

## PLANT–SOIL–MICROORGANISM INTERACTIONS: HERITABLE RELATIONSHIP BETWEEN PLANT GENOTYPE AND ASSOCIATED SOIL MICROORGANISMS

JENNIFER A. SCHWEITZER,<sup>1,2,4</sup> JOSEPH K. BAILEY,<sup>2,3,5</sup> DYLAN G. FISCHER,<sup>1,2,6</sup> CARRI J. LE ROY,<sup>2,3,6</sup>  
ERIC V. LONSDORF,<sup>2,3,7</sup> THOMAS G. WHITHAM,<sup>2,3</sup> AND STEPHEN C. HART<sup>1,2</sup>

<sup>1</sup>*School of Forestry, Northern Arizona University, Flagstaff, Arizona 86011 USA*

<sup>2</sup>*Merriam-Powell Center for Environmental Research, Flagstaff, Arizona 86011 USA*

<sup>3</sup>*Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011 USA*

**Abstract.** Although soil microbial communities are known to play crucial roles in the cycling of nutrients in forest ecosystems and can vary by plant species, how microorganisms respond to the subtle gradients of plant genetic variation is just beginning to be appreciated. Using a model *Populus* system in a common garden with replicated clones of known genotypes, we evaluated microbial biomass and community composition as quantitative traits. Two main patterns emerged. (1) Plant genotype influenced microbial biomass nitrogen in soils under replicated genotypes of *Populus angustifolia*, F<sub>1</sub>, and backcross hybrids, but not *P. fremontii*. Genotype explained up to 78% of the variation in microbial biomass as indicated by broad-sense heritability estimates (i.e., clonal repeatability). A second estimate of microbial biomass (total phospholipid fatty acid) was more conservative and showed significant genotype effects in *P. angustifolia* and backcross hybrids. (2) Plant genotype significantly influenced microbial community composition, explaining up to 70% of the variation in community composition within *P. angustifolia* genotypes alone. These findings suggest that variation in above- and belowground traits of individual plant genotypes can alter soil microbial dynamics, and suggests that further investigations of the evolutionary implications of genetic feedbacks are warranted.

**Key words:** community genetics; community heritability; phospholipid fatty acid analyses; plant–soil microorganism interactions; *Populus*.

### INTRODUCTION

Soil microbial communities can change in response to many different ecological factors. For example, associations with plants, including plant productivity gradients (Horner-Devine et al. 2003), susceptibility to herbivores (Kuske et al. 2003), and even biological invasions (Kourtev et al. 2002, Hawkes et al. 2005) have all been demonstrated to alter soil microbial communities. While several studies have documented the community response of microbes to plant functional groups and plant species (Templer et al. 2003, Kang and Mills 2004), little is known about how soil microbial communities may respond to subtle gradients of plant genetic factors such as tree genotype in natural systems

(Bever et al. 1996, Madritch and Hunter 2002, 2003, Kasurinen et al. 2005).

Feedbacks between plants and soils in natural systems have commonly been shown to influence soil microbial community composition or activity and even ecosystem processes (Hobbie 1992, Bever et al. 1997, Hooper et al. 2000, Wardle et al. 2004, Wardle 2006); however, most of this work has been focused at the level of plant functional group or species. An appreciation of the links between plant traits and belowground processes in terrestrial ecosystems (Hooper et al. 2000, Wardle et al. 2004) suggests that individual plant genotypes could influence the associated belowground soil microbial community (and also vice versa). The quantity and quality of plant litter inputs to the soil (both above and below ground) influences substrate availability for soil microorganisms and may control microbial community structure (Paul and Clark 1996, Horwath 2007). Variation in the aboveground plant community (regardless of the mechanism), and subsequent changes in plant traits at the plant species and functional group scales (Kang and Mills 2004, Wardle et al. 2004, Wardle 2006 and references therein) can lead to shifts in microbial community structure. For example, many studies have documented that soil microbial community composition or microbial biomass differs in soils beneath different

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<sup>4</sup> Present address: Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996 USA. E-mail: Jen.Schweitzer@utk.edu

<sup>5</sup> Present address: Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996 USA.

<sup>6</sup> Present address: Evergreen State College, Olympia, Washington 98505 USA.

<sup>7</sup> Present address: Conservation and Science Department, Lincoln Park Zoo, Chicago, Illinois 60614 USA.

dominant tree species. These differences are likely due to differences in leaf and root chemistry or production, root exudation, or abiotic effects due to differences in canopy structure (Bever et al. 1996, Priha et al. 2001, Templer et al. 2003, Grayston and Prescott 2005). Bartelt-Ryser et al. (2005) found legacy effects of microbial community composition in soils after plants of different species were grown and then removed from plots, suggesting that plant species can have long-term effects on microbial community composition even after the plant itself is gone. At the level of plant phenotype or genotype, short-term experiments have found variation in total phospholipid fatty acid (PLFA) as well as ectomycorrhizal colonization between two clones (Kasurinen et al. 2005), but few differences in either arbuscular mycorrhizal fungi, bacterial communities, or microbial biomass (Bever et al. 1996, Madritch and Hunter 2002, 2003). These studies suggest that microbial dynamics may have the capacity to respond to plant traits at both inter- and intraspecific scales.

We hypothesized that after 13 years of leaf and root inputs to the soils beneath replicated *Populus* spp. genotypes in a common garden, plant genetic effects would result in distinct microbial biomass (i.e., active biomass of fungi and bacteria in the soil) and microbial community compositions among replicate copies of each genotype. We tested this hypothesis within two *Populus* species and their naturally occurring hybrids. We considered microbial biomass nitrogen (N), PLFA biomarkers, and microbial community composition (measured by Bray-Curtis dissimilarity of PLFA biomarkers) to respond like a quantitative trait of their associated tree similar to other plant phenotypes displaying quantitative inheritance. When these estimates are generated according to standard breeding designs (e.g., among lineages of genotypes), quantitative genetic methods are appropriate for relating the observable components of phenotypic variance of the microbial community to the measurable components of plant genetic variance (i.e., broad-sense heritability or clonal repeatability; Falconer and McKay 1996, Conner and Hartl 2004). We define community heritability as the tendency of replicated individuals of the same genotype to support the same biomass or community of microorganisms in soils directly beneath that tree (Shuster et al. 2006, Whitham et al. 2006).

#### METHODS

Using a common garden of known genotypes planted in 1991, we quantified the soil microbial community beneath three to five replicate clones each of multiple genotypes of *Populus fremontii* (four genotypes), *P. angustifolia* (five genotypes), and their natural  $F_1$  (five genotypes) and backcross hybrids (six genotypes), for a total of 70 trees. Tree genotypes were previously collected and replicated as stem cuttings from natural populations along a 105-km transect along the Weber River, in northern Utah, USA, and planted randomly in

a 1-ha common garden in Ogden, Utah, on 5-m centers (see Plate 1). Randomization of replicate clones within the garden minimized site bias and maximized our ability to detect genetic effects. Soils at the garden were typical of low-elevation alluvial riparian sites and were characterized as coarse-loamy, mixed, mesic Oxyaquic Haploxerolls. Due to 13 years of *Populus* litter accumulation and shading, the understory is sparse immediately adjacent to the tree trunk but includes *Bromus tectorum*, *Ambrosia* spp., *Tragopogon dubious*, *Poa bulbosa*, and *Lactuca serriola*; the understory community showed no pattern of abundance relative to the tree genotypes or cross types (Z. Kovacs, J. Lamit, T. Wojtowicz, and S. Wooley, *personal communication*). Tree genotypes and specific cross type status (i.e., pure species,  $F_1$  or backcross hybrids) were previously determined with restriction fragment length polymorphism (RFLP) analyses (Martinsen et al. 2001). While genetic  $\times$  environment interactions ( $G \times E$ ) are clearly important to microbial dynamics, common garden approaches to elucidate the role of the genetic vs. environmental components are valuable to help tease apart the genetic interactions that may tie plants to their associated communities in ways that are only beginning to be appreciated.

#### Microbial analyses

In October 2004, when the trees were 13 years old (and 10–15 m tall), we sampled bulk mineral soils beneath each tree using polycarbonate soil cores (4.8  $\times$  15 cm). We chose this season as previous research in this system indicated that fall was an optimal time to quantify microbial activity due to increased rainfall and pulses of carbon from litterfall (as demonstrated by highest rates of net nitrogen mineralization, relative to other seasons in the year; data from Schweitzer et al. [2004]). To minimize disturbance beneath each tree in this long-term common garden, soil core placement was standardized; a 15 cm deep soil core was placed and removed on the north side of each tree within 0.25 m of the trunk. The soils were kept cool, sieved (<2 mm) to remove coarse fragments and roots, and a subsample was immediately freeze-dried. From these samples, we assessed microbial community composition with PLFA biomarkers using the method of White and Ringleberg (1998). The freeze-dried soil was extracted with a phosphate-buffered chloroform–methanol solvent (Bligh and Dwyer 1959) and separated into functional classes of signature fatty acids after methylation of the polar lipids and analysis by gas chromatography (White et al. 1979; Agilent Technologies GC-Mass Spectrometer [6890N GC/ 5973N MSD], Palo Alto, California, USA). The PLFAs are unique to major taxonomic groups (e.g., gram positive and gram negative bacteria, and fungi; White et al. 1979, Zelles 1999, Leckie 2005). While this is a coarse measure of microbial community composition, recent evidence suggests it is just as effective in differentiating treatment patterns as func-

tional analyses (i.e., community-level physiological profiling) and molecular techniques (Ramsey et al. 2006). We conservatively used common PLFAs between 14 and 18 carbon atoms long to include in the analysis as those are PLFAs exclusively identified as microbial. Total PLFA was determined as the sum of all PLFA biomarker concentrations (nmol/g; Zelles 1999, Bailey et al. 2002). PLFA compounds identified as general bacterial and fungal biomarkers (Appendix) were used to calculate the ratio of bacterial to fungal PLFA concentration in the soils.

We also quantified microbial biomass N using the chloroform fumigation extraction method (Haubensak et al. 2002). A subsample of field moist soil (~25 g) was extracted with 50 mL 0.5 mol/L K<sub>2</sub>SO<sub>4</sub>, shaken, gravity-filtered with Whatman filter paper no. 1 (leached with deionized water and K<sub>2</sub>SO<sub>4</sub>), and stored in a freezer until chemical analysis. Another 25-g field-moist subsample was exposed to chloroform for five days in a glass vacuum desiccator with 30 mL ethanol-free chloroform. After the fumigation period the samples were extracted with 50 mL of 0.5 mol/L K<sub>2</sub>SO<sub>4</sub> in the same manner as above. Total microbial biomass N was determined on the thawed extracts with a micro-Kjeldahl digestion followed by colorimetric analysis using the salicylate method on a Lachat AE auto-analyzer (Lachat Industries, Loveland, Colorado, USA). Microbial biomass N was determined by subtracting the unfumigated sample from the fumigated sample. A third subsample of field moist soil was oven dried (48 h at 105°C) to determine soil moisture content. All PLFA and microbial biomass values are expressed on an oven-dry mass basis.

#### Statistical analyses

To detect differences in microbial biomass and microbial communities (based on the nonmetric multidimensional scaling [NMDS] scores) among tree cross types, we performed a line-cross analysis of the size of the microbial biomass and the composition of the microbial community across the four cross types (*Populus fremontii*, *P. angustifolia*, and their natural F<sub>1</sub> and backcross hybrids). A line-cross analysis estimates the additive (i.e., parent to offspring genetic effects), dominant (i.e., nonadditive, one allele effects), and epistatic (i.e., effects of nonadditive novel combinations of genes) components of phenotypic variance associated with plant cross type and microbial dynamics across the hybridizing complex (Lynch and Walsh 1998). This allowed us to make inferences on the relative contribution of plant genotype vs. hybrid cross type to microbial dynamics as well as how genetic introgression may affect microbial community dynamics.

We assessed plant genetic effects on microbial communities across the hybridizing complex following the methods of Shuster et al. (2006). We calculated a dissimilarity matrix of the PLFA from soils collected beneath individual tree genotypes using the Bray-Curtis

dissimilarity coefficient (Legendre and Legendre 1998). The major directions of microbial compositional change were summarized and quantified using NMDS with a one-dimensional solution (Minchin 1987). We extracted the NMDS score, which represents an estimate of the combined PLFA richness (i.e., functional classes) and abundance (based on nmol PLFA/g of dry soil) for each replicated clone of each tree genotype.

To analyze differences in microbial biomass and microbial community composition among genotypes within each cross type, we performed a univariate ANOVA of microbial data within each cross type using JMP 5.1 (SAS 2003). The data showed no evidence for violation of assumptions of normality and homogeneity of variance. From the variance component estimates from the ANOVA analyses, we calculated the broad-sense heritability (i.e., clonal repeatability) of microbial biomass ( $H_B^2$  with 95% confidence limits) and microbial community composition ( $H_C^2$  with 95% confidence limits) as the among-genotype component of variation in the community phenotype ( $\sigma_G^2$ ) divided by the total variance in the community phenotype for all replicate trees ( $\sigma_{\text{tot}}^2$ ), where the total variance in the phenotype was the sum of the among genotype and the error component ( $\sigma_e^2$ ; Falconer and McKay 1996, Conner and Hartl 2004, Shuster et al. 2006).

To determine if particular PLFAs were structuring the microbial community (as opposed to a whole community response), we used "indicator species analysis" (Dufrêne and Legendre 1997, Legendre and Legendre 1998) using PC-ORD 4.02 (McCune and Mefford, 1999). This analysis calculates an indicator value based on fidelity and relative abundance of species (i.e., in this case, PLFA) to each group (i.e., in this case, plant genotype). Indicator species (those with significant indicator values) are defined as those expressing the highest degree of specificity and fidelity to a group or site, independent of abundance, following this basic formula:  $A_{ij} \times B_{ij} \times 100 = \text{IndVal}_{ij}$  and  $\max[\text{IndVal}_{ij}] = \text{IndVal}_i$ .  $A_{ij}$  is a specificity measure ( $A_{ij} = N$  individuals<sub>*ij*</sub>/ $N$  individuals<sub>*i*</sub>) and  $B_{ij}$  is a fidelity measure ( $B_{ij} = N$  sites<sub>*ij*</sub>/ $N$  sites<sub>*j*</sub>; Dufrêne and Legendre 1997, Legendre and Legendre 1998, Wimp et al. 2005). A significant indicator species for a particular group was determined by a Monte Carlo test based on Bray-Curtis distance (Dufrêne and Legendre 1997).

## RESULTS

### Microbial biomass

The line-cross analyses used to estimate the plant genetic contributions to microbial dynamics across the hybridizing complex show that the model incorporating additive genetic effects was not rejected ( $\chi^2 = 5.98$  and  $\chi^2 = 0.05$ ; df = 2, for total PLFA and microbial biomass N, respectively). However, the additive genetic coefficient was not significant, indicating that variation in microbial biomass within cross types overwhelms the variation among tree cross types (data not shown). In other

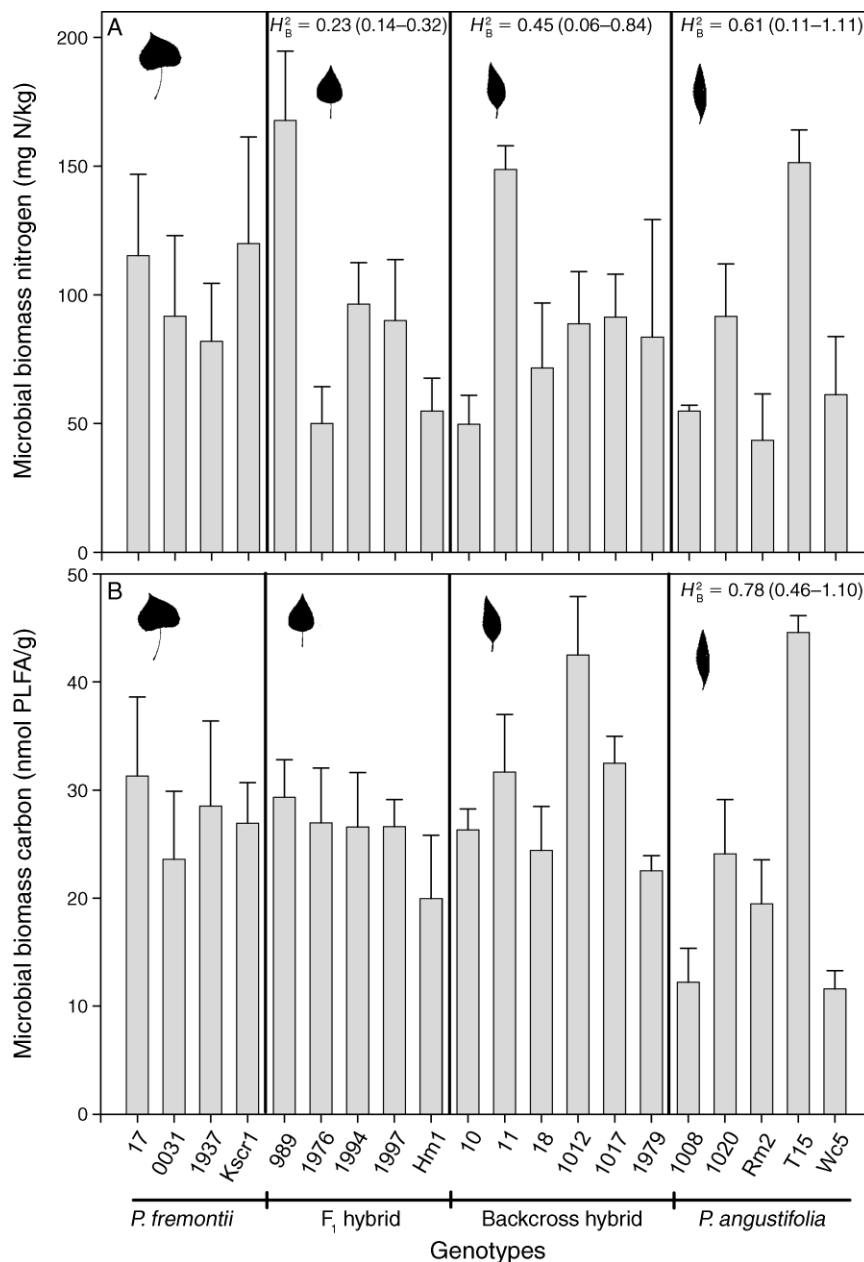


FIG. 1. (A) Microbial biomass nitrogen (N; mean + SE) and (B) total phospholipid fatty acid (PLFA; mean + SE) biomarkers beneath replicated genotypes of *Populus fremontii*, F<sub>1</sub> hybrids, backcross hybrids, and *Populus angustifolia* in a common garden. Broad-sense heritabilities ( $H_B^2$  with 95% confidence intervals) are listed where significant.

words, plant genotype explained an overwhelming proportion of the variance in total PLFA and microbial biomass N relative to the differences between the species and their naturally occurring hybrids.

Consistent with this observation, we found significant differences in microbial biomass N within cross types. Microbial biomass N differs among genotypes within *P. angustifolia* ( $F_{4,14} = 4.95$ ,  $P = 0.026$ ), as well as the F<sub>1</sub> ( $F_{4,16} = 6.01$ ,  $P = 0.007$ ) and backcross hybrids ( $F_{5,19} = 3.67$ ,  $P = 0.025$ ). While there was variation, there were

no significant differences in microbial biomass N among genotypes within *P. fremontii* ( $F_{3,13} = 0.28$ ,  $P = 0.84$ ). From the variance estimates for microbial biomass N, we found significant broad-sense heritability among genotypes within *P. angustifolia* ( $H_B^2 = 0.61$  (0.02–1.20); [95% confidence intervals]), the F<sub>1</sub> hybrids ( $H_B^2 = 0.23$  (0.20–0.32)), and the backcross hybrids ( $H_B^2 = 0.45$  (0.06–0.84); Fig. 1A).

Similarly, for total PLFA (another estimate of microbial biomass), we found significant differences

among genotypes in *P. angustifolia* ( $F_{4,14} = 15.57$ ,  $P = 0.0002$ ) and the backcross hybrids ( $F_{5,19} = 3.25$ ,  $P = 0.04$ ). There were no differences among genotypes for *P. fremontii* or the  $F_1$  hybrids. The variance estimates for total PLFA suggest that plant genotype explained 78% ( $H_B^2 = 0.78$  (0.748–0.812)) of the variation within *P. angustifolia* genotypes and 43% of the variation within backcross hybrids ( $H_B^2 = 0.43$  (0.0–0.86)), although this is not significant due to overlapping of confidence intervals (Fig. 1B). These results indicate that tree genotypes within species (i.e., intraspecific genetic variation) can create environments to which soil microorganisms consistently respond, leading to potential feedbacks in nutrient availability and carbon storage in soils beneath individual trees.

#### Microbial community composition

A line-cross analysis revealed that additive ( $\chi^2 = 9.79$ ) and additive plus dominance models ( $\chi^2 = 8.63$ ) were rejected ( $df = 2$ ; data not shown), suggesting that variation in microbial community composition within tree cross types was higher than differences among tree cross types. These results also suggest that epistatic genetic interactions across the hybridizing complex likely have a large effect on the microbial community dynamics among the cross types.

When we examined microbial community composition of genotypes within each cross type, we only found significant differences in microbial community composition among *P. angustifolia* genotypes ( $F_{4,14} = 8.31$ ,  $P = 0.0002$ ). Plant genetic factors explained 70% of the variation (i.e.,  $H_C^2 = 0.70$  (0.30–1.10); Fig. 2) in soil microbial communities within *P. angustifolia*. Furthermore we found no significant patterns in the types of microorganisms associated with the tree cross types, indicating that our patterns were not driven by specific categories or “blooms” of microorganisms in the soil. There were no differences within any of the cross types in the PLFA bacterial:fungal ratio among genotypes (data not shown). Similarly, when we examined the effects of plant genetic factors on individual PLFA biomarkers we only found patterns within backcross hybrids, suggesting that in the pure species and  $F_1$  hybrids no single PLFA was specific to those tree types. PLFA i15:0, a15:0, 17:0, cy17:0, and 18:2 $\omega$ 6t were significant indicators for backcross hybrids (see Appendix for PLFA identification), suggesting strong associations of the soil microorganisms containing these biomarkers with backcross tree genotypes alone. However, as there were no overall significant differences in community composition between genotypes in backcross hybrids, the meaning of this result is unclear.

#### DISCUSSION

After 13 years of plants conditioning the soils beneath them, we found that intraspecific plant genetic variation can affect soil microbial biomass and microbial community composition. These data support the hypothesis that

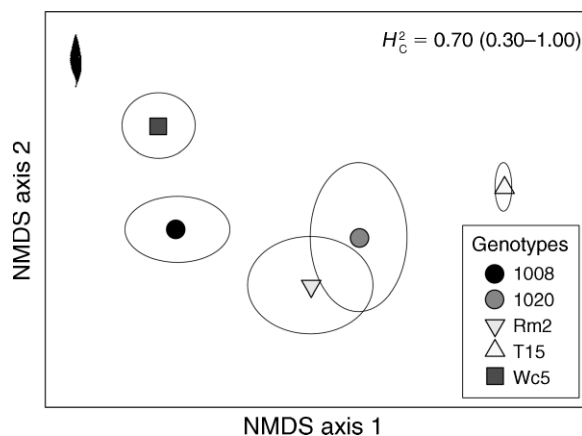


FIG. 2. Mean microbial community composition (i.e., Bray-Curtis dissimilarity) of an individual plant genotype (symbols surrounded by ellipses representing  $\pm$  SE;  $N = 3$ –5 replicates per genotype) underneath replicated tree genotypes of *Populus angustifolia* in a common garden. As this is a distance measure, the NMDS (nonmetric multidimensional scaling) axes are unitless.  $H_C^2$  (with 95% confidence intervals) represents the broad-sense heritability for the microbial community composition.

feedbacks from plant genotype may be an important ecological factor affecting microbial dynamics. Overall, we found that plant genotype influenced microbial biomass N beneath replicated genotypes of *P. angustifolia*,  $F_1$  and backcross hybrids (but not *P. fremontii* genotypes), and demonstrated significant broad-sense heritability. Plant genetic factors explained 61%, 23%, and 41% of the variation in microbial biomass N, respectively. Total PLFA, as another measure of microbial biomass, demonstrated significant genotype effects in soils associated with *P. angustifolia* and backcross hybrid genotypes. Genotype was a significant predictor of soil microbial community composition within genotypes of *P. angustifolia*, with plant genotype explaining 70% of the variation in community composition. Among cross types (excepting backcross hybrids) we found no specific PLFA were associated or specific to tree cross type; the bacteria:fungi ratio also did not differ among genotypes in any of the tree cross types. The lack of significant indicators suggests that the observed patterns were not driven by any particular microbial group. In total, these results demonstrate that microbial biomass N and sometimes microbial community composition vary by plant genotype, which suggests that plant genotype can select for particular soil microbial characteristics (Kukui 2003).

#### Genotypic variation

While the studies to date in this system have focused on the pure and hybrid cross type level (Whitham et al. 2003, 2006), only recently have we begun to investigate the within-class variation (intraspecific genetic variation). For example, when plant genotype was examined, genotype explained a greater proportion of the variance



PLATE 1. Common garden, Ogden Nature Center, Ogden, Utah, USA. Photo credit: T. G. Whitham.

in arthropod community composition than species or hybrid cross type (Shuster et al. 2006). Moreover, the patterns of broad-sense heritability of arthropod community composition followed a similar gradient with the highest  $H_C^2$  occurring in the *P. angustifolia* and backcross hybrids and the lowest  $H_C^2$  in *P. fremontii* (Shuster et al. 2006). The consistency of these two studies suggests that there are similar patterns of genetic variation within the pure and hybrid cross types (i.e., low genetic variation in *P. fremontii* and high genetic variation in *P. angustifolia*), which is consistent with past research (Keim et al. 1989).

Results from these two studies indicate that, regardless of the response variable (i.e., arthropod communities [see Shuster et al. 2006]; and microbial biomass N and microbial community composition from this study), intraspecific plant genetic variation explains a greater proportion of the variance than do the differences between species and their hybrids. From an evolutionary perspective this should not be unexpected. Populations of genotypes that are distributed across the landscape face a variety of biotic and abiotic selective forces that results in genotypes with unique suites of traits, genetic architecture, and therefore unique evolutionary dynam-

ics (Thompson 2005). Other studies of genotype-specific responses in many response variables (e.g., arthropod communities, trophic-level interactions, and litter decomposition) are being demonstrated across plant species (see Johnson and Agrawal 2004, Schweitzer et al. 2005, Bailey et al. 2006, Crutsinger et al. 2006, Madritch et al. 2006, Shuster et al. 2006) and suggest that there may be much genetic variation (for any given trait) within species.

Demonstration of broad-sense heritability, or clonal repeatability, in microbial biomass and community composition (at least in *P. angustifolia*) suggests that tree genotypes within species (i.e., intraspecific genetic variation) can create environments to which soil microorganisms may respond. However in natural systems, experiments at the genotype level examining plant-microbial interactions have been inconclusive, mostly due to lack of replication. The studies to date also suggest that belowground components (i.e., roots and/or root exudates) may play a large role in structuring the soil microbial community. For example, Bever et al. (1996) found no effect of plant genotype on arbuscular mycorrhizal communities in a greenhouse study, although the study only used one genotype each of four different plant species. Similarly, Madritch and Hunter (2002, 2003) found that after 24 and 36 months, respectively, of litter additions by individual oak phenotypes into experimental microcosms, there was no effect of litter phenotype on either soil microbial biomass or microbial community composition. A study that examined the effects of both above- and belowground inputs (i.e., leaf litter and roots) across replicates of two clones found significant clonal effects on total PLFA and on ectomycorrhizal colonization (averaged across the experimental treatments; Kasurinen et al. 2005). The latter studies support the idea that clonal variation in belowground properties may impact soil microbial characteristics, which is supported by our data. Similarly, in agricultural systems, variation in the soil microbial community size or composition has been correlated with plant cultivar (Smith and Goodman 1999, Diab el Arab et al. 2001, Mazzola and Gu 2002). Using clonal replicates of 20 plant genotypes (across four cross types) we found that not only is clonal variation important to some microbial characteristics but that sometimes there is predictable variation in the community signature.

#### *Heritability of microbial dynamics*

Heritability of community composition was recently empirically demonstrated and mathematically defined as  $H_C^2$  in Shuster et al. (2006). Based on theory outlined by Shuster et al. (2006), the tendency of replicated individuals of the same genotype to support the same biomass or community of microorganisms in soils directly beneath that tree (i.e., community heritability) suggests that there are correlated fitness effects arising from interspecific indirect genetic effects (IIGEs) be-

tween the associated host plant and associated interacting species in a community. However, just like traditional studies of phenotypic heritability, the amount of variance explained by plant genetic factors may be largely context dependent.

Interspecific indirect genetic effects provide the theoretical basis for individual plant genotypes having heritable community phenotypes such as what has been observed with arthropod communities (Bailey et al. 2006, Shuster et al. 2006) and now some microbial characteristics. For example, Shuster et al. (2006) found significant heritable variation in arthropod communities within *P. fremontii*, *P. angustifolia*, and their backcross hybrids. Similarly, here we found significant heritable variation in microbial community composition, but only within *P. angustifolia*. These data suggest that there may be feedbacks at the genotype level to associated soil microorganisms (and potentially back to the plants), even though our test of microbial community composition (PLFA) may have been too coarse to detect differences that may actually exist. IIGEs account for the interactions of the whole community (i.e., the plant and each microbial community member) in which fitness of individual members is affected by their interactions within the community (for details on theory, see Moore et al. 1997, Agrawal et al. 2001, Wade 2003, Shuster et al. 2006). While one may be able to identify all of the interacting phenotypes and their fitness consequences within a simple two or three species community, identifying all of the interacting phenotypes in a complex community is just not tractable, particularly in a microbial system. Therefore all that can be said at present is that IIGEs represent the likely mechanism for the heritable variation in *P. angustifolia* genotypes' microbial community composition patterns (but see the following section).

#### *Above- and belowground mechanisms*

Our demonstration of broad-sense heritability, or clonal repeatability, in microbial biomass and community composition demonstrates that soil microorganisms can consistently respond (within a genotype) to the environment related to intraspecific genetic variation. Whether the plant genetic factors affecting microbial dynamics are related to above- or belowground processes is currently unknown. Previous work in this *Populus* system has shown that tree cross types (and genotypes) differ in many traits that could impact associated soil microorganisms. For example, plant secondary chemistry (condensed tannins, phenolics) and nutrients (nitrogen and phosphorus; Schweitzer et al. 2004, Bailey et al. 2006, LeRoy et al. 2006, Rehill et al. 2006) differ between the foliage of different cross types. *P. fremontii* and F<sub>1</sub> hybrids typically demonstrate low concentrations of condensed tannin while the backcross hybrids and *P. angustifolia* genotypes generally have high concentrations of condensed tannin in their leaf, twig, and bark tissues. Furthermore, the

concentration of foliar condensed tannin demonstrates broad-sense heritability within *P. angustifolia* (Bailey et al. 2006). Similarly, fine root production (Fischer et al. 2006), soil CO<sub>2</sub> efflux, and total belowground carbon allocation (TBCA; Fischer et al. 2007), and aboveground net primary productivity (AGNPP; Lojewski 2007) differ across the hybridizing complex and demonstrate broad-sense heritability. Trees producing high condensed tannin in their tissues have higher fine root biomass but lower overall TBCA and AGNPP than trees with low condensed tannins in their tissues. It is likely a combination of these genetic-based above- and belowground trait differences that result in specific microbial phenotypes across replicate clones, especially with respect to microbial biomass. As Madritch and Hunter (2002, 2003) showed no effect of a three-year litter phenotype manipulation, and a similar short-term litter manipulation experiment in the *Populus* system also show no effect of litter cross type (D. G. Fischer, unpublished data), variation in belowground traits could be responsible for the patterns in microbial dynamics that we documented.

#### *Conclusions*

Genetic links between above- and belowground factors, as those documented here, may commonly exist. However, the strength of these interactions at the genetic level may depend upon the genetic variation within the plant population or subpopulation of interest. For example, microbial biomass varied beneath replicated clones of *P. angustifolia*, F<sub>1</sub>, and backcross hybrid genotypes indicating that there was significant genetic variation in traits that impact soil microbial biomass in these trees. We did not find that microbial characteristics were predictably related to plant genotype in *P. fremontii*, which suggests low genetic variation for traits that impact soil microorganisms. Previous work has demonstrated much less variation in aboveground net primary productivity, leaf secondary chemistry (i.e., condensed tannins), litter decomposition, or fine root production within *P. fremontii* than in the other cross types (Schweitzer et al. 2004, Fischer et al. 2006, LeRoy et al. 2007, Lojewski et al. 2007). Several studies have now demonstrated a genetic basis to community structure and ecosystem processes (Crutsinger et al. 2006, Whitham et al. 2006 and references therein, Johnson and Stinchcombe 2007). Because the soil microbial community is so intimately linked with fundamental ecosystem processes (e.g., litter decomposition, nutrient mineralization), these results suggest that important evolutionary linkages may exist between above- and belowground communities. Our results of heritable variation in microbial biomass and microbial community composition, at least in some cross types, suggest that this could be an important but understudied aspect of plant–microorganism interactions that deserves more attention.

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#### APPENDIX

A table listing the identified phospholipid fatty acid (PLFA) biomarkers found in the soil samples (*Ecological Archives* E089-043-A1).