SOIL BACTERIAL DIVERSITY AND COMMUNITY STRUCTURE IN A RIVER FLOODPLAIN CONTINUUM

By

YANG SONG

Bachelor of Science

Beijing Agricultural College

Beijing, People's Republic of China

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STRUCTURE IN A RIVER FLOODPLAIN

CONTINUUM

Thesis Approved:

Shiping Deng
Thesis Adviser

Jacqueline Fletcher

Babu Z. Fathepure

Gorson Emslie
Dean of the Graduate College

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FORMAT OF THESIS

This thesis is presented in a combination of Soil Biology and Biochemistry, Applied and Environmental Microbiology, and formats outlined by the Oklahoma State University graduate college style manual. The use of this format allows the independent chapters to be suitable for submission to scientific journals. Each paper is complete in itself with an abstract, introduction, materials and methods, results, discussion, and reference section.

Chapter I

Introduction

Floodplain refers to a depositional landform that temporarily stores excess water from streams or lakes that overflow banks. Sedimentation processes lead natural floodplains to be sinks, sources, or transformers of dissolved and particulate organic matter, inorganic nutrients, biota and even heavy metals, pesticides, and other potential agricultural and environmental contaminants (Macklin and Klimek, 1992; Walling et al., 1996). Such processes can have adverse effects on water quality, aquatic habitats, and agricultural use of floodplain areas.

The increased anthropogenic inputs of heavy metals in terrestrial environments have caused considerable concern. It is well known that heavy metals are toxic to most organisms when they are present in high concentrations in the environment (Aoyama and Nagumo, 1997; Barajas-Aceves et al., 1999; Kizilkaya, et al., 2004). They affect growth, morphology and metabolism of microbes by causing protein denaturation or destruction of the integrity of cell membranes (Leita et al, 1995). However, there is discrepancy between total heavy metal concentration and the bioavailable portion. Only a small portion of trace elements in soil is bioavailable. Bioavailability of these elements is

controlled by many factors, including pH, redox potential, and organic matter content.

Microorganisms are important in promoting nutrient cycling and biodegradation of contaminants. Although less organic matter and microbial population reside in subsoil, the subsurface plays an important role in retaining contaminants and preventing them from leaching into ground water. Understanding of microbial community structure and metabolic activity of subsoil microbes would enhance understanding of the transformation and degradation of natural and xenobiotic compounds in the soil environment. The objectives of this study were (1) to evaluate the impact of chronic flooding with accidental spills of heavy metals on soil biota in a river floodplain continuum; and (2) to assess microbial responses to redox states in different soil depth. Microbial communities were evaluated by measuring the microbial biomass carbon and nitrogen contents, dehydrogenase activity, and culturable bacterial populations. Depth- and redox-related variations in microbial community structure were evaluated using 16S rRNA-based approaches, culture-based methods, and Fatty Acid Methyl Ester (FAME) analysis.

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Chapter II

LITERATURE REVIEW

Characteristics of river floodplains

Floodplain refers to a depositional landform that temporarily stores excess water from streams or lakes that overflow banks (Bridge, 2003). Floodplain is composed of complex assemblages of landforms (Fig.1). It results from the long-term cumulative action of flow, erosion and depositional processes. Floodplains usually include channel features (bars, bedforms), channel edge features (banks, benches, and levees), and floodplain features (e.g. oxbows, backswamps). As the land adjacent to a waterway, it can provide records of past hydrologic conditions because they are reflected in the sediments (Brown, 1996; Ward and Stanford, 1995a, b). The driving force of exchange processes between the river and its floodplains is hydrological connectivity, via both surface and groundwater inflow (Heiler et al., 1995; Ward and Stanford, 1995a). The sedimentation on floodplains, caused by discrete overbank flooding, usually ranges from millimeter-thick to decimeter-thick (Bristow et al., 1999; Perez-Arlucea and Smith, 1999). During flooding, the flow is relatively slower and shallower over the floodplain than in the channel, so the suspended sediment carried is also lower. This leads to the coarser-grained sediment falling out of suspension near the

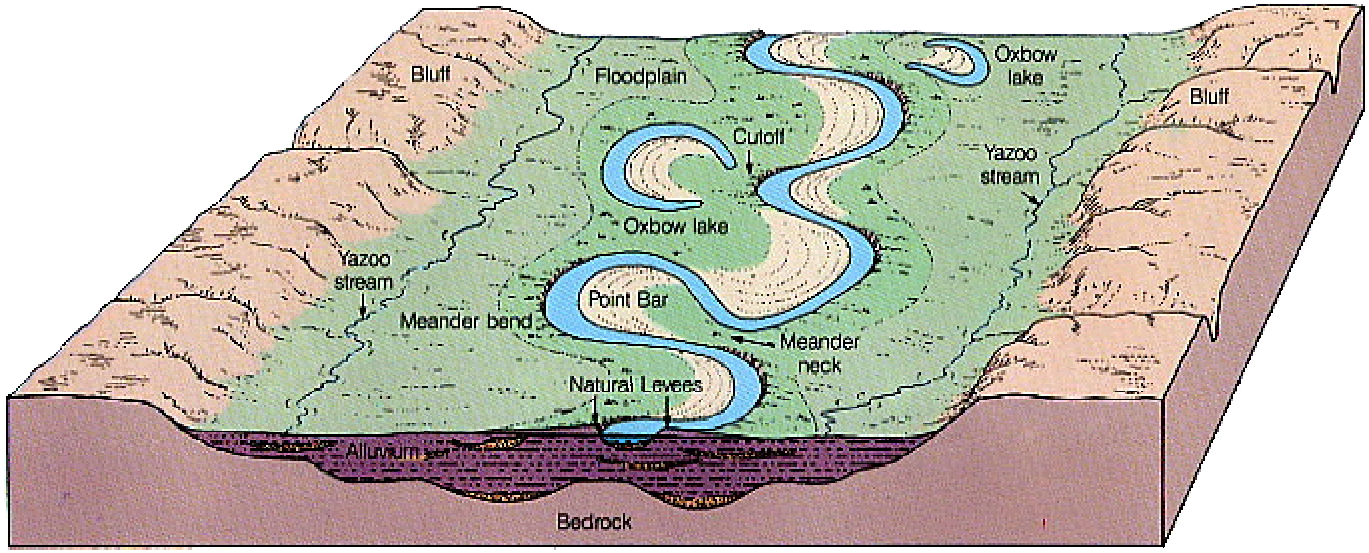

Figure 1. Schema of a floodplain.

(Adapted from http://shiro.wustl.edu/210A/transparencies.html)

channel and a gradual decrease in the mean grain size of deposited material as distance from the channel increases (Phillips, 1991; Meade, 1994; Mertes, 1994).

Due to constant fluctuation in the availability of water and nutrients, a floodplain often contains diverse habitats that harbor diverse biota (Duncan, 1993; Ward and Stanford, 1995b; Planty-Tabacchi et al., 1996). There are three broad hydrogeomorphological categories of floodplains (Brown, 1996): (1) Relatively free-draining and coarse-textured soil environments, where little soil development and low levels of nutrients are found. Plant communities occupying this area have relatively short life cycles, spend much energy for reproduction and are tolerant of disturbance and frequent inundation. (2) Levees and floodplain flats, which are often used for agricultural production. (3) Backswamps and floodplain cutoffs contain high organic matter because of their waterlogged conditions. Because of the lack of available nitrogen and oxygen, this area is favored by rushes, reeds and grasses. Moving from relatively high ground within the flood plain to the river edge, there are different plant communities: flood plain forests, sedge meadows and wet prairies occupying the less saturated soil, and the aquatic plant communities occupying the saturated soils. Wet, poorly drained floodplains (marshes, swamps, forests) typically have chemically reducing conditions at and below the surface, and are favorable sites for preservation of tree stumps, leaves, peat, seeds, microflora, foots and insect parts. In well drained floodplains, organic debris is readily oxidized and organic remains are incorporated into floodplain soil by burial during seasonal floods. Some organic

remains (tree stumps and roots) can be preserved as casts in soil calcium carbonate or as casts of sandstone or mudstone.

Recently, there has been increasing interest to evaluate impacts of inorganic and organic contamination on floodplain ecosystem health. Erosion and sedimentation processes lead natural floodplains to be sinks, sources, or transformers of dissolved and particulate organic matter, inorganic nutrients, biota and even heavy metals, pesticides, and other potential agricultural and environmental contaminants (Macklin and Klimek, 1992; Walling et al., 1996). Such processes can have adverse effects on water quality, aquatic habitats, and the agricultural use of floodplain areas. Remobilization of contaminated overbank sediment deposits stored on floodplains can reintroduce nutrients and contaminants into the river system again (Bradley and Cox, 1990; Pinay et al., 1992; Foster and Charlesworth, 1996, Steiger et al., 2005).

Heavy metals in the environment

Heavy metals are a group of elements that have specific gravities greater than 5g cm^{-3}. The two important sources of heavy metals in soil include mineral rock weathering and anthropogenic sources. The main anthropogenic sources of heavy metals generally include five main groups: (1) mining and smelting (arsenic, cadmium, lead and mercury); (2) industry (arsenic, cadmium, chromium, cobolt, copper, mercury, nickel, zinc); (3) atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury, uranium); (4) agriculture fertilizers (arsenic, cadmium, copper, lead, selenium, uranium, zinc); and (5) waste disposal (arsenic, cadmium, chromium, copper, lead, mercury, zinc)

(Ross, 1994). Heavy metals are stable and persistent in the environment since they cannot be easily degraded or destroyed. In soil, there are several main processes associated with heavy metals: (1) Precipitation and co-precipitation of inorganic compounds in soluble species, for example, carbonates and sulfides; (2) Uptake by plant roots and immobilization by soil organisms; (3) Exchange with cations of clays or soil organic matter; and (4) Chelation and complex formation by soil organic matter, and (5) Leaching of soluble organic-metallic chelates or mobile ions (Fig. 2) (Ross, 1994).

In recent years, increased anthropogenic inputs of heavy metals in terrestrial environments have caused considerable concern relative to their impact on groundwater contamination (Alloway, 1995). Although most heavy metals are considered to be relatively immobile in most soils for short periods of time, their mobility under certain solution and soil chemical conditions may pose a serious threat to groundwater quality (Scokart et al., 1983; Maskall et al., 1995). This threat was supported by recent research evidence showing that water-dispersed colloidal particles migrating though soil macropores and fractures can significantly enhance metal mobility (Puls and Powell, 1992; Ouyang et al., 2002; Ryan and Elimelech, 1996).

Factors affect bioavailability of heavy metals.

In soils and sediments, metals usually exist as six fractions, including dissolved, exchangeable, carbonate, iron-manganese oxide, organic, and crystalline form (Salomons, 1995). There is discrepancy between the total and

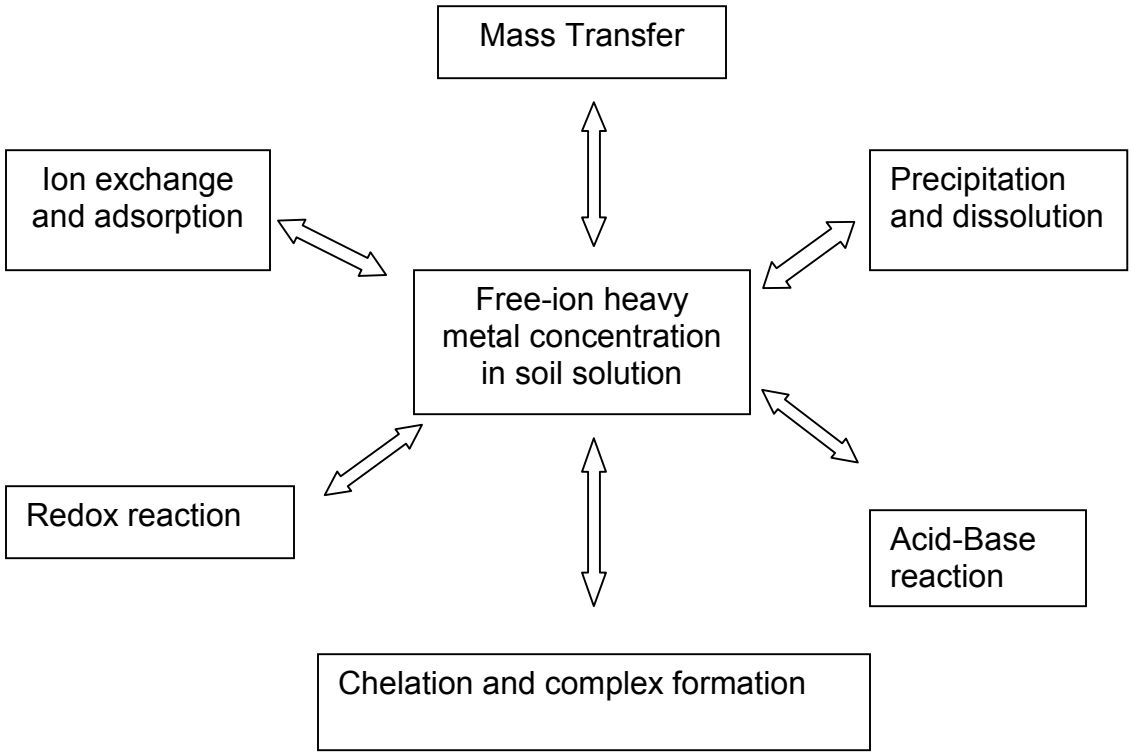

Figure 2. Factors control the concentrations of free-ion metals in soil solution (Adapted from Mattigod, et al., 1981)

the bioavailable amount of metal present. Bioavailabile metals usually refer to metals that are present as, or can be transformed readily to, free-ion species. Bioavailable metals are soluble, nonsorbed, and mobile in soil. In reality, the bioavailability of heavy metals is difficult to quantify. It is usually assessed by measuring the growth of microorganisms or the uptake of heavy metals by plants or microbes. Metal availability is strongly dependent on environmental conditions, such as pH, redox potential, and organic matter content (Fig. 2). pH plays an important role in governing metal speciation, solubility from mineral surfaces, transport, and eventual bioavailability of metals. In general, heavy metal cations are most mobile under acid conditions. Increasing pH values usually leads to reduction of metal bioavailability. A number of authors have found positive correlations between copper (Cu) retention and pH (Tyler and McBride, 1982; Harter, 1983; King, 1988). Heavy metals present in soil will not all be bioavailable because solid-phase humic substances such as humic acids absorb metals by forming chelated complexes. Important chemical processes affecting bioavailability of metals in soil include cation exchange, specific exchange of heavy metal cations with surface ligands and co-precipitation with other elements. Redox potential is another factor that influences the bioavailability of heavy metals by promoting their precipitation. In saturated soils (lower redox potential), oxygen is quickly depleted, SO_4^{2-} is reduced to sulfide, and heavy metals may precipitate with sulfide.

Effects of heavy metals on microbial life

Some heavy metals are trace elements that play an important role in

biochemical reactions. These metals may function as catalysts for biochemical reactions and stabilizers of protein structures and bacterial cell walls, and may serve in maintaining osmotic balance (Hughes and Poole, 1989; Ji and Silver, 1995). Some heavy metals, including cadmium (Cd), lead (Pb) and mercury (Hg), have no essential biological functions and are potentially toxic to microorganisms. These metals bind to SH groups, and thus inhibit the activity of enzymes (Nies, 1999). However, at high levels, both essential and nonessential metals may be toxic to organisms by affecting their growth, damaging cell membranes, altering enzyme specificity, disrupting cellular functions, and damaging the structure of DNA and proteins of micro-organisms (Fliessbach et al., 1994; Leita et al., 1995; Giller et al., 1998).

At high concentrations of heavy metals, some microorganisms develop resistance to protect themselves. Several mechanisms are utilized by microorganisms to resist heavy metals (Bruins et al, 2000). These include (1) metal exclusion by a permeability barrier, such as an alteration in the cell wall or membrane to allow bio-absorption of metal ions in the extra-cellular poly-saccharide coating, (2) active transport of the metal away from the microorganism, via chemicals encoded by genes located on the chromosome or plasmid, (3) intra- cellular sequestration by accumulation of metals within the cytoplasm or extra-cellular sequestration, (4) enzymatic detoxification of a metal, and (5) reduction cellular sensitivity to metal ions.

Although some microbes develop resistance against heavy metals, high concentrations of metals can negativelly affect soil microbial activity, abundance

and associated processes. As a result, significant reduction in microbial biomass and activity had been found in metal contaminated soils (Barajas-Aceves, et.al, 1999, Kizikaya, et al. 2004). Meanwhile, many studies have shown that the heavy metal contamination cause a shift of soil microbial community (Kelly and Tate, 1998). Studies showed that fungi were more resistant to heavy metals than bacteria (Hiroki, 1992; Frostegård et al., 1993; Khan and Scullion, 2000). The number of gram-negative bacteria increased and the number of gram-positive bacteria decreased in metal-contaminated soils (Zelles et al., 1994). Moreover, an increase in actinomycetes in contaminated soil was reported by Frostegård et al. (1993). In addition, carbon mineralization and nitrogen fixation were also influenced by different heavy metals. Metal contamination led to increased carbon respiration and N mineralization (Bååth, 1989).

Characteristics of the subsurface soil environment

As a large pool of terrestrial organic carbon resources in the biosphere, soil provides a heterogenic environment for microbial life. There are significant quantities of organic carbon in both surface and subsurface soil horizons. Although organic C concentrations are generally much lower in subsurface than surface horizons, the total mass of the subsurface soil is large. On a global basis, more than 50% of the organic C is found in the upper 1 m depth of soil profiles. This suggests that about 50% of organic C can be found in subsurface soil horizons below 1 m depth (Batjes, 1996). The rate of microbial mineralization of the organic C in subsurface horizons is generally low and subsurface organic C pools have long mean residence times (Trumbore, 2000). This is due, in part, to

differences in aeration. Subsurface soils may be oxygen limited, while surface soils often provide an oxic environment. During biotic or abiotic processes, changes of redox potential are important for potential energy requirements that transform chemical compounds in the environment. In contrast of oxic respiration, the metabolic process in the anoxic layer is dominant by denitrification, sulfate reduction and methanogenesis. NO^{3-}, Fe^{3+}, Mn^{4+}, SO^{4-}, CO_2, and organic intermediates act as the main electron acceptors. Less energy is released when these terminal electron acceptors are used instead of oxygen.

Soil profiles are often many meters deep and large numbers of microorganisms reside in subsurface horizons (Van Gestel et al., 1992; Dodds et al., 1996; Fritze et al., 2000; Blume et al., 2002). These subsurface microbes play an important role in soil formation, ecosystem biogeochemistry, contaminant degradation, and the maintenance of groundwater quality (Konopka and Turco, 1991; Hiebert and Bennett, 1992; Madsen, 1995). Under oxygen limiting subsurface conditions, several major factors regulated microbial diversity and function. Spatial limitation (texture, porosity, and permeability of the subsurface material) affects the water availability and determines pore space, which in turn limit the types and sizes of organisms present. Nutrients availability is another limiting factor that regulates microbial diversity and function. The types of organic compounds in the subsurface zone usually depend on the parent material. Readily metabolized compounds are largely consumed by the surface microflora before they reach subsurface horizons. Nitrogen, phosphorous, oxygen, and other electron acceptor may also be limiting. Numerous microorganisms are

forced to use different electron acceptors and to operate different metabolic pathway in an oxygen limited environment (Table 1) (Aelion et al., 1987; Swindoll et al. 1988).

While the existence and activity of subsurface microorganisms, particularly in deep soils and aquifers, have been demonstrated (Ghiorse and Wilson, 1988; Crocker et al. 2000; Dobbins et al., 1992), relatively little information is available concerning such subsurface microbial life.

Microbial life in soil

Soil provides a heterogeneous habitat for microbial life and is inhabited by a wide range of microorganisms whose numbers reach as high as million to billion g^{-1} of soil. There are six groups of organisms are found in soil. These include bacteria, fungi, protozoa, nematodes, arthropods and earthworms. Bacteria are the most abundant of the soil organisms and they existed in as many as a trillion species (Dykhuizen, 1998). As a diverse group of organisms, they can use organic or inorganic compound or sunlight as the source of energy and thrive in oxic or anaerobic environment.

Fungi are eukaryotic microbes and many have long hyphae able to penetrate soil systems and push their way between soil particles, roots, and rocks. They contribute a great portion of the soil biomass. Fungi perform important services related to water dynamics, nutrient cycling, and disease suppression. Along with bacteria, fungi are important as decomposers in the soil nutrient resource. They convert hard-to-digest organic material into forms that

Table 1. Common functional microbial groups in surface and subsurface soils

Functional group	Electron donor	Electron acceptor	Environment
Aerobic heterotrophs	Organic compounds	Oxygen	Aerobic
Denitrifiers	Organic compounds	Nitrate	Hypoxic/anaerobic
Manganese-Ion reducers	Organic compounds	Fe^{3+}, Mn^{4+}	Anaerobic
Sulfate reducers	Organic compounds	SO_4^{2+}	Anaerobic
Methanogens	Acetate, H2	CO2	Anaerobic
Nitrifiers	Ammonia	Oxygen(several steps)	Aerobic, aerobic-anaerobic interface
Sulfur oxidizers	Sulfide, S, thiosulfate	Oxygen, others like Fe^{3+}	Aerobic, aerobic-anaerobic interface
Iron oxidizer	Fe^{2+}	Oxygen	Aerobic, near-anaerobic interface
Fermenters	Reduced organic compounds	Oxidized organic compounds	Prevalent in anaerobic

other organisms can use. Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity.

Soil protozoa are single-celled eukaryotic organisms. They contribute a small percentage of total soil biomass compared with fungi and bacteria. As a major predator of bacteria, protozoa play an important role in regulation and modification of the size and composition of the microbial community. Moreover, they also accelerate the turnover of microbial biomass over soil organic matter and direct excretion of nutrients. The number of protozoa decreases with increasing soil depth.

Nematodes are the most abundant, non-segmented metazoans in the soil. Like protozoa, nematodes play an important role in regulation and modification of the size of bacteria and on promoting nutrient cycling through feeding on bacteria, fungi hyphae, plants, fauna, and other nematodes. At low nematode densities, feeding by nematodes stimulates the growth of prey populations.

Arthropods are invertebrates. Based on their functions in soil, they include four groups: shredders, predators, herbivores, and fungal feeders. They survive in the soil surface or in the upper three inches. Their numbers range from 100-25,000 per ft^2 in different soil types and managed systems. Their activities also play an important role on stimulating microbial activity, affecting soil aggregation and physical properties.

Earthworms live in non-acidic soils and feed on dead organic matter and plant debris. Through burrowing extensively in soil, they create passages for air

and water movement, and nutrient transport in soil. Soil and organic matter are moved from surface to lower layers by earthworm activity.

In summary, the heterogenic soil environment supports diverse microbes. These organisms contribute to development of soil physical and chemical properties, and soil structure. Understanding the characteristics and functions of soil microbes is important in maintaining soil quality and productivity.

Linking microbial community to its ecological function

Although the living portion of soil organic carbon is less than 5%, this portion is the most active. Activities of soil biota regulate transformations of nutrients, metals and gases, and impact availability of oxygen and water. Therefore, microorganisms are important components of an ecosystem. Changes in microbial activity would impact ecosystem health and function.

The main objective of this research was directed to evaluate soil microbial diversity and community structure in a floodplain continuum. Tisza river floodplain continuum in Hungary was chosen as the study site. This site was chosen because it is a diverse freshwater ecosystem, and is the home for many unique flora and fauna in Europe. This river ecosystem provides drinking water, irrigation and supports an agriculture-, fishing-, and tourism-based economy. Unfortunately, chronic flooding with accidental spills of contamination from upstream old mining sites jeopardizes the sustainability of river ecosystem. For example, when a tailing dam broke due to an overflow, about 100,000 m^3 of wastewater containing cyanide as well as heavy metals was released into closest river system and moved down stream into Tisza River which caused an acute

environmental and ecological damage (Fleit and Lakatos, 2003). Another major accidental spill happened in March 2000. An overflow and breach of the Novat tailings dam in Baia Borsa, Romania led to release of 100,000 m^3 of sludge into a tributary of Tisza River. The sludge included about 20,000 tons of solid tailings containing elevated amounts of heavy metals. These catastrophic events led to dead fish floating in the river, attracted international attention for ecosystem evaluation and restoration. Understanding relationships and complexity of components in an ecosystem is important in developing management strategies to maintain sustainability of a functioning ecosystem.

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Chapter III

CHRONIC FLOODING WITH HEAVY METAL CONTAMINATIONS IMPACTED SOIL BIOTA IN A RIVER FLOODPLAIN CONTINUUM

Abstract

Metal dynamics and redox states in an ecosystem play crucial roles in regulating microbial abundance and activity. We evaluated relationships between the soil microbial community and concentrations of six metals under three redox conditions in a river floodplain continuum that was subjected to seasonal flooding and metal contamination. Soils were collected from four sites at each of two locations along Tisza River in Hungary. Each soil profile was divided into three redox horizons: aerobic (oxic), seasonally reduced (intermittent), and permanently reduced (anoxic). Microbial communities were evaluated by microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) contents, dehydrogenase (DH) activity, and culturable bacterial populations (CBP). Microbial biomass and DH activity were significantly lower in the anoxic layers when compared with those in the oxic horizon. However, trends for culturable bacterial populations among the three redox layers from different sites were not consistent. Four out of eight tested sites showed slightly or significantly higher culturable bacterial population in the seasonally flooded horizons than in the oxic or anoxic layers. The vertical distribution of these microbes might largely correlate with the decline

in C availability with soil depth. There were significant correlations between heavy metals and microbial properties in the oxic layer. Of the six metals evaluated, microbial activity and population in oxic horizons were closely related to total concentrations of Cu and Cd, followed by Pb and Zn, and least to Cr and Ni. Microbial metabolic activity decreased with increasing metal concentrations. There were significant negative correlations between metal concentrations and DH activity ($r > -0.9^{***}$ for Cu and Cd). However, microbial populations were higher in soils with higher metal concentrations, evidenced by significant positive correlations between metal concentrations and C_{mic}, N_{mic}, cultural bacterial populations, and soil DNA concentrations, respectively ($r > 0.9^{***}$ for Cu and Cd). Exposure of microbes to heavy metals at the concentrations evaluated suppressed microbial metabolic activity, but did not cause acute toxicity, cell death, or population reduction. In fact, some microbes increased their population in response to stress conditions. These results suggest that microbial properties were significantly impacted by C availability. However, microbial communities living in anoxic horizons demonstrated little response to variations in metal concentrations, suggesting that microbial communities thriving in reduced conditions was either more tolerant to environmental stress, or that stress factors were markedly suppressed under oxygen limiting conditions.

Keywords: redox states, microbial biomass, dehydrogenase activity, culturable population, heavy metals

1. Introduction

Flooding usually leads to the transfer of sediments and biota from a river channel to a floodplain (Thoms, 2003). The influence of river flooding on floodplain soils is mainly through erosion and deposition effects, which often alter carbon (C), nitrogen (N) or even heavy metal distributions in soils (Pinay et al., 1992). Metal contents drop sharply with increasing distance from the river channel (Martin, 1995).

Tisza River in Hungary is located almost exactly in the geographical center of Europe. It is not only the home for many unique flora and fauna in Europe; but also provides water for drinking, and irrigation, and supports an agriculture-, fishing-, and tourism-based economy. Unfortunately, chronic flooding and several major recent accidental spills from upstream old mining sites in Romania released over 100 tons of cyanide and equal amounts of heavy metals, which led to the death of fish and other major impacts on the biosphere (Fleit and Lakatos, 2003).

Heavy metals are toxic to most organisms when they are present in high concentrations in the environment (Kizilkaya, et al., 2004; Aoyama and Nagumo, 1997; Barajas-Aceves et al., 1999). They affect growth, morphology and metabolism of microbes by causing protein denaturation or destruction of the integrity of cell membranes (Leita et al, 1995). The degree of exposure of microorganisms to heavy metals depends on metal concentration, soil properties (for example, pH, organic matter content, redox states) and characteristics of microorganisms. Most research has evaluated the effects of heavy metals at high

concentrations in terrestrial areas or under laboratory conditions. Little information is available to demonstrate to what extent heavy metals introduced by flooding event affect microbial communities in floodplain ecosystems.

The microbial community plays a key role in processes of organic matter decomposition, nutrient cycling, and biodegradation of contaminants in soil. Therefore, microbial community properties, including microbial population, microbial biomass, and enzyme activities, have been widely used as indicators to evaluate changes in the soil environment (Sparling, 1997). Although soil microbial biomass is only 1-5% of the total organic C (C_{org}) and around 5% of the organic N (N_{mic}), changes of this microbial parameter has been used as an early indicator of changes in soil processes because of its faster turnover rate than total organic matter (Jenkinson and Ladd, 1981; Powlson et al., 1987). Activity of dehydrogenase, an enzyme that is active only in living cells, was widely used in evaluating metabolic activity of soil microbes, especially under stress or toxic conditions (Lenhard, 1963; Chander and Brookes, 1991; Chander et al., 1995). However, most studies focused on microbial properties in surface soils in the oxic layer. A large portion of soil profiles is associated with alternating aerobic and reduced conditions; it is not possible to infer subsurface community structure from studying the surface layer. Since the subsurface microbial community under seasonal or permanently flooding condition plays a crucial role in regulating soil formation, contaminant degradation and ground water quality (Konopka and Turco, 1991; Madsen, 1995), it is of interest to understand microbial community structure and activity below the surface layer.

Therefore the main objective of this study was to evaluate changes of soil microbial properties in a river floodplain continuum that were induced by flooding and contamination of heavy metals. Microbial properties tested included DH activity, microbial biomass, culturable bacteria population and DNA content.

2. Materials and methods

2.1. Soil

Soil samples were taken from two locations along Tisza River floodplain continuum in October 2003 (Fig. 1). At each location, four sampling sites were identified to reflect different structural properties of floodplain (Table 1). The sites were covered by different vegetation, including, pasture, cultivated field and trees. L1S1 was the lowest and wettest point of the floodplain. Site 4 at each location was further away from the river and served as a control. Presumably these two sites have not been flooded for over 100 years. At each sampling site, soils were composited based on redox states, including oxic, seasonally flooded (intermittent), and permanently saturated (anoxic) horizons.

Field-moist soil samples were stored and transported on ice. Following passing through a 2 mm sieve, the samples were mixed thoroughly and stored in sealed plastic bags at 4^{o}C. A portion of each sample was air-dried and a portion of each air-dried sample was ground to pass through an 80-mesh (180 μm) sieve. Air-dried soils were stored in sealed containers at room temperature.

2.2. Chemical properties

Soil pH values were determined using a combination glass electrode (soil:

0.01 M $CaCl_2$ ratio = 1:2.5). Organic C (C_{org}) was determined according to Walkley-Black procedure (Nelson and Sommers, 1996). Total metal contents were extracted by digesting soils with HNO_3 and H_2O_2, and determined by inductively coupled plasma atomic emission spectrometry (ICP) (Kovács et al., 2000). Particle size distribution was determined by a pipette analysis (Kilmer and Alexander, 1949).

C_{org}, total metal contents were determined using samples with particle size less than 180 μm. Soil pH and particle-size distribution were determined with air-dried samples that were < 2 mm.

2.3. Microbial characteristics

Microbial biomass C (C_{mic}) was determined by the chloroform-fumigation-incubation method described by Jenkinson and Ladd (1981) using a K_c factor of 0.45 with subtraction of the control. Microbial biomass N (N_{mic}) was determined as described by Brookes et al. (1985) using a K_n factor of 0.54 with subtraction of control.

Dehydrogenase activity (DH) was assayed as described by Casida et al. (1964). The C_{mic} content, DH activity, and other microbiological determinations were done using < 2 mm field-moist samples. All results are expressed on a moisture-free basis. Moisture was determined after drying at 105^{o}C for 48 h. Soil bacterial population was determined on a 0.1-strength trptone soya agar

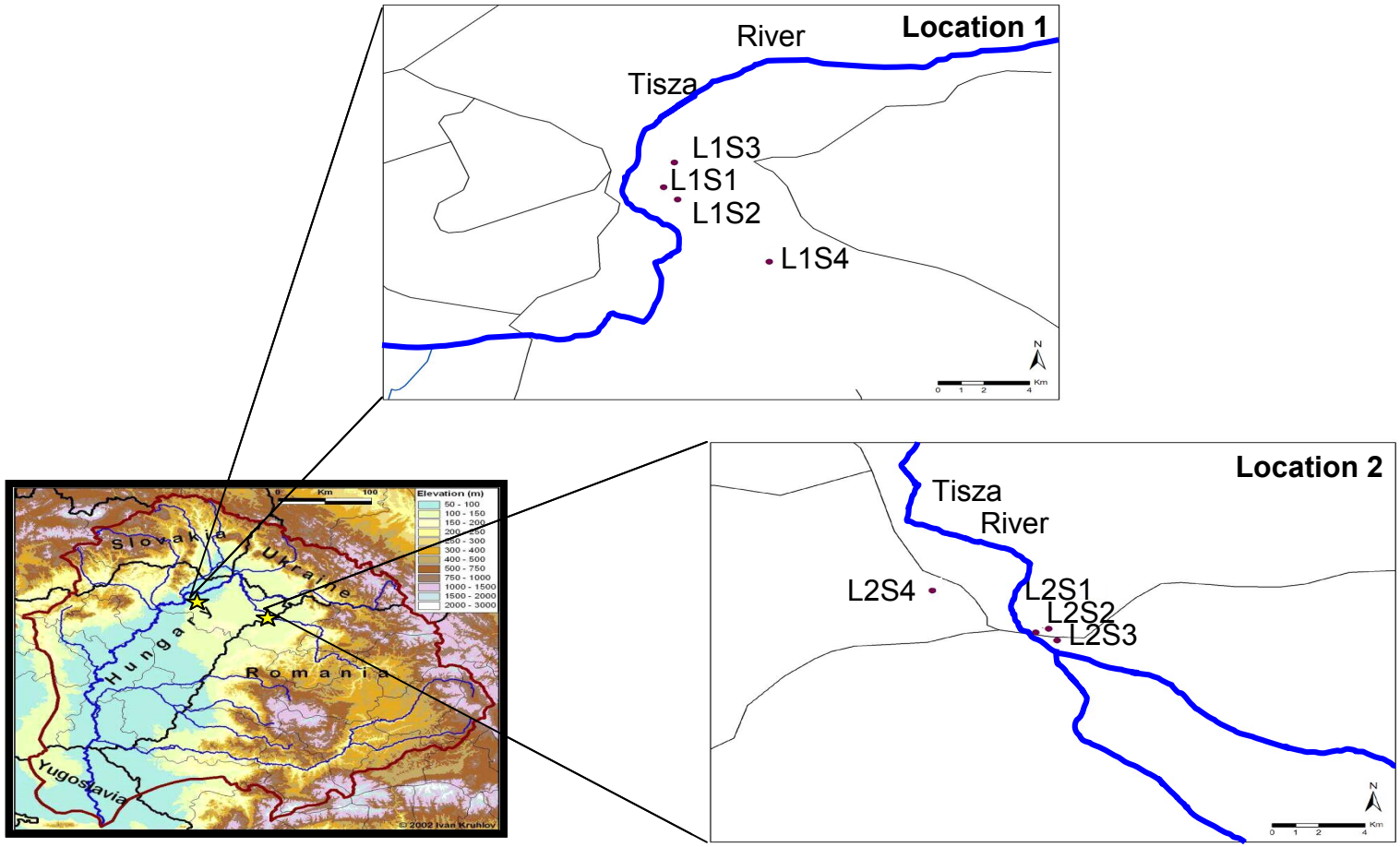

Fig. 1. Geographic locations of the sampling sites. Location 1 is in the downstream and Location 2 is in the upstream of the Tisza River. Location 2 is closer to the heavy metal contamination source than Location 1.

Table 1. Description of the sampling sites.

Sample Site[†]	Vegetation	Sites description
L1S1	unimproved pasture	Located in the midway between the river and the boundary to the first terrace . It is in the local low point of the floodplain
L1S2	cultivated field	Located on natural levee about 80 m from Tisza River
L1S3	unimproved pasture	This location is near an oxbow that lies at the boundary between the floodplain and first terrace
L1S4	pasture or hay meadow	Located on a terrace that is not subject to flooding
L2S1	unimproved pasture.	Located about 100 m upslope (~2% slope) of an oxbow/drainageway near the boundary between the floodplain and first terrace
L2S2	hardwood trees	Located on a high area about 200 m from Tisza river
L2S3	cultivated field	Located about 75 m from Tisza river on opposite site of road from L2S1 and L2S2
L2S4	orchard	Located on first terrace above floodplain in an apple orchard. It has not flooded for at least 170 years

[†] L-location, S-site. There were two locations and four sites at each location.

(TSA) medium at 23^{o}C (Katoh and Itoh, 1983; Lawley et al., 1983). Colonies were enumerated on a daily basis for 5 consecutive days and on day 10. Plates were examined at low magnification (1.5X), and each visible colony was marked and enumerated. Thus 6 counts were generated per plate. When plates became too crowded, the next dilutions were used for enumeration. All the experiments were conducted in triplicate treatments with four replicate plates for each dilution.

Soil DNA was extracted using an UltraClean^{TM} soil DNA kit (MoBio Laboratories, Inc., Solana Beach, CA, USA). DNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, Delaware, USA).

2. 4. Statistical analysis

Significant differences among treatments were determined using one-way analysis of variance (ANOVA). Comparison of treatment means was done using the least significant difference (LSD) test. Percentage data were transformed using the Arc Sine $\sqrt{\text{Percentage}}$ transformation before analysis (Gomez and Gomez, 1984). All results reported are averages of triplicate assays and analyses.

3. Results

3.1. Soil properties

Although sampling depths were all around 280 cm, the depth of different redox horizons varied considerably (Table 2).The oxic horizons ranged from 30

Table 2. Properties of soils tested.

Sample site	Depth (cm)			pH			Texture (%) Sand			Texture (%) Clay			C_{org} (%)		
	Oxic	Intermittent	Anoxic	Ar	Rx	Rd	Ar	Rx	Rd	Ar	Rx	Rd	Ar	Rx	Rd
L1S1	0-30	31 - 180	181-270	6.3	7.3	7.1	8.4	13.7	49.6	38.8	34.8	16.0	2.99	0.83	0.42
L1S2	0-127	128 - 180	181-280	7.2	7.3	6.8	42.1	7.7	17.9	14.6	31.7	21.9	0.59	0.90	0.29
L1S3	0-74	75 - 125	126-280	7.1	6.8	6.7	4.0	3.5	9.7	32.6	47.0	48.2	1.46	1.09	1.13
L1S4	0-95	96 - 184	185-270	7.4	7.9	7.9	11.4	34.9	49.9	30.1	15.0	16.7	1.25	0.24	0.35
L2S1	0-107	108 - 198	199-261	7.4	7.5	7.6	7.0	62.8	68.0	33.2	11.5	9.1	1.37	0.68	0.37
L2S2	0-145	146 - 195	196-255	7.4	7.5	7.6	55.1	74.6	75.0	12.9	8.0	7.5	0.70	0.31	0.72
L2S3	0-85	86 - 281	ND[†]	7.2	7.0	ND	6.9	22.3	ND	33.1	22.8	ND	1.49	0.75	ND
L2S4	0-128	129 - 188	189-277	7.5	7.7	7.7	13.7	15.5	13.8	24.4	18.6	19.0	0.75	0.26	0.31

[†]not determined

to 145 cm, with the thinnest one found in L1S1 and deepest in L2S2. In general, sample sites under unimproved pasture and hardwoods had thinner oxic layers than did cultivated sites. The intermittent horizons for L1S1 and L2S3 were significantly thicker (2 to 4-fold) than others tested. In fact, the anoxic horizon for L2S3 was not reached following sampling up to 281 cm.

pH values ranged from 6.3 to 7.5, 6.8 to 7.7, and 6.7 to 7.7 for the oxic, intermittent and anoxic layers, respectively. The two control soils had higher pH values than other soils tested (Table 2). Considerable variations in soil texture were observed. Sand contents varied from 4 to 75% and clay contents varied from 7.5 to 48%. With the exception of L1S2 and L1S3, clay contents were higher in the oxic horizons than in the intermittent and anoxic horizons tested. C_{org} contents in the oxic horizons varied from 0.6% to 3% with the highest in L1S1 and the lowest in L2S2. In general, organic C (C_{org}) contents were higher in soils sampled from the oxic horizons than those sampled from the intermittent and anoxic horizons.

Moreover, contents of C_{org} and clay in the oxic horizones were correlated with soil depth. Thinner oxic horizons (< 107cm) had relatively higher C_{org} content (>1.3%) and clay content (>30%). L1S1 had the thinnest oxic layer (30 cm), but the highest C_{org} (3%) and clay (38.8%) contents. When oxic layers exceeded 107 cm depth, their C_{org} content was less than 1% while clay content was under 25%. In the intermittent and anoxic layers, C_{org} contents in the control sites were relatively lower than those in sites that were subjected to flooding. Sites with more than 30% clay content had relatively higher C_{org} (around 1%) than others.

3.2. Heavy metal contents

Of the six metals determined, Zn had the highest concentration, ranging from 53 to 310 mg kg^{-1} soil (Fig. 2). Concentrations of Cu, Cr, and Pb were in the same range, ranging from 11 to 80 mg kg^{-1} soil. Total Ni concentrations ranged from 11 to 37 mg kg^{-1} soil, while those of Cd from 0.7 to 2.8 mg kg^{-1} soil. In general, total metal contents in soils decreased with increasing soil depth and were higher in soils sampled from sites in closer proximity to the river bed than those that were further away. Heavy metal concentrations in the upstream and downstream site were not significantly different.

Some of the tested metal contents were significantly correlated with each other. The correlation coefficients (r) between Zn and Cd, and between Zn and Pb were 0.95 and 0.96, respectively. The r values between Cd and Cu, or Cd and Pb were 0.94, 0.92, respectively. The r value between Cr and Ni was 0.98.

3.3. DH activity, culturable population, microbial biomass, and DNA content

DH activity was markedly reduced with increasing soil depth (Fig. 3). Of the soils tested, L1S1 showed the highest DH activity in all three redox horizons. With the exception of L1S1, control soils had significantly higher DH activity in the oxic horizons comparing to those sites that were subject to flooding. Differences in DH activity between different soils in similar redox state decreased with increasing soil depths. In the anoxic horizon, there was no detectable difference in DH activity in four out of seven soils tested.

The trend for culturable bacterial populations (CBP) was somewhat

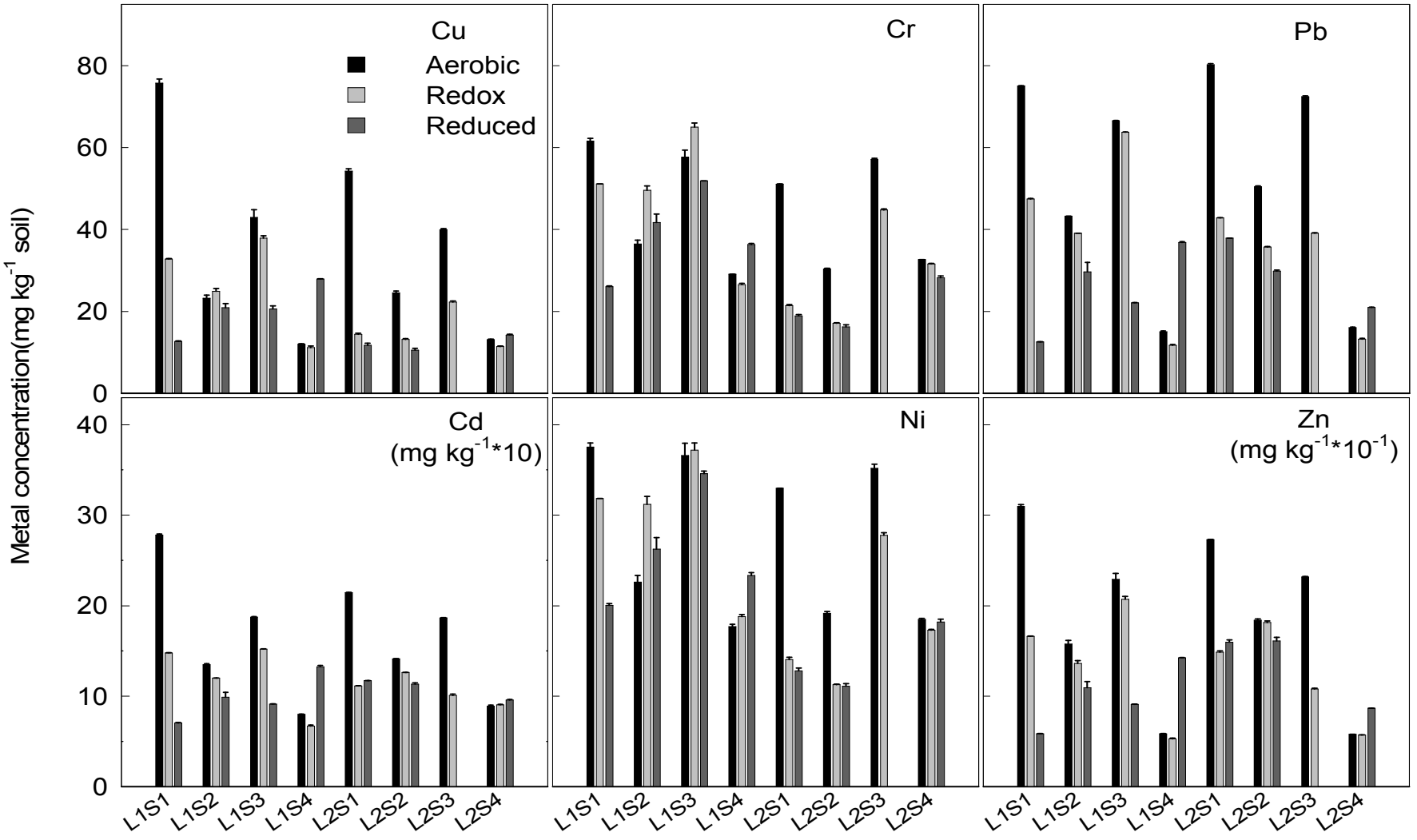

Fig. 2. Total heavy metal (Cd, Cu, Pb, Zn, Cr, and Ni) concentration in soils tested

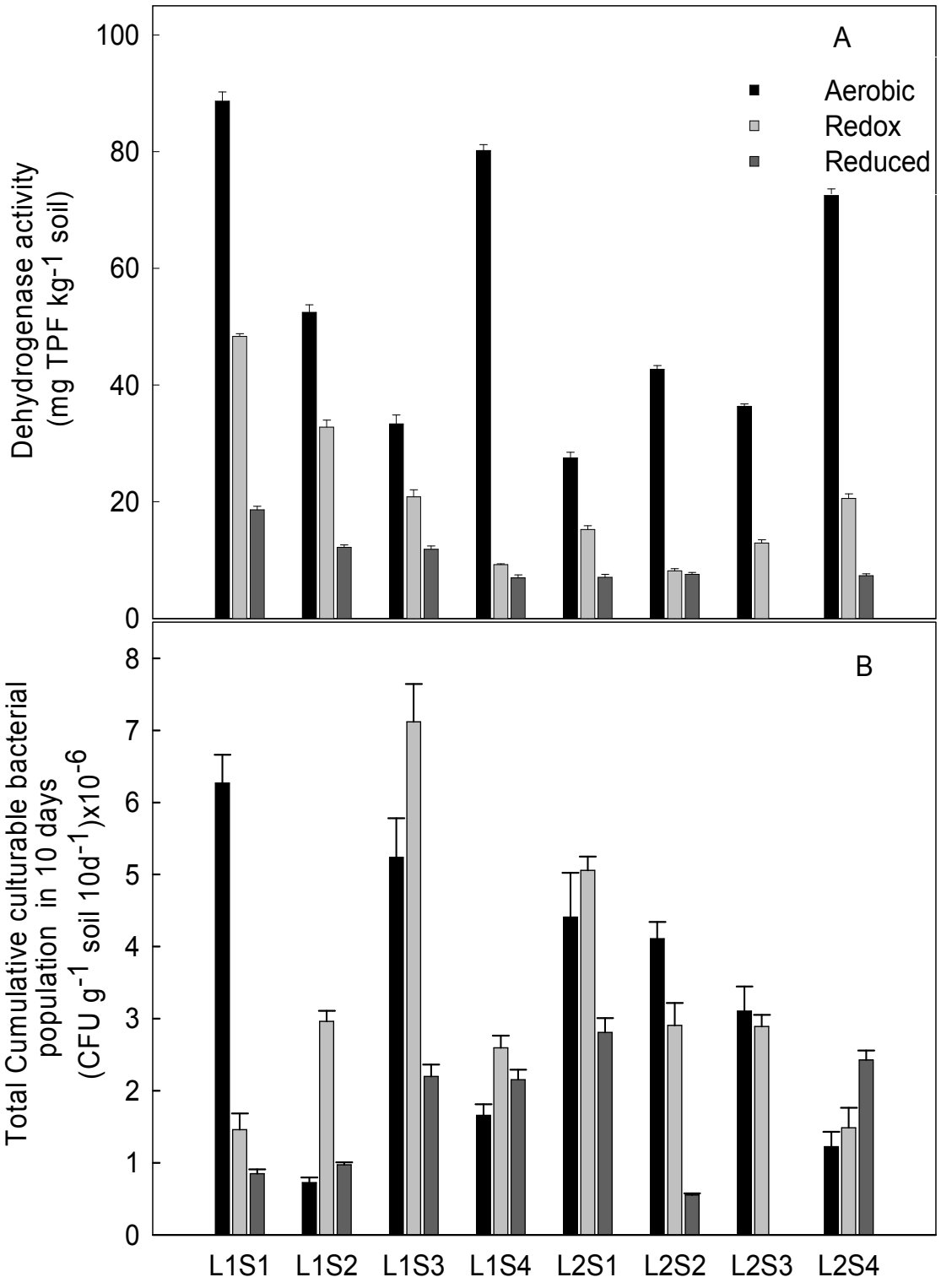

Fig. 3. Dehydrogenase activities (A) and cumulative bacteria population (B) in soils tested.

different from those found for DH (Fig. 3). In the oxic soils, the highest CBP was also found in L1S1. CBP decreased with increasing soil depth in two of the sites tested. In the remaining 6 sites, CBP in the intermittent layers were significantly higher than or similar to those found in the oxic layers within the site. In fact, CBP increased with increasing soil depth in soils sampled at L2S4. With the exception of two control sites, CBP was lower in the anoxic layers than the oxic and intermittent layers (Fig. 3). When compared among soils taken in the oxic and intermittent layers, CBP in the control soils were in the similar range or were significantly lower than those in other sites within each location. CBP in the anoxic layers of the control sites were higher or in the same range as those in the rest of sites tested.

Microbial biomass and recovered soil DNA concentration also decreased with increasing soil depth (Fig. 4). Microbial biomass C (C_{mic}), N (N_{mic}) and DNA contents were highest in the oxic layer of L1S1. Similar trends for C_{mic} and DNA contents were detected for all soils tested. However, with the exception of oxic soils, considerably lower levels of N_{mic} were detected. There was little difference in N_{mic} content between intermittent and anoxic layers. In general, decreases of microbial biomass, DNA concentration and DH activity were accompanied by decreasing C_{org} content from oxic to anoxic horizons. However, the correlations are not statistically significant ($r<0.38$, $n=23$). C_{org} and clay contents oxic and intermittent layers were highly correlated with C_{mic}, with r values ranging from 0.82 to 0.88 (data not shown).

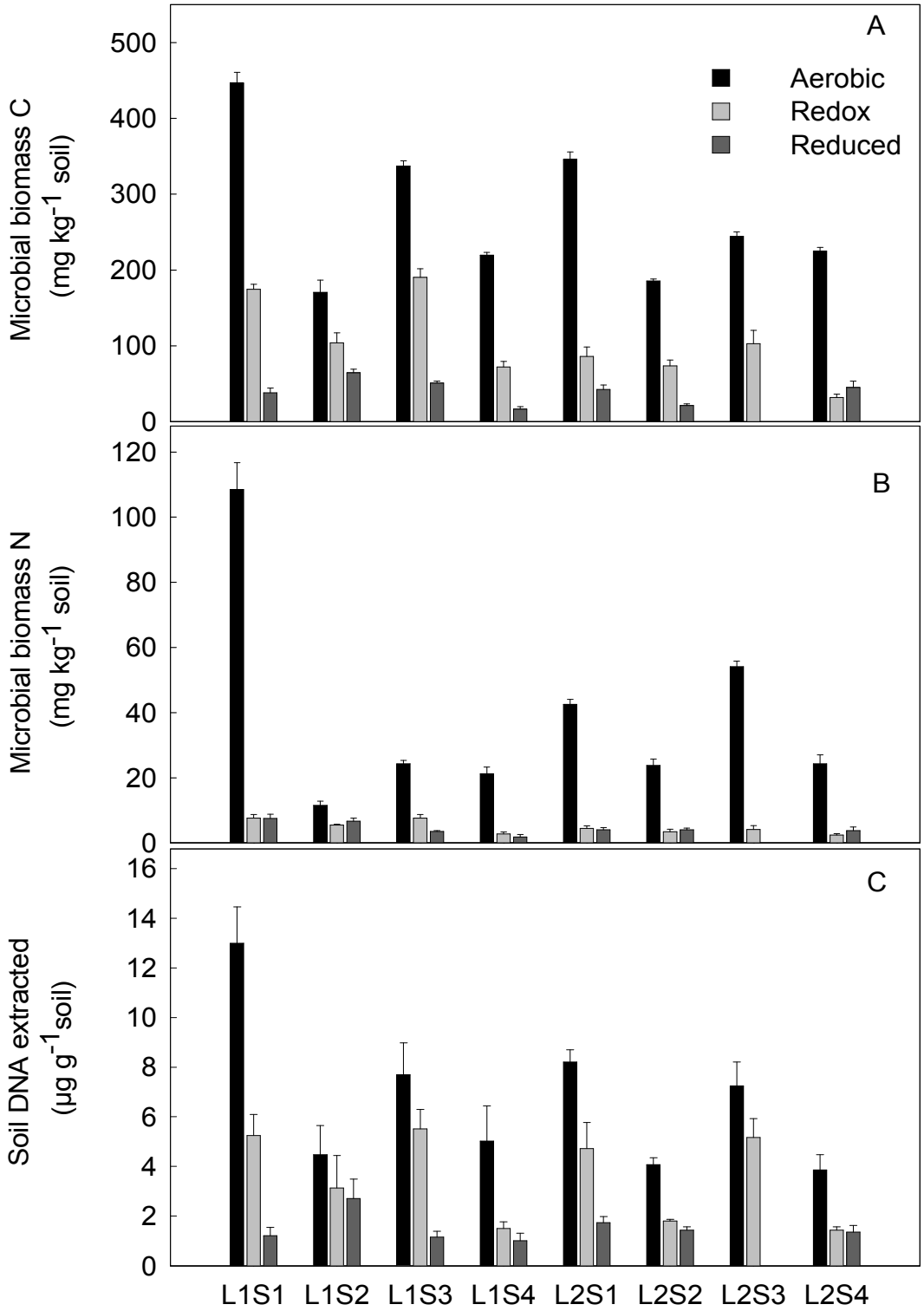

Fig. 4. Microbial biomass C and N, and total DNA concentration in soils tested. (A) Microbial biomass carbon, (B) microbial biomass nitrogen, and (C) total soil DNA concentration.

Percentage of C_{org} presented as C_{mic} ranged from 0.29% to 3.02% (Table 4). In general, these percentages decreased with increasing soil depth with the exception of L1S1 and L1S4 in which the highest values were detected in the intermittent layers. On the contrary, the ratio of DH activity to C_{mic} in intermittent or anoxic layers was not consistently lower than that in the oxic layer. In the oxic layer, DH/C_{mic} was higher in control sites when compared with those that were subjected to flooding. C_{mic}:N_{mic} ranged from 4 to 15, 13 to 25, and 5 to 12 in oxic, intermittent and anoxic layers, respectively.

3.4. Relationships between heavy metals and microbial characteristics or C_{org}

DH activity was markedly lower in proportion to increasing Cu concentration in the oxic horizon ($r>-0.9$, $P<0.001$). Microbial biomass C and N and the amount of DNA extracted were also significantly correlated with Cu concentrations ($r>0.9$, $P<0.001$). Correlations between total Cu and culturable bacterial population were 0.88 ($P\leq0.01$). Little relationship was observed for microbial activity or population and metal concentrations in soils under anoxic condition. For soils from the intermittent layer, some significant correlations between metal concentrations and microbial activity and population were observed. Cd concentrations were also significantly correlated to soil microbial activity and population ($r>0.9$*** for Cd and DH; $r=0.8$-0.9** for Cd and C_{mic} or N_{mic}.

In general, there were significant correlations in the oxic layers between heavy metal concentrations and the microbial characteristics tested (Table 5).

Table 3. Percentage of microbial biomass carbon (C_{mic}) in organic carbon (C_{org}), dehydrogenase activity per unit of microbial biomass carbon (DH/C_{mic}), and C_{mic} to N_{mic} ratios.

Sample site	C_{mic}/C_{org}				DH / C_{mic}				C_{mic}/N_{mic}			
	%				mg TPF $mg^{-1}C$							
	Oxic	Intermittent	Anoxic	LSD	Oxic	Intermittent	Anoxic	LSD	Oxic	Intermittent	Anoxic	LSD
L1S1	1.49	2.11	0.90	0.20	0.20	0.28	0.46	0.07	4	21	5	2
L1S2	2.89	1.15	2.23	0.45	0.31	0.32	0.19	0.09	15	19	10	3
L1S3	2.30	1.74	0.45	0.44	0.10	0.11	0.23	0.11	14	25	15	2
L1S4	1.76	3.01	0.47	0.12	0.36	0.13	0.42	0.05	10	26	9	5
L2S1	2.53	1.27	1.14	0.33	0.08	0.18	0.16	0.04	8	19	11	3
L2S2	2.65	2.39	0.29	0.37	0.23	0.11	0.35	0.03	8	22	5	4
L2S3	1.64	1.38	nd†	0.36	0.15	0.12	nd	0.03	5	25	nd	6
L2S4	3.02	1.23	1.45	0.47	0.32	0.67	0.14	0.12	9	13	12	3
LSD	0.23	0.41	0.31		0.03	0.08	0.08		2	5	3	

† not determined

Table 4. Correlation coefficient (r) between metal concentration and dehydrogenase activity (DH), microbial biomass (C_{mic}, N_{mic}), culturable bacterial population (CBP), DNA concentration, DH/C_{mic} ratio, or C_{org}.[†]

	Redox states	Cd		Cr		Cu		Ni		Pb		Zn	
DH	Oxic	- 0.97	***	- 0.77	*	- 0.93	***	- 0.82	**	- 0.98	***	- 0.99	***
	Intermittent	0.50		0.61		0.61		0.60		0.33		0.24	
	Anoxic	- 0.39		0.08		0.16		- 0.01		- 0.55		- 0.53	
C_{mic}	Oxic	0.89	**	0.85	**	0.94	***	0.84	**	0.71	*	0.79	*
	Intermittent	0.73	*	0.19		0.49		0.17		0.52		0.65	
	Anoxic	- 0.36		- 0.10		- 0.28		0.00		- 0.31		- 0.35	
N_{mic}	Oxic	0.82	**	0.70	*	0.85	**	0.66		0.57		0.69	*
	Intermittent	0.88	**	0.80	**	0.95	***	0.81	**	0.87	**	0.75	*
	Anoxic	- 0.58		- 0.06		- 0.22		- 0.01		- 0.36		- 0.30	
CBP	Oxic	0.87	**	0.67	*	0.88	**	0.68	*	0.77	*	0.85	**
	Intermittent	0.26		0.04		0.20		0.06		0.54		0.45	
	Anoxic	0.20		0.19		0.26		0.15		0.10		- 0.10	
DNA	Oxic	0.92	***	0.85	**	0.95	***	0.84	**	0.72	*	0.81	**
	Intermittent	0.65		0.64		0.77	*	0.65		0.85	**	0.6	
	Anoxic	0.13		0.07		0.10		- 0.03		0.40		0.22	
DH/C_{mic}	Oxic	- 0.74	*	- 0.79	**	- 0.71	*	- 0.83	**	- 0.91	***	- 0.83	**
	Intermittent	- 0.22		- 0.01		- 0.22		- 0.10		- 0.46		- 0.48	
	Anoxic	- 0.40		- 0.20		- 0.09		- 0.09		- 0.49		- 0.28	
C_{org}	Oxic	0.80	**	0.75	*	0.85	**	0.73	*	0.55		0.66	
	Intermittent	0.73	*	0.84	**	0.90	***	0.84	**	0.88	**	0.66	
	Anoxic	0.01		0.23		0.44		0.16		0.24		0.1	

[†] *,**,*** indicate significant at $p<0.05$, 0.01,0.001, respectively. n=8 for soils sampled from the oxic and intermittent layers; and n=7 for soil sampled from the anoxic layers.

DH activity was negatively, while other tested microbial parameters were positively correlated with different heavy metal concentrations. Correlations between DH activity and concentrations of Cd, Cu, Zn and Pb were greater than 0.93 at $p<0.001$, while those between DH activity and concentrations of Ni and Cr were 0.82 at $p<0.01$ and 0.77 at $p<0.05$ for Ni and Cr, respectively. Correlations between C_{mic} and Cu concentrations were 0.94***, followed by Cd, Cr, and Ni at 0.89**, 0.85**, and 0.84**, respectively. Correlations between C_{mic} and concentrations of Pb and Zn were 0.71* and 0.79*, respectively. Correlations between N_{mic} and total metal concentrations were in the order of Cu>Cd>Cr>Zn (Table 3). Correlation sensitivity orders for the amount of DNA and concentration of metals were similar to those between C_{mic} or N_{mic} and metal concentrations. However, the trend between CBP and metal concentrations were somewhat different, shown as Cu>Cd>Pb>Zn>Ni>Cr. was observed for relationships between metal and DNA concentrations. Of the six metals tested, Cu and Cd showed relatively higher correlations with all microbial properties evaluated ($r>0.82$).

DH/C_{mic} was negatively correlated with Pb ($r=0.91$***, $p<0.001$), followed by Zn and Ni ($r=0.83$**, $p<0.01$) and was least correlated with Cd, Cr and Cu ($r>0.71$*, $p<0.05$). On the contrary, C_{org} was positively correlated with Cu and Cd ($r>0.82$**, $p<0.01$), followed by Cr and Ni content ($r<0.73$**, $p<0.01$). In the intermittent layer, N_{mic} and C_{org} were both positively correlated with Cu ($r>0.90$***, $p<0.001$), followed by Cr, Ni and Pb ($r<0.88$**, $p<0.01$).

4. Discussion

4.1. Relationships between total heavy metal concentration and microbial community.

Of the microbial parameters determined, DH was the most sensitive indicator of metal stress in the environment, and was negatively correlated with concentrations of metals tested. On the other hand, microbial biomass, DNA content, and culturable bacterial population increased with increasing heavy metal concentrations in the oxic layer. Dai et al. (2004) also reported positive correlations between microbial biomass and concentrations of Cd, Cu, Pb, and Zn). The positive correlations may be due to relatively low metal concentrations in these environments. Concentrations of Cd, Cu, Ni, Pb, and Zn reported in other literatures ranged from 0.3-10, 23-690, 8-72, 128-1543, and 91-6488 mg kg^{-1} soil (Flieβbach, 1994; Chander and Brooks, 1993, Barajas-Aceves et al., 1999). In these studies, negative correlations between microbial biomass and metal concentrations were observed. Unlike DH activity, microbial biomass and DNA contents provide measures of not only the active portion of microbial community, but also the dormant and dead cells. It is, therefore, not surprising that DH activity was the most sensitive microbial parameter in response to metal stress.

DH activity plays an important role in the initial stages of oxidizing organic matter by transferring hydrogen and electron from substrates to acceptors (Ross, 1971). Inhibition of DH activity by metal stress could lead to reduced rates of organic matter degradation, which would result in C_{org} accumulation. This, in part,

was supported by positive relationships between heavy metal concentrations and soil C_{org} content (Valsecchi et al., 1995; Dai et al., 2004).

Of the metals tested, Cd and Cu exhibited a relatively greater effect on the tested properties of the microbial community. Dai et al. (2004) and Hassen et al. (1998) also showed relatively higher toxic effects of Cd and Cu on N mineralization when compared with several other heavy metals tested. The relatively greater effects of Cd are not surprising because Cd carries no known biological function and is relatively mobile in the environment. However, Cu is an essential element for living organisms, required for the functioning of more than 30 enzymes (Weser et al., 1979). Cu concentrations in these soils must have exceeded the trace concentration required for microbiological functions and led to toxic and inhibitory effects on the microbial community. Moreover, the presence and interactions of multiple metals could lead to additive effects to the microbial community (Chander and Brookes, 1993; Chander et al., 1995; Khan and Scullion, 1999).

Samples from L1S1 behaved somewhat differently from others tested. DH activity, microbial biomass, DNA concentrations, and culturable bacterial population in soils sampled from L1S1 were similar to, or significantly higher than, those from other sites. However, its heavy metal concentration was also highest among samples tested. Soils from oxic layer of L1S1 contained relatively high moisture content and this site had a shallow oxic layer (31 cm). It has been reported that high water content affected the soil oxidation-reduction status that led to an increase in DH activity (Brzezińska et al., 1998). In addition, relatively

high C_{org} in soils from L1S1 could also contribute to establishing relatively high C_{mic} and CBP in the oxic layer.

4.2. Responses of the microbial community to redox states along a soil profile

Bioavailability of metals varies with redox states. Lower redox potential often leads to precipitation of heavy metals, resulting in reduced toxicity. Other soil properties, including high pH, organic matter, and clay content, also affect bioavailability of heavy metals (Babich and Stotzky, 1980). Little correlation was found between metal concentrations and microbial activity or populations in the anoxic layers. Since organic C could protect microbial mediated processes by reducing metal toxicity through chelation or complex reactions, it was also expected that metal toxicity would increase along soil profile as C_{org} content decreased with increasing soil depth. However, this was not observed in this study, suggesting that C_{org} was not a dominant factor that regulated metal toxicity in these environments.

Organic matter contents in soils that were sampled from the oxic layers were affected by the thickness of the oxic layers and the vegetation of the fields. Relatively higher C_{org} contents were detected in soils that were sampled from thinner oxic layers and pasture field when compared with other soils tested. This is because 80% of C_{org} accumulates in top 25 cm soil (Fierer et al., 2003), and soils under perennial pastures often containe significantly higher soil C_{org} when compared with those under continuous cotton (Acosta-Martínez, 2004). C_{org} content in the oxic layers was significantly correlated with microbial biomass ($r=0.84^{**}$, $r=0.87^{**}$, $p<0.01$, data not shown). Moreover, the clay content also

showed significant correlation with C_{mic} ($r=0.82$, $p<0.01$, data was not shown). Therefore, the observed increase of microbial biomass with increasing metal concentrations in the oxic layer might not indicate a positive impact of heavy metals on the microbial community, but could be related to C_{org} and clay content in the oxic layer. Significant correlations between C_{org} and C_{mic} were also reported in other studies (Taylor et al., 2002; Fierer et al., 2003). Contents of C_{org} and C_{mic} in the intermittent and anoxic layers were not significantly correlated, indicating that C might not be the most limiting factor in these environments. Under oxygen limiting conditions, C use efficiency was reduced significantly because more substrate was diverted towards catabolic processes leading to reduced microbial biomass in an anabolic process (Chander and Brookes, 1991; Lavahun et al., 1996). This was evidenced by significantly reduced C_{mic}:C_{org} ratios.

Although microbial biomass and activity decreased with increasing soils depth, specific DH activity per unit of microbial biomass in five out of eight soils was higher in the anoxic layers when compared with those in the oxic layers. This result might indicate that C limited environment selected proliferation of more active microbes, as reported by other studies (Tate, 1979; Blume et al., 2002). This was also evidenced by significantly higher culturable bacterial population in the intermittent layers than that in the oxic layer. Studies by Fierer et al. (2003) also showed that abundance of gram-positive bacteria increased with increasing soil depth. The higher C_{mic}:N_{mic} (>10) in the intermittent layers than other redox states suggested that there were considerable changes in

composition of microbial communities due to changes in redox states in the environment. Although it is not clear what specific groups of microbes proliferated in the intermittent oxic-reducing conditions, it demonstrated the complexity of the microbial community in the environment.

Conclusion

Of the microbial parameters tested, DH activity, which decreased with increasing metal concentrations in the soil, was the most sensitive indicator of metal contamination and environmental stress. However, the relatively low metal concentrations in these soils did not measurably change microbial biomass, DNA content, or culturable bacterial population. Microbial activity and abundance along the soil profile was correlated with C_{org} content and oxygen availability. When compared with soils from other redox states, soils from intermittent layers showed relative higher culturable bacterial population and higher C_{mic}:N_{mic} ratios.

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Chapter IV

CHARACTERIZATION OF DEPTH- AND REDOX-RELATED MICROBIAL COMMUNITY STRUCTURE OF A RIVER FLOODPLAIN CONTINUUM USING a 16S rRNA-BASED APPROACHE AND FATTY ACID METHYL ESTER (FAME) ANALYSIS

ABSTRACT

Substrate availability and redox states in an ecosystem play crucial roles in regulating microbial abundance and community structure. We evaluated changes of microbial community under three redox conditions in a river floodplain continuum that was subject to seasonal flooding and metal contamination. Soils were collected from four sites at each of two locations along Tisza River in Hungary. Each soil profile was divided into three redox horizons: oxic, intermittent, and anoxic. Microbial communities were evaluated using 16S rRNA-based terminal-restriction fragment length polymorphism (T-RFLP) and fatty acid methyl ester (FAME) analysis. Abundance of Gram-positive bacteria increased with increasing depth and shifting from oxic to anoxic conditions. Gram-negative bacteria, fungi and actinomyces were more abundant in the oxic layers than in the intermittent and anoxic layers. Protozoa were detected only in the oxic layer. Despite

wide variation in vegetation between sampling sites, bacterial communities in the oxic layers shared >50% similarity among sites. Considerable variation was shown among bacterial communities in the intermittent or anoxic layers. Bacterial richness (total numbers of T-RFLP bands) decreased with increasing soil depth. Bacterial communities were distributed more evenly in the oxic layers than the anoxic layers. The loss of diversity with increasing soil depth was due primarily to variation in redox states. Under oxygen limiting conditions, bacterial community structure was regulated by other stress factors such as organic carbon and water content.

INTRODUCTION

As the land adjacent to a waterway or drain that is prone and subject to periodical flooding, a floodplain serves as the transformer or sink of nutrients and biota in the river environment through hydrological connectivity, via surface and groundwater inflow (53). Erosion and sedimentation processes also lead to remobilization of heavy metals, pesticides, and other potential agricultural and environmental contaminants (38, 55). Such processes can have adverse effects on water quality, aquatic habitats, and the agricultural use of floodplain areas.

Much of the associated effects are related to the microbial communities inhabiting that environment. As an important factor controlling substrate availability and energy transformation, redox states play a crucial role in regulating microbial activity and community structure (36). With the development

of microhabitats from the oxic surface layers to anoxic subsurface zones, microbial communities stratify along the soil profile. Although numerous studies have been conducted to evaluate microbial communities in surface soils or sediments (2, 52, 54), or in deep subsurface soils that hundreds of meters below the surface (4, 9, 10, 12), few have been directed to characterize microbial communities in the transitional redox zones that are several meters below the surface. Since a large part of soil profiles is influenced by alternating oxic and anoxic conditions, it is not possible to infer subsurface community structure from studying surface layer. Therefore, understanding microbial communities in the subsurface habitats require targeted investigation.

Past research effort has been focused on evaluation of microbial community in sediments (22, 25, 30, 44); little study demonstrated depth- and redox-related changes of whole microbial communities in a floodplain.

As sensitive and powerful tools for characterizing complex microbial community structure, culture-independent methods had been widely adapted to evaluate microbial community structure in soil (5, 8, 30). Previous studies have reported that the fatty acid methyl ester (FAME) profiles are affected by heavy metal contamination (20, 26, 27, 59). Within the soil FAME profiles, certain marker FAMEs of bacterial (i.e., *a*15:0, *i*15:0, *a*17:0, and *i*17:0), fungal (i.e., 18:1ω9c, 18:2 ω6c, 18:3 ω6c and 16:1 ω5c), and/or protozoan groups (i.e., 20:4 ω6c) have been used to provide insight into the microbial community composition (1, 41, 48). More recently, 16S rRNA gene-base technique, such as terminal-restriction fragment length polymorphism (T-RFLP), has been developed to

reveal phylogenetic diversity of a microbial community. T-RFLP analysis is highly reproducible and could quantify the community diversity by analysis of fragments of precise size (40). Richness and evenness are two essential parameters for defining community structure through analyzing T-RFLP fingerprint. The richness and evenness of biological communities reflect selective pressures from environment on shaping diversity within communities. Measuring these parameters is useful when assessing treatment effects (e.g., pollution, nutrient addition, physical disturbance) on community diversity. Diversity analysis can also indicate the stability of a community (11).

The major goal in this study was to assess depth-related microbial community structure in a floodplain continuum by using FAME analysis and T-RFLP fingerprinting.

MATERIALS AND METHODS

Soils

Soil samples were taken from two locations along Tisza River in October 2003 (Fig. 1. Chapter III). At each location, four sampling sites were identified to reflect different structural components of a floodplain continuum. Site 4 at each location was farther away from the river and served as a control. These two sites have not been flooded for over 100 years. At each sampling site, soils were composited to three samples that corresponded to oxic, seasonally flooded (intermittent), and permanently saturated (anoxic) horizons.

Field-moist soil samples were stored and transported on ice. Following passing through a 2 mm sieve, the samples were mixed thoroughly and stored in sealed plastic bags at 4^{o}C. A portion of each sample was freeze-dried and kept in sealed containers.

The site description and selected characteristic of samples in three redox layers are shown in chapter III. Briefly, soil pH ranged from 6.3 to 7.5, 6.8 to 7.9, and 6.7 to 7.9 in the oxic, intermittent and anoxic layers, respectively. Organic C ranged from 0.59 to 2.99, 0.24 to 1.09, and 0.29 to 1.13 in the oxic, intermittent and anoxic layers.

FAME analysis

Fatty acids were extracted from the soils using the procedure described for pure culture isolates by the Microbial Identification System (MIS, Microbial ID, Inc., Newark, DE) as previously applied for soil analyses (1, 8, 24). Briefly, the method consists of four steps: (1) saponification of fatty acids by treating the soil (3 g of <2 mm freeze dried soil) with 3 ml 3.75 M NaOH (methanol: water, 1:1) solution at 100^{o}C for 30 min; (2) methylation of fatty acids by treating the samples with 6 ml of 6 M HCl in aqueous methanol (1:0.85) at 80^{o}C for 10 minutes; (3) extraction of fatty acids methyl esters (FAMEs) with 3 ml of 1:1 hexane: methyl-*tert* butyl-ether solution and rotating the samples end-over-end for 10 min, and (4) washing of the organic phases with 3 ml of 1.2 % diluted NaOH by rotating the tubes end-over-end for 5 min. The organic phase (top phase), containing FAMEs, was analyzed in a 5890 Gas Chromatograph series II (Hewlett Packard, Wilmington, Del.). Peaks in a sample were compared to standard fatty acids

(Microbial ID, Newark, Del.) and their relative peak areas (percentages over total detected areas) were determined with respect to other fatty acids in a sample using the MIS Aerobe method of the MIDI system (Microbial ID, Inc., Newark, DE).

FAMEs are described by the number of C atoms, followed by the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules. Isomers *cis* and *trans* are indicated by c and t, respectively. Branched fatty acids are indicated by the prefixes *i* and *a,* for *iso* and *anteiso*, respectively. Other abbreviated notations are *Me* for methyl and *cy* for cyclopropane.

Within the FAME profiles obtained, individual FAME markers were investigated to compare the relative abundance of specific microbial groups. Information of the relative abundance of Gram-positive (G^+) bacterial populations in soils was obtained from FAME markers such as *i*15:0, *a*15:0, 15:0, *i*16:0, *i*17:0, and *a*17:0; whereas information about the abundance of Gram-negative(G^-) bacterial populations was obtained from 18:1ω7c, 18:1 ω9c, and cy17:0 (55, 58, 59). Information about actinomycete (Actino.) abundance was obtained from 10Me16:0, 10Me17:0, and 10Me18:0 (59), and the FAME markers (20:4ω6c) was used for the protozoan abundance (55). Similarly, the relative abundance of fungal populations was obtained using 18:3ω6c, 18:2ω6c, and 16:1ω5c (20, 58). Total saturated/total monounsaturated fatty acid ratios (12:0+13:0+14:0+15:0+ 16:0+17:0+18:0+20:0)/(14:1ω5c+15:1ω6c+16:1ω7c+16:1ω5c+17:1ω9c+18:1ω9

c+18:1ω7c) were calculated to evaluate the nutritional stress in bacterial communities as proposed by several other studies (23, 28).

DNA extraction and PCR amplification

Soil DNA was extracted using an UltraClean™ Soil DNA kit (MoBio Laboratories, Inc., Solana Beach, CA, USA). Prior to PCR amplification, DNA concentration and purity were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware, USA).

Two PCR primers, BF8 and 926R (BF8 5'-AGAGTTTGATCCTGGCTCAG-3' and 926R 5'-CCGTCAATTCCTTTRAGTTT-3') were used to amplify an internal region (927 bp) of 16S rRNA genes in the Bacterial Domain (34). BF8 was fluorescently labeled with IRDye™ 700 at the 5' end. Each 100 µl PCR mixture contained 25 ng of soil DNA extract, 0.5 µM of each primers, 500 µM of PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific Inc., Pittsburgh, PA, USA), 1.75 mM $MgCl_2$, 1.5 µg bovine serum albumin and 2.5 Units Taq DNA polymerase in PCR buffer A (Promega Chemicals, Madison, WI, USA). PCR reactions were conducted at 94°C for 2 mins, followed by 94°C for 1 min, 52°C for 1 min and an extension at 72°C for 2 mins for 31 cycles and a final extension at 72°C for 10 mins.

T-RFLP analysis

Fluorescently labeled PCR products were digested by five restriction enzymes, *Alu*I, *Hae*III, *Hha*I, *Hpa*II, and *Rsa*I. Each 20 µl digestion mixture

contained 10 U of enzyme and the recommended buffer. Samples were digested at 37^{o}C for 5 h.

Digested products were denatured by heating at 95°C for 5 min and then immediately quenched on ice. Aliquots (1.0 μl) were loaded onto a 6.5% denaturing polyacrylamide gel and DNA fragments were separated by electrophoresis for up to 5 h at 2,500 V and 40 mA. The sizes of the terminal restriction fragments (TRFs) were estimated by reference to internal standards of 50-700bp.

Banding patterns of three replicated experiments were analyzed by GeneProfiler database manager with the TreeCon software (GeneProfiler Version 4.01, Scanalytics Inc., Fairfax VA). The lower limit for band detection was set by the band peak height threshold of 0.1% of the total detected optical density in the lane. Each sample was scored based on the presence or absence of each band in its profile compared with the profile of each of the other samples. The total numbers of bands were determined. Shannon diversity indices (H') (51) were calculated to reflect species richness and evenness of the bacterial community using the following equation:

$$H' = -\Sigma(\%IntOD \times \log(\%IntOD))$$

Where %IntOD represented the integrated density of a band, relative to the sum of all the other bands in a lane (GeneProfiler Version 4.01, Scanalytics Inc. Fairfax VA).

Species evenness of the bacterial community was also indicated by the Pielou's diversity (J) (25), which were calculated using the following equation:

J=H' x log (total number of bands in a lane)

Similarity among the microbial communities was assessed by pair-wise matching, in which matching bands were identified by comparing two samples at a time. The band sharing coefficient (S) was used as a measure of similarity between two treatments and was determined from the following equation:

S=2Ns/NT

where Ns is the number of shared bands in samples A and B, NT is the total number of bands in sample A and B. Relatedness of bacterial communities was evaluated by determining similarity coefficients for bands common to two samples. Two bands were common if they migrated the same distance on a gel.

Based on the number, intensity values, and positions of detected bands, cluster analyses and statistical analyses were made. Dendrograms were constructed from "0/1"string data using Gene Profiler 4.01 software (Scanalytics Inc., Fairfax VA). Bands were marked as matching if the molecular weight (WM) of one of the bands was within the upper and lower MW limits of the other bands. The upper and lower MW limits of a band were determined by multiplying its MW by 1±match tolerance. The dendrograms were created with 100 iterations, with bootstrap values providing a confidence level for each branch point. The higher the bootstrap value, the more reliable is the measurement of the branch point value.

Statistic analysis

Significant differences among treatments were determined using one-way analysis of variance (ANOVA). Comparison of treatment means was done using the least significant difference (LSD) test. Indicators FAMEs of fungal (18:1ω9c, 18:2ω6c, 16:1ω5c and 18:3ω6c) and bacterial (Gm^+: *a*15:0, *i*15:0, *i*17:0; Gm-: cy17:0, cy19:0; actinomycetes: 10Me16:0, 10Me17:0) groups were evaluated with the PC-ORD statistical software (version 4) to determine differences in the microbial community composition of the soil redox layers studied (39). The data obtained was examined using Principal Component Analyses (PCA). This type of PCA can indicate higher relative abundance of major microbial groups when soil samples are grouped close to vector lines representing FAMEs indicators of fungal (18:1ω9c, 18:2 ω6c, and 18:3 ω6c) populations, bacterial (Gm^+: 15:0, *a*15:0, i15:0, *a*17:0, *i*17:0; Gm-: cy17:0, cy19:0; actinomycete: 10Me16:0, 10Me17:0) populations, or the F:B (fungal :bacteria) ratios, respectively.

RESULTS

FAME analysis.

Among FAMEs possibly named with the MIDI method, 90 FAMEs were extracted from soils tested. Of FAMEs extracted, 25 were present in most of the soils, which accounted for approximately 66.3% of the total peak area in the FAME profiles.

Using individual FAMEs as biomarkers, the relative abundance of specific microbial groups was determined (Table 1). The protozoa (20:4 ω6c) were

TABLE 1. Percentage of each fatty acids methyl esters (FAME) in total detected FAMEs in soils tested.

FAME indicator	Oxic	Intermittent	anoxic
	---------- % ----------		
Protozoa			
20:4ω6c	0.79±0.19 a	0.00 b	0.00 b
Fungi			
18:3ω6c	1.09±0.36 a	1.98±1.31 a	1.58±0.97 a
18:2ω6c	4.21±0.90 a	2.87±1.70 b	1.70±0.52 c
16:1ω5c	11.60±4.37 a	6.03±3.80 b	2.61±0.79 b
G^+ bacteria			
i 15:0	4.02±0.65 a	4.31±0.53 a	4.75±1.29 a
a 15:0	3.18±0.55 b	3.98±0.27 a	4.62±0.91 a
15:0	0.76±0.12 b	1.03±0.26 ab	1.28±0.44 a
i 17:0	0.96±0.28 b	1.02±0.51 b	1.91±0.24 a
a 17:0	0.95±0.25 b	1.36±0.21 a	1.56±0.16 a
i 16:0	2.23±0.72 a	2.13±0.30 a	2.11±0.70 a
G^- bacteria			
18:1 ω7c	4.09±1.78 a	2.65±0.52 ab	1.63±0.45 b
cy 17:0	0.95±0.26 a	0.62±0.52 a	0.00 b
18:1 ω9c	7.98±2.68 a	5.80±1.25 b	3.65±0.78 b
Actinomycetes			
10Me17:0	0.50±0.11 a	0.27±0.20 b	0.00 c
10Me18:0	0.40±0.20 a	0.21±0.30 ab	0.00 b
10Me16:0	2.94±0.61 a	2.89±0.89 a	2.36±1.65 a

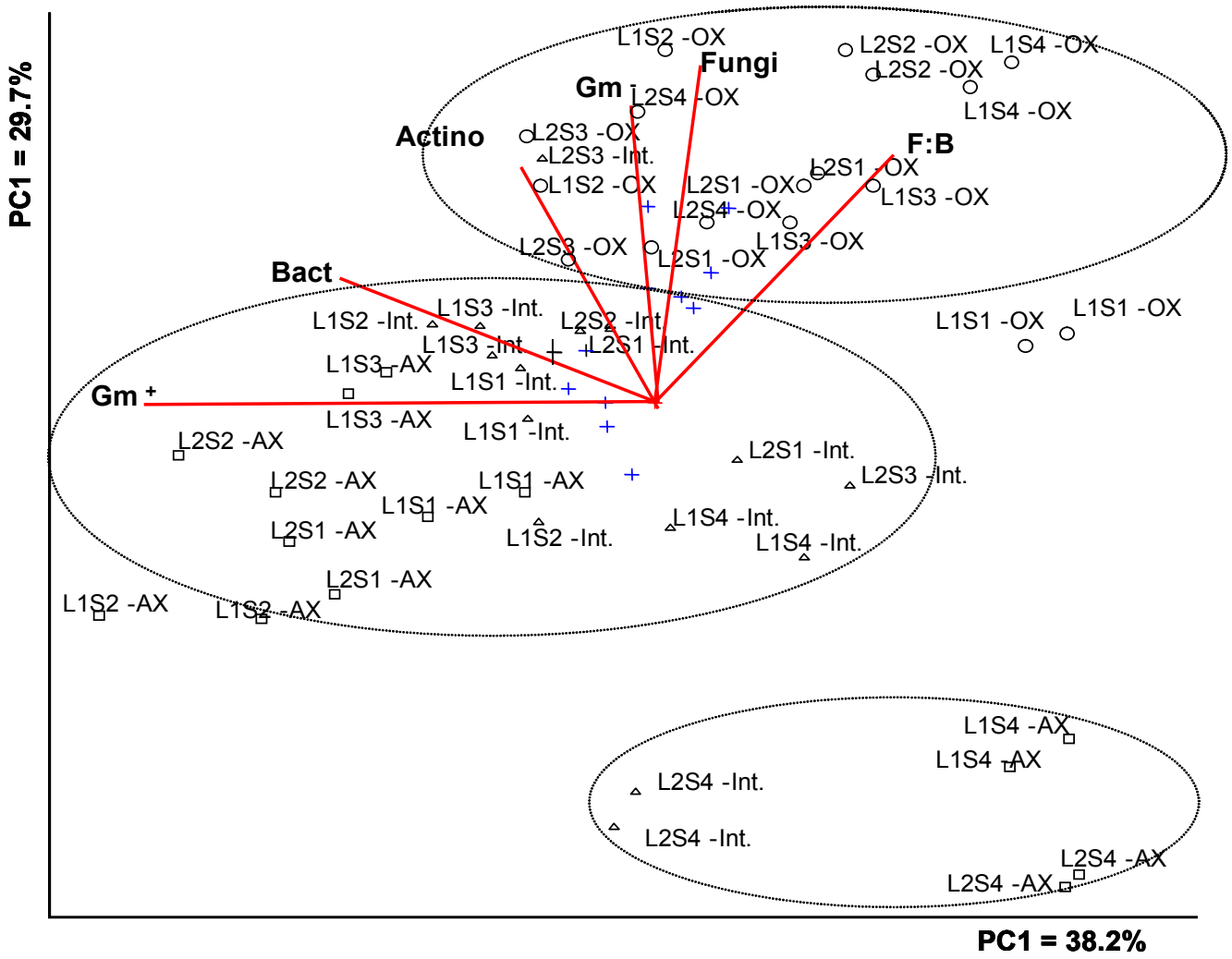

FIG. 1. Principal component analyses (PCA) of fatty acid methyl ester (FAME) for soils from three redox layers. (OX-oxic; Int.- intermittent; AX-anoxic)

detected only in the oxic layer. The relative abundance of fungal FAMEs (18:2 ω6c, and 16:1 ω5c) was higher in oxic layers when compared with those in anoxic layers. Gram+ bacteria were less abundant in the oxic layer compared to the intermittent and anoxic layers. Actinomyctes were relatively abundant in oxic layer when compared with anoxic layer. Ratios of total saturated/total monounsaturated fatty acids were 0.94 ±0.98, 1.37 ±0.38, and 3.93 ±0.48 (data not shown) for oxic, intermittent and anoxic layers, respectively.

Principal Component Analysis (PCA) of the FAME was used to reveal differences between microbial communities in different redox layers. The first principal component (PC1) explained 38.2% of the variance while the second (PC2) explained 29.7% for a total of 67.9 % of the variability in the data being explained. The result showed three distinct groups of microbial communities that were separated according to the redox state of the soils (Fig. 1). Greater abundance of fungi, actinomycetes, and G^- bacterial populations were found in microbial communities from the oxic layers when compared with those from the intermittent and anoxic layers. The microbial communities from the intermittent and anoxic layers were clustered together as a group and show higher G^+ bacterial population when compared with those from the oxic layers. The microbial communities in the control soils of different redox states grouped together, and were distinctively different from those in soils under the influence of flooding.

T-RFLP analysis of bacterial community

Among the T-RFLP fingerprints obtained, terminal fragments (TRF) in the

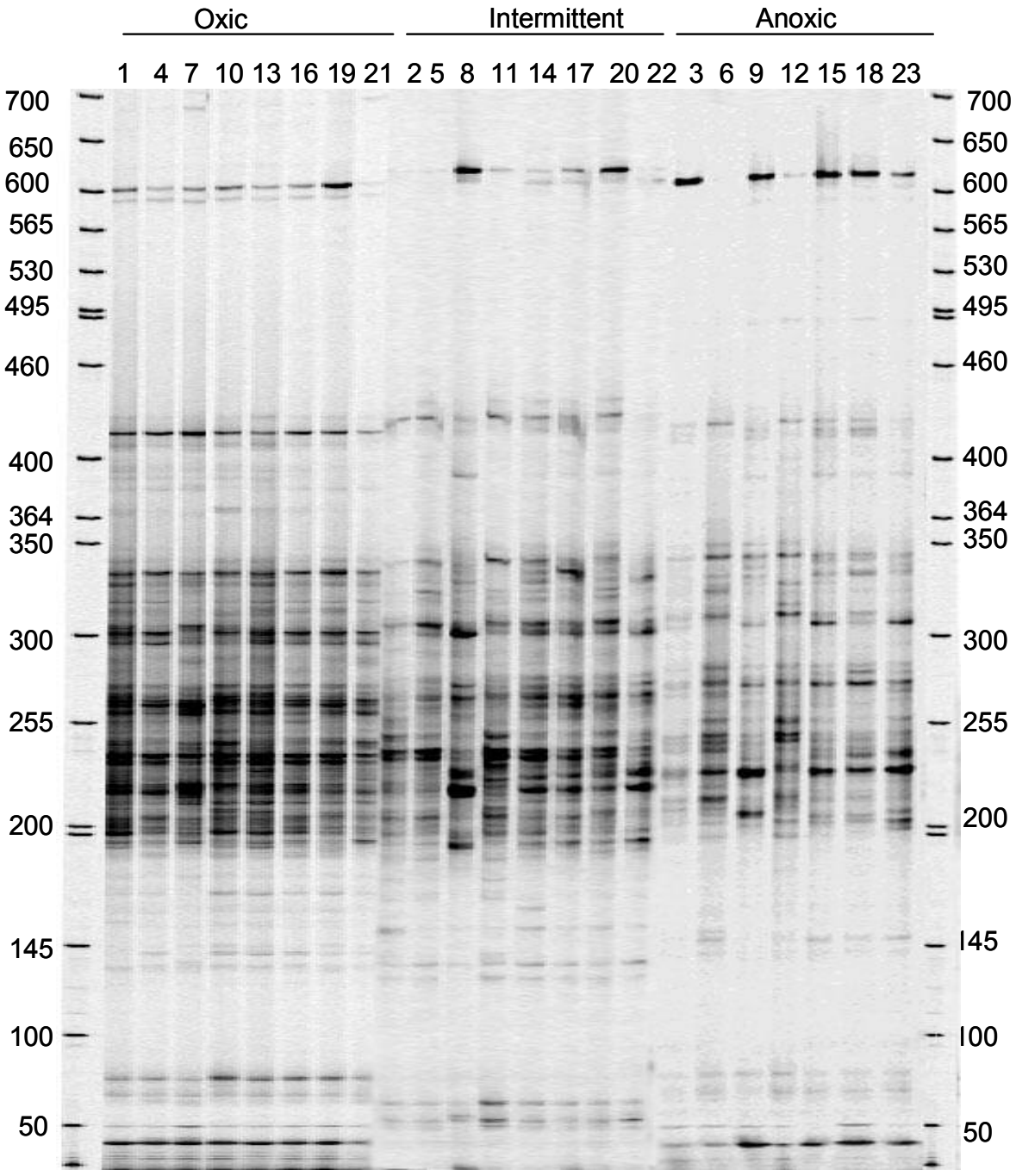

FIG. 2. 16S rRNA gene fingerprints of bacterial communities by PCR amplification, *Hae*III digestion, and T-RFLP separation. For each redox layer, samples are in the order of L1S1, L1S2, L1S4, L1S3, L2S1, L2S2, L2S3, and L2S4.

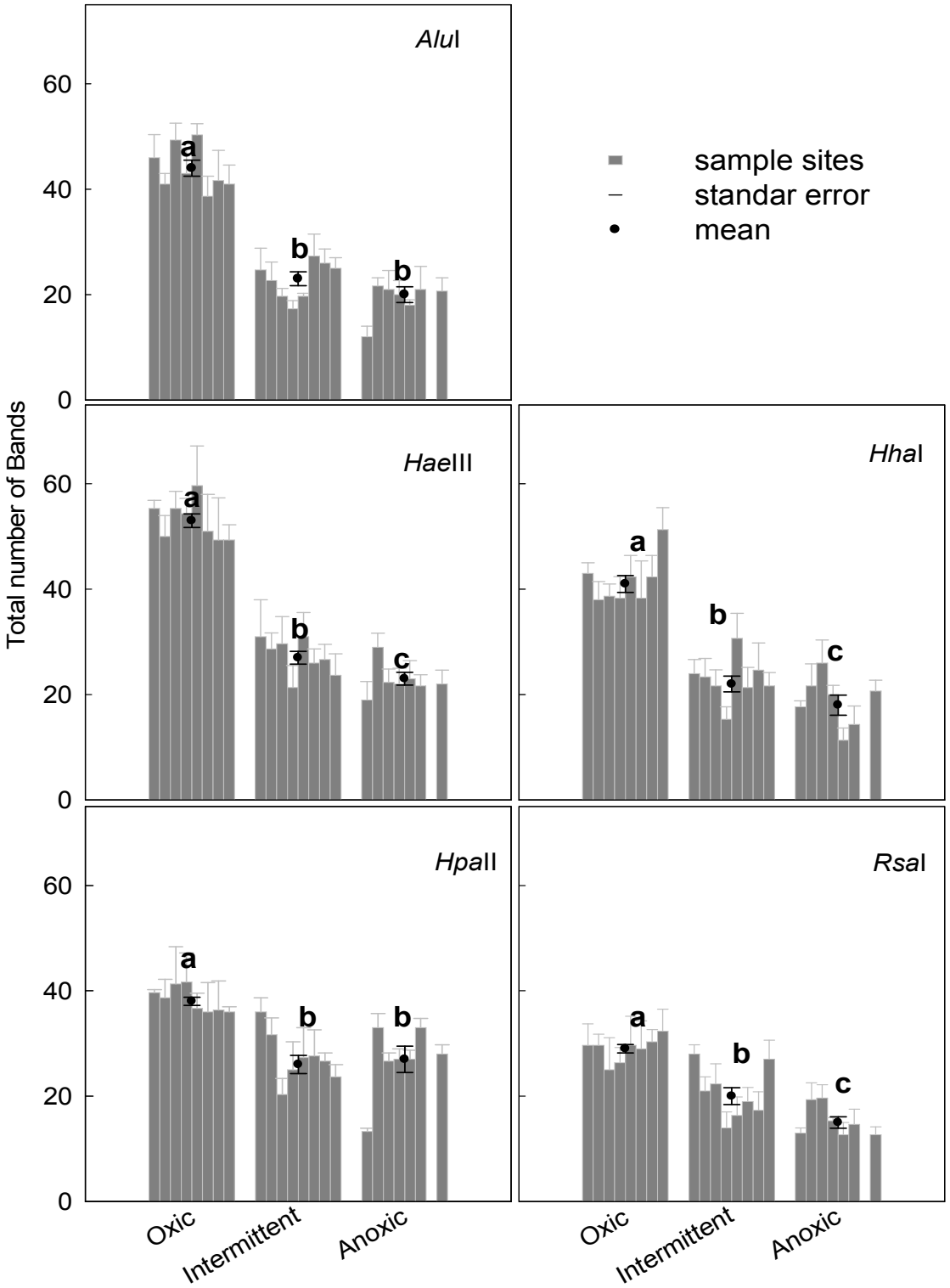

FIG. 3. Total number of bands detected in each lane of a T-RFLP fingerprint. Different letters indicate significantly different means at P<0.05 according to least significance test.

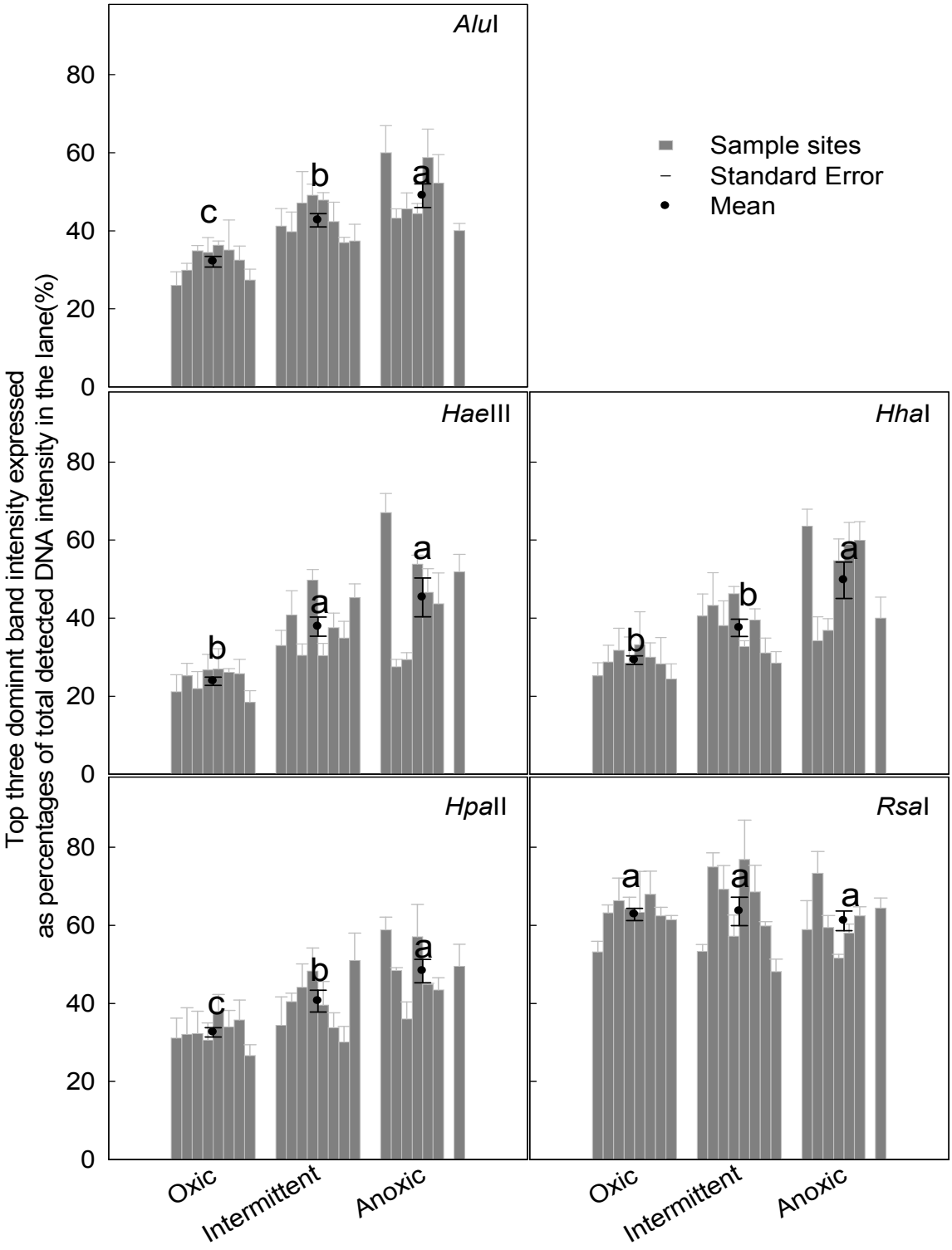

FIG. 4. The sum of the top three dominant bands intensity expressed as percentages of total detected DNA intensity in a lane of a T-RFLP fingerprint. Different letters indicate significantly different means at P<0.05 according to least significance test.

TABLE 2. Shannon diversity (H') indices of bacteria community in soils tested[†].

	H'		
Restriction Enzyme	Oxic	Intermittent	Anoxic
Alu I	1.37±0.01 a	1.12±0.03 b	1.07±0.04 b
Hae III	1.50±0.01 a	1.24±0.05 b	1.11±0.05 c
Hha I	1.42±0.02 a	1.19±0.03 b	1.05±0.06 b
Hpa II	1.36±0.01 a	1.20±0.03 b	1.14±0.04 b
Rsa I	0.97±0.02 a	0.91±0.04 a	0.91±0.01 a

[†] n=8 for soils sampled from oxic and intermittent layers, respectively, n=7 for soils sampled from anoxic layers. Different letters indicate significantly different means within the row at $\rho<0.05$ according to least sigificiant difference test.

size range of 50 to 700bp were clearly distinguishable. The electropherograms of the 5' TRFs of HaeIII-digested 16S rRNA are shown in Figure 2 as an example. The richness of ribotypes in each of the redox states were estimated by the number of terminal restriction fragment (TRFs) (Fig. 2). Among the five enzymes used, HaeIII, AluI, HhaI and HpaI revealed more ribotypes, while RsaI revealed the least number of ribotypes (Fig. 2). The average TRFs estimated from five restriction enzymes were higher in the oxic layer than in the intermittent and anoxic layer (Fig. 2).

The Shannon index (H') indicated that bacterial diversity was significantly higher in the oxic layers than in the intermittent or anoxic layers ($p<0.05$) (Table 2). The indices for evenness (J) of the bacterial community were 0.82±0.04, 0.82±0.03, and 0.80±0.02 for oxic, intermittent and anoxic layer respectively (data not shown). These indices were not significantly different along the soil profile. However, the intensity of the top three dominant bands, expressed as a percentage of total DNA intensity in a lane, was significantly lower in the oxic layers than in the intermittent and anoxic layers in four out of five T-RFLP fingerprints generated with different restriction enzymes (Fig. 4). The mean intensity for the top three dominant bands that were generated using *Alu*I, *Hae*III, *HhaI*, or *HpaII* digestion were around 48% in the anoxic horizon, which was significantly higher than that for the oxic layer (29%).

Similarity of bacterial communities in the soils tested was evaluated in pair-wise comparisons based on band sharing coefficients. A total of 253 band

TABLE 3. Number of sampling sites within each location or between two locations that had greater than 0.5 similarity coefficient between paired terminal restriction fragment length polymorphism (T-RFLP) fingerprints[†].

Paired redox states[‡]	Location comparision			
	L1 with L1	L2 with L2	L1 with L2	Sum
OX with OX	6/6	6/6	15/16	27/28
Int with Int	3/6	5/6	2/16	10/28
AX with AX	0/6	1/3	2/12	3/21
OX with AX	0/16	2/16	0/32	2/64
OX with Int	0/16	0/12	0/28	0/56
Int with AX	0/16	0/12	0/28	0/56

[†] A total of 253 band sharing coefficents were obtained; 28 were within the oxic layers, 28 within the intermittent layers, 21 within the anoxic layers, and 176 between layers of different redox states.

[‡] L1-Location 1, L2-Location 2, OX-oxic, Int-Intermittent, and AX-anoxic.

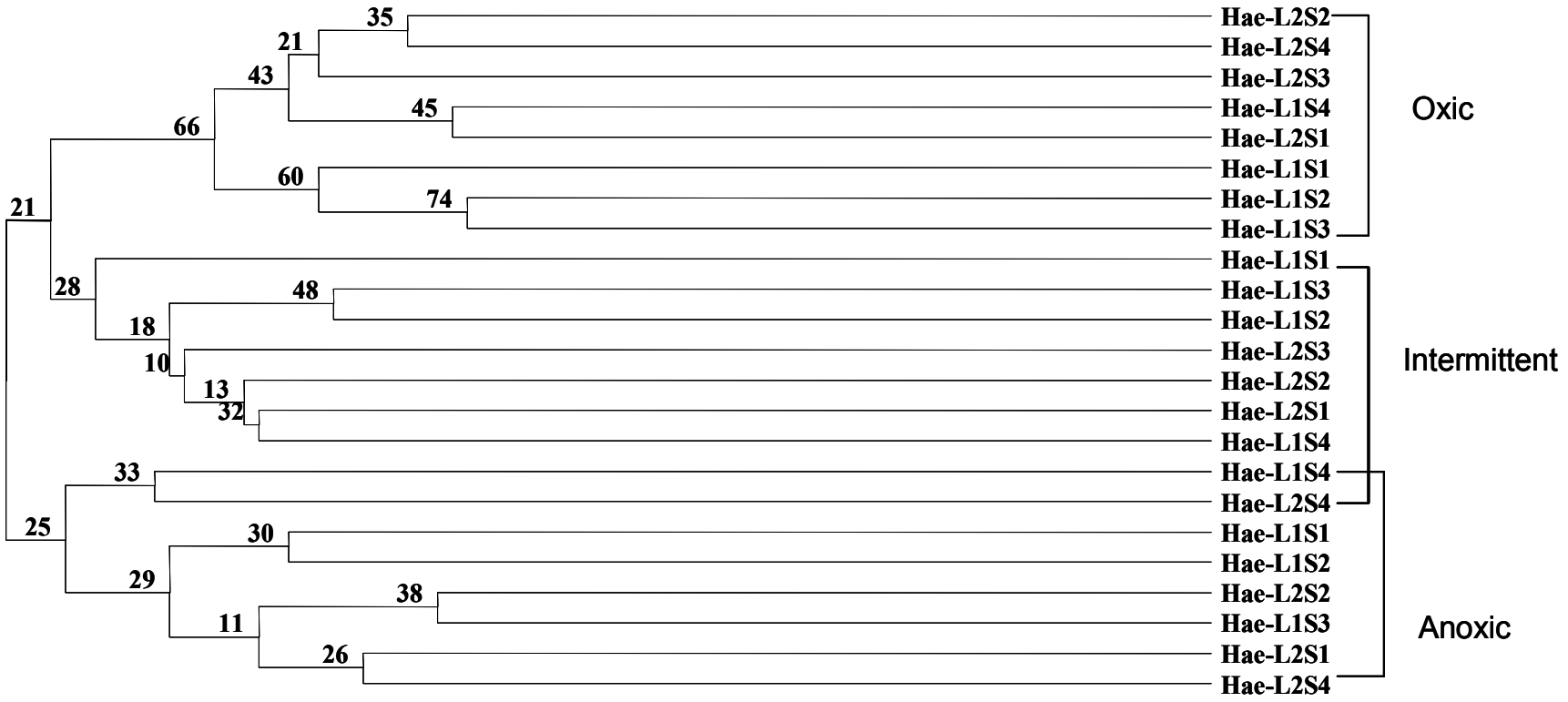

FIG. 5. Dendrogram for T-RFLP fingerprints that were generated with *Hae*III digestion and constructed from "0/1" string data at 0.5% match tolerance.

sharing coefficients were obtained. In general, band sharing coefficients were greater than 0.5 between bacterial communities in oxic layers when compared within each location or between two locations (Table 3). Considerable variations were shown among bacterial communities in the intermittent layers, and bacterial communities in the anoxic layers showed little in common. There was less similarity between different redox states when compared within each location or between two locations.

Similarity of bacterial communities was further revealed by cluster analysis of dendrograms generated based on five T-RFLP fingerprints compared at three different tolerance levels (0.5, 0.8, and 1.0). When bacterial communities were clustered based on redox states, three clusters emerged (Fig. 5).

DISCUSSION

Abundance of G^+ bacteria increased and those of G^- bacteria decreased with increasing soil depth up to 288 cm. Similar results were reported by Fierer et al. (18). Sinclair et al. (50) observed that G^+ was associated with high clay content while G^- was associated with sandy sediments, a correlation that is not supported by data obtained from this study. Perhaps clay and sand content affected C availability in soil, thus indirectly affected soil microbial community. Data obtained from this study indicated that C availability was an important factor regulating the bacterial communities in the subsurface soil environment. Similar conclusions have been reported previously (18, 29, 44). Some FAME indicators of G^+ bacteria dominated in the oxygen limited or anoxic soil environment. This might be related to the sporulating bacteria, such as *Clostridium* and *Bacillus*,

which, when conditions are stressful, produce oval endospores that can remain dormant for extended periods. Dominance of G^+ bacteria in the oxygen limiting environment is also evidenced by isolation of G^+ sulfate-reducing bacteria and *Thermodesulfobacterium* from anoxic marine environments (51). The increase of G+ bacterial populations in the reduced soil environment was often accompanied by the decrease of protozoa population in the intermittent and anoxic layers (16, 50).

Microbial community structures of the intermittent and anoxic layers shared more similarity with each other, but little with those in the oxic layers, which indicated that subsurface microbial communities had distinctly different compositions from the surface communities (6, 19). This could be, in part, due to the fact that surface soils experience a wide range of environmental factors, such as moisture, temperature, and substrate availability (37, 48, 57).

Not only did the abundance of specific groups of bacterial communities, such G^+ and G^- bacteria, vary along soil depth, but bacterial diversity did as well. Based on analysis of T-RFLP fingerprints, bacterial diversity decreased with increasing soil depth. The observed differences may reflect selective pressures that shape diversity within communities. In this study, an important selective pressure was soil redox states, evidenced by distinctive groupings of bacterial communities based on soil redox states as shown by FAME analysis and cluster analysis of T-RFLP fingerprints. Direct linkage between bacterial diversity and oxygen supply had been reported before (35, 54). Low diversity at deep soil

layers is also consistent with species-energy theory, which predicted relatively low diversity in the low organic matter horizon (33, 58).

Although numerous previous studies demonstrated that microbial community diversity decreased with increasing soil depth (2, 19, 54), the reported differences between microbial communities were not as significant as those shown in this study. In part, most studies used surface soils that were taken from less than 15 cm depth, where there was little variation in soil redox state.

With wide variations of environmental conditions on the surface, one might expect wider variations of bacterial communities in surface soils when compared with subsurface soils. Data obtained from this study showed the opposite trend despite the wide variation in vegetation that existed between fields. These results were not consistent with those reported previously (13, 42). The discrepancies among studies could be attributed to sampling depth. Most studies reported data obtained from soils sampled from the surface 15 cm depth. Perhaps the redox state is a predominant factor regulating bacterial community structures in the subsurface. With adequate oxygen supply, bacterial communities in the subsurface might share considerable similarity, a hypothesis supported by previous studies showing considerable similarity of bacterial communities among sediments (30, 31, 54). However, variations in bacterial community structure increased with decreasing oxygen supply in subsurface soils, suggesting emergence of other stress factors, possibly organic C and/or water content, in these environments that regulated bacterial community. The distinct grouping of bacterial communities from control sites, based on FAME

analysis, which suggested dominance of flooding in regulating bacterial community structure, is consistent with the latter explanation.

Although oxic layers were subjected to flooding and heavy metal contamination, there were no detectable differences induced by these environmental disturbances on bacteria diversities when compared with the control sites. Similar findings were reported by several other investigators (15, 32, 61). However, Sandaa et al. (46) reported that the α-subdivision of *Proteobacteria* was dominant in heavy metal contaminant sites. These results suggested that the overall bacterial community might be resilient to environment disturbance, but a specific bacterial group could be sensitive to these changes.

In summary, the abundance of G^+ bacteria increased and those of G^- bacteria decreased with increasing soil depth. Microbial community diversity decreased with increasing soil depth. Despite wide variations in vegetation between sampling sites, bacterial communities in oxic layers shared more similarity than those in the intermittent and anoxic layers. The loss of diversity with increasing soil depth was due, in part, to variations in redox states. Under oxygen limiting conditions, bacterial community structure was regulated by other stress factors such as organic C and water content.

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Chapter V

SUMMARY AND CONCLUSION

Results from this study indicated that DH activity decreased with increasing metal concentrations in the soil. Of the microbial parameters tested, DH activity was the most sensitive indicator of metal contamination and environmental stress. However, changes in microbial biomass, DNA content, culturable population were not detectable in the relatively low metal concentrations. Microbial activity and abundance along soil profile was correlated with C_{org} content and oxygen availability. When compared with soils from other redox states, soils from intermittent layers showed relative higher culturable bacterial population and higher C_{mic}:N_{mic} ratios. Data suggested that nutrient and oxygen limitation regulated microbial distribution along soil profile. Abundance of G^+ bacteria increased and those of G^- bacteria, fungi decreased with increasing soil depth. Microbial community diversity was higher in oxic layer when compared with intermittent of reduce layer. Heavy metal contamination did not lead to detectable changes in the abundance of bacteria, fungi, and protozoa, and/or distribution evenness and richness of the bacterial community. Oxygen limitation reduced bacterial diversity and distribution evenness in the environment.

APPENDIX

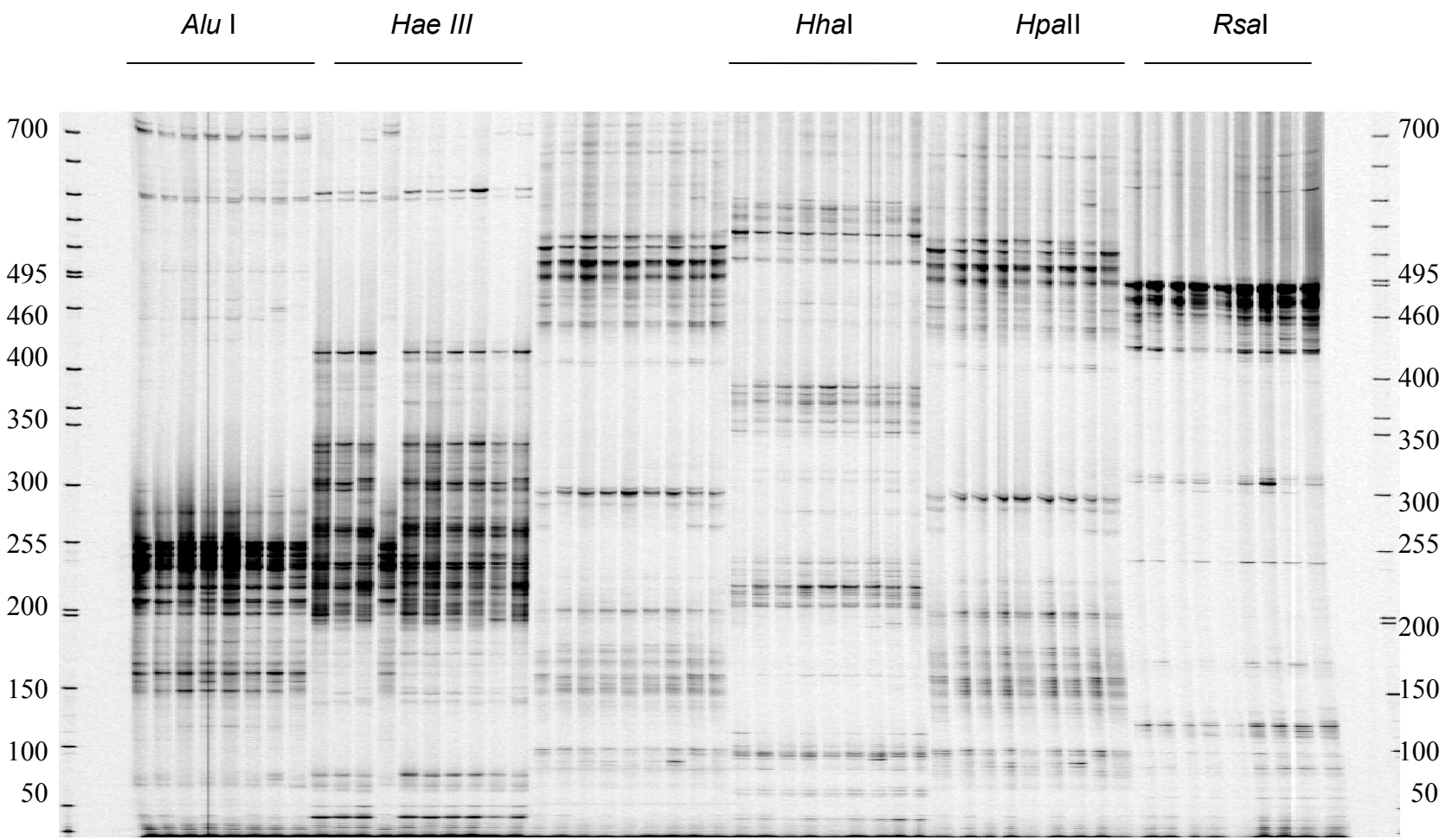

FIG. 1. 16S rRNA gene fingerprints of bacterial communities in soils of oxic layers by PCR amplification, restriction enzyme digestion, and T-RFLP separation. Samples were in the order of L1S1, L1S2, L1S4, L1S3, L2S1, L2S2, L2S3, and L2S4.

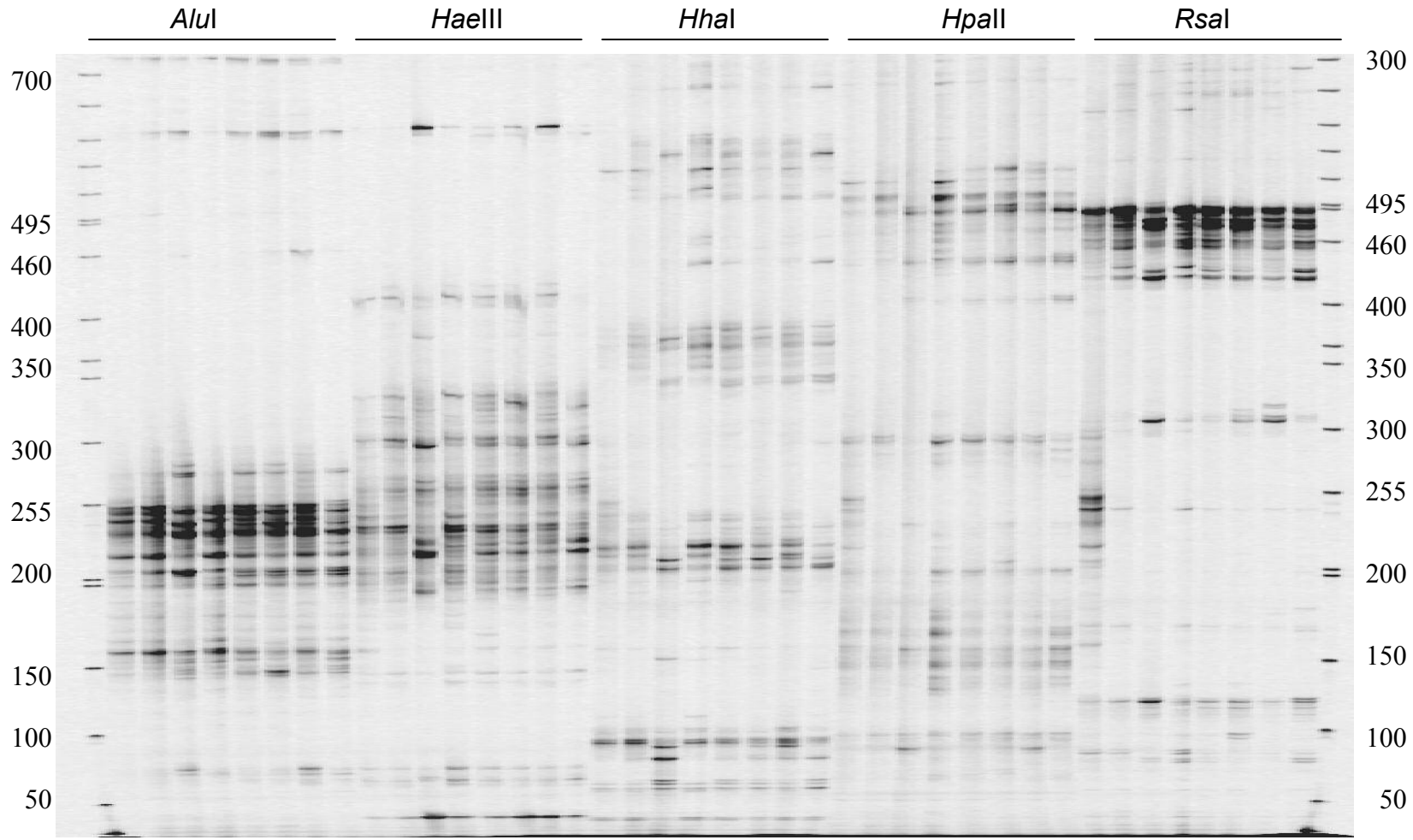

FIG. 2. 16S rRNA gene fingerprints of bacterial communities in soils of intermittent layers by PCR amplification, restriction enzyme digestion, and T-RFLP separation. Samples were in the order of L1S1, L1S2, L1S4, L1S3, L2S1, L2S2, L2S3, and L2S4.

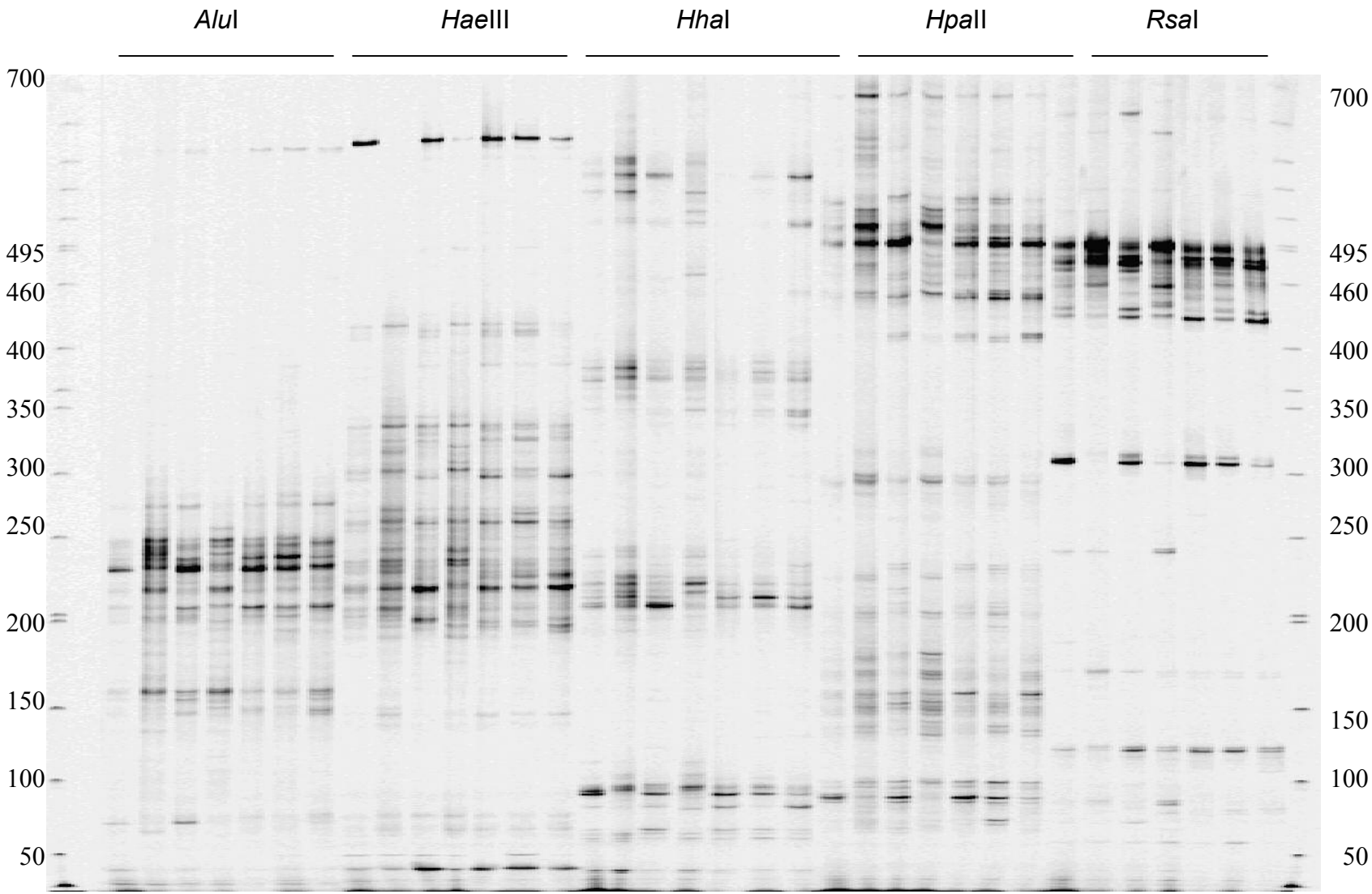

FIG. 3. 16S rRNA gene fingerprints of bacterial communities in soils of anoxic layers by PCR amplification, restriction enzyme digestion, and T-RFLP separation. Samples were in the order of L1S1, L1S2, L1S4, L1S3, L2S1, L2S2, L2S3, and L2S4.

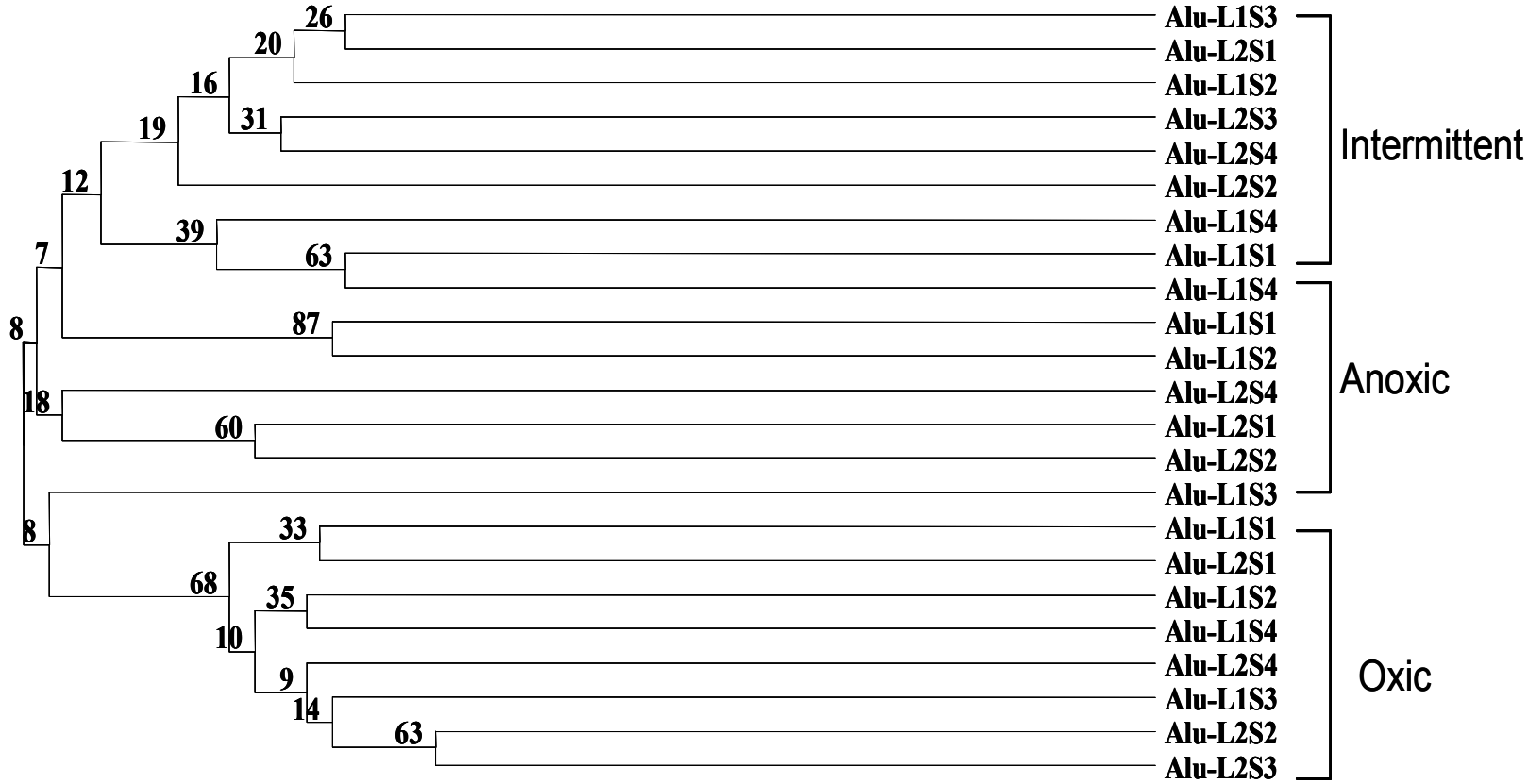

FIG. 4. Dendrogram for T-RFLP fingerprints that were generated with *Alu*I digestion and constructed from "0/1" string data at 0.5% match tolerance.

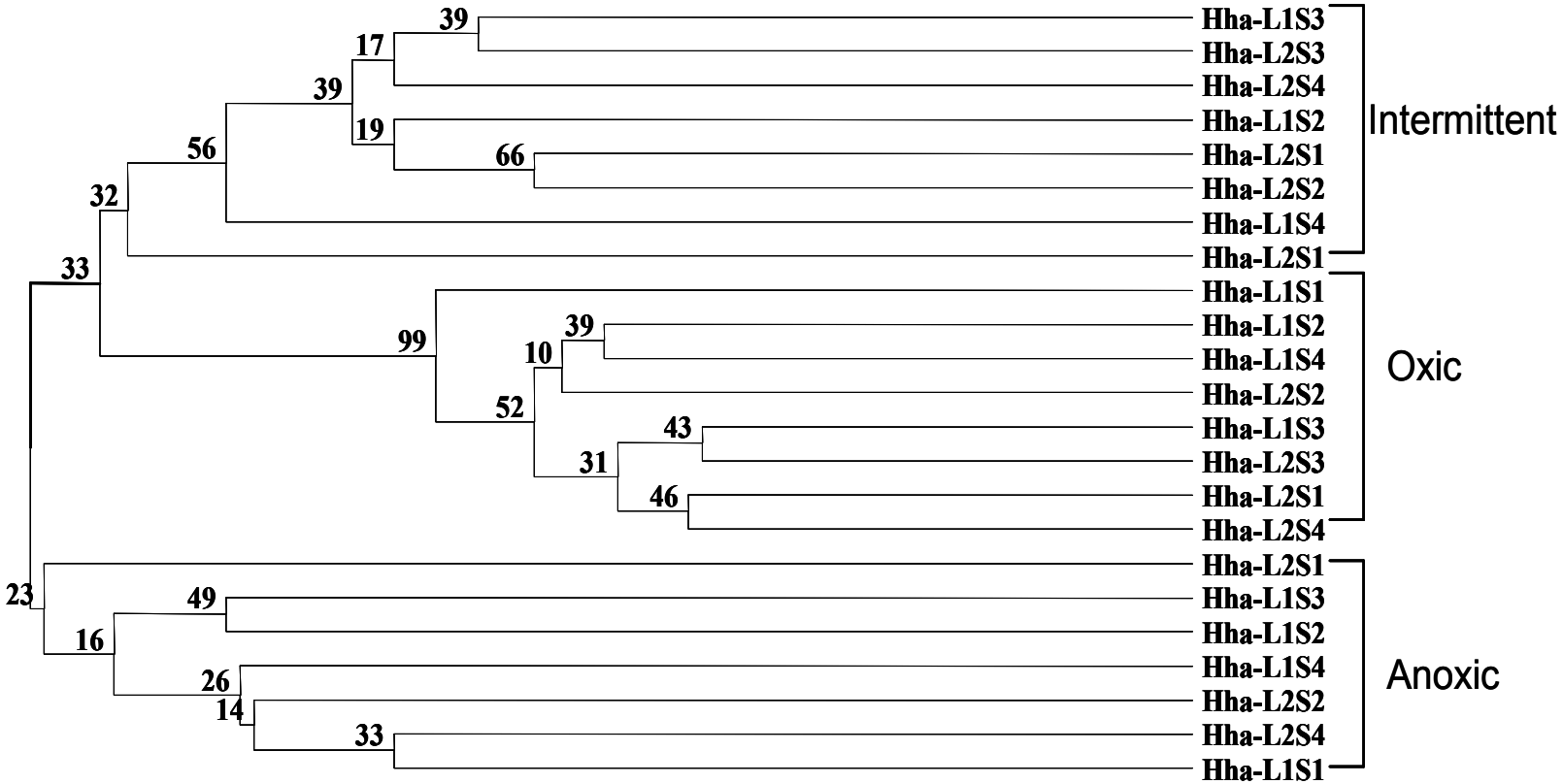

FIG. 5. Dendrogram for T-RFLP fingerprints that were generated with *Hha*I digestion and constructed from “0/1” string data at 0.5% match tolerance.

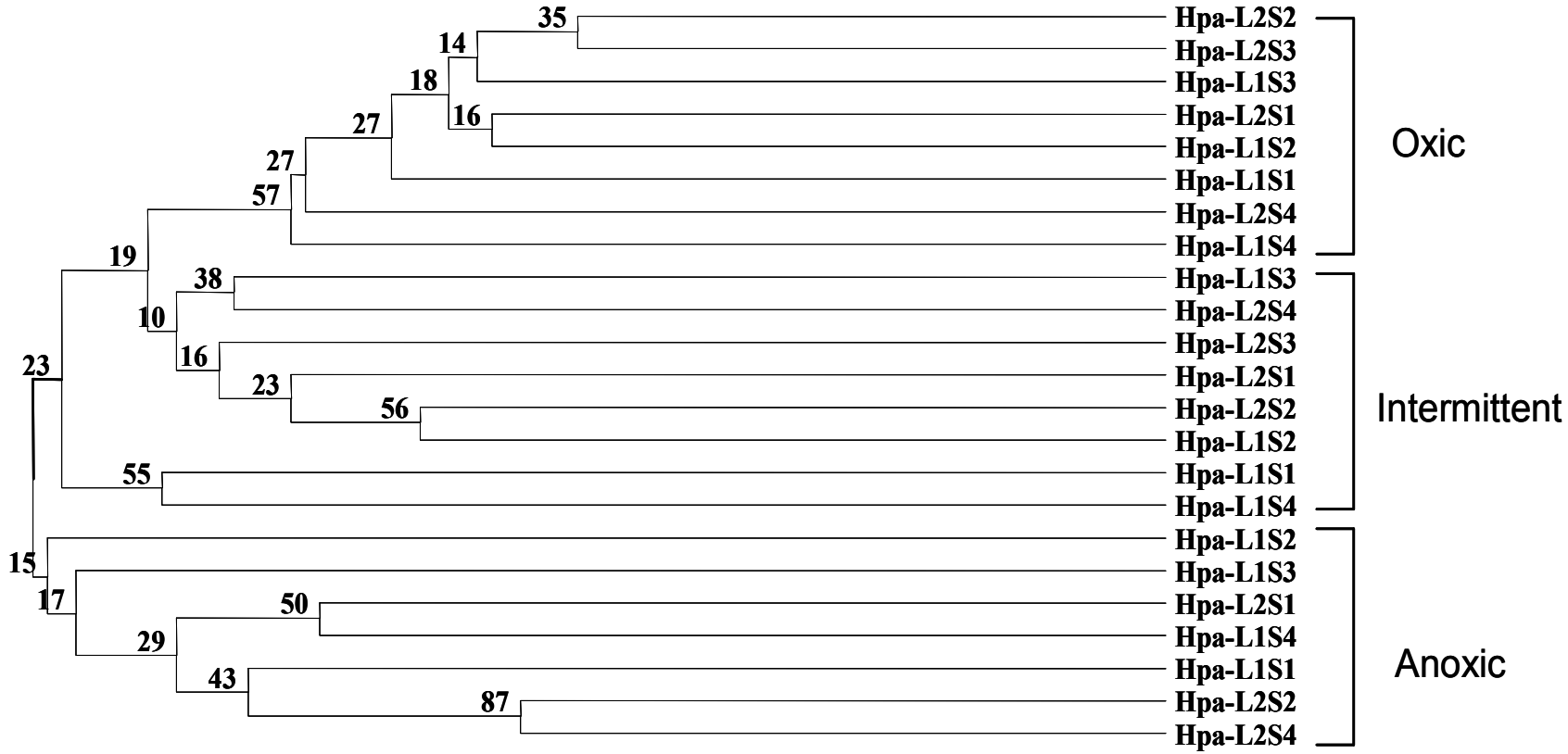

FIG. 6. Dendrogram for T-RFLP fingerprints that were generated with *Hpa*II digestion and constructed from "0/1" string data at 0.5% match tolerance.

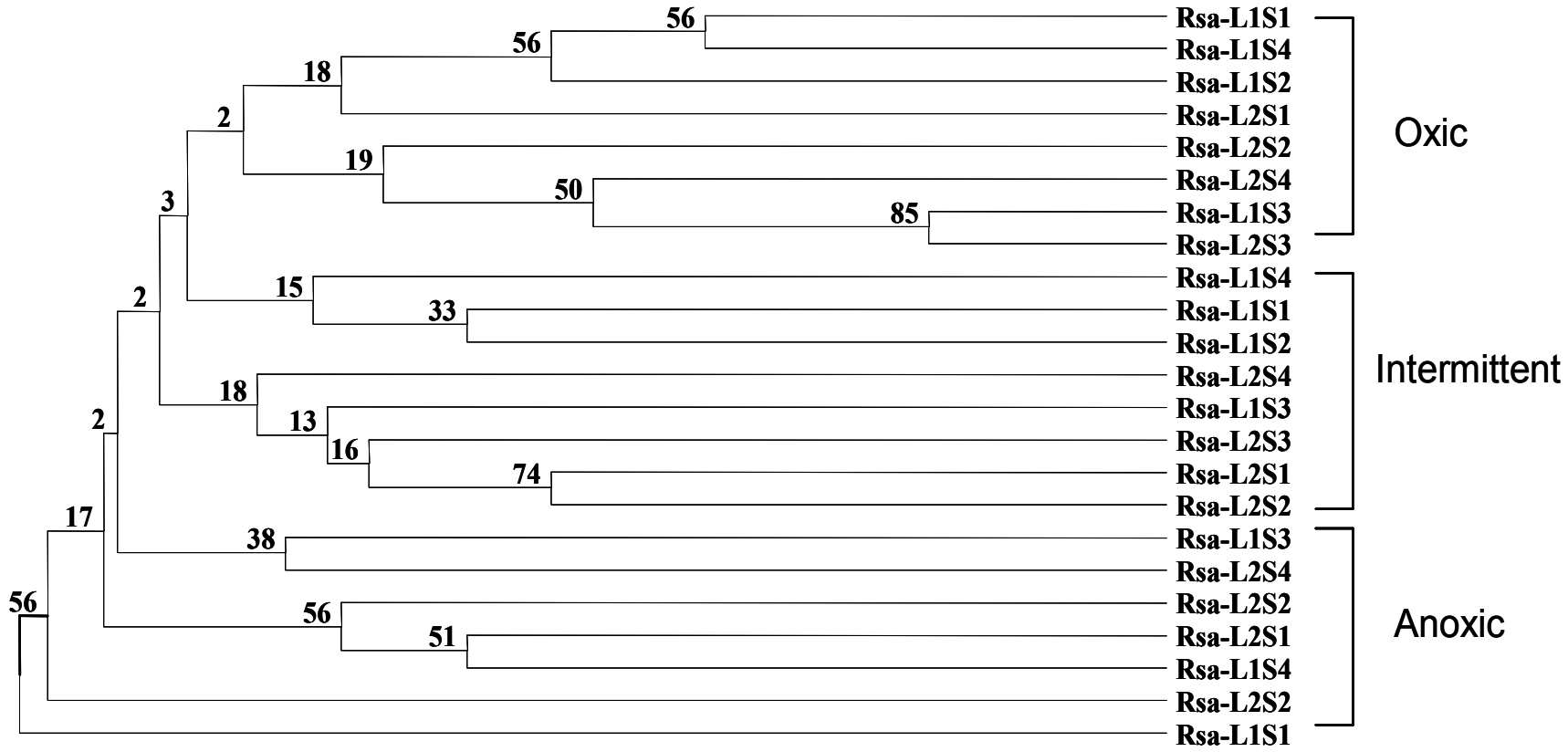

FIG. 7. Dendrogram for T-RFLP fingerprints that were generated with *Rsa*I digestion and constructed from "0/1" string data at 0.5% match tolerance.

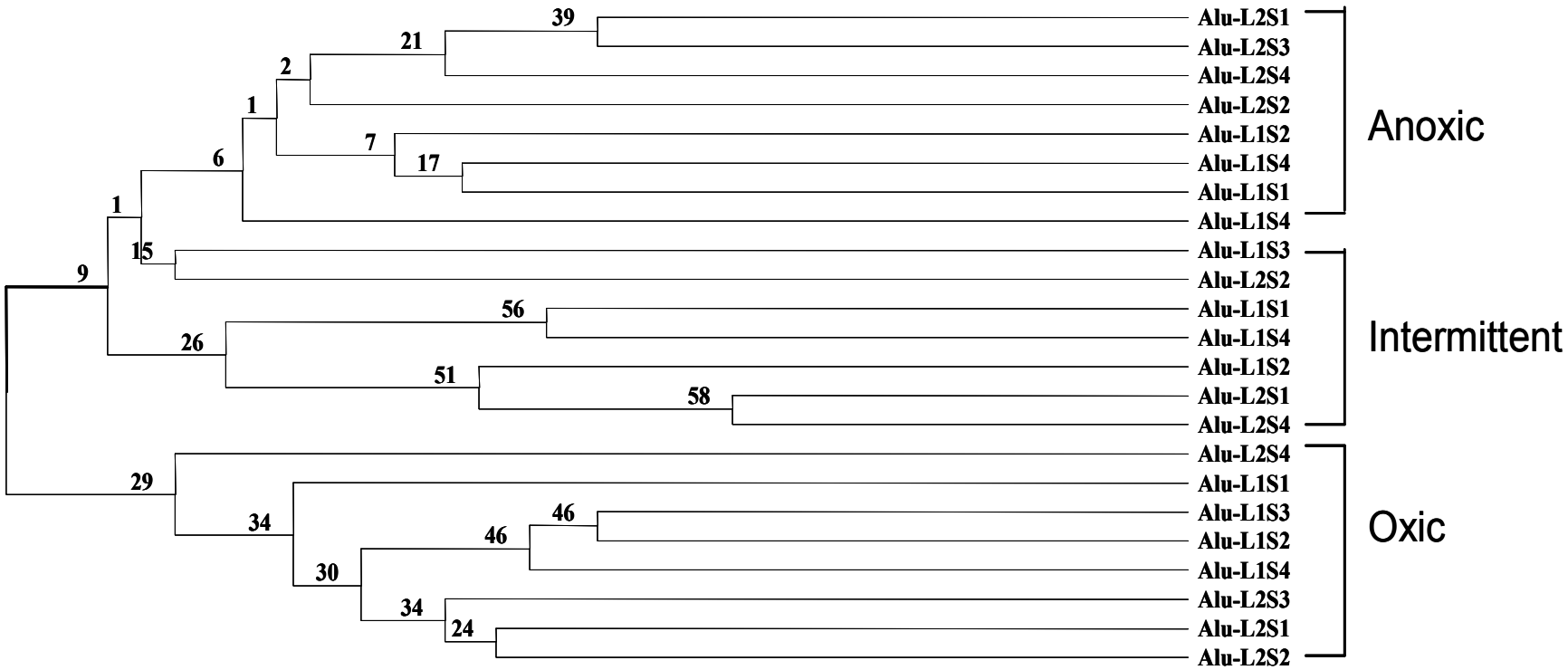

FIG. 8. Dendrogram for T-RFLP fingerprints that were generated with *Alu*I digestion and constructed from "0/1" string data at 0.8% match tolerance.

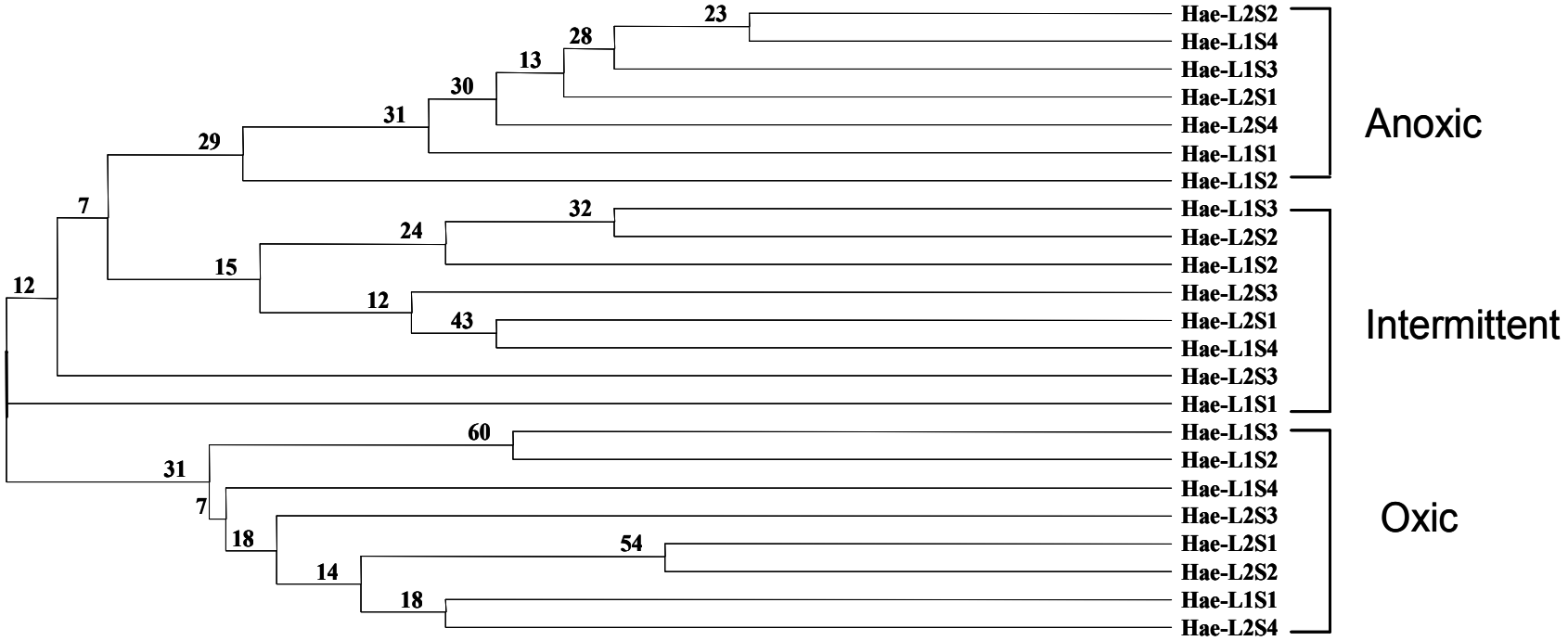

FIG.9. Dendrogram for T-RFLP fingerprints that were generated with *Hae*III digestion and constructed from "0/1" string data at 0.8% match tolerance.

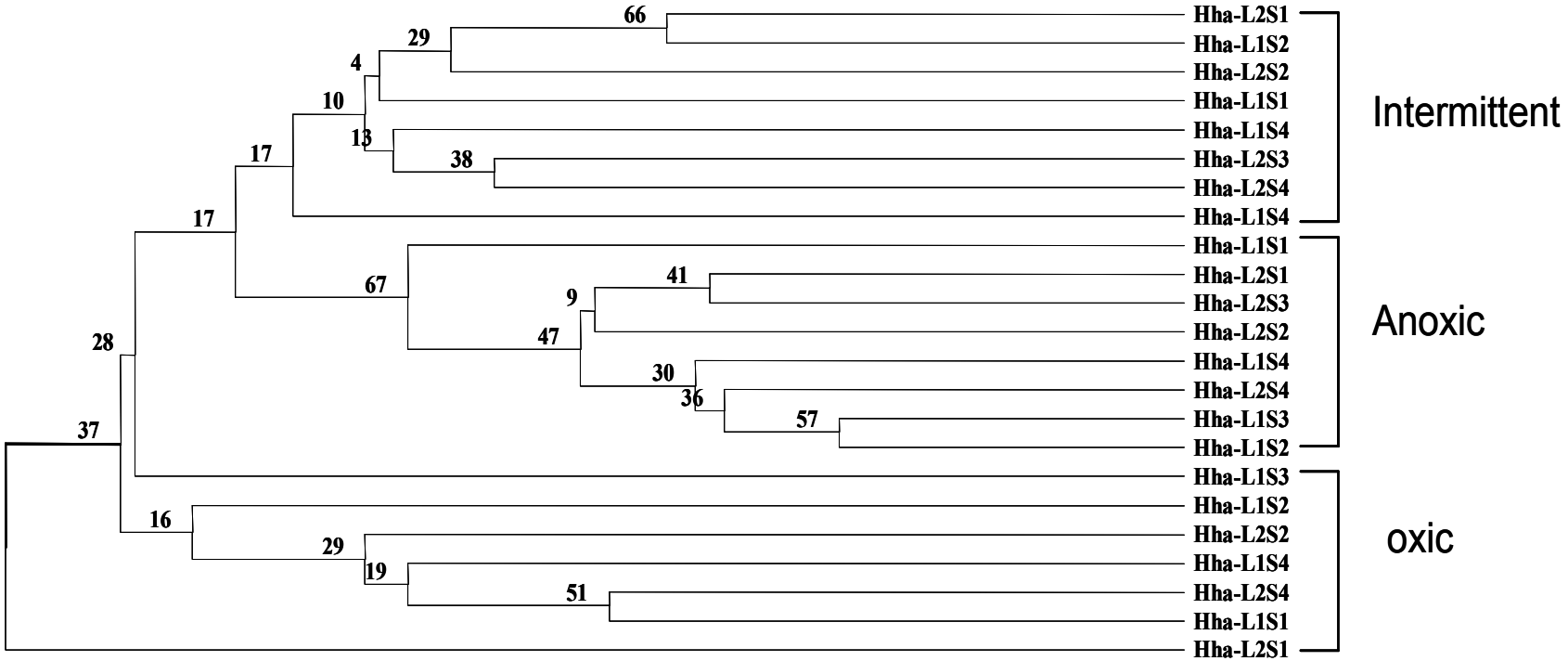

FIG. 10. Dendrogram for T-RFLP fingerprints that were generated with *Hha*I digestion and constructed from "0/1" string data at 0.8% match tolerance.

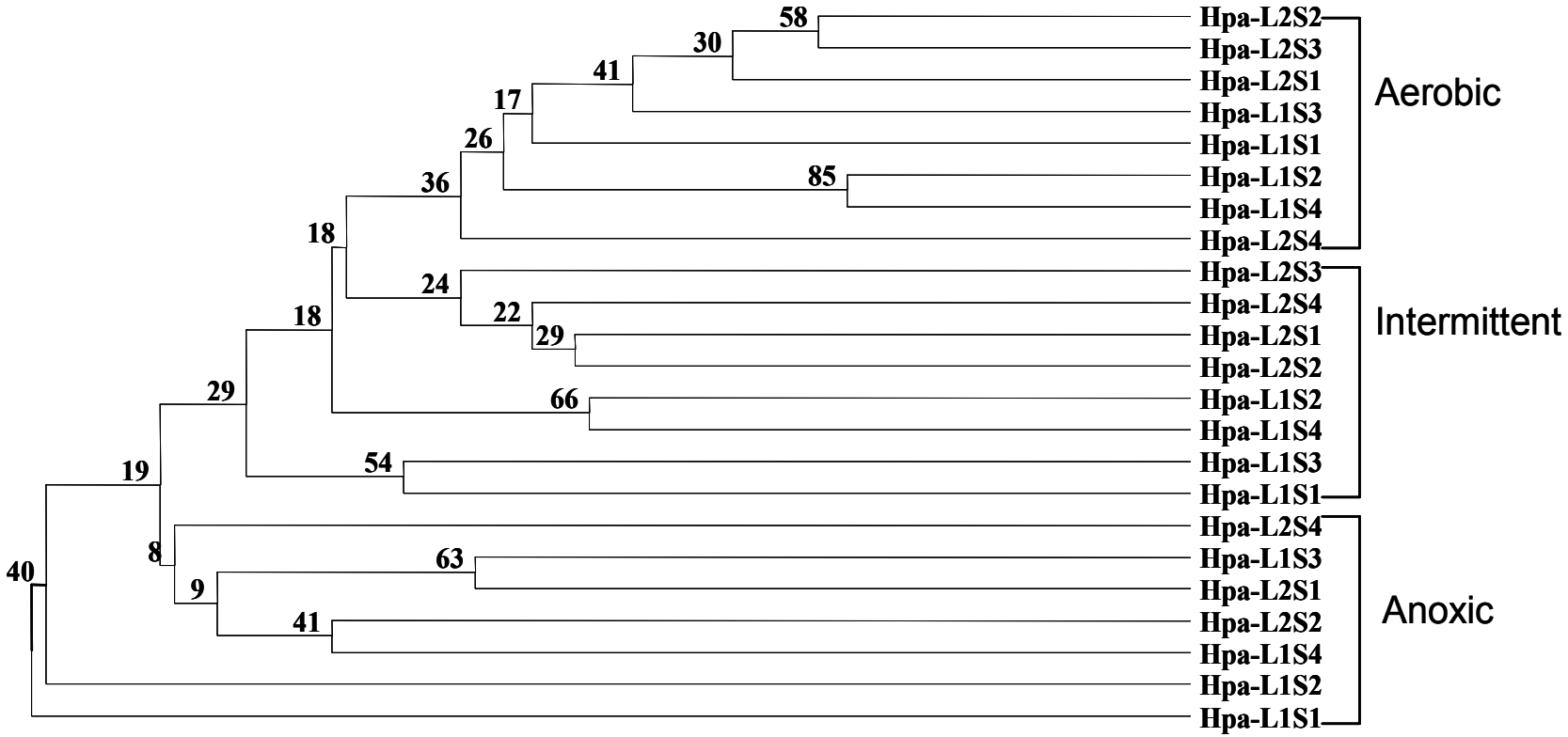

FIG. 11. Dendrogram for T-RFLP fingerprints that were generated with *Hpa*II digestion and constructed from "0/1" string data at 0.8% match tolerance.

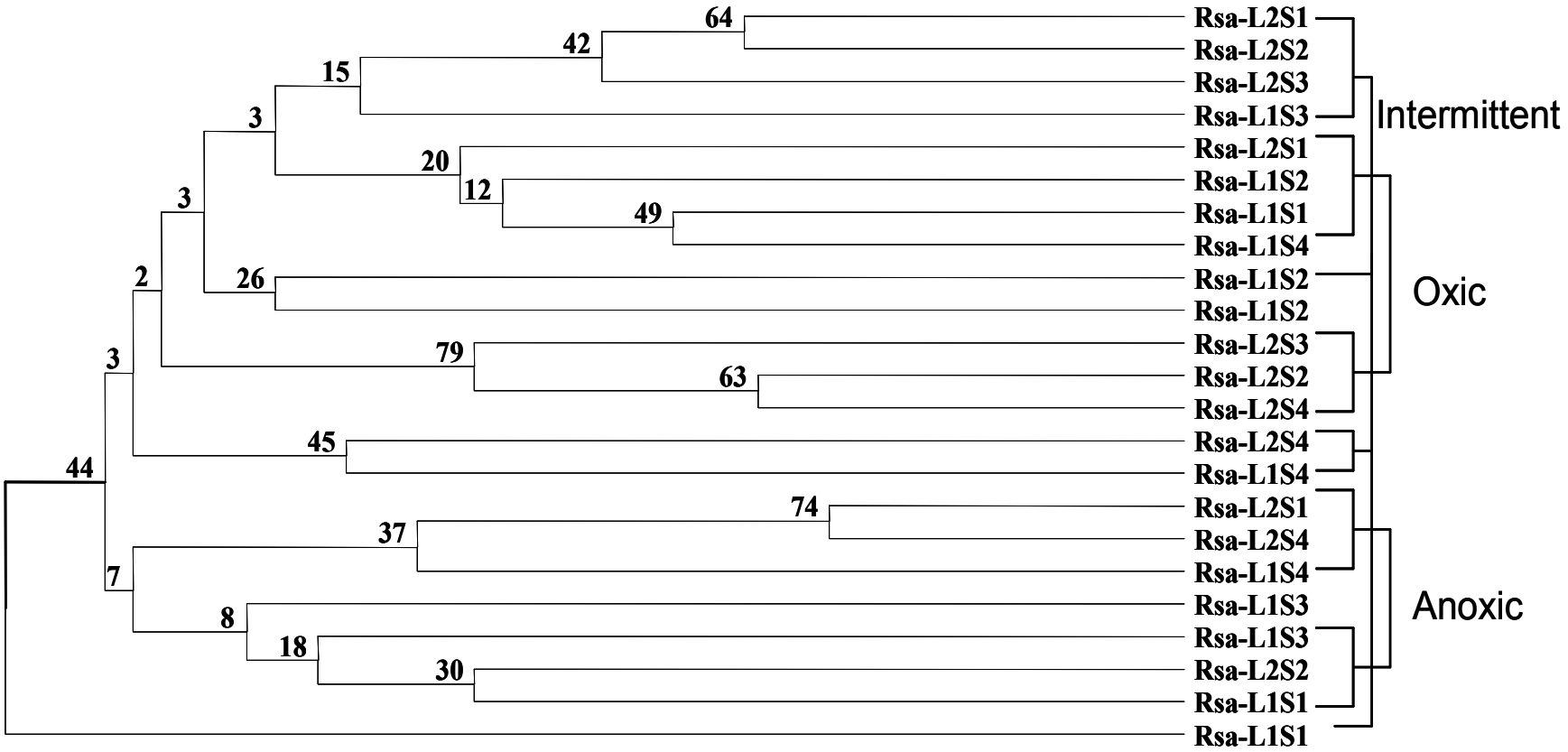

FIG. 12. Dendrogram for T-RFLP fingerprints that were generated with *Rsa*I digestion and constructed from "0/1" string data at 0.8% match tolerance.

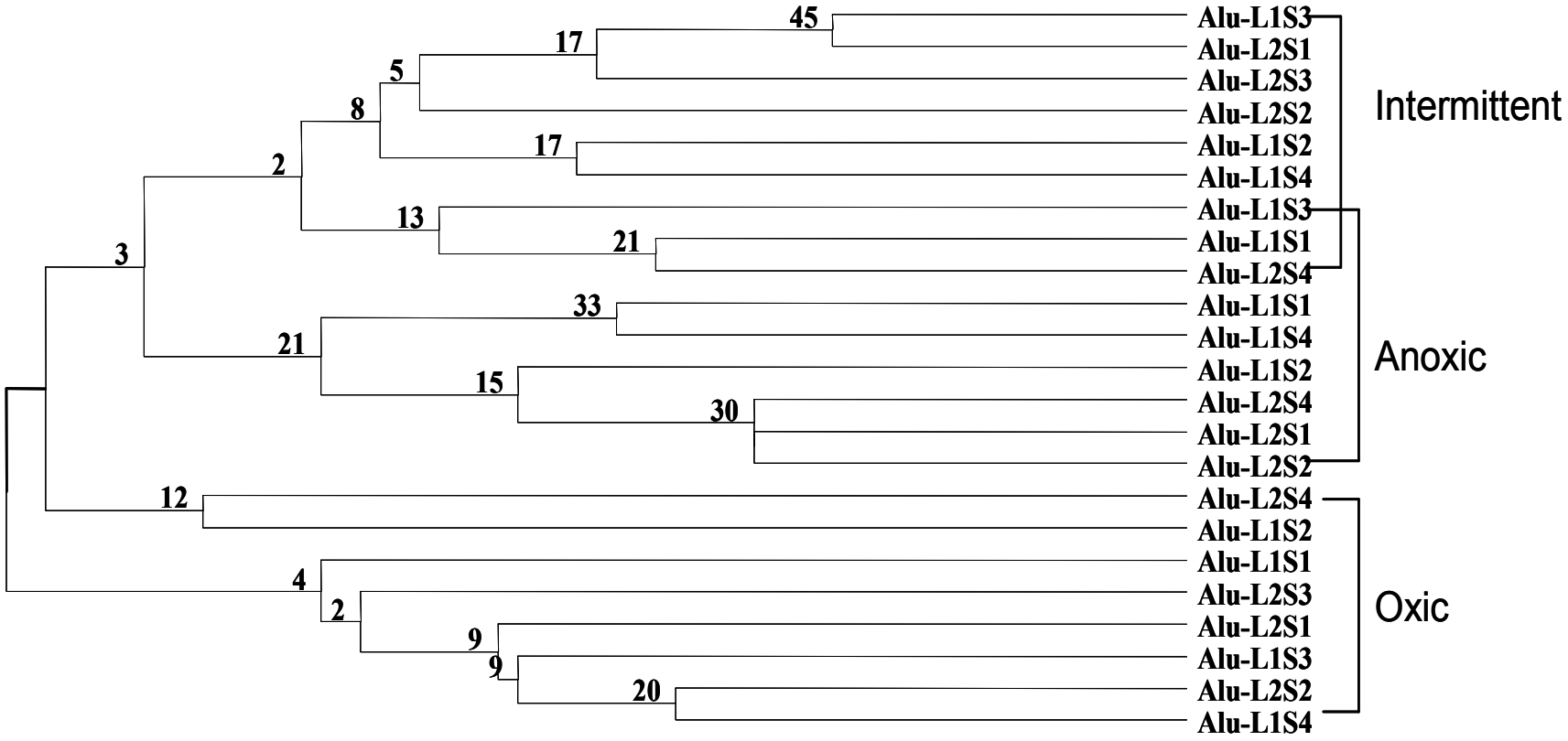

FIG. 13. Dendrogram for T-RFLP fingerprints that were generated with *Alu*I digestion and constructed from "0/1" string data at 1.0% match tolerance.

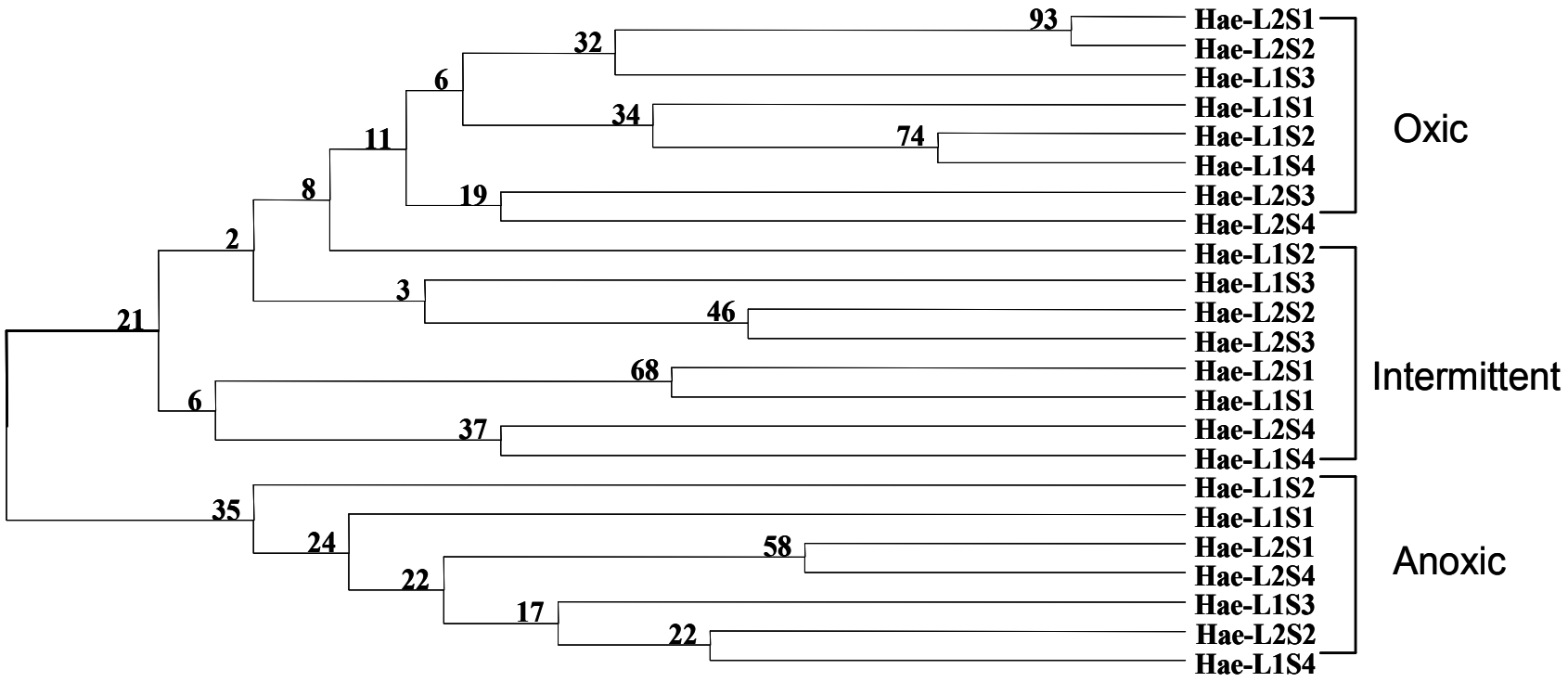

FIG. 14. Dendrogram for T-RFLP fingerprints that were generated with *Hae*III digestion and constructed from "0/1" string data at 1.0% match tolerance.

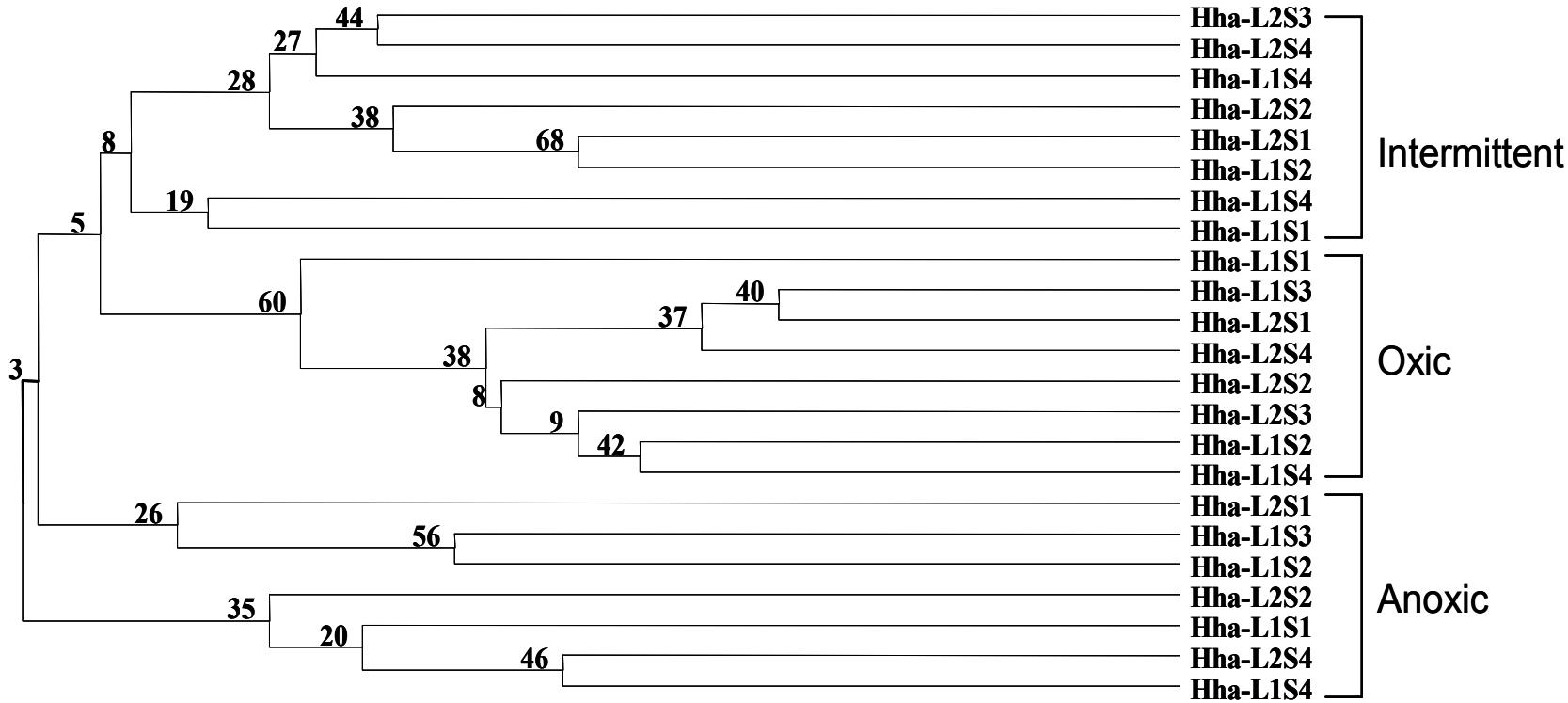

FIG. 15. Dendrogram for T-RFLP fingerprints that were generated with *Hha*I digestion and constructed from "0/1" string data at 1.0% match tolerance.

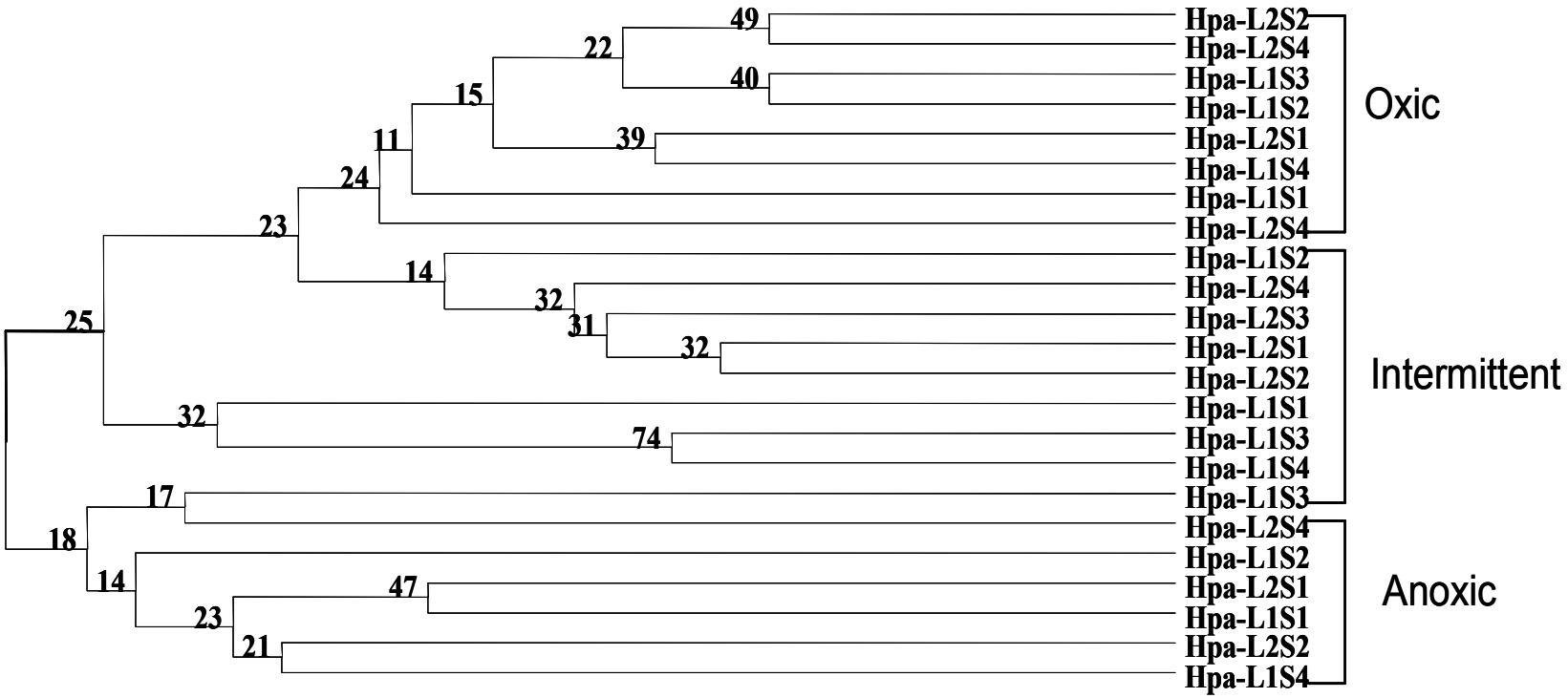

FIG. 16. Dendrogram for T-RFLP fingerprints that were generated with *Hpa*II digestion and constructed from "0/1" string data at 1.0% match tolerance.

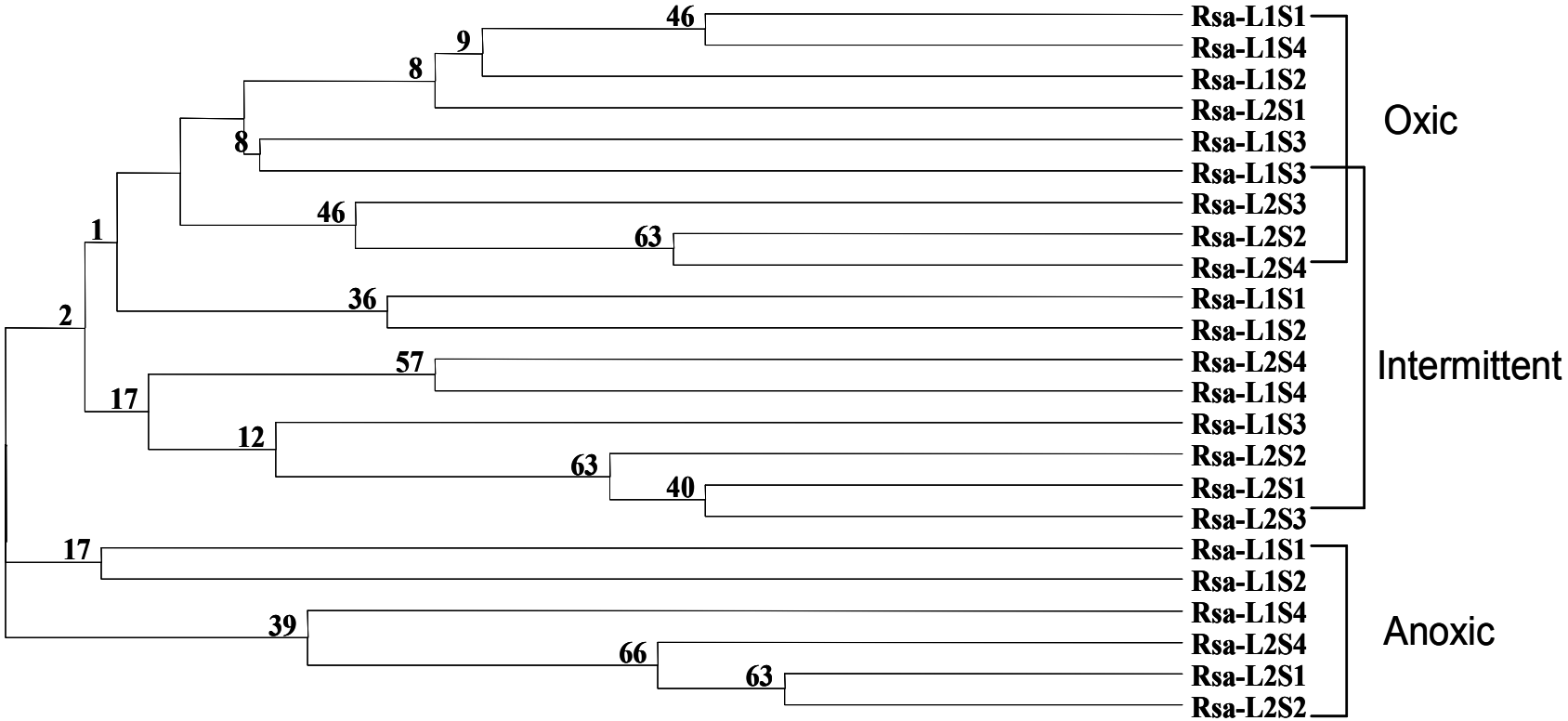

FIG. 17. Dendrogram for T-RFLP fingerprints that were generated with *Rsa*I digestion and constructed from "0/1" string data at 1.0% match tolerance.

VITA

Yang Song

Candidate for the Degree of

Master of Science

Thesis: SOIL BACTERIAL DIVERSITY AND COMMUNITY STRUCTURE IN A RIVER FLOODPLAIN CONTINUUM

Major Field: Plant and Soil sciences

Biographical:

Education: Graduated from HuaRou High School in the People's Republic of China in July 1986; received Bachelor of Science degree in Plant Protection from Beijing Agricultural College, P. R. China, in July 1996; and completed the requirements for the Master of Science degree with a major in Plant and Soil Sciences at Oklahoma State University in December, 2005.

Experience: Employed as an Assistant Manager in Beijing Food Production and Processing Center from 1996 to 2002 and as a graduate research assistant in the Department of Plant and Soil Sciences at Oklahoma State University since 2003. Research techniques include soil DNA extraction, polymerase chain reaction (PCR) for DNA amplification, cloning and expression of 16S rRNA genes of bacterial community, and denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphisms (T-RFLP) for fingerprinting soil bacterial communities.

Name: Yang Song Date of Degree: December, 2005

Institution: Oklahoma State university Location: Stillwater, Oklahoma

Title of Study: SOIL BACTERIAL DIVERSITY AND COMMUNITY

STRUCTURE IN A RIVER FLOODPLAIN CONTINUUM

Page in study: 113 Candidate for the Degree of Master of Science

Major Field: Plant and Soil Science

Scope and Method of Study: The objectives were (1) to evaluate impact of chronic flooding with heavy metal contaminations on soil biota in a river floodplain continuum; and (2) to assess microbial responses to soil redox states. Soils were collected from four sites at each of two locations along Tisza River in Hungary. Each soil profile was divided into three redox horizons: oxic, intermittent, and anoxic. Microbial communities were evaluated by microbial biomass carbon and nitrogen contents, dehydrogenase activity, and culturable bacterial populations. Depth- and redox-related variation in microbial community structure was evaluated using a 16S rRNA-based approach and fatty acids methyl esters (FAME) analysis.

Findings and Conclusions: Results from this study indicated that nutrient and oxygen availability regulated microbial distribution along a soil profile. Oxygen and carbon limitation reduced bacterial diversity and distribution evenness in the environment. Microbial activity, microbial biomass, and the amount of DNA extracted were higher in oxic layers than anoxic layers. Abundance of gram-positive bacteria increased and those of gram-negative bacteria and fungi decreased with increasing soil depth. Heavy metal contamination in the detected concentrations did not lead to significant changes in the abundance of bacteria, fungi, and protozoa, and/or distribution evenness and richness of the bacterial community. Among microbial parameters tested, dehydrogenase activity was most sensitive and responsive to heavy metal contaminations and environmental stress.

ADVISER'S APPROVAL: