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Seasonal Changes in the Rhizosphere Microbial Communities Associated with Field-Grown Genetically Modified Canola (*Brassica napus*)

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The introduction of transgenic plants into agricultural ecosystems has raised the question of the ecological impact of these plants on nontarget organisms, such as soil bacteria. Although differences in both the genetic structure and the metabolic function of the microbial communities associated with some transgenic plant lines have been established, it remains to be seen whether these differences have an ecological impact on the soil microbial communities. We conducted a 2-year, multiple-site field study in which rhizosphere samples associated with a transgenic canola variety and a conventional canola variety were sampled at six times throughout the growing season. The objectives of this study were to identify differences between the rhizosphere microbial community associated with the transgenic plants and the rhizosphere microbial community associated with the conventional canola plants and to determine whether the differences were permanent or depended on the presence of the plant. Community-level physiological profiles, fatty acid methyl ester profiles, and terminal amplified ribosomal DNA restriction analysis profiles of rhizosphere microbial communities were compared to the profiles of the microbial community associated with an unplanted, fallow field plot. Principal-component analysis showed that there was variation in the microbial community associated with both canola variety and growth season. Importantly, while differences between the microbial communities associated with the transgenic plant variety were observed at several times throughout the growing season, all analyses indicated that when the microbial communities were assessed after winter, there were no differences between microbial communities from field plots that contained harvested transgenic canola plants and microbial communities from field plots that did not contain plants during the field season. Hence, the changes in the microbial community structure associated with genetically modified plants were temporary and did not persist into the next field season.

The global transgenic crop acreage has increased yearly since the commercial introduction of transgenic crops in 1996. In fact, approximately 59 million hectares of genetically modified crops were grown worldwide in 2002 (20). However, the majority (99%) of these genetically modified crops were grown in four countries (United States, Argentina, Canada, and People's Republic of China) (20). Many countries are still in the process of assessing the environmental risks of genetically modified plants and drafting legislation to regulate the use of commercial genetically modified crops. The possibility of nontarget effects of transgenic plants on native flora and fauna, including soil microorganisms, is one cause of concern in environmental risk assessments of genetically modified plants (30, 35). Several studies have shown that genetically modified plants can affect the rhizosphere microbial community (1, 8, 9, 12, 17–19, 26, 32, 33).

A complicating issue in environmental risk assessments is that seasonal shifts in rhizosphere microbial communities have been documented. For example, plant development has been shown to significantly affect the biodiversity of a *Burkholderia cepacia* population associated with maize roots (7). Also, using a PCR-based community profiling technique, denaturing gradient gel electrophoresis, Smalla et al. (34) recently presented evidence showing that the abundance and composition of bacterial rhizosphere populations associated with strawberry, potato, and oilseed rape changed over the field season. The composition and activity of rhizosphere microflora are likely to be altered as a function of time because of changes that occur in the exudation patterns of roots as plants age, and as a consequence, genotype selection may occur as microorganisms adapt to these changes (7). Furthermore, soil microbial growth and metabolic activity often increase in the spring and summer in conjunction with higher soil temperatures, mobilization of accumulated soil organic matter, and accelerated root growth (16). For these reasons, Grayston et al. (16) suggested that caution should be taken when conclusions are drawn about root-associated microbial community structure based on results for a single time point.

Previously described studies that examined the influence of transgenic plants on the root-associated microbial communities throughout a field season showed that the effects of transgenic plants on these microbial communities are subject to seasonal variation. Lottmann et al. (25) found that a transgenic line of potato expressing T4 lysozyme influenced the composition of root-associated bacterial antagonists; however, this was dependent both on the field year and on the time of sampling. Denaturing gradient gel electrophoresis analysis of

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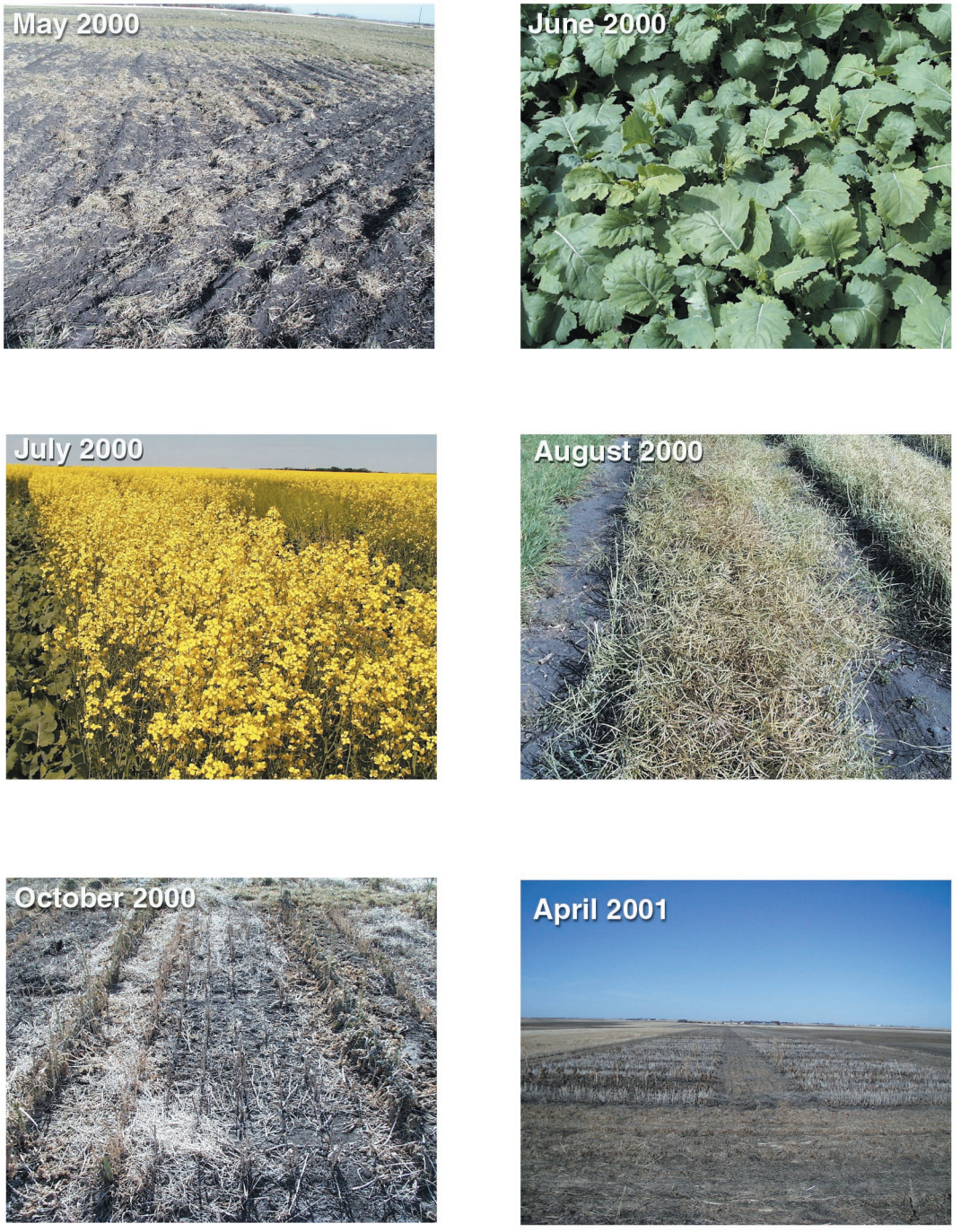

FIG. 1. Growth stages of canola sampled during the study. Microbial community samples were obtained at designated stages of growth corresponding to preseeding, rosette, flowering, maturity, fall stubble, and overwintered stubble. The sampling dates were in May, June, July, August, October, and the following April.

the bacterial rhizosphere community associated with the transgenic potatoes revealed seasonal shifts in the composition of the microbial community (26). Furthermore, the community-level physiological profiles (CLPP) of the microbial community associated with another transgenic potato that produced *Galanthus nivalis* agglutinin and concanavalin A lectins were also subject to seasonal variation (17).

Canada is currently the third largest producer of genetically modified crops in the world behind the United States and Argentina. Canola (*Brassica* sp., oilseed rape) is the most important genetically modified crop in Canada, and to date nearly 80 varieties of herbicide-resistant canola have been granted unrestricted registration by the Canadian Food Inspection Agency (5). Previous studies done our lab that examined genetically modified canola grown at field sites in Saskatchewan, Canada, showed that both rhizosphere and root

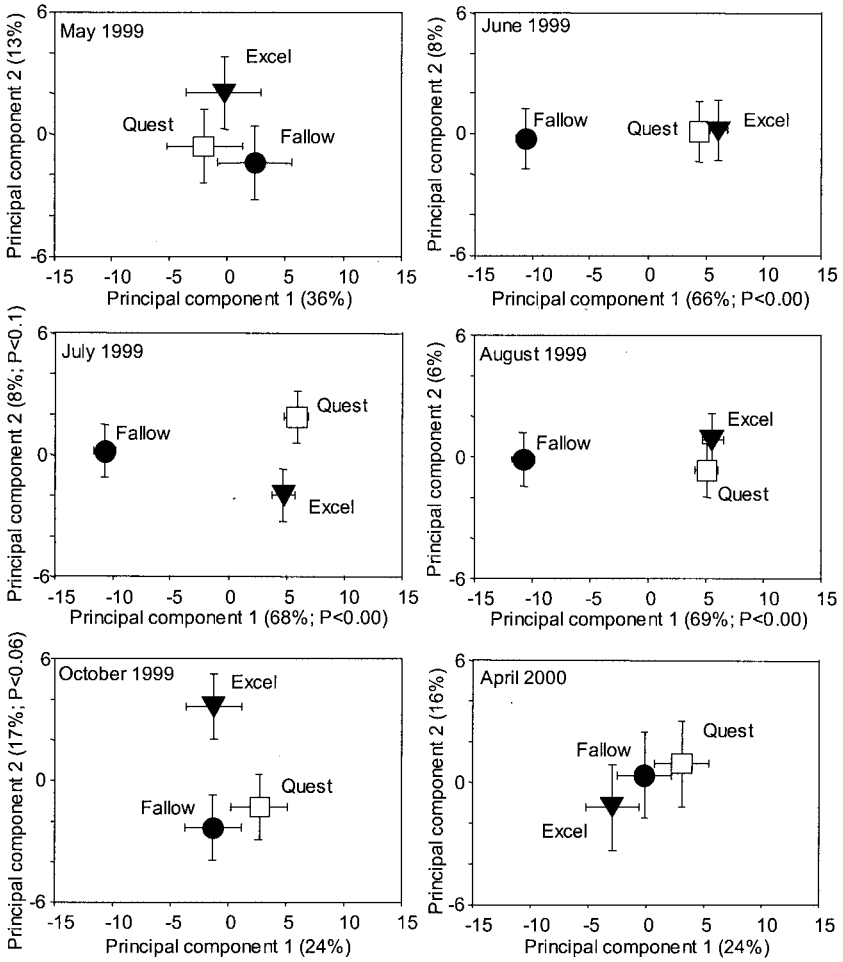

FIG. 2. PCA of CLPP obtained for microbial communities from fallow soil and rhizosphere microbial communities of canola varieties grown at Watson, Saskatchewan, sampled in May, June, July, August, and October 1999 and April 2000. Symbols: ●, fallow soil ($n = 4$); ▲, conventional variety Excel ($n = 4$); □, genetically modified variety Quest ($n = 4$). The error bars indicate the standard errors of the means. The level of variation explained by each principal component is indicated in parentheses. *P* values are indicated when there was a significant variety effect, as determined by ANOVA.

interior microbial populations associated with a transgenic canola variety, Quest, have altered CLPP and fatty acid methyl ester (FAME) profiles compared to the profiles of a nontransgenic counterpart (12, 32, 33). However, these studies examined bacterial populations associated with canola plants at one stage of growth (flowering). The objectives of the present study were to identify changes in the soil microbial community associated with growing genetically modified canola and to determine whether these changes persisted in the soil over the growing season or were temporary and dependent on the presence of the plant. A 2-year study was conducted at two field sites in Saskatchewan during the 1999 and 2000 field seasons. The microbial communities associated with a genetically modified canola variety, a conventional canola variety, and a fallow soil were assessed at six times during the field season by using three different methods of microbial community analysis, FAME analysis, CLPP analysis, and terminal amplified ribosomal DNA (rDNA) restriction analysis (T-ARDRA).

MATERIALS AND METHODS

Experimental design. The treatments included a commercially available transgenic canola variety (Quest), a conventional canola variety (Excel), and an unplanted fallow control plot. Quest is a glyphosate-resistant variety generated by *Agrobacterium tumefaciens*-mediated transformation to include 5-enolpyruvylshikimate-3-phosphate synthase and glyphosate oxidoreductase (29). Quest was developed from Excel parental material, and therefore these varieties were considered nearly isogenic lines in this study (32).

Field plots were located at two field sites, in Watrous and Watson, Saskatchewan, Canada. Access to field plots was provided by the Saskatchewan Wheat Pool. The plots were seeded at each site in a replicated ($n = 4$) randomized complete block design. The studies at Watrous were performed for two field years (1999 and 2000), while the field studies at Watson were terminated after the first field year (1999) due to loss of access to the field site. Canola seeds were planted on 8 May 1999 and 12 May 2000, and the plants were harvested in the first week of September in both field years. Canola was planted according to a standard crop rotation in Saskatchewan consisting of a maximum of one canola crop every 4 years; therefore, canola was not planted in a field in which canola would be planted within 4 years.

Sample processing. Both field soil and rhizosphere samples were collected on various dates corresponding to preseeding and when plants were at the rosette, flowering, maturity, postharvest fall stubble, and overwintered stubble stages

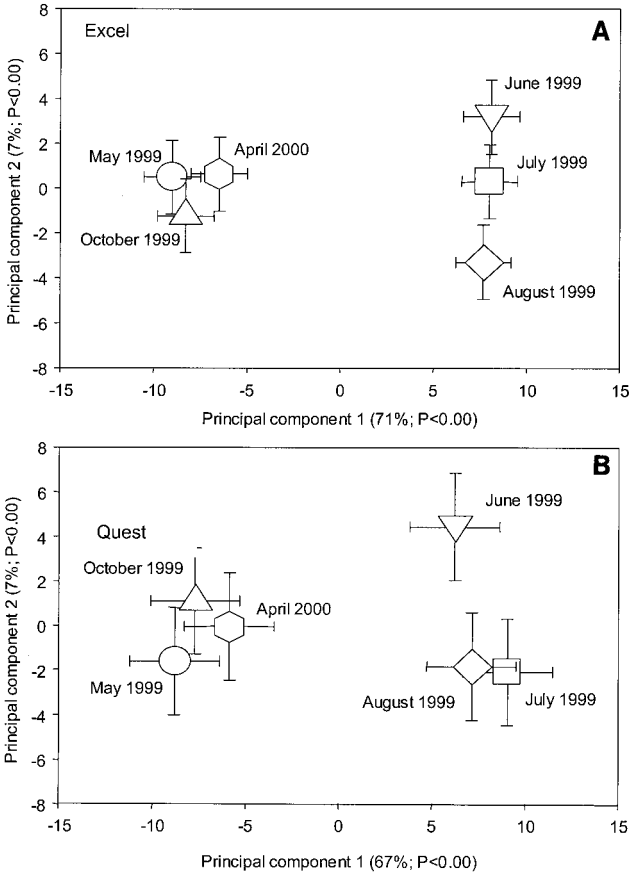

FIG. 3. PCA of CLPP obtained for rhizosphere microbial communities of conventional canola variety Excel (A) and genetically modified canola variety Quest (B) grown at Watrous, Saskatchewan, in 1999. Each symbol indicates the average for four replicates at one field site ($n = 4$). The error bars indicate the standard errors of the means. The levels of variation explained by individual principal components are indicated in parentheses. *P* values are indicated when there was a significant sampling time effect, as determined by ANOVA.

(universal growth stages 0.0, 1.4, 6.5, 8.9, 9.7, and 9.7) (23) (Fig. 1). The sampling dates for the 1999 field study were 8 May, 22 June, 15 July, 17 August, and 7 October 1999 and 19 April 2000. The sampling dates for the 2000 field study were 12 May, 22 June, 20 July, 21 August, and 17 October 2000 and 25 April 2001. At growth stages at which plants were present (i.e., rosette, flowering, maturity) 10 plants with adhering soil were taken randomly from throughout each of the four replicate plots and combined; each grouped sample was considered a replicate of rhizosphere soil. At growth stages at which no root system was present (i.e., preseeding, fall stubble, and overwintered stubble) 10 soil samples were taken randomly from throughout each of the four replicate plots and combined; the grouped samples were considered a replicate of fallow soil. The method used to process soil samples was described previously (12).

On each processing day (24 h after soil sampling) soil (5 g) was placed into a dilution bottle containing 95 ml of phosphate-buffered saline (PBS) (1.2 g of Na_2HPO_4 per liter, 0.18 g of NaH_2PO_4 per liter, 8.5 g of NaCl per liter) and shaken on a rotary shaker (200 rpm) at 22°C for 20 min. In addition, a portion (5 g) of roots with adhering soil was placed into a 1-liter Erlenmeyer flask containing 495 ml of PBS and shaken on a rotary shaker (200 rpm) at 22°C for 20 min. Dilutions (1:10) of the resulting slurry were made in sterile PBS, and the 10^{-4} dilution was saved for inoculating Biolog GN2 plates. The slurry was transferred to a 500-ml centrifuge tube and centrifuged at 2,000 × *g* for 5 min to pellet the rhizosphere soil and to remove excess water. A sample of rhizosphere soil was transferred to microcentrifuge tubes (1.5 ml) and stored at −20°C for DNA and FAME extraction.

CLPP. The CLPP analysis was performed as described by Siciliano and Germida (31) with Biolog gram-negative (GN2) microplates (Biolog, Inc., Hayward, Calif.). Briefly, 100 μl of a 10^{-4} dilution was inoculated into each well, and the plates were incubated at 28°C for 5 days. Color development was measured by determining the optical density at 590 nm with an automated microplate reader (Molecular Devices, Inc., Sunnyvale, Calif.) and Microlog 3E software (Biolog, Inc.). The average well color development (AWCD) was calculated as described by Garland and Mills (15).

FAME analysis. FAME analysis of the soil microbial community was performed as described by Cavigelli et al. (6) and Siciliano et al. (33). Briefly, 5 g of soil was mixed with 5 ml of methanoic NaOH (15% [wt/vol] NaOH in 50% [vol/vol] methanol) and saponified at 100°C for 30 min. Esterfication of fatty acids was performed with 10 ml of 3.25 N HCl in 46% (vol/vol) methanol at 80°C for 10 min. The FAMEs were extracted in 1.5 ml of methyl-*tert*-butyl ether–hexane(1:1, vol/vol) and centrifuged at 110 × *g* for 5 min, and the top phase was transferred to a 10-cm test tube. This organic extract was washed with 3 ml of 1.2% (wt/vol) NaOH and analyzed with a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.). FAME peaks were automatically integrated with the Hewlett-Packard 3365 ChemStation software, and FAMEs were identified by using the MIDI microbial identification system software (Sherlock TSBA Library, version 3.80; Microbial ID, Inc., Newark, Del.). In order to minimize the fatty acids derived from plant and animal sources, fatty acids with chains longer than 20 carbons, which are generally more characteristic of eukaryotic organisms than prokaryotes, were not included in the statistical analysis (6, 14).

T-ARDRA. Soil DNA was extracted directly from 0.5 g of rhizosphere soil by using a FASTDNA spin kit for soil (Bio 101, Carlsbad, Calif.) as described by Borneman et al. (2). DNA (50 μl) was purified by using a Sephracryl-400h MicroSpin column (Pharmacia Biotech Inc.). The presence of DNA was confirmed by electrophoresis on a 1% agarose gel stained with ethidium bromide.

Bacterial 16S rDNA was selectively amplified by PCR performed with oligonucleotide primers designed to anneal to conserved positions in the 3′ and 5′ regions of bacterial 16S rDNA. The forward primer, 8F (AGAGTTTGATCCT GGCTCAG), corresponded to positions 8 to 27 of *Escherichia coli* 16S rRNA (24), and the reverse primer, R10 (CATTGTAGCATCCGTTGAAG), corresponded to positions 1224 to 1242 of *E. coli* 16S rRNA (10). The forward primer was end labeled with [γ-^{32}P]ATP by using polynucleotide kinase. The amplification mixtures (final volume, 12.5 μl) contained 6.25 pmol of primer R10, 0.025 pmol of primer 8F, each deoxynucleoside triphosphate at a concentration of 1 mM, 5 mM magnesium chloride, 1.25 μl of 10× PCR buffer, and 0.625 U of *Taq* polymerase (Invitrogen, Burlington, Ontario, Canada). The PCR was performed with an automated thermal cycler (Robocycler; Stratagene, La Jolla, Calif.). The reaction began with a denaturation step at 95°C for 5 min, and this was followed by 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 45 s and then a final extension step at 72°C for 15 min. PCR products were restricted by using the *Cfo*I (GCG′C) and *Msp*I (C′CGG) restriction enzymes (Invitrogen) for 90 min at 37°C.

T-ARDRA was carried out with a Hoeffer SQ3 Sequencer vertical gel electrophoresis system (Amersham Pharmacia Biotech, Baie d'Urfé, Quebec, Canada). Aliquots (12.5 μl) of each digested product were mixed with 10 μl of loading dye buffer and resolved by electrophoresis through a 6% (wt/vol) nondenaturing acrylamide gel (ratio of acrylamide to *N*,*N*-methylenebisacrylamide, 19:1). Electrophoresis was carried out at 65 W for 4 to 5 h. In addition, an end-labeled [γ-^{32}P]ATP ladder (30 to 330 bp) was included on each gel.

In order to visualize the banding patterns, gels were transferred to 3-mm chromatography paper and placed along with Kodak XAR autoradiography film into an autoradiography film exposure cassette for 24 h. The film was developed and photographed with a digital camera.

Statistical analysis. To standardize FAME data, the adjusted response area of each sample was calculated by multiplying the percentage of each individual FAME by the total named area for the chromatogram (13). Soil FAME profiles were compared by principal-component analysis (PCA) by using a correlation matrix (Minitab v. 12; Minitab Inc., State College, Pa.). The principal-component data were analyzed by using analysis of variance (ANOVA).

The AWCD was used as a standardized reference point in color development (8, 15). Absorbance data (A_{590}) from microplates having AWCD of approximately 0.75 were used for statistical analysis. PCA was performed as described above.

Because DNA quantity cannot be consistently and accurately represented by band intensity on an autoradiograph, band intensity was not assessed; instead, T-ARDRA gels were scored for the presence or absence of bands as described in Konopka et al. (22). Molecular weights of bands were determined by using the

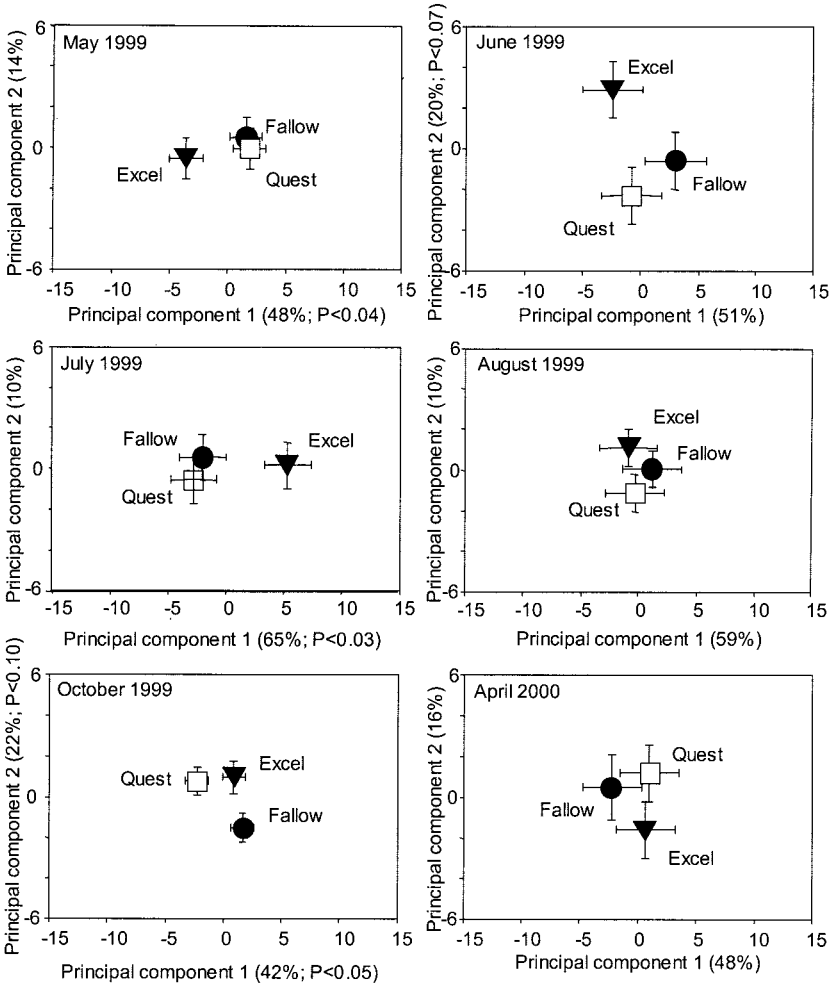

FIG. 4. PCA of FAME profiles obtained for microbial communities from fallow soil and for rhizosphere microbial communities of canola varieties grown at Watrous, Saskatchewan, sampled in May, June, July, August, and October 1999 and April 2000. Symbols: ●, fallow soil (n = 4); ▲, conventional variety Excel (n = 4); □, genetically modified variety Quest (n = 4). The error bars indicate the standard errors of the means. The level of variation explained by each principal component is indicated in parentheses. P values are indicated when there was a significant variety effect, as determined by ANOVA.

Geneprofiler software (Scanalytics Inc., Fairfax, Va.). Digital images of gels were analyzed by PCA.

RESULTS

CLPP of microbial communities. The plant variety significantly affected the CLPP of microbial communities in all soils and in all years tested. PCA indicated that between June and October there were significant differences between the carbon substrate utilization of microbial communities associated with the rhizosphere of canola plants and the carbon substrate utilization of the communities associated with a fallow unplanted field plot. For example, at Watson in the 1999 field year, there were significant differences in the CLPP of communities associated with the genetically modified variety Quest and the conventional variety Excel at the July and October sampling times (Fig. 2). Similar results were seen at the Watrous field site in the 1999 and 2000 field years (data not shown).

The microbial communities associated with the rhizospheres of both the conventional variety of canola, Excel, and the genetically modified variety of canola, Quest, grown at Watrous in 1999 resulted in significant differences in the CLPP over the field season (Fig. 3). However, there were no significant differences between the CLPP in May 1999 (preseeding stage) and the CLPP in April 2000 (overwintered stubble stage). This may indicate that while the carbon substrate utilization pattern of the microbial community does change over the field season, the changes do not reflect permanent changes in the microbial community structure. Similar CLPP for plants grown at Watson in the 1999 field season and at Watrous in the 2000 field season were observed (data not shown).

FAME profiles of microbial communities. Significant differences in the fatty acid compositions of the rhizosphere microbial communities and the communities from fallow soil were observed (Fig. 4 and 5). PCA of the fatty acid profiles of microbial communities associated with canola plants grown at Watrous in 1999 showed that in May, June, July, and October there were significant differences between the fatty acid profiles of the rhizosphere of the conventional canola variety (Excel) plants and the fatty acid

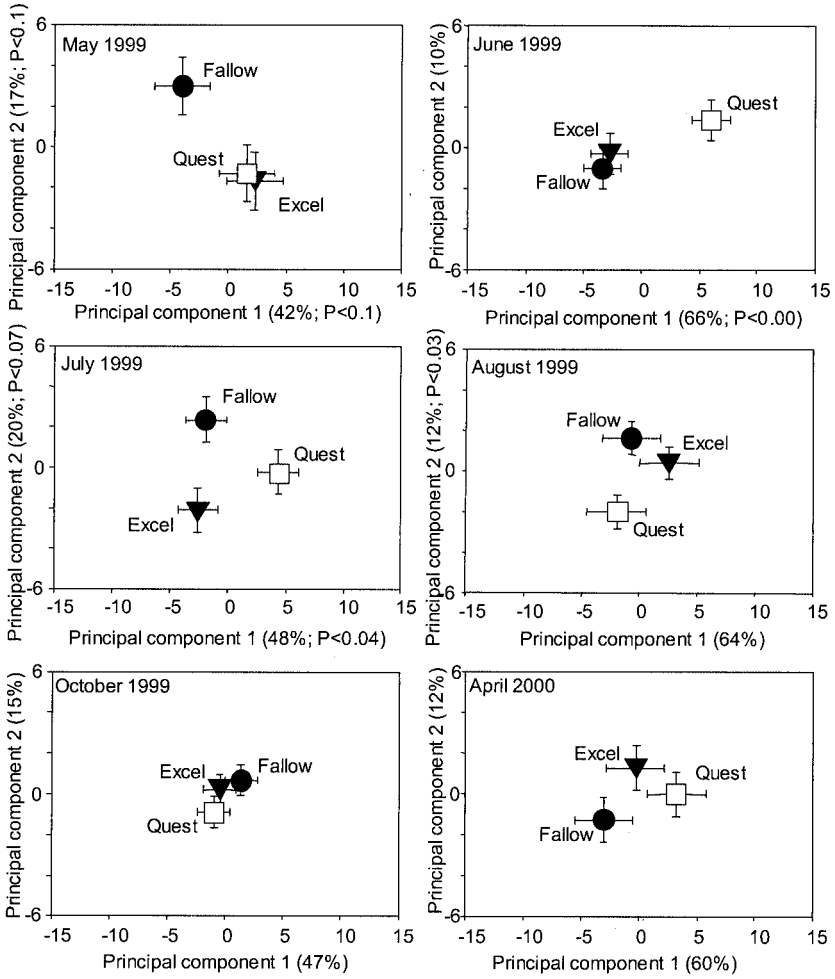

FIG. 5. PCA of FAME profiles obtained for microbial communities from fallow soil and for the rhizosphere microbial communities of canola varieties grown at Watson, Saskatchewan, sampled in May, June, July, August, and October 1999 and April 2000. Symbols: ●, fallow soil ($n = 4$); ▲, conventional variety Excel ($n = 4$); □, genetically modified variety Quest ($n = 4$). The error bars indicate the standard errors of the means. The level of variation explained by each principal component is indicated in parentheses. *P* values are indicated when there was a significant variety effect, as determined by ANOVA.

profiles of the rhizosphere of the genetically modified Quest plants and the unplanted fallow field plot (Fig. 4). Similarly, PCA of the FAMEs of microbial communities from plants grown at Watson in 1999 showed that there were significant differences between the fatty acid profiles of the rhizosphere communities and the profiles of the unplanted fallow plots for the May, June, July, and August sampling times (Fig. 5). In contrast, at Watrous in 2000, significant differences were observed only between the microbial communities for the preseeding sampling time (data not shown). Importantly, no significant differences between the fatty acid profiles of microbial communities from unplanted field plots and the fatty acid profiles of microbial communities from plots that contained canola plants were observed for the April sampling (overwintered stubble) for either field site or field year. In addition, the fatty acid composition of the rhizosphere community associated with the conventional variety, Excel, was significantly different from the fatty acid composition of the community associated with the genetically modified variety, Quest. This difference was revealed by PCA of the communities sampled in May, June, July, and October at Watrous in 1999 and in June, July, and August at Watson in 1999 (Fig. 6).

The fatty acid compositions of the rhizosphere community associated with canola and the community in fallow soil changed significantly over the field season (Fig. 6). In some cases the changes were temporary, and the fatty acid profile of the microbial community associated with overwintered stubble (April 2001) was not significantly different from the fatty acid profile of the initial microbial community assessed before seeding. An example is the microbial communities associated with Excel canola grown at Watrous in 2000 (Fig. 6A). In contrast, there were significant differences between the fatty acid composition of the microbial community associated with Quest plants sampled at the overwintered stubble growth stage and the fatty acid composition of the microbial community at the preseeding stage at Watrous in 1999 and 2000 (Fig. 6B and data not shown).

T-ARDRA profiles of microbial communities. PCA revealed no significant influence of plant growth stage on the T-ARDRA banding patterns associated with canola plants (data not shown).

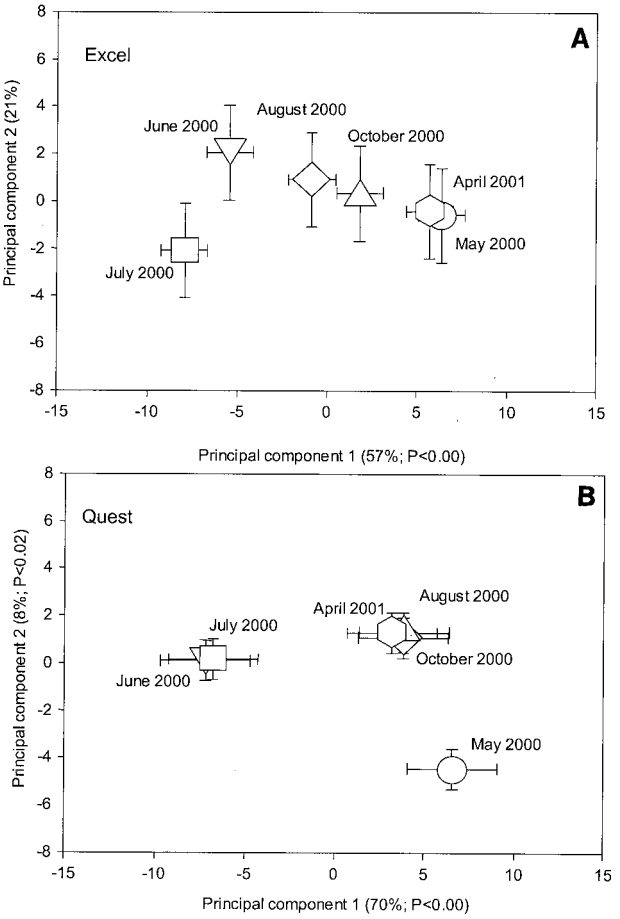

FIG. 6. PCA of FAME profiles obtained for rhizosphere microbial communities of conventional canola variety Excel (A) and genetically modified canola variety Quest (B) grown at Watrous, Saskatchewan, in 2000. Each symbol indicates the average for four replicates at one field site ($n = 4$). The error bars indicate the standard errors of the means. The levels of variation explained by individual principal components are indicated in parentheses. *P* values are indicated when there was a significant sampling time effect, as determined by ANOVA.

Moreover, at one of the field sites, Watrous, PCA of T-ARDRA profiles indicated that there were no significant differences between the banding patterns of microbial communities associated with canola plants and the banding patterns of the microbial communities associated with the unplanted fallow plot. Similarly, at the second field site, Watson, at five of the six sampling times there were no differences in the banding patterns of the microbial communities. The one exception was the June 1999 sampling time; at this time the T-ARDRA banding patterns of the microbial communities associated with genetically modified Quest plants and the communities from fallow soil were significantly different from the T-ARDRA banding patterns of the rhizosphere communities associated with Excel plants (Fig. 7).

DISCUSSION

The question posed in this study was whether changes in the bacterial community associated with growing a genetically modified canola variety, Quest, were permanent or depended on the presence of viable plants. This study showed that plant variety had a significant influence on CLPP, FAME, and T-ARDRA results at different field sites and in different years. There were no significant differences between the microbial communities associated with over-wintered canola stubble at the April sampling time (after plants were harvested in the preceding September) and the microbial communities associated with canola plants at the preseeding sampling time. This suggests that changes in the microbial community structure associated with genetically modified plants were not permanent. Two exceptions were observed. First, the fatty acid profiles of the rhizosphere communities and communities from fallow soil from Watrous in 1999 changed significantly over the field season. Environmental factors, such as moisture content, have previously been shown to influence the fatty acid profiles of soil microbial communities (3). Therefore, the environment may have played a role in changing the fatty acid profiles of the soil communities in this location, suggesting that differences in the microbial communities not related specifically to the growth of plants could have occurred. Second, at Watrous in 2000, the fatty acid profiles of the microbial community associated with cultivar Quest at the April sampling time, after plants were harvested, were different from the fatty acid profiles of the community associated with this cultivar in the previous spring, at the preseeding stage. However, it is important to note that at this field site, while there were differences in the microbial communities associated with the Quest and Excel field soils at the seeding stage, there were no significant differences between the microbial communities associated with Quest and Excel at the April sampling time, after harvest. Therefore, there were differences in the microbial communities before seeds were planted. Spatial variability has been documented in agricultural field plots containing transgenic potato plants (27).

Previous studies in which microbial communities were examined throughout a field season have often indicated that there is seasonal variability (7, 16, 17, 19, 25, 26, 34). The results of the present study also show that microbial community structure was influenced by seasonal variation, as indicated by significant changes in the CLPP and fatty acid composition of the microbial community associated with the time of sampling.

The microbial community associated with the rhizosphere of the transgenic Quest plants was significantly different from microbial community associated with the rhizosphere of conventional Excel plants; however, the differences depended on the time of sampling, and communities were not consistently different throughout the entire field season. Most of the observed differences occurred at the July sampling time, at the midflowering stage of growth. Previous studies have shown that there are differences in the composition of the rhizosphere microbial community associated with transgenic Quest plants compared to the compositions of the communities associated with Excel plants and six other canola varieties tested (12, 32, 33). It is interesting that July was the time period selected for these studies. By comparison of the relative abundance of 16S rDNA targets, Smalla et al. (34) also found that enrichment of bacterial populations associated with canola was most pronounced when canola was flowering.

In this study, the CLPP of the microbial communities, rather

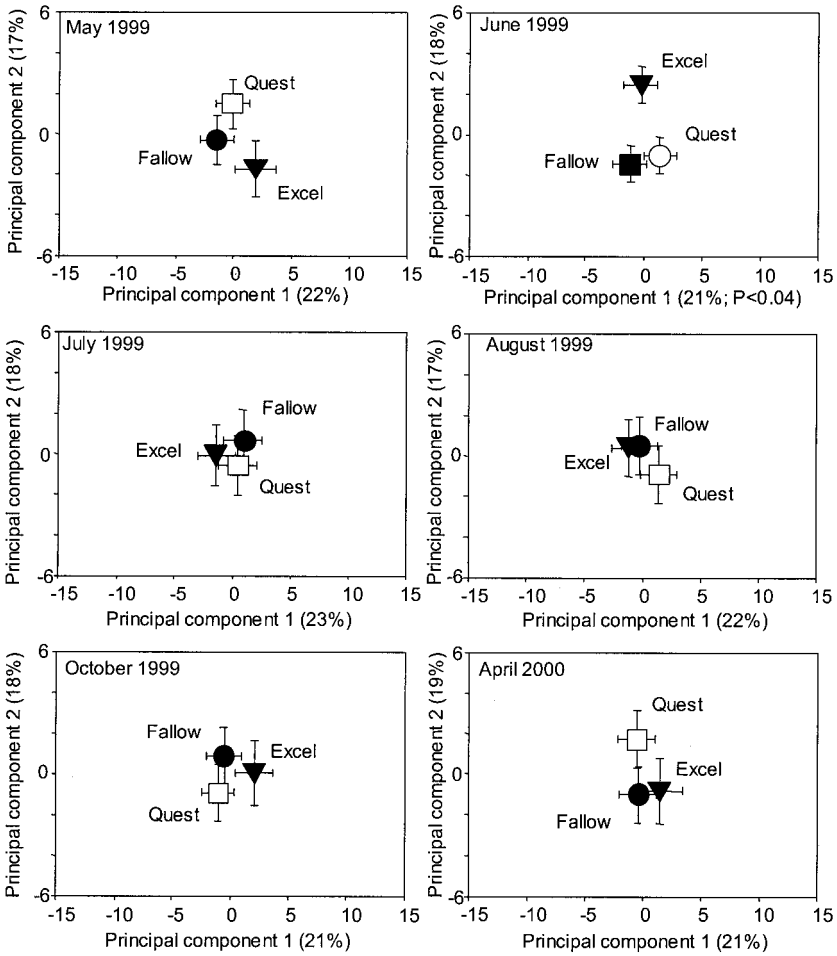

FIG. 7. PCA of T-ARDRA profiles obtained for microbial communities from fallow soil and for rhizosphere microbial communities of canola varieties grown at Watson, Saskatchewan, sampled in May, June, July, August, and October 1999 and April 2000. Symbols: ●, fallow soil ($n = 4$); ▲, conventional variety Excel ($n = 4$); □, genetically modified variety Quest ($n = 4$). The error bars indicate the standard errors of the means. The level of variation explained by each principal component is indicated in parentheses. *P* values are indicated when there was a significant variety effect, as determined by ANOVA.

than the fatty acid composition or the genetic diversity, was affected more by seasonal variation. Slight changes in bacterial diversity are sometimes not revealed by FAME analysis because fatty acids can be present in a wide range of bacteria, FAME profiles may be dominated by fatty acids from numerically dominant bacteria, and rare microbial populations may be missed in a profile. Therefore, slight differences in community structure may not translate to significant differences in fatty acid profiles (28). Similarly, microbial communities with similar structures as determined by T-ARDRA may still have ecologically significant differences in community composition, as this method is not sensitive to changes in community composition that may occur at the level of individual strains or species (4). In addition, T-ARDRA assesses changes in the numerically dominant populations of bacteria in a population. Rare microbial populations are not represented because the template DNAs from these populations represent a small fraction of the total community and are not amplified by PCR or are present at levels that are not detected above the background (24). Furthermore, in our study T-ARDRA banding patterns were analyzed by considering the presence or absence of bands rather than the intensity. Duineveld et al. (11) pointed out that bands can be present at all times but differ in intensity. The differences in intensity indicate that bacterial numbers are changing and hence altering the diversity of the community. In the present study, there may have been differences in the numbers of bacteria present in the rhizosphere that caused a shift in the functional diversity of the rhizosphere microbial community that was not identified as a shift in the genetic diversity of the microbial population. For these reasons, differences in the CLPP of the community over the field season did not coincide with significant changes in the FAME or T-ARDRA banding patterns.

In the present study, both CLPP and FAME analyses indicated that there were differences between the microbial communities associated with fall stubble from Quest and Excel plants. Other authors have found differences in microbial communities associated with transgenic plants at the senescence plant growth stage. For example, Lukow et al. (27) found differences in the community fingerprint patterns of rhizosphere soil samples associated with senescent potato plants. Similarly, Lottmann et al. (25, 26) only found differences in the composition of the population of the

beneficial bacteria associated with senescent potato plants. One critical role of soil microorganisms in soil ecosystem functioning is the decomposition of plant residues and nutrient cycling (21). It is possible that decomposition of genetically modified plant tissue affects the composition of the soil microbial community. Donegan et al. (9) investigated the potential ecological impact of genetically engineered plants on soil ecosystems by burying litterbags containing leaves of transgenic tobacco that expressed proteinase inhibitor I in field plots. They found differences in carbon content between the decomposing parental plant litter and the transgenic plant litter along with differences in the numbers of nematodes and Collembola in the soil surrounding the transgenic plant litterbags.

Seasonal variation in microbial community structure is a complicating factor in the environmental assessment of transgenic plants. The question arises whether differences in microbial communities associated with transgenic plants at a single time point are ecologically significant. This study affirmed that future ecological assessments of genetically modified plants should be conducted at several time points in a field season. Furthermore, this study suggests that in the Canadian agricultural system, in which plants are harvested in early fall and senescent plant roots are left over a long winter season, any differences in the microbial communities associated with transgenic plants are minimized or eliminated. This suggests that changes in the soil microbial community are temporary and dependent on the presence of the transgenic plants.

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