The Journal of Microbiology, December 2004, p.278-284 Vol. 42, No. 4

Monitoring of Bacterial Community in a Coniferous Forest Soil After a Wildfire

Ok-Sun Kim[1], Jae-Jun Yoo[1], Dong-Hun Lee[2], Tae-Seok Ahn[1] and Hong-Gyu Song[3,*]

[1]*Department of Environmental Science, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[2]*Division of Life Sciences, Chungbuk National University, Cheongju 361-763, Republic of Korea*
[3]*Division of Biological Sciences, Kangwon National University, Chuncheon 200-701, Republic of Korea*

(Received August 2, 2004 / Accepted October 26, 2004)

Changes in the soil bacterial community of a coniferous forest were analyzed to assess microbial responses to wildfire. Soil samples were collected from three different depths in lightly and severely burned areas, as well as a nearby unburned control area. Direct bacterial counts ranged from 3.3-22.6 × 10^8 cells/(g · soil). In surface soil, direct bacterial counts of unburned soil exhibited a great degree of fluctuation. Those in lightly burned soil changed less, but no significant variation was observed in the severely burned soil. The fluctuations of direct bacterial count were less in the middle and deep soil layers. The structure of the bacterial community was analyzed via the fluorescent *in situ* hybridization method. The number of bacteria detected with the eubacteria-targeted probe out of the direct bacterial count varied from 30.3 to 84.7%, and these ratios were generally higher in the burned soils than in the unburned control soils. In the surface unburned soil, the ratios of α-, β- and γ-proteobacteria, *Cytophaga-Flavobacterium* group, and other eubacteria groups to total eubacteria were 9.9, 10.6, 15.5, 9.0, and 55.0%, respectively, and these ratios were relatively stable. The ratios of α-, β- and γ-proteobacteria, and *Cytophaga-Flavobacterium* group to total eubacteria increased immediately after the wildfire, and the other eubacterial proportions decreased in the surface and middle layer soils. By way of contrast, the composition of the 5 groups of eubacteria in the subsurface soil exhibited no significant fluctuations during the entire period. The total bacterial population and bacterial community structure disturbed by wildfire soon began to recover, and original levels seemed to be restored 3 months after the wildfire.

Key words: wildfire, bacterial community structure, fluorescent *in situ* hybridization (FISH)

Soil microorganisms are critical for the maintenance of belowground systems, which are, in turn, essential for the sustainability of all terrestrial ecosystems (Neary *et al.*, 1999). The physical, chemical, and biological characteristics of soil which support aboveground plant growth, including soil structure, nutrient and water holding capacity, aeration, water infiltration, and others, are deeply involved with the activity of soil microbes. Therefore, disruption of the soil microbial community may have immediate and long-lasting deleterious effects on plant growth in general. Fire is one of the many disruptive forces at play in the forest ecosystem. It destroys the physicochemical properties of soil, and kills animals and plants. It also disrupts the community of soil microorganisms (Neary *et al.*, 1999). Hazardous compounds, such as dioxins, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls are also produced during wildfire, and also tend to inhibit the growth and survival of soil organisms (Martínez *et al.*, 2000; Gabos *et al.*, 2001). Although the importance of microbes in the soil ecosystem is well established, and numerous forest fires occur everywhere around the world, the effects of fire on microbial communities have not been investigated in details. Several reports have focused on the effects of fire on soil microbes, but they have been, unfortunately, limited only to the population changes of some large taxonomic groups or physiological groups (Sharma, 1981; Vázquez *et al.*, 1993; Harris *et al.*, 1995; Acea and Carballas, 1996; Acea *et al.*, 2003). Moreover, in those studies microbial populations were determined by plate counting, which significantly underestimates the actual soil microbial population (Amann *et al.*, 1995). Bååth *et al.* (1995) investigated the soil microbial community structure using phospholipid fatty acid (PLFA) analysis, demonstrating that burning induced the largest treatment effect on the PLFA pattern. However, they were not able to characterize in detail the changes occurring in the soil microbial community as a result of fire.

Recently, the fluorescent *in situ* hybridization (FISH) method was developed, and has been applied to the anal-

* To whom correspondence should be addressed.
(Tel) 82-33-250-8545; (Fax) 82-33-241-4627
(E-mail) hgsong@kangwon.ac.kr

ysis of microbial communities in a variety of environments (Hicks *et al.*, 1992; Trevesius *et al.*, 1994). The FISH method is easier and more rapid than other molecular biological techniques for the analysis of microbial community structure. However, it has been used primarily in analyses of aquatic systems (Alfreider *et al.*, 1996; Belkova *et al.*, 2003). In this study, the microbial community of a coniferous forest's soil after a wildfire was monitored by direct counting and the FISH method, during the 90 days after the fire. We have comparatively analyzed the effects of fire severity and soil depth on wildfire-induced changes in the microbial community.

Materials and Methods

Study area

The study area was a temperate coniferous forest (30-year old *Pinus densiflora*) located in Kangnung, Republic of Korea. The wildfire occurred on March 21-22, 2001 during a dry spring, and 25.4 ha of forest was burned. The research areas were classified into lightly burned sites damaged by surface fire, severely burned sites damaged by crown fire, and the nearby control site, which was unaffected by wildfire. Each site was randomly divided into triplicate plots (10 m×10 m each).

Soil sampling and treatment

Soil collection commenced on April 9, 17 days after the fire, and continued until July 5 (total 5 collections). From each plot, 3 random subsites were selected, ash and unburned plant residue was removed, and underlying soil was collected separately from the surface layer (0-5 cm depth), middle layer (6-15 cm) and deep layer (16-25 cm) soils. Soil samples of each depth from 3 subsites were combined, constructing composite samples for each plot. Samples were placed in a sterile plastic bag, and immediately transferred to the laboratory. The fresh soil was then filtered through a 2-mm mesh sieve, and the moisture contents were measured. The soil was sandy loam at a pH of 5.2, and organic matter content was measured as 3.85% by the loss-on-ignition method. Sterile distilled water (100 ml) was added to 10 g soil samples and homogenized for 45 sec in a mechanical blender. This soil suspension was ultrasonicated for 30 min (28 KHz, 400 W) in order to detach the adsorbed bacteria from the mineral and organic particles.

Microbial community analyses

The soil suspension was diluted in a 10-fold series, and the properly diluted soil suspensions were fixed with neutral formalin (final conc. 2%). For the direct counting of total bacteria, 10 μl of fixed sample was filtered through black polycarbonate membrane filters (Nuclepore, pore size 0.2 μm, dia. 25 mm), and stained with acridine orange (final conc. 0.01%) (Hobbie *et al.*, 1977). The stained bacterial population on the membrane filter was counted under an epifluorescent microscope (Olympus BX60, Japan). Total bacterial count was averaged from the bacterial numbers obtained in 20 microscopic fields.

In order to analyze of the structure of the bacterial community by the FISH method, diluted soil suspensions were fixed by the addition of freshly-prepared 4% paraformaldehyde, and 30 μl was passed through 0.2 μm pore-sized polycarbonate filters. The filters were washed free of paraformaldehyde three times with 0.5 ml phosphate buffered saline and then sterile water, and dried in the air. The rRNA probes used included: EUB338 for the detection of eubacteria, ALF1b for α-proteobacteria, BET42a for β-proteobacteria, GAM42a for γ-proteobacteria, and CF319a for the *Cytophaga-Flavobacterium* group (Table 1), all of which have been designed and utilized frequently for the FISH analysis of environmental bacteria (Manz *et al.*, 1992; Alfreider *et al.*, 1996; Glöckner *et al.*, 1999). Oligonucleotide probes were commercially constructed and labeled with tetramethylrhodamine (TaKaRa, Japan). The filter was placed on a gelatin-coated slide glass, and 16 μl of hybridization solution [0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% SDS, formamide (concentrations for EUB, ALF, BET, GAM and CF were 0, 20, 35, 35, and 15%, respectively)] was added. At that time, 2 μl of probe solution (25 ng) was added (Alfreider *et al.*, 1996). After mixing the solutions, each slide was placed into a hybridization chamber, and incubated at 46°C for 90 min. The hybridization mixture was then removed by immersing each filter in the wash buffer [20 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.01% SDS, NaCl

Table 1. The oligonucleotide probes used in this study

Probe	Probe structure (5'-3')	rRNA type, Detected	positions bacterial group
EUB338	GCTGCCTCCCGTAGGAGT	16S, 338-355	Total eubacteria
ALF968	GGTAAGGTTCTGCGCGTT	16S, 968-986	Alpha-Proteobacteria
BET42a	GCCTTCCCACTTCGTTT	23S, 1027-1043	Beta-Proteobacteria
GAM42a	GCCTTCCCACATCGTTT	23S, 1027-1043	Gamma-Proteobacteria
CF319a	TGGTCCGTGTCTCAGTAC	16S, 319-336	*Cytophaga-Flavobacterium*
NON338	ACTCCTACGGGAGGCAGC	-	Negative control

solution (centrations for EUB, ALF, BET, GAM and CF were 0.9 M, 0.225 M, 80 mM, 80 mM and 80 mM, respectively)] at 48°C for 20 min. The slide was then gently rinsed with distilled water and air-dried. Since the β- and γ-group of proteobacteria tend to interfere with each other, 2 μl each of non-labeled GAM42a and BET42a were used, together with labeled BET and GAM probes, for the detection of the β and γ group, respectively, to prevent interference (Manz *et al.*, 1992). For the counterstaining of total bacteria, a subset of filters was stained solely with 4', 6-diamidino-2-phenylindole (DAPI). DAPI and rhodamine-stained bacteria on the filter were counted from over 20 microscopic fields under an epifluorescent microscope, and the cell numbers were averaged.

All experiments were carried out in triplicate, and the mean values are presented.

Results and Discussion

Direct counts of soil bacteria

Soil microorganisms are involved in soil genesis, the development of soil structure, and a variety of other soil processes. As the microbial population and distribution after wildfire can be used as indicators for the ecological state of the soil, the structure of the microbial community can be used as an indicator for the degree to which the burned forest has recovered (Ahn *et al.*, 2002). In this study, the direct bacterial count of the surface layer (0-5 cm depth) of the unburned control soil was 8.7×10^8 cells/(g · soil) on April 9 (Fig. 1A). This count increased by a factor of 3 by April 21, but this was probably largely attributable to rainfall (11.9 mm) occurring on April 11. It has been reported that remoistening increases the microbial activity in dried soil, probably by the liberation of absorbed organic nutrients (Stevenson, 1956). The study area, located in the eastern Taebaek Mountain region, was experiencing very dry weather due to the Föhn phenomenon, which leads to low precipitation. Therefore, the microflora in the dry soil of this area might have been significantly affected by this rainfall. Lund and Goksøyr (1980) reported a 5-6 fold increase in the direct bacterial counts after 5 to 10 days of rewetting in podzol soil.

The total bacterial count in the lightly burned soil, 12.3×10^8 cells/(g · soil) April 9, was higher than that recorded in the control soil, and these increased levels persisted for about 1 month (Fig. 1A). Vázquez *et al.* (1993) also reported an increase in microbial population, including aerobic heterotrophic bacteria, after 1 month of wildfire in an Atlantic forest of 30-year old *Pinus pinaster*. However, in this case, the positive effect of the fire did not persist for as long a time. It has been established that low-intensity fire does not have a major effect on microbial populations, and in some cases, the number of heterotrophic microbes can increase as a consequence of bacterial utilization of fire-killed vegetation as a growth substrate (Neary *et al.*, 1999). The bacterial counts in the severely burned soil persisted at a lower level than those of the control and lightly burned soils, and have not changed significantly during the study period (Fig. 1A). Moreover, there might be some fire-killed or fire-inactivated cells in our samples, which can be enumerated by direct counting. Although most heat is lost to the atmosphere during a fire, some of the heat is transferred into the soil, killing or damaging soil organisms (Walstad *et al.*, 1990). Therefore, the severe fire in this site had a massive effect on microbes, especially in the surface soil, and the recovery of bacterial populations appeared to be arduous during the short period immediately following the wildfire.

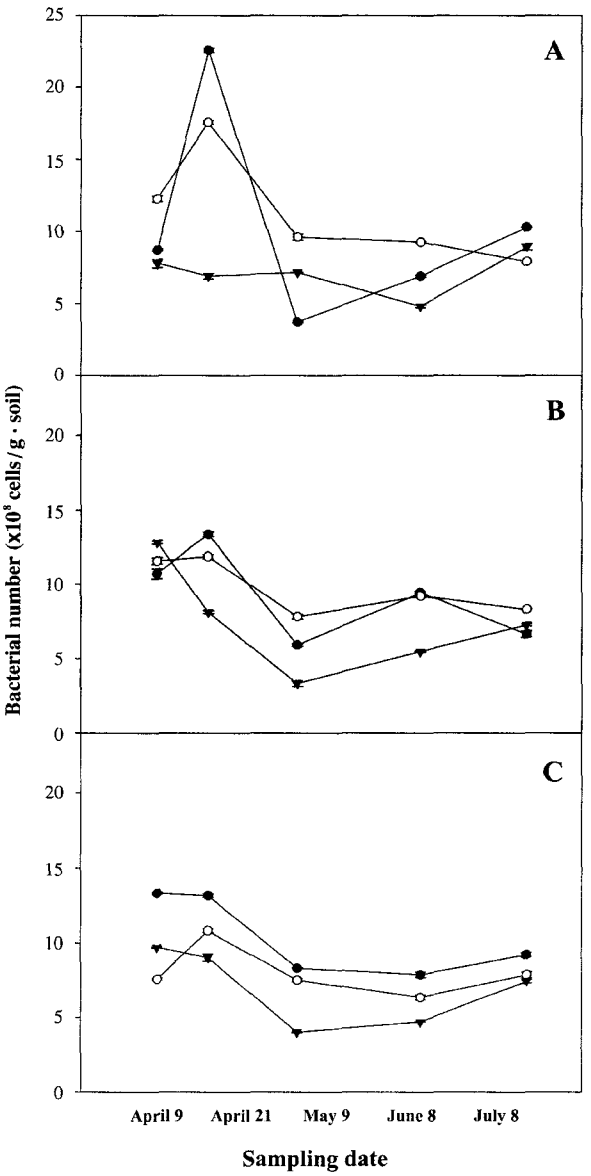

Fig. 1. Changes of bacterial counts measured by acridine orange direct counting at 3 different soil depths (A : 0-5 cm, B: 6-15 cm, C: 16-25 cm) after a wildfire in a coniferous forest in Kangnung, Korea. ●; unburned control soil, ○; lightly burned soil, ▼; severely burned soil.

The patterns of change with regard to the bacterial counts in the control, lightly burned and severely burned soils were quite similar in the middle layer (6-15 cm depth) and deep layer (16-25 cm) soils (Fig. 1B and 1C). Total bacterial counts of the severely burned soils at both depths were fairly consistently lower than those of the control and lightly burned soils. The fluctuations occurring in subsurface soils during the study period tended to be less pronounced than those in the topsoil, and the differences between the subsurface soils of the control, lightly burned, and severely burned sites were also less dramatic than those observed in the surface soil. It has been well-established that subsurface soil is less affected by fire, and fires effects on microorganisms are greatest in the organic horizon and/or the top 1-2 cm of soil (Neary *et al.*, 1999). On July 8, 3½ months after the wildfire, the direct counts of all soil samples had become similar, indicating a recovery of the pre-fire soil microbial population levels.

Although the direct bacterial count may reveal changes in soil bacterial populations, it can also include many dead cells. Furthermore, many fluorescent mineral particles tend to be included in soil suspensions, making the enumeration of soil bacteria by direct counting more difficult and less accurate. Therefore, other enumerating methods should be applied in any precise examination of the structure of a soil bacterial community.

Changes of bacterial community structure after wildfire

Investigation of the structure of microbial communities in a given environment is crucial in the examination of fundamental ecosystem processes. Although the importance of soil microorganisms in ecological processes is well known, the role of microbial diversity and community structure in maintaining the sustainability of an ecosystem is not yet so well understood (Neary *et al.*, 1999). The ratio of eubacteria, determined by the FISH method, to total bacteria, enumerated by direct counting, was highly variable, and in most cases no definite pattern of change could be discerned between soil samples from different depths and fire intensities (Figs. 2, 3 and 4). In this study, the range of these ratios was 30.3-84.7%, and these ratios were generally higher in the burned soils than in the control soils. Hicks *et al.* (1992) reported that 35-67% of direct counts of natural bacterioplankton were detectable by the FISH method with a EUB probe. Ratios between 40.2-80.7% were determined in snow and in a mountain lake (Alfreider *et al.*, 1996). Although some Archaea, which can not be detected with a EUB probe, might be in soil, these somewhat low ratios of eubacteria to direct counts were primarily due to the differences between the two enumeration techniques. Hicks *et al.* (1992) reported that about 30% of total count was lost during the transfer from filter to slides in the FISH method. Probe permeability is another problem with FISH analysis, as not all bacterial cells, especially Gram-positive bacteria and bacteria with capsules or slime layers take up the rRNA probe including EUB easily. This is another factor which may effectively lower the measured percentage of eubacteria (MacGregor, 1999).

In this study, the soil microbial community was inves-

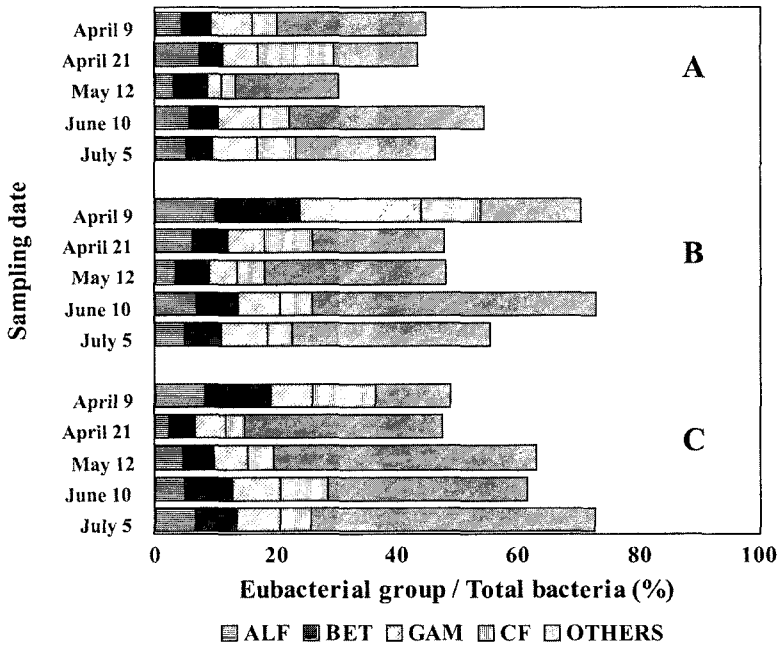

Fig. 2. Changes in bacterial community structure in surface layer soils (0-5 cm depth) of the unburned control site (A), lightly burned site (B) and severely burned site (C) after wildfire in a coniferous forest. ALF; α-Proteobacteria, BET; β-Proteobacteria, GAM; γ-Proteobacteria, CF; *Cytophaga-Flavobacterium* group, OTHERS; Other eubacteria.

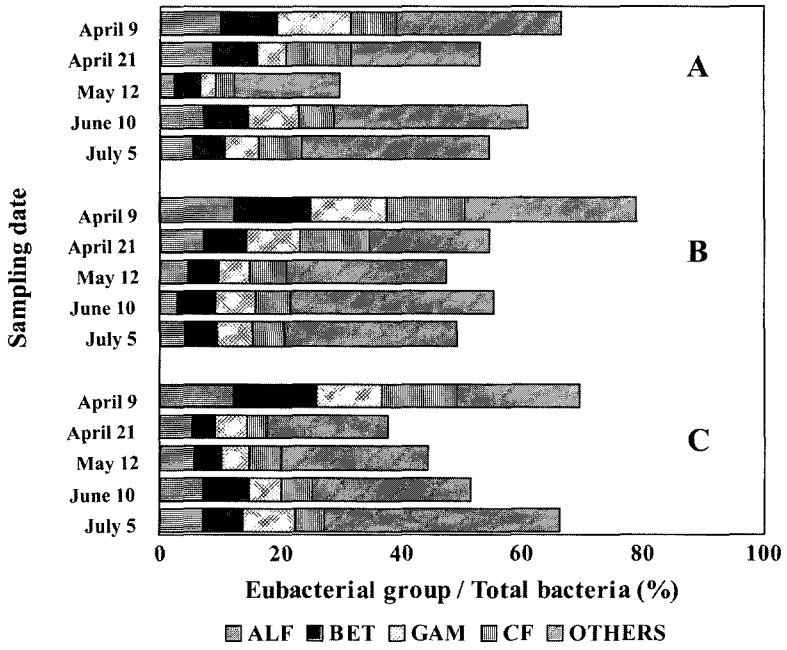

Fig. 3. Changes in bacterial community structure in middle layer soils (6-15 cm depth) of the unburned control site (A), lightly burned site (B) and severely burned site (C) after wildfire in a coniferous forest. ALF; α-Proteobacteria, BET; β-Proteobacteria, GAM; γ-Proteobacteria, CF; *Cytophaga-Flavobacterium* group, OTHERS; Other eubacteria.

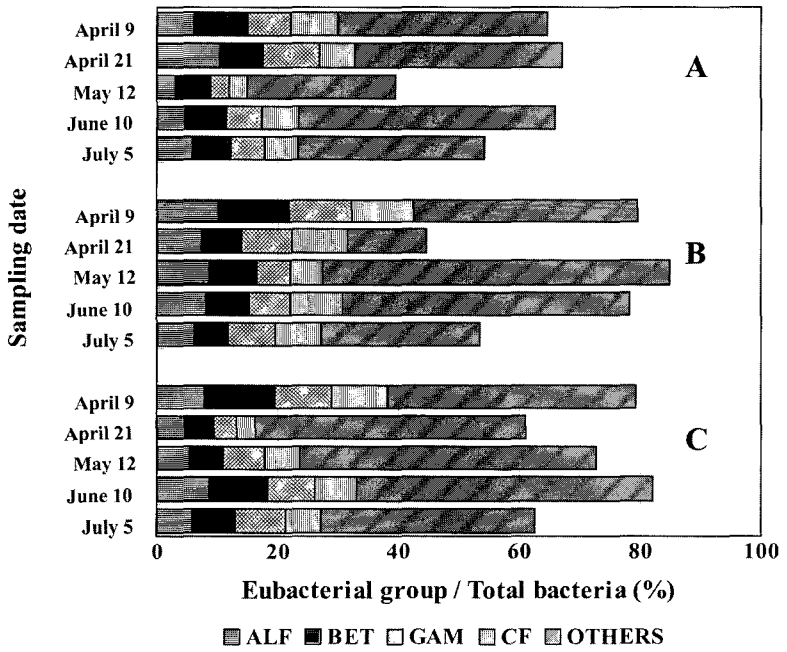

Fig. 4. Changes in bacterial community structure in deep layer soils (16-25 cm depth) of the unburned control site (A), lightly burned site (B) and severely burned site (C) after wildfire in a coniferous forest. ALF; α-Proteobacteria, BET; β-Proteobacteria, GAM; γ-Proteobacteria, CF; *Cytophaga-Flavobacterium* group, OTHERS; Other eubacteria.

tigated with 5 different rRNA probes. The eubacteria were divided into the α-, β-, and γ-proteobacteria, and the *Cytophaga-Flavobacterium* group, and others, which were not hybridized with the probes targeted to the previous 4 groups among the eubacteria, were classified as "Other eubacteria". These 4 groups of bacteria are the most abundant group in a variety of natural environments, and the pioneering investigations of microbial community structure were performed with these groups of rRNA probes (Hicks *et al.*, 1992; Alfreidner *et al.*, 1996). In surface soils from unburned control sites, the ratios of α-, β-, and γ-proteobacteria, *Cytophaga-Fla-*

vobacterium group, and the Other eubacteria group to total eubacteria were 9.9, 10.6, 15.5, 9.0, and 55.0%, respectively, on April 9 (Fig. 2). Those ratios might represent pre-fire values, although they were not examined on or before the wildfire which occurred on March 21. The ratios of α-, β-, and γ-proteobacteria and the *Cytophaga-Flavobacterium* group ranged from 7.3-11.0%, 7.8-10.6%, 7.5-16.05%, and 8.22-8.37%, respectively, during the study period. In contrast, Other eubacteria accounted for 32.2-59.1% in the control surface soil, which is a much larger percentage than the previous 4 groups. The ratios of each group were relatively stable, with the exception of the larger *Cytophaga-Flavobacterium* group and the smaller Other eubacteria group, on April 21, possibly due to the rainfall on April 11. When the bacterial community structure in the same soil was analyzed by terminal restriction fragment length polymorphism (T-RFLP), the proportions of Actinobacteria, α-proteobacteria, Bacilli, β-proteobacteria, γ-proteobacteria, Clostridia and Sphingobacteria were suggested to be 31, 16, 13, 10, 10, 6, and 4%, respectively. Among these groups, Gram-positive bacteria comprised 50%, and Gram-negative bacteria only 40% (Kim, 2002). The results obtained from T-RFLP should be corroborated by other tests, however, the ratio of other unknown eubacteria seemed to be higher because of the high proportion of Gram-positive bacteria in this soil.

The ratios of α-, β-, and γ-proteobacteria and *Cytophaga-Flavobacterium* group to total eubacteria increased in the surface and middle layer soils immediately after the wildfire, whereas the Other eubacterial proportion decreased right after wildfire (on April 9) (Figs. 2 and 3), but they returned to pre-fire levels thereafter. In contrast, the proportions of the 5 groups of eubacteria in the subsurface soil layer (16-25 cm) exhibited no significant fluctuations during the study period (Fig. 4), indicating that the fire effect was not strong enough to change the structure of the microbial community in this area. This is also likely due to the temperature insulating effect of soil (Vasander and Lindholm, 1985; Swift *et al.*, 1993). The increases in the α-, β-, and γ-proteobacteria and *Cytophaga-Flavobacterium* groups in the surface soils after wildfire may be partially due to increases in the population of bacteria which can degrade several recalcitrant organic compounds produced during forest fires. These compounds include polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated dibenzodioxins, and dibenzofurans (Martínez *et al.*, 2000; Gabos *et al.*, 2001). Although there are some exceptional bacterial groups, such as *Bacillus* and *Mycobacterium*, with the ability to degrade such compounds, most representative bacteria in this category are included in the proteobacteria: *Sphingomonas* in the α-proteobacteria, *Alcaligenes*, *Burkholderia* and *Commamonas* in the β-proteobacteria, *Pseudomonas*, *Acinetobacter* and *Shewanella* in the γ-proteobacteria, and *Flavobacterium* in the *Cytophaga-Flavobacterium* group (Wackett and Hershberger, 2001; Madigan *et al.*, 2003).

It has been established that fire effects depend on fire intensity and duration, soil water content, and organic matter content (Neary *et al.*, 1999). As soil environments are quite heterogeneous in terms of texture, water content, and organic matter content, fire effects are highly variable, rendering the effects of fire on soil microbial communities even harder to examine and analyze. In this study, we monitored the soil bacterial community after wildfire by both direct counting, and the FISH method. It was demonstrated that the wildfire affected mostly surface soil, and that the total bacterial population and bacterial community structure, after disruption by fire, began to recover quite quickly, such that the original level seemed to be restored only 3 months after the wildfire. The FISH method is known to be easier and more rapid than other molecular biological techniques for the detection of bacteria of various taxonomic levels (Manz *et al.*, 1992; Amann *et al.*, 1995). Although the microscopic observation inherent in FISH analysis is difficult to use with soil samples, due to background fluorescence and interference by mineral particles in the soil, it was carefully, but effectively, used in this study. The proportion of bacteria in the total bacterial population which could not be detected by FISH was quite large in some cases, and the reasons for this should be revealed in the near future. The proportion of other unknown eubacteria, which appeared to be largely Gram-positive bacteria, was higher than expected, but all of the oligonucleotide probes used in this study targeted for subgroups of eubacteria were for Gram-negative bacteria. To date, the FISH method has been applied mainly to studies of aquatic systems and biofilms (Trebesius *et al.*, 1994; Alfreider *et al.*, 1996), and the probes used in this study were also developed for those purposes (Ahn *et al.*, 2002; Belkova *et al.*, 2003). The thickness of bacterial cell walls may sometimes inhibit the penetration of probes into the cytoplasm, and reliable pretreatment techniques for Gram-positive bacteria still need to be developed (Hartmann *et al.*, 1997; MacGregor, 1999). Therefore, all the problems in the detection of Gram-positive bacteria should be resolved before the pursuit of a comprehensive FISH analysis of the soil bacterial community.

Acknowledgments

This research was performed for the Natural Hazards Prevention Research Project, one of the Critical Technology-21 Programs, funded by the Ministry of Science and Technology of Korea. This study was also partially supported by Research Institute for Basic Science, Kangwon National University.

References

Acea, M. and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. *FEMS Microbiol. Ecol.* 20, 33-39.

Acea, M., A. Prieto-Fernández, and N. Diz-Cid. 2003. Cyanobacterial inoculation of heated soils: effect on microorganisms of C and N cycles and on chemical composition in soil surface. *Soil Biol. Biochem.* 35, 513-524.

Ahn, T., J. Lee, D. Lee, and H. Song. 2002. Ecological monitoring of soil microbial community after forest fire, p. 144-175. *In* Proceedings of Symposium on Prevention of large forest fire and remediation of ecosystem. Korea Forest Research Institute, Seoul, Korea.

Alfreider, A., J. Pernthaler, R. Amann, B. Sattler, F. Glöckner, A. Wille, and R. Psenner. 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by *in situ* hybridization. *Appl. Environ. Microbiol.* 62, 2138-2144.

Amann, R., W. Ludwig, and K. Schleifer. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59, 143-169.

Bååth, E., A. Frostegard, T. Pennanen, and H. Fritze. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biol. Biochem.* 27, 229-240.

Belkova, N.L., V.V. Dryukker, S.H. Hong, and T.S. Ahn. 2003. A study of the composition of the aquatic bacterial community of Lake Baikal by the in situ hybridization method. *Microbiol.* 72, 244-245.

Gabos, S., M. Ikonomou, D. Schopflocher, B. Fowler, J. White, E. Prepas, D. Prince, and W. Chen. 2001. Characteristics of PAHs, PCDD/Fs and PCBs in sediment following forest fires in Northern Alberta. *Chemosphere.* 43, 709-719.

Glöckner, F.O., B.M. Fuchs, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl. Environ. Microbiol.* 65, 3721-3726.

Harris, P., H. Schomberg, P. Banks, and J. Giddens. 1995. Burning, tillage and herbicide effects on the soil microflora in a wheat-soybean double-crop system. *Soil Biol. Biochem.* 27, 153-156.

Hartmann, A., B. Aâmus, G. Kirchhof, and M. Schloter. 1997. Direct approaches for studying soil microbes, p. 279-309.*In* J. van Elsas, J. Trevors, and E. Wellington (eds.) Modern Soil Microbiology, Marcel Dekker, Inc., New York.

Hicks, R., R. Aβmann, and D. Stahl. 1992. Dual staining of natural bacterioplankton with 4, 6-diamidino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom level 16S rRNA sequences. *Appl. Environ. Microbiol.* 58, 2158-2163.

Hobbie, J., R. Daley, and S. Japer. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33, 1225-1228.

Kim, J.-B. 2002. Molecular ecological analysis of the structural changes of bacterial community in forest soil stressed by fire. M.S. Thesis, Chungbuk National University, Cheongju, Korea.

Lund, V. and J. Goksøyr. 1980. Effects of water fluctuations on microbial mass and activity in soil. *Microb. Ecol.* 6, 115-123.

MacGregor, B. 1999. Molecular approaches to the study of aquatic microbial communities. *Curr. Opin. Biotechnol.* 10, 220-224.

Madigan, M., J. Martinko, and J. Parker. 2003. Brock Biology of Microorganisms, p. A5-A13. Prentice Hall, Upper Saddle River.

Manz, W., R. Amann, W. Ludwig, and M. Wagner. 1992. Phylogentic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: Problems and solutions. *Syst. Appl. Micorbiol.* 15, 593-600.

Martínez, M., J. Díaz-Ferrero, R. Martí, F. Broto-Puig, L. Comellas, and M. Rodríguez-Larena. 2000. Analysis of dioxin-like compounds in vegetation and soil samples burned in Catalan forest fire. Comparison with the corresponding unburned material. *Chemosphere* 41, 1927-1935.

Neary, D., C. Klopatek, L. DeBano, and P. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. *Forest Ecol. Manage.* 122, 51-71.

Sharma, G.D. 1981. Effect of fire on soil microorganisms in a Meghalaya pine forest. *Folia Microbiol.* 26, 321-327.

Stevenson, I.L. 1956. Some observations on the microbial activity in remoistened air-dried soils. *Plant and Soil* 8, 171-182.

Swift, L., K. Elliott, R. Ottmar, and R. Vihnanek. 1993. Site preparation burning to improve Southern Appalachian pine-hardwood stands: fire characteristics and soil erosion, moisture, and temperature. *Can. J. Forest Res.* 23, 2242-2254.

Trebesius, K., R. Amann, W. Ludwig, K. Mühlegger, and K. Schleifer. 1994. Identification of whole fixed bacterial cells with nonradioactive rRNA targeted transcript probes. *Appl. Environ. Microbiol.* 60, 3228-3235.

Vasander, H. and T. Lindholm. 1985. Fire intensities and surface temperatures during prescribed burning. *Silva Fennica.* 19, 1-15.

Vázquez, F., M. Acea, and T. Carballas. 1993. Soil microbial populations after wildfire. *FEMS Microbiol. Ecol.* 13, 93-104.

Wackett, L. and C. Hershberger. 2001. Biocatalysis and Biodegradation : Microbial transformation of organic compounds, p. 39-69. ASM Press, Washington, D.C.

Walstad, J., S. Radosevich, and D. Sandberg. 1990. Introduction to natural and prescribed fire in Pacific Northwest forests, p. 3-5. *In* J.D. Walstad, S.R. Radosevich, and D.V. Sandberg (eds.), Natural and Prescribed Fire in Pacific Northwest Forests Oregon State University Press, Corvallis.