No Evidence of an Impact on the Rhizosphere Diazotroph Community by the Expression of *Bacillus thuringiensis* Cry1Ab Toxin by Bt White Spruce

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Received 11 April 2007/Accepted 17 July 2007

Nitrogen fixation is one of the most important roles played by soil bacterial communities, as fixation supplies nitrogen to many ecosystems which are often N limited. As impacts on this functional group of bacteria might harm the ecosystem’s health and reduce productivity, monitoring that particular group is important. Recently, a field trial with Bt white spruce, which constitutively expresses the Cry1Ab insecticidal toxin of *Bacillus thuringiensis*, was established. The Bt white spruce was shown to be resistant to spruce budworm. We investigated the possible impact of these genetically modified trees on soil nitrogen-fixing bacterial communities. The trial consisted of untransformed controls, GUS white spruce (transformed with the β-glucuronidase gene), and Bt/GUS white spruce (which constitutively expresses both the Cry1Ab toxin and β-glucuronidase) in a random design. Four years after planting, soil samples from the control and the two treatments from plantation as well as from two natural stands of white spruce were collected. Diazotroph diversity was assessed by extracting soil genomic DNA and amplifying a region of the nitrogenase reductase (*nifH*) gene, followed by cloning and sequencing. Analysis revealed that nitrogen-fixing communities did not differ significantly among the untransformed control, GUS white spruce, and Bt/GUS white spruce. Nevertheless, differences in diazotroph diversity were observed between white spruce trees from the plantation site and those from two natural stands, one of which grew only a few meters away from the plantation. We therefore conclude, in the absence of evidence that the presence of the *B. thuringiensis* cry1Ab gene had an effect on diazotrophic communities, that either site and/or field preparation prior to planting seems to be more important in determining diazotroph community structure than the presence of Bt white spruce.

Plant genetic engineering is a field of biotechnology that has experienced significant development over the last decades. Genetic modifications have been performed with several different plant species, mainly crops (e.g., maize, cotton, wheat, and rice), with different goals such as resistance to insect pests or herbicides, increased growth, and increased nutritional quality. However, plant genome modification might affect some important ecosystem components, such as soil microbial communities. Recent studies have been conducted to assess the potential beneficial and/or detrimental effects of genetically engineered plants on soil microbiota. Particular attention was paid to studying the impact of Bt plants, which constitutively express the Cry1Ab insecticidal toxin of *Bacillus thuringiensis*, on rhizosphere microbial communities. Although studies demonstrating a negative impact of Bt crops on soil microbiota exist (1, 4, 10, 12, 23, 26, 32), more studies showing no effect have been published (1, 3, 4, 10, 12, 23, 26, 32).

Developments with regard to tree genetic engineering have made it possible to produce transgenic conifers. In one instance, Bt/GUS white spruce (*Picea glauca* (Moench) Voss) (which constitutively expresses both the Cry1Ab toxin and β-glucuronidase), resistant to the spruce budworm, *Choristoneura fumiferana* (Clemens), was produced and tested in the laboratory and in an experimental plantation (D. Lachance and A. Séguin, cited in reference 27). Compared with crops, trees can have a long-lasting impact on their ecosystems: their root network is more widespread, they are generally present in the ecosystem for several decades, and they naturally harbor an important microbial community (17, 38), which might be more or less sensitive to the presence of the *Bacillus thuringiensis* cry1Ab gene. Thus, genetically modified trees could have impacts on soil microbial diversity that are different from those studied in agricultural crops, and it is crucial to investigate further these effects before their extensive release.

Studies of microbial diversity have generally used the 16S rRNA genes. However, to globally understand the microbial ecology of a particular ecosystem, the study of genes with important functions in an ecosystem is necessary, since that approach provides more information about the biological and/or ecological functions carried out by microbial communities. Soil microorganisms are responsible for different key functions in ecosystems as they are involved in many decomposing processes as well as in all major biogeochemical cycles, in the recycling of essential elements. Studies of the impact of genetically modified organisms should therefore also focus on microbial community functions as they are key elements in a healthy ecosystem.

One crucial function carried out by soil microorganisms is...
nitrogen fixation, which is the major source of nitrogen for many natural ecosystems. It is important primarily because nitrogen often is the limiting nutrient in many terrestrial ecosystems (43). Moreover, nitrogen fixation is a function performed by a wide diversity of bacteria belonging to many different taxa (46, 47). Hence, the study of one of the most conserved genes involved in nitrogen fixation, the nifH gene (29), is an interesting tool with which to evaluate microbial functional diversity, as it represents the diversity of a group of microorganisms, the nitrogen fixers, that are crucial to ecosystem productivity. Moreover, it has been demonstrated that nifH phylogenies are generally congruent with 16S rRNA gene phylogenies (6, 46).

The present study reports on diazotroph communities from the white spruce rhizosphere, and it is the first study to assess the impact of genetically modified organisms, for instance, Bt white spruce, on N2-fixing communities. The objectives of this study were (i) to evaluate the functional diversity of the diazotroph community in the rhizospheres of genetically modified (GM) and non-GM spruce and (ii) to determine if genetic transformation has an effect on the composition of the diazotroph community in spruce rhizospheres.

MATERIALS AND METHODS

Study site, experimental design, and soil sampling. Rhizosphere soil samples were collected in August 2004 at Valcartier (approximately 25 km north of Quebec City), from the only transgenic tree plantation in Canada. The Bt/GUS white spruce [Picea glauca (Moench) Voss] plantation was established in June 2000 and comprises eight replicates in a randomized complete block design of three different treatments—untransformed control, GUS white spruce, and Bt/GUS white spruce—surrounded by two guard rows of Norway spruce [Picea abies (L.) Karst.]. GUS white spruce contained the gene uidA encoding β-glucuronidase, a selective marker. Bt/GUS white spruce contained the gene cry1Ab and contained the neomycin phosphotransferase gene (nptII), which is a widely used selective genetic marker in plant transformation. Soil samples were also collected from the rhizospheres of white spruce growing around the plantation site (less than 50 m away) (Wild-VC; 3.72% total C; 0.20% total N; and pH 6.08) and from the rhizospheres of white spruce growing around the plantation site (less than 50 m away) (Wild-SF; 48.93% total C; 1.68% total N; and pH, 3.20).

DNA extraction and PCR amplification. Total DNA was extracted from approximately 5 to 10 g (wet weight) of soil from the plantation site) (Wild-SF; 48.93% total C; 1.68% total N; and pH, 3.20). DNA extraction and PCR amplification were performed using the QIAamp DNA extraction kit (QIAGEN). DNA extraction was performed using silica bead lysis and the provided lysis buffer. Samples were kept at −80°C until further analysis.

DNA extraction and PCR amplification. Total DNA was extracted from approximately 250 mg (dry weight) of rhizosphere by using a PowerSoil DNA kit from MoBio (MoBio Laboratories Inc., Solana Beach, CA) according to the manufacturer's instructions. Universal nifH primers Pof (5′-TGC GAC GTC AAC AGB GCA GAC TC-3′) and PolR (5′-ATS GCC ATC ATY TCR CCG GA-3′) were used to amplify a 360-bp portion of the nifH gene. PCR reactions were performed with a final volume of 25 μL and contained 1× PCR buffer (QiAGEN), 1.5 mM MgCl2, 200 μM of each deoxynucleotide triphosphate, 0.6 μM of each primer, 1 U of Taq platinum DNA polymerase (Invitrogen), and 1 μL of extracted DNA. The PCR program was as follows: initial denaturation at 94°C for 3 min and 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. Final extension was performed at 72°C for 5 min. For each DNA sample, PCR was repeated twice. To confirm amplification, aliquots (5 μL) of community nifH amplicons were run in a 1.5% agarose gel in 1× Tris-acetate-EDTA and stained with ethidium bromide. PCRs from the same sample were then pooled, purified using a QIAquick PCR purification kit (QiAGEN), and quantified using a Picoquant double-strand DNA quantitation kit (Molecular Probes, Eugene, OR).

Cloning and sequencing. Five clone libraries were constructed (control, GUS, Bt/GUS, Wild-VC, and Wild-SF) by cloning purified products using a QIAGEN QIAquick PCR purification kit (QIAGEN), and quantified using a Picogreen double-strand DNA quantitation kit (Molecular Probes, Eugene, OR).

RESULTS

DOTUR analysis. Using DOTUR software, we calculated richness estimators (bias-corrected Chao1 and bootstrap values) and diversity indices (Shannon and Simpson), and they are presented in Table 1. Ninety clones were analyzed for each library. By performing DOTUR analysis at the 3% distance level, we obtained a much lower abundance of OTUs with wild (VC and SF) stands than with the plantation stand (including the control, GUS, and Bt/GUS white spruce), supported by
bias-corrected Chao1 and bootstrap richness estimators, and diversity indices (Shannon and Simpson) are shown for each treatment. Values ± standard deviations (SD) were obtained by use of the DOTUR program, using a 3% distance level. Groups with the same letters, a, b, and c, are not significantly different at α = 0.05.

**Dendrogram analysis.** We performed a dendrogram analysis using the *nifH* OTU sequences and the *nifH* sequences from known organisms to infer the possible affiliations of our different OTUs (see Fig. S1 in the supplemental material). The majority of clones (>75%) were related to the classes *Alphaproteobacteria, Betaproteobacteria,* and *Rhizobium* rhizobia genera (e.g., *Rhizobium, Bradyrhizobium,* and *Mesorhizobium*) of *Alphaproteobacteria* and the genera *Burkholderia* and *Herbaspirillum* of *Betaproteobacteria.* Even though we could not clearly separate those two classes of proteobacteria, we observed that the OTUs found in that cluster were more closely related to *Alphaproteobacteria.* The second most well-represented group to which clones were related was the class *Deltaproteobacteria,* which includes the genera *Geobacter,* *Methylosinus,* and *Methylocella* (in cluster A) and *Desulfovibrio* and *Desulfovibrio* (in cluster B) (Table 2 and see Fig. S1 in the supplemental material). According to the recent classification (11, 30), the majority of OTUs (>80%) belong to the *nifH* group I gene, which corresponds to typical molybdenum-iron (Mo-Fe) nitrogenase. Cyanobacteria were absent from wild stands, and there were more clones related to *Deltaproteobacteria* cluster B in the Wild-VC stand. No major trend was observed for the control and the two treatment groups from the plantation stand.

**SONS analysis.** We used SONS software to obtain OTU distribution across libraries (Fig. 1). The control, GUS, and Bt/GUS white spruce stands shared approximately 25% of their OTUs, whereas none of the OTUs from either the Wild-VC or the Wild-SF stands were shared with any other library.

**LIBSHUFF analysis.** Clone libraries were compared in a pair-wise manner between treatments. Comparisons between wild stands (Wild-VC and Wild-SF) of white spruce and the control, GUS, and Bt/GUS white spruce yielded small P values (<0.0001) (Table 3). Wild-VC and Wild-SF were also significantly different from each other (Table 3). The significance of those differences was supported by the Monte Carlo procedure (P < 0.001).

**UniFrac analysis.** We first used UniFrac to perform significance tests (Table 4). The UniFrac P value is the proportion, out of the 1,000 randomized trees, that had at least as many unique branch lengths as the true tree, whereas the phylogenetic P value is the proportion, out of the 1,000 randomized trees, that had at least as many parsimony changes as the true tree. Both the UniFrac and phylogenetic tests gave significant P values for overall differences. The UniFrac test, investigating individual differences, shows that the control, GUS, and Bt/GUS white spruce have significantly more unique branch lengths than expected by chance, whereas for pair-wise differences, both the UniFrac and the phylogenetic tests present significant differences between all pairs of libraries, except for control versus Bt/GUS white spruce and GUS versus Bt/GUS white spruce. Both wild white spruce libraries (VC and SF) were significantly different from all other libraries.

We then used the clusterEnvs function to compare diazotrophic communities from each library. Samples were clustered using UPGMA (Fig. 2). Diazotroph communities originating from the rhizospheres of both natural stand sites (Wild-VC and Wild-SF) were on a completely different branch than those

**Table 1. Comparison of diazotroph diversity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of DNA sequences</th>
<th>No. of OTUs</th>
<th>Bias-corrected Chao1</th>
<th>Bootstrap value</th>
<th>Shannon (H)</th>
<th>Simpson (1-D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
<td>53</td>
<td>77.17 ± 37.95 b</td>
<td>26.81 ± 4.31 b</td>
<td>2.85 ± 0.23 b</td>
<td>0.958 ± 0.022 b</td>
</tr>
<tr>
<td>GUS</td>
<td>90</td>
<td>52</td>
<td>59.70 ± 13.58 a, b</td>
<td>29.66 ± 1.40 c</td>
<td>2.98 ± 0.12 b</td>
<td>0.970 ± 0.014 b</td>
</tr>
<tr>
<td>Bt/GUS</td>
<td>90</td>
<td>55</td>
<td>66.28 ± 38.87 a, b</td>
<td>28.31 ± 5.83 b, c</td>
<td>2.91 ± 0.29 b</td>
<td>0.963 ± 0.030 b</td>
</tr>
<tr>
<td>Wild-VC</td>
<td>90</td>
<td>34</td>
<td>36.50 ± 20.23 a, b</td>
<td>16.42 ± 7.48 a, b</td>
<td>1.84 ± 0.66 a, b</td>
<td>0.726 ± 0.193 a, b</td>
</tr>
<tr>
<td>Wild-SF</td>
<td>90</td>
<td>23</td>
<td>15.94 ± 16.73 a</td>
<td>11.74 ± 10.32 a</td>
<td>1.34 ± 1.27 a</td>
<td>0.508 ± 0.439 a</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of *nifH* diversity distribution among bacterial groups in GM and non-GM white spruce rhizospheres**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of clones</th>
<th>No. of DNA sequences</th>
<th>% of clones</th>
<th>No. of DNA sequences</th>
<th>% of clones</th>
<th>No. of DNA sequences</th>
<th>% of clones</th>
<th>No. of DNA sequences</th>
<th>% of clones</th>
<th>No. of DNA sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>53</td>
<td>3.3</td>
<td>3</td>
<td>37.7</td>
<td>34</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GUS</td>
<td>2.2</td>
<td>2</td>
<td>58.9</td>
<td>53</td>
<td>3.3</td>
<td>3</td>
<td>63.3</td>
<td>57</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bt/GUS</td>
<td>3.3</td>
<td>3</td>
<td>31.1</td>
<td>28</td>
<td>3.3</td>
<td>3</td>
<td>45.5</td>
<td>41</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>Wild-VC</td>
<td>0</td>
<td>2</td>
<td>50.0</td>
<td>45</td>
<td>0</td>
<td>45.5</td>
<td>75.5</td>
<td>68</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wild-SF</td>
<td>0</td>
<td>75</td>
<td>22.2</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>12.2</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a Percentages of clones (total of 90 per treatment) in each bacterial group are shown. There was one Wild-VC OTU that was not included (it is in the uncultured bacterial clade).
of the control, GUS, and Bt/GUS white spruce trees, located in plantation, all with high jackknife values. Finally, we performed a principal coordinate analysis (UniFracPCA) showing the mean coordinate values with standard deviations of the three sublibraries (i.e., from the three trees sampled) (Fig. 3). The first two principal coordinates for the analysis including all five libraries (Fig. 3a) explained 15.0 and 14.2% of the variance. The first principal component separates samples originating from the rhizospheres of white spruce in plantation versus those originating from natural white spruce stands. As wild samples had an important impact, we also performed the analysis by including only the control, GUS, and Bt/GUS white spruce stands to have a closer look at the possible impact of Bt/GUS white spruce (Fig. 3b). No clear segregation was observed.

**DISCUSSION**

Our study describes diazotroph communities present in white spruce rhizospheres. This study is the first to evaluate the impact of Bt white spruce, or any other Bt plant, on nitrogen-fixing communities. No effect associated with the presence of the *B. thuringiensis* transgene was observed in this study, which is in agreement with other recent studies evaluating the impact on overall bacterial diversity (1, 3, 4, 10, 12, 23, 26, 32). On the other hand, significant differences were found between diazotroph communities associated with white spruce in natural stands and those associated with trees in a plantation. This result is specifically in agreement with those obtained in a previous study we carried out (J. Lamarche and R. C. Hamelin, submitted), where terminal restriction fragment length polymorphism profiles of 16S rRNA genes showed that the tree’s growing site had a greater impact on bacterial diversity than the treatment of soil.

One main objective was to characterize the N$_2$-fixing community of the white spruce rhizosphere. To do so, we used tree reconstruction with sequences of well-known N$_2$ fixers (see Fig. S1 in the supplemental material). It revealed that the vast majority of OTUs were clustering within the group I nitrogenase. Group I nitrogenases are typical Mo-Fe nitrogenases and are found predominantly in proteobacteria and cyanobacteria, whereas group II nitrogenases are anaerobic Mo-Fe nitrogenases, predominantly found in methanogens and anaerobic bacteria (30). There exist three other nitrogenase groups according to Raymond et al. (30), but none of the clones we obtained seemed to belong to those groups. Moreover, the results showed that most of the OTUs belonged to the *Deltaproteobacteria* cluster A, where the only known sequences are those from *Geobacter metallireducens* and *Geobacter sulfurreducens*. This phenomenon has already been reported in other studies (cluster A in the study by Hamelin et al. [14], *nifH* cluster 3 in Bürgmann et al. [6], and cluster

![FIG. 1. OTU distribution for each treatment.](image-url)
NF5 in Yeager et al. [45]). As the diversity within that group is quite important and is noticeable in many different ecosystems (47), thus suggesting it might be a major nitrogen-fixer group, it should be investigated further. More sequences of known species belonging to that group would be required for a better characterization.

As for the effect of Bt/GUS white spruce, the dendrograms as well as the distributions of the different OTUs throughout the different bacterial groups did not appear different between libraries. We also looked at diversity and more specifically at richness, one of the two major components of diversity, and we observed that both were much lower and more variable in the rhizospheres of white spruce in natural stands. Lower diversity is the result of unevenness, as there is some redundancy in cloned sequences, with some OTUs being represented by up to 53 clones in Wild-SF but only 10 clones per OTU in plantation. Since the natural stands were around 25 and 65 years old for Wild-VC and Wild-SF, respectively, diazotroph communities might have reached equilibrium with their environment. Selection toward the "best performers" may have occurred, resulting in the overrepresentation of those species or bacterial groups. Diazotroph communities in plantation (which were approximately 4 years old when sampled) might still be in their "adaptation period," with a greater abundance of species and no species dominating the communities. There was also a large variability among the plots in the natural stands compared to that of samples in plantation. Two main hypotheses might explain that result: (i) soil under plantation treatment has been plowed before tree transplantation, therefore homogenizing the site, and (ii) a greater abundance of soil microsites is present in natural stands; each microsite could then have various degrees of diversity.

The overall distribution of OTUs according to each library was analyzed using SONS (34), and results indicate that many sequences from a particular library are attributed to each OTU (OTU number in Fig. 1). We clearly see that some OTUs from samples in plantation (control, GUS, and Bt/GUS white spruce) are shared, whereas Wild-VC and Wild-SF have a completely distinct sets of OTUs, which was quite unexpected. Even though the sites were quite different, we were expecting to observe at least some shared OTUs. These results suggest that diazotroph groups are very diverse and that they may be site specific. It also demonstrates that the soil environment in plantation is quite different from that of both the wild sites. The Wild-VC site was only a few meters away from the plantation, but the immediate surroundings were quite different; there was the presence of understory vegetation as well as other tree species growing around. As for the Wild-SF site, it was in a completely different environment (as described in Materials and Methods), approximately 35 km from the plantation site in the St. Lawrence Plain.

To further investigate the differences in diazotroph communities, and more specifically that of Bt/GUS white spruce, we used three different microbial community analysis tools (FLIBSHUFF, UniFracP, and PhylogeneticTestP). In general, they led us to the same conclusions, with some minor disparities. Nevertheless, the major conclusions drawn from those analyses were that (i) there is an absence of evidence that cry1Ab insertion (as well as nptII and uidA insertion) in white spruce has an effect on nitrogen-fixing bacterial communities and that (ii) the differences between treatments in plantation and natural stands are highly significant, which is consistent with the results previously discussed.

The absence of evidence of the impact of Bt/GUS white spruce suggests that there is no direct impact of the toxin itself even if bacteria can utilize the B. thuringiensis toxin as a source

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**TABLE 4. Comparison of nifH gene libraries, done using UniFracP and PhylogeneticTestP**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>GUS</th>
<th>Bt/GUS</th>
<th>Wild-VC</th>
<th>Wild-SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.007</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>GUS</td>
<td>0.014</td>
<td>0.003</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Bt/GUS</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Wild-VC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-SF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Comparisons of nifH gene libraries from GM and non-GM white spruce rhizospheres were made, using UniFracP and PhylogeneticTestP implemented in UniFrac.

b P values were calculated using 1,000 permutations. Correction for multiple comparison (20) tests: for a family-wise error rate of 0.05, the minimum P value is 0.0026 (significant results are followed by an asterisk). Values for overall treatments were <0.001 for both tests, whereas UniFrac P values for each treatment were 0.022 (control), 0.043 (GUS), 0.047 (B. thuringiensis), 0.780 (Wild-VC), and 0.998 (Wild-SF). The minimum P value for the latter test is 0.05 as there is no multiple comparison.
of carbon and/or nitrogen (21). It is in agreement with the demonstration that the toxin itself does not have any microbi-cidal or microbiostatic activity (20). No indirect impact triggered by *B. thuringiensis* gene insertion within the white spruce genome was detectable in the present study. This absence of evidence of Bt white spruce impact, however, is not the result of low or no *B. thuringiensis* toxin production by trees, as the expression of the toxin was confirmed in 2004 by Northern blotting and enzyme-linked immunosorbent assay (D. Lachance and A. Séguin, personal communication). Nonetheless, the available concentrations of free toxins in the soil might have been low due to the presence of humic acids and/or clay particles to which the toxin is known to adhere (9, 39–42). Furthermore, it was established that bacteria are not able to use the *B. thuringiensis* toxin when the latter is bound to clay (21). The proportion of available toxin might have been in concentrations insufficient to induce changes in nitrogen-fixing bacterial communities.

As for the presence of the *nptII* and *uidA* genes, which encode a neomycin phosphotransferase and a β-glucuronidase, respectively, we could have suspected some impact since they are not normally found in plants (2, 16, 18). The concern was not for the persistence of the genes themselves in soil, as it has already been demonstrated that they did not persist over 4 months (15), but rather for their products. Indeed, neomycin phosphotransferase is an enzyme able to inactivate different aminoglycoside antibiotics, such as kanamycin, neomycin, G418 (Geneticin), and paromomycin. These antibiotics might affect prokaryotic and/or eukaryotic organisms. Their inactiva-
tion might therefore influence the overall microbial community. Nevertheless, no significant difference triggered by the presence of those genes and their product was observed in the present study.

The differences observed between samples in plantation and those in natural stands might be the result of different factors, such as the difference in tree genetic background, which might have triggered variations in root exudates and therefore have affected diazotroph communities (5), or stand age (approximately 4 years old for the plantation and 25 and 65 years old for natural stands). Finally, field preparation prior to planting might have caused a lot of modifications to the soil structure and physicochemical properties, thereby possibly driving changes in nitrogen-fixing communities.

Our study shows that there already are, under natural conditions, great differences in diazotroph communities, driven only by stand sites. However, although no significant impact of Bt white spruce was observed for a particular bacterial functional group, further studies might be required to evaluate, among other things, potential horizontal gene transfer between transgenic white spruce and microbial communities. GM trees are novel organisms with particular characteristics that distinguish them from the more widely studied GM crops. Therefore, specific attention should be given to GM trees in general as they are long lived and might have long-term impacts.

**ACKNOWLEDGMENTS**

We thank A. Séguin and D. Lachance for the production of transgenic white spruce and for setting up the experimental design, and we thank all field assistants. We also thank N. Feau, F. O. Stefani, and I. Lamarre for the revision of the manuscript. We also thank those who developed the different statistical tools we used; their work is very valuable.

This work was supported by grants from the Canadian Biotechnology Strategic Funds.

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