Effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*)

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**Summary**

- The focus of many fungal endophyte studies has been how plants benefit from endophyte infection. Few studies have investigated the role of the host plant as an environment in shaping endophyte community diversity and composition. The effects that different attributes of the host plant, that is, host genetic variation, host variation in resistance to the fungal pathogen *Ustilago maydis* and *U. maydis* infection, have on the fungal endophyte communities in maize (*Zea mays*) was examined.
- The internal transcribed spacer (ITS) region of the rDNA was sequenced to identify fungi and the endophyte communities were compared in six maize lines that varied in their resistance to *U. maydis*.
- It was found that host genetic variation, as determined by maize line, had significant effects on species richness, while the interactions between line and *U. maydis* infection and line and field plot had significant effects on endophyte community composition. However, the effects of maize line were not dependent on whether lines were resistant or susceptible to *U. maydis*.
- Almost 3000 clones obtained from 58 plants were sequenced to characterize the maize endophyte community. These results suggest that the endophyte community is shaped by complex interactions and factors, such as inoculum pool and microclimate, may be important.

**Key words:** community composition, community diversity, fungal endophytes, interspecific interactions, microbial communities, plant–fungal interactions, symbioses.

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**Introduction**

Many microbial symbionts have strong interactions with hosts, such that microbial communities and microbial biodiversity should be heavily influenced by variation within and among macroorganism hosts, such as plants. Host plants are likely to have particularly strong effects on fungal endophytes since endophytes live asymptptomatically in the above-ground tissues of plants and rely completely on host plants for resources (Pan & Clay, 2004). Here, we examine the role of the host plant environment in shaping the endophyte community by using host plants with known genotypes and phenotypes; attributes of the host plant environment include host genotype, traits, and biotic interactions.

The primary focus of many ecological studies on fungal endophytes has been to understand how plants benefit from this symbiotic interaction. Endophytes have been shown to positively affect host growth, morphology, physiology, and interspecific interactions (Clay & Schardl, 2002; Pan & Clay, 2003, 2004; Schulz & Boyle, 2005). The endophytes of C\(_3\)
grasses have also been found to have larger-scale effects, which include reducing plant community diversity (Clay & Holah, 1999) and reducing energy transfer to consumers (Omacini et al., 2001). Although the interaction between the C3 grasses and their host-specific fungal endophytes are the best studied of all endophyte systems, such specific interactions are the exception rather than the rule. Most host plants are infected by many nonspecific fungal endophyte species, which can have effects that are similar to what has been observed in the C3 grass–endophyte systems. For example, it has been shown that these nonspecific fungal endophytes can also affect other interspecific interactions by preventing host infection by root pathogens (Carroll, 1988; Arnold et al., 2003).

The impact of host plants on endophyte communities has not been examined to the same extent as have the effects of fungal endophytes on their hosts. Host environment has been found to affect mycorrhizal communities (Korkama et al., 2006), microbial communities in the gut (Zoetendal et al., 2001; Vaahhtovuo et al., 2003; Stewart et al., 2005), and bacterial and fungal endophytes (Todt, 1988; Adams & Kloeper, 2002; Ahlholm et al., 2002; Bailey et al., 2005). Ahlholm et al. (2002) found that host genetic variation can affect endophyte genetic variation. Birch (Betula pubescens ssp. cerepanovii) tree families that were highly susceptible to the fungal endophyte, Venturia diricha, had not only greater infection levels, but were also infected by V. diricha genotypes that were genetically similar. Almost 10% of the genetic variation found in V. diricha can be attributed to among-host family differences. Understanding the role of the host environment on endophyte species diversity is important because current estimates of fungal biodiversity are partially based on the interaction between fungi and their hosts (Zhou & Hyde, 2001).

Despite the widespread presence of fungal endophytes in almost all plants (Saikkonen et al., 1998), we have a limited understanding of factors that can affect endophyte communities. In this study, we used the host Zea mays (maize) and its host-specific fungal pathogen Ustilago maydis (corn smut), to examine the role of the host plant environment in shaping the endophyte community. We chose to examine the effects of U. maydis because it is a common fungus on maize and its presence or absence on a host has the potential to directly facilitate or inhibit host infection by endophytes. We addressed the following questions: Is fungal endophyte community diversity and composition affected by genetic differences between host plants? Is fungal endophyte community diversity and composition affected by host resistance to U. maydis? Does U. maydis infection affect the endophyte community? We hypothesized that endophyte communities would be similar on plants with the same host attributes. To test our hypothesis, we compared endophyte communities on genetically distinct maize recombinant inbred lines that varied in their resistance to U. maydis, between resistant and susceptible plants, and between plants infected or not infected by U. maydis using a polymerase chain reaction (PCR)-based, culture-independent approach.

**Materials and Methods**

**Field experiment and sampling**

A fully factorial experimental design was used to examine the effects of host plant environment. Three U. maydis resistant and three susceptible F2 generation, W23 × CMV3 recombinant inbred (RI) maize lines were chosen from a set of lines generated in a larger quantitative trait loci (QTL) study of U. maydis resistance and susceptibility (Baumgarten et al., 2007). Since individuals of each RI line represent a particular set of alleles and lines are genetically different, lines can be considered different genotypes. The six lines were chosen from the extremes of resistance and were either highly resistant (R1, R2 and R3) or highly susceptible (S1, S2 and S3) to infection by U. maydis. Seeds representing each RI line came from two F1 plants (numbers in parentheses are R. L. Phillips’ laboratory accession numbers): R1 (56852-5 and 57001-17), R2 (56672-10 and 56967-2) and R3 (56701-6 and 57065-2); S1 (56602-17 and 56617-4), S2 (56921-14 and 57101-1) and S3 (56576-10 and 57059-8).

Seeds were planted into two plots in one agriculture field at the St Paul campus at the University of Minnesota, USA, in 2003. Plots were in different planting sections of the field and were diagonally separated from each other by > 50 m. Each plot consisted of 36 rows that were approx. 5.4 m long and 0.9 m apart. Eighteen seeds were planted into each row, with 0.3 m between seeds. Three seeds from each line were randomly assigned to and planted in three locations within a row. Over 200 seeds per line were planted across the two plots, for a total of almost 1,300 seeds. To increase the probability that some plants in each line would be infected by U. maydis, half of all seedlings (every other row) were inoculated 7 wk after planting with seven compatible haploid strains using the protocol described in Baumgarten et al. (2007). Plants not inoculated with U. maydis received 1 ml of water as a control for the inoculation procedure. After inoculation, all plants were assessed for U. maydis infection weekly. Plants were scored as infected if they showed U. maydis disease symptoms (galls) on any part of the plant during any of the weekly censuses; plants without U. maydis galls were considered to be uninfected. The source of U. maydis infection was from artificial inoculation (inoculated plants) or natural infection (inoculated and uninoculated plants).

At the end of the growing season (August 2003), leaf, ear and stalk tissues were collected from five U. maydis infected and five U. maydis uninfected plants for each of five lines across the field plots. For the sixth line, S3, eight plants were sampled because only three uninfected plants could be found. A total of 58 plants were assessed across the six maize lines. All tissue samples were surface-sterilized in the field by rinsing in
water, submerging within a 50% bleach solution for 2 min, and rinsing with water again. Surface-sterilized tissues were kept on dry ice in the field and then stored at −80°C until DNA extraction. Leaf tissues were lyophilized before DNA extraction.

**Molecular methods**

Total DNA was extracted from corn tissues using a modified cetyltrimethylammonium bromide (CTAB) procedure obtained from Dr R. L. Phillips' laboratory (Agronomy and Plant Genetics, University of Minnesota, MN, USA), which was based on a previously described procedure (Saghai-Marooof et al., 1984). DNA was extracted from kernels, the stalk, and each of two leaves separately and then equal concentrations of the DNA were added together to produce an overall sample for each plant. Approximately 400 µg of ground, lyophilized leaf tissue was used for each leaf extraction. DNA from ear (kernel) and stalk tissues (pith) were extracted from frozen samples, which were ground in liquid nitrogen using a mortar and pestle, and then immediately suspended in 800 µl of CTAB prewarmed to 65°C. All DNA concentrations were determined using PicoGreen, following the manufacturer’s directions (Molecular Probes, Eugene, OR, USA).

Fungal-specific PCR primers ITS1F and ITS4 (Gardes & Bruns, 1993) were used to amplify the ITS region of the rDNA (ITS1, 5.8S, and ITS2) from the total extracted DNA. Each 25-µl PCR reaction contained the following: 20–30 ng of genomic DNA, 1× Takara Ex Taq PCR buffer with MgCl₂, 200 µM dNTPs, 0.25 µM of each primer, 1.25 U of Ex Taq (Panvera, Madison, WI, USA), and sterile water. The PCR conditions were: 94°C for 1 min, 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1.5 min, with a final extension at 72°C for 3 min. All PCR products were run in 0.8% 0.5× TAE agarose gels and visualized using ethidium bromide under UV light. For each set of PCR reactions, a negative control was included (sterile water added instead of DNA). Only sets of reactions with no bands in the negative control were used for cloning.

The PCR products were then cloned using the TOPO TA Cloning kit following the instructions provided by the manufacturer (Invitrogen, Carlsbad, CA, USA). One-hundred bacterial colonies from each cloning reaction were chosen for each plant sample, grown at 37°C overnight in liquid Luria-Bertani (LB) and plasmids were extracted as described in Surzycki (2000). Plasmids were assessed for fungal rDNA inserts with PCR using the ITS1F and ITS4 primers under the PCR conditions described above. DNA sequence was obtained from at least 50 clones per plant to characterize the fungal endophyte community on each plant. Clones were sequenced in one direction using either the ITS1F or ITS4 primer with the ABI BigDye terminator chemistry on an ABI 3700 capillary sequencer at the University of Minnesota Sequencing Center (AGAC). Approximately 3000 clones were sequenced across all sampled plants.

**Sequence analysis**

Sequences were edited using SEQUENCER 3.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Complete, full-length sequences ranged from c. 450 to over 750 bp and included ITS1, 5.8S and ITS2. Chimeric sequences, which are PCR-generated artifacts (Vilgalys, 2003), were detected using BLAST. All edited full-length sequences were compared to the ‘nr’ database in GenBank with BLAST using the default parameters (filter = 'low complexity', expect = '10', word size = '11'). Chimeric sequences were identified when ITS1 was a significant match (E-value < 0.001 and alignment score > 200) to one set of taxonomically related sequences and ITS2 was a significant match to a different set of taxonomically-related sequences in GenBank (O’Brien et al., 2005). All sequences identified as chimeras, on average less than two sequences per plant, were removed from the dataset and not analysed further. Nonidentical, representative sequences were deposited in Genbank (accession numbers EF504318–EF505893).

**Sequence similarity operational taxonomic units (OTUs)**

Because of the extensive variability in ITS1 and ITS2, it was not possible to put all sequences into one alignment. Instead, edited, full-length sequences were grouped into smaller alignments, or OTUs (phylootypes), based on nucleotide similarity using the ‘assemble automatically’ function in SEQUENCER 3.1. The ‘dirty data’ assembly algorithm was used for groupings, with a value of 55 (nucleotides) for the minimum overlap. The minimum percentage (sequence) match was adjusted to reflect the stringency of our groupings (see below). There were no significant gaps in any of our assembled alignments and sequences were similar in length within an alignment, although the assembly algorithm used by SEQUENCER did not seem to be heavily affected by small differences in sequence lengths at the 5’ or 3’ ends.

In order to take into account errors that occurred during PCR, cloning, or sequencing and to determine whether groupings were robust, different minimum percentage match (referred to as sequence similarity from hereon) levels were used to define the OTUs in SEQUENCER. Sequence similarity values were 90, 95, 98 or 99%. For example, to obtain the 90% sequence similarity dataset, sequences were assembled using a minimum per cent match value of 90, which produced OTUs composed of sequences that were ≥ 90% similar to each other. We repeated this procedure for each sequence similarity level, 95, 98 or 99%, and thus obtained four datasets. Datasets based on these OTU groupings will be referred to as ‘sequence similarity’ data. Because the different groupings led to similar results statistically, further consideration of the data will be limited to the 98% sequence similarity dataset.
Genera OTUs
BLAST was used to generate a second dataset based on taxonomic affiliations at the generic-level, which will be referred to as ‘genera’ OTU data. For each 98% sequence similarity OTU, a consensus sequence was generated by plurality from SEQUENCER and compared with the ‘nr’ database in GenBank using the default BLAST settings as already described. In order for a sequence to be considered a match to a particular genus, a majority of the query sequence (ITS1, 5.8S and ITS2) needed to match the database sequence with an E-value < 0.001 and an alignment score > 200. If more than one sequence similarity OTU BLASTed to the same fungal genus, then they were combined into the same genus OTU, which led to fewer groups based on taxonomic affiliation than sequence similarity. Some OTUs could not be assigned to a genus because they matched sequences that had no taxonomic affiliation, i.e. unidentified environmental samples, or did not match any of the sequences in GenBank.

Assessment of endophyte communities and statistical analyses
The effects of the experimental factors on endophyte species diversity were assessed by examining: an overall measure of species diversity with Simpson’s index (1/D) and two components of species diversity, species richness and evenness (Magurran, 2004). Species richness was calculated as the number of unique OTUs per plant. Species evenness was calculated using Simpson’s evenness, E_{1/D} (Magurran, 2004). Univariate ANOVAs were used to analyse these species diversity response variables.

Because the S3 line had fewer sampled plants than the other five lines, rarefaction curves were used to compare total species richness across lines for a particular sample size. The program ESTIMATES 7.5 (http://purl.oclc.org/estimates; Colwell, 2005) was used to generate sample-based rarefaction curves for each of the maize lines. Rarefaction curves were generated by sampling without replacement (Colwell, 2005).

Assessments of species diversity take into account only the number of species in the fungal community but do not consider the community composition. Community composition was first analysed using DISTLM, the nonparametric multiple analysis of variance (NPMANOVA) program for unbalanced designs (http://www.stat.auckland.ac.nz/~mja/Programs.htm; Anderson, 2001, 2004). This procedure considered all the species abundance data (number of clones per OTU) at once (Anderson, 2001). NPMANOVAS were run with 5000 permutations, pseudo-F-ratios were obtained, and the Monte Carlo asymptotic P-values interpreted for all analyses (Anderson, 2005). PC-ORD (version 4.38) was then used to analyse endophyte community composition using the nonmetric multidimensional scaling (NMDS), an ordination technique (McCune & Mefford, 1999). This was used to visualize whether fungal endophyte community composition was more similar among different host factors (Anderson, 2001), for example maize line. Plants with a similar fungal community composition should be closer in the ordination space than plants with dissimilar fungal communities. For both NPMANOVA and NMDS, abundance data were log_{10}(x + 1) transformed and statistical analyses were conducted on Bray–Curtis dissimilarities.

All response variables for species diversity and community composition data were calculated on a per plant basis with the OTUs from the sequence similarity or genera datasets treated as species. The statistical model of field plot (P), resistance or susceptibility to U. maydis (RS), maize line nested within resistance or susceptibility (L(RS)), U. maydis infection status (Inf), inoculation with U. maydis (Inc), all two-way interactions, and all three-way interactions was tested in all analyses. Higher-order interactions were not tested in order to maintain a reasonable number of error degrees of freedom and because they are difficult to interpret (Cox, 1958).

Results
Identification of fungal genera
We matched most of our OTUs to a fungal genus or a small group of fungal genera using BLAST (Table 1). Any OTUs that matched multiple genera usually arose because the consensus sequence had significant hits along the majority of the length of the sequence to sequences from different genera. Such a result could occur if the ITS lacked taxonomic resolution for particular genera or if GenBank sequences were misidentified (Vilgalys, 2003).

Operational taxonomic units from the 98% sequence similarity dataset matched 19 fungal genera (Table 1). Most genera were found on more than one plant sample and representatives of Alternaria, Cladosporium, Cryptococcus, and Puccinia were found on plants from all lines, regardless of U. maydis infection status. Acrothecium, Aureobasidium, Curvularia, Filobasidium and Rhodotorula were the least common fungal genera but were found on plants from more than one maize line. Ustilago was also found on all of the maize lines, both U. maydis infected and uninfected plants. While this genera OTU contained sequences that matched U. maydis, it also contained sequences from other Ustilago species and close relatives.

Species diversity
Genetic variation among maize lines had a significant effect on endophyte species richness as assessed by the number of genera OTUs ($F_{5,7} = 2.99, P = 0.037$). The mean number of OTUs per plant ranged from just over 6 to almost 9 (Fig. 1).
None of the main effects or interactions were statistically significant for the sequence similarity datasets.

With rarefaction, we found that species richness of S3 was comparable to the other lines at its given sample size (Fig. 2). Moreover, there was no pattern for total species richness across resistant and susceptible maize lines, consistent with what was seen for mean species richness per plant.

In contrast to the results for species richness, species evenness was not affected by any of the factors, with the exception of field plot in the sequence similarity data ($F_{1,57} = 6.0$, $P = 0.028$). We found that the endophyte community on plants grown in one field plot had higher evenness than plants in the second field plot (mean ± SE: 0.564 ± 0.115 vs 0.448 ± 0.107, respectively). For the genera data, there was greater evenness in field plot 1 than field plot 2 (mean ± SE: 0.453 ± 0.123 vs 0.355 ± 0.091, respectively), but field plot was only marginally significant ($F_{1,57} = 2.95$, $P = 0.097$).

No factors from our model had significant effects on overall species diversity, as measured by the Simpson's index, for any of the datasets (Fig. 3). Although none of the factors was statistically significant, there was greater variation in species diversity within plants that did not have disease symptoms of *U. maydis* (uninfected) than plants with symptoms (infected) (Fig. 3).

### Table 1 Incidence of fungal genera on six maize (*Zea mays*) lines and for all plants sampled ('Total')

<table>
<thead>
<tr>
<th>98% Genera</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>Total</th>
</tr>
</thead>
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<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Alternaria</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>58</td>
</tr>
<tr>
<td>Aureobasidium</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Bullera/Bulleromyces</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Cladosporium</td>
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<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>3</td>
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<td>15</td>
</tr>
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<td>0</td>
<td>1</td>
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<td>12</td>
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<td>2</td>
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<td>39</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>3</td>
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<td>1</td>
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<td>Leptosphaeria/Phaeosphaeria</td>
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<td>1</td>
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<td>2</td>
<td>20</td>
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</table>

Numbers given are based on the presence of a genus on *Ustilago maydis* infected (I) and uninfected (U) plants for each maize recombinant inbred (RI) line. R1, R2, R3 were resistant and S1, S2, and S3 were susceptible to *U. maydis* infection. The maximum number of plants for each line-infection status combination is five, except for the S3 line, where only three uninfected plants could be sampled. For groupings that include two genera (e.g. *Bullera/Bulleromyces*), sequences had low E-values and alignment scores > 200 to both genera from BLAST. The *Ustilago* group includes *U. maydis*, as well as other *Ustilago* species and close relatives.

Fig. 1 Species richness of endophyte communities by maize (*Zea mays*) recombinant inbred (RI) lines. Mean (± SE) number of genera operational taxonomic units (OTUs) based on 98% sequence similarity. Maize lines resistant to the fungal pathogen *Ustilago maydis* have white backgrounds and lines susceptible to *U. maydis* have gray backgrounds.
Species composition

Multivariate approaches were used to assess species composition because host plants with similar endophyte species richness may have very different sets of species in their communities. Endophyte community composition was significantly affected by the interactions between maize line with *U. maydis* infection status and maize line with field plot for both the genera and the sequence similarity OTU datasets (Table 2).

From the NMDS ordination, we found that, within a maize line, endophyte communities were generally more similar among plants with the same *U. maydis* infection status (Fig. 4a,b; for resistant plants, 33.2% of the variance explained for axis 1 and 27.1% for axis 2; for susceptible plants, 20.5% for axis 1 and 40.8% for axis 2). However, *U. maydis*-infected plants of one line sometimes had communities that were more similar to uninfected plants of another line. For example, endophyte communities on uninfected plants of R1 were more similar to infected plants of R2 than to infected plants from R1 (Fig. 4a).

We did not find a consistent effect of field plot on the endophyte communities of the six lines from the NMDS ordination. Resistant lines had greater variation in endophyte communities across plots than susceptible lines. The endophyte communities of resistant lines did not cluster by either line or plot for any of the datasets (data not shown). On the other hand, plants of one susceptible line (S3) clustered together by line but not by plot, while plants of the other two susceptible lines were intermixed and did not cluster by either line or field plot for any analyses (Fig. 4c).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>98% OTUs F</th>
<th>98% OTUs P</th>
<th>98% genera F</th>
<th>98% genera P</th>
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<td>0.0002</td>
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<tr>
<td>P × Inf</td>
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<td>0.99</td>
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</tr>
<tr>
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<td>1.27</td>
<td>0.24</td>
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<td>P × L(RS)</td>
<td>4</td>
<td>1.43</td>
<td>0.061</td>
<td>1.74</td>
<td>0.029</td>
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<tr>
<td>Inf × L(RS)</td>
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<td>2.36</td>
<td>0.0002</td>
<td>3.14</td>
<td>0.0002</td>
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<tr>
<td>Inc × L(RS)</td>
<td>4</td>
<td>1.29</td>
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<td>1.46</td>
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<td>P × Inf × Inc</td>
<td>1</td>
<td>0.60</td>
<td>0.60</td>
<td>0.75</td>
<td>0.61</td>
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<td>P × Inf × RS</td>
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<td>0.91</td>
<td>0.51</td>
<td>0.51</td>
<td>0.80</td>
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<tr>
<td>P × Inc × RS</td>
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<td>0.62</td>
<td>0.71</td>
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<tr>
<td>Inf × Inc × RS</td>
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<td>1.62</td>
<td>0.10</td>
<td>2.76</td>
<td>0.019</td>
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</table>

Factors in the analysis were field plots (P), *Ustilago maydis* infection status (Inf), inoculation status (Inc), resistance or susceptibility to *U. maydis* infection (RS), maize line nested within resistance or susceptibility to *U. maydis* (L(RS)), and two- and three-way interactions. The F-value (F) shown is a pseudo F-value and the Monte Carlo P-value is provided. df, Degrees of freedom. Significant effects (P < 0.05) are in bold type.
The three-way interaction between U. maydis resistance or susceptibility, smut infection status, and smut inoculation was statistically significant for the 98% genera OTU dataset and approached significance ($P = 0.10$) for the 98% sequence similarity OTUs (Table 2). However, plants did not obviously cluster by any combination of these factors with the first three dimensions of NMDS, possibly owing to the complicated nature of the three-way interaction.

**Discussion**

The host plant environment had significant effects on composition of the endophyte community on maize, as measured by the number of OTUs. Host plant genotype, represented by six maize RI lines, had effects on endophyte richness, endophyte diversity, and community composition; endophyte community composition was more similar within a line than across lines for U. maydis susceptible lines. Conversely, maize resistance and susceptibility to U. maydis and U. maydis infection had no significant effects on endophyte species diversity and had few effects on community composition.

**Attributes of the host plant environment–host genotype, U. maydis resistance and infection**

We predicted that endophyte communities would be more similar on host plants that had similar attributes. Interestingly, we found that the host attributes examined did not have measurable effects on the endophyte community, except for host genotype, which had significant effects on genera species richness and community composition. Host genotype has been found to affect other microbial systems, such as mycorrhizae (Korkama et al., 2006), bacterial endophytes (Adams & Kloepper, 2002), soil bacteria (Smith & Goodman, 1999), and gut communities (Zoetendal et al., 2001; Vahtovuo et al., 2003; Stewart et al., 2005). In these studies, microbial communities on closely related hosts were more similar than on distantly related hosts. We did not observe greater similarity of endophyte communities among more closely related maize lines (Fig. 4; see the Supplementary Material, Table S1). Assessments of our maize lines with 90 simple sequence repeats (SSRs) showed that the genetic similarity between lines ranged from 34% to 68% (Table S1; Baumgarten et al., 2007). If endophyte species richness and species diversity were correlated with genetic similarity of maize lines, we would predict that line R2 would be the most genetically similar to lines S2 and S3. Instead, we found that from the SSR data, R2 had the greatest genetic similarity to S1 (68%). Based on the community composition data, we would have predicted that lines S1 and S2 were genetically more similar to each other than to S3 (Fig. 4b). However, genetic similarity between S1 and S2 was not substantially different from comparisons for other lines (Table S1). Rather than overall genetic similarity, endophytes may be influenced by particular loci specifically affecting these associations.

We expected that traits for U. maydis resistance and susceptibility would have significant effects on the endophyte communities of maize. For example, Korkama et al. (2006) found that the growth rate of Picea abies affected ectomycorrhizal community composition, with slow-growing trees having distinct communities from fast-growing trees. Bailey et al. (2005) found endophyte infection levels were negatively correlated with tannin concentrations but positively correlated with the introgression of Populus fremontii genetic markers in hybrids between P. fremontii and Populus.
angustifolia. In contrast to what we predicted, the U. maydis resistance phenotype had no measurable effects on the endophyte community. Our RI lines were derived from the same initial parental cross and represent different genetic combinations of two diploid sets of alleles. Despite being from the extremes of the resistance continuum – either highly resistant or highly susceptible to U. maydis – there were no consistent host growth differences associated with resistance or susceptibility (J. J. Pan, data not shown). Because we observed different results by maize line but not by U. maydis resistance, the results together suggest that loci affecting the endophyte community on maize were not strongly associated with U. maydis resistance phenotypes or host growth.

The presence of one microbial species has been shown to affect host infection by other microbes (Saikkonen et al., 1998). Infection levels of many endophyte species on Cirsium arvense were negatively correlated with each other, suggesting antagonistic interactions between fungal species (Gange et al., 2007). Because of the possible interactions between U. maydis and fungal endophytes, it was expected that U. maydis infection would have a large effect on endophyte community diversity. Instead, U. maydis infection had few statistically significant effects, although there was lower variation in endophyte community diversity on plants infected by U. maydis compared with uninfected plants (Fig. 3). Since U. maydis causes localized infections on maize plants (Christensen, 1963), its effects on the host plant and its interaction with other fungi may be limited only to the area of infection, leading to variable effects on the endophyte community.

The fungal endophyte community on maize is likely to be determined by a combination of factors or host attributes, rather than single factors. We found that maize line had significant interactions with field plot and U. maydis infection (Table 2). That is, similarity of endophyte community composition between lines differed with field plot and with U. maydis infection. Many of the host attributes examined did not have significant influences on the endophyte community either independently or in conjunction with other effects. Other factors, such as interspecific interactions (e.g. host–insect), fungal inoculum pool, abiotic conditions (e.g. moisture) and microclimate, may also be important in shaping the endophyte community (Saikkonen et al., 1998; Vahtovuo et al., 2003; Korkama et al., 2006). Both fungal pathogens and arbuscular mycorrhizal fungi have been found to have patchy distributions in the field (Griffin et al., 2001; Carvalho et al., 2003), which could lead to microbial communities being spatially autocorrelated based on host proximity (Gange et al., 2007). Alternatively, it may be that endophytes opportunistically infect hosts and are not systematically influenced by any particular factor, although this seems unlikely considering what has been found in other microbial systems (Vahtovuo et al., 2003; Korkama et al., 2006). Given the variation in endophyte community diversity and composition within maize lines, greater sampling may be needed to tease apart the effects of different contributing factors.

Asymptomatic hosts as pathogen reservoirs

Our findings suggest that fungal pathogen species may associate with some host plants (e.g. maize) asymptptomatically, causing few if any negative effects on hosts. These hosts could potentially act as pathogen reservoirs with alternative modes of transmission (Hepperly et al., 1985; León-Ramírez et al., 2004). Many of the fungal genera found on our maize plants contain species that are well-known pathogens on maize and on other hosts (Table 2). Because we purposely sampled plant tissues with little or no apparent disease symptoms, we did not expect to find a high number of OTUs that matched sequences of fungal pathogens. With our molecular approach, we found sequences for U. maydis on two plants rated as uninfected because they exhibited no disease symptoms (galls) of U. maydis. Using a second locus, Fusarium graminearum was also identified among endophytes cultured from maize (J. J. Pan & K.-S. Lee, unpublished), which is one of the causal agents of head blight and one of the most economically damaging pathogens on wheat (McMullen et al., 1997). The pathogen groups detected may be truly asymptomatic on maize or latent pathogens that will lead to disease under stress or at a later time (Carroll, 1988; Schulz & Boyle, 2005), for example Epichloë endophytes on C₃ grasses that are visibly apparent only when hosts are reproductive (Clay & Schardl, 2002). The likelihood of asymptomatic pathogens causing disease on maize plants in this study are low because maize is an annual and tissues were collected near the end of the growing season, just before plants senesced. Thus, management plans for the control of fungal pathogens should include all potential host species, even those not exhibiting disease symptoms.

Implications for microbial biodiversity

We are currently experiencing an unprecedented rate of global species extinction. The magnitude of the extinction threat is likely to be underestimated for microbes, which suffer from being ‘poorly studied taxa’ (McKinney, 1999). The results of this study suggest that host plants as an environment, particularly host genotype, can play a role in the maintenance of fungal endophyte biodiversity. The effects of host species loss on endophyte biodiversity should be more dramatic, where the extinction of one host species could lead to the loss of whole endophyte communities. Thus, changes in plant communities through the addition or loss of plant species can have unforeseen consequences for many microbial communities (Zhou & Hyde, 2001).

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References


Supplementary Material

The following supplementary material is available for this article online:

Table S1 Genetic similarities of six maize recombinant inbred lines as determined by 90 simple sequence repeat loci

This material is available as part of the online article from:

(This link will take you to the article abstract).

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