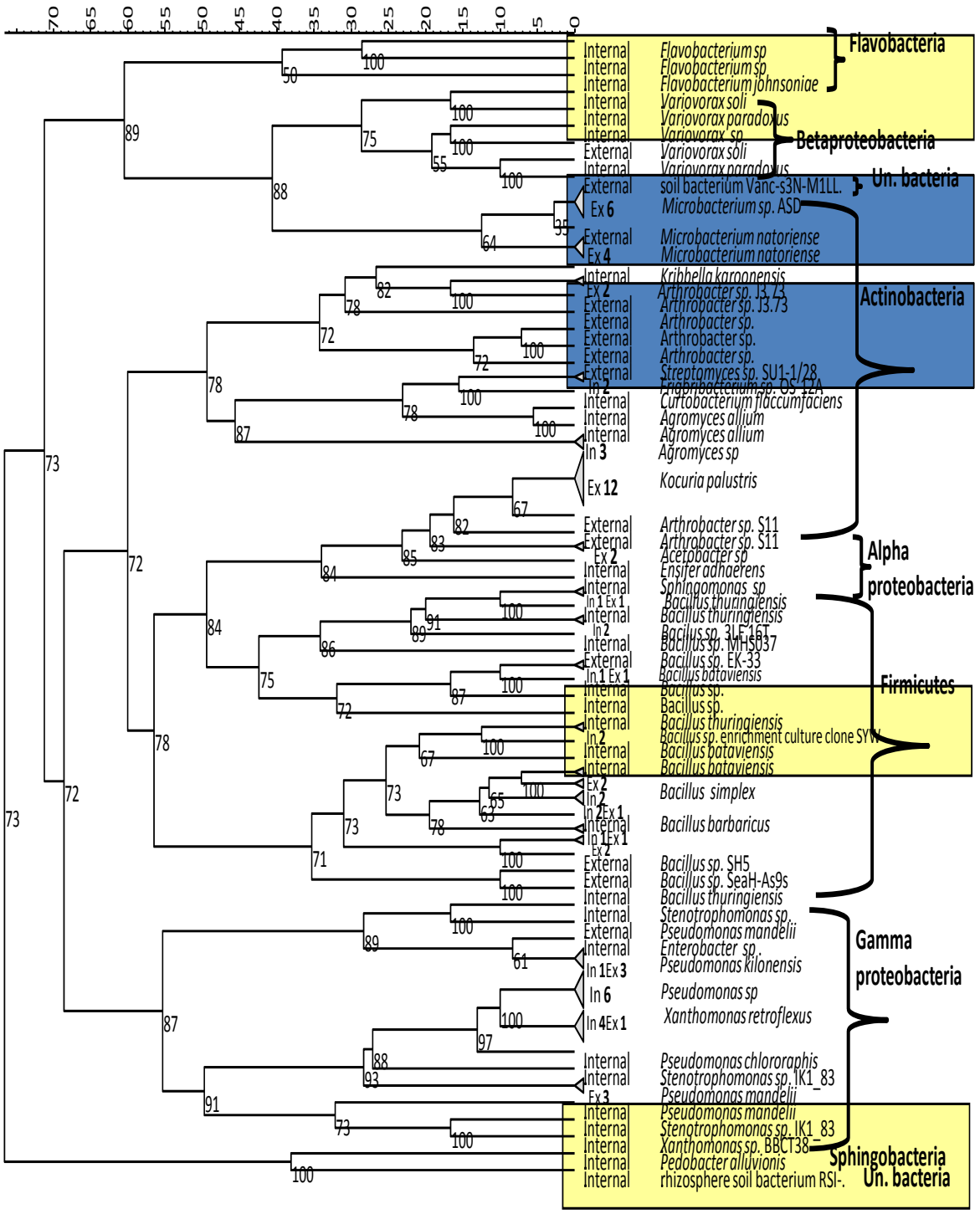


Fig 4.19. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external surface soil of October 09. Sequences marked **In** are those from internal and those marked **Ex** are from external soil. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

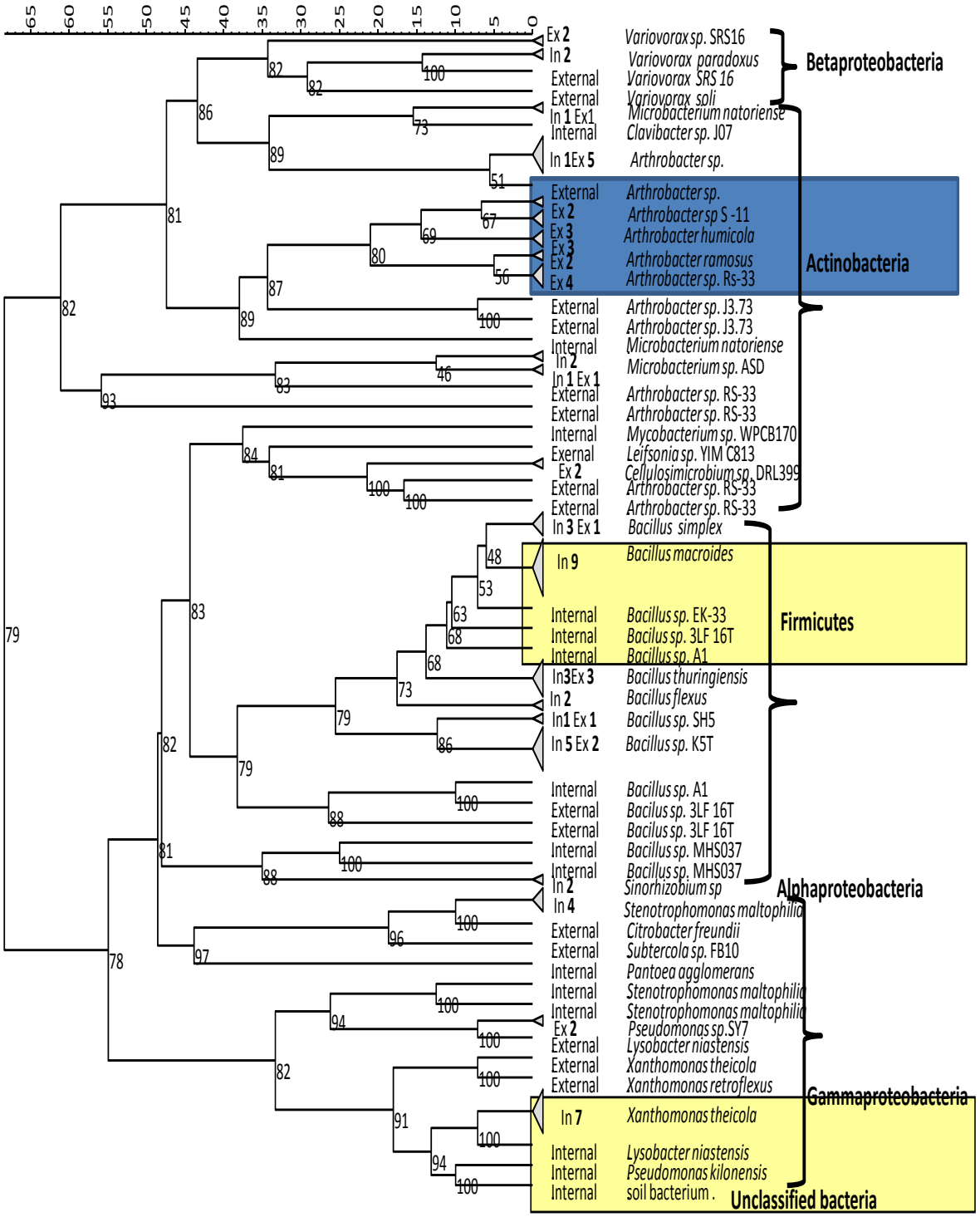


Fig 4.20. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external medium depth soil of October 09. Sequences marked **In** are those from internal and those marked **Ex** are from external soil. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

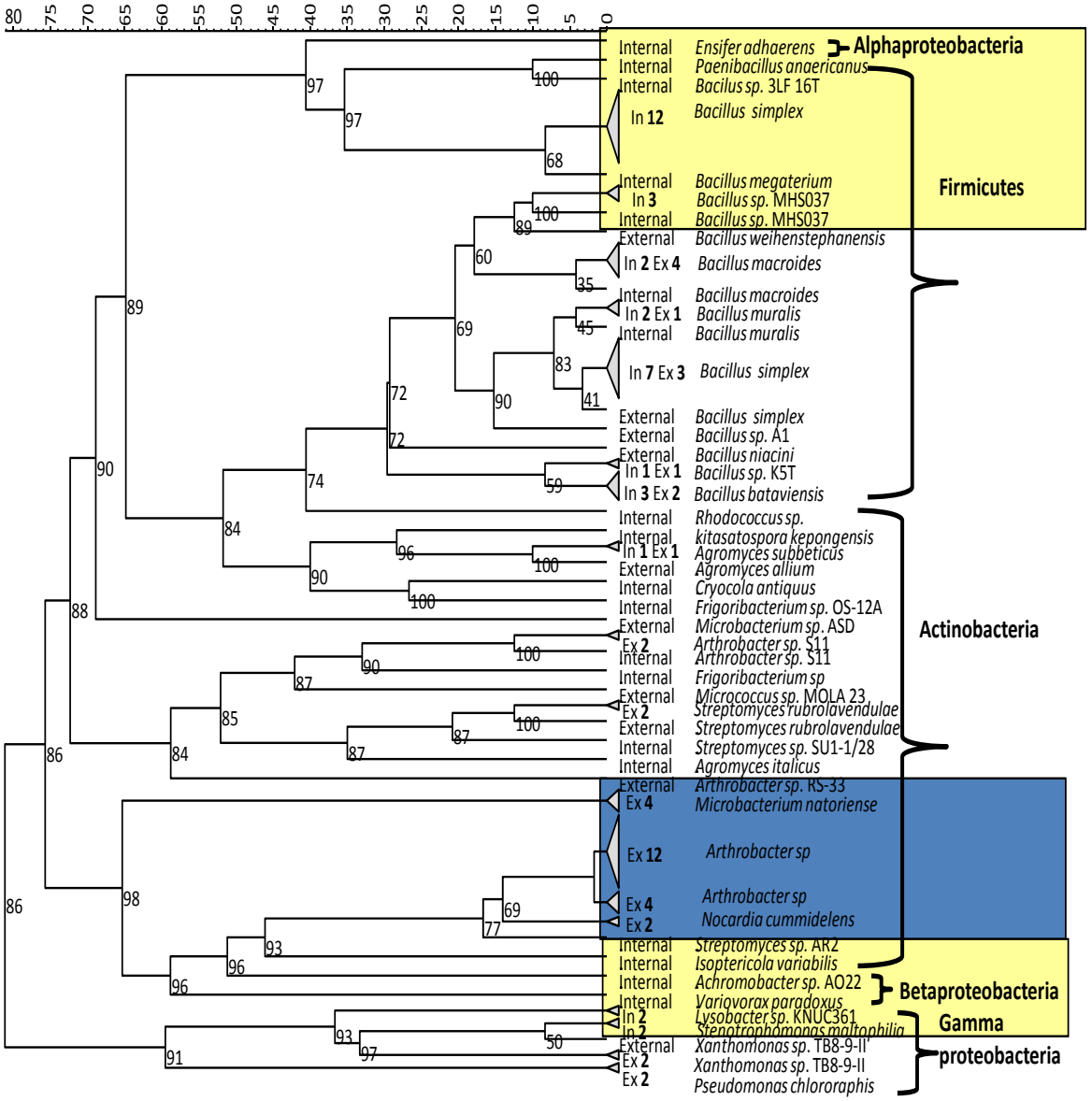


Fig 4.21. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of culturable bacteria from internal and external deep soil of October 09. Sequences marked **In** are those from internal and those marked **Ex** are from external soil. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of isolates the sequences are coming from. Yellow and blue colors indicate some groups recovered in the internal and external soils, respectively.

4.3.6. Phylotypes distribution of culturable communities

The Phylotypes distributions of the March and July 2008 soil bacterial communities for the internal samples collected at different depths are shown in Table 4.3.

From surface soil samples the 16S rDNA Phylotypes were affiliated with seven major bacterial divisions, namely Firmicutes, Gammaproteobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Flavobacteria and Unclassified bacteria (Table 4. 3). The Flavobacteria division (2.6% OTUs and 1.3% colonies) was found only in March.

Bacteria isolated from medium depth soil were also affiliated with seven major bacterial lineages, namely Firmicutes, Gammaproteobacteria, Actinobacteria, Alphaproteobacteria, Flavobacteria, Aquificae and Unclassified bacteria (Table 4.3). Firmicutes division presented high percentage of OTUs (45.8% and 43.2%) and high percentage of colonies (47.8% and 52.5%) in March and July 08 medium depth soil, respectively. The Flavobacteria division (2.6% OTUs and 1.3% colonies) and Aquificae division (2.3% OTUs and 1.6% colonies) were found only in March and July 08.

The isolates from deep soil were affiliated with five major bacterial lineages, namely Firmicutes, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Actinobacteria (Table 4.3). Firmicutes were the most dominant division (58.8% and 61.0% OTUs, 71.4% and 63.8% colonies) in March and July 2008, respectively. Flavobacteria and Aquificae were absent in deep soil.

Some interesting data are referred to the most representative groups. Particularly, it clearly results that while the number of OTUs are increasing with the depth for the Firmicutes, such a number is decreasing for the Actinobacteria group. It seems to happen both in March and July 2008 within the riparian buffer soil (Tab 4.3). This finding may be considered as a microbiological picture of the riparian soil, here always mentioned as “internal soil”, exposed to the artificial pressure deriving from the forced hydrology of the site and from the vegetation there introduced.

Indeed, the Firmicutes are generally gram positive, spore forming bacteria often able to adapt to extreme conditions and sometimes capable of carry on photosynthesis. These characteristics make this bacterial division more able to colonize deep soil where organic carbon, nitrogen and oxygen are less available. On the other hand, Actinobacteria that are

much more involved in the organic carbon utilization, such as cellulose and lignin degradation, results as more distributed toward the soil surface.

		Surface		Medium		Deep	
Sampling	**Divisions**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**
March 08 Internal	Firmicutes	34.2	37.3	45.8	47.8	58.8	71.4
	Actinobacteria	29.0	22.7	20.8	17.4	17.7	14.3
	Alphaproteobacteria	2.6	1.3	8.3	6.5	5.9	3.6
	Gammaproteobacteria	21.1	30.7	16.7	21.7	11.8	7.1
	Betaproteobacteria	5.3	4.0	4.2	2.2	5.9	3.6
	Flavobacteria	2.6	1.3	0	0	0	0
	Unclassified bacteria	5.3	2.7	4.2	4.4	0	0
July 08 Internal	Firmicutes	31.3	32.8	43.2	52.5	61.0	63.8
	Actinobacteria	41.7	41.4	34.1	31.2	29.3	27.6
	Alphaproteobacteria	4.2	3.5	2.8	1.6	2.4	3.5
	Gammaproteobacteria	12.5	13.8	13.6	9.8	4.9	3.5
	Betaproteobacteria	6.3	5.2	0	0	0	0
	Flavobacteria	0	0	0	0	0	0
	Unclassified bacteria	4.2	3.5	4.6	3.2	2.4	1.7
	Aquificae	0	0	2.2	1.6	0	0

Table 4.3. Phylotypes distributions of culturable communities of March and July 2008 for the Internal soil.

Considering that the vegetation in the riparian zone has been introduced in the recent years, the contribution of the growing plants to the soil organic carbon budget is increasing every year. This may account for the results shown in Tab 4.5 and 4.6 showing that the trend of the Actinobacteria biodiversity seems to change gradually. The distribution of bacteria belonging to this division, although remaining abundant in the surface soil, seems to extend to the medium depth and deep soil in 2009. This behavior may be ascribed to the increasing development of the plant roots that can extend to deeper soil providing there organic carbon exudates to the bacteria, and in some extent to the increasing organic matter input due to the green fraction decay of the growing plants. As a consequence, the Firmicutes could have reduced chances to colonize these environments and their high biodiversity observed in 2008 and 2009 in the deeper soil could tend to be reduced in the future.

		Surface		Medium		Deep	
Sampling	**Divisions**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**
October 08 External	Firmicutes	9.4	14.4	31.9	39.4	18.2	18.5
	Actinobacteria	50.9	54.8	46.8	46.1	50.9	55.6
	Alphaproteobacteria	1.9	1.0	4.35	2.6	7.3	6.2
	Gammaproteobacteria	9.4	6.7	12.8	9.2	16.3	14.8
	Betaproteobacteria	11.3	12.5	4.4	2.6	7.3	4.9
	Flavobacteria	5.7	3.85	0	0	0	0
	Unclassified bacteria	5.7	2.85	0	0	0	0
	Sphingobacteria	5.7	3.85	0	0	0	0

Table 4.4. Phylotypes distributions of culturable communities of October 2008 for the External soil.

By comparing these data with those referred to an external soil, the above considerations seem to assume more and more consistency. Indeed, the data reported in Tab 4.3, 4.4, 4.5 and 4.6 indicate that Actinobacteria are much more present in terms of biodiversity in all the soil layers, while Firmicutes experienced a clear decrease as compared to the internal soil data. The reason of that may be found on the history of the soil itself: the external soil adopted for this study has been periodically subjected to plough, thus mixing the soil layers.

		Surface		Medium		Deep	
Sampling	**Divisions**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**	**% OTUS**	**% colonies**
April 09 Internal	Firmicutes	35.7	54.2	52.0	76.1	52.0	62.2
	Actinobacteria	32.1	23.0	28.0	10.9	28.0	22.2
	Alphaproteobacteria	10.7	6.3	4.0	2.2	0	0
	Gammaproteobacteria	10.7	10.4	0	0	12.0	8.9
	Betaproteobacteria	7.1	4.2	12.0	8.7	4.0	2.2
	Flavobacteria	3.5	2.1	0	0	0	0
	Unclassified bacteria	0	0	4	2.2	4.0	4.4
	Sphingobacteria	0	0	0	0	0	0
April 09 External	Firmicutes	31.0	40.8	37.5	47.8	50.0	42.6
	Actinobacteria	44.8	40.8	50.0	45.7	45.0	48.9
	Alphaproteobacteria	0	0	4.2	2.2	0	0
	Gammaproteobacteria	6.9	4.1	4.2	2.2	5	8.5
	Betaproteobacteria	3.5	4.1	0	0	0	0
	Flavobacteria	6.9	6.1	4.2	2.2	0	0
	Unclassified bacteria	0	0	0	0	0	0
	Sphingobacteria	7.0	4.1	0	0	0	0

Table 4.5. Phylotypes distributions of culturable communities of April 2009 for Internal and External soils.

Sampling	Divisions	Surface		Medium		Deep	
		% OTUS	% colonies	% OTUS	% colonies	% OTUS	% colonies
October 09 Internal	Firmicutes	34.1	30.4	42.4	51.7	46.7	64.8
	Actinobacteria	17.1	17.9	18.2	12.1	36.7	22.2
	Alphaproteobacteria	4.9	3.6	3.0	3.5	3.3	1.9
	Gammaproteobacteria	22.0	32.1	30.3	27.6	6.7	7.4
	Betaproteobacteria	9.8	7.1	3.0	3.5	6.7	3.7
	Flavobacteria	7.3	5.4	0	0	0	0
	Unclassified bacteria	2.4	1.8	0	0	0	0
	Sphingobacteria	2.4	1.8	3.0	1.7	0	0
October 09 External	Firmicutes	26.5	19.3	12.9	13.7	33.3	28.9
	Actinobacteria	52.9	59.7	54.8	64.7	50.0	59.6
	Alphaproteobacteria	2.9	3.5	0	0	0	0
	Gammaproteobacteria	11.8	14.0	22.6	13.7	16.7	11.5
	Betaproteobacteria	2.9	1.8	9.7	7.8	0	0
	Flavobacteria	0	0	0	0	0	0
	Unclassified bacteria	0	0	0	0	0	0
	Sphingobacteria	2.9	1.8	0	0	0	0

Table 4.6. Phylotypes distributions of culturable community of October 2009 for Internal and External soils.

4.3.7. Principal component analysis (PCA) of culturable isolates

All the above data were analyzed by PCA, studying the relationships among microbial culturable communities.

Phylotypes distributions of March and July 08 internal soil are shown in Fig 4.22**.**

A distinct difference in microbial community composition was observed in relation to seasons and different soil levels. In fact, the microbial communities of March and July 08 surface soil were significantly different from those recovered in the medium and deep soil.

The relationships among microbial communities distribution of April 2009, internal and external soils, are shown in Fig 4.23A. The samples from April 2009, internal soil, were well separated from the samples of April 2009, external soil, at all the soil depths. These results clearly confirm the considerations made above that the overall community structure of the riparian zone experienced conditions significantly affecting its composition and biodiversity.

Similar results were obtained when the relationship among microbial communities where carried out for the data related to October 2009, internal and external soils (Fig 4.23B).

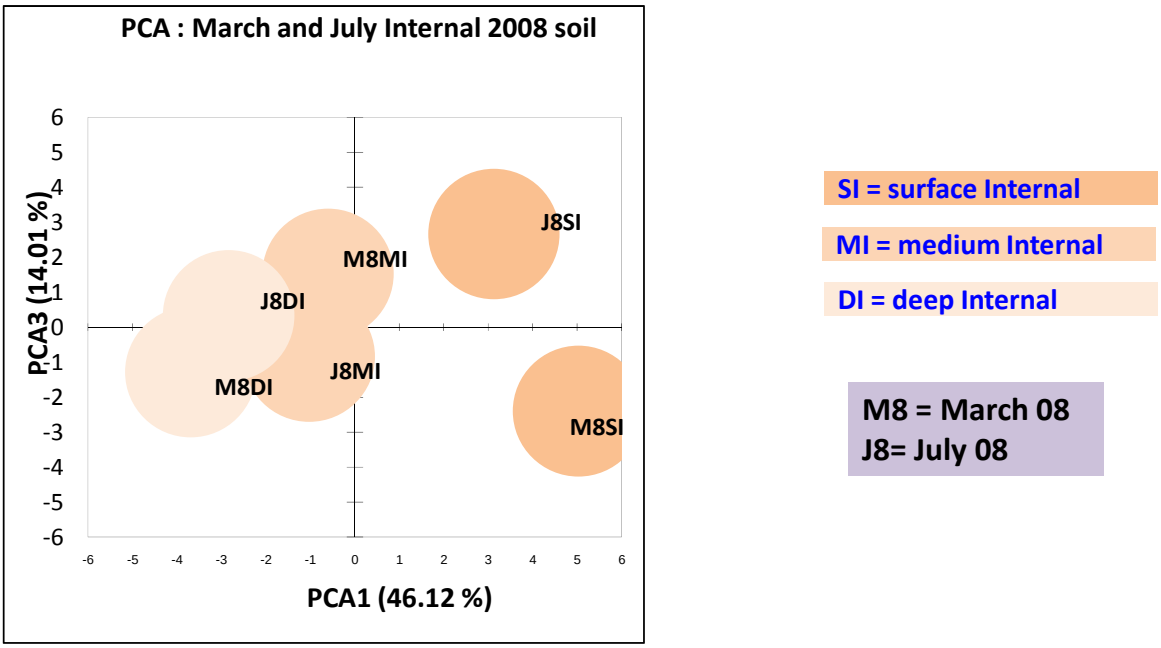
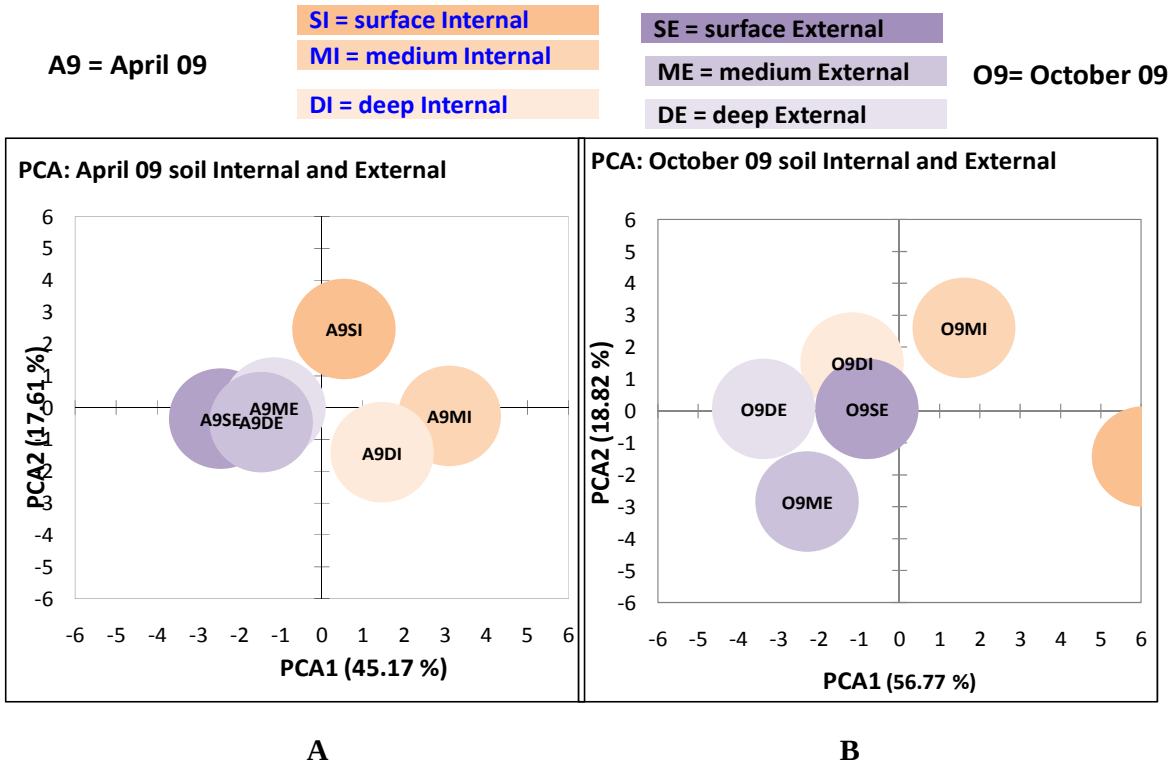


Fig 4.22. Principal component analysis (PCA) of surface, medium depth and deep Internal soil of March and July 08, based on data of 16S rDNA Phylotypes distribution.



A **B**

Fig 4.23. Principal component analysis (PCA) of Internal and External surface, medium depth and deep soils (April 09 and October 09) based on 16S rDNA Phylotypes distribution. A: April 2009, B: October 2009.

4.3.8. Diversity index of culturable bacteria

Shannon-Wiener Diversity Index (H) was determined for the microbial communities of the surface, medium depth and deep soil for 2008 and 2009, as shown in Table 4.7. The results seem to reinforce the observations made in the above sections, clearly indicating a decrease in the diversity for deep soil as compared to the medium and surface ones, in both internal and external samples.

Simpson's dominance index (D) was also calculated and the data are shown in Table 4.7. Index values are lower for surface soil and increase according to the depth.

	Surface		Medium		Deep	
Sampling	**H**	**D**	**H**	**D**	**H**	**D**
Internal 08						
March 08 Internal	1.56	0.25	1.45	0.29	1.20	0.40
July 08 Internal	1.43	0.29	1.31	0.32	1.20	0.46
Internal and External 09						
April 09 Internal	1.52	0.26	1.27	0.34	1.21	0.37
April 09 External	1.39	0.31	1.11	0.40	0.86	0.46
October 09 Internal	1.75	0.21	1.35	0.31	1.20	0.36
October 09External	1.20	0.36	1.16	0.38	1.01	0.39

Table 4.7. Diversity Index of culturable bacterial communities. H: Shannon-Wiener Diversity Index, D: Simpson's dominance index

4.4. Further considerations

In the current study, culture independent and culture dependent approaches were used to analyze soil microbial diversity inside and outside the riparian strip at different soil depths and seasons.

In general it can be observed that in accordance with literature (Braun et al., 2006; Fierer et al., 2003; Fritze et al., 2000), in deep soil all the analyzed parameters (total, CTC+ and culturable cells) have lower values if compared with those of surface soil. These results clearly indicate that in surface soil microorganisms are present in higher number and are more metabolically active than deeper in the soil (Fig 4.7–Fig 4.8). Moreover, from colony

morphologies observation, the microbial diversity seems to be more intense in surface soil (Fig 4.10). These evidences are confirmed by DNA based analysis.

In July, when the temperature was higher, a lower number of living and metabolically active cells was observed when compared with April-October sampling time (Fig 4. 5). This result is confirming that metabolic activity of microorganism strongly depends upon a number of environmental factors such as temperature, as described by Promeroy & Wiebe (2001) and Price & Sowers (2004).

In this study, in all samples, at any level and at any time, the culturable cells are a small percentage of CTC+ that are themselves a percentage of total living population. These results confirm the well-known evidence that in natural environments, such as soil, culturable bacteria are only a small percentage of the totals (Janssen et al., 2002; Zdanowski et al., 2001; Sigler et al., 2002; Aislabie et al., 2006; Edenbor and Sexstone, 2007; Stress, 2007).

More metabolic active cells were obtained in internal samples at three different soil depths as compared with the external ones (Fig 4.7 and Fig 4.8). These results could be explained by the fact that the external soil was left uncultured, while in the internal soil several plants were grown. Root exudates contain organic, inorganic and growth stimulating substances that enrich soil quality and the nitrogen flux coming from the Zero river can further support their growth. These clearly confirm that the plants can play an important role for supporting and developing microbial diversity in soil (Kuske et al., 2002; Smalla et al., 2001; Grayston et al., 2001; Curl and Truelove, 1986; Garbeva et al., 2004).

Molecular techniques based on ARDRA and 16S rDNA sequencing have been widely used to evaluate changes in microbial community structures. For example Smit et al. (1997) and Torsvik et al. (1998) found a distinct difference in microbial community structure in soil contaminated with heavy metals compared to uncontaminated soil. The ARDRA technique provided information on the genetic diversity among the culturable bacteria in soil populations revealing a higher biodiversity in the riparian strip soil as compared to the outside soil (Table 4.1B and Table 4.1C).

A number of studies have found 16S rDNA sequences that identify a single bacterial species or differentiate among a limited number of different species or genera (Becker et al., 2004; Bertilsson et al., 2002; Choi et al., 1996; Clarridge, 2004; Kataoka et al., 1997;

Lu et al., 2000; Marchesi et al., 1998; Maynard et al., 2005; Rothman et al., 2002; Yang et al., 2002). On the basis of 16S rDNA analyses, Hugenholtz et al. (1998) reported that the phyla Proteobacteria, Cytophagales, Actinobacteria and Firmicutes are well represented among cultivated microorganisms ccounting for 90%. In the present study, 16S rDNA Phylotypes distribution within soils from March 08 and July 08 internal samples were affiliated with five to seven major bacterial divisions, namely Firmicutes, Gammaproteobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Flavobacteria and Unclassified bacteria (Table 4.3).

Bacterial divisions were slightly more in internal soil as compared to the external. Five to eight major bacterial divisions were found in internal soil samples in April 09 and October 09 (Table 4.4 and Table 4.5), whereas three to six major divisions were found in external soil samples of April 09 and October 09 (Table 4.4 and Table 4.5). These results indicate that in the riparian strip soil the microflora biodiversity increased if compared with that of the external area. Moreover, the numbers of divisions seem to be higher within the surface soil layer and to decrease according to soil depth. In this analysis, Acidobacteria and Verrucomicrobia were not found within the culturable bacterial community. These phyla such are mainly revealed only by clonal analysis, and are poorly represented by sequences from cultivated organisms. For example, Acidobacteria appear to be numerically dominant and active in most soils and form up to 52% of 16S rRNA gene sequences in clone libraries (Kuske et al., 1997; Nogales et al., 1999; Felske et al., 2000); however, only few isolates have been obtained from soil (Kishimoto and Tano, 1987; Sait et al., 2002).

In the riparian strip ecosystem, Firmicutes were dominant with high percentage of colonies and OTUs as compared to the external soil (Table 4.4 and Table 4.5). Similarly, many researchers have found Firmicutes to constitute a dominant portion of culturable bacterial populations in metal-polluted soils (Gans et al., 2005; Desai et al., 2009). It has been also established that Firmicutes contribute to a mere 12% to the total pristine soil bacterial community (Kapley et al., 2007) an, recently Ishii et al. (2009) demonstrated that they are dominant in rice paddy soils and potential key players in nitrate respiration and denitrification; these results were obtained by comparing the community structures of soils with strong denitrifying activity to those of soils without denitrification. The riparian strip soils described in this research are in a water management systems carrying dissolved

nitrogen from Zero river through specifically designed ditches thus supporting a potential selection of Firmicutes bacteria as dominant members of the community.

Bacillus and *Paenibacillus* are members of Firmicutes and were well represented in the isolates from our soils. Previous researches reported that the genera *Bacillus* are able to remove nitrogen and phosphorus as well as organic matter efficiently (Choi et al., 2000, Kim et al., 2005).

Proteobacteria found in the internal surface soil in March and July 08 shared common lineages with different species of the genera *Pseudomonas, Lysobacter, Xanthomonas, Stenotrophomonas, Ensifer, Bosea,* and *Variovorax.* It must be noted that *Ensifer sp* was present only in surface soil in July 08, while *Bosea* sp was present in March 08 (Fig 4.13). This result is indicating seasonal and spatial variations in microbial community structure as previously reported in other studies (Tan et al., 2009).

Alphaproteobacteria found in deep soil in March and July 08 shared common lineages with Rhizobium*, Sphingomonas, Enterobacter, Stenotrophomonas* and *Xanthomonas* that are completely different than those found in both surface and medium soils in March and July 08 (Fig 4.15). This means that the bacterial groups present in the surface and medium depth soils are not always found in the deeper horizons, suggesting that this deeper level produces a selective pressure on the microbial community reducing or suppressing one or more fractions of the bacterial population. On the other hand, some microbial groups dominate only in the deeper soil horizons, thus demonstrating that this deep environment can even support some bacterial species which has to be specifically adapted. On the same line are some previous observations (Tsai et al., 2007, Tsai et al., 2009).

Phylogenetic analysis of Proteobacteria division revealed that more diverse genera were present in April and October 09 in the internal soil as compared to the external, Some of these genera, especially found in the internal samples, are detected as denitrifiers, like *Bosea sp* (Falk et al., 2010) and *Rhodovulum* (Srinivas et al., 2007), while *Sphingomonas* is reported as organic matter (OM) degrader (Leys et al., 2004; Stolz, 1999; Cassidy et al., 1999; Keim et al., 1999).

The genera *Pseudomonas* and *Xanthomonas* were common in both sites (Internal and External) at all the soil depths (Fig 4.16 and Fig 4.21). A similar result was obtained by

Shuai et al. (2010). These evidences could be explained by the fact that some genera are well distributed in many environments due to their nutritional flexibility.

Proteobacteria division revealed that the genera *Lysobacter* and *Stenotrophomonas* were well distributed in the internal soil in April and October 09. This abundance could be expected in soils with adequate organic carbon content (Maeda et al. 2009, Yu et al., 2009) Phylogenetic analyses of Proteobacteria have also indicated that some genera are typical for specific soil sites, seasons and levels. For example, the genera *Variovorax, Acetobacter* and *Stenotrophomonas* were present only in the medium depth layer of the internal soil in April 09, while *Ochrobacterium* sp was present in the external (Fig 4.17). Similarly, in October 09, *Sinorhizobium* sp*, Lysobacter* sp and *Stenotrophomonas* sp were present only in the medium depth internal soil, while *Citrobacter* and *Subtercola* were present in the same layer of the external soil (Fig 4.20)

Betaproteobacteria were absent in the medium depth external soil of April 09 and deep soil of both April 09 and October 09 (Fig 4.18 and Fig 4.21) and in the internal soil were present with different strains of *Variovorax* and *Achromobacter* sp (Fig 4.21). In other studies, Betaproteobacteria predominance coincided with that found in other heavy metal contaminated soils, gold tailings, and uranium mine sediments (Akob et al., 2007; Chang et al., 2001; Fields et al., 2005; Peacock et al., 2004; Reardon et al., 2004).

The Actinobacteria showed less percentage in both March and July 08 for the internal soil as compared to Firmicutes division (Table 4.3) even if the numbers of genera were variable with soil depth (Fig 4.13-Fig 4.15). These evidences agree with previously published works on the average abundance of 16S rDNA clones of Actinobacteria, originating from various soils and depths (Girvan et al., 2003; Borneman and Triplett**,** 1997; Lee et al., 1996; McCaig et al., 1999).

The Actinobacteria division were the most dominant in the external (46.8% - 48.9% colonies in April 09 and 59.7% - 64.7% colonies in October 09) as compared to the internal soil (10.9% - 22.9% colonies in April 09 and 12.1% - 17.9% colonies in October 09) (Table 4.4 and Table 4.5). This may reflect the lack of organic carbon and energy sources in the external soils. Similarly, Yendi et al. (2010) detected Actinobacteria as the most abundant group in bulk soils with a high concentration of heavy metal-sulfur complexes, and lacking carbon and organic energy sources. This is in accordance with other studies where

Actinobacteria phylum was largely represented in ribosomal libraries from heavy metal polluted soils (Gremion et al., 2003; Herrera et al., 2007; Mendez et al., 2008).

Among the isolates from external soil on April and October 09, the majority of the isolates belong to the genus *Arthrobacter* of the Actinobacteria division. Some researcher reported that these bacteria are considered to be very common in soil and have been found to be among the predominant members of culturable communities from several terrestrial subsurface environments (Crocker et al., 2000; Overhage et al., 2005). Among the explanations advanced for their ubiquity or even predominance in soil are their resistance to desiccation and nutrient depletion, and their nutritional versatility.

The genera *Microbacterium* and *Streptomyces* were other abundant genera in the external soils (Fig 4.16-Fig 4.21). Similar results are obtained by Niva et al. (2006) from boreal forest with acidic and poor nutrient containing soil. *Agromyces* and *Isoptericola* were most abundant in April 09 internal soils at surface and deep soil levels (Fig 4.16).

In addition several rare and novel Actinobacteria genera were detected, including *Frigoribacteria, Kitasatospora* sp. and *Isoptericola* sp in the internal deep soil of April and October 09, whereas *Leifsonia* sp. and *Cellulosimicrobium* sp. were found only at medium soil depth of external samples. For instance, *Kitasatospora* is reported as a novel species (Liu et al., 2005), *Isoptericola* sp was described as a new genus (Zhang et al., 2005) and *Leifsonia* sp as a novel species (Pindi et al., 2009).

Differences in Flavobacteria community complexity were observed between the internal and external soils. For example, Flavobacteria with *Flavobacterium* sp was found at low percentage (2.6% OTUs and 1.3% colonies in March 08, 3.5% OTUs and 2.1% colonies in April 09 and 7.3% OTUs and 5.3% colonies in October 09) in the internal surface soil. On the other hand, Flavobacteria with *Chyseobacterium* sp. was observed only in the April 09 external surface and medium depth soil with less percentage (6.9% OTUs and 6.1% colonies in surface, and 4.2% OTUs and 2.2% colonies in medium depth soil) (Table 4.4 and Fig 4.16 and 4.17). The presence of the Flavobacteria subdivision is less common according to some authors (Dunbar et al., 1999; Chow et al., 2002; Fierer et al., 2005; Tsai et al., 2009).

The bacterial division Sphingobacteria, with *Sphingobacterium*, was found in the external surface soil of April 09 (Fig 4.16), while *Pedobacter* was only found in the internal surface

soil of October 09 (Fig 4.19). The type genus *Sphingobacterium* was proposed by Liu et al. (2008), while the genus *Pedobacter* was detected from forest soil as a novel sp. by Gordon et al. (2009).

Principal components analysis (PCA) is a very useful tool in ecosystem studies (Clement et al., 1998; Tiquia, 2005). In this study, PCA was used to determine the relationships among microbial communities by 16S rDNA Phylotypes distribution. The results of PCA indicate a completely different microbial community composition between internal and external soils (Fig 4.23), with enhanced differences for the surface soil (Fig 4.22).

A range of diversity indexes have been used with bacterial communities, in particular the ubiquitous Shannon index, the evenness index derived from it, and Simpson's dominance index (Dunbar et al., 1999; Cho and Kim, 2000; McCaig et al., 1999). The Shannon index (H) seems to be a useful general diversity index that is influenced by both richness and evenness and is more sensitive to changes in abundance of the rare groups (Hill et al., 2003). In contrast, the reciprocal Simpson's indexes (D) are heavily weighted by the dominant(s) and it has been widely used for microbial ecological studies and has good discriminating ability (Zhou et al., 2002). In this study, Diversity index was determined by two alternative ways for comparative diversity analysis. Higher Shannon–Wiener or lower reciprocal Simpson's index values describe a community with greater numbers of species and a more even distribution of species (Gomez-Alvarez et al., 2007). In this study an increased species evenness index and a decreased species dominance index was found for the internal soil samples as compared to the external one (Table 4.7). Moreover, a lower evenness index and higher dominance index for the deep and medium depth as compared to that of the surface soil indicate a possible effect of plant and organic matter on bacterial community structures. Aeration and organic substrate supply decreased with the increasing soil depth, leading to reduction of the bacterial species number and disappearance of bacteria unable to survive such adverse conditions. Other studies using, 16S rRNA gene (Tsai, et al., 2009), phospholipid fatty acid analysis (Blume et al., 2002), fluorescence *in situ* hybridization (Kobabe et al., 2004), and terminal restriction fragment length polymorphism analysis (LaMontagne et al., 2003) have also shown a significant reduction in the species number of soil microbial communities with changes in soil depths.

5. Microbial Community Analysis in Wooded Riparian Strip Soil by 16S rDNA PCR-DGGE.

5.1. Introduction

Despite considerable interest in the microbiology and biochemistry of soils, relatively little is know about the diversity and ecology of the microbial community (Nakatsu et al., 2000). Studies have demonstrated that microbial community is dependent on available organic matter, macro flora, and fauna. .The enormous range of complexity in soil microbial communities has made it an incredibly challenging ecosystem to study (Torsvik and Øvreås, 2002; Torsvik et al., 2002).

A number of molecular biological approaches are now being used to gain a better understanding of the ecology of soil microbiota (Nakatsu, 2004). This has helped soil scientists to evaluate differences in microbial communities with respect to their environment. It has enabled advancement beyond the traditional laboratory cultivation approaches that were able to capture only about 1% of the community in the past (Staley and Konopka, 1985). The majority of molecular methods currently being used for community analysis examine nucleic acids, whereas PCR amplification has been used to increase copies of a target gene for easier detection. Examples of methods being used to directly analyze nucleic acids are DNA:DNA reassociation kinetics (Torsvik et al., 1990), nucleic acid hybridization (Buckleyet al., 1998), fluorescent in situ hybridization (Christensen et al., 1999; Ravenschlag et al., 2000), microarrays (Rhee et al., 2004; Small et al., 2001), and metagenome sequence analysis (Handelsman, 2004).

The sequence composition based separation technique of PCR products of the same length utilising chemical denaturants (urea and formamide) has become a standard method for rapid and accurate screening of complex microbial communities in clinical microbiology, food safety, and environmental microbiology (Muyzer et al. 1993). Specific primers have been designed for assessing microbial communities, functional groups, genus, or species of complex ecosystems (Muyzer et al. 1993; Nakatsu et al. 2000; Vainio and Hantula 2000; Kowalchuk et al. 2006; Mühling et al. 2008; Cheng et al. 2009). The strength of the DGGE method applied to soil ecology is the rapid, simple, reproducible, efficient (contemporary processing of a large number of samples), and low-cost way to assess microbial populations in complex communities and to monitor their response to environmental changes (Agnelli et al. 2004; Lynch et al. 2004; Ascher et al. 2009a).

Bacterial communities have been examined by PCR-DGGE from almost every soil environment, including fresh soil (Jolanda et al., 2008), archived soil (Tzeneva et al., 2009), agricultural soils (Nakatsu et al., 2000), plant rhizospheres (Duineveld et al., 1998; Marschner et al., 2001; Yang and Crowley, 2000), forests (Jaatinen et al., 2004; Laverman et al., 2005; Marschner and Timonen, 2005, Toms et al., 2010), grasslands (Felske and Akkermans, 1998; Griffiths et al., 2003; Ritz et al., 2004), upland (Dilly et al., 2004; Sun et al., 2004), rice paddy agricultural soils (Weber et al., 2001), oil-contaminated paddy soil (Zhang et al., 2005), orchards (Yao et al., 2005), wetlands (Ibekwe et al., 2003; Jaatinen et al., 2005; Wartiainen et al., 2003), industrial sites (Zocca et al., 2004), desert soil crusts (Nagy et al., 2005), sand dunes (Kowalchuk et al., 1997b; Smith et al., 2004), landfills (Wise et al., 1999), nematode egg masses (Papert et al., 2004), and plant endophytes (Kuklinsky-Sobral et al., 2005; Seghers et al., 2004). These studies were able to illustrate and compare the complexity of communities in these various environments. Because DGGE does not depend on cultivation, it has revealed greater diversity than previously uncovered using traditional cultivation methods (El Fantroussi, 2000; Nakatsu et al., 2005; Wise et al., 1999).

Taking into account the above reason, the distribution and dynamic of the bacterial communities in the soil systems under study have been here investigated by PCR-DGGE analysis.

5.2. Materials and Methods

5.2.1. Collection of samples

(Same as Chapter I, material and methods section)

5.2.2. DNA extraction and purification from dry soil

DNA from soil was extracted using the Power Soil ™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA). DNA isolation was performed from 0.50 g dry soil according to the manufacturers' instructions, modified as follows: extra glass beads (0.15 – 0.30 g., bead size 0.1 mm) were added to the soil samples and the cells were disrupted by bead beating (Mini-bead beater™, Bio Spec products, USA). To obtain pure DNA, the samples were incubated at 37°C with RNase A (Sigma) at a final concentration of 100 μg ml^{-1} for 10 min (Griffiths et al., 2000).This purified DNA was used for PCR.

5.2.3. Polymerase Chain Reaction (PCR) of 16SrDNA from total DNA of soil samples

PCR was performed for 16S r DNA in a final volume of 25 μl containing 10× assay buffer, 1.0 unit of *Taq*DNA polymerase (Amersham Biosciences), 0.2 mM each of dNTPs, 200 nM of each primer 63F5'CAGGCCTAACACATGCAAGTC (Marchesi et al.,1998) and 1389R5'ACGGGCGGTGTGTACAAG (Osborn et al., 2000) and 100 ng template DNA. The thermal cycler (Bio Rad ICycler 170-8740) was programmed for the initial denaturation step (94°C) of 5 min, followed by 44 cycles of 1 min denaturation along with 1 min primer annealing (37°C) and 2 min primer extension (72°C), followed by the 7 min primer extension (72°C) step. Fragments were analyzed by 1% agarose gel electrophoresis in 0.5% tris Borate EDTA buffer, at a constant voltage of 100V. Gel images were acquired in digital format using an EDAS 290 Image capturing system (Kodak, Rochester,NY).

5.2.4. Denaturing Gradient Gel Electrophoresis (DGGE) analysis of PCR-amplified 16S rDNA fragments

A 433 bp fragment (V6-V8 region) of the 16S rDNA sequence was amplified by using the primer pair F984GC/R1378, which is suitable for total community fingerprinting (Heuer et al., 1997). Each 25 μl reaction contained 10x buffer, 3.75 mM $MgCl_2$, 4% (w/v) acetamide, 0.2 mM deoxynucleoside triphosphates (Roche Diagnostics, Mannheim, Germany), 0.2 μM

of each primer (GC-F968-984, R1378-1401, synthesized by TIB-MolBiol, Berlin, Germany) and 2.5 U Taq DNA polymerase fragment (Applied Biosystems, Darmstadt, Germany), to which 1 µl template DNA (ca. 20 ng) was added. After 5 min of denaturation at 94 °C, 35 thermal cycles including 1 min at 94 °C, 1 min at 53 °C and 2 min at 72 °C were performed, followed by a final extension at 72 °C for 10 min. At least two independent PCRs were done per sample and analyzed separately. Amplicons were checked on 1% agarose gels. DGGE analysis was performed as described by Heuer et al. (2002) with a denaturing gradient of 26 to 58% (where 100% denaturant contains 7 M urea and 40% formamide) but with an additional acrylamide gradient of 6 to 9%to enhance the bands resolution and sharpness (Gomes et al., 2005). The DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, München, Germany) was used. Approximately equal DNA amounts of the PCR products were loaded on the DGGE either in blocks of samples from the same site, or randomly. The products were separated during the running in 1× TAE buffer (40 mMTris–acetate, 1 mM EDTA, pH 8.0) for 6 hours at a constant voltage of 220 V and temperature of 58 °C. The gels were silver stained, dried at 37 °C and scanned. At least two different DGGE runs were carried out for all samples and for both loading orders of the samples on gel, in order to estimate the reproducibility of DGGE profiles generated with different loading schemes of samples.

5.2.5. Identification of DGGE bands and cluster analysis of profile

DGGE bands were identified by visually inspecting gel images in BioNumerics version 4.5 software program through band intensity. Brightness and contrast were adjusted for each image to facilitate band identification. Similarities between microbial communities of DGGE profiles generated by Dice similarity index were based on UPGAMA (Unweighted Pair Group Method using Arithmetic Averages) analysis using the BioNumerics version 4.5 software (www.applied-maths.com).

5.2.6. Statistical analysis of DGGE banding patterns

Diversity index was measured by Shannon diversity index (H) (Shannon and Weaver, 1963, Ibekwe et al., 2010). The Shannon diversity index (H) is commonly used to characterize species diversity in a community. In this analysis we used single band as a species. Because as different species may have same melting behavior, a single band in DGGE profiles could

be identified as different bacterial species (Wang et al., 2007). Shannon's index accounts for both abundance and evenness of the species present. The proportion of species *i* relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1:

$$H = -\sum p_i \, ln \, p_i$$

Principal components analysis (PCA) was performed by XLSTAT 2007 software to determine the possible relationships between microbial communities on the basis of the DGGE banding patterns.

5.3. Results and Discussion

5.3.1. PCR amplification of 16S rDNA of soil microbial communities

Total DNA was extracted directly from the dry soils of inside (Internal) and outside (External) the riparian zone, at different depths and at different seasons. 16S rDNA was amplified from the extracted DNA by PCR. The extracted DNA from surface and medium depth soil produced satisfactory amplification in both internal and external samples (examples of amplification 16S rDNA of October 09 are shown in Fig 5.1). Different dilutions (no dilution, 1/10, 1/100) of extracted DNA were used for PCR. 1/100 dilutions were proved reliable and produced high yields of PCR products compared to undiluted and 1/10 diluted DNA (Fig 5.I). 16SrDNA PCR products from surface and medium levels resulted more intense as compared to those from deep levels (i.e. see Fig 5.1), indicating that the microbial population is more abundant in the surface levels.

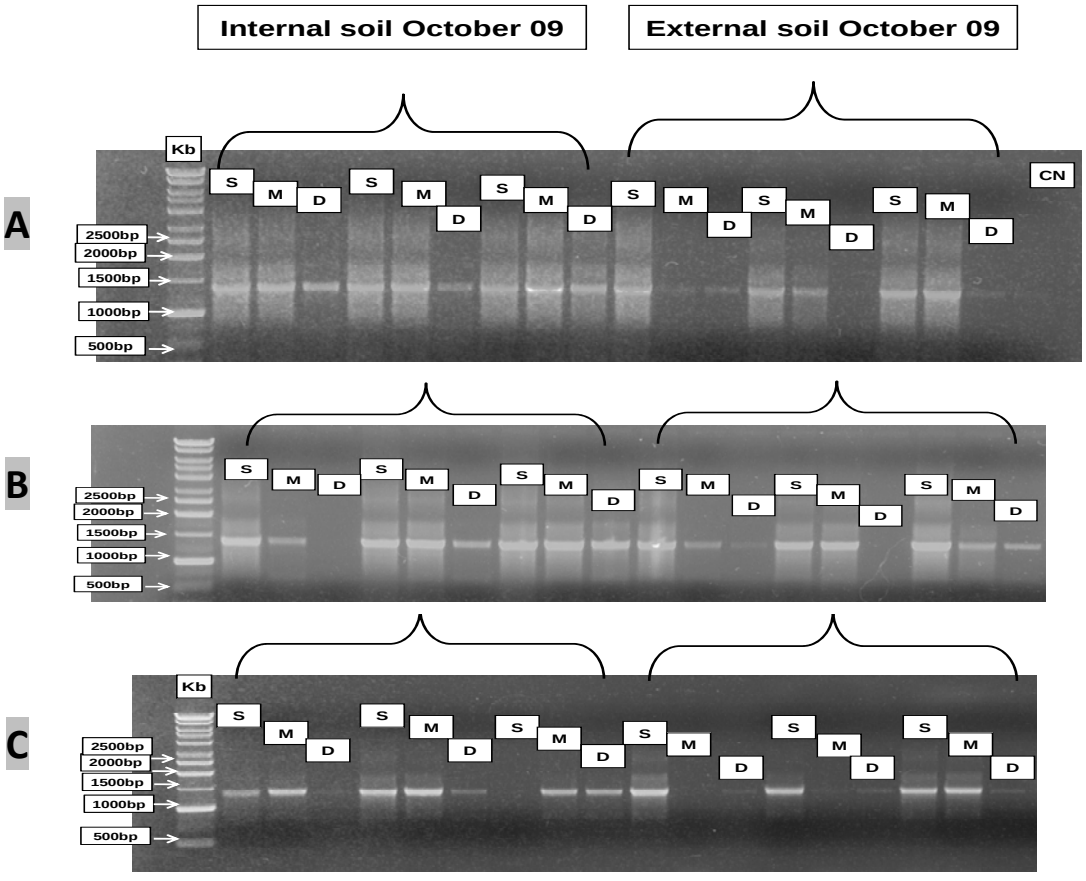


Fig 5.1. 16SrDNA amplification of DNA extracted from 0.5g dry soil. A, B, & C indicate no Dilution, 1/10 dilution and 1/100 dilution, respectively. S, M, &D indicate surface, medium and deep level soil, respectively. Kb is 1000 base pair marker and CN mean negative control.

5.3.2. DGGE band analysis of microbial communities of soil samples

In the current study, 16S rDNA PCR-DGGE based approaches were used to analyze the microbial populations in the riparian strip.

The variable region V6-V8 of 16S rDNA was amplified from the 16S rDNA and produced good amplification in all the soil samples (as an example see Fig 5.2).

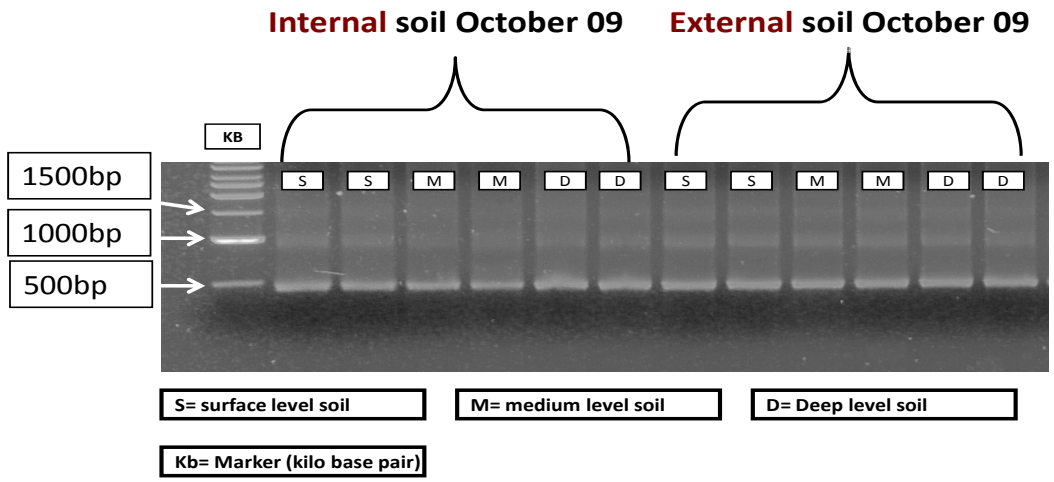
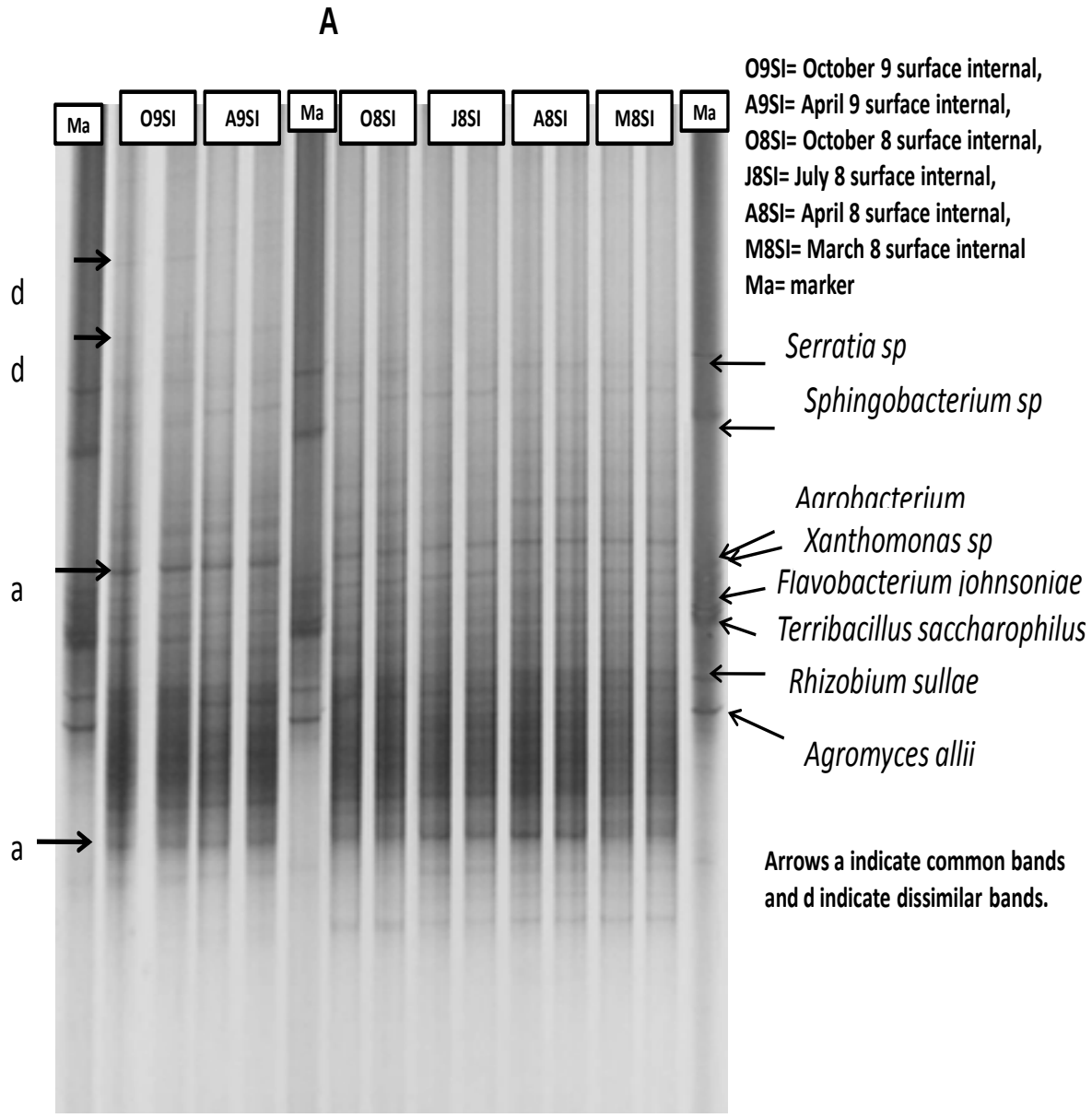


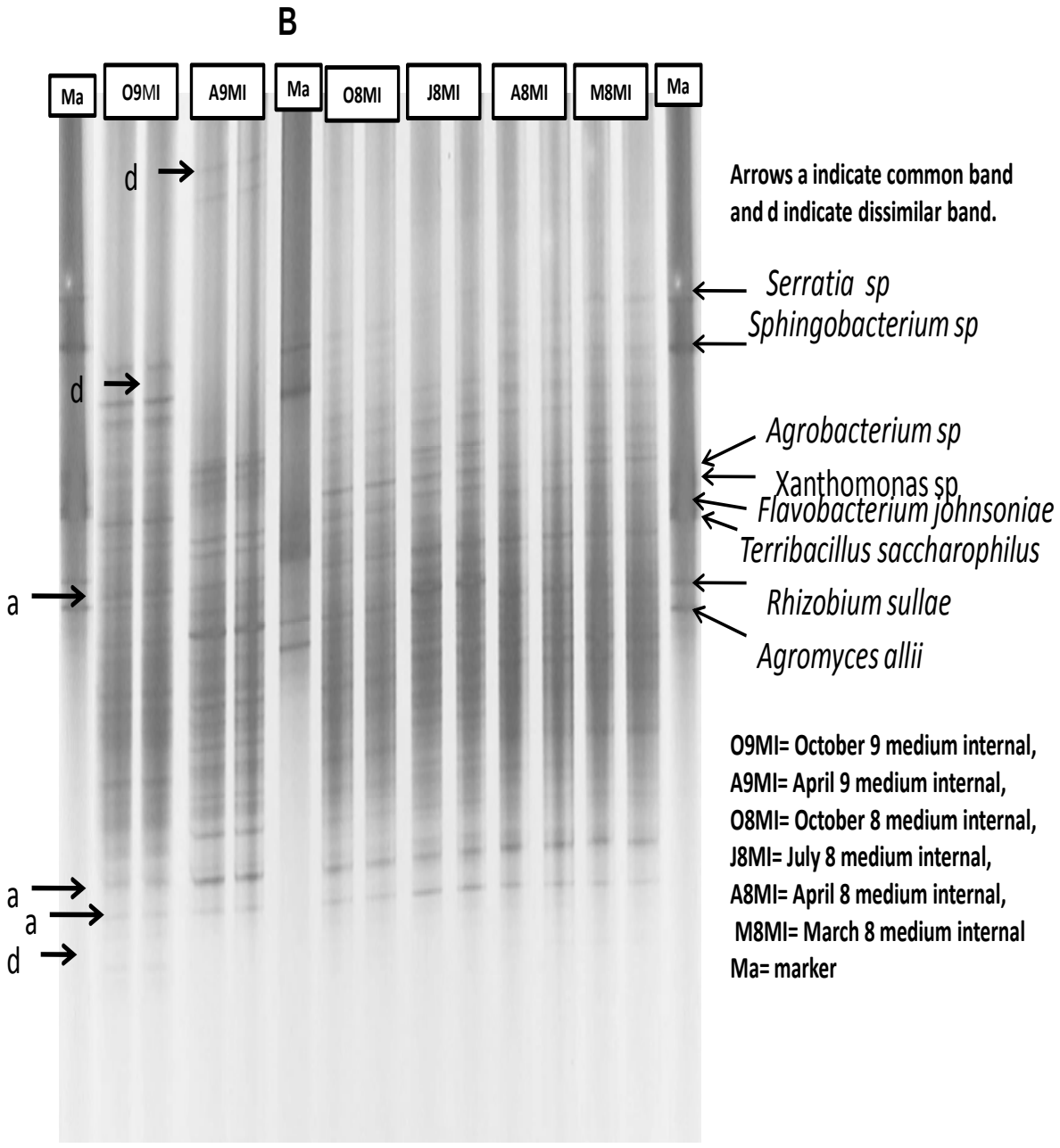
Fig 5. 2 Amplification of the V6-V8 variable regions of 16S rDNA for DGGE analysis.

In DGGE pattern of amplified variable region V6-V8 of 16S rDNA, each DNA band at different locations and its relative concentration (brightness), may represent a particular microbial species and its relative abundance/richness within the microbial community (Muyzer et al, 1993, Wenhui et al, 2007). Because the PCR template is the total soil DNA, which included the DNA of culturable and unculturable microorganisms, PCR-DGGE can reflect more microbial species than culturable microorganisms.

In the present study this technique produced good amplification in all the soil samples (as an example see Fig 5.2). The amplified variable region was used in DGGE analysis to identify the total microbial communities of internal and external soils at three different depths (surface, medium depth and deep soil) for years 2008 and 2009. Band positions were measured by percentage of denaturant concentration through BioNumerics version 4.5 software and the results are shown in Fig 5.3 and Fig 5.4.

Some bands were found only in the internal soils, whereas some ones were present only in the external one (Fig 5.4).




B
Ma
O9MI
A9MI
Ma
O8MI
J8MI
A8MI
M8MI
Ma
d
d
a
a
a
d
Arrows a indicate common band and d indicate dissimilar band.
Serratia sp
Sphingobacterium sp
Agrobacterium sp
Xanthomonas sp
Flavobacterium johnsoniae
Terribacillus saccharophilus
Rhizobium sullae
Agromyces allii
O9MI= October 9 medium internal,
A9MI= April 9 medium internal,
O8MI= October 8 medium internal,
J8MI= July 8 medium internal,
A8MI= April 8 medium internal,
M8MI= March 8 medium internal
Ma= marker

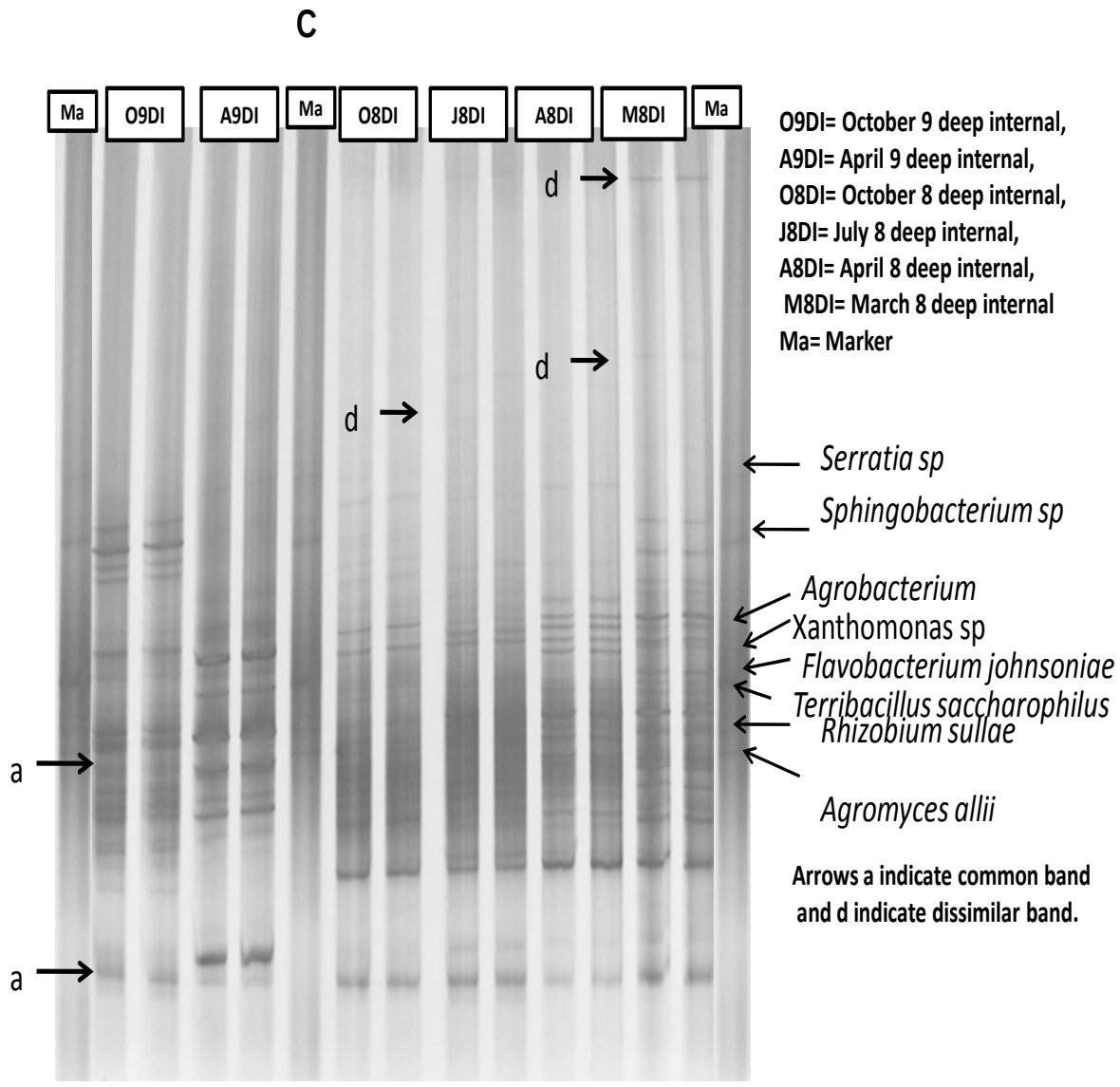
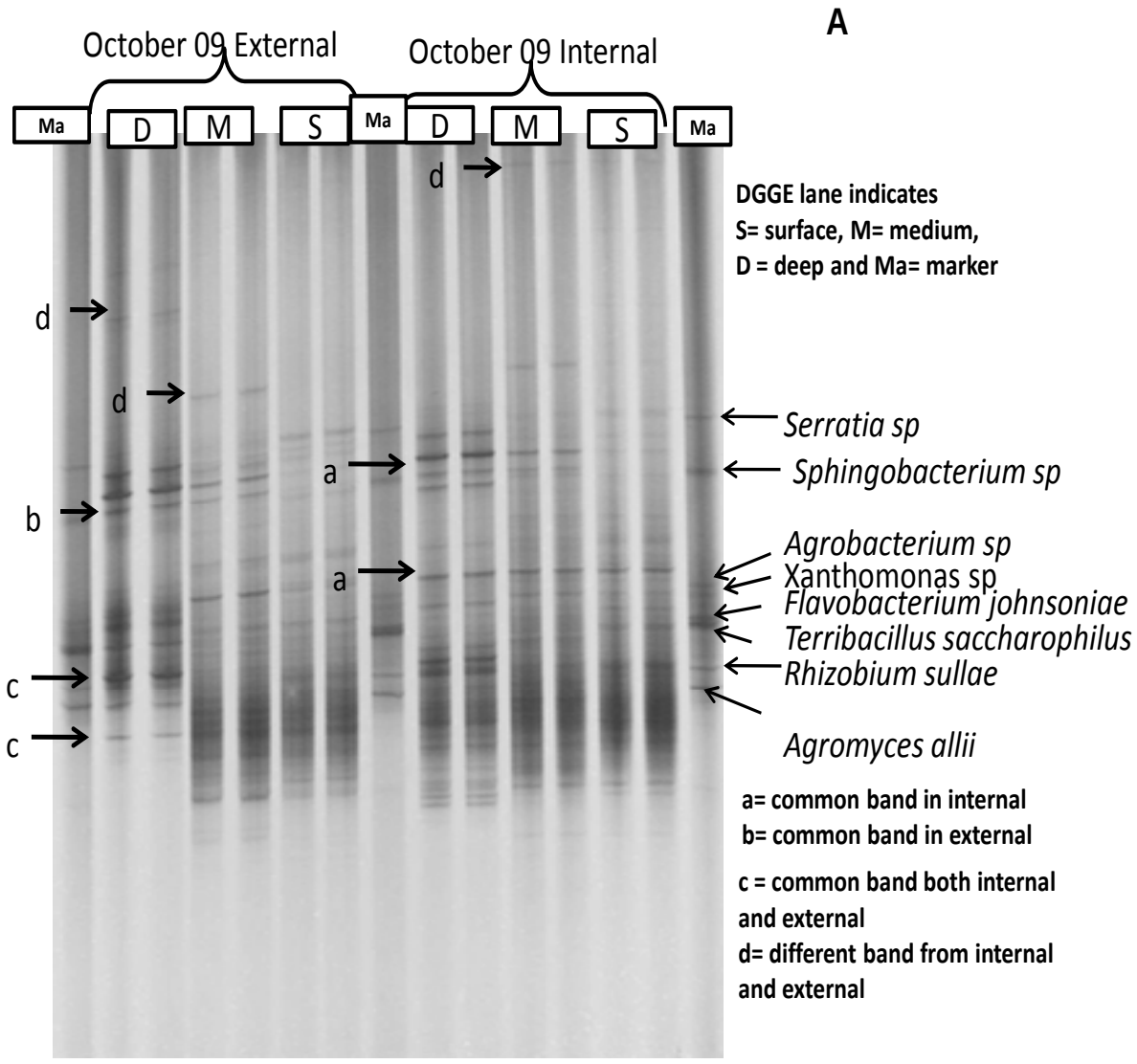
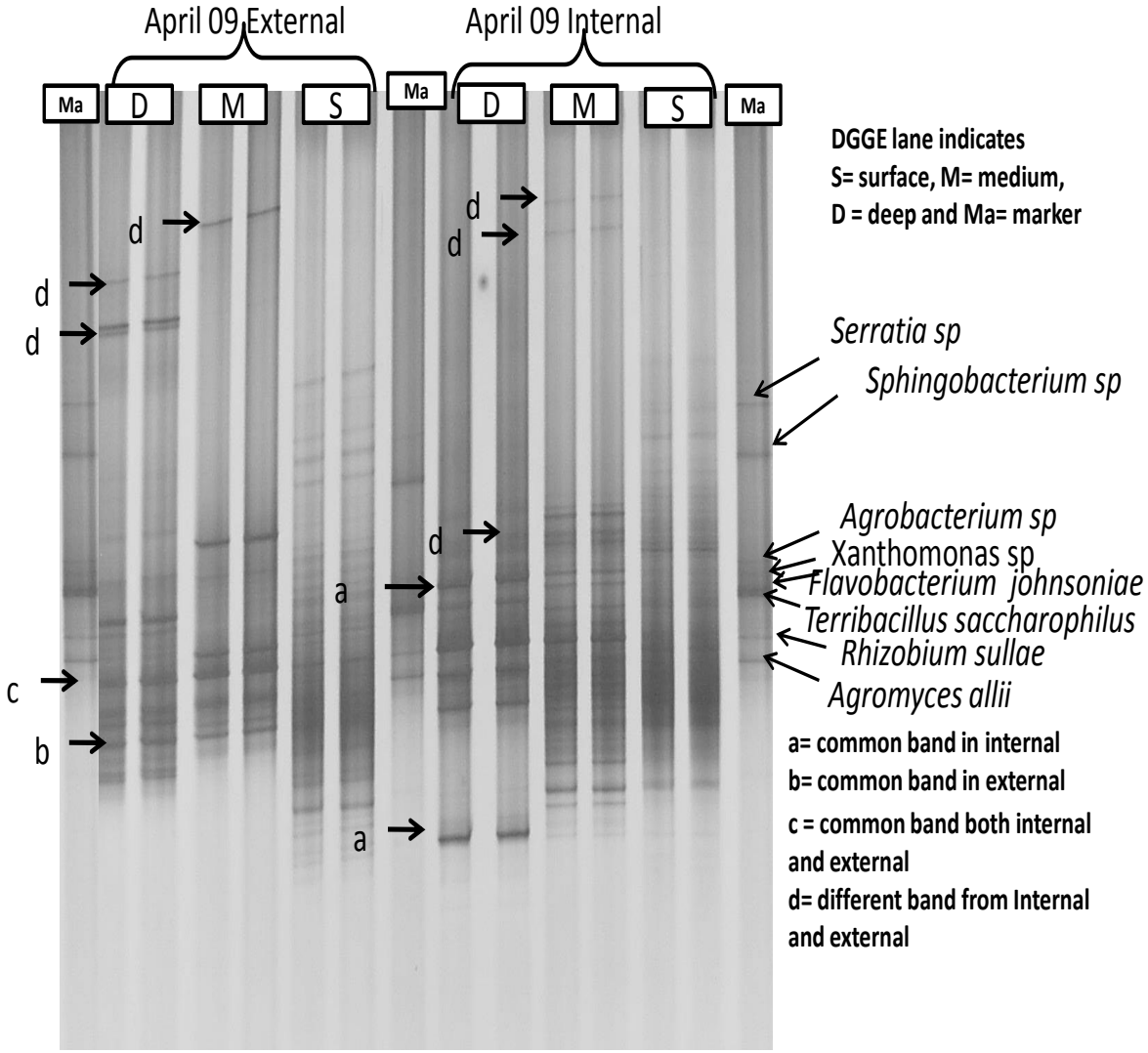


Fig 5.3. DGGE band patterns of microbial communities at different seasons and depths of soil. A, B and C indicate surface, medium depth and deep of internal 2008 and 2009 soil, respectively


A
October 09 External
October 09 Internal
Ma
D
M
S
Ma
D
M
S
Ma
d
d
d
a
b
a
c
c
DGGE lane indicates
S= surface, M= medium,
D = deep and Ma= marker
Serratia sp
Sphingobacterium sp
Agrobacterium sp
Xanthomonas sp
Flavobacterium johnsoniae
Terribacillus saccharophilus
Rhizobium sullae
Agromyces allii
a= common band in internal
b= common band in external
c = common band both internal and external
d= different band from internal and external

B


April 09 External
April 09 Internal
Ma
D
M
S
Ma
D
M
S
Ma
DGGE lane indicates
S= surface, M= medium,
D = deep and Ma= marker
d
d
d
d
d
d
a
a
c
b
Serratia sp
Sphingobacterium sp
Agrobacterium sp
Xanthomonas sp
Flavobacterium johnsoniae
Terribacillus saccharophilus
Rhizobium sullae
Agromyces allii
a= common band in internal
b= common band in external
c = common band both internal and external
d= different band from Internal and external

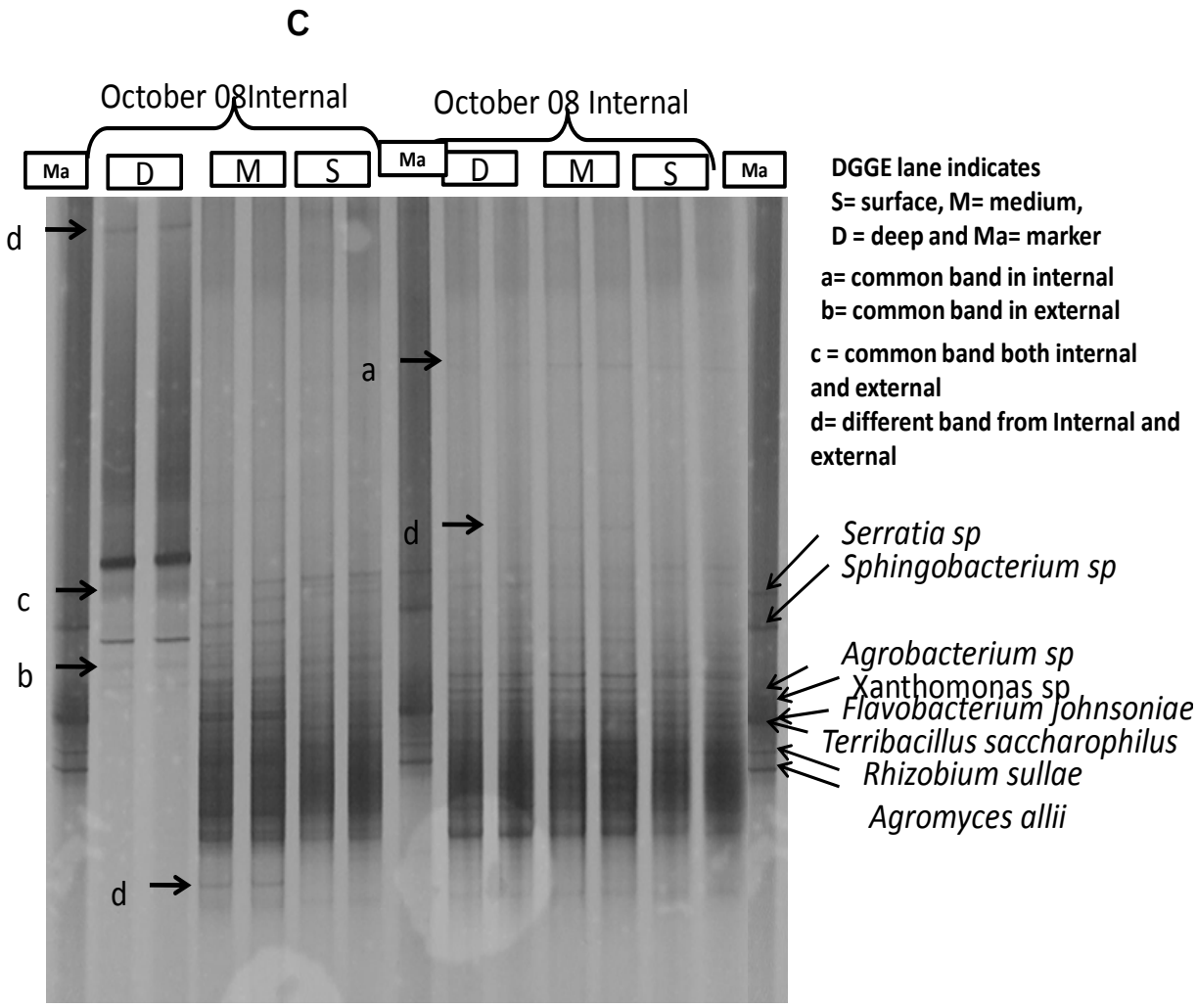


Fig 5.4. DGGE band patterns of microbial communities at different seasons and depths of internal and external soil samples. A, B and C indicate October 09, April 09 and October 08 soil samples (internal and external), respectively.

The number of bands obtained (Table 5.1) are in accordance with previous research: Nakatsu (2007) reported that, from soil samples, 20 to 40 bands in a DGGE profile can be clearly resolved in a gel.

A rapid observation of the number of bands, as reported in Table 5.1, confirms what it was observed for the culturable fraction of the microbial community: in both internal and external soils the numbers of species increase from deep to medium depth and from medium depth to surface soils. Indeed, the surface soil seems to hold always higher numbers as compared to the other two soil layers, with the deeper one showing the lowest diversity. Moreover, the samples collected in October 2008, April and October 2009 enable to compare external and internal soils and show that higher numbers of bacterial species were present in the internal soil microbial communities: 25-44 bands were obtained for internal soil at three different soil depths, while only 12-35 bands were found for the external soil. Once again, these results indicate that the treatment applied to the buffer zone is positively affecting the soil microbial biodiversity.

		Surface		**Medium**		**Deep**	
		Internal	**External**	**Internal**	**External**	**Internal**	**External**
2008	**March**	41		28		25	
	April	39		27		25	
	July	36		30		27	
	October	37	31	32	26	27	14
2009	**April**	39	30	29	25	27	22
	October	44	31	35	26	31	20

Table 5.1. Difference in band numbers obtained from microbial communities present in internal and external soil samples.

5.3.3. Identification of the main microbial groups

Eight know bacterial species *(Serratia* sp*, Sphingobacterium* sp*, Agrobacterium* sp*, Xanthomonas* sp*, Flavobacterium johnsoniae, Terribacillus saccharophilus, Rhizobium sullae* and *Agromyces allii)* were used as a marker to identify the microbial communities of soil. The results shown in Fig 5.3 and 5.4 may be used in the future to better understand the key bacterial genera living in these soils and to try to identify some particular microbial groups showing specific properties related to the buffer zone developed for water remediation. This intent is out of the main objectives of this study and will be extended in the coming years.

5.3.4. Cluster analysis

The presence and absence of bands in two PCR-DGGE profiles have been used to create a binary matrix for quantitative comparisons between two communities (Kropf et al., 2004; Wilbur et al., 2002). In this current study, similarities between microbial communities of DGGE profiles were generated by Dice similarity index based UPGAMA (Unweighted Pair Group Method using Arithmetic Averages) analysis using the BioNumerics version 4.5 software.

The related results are shown in Fig 5.5, Fig 5.6 and Fig 5.7.

As clearly shown in Fig 5.5, distinct and separate cluster groups were obtained from different soil depths for the samples collected in the buffer zone, further confirming that microbial community composition is different for each soil layer, probably according to the sources and availability of nutrients and oxygen accessibility. This is also in agreement with a number of previous studies showing that subsurface microbial communities are distinct in composition from the surface communities (Ghiorse and Wilson, 1988; Zvyagintsev, 1994; Fritze et al., 2000; Blume et al., 2002). Similarly, other Authors (Fierer et al., 2003; Steenwerth et al., 2008; Thoms et al., 2010) reported that the microbiota is more abundant within the first few centimeters of the topsoil, due to the possible copious food supply deriving from the decomposition of plant litter and plant residues.

On the other hand, profiles obtained from the external soil samples revealed that they do not cluster separately in different groups (Fig 5.6), indicating that outside the buffer zone under study no significant differences in the microbial community compositions occur at all

the soil depths tested. This is probably due to agronomic treatments of ploughing, applied to the external soil. Some authors reported, for several soils, that the microbial population structure and activity are affected by plough (Lynch and Panting, 1980; Patra et al., 1990; Buchanan and King, 1992; Kaiser and Heinemeyer, 1993; Blume et al., 2002).

Finally, by clustering together the DGGE profiles related to the internal and the external soils, the groups remain perfectly separated. (Fig 5.7), thus reflecting the different history of the two soils in terms of assessment, treatments and vegetation there developed (root exudates contain organic, inorganic and growth stimulating substances that enrich soil quality, and the nitrogen flux coming from the Zero River can further support their growth). This clearly confirms that the plants play an important role in supporting and developing microbial diversity in soil, as previously reported (Kuske et al., 2002; Smalla et al., 2001; Grayston et al., 2001).

The above observations definitely confirm that the treatments applied to the wooded riparian zone in terms of water flux and vegetation, unambiguously affect the soil microbial composition by selecting some specific bacterial groups for each soil depths, thus assuming a very different structure as compared to the bacterial community of an untreated soil located outside the riparian buffer strip.

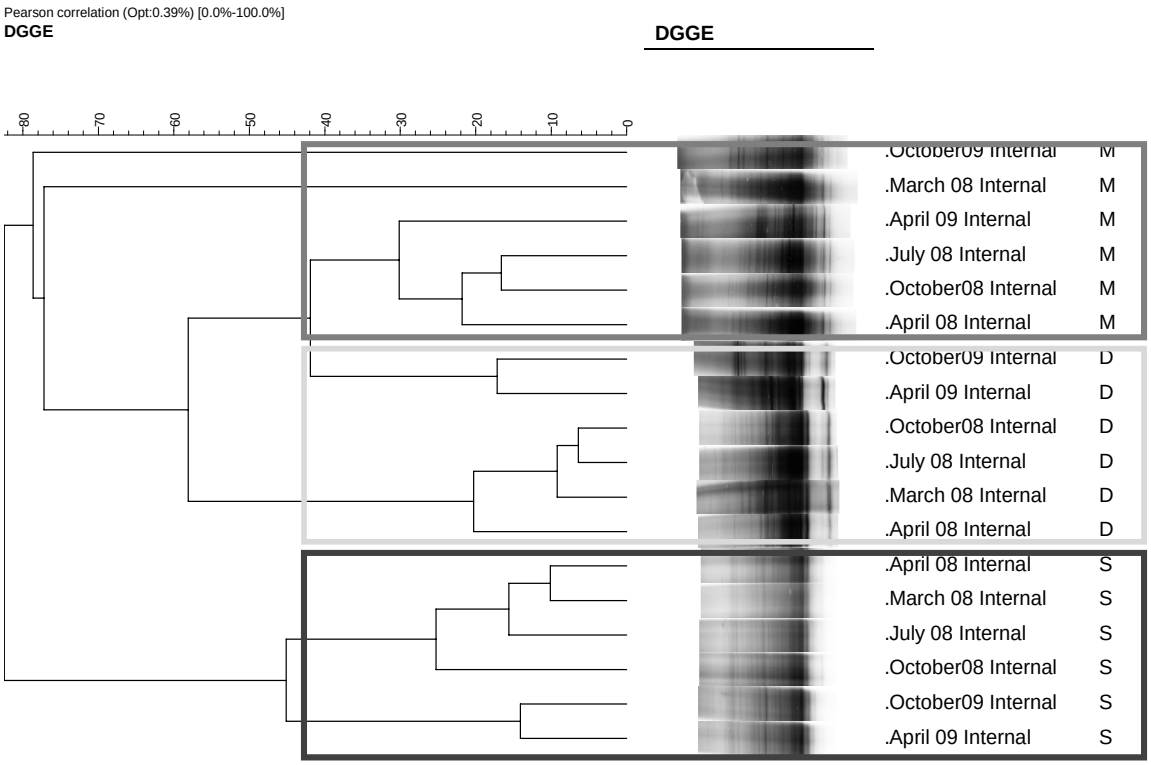


Fig 5.5. DGGE clusters analysis of microbial communities of internal soil samples (2008 and 2009). S, M and D indicate surface, medium depth and deep soil, respectively. The dissimilarity matrix for each sample was determined using Pearson correlation, and clustering was performed by BioNumerics.

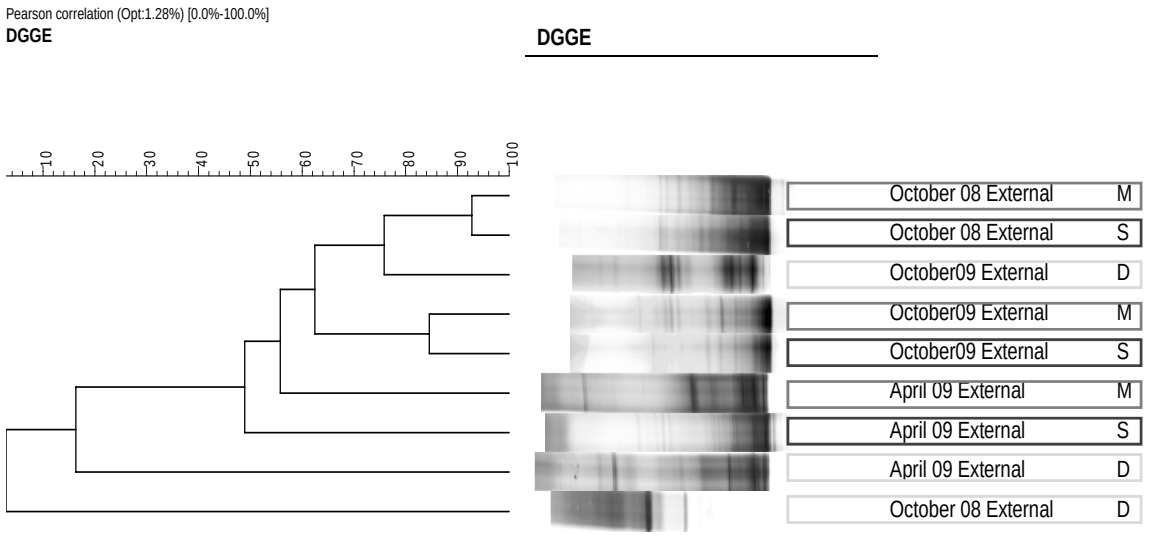


Fig 5.6. DGGE clusters analysis of microbial communities of external soil samples (2008 and 2009). S, M and D indicate surface, medium depth and deep soil, respectively. The dissimilarity matrix for each sample was determined using Pearson correlation, and clustering was performed by BioNumerics.

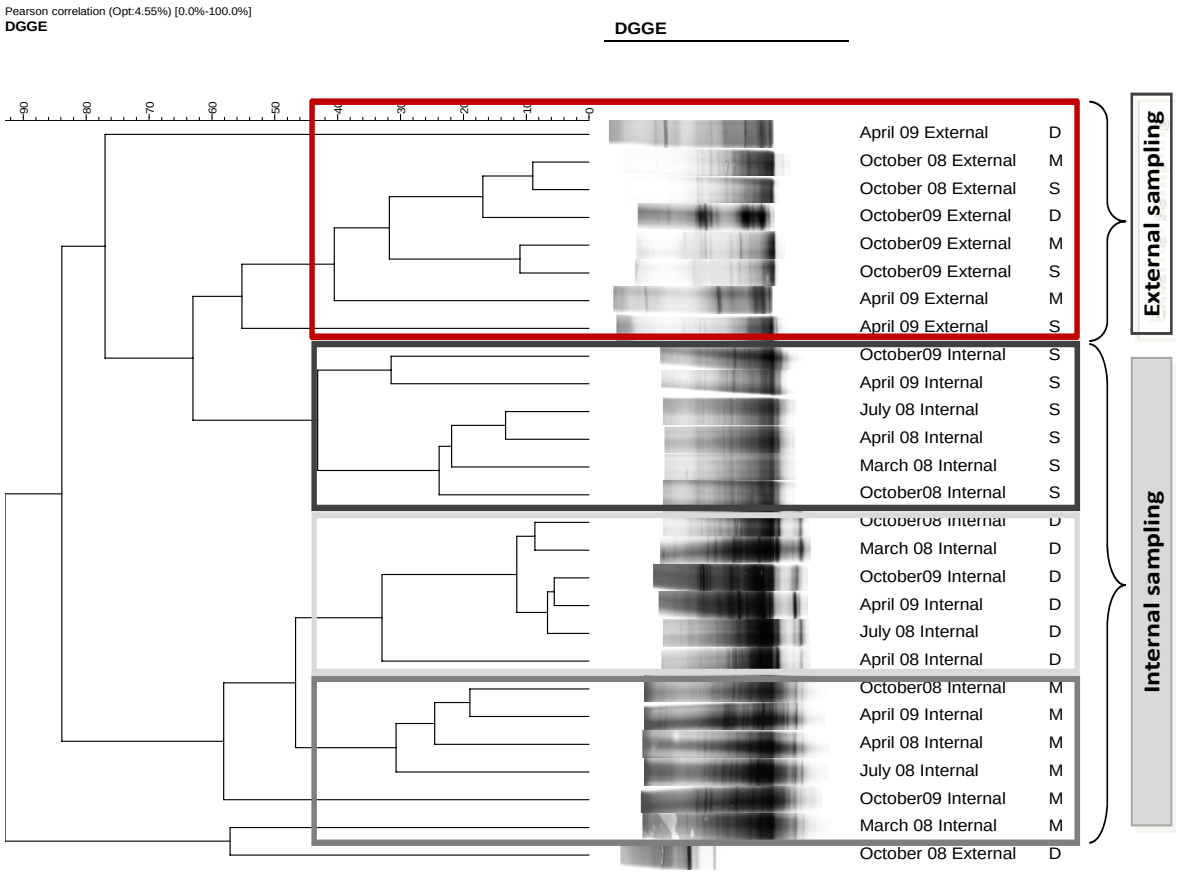


Fig 5.7. DGGE clusters analysis of microbial communities of internal and external soils for both year 2008 and 2009. S, M and D indicate surface, medium depth and deep soil, respectively. The dissimilarity matrix for each sample was determined using Pearson correlation, and clustering was performed by BioNumerics.

5.3.5. Principal component analysis (PCA) of the soil microbial communities

Multivariate analysis methods, such as principal components analysis (PCA; Pielou, 1969), have been used to analyze large data sets with greater sources of variation (Gremion et al., 2004; Joynt et al., 2006). A PCA calculates and ranks the contribution of each variable in a profile, and the approach can be used to identify the main sources of variation observed between profiles (Wilbur et al., 2002). For example, in DGGE profiles, the source (band) contributing to the greatest variability can be statistically determined, then the bands can be extracted from the gel, and their nucleotide sequences determined to identify specific components of the bacterial population.

The results here obtained are shown in Fig 5.8.

PCA analysis, while confirming the results discussed above, gives further information on the composition and dynamic of the microbial community of these soils. Fig 5.8A shows the behavior of the principal components, as revealed by the DGGE band distribution, of the population living in the soil inside the buffer zone. It is evident that the surface soil of this strip, characterized by higher number of species, shows a distinct collocation in the panel, thus confirming that the microflora living in this soil layer received a quite different selective pressure as compared to the other communities living deeper in the soil. However, the other two soil layers, although less clearly, tend to separate in different parts of the PCA box. The same behavior is not occurring in the soil outside the riparian strip. Indeed, Fig 5.8B shows that in this case the different soil layers are not clearly separated.

More interesting is Fig 5.8C, showing in the same box the PCA analysis of all the samples. Once again the profiles related to the internal soil remain as definitely separate from those of the external ones and maintain an excellent separation among the three soil layers.

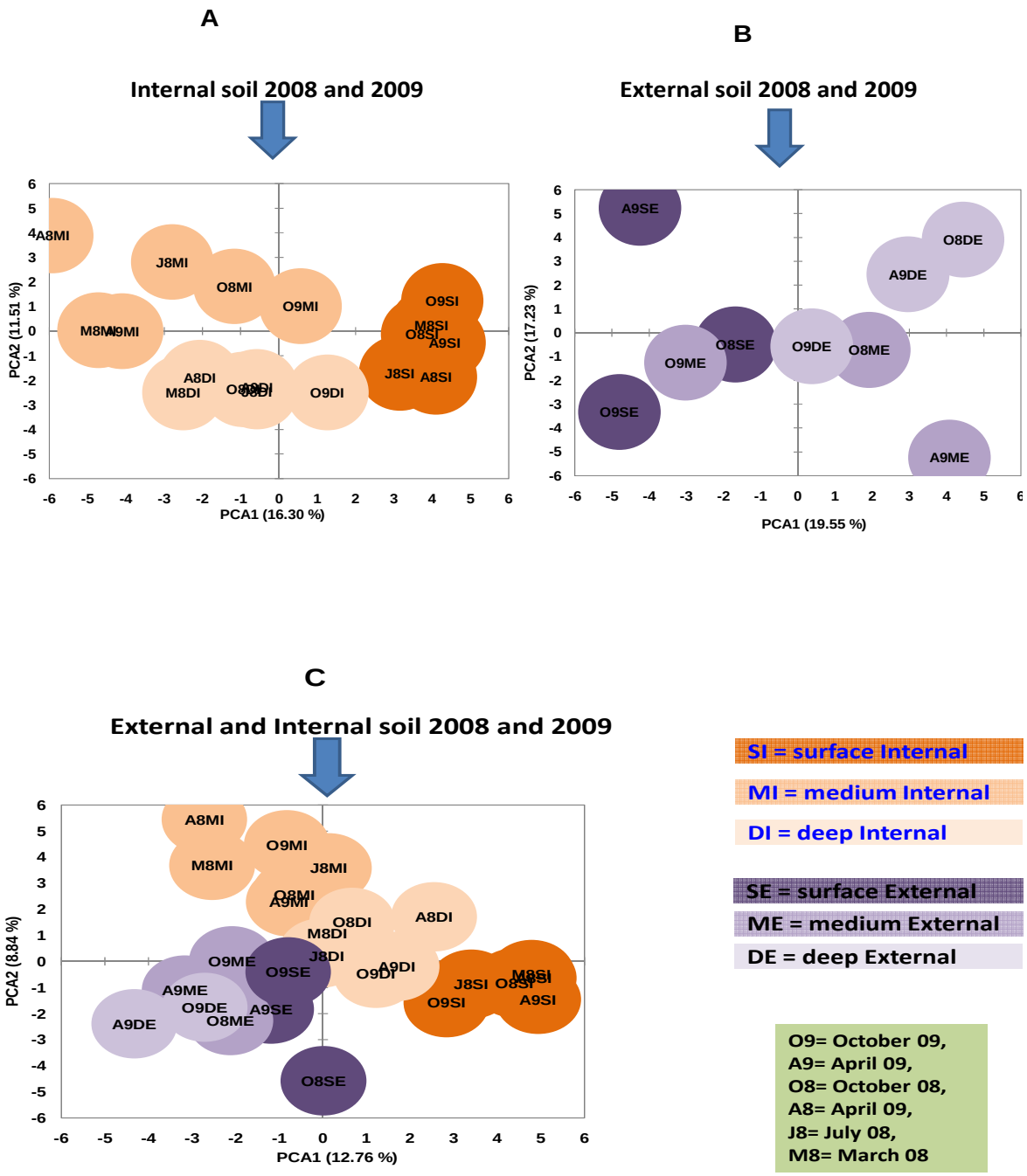


Fig 5.8. Principal component analysis based on DGGE band distribution. Fig A, B and C indicate internal, external and both internal and external soils, respectively.

5.3.6. Diversity index of soil microbial communities

Shannon-Wiener Diversity Index (H) were determined for the microbial community of surface, medium depth and deep soil for both years 2008 and 2009, as shown in Table 5.2. Nübel et al. (1999) reported that the number of bands and the Shannon–Wiener diversity index can be used as an estimate of the bacterial diversity. Shannon–Wiener diversity index (H) Shannon–Wiener diversity index provides a numerical indicator to evaluate the structure of the microbial community (Sigler and Turco, 2002). Since H is based on the entire proportional abundances of individual organism types (DGGE bands and their intensity), a decrease in H either implies a decrease in the number of organism types (bands) or a decrease in the proportional abundance of a given type (band intensity).

In the current study, Shannon– Wiener Index (H) were clearly indicating a decrease in the diversity in deep soil levels compared to the medium and surface ones in both internal and external samples. High microbial diversity was present in the internal soil as compared to the external soil. These values represent a further validation of the results described above, indicating that the microbial diversity is higher in the soil subjected to the assessment of the buffer strip as compared to an external soil. It is interesting to underline that in a normally managed soil such as the external one, the deeper layer shows a very reduced biodiversity, but on the other hand the particular hydrology applied to the buffer strip and especially the vegetation there introduced made the goal of strongly enhancing the microbial colonization of the deeper portion of the soil. Finally, the different distribution among the three layers is confirmed for both internal and external soils.

Sampling	Surface	Medium	Deep
		Diversity Index(H)	
Internal			
March 08	3.578	3.332	3.052
April 08	3.387	3.244	3.052
July 08	3.284	3.262	3.193
October 08	3.386	3.335	3.193
April 09	3.423	3.158	3.093
October 09	3.646	3.476	3.255
External			
October 08	3.299	3.151	2.639
April 09	3.152	3.107	2.838
October 09	3.299	3.204	2.920

Table 5.2. Shannon-Wiener Diversity Index for microbial communities of the internal and external soils.

6. Characterization of the microbial diversity in the water fluxes of the wooded riparian strip set up for nitrogen removal.

6.1. Introduction

Bacteria are recognized as important agents in biogeochemical processes in all aquatic ecosystems. It is well known that heterotrophic prokaryotes play relevant roles in the structure and dynamics of trophic web networks and in the remineralization of organic matter (Azam et al., 1983; Azam and Long**,** 2001). Surveys have already been performed in many marine environments, including oligotrophic open ocean (Fuhrman et al., 1993), coastal temperate (Kelly and Chistoserdov, 2001; Acinas et al., 2004) and marine sediments (Li et al., 1999; Bowman et al., 2003; Heijs et al., 2008). These studies have helped to discover the spatial distribution of bacterial populations (Sala et al., 2008; Vieira et al., 2008), improved the overall understanding of the global patterns of aquatic bacterial diversity (Pommier et al., 2007) and even helped to comprehend local and global biogeochemical processes (Zehr and Ward, 2002; DeLong and Karl, 2005).

The wooded riparian strips or buffer strips consist in wooded areas interposed between terrestrial and aquatic ecosystems. They play an important role in the regulation of nutrient dynamics and in particular in nitrogen removal. The nitrogen cycle strongly depends upon microbial activities and it is important to note that combined nitrogen is considered one of the most important limiting factors for plant growth. While nitrogen fixation can be considered as a positive activity for the whole ecosystem, denitrification may play a double role in relation to the context it takes place: while nitrogen removal from a NO_3^--fertilized soil is an absolute detrimental process, the gasification of nitrate from surface and subsurface water flows of terrestrial and aquatic ecosystems, may result as a very useful tool for reducing nitrate and nitrite pollution (Knowles, 1982).

Most of the heterotrophic denitrifiers prefer to use oxygen if it is present, but removal of nitrate via denitrification is assumed to take place typically under anoxic condition (Tiedje, 1989). For this reason, the most important factor to be investigated is the site hydrology which regulates the succession of anoxic and aerobic conditions together with nitrate input to the system, thus also affecting the distribution of bacterial communities in the riparian strip. Our understanding of microbial diversity in aquatic environments is still limited due to the highly variable physical and biogeochemical conditions. In this study, we determined the composition of bacterial assemblages in the water passing through the riparian strip and coming from Zero river during different seasons, using 16S rDNA sequences and DGGE

analysis. Our primary focus was to find spatial and seasonal patterns of bacterial communities and to gain an overall understanding of the bacterial diversity.

6.2. Materials and Methods

6.2.1. Sample collection

The particular structure of the experimental field described above (see experimental site section Fig 2.4), which is characterized by ridges and furrows, facilitates surface and sub-surface flows of water through the woodland strips. The water drains through the field from the irrigation ditch (located on a small ridge) towards the parallel network of drainage ditches. Water samples were collected on March 08 (M), April 08 (A) and July 08 (J) from the three different sites: Zero river (Z), irrigation ditch (S) and drainage ditch (E) (Fig 2.4). The water of the Zero river has a pH of 8 and chemical characteristics are given in Table 6.1.

6.2.2. Microbial enumeration

0.1 ml aliquots of the serial dilution (1:10) were dropped and spread onto the solid media (PCA) for determinations of total culturable aerobic bacteria. Petri-dishes were incubated aerobically at 30 °C. After 14 days the number and the morphology (shape, size, colour etc) of colonies were recorded. Representative colonies of different morphologies were isolated and stored in glycerol at -20° for molecular analyses.

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Sampling	Nitrate (N-No3) mg/L	Nitrite(N-No2)mg/L	Ammonium(N-NH4) mg/L	N Total mg/L	Dissolved Organic Carbon (DOC)mg/L	Chloride(Cl) mg/L	Sulphate(SO4) mg/L
March 08 (M)							
Zero river (Z)	1.80	0.03	0.29	2.50	2.80	11.20	51.70
Irrigation ditch (S)	1.70	0.03	0.01	2.10	3.70	11.90	47.20
Drainage ditch (E)	0.70	< 0,010	0.02	1.20	4.10	13.10	44.50
April 08 (A)							
Zero river (Z)	1.60	0.04	< 0.20	2.30	4.70	10.60	44.40
Irrigation ditch (S)	1.80	0.05	0.19	2.50	1.70	12.00	44.20
Drainage ditch (E)	0.35	0.01	0.10	1.20	3.60	12.80	40.30
July 08 (J)							
Zero river (Z)	1.70	0.10	0.07	2.50	5.90	11.40	33.40
Irrigation ditch (S)	1.50	0.05	0.15	2.20	3.80	12.50	30.00
Drainage ditch (E)	0.59	0.02	0.53	1.50	12.30	23.70	22.60

Table 6.1. Chemical properties of water at different sites in different seasons

6.2.3 DNA extraction from isolated colonies

DNA was extracted from single colony by alkaline lysis. Thus, one colony was suspended in an Eppendorf tube with 50 µL of lysis buffer (2.5 ml 10% sodium dodecyl sulfate, 5 ml 1 M NaOH, 92.5 ml MilliQ water). After 15 min the suspension was heated at 95°C, then the tube was centrifuged for 5 min at 13,000xg, the supernatant was transferred to a new tube and 90 µl MilliQ water was added. Extracted DNA was stored at -20°C for further molecular analyses.

6.2.4. DNA extraction directly from water

250 ml water was filtered through polyethersulfone filters (0.2µm pore size; Pall Corporation). 1/2 (half) filters was used to extract DNA using the Power Soil ™ DNA Isolation Kit (Mo Bio Laboratories Inc., USA). DNA isolation was performed according to the manufacturers' instructions, modified as follows: extra glass beads (0.15 – 0.30 g., bead size 0.1 mm) were added to the half filter and the cells were disrupted by bead beating (mini-bead beater™, Bio Spec products, USA). Final purification of the extracted DNA was performed using the Wizard® DNA clean-up system (Promega, USA).

6.2.5. Amplified ribosomal DNA restriction analysis (ARDRA) of culturable bacteria

(same as Chapter I, Material and Methods section)

6.2.6. Sequencing 16S rDNA from culturable bacteria.

(same as Chapter I, Material and Methods section)

6.2.7. DGGE analysis

(same as Chapter II, Material and Methods section)

6.2.8. Diversity index and principal component analysis (PCA)

(same as Chapter I, Material and Methods section).

6.3. Results and Discussion

A first morphological characterization was performed on the basis of the colony shape, color and margins as shown in Fig 6.1. Such a preliminary analysis demonstrated 18-25 different morphologies that are indicative of the abundant bacterial diversity among the isolates. The samples coming from the drainage ditches (E) (Fig 6.1) showed higher microbial diversity. Seasonal fluctuations of culturable microbial populations are reported in Fig 6.2. The number of CFU seems to increase from March 08 to July 08, especially for the drainage ditch. As expected, the values related to the Zero river and the irrigation ditches are not significantly different.

Discussion 1- The microbial diversity of the water coming from the drainage ditch seems to be higher as compared to the river water and to the irrigation ditches. It was expected, at least in some extent, because the output water could contain microbial populations also collected during the transit through the soil.

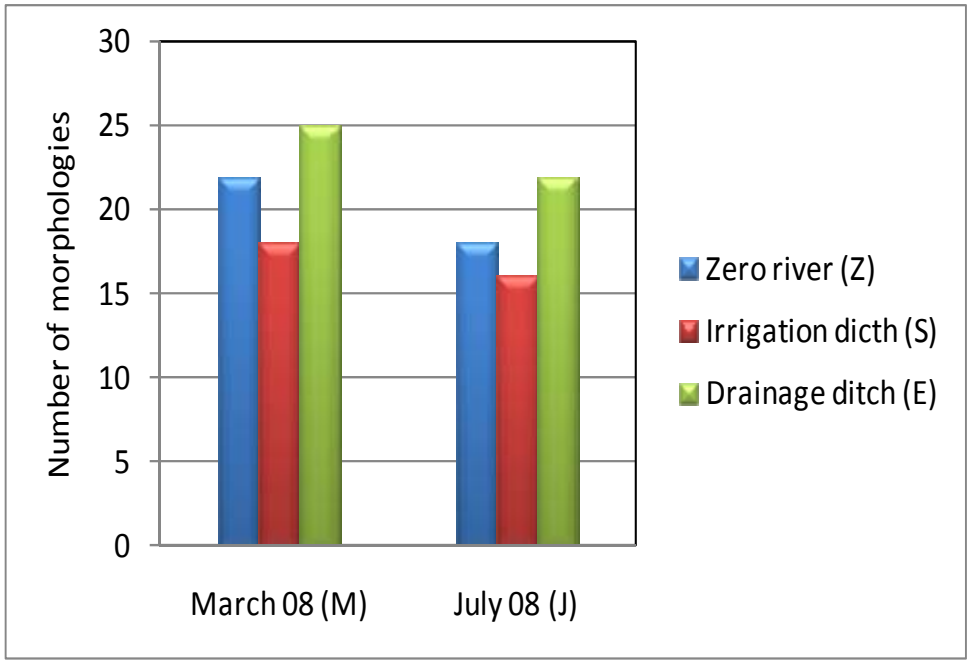


Fig 6.1. Number of morphologies of culturable bacteria of water in the buffer strips at three sites.

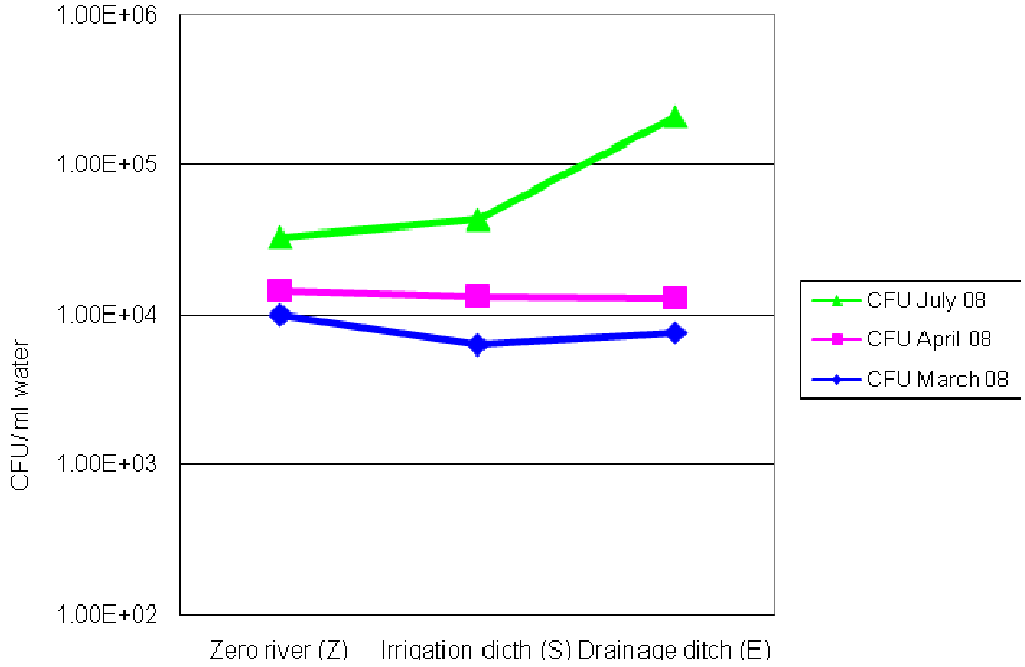


Fig 6.2. Colony forming units/ ml water at different sites in different seasons.

6.3.1. Amplified ribosomal DNA restriction analysis (ARDRA)

To investigate on microbial diversity of the culturable bacteria present in the water flux of the wooded riparian strip, several hundred colonies were isolated from March 08 (M) and

July 08 (J) at the three different sites: Zero river (Z), irrigation ditch (S) and drainage ditch (E). DNA was extracted from the isolated colonies and 16S rDNA was amplified by PCR. Amplified 16S rDNA was digested by two restriction enzymes (*Hinf*I and *Hpa*II). A variety of different DNA fragment patterns were revealed. ARDRA patterns, clustered by BioNumerics version 4.5 software, discerned high numbers of OTUs in both seasons. The 16S rDNA clustering consisted of 83 and 85 colonies and comprised 57 and 56 different ARDRA patterns in water samples of March 08 and July 08, respectively.

6.3.2. The sequences of 16S rDNA of culturable bacteria

For sequencing of 16S rDNA of culturable bacteria, total soil and water sample were clustered together (Appendix.1). Then representative colonies of each unique ARDRA pattern were sequenced. A list of 16S rDNA sequences of culturable bacteria are reported in Table 6. 2 according to their source, closest relatives, similarity percentage, accession number, phylogenetic group, and inherited blast name as identified by using the BLAST program in the GenBank databases. All of the culturable bacteria sequenced (except one isolate) had sequences that were less than 5% different from those in the current databases.

Isolate and Isolation sources	Closest relative	% Similarity	Accession number	Phylogenetic group(division, subdivision)	Inherited blast name
Z1, MZ	*Duganella zoogloeoides*	98	NR_025833	Betaproteobacteria	b-proteobacteria
E6, ME	*Zoogloea ramigera*	93	X74914	Betaproteobacteria	b-proteobacteria
S2, MS	*Janthinobacterium sp.*	100	FJ006906	Betaproteobacteria	b-proteobacteria
S36, MS	*Exiguobacterium undae*	100	DQ019165	Firmicutes	firmicutes
E37, ME	*Aeromonas sp.* AKB-2008-	100	AM989245	Gammaproteobacteria	g-proteobacteria
S7, MS	*Aeromonas punctata*	100	FJ646664	Gammaproteobacteria	g-proteobacteria
E10, ME	*Agreia pratensis*	100	NR_025460	Actinobacteria	high GC Gram+
E14, ME	*Sphingomonas azotifigens*	98	AB217472	Alphaproteobacteria	a-proteobacteria
E4, ME	*Pedobacter alluvionis*	99	EU030688	Sphingobacteria	CFB group bacteria
E18, ME	*Labedella kawkjii*	99	DQ533552	Actinobacteria	high GC Gram+
Z72, MZ	*Plantibacter flavus*	100	NR_025462	Actinobacteria	high GC Gram+
S50, MS	*Subtercola sp.* FB10	99	AM940948	Actinobacteria	high GC Gram+
E53, ME	*Brevundimonas sp.* GOBB3	99	AF321047	Alphaproteobacteria	a-proteobacteria
E19, ME	*Nocardioides sp.* Tibet-IIR12	99	DQ177472	Actinobacteria	high GC Gram+
S18, MS	*Pseudomonas libanensis*	98	NR_024901	Gammaproteobacteria	g-proteobacteria
E21, ME	*Flavobacterium sp.* AKB	99	AM988928	Flavobacteria	CFB group bacteria
E55, ME	*Xanthomonas cynarae*	100	AF208315	Gammaproteobacteria	g-proteobacteria
S9, MS	*Acinetobacter johnsonii*	100	EU275352	Gammaproteobacteria	g-proteobacteria
S35, MS	*Flavobacterium sp.* WB4.3-51	98	AM934670	Flavobacteria	CFB group bacteria
S60, MS	*Klebsiella oxytoca*	99	AB053117	Gammaproteobacteria	enterobacteria

Z9, MZ	*Terrabacter sp.* DFA1	100	AB180233	Actinobacteria	high GC Gram+
Z66, MZ	*Xanthomonas theicola*	100	Y10763	Gammaproteobacteria	g-proteobacteria
Z8, MZr	*Rheinheimera sp.* W2	99	EU794394	Gammaproteobacteria	g-proteobacteria
Z60, MZ	*Janthinobacterium sp.* Man12	99	AY788973	Betaproteobacteria	b-proteobacteria
Z7a, MZ	*Tiedjeia arctica*	99	DQ107523	Gammaproteobacteria	enterobacteria
Z19, MZ	*Citrobacter farmeri*	98	EU030438	Gammaproteobacteria	enterobacteria
S1, MS	*Acidovorax sp.* PPs-5	99	FJ605421	Betaproteobacteria	b-proteobacteria
E56, ME	*Bosea sp.* AKB-2008-KK9	98	AM988982	Alphaproteobacteria	a-proteobacteria
E24, ME	*Sphingomonas sp.* BR12262	98	FJ455075	Alphaproteobacteria	a-proteobacteria
Za34, JZ	*Streptomyces sp.* 331H08	96	AB124219	Actinobacteria	high GC Gram+
Sa29, JS	*Enterobacter sp.* 253a	100	AY082447	Gammaproteobacteria	enterobacteria
Ea24, JE	*Pantoea vagans*	99	EF688012	Gammaproteobacteria	enterobacteria
Ea8, JE	*Pseudomonas cannabina*	100	NR_025550	Gammaproteobacteria	g-proteobacteria
Ea36, JE	*Microbacterium sp.* VTT E-	100	EU438940	Actinobacteria	high GC Gram+
Sa41, JS	*Rahnella sp.* BIHB 783	99	DQ885948	Gammaproteobacteria	enterobacteria
Za6, JZ	*Sphingomonas sp.* AKB-2008-	99	AM989050	Alphaproteobacteria	a-proteobacteria
Sa42, JS	*Arthrobacter sp.* HK-2	100	FJ477042	Actinobacteria	high GC Gram+
Ea29, JE	*Microcella putealis*	97	AJ717388	Actinobacteria	high GC Gram+
Ea30, JE	*Paenibacillus sp.* HDDMM03	100	EU723825	Firmicutes	firmicutes
Ea37, JE	*Sphingobacter sp*	99	EU723088	Sphingobacteria	CFB group bacteria
Za14, JZ	*Pedobacter borealis*	98	EU030687	Sphingobacteria	CFB group bacteria
Ea15, JE	*Curtobacterium flaccumfaciens*	98	DQ512787	Actinobacteria	high GC Gram+
Sa45, JS	*Curtobacterium sp.* K6-02	99	EF612296	Actinobacteria	high GC Gram+
Ea47, JE	*Arthrobacter sp.* 4C1-b	98	AM409362	Actinobacteria	high GC Gram+
Za28, JZ	*Chryseobacterium sp.* H-6-6	98	AB164637	Flavobacteria	CFB group bacteria
Za8, JZ	*Chryseobacterium piscium*	97	DQ862541	Flavobacteria	CFB group bacteria
Za7, JZ	*Chryseobacterium indoltheticum*	99	AY468444	Flavobacteria	CFB group bacteria
Sa3, JS	*Kocuria palustris*	100	NR_026451	Actinobacteria	high GC Gram+
Ea10, JE	*Chryseobacterium sp.* CI44	100	DQ530096	Flavobacteria	CFB group bacteria
Ea1, JE	*Curtobacterium sp.* Fon07	99	AY788956	Actinobacteria	high GC Gram+
Ea46, JE	*Flavobacterium johnsoniae*	97	DQ256490	Flavobacteria	CFB group bacteria
Ea45, JE	*Pantoea agglomerans*	99	FM202484	Gammaproteobacteria	enterobacteria
Ea6, JE	*Burkholderia caryophylli*	98	AM184283	Betaproteobacteria	b-proteobacteria
Ea34, JE	*Acinetobacter calcoaceticus*	99	AY346314	Gammaproteobacteria	g-proteobacteria
Za38, JZ	*Caulobacter sp.* AKB-2008-	99	AM989012	Alphaproteobacteria	a-proteobacteria
Sa21, JS	*Caulobacter sp.*	100	AJ227777	Alphaproteobacteria	a-proteobacteria
Sa31, JS	*Aeromonas veronii*	99	FJ940834	Gammaproteobacteria	g-proteobacteria
Sa16, JS	*Acidovorax sp.* 75	99	EU304287	Betaproteobacteria	b-proteobacteria
Ea16, JE	actinobacterium kmd_323	99	EU723164	Actinobacteria	high GC Gram+
Za13, JZ	*Kurthia gibsonii*	100	EF204299	Firmicutes	firmicutes
Za15, JZ	*Arthrobacter sp.* AE05102002	100	AM260537	Actinobacteria	high GC Gram+
Sa18, JS	*Microbacterium sp*	99	AB362420	Actinobacteria	high GC Gram+
Sa32, JS	*Sphingobacterium sp.* F1	98	AF380159	Sphingobacteria	CFB group bacteria
Za35, JZ	*Sphingomonas sp.* lxb-8	99	GQ249218	Alphaproteobacteria	a-proteobacteria
Sa34, JS	*Agrobacterium sp.* RF-152	99	GQ205108	Alphaproteobacteria	a-proteobacteria

Table 6.2. Analysis of isolates from water samples on the basis of partial 16S rDNA sequences, including the closest relatives as identified by BLAST program in the GenBank databases.(MZ = March-Zero river, MS = March-irrigation ditch, ME = March-drainage ditch, JZ = July-Zero river, JS = July-irrigation ditch, JE = July-drainage ditch).

6.3.3. Distribution of culturable community

The Phylotypes distributions of the bacterial community were reconstructed from different sites of March 08 and July 08 water samples as shown in Table 6.3. 16S rDNA sequences Six major bacterial lineages, namely Firmicutes, Gammaproteobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria and Flavobacteria, seem to be present in MZ and JZ samples, with a seventh one, Sphingobacteria, present only in the JZ samples. The Gammaproteobacteria were the most dominant in both seasons with high percentage of colonies (58.6% in MZ and 32.1% in JZ) and OTUs (40.1% in MZ and 22.7% in JZ) (Table `6.3). With some differences, the same groups were also present in the MS and AS samples (irrigation ditches).

Concerning the drainage ditches, ME samples exhibited six major bacterial phylogenetic affiliations with Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, Flavobacteria and Sphingobacteria, while JE samples showed also members of the Firmicutes and of an unclassified group bacteria (Table 6.3).

Sampling	Divisions	Zero river (Z)		Irrigation ditch (S)		Drainage ditch (E)	
		OTUs %	Colonies %	OTUs %	Colonies %	OTUs %	Colonies %
March 8 (M)	Firmicutes	13.6	10.3	6.3	3.3	0	0
	Actinobacteria	22.7	17.2	18.8	10	23.5	20.8
	Alphaproteobacteria	4.5	3.5	0	0	11.8	16.7
	Gammaproteobacteria	40.1	58.6	50	66.7	29.4	33.3
	Betaproteobacteria	4.5	3.4	12.5	13.3	11.8	12.5
	Flvobacteria	9.1	6.9	12.5	6.7	17.7	12.2
	Sphingobacteria	0	0	0	0	5.9	4.2
	Unclassified bacteria	0	0	0	0	0	0
July 08 (J)	Firmicutes	13.6	10.7	14.3	20	9.1	6.2
	Actinobacteria	22.7	21.4	19.0	16	22.7	15.6
	Alphaproteobacteria	9.1	7.1	14.2	12	4.5	3.1
	Gammaproteobacteria	22.7	32.1	33.3	36	31.8	40.6
	Betaproteobacteria	4.5	3.5	4.7	4	9.1	18.7
	Flvobacteria	22.7	21.4	0	0	9.1	6.2
	Sphingobacteria	4.5	3.5	4.7	4	9.1	6.2
	Unclassified bacteria	0	0	9.5	8	4.5	3.1

Table 6.3. Phylotypes distributions of culturable communities of water samples in March and July 08.

Discussion 2- It is interesting to note that Gammaproteobacteria are much more represented, in terms of different species, as compared to the analysis related to the soil, as described in the previous sections (Chapter 4). This may indicate that the water flow from the Zero river uses to abundantly carry several bacterial species that are originally absent into the riparian soil. Since Gammaproteobacteria, together with some important phototrophic species, include also a number of potentially pathogens, it may represent a possible indication of the degree of contamination of the river. Of course, in order to confirm such a hypothesis, learning much more on the nature of such a population at species level would be strongly required. However, it is worthy of notice the fact that the drainage ditches seem to contain a lower amount of bacteria belonging to this class, at least for the March season, thus suggesting a possible role of the buffer strip in the biological abatement of water contamination. Since this is not happening with the same trend in the July samples, it would be important to verify whether this action is a casual effect or it is linked to the seasonal variations.

Finally, no significant content and oscillation of Actinobacteria can be observed for the three different water sampling (Z, S and E), and that Firmicutes seem to be reduced in the drainage ditch water. This last observation may be explained by considering that a fraction of the bacterial population the river water uses to convey may have a soil origin and would find back in the soil a suitable ecological niche to colonize.

6.3.4. Phylogenetic analysis of culturable bacteria of water samples

Phylogenetic analysis of 16S rDNA sequences of March and July 08 water samples are outlined in Fig 6.4 and Fig 6.5. As an example, the 16S rDNA sequences of Firmicutes of March 08 samples revealed that the genera *Bacillus, Exiguobacterium* and *Kurthia* were found from MZ and MS (Fig 6.4). On the other hands, in July 08 samples, the genera *Bacillus* and *Paenibacillus* were found from culturable isolates at three sites (Fig 6.5). The genus *Bacillus* was common in both seasons (March and July 08), while *Exiguobacterium* (from MZ) and *Kurthia* (from MS) were found only in March 08, and *Paenibacillus* only in JE.

All these results give an insight of the community composition for each class of bacteria allowing to verify the presence or the absence of the most common bacterial groups. Some interesting examples can be extracted from these results, supporting in some extent the

considerations made in the previous section (see par. 6.3.3). For instance, the genus *Xanthomonas* in the gamma subdivision consists of 27 plant-associated species, many of which are conducive of important diseases on at least 124 monocot species and 268 dicot species, including fruit and nut trees, solanaceous and brassicaceous plants, and cereals (Hayward, 1993). Moreover, individual species comprise multiple pathogenic variants (pathovars, pv.) able to cause a variety of symptoms including necrosis, cankers, spots, and blight, and they affect a variety of plant parts including leaves, stems, and fruits. Interestingly, as shown in Fig 6.4 this genus is well represented in March, but it is completely absent in July, thus suggesting a reduction of the contamination of the river or, possibly, a sort of decontamination role of the buffer zone. The same observations can be drawn for the genus *Aeromonas* that includes fourteen species, most of which have been associated with human diseases. Even this genus is well represented in March and reduced in July. On the other hands, the genus *Pantoea*, generally isolated from plant surfaces, seeds, soil, and water, as well as from animals and human wounds, blood, and urine, seems to be sporadically present in March and to enrich the water flux in July.

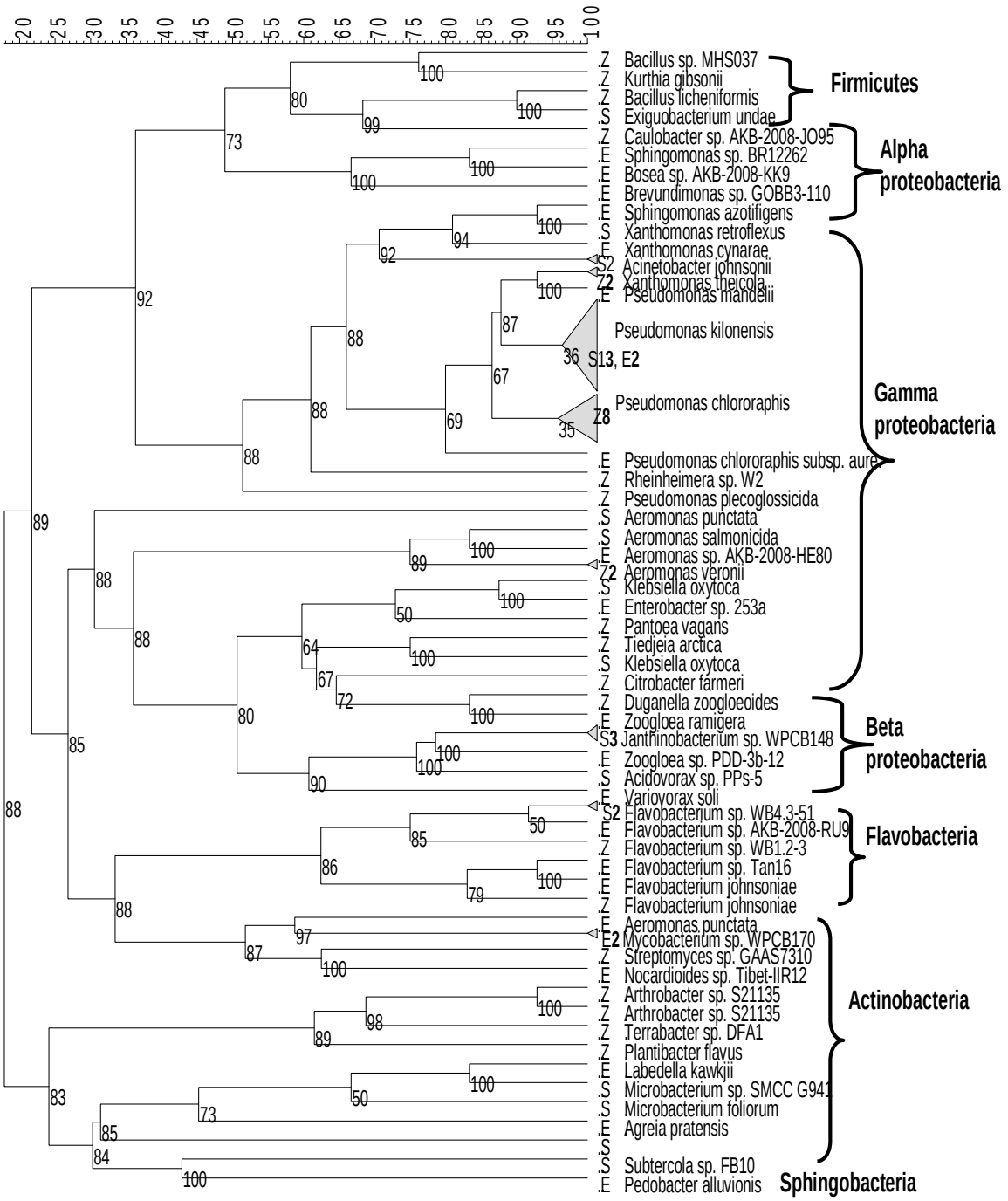


Fig 6.4. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of bacteria isolated from March 08 water samples and selected at random. Sequences marked as Z are those from Zero river, S from irrigation ditch and E from drainage ditch. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of sequences in their respective isolates.

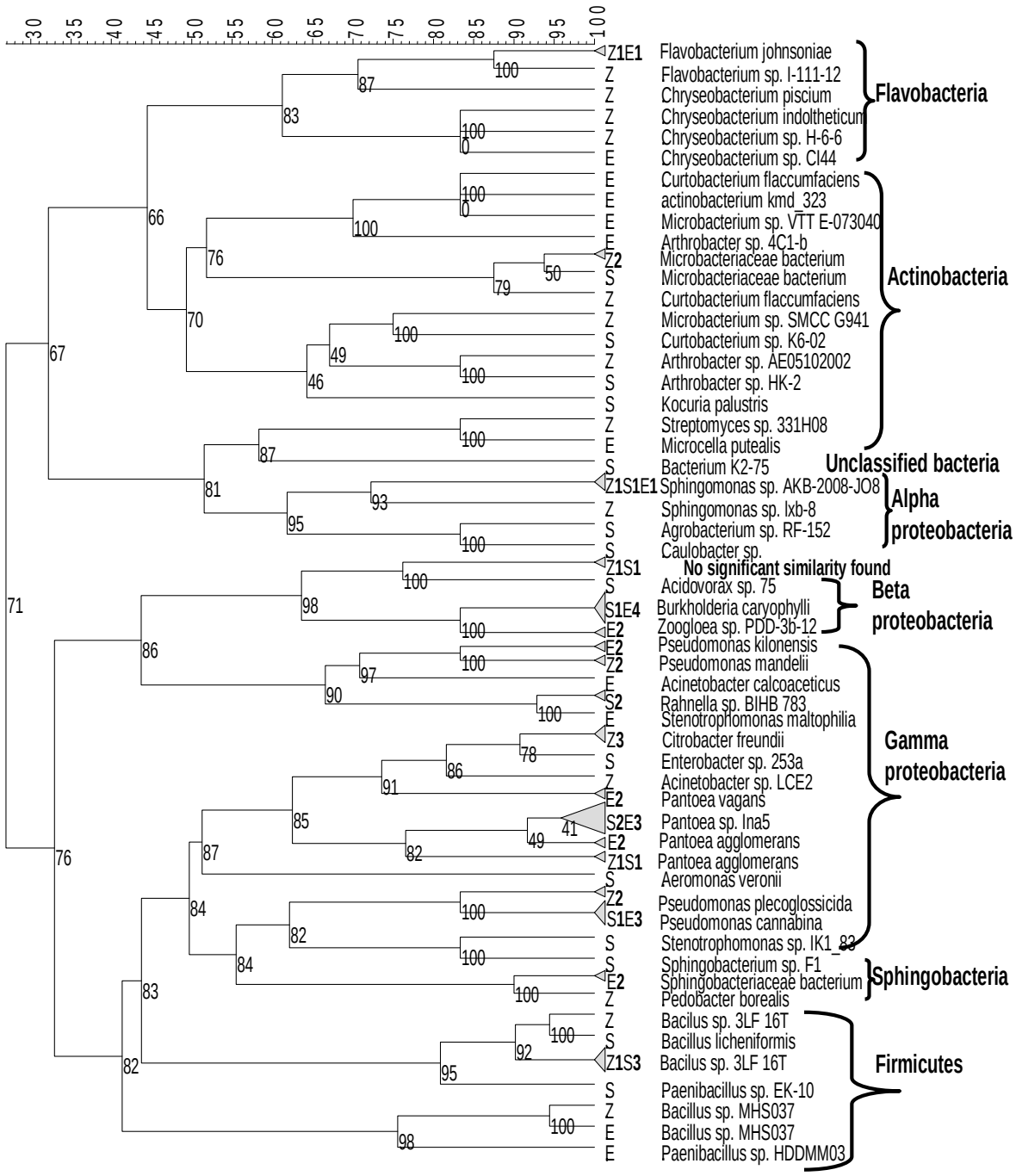


Fig 6.5. Neighbor-joining tree showing phylogeny of 16S rDNA sequences of bacteria isolated from July 08 water samples and selected at random. Sequences marked Z are those from Zero river, S from irrigation ditch and E from drainage ditch. Bar indicates 5% sequence divergence. Aligned sequences were 1389 bp in length. Additional number shows the number of sequences in their respective isolates

6.3.5. DGGE analysis of the microbial community of water

The 16S rDNA variable region V6-V8 was used in DGGE analysis to identify the total microbial communities of March 08 (MZ, MS and ME), April 08 (AZ, AS and AE) and July 08 (JZ, JS and JE) in water and the results are shown in Fig 6.6. Some common bands and some dissimilar bands where obtained from the water samples coming from different sites. Band positions were measured by percentage of denaturant concentration through BioNumerics version 4.5 software. 22-26 bands in drainage ditches (ME, AE and JE), 12-24 bands in irrigation ditches (MS, AS and JS) and 16-21 bands in Zero river (MZ, AZ and JZ) were found, indicating higher microbial diversity for the water of the drainage ditches as compared to irrigation ditches and Zero river. Once again, this confirms the bacterial enrichment of the water flux during the transit throughout the buffer strip soil.

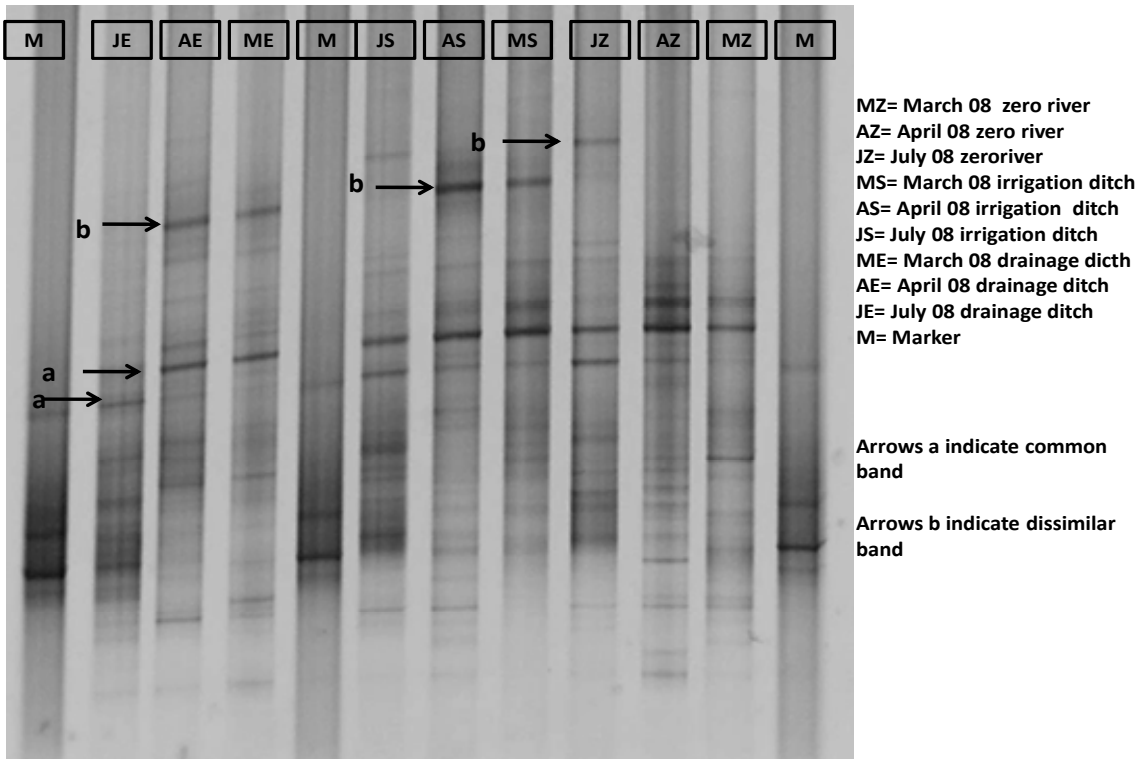


Fig 6.6. DGGE analysis of water samples at different sites and in different seasons

6.3.6. Comparison of microbial communities in water at different seasons and sites

DGGE profiles were clustered by BioNumerics software and three main clusters where found, as shown in Fig 6.7. The main difference was observed between the profiles of July 08 and all other samples. Indeed, July 08 samples clustered separately with about 25% similarities. The profile of samples AZ and MZ fall into the same cluster and those of AE, AS, ME, and MS were included into another cluster. These observations confirm that in July the composition of the microbial community of the water flux is changed.

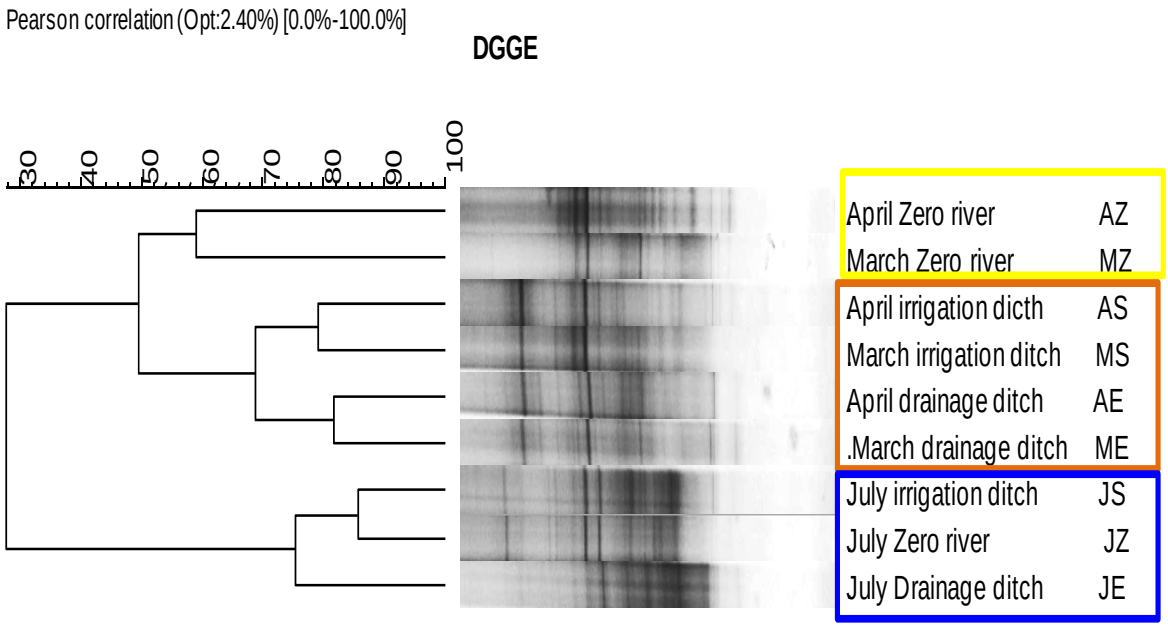


Fig 6.7. Comparison of the bacterial community compositions in different sites of the riparian strip by cluster analysis of DGGE profiles prepared with 16S rDNA fragments (V6-V8). The dissimilarity matrix for each sample was determined using Pearson correlation, and clustering was performed by BioNumerics. The highest dissimilarity value between samples was set at 100%.

6.3.7. PCA analysis

PCA was analyzed on the basis of 16S rDNA Phylotypes distribution and the results are reported in Fig 6.8. PCA1 shows a variability of 33.35% and PCA2 of 25.61%. Drainage ditches (JE and ME) were well separated from irrigation ditches (JS and MS) and Zero river (JZ and MZ) in both seasons. This analysis confirms once again that a distinct microbial community was present at the drainage ditches as compared to the Zero river and irrigation ditches water, at both the seasons.

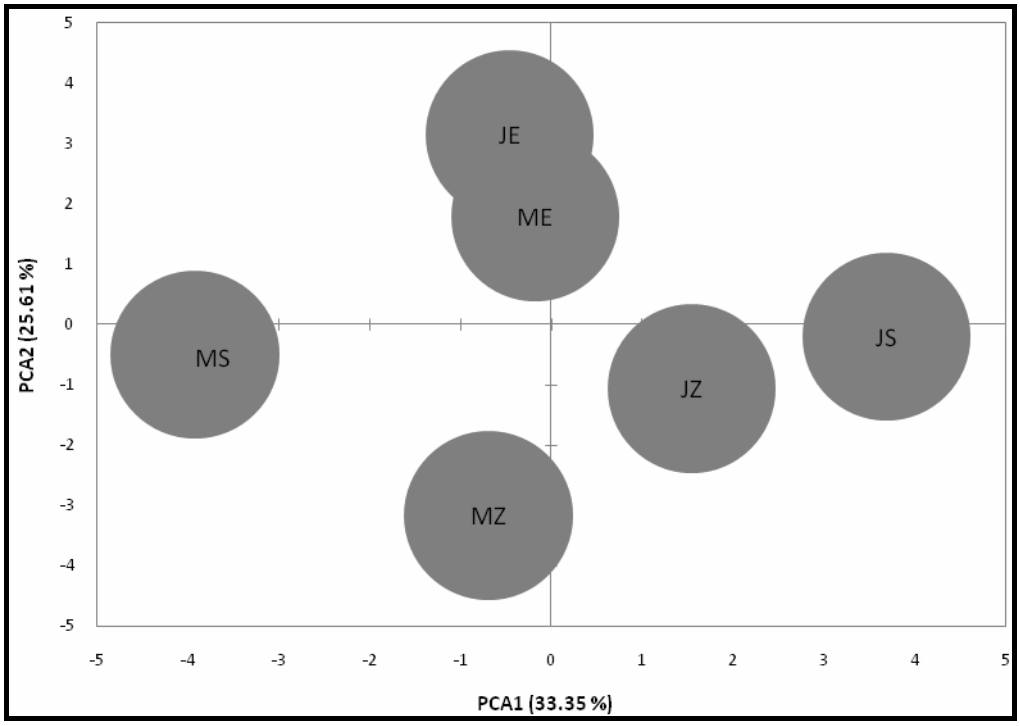


Fig 6.8. PCA biplot of the 16S rDNA band patterns from water samples from all seasons and sites. Samples of March from Zero river, irrigation ditch and drainage ditch are represented by MZ, MS and ME and samples of July by JZ, JS and JE, respectively. Gray circles indicate the Phylotypes distribution. The percentages in the axis labels represent the percentage of variation explained by the principal coordinates.

PCA analysis was also performed for the chemical properties of water samples from all seasons and sites, as shown in Fig 6.9. The results show that there were no significant differences between Zero river and irrigation ditches among all the seasons (March, April and July 08) although drainage ditches were well separated from irrigation ditches and Zero river. These results suggested that a particular variation of the chemical properties occurred in the drainage ditches as compared to the irrigation ditches and the Zero river, particularly during the hot season.

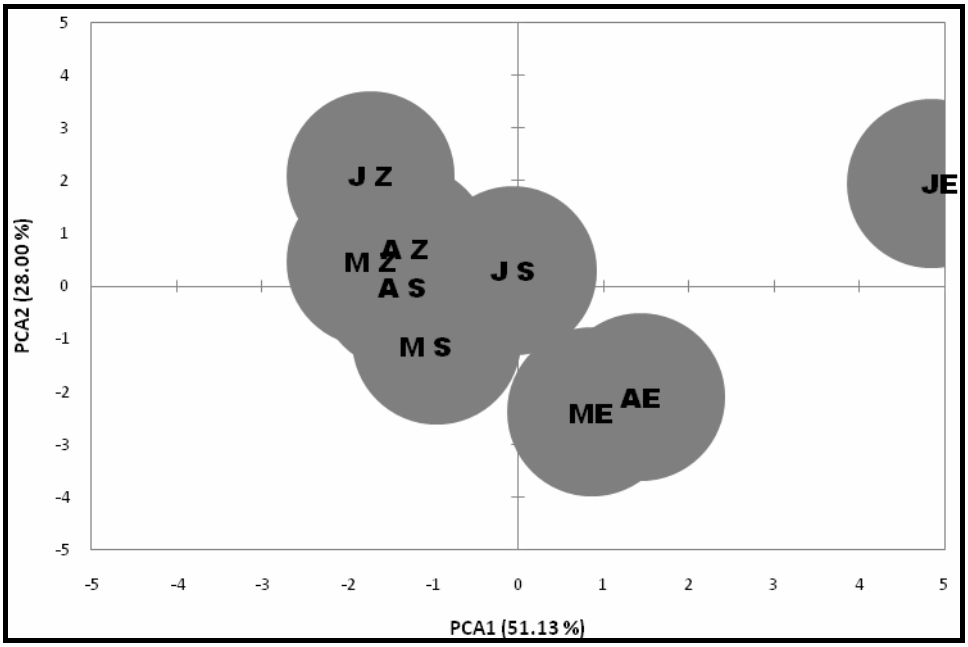


Fig 6.9. PCA biplot of the chemical properties of water from all seasons and sites. Samples from Zero river, irrigation ditch and drainage ditch of March 08 are represented by MZ, MS and ME, those of April 08 by AZ, AS and AE and samples of July 08 by JZ, JS and JE, respectively. Gray circles indicate the Phylotypes distribution. The percentages in the axis labels represent the percentage of variation explained by the principal coordinates.

6.3.8. Diversity index

Shannon-Wiener Diversity Index (H) was determined for Zero river, irrigation ditches and drainage ditches water of March and July 08, as shown in Table 6.4. These values indicate an increase in the diversity for the drainage ditches as compared to the irrigation ditches and the Zero river, for both the seasons. Simpson's dominance index (D) was also calculated for March and July 08. Simpson's dominance index values were lower at drainage ditches. A decreased species evenness index and increased species dominance index were recorded for Zero river and irrigation ditches as compared to that of drainage ditches. These results further confirm that a higher microbial diversity is present in the drainage ditches as compared to Zero river and irrigation ditches, and this is a constant for all the seasons taken into account

Sampling names	Shannon-Wiener Diversity Index	Simpson Diversity Index
March 08		
Zero river (MZ)	1.50	0.27
Irrigation ditch (MS)	1.35	0.32
Drainage ditch (ME)	1.68	0.20
July 08		
Zero river (JZ)	1.76	0.19
Irrigation ditch (JS)	1.81	0.20
Drainage ditch (JE)	1.97	0.17

Table 6.4. Diversity Index of culturable bacterial communities of water at three different sites.

6.4. Further considerations

The bacterial diversity of water in the riparian buffer zone was investigated by both culture dependent (conventional) and culture-independent molecular methods. The culture-independent molecular approaches based on small-subunit rDNA have also been used for studies of microbial ecology in freshwater sediments (Tamaki et al., 2005; Altmann et al., 2003; MacGregor et al., 2001; Purdy et al., 2003; Spring et al., 2000). In the current study, morphological analysis of culturable colonies demonstrated diverse forms, colors, margins and shapes suggesting a great bacterial diversity among the isolates. The analysis of culturable bacteria suggested that fluctuations are also depending upon seasonal factors, thus supporting previous studies (Lindstrom et al. 2005).

The chemical properties of water samples showed lower nitrogen content in the drainage ditches than Zero river and irrigation ditches, whereas higher amount of organic carbon was found in the drainage ditches (4.1-12.3 mg/l) compared to irrigation ditches (1.7-3.8 mg/l) (Table 6.1). These data suggest that nitrogen compound may be reduced in drainage ditches by denitrification process and carbon compounds may play a vital role to enhance denitrification process. In agreement with our results, several studies reported that carbon compounds can be used as the substrates by denitrifying bacteria (Kutako et al., 2009; Sobieszuk and Szewczyk, 2006; Shrimali and Singh, 2001; Ferguson, 1994; Mateju et al., 1992).

In the current study, a 16S rDNA based molecular approach was also applied to characterize the total microbial population of water samples at different sites in the riparian buffer zone

Sekiguchi et al (2002) reported that 16S rDNA sequences affiliated to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes and Actinobacteria were most abundant in water. In this study, six to seven Phylotypes divisions from Zero river, five to seven divisions from irrigation ditches and six to eight divisions from drainage ditches were found in the March and July 08 water samples respectively (Table 6.3). Overall results showed that bacterial divisions are slightly high in July 08 water sample, especially in JE (drainage ditch of July 08). This could be explained with the fact that drainage ditches can pick up soil sediments from runoff water flowing over the soil surface. Feng et al. (2009) reported that more microflora was present in the sediment compared to water samples.

Gammaproteobacteria were the most dominant division at Zero river and irrigation ditches water samples in both seasons. Similarly, other reports showed that Gammaproteobacteria, which are known to be water organisms, formed large clusters in all of the sea areas surveyed (Fuhrmanet al., 1993; Suzuki et al., 1997; Acinas et al., 1999; Crump et al., 1999). Betaproteobacteria is commonly detected in freshwater lakes worldwide, where they are the most numerically dominant cell and clone type (Mueller-Spitz et al., 2009; Zwart et al., 2002). Interfaces between riverine and marine systems also appear to be dominated by Betaproteobacteria (Crump et al., 1999; Cottrell et al., 2005). In this study, the Betaproteobacteria were slightly high in JE samples compared to JZ and JS samples indicating that the higher presence of Betaproteobacteria is due to the influence between soil and water on the structure of bacterial community.

The phylogenetic analysis of Proteobacteria division in March 08 water samples showed that some genera seem to be a distinctive for a specific site. For example, the genera *Duganell, Citrobacter, Tiedjeia, Rheinheimera* and *Caulobacter* were found only in MZ water sample; *Acinetobacter, Klebsiella, Janthinobacterium* and *Acidovorax* in MS water sample, and *Bosea, Brevundimonas*, *Enterobacter, Zoogloea* and *Variovorax* in ME water sample (Fig 6.4). Interestingly, some isolates from drainage ditch (ME) appeared as soil microorganism. In this study, *Bosea* and *Variovorax* were isolated from riparian soil. Falk et al. (2010) classified *Bosea sp* as denitrifying and *Variovorax* as a soil bacterium frequently associated with important biodegradation processes in nature.

Actinobacteria were the second abundant division in March 08 and July 08 water samples at three different sites. *Arthrobacter and Streptomyces* were common at Zero river and the

genus *Microbacterium* was more common at irrigation ditches and drainage ditches in both seasons. It was not possible to find a common genus in both seasons at three different sites. For example, in Zero river, *Terrabacter and Plantibacter* were found only in MZ samples, while *Curtobacterium and Microbacterium* were found only in JZ. Likewise, in irrigation ditches, *Subtercola* was only found in MS samples and *Curtobacterium, Arthrobacter* and *Kocuria* were found only in JS water samples. Moreover, in drainage ditches, *Aeromonas, Nocardioides* and *Labedella* were found only in ME samples, and *Curtobacterium, Actinobacterium, Arthrobacter* and *Microcella* were found in JE samples (Fig 6.4 and Fig 6.5). These evidences agree with previously published works on the average abundance of 16S rDNA sequence of Actinobacteria, originating from diverse water (Jensen et al., 2005, 2007; Jiang et al., 2008).

Bacterial subdivision Flavobacteria with *Flavobacterium sp.* and *Chryseobacterium sp.* were found both in March and July 08 water samples at the sites (Zero river and drainage ditch) (Fig 6.4 and Fig 6.5). These distributions of Flavobacteria community could confirm the fact that soil certainly contributes significantly to the Flavobacteria richness and abundance in river water. Similarly, von Wintzingerode et al (1997) assumed that the Flavobacteria populations are universal populations, spreading from soil to aquatic environments.

The bacterial subdivision Sphingobacteria with *Sphingobacterium* and *Pedobacter* were more abundance at three different sites in July 08 water samples compared to March 08 water samples. For example, Sphingobacteria with *Pedobacter sp* represented 4.2% of the bacterial community in ME water samples (Fig 6.4). On the other hand, Sphingobacteria were found in sites JZ, JS and JE water samples and shared common lineages with genera *Sphingobacterium* and *Pedobacter* (Fig 6.5).

DGGE band numbers suggest that drainage ditches seem to be more diverse compared to irrigation ditches and Zero river at different seasons. Cluster analysis of the DGGE profiles (Fig 6.7) demonstrated that a distinct microbial community was present in the July 08 water sample compared to March 08 and April 08 water samples. There were no significant differences between irrigation ditches and drainage ditches, although a significant difference was found from irrigation ditches and drainage ditches by PCA based on 16S rDNA of the culturable fraction. So, it is reasonable to speculate that culturable bacteria are

metabolically more active and they can actively work on nitrate reduction. Nitrogen removal may be directly influenced by soil type, watershed hydrology (e.g., soil saturation, groundwater flow paths, etc.) and drainage ditches biogeochemistry (decomposition of plant materials, organic carbon supply, high NO_3^- inputs) through cumulative effects.

Diversity index was calculated by two alternative ways for comparative diversity analysis. Higher Shannon–Wiener or lower reciprocal Simpson's index values describe a community with greater numbers of species and a more even distribution of species (Gomez-Alvarez et al., 2007). An increased species evenness index and decreased species dominance index was found in drainage ditches in comparison to irrigation ditches and Zero river (Table 6.4). The bacterial diversity appears to be much more complex in drainage ditches than that of other water samples.

7. CONCLUSIONS

Agricultural practices are specifically directed toward the achievement of the conditions required for optimal plant growth. The use of rationalized water supply and fertilizers, and especially the treatments operated on an agricultural soil, are part of a strategy to pursue the goal. However, a main role in the soil fertility achievement is played by soil microorganisms that can properly operate if allowed to live in a suitable environment. Their activities can support plant health and growth, making available many nutrients and contributing to make suitable the physical structure of the soil for root development.

If the main objective of soil management is not the cultivation of plants of agricultural interest, but the establishment of a buffer zone specifically designed to remove nitrogen excess from a water flux, the soil management strategy may change in order to take into account the conditions needed to make the soil in the optimal situations to carry out this task. In the present study, the main objective of the overall project was to reduce the nitrogen input into the Venice lagoon through a specifically-designed afforested riparian zone. Therefore, the site management adopted was quite different from that applied to a normal agricultural soil. Particularly, the buffer zone was set with irrigation ditches carrying water from the river Zero and producing a sub-surface water flow, running through the entire buffer strip to the drainage ditches, thus creating a semi-natural floodplains where water flows can be efficiently managed. As a consequence of the irrigation, a perched aquifer was created on the clay-calcic layer at around 90-150 cm depth. Thus, the water level in the experimental site was always between 25 to 60 cm below the soil surface, with a slope of 4%. While the 0-15 cm soil layer was subjected to the normal seasonal cycle, the 40-55 cm and 80-95 cm layers were often saturated. This particular water flow management became conducive for reducing oxygen availability and, in some seasons and deeper in the soil, for promoting anoxic conditions, thus supporting the co-occurrence of two main processes: vegetation and microbial uptake and denitrification.

In other words, the translation of an agricultural soil into a buffer zone should have a precise effect on a key microbial community specifically tailored to carry out the main activities required by the system. Therefore, in order to verify the possible changes occurred in the composition of the microbial populations of this soil, proper microbiological analysis have been carried out on both soil and water, taking also into account a soil outside the buffer strip, as a control. The main results obtained, although far

to be definitive, provided interesting advances of knowledge related to the bacterial population dynamic and biodiversity, by using both conventional and advanced methods. Specifically, it can be stated that:

a) The microbial community found into the buffer zone appears to be much more active as compared to that of the external soil

b) The microbial biodiversity found in the buffer zone is more pronounced than that typical of the external soil

c) Microbial activities and bacterial biodiversity follow the soil profile: the surface layer was found as overactive and more rich in bacterial taxa as compared to the medium depth, but especially to the deeper soil layer; a number of bacterial species appear to be typical for a specific soil layer.

d) Seasonal fluctuations related to soil bacterial populations can be monitored and follows different trends within the buffer zone as compared to an external soil; a number of bacterial species appear to be typical for a specific season.

e) The conditions created by the specific management of the site were found effectively conducive of an increase in bacterial biodiversity and proficient in supporting bacterial denitrification.

f) The introduced vegetation was able to develop abundantly and to contribute to the nitrogen removal by (i) direct plant uptake and (ii) by indirect support to the soil microbiota through the organic matter release.

g) The microbial diversity of the water increases as a consequence of the transit through the soil; the bacterial population of the output water could be studied to understand a possible “filter effect” or the degree of bacterial release by the soil.

h) Water decontamination of undesired bacterial taxa might be proposed after a deeper analysis of the in-stream and out-stream, in terms of composition of the bacterial population.

To sum up, the above observations clearly indicate that microbial analysis can be successfully performed in order to obtain a good picture of the soil/water ecosystem when a different management was applied.

It is worthy of our attention a parallel study carried out within the same project, and aimed at obtaining further information on the chemical changes occurring in the buffer soil and water flux, especially in terms of combined nitrogen removal. The results there obtained can be successfully combined with the microbiological analysis here performed. Taken together, these data unambiguously indicate that the wooded riparian buffer zone assessed for water remediation is effectively working, confirming that the soil management adopted was appropriate.

As an important side result, it was ascertained that these analysis could be used to design *ex novo* special DNA primers and probes to target bacterial groups of interest. For instance, the system here described showed to effectively enhance denitrification processes and it was possible to identify several species of denitrifiers by 16S rDNA sequence analysis. Even more interesting, novel and rare bacterial species could be also identified, thus adding new information to our knowledge.

8. REFERENCES

Abawi, G. S., and T. L. Widmer (2000). Impact of soil health managment practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Appl. Soil Ecol. **15**: 37–47.

Acinas, S. G., J. Anton and F. Rodriguez-Valera (1999). Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl. Environ. Microb. **65**: 514–522.

Agnelli, A., J. Ascher, G. Corti, M. T. Ceccherini, P. Nannipieri, and G .Pietramellara (2004). Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil. Biol. Biochem. **36**: 859–868.

Ahmad, S., A. Selvapandiyan, and R. K. Bhatnagar (2000). Phylogenetic analysis of Gram-positive bacteria based on grpE, encoded by the dnaK operon. Int. J. Syst. Evol. Microbiol. **50**: 1761–1766.

Aislabie, J. M., K. M. Chhour, D. J. Saul, S. Miyauchi, J. Ayton, R. F. Paetzold, and M. R. Balks (2006). Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biol. Biochem. **38**: 3041–3056.

Akob, D. M., H. J. Mills and J. E. Kostka (2007). Metabolically active microbial communities in uranium-contaminated subsurface sediments. FEMS Microbiol Ecol. **59**: 95–107.

Akratos, C. S., and V. A. Tsihrintzis (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilotscale horizontal subsurface flow constructed wetlands, Ecol. Eng. **29**: 173–191.

Altmann, D., P. Stief, R. Amann, D. De. Beer, and A. Schramm (2003). In situ distribution and activity of nitrifying bacteria in freshwater sediment. Environ. Microbiol. **5**: 798–803.

Alvey, S., C. H. Yang, A. Buerkert, and D. E. Crowley (2003). Cereal/ legume rotation effects on rhizosphere bacterial community structure in West African soils. Biol. Fertil. Soil. **37**: 73–82.

Ascher, J., M. T. Ceccherini, L. Landi, M. Mench, G. Pietramellara, P. Nannipieri, and G. Renella (2009). Composition, biomass and activity of microflora, and leaf yields and foliar elemental concentrations of lettuce, after in situ stabilization of an arsenic contaminated soil. Appl. Soil. Ecol. **41**: 351–359.

Axelrood, P. E., M. L. Chow, C. C. Radomski, J. M. McDermott, and J. Davies (2002). Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. Can. J. Microbiol. **48**: 655–667.

Azam, F., and R. A. Long (2001). Sea snow microcosms. Nature. 414: 497–498.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyerreil, and F. Thingstad (1983). The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10**: 257–263.

Bach, P. A. M., and A. J. Horne (2000). Denitrification in constructed free-water surface wetlands, II: Effects of vegetation and temperature. Ecol. Eng. **14**: 17–32.

Bailey, V. L., and W. B. McGill (2002). Fate of 14Clabeled pyrene in a creosote- and octadecane in an oil-contaminated soil. Soil Biol. Biochem. **34**: 423–33.

Barling, R. D, and I. D. Moore (1994). Role of buffer strips in management of waterway pollution: a review. Environ. Management. **18:** 543-558.

Basaglia M., F. Fontana, M. Paro, N. Cendron, A. Squartini, and S. Casella (2003). Metabolic state of microbial populations as an efficiency index of a biopile. Second European bioremediation Conference. June 30-July 4, 2003. (vol. 1, pp. 135-138). Chania, Crete, Greece

Basaglia M., S. Povolo, and S. Casella (2007). Effect of oxygen and host plant on resuscitation of viable not culturable *Sinorhizobium meliloti* 41. Cur. Microbiol. **54**: 167-174.

Basaglia, M., A. Toffanin, E. Baldan, M. Bottegal, J. P. Shapleigh, and S. Casella (2007). Selenite-reducing capacityof the copper-containing nitrite reductase of *Rhizobium sullae*. FEMS Microbiol. Lett. 269: 124–130.

Bastviken, S. K., and P. G. Eriksson (2005). A. Premrov, K. Tonderski, Potential nitrification and denitrification in wetland sediments with different plant species detritus. Ecol. Eng. 25: 183–190.

Bater, J. E (1996). Micro- and macro-arthropods. In: Hall GS (ed) Methods for the examination of organismal diversity in soils and sediments. CAB International, Wallingford, pp 163–174.

Becker, K., D. Harmsen, A. Mellmann, C. Meier, P. Schumann, G. Peters, and C. von Eiff (2004). Development and evaluation of a quality-controlled ribosomal sequence database

for 16S ribosomal DNA-based identification of *Staphylococcus* species. J. Clin. Microbiol. **42**: 4988–4995.

Becker, M. R., B. J. Paster, E. J. Leys, M. L. Moeschberger, S. G. Kenyon, J. L. Galvin, S. K. Boches, F. E. Dewhirst, and A. L. Griffen (2002). Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol. **40**: 1001–1009.

Bedmar E. J., E. F. Robles, and M. J. Delgado (2005). The complete denitrification pathway of the symbiotic, nitrogen-fixing bacterium Bradyrhizobium japonicum. Biochem Soc Trans. **33**: 141–144.

Benizri, E., E. Baudin, and A. Guckert (2001). Root colonization by plant growth promoting Rhizobacteria. Biocontrol Sci. Technol. **5:** 557–574.

Bertilsson, S., C. M. Cavanaugh, and M. F. Polz (2002). Sequencing-independent method to generate oligonucleotide probes targeting a variable region in bacterial 16S rRNA by PCR with detachable primers. Appl. Environ. Microbiol. **68**: 6077–6086.

Bertrand, H., R. Nalin, R. Balli, and J. C. Cleyet- Marel (2001). Isolation and identification of the most efficient plant growthpromoting bacteria associated with canola (*Brassica mapus*). Biol. Fertil. Soils. **33**: 152–56.

Betancourt, D. A., T. M. Loveless, J. W. Brown, and P. E. Bishop (2008). Characterization of diazotrophs containing Mo-independent nitrogenases, isolated from diverse natural environments. Appl. Environ. Microbiol. **74**:3471–3480.

Blume, E., M. Bischoff, J. Reichert, T. Moorman, A. Konopka, and R. Turco (2002). Surface and subsurface community structure and metabolic activity as a function of soil depth and season. Appl. Soil. Ecol. **592**: 1-11.

Boddington, C. L., and J. C. Dodd (2000). The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian Ultisol. Plant Soil. **218**: 137–144.

Bolton Jr, H., L. F. Elliott, R. I. Papendick, and. D. F. Bezdicek (1985). Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biol. Biochem. **17**: 297–302.

Boothroyd, I. K. G and E. R. Langer (1999). Forest harvesting and riparian management guidelines: a review. NIWA Technical Report 56.

Borneman, J., and E. W. Triplett **(**1997). Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl. Environ. Microbiol. **63:** 2647–2653.

Bossio, D. A., K. M. Scow, N. Gunapala, and K. J. Graham (1998). Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb. Ecol. **36**: 1 –12.

Boulos, L., M. Prevost, B. Barbeau, J. Coallier, and R. Desjardins (1999). LIVE/ DEAD. BacLightE: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. J. Microbiol. Methods. **37**: 77–86.

Bowman, J. P., S. A. McCammon, J. A. E. Gibson, L. Robertson and P. D.Nichols (2003). Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Appl. Environ. Microb. **69**: 2448–2462.

Brandt, K. K., A. Petersen, P. E. Holm, and O. Nybroe (2006). Decreased abundance and diversity of culturable Pseudomonas spp. Populations with increasing copper exposure in the sugar beet rhizosphere. FEMS Microbiol. Ecol. **56**: 281- 291.

Braun, B., U. Böckelmann, E. Grohmann and U. Szewzyk (2006). Polyphasic characterization of the bacterial community in an urban soil profile with in situ and culture dependent methods. Appl. Soil Ecol. **31**: 267–279.

Brettar, I., E. R. B. Moore, and M.G. Höfle (2001). Phylogeny and abundance of novel denitrifying bacteria isolated from the water column of the central Baltic Sea. Microbial Ecol. **42**: 295-305.

Brinson, M. M., L. J. MacDonnell, D. J. Austen, R. L. Beschta, T. A. Dillaha, D. L. Danahue, S. V. Gregory, J. W. Harvey, M. C. Molles, E. I. Rogers, and J. A. Stanford (2002). Riparian AreasFunctions and Strategies for Management. National Academy Press, Washington, DC, USA.

Buchanan, M., and L. D. King (1992). Seasonal fluctuations in soil microbial biomass carbon, phosphorus, and activity in no-till and reduced-chemical-input maize agroecosystems. Biol. Fertil. Soils. **13**: 211–217.

Buckley, D. H., and T. M. Schmidt (2003). Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ. Microbiol. **5**: 441–52.

Buckley, D. H., J. R. Graber, and T. M. Schmidt (1998). Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. Appl. Environ. Microbiol. **64**: 4333–4339.

Bundt, M., F. Widmer, M. Pesaro, J. Zeyer, and P. Blaser (2001). Preferential flow paths: biological 'hot spots' in soils. Soil Biol. Biochem. **33**: 729–738.

Burdman, S., J. Kigel, and Y. Okon (1997). Effects of *Azospirillum brasilense* on nodulation and growth of common bean (*Phaseolus vulgaris* L.). Soil Biol. Biochem. **29**: 923–29.

Burgmann, H., F. Widmer, W. Von Sigler, and J. Zeyer (2004). New molecular screening tools for analysis of free-living diazotrops in soil. Appl. Environ. Microbiol. **71**: 2079-2085.

Buyer, J. S., D. P. Roberts, and E. Russek-Cohen (1999). Microbial community structure and function in the spermosphere as affected by soil and seed type. Can. J. Microbiol. **45**: 138–44.

Carpenter, S., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. Issues in Ecol. **3**: 1–12.

Carter, V (1986). An overview of the hydrological concerns related to wetlands in the United States. Canadian. J. Botany. **64**: 363–374.

Casella S., F. Fontana, S. Povolo, and. M. Basaglia (2001). VBNC induction and resucitation of *Sinorhizobium meliloti* strain 41. 9th International Symposium on Microbial Ecology, Interactions in the Microbial World, Amsterdam (The Netherlands), August 26-31, P.01.031, pp. 191.

Casella, S., J. P. Shapleigh and W. J. Payne (1986). Nitrite reduction in *Rhizobium* 'hedysari' strain HCNT1. Arch Microbiol. **146**: 233–238.

Cassidy, M., H. Lee, J. Trevors, and R. Zablotowicz (1999). Chlorophenol and nitrophenol metabolism by *Sphingomonas* sp UG30. J. Ind. Microbiol. Biotechnol. **23**: 232–241.

Chang ,Y-J., A. D. Peacock, P. E. Long, J. R. Stephen, and J. P. McKinley, S. J. Macnaughton, A. K. M. Anwar Hussain, A. M. Saxton, and D. C. White (2001). Diversity and characterization of sulfate- reducing bacteria in groundwater at a uranium mill tailings site. Appl. Environ. Microbiol. **67**: 3149–3160.

Chapman, S. J., C. D. Campbell, and R. R. E. Artz (2007). Assessing CLPPs using MicroResp™. A comparison with Biolog and multi-SIR. J. Soils Sediments. **7**: 406–410.

Chiarini, L., A. Bevivino, C. Dalmastri, C. Nacamulli, and S. Tabacchioni (1998). Influence of plant development, cultivar and soil type on microbial colonization of maize root. Appl. Soil Ecol. **8**: 11–18.

Cho, J.-C., and S-J. Kim (2000). Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. Appl. Environ. Microbiol. **66**: 956-965.

Choi, B. K., C. Wyss and U. B. Gobel (1996). Phylogenetic analysis of pathogen-related oral spirochetes. J. Clin. Microbiol. **34**: 1922–1925.

Choi, S. C., K. K. Kwon, J. H. Sohn, and S. J. Kim (2002). Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mescocosms. J. Microbiol. Biotechnol. **12**: 431-436.

Christensen, H., M. Hansen, and J. Sorensen (1999). Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe. Appl. Environ. Microbiol. **65**: 1753–1761.

Christian, L. L., M. S. Strickland, M. A. Bradford, and N. Fierer (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. **40**: 2407–2415.

Clarridge III, J. E (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev. **17**:840–862.

Clegg, C. D., R. D. L. Lovell, and P. J. Hobbus (2003). The impact of grassland management regime on the community structure of selected bacterial groups in soil. FEMS Microbiol. Ecol. **43**: 263–70.

Clement, B. G., L. E. Kehl, K. L. DeBord, and C. L. Kitts (1998). Terminal restriction fragment patterns (TRFPs), a rapid, PCR-based method for the comparison of complex bacterial communities. J. Microbiol. Method. **31**: 135–142.

Cole, J. A, and C. M. Brown (1980). Nitrite reduction to ammonia by fermentative bacteria: a short circuit in the biological nitrogen cycle. FEMS Microbiol. Lett. **7**: 65–72.

Colles, F. M., K. E. Dingle, A. J. Cody, and M. C. J. Maiden (2008). Comparison of Campylobacter populations in wild geese with those in starlings and free-range poultry on the same farm. Appl Environ Microbiol. **74**: 3583–3590.

Collins, H. P., L. F. Elliott, R. W. Rickman, D. F. Bezdicek, and R. I. Papendick (1990). Decomposition and interaction among wheat residue components. Soil Sci. Soc. Am. J. **54**: 780–785.

Compant, S., B. Duffy, J. Nowak, C. Clément, and E. Ait Barka (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. **71**: 4951–4959.

Connolly, R., Y. Zhao, G. Sun, and S. Allen (2004). Removal of ammoniacal-nitrogen from an artificial landfill leachate in downflow reed beds. Process Biochem. **39**: 1971–1976.

Cooper, A. B (1990). Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiol. **202**: 13–26.

Cooper, P. F, and A. G. Boon (1987). The use of Phragmites for wastewater treatment by the root zone method: The UK approach, in: Aquatic Plants for Water Treatment and Resource Recovery (Eds. K. R. Reddy, W. H. Smith), Magnolia Publishing, Orlando. 153–174.

Costa, A. L., S. M. Paixão, I. Caçador, and M. Carolino (2007). CLPP and EEA profiles of microbial communities in salt marsh sediments. J. Soils Sediments. **7**: 418–425.

Costa, R., M. Gotz, N. Mrotzek, J. Lottmann, G. Berg, and K. Smalla (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol. Ecol. **56**: 236–249.

Cottrell, M. T., L. A. Waidner, L. Y. Yu, and D. L. Kirchman (2005). Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environ Microbiol **7**: 1883–1895.

Cowardin, L. M., V. Carter, F. C. Golet, and E. T. Laroe (1979). Classification of Wetlands and Deepwater Habitats of the United States. US Fish Wildl. Serv. Off. Biol. Serv. FWS/OBS 79/31, pp.1–103.

Cragg, R. G, and R. D. Bardgett (2001). How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biol. Biochem. **33**: 2073–2081.

Crocker, F. H., J. K. Fredrickson, D. C. White, D. B. Ringelberg, and D. L. Balkwill (2000). Phylogenetic and physiological diversity of Arthrobacter strains isolated from unconsolidated subsurface sediments. Microbiol. **146**: 1295–1310.

Crump, B. C., E. V. Armbrust, and J. A. Baross (1999). Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol **65**: 3192–3204.

Culley, J. L. B, and E. F. Bolton (1983). Suspended solids and phosphorus loads from a clay soil: II. Watershed study. J. Environ. Qual. **12**: 498-503.

Curci, M., M. D. R. Pizzigallo, C. Crecchio, R. Mininni, and P. Ruggiero (1997). Effects of conventional tillage on biochemical properties of soils. Biol. Fertil. Soil. 25:1–6.

Da Silva, K. R. A., J. F. Salles, L. Seldin, and J. D. van Elsas (2003). Application of a novel *Paenibacillus*-specific PCR-DGGE method and sequence analysis to assess the diversity of *Paenibacillus* spp. in the maize rhizosphere. J. Microbiol. Methods. **54**: 213–31.

Dahl, M., B. Nilsson, J. H. Langhoff, and J. C. Refsgaard (2007). Review of classification systems and new multi-scale typology of groundwater–surface water interaction. J. Hydrol. **344**: 1– 16.

Daniell, T. J., R. Husband, A. H. Fitter, and J. P. W. Young (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiol. Ecol. 36: 203–209.

De Deyn, G. B., C. E. Raaijmakers, H. R. Zoomer, M. P. Berg, P. C. Rulter, H. A. Verhoef, T. M. Bezemer, and W. H. Van der Putten (2003). Soil invertebrate fauna enhances grassland succession and diversity. Nature. **422**: 711–713.

De Ridder-Duine, A. S., G. A. Kowalchuk, P .J. A. K. Gunnewiek, W. Smant, J. A. van Veen, and W. de Boer (2005). Rhizosphere bacterial community composition in natural stands of Carex arenaria (sand sedge) is determined by bulk soil community composition. Soil Biol. Biochem. **37**: 349–357.

DeLong, E. F., and D. M. Karl (2005). Genomic perspectives in microbial oceanography. Nature. **437**: 336–342.

Desai. C, R. Y. Parikh, T. Vaishnav, Y. S. Shouche, and D. Madamwar (2009). Tracking the influence of long-term chromium pollution on soil bacterial community structures by comparative analyses of 16S rRNA gene phylotypes. Res. Microbiol. **160**: 1- 9.

Dilly, O., J. Bloem, A. Vos, and J. C. Munch (2004). Bacterial diversity in agricultural soils during litter decomposition. Appl. Environ. Microbiol. **70**: 468–474.

Dix, N. J, and J. Webster (1995). Fungal Ecology. Chapman & Hall, London.

Drijber, R. A., J. W. Doran, A. M. Parkhurst, and D. J. Lyon (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. **32**: 1419–30.

Duineveld, B. M., G. A. Kowalchuk, A. Keijzer, J. D. van Elsas, and J. A. van Veen (2001). Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Appl. Environ. Microbiol. **67**: 172–178.

Dunbar, J., L. O. Ticknor, and C. R. Kuske (2000). Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Appl. Environ. Microbiol. **66**: 2943–2950.

Dunbar, J., S. Takala, S. M. Barns, J. A. Davis, and C. R. Kuske (1999). Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl. Environ. Microbiol. **65**: 1662–1669.

Edenborn, S., and A. Sexstone (2007). DGGE fingerprinting of culturable soil bacterial communities complements culture-independent analyses. Soil Biol. Biochem. **39**: 1570–1579.

Ehrenreich, P., A. Behrends, J. Harder, and F. Widdel (2000). Anaerobic oxidation of alkanes by newly isolated denitrifying bacteria. Arch. Microbiol. **173**(1): 58-64.

El Fantroussi, S (2000). Enrichment and molecular characterization of a bacterial culture that degrades methoxy-methyl urea herbicides and their aniline derivatives. Appl. Environ. Microbiol. **66**: 5110–5115.

El Fantroussi, S., L. Verschuere, W. Verstraete, and E. M. Top (1999). Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community- level physiological profiles. Appl. Environ. Microbiol. **65**: 982– 988.

Falk, S., B. Liu, and G. Braker (2010). Isolation, genetic and functional characterization of novel soil nirK-type denitrifiers. Syst. Applied. Microbiol. **33**: 337–347.

Fang , M., P. P. Motavalli, R. J. Kremer and K. A. Nelson (2007). Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils. Applied. Soil Ecol. **37**: 150–160.

Fang, M., R. J. Kremer, P. P. Motavalli, and G. Davis (2005). Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. Appl. Environ. Microbiol. **71**: 4132–4136.

Felske, A., A. Wolterink, R. Van Lis, W. M. De Vos, and A. D. Akkermans (2000). Response of a soil bacterial community to grassland succession as monitored by 16S RNA levels of the predominant ribotypes. Appl Environ Microbiol. **66**: 3998–4003.

Felske, A., and A. D. L.Akkermans (1998). Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils. Microb. Ecol. **36**: 31–36.

Feng, B- W., X-R. Li1, J-H. Wang, Z-Y. Hu, H. Meng, L-Y Xiang, and Z-X. Quan (2009). Bacterial diversityofwaterand sediment in theChangjiang estuary and coastal area of the EastChina Sea. FEMS Microbiol. Ecol. 70: 236–248.

Ferguson, S. J (1994). Denitrification and its control. A Van Leeuw. **66**: 89-110.

Fields, M. W., T. Yan, S-K, Rhee, S. L. Carroll, P. M. Jardine, D. B. Watson, C. S. Criddle, and J. Zhou (2005). Impacts on microbial communities and cultivable isolates from groundwater contaminated with high levels of nitric acid–uranium waste. FEMS Microbiol. Ecol. **53**: 417–428.

Fierer, N., J. P. Schimmel and P.A. Holden (2003). Influence of drying–rewetting frequency on soil bacterial community structure. Microb. Ecol. **45**: 63–71.

Fierer, N., J.A. Jackson, R. Vilgalys, and R.B. Jackson (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. **71**: 4117-4120.

Fritze, H., J. Perkiomaki, U. Saarela, R. Katainen, P. Tikka, K. Yrjala, M. Karp, J. Haimi, and M. Romantschuk (2000). Effects of Cd-containing wood-ash on the microflora of coniferous forest humus. FEMS Microbiol. Ecol. **32**: 43–51.

Fuhrman, J. A., K. McCallum, and A. A. Davis (1993). Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl Environ Microb **59**: 1294–1302.

Furlong, M. A., D. R. Singleton, D. C. Coleman, and W. B. Whitman (2002). Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and casts of the earthworm Lumbricus rubellus. Appl. Environ. Microbiol. **68**: 1265–1279.

G.B. De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten (2004). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos. **106**: 576–586.

Gamble, T. N., M. R. Betlach, and J. M. Tiedje (1977). Numerically Dominant Denitrifying Bacteria from World Soils. Appl. Environ. Microbiol. **33**(4): 926-939.

Gans, J., M. Wolinsky, and J. Dunbar (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science. **309**: 1387-1390.

Garbaye, J (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. **128**: 197–210.

Garbeva, P., J .A. van Veen, and J. D. van Elsas (2004). Microbial biodiversity in soil: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. Annu. Rev. Phytopathol. **42**: 243–70.

Garbeva, P., J. D. van Elsas, and J. A. van Veen (2008). Rhizosphere microbial community and its response to plant species and soil history. Plant Soil. **302**: 19–32.

Garland, J. L (1996). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. **28**: 213–221.

Garland, J. L (1996). Patterns of potential C source utilization by rhizosphere communities. Soil Biol. Biochem. **28**: 223– 230.

Garland, J. L, and A. L. Mills (1991). Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source utilization. Appl. Environ. Microbiol. **57**: 2351– 2359.

Garrity, G. M, and J. G. Holt (2001). Phylum BVI. *Chloroflexi* phy. nov. In Boone, D. R. & R.W. Castenholz (eds.), Vol. 1: The Archaea and the Deeply Branching and Phototrophic Bacteria. In G.M. Garrity (ed.), Bergey's Manual of Systematic Bacteriology, 2nd ed., Springer-Verlag, New York, p427-446.

Gelsomino, A., A. C. Keijzer-Wolters, G. Cacco, and J. D. van Elsas (1999). Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J. Microbiol. Methods. **38**: 1 –15.

George, E., H. Marschner, and I. Jakobsen (1995). Role of arbuscular mycorrhizal fungi in uptake of phosphorous and nitrogen from soil. CritCrypt. Rev. Biotechnol. **15**: 257–270.

Germida, J. J., S. D. Siciliano, J. R. Freitas, and A. M. Seib (1998). Diversity of rootassociated bacteria associated with fieldgrown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol. Ecol. **26**: 43–50.

Gersberg, R. M., B. V. Elkins, S. R. Lyons, and C. R. Goldman (1985). Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. **20**: 363367.

Ghiorse, W., and J. Wilson (1988). Microbial ecology of the terrestrial subsurface. In: Laskin, A., (Ed.), Advances in Applied Microbiology, Academic Press, New York, p. 107–172.

Girvan, M. S., J. Bullimore, J. N. Pretty, A. M. Osborn, and A. S. Ball (2003). Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. Appl. Environ. Microbiol. **69**: 1800–1809.

Gomes, N. C. M., I. A. Kosheleva, W.-R. Abraham, and K. Smalla (2005). Effects of the inoculant strain *Pseudomonas putida* KT2442 (pNF142) and of naphthalene contamination on the soil bacterial community. FEMS Microbiol. Ecol. **54**: 21–33.

Gomez-Alvarez, V., G. M. King, and K. Nusslein (2007). Comparative bacterial diversity in recent Hawaiian volcanic deposits of di¡erent ages. FEMS Microbiol. Ecol. **60**: 60–73.

Gorbushina, A. A., and W. E. Krumbein (2005). Role of microorganisms in wear down of rocks and minerals. In: Buscot F, Varma A (Eds.) Microorganisms in Soils: Roles in Genesis and Functions, Springer, Berlin, Germany, pp 59–84.

Gordon, S. N., A. Valenzuela, S. M. Adams, P. W. Ramsey, J. L. Pollock, W. E. Holben, and J. E. Gannon (2009). *Pedobacter nyackensis* sp. nov., *Pedobacter alluvionis* sp. nov. and *Pedobacter borealis* sp. nov., isolated from Montana flood-plain sediment and forest soil. Int. J. Syst. Evol. Microbiol. **59**: 1720-1726.

Gosselink, J. G, and R. E. Turner (1978). The role of hydrology in freshwater wetland ecosystems. In: Good, R.E., D. F. Whigham, and R. L. Simpson (Eds.). Freshwater

Wetlands: Ecological Processes and Management Potential. Academic Press, New York, USA, pp. 63–78.

Graham, J. H., N. C. Hodge, and J. B. Morton (1995). Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. Appl. Environ. Microbiol. **61**: 58– 64.

Grayston, S. J., G. S. Griffith, J. L. Mawdsley, C. D. Campbell, and R. D. Bardgett (2001). Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol Biochem. **33**: 533–551.

Grayston, S. J., S. Wang, C. D. Campbell, and A. C. Edwards (1998). Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. **30**: 369–78.

Green, S. J., M. B. Leigh, and J. D. Neufeld (2009). Denaturing gradient electrophoresis (DGGE) for microbial community analysis. Pages 4137-4158 *in*: Timmis, K.N. (Ed) Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds. Springer (Heidelberg, 5 Germany).

Gregory, S.V., F. J. Swanson, W. A. McKee, and K. W. Cummins (1991). An ecosystem perspective on riparian zones. Bioscience. **41**: 540-551.

Gremion, F., A. Chatzinotas, and H. Harms (2003). Comparative 16S rRNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. Environ. Microbiol. **10**: 896–907.

Gribbon, L. T, and M. R. Barer **(**1995**).** Oxidative metabolism in nonculturable Helicobacter pylori and Vibrio vulnificus cells studied by substrate-enhanced tetrazolium reduction and digital image processing. Appl. Environ. Microbiol. **61**: 3379-3384.

Griffiths, R. I., A. S. Whiteley, A. G. O'Donnell, and M. J. Bailey (2000). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl. Environ. Microbiol. **66**: 5488–5491.

Gumiero, B., B. Boz, P. Cornelio, and S. Casella (2011). Shallow groundwater nitrogen and denitrification in a newly afforested, sub-irrigated riparian buffer. J. Appl. Ecol. (in press)

Gupta, R. S (2000). The phylogeny of Proteobacteria: relationships to other eubacterial phyla and eukaryotes. FEMS Microbiol. Rev. **24**: 367–402.

Hackl, E., S. Zechmeister-Boltenstern, L. Bodrossy, and A. Sessitsch (2004). Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl. Environ Microbiol. **70**: 5057–5065.

Hafez, E. E and E. Elbestawy (2009). Molecular characterization of soil microorganisms: effect of industrial pollution on distribution and biodiversity. World J Microbiol. Biotechnol. **25**: 215–224.

Hammer, D. A (1992). Designing constructed wetlands systems to treat agricultural non-point source pollution. Ecol. Eng. **1**: 49–82.

Handelsman, J (2004). Metagenomics: Application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. **68**: 669–685.

Hanson, G.C., P.M. Groffman, and A.J. Gold. (1994). Symptoms of nitrogen saturation in a riparian wetland. Ecol. Applications. **4**: 750-756.

Hatamoto, M., H. Imachi, Y. Yashiro, A. Ohashi, and H. Harada (2008). Detection of active butyrate-degrading microorganisms in methanogenic sludges by RNA-based stable isotope probing. Appl Environ Microbiol. **74**: 3610–3614.

Haycock, N. E, and G. Pinay (1993). Groundwater nitrate dynamics in grass and poplar vegetated riparian buffer strips during winter. J. Environ. Qual. **22**: 273–278.

Hayward, A. C. (1993). The hosts of *Xanthomonas*. In *Xanthomonas*, J.G. Swings, and E.L. Civerolo, eds. (London, United Kingdom, Chapman and Hall), pp. 1–119.

He, Q., and K. Mankin (2002). Performance variations of COD and nitrogen removal by vegetated submerged bed wetlands. J. Am. Water Res. Assoc. **38**: 1679–1689.

Hedrick, D. B., A. Peacock, J. R. Stephen, S. J. Macnaughton, J. Bruggemann, and D. C. White (2000). Measuring soil microbial community diversity using polar lipid fatty acid and denaturing gradient gel electrophoresis data. J. Microbiol. Methods. **41**: 235–48.

Hefting, M. M., and J. J.M. de Klein (1998). Nitrogen removal in buffer strips along a lowland stream in the Netherlands: a pilot study. Environ. Pol .**102**: 521-526

Heijs, S. K., A. M. Laverman, L. J. Forney, P. R. Hardoim, and J. D.van Elsas (2008). Comparison of deep-sea sediment microbial communities in the Eastern Mediterranean. FEMS Microbiol. Ecol. **64**: 362–377.

Hendrix,P. F., R. W. Parmelee, J. Crossley, D. C. Coleman, E. P. Odum, and P. M. Groffman (1986). Detritus food webs in conventional and no-tillage agro ecosystems. BioScience. **36**: 374–380.

Herkowitz, J (1986). Listowel Artificial Marsh Project Report, Ontario Ministry of the Environment, Water Resources Branch, Toronto.

Herrera, A., M. He'ry, J. E. M. Stachc, T. Jaffr'ed, P. Normand, and E. Navarro (2007). Species richness and phylogenetic diversity comparisons of soil microbial communities affected by nickel-mining and revegetation efforts in New Caledonia. Eur. J. Soil Biol. **43**: 130–139.

Heuer, H., M. Krsek, P. Baker, K. Smalla, and E. M. H. Wellington (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl. Environ. Microbiol. **63**: 3233–3241.

Heuer, H., R. M. Kroppenstedt, G. Berg and K. Smalla (2002).Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. Appl. Environ. Microbiol. **68**: 1325–1335.

Heylen, K., B. Vanparys, L. Wittebolle, W. Verstraete, N. Boon, and P. De Vos (2006). Cultivation of Denitrifying Bacteria: Optimization of Isolation Conditions and Diversity Study. Appl. Environ. Microbiol. **72**: 2637–2643.

Heywood, V. H (1995). Global biodiversity assessment. Cambridge University Press, Cambridge.

Hill, G. T., N. A. Mitkowski**,** L. Aldrich-Wolfe**,** L. R. Emele**,** D. D. Jurkonie**,** A. Ficke, S. Maldonado-Ramirez**,** S. T. Lynch, and E. B. Nelson (2000). Methods for assessing the composition and diversity of soil microbial communities Appl. Soil Ecol. **15**: 25–36.

Hoffmann, C. C., P. Berg, M. Dahl, S. E. Larsen, H. E. Andersen, and B. Andersen (2006). Groundwater flow and transport of nutrients through a riparian meadow – Field data and modeling. J. Hydrol. **331**: 315– 335.

Hoffmann, C. C., S. Rysgaard and P. Berg (2000). Denitrification rates predicted by Nitrogen-15 labeled nitrate microcosm studies, in situ measurements, and modeling. J. Environ. Qual. **29**(6): 2020–2028.

Huang, J., R. Reneau, and C. Hageborn (2000). Nitrogen removal in constructed wetlands employed to treat domestic wastewater. Water Res. **34**: 2582–2588.

Hugenholtz, P., B .M. Goebel, and N. R. Pace (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. **180**: 4765–4774.

Hugenholtz, P., B. M. Goebel, and N. R. Pace (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol. **180**: 4765–4774.

Hunter, R.G., and S. P. Faulkner (2001). Denitrification Potentials in Restored and Natural Bottomland Hardwood Wetlands. Soil Sci. Soci. Am. J. **65**: 1865-1872.

Ibekwe, A. M, and A. C. Kennedy (1998). Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiol. Ecol. **26**: 151–163.

Ibekwe, A. M, and A. C. Kennedy (1999). Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant Soil. **206**: 151–161.

Ibekwe, A. M., C. M. Grieve, and S. R. Lyon (2003). Characterization of microbial communities and composition in constructed dairy wetland wastewater effluent. Appl. Environ. Microbiol. **69**: 5060–5069.

Ibekwe, A. M., J. A. Poss, S. R. Grattan, C. M. Grieve, and D. Suarez (2010). Bacterial diversity in cucumber (Cucumis sativus) rhizosphere in response to salinity, soil pH, and boron Soil Biology & Biochem. **42**: 567-575.

Ingram, H. A. P (1983). Hydrology. In: Gore, A.J.P. (Ed.), Ecosystems of the World 4A. Mires: Swamps, Bog, Fen and Moor. Elsevier Scientific Publication Company, Amsterdam, the Netherlands, pp. 67–159.

Insam, H (2001). Development in soil microbiology since mid 1960s. Geoderma. **100**: 389–402.

Ishii, S., M. Yamamoto, M. Kikuchi, K. Oshima, M. Hattori, S. Otsuka, and K. Senoo (2009). Microbial Populations Responsive to Denitrification-Inducing Conditions in Rice Paddy Soil, as Revealed by Comparative 16S rRNA Gene Analysis. Appl. Environ. Microbiol. **75**(22): 7070–7078.

Iwamoto, T., K. Tani, K. Nakamura, Y. Suzuki, N. Kitagawa, M. Eguchi, and M. Nasu (2000). Monitoring impact of in situ biostimulation treatment on groundwater bacterial community by DGGE. FEMS Microbiol. Ecol. **32**: 129– 141.

Jaatinen, K., E. S. Tuittila, J. Laine, K. Yrjälä and H. Fritze (2005). Methane oxidizing bacteria in Finnish raised mire complex: Effects of site fertility and drainage. Microb. Ecol. **50**: 429–439.

Jaatinen, K., K. Knief, K. Yrjälä, P. Dunfield, and H. Fritze (2004). Methanotrophic bacteria in boreal forest soil—effects of fire. FEMS Microbiol. Ecol. **50**: 195–202.

Jacobs, T. C., and J. W. Gilliam (1985). Riparian losses of nitrate from agricultural drainage waters. J. Environ. Qual. **14**: 472–478.

James, J. B., T. D. Sherman, and R. Devereux (2006). Analysis of bacterial communities in sea grass bed sediments by double-gradient denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA genes. Microb. Ecol. **52**(4): 655-61.

Janssen, P. H., P. S. Yates, B. E. Grinton, P. M. Taylor, and M. Sait (2002). Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. Appl. Environ. Microbiol. **68**: 2391–96.

Jensen, P. R., T. J. Mincer, P. G. Williams, and W. Fenical (2005). Marine actinomycete diversity and natural product discovery. Antonie Van Leeuwenhoek **87**: 43–48.

Jensen, P.R., P. G. Williams, D. C. Oh, L. Zeigler, and W. Fenical (2007). Species specific secondary metabolite production in marine actinomycetes of the genus Salinispora. Appl Environ Microbiol **73**: 1146–1152.

Jiang, S., L. Xiang, L. Zhang, W. Sun, S. Dai, L. Xie (2008). Culturable actinobacteria from marine sponge Iotrochota sp. Mar Biol **153**: 945–952.

Jin, H., R. Zhou, M. S. Kang, R. Luo, X. W. Cai, and H. C.Chen (2006). Biofilm formation by field isolates and reference strains of Haemophilus parasuis. Veterin Microbiol. **118**: 117–123.

John, M. G. St., D. H. Wall, and V.M. Behan-Pelletier (2006).Does plant species co-occurrence influence soil mite diversity? Ecology. **87**: 625–633.

Jolanda, K. B, and J. D. van Elsas (2008). Analysis of Bacterial Communities in Soil by Use of Denaturing Gradient Gel Electrophoresis and Clone Libraries, as Influenced by Different Reverse Primers Appl Environ Microbiol. **74**: 2717–2727**.**

Jones, D. L., A. Hodge, and Y. Kuzyakov (2004). Plant and mycorrhizal regulation of rhizodeposition. New Phytol. **163**: 459–480.

Joo, H. Z., M. Hirai, and M. Shoda (2005). Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by Alcaligenes faecalis no. 4. J. Biosci. Bioeng. **100**: 184–191.

Joseph, S. J., P. Hugenholtz, P. Sangwan, C. A. Osborne, and P. H. Janssen (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. Appl. Environ. Microbiol. **69**: 7210-7215.

Joynt, J., M. Bischoff, R. F. Turco, A. Konopka, and C. H. Nakatsu (2006). Microbial community analysis of soils contaminated with lead, chromium and organic solvents. Microb. Ecol. **51**: 209–219.

Kadlec, R. H., and R. L. Knight (1996). Treatment Wetlands, CRC Press, Boca Raton

Kadlec, R. H., R. L. Knight, J. Vymazal, and H. Brix. (2000). Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation, IWA specialist group on use of macrophytes in water pollution control, Scientific and Technical Report, No. 8, IWA Publishing, London.

Kaiser, E. A., and O. Heinemeyer (1993). Seasonal variations of soil microbial biomass carbon within the plough layer. Soil Biol. Biochem. **25**: 1649–1655.

Kandeler, E., M. Stemmer, and M. H. Gerzabek (2005). Role of microorganisms in carbon cycling in soil. In: Buscot F, Varma A (Eds.) Microorganisms in Soils: Roles in Genesis and Functions, Springer, Berlin, Germany, pp 139–157

Kapley, A., T. D. Baere, and H. J. Purohit (2007). Eubacterial diversity of activated biomass from a common effluent treatment plant. Res. Microbiol. **158**: 494- 500.

Kataoka, M., K. Ueda, T. Kudo, T. Seki, and T. Yoshida (1997). Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus *Streptomyces*, FEMS Microbiol. Lett**.** **151**: 249–255.

Kelly KM & Chistoserdov AY (2001) Phylogenetic analysis of the succession of bacterial communities in the Great South Bay (Long Island). FEMS Microbiol Ecol 35: 85–95.

Kelly, J. J., M. Haggblom, and R. L. Tate III (1999). Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. Soil Biol. Biochem. **31**: 1455– 1465.

Kennedy, A. C., and K. L. Smith (1998). Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil. **170**: 75–86.

Kent, A. D., and E. W. Triplett (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. Annual Rev. Microbiol. **56**: 211–236.

Killingstad, M. W., M. A. Widdowson, and R. L. Smith (2002). Modeling enhanced in situ denitrification in groundwater. J. Environ. Eng. **128**: 491–504.

Kim, E., and G. J. Zylstra (1999). Functional analysis of genes involved in biphenyl, naphthalene, phenanthrene, and m-xylene degradation by Sphingomonas yanoikuyae B1. J. Ind. Microbiol. Biotechnol. **23**: 294–302.

Kim, S., D. H. Choi, D. S. Sim and Y. Oh (2005). Evaluation of bioremediation effectiveness on crude oil–contaminated sand. Chemosphere. **59**: 845-852.

Kim, S., H. Jung, K. S. Kim, and I. S. Kim (2004). Treatment of high nitrate containing waste waters by sequential heterotrophic and autotrophic denitrification. J. Environ. Eng. **130**: 1475–1480.

Kirchner, M. J., A. G. Wollum II, and L. D. King (1993). Soil microbial populations and activities in reduced chemical input agroecosystem. Soil Sci. Soc. Am. J. **57**: 1289–1295.

Kirk, J. L., L. A. Beaudette, M. Hart, P. Moutoglis, J. N. Klironomos, H. Lee, and J. T. Trevors (2004). Methods of studying soil microbial diversity. J. Microbiol Methods. **58**: 169– 188.

Kishimoto, N., and T. Tano (1987). Acidophilic heterotrophic bacteria isolated from acidic mine drainage, sewage and soils. J. Gen. Appl. Microbiol. **33**: 11–25.

Knief, C., A. Lipski, and P. F. Dunfield (2003). Diversity and activity of methanotrophic bacteria in different upland soils. Appl. Environ. Microbiol. **69**: 6703– 6714.

Knowles R (1982). Denitrification. Microbiol. Rev. **46**: 43–70.

Kobabe, S., D. Wagner, and E. M. Pfeiffer (2004). Characterisation of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridization. FEMS Microb. Ecol. **50**: 13-23.

Kowalchuk, G. A., B. Drigo, E. Yergeau, and. J. A. van Veen (2006). Assessing bacterial and fungal community structure in soil using ribosomal RNA and other structural gene markers. In: P. Nannipieri, K. Smalla (eds) Nucleic acids and proteins in soil, chap 8. Springer, Berlin, pp 159–188.

Kowalchuk, G. A., D. S. Buma, W. de Boer, P. G. L. Klinkhamer, and J. A. van Veen (2002). Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. AV Leeuwenhoek Internat. J. Gen. Mol. Microbiol. **81**: 509–520.

Kowalchuk, G. A., J. R. Stephen, W. Deboer, J. I. Prosser, T. M. Embley, and J. W. Woldendorp (1997.b). Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. Appl. Environ. Microbiol. **63**: 1489–1497.

Kropf, S., H. Heuer, M. Gruning, and K. Smalla (2004). Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. J. Microbiol. Methods. **57**:187–195.

Kuklinsky-Sobral, H. L., W. L. Araujo, R. Mendes, A. A. Pizzirani-Kleiner, and J. L. Azevedo (2005). Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil. **273**: 91–99.

Kuschk, P., A. WieXner, U. Kappelmeyer, and E. WeiXbrodt (2003). Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. Water Res. **37**: 4236–4242.

Kuske, C. R., L. O. Ticknor, and M. E. Miller (2002). Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in arid grassland. Appl Environ Microbiol. **68**: 1854-1863.

Kuske, C. R., S. M. Barns, and J. D. Busch (1997). Diverse uncultivated bacterial groups from soils of the arid southwestern United States that is present in many geographic regions. Appl. Environ. Microbiol. **63**: 3614–3621.

Kutako, M., T. Limpiyakorn, E. Luepromchai, S. Powtongsook, and P. Menasveta (2009). Inorganic nitrogen conversion and changes of bacterial community in sediment from shrimp pond after methanol addition. J. Applied Sci. **9**: 2907-2915.

Lagomarsino, A., M. C. Moscatelli, A. Di Tizio, R. Mancinelli,S. Grego, and S. Marinari (2009). Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. Ecologic. Indicat. **9**: 518–527.

LaMontagne, M. G., J. P. Schimel, and P. A. Holden (2003). Comparison of subsurface and surface soil bacterial communities in California grassland as assessed by terminal restriction fragment length polymorphisms of PCRamplified 16S rRNA genes. Microb. Ecol. **46**: 216-227.

Langergraber, G (2007). Simulation of the treatment performances of outdoor subsurface flow constructed wetlands in temperature climates. Sci. Total Environ. **380**: 210–219.

Latour, X., T. Corberand, G. Laguerre, F. Allard, and P. Lemanceau (1996). The composition of fluorescent *Pseudomonas* population associated with roots is influenced by plant and soil type. Appl. Environ. Microbiol**. 62**: 2449–56.

Lauber, C. L., M. S. Strickland, M. A. Bradford and N. Fierer (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. **40**: 2407– 2418.

Laverman, A. M., M. Braster, W. F. M. Röling, and H. W. van Verseveld (2005). Bacterial community structure and metabolic profiles in a forest soil exhibiting spatially variable net nitrate production. Soil Biol. Biochem. **37**: 1581–1588.

Lee, C- G., T. D. Fletcher, and G. Sun (2009). Nitrogen removal in constructed wetland systems. Eng. Life Sci. **9**: 11–22.

Lee, S.-Y., J. Bollinger, D. Bezdicek, and A. Ogram **(**1996). Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method. Appl. Environ. Microbiol. **62:**3787–3793.

Lepeuple, A. S., S. Gilouppe, E. Pierlot, and M. R. de Roubin (2004). Rapid and automated detection of fluorescent total bacteria in water samples. Int. J. Food Microbiol. **92**: 327–332.

Leys, N. M., A. Ryngaert, L. Bastiaens, W. Verstraete, E. M.Top, and D. Springael (2004). Occurrence and phylogenetic diversity of Sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. **70**: 1944–1955.

Li, L., C. Kato and K. Horikoshi (1999). Microbial diversity in sediments collected from the deepest cold-seep area, the Japan Trench. Mar. Biotechnol. **1**: 391–400.

Li, Z., J. Xu, C. Tang, J. Wu, A. Muhammad, and H. Wang (2006). Application of 16S r DNA-PCR amplification and DGGE fingerprinting for detection of shift in microbial community diversity in Cu-, Zn-, and cd-contaminated paddy soils. Chemosphere. **62:** 1374- 1380.

Lindström, E. S., M. P. K-V. Agterveld, G. Zwart (2005). Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Appl. Environ. Microbiol. **71**: 8201-8206.

Liu, R., H. Liu, C-X. Zhang, S-Y. Yang, X-H Liu, K-Y. Zhang, and R. Lai (2008). *Sphingobacterium siyangense* sp. nov., isolated from farm soil. Int. J. Syst. Evol. Microbiol. .**58**: 1458–1462.

Liu, Z., C. Rodriguez, L. Wang, Q. Cui, Y. Huang, E. T. Quintana, and M. Goodfellow (2005). Kitasatospora viridis sp. nov., a novel actinomycete from soil. Int. J. Syst. Evol. Microbiol. **55**: 707-711.

Lockeretz, W., G. Shearer, and D. H. Kohl (1981). Organic farming in the Corn Belt. Science. **211**: 540–547.

Lovely, D. R., and J. D. Coates (1997). Bioremediation of metal contamination. Curr. Opin. Biotechnol. **8**: 285–289.

Lowrance, R. R., R. L. Todd, J. Fail Jr, O. Hendrickson Jr, R. Leonard, and L. E. Asmussen (1984). Riparian forest as nutrient filters in agricultural watersheds. Bioscience. **34**: 374–377.

Lozupone, C. A., and R. Knight (2008). Species divergence and the measurement of microbial diversity. FEMS Microbiol. Rev. **32**: 557–568.

Lu Yu, L., Y. Liu and G. Wang (2009). Identification of novel denitrifying bacteria *Stenotrophomonas* sp. ZZ15 and *Oceanimonas* sp. YC13 and application for removal of nitrate from industrial wastewater. Biodegradation. **20**: 391–400.

Lu, J. J., C. L. Perng, S. Y. Lee, and C. C. Wan (2000). Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J. Clin. Microbiol. **38**: 2076–2080.

Lynch, J. M., A. Benedetti, H. Insam, M. P. Nuti, K. Smalla, V. Torsvik, and P. Nannipieri (2004). Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol Fertil Soils. **40**: 363–385.

Maarit-Niemi, R., L. Heiskanen, K. Wallenius, and K. Lindstrom (2001). Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. J. Microbiol. Methods. **45**: 155–165.

MacGregor, B. J., D. P. Moser, B. J. Baker, E. W. Alm, M. Maurer, K. H. Nealson, and D. A. Stahl. (2001). Seasonal and spatial variability in Lake Michigan sediment small-subunit rRNA concentrations. Appl. Environ. Microbiol. **67**: 3908–3922.

MacNaughton, S. J., J. R. Stephen, A. D. Venosa, G. A. Davis, Y. J. Chang, and D. C. White (1999). Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microbiol. **65**: 3566–3574.

Madigan, M, and J. Martinko (ed) (2005). Brock biology of microorganisms, 11 edn. Prentice Hall, Upper Saddle River, NJ

Maeda, R., H. Nagashima, J. Widada, K. Iwata, and T. Omori (2009). Novel marine carbazole-degrading bacteria. FEMS. Microbiol. Letters. **292**: 203–209.

Marchesi, J. R., T. Sato, A. J. Weightman, T. A. Martin, J. C. Fry, S. J. Hiom and W. G. Wade (1998). Design and Evaluation of useful Bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. **64**: 795-799.

Mariotti, A., A. Landreu, and B. Simon, (1988). 15^N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. Geochim. Cosmochim. Acta. **52**: 1869–1878.

Marschner, P., and S. Timonen (2005). Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Appl. Soil. Ecol. **28**: 23–36.

Marschner, P., and S. Timonen S (2004). Interactions between plant species,mycorrhizal colonization and light intensity on the bacterial community composition in the rhizosphere. Appl. Soil Ecol. **28**: 23–36.

Marschner, P., C. H. Yang, R. Lieberei, and. D. E. Crowley (2001). Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. **33**: 1437–1445.

Martin, T. L., N. K. Kaushik, J. T. Trevors, and H. R. Whiteley (1999). Review: denitrification in temperate climate riparian zones. Water Air soil pollution. **111**: 171-186.

Martins-Loução, M. A., and C. Cruz (2007). Microbial communities (Editorial). J. Soils Sediments. **7**(6): 398.

Mateju, V. S., J. Cizinska, Krejci, and T. Janoch (1992). Biological water denitrification -a review. Enzyme Microb. Technol. **14**: 170-183.

Maynard,C., F. Berthiaume, K. Lemarchand, J. Harel, P. Payment, P. Bayardelle, L. Masson, and R. Brousseau (2005). Waterborne pathogen detection by use of oligonucleotide-based microarrays. Appl Environ. Microbiol. **71**: 8548–8557.

Mayo, A. W., and J. Mutamba (2004). Effect of HRT on nitrogen removal in a coupled HRP and unplanted subsurface flow gravel bed constructed wetland. Phys. Chem. Earth. **29**: 1253–1257.

McCaig A. E., L. A. Glover and J. J. Prosser (2001). Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. Appl. Environ. Microbiol. **67**: 4554– 59.

McCaig, A. E., L. A. Glover, and J. I Prosser (1999). Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. **65**:1721-1730.

McCaig, A. E., L. A. Glover, and J. I. Prosser (1999). Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. **65**:1721-1730.

Mendez, M. O., J. W. Neilson, and R. M. Maier (2008). Characterization of a bacterial community in an abandoned semiarid lead zinc mine tailing site. Appl. Environ. Microbiol. **74**: 3899–3907.

Mene´ndez, A. B., J. M. Scervino, and A. M. Godeas (2001). Arbucular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. Biol. Fertil. Soil. **33**: 373–381.

Metcalf and Eddy, revised by G. Tchobanoglous, and F. L. Burton (1991). Wastewater Engineering – Treatment, Disposal, and Reuse, 3rd ed., McGraw-Hill, New York.

Millar, L. C, and M. E. Barbercheck (2002). Effects of tillage practices on entomopathogenic nematodes in a corn agro ecosystem. Bio. Control. **25**: 1–11.

Miller, K. M., T. J. Ming, A. D. Schulze and R. E. Withler (1999). Denaturing gradient gel electrophoresis (DGGE): A rapid and sensitive technique to screen nucleotide sequence variation in populations. Bio. Techniques. **27**: 1016–1030.

Mills, D. K., K. Fitzgerald, C. D. Litchfield, and P. M. Gillevet (2003). A comparison of DNA profiling techniques for monitoring nutrient impact on microbial community composition during bioremediation of petroleum-contaminated soils. J Microbiol Methods. **54**: 57–74.

Mitsch, W. J, and J. G. Gosselink (1986). Wetlands. Van Nostrand Reinhold Company, New York, USA.

Mocali, S., D. Paffetti, G. Emiliani, A. Benedetti, and R. Fani (2008). Diversity of heterotrophic aerobic cultivable microbial communities of soils treated with fumigants and dynamics of metabolic, microbial, and mineralization quotients. Biol Fertil Soils. **44**: 557–569.

Molla, A. H, Z. H. Shamsuddin, and H. M.Saud (2001). Mechanism of root growth and promotion of nodulation in vegetable soybean by *Azospirillum brasilense.* Commun. Soil Sci. Plant Ana. **32**: 2177– 87.

Monteiro, J. M., R. E. Vollu, M. R. R. Coelho, C. S. Alviano, A. F. Blank, and L. Seldin (2009). Culture-dependent and -independent approaches to analyze the bacterial community of different genotypes of Chrysopogon zizanioides (L.) Roberty (vetiver) rhizospheres. J. Microbiol. **47**: 363–370.

Mougel, C., P. Offre, L. Ranjard, T. Corbeand, E. Gamalero, C. Robin, and P. Lemanceau (2006). Dynamics of the genetic structure of bacterial and fungal communities at different developmental stages of Medicago truncatula Gaertn.cv.jemalong line J5. New Phytol. **170**: 165–175.

Mueller-Spitz, S. R., G. W. Goetz, and S. L. McLellan (2009). Temporal and spatial variability in near shore bacterioplankton communities of Lake Michigan. FEMS Microbiol Ecol **67**: 511–522.

Mühling, M., J. Woolven-Allen, C. Murrell, and I. Joint (2008). Improved group-specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. ISME J. **2**: 379–392.

Mullen, M. D., C. G. Melhorn, D. D. Tyler, and B. N. Duck (1998). Biological and biochemical soil properties in no-till corn with different cover crops. J. Soil Water Conserv. **53**: 219–224.

Mungai, N.W., P. P Motavalli, K. A. Nelson, and R. J. Kremer (2005). Differences in yields, residue composition and N mineralization dynamics of Bt and non-Bt maize. Nutr. Cycl. Agroecosyst. **73**: 101–109.

Murty, D., M. U. F. Kirschbaum, R. E. McMurtrie, and A. McGilvray (2002). Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. Global Change Biol. **8**: 105–123.

Muscutt, A. D., G. L. Harris, S. W. Bailey, and D. B. Davies (1993). Buffer zones to improve water quality: a review of their potential use in UK agriculture. Agricul. Ecosyst. Environ. **45**: 59-77.

Muyzer, G (1999). DGGE/TGGE, a method for identifying genes from natural ecosystems. Curr. Opin. Microbiol. **2**: 317–322.

Muyzer, G, and K. Smalla (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek. **73**: 127–141.

Muyzer, G., E. C. de Waal, and A. G. Uitterlinden (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ Microbiol. **59**(3): 695-700.

Nagy, M. L., A. Perez, and F. Garcia-Pichel (2005). The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiol. Ecol. **54**: 233–245.

Nakatsu, C. H (2004). Microbial community analysis. In D. Hillel et al. (ed.) Encyclopedia of soils in the environment. Elsevier, Oxford, UK. p. 455–463.

Nakatsu, C. H (2007). Soil microbial community analysis using denaturing gradient gel electrophoresis. Soil Sci. Soc. Am. J. **71**: 562–571.

Nakatsu, C. H., N. Carmosini, B. Baldwin, F. Beasley, P. Kourtev, and A. Konopka (2005). Soil microbial community responses to additions of organic carbon substrates and heavy metals (Pb and Cr). Appl. Environ. Microbiol. **71**: 7679–7689.

Nakatsu, C. H., V. Torsvik, and L. Øvreas (2000). Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil. Sci. Soc. Am. J. **64**: 1382–1388.

Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Loretta, P. Giacomo, and R. Giancarlo (2003). Microbial diversity and soil functions. Eur. J. Soil. Sci. **54**: 655–670

Niva, M., A. Hernesmaa, K. Haahtela, M. S-Salonen, K. Sivonen, and K. Haukka (2006). Actinobacterial communities of boreal forest soil and lake water are rich in mycobacterium. B. Environ. Res. **11**: 45-53.

Nogales, B., E. R. Moore, W. R. Abraham, and K. N. Timmis (1999). Identification of the metabolically active members of a bacterial community in a polychlorinated biphenyl-polluted moorland soil. Environ Microbiol. **1**: 199–212.

Nogales, B., K. N. Timmis, D. B. Nedwell, and A. M. Osborn (2002). Detection and diversity of expressed denitrification genes in estuarine sediments after reverse transcription-PCR amplification from mRNA. Appl. Environ. Microbiol. **68**: 5017-5025.

Norton, J. M., M. G. Klotz, L. Y. Stein, D. J.Arp, P. J. Bottomley, P. S. G. Chain, L. J.Hauser, M. L. Land, F. W. Larimer, M. W. Shin, ands. S. R. Starkenburg (2008). Complete genome sequence of Nitrosospira multiformis, an ammonia-oxidizing bacterium from the soil environment. Appl Environ Microbiol. **74**(11): 3559–3572.

Nübel, U., F. Garcia-Pichel, M. Kühl, and G. Muyzer (1999) .Quantifying microbial diversity: morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. App Env Microbiol **65**: 422–430.

Nunan, N., T. J. Daniell, B. K. Singh, A. Papert, J. W. McNicol, and J. I. Prosser (2005). Links between plant and rhizoplane bacterial communities in grassland soils characterized using molecular techniques. Appl. Environ. Microbiol. **71**: 6784–6792.

Nusslein, K, and J. M. Tiedje (1999). Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl. Environ. Microbiol**. 65**: 3622–26.

NWWG (1997). The Canadian wetland classification system. In: Warner, B. G, and C. D. A. Rubec (Eds.), National Wetland Working Group, University of Waterloo, Wetland Research Centre, pp. 1– 68.

Ogram, A., G. S. Sayler, and T. Barkay (1987). The extraction and purification of microbial DNA from sediments. J Microbiol Methods. **7**: 57–66.

Ohtonen. R., S. Aikio, and H. Vare (1997). Ecological theories in soil biology. Soil Biol. Biochem. **29**: 1613–1619.

Osborn A. M., E. R. B. Moore, and K. N. Timmis (2000). An evaluation of terminal restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ. Microbiol. **2**: 39-50.

Ostrom, N. E., L. O. Hedin, J. C. von Fischer, and G. P. Robertson (2002). Nitrogen transformations and NO^-_3 removal at a soil stream interface: a stable isotope approach. Ecol. Appl. **12**(4): 1027–1043.

Overhage. J., S. Sielker, S. Homburg, K. Parschat, and S. Fetzner (2005). Identification of large linear plasmids in Arthrobacter spp. encoding the degradation of quinaldine to anthranilate. Microbiol. **151**: 491–500.

Pace, N. R. (1997). A molecular view of microbial diversity and the biosphere. Science. **276**: 734– 740.

Palmer, K. M., and J. P. W. Young (2000). Higher diversity of *Rhizobium leguminosarum* biovar *viciae* populations in arable soils than in grass. Soils Appl. Environ. Microbiol. **66**: 2445–50.

Papert, A., C. J. Kok, and J. D. van Elsas (2004). Physiological and DNA fingerprinting of the bacterial community of *Meloidogyne fallax* egg masses. Soil Biol. Biochem. **36**: 1843–1849.

Patra, D. D., P. C. Brookes, K. Coleman, and D. S. Jenkinson (1990). Seasonal changes of soil microbial biomass in an arable and a grassland soil which have been under uniform management for many years. Soil Biol. Biochem. **22**:739–742.

Peacock, A. D., Y-J. Chang, J. D. Istok, L. Krumholz, R. Geyer, B. Kinsall, D. Watson, K. L. Sublette, and D.C. White (2004). Utilization of microbial biofilms as monitors of bioremediation. Microb. Ecol. **47**: 284–292.

Peterjohn, W. T, and. L. Correll (1984). Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecol. **65**: 1466-1475.

Phillips, D. A., H. Ferris, D. R. Cook, and D. R. Strong (2003). Molecular control points in rhizosphere food webs. Ecol. **84**: 816– 826.

Phipps, R. G. and W. G. Crumpton (1994). Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. Ecol. Eng. **3**: 399–408.

Pielou, E. C. (1969). An Introduction to Mathematical Ecology. John Willy & Sons. Inc., New York.

Pinay, G., L. Roques, and A. Fabre (1993). Spatial and temporal patterns of denitrification in a riparian forest. J. Appl Ecol. **30**: 581–591.

Pindi, P. K., K. H. Kishore, G. S. Reddy, and S. Shivaji (2009). Description of Leifsonia kafniensis sp. nov. and Leifsonia antarctica sp. nov. Int. J. Syst. Evol. Microbiol. 59:1348-1352.

Pomeroy, R. L., and W. J. Wiebe (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat Microb Ecol. **23**: 187–204.

Pommier, T., B. Canback, and L. Riemann (2007). Global patterns of diversity and community structure in marine bacterioplankton. Mol. Ecol. **16**: 867–880.

Price, P. B., and T. Sowers (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. PNAS. **101**(13): 4631-4636.

Prosnansky, M., Y. Sakakibarab, and M. Kuroda (2002). High-rate denitrification and SS rejection by biofilm-electrode reactor (BER) combined with microfiltration. Water Res. **36**: 4801–4810.

Purdy, K. J., D. B. Nedwell, and T. M. Embley **(**2003). Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Appl. Environ. Microbiol. **69**: 3181–3191.

Pyle, B. H., S. C. Broadway, and G. A. McFeters (1995). Factors affecting the determination of respiratory activity on the basis of cyanoditolyl tetrazolium chloride reduction with membrane filtration. Appl. Environ. Microbiol. **61**: 4304–4309.

Quinn, J. M.; G. L. Steele, C. W. Hickey, and M. L. Vickers (1994). Upper thermal tolerances of twelve common New Zealand stream invertebrate species. New Zealand J. Marine. Freshwater Research .**28**: 391-397.

Ranjard, L., F. Poly, J. C. Lata, C. Mougel, J. Thioulouse, and S. Nazaret (2001). Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Appl. Environ. Microbiol. **67**: 4479–87.

Ravenschlag, K., K. Sahm, C. Knoblauch, B. B. Jorgensen and R. Amann (2000). Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine Arctic sediments. Appl. Environ. Microbiol. **66**: 3592–3602.

Reardon, C. L., D. E. Cummings, L. M. Petzke, B. L. Kinsall, D. B. Watson, B. M. Peyton, and G. G. Geesey (2004). Composition and diversity of microbial communities recovered from surrogate minerals incubated in an acidic uranium-contaminated aquifer. Appl Environ Microbiol. **70**: 6037–6046.

Reddy, K. R, and W. H. Patrick (1984). Nitrogen transformations and loss in flooded soils and sediments. CRC Crit Rev Environ Control. **13**: 273–309.

Reganold, J. P., A. S. Palmer, J. C. Lockhart, and A. N. Macgregor (1993). Soil quality and financial performance of biodynamic and conventional farms New Zealand. New Zealand. Science .**260**: 344–349.

Rhee, S.-K., X. Liu, L. Wu, S. C. Chong, X. Wan, and J. Zhou (2004). Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Appl. Environ. Microbiol. **70**: 4303–4317.

Richardson, W., E. Strauss, E. Bartsch, L. Monroe, E. Cavanaugh, and J. Vingum (2004). Denitrification in the upper Mississippi River: Rates, controls, and contribution to nitrate flux. Can. J. Fish. Aquat. Sci. **61**: 1102–1112.

Rijn, J. V., Y. Tal, and H. J. Schreier (2006). Denitrification in re-circulating systems: theory and applications. Aquacul. Eng. **34**: 364–376.

Ritchie, J. T (1972). A model for predicting evaporation for a row crop with incomplete cover. Water Res. Research. **8**: 1204-1213.

Ritz, K., J. W. McNicol, N. Nunan, S. Grayston, P. Millard, D. Atkinson, A. Gollotte, D. Habeshaw, B. Boag, and C. D. Clegg (2004). Spatial structure in soil chemical and microbiological properties in upland grassland. FEMS Microbiol. Ecol. **49**:191–205.

Rothman, R. E., M. D. Majmudar, G. D. Kelen, G. Madico, C. A. Gaydos, T. Walker, and T. C. Quinn (2002). Detection of bacteremia in emergency department patients at risk for infective endocarditis using universal 16S rRNA primers in a decontaminated polymerase chain reaction assay. J. Infect. Dis. **186**: 1677–1681.

Russell, J. M., A. J. van Oostrom, and S. B. Lindsey (1994). Denitrifying sites in constructed wetlands treating agricultural industry waste – a note. Environ. Technol. **15:** 95–99.

Sait, M., P. Hugenholtz., and P. H Janssen (2002). Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. Environ. Microbiol. **4**: 654–666.

Sakamoto, M., Y.Takeuchi, M. Umeda, I. Ishikawa, and Y. Benno (2003). Application of terminal RFLP analysis to characterize oral bacterial flora in saliva of healthy subjects and patients with periodontitis. Journal of Medical Microbiology. **52**: 79–89.

Sala, M. M., R. Terrado, C. Lovejoy, F. Unrein, and C. Pedros-Alio (2008). Metabolic diversity of heterotrophic bacterioplankton over winter and spring in the coastal Arctic Ocean. Environ. Microbiol. **10**: 942–949.

Sallam, A, and A. Steinbüchel (2008). Anaerobic and aerobic degradation of Cyanophycin by the denitrifying bacterium Pseudomonas alcaligenes strain DIP1 and role of three other coisolates in a mixed bacterial consortium. Appl Environ Microbiol. **74**: 3434–3443.

Saul, D. J., J. M. Aislabie, C. E. Brown, L. Harris, and J. M. Foght (2005). Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. FEMS Microbiol. Ecol. **53**: 141–155.

Schipper, L. A., A. B. Cooper, C. G. Harfoot, and W. J. Dyck (1993). Regulators of denitrification in an organic riparian soil. Soil Biol. Biochem. **25**: 925–933.

Schmalenberger, A., and C. C. Tebbe (2002). Bacterial community composition in the rhizosphere of transgenic, herbicideresistant maize (*Zea mays*) and comparison to its non-transgenic cultivar Bosphore. FEMS Microbiol. Ecol. **40**: 29– 37.

Seghers, D., L. Wittebolle, E. M. Top, W. Verstraete, and S. D. Siciliano (2004). Impact of agricultural practices on the *Zea mays* L. endophytic community. Appl. Environ. Microbiol. **70**: 1475–1482.

Sekiguchi, H. M., M. Watanabe, T. Nakahara, B. Xu, and H. Uchiyama (2002). Succession of bacterial community structure along the Changjiang River determined by denaturing gradient gel electrophoresis and clone library analysis. Appl Environ Microbiol **68**: 5142-5150.

Sessitsch, A., A. Weilharter, M. Gerzabek, H. Kirchmann, and E. Kandeler (2001). Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl. Environ. Microbiol. **67**: 4215–4224.

Shannon, C. E. and W. Weaver (1963). The Mathematical Theory of Communication. In: University Illinois Press, Urbana, p. 117.

Sharma, N., Y. Sudarsan, R. Sharma, and G. Singh (2008). RAPD analysis of soil microbial diversity in western Rajasthan. Cur. Sci. **94**: 1058-1061.

Shrimali, M., and K. P. Singh (2001). New methods of nitrate removal from water. Environ. Pollut. **112**: 352-359.

Shuai, J. J., Y. S. Tian, Q. H. Yao, Ri. H. Peng, F. Xiong and A. S. Xiong (2010). Identification and Analysis of Polychlorinated Biphenyls (PCBs)- Biodegrading Bacterial Strains in Shanghai. Curr. Microbiol. **61**: 477–483.

Siciliano, S. D., and J. J. Germida (1998). Biolog analysis and fatty acid methyl ester profiles indicate that Pseudomonad inoculants that promote phytoremediation alter the root-associated microbial community of Bromus biebersteinii. Soil Biol. Biochem. **30**: 1717–1723.

Sigler, W. V., and R. F. Turco (2002). The impact of chlorothalonil application on soil bacterial and fungal populations as assessed by denaturing gradient gel electrophoresis. Appl. Soil Ecol. **21**: 107–118.

Sigler, W. V., S. Crivii, and J. Zeyer (2002). Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb. Ecol. **44**: 306–316.

Singh, B. K., P. Millard, A. S. Whiteley, and J. C. Murrell (2004). Unravelling rhizosphere–microbial interactions: opportunities and limitations. Trends Microbiol. **12**: 386– 393.

Singh, B. K., S. Munro, J. M. Potts, and P. Millard (2007). Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Applied soil ecology. **36**: 147–155.

Sirivedhin, T., and K. A. Gray (2006). Factors affecting denitrification rates in experimental wetlands: Field and laboratory studies. Ecol. Eng. **26**: 167–181.

Small, J., D. R. Call, F.J. Brockman, T. M. Straub, and D. P. Chandler (2001). Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. Appl. Environ. Microbiol. **67**: 4708–4716.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microbiol. **67**: 4742–4751.

Smalla, K., U. Wachtendorf, H. Heuer, W. Liu, and L. Forney (1998). Analysis of BIOLOG GN substrate utilization patterns by microbial communities. Appl. Environ. Microbiol**. 64**: 1220–25.

Smit, E., P. Leeflang, and K. Wernars (1997). Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. FEMS Microbiol. Ecol. **23**: 249-261.

Smith, C. M (1989). Riparian pasture retirement effects on sediment, phosphorus and nitrogen in channelised surface run-off from pastures**.** New Zealand J. Marine. Freshwater Research. **23**: 139-146.

Smith, K. P., and R. M. Goodman (1999). Host variation for interactions with beneficial plant-associated microbes. Annu. Rev. Phytopathol**. 37**: 473–491.

Smith, R. L., S. P. Garabedian, and M. H. Brooks (1996). Comparison of denitrification activity measurements in groundwater using cores and natural-gradient tracer tests. Environ. Sci. Technol. **30**: 3448–3456.

Smith, S. M., R. M. M. Abed, and F. Garcia-Pichel (2004). Biological soil crusts of sand dunes in Cape Cod National Seashore, Massachusetts, USA. Microb. Ecol. **48**: 200–208.

Sobieszuk, P, and K. W. Szewczyk (2006). Estimation of (C/N) ratio for microbial denitrification. Environ. Technol. **27**: 103-108.

Soderberg, K. H, P. A.Olsson, and E. Baath (2002). Structure and activity of the bacterial community in the rhizosphere of deferent plant species and the effect of arbuscular mycorrhizal colonisation. FEMS Microbiol. Ecol. **40**: 223-231.

Spring, S., R. Schlze, J. Overmann, and K. H. Schleifer **(**2000). Identification and Mcharacterization of ecologically significant prokaryotes in the sediment of freshwater lakes: molecular and cultivation studies. FEMS Microbiol. Rev. **24**: 573–590.

Spruill, T. B (2004). Effectiveness of riparian buffers in controlling ground-water discharge of nitrate to streams in selected hydrogeologic settings of the North Carolina Coastal Plain. Water Sci Tech. **49**: 63-70.

Srinivas T. N. R., P. Anil Kumar, Ch. Sasikala, and Ch. V. Ramana (2007). Rhodovulum imhoffii sp. nov. In. J. Syst. Evol. Microbiol. **57**: 228–232.

Staley, J. T., and A. Konopka (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. **39**: 321–346.

Steenwerth, K. L., L. E. Jackson, F. G. Calderon, and M. R. Stromberg (2003). Soil microbial community and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. **35**: 489–500.

Steer, J., and J. A. Harris (2000). Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of Agrostis stolonifera. Soil Biol. Biochem. **32**: 869–878.

Stolz A (1999) Degradation of substituted naphthalenesulfonic acids by Sphingomonas xenophaga BN6. J. Ind. Microbiol. Biotechno. **23**: 391–399.

Stres, B. (2007). The relationship between total and cultural bacteria in cold soils. Acta Agri Slovenica. **90**: 25–31.

Sturz, A. V., and J. Nowak (2000). Endophytic communities of rhizobacteria and the strategies to create yield enhancing associations with crops. Appl. Soil. Ecol. **15**: 183–190.

Sun, G., Y. Zhao, and S. Allen (2006). Generating 'tide' in pilot-scale constructed wetlands to enhance agricultural wastewater treatment. Eng. Life Sci. **6**: 560–565.

Sun, G., Y. Zhao., and S. Allen (2005). Enhanced removal of organic matter and ammoniacal-nitrogen in a column experiment of tidal flow constructed wetland system. J. Biotechnol. **115**: 189–197.

Sun, H. Y., S. P. Deng and R. Raun (2004). Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Appl. Environ. Microbiol. **70**: 5868–5874.

Suzuki, M. T., M. S. Rappe, Z. W. Haimberger, H. Winfield, N. Adair, J. Strobel, and S. J. Giovannoni (1997). Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. Appl Environ Microb **63**: 983–989.

Swackhamer, D. L., H. W. Paerl, S. J. Eisenreich, J. Hurley, K. C. Hornbuckle, M. McLachlan, D. Mount, D. Muir, and D. Schindler (2004). Impacts of atmospheric pollutants on aquatic ecosystems. Issues in Ecol. **12**: 1–24.

Swift, M. J (1999). Towards the second paradigm: integrated biologicalì management of soil. In: Siqueira JO, Moreira FMS, Lopes AS, Guilherme LRG, Faquin V, Furtani Neto AE, Carvalho JG (eds) Interrelacao Fertilidade Biologia do Solo e Nutric¸ao de Plantas. UFLA, Lavras, Brazil, pp 11–24.

Szekeres, S., I. Kiss, M. Kalman, and M. I. M. Soares (2002). Microbial population in a hydrogen-dependent denitrification reactor. Water Res. **36**: 4088–4094.

Tabacchioni, S., L. Chiarini, A. Bevivino, C. Cantale, and C. Dalmastri (2000). Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. Microb. Ecol. **40**: 169– 176.

Tamaki, H., Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, and Y. Kamagata (2005). Comparative Analysis of Bacterial Diversity in Freshwater Sediment of a Shallow Eutrophic Lake by Molecular and Improved Cultivation-Based Techniques. Appl. Environ. Microbiol. **71**: 2162–2169.

Tan, G-L., W. S. Shu, W. H. Zhou, X. L. Li, C. Yu. Lan, and L. N. Huang (2009). Seasonal and spatial variations in microbial community structure and diversity in the acid stream draining across an ongoing surface mining site. FEMS Microbiol. Ecol. **70**: 277–285.

Taylor, G. D., T. D. Fletcher, T. H. F. Wong, and H. P. Duncan (2006). Base flow water quality behavior: Implications for wetland performance monitoring. Aust. J. Water Res. **10**: 293–302.

Taylor, G. D., T. D. Fletcher, T. H. F. Wong, and P. F. Breen (2005). Nitrogen composition in urban runoff – implications for storm water management. Water Res. **39**: 1982–1989.

Theron, J, and T. E. Cloete (2000). Molecular techniques for determining microbial diversity and community structure in natural environments. Critical Review Microbiol. **26**: 37–57.

Thoms, C., A. Gattinger, M. Jacob, F. M. Thomas and G. Gleixne (2010). Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biol. Biochem. **42**: 1558-1565.

Throbäck, A. N., K. Enwall, Å. Jarvis, and A. Hallin (2004). Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol. Ecol. **49**: 401-417.

Tiedje, J. M., J. C. Cho, A. Murray, D. Treves, B. Xia, and J. Zhou (2001). Soil teeming with life: new frontiers for soil science. In: Sustainable Management of Soil OrganicMatter (eds R.M. Rees, B.C. Ball, C.D. Campbell & C.A. Watson), CAB International, Wallingford. pp. 393–412.

Tiedje, J. M., S. Asuming-Brempong, K. Nusslein, T. L. Marsch, and S.J. Flynn (1999). Opening the black box of soil microbial diversity. Appl. Soil Ecol. **13**: 109–22.

Tiedje, J. M., S. Simkins, and P. M. Groffman (1989). Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Plant Soil. **115**: 261-284.

Tiehang, W., D. O. Chellemi, J. H. Graham, K. J. Martin, and E. N. Rosskopf (2008). Comparison of Soil Bacterial Communities Under Diverse Agricultural Land Management and Crop Production Practices. Microb Ecol. **55**: 293–310.

Tilak, K. V. B. R. , N. Ranganayaki, K. K. Pal, R. De, A. K. Saxena, C. S. Nautiyal, S. Mittal, A. K. Tripathi, and B. N. Johri (2005). Diversity of plant growth and soil health supporting bacteria. Curr. Sci. **89**: 136–150.

Tiquia, S. M (2005). Microbial community dynamics in manure compost based on 16S and 18S rDNA T-RFLP profiles**.** Environ. Technol. **26**: 1101-1113.

Tisdall, J. M., B. Cockroft, and N. C. Uren (1978). The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. Aust. J. Soil. Res. **10**: 9–18.

Toffanin A., M. Basaglia, C. Ciardi, P. Vian, S. Povolo, and S. Casella (2000). Energy content decrease and viable-not-culturable status induced by oxygen limitation coupled to the presence of nitrogen oxides in *Rhizobium 'hedysari'*. Biol. Fertil. Soils .**31**(6): 484-488.

Torsvik, V., and L. Ovreas (2002). Microbial diversity and function in soil: from genes to ecosystems.Curr. Opin. Microbiol**.** **5**: 240–245.

Torsvik, V., F. Daae, R-A. Sandaa, and L. Ovreås (1998). Novel techniques for analysing microbial diversity in natural and perturbed environments. J Biotechnol. **64**: 53-62.

Torsvik, V., K. Salte, R. Soerheim, and J. Goksoeyr (1990). Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. Appl. Environ. Microbiol. **56**: 776– 781.

Torsvik, V., L. Øvreås, and T. F. Thingstad (2002). Prokaryotic diversitymagnitude, dynamics and controlling factors. Science. **296**: 1064–1066.

Torsvik,V., J. Goksøyr, and F. L. Daae (1990). High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. **56**: 782–787.

Trevors, J. T (1998). Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. Water Air Soil Pollut. **101**: 45– 67.

Tsai, S. H., A. Selvam, and S. S. Yang (2007). Microbial community of topographical gradient profiles in Fushan forest soils of Taiwan. Ecol. Res. **22**: 814-824.

Tsai, S. H., A. Selvam, Y. P. Chang, and S. S. Yang (2009). Soil bacterial community composition across different topographic sites characterized by 16S rRNA gene clones in the Fushan Forest of Taiwan. Botanical Studies. **50**: 57-68.

Urbach, E., K. L. Vergin, and S. J. Giovannoni (1999). Immonochemical detection and isolation of DNA from metabolically active bacteria. Appl. Environ. Microbiol. 65: 1207–13.

USDA-SCS (U.S. Department of Agriculture, Soil Conservation Service) (1984). User's guide for the CREAMS computer model—Washington Computer Center version. Washington, D.C. USDA-SCS TR-72.

USEPA (1993). Subsurface Flow Constructed Wetlands for Wastewater Treatment: A Technology Assessment, Office of Water, EPA 832-R-93-008, Washington, D.C

USEPA (2002a). National water quality inventory: 2000 Rep., EPA/ 841/R-02/001. Office of Water, Washington, DC.

USEPA (2002b). National primary drinking water standards, EPA/ 816/F-02/013. Office of Water, Washington, DC.

Vainio, E. J., and J. Hantula (2000). Genetic differentiation between European and North American populations of *Phlebiopsis gigantea*. Mycologia. **92**: 436–446.

Van der Heijden, M. G. A., J. N. Klinonomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. **396**: 69–7.

Van Elsas, J. D, and J. T. Trevors (1997). Wellington E M H, editors. Modern soil microbiology. New York, N.Y: Marcel Dekker, pp. 353–373.

van Elsas, J. D., G. E. Duarte, A. S. Rosado, and K. Smalla (1998). Microbiological and molecular methods for monitoring microbial inoculants and their effects in the environment. J. Microbiol. Methods. **32:** 133–54.

van Elsas, J. D., P. Garbeva and J. Salles (2002). Effects of agronomical pressures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation. **13**: 29–40.

van Oostrom, A. J and J. M. Russell (1994). Denitrification in constructed wastewater wetlands receiving high concentrations of nitrate. Water Sci. Technol. **29:** 7–14.

Vidon, P., and A. R. Hill (2004a). Denitrification and patterns of electron donors and acceptors in eight riparian zones with contrasting hydrogeology. Biogeochem. **71:** 259–283.

Vieira, R. P, A. M. Gonzalez, and A. M. Cardoso (2008). Relationships between bacterial diversity and environmental variables in a tropical marine environment, Rio de Janeiro. Environ. Microbiol. **10**: 189–199.

Vitousek, P. M., J. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman (1997). Human alteration of the global nitrogen cycle: Causes and consequences. Issues in Ecol. **1:** 1–15.

von Wintzingerode, F., U. B. Gobel, and E. Stackebrandt (1997). Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. **21**: 213–229.

Vymazal, J (1995). Algae and Element Cycling in Wetlands, Lewis Publishers, Chelsea. 698.

Vymazal, J (2007). Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. **380**: 48–65.

Wang, J., T. Ma, L. Zhao, J. Lv, G Li, H. Zhang, B. Zhao, F. Liang, and R. Liu (2008). Monitoring exogenous and indigenous bacteria by PCRDGGE technology during the process of microbial enhanced oil recovery. J Ind Microbiol Biotechnol. **35**: 619–628.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole (2007). A naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. **73**: 5261-5267.

Wani, A. A., V. P. Surakasi, J. Siddharth, R. G. Raghavan, M.S. Patole, D. Ranade, and Y. S. Shouche (2006). Molecular analyses of microbial diversity associated with the Lonar soda lake in India: An impact crater in a basalt area. Res. Microbiol. 157, 928- 937.

Ward, R. D., I. E. Morton, R. P. Brazil, S. Trumper, and A. L. Falcao (1990). Preliminary laboratory and field trials of a heated pheromone trap for the sand fly Lutzomyia longipalpis (Diptera: Psychodidae). Mem Inst Oswaldo Cruz. **85**: 445–452.

Wartiainen, I., A. G. Hestnes, and M. M. Svenning (2003). Methanotrophic diversity in high arctic wetlands on the islands of Svalbard (Norway): Denaturing gradient gel electrophoresis analysis of soil DNA and enrichment cultures. Can. J. Microbiol. **49**: 602–612.

Weber, S., S. Stubner, and R. Conrad (2001). Bacterial populations colonizing and degrading rice straw in anoxic paddy soil. Appl. Environ. Microbiol. **67**: 1318–1327.

Webster, G., T. M. Embley and J. Prosser (2002). Grassland management regimes reduce small-scale heterogeneity and species diversity of Beta-proteobacterial ammonia oxidizer population. Appl. Environ. Microbiol. **68**: 20–30.

Welch, D. J (1991). Riparian forest buffers–Functional and design protection and enhancement of water resources. Tech. Rep. NA-PR- 07-91. USDA Forest Service, Radnor, Pennsylvania.

Wenhui, Z., C. Zucong, Y. Lichu, and Z. He (2007). Effects of the long-term application of inorganic fertilizers on microbial community diversity in rice-planting red soil as studied by using PCR-DGGE. Acta Ecol. Sinica. **27**: 4011-4018.

Wetzel, R. G (2000). Fundamental processes within natural and constructed wetland ecosystems: Short-term vs. long-term objectives, in Proc. of the 7th Int. Conf. on Wetland Systems for Water Pollution Control, 11–16 November, Lake Buena Vista 3–12.

Whiteley, A. S, and M. J. Bailey (2000). Bacterial community structure and physiological state within an industrial phenol bioremediation system. Appl. Environ. Microbiol. **66**: 2400– 2407.

Wieland, G., R. Neumann, and H. Backhaus (2001). Variation of microbial communities in soil, rhizosphere and rhizoplane in response to crop species, soil type, and crop development. Appl. Environ. Microbiol. **67**: 5849–54.

Wilbur, J. D., J. K. Ghosh, C. H. Nakatsu, S. M. Brouder, and R. W. Doerge (2002). Variable selection in high-dimensional multivariate binary data with application to the analysis of microbial community DNA fingerprints. Biometrics. **58**: 378–386.

Winding, A., and N. B. Hendriksen (2007). Comparison of CLPP and enzyme activity assay for functional characterization of bacterial soil communities. J. Soils Sediments**. 7**: 411–417.

Wise, M. G., J. V. McArthur, and L. J. Shimkets (1999). Methanotroph diversity in landfill soil: Isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Appl. Environ. Microbiol. **65:** 4887–4897.

Wolters V (2001). Biodiversity of soil animals and its function. Eur J Soil Biol. **37:** 221–227.

Xu, Q., P. Jiang, and Z. H. Xu (2008a). Soil microbial functional diversity under intensively managed bamboo plantations in southern China. J. Soils Sediments. **8**: 177–183.

Xu, Z., S. Ward, C. Chen, T. Blumfield, N. Prasolova, and J. Liu (2008b). Soil carbon and nutrient pools, microbial properties and gross nitrogen transformations in adjacent natural forest and hoop pine plantations of subtropical Australia. J Soils Sediments. **8**: 99– 105.

Yang, S., S. Lin, G. D. Kelen, T. C. Quinn, J. D. Dick, C. A. Gaydos, and R. E. Rothman (2002). Quantitative multiprobe PCR assay for simultaneous detection and identification to species level of bacterial pathogens. J. Cli. Microbiol. **40**: 3449–3454

Yao, S., I. A. Merwin, G. W. Bird, G. S. Abawi, and J. E. Thies (2005). Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. Plant Soil. 271: 377–389.

Yendi, E. N-N., J- R. Janet, del. C. G-C. Maria, H-G. Regina, and H-R. Cesar (2010). Bacterial communities associated with the rhizosphere of pioneer plants (Bahia xylopoda and Viguiera linearis) growing on heavy metals-contaminated soils. Antonie van Leeuwenhoek. **97**: 335–349.

Yin, B., D. Crowley, G. Sparovek, W. J. De Melo, and J. Borneman (2000). Bacterial functional redundancy along a soil reclamation gradient. Appl. Environ. Microbiol. **66**: 4361–65.

Yu, F. P, and G. A. McFeters (1994). Rapid in situ assessment of physiological activities in bacterial biofilms using fluorescent probes. J. Microbiol Methods. **20**: 1–10.

Yu, Z., and M. Morrison (2004). Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **70**: 4800-4806.

Yu, Z., R. Garcia-Gonzalez, F. L. Schanbacher, and M. Morrison (2008). Evaluations of different hypervariable regions of archaeal 16S rRNA genes in profiling of methanogens by Archaea-specific PCR and denaturing Gradient gel electrophoresis. Appl. Environ. Microbiol.**74**: 889-893.

Yu. L., Y. Liu, and G. Wang (2009). Identification of novel denitrifying bacteria Stenotrophomonas sp. ZZ15 and Oceanimonas sp. YC13 and application for removal of nitrate from industrial wastewater. Biodegradation. **20**: 391–400.

Zdanowski, M. K, and P. Weglenski (2001). Ecophysiology of soil bacteria in vicinity of Henryk Arctowski Station, King George Islan, Antarctica. Soil Biol. Biochem. **33**: 819–829.

Zehr, J. P., and B. B. Ward (2002). Nitrogen cycling in the ocean: new perspectives on processes and paradigms. Appl. Environ. Microb. **68**: 1015–1024.

Zelles, L (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fertil. Soils. **29**: 111 – 129.

Zhang, H. L. Y., C. G. Zhang, and G. X. Chen (2005). Effect of petroleum-containing wastewater irrigation on bacterial diversities and enzymatic activities in a paddy soil irrigation area. J. Environ. Qual. **34**: 1073–1080.

Zhang, Y., D. Junde, Y. Zhihao, Z. Si, and W. Youshao (2008). Phylogenetic diversity of nitrogen-fixing bacteria in mangrove sediments assessed by PCR- denaturing gradient gel electrophoresis. Arch. Microbiol. **190**: 19-28.

Zhao, X., L. Yang, and C. Chen C (2006). Microbial diversity in lake sediments detected by PCR-DGGE. Front Biol China. **26**(11): 3610–3616.

Zhao, X; L. Yang, Z. Yu, N. Peng, L. Xiao, D. Yin, and B. Qin (2008). Characterization of depth-related microbial communities in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rRNA fragments. J. Environ Sci (China). **20**: 224-30.

Zhao, Y., G. Sun, and S. Allen (2004). Anti-sized reed bed system for animal wastewater treatment: A comparative study. Water Res. **38**: 2907–2917.

Zhao, Y., G. Sun, and S. Allen (2004). Purification capacity of a highly loaded laboratory scale tidal flow reed bed system with effluent recirculation. Sci. Total Environ. **330**: 1–8.

Zhou, J., B. Xia, D. S. Treves, L-Y. Wu, T. L. Marsh, R. V. O'Neill, A. V. Palumbo and J. M. Tiedje (2002). Spatial and resource factors influencing high microbial diversity in soil. Appl. Environ. Microbiol. **68**: 326–334.

Zimmerman, R., R. Iturriaga, and J. Becker-Birck (1978). Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol. **36**: 926–935.

Zocca, C., S. Di Gregorio, F. Visentini, and G.Vallini (2004). Biodiversity amongst cultivable polycyclic aromatic hydrocarbon-transforming bacteria isolated from an abandoned industrial site. FEMS Microbiol. Lett. **238**: 375–382.

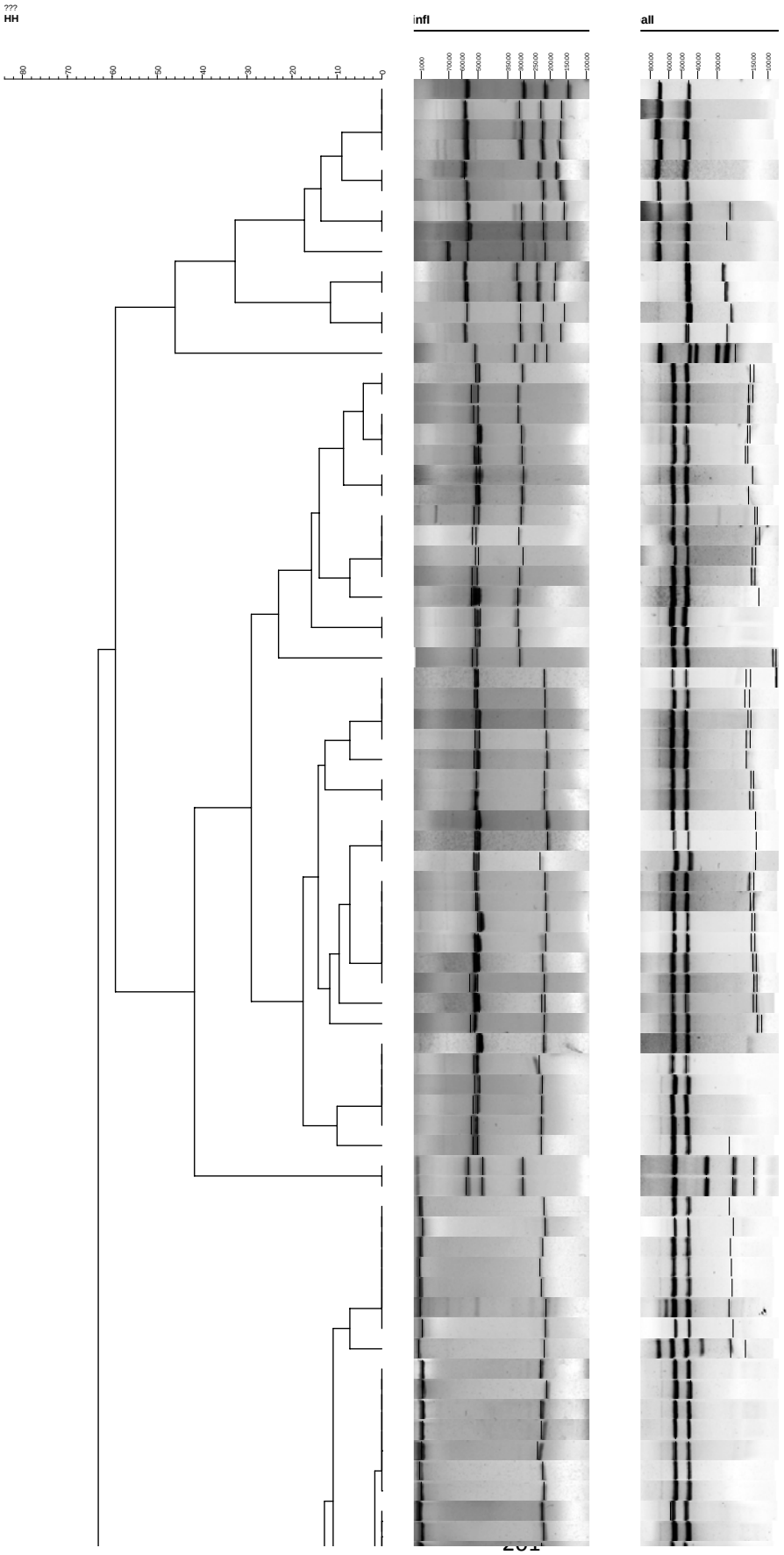
Zoltai, S. C., F. C. Pollett, J. K. Jeglum, and D. G. Adams (1975). Developing a wetland classification for Canada. In: Bernier, B., Winget, C.H. (Eds.), Forest Soils and Forest Land Management. Proceedings of the Fourth Forest Soils Conference. Laval University, Canada, pp. 497–511.

Zvyagintsev, D. (1994). Vertical distribution of microbial communities in soils. In: Ritz, K., Dighton, J., Giller, K. (Eds.), Beyond the Biomass, Wiley, West Sussex, UK, p. 29–37.

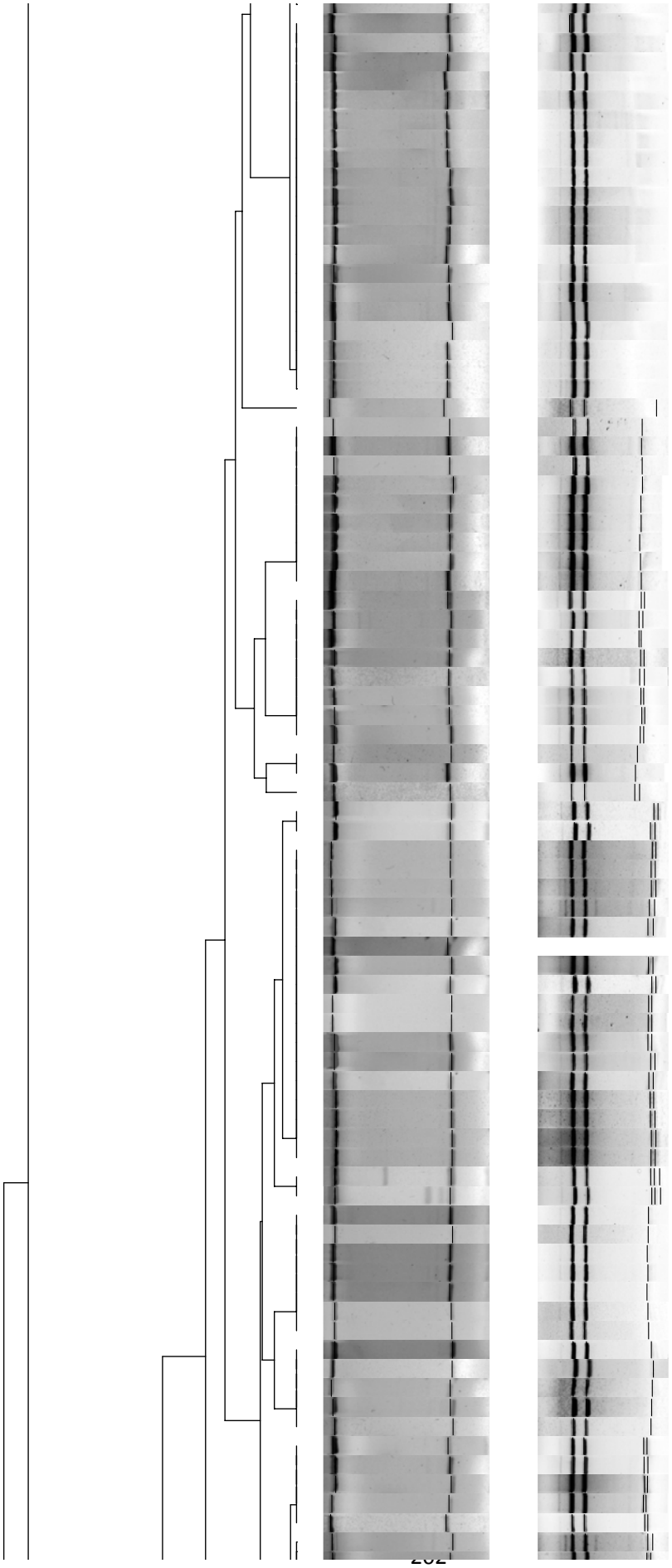
Zwart, G., B. C. Crump, M. Agterveld, F. Hagen, and S. K. Han (2002). Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol **28**: 141–155.

9. Appendix

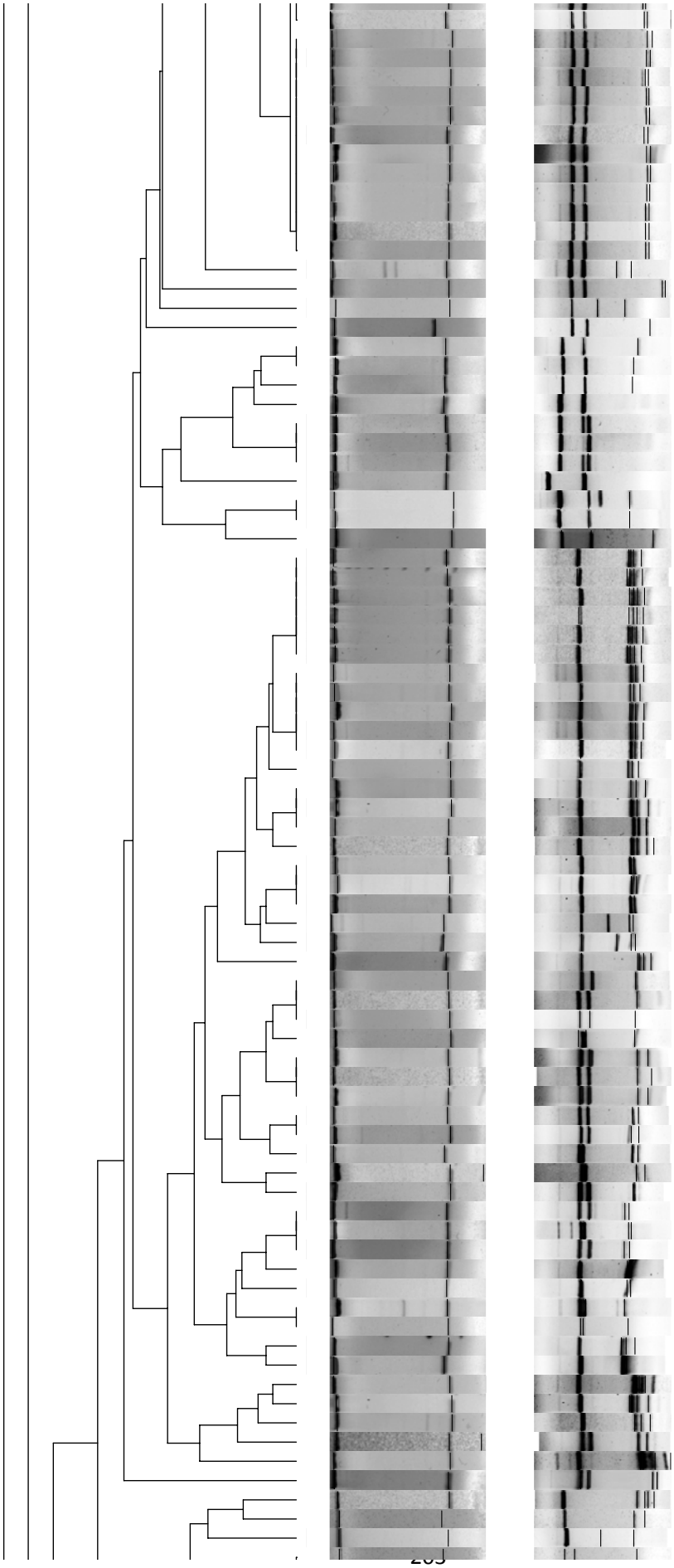
Appendix 1. BioNumerics similarity dendrogram



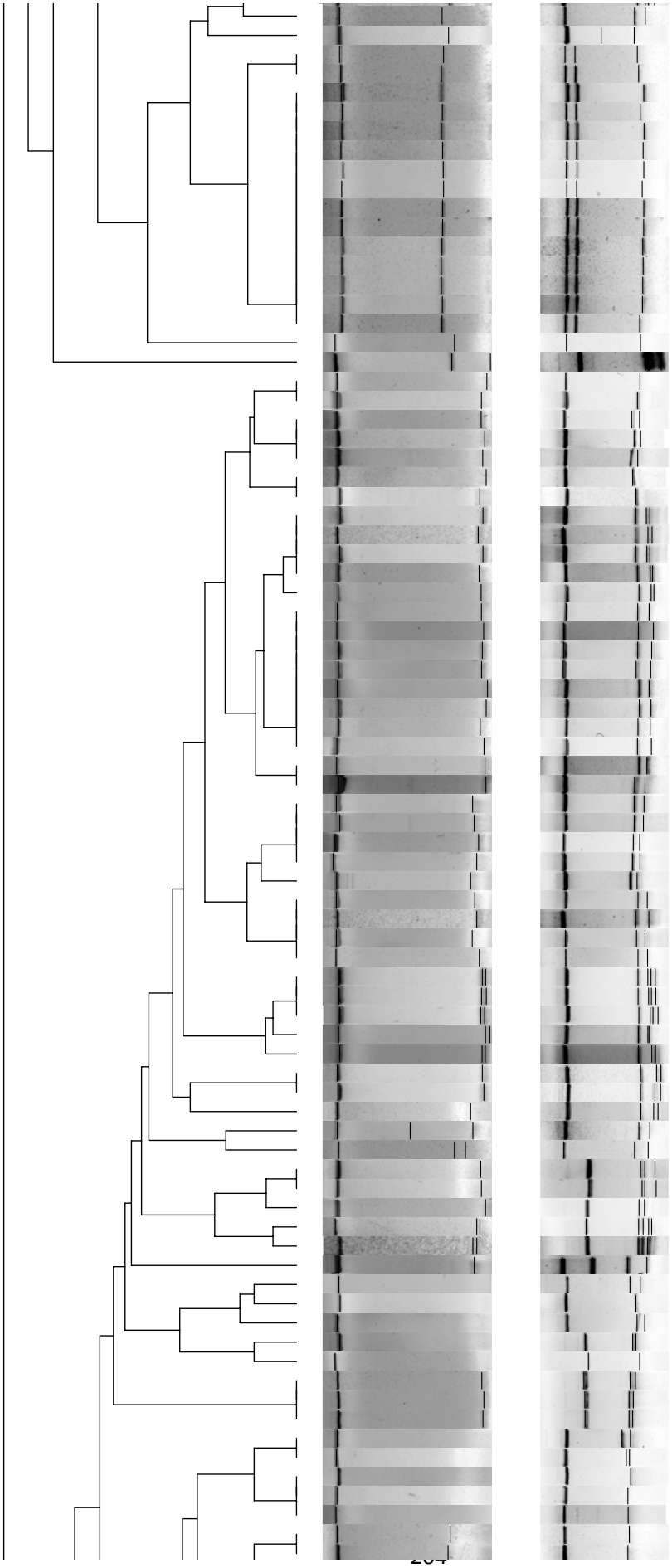
Z10	ZR	March 08	
Za37	ZR	July 08	
Ea46	DD	July 08	
E27	DD	March 08	
A15	S	March 08	Internal
E17	DD	March 08	
Za33	ZR	July 08	
Gb22	S	October 08	External
Gb25	S	October 08	External
S35	IR	March 08	
S66	IR	March 08	
Z50	ZR	March 08	
E21	DD	March 08	
2Ba17	M	October 09	External
Ab28	S	October 08	External
Gb55	S	October 08	External
Ga24	S	July 08	Internal
Cb18	S	October 08	External
Gb5	S	October 08	External
A24	S	March 08	Internal
2C1	S	April 09	External
G26	S	March 08	Internal
C11	S	March 08	Internal
Db33	M	October 08	External
Gb40	S	October 08	External
Fb6	D	October 08	External
2Ha3	M	October 09	External
2Ha4	M	October 09	External
Ga2	S	July 08	Internal
Aa16	S	July 08	Internal
Jb28	D	October 08	External
Gb29	S	October 08	External
Ab36	S	October 08	External
2A23	S	April 09	External
B76	M	March 08	Internal
B79	M	March 08	Internal
Z5	ZR	March 08	
1A15	S	April 09	Internal
1Ca8	S	October 09	Internal
B73	M	March 08	Internal
B75	M	March 08	Internal
Za5	ZR	July 08	
Za11	ZR	July 08	
G31	S	March 08	Internal
Gb45	S	October 08	External
Ha30	M	July 08	Internal
Ga8	S	July 08	Internal
Sa19	IR	July 08	
Ea4	DD	July 08	
Ea11	DD	July 08	
Ea6	DD	July 08	
Ea33	DD	July 08	
1Ca15	S	October 09	Internal
Ha12	M	July 08	Internal
1H12	M	April 09	Internal
1Ca11	S	October 09	Internal
2Aa30	S	October 09	External
1Ca12	S	October 09	Internal
1Ca14	S	October 09	Internal
1Ca16	S	October 09	Internal
1Ha18	M	October 09	Internal
2Aa29	S	October 09	External
2Da1	M	October 09	Internal
S13	IR	March 08	
1Aa8	S	October 09	Internal
S14	IR	March 08	
S16	IR	March 08	
E8	DD	March 08	
1Aa2	S	October 09	Internal
1Aa6	S	October 09	Internal
Hb25	M	October 08	External
1Ha21	M	October 09	Internal



Hb25	M	October 08	External
1Ha21	M	October 09	Internal
Hb37	M	October 08	External
S4	IR	March 08	
S23	IR	March 08	
S40	IR	March 08	
S40a	IR	March 08	
S47	IR	March 08	
S53	IR	March 08	
S56	IR	March 08	
S57	IR	March 08	
S59	IR	March 08	
S67	IR	March 08	
Ea13	DD	July 08	
Ea21	DD	July 08	
E28	DD	March 08	
1Ca19a	S	October 09	Internal
1Aa3	S	October 09	Internal
1Aa4	S	October 09	Internal
1Aa5	S	October 09	Internal
Fb3	D	October 08	Exernal
Z51	ZR	March 08	
1Da9	M	October 09	Internal
Z66	ZR	March 08	
1Ha1	M	October 09	Internal
1Ha5	M	October 09	Internal
1Ha6	M	October 09	Internal
1Ha8	M	October 09	Internal
1Ha10	M	October 09	Internal
1Da8	M	October 09	Internal
B20	M	March 08	Internal
Jb41	D	October 08	External
B70	M	March 08	Internal
A42	S	March 08	Internal
Aa6	S	July 08	Internal
Cb4	S	October 08	External
Cb5	S	October 08	External
E20	DD	March 08	
1F1	D	April 09	Internal
1Da14	M	October 09	External
Da14	M	July 08	Internal
G12	S	March 08	Internal
G19	S	March 08	Internal
B31	M	March 08	Internal
B32	M	March 08	Internal
B34	M	March 08	Internal
B35	M	March 08	Internal
Ha28	M	July 08	Internal
F34	D		
C22	S	March 08	Internal
G25	S	March 08	Internal
C25	S	March 08	Internal
C37	S	March 08	Internal
Fb22	D	October 08	External
Fb29	D	October 08	External
1H28	M	April 09	Internal
2J5	D	April 09	External
2J6	D	April 09	External
2J9	D	April 09	External
2J10	D	April 09	External
Ga29	S	July 08	Internal
Ga38	S	July 08	Internal
Z16a	ZR	March 08	
Z42	ZR	March 08	
Z17	ZR	March 08	
Z16b	ZR	March 08	
Z18	ZR	March 08	
Z20	ZR	March 08	
Z24	ZR	March 08	
Z14	ZR	March 08	
1Aa25	S	October 09	Internal
Fb10	D	October 08	External
Fb11	D	October 08	External
2H1	M	April 09	External
Za12	ZR	July 08	
E25	DD	March 08	
C9	S	March 08	Internal
C53	S	March 08	Internal
Aa31	S	July 08	Internal
B36	M	March 08	Internal



Aa31	S	July 08	Internal
B36	M	March 08	Internal
Gb50	S	October 08	External
B68	M	March 08	Internal
Ga16	S	July 08	Internal
Fb43	D	October 08	External
A29	S	March 08	Internal
Za31	ZR	July 08	
C28	S	March 08	Internal
C30	S	March 08	Internal
C34	S	March 08	Internal
Da28	M	July 08	Internal
Gb41	S	October 08	External
2Da5	M	October 09	External
Ga3	S	July 08	Internal
Xb43	D	October 08	External
Z8	ZR	March 08	
Za16	ZR	July 08	
S9	IR	March 08	
S3	IR	March 08	
2Ja18	D	October 09	External
2Aa5	S	October 09	External
2Aa8	S	October 09	External
2Aa6	S	October 09	External
Ea34	DD	July 08	
2Fa10	D	October 09	External
2Fa11	D	October 09	External
Jb12	D	October 08	External
A13	S	March 08	Internal
A47	S	March 08	Internal
A31	S	March 08	Internal
A32	S	March 08	Internal
A35	S	March 08	Internal
A40	S	March 08	Internal
Ab3	S	October 08	External
Fb46	D	October 08	External
C14	S	March 08	Internal
2C8	S	April 09	External
2Ha20	M	October 09	External
Gb6	S	October 08	External
Cb26	S	October 08	External
1A15	M	April 09	Internal
Db26	M	October 08	External
Ba20	M	July 08	Internal
Ab40	S	October 08	External
2Ha16	M	October 09	External
2C11	S	April 09	External
Cb44	S	October 08	External
E7	DD	March 08	
F27	D	March 08	Internal
Ga19	S	July 08	Internal
1X3	D	April 09	Internal
Ea14	DD	July 08	
Jb30	D	October 08	External
Sa2	IR	July 08	
X3	D	July 08	Internal
Sa13	IR	July 08	
Xb19	D	October 08	External
Jb45	D	October 08	External
Xb38	D	October 08	External
Sa27	IR	July 08	
1Ga11	S	October 09	Internal
Hb27	M	October 08	External
S21	IR	March 08	
Hb28	M	October 08	External
2Ba18	M	October 09	External
1Ga7	S	October 09	Internal
E55	DD	March 08	
E14	DD	March 08	
Hb39	M	October 08	External
1Ba15	M	October 09	Internal
B23	M	March 08	Internal
Ha18	M	July 08	Internal
Fb16	D	October 08	External
Fa14	D	July 08	Internal
A50	S	March 08	Internal
F16	D	March 08	Internal
Ba18	M	July 08	internal
1J13	D	April 09	Internal
1Ca19	S	October 09	Internal
1J14	D	April 09	Internal



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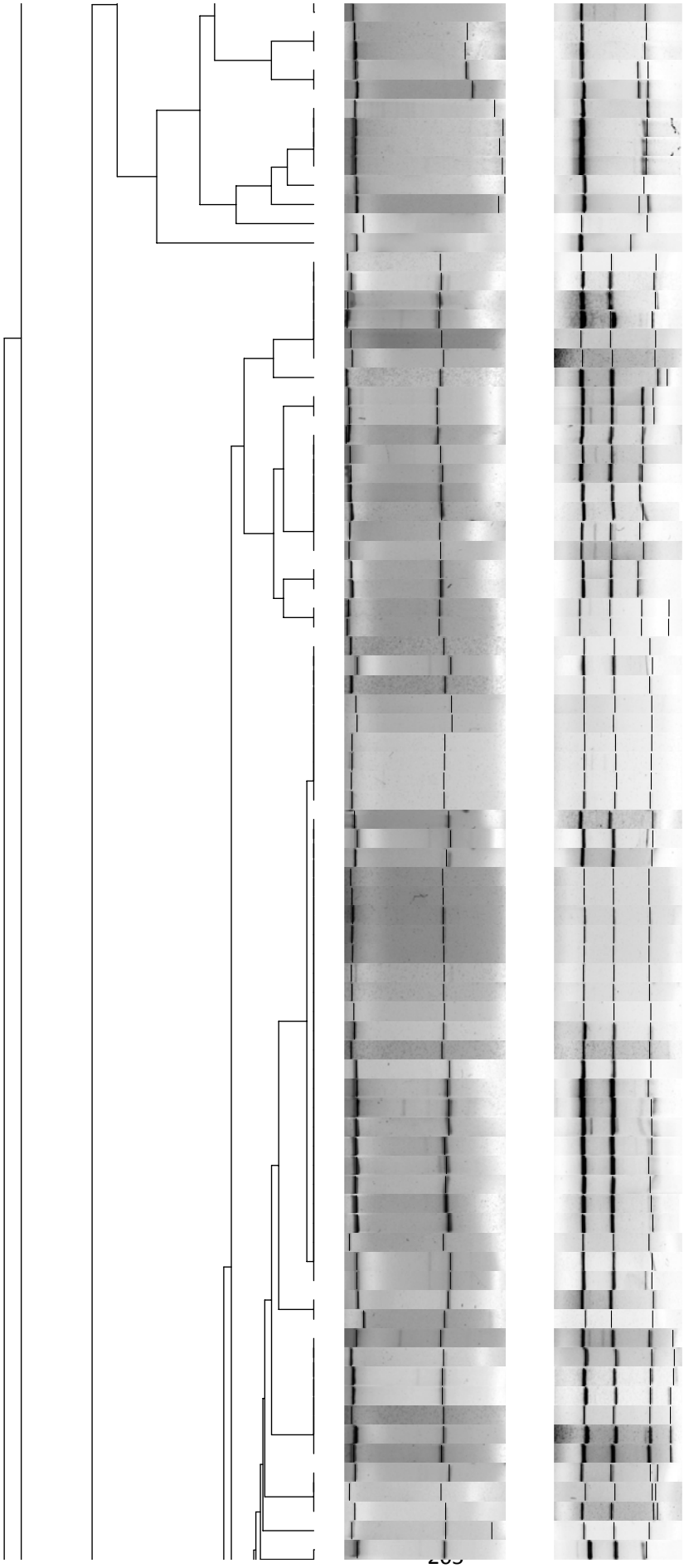
1Ca19	S	October 09	Internal
1J14	D	April 09	Internal
1J15	D	April 09	Internal
1H5	M	April 09	Internal
1J19	D	April 09	Internal
1H17	M	April 09	Internal
1H11	M	April 09	Internal
1F15	D	April 09	Internal
1F16	D	April 09	Internal
2B5	M	April 09	External
2B6	M	April 09	External
2J1	D	April 09	External
2J3	D	April 09	External
2J4	D	April 09	External
2J11	D	April 09	External
1J16	D	April 09	Internal
1Ja30	D	October 09	Internal
A48	S	March 08	Internal
1Fa22	D	October 09	Internal
2Ja9	D	October 09	External
B50	M	March 08	Internal
1Ga12	S	October 09	Internal
Hb4	M	October 08	External
1Ga9	S	October 09	Internal
2Ga13	S	October 09	External
Ha27	M	July 08	Internal
X2	D	July 08	Internal
1D24	M	April 09	Internal
2X10	D	April 09	External
Db15	M	October 08	External
Db20	M	October 08	External
Jb18	D	October 08	External
Cb33	S	October 08	External
Cb36	S	October 08	External
Gb48	S	October 08	External
1G16	S	April 09	Internal
1G24	S	April 09	Internal
2Ja1	D	October 09	External
Db27	M	October 08	External
Gb20	S	October 08	External
X17	D	July 08	Internal
Fb47	D	October 08	External
B81	M	March 08	Internal
Cb42	S	October 08	External
Hb44	M	October 08	External
Fb27	D	October 08	External
1X2	D	April 09	Internal
Fb40	D	October 08	External
1G12	S	April 09	Internal
Ga32	S	July 08	Internal
Ga33	S	July 08	Internal
Ga37	S	July 08	Internal
Ga14	S	July 08	Internal
Jb17	D	October 08	External
G2	S	March 08	Internal
G16	S	March 08	Internal
1Aa20	S	October 09	Internal
Fb8	D	October 08	External
1F2	D	April 09	Internal
1Aa23	S	October 09	Internal
1Aa24	S	October 09	Internal
1Fa21	D	October 09	Internal
Aa26	S	July 08	Internal
Fa16	D	July 08	Internal
1X17	D	April 09	Internal
Z72	ZR	March 08	
2Ha8	M	October 09	External
E10	DD	March 08	
E18	DD	March 08	
1Fa4	D	October 09	Internal
Jb23	D	October 08	External
Jb38	D	October 08	External
Jb40	D	October 08	External
Ea29	DD	July 08	
1Ga3	S	October 09	Internal
S50	IR	March 08	
1Ga6	S	October 09	Internal
2Ba15	M	October 09	External
2Ca6	S	October 09	External
2Ca8	S	October 09	External



2Ba15	M	October 09	External
2Ca6	S	October 09	External
2Ca8	S	October 09	External
Sa44	IR	July 08	
Jb34	D	October 08	External
Ab42	S	October 08	External
1Ha12	M	October 09	Internal
1Ha13	M	October 09	Internal
1Ha14	M	October 09	Internal
1Aa9	S	October 09	Internal
Jb33	D	October 08	External
2Ca2	S	October 09	External
2Ha1	M	October 09	External
Ba11	M	July 08	Internal
2Ha21	M	October 09	External
Fb2	D	October 08	External
Fb5	D	October 08	External
Gb31	S	October 08	External
2G6	S	April 09	External
Ba23	M	July 08	Internal
Hb42	M	October 08	External
Hb43	M	October 08	External
D14	M	March 08	Internal
2Ba12	M	October 09	External
Bb8	M	October 08	External
Hb21	M	October 08	External
1Ba18	M	October 09	Internal
2Ga8	S	October 09	External
2Ba7	M	October 09	External
1Da15	M	October 09	Internal
1Da16	M	October 09	Internal
B10	M	March 08	Internal
B11	M	March 08	Internal
1A9	S	April 09	Internal
2Xa8	D	October 09	External
1A13	S	April 09	Internal
1J9	D	April 09	Internal
1F10	D	April 09	Internal
2H7	M	April 09	External
2H8	M	April 09	External
2H9	M	April 09	External
2H12	M	April 09	External
A46	S	March 08	Internal
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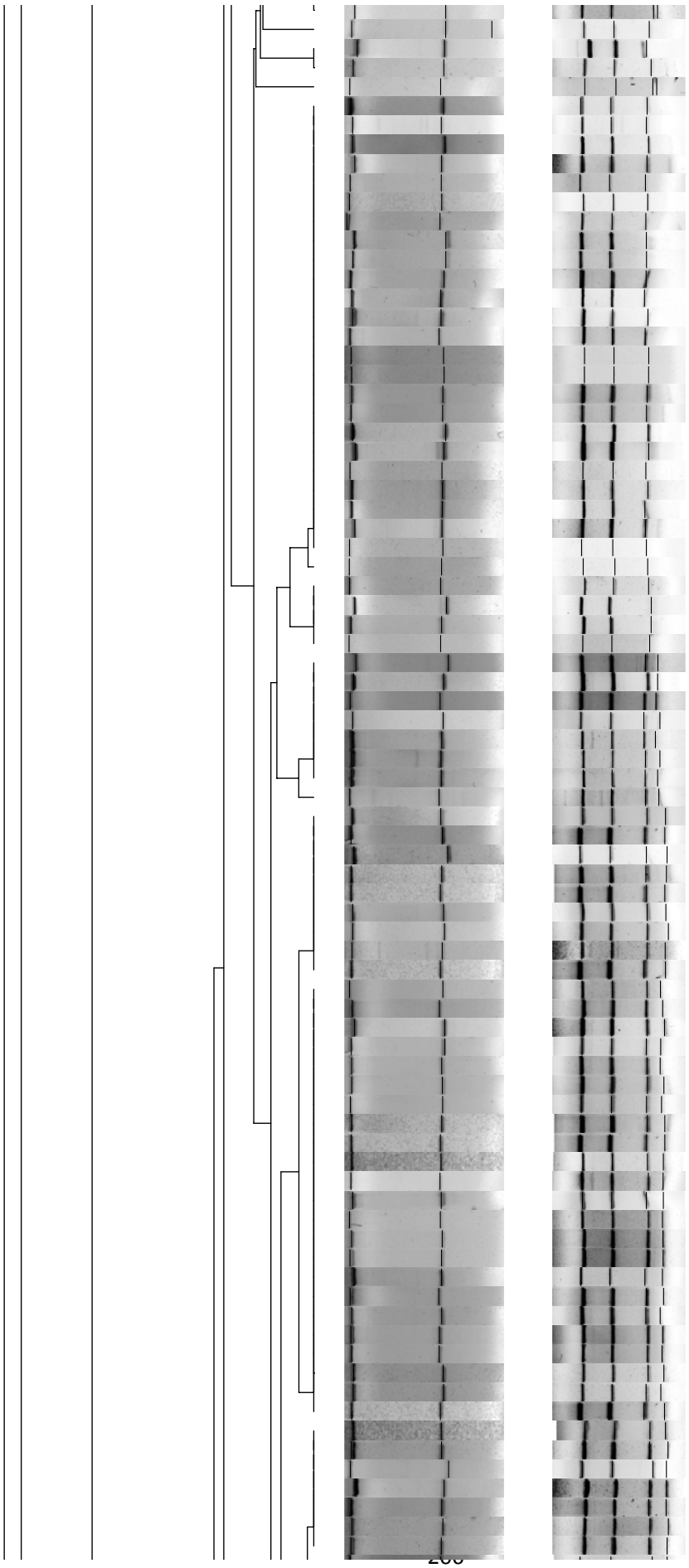
Bb30	M	October 08	External
1D6	M	April 09	Internal
1D14	M	April 09	Internal
1D15	M	April 09	Internal
1D22	M	April 09	Internal
1D23	M	April 09	Internal
1H21	M	April 09	Internal
1H22	M	April 09	Internal
1F12	D	April 09	Internal
2H4	M	April 09	External
2J19	D	April 09	External
1Ga2	S	October 09	Internal
1Ha15	M	October 09	Internal
1Ha17	M	October 09	Internal
1Ba1	M	October 09	Internal
1Ba3	M	October 09	Internal
1Ba11	M	October 09	Internal
1Ba17	M	October 09	Internal
1Ba23	M	October 09	Internal
1Ba29	M	October 09	Internal
1Ja14	D	October 09	Internal
2Xa7	D	October 09	External
2Xa9	D	October 09	External
1Xa7	D	October 09	Internal
1Ja27	D	October 09	Internal
Ga10	S	July 08	Internal
1Ca2	S	October 09	Internal
G8	S	March 08	Internal
G17	S	March 08	Internal
1D34	M	April 09	Internal
2G15	S	April 09	External
2X7	D	April 09	External
D2	M	March 08	Internal
1Ca9	S	October 09	Internal
C5	S	March 08	Internal
2Ja2	D	October 09	External
E53	DD	March 08	



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2Ja2	D	October 09	External
E53	DD	March 08	
2H5	M	April 09	External
1Ca10	S	October 09	Internal
B48	M	March 08	Internal
2Ha13	M	October 09	External
Z6	ZR	March 08	
Za22	ZR	July 08	
J4	D	March 08	Internal
Aa24	S	July 08	Internal
D17	M	March 08	Internal
Bb4	M	October 08	External
Bb5	M	October 08	External
Bb18	M	October 08	External
S68	IR	March 08	
Ea19	DD	July 08	
Bb38	M	October 08	External
1D18	M	April 09	Internal
1D19	M	April 09	Internal
2A8	S	April 09	External
2A9	S	April 09	External
1Ga14	S	October 09	Internal
1Ba14	M	October 09	Internal
1Da10	M	October 09	Internal
1Da12	M	October 09	Internal
1Ba27	M	October 09	Internal
1Xa8	D	October 09	Internal
1Fa16	D	October 09	Internal
1Ja28	D	October 09	Internal
D42	M	March 08	Internal
2Aa16	S	October 09	External
Ea28	DD	July 08	
Xb25	D	October 08	External
Jb3	D	October 08	External
1Ha4	M	October 09	Internal
Jb5	D	October 08	External
Ca13	S	July 08	Internal
Hb2	M	October 08	External
Hb5	M	October 08	External
Hb8	M	October 08	External
Xb28	D	October 08	External
Ja15	D	July 08	Internal
1X19	D	April 09	Internal
A43	S	March 08	Internal
X1	D	July 08	Internal
X8	D	July 08	Internal
Cb14	S	October 08	External
1G4	S	April 09	Internal
2G3	S	April 09	External
1X8	D	April 09	Internal
B41	M	March 08	Internal
Ga23	S	July 08	Internal
Ha7	M	July 08	Internal
X19	D	July 08	Internal
Ab2	S	October 08	External
Ab15	S	October 08	External
Ab16	S	October 08	External
Ba31	M	July 08	Internal
X9	D	July 08	Internal
Fa5	D	July 08	Internal
C10	S	March 08	Internal
Cb1	S	October 08	External
Db24	M	October 08	External
Ab5	S	October 08	External
Ab6	S	October 08	External
Hb14	M	October 08	External
Gb54	S	October 08	External
2A1	S	April 09	External
2F9	D	April 09	External
2F10	D	April 09	External
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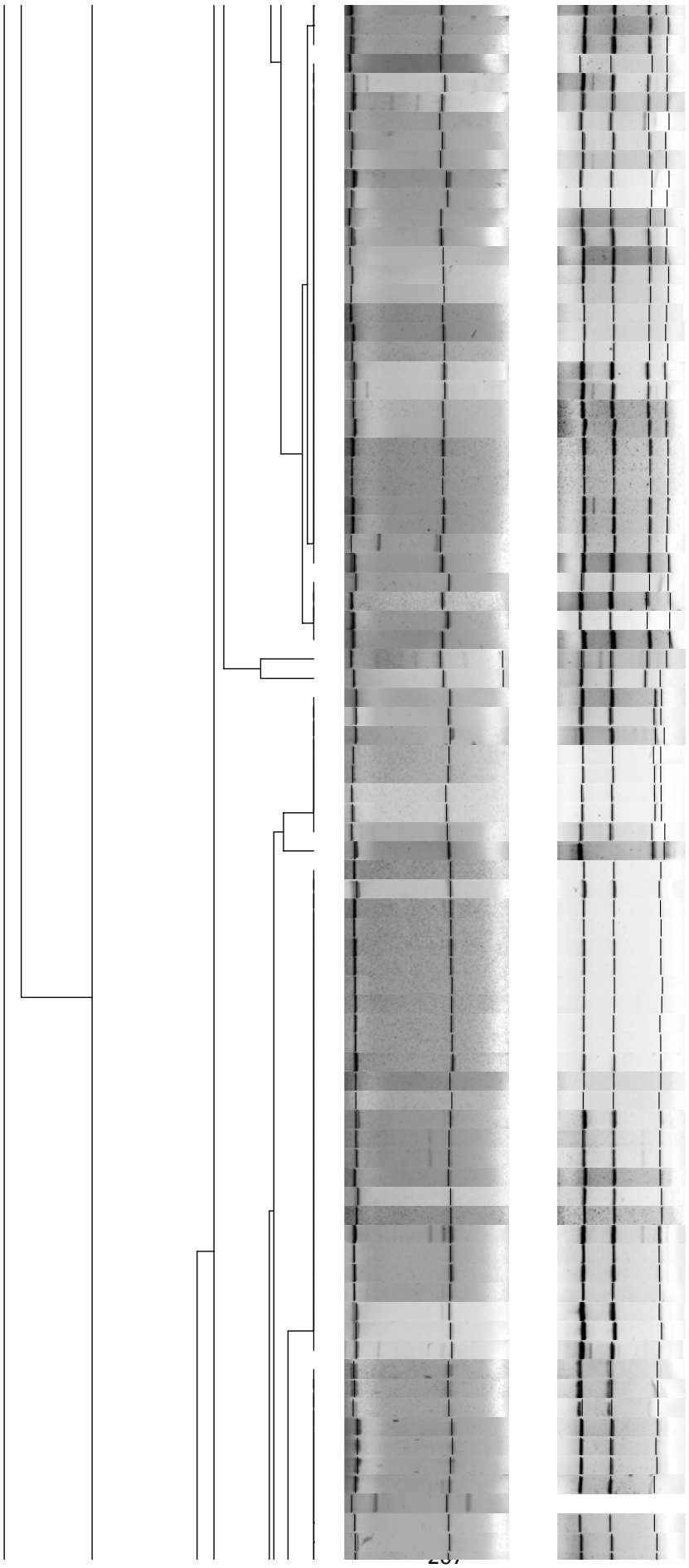
1J20	D	April 09	Internal
1X9	D	April 09	Internal
Fa12	D	July 08	Internal
2X3	D	April 09	External
Gb1	S	October 08	External
2G14	S	April 09	External
2D15	M	April 09	External
2J26	D	April 09	External
2X2	D	April 09	External



2D15	M	April 09	External
2J26	D	April 09	External
2X2	D	April 09	External
F1	D	March 08	Internal
Ha14	M	July 08	Internal
Ja20	D	July 08	Internal
Db5	M	October 08	External
Db17	M	October 08	External
Xb11	D	October 08	External
B37	M	March 08	Internal
B72	M	March 08	Internal
Ba22	M	July 08	Internal
Fb23	D	October 08	External
Db32	M	October 08	External
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1D2	M	April 09	Internal
1D25	M	April 09	Internal
1D36	M	April 09	Internal
1H7	M	April 09	Internal
1H14	M	April 09	Internal
2G2	S	April 09	External
2G10	S	April 09	External
2H17	M	April 09	External
2H18	M	April 09	External
2H20	M	April 09	External
2B2	M	April 09	External
2B4	M	April 09	External
2X1	D	April 09	External
2X11	D	April 09	External
Db9	M	October 08	External
1X1	D	April 09	Internal
B5	M	March 08	Internal
2X5	D	April 09	External
Ha31	M	July 08	Internal
Ca9	S	July 08	Internal
B52	M	March 08	Internal
X13	D	July 08	Internal
D6	M	March 08	Internal
Aa21	S	July 08	Internal
Aa23	S	July 08	Internal
Ba7	M	July 08	Internal
Ba9	M	July 08	Internal
Cb41	S	October 08	External
1X13	D	April 09	Internal
1A2	S	April 09	Internal
2Ja7	D	October 09	External
1A4	S	April 09	Internal
1A5	S	April 09	Internal
1A6	S	April 09	Internal
1A8	S	April 09	Internal
1A10	S	April 09	Internal
1A11	S	April 09	Internal
1C2	S	April 09	Internal
1C3	S	April 09	Internal
1C5	S	April 09	Internal
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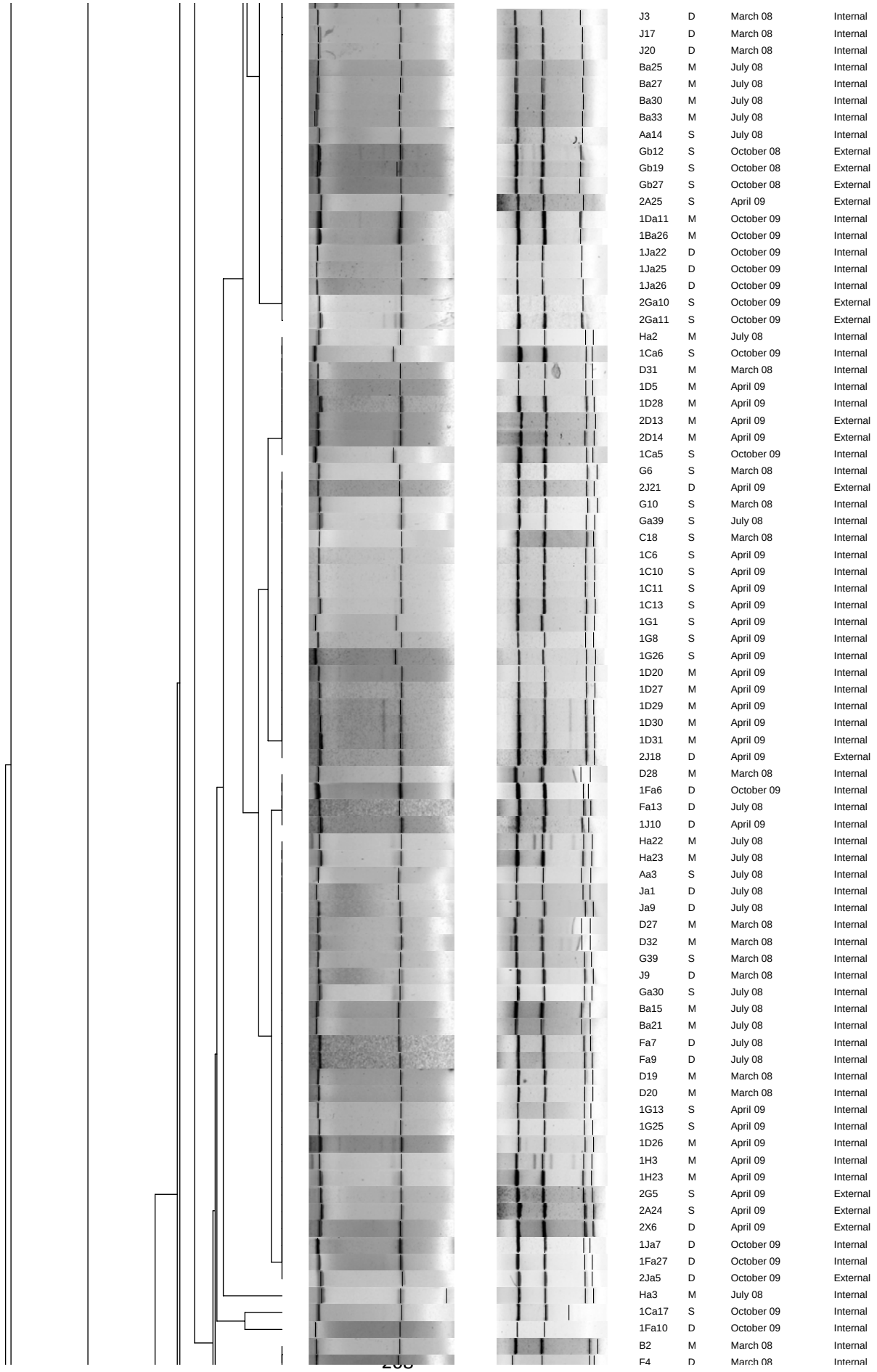
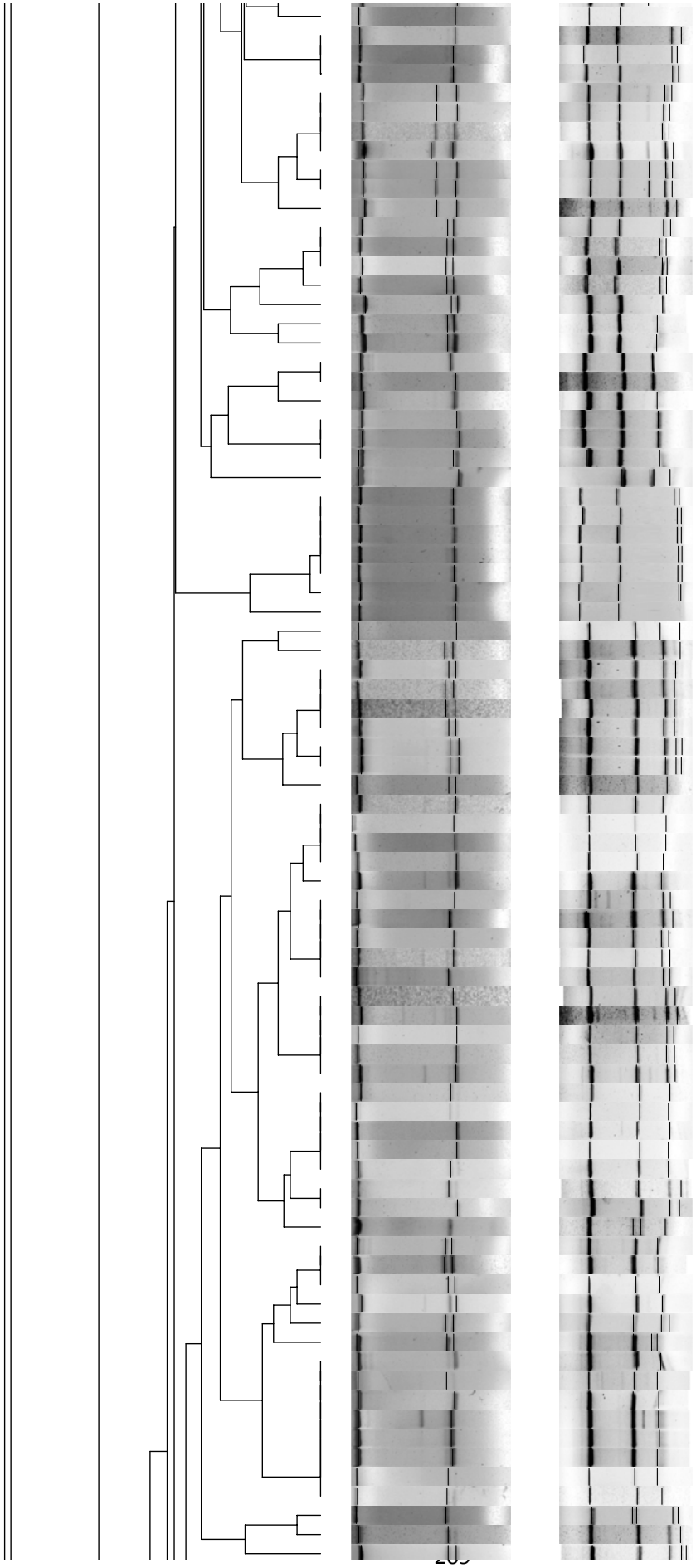
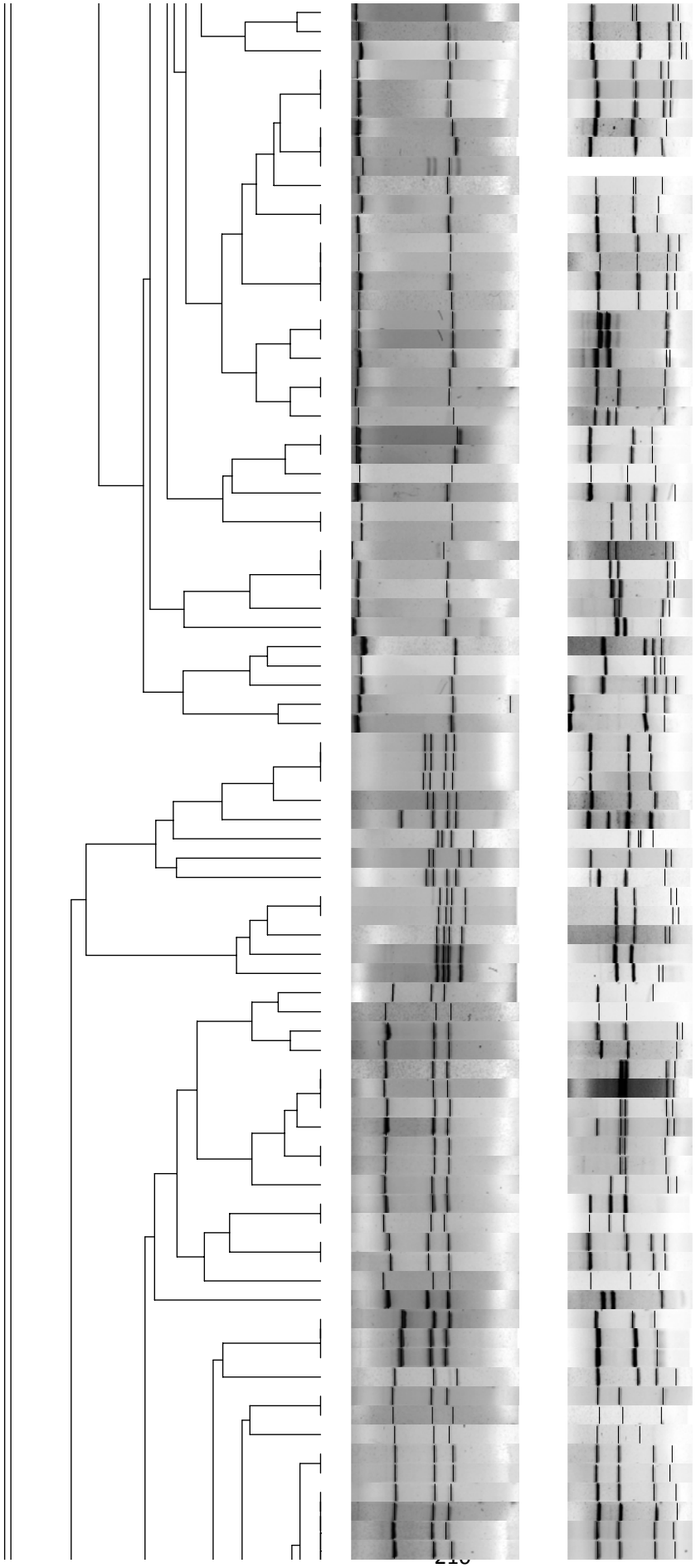
1J3	D	April 09	Internal
2D7	M	April 09	External
2H3	M	April 09	External
2H15	M	April 09	External
1Ja5	D	October 09	Internal
1Ja8	D	October 09	Internal
1Ja9	D	October 09	Internal
1Ja17	D	October 09	Internal
2Fa4	D	October 09	External
2Fa8	D	October 09	External
2Fa17	D	October 09	External
A1	S	March 08	Internal
A2	S	March 08	Internal
A9	S	March 08	Internal
A25	S	March 08	Internal
A27	S	March 08	Internal
A28	S	March 08	Internal
A41	S	March 08	Internal
Db11	M		
J3	D	March 08	Internal
J17	D	March 08	Internal
J20	D	March 08	Internal



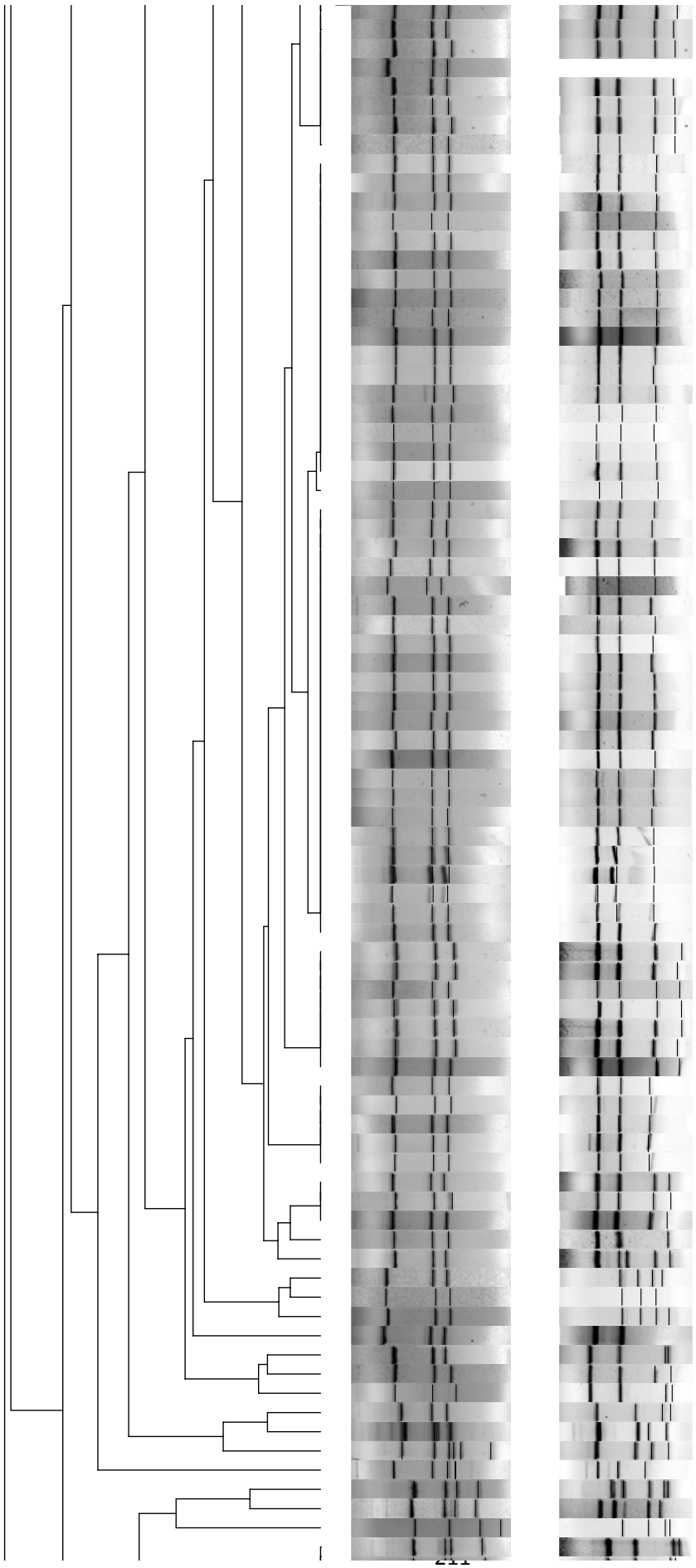
J3	D	March 08	Internal
J17	D	March 08	Internal
J20	D	March 08	Internal
Ba25	M	July 08	Internal
Ba27	M	July 08	Internal
Ba30	M	July 08	Internal
Ba33	M	July 08	Internal
Aa14	S	July 08	Internal
Gb12	S	October 08	External
Gb19	S	October 08	External
Gb27	S	October 08	External
2A25	S	April 09	External
1Da11	M	October 09	Internal
1Ba26	M	October 09	Internal
1Ja22	D	October 09	Internal
1Ja25	D	October 09	Internal
1Ja26	D	October 09	Internal
2Ga10	S	October 09	External
2Ga11	S	October 09	External
Ha2	M	July 08	Internal
1Ca6	S	October 09	Internal
D31	M	March 08	Internal
1D5	M	April 09	Internal
1D28	M	April 09	Internal
2D13	M	April 09	External
2D14	M	April 09	External
1Ca5	S	October 09	Internal
G6	S	March 08	Internal
2J21	D	April 09	External
G10	S	March 08	Internal
Ga39	S	July 08	Internal
C18	S	March 08	Internal
1C6	S	April 09	Internal
1C10	S	April 09	Internal
1C11	S	April 09	Internal
1C13	S	April 09	Internal
1G1	S	April 09	Internal
1G8	S	April 09	Internal
1G26	S	April 09	Internal
1D20	M	April 09	Internal
1D27	M	April 09	Internal
1D29	M	April 09	Internal
1D30	M	April 09	Internal
1D31	M	April 09	Internal
2J18	D	April 09	External
D28	M	March 08	Internal
1Fa6	D	October 09	Internal
Fa13	D	July 08	Internal
1J10	D	April 09	Internal
Ha22	M	July 08	Internal
Ha23	M	July 08	Internal
Aa3	S	July 08	Internal
Ja1	D	July 08	Internal
Ja9	D	July 08	Internal
D27	M	March 08	Internal
D32	M	March 08	Internal
G39	S	March 08	Internal
J9	D	March 08	Internal
Ga30	S	July 08	Internal
Ba15	M	July 08	Internal
Ba21	M	July 08	Internal
Fa7	D	July 08	Internal
Fa9	D	July 08	Internal
D19	M	March 08	Internal
D20	M	March 08	Internal
1G13	S	April 09	Internal
1G25	S	April 09	Internal
1D26	M	April 09	Internal
1H3	M	April 09	Internal
1H23	M	April 09	Internal
2G5	S	April 09	External
2A24	S	April 09	External
2X6	D	April 09	External
1Ja7	D	October 09	Internal
1Fa27	D	October 09	Internal
2Ja5	D	October 09	External
Ha3	M	July 08	Internal
1Ca17	S	October 09	Internal
1Fa10	D	October 09	Internal
B2	M	March 08	Internal
F4	D	March 08	Internal



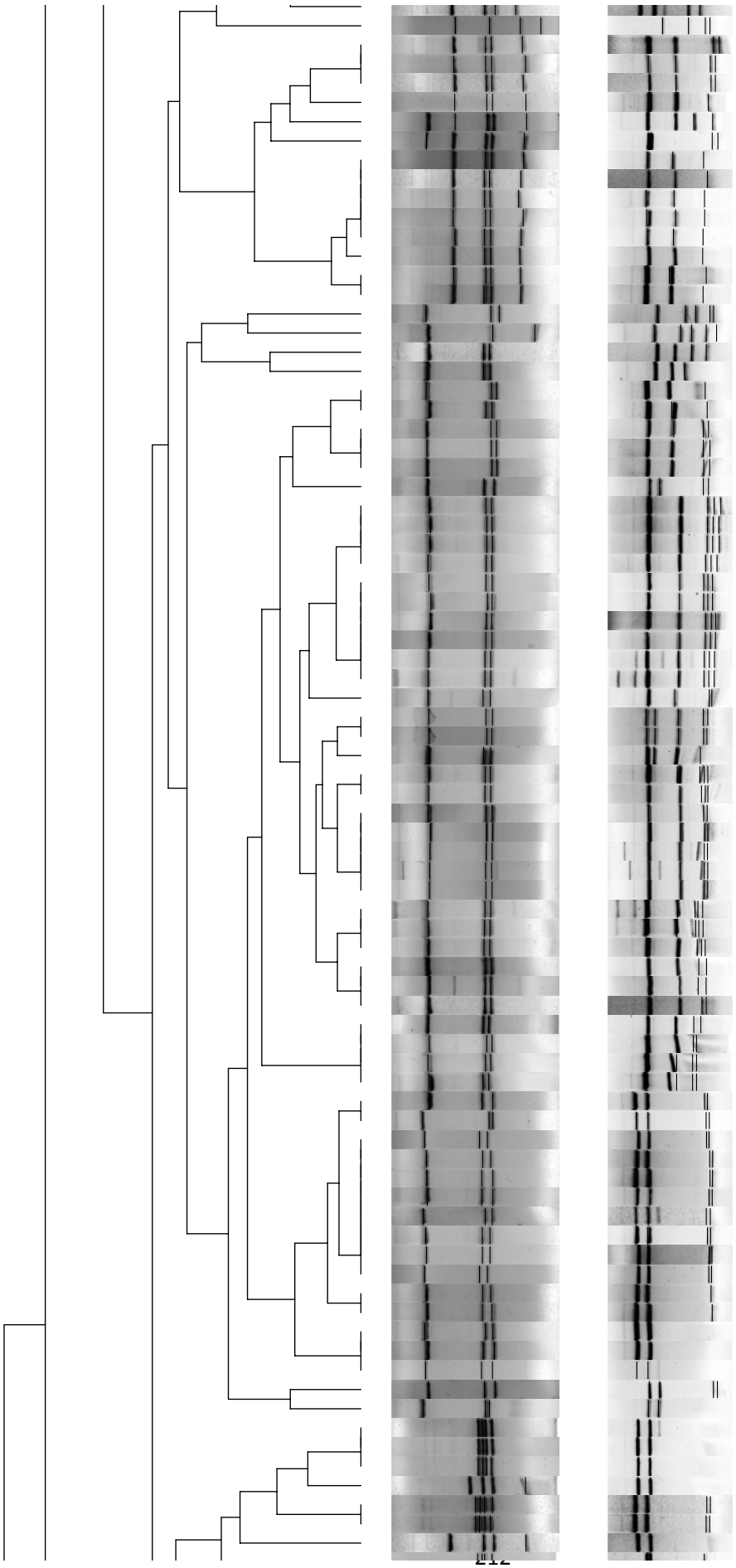
1Fa10	D	October 09	Internal
B2	M	March 08	Internal
F4	D	March 08	Internal
F31	D	March 08	Internal
G41	S	March 08	Internal
Ca2	S	July 08	Internal
Da16	M	July 08	Internal
2Fa20	D	October 09	External
Xb7	D	October 08	External
Xb9	D	October 08	External
2A22	S	April 09	External
X24	D	July 08	Internal
A11	S	March 08	Internal
C7	S	March 08	Internal
A10	S	March 08	Internal
1Ha3	M	October 09	Internal
1Ba19	M	October 09	Internal
1Xa11	D	October 09	Internal
Bb33	M	October 08	External
2D3	M	April 09	External
1Ha16	M	October 09	Internal
Ab9	S	October 08	External
2A4	S	April 09	External
Bb20	M	October 08	External
Fb42	D	October 08	External
F8	D	March 08	Internal
F25	D	March 08	Internal
F14	D	March 08	Internal
F19	D	March 08	Internal
F21	D	March 08	Internal
F17	D	March 08	Internal
F18	D	March 08	Internal
Jb21	D	October 08	External
1X4	D	April 09	Internal
Ha25	M	July 08	Internal
X4	D	July 08	Internal
Fa8	D	July 08	Internal
1H25	M	April 09	Internal
Ha13	M	July 08	Internal
1H13	M	April 09	Internal
2D2	M	April 09	External
Fa3	D	July 08	Internal
2Fa23	D	October 09	External
1Fa12	D	October 09	Internal
1Fa19	D	October 09	Internal
2Aa11	S	October 09	External
C48	S	March 08	Internal
1X15	D	April 09	Internal
C49	S	March 08	Internal
Da21	M	July 08	Internal
Hb13	M	October 08	External
Fa19	D	July 08	Internal
2G1	S	April 09	External
C24	S	March 08	Internal
1G9	S	April 09	Internal
1G19	S	April 09	Internal
2H6	M	April 09	External
2Fa7	D	October 09	External
1Fa3	D	October 09	Internal
1Fa23	D	October 09	Internal
2Fa1	D	October 09	External
G3	S	March 08	Internal
1Ca4	S	October 09	Internal
Aa9	S	July 08	Internal
Hb46	M	October 08	External
1Ba22	M	October 09	Internal
2Ca4	S	October 09	External
2Ha11	M	October 09	External
Aa2	S	July 08	Internal
Bb28	M	October 08	External
Bb31	M	October 08	External
2Ba14	M	October 09	External
1Da4	M	October 09	Internal
1Ba5	M	October 09	Internal
1Ba6	M	October 09	Internal
1Ba7	M	October 09	Internal
2Ca1	S	October 09	External
2Ha19	M	October 09	External
F6	D	March 08	Internal
2J22	D	April 09	External
G14	S	March 08	Internal



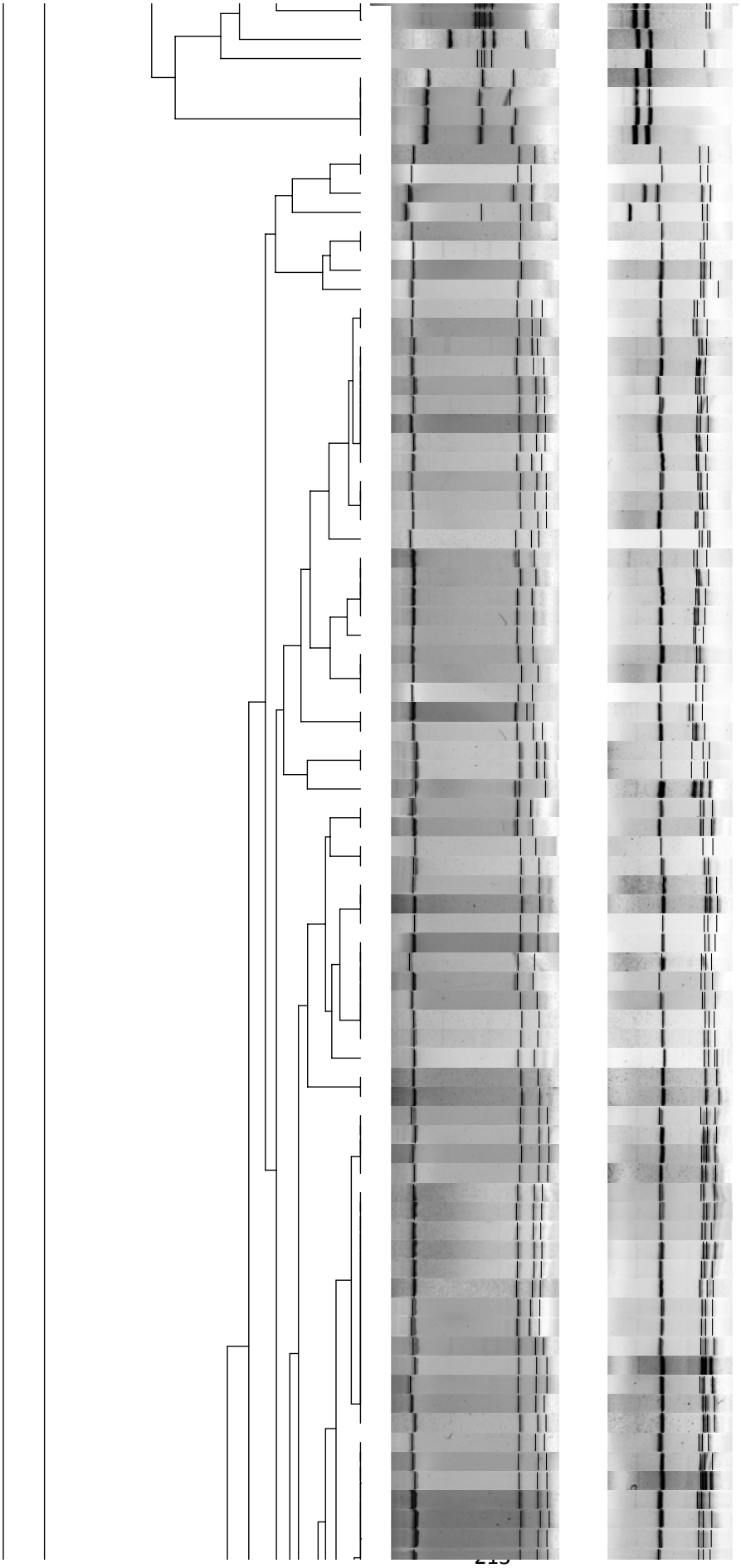
F6	D	March 08	Internal
2J22	D	April 09	External
G14	S	March 08	Internal
X15	D	July 08	Internal
Ja17	D	July 08	Internal
J22	D	March 08	Internal
A39	S	March 08	Internal
1Da5	M	October 09	Internal
Db12	M		
Da17	M	July 08	Internal
Aa22	S	July 08	Internal
2Aa3	S	October 09	External
1C15	S	April 09	Internal
2A21	S	April 09	External
1G14	S	April 09	Internal
1H15	M	April 09	Internal
Ab21	S	October 08	External
2A13	S	April 09	External
D7	M	March 08	Internal
Ba28	M	July 08	Internal
X6	D	July 08	Internal
F32	D	March 08	Internal
Hb33	M	October 08	External
Hb36	M	October 08	External
2Ja4	D	October 09	External
Db8	M	October 08	External
Ab32	S	October 08	External
2C4	S	April 09	External
D8	M	March 08	Internal
1G23	S	April 09	Internal
1Ca7	S	October 09	Internal
D33	M	March 08	Internal
Ea30	DD	July 08	
Sa21	IR	July 08	
2Ha10	M	October 09	External
Za38	ZR	July 08	
Ja26	D	July 08	Internal
Bb2	M	October 08	External
Bb36	M	October 08	External
Bb37	M	October 08	External
Bb40	M	October 08	External
2D5	M	April 09	External
2Ba6	M	October 09	External
1Aa16	S	October 09	Internal
Xb10	D	October 08	External
2Ga20	S	October 09	External
Z23	ZR	March 08	
Z48	ZR	March 08	
Sa31	IR	July 08	
E37	DD	March 08	
S7	IR	March 08	
S36	IR	March 08	
1A12	S	April 09	Internal
Cb29	S	October 08	External
2D11	M	April 09	External
Ba29	M	July 08	Internal
D35	M	March 08	Internal
Ca4	S	July 08	Internal
Ja4	D	July 08	Internal
D34	M	March 08	Internal
D35	M	March 08	Internal
X22	D	July 08	Internal
1Ba10	M	October 09	Internal
1Ba12	M	October 09	Internal
X14	D	July 08	Internal
Ja25	D	July 08	Internal
1Fa5	D	October 09	Internal
1X14	D	April 09	Internal
Hb38	M	October 08	External
1Ba2	M	October 09	Internal
1Ba4	M	October 09	Internal
1C7	S	April 09	Internal
Xb14	D	October 08	External
1Ja3	D	October 09	Internal
1Xa9	D	October 09	Internal
Ja23	D	July 08	Internal
Ca7	S	July 08	Internal
Ha17	M	July 08	Internal
2X4	D	April 09	External
Ja2	D	July 08	Internal
Ja12	D	July 08	Internal



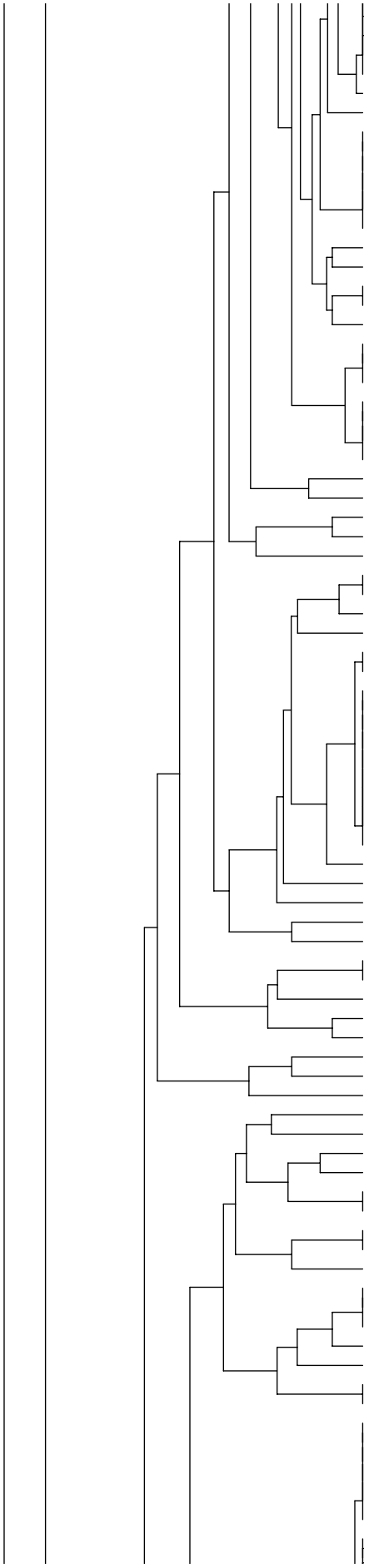
2X4	D	April 09	External
Ja2	D	July 08	Internal
Ja12	D	July 08	Internal
Db7	M		
J2	D	March 08	Internal
J7	D	March 08	Internal
J11	D	March 08	Internal
Da32	M	July 08	Internal
G7	S	March 08	Internal
2Ja21	D	October 09	External
Fb19	D	October 08	External
Db22	M	October 08	External
1H17	M	April 09	Internal
1J4	D	April 09	Internal
2A5	S	April 09	External
2B1	M	April 09	External
2J24	D	April 09	External
2F4	D	April 09	External
1Xa1	D	October 09	Internal
1Xa2	D	October 09	Internal
1Ja4	D	October 09	Internal
1Ja6	D	October 09	Internal
1Fa14	D	October 09	Internal
1Xa12	D	October 09	Internal
2Fa6	D	October 09	External
1Fa1	D	October 09	Internal
X21	D	July 08	Internal
X28	D	July 08	Internal
Za23	ZR	July 08	
Ba1	M	July 08	Internal
D3	M	March 08	Internal
Bb26	M	October 08	External
Sa36	IR	July 08	
Sa40	IR	July 08	
Sa9	IR	July 08	
Ab38	S	October 08	External
2A6	S	April 09	External
2F11	D	April 09	External
1Xa4	D	October 09	Internal
1Fa7	D	October 09	Internal
2Ba8	M	October 09	External
2Ba9	M	October 09	External
2Ba10	M	October 09	External
2Ja11	D	October 09	External
2Ja12	D	October 09	External
2Ja14	D	October 09	External
2Ja16	D	October 09	External
2Ja19	D	October 09	External
2Ja20	D	October 09	External
Ha5	M	July 08	Internal
Ha11	M	July 08	Internal
Ja8	D	July 08	Internal
Ga36	S	July 08	Internal
1H5	M	April 09	Internal
1D35	M	April 09	Internal
2F3	D	April 09	External
1Ca13	S	October 09	Internal
2Ha7	M	October 09	External
1Ba25	M	October 09	Internal
2Ha5	M	October 09	External
2Ha6	M	October 09	External
Sa8	IR	July 08	
Ja24	D	July 08	Internal
Ba13	M	July 08	Internal
Aa7	S	July 08	Internal
Za21	ZR	July 08	
Da33	M	July 08	Internal
1A16	S	April 09	Internal
1H27	M	April 09	Internal
Ba10	M	July 08	Internal
Ja3	D	July 08	Internal
1J17	D	April 09	Internal
2Ha23	M	October 09	External
X26	D	July 08	Internal
1Fa26	D	October 09	Internal
X16	D	July 08	Internal
1Xa10	D	October 09	Internal
Cb34	S	October 08	External
Sa32	IR	July 08	
Z9	ZR	March 08	
Za20	ZR	July 08	



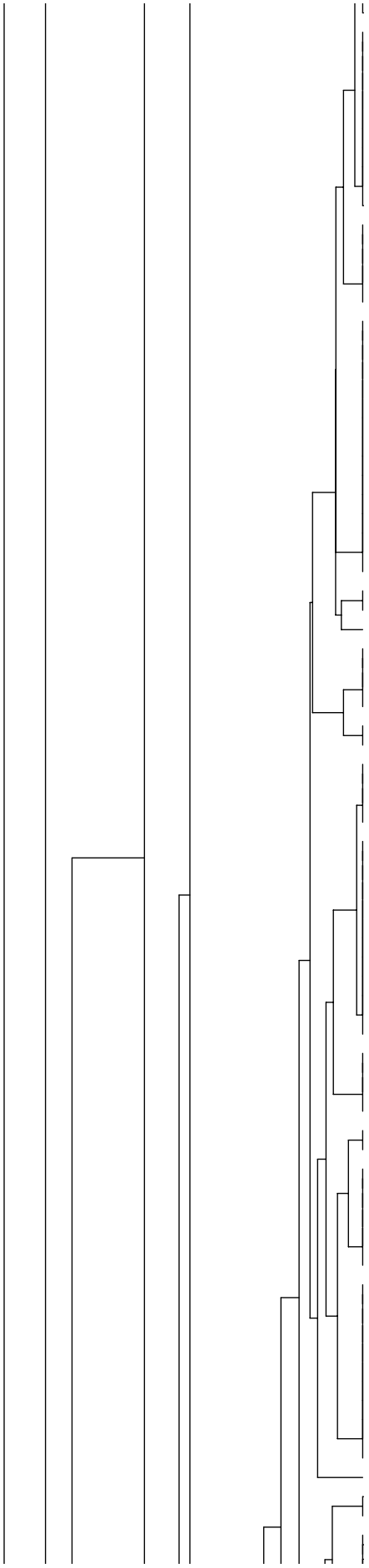
Z9	ZR	March 08	
Za20	ZR	July 08	
Cb13	S	October 08	External
Sa15	IR	July 08	
1Ha2	M	October 09	Internal
Z7a	ZR	March 08	
Z19	ZR	March 08	
Hb31	M	October 08	External
Sa30	IR	July 08	
Ea1	DD	July 08	
Ea9	DD	July 08	
Ea18	DD	July 08	
Sa24	IR	July 08	
Ea20	DD	July 08	
Ea45	DD	July 08	
D46	M	March 08	Internal
Cb8	S	October 08	External
Sa33	IR	July 08	
Sa41	IR	July 08	
Ea17	DD	July 08	
Ea24	DD	July 08	
Gb3	S	October 08	External
Ab37	S	October 08	External
2C9	S	April 09	External
Jb29	D	October 08	External
2Da10	M	October 09	External
2Da11	M	October 09	External
2Da13	M	October 09	External
2Da14	M	October 09	External
Za2	ZR	July 08	
Za4	ZR	July 08	
Za27	ZR	July 08	
Cb27	S	October 08	External
2Da16	M	October 09	External
2Da18	M	October 09	External
Za18	ZR	July 08	
Ab20	S	October 08	External
2A12	S	April 09	External
Za34	ZR	July 08	
2Da8	M	October 09	External
2Da9	M	October 09	External
E13	DD	March 08	
2Xa14	D	October 09	External
2Xa11	D	October 09	External
2Xa12	D	October 09	External
2Xa13	D	October 09	External
2Da3	M	October 09	External
2Da4	M	October 09	External
2Da7	M	October 09	External
Hb20	M	October 08	External
S60	IR	March 08	
Sa29	IR	July 08	
S31	IR	March 08	
2Da20	M	October 09	External
2Da21	M	October 09	External
2Da22	M	October 09	External
Cb21	S	October 08	External
E23	DD	March 08	
Ga20	S	July 08	Internal
Ab11	S	October 08	External
Ab12	S	October 08	External
Xb3	D	October 08	External
A5a	S	March 08	Internal
Za17	ZR	July 08	
Ab7	S	October 08	External
Gb53	S	October 08	External
2G16	S	April 09	External
2C10	S	April 09	External
Ea5	DD	July 08	
Ea50	DD	July 08	
E15	DD	March 08	
Z1	ZR	March 08	
E6	DD	March 08	
S2	IR	March 08	
S37	IR	March 08	
S49	IR	March 08	
S1	IR	March 08	
A7	S	March 08	Internal
A8	s	March 08	Internal
Sa16	IR	July 08	



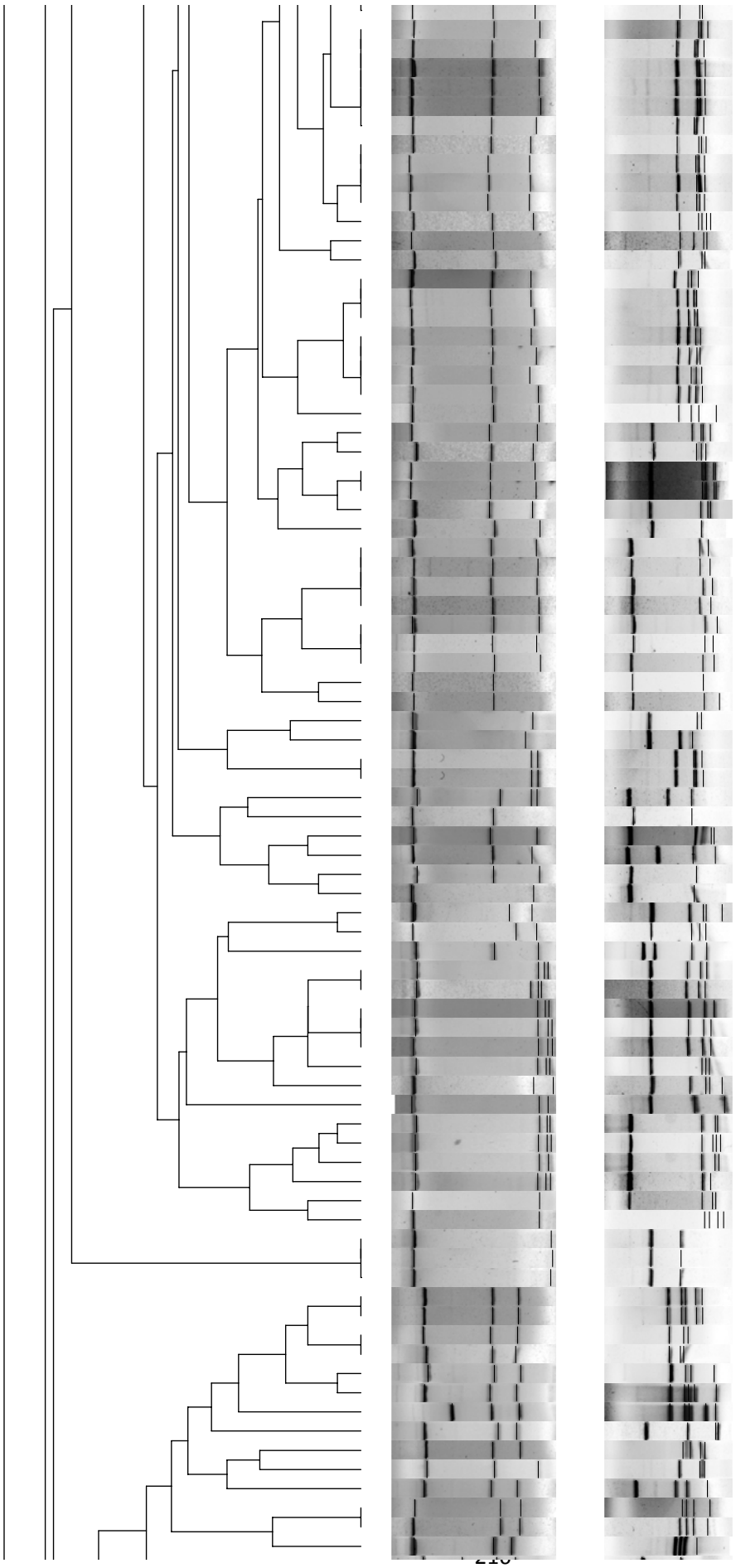
A8	s	March 08	Internal
Sa16	IR	July 08	
Z60	ZR	March 08	
Sa17	IR	July 08	
Ea8	DD	July 08	
Ea27	DD	July 08	
Ea40	DD	July 08	
1X5	D	April 09	Internal
2Aa27	S	October 09	External
Cb7	S	October 08	External
Za13	ZR	July 08	
1Ja1	D	October 09	Internal
2Ga14	S	October 09	External
2A10	S	April 09	External
2Da17	M	October 09	External
Ab45	S	October 08	External
Jb24	D	October 08	External
Fb45	D	October 08	External
Xb26	D	October 08	External
Cb22	S	October 08	External
Gb11	S	October 08	External
Gb16	S	October 08	External
Ab29	S	October 08	External
Ab41	S	October 08	External
Gb4	S	October 08	External
Xb17	D	October 08	External
Ab35	S	October 08	External
Ba3	M	July 08	Internal
Hb3	M	October 08	External
2C2	S	April 09	External
2C13	S	April 09	External
2C15	S	April 09	External
2C16	S	April 09	External
Bb21	M	October 08	External
2C7	S	April 09	External
2Aa31	S	October 09	External
Hb34	M	October 08	External
Ab44	S	October 08	External
Ha6	M	July 08	Internal
1H6	M	April 09	Internal
Aa10	S	July 08	Internal
Fb26	D	October 08	External
Hb15	M	October 08	External
Z26	ZR	March 08	
2H2	M	April 09	External
Fb14	D	October 08	External
2H23	M	April 09	External
1F18	D	April 09	Internal
Z11	ZR	March 08	
Fb1	D	October 08	External
Hb16	M	October 08	External
2A7	S	April 09	External
2D21	M	April 09	External
2D23	M	April 09	External
G20	S	March 08	Internal
2H19	M	April 09	External
2H22	M	April 09	External
Cb28	S	October 08	External
2J17	D	April 09	External
Gb47	S	October 08	External
2G4	S	April 09	External
Ha29	M	July 08	Internal
Ja6	D	July 08	Internal
Ja18	D	July 08	Internal
J5	D	March 08	Internal
J25	D	March 08	Internal
Da24	M	July 08	Internal
Fb36	D	October 08	External
Fb37	D	October 08	External
Cb35	S	October 08	External
Gb38	S	October 08	External
Gb44	S	October 08	External
2D16	M	April 09	External
2J2	D	April 09	External
Ab4	S	October 08	External
Gb56	S	October 08	External
Gb37	S	October 08	External
Gb18	S	October 08	External
Gb21	S	October 08	External
Gb26	S	October 08	External
Gb30	S	October 08	External



Gb21	S	October 08	External
Gb26	S	October 08	External
Gb30	S	October 08	External
Hb47	M	October 08	External
Xb16	D	October 08	External
Ga12	S	July 08	Internal
Ga25	S	July 08	Internal
X20	D	July 08	Internal
Ab13	S	October 08	External
Ab17	S	October 08	External
B85	M	March 08	Internal
Hb9	M	October 08	External
Jb36	D	October 08	External
2D17	M	April 09	External
B42	M	March 08	Internal
B67	M	March 08	Internal
2A3	S	April 09	External
Ha8	M	July 08	Internal
1H8	M	April 09	Internal
Sa22	IR	July 08	
Ga7	S	July 08	Internal
G9	S	March 08	Internal
Ba12	M	July 08	Internal
Ba14	M	July 08	Internal
Xb2	D	October 08	External
Z75	ZR	March 08	
Za15	ZR	July 08	
1Ga13	S	October 09	Internal
Cb19	S	October 08	External
2Fa2	D	October 09	External
2Fa5	D	October 09	External
1Da3	M	October 09	Internal
2Aa21	S	October 09	External
2Aa4	S	October 09	External
2Ga7	S	October 09	External
Sa3	IR	July 08	
2Aa12	S	October 09	External
2Ca5	S	October 09	External
2Ca9	S	October 09	External
2Ca11	S	October 09	External
2Ca13	S	October 09	External
2Ca14	S	October 09	External
2Ca15	S	October 09	External
2Ga3	S	October 09	External
1Ba20	M	October 09	Internal
2Ha15	M	October 09	External
Bb29	M	October 08	External
Sa42	IR	July 08	
1J7b	D	April 09	Internal
Ea47	DD	July 08	
2Ga9	S	October 09	External
1X20	D	April 09	Internal
Da31	M	July 08	Internal
Hb1	M	October 08	External
A51	S	March 08	Internal
2H20a	M	April 09	External
2Fa22	D	October 09	External
E4	DD	March 08	
1Ga4	S	October 09	Internal
Bb6	M	October 08	External
2Ja17	D	October 09	External
1Da1	M	October 09	Internal
1Da2	M	October 09	Internal
2Aa15	S	October 09	External
2Aa19	S	October 09	External
2Ca12	S	October 09	External
Jb4a	D	October 08	External
E57	DD	March 08	
E54	DD	March 08	
2Ba1	M	October 09	External
Ea15	DD	July 08	
Ea37	DD	July 08	
Ea38	DD	July 08	
Fa4	D	July 08	Internal
1J6	D	April 09	Internal
Cb37	S	October 08	External
Db29	M	October 08	External
1G3a	S	April 09	Internal
1G11	S	April 09	Internal
Db10	M	October 08	External
Jb19	D	October 08	External



1G11	S	April 09	Internal
Db10	M	October 08	External
Jb19	D	October 08	External
1J5	D	April 09	Internal
1J7a	D	April 09	Internal
2H16	M	April 09	External
2H24	M	April 09	External
2J7	D	April 09	External
2J14	D	April 09	External
2J27	D	April 09	External
1Ja16	D	October 09	Internal
F3	D	March 08	Internal
2F1	D	April 09	External
Fa10	D	July 08	Internal
Fa11	D	July 08	Internal
2J30	D	April 09	External
X12	D	July 08	Internal
Ja11	D	July 08	Internal
Ab22	S	October 08	External
J10	D	March 08	Internal
Aa25	S	July 08	Internal
Ba16	M	July 08	Internal
Ca6	S	July 08	Internal
Fa15	D	July 08	Internal
Cb11	S	October 08	External
Cb16	S	October 08	External
Db23	M	October 08	External
2A14	S	April 09	External
2F7	D	April 09	External
2F8	D	April 09	External
Db16	M	October 08	External
Da25	M	July 08	Internal
Z79	ZR	March 08	
Ga28	S	July 08	Internal
Ha4	M	July 08	Internal
Ga35	S	July 08	Internal
Ha1	M	July 08	Internal
Jb15	D	October 08	External
Ga31	S	July 08	Internal
Da29	M	July 08	Internal
1F4	D	April 09	Internal
2D18	M	April 09	External
2J15	D	April 09	External
A12	S	March 08	Internal
A37	S	March 08	Internal
Db1	M		
Db3	M		
Db6	M		
Ca1	S	July 08	Internal
Cb23	S	October 08	External
Ab48	S	October 08	External
Xb54	D	October 08	External
2Ga12	S	October 09	External
2Ha18	M	October 09	External
A16	S	March 08	Internal
A19	S	March 08	Internal
A36	S	March 08	Internal
A38	S	March 08	Internal
1C12	S	April 09	Internal
2J28	D	April 09	External
Ha16	M	July 08	Internal
Db19	M	October 08	External
G23	S	March 08	Internal
Aa29	S	July 08	Internal
D30	M	March 08	Internal
1H16	M	April 09	Internal
A23	S	March 08	Internal
2Ja8	D	October 09	External
1G15	S	April 09	Internal
1G18	S	April 09	Internal
1H20	M	April 09	Internal
2D10	M	April 09	External
2D19	M	April 09	External
2J8	D	April 09	External
2J25	D	April 09	External
2Ja3	D	October 09	External
Za14	ZR	July 08	
E19	DD	March 08	
2Aa28	S	October 09	External
C13	S	March 08	Internal
2C17	S	April 09	External



2Aa28	S	October 09	External
C13	S	March 08	Internal
2C17	S	April 09	External
Gb13	S	October 08	External
Gb14	S	October 08	External
Gb15	S	October 08	External
Ab46	S	October 08	External
Da20	M	July 08	Internal
Xb37	D	October 08	External
Xb33	D	October 08	External
Xb36	D	October 08	External
Da30	M	July 08	Internal
2D1	M	April 09	External
1Ga8	S	October 09	External
Hb29	M	October 08	External
Bb34	M	October 08	External
Bb32	M	October 08	External
Bb22	M	October 08	External
2C19	S	April 09	External
Bb27	M	October 08	External
Gb8	S	October 08	External
1Fa18	D	October 09	Internal
Ga17	S	July 08	Internal
Fa2	D	July 08	Internal
D47	M	March 08	Internal
D48	M	March 08	Internal
Ja16	D	July 08	Internal
1Ga15	S	October 09	Internal
Cb38	S	October 08	External
1F5	D	April 09	Internal
1G20	S	April 09	Internal
1J11	D	April 09	Internal
Cb9	S	October 08	External
2H11	M	April 09	External
1G2	S	April 09	Internal
1C1	S	April 09	Internal
2B3	M	April 09	External
Ea16	DD	July 08	
Sa45	IR	July 08	
Ab33	S	October 08	External
2C5	S	April 09	External
Aa17	S	July 08	Internal
1Aa1	S	October 09	Internal
Jb4b	D	October 08	External
1X12	D	April 09	Internal
Aa20	S	July 08	Internal
1Ha11	M	October 09	Internal
1Aa21	S	October 09	Internal
2Aa20	S	October 09	Internal
Za8	ZR	July 08	
Za3	ZR	July 08	
Sa18	IR	July 08	
Jb16	D	October 08	External
Za10	ZR	July 08	
Gb39	S	October 08	External
Za1	ZR	July 08	
1Aa19	S	October 09	Internal
Ga1	S	July 08	Internal
Ha15	M	July 08	Internal
Ga34	S	July 08	Internal
1H1	M	April 09	Internal
Aa13	S	July 08	Internal
C27	S	March 08	Internal
1Fa20	D	October 09	Internal
1Aa12	S	October 09	Internal
1Aa14	S	October 09	Internal
1Aa17	S	October 09	Internal
Jb26	D	October 08	External
Jb27	D	October 08	External
E24	DD	March 08	
1Aa18	S	October 09	Internal
Za6	ZR	July 08	
Sa 12	IR	July 08	
Za35	ZR	July 08	
G24	S	March 08	Internal
Jb31	D	October 08	External
2Ha9	M	October 09	External
J1	D	March 08	Internal
2F2	D	April 09	External
1Ja2	D	October 09	Internal
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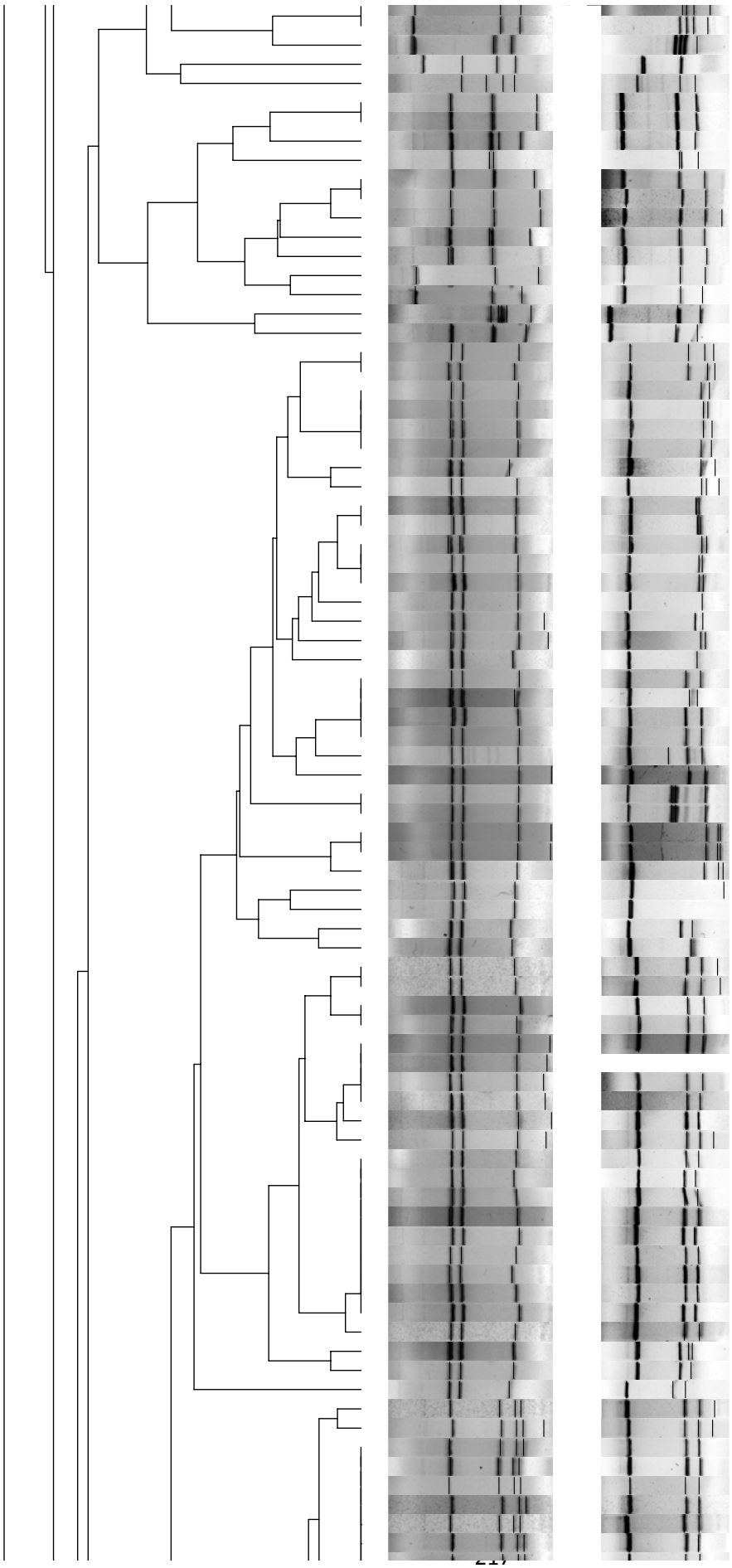

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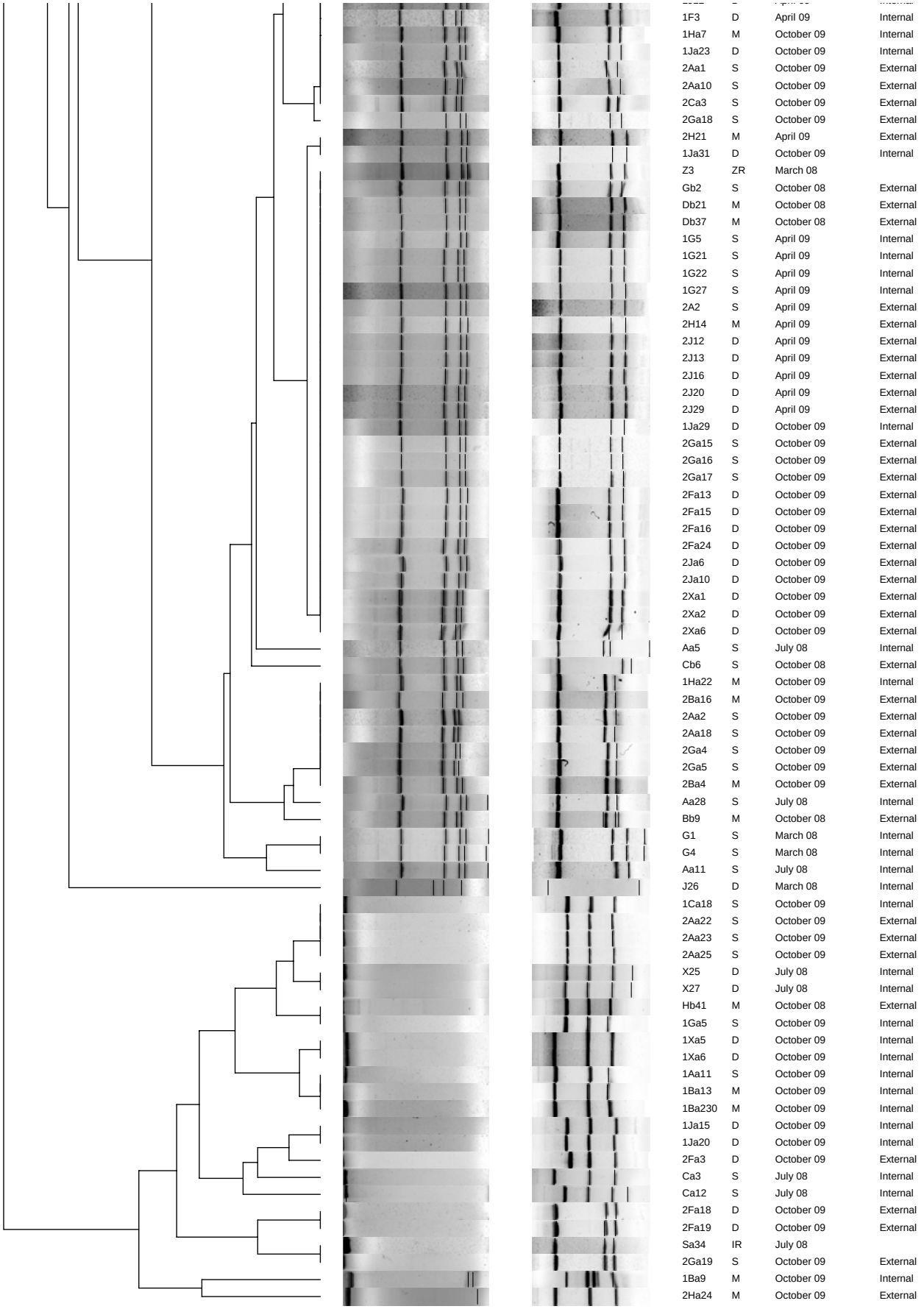
1Ja2	D	October 09	Internal
1Ba16	M	October 09	Internal
E56	DD	March 08	
2Ba2	M	October 09	External
Ab30	S	October 08	External
2C3	S	April 09	External
S61	IR	March 08	
1Ga1	S	October 09	Internal
Za28	ZR	July 08	
2G20	S	April 09	External
2G7	S	April 09	External
Fb24	D	October 08	External
2D24	M	April 09	External
Za7	ZR	July 08	
Ea10	DD	July 08	
A22	S	March 08	Internal
Hb40	M	October 08	External
Xb6	D	October 08	External
Xb12	D	October 08	External
Ab24	S	October 08	External
Db18	M	October 08	External
Fb33	D	October 08	External
2A17	S	April 09	External
Fb7	D	October 08	External
2Da15	M	October 09	External
Hb7	M	October 08	External
1Xa3	D	October 09	Internal
Fb39	D	October 08	External
Gb10	S	October 08	External
Cb24	S	October 08	External
1Ja19	D	October 09	Internal
Cb17	S	October 08	External
C1	S	March 08	Internal
2Aa13	S	October 09	External
Ab18	S	October 08	External
Hb30	M	October 08	External
Hb10	M	October 08	External
2A11	S	April 09	External
Ab1	S	October 08	External
Jb11	D	October 08	External
Ab23	S	October 08	External
2A16	S	April 09	External
Jb13	D	October 08	External
Jb14	D	October 08	External
1Aa22	S	October 09	Internal
1Aa10	S	October 09	Internal
1Aa15	S	October 09	Internal
2Aa17	S	October 09	External
2Ga6	S	October 09	External
Ba17	M	July 08	Internal
X10	D	July 08	Internal
Z4	ZR	March 08	
Fb31	D	October 08	External
Jb6	D	October 08	External
Db2	M		
Za36	ZR	July 08	
Sa28	IR	July 08	
1Ga10	S	October 09	Internal
Ja28	D	July 08	Internal
S17	IR	March 08	
S43	IR	March 08	
Ea3	DD	July 08	
Gb17	S	October 08	External
Ab34	S	October 08	External
Xb18	D	October 08	External
Xb35	D	October 08	External
Hb6	M	October 08	External
2C6	S	April 09	External
1X10	D	April 09	Internal
Hb26	M	October 08	External
Ea36	DD	July 08	
2Xa5a	D	October 09	External
X5	D	July 08	Internal
Ca8	S	July 08	Internal
Cb43	S	October 08	External
2Xa3	D	October 09	External
Xb27	D	October 08	External
1J12	D	April 09	Internal
1F3	D	April 09	Internal
1Ha7	M	October 09	Internal
1Ja23	D	October 09	Internal



1F3	D	April 09	Internal
1Ha7	M	October 09	Internal
1Ja23	D	October 09	Internal
2Aa1	S	October 09	External
2Aa10	S	October 09	External
2Ca3	S	October 09	External
2Ga18	S	October 09	External
2H21	M	April 09	External
1Ja31	D	October 09	Internal
Z3	ZR	March 08	
Gb2	S	October 08	External
Db21	M	October 08	External
Db37	M	October 08	External
1G5	S	April 09	Internal
1G21	S	April 09	Internal
1G22	S	April 09	Internal
1G27	S	April 09	Internal
2A2	S	April 09	External
2H14	M	April 09	External
2J12	D	April 09	External
2J13	D	April 09	External
2J16	D	April 09	External
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2Ga16	S	October 09	External
2Ga17	S	October 09	External
2Fa13	D	October 09	External
2Fa15	D	October 09	External
2Fa16	D	October 09	External
2Fa24	D	October 09	External
2Ja6	D	October 09	External
2Ja10	D	October 09	External
2Xa1	D	October 09	External
2Xa2	D	October 09	External
2Xa6	D	October 09	External
Aa5	S	July 08	Internal
Cb6	S	October 08	External
1Ha22	M	October 09	Internal
2Ba16	M	October 09	External
2Aa2	S	October 09	External
2Aa18	S	October 09	External
2Ga4	S	October 09	External
2Ga5	S	October 09	External
2Ba4	M	October 09	External
Aa28	S	July 08	Internal
Bb9	M	October 08	External
G1	S	March 08	Internal
G4	S	March 08	Internal
Aa11	S	July 08	Internal
J26	D	March 08	Internal
1Ca18	S	October 09	Internal
2Aa22	S	October 09	External
2Aa23	S	October 09	External
2Aa25	S	October 09	External
X25	D	July 08	Internal
X27	D	July 08	Internal
Hb41	M	October 08	External
1Ga5	S	October 09	Internal
1Xa5	D	October 09	Internal
1Xa6	D	October 09	Internal
1Aa11	S	October 09	Internal
1Ba13	M	October 09	Internal
1Ba230	M	October 09	Internal
1Ja15	D	October 09	Internal
1Ja20	D	October 09	Internal
2Fa3	D	October 09	External
Ca3	S	July 08	Internal
Ca12	S	July 08	Internal
2Fa18	D	October 09	External
2Fa19	D	October 09	External
Sa34	IR	July 08	
2Ga19	S	October 09	External
1Ba9	M	October 09	Internal
2Ha24	M	October 09	External

ACKNOWLEDGEMENTS

At first I express my deep sense of gratitude to my supervisor, Professor Sergio Casella and Professor Marina Basaglia, Department of Agrobiotechnology, School of Crop Science, University of Padova, Italy, for their guidance, inspiration, encouragement, advice, facilities and constant sympathetic co-operation helped me to complete this work successfully.

I would like to thanks University of Padova for financial support during 3 years of my PhD work.

I am also highly grateful to Professor Andrea Squartini, Professor Alessio Giacomini and Professor Viviana Corich, Department of Agrobiotechnology, University of Padova, Italy.

I am indebted to Dr. F. Fontana, for his friendly behavior and technical supporting during to my thesis. I would like to thanks Dr. E. Vendramin and Dr. E. Baldan for their valuable suggestion and for helping me to complete this work and my thanks are toward my research partner and friend Dr. Bruno Boz for his kind assistance.

I express my thanks to Dr. S. Povolo, Dr. L. Favaro, Dr. M. Bottegal, Dr. E. Polone, Dr. A. Tondello, Dr. P. Maragkoudakis, Dr. D.M. Kagli , Dr B. Bovo, L. Treu, A. Trento and other members of this laboratory for their friendly behaviour and continuous co- operation.

My thanks are toward my friends Mohamed, Ibrahim, Maha, Eriona, Andrea, Federico, Pietro and other friends for their necessary help and encouragement.

I would like to express my grateful thank to my family members , mother, father in law, mother in law, sisters and brothers, and especially to my wife Munni, my little son Arosh, and Mitu for their cordiality, inspirations, sacrifice and mental support to complete my PhD.

Eventually, my thanks are also to all my well wishers.

The Author