2015

# Fungal and Bacterial Communities of Woody Plants at the Morris Arboretum

Jacob Golan
*University of Pennsylvania*



An independent study project report by The John J. Willaman and Martha Haas Valentine Endowed Plant Protection Intern (2014-2015)

# Fungal and Bacterial Communities of Woody Plants at the Morris Arboretum


## Abstract

Microbial communities in the rhizosphere of plants play a critical role in terrestrial nutrient cycling. The composition and abundance of bacteria and fungi has been attributed to a plant host effect and to microbial host preference, as well as to the chemical and physical properties of soils. The goal of this study was to better understand how the influence of plant host and soil properties affect the distribution of bacterial and fungal communities across a variety of native (Eastern North America) and non-native (East Asian) plant taxa planted in a single artificial setting. Soil samples from the Morris Arboretum of the University of Pennsylvania (Philadelphia, PA USA) were selected from one native and non-native species from each of the following genera—Acer, Quercus, and Pinus. To characterize a profile of microbial species associated with each tree, DNA was extracted directly from soil samples in preparation for Illumina amplicon sequencing of loci targeting fungi (ITS & LSU rDNA) and bacteria (V4, 16S, SSU). A low concentration of high molecular weight genomic DNA was extracted, suggesting that soil amendments, pesticide application, and slightly elevated pH may be inhibiting microbial growth, and/or that seasonal fluctuations may considerably affect microbial abundance at the Arboretum. Future work should aim to purify extracted DNA of any contaminant prior to sequencing. Suggestions for more efficient soil maintenance are described in order to promote soil microbes that facilitate nutrient uptake by their plant hosts.



## Disciplines

Botany | Horticulture

## Comments

An independent study project report by The John J. Willaman and Martha Haas Valentine Endowed Plant Protection Intern (2014-2015)

**TITLE:** Fungal and Bacterial Communities of Woody Plants at the Morris Arboretum

**AUTHOR:** Jacob Golan
The John J. Willaman & Martha Haas Valentine Endowed Plant Protection Intern

**DATE:** April 2015

**ABSTRACT:**

Microbial communities in the rhizosphere of plants play a critical role in terrestrial nutrient cycling. The composition and abundance of bacteria and fungi has been attributed to a plant host effect and to microbial host preference, as well as to the chemical and physical properties of soils. The goal of this study was to better understand how the influence of plant host and soil properties affect the distribution of bacterial and fungal communities across a variety of native (Eastern North America) and non-native (East Asian) plant taxa planted in a single artificial setting. Soil samples from the Morris Arboretum of the University of Pennsylvania (Philadelphia, PA USA) were selected from one native and non-native species from each of the following genera—Acer, Quercus, and Pinus. To characterize a profile of microbial species associated with each tree, DNA was extracted directly from soil samples in preparation for Ilumina amplicon sequencing of loci targeting fungi (ITS & LSU rDNA) and bacteria (V4, 16S, SSU). A low concentration of high molecular weight genomic DNA was extracted, suggesting that soil amendments, pesticide application, and slightly elevated pH may be inhibiting microbial growth, and/or that seasonal fluctuations may considerably affect microbial abundance at the Arboretum. Future work should aim to purify extracted DNA of any contaminant prior to sequencing. Suggestions for more efficient soil maintenance are described in order to promote soil microbes that facilitate nutrient uptake by their plant hosts.

**TABLE OF CONTENTS**

## INTRODUCTION

Soils are generally defined as the uppermost layer of Earth's surface formed through the accumulation of eroded rock material and degraded organic matter (Wild 1993). This layer can be subdivided vertically into the organic horizon, the minerals and clay-organic horizon and the weathered rock material and mineral horizon (Bruns & Slatar 1982; Subba Rao 1997). The rhizosphere, or area of primary root influence, develops across these layers and provides a space for the development of interactions between soil fungi, bacteria, and plant roots (Barea et al 2005).

The rhizosphere is not only an important region of soil biodiversity, but also the primary region of nutrient exchange between plants and their microbial symbionts. The natural cycling of carbon (C), nitrogen (N), and phosphorous (P) and the ability of a plant to uptake these nutrients are often largely determined by the microbial community structure and species diversity in a particular soil (Treseder 2004). It is generally understood that soil fungi and bacteria work together to facilitate the uptake of P and N by plants, and in exchange a significant portion of photosynthesis-derived carbon is transferred to soil microbes (Fajardo-López et al 2007; Deveau et al 2008; Lamhamedi et al 1994; Leake et al 2004).

Specifically, plant-fungi-bacteria symbioses usually make use of available nitrate ($NO_3$-) or ammonium ($NH_4^+$) for their N intake. Bacteria (eubacteria and archaea) play a dominant role in atmospheric N2 fixation, nitrification, and denitrification, while they facilitate ammonification at more minor levels (Postgate 1987). The most common examples of N-fixing bacterial include Rhizobium, Bradyrhizobium in association with legumes, and Frankia actinomycetes associated with Alnus, Casuarina, Ceanothus and Myrica (Giri et al 2005). Fungal symbionts, specifically those forming ectomycorrhizal (ECM) associations, play a more important role in soil ammonification (Wainwright 1992; Lakhanpal 2000), while ECM participation in nitrification remains less substantial (Giri et al 2005).

Phosphorous represents a more significant growth-limiting factor as unlike in the case of nitrogen, it is not abundantly present in the atmosphere (Ezawa et al 2002). To overcome the difficulties involved in P uptake, both bacteria and (more significantly) fungi release several classes of enzymes that facilitate the uptake of phosphates (Tabatabai 1982; Schachtman et al 1998). In effect, soil bacteria and fungi can directly affect the ability of plants to occupy ecosystems with soil chemistries otherwise inhospitable to their survival.

In a meta-analysis of mycorrhizal response to N, P, and atmospheric CO2, Treseder (2004) showed that mycorrhizal abundance across studies decreased on average by 15% under N fertilization and by 32% under P fertilization, while elevated CO2 led to a 47% increase in colonization. This supports the hypothesis that mycorrhizal fungi are more abundant where plants have limited access to N and P (Treseder et al 2004). Additionally, a lower soil pH between 6.0 to 5.0 appears to positively affect ECM root colonization (Read 1991), and a lower C:N ratio paired with increased soil acidity seems to have a similar effect on the presence of ECM (Soudzilovskaia et al 2015).

Bacterial taxa have also been shown to be optimally present at discrete pH intervals (Fierer & Jackson 2006; Hartman et al 2008), although even within soils of similar pH host specificity seems to play a larger role in moderating community structure (Bonito et al 2014).

Although the soil physical and chemical properties have been shown to affect the richness and structure of bacterial-fungal-plant relationships, the effect of phylogenetic specificity on host-microbe partnerships has yet to be fully understood across a wider variety of both plant and microbe taxa (Bruns 1995; Huggins et al 2014; Öpik et al 2010). The difficulty in better understanding phylogenetic specificity in these partnerships lies in the volumes of environmental, geographic and species-related data required to do so. The last of these data are particularly problematic because a high proportion of taxa operating within soil systems are still unknown to science. It is estimated that only 10% of soil bacteria and 5% of soil fungi have been described, and concerning known species it is still poorly understood how individual taxa function within a single symbiotic network (Giri et al 2005, 48). Major questions preventing a fuller understanding of bacteria-fungi-plant symbioses involve bridging host specificity and biogeographic range with local environmental conditions affecting microbial populations in the soil (Peay et al 2010; Tedersoo et al 2012; Öpik et al 2013).

The Morris Arboretum of the University of Pennsylvania was selected as an ideal location to better understand how soil bacteria and fungi symbioses are formed with respect to plant host-specificity and soil type, as well as how microbial rhizosphere communities develop across a variety of plant taxa in a single location. Relatively few studies focus on artificially planted areas, with the vast majority concentrating instead on natural settings, such as forests or grasslands. Studies of tree nurseries (Bahram et al 2013) and agricultural plots (Liu et al 2014; Williams et al 2014) begin to address questions related to microbial community structure in disturbed or artificial settings, although they usually target a single plant host growing in approximately uniform soil conditions. For example, Helgason et al (2013) made use of an arboretum setting to quantify mycorrhizal activity across Acer L. (maple) specimens. However, no study has conducted a diversity and community structure analysis in a similar setting across several plant taxa.

One objective of this study was to conduct a preliminary survey of the soil microbial diversity of the Morris Arboretum using high-throughput amplicon sequencing of soil samples from Acer, Pinus (pine), and Quercus (oak) specimens. It is expected that fungal species richness will be directly affected by soil pH and seasonal fluctuations. Fungal specificity to certain plant taxa is predicted to vary at the genus level, while intragenerically fungal diversity is expected to vary minimally between native and non-native plant hosts (Tedersoo et al 2013). Bacterial communities are expected to be structured by host genus, although intrageneric differences in bacterial community compositions are expected to be a result of local soil chemistry and pH (Talbot et al 2014).

A secondary goal of this study was to show how a fungal-bacterial species diversity profile can be utilized in horticulture and integrated pest management protocols, using the Morris Arboretum as a case study.

## MATERIALS AND METHODS

### Study Site and Sampling

Sampling was conducted in early November 2014 at the Morris Arboretum. Soil samples were collected from ten individuals in Acer, Pinus and Quercus for a total of 30 specimens (Map A, B). For each genus, one native and one non-native species were chosen for sampling based on the criterion that each species was well distributed throughout the arboretum. The species pairs were as follows (with the native species listed first in each pair): A. rubrum and A. griseum; P. strobus and P. bungeana; Q. velutina and Q. dentate (Figure 1). Non-native specimens were sourced from East Asia (Japan, Korea, and China), and eastern North American species were considered to be native. Sampling redundancy between soil types was avoided by choosing specimens as spatially separate from one another as possible.

Samples from five accessions for each of the six species were collected in November 2014. For each accession, soil cores were taken at three equidistant points from around the circumference of the tree's trunk. A major root was traced from the tree base to approximately one to three meters radially outwards, so as to ensure that soil be collected from the rhizosphere of the target specimen. The surface litter was removed and a mass of short roots was extracted at approximately five to ten centimeters in depth. A total of three samples (labeled A, B, and C) were collected for each tree specimen, for a total of 90 samples across all three genera. Each sample was immediately stored in a cooler to prevent further microbial growth. Prior to being processed for extraction, samples were stored in a freezer at -6° C.

### Soil Preparation, Testing & DNA Extraction

Each soil sample was allowed to thaw and then immediately sieved through a 2mm mesh to remove plant debris and rocks. Soil samples were then refrozen for downstream processes.

Soil pH was measured randomly for one of the three samples per specimen. The pH was measured using a Mettler Toledo pH Meter and Sensor Samples by adding 2.5g of soil from a thawed sample to 50mL of deionized H20.

To extract soil DNA, a subsample of approximately 0.15 to 0.25g was placed directly into a bead tube from the PowerSoil® DNA Extraction Kit (MoBio, Carlsbad, CA USA). A gel electrophoresis analysis was conducted to estimate the relative concentration of high molecular weight genomic DNA. This DNA was frozen and stored at the Vilgalys Lab at Duke University, Durham NC

### PCR

PCRs were carried out in 20 µL reactions that included 10X REDTaq® PCR buffer (Sigma-Aldrich Co. LLC, St. Louis, MO), 1.0 mM deoxynucleoside triphosphates (dNTPs), 10 mM forward and reverse primers, a 10% bovine serum albumin (BSA) solution, and one unit of REDTaq® DNA Polymerase (Sigma-Aldrich Co. LLC, St. Louis, MO).

To each 20 µL reaction mixture, 7µL of template DNA (undiluted) was added. Thermocycler settings were 5 minutes at 95 °C, then 30 cycles at 95 °C for 1 minute, 52–62 °C for 45 seconds and 72 °C for 1 minute, with a final extension for 7 minutes at 72 °C. Annealing temperatures were 62 °C for ITS and LSU. PCR products were visualized through gel electrophoresis (Figure 3).

Joining Frameshift Primers and Sequencing Adaptors

After target genes were successfully amplified, a modified procedure from Lundberg et al (2013) was employed. Normal PCR was used to join Ilumina sequencing primer with frameshift nucleotides and the initial DNA template. The use of frameshifts enhances library diversity, and allows the Ilumina reading frame to begin at different starting positions, thereby mitigating amplification biases. PCR attempts at this stage were unsuccessful, and future work should aim to optimize initial PCR product for downstream applications.

Future Work

*Sequencing*

An Ilumina sequencing adaptor and a unique barcode per sample need next be joined with the PCR product from the above procedures. Once samples have been barcoded, they can all be pooled into a single tube. Next a clean-up should be performed to remove primer dimer and small fragments. An Ampure XP bead clean up kit can be used to target 800 bp size fragments as is appropriate for LSU. The clean-up protocol follows that described by Keats lab, see http://www.keatslab.org/blog. Samples can finally be submitted to a sequencing facility.

*Physical and Chemical Properties Testing*

To determine other physical and chemical soil characteristics, samples should be sent to the Pennsylvania State University College of Agricultural Sciences Agricultural Analytical Services Lab for Soil Fertility Testing. Samples are analyzed for Mehlich buffer lime requirement, and for phosphorus, potassium, magnesium, and calcium by the Mehlich 3 (ICP) test. Additional analyses should be purchased to test for total nitrogen (combustion).

## RESULTS

pH analysis

A soil pH analysis revealed that the average pH for Acer, Quercus, Pinus specimens was 6.84, 6.79, 6.73, respectively. The lowest overall pH was recorded from P. bungeana 1996-584*A (5.90), while the highest overall pH was recorded from A. rubrum 1963-007*A (7.37). Interestingly, native and non-native species separated into two approximately equal pH groups. The average pH of native Acer, Quercus, Pinus specimens was 6.57, 6.51, and 6.53 respectively, while the average pH on non-native Acer, Quercus, Pinus specimens was 7.11, 7.07, and 6.93, respectively (Figure 2).

PCR and Ilumina Sequencing

A gel electrophoresis analysis of DNA directly extracted from each soil sample suggested an overall low concentration of high molecular weight genomic DNA (Figure 3A)

While the LSU, ITS, and V4 gene regions were successfully amplified, downstream PCRs in preparation for Ilumina sequencing were not successful. The failure of PCR in joining of a frameshift section and a sequencing adaptor for all samples may have been caused by inhibiting factors present in the initially extracted soil DNA.

Suggestions for Generating Future Results

Future tests should make use of high throughput amplicon sequencing to delineate the assembly of bacteria and fungi in the rhizosphere of sampled Acer, Quercus, Pinus species. For fungi, the LSU and ITS loci should be favored as they have proven useful in many fungal phylogenetic studies (Bonito et al 2014). The LSU region (28S rDNA) can be aligned across the Kingdom and can be easily sequenced to identify taxa at the level of genus and/or species. Likewise, the ITS rDNA locus is useful in discerning fungal species in community analyses due to the high variability of the ITS1 and ITS2 regions (Schoch et al 2012). Additionally, the reference databases for taxonomic assignment based on the ITS region is more developed and robust than those for LSU (Kõljalg et al 2013). For bacteria, the V4 region of the 16S rDNA (SSU) was successfully amplified to selectively detect any bacteria present (Bonito et al 2014).

Future efforts should concentrate on optimizing PCR reagent volumes, as well as to better purify DNA using Sephadex G-200 gel purification, in order to successfully prepare templates for sequencing (Miller et al 1999; Tsai & Olson 1992).

## DISCUSSION

Some patterns of microbial host specificity to the genera Acer, Quercus, and Pinus are are already known and are expected to be present on representative specimens at the Morris Arboretum. The families Fagaceae and Pinaceae (in which Quercus and Pinus are situated, respectively) are known to form ECM associations by majority, while the family Sapindaceae (in which Acer is situated) forms predominantly AMF associations (Wang & Qiu 2006). Suillus and Rhizopogon species are expected to be significantly represented on Pinus species, as several studies have indicated a relatively high host preference between pine species and suilloid fungi (Richardson & Higgins 1998; del Lungo et al, 2006). A study of European oaks suggests that Lactarius, Russula, and Cenococcum may be significantly represented on Quercus species at the Arboretum, although no biogeographic study of northeastern North American Quercus species has been conducted on a similar scale (Suz et al 2014). As Acer species form predominantly AMF partnerships, various genera in the Glomeromycota are expected to be present with the genus Glomus being the most represented (Cooke et al 1992; Helgason et al 2002). Important bacterial microsymbionts are expected to be Pseudomonas and Bacillus species across all three genera (Barea et al 2005).

In order to determine the bacterial and fungal rhizosphere composition of Acer, Quercus, and Pinus samples, Ilumina sequencing could be conducted using the DNA successfully extracted for this project. Due to a low concentration of high molecular weight genomic DNA, future manipulation should aim to maximize PCR products for downstream procedures in preparation for the Ilumina sequencing.

Difficulties in amplifying extracted DNA may be explained by the presence of humic acid present in the soil that inhibits TAQ polymerase functioning during PCR (Tebbe & Vahjen 1993). Humic acid is known to accumulate through the degradation of organic compounds, and usually indicates good soil health. Data on pH and low microbial abundance suggests good soil quality at the Morris Arboretum, and it can be inferred that humic acid is present at significant levels through the arboretum. Various studies have shown successful removal of humid acid, as well as other PCR-inhibiting substances, with minimal DNA loss, by using Sephadex G-200 gel purification (Miller et al 1999; Tsai & Olson 1992). While there was not sufficient time to remove contaminants from DNA, future analyses should aim to purify the stored DNA for all 90 samples.

The low concentration of extracted genomic DNA might be explained by the timing of sampling. Many studies have shown that the composition of microorganism in the rhizosphere of plants fluctuates across seasons (Smalla et al 2001; Palomino et al 2005; Baum & Hrynkiewicz 2006). For example, Yu et al (2015) shows that bacterial and fungal abundance can substantially vary over the course of a single year. It is important to determine how similar fluctuations in overall microbial abundance affect the yield of extractable soil DNA, and if a sampling date in November coincided with a seasonal decrease in microbial populations at the Morris Arboretum.

Local chemical and physical soil properties may have also affected bacterial and fungal rhizosphere communities (Bonito et al 2014; Talbot et al 2014). While host specificity can explain some of the predicted variation between rhizosphere microbial communities associated with Acer, Quercus, and Pinus at the Morris Arboretum, several chemical and physical properties of the soil should be considered as having a greater impact on the overall microbial assembly. Further analyses of soil physical and chemical properties such as N, P, and C content should be conducted.

While gel electrophoresis and pH analyses indirectly suggest good soil quality at the Arboretum, the means by which soil fertility has been achieved may be unsustainable or uneconomic (Soudzilovskaia et al 2015). Beneficial soil bacteria and fungi have been shown by numerous studies to decrease the reliance of plants on artificial amendments by facilitating nutrient uptake, as well by providing protection from pathogenic microbes (Azcón-Aguilar & Barea 1997a, b; Barea et al 2005). Fertilizers and pesticides at the Arboretum may be adversely affecting microbial populations, especially if applied during seasonal periods of high microbial diversity (Trappe 1984; Marin 2011). As N- and P-fertilization, specifically, have been shown to decrease soil microbial communities, future soil treatments should be scheduled during seasonal lows in microbial diversity (Cooke et al 1992; Treseder 2004; Tedersoo & Nara 2010; Soudzilovskaia et al 2015; Yu et al 2015). The pH level of Arboretum soils should also be slightly lowered to an optimal 5.0 to 6.0 in order to promote mycorrhizae that would reduce the need for artificial fertilization (Treseder 2004; Soudzilovskaia et al 2015). More careful

management of microbial communities is encouraged as part of the Morris Arboretum integrated pest management protocols.

The use of microbial inocula may also benefit the Arboretum. While most bacterial and mycorrhizal inocula are not specific to any particular taxon, the majority of products include a large mixture of plant-beneficial bacteria and fungi. Although the diversity of organisms appears to increase the probability that an inoculum will benefit plant growth, it is still undetermined if artificially placed microbes will persist through many growing seasons. It is known that introduced ECM species typically survive for short periods in the field (Molina et al 1992; Thomson et al 1996; Dell et al 2002), and that these species generally remain in association with their exotic host plant without spreading to native hosts (Vellinga et al 2009). Specific Hamamelis and Corylus specimens at the Morris Arboretum have been inoculated with the product DIEHARD™ Transplant (Horticultural Alliance, Inc. Sarasota FL) in Summer 2013, and have all shown more robust growth. In the coming years these specimens and neighboring plants should be examined for the presence of organisms included in DIEHARD™ Transplant. Additional plants should also be inoculated with the product to test the longevity of growth promotion and its effect on a variety of taxa grown by the Arboretum.

## CONCLUSION

This study offers preliminary details about the soil quality and microbial community structure at the Morris Arboretum of the University of Pennsylvania. The low relative abundance of extracted DNA from Arboretum soil samples may have been a result of seasonal decreases in microbial communities, and/or unfavorable chemical and physical soil properties for bacteria and fungi. Initial data on microbial abundance suggests that soil pH might be slightly reduced to better promote beneficial bacterial and fungal symbionts in the rhizosphere of Acer, Quercus, and Pinus specimens. However, greater efforts to optimize a more efficient means of achieving adequate soil quality should be directed at promoting plant growth promoting rhizobacteria, pathogen antagonistic fungi and bacteria, and mycorrhizae through moderated fertilizer and pesticide usage. Further analyses to determine the nature of fluctuations in microbial populations should also be conducted over several seasonal cycles. Additionally, an expanded application of microbial inocula should be employed across a wider variety of taxa. Careful monitoring of the relative success of inoculated plants over the course of many growing seasons will be important in developing a more informed and species-specific soil amendment protocol at the Morris Arboretum.

## BIBLIOGRAPHY

**Azcón-Aguilar**, C., and J. M. Barea. 1997b. Arbuscular Mycorrhizas and Biological Control of Soil-Borne Plant Pathogens - an Overview of the Mechanisms Involved. *Mycorrhiza* 6 (6): 457–64. doi:10.1007/s005720050147

**Azcón-Aguilar**, C., and J.M. Barea. 1997a. Applying Mycorrhiza Biotechnology to Horticulture: Significance and Potentials. *Scientia Horticulturae* 68 (1-4): 1–24. doi:10.1016/S0304-4238(96)00954-5

**Bahram**, Mohammad, Urmas Kõljalg, Petr Kohout, Shahab Mirshahvaladi, and Leho Tedersoo. 2013. Ectomycorrhizal Fungi of Exotic Pine Plantations in Relation to Native Host Trees in Iran: Evidence of Host Range Expansion by Local Symbionts to Distantly Related Host Taxa. *Mycorrhiza* 23 (1): 11–19. doi:10.1007/s00572-012-0445-z

**Barea**, Jose Miguel, R. Azcón, and C. Azcón-Aguilar. Interactions Between Mycorrhizal Fungi and Bacteria to Improve Plant Nutrient Cycling and Soil Structure. 2005. In *Microorganisms in Soils : Roles in Genesis and Functions*, 195-212. Berlin: Springer-Verlag.

**Baum**, Christel, and Katarzyna Hrynkiewicz. 2006. Clonal and Seasonal Shifts in Communities of Saprotrophic Microfungi and Soil Enzyme Activities in the Mycorrhizosphere ofSalix Spp. *Journal of Plant Nutrition and Soil Science* 169 (4). WILEY-VCH Verlag: 481–87. doi:10.1002/jpln.200521922

**Bonito**, Gregory, Hannah Reynolds, Michael S. Robeson, Jessica Nelson, Brendan P. Hodkinson, Gerald Tuskan, Christopher W. Schadt, and Rytas Vilgalys. 2014. Plant Host and Soil Origin Influence Fungal and Bacterial Assemblages in the Roots of Woody Plants. *Molecular Ecology* 23 (13): 3356–70. doi:10.1111/mec.12821

**Bruns** RG, Slatar JH. 1982. Experimental microbial ecology. Blackwell, Oxford, 683 pp

**Bruns**, Thomas D. 1995. Thoughts on the Processes That Maintain Local Species Diversity of Ectomycorrhizal Fungi. *Plant and Soil* 170 (1): 63–73. doi:10.1007/BF02183055

**Cooke**, Margaret A., Paul Widden, and Ivan O'Halloran. 1992. Morphology, Incidence and Fertilization Effects on the Vesicular-Arbuscular Mycorrhizae of Acer Saccharum in a Quebec Hardwood Forest. *Mycologia* 84 (3): 422. doi:10.2307/3760195

**del Lungo** A, Ball J, Carle J. 2006. Global planted forests thematic study: results and analysis. *Planted Forests and Trees Working Paper* 38. Rome, Italy: FAO

**Dell** B, Malajczuk N, Dunstan WA. 2002. Persistence of some Australian Pisolithus species introduced into eucalypt plantations in China. *Forest Ecology and Management* 169: 271–281

**Deveau** A, Kohler A, Frey-Klett P, Martin F. 2008. The major pathways of carbohydrate metabolism in the ectomycorrhizal basidiomycete *Laccaria bicolor* S238N. *New Phytologist* 180:379–390

**Ezawa** T, Smith SE, Smith FA. 2002. P metabolism and transport in AM fungi. *Plant Soil* 244:221–230

**Fajardo** López M, Männer P, Willmann A, Hampp R, Nehls U. 2007. Increased trehalose biosynthesis in Hartig net hyphae of ectomycorrhizas. *New Phytologist* 174, 389–398.

**Fierer**, Noah, and Robert B Jackson. 2006. The Diversity and Biogeography of Soil Bacterial Communities. *Proceedings of the National Academy of Sciences of the United States of America* 103 (3): 626–31. doi:10.1073/pnas.0507535103

**Giri**, Bhoopander, Pham Huong Giang, Rina Kumari, Ram Prasad, and Ajit Varma. Microbial Diversity in Soils. In *Microorganisms in Soils* : Roles in Genesis and Functions, 19-55. Berlin: Springer-Verlag, 2005

**Gottel**, Neil R, Hector F Castro, Marilyn Kerley, Zamin Yang, Dale A Pelletier, Mircea Podar, Tatiana Karpinets, et al 2011. Distinct Microbial Communities within the Endosphere and Rhizosphere of *Populus deltoides* Roots across Contrasting Soil Types. *Applied and Environmental Microbiology* 77 (17). American Society for Microbiology: 5934–44. doi:10.1128/AEM.05255-11

**Hartmann**, Anton, Michael Schmid, Diederik van Tuinen, and Gabriele Berg. 2008. Plant-Driven Selection of Microbes. *Plant and Soil* 321 (1-2): 235–57. doi:10.1007/s11104-008-9814

**Helgason**, T., J. W. Merryweather, J. Denison, P. Wilson, J. P. W. Young, and A. H. Fitter. 2002. Selectivity and Functional Diversity in Arbuscular Mycorrhizas of Co-Occurring Fungi and Plants from a Temperate Deciduous Woodland. *Journal of Ecology* 90 (2): 371–84. doi:10.1046/j.1365-2745.2001.00674.x

**Helgason**, Thorunn, Huyuan Feng, David J Sherlock, J Peter W Young, and Alastair H Fitter. 2014. Arbuscular Mycorrhizal Communities Associated with Maples (Acer Spp.) in a Common Garden Are Influenced by Season and Host Plant. *Botany* 326 (February): 321–26

**Huggins**, Julia A., Jennifer Talbot, Monique Gardes, and Peter G. Kennedy. 2014. Unlocking Environmental Keys to Host Specificity: Differential Tolerance of Acidity and Nitrate by Alnus-Associated Ectomycorrhizal Fungi. *Fungal Ecology* 12 (December): 52–61. doi:10.1016/j.funeco.2014.04.003

**Lamhamedi** MS, Godbout C, Fortin JA. 1994. Dependence of Laccaria bicolor basidiome development on current photosynthesis of *Pinus strobus* seedlings. *Canadian Journal for Forest Research* 24:1797–1804

**Leake** JR, Johnson D. 2004. Networks of Power and Influence: The Role of Mycorrhizal

Mycelium in Controlling Plant Communities and Agro Ecosystem functioning. *Canadian Journal of Botany* 82(8):1016–1045

**Liu**, Wei, Shanshan Jiang, Yunlong Zhang, Shanchao Yue, Peter Christie, Philip J Murray, Xiaolin Li, and Junling Zhang. 2014. Spatiotemporal Changes in Arbuscular Mycorrhizal Fungal Communities under Different Nitrogen Inputs over a 5-Year Period in Intensive Agricultural Ecosystems on the North China Plain. *FEMS Microbiology Ecology* 90 (2): 436–53. doi:10.1111/1574-6941.12405

**Lundberg**, Derek S, Scott Yourstone, Piotr Mieczkowski, Corbin D Jones, and Jeffery L Dangl. 2013. Practical Innovations for High-Throughput Amplicon Sequencing. *Nature Methods* 10 (10). Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. 999–1002. doi:10.1038/nmeth.2634

**Marin**, Miguel. Effects of Pesticides on the Growth of Ectomycorrhizal Fungi and Ectomycorrhiza Formation. In *Diversity and Biotechnology of Ectomycorrhizae* 323-346. Berlin: Springer-Verlag, 2011

**Miller**, D. N., J. E. Bryant, E. L. Madsen, and W. C. Ghiorse. 1999. Evaluation and Optimization of DNA Extraction and Purification Procedures for Soil and Sediment Samples. *Applied and Environmental Microbiology* 65 (11): 4715–24. http://aem.asm.org/content/65/11/4715.abstract

**Molina** R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF, ed. *Mycorrhizal Functioning: an Integrative Plant–Fungal Process*. New York, NY, USA: Chapman & Hall, 357–423

**Morris**, Melissa H, Matthew E Smith, David M Rizzo, Marcel Rejmánek, and Caroline S Bledsoe. 2008. Contrasting Ectomycorrhizal Fungal Communities on the Roots of Co-Occurring Oaks (*Quercus* Spp.) in a California Woodland. *The New Phytologist* 178 (1): 167–76. doi:10.1111/j.1469-8137.2007.02348.x

**Öpik**, Maarja, E Vanatoa, M Moora, J Davison, J M Kalwij, U Reier, and M Zobel. 2010. The Online Database *MaarjAM* Reveals Global and Ecosystemic Distribution Patterns in Arbuscular Mycorrhizal Fungi (Glomeromycota). *The New Phytologist* 188 (1): 223–41. doi:10.1111/j.1469-8137.2010.03334.x

**Öpik**, Maarja, Martin Zobel, Juan J Cantero, John Davison, José M Facelli, Inga Hiiesalu, Teele Jairus, et al 2013. Global Sampling of Plant Roots Expands the Described Molecular Diversity of Arbuscular Mycorrhizal Fungi. *Mycorrhiza* 23 (5): 411–30. doi:10.1007/s00572-013-0482-2

**Peay**, Kabir G, Martin I Bidartondo, and A Elizabeth Arnold. 2010. Not Every Fungus Is Everywhere: Scaling to the Biogeography of Fungal-Plant Interactions across Roots, Shoots and Ecosystems. *The New Phytologist* 185 (4): 878–82. doi:10.1111/j.1469-8137.2009.03158.x

**Postgate** JR. 1987. Nitrogen fixation, 2nd edn. Arnold, London

**Read** DJ. 1991. Mycorrhizas in ecosystems. *Experientia* 47(4):376–390

**Read**, D. J., and S. E. Smith. 2008. Mycorrhizal symbiosis Rev. ed. of: Mycorrhizal symbiosis. Amsterdam: Academic Press

**Richardson** DM, Higgins SI. 1998. Pines as invaders in the southern hemisphere. In: Richardson DM, ed. Ecology and biogeography of *Pinus*. Cambridge, UK: Cambridge University Press, 243–266

**Rineau**, François, Jean-Paul Maurice, Claude Nys, Hubert Voiry, and Jean Garbaye. 2010. Forest Liming Durably Impact the Communities of Ectomycorrhizas and Fungal Epigeous Fruiting Bodies. *Annals of Forest Science* 67 (1): 110–110. doi:10.1051/forest/2009089

**Schachtman** DP, Reid RJ, Ayling SM. 1998. Phosphorus Uptake by Plants: from Soil to Cell. *Plant Physiology* 116:447–453

**Schoch**, Conrad L, Keith A Seifert, Sabine Huhndorf, Vincent Robert, John L Spouge, C André Levesque, and Wen Chen. 2012. Nuclear Ribosomal Internal Transcribed Spacer (ITS) Region as a Universal DNA Barcode Marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America* 109 (16): 6241–46. doi:10.1073/pnas.1117018109.

**Smalla**, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-Dependent Enrichment and Seasonal Shifts Revealed. Applied and Environmental Microbiology 67 (10). *American Society for Microbiology*: 4742–51. doi:10.1128/AEM.67.10.4742-4751.2001

**Soudzilovskaia**, Nadejda A., Jacob C. Douma, Asem A. Akhmetzhanova, Peter M. van Bodegom, William K. Cornwell, Esther J. Moens, Kathleen K. Treseder, Mark Tibbett, Ying-Ping Wang, and Johannes H. C. Cornelissen. 2015. Global Patterns of Plant Root Colonization Intensity by Mycorrhizal Fungi Explained by Climate and Soil Chemistry. *Global Ecology and Biogeography* 24 (3): n/a – n/a. doi:10.1111/geb.12272

**Subba Roa** NS. 1997. Soil microbiology. IBH Publ, Oxford

**Suz**, Laura M, Nadia Barsoum, Sue Benham, Hans-Peter Dietrich, Karl Dieter Fetzer, Richard Fischer, Paloma García, et al 2014. Environmental Drivers of Ectomycorrhizal Communities in Europe's Temperate Oak Forests. *Molecular Ecology* 23 (22): 5628–44. doi:10.1111/mec.12947

**Tabatabai** MA. 1982. Soil enzymes. In: Page AL, Miller Rh, Keeney DR *Methods of Soil Analysis*, part 2. Chemical and Microbiological Properties – Agronomy monograph, No 9, 2nd edn. Wisconsin, pp 903–947

**Talbot**, Jennifer M, Thomas D Bruns, John W Taylor, Dylan P Smith, Sara Branco, Sydney I Glassman, Sonya Erlandson, et al 2014. Endemism and Functional Convergence across the North American Soil Mycobiome. *Proceedings of the National Academy of Sciences of the United States of America* 111 (17): 6341–46. doi:10.1073/pnas.1402584111

**Tebbe**, C C, and W Vahjen. 1993. Interference of Humic Acids and DNA Extracted Directly from Soil in Detection and Transformation of Recombinant DNA from Bacteria and a Yeast. *Applied and Environmental Microbiology* 59 (8): 2657–65. http://www.pubmedcentralnih.gov/articlerender.fcgi?artid=182335&tool=pmcentrez&rendertype=abstract

**Tedersoo**, Leho, and Kazuhide Nara. 2010. General Latitudinal Gradient of Biodiversity Is Reversed in Ectomycorrhizal Fungi. *The New Phytologist* 185 (2): 351–54. doi:10.1111/j.1469-8137.2009.03134.x

**Tedersoo**, Leho, Marit Mett, Takahide A Ishida, and Mohammad Bahram. 2013. Phylogenetic Relationships among Host Plants Explain Differences in Fungal Species Richness and Community Composition in Ectomycorrhizal Symbiosis. *The New Phytologist* 199 (3): 822–31. doi:10.1111/nph.12328

**Tedersoo**, Leho, Mohammad Bahram, Märt Toots, Abdala G Diédhiou, Terry W Henkel, Rasmus Kjøller, Melissa H Morris, et al 2012. Towards Global Patterns in the Diversity and Community Structure of Ectomycorrhizal Fungi. *Molecular Ecology* 21 (17): 4160–70. doi:10.1111/j.1365-294X.2012.05602.x

**Thomson** BD, Hardy GES, Malajczuk N, Grove TS. 1996. The Survival and Development of Inoculant Ectomycorrhizal Fungi on Roots of Outplanted *Eucalyptus globulus* Labill. *Plant and Soil* 178: 247–253

**Trappe**, JM, and R Molina, and M Castellano. 2003. Reactions of Mycorrhizal Fungi and Mycorrhiza Formation to Pesticides. Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA. http://www.annualreviews.org/doi/abs/10.1146/annurev.py.22.090184.001555

**Treseder**, Kathleen K. 2004. A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO2 in Field Studies. New Phytologist 164 (2): 347–55. doi:10.1111/j.1469-8137.2004.01159.x.

**Tsai**, Y L, and B H Olson. 1992. Rapid Method for Separation of Bacterial DNA from Humic Substances in Sediments for Polymerase Chain Reaction. *Applied and Environmental Microbiology* 58 (7): 2292–95. http://www.pubmedcentralnih.gov/articlerender.fcgi?artid=195770&tool=pmcentrez&rendertype=abstract

**Vellinga**, Else C., Benjamin E. Wolfe, and Anne Pringle. 2009. Global Patterns of Ectomycorrhizal Introductions. *New Phytologist* 181 (4): 960–73. doi:10.1111/j.1469-8137.2008.02728.x

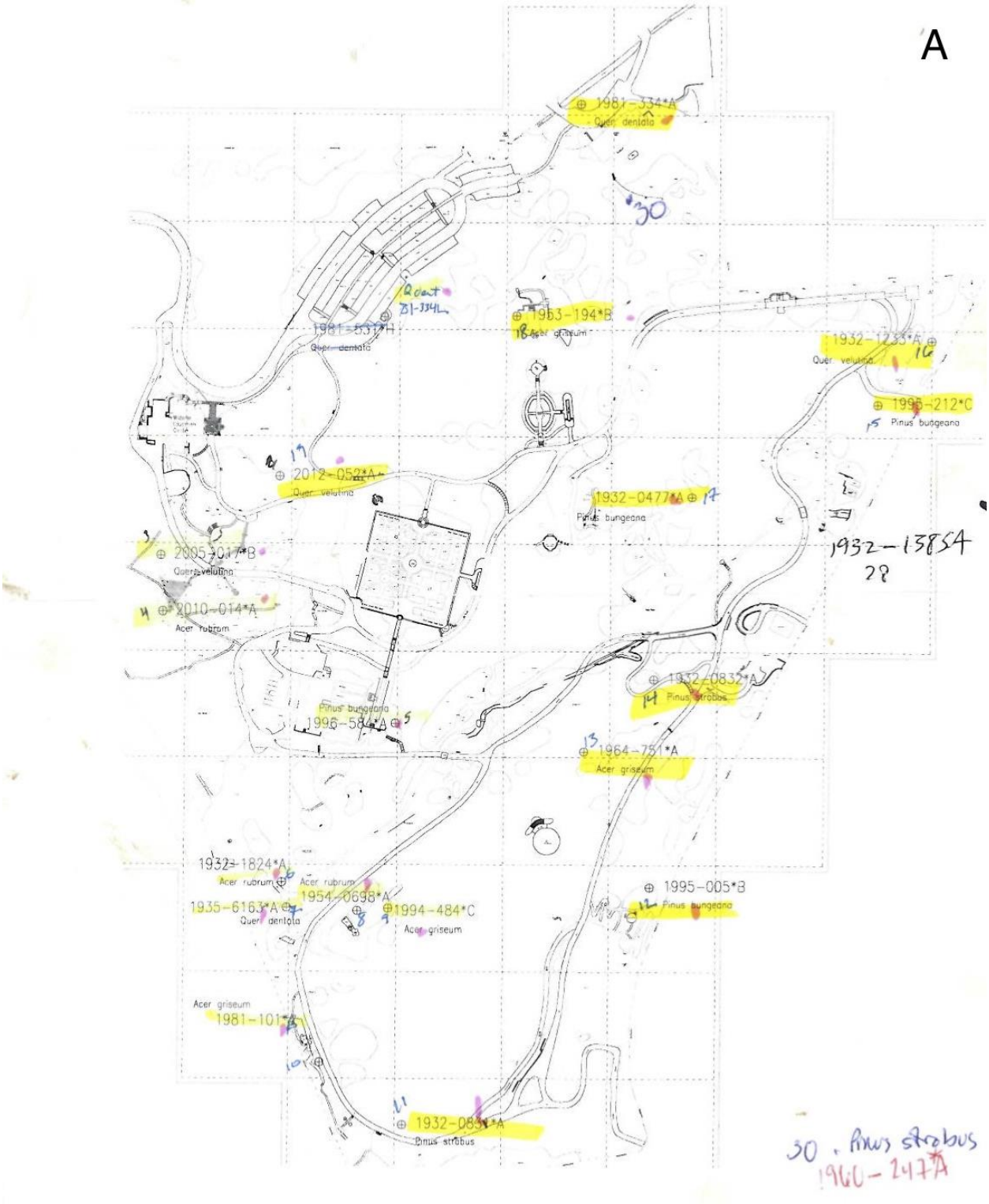
**Wang**, B, and Y-L Qiu. 2006. Phylogenetic Distribution and Evolution of Mycorrhizas in Land Plants. *Mycorrhiza* 16 (5): 299–363. doi:10.1007/s00572-005-0033-6

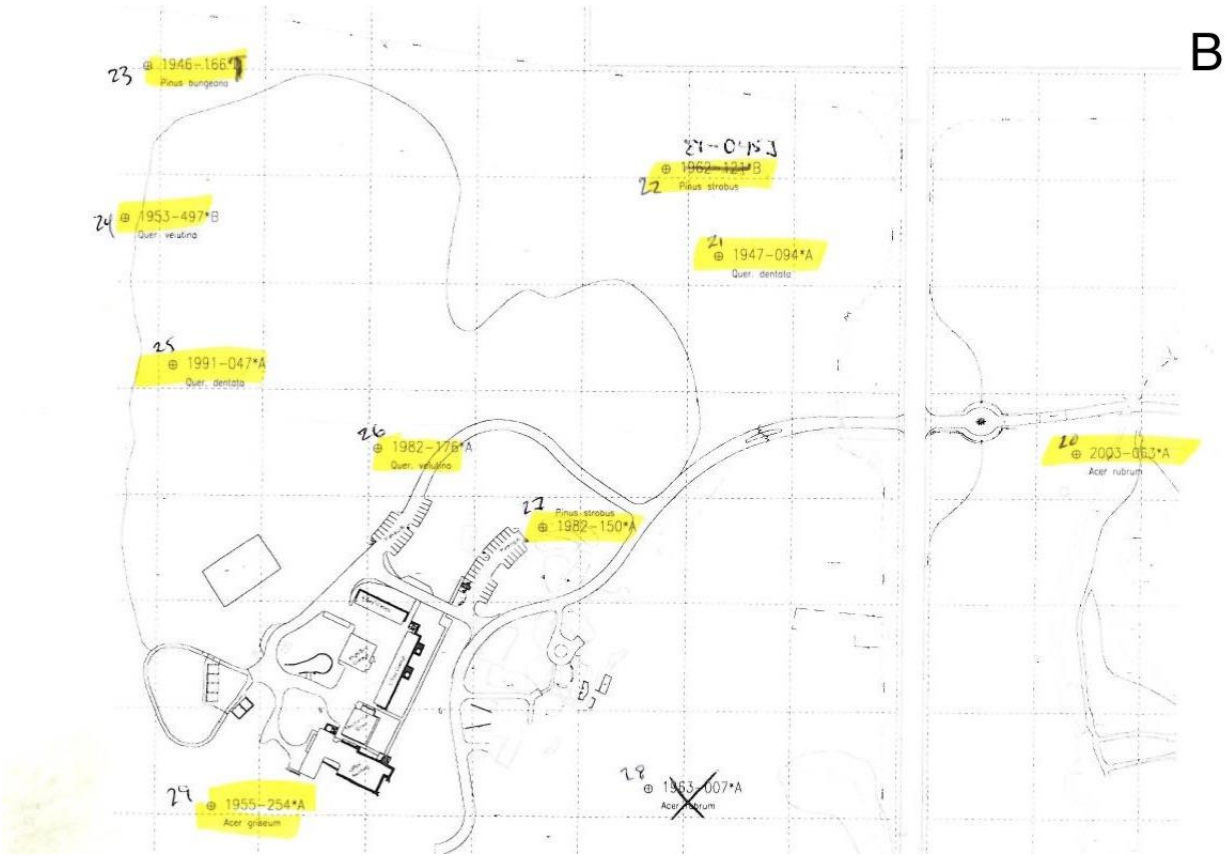
**Wild** A. 1993. Soils and environment. Cambridge Univ Press, Cambridge

**Williams**, Alwyn, and Katarina Hedlund. 2014. Indicators and Trade-Offs of Ecosystem Services in Agricultural Soils along a Landscape Heterogeneity Gradient. *Applied Soil Ecology* 77 (May): 1–8. doi:10.1016/j.apsoil.2014.01.001

**Yu**, C., X.M. Hu, W. Deng, Y. Li, C. Xiong, C.H. Ye, G.M. Han, and X. Li. 2015. Changes in Soil Microbial Community Structure and Functional Diversity in the Rhizosphere Surrounding Mulberry Subjected to Long-Term Fertilization. *Applied Soil Ecology* 86 (February): 30–40. doi:10.1016/j.apsoil.2014.09.013

## TABLES AND FIGURES






**Map A and B**: Maps of the Morris Arboretum: Map A is a map of the Compton section, and Map B is a map of the Bloomfield Farm section. All 30 tree specimens included in the study are mapped by acquisition number (listed in Figure 1)

**Figure 1**: A list of tree specimen acquisition numbers across all 6 species. Non-native species are labeled yellow, native species are labeled green

**Q. dentata-Q. velutina**

1947-094*A
1991-047*A
1935-6163*A
1981-334*L
1981-334*A
1953-497*B
1982-176*A
2005-017*B
1932-1233*A
2012-052*A

**A. griseum-A. rubrum**

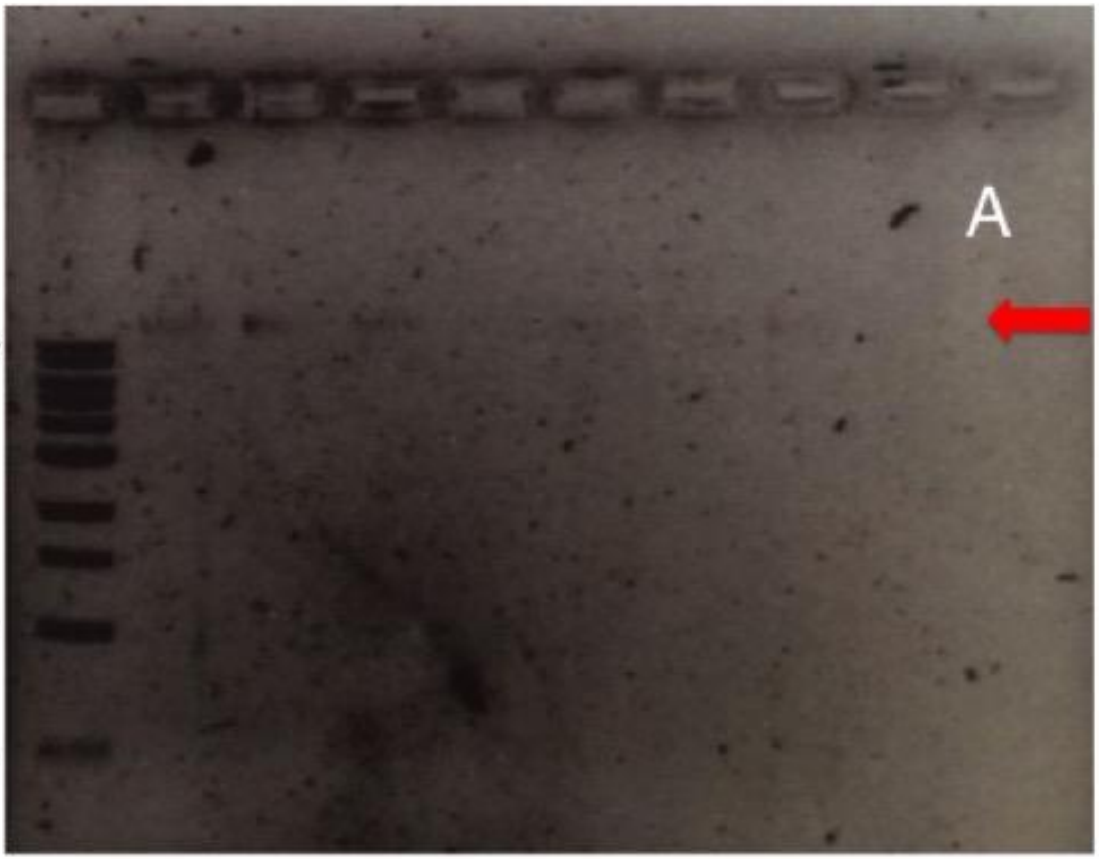
1955-254*A
1981-101*A
1964-751*A
1953-194*B
1994-484*C
1963-007*A
2003-063*A
1932-1824*A
1954-0698*A
2010-014*A

**P. bungeana-P.strobus**

1946-166*O
1995-212*C
1995-005*B
1996-584*A
1932-0477*A
1960-247*A
1932-0832*A
1982-150*A
1962-121*B
1932-0831*A

| ***Acer*** | **pH** | ***Quercus*** | **pH** | ***Pinus*** | **pH** |
|---|---|---|---|---|---|
| *A. griseum* | 6.8 | *Q. dentata* | 5.91 | *P. bungeana* | 5.9 |
| | 6.19 | | 6.64 | | 6.5 |
| | 6.51 | | 6.54 | | 6.5 |
| | 6.62 | | 6.42 | | 6.93 |
| | 6.76 | | 7.06 | | 6.84 |
| ***A. rubrum*** | 7 | *Q. velutina* | 6.98 | *P. strobus* | 6.85 |
| | 7.2 | | 6.95 | | 6.78 |
| | 7.15 | | 7.16 | | 6.99 |
| | 7.37 | | 7.1 | | 6.85 |
| | 6.84 | | 7.14 | | 7.2 |
| **AVE** | **6.844** | | **6.79** | | **6.734** |

**Figure 2**: Table of measured pH for all 30 tree specimens. 2.5g of soil was added to 50mL of deionized $H_20$. The pH was measured using a Mettler Toledo pH Meter and Sensor




(SSU) was successfully extracted and amplified (indicated by bands pointed to by red arrow).