LEAF ELEMENTAL ANALYSIS AND GROWTH CHARACTERISTICS OF MYCORRHIZAL TREATED POST OAK SEEDLINGS VIA PARTICLE INDUCED X-RAY EMISSION SPECTROSCOPY

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Growth and element assimilation was investigated in post oak seedlings exposed to four different treatment combinations of fertilization and ectomycorrhizal inoculation. Element concentration in excised leaves was analyzed via particle induced X-ray emission spectrometry with a 1.8 MeV proton macrobeam. Mean growth was significantly different across the treatment groups as well as mean concentration of Mg, Al, S, K, Ca, Fe, Cu, and Zn. The data suggest that fertilization rather than mycorrhizal inoculation had a stronger influence on plant growth and nutrient uptake. A follow up study was conducted with a 3 MeV microbeam. A 850 μm^2 scanned area of a post oak leaf produced topographical maps of 11 elements.

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INTRODUCTION

Mycorrhizal Symbiosis

In 1885, German botanist, Albert Bernard Frank, introduced the word mycorrhiza, a word derived from Greek meaning “fungus root”. Frank used this word to describe the mutually beneficial relationship between specialized soil fungi and plant roots. The fungus provides vital nutrients as well as water while the plant provides carbohydrates produced by photosynthesis. The mycorrhizal fungus is completely dependent on the plant for organic carbon (1997).

Approximately 80-90% of all plants are associated with mycorrhizae (Moore et al. 1998, Maetz et al. 1999). It is estimated that one category alone of mycorrhizal fungus, the vesicular-arbuscular mycorrhizae, can be found in 1000 genera of plants representing about 200 families (Bagyaraj 1991). Remy et al. (1994) published their discovery of fossilized mycorrhizae within a fossil of *Aglaophyton major*, an early Devonian land plant. This discovery provides strong evidence that the mycorrhizal relationship was established more than 400 million years ago. Furthermore, there is fossil evidence of mycorrhizal fungi in roots from the more recent Pennsylvanian subdivision of the Carboniferous Period (Wagner and Taylor 1981). It is believed that this unique fungus-root relationship helped plants move from the nutrient rich soup of the ancient seas to the comparatively inhospitable terra firma (Simon et al. 1993). Indeed, invasion of land by plants

may have depended upon the evolution of mycorrhizae, and this relationship moved with the plants to the present age (Allen 1991, Remy et al. 1994).

Mycorrhizal fungi can be grouped into seven divisions based on the extent of hyphal penetration and host taxonomic preference: vesicular-arbuscular, ectomycorrhizal, ectendomycorrhizal, arbutoid, monotropoid, ericoid, and orchid.

The arbuscular mycorrhiza (AM), also known as endomycorrhiza, is by far the most common mycorrhizal symbiosis. AM fungi are aseptate obligate symbionts belonging to the order Glomales. Host plants include the angiosperms, gymnosperms, and pteridophytes as well as the gametophytes of some mosses, lycopods and Psilotales (Smith et al. 1997). The description of fossilized mycorrhizae by Remy et al. (1994) showed physical characteristics of AM. The AM is so called because of characteristic fungal vesicles that form within or between root cortical cells and arbuscules (highly branched structures) that penetrate root cortical cells from intercellular hyphae (Smith et al. 1997).

The ecto-, ectendo-, ericoid, arbutoid, and monotropoid mycorrhizal fungi are septate and are members of Basidiomycetes and Ascomycetes. Ectendomycorrhizae hyphae penetrate the cortical cells and an intercellular network of hyphae, called the Hartig net, is usually well developed. They form a sheath that encases the individual rootlets from which hyphae radiate outwards through the soil, but it may be reduced or absent. Ectendomycorrhizae, arbutoid, and monotropoid mycorrhizae have several features in common. However, arbutoid and monotropoid mycorrhizae are limited to Ericaceae and

Monotropaceae, respectively. Ericoid and orchid mycorrhizal fungi are limited to members of Ascomycetes and Basidiomycetes, respectively. As their name implies, they are found in hosts belonging to Ericaceae and Orchidaceae.

Ectomycorrhizae (EM) hyphae do not normally penetrate root cortical cells to the same extent as AM. Instead, the defining characteristic of the EM fungus is the well defined sheath. Species are usually defined by physical characteristics of the fungal sheath. Fungal hyphae of this category also penetrate root cortical cells, but they are limited intercellularly and form a Hartig net. Approximately 3% of seed plants are ectomycorrhizal (Meyer 1973). The plant hosts of ectomycorrhizae belong mainly to Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, and Pinaceae. The plants in the latter four families are almost exclusively ectomycorrhizal (Smith et al. 1997).

The *Quercus* genus is known to be associated with a wide variety of EM fungi. Valentine et al. (2004) described 39 different morphotypes of ectomycorrhizas, each representing known and unknown ectomycorrhizae, that were all associated with just one species of oak, *Q. garryana*, in southern Oregon. Agerer (1987) in his excellent color atlas of ectomycorrhizae describes 24 morphotypes of ectomycorrhizae associated with European species of oak. Furthermore, Tyler (1992) described 12 morphotypes associated with *Q. robur* in Sweden. Berman and Bledsoe (1998) described nine morphotypes associated with *Q. lobata;* Urban (1998) described five ectomycorrhizal species (*Scleroderma areolatum, Amanita citrina, Tricholoma album, Lactarius quietus,*

and *Craterellus cornucopioides*) associated with *Q. petraea* and *Q. robur*; and Oh et al. (1995) showed that *P. arhizus* and *Hebeloma cylindrosporum* form ectomycorrhizae with *Q. serrata* and *Q. acutissima*. Besides naturally occurring associations, a wide variety of mycorrhizae associated with the *Quercus* genus have been successfully cultured in the lab. For example, Dixon et al. (1984) described 5 fungal species (*Pisolithus arhizus*, *Suillus granulatus, Suillus luteus, Thelephora terrestris*, and *Cenococcum geophilum*) associated with *Q. robur*, *Q. velutina*, and *Q. alba* in culture. Similarly, *P. arhizus* and *Scleroderma auranteum* form ectomycorrhizas with *Q. rubra* under laboratory conditions (Beckjord et al. 1985).

The ectomycorrhizal fungus *Pisolithus arhizus* (Scopoli : Persoon) Rauschert (synonym *Pisolithus tinctorius* (Persoon) Coker & Couch), a member of order Boletales and family Sclerodermataceae, is a common component of soil treatment products containing mycorrhizal fungal spores, and is commonly used in forestry inoculation programs worldwide (Landis et al. 1989a). Its popularity is no doubt due in part to its broad host range and its ability to tolerate adverse soil conditions such as drought, high temperature, soil contamination, extreme acidity, and low fertility (Marx et al. 1982, Smith et al. 1997). *P. arhizus* has the ability to form ectomycorrhizae with species in at least 20 different genera and over 50 tree species, *Pinus* and *Quercus* being the two most important commercially. It has been reported from over 33 countries and 38 states in the United States (Marx 1977).

P. arhizus establishment has been observed in *Q. acutissima, Q. agrifolia, Q. alba, Q. coccinea, Q. palustris, Q. robur*, *Q. rubra, Q. serrata, Q. shumardii, Q. velutina*, and the hybrid *Q. gambelii* X *Q. turbinella* (Dixon et al. 1981, Anderson et al. 1983, Daughtridge et al. 1986, Walker 1990, Tyler 1992, Oh et al. 1995, Egerton-Warburton and Allen 2001, Rieske 2001, Appleton et al. 2003, Martin et al. 2003).

A literature search has failed to locate any published articles concerning *Quercus stellata* (post oak) mycorrhizae. This work may be the first to investigate the growth benefits and element uptake of post oak mycorrhizae. *P. arhizus* was chosen as the symbiont because of its wide host range and its popularity as a soil inoculum in nursery production, outplanting, and reforestation.

Mycorrhizal hyphae act as extensions of the root system, increasing its surface area. In addition, hyphae are more effective in nutrient and water absorption than roots alone (Amaranthus et al. 1996). Compared to non-mycorrhizal roots of some plants, mycorrhizal roots are able to accumulate significantly more of the major and minor elements such as nitrogen, phosphorous, potassium, iron, manganese, copper, and zinc (Harley and Smith 1983, Tinker and Gildon 1983, Quoreshi and Timmer 1998). Furthermore, fungal colonization may help protect the plant from herbivores and pathogens, either from physical protection by the fungal sheath surrounding the rootlets or by altering investments in growth, defense, and reproduction (Amaranthus et al. 1996, Rieske 2001). Colonization enhances the nutritional status of the host

plant, increasing its ability to sustain damage without compromising productivity (Rieske 2001). For example, gypsy moth predation was found to be lowest on northern red oak seedlings inoculated with *P. arhizus*. In addition, these inoculated seedlings showed enhanced productivity relative to nonmycorrhizal seedlings in terms of number of leaves produced, leaf area, and stem diameter (Rieske 2001).

Trees planted and growing in an urban environment often are subjected to a variety of stresses including poor quality soil. Trees planted in poor quality soils will be more susceptible to pathogens and insect herbivores (Pirone 1978). Furthermore, they often require intensive maintenance and often die within a few years of outplanting (Anderson et al. 1983).

Urban soils often have an elevated pH due to concrete rubble, crushed limestone, or calcareous subsoil intruding into the root zone (Hauer and Dawson 1996). Calcareous soil in particular is typical of soils of the Southwest (Herrera 1998). Furthermore, urban soils suffer from increased compaction, high levels of inorganic and organic contaminants, elevated soil temperatures, mixed soil profiles, disrupted hydrologic flow, and interrupted nutrient and carbon cycles (Hauer and Dawson 1996). The major pH effect is to decrease the availability of micronutrients such as iron, manganese, and zinc. Soil application of micronutrients is usually ineffective unless it is in chelated form because it is rendered unavailable by soil chemical action. The macronutrients are usually

available for root absorption at most pH ranges except in highly acidic soils (Herrera 1998).

The problem of soil compaction has been a problem in the urban environment and beyond into managed forests since the advent of the bulldozer (Bridges 1991, Amaranthus et al. 1996). Considering that the lingering effects of soil compaction can last up to 45 years, it is indeed an important problem (Amaranthus et al. 1996). Woody plants exhibit different degrees of tolerance to soil compaction. Hartman et al. (2000) listed *Quercus* spp. as being the most severely injured from soil compaction (Table 1).

The induction of mycorrhizae to ameliorate the harmful effects of urban stress is becoming increasingly common. There are now soil amendment products on the consumer market that claim to help improve plant nutrient uptake and growth. For example, Plant Health Care Inc. produces Mycor™ Plant Saver®, a line of general-purpose fertilizer containing spores of endomycorrhizal fungi (Plant Health Care Inc., 440 William Pitt Way, Pittsburgh, PA, www.planthealthcare.com/index.html).

A great number of studies have been conducted to investigate the role of mycorrhizae in nutrient uptake. Two aspects are often studied: effects of mycorrhizae on trace element toxicity resistance and enhancement of nutrient absorption by mycorrhizae (Leyval et al. 1997). However, a few studies have focused upon the use of mycorrhizal inoculation to combat stress caused by soil disturbances such as soil compaction and contamination, and a smaller

collection of studies have investigated mycorrhizae in oaks subjected to soil contamination, soil disturbance, drought or the stresses of an urban environment (Anderson et al. 1983, Garbaye and Churin 1997, Walker and McLaughlin 2002a, b, Appleton et al. 2003). Studies involving post oak have not been located. In fact, there is very little literature concerning post oak in any regard.

Nutrient Requirements in Plants

Table 3 lists the essential elements required for normal plant growth along with the typical percentage of these elements in dry tissue and their important physiological function.

Macronutrients and micronutrients combined constitute about 4% of a plant's total weight (Moore et al. 1998). The remaining component of a plant consists of the light elements hydrogen, carbon, and oxygen. These three elements constitute the bulk of a plant's mass (i.e. matrix). They are major components of structural molecules as well as carbohydrates and other organic compounds and of course water. Since these elements are absorbed from the atmosphere and soil as water, carbon dioxide, and as molecular oxygen, they generally are not considered limiting (Campbell and Plank 2000).

Macronutrients and micronutrients are equally essential for growth. However, they are required in different proportions. The macronutrients are required in relatively large amounts, about 3.5% of a plant's dry weight (Moore et al. 1998). Micronutrients are required in quantities of a few parts per million.

Macronutrients are constituents of organic compounds vital to plants, such as proteins and nucleic acids, or are important for osmotic regulation. The micronutrients are primarily components of enzymes (Landis et al. 1989c).

A few more elements are essential but only for certain groups of plants. Silicon is essential for some grasses and horsetails. Sodium is essential for CAM plants, C4 plants, and halophytes. Nickel may be required for soybeans, and selenium may be required for some halophytes (Moore et al. 1998). Cobalt is necessary for a significant number of plants, albeit indirectly. Cobalt is essential for symbiotic N_2-fixing bacteria associated with legumes (Campbell and Plank 2000).

Very little information exists concerning elemental content in oaks, especially post oak. A literature search has failed to find any published material concerning element content in post oak. However, the Agricultural Analytical Services Laboratory at Penn State University has analyzed the elemental requirements of pin oak and listed 11 elements in terms of a sufficiency range, see Table 2 (Agricultural Analytical Services Laboratory, http://www.aasl.psu.edu/plant%20recs/Oak,%20Pin.pdf). These reported values are similar to the typical values for most plants given in Table 3.

Table 1. Differential vulnerability to soil compaction (Hartman et al. 2000).

most severely injured	sugar maple (*Acer saccharum* Marsh.) , beech (*Fagus* spp.), flowering dogwood (*Cornus florida* L.), oak (*Quercus* spp.), tulip poplar, pines (*Pinus* spp.) and spruce (*Picea* spp.)
less severely injured	birch (*Betula* spp.), hickory (*Carya* spp.), and hemlock (*Tsuga* spp.)
least injured	elm (*Ulmus* spp.), poplar (*Populus* spp.), willow (*Salix* spp.), plane tree (*Platanus* spp.), pin oak (*Quercus palustris* Muenchh.) and locust (*Gleditsia* spp., *Robinia* spp.)

Table 2. Interpretive element levels for pin oak in terms of a sufficiency range (Agricultural Analytical Services Laboratory, http://www.aasl.psu.edu/plant%20recs/Oak,%20Pin.pdf).

	Low	Normal	High	Excessive
N (% DW)	0.80	1.00	2.33	3.50
P (% DW)	0.12	0.16	0.39	0.50
K (% DW)	0.50	0.76	1.25	2.00
Ca (% DW)	0.30	0.40	1.36	2.00
Mg (% DW)	0.10	0.14	0.28	0.40
S (% DW)	0.05	0.11	0.30	0.50
Mn (ppm DW)	100	218	633	2000
Fe (ppm DW)	30	45	180	500
Cu (ppm DW)	3	7	38	50
B (ppm DW)	10	19	122	200
Zn (ppm DW)	15	29	88	100

Table 3. Typical percentage of essential elements in plants and their important function. Macronutrients are in bold (Landis et al. 1989c, Moore et al. 1998).

Element	% of dry tissue	Form available to plant	Function
Sulfur	0.1	SO_4^{2-}	Part of coenzyme A, cystine, methionine.
Phosphorous	0.2	H_2PO_4 -, HPO_4^{2-}	Part of nucleic acids, sugar phosphates, and ATP. Component of phospholipids
Magnesium	0.2	Mg^{2+}	Part of cholophyll. Required for enzyme activators and protein synthesis
Calcium	0.5	Ca^{2+}	Functions as second messenger to coordinate plant's response to environmental stimuli. Required for membrane integrity
Potassium	1.0	K^+	Regulates osmotic pressure of guard cells. Activates enzymes and necessary for starch formation
Nitrogen	1.5	NO_3 -, NH_4 +	Part of nucleic acids, chlorophyll, amino acids, protein, nucleotides, and coenzymes
Molybdenum	$1.0*10^{-5}$	MoO_4^{2-}	Part of nitrate reductase and essential for nitrogen fixation
Copper	$6.0*10^{-4}$	Cu^{2+}, Cu^+	Component of plastocyanin and lignin. Activates enzymes
Zinc	0.002	Zn^{2+}	Necessary for formation of pollen. Involved in auxin synthesis. Maintenance of ribosome structure
Manganese	0.005	Mn^{2+}	Involved in photosynthetic oxygen evolution. Enzyme activator. Electron transfer
Boron	0.002	H_3BO_3	Essential for growth of pollen tubes. Regulation of enzyme function
Iron	0.01	Fe^{3+}, Fe^{2+}	Required for synthesis of chlorophyll. Componenet of cytochromes and ferredoxin
Chlorine	0.01	Cl^-	Activates photosynthetic elements. Functions in water balance

PIXE Analysis

Particle induced X-ray emission spectrometry (PIXE) is a nuclear technique for multi-element analysis. Protons, or occasionally heavier ions, are accelerated to an energy of a few mega electron volts (MeV) (Johansson et al. 1995). A beam of these ions is focused onto the sample in a perpendicular orientation to the beam direction. Characteristic X-rays are produced when the beam's ions interact with the electron shells of the sample's atoms. The protons eject electrons from the atom's innermost electron shell. When an electron from an outer shell fills the vacancy, an X-ray quantum is produced (Johansson et al. 1995). Every element emits a characteristic X-ray upon interaction. These X-rays are detected by means of a Si(Li) detector. There are two series of X-rays, the K series and L series. K series X-rays are produced when electrons fall from the L-level to the K-level (Kα X-rays) and from the M-level to the K-level (Kβ X-rays). L series X-rays are produced when electrons fall from the M-level to the L-level. The L series X-rays have several different components (Johansson et al. 1995).

The detection of an X-ray is represented graphically on the spectrum as a peak. The number of pulses (counts) is a measure of the concentration of the corresponding element. For thin samples, it is possible to detect elements down to the picogram level. PIXE can be more sensitive if the beam size is reduced from a few square millimeters to a few square micrometers. Micro-PIXE is the term used to describe analyses using the micrometer beam. Analyses with the larger beam is called macro-PIXE. The highest sensitivity is achieved for

elements with an atomic number of $20 < Z < 40$ and $Z > 75$. For biological analyses, the poor sensitivity for the rare heavy elements is usually not a disadvantage (Johansson et al. 1995).

Most biological samples are composed of an organic matrix. Therefore, the matrix elements are light elements, such as carbon, hydrogen, and oxygen (Malmqvist 1995). The light element matrix of organic materials make them favorable for PIXE analysis. Protons moving through a light element organic matrix will lose little energy. The detection limit of PIXE analysis in organic samples is well below 1 μg/g. This limit makes PIXE analysis in organic matrices a genuine trace element analysis (Malmqvist 1995). However, organic matrices are sensitize to radiation and heat damage. Furthermore, the loss of some light elements (i.e. C, H, O) is to be expected, but corrections can be applied to ensure a good quantification procedure (Malmqvist 1995).

The advantage of PIXE for some types of biological samples is the need for no or very little sample preparation. A variety of instrumental techniques have been applied to determine elements in plant tissue: atomic absorption with flame or electrothermal vaporizer, inductively coupled plasma combined with optical emission spectrometry or mass spectrometry. However, in most cases these techniques require the samples to be dried and ashed and then the ash is dissolved (Orlic et al. 2002). Such lengthy preparation procedures introduce numerous opportunities for contamination. Hard botanical tissue, on the other hand, can be analyzed via PIXE directly. Botanical samples that are good

candidates for PIXE include leaves, wood, bark, or tree rings. For example, the tree rings in core samples can be analyzed so that the variation of elemental concentrations in the different rings can be examined (Legge et al. 1984). Soft biological specimens, on the other hand, require a significant amount of preparation and are the most difficult part of analysis. Only cryotechniques are recommended for all types of PIXE analytical studies (Mesjasz-Przybylowicz 2001). The leaf as a specimen for PIXE is ideal for a few reasons: it is thin, hard, easy to collect, and easy to prepare.

MATERIALS AND METHODS

Seedling Inoculation and Growth

In the fall of 2004 four hundred and eighty plants were grown from acorns (single tree parentage) in a temperature-controlled (~22 °C set point) greenhouse. The acorns were collected in the fall of 2004 from under the canopy of a single large post oak tree growing in a parking lot located on the campus of the University of North Texas on the east side of McConnell Hall. This individual tree was chosen based on two criteria: health and isolation from other post oaks. The tree appeared to be healthy, and the nearest other post oak was approximately 75 m away. Care was taken to ensure acorns were collected from a single tree to reduce genetic diversity.

Acorns were tested for viability using a float test. Acorns that floated in a bucket of water were discarded. Before planting, acorns were washed with tap water then stored in a closed lid container until sowed. The acorns were stored at approximately 4 °C for no more than 1 week before being planted. Unlike the red oaks, the white oaks, of which post oak is a member, do not require stratification. Post oak acorns germinate easily in warm moist soil or in any other moist medium. An artificial growth medium of vermiculite and peat moss (1:1) was chosen based on published recommendations from the U.S. Department of

Agriculture (Landis et al. 1989b) and from published research involving the growing of oak seedlings (Anderson et al. 1983), as well as the combined characteristics of water retention and porosity of this growth medium.

In October of 2004 the acorns were planted in 3.5" (470 ml) square pots, which were placed in compartmentalized trays. Each tray held 18 pots. The pots and trays had manufactured drainage holes. The acorn was placed on top of the moist growing medium and subsequently covered with plastic sandwich bags. The sandwich bags retained moisture inside the pot and allowed for a convenient visual inspection for signs of germination, which usually occurred within a week. The sandwich bags were removed when the seedling's shoot grew approximately 3 cm above the growing medium.

All seedlings were watered either daily or at times every other day depending upon need, and grown without fertilization until the plants were ready to be placed into treatment groups. An effort was made to keep the growing medium moist to slightly moist. The maximum air temperature recorded was 47°C in direct sunlight. Therefore, vigilance was needed during the hot summer months.

In May of 2005 the plants were randomly assigned to four different treatment groups: 1) mycorrhizal/fertilized (MF), 2) mycorrhizal/non-fertilized (M-NF), 3) non-mycorrhizal/fertilized (NM-F), and 4) non-mycorrhizal/non-fertilized (NM-NF). The plants were placed into these treatment groups when it was judged that they had reached a certain level of maturity. At this point, the

cotyledon, or the remnants of, was cut off, if it had not already dropped off. Plants of each treatment were grown together. These four plants together represented a block for statistical purposes.

Mycorrhizal plants were inoculated with spores of Pisolithus arhizus (Scopoli : Persoon) Rauschert. Approximately, 0.69 g of spores were suspended in 7200 ml of water with 26.16 ml of surfactant (spores and surfactant courtesy of Plant Health Care, Inc. 440 William Pitt Way, Pittsburgh, PA 15238). Mycorrhizal treatment groups (240 plants) were given 30 cc of inoculant suspension. Fertilized plants were given ~5.5 g of Plant-tone® organic bone and blood meal based fertilizer (The Espoma Co., 6 Espoma Rd., Millville, NJ, http://www.espoma.com; see for guaranteed analysis). The plants were fertilized again in June 2005.

The seedlings were ~10 months old when forty plants were randomly selected for elemental analysis, ten from each group, and one leaf was chosen based upon size and apparent health. Discoloration, chlorosis, necrosis, and stunted growth are all symptoms of nutrient deficiencies. For this reason, only the largest and healthiest leaf was chosen and excised for analysis.

Every month for three months beginning about May, the height of the plant was measured with a T-shaped ruler. In order for the height to be measured from the same point on all of the plants, the base of the T was placed on the top edge of the pot when taking the measurements. If necessary, the plant was carefully

straightened out. The height of the plant was measured at its tip. If the plant was dead, no measurements were taken.

Statistical analysis of growth across treatment groups was conducted via parametric block ANOVA. This was followed by a Student-Newman-Keuls multiple range test as well as a Dunnett's multiple range test to compare the different treatments against the control (NM-NF).

Macro-PIXE Experimental Procedure

About three months after the plants had been assigned to treatment groups, ten were randomly chosen from each treatment group. The leaves chosen for analysis were cut off at the petiole, washed briefly with 70% ethanol made from distilled water, then dried overnight in an incubator at 60 °C and placed onto an aluminum frame (19 mm x 30 mm) with double faced carbon tape inside a high vacuum (~10^{-6} torr) chamber to perform the analysis. The leaf was trimmed to its central part, an area of ~0.75" x 0.75". The underside of the leaf was chosen for analysis so the beam could be oriented with respect to the central vascular bundle (CVB).

The leaf venation of post oak is netted with a thick central vascular bundle, typical of most dicot leaves. Each sample was irradiated with a ~9 mm^2 beam across the sample, avoiding the CVB, where the venation is finer and where most of the tissue is composed of mesophyll. For the results shown in Figures 4-7, each sample was irradiated only once. Due to the dispersion in the

concentration of some elements, in particular Si, an effort was made to find the basis of this dispersion. To this end, element concentration dispersion was measured by comparing the following multiple sets of spectra: 1) spectra collected from the same spot on the sample in order to check for beam induced element loss, 2) spectra from different spots on the same leaf to check for spatial variation of element concentration, 3) spectra from different leaves belonging to the same plant to check for variation among different leaves of the same plant.

A 2.5 MV Van de Graaff accelerator was used to accelerate a helium cation beam with an energy of 1.8 MeV (Naab 2006b). Figure 8 shows a Rutherford Backscattering Spectrum (RBS) analysis from these samples depicting the percentage of the major elements H, C, N, O, and Ca. RBS is used in conjunction with PIXE for major light element (matrix) determination and depth profiling, and PIXE is used simultaneously for minor and trace element analysis (Watt and Grime 1995). The RBS/PIXE combination is useful for thin specimens because the peaks from light elements (i.e. C, N, and O) can be easily resolved in the RBS spectrum, and the minor/trace elements can be resolved in the PIXE spectrum (Watt and Grime 1995).

RBS provides matrix information necessary to determine the mass attenuation coefficient (μ) of the characteristic X-rays and the stopping power of the matrix (S_M) which is the concentration weighted sum of the individual stopping powers of the matrix elements comprising the sample (Campbell 1995). The mass attenuation coefficient for each major element (i) was calculated using Equation 1

(Naab 2006b). The attenuation of the X-rays along the outward path originating from within the sample to the detector is determined by the transmission factor (T_Z), which is a function of μ, see Equation 2 (Naab et al. 2005). This information is used to determine the average mass concentration (C_z) for each element given for every measurable element, see

Equation 3 (Naab et al. 2005).

Equation 1

$$\mu = \sum_i w_i \, \mu_i$$

w_i = mass fraction by weight of the element in the sample
μ_i = values from the table of mass attenuation coefficients for elemental media (Hubbell and Seltzer)

Equation 2

$$T_Z = e^{-\mu(E)\delta X}$$

$\mu(E)$ = mass attenuation coefficient for the characteristic X-ray of energy E
δ = sample density and x is
X = length that the X-rays travel in the sample

Equation 3

$$C_Z = \frac{N_X \; A_Z}{\varepsilon_Z \; b_Z \; N_p \int_{E_0}^{0} \frac{\sigma_Z(E) T_Z(E)}{S_M(E)} \, dE}$$

N_X = number of counts for the Kα or Lα transition in the X-ray spectrum
A_Z = atomic mass of the element
E_Z = detector's absolute efficiency for the Kα or Lα transition
b_Z = intensity fraction of the Kα or Lα transition (values from Firestone and Shirley 1996)
N_P = number of incident protons
σ_Z = elemental K-shell or L-shell X-ray production cross section
T_Z = transmission in the sample for the Kα or Lα x-ray
S_M = the stopping power of the matrix of the sample.

RBS was done on five samples to determine the mass attenuation coefficient and stopping power in each of them according to the matrix composition. The average values were used to evaluate C_z. The dispersion of data for both the mass attenuation coefficient and stopping power for the five samples was within 5% with respect to the averages.

A Princeton Gamma-Tech Inc. X-ray detector with a ~60 mm thick aluminum foil “funny filter” was used. It had a resolution of 135 eV at 5.9 keV. It was positioned at 150° with respect to the beam direction. This detector has an ultra thin window supported on a high transmission mesh (Naab 2006b). As performed in Naab et al., 2005, an electrically insulated tungsten mesh was placed ~70 cm upstream from the target to measure the number of ions striking the sample (N_p) during spectrum acquisition. The current measured by the mesh was 25% of the beam current reaching the target. Each spectrum was acquired for 20 μC.

The thickness of the leaves was about ~120 μm. Protons with an energy of 1.8 MeV will travel ~40 μm through this thickness (Naab 2006b). Since electronic excitation occurs along much of the ion range, the total X-ray yield is a result of depth-dependent factors that must be integrated to yield the elemental concentration in the bulk material (Naab et al. 2005).

Mean concentration was analyzed via parametric ANOVA if the element’s concentrations were normally distributed across treatment groups. Otherwise, a nonparametric Kruskal-Wallis analysis of variance was conducted. As a follow up

for the parametric data set, a Tukey's multiple comparison test was conducted and a Tukey's multiple comparison test on ranked data was conducted for the nonparametric data set.

Micro-PIXE Experimental Procedure

A healthy nonmycorrhizal post oak leaf was randomly chosen for analysis. The leaf was excised at the petiole and washed briefly with 70% ethanol, then dried overnight in an incubator at 60 °C. After desiccation the leaf was placed on an aluminum frame and secured to the frame with double-faced carbon tape. The underside of the leaf was chosen for analysis. The mounted leaf was placed inside a high vacuum (~10-7 torr) spherical chamber to perform the analysis. For a more detailed description of the spherical microprobe vacuum chamber used at the Ion Beam Modification and Analysis Laboratory see Pelicon et al., 2005. The sample thickness was ~100 μm. A proton particle will travel a similar amount,~100 μm, through the sample (Naab 2006a).

Elemental concentrations using the microbeam were achieved using a high-energy, heavy ion microprobe system installed on a 3 MV National Electrostatics Corporation 9SDH-2 Pelletron® tandem accelerator at the Ion Beam Modification and Analysis Laboratory at the University of North Texas (National Electrostatics Corp., 7540 Graber Road, Middleton, WI, www.pelletron.com). This system has a probe-forming lens system with a Russian quadruplet configuration and a demagnification factor of 60 (Naab 2006a). For a more detailed description of the microprobe system at UNT, see McDaniel 2001. For this analysis, we used a

proton microbeam with a spatial resolution of 10 μm and with an energy of 3 MeV. Two areas (850 μm^2 or 200 $pixels^2$) adjacent to each other were scanned on the same leaf (Figure 10).

A Princeton Gamma-Tech Inc. X-ray detector was used to acquire the spectra. The X-ray detector resolution was 135 eV at 5.9 keV and positioned at 150° with respect to the beam direction. The sample surface was perpendicular to the beam direction. The X-ray detector has an ultra thin window supported on a high transmission mesh that allows detecting X-rays energies down to the carbon Kα X-ray transition (Naab 2006a). The beam current on the target was ~100 pA and the spectra were taken for a total integrated charge of ~1 μCoulomb.

RESULTS

Growth Analysis

Total growth was calculated as the difference between baseline height and height taken on the last day of data collection. A parametric block ANOVA of the growth over the three-month period revealed that the H_0 (H_0: Mean growth across the four treatments is not significantly different) can be rejected. Mean growth is significantly different across the four different treatment groups (parametric block ANOVA $p<0.0001$, $\alpha=0.05$). A follow up analysis with a Student-Newman-Keuls multiple range test revealed that the difference is probably among the fertilized and non-fertilized groups regardless of mycorrhizae inoculation (Table 4). A comparison to the control (NM-NF) via a Dunnett's test reveals again that the difference among the means is primarily between the fertilized groups and the non-fertilized groups. The two fertilized groups are significantly different from the control, which was not significantly different from the M-NF group (Table 5).

Figure 1 depicts a box plot of change in plant height over time. A small circle represents statistical outliers and an asterisk represents extreme values. As expected, this figure suggests that an upward trend exists within each treatment. Notice that all of the extreme values belong to the M-F group. The upward trend in plant height is also evident when treatment groups are grouped together (Figure 2).

Table 4. Student-Newman-Keuls multiple range test revealed that the difference is probably among the fertilized and non-fertilized groups regardless of mycorrhizae inoculation. Means with the same letter are not significantly different from each other.

Treatment	Grouping	Mean (cm)
M-F	A	2.3096
NM-F	A	2.2890
NM-NF	B	1.3569
M-NF	B	1.2155

Table 5. A comparison to the control (NM-NF) via a Dunnett's test reveals again that the difference among the means is primarily between the fertilized groups and the non-fertilized groups. Comparisons significant at α=0.05 are indicated by ***.

Treatment Comparison	Difference between means	
M-F - NM-NF	0.9528	***
NM-F - NM-NF	0.9321	***
M-NF - NM-NF	-0.1414	-

Figure 1. A box plot of change in plant height over time. A small circle represents statistical outliers and an asterisk represents extreme values. Notice that all of the extreme values belong to the M-F group.

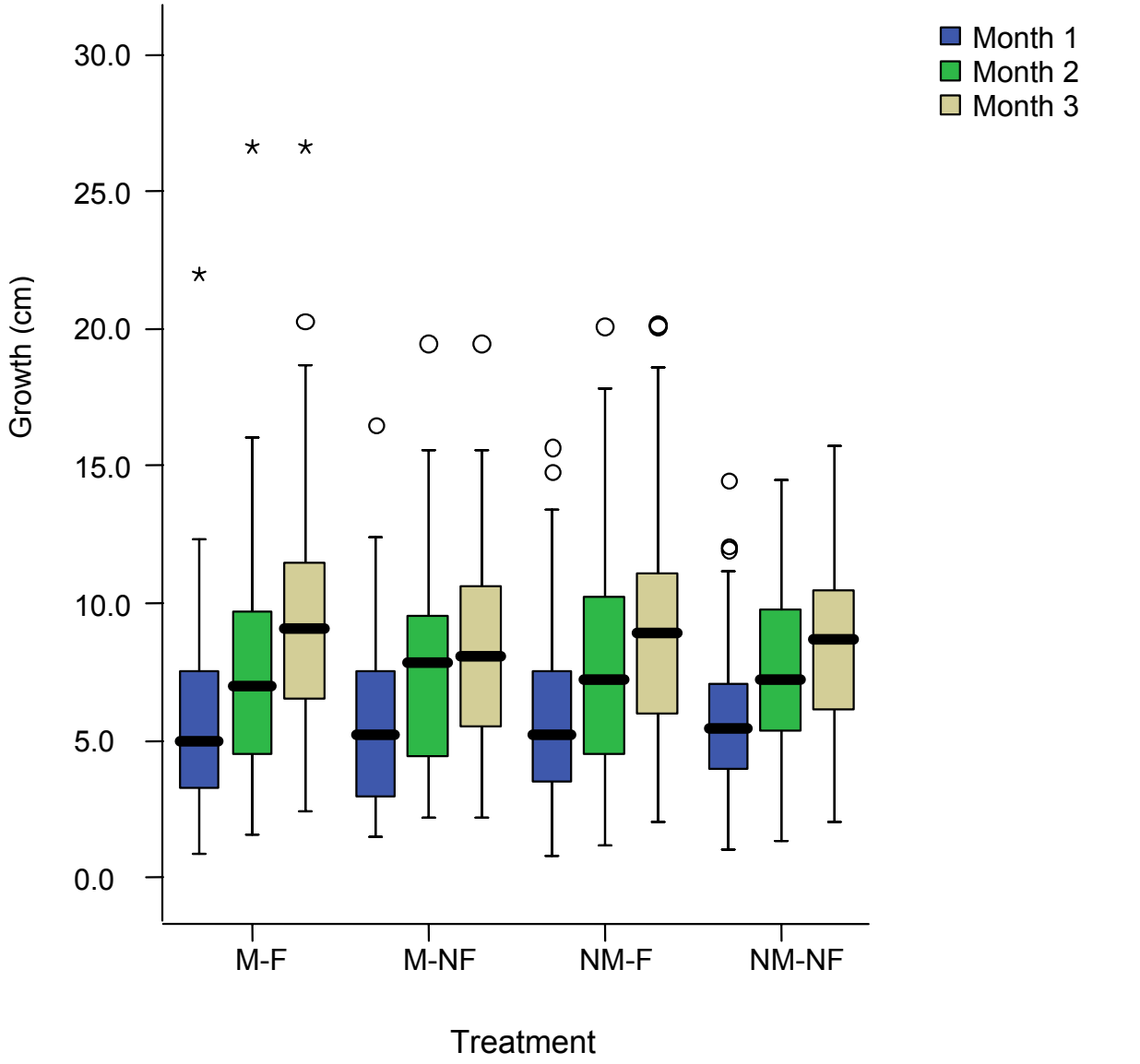
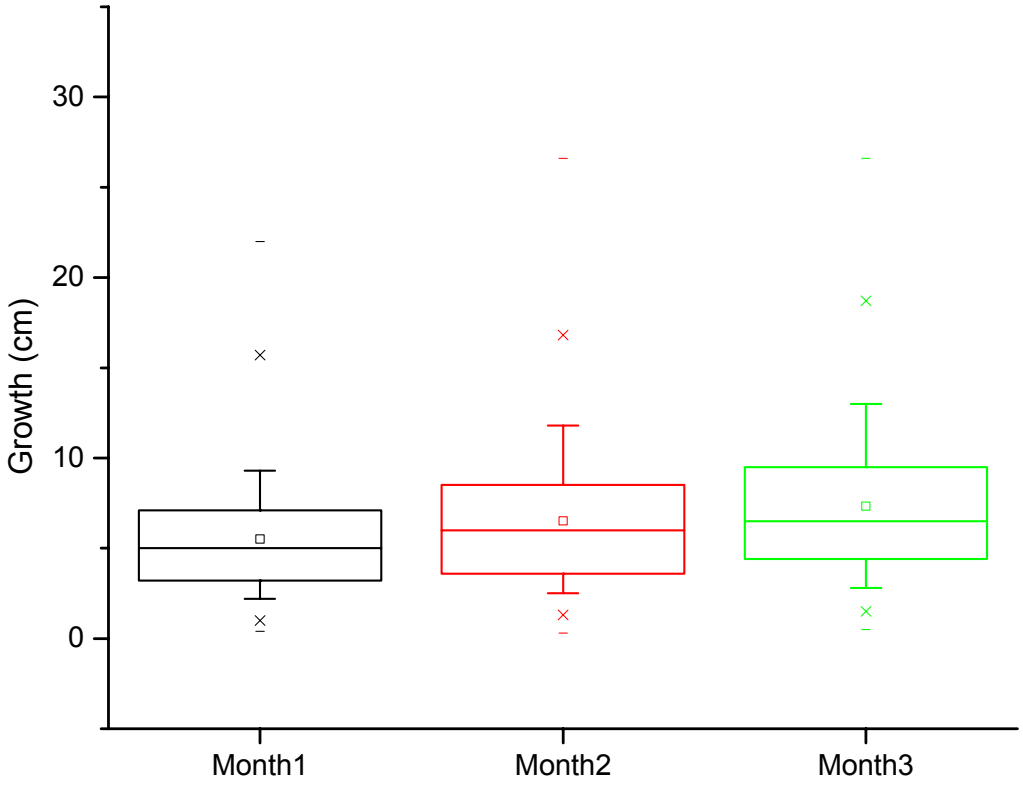


Figure 2. Box plot of changes in plant height over time. As expected, this graph depicts an upward trend in growth.



Macro-PIXE Elemental Analysis

Eighteen elements were detected by PIXE and RBS. Of these eighteen, Rb, Ni, Cr, Br, and Ba had insufficient counts to be analyzed statistically. An examination of Figures 3-6 reveals a paucity of data for these elements, and it was judged that these elements be filtered from the analysis. The remaining elements were tested for normality. Only five elements (Mg, Al, S, K, and Zn)

were found to be not significantly different from a normal distribution (Table 6). As a result, the mean concentrations of these 5 elements were analyzed via parametric ANOVA (Table 7). The treatment means of the remaining elements were analyzed via Kruskal-Wallis, a nonparametric analysis of variance (Table 7).

Parametric ANOVA revealed that the means of all five elements with normal distributions have mean concentrations significantly different across the treatment groups (Table 7). As a follow up, a Tukey's studentized multiple comparison test was employed to detect among group differences. The results indicate group overlap for all but one element, Al (Figure 3).

Of the eight remaining elements with sufficient data for analysis, a Kruskal-Wallis non-parametric analysis of variance detected a significant difference across the treatments for three elements: Fe, Ca, and Cu (Table 7). Because of the nonparametric nature of the data, a follow up with a Tukey's studentized range test on ranked data was conducted. This test placed the treatment means into overlapping groups (Table 9).

In summary, eighteen elements were detected in post oak leaf using macro-PIXE as an analytical tool. Of these eighteen, five elements not required by plants (Rb, Ni, Cr, Br, and Ba) were not analyzed statistically because of insufficient data. However, they are represented graphically in Figures 4-7. Eight of the 13 remaining elements (Mg, Al, S, K, Zn, Fe, Ca, and Cu) were found to have mean element concentrations significantly different across treatment groups. Multiple comparison tests did not find distinct group differences. The only

exception was aluminum. The mean concentration of Al in the NM-F group (1780 ppm) was significantly different from the three other groups, which were not significantly different from each other: M-F (950 ppm), M-NF (880 ppm), NM-NF (710 ppm), see Table 8. Because of the lack of distinct treatment group differences, a clear treatment influenced trend is not discernable. However, the results of the multiple comparison tests suggest fertilization may have had a stronger influence on element uptake versus mycorrhizal inoculation. The fertilized groups tended to have higher mean element concentrations, the exceptions being Mg, Ca, and Fe (see Table 6).

Micro-PIXE Elemental Analysis

Figure 11 and Figure 12 show the elemental topographies generated from the micro-PIXE analysis. Thirteen elements were detected in Spot A (Mg, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, and As). Eleven elements were detected in Spot B (Mg, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, and Zn). The topographies for potassium and calcium are striking. These elements tend to be concentrated in the vascular tissue. For potassium, the minor veins tend to have a higher concentration rather than the dominate vein located along the upper and left edge of the graph for Spot A and down the right diagonal for Spot B. Calcium on the other hand is more concentrated along the margins of the dominant vein. This may also be the case for magnesium but to a lesser extent. Mesophyll tends to have a higher concentration of phosphorous with perhaps slightly more near the margins of the minor veins. This is more evident in Spot A. The area of low element

concentration on the far left edge of the dominant vein in Spot A is the result of a shadow effect. This is an area not scanned by the micro-beam because the area was blocked by the relatively high protrusion of the dominant vein. For magnesium and phosphorous at least in Spot B, the shadow effect may have had a more significant affect. The concentrations for these two elements are higher on the right side (windward side) of the dominant vein.

Table 6. Mean±SD and Shapiro-Wilks probabilities for all detected elements. Mg, Al, S, K, and Zn are normally distributed across all treatment groups. Ba, Cr, Ni, Br, and Rb have insufficient data for analysis. Mean concentrations are in weight fraction. 10 plants for each treatment were analyzed.

Element	M-F	M-NF	NM-F	NM-NF
Mg*	3.32E-03±1.52E-03 p=0.8938	4.18E-03±1.37E-03 p=0.5673	2.22E-03±9.08E-04 p=0.8922	3.47E-03±7.96E-04 p=0.9972
Al*	9.48E-04±6.34E-04 p=0.4566	8.80E-04±4.06E-04 p=0.2577	1.78E-03±5.07E-04 p=0.0990	7.07E-04±4.15E-04 p=0.5272
Si	1.14E-02±2.42E-02 <0.0001	1.66E-02±1.54E-02 p=0.0939	9.14E-03±9.11E-03 p=0.0795	1.84E-02±1.73E-02 p=0.0257
P	2.04E-03±8.04E-04 p=0.3866	3.62E-03±2.14E-03 p=0.0010	3.12E-03±1.30E-03 p=0.3401	3.51E-03±2.56E-03 p=0.0363
S*	2.26E-03±8.45E-04 p=0.5785	1.69E-03±3.28E-04 p=0.1707	2.08E-03±4.60E-04 p=0.7895	1.31E-03±5.00E-04 p=0.5036
Cl	9.26E-04±5.27E-04 p=0.0036	1.45E-03±9.15E-04 p=0.0006	1.32E-03±6.45E-04 p=0.9627	1.57E-03±7.01E-04 p=0.2812
K*	1.30E-02±5.34E-03 p=0.5843	8.17E-03±4.16E-03 p=0.0562	9.64E-03±2.11E-03 p=0.1014	7.00E-03±3.25E-03 p=0.8779
Ca	7.09E-03±3.57E-03 p=0.9073	1.74E-02±6.55E-03 p=0.0426	1.46E-02±7.71E-03 p=0.3556	1.83E-02±7.22E-03 p=0.7345
Ba†	0±0 -	6.00E-01±5.16E-01 p=0.0002	4.00E-01±5.16E-01 p=0.0002	5.00E-01±5.27E-01 p=0.0003
Cr†	9.97E-07±3.15E-06 p=<0.0001	4.01E-05±8.29E-05 p=<0.0001	0±0 -	4.95E-06±1.57E-05 p=<0.0001
Mn	1.46E-03±2.50E-03 p=<0.0001	1.08E-03±6.05E-04 p=0.2184	8.87E-04±4.84E-04 p=0.7049	1.22E-03±9.45E-04 p=0.0188
Fe	1.20E-04±9.41E-05 p=<0.0001	2.40E-04±2.17E-04 p=0.0019	7.81E-05±3.30E-05 p=0.4873	1.05E-04±8.77E-05 p=0.3641
Ni†	5.06E-06±1.05E-05 p=<0.0001	1.23E-05±2.39E-05 p=0.0001	2.98E-6±3.29E-06 p=0.0383	1.85E-06±3.37E-06 p=0.0002
Cu	3.17E-05±4.57E-05 p=<0.0001	1.44E-05±5.78E-06 p=0.5380	2.03E-05±7.73E-06 p=0.7000	7.75E-06±3.35E-06 p=0.2102
Zn*	5.96E-05±1.96E-05 p=0.2102	3.76E-05±7.96E-06 p=0.1174	4.93E-05±2.10E-05 p=0.1671	4.31E-05±1.45E-05 p=0.7854
Br†	0±0 -	9.57E-06±1.33E-05 p=0.0060	8.68E-06±8.37E-06 p=0.0785	8.71E-06±7.47E-06 p=0.2043
Rb†	1.42E-05±1.81E-05 p=0.0062	1.72E-06±3.77E-06 p=<0.0001	1.51E-06±3.18E-06 p=<0.0001	5.83E-06±1.46E-05 p=<0.0001
Sr	1.61E-05±2.34E-05 p=0.0031	4.78E-05±2.23E-05 p=0.4017	4.00E-05±1.99E-05 p=0.1472	4.35E-05±3.16E-05 p=0.7504

* Element has a distribution across all treatment groups that is not significantly different from a normal distribution (α=0.05).
† Element has insufficient data for analysis.

Table 7. Treatment means of normally distributed elements were analyzed via parametric ANOVA. Non-normal elements were analyzed via Kruskal-Wallis. Elements with an asterisk have means significantly different across treatment groups (α=0.05).

Element	ANOVA Probability	Kruskal-Wallis Probability
Mg*	0.0074	-
Al*	0.0001	-
K*	0.0087	-
S*	0.0030	-
Zn*	0.00336	-
P	-	0.1156
Si	-	0.0907
Cl	-	0.0664
Ca*	-	0.0019
Mn	-	0.5904
Fe*	-	0.0235
Cu*	-	0.0017
Sr	-	0.0566

Table 8. Tukey's studentized range test for Mg, Al, S, K, and Zn. Means with the same letter are not significantly different (α=0.05). Mean concentrations are in weight fraction.

Element	Grouping	Mean Concentration	Treatment
Mg	A	0.00418	M-NF
	B A	0.00347	NM-NF
	B A	0.00332	M-F
	B	0.00222	NM-F
Al	A	0.00178	NM-F
	B	0.00095	M-F
	B	0.00088	M-NF
	B	0.00071	NM-NF
S	A	0.00226	M-F
	A	0.00208	NM-F
	B A	0.00169	M-NF
	B	0.00131	NM-NF
K	A	0.01305	M-F
	B A	0.00964	NM-F
	B	0.00817	M-NF
	B	0.00700	NM-NF
Zn	A	0.000060	M-F
	B A	0.000049	NM-F
	B A	0.000043	M-NF
	B	0.000038	NM-NF

Table 9. Tukey's studentized range test on ranked data for Ca, Fe, and Cu. Mean of ranks with the same letter are not significantly different (α=0.05).

Element	Grouping	Mean of Ranks	Treatment
Ca	A	26.4	NM-NF
	A	26.3	M-NF
	B A	20.4	NM-F
	B	8.9	M-F
Fe	A	29.5	M-NF
	B A	20.1	M-F
	B A	18.6	NM-NF
	B	13.8	NM-F
Cu	A	27.8	M-F
	A	25.1	NM-F
	B A	20.1	NM-NF
	B	9.0	M-NF

Figure 3. Box plants for 14 elements with reportable statistics. Treatments with the same letter are not significantly different (Tukey's studentized range test and Tukey's studentized range test on ranked data, α=0.05). Weight fraction: 0.001=1000 ppm, 0.0001=100 ppm, etc

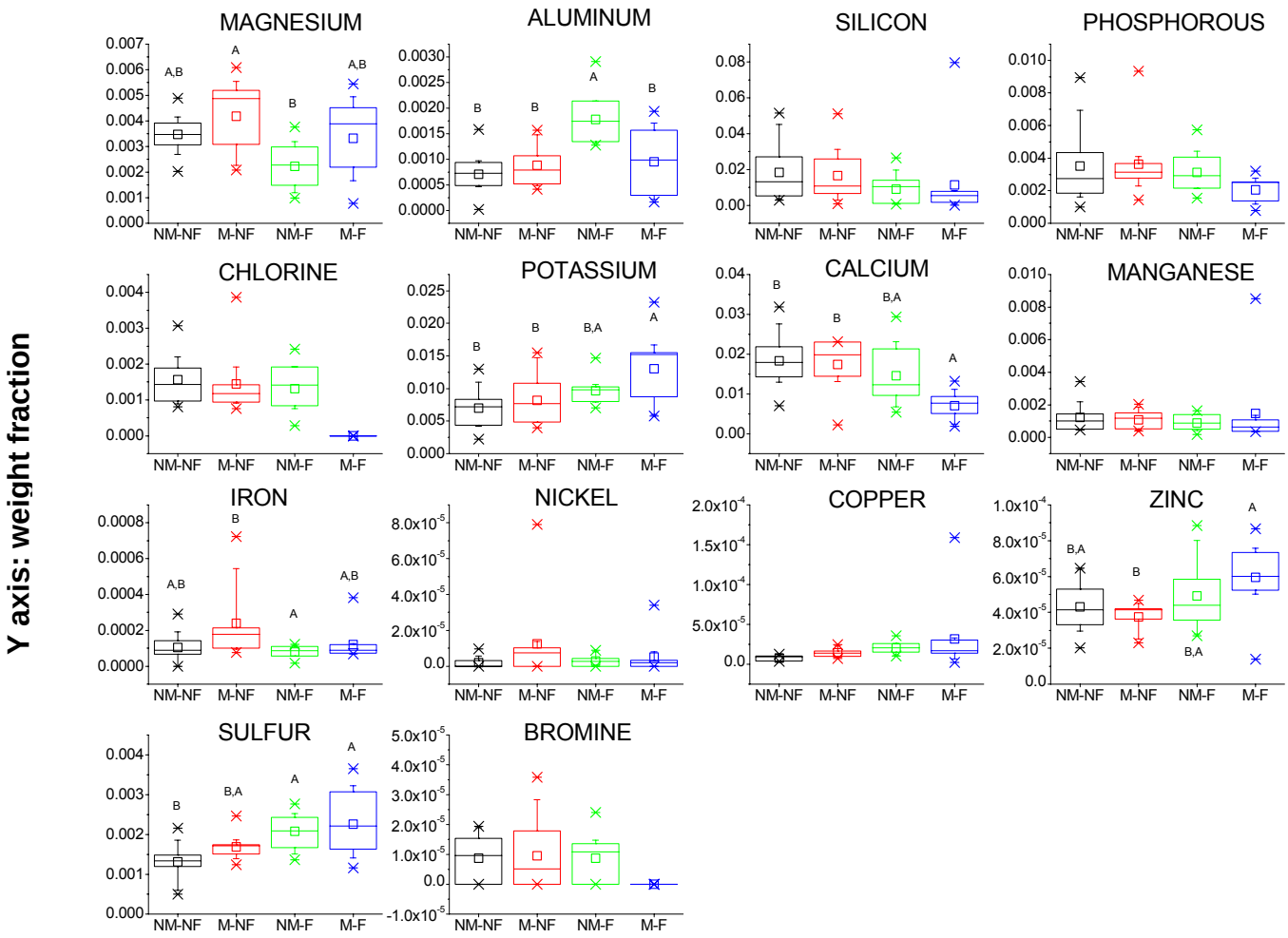


Figure 4. Concentration of all detected elements for NM-NF. Ten different leaves were analyzed for each treatment. Weight fraction: 0.001=1000 ppm, 0.0001=100 ppm, etc

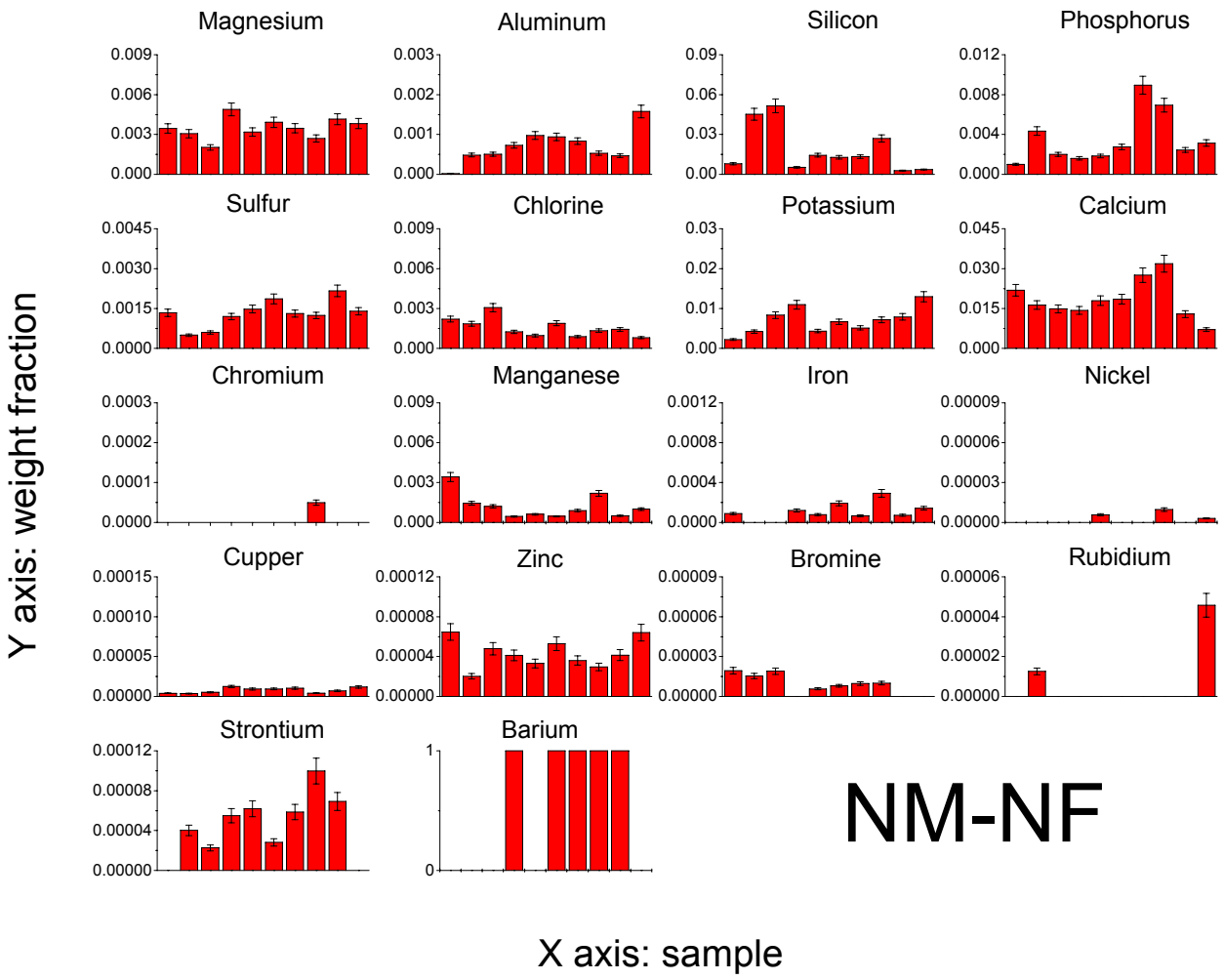


Figure 5. Concentration of all detected elements for NM-F treatment. Ten different leaves were analyzed for each treatment. Weight fraction: 0.001=1000 ppm, 0.0001=100 ppm, etc

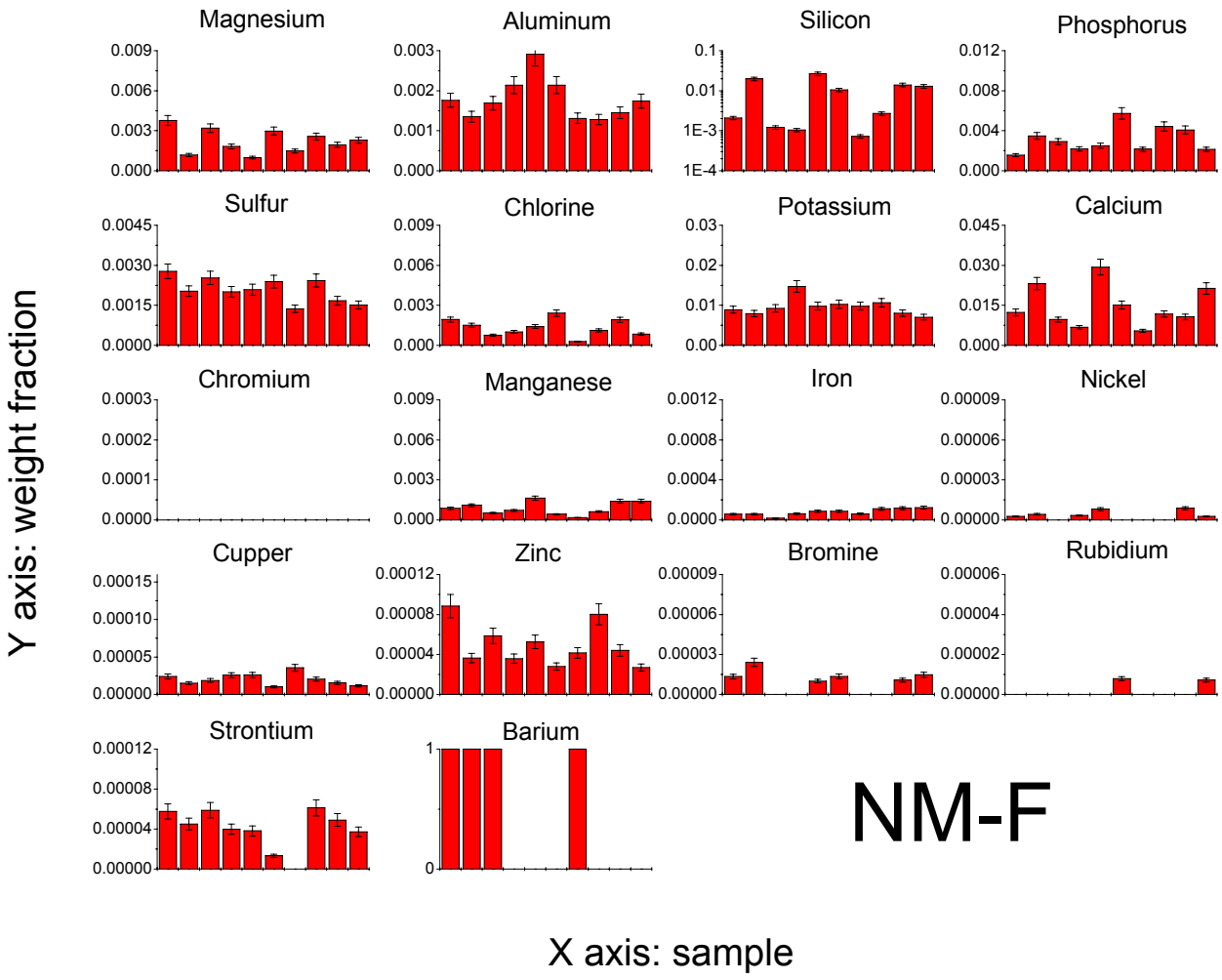


Figure 6. Concentration of all detected elements for M-NF treatment. Ten different leaves were analyzed for each treatment. Weight fraction: 0.001=1000 ppm, 0.0001=100 ppm, etc

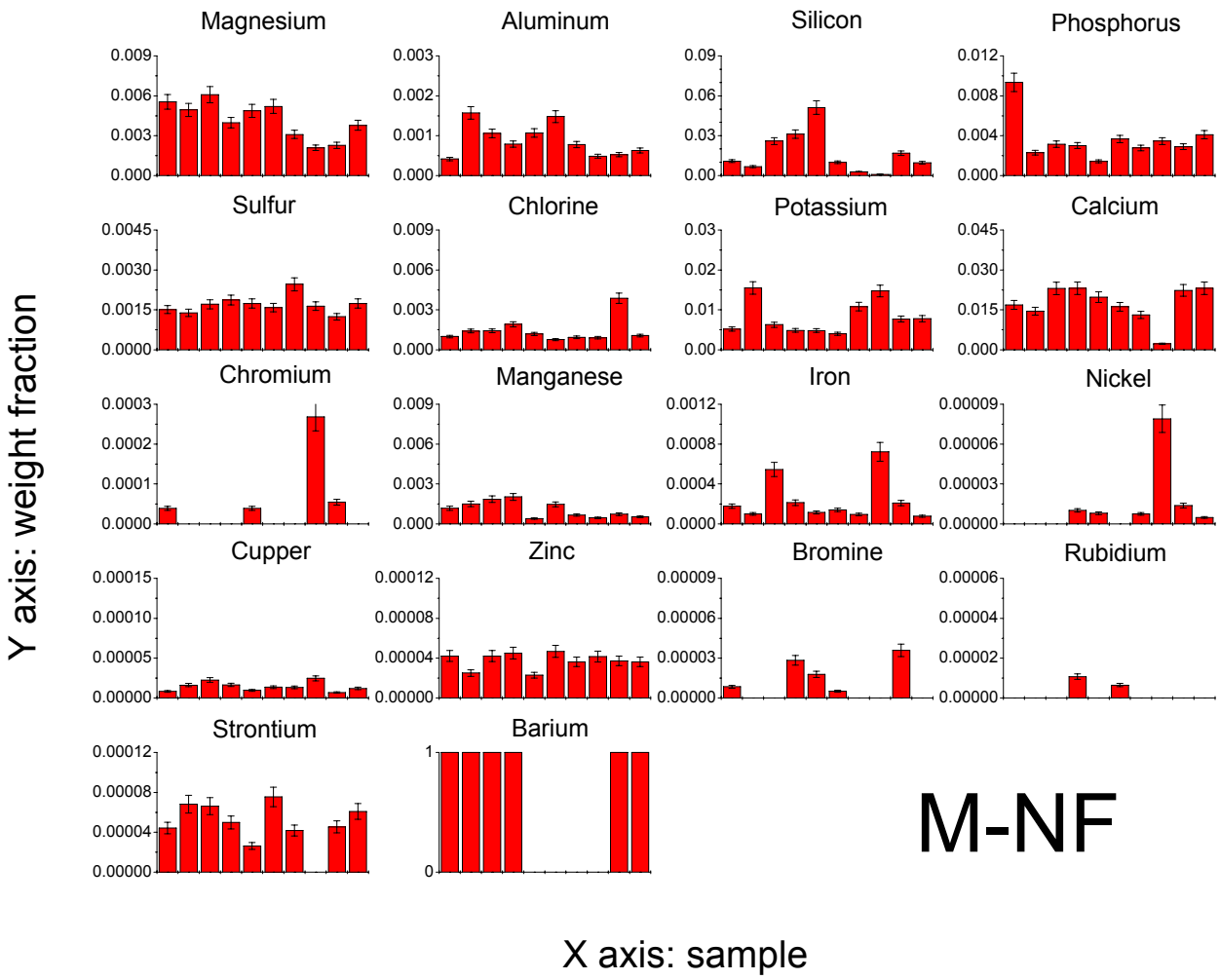


Figure 7. Concentration of all detected elements for M-F treatment. Ten different leaves were analyzed for each treatment. Weight fraction: 0.001=1000 ppm, 0.0001=100 ppm, etc

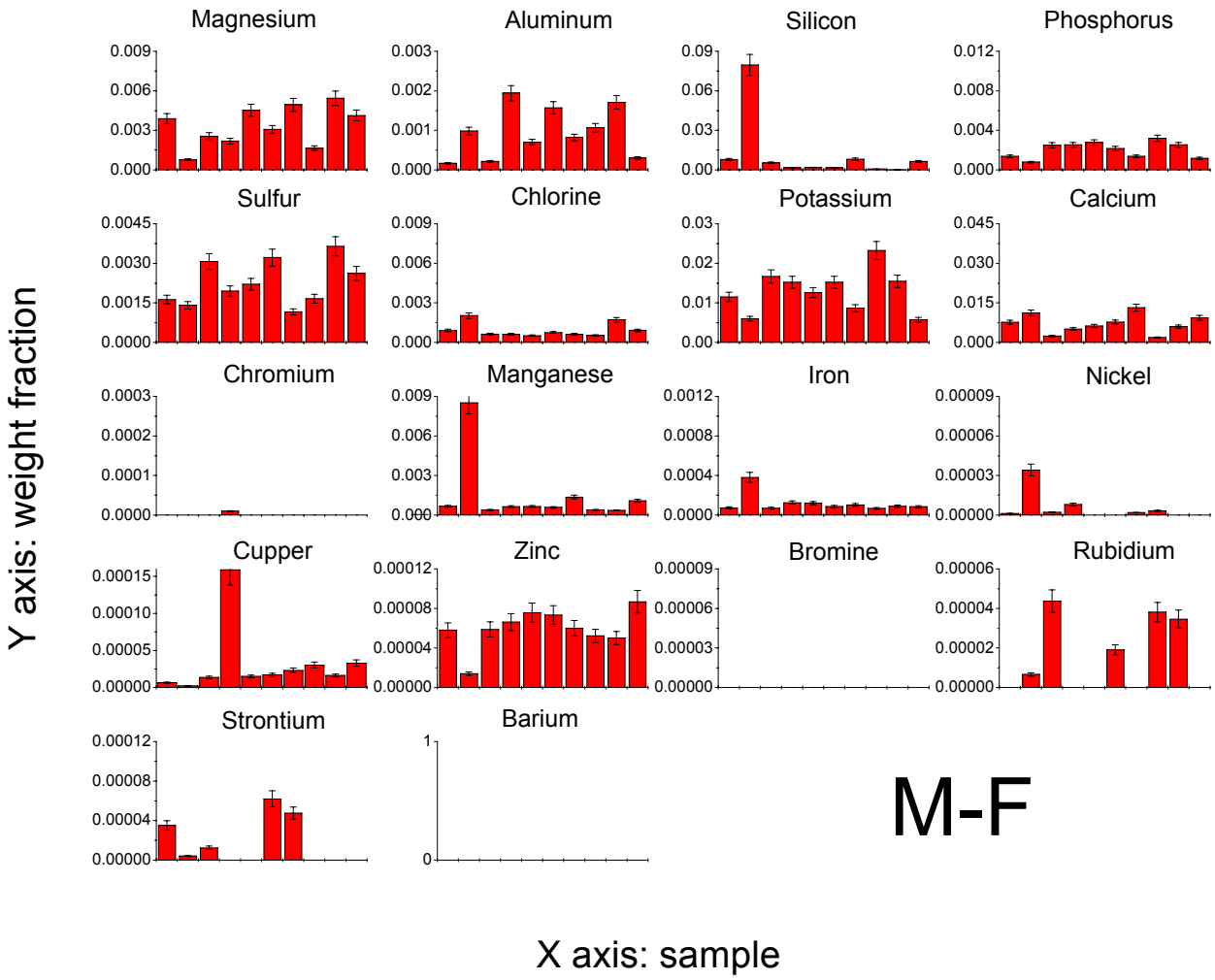


Figure 8. RBS spectrum. Atomic concentration of major elements: H= 5 %, C= 80 %, N= 3 %, O= 11 %, and Ca= 1 %.

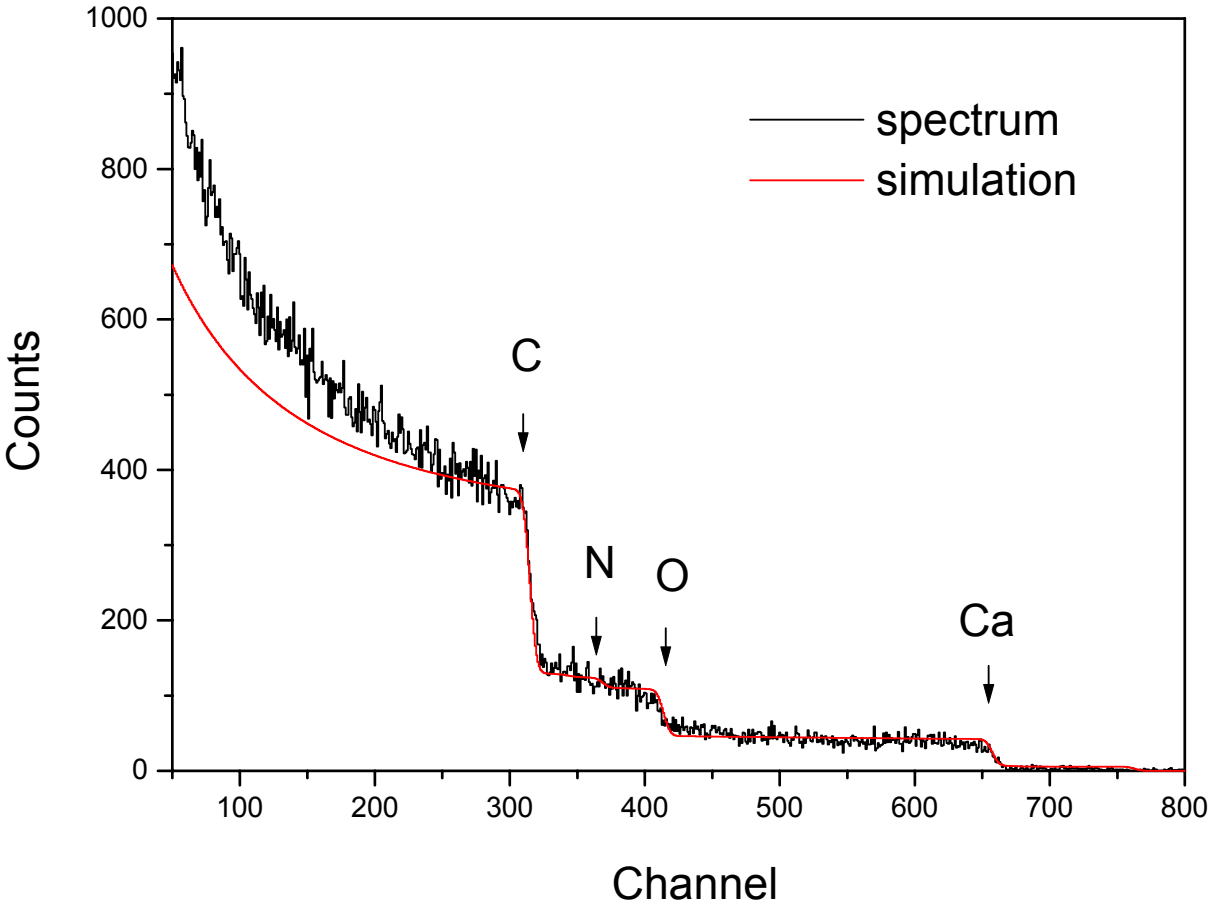


Figure 9. A typical PIXE spectrum of detected elements. Spectra for each sample will be different depending on element composition. Element concentration was calculated from Equation 3. Count is directly proportional to concentration.

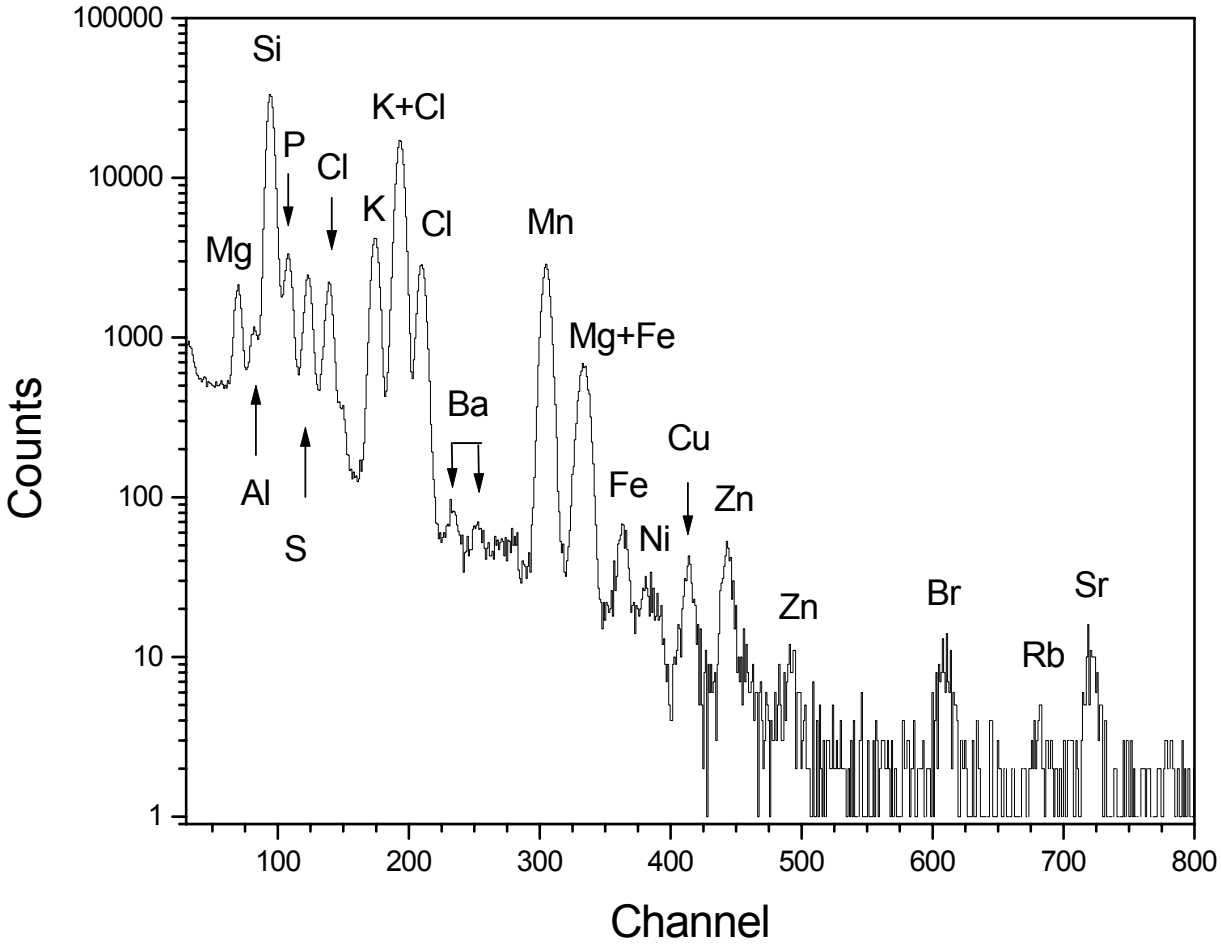


Figure 10. Location of microbeam scanning areas. Left=Spot A, Right=Spot B

Figure 11. Microbeam topographies of post oak leaf Spot A (images courtesy of Dr. Naab, Ion Beam Modification and Analysis Laboratory, University of North Texas, Denton, Texas). Images are 850 μm x 850 μm.

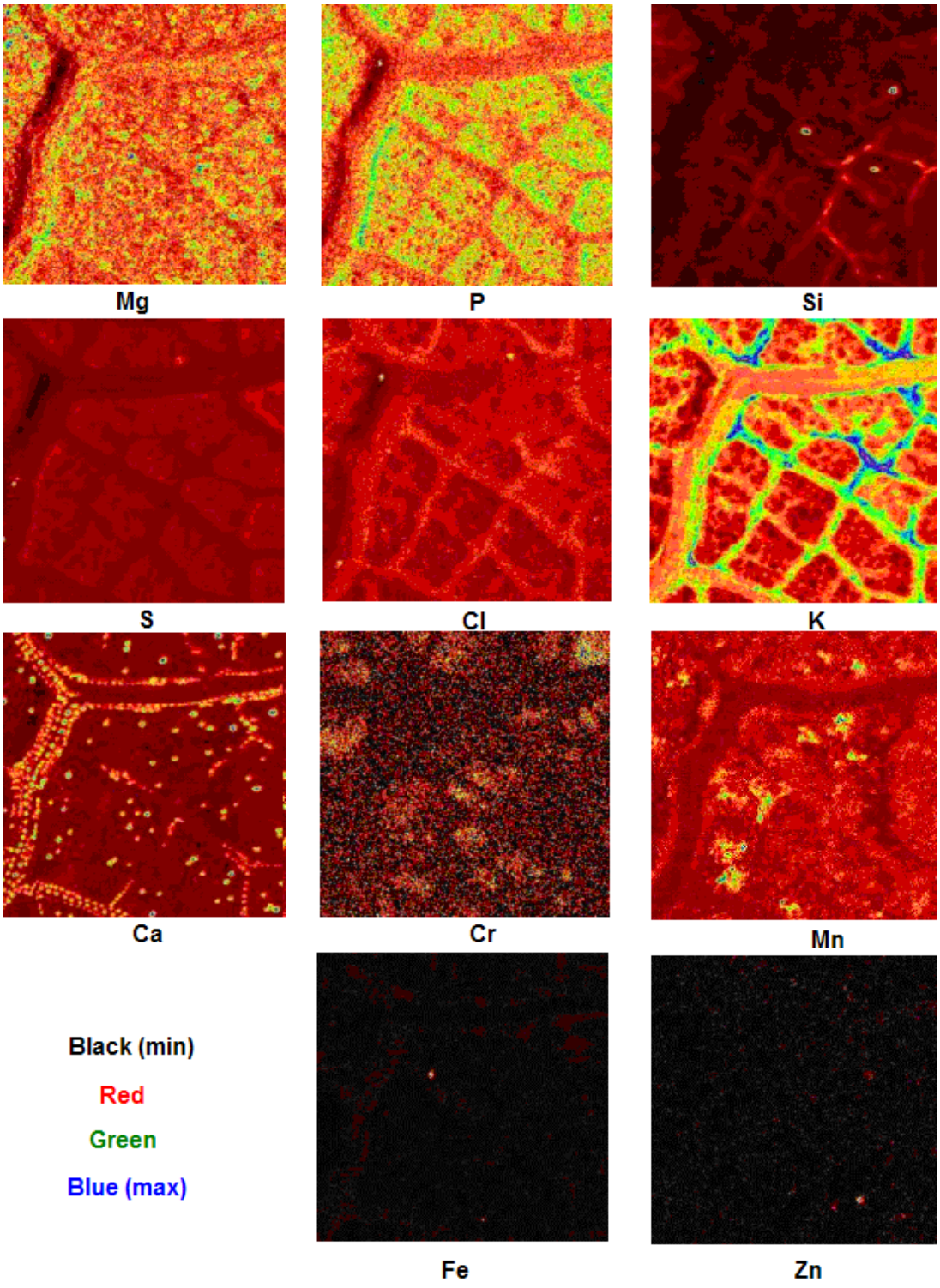
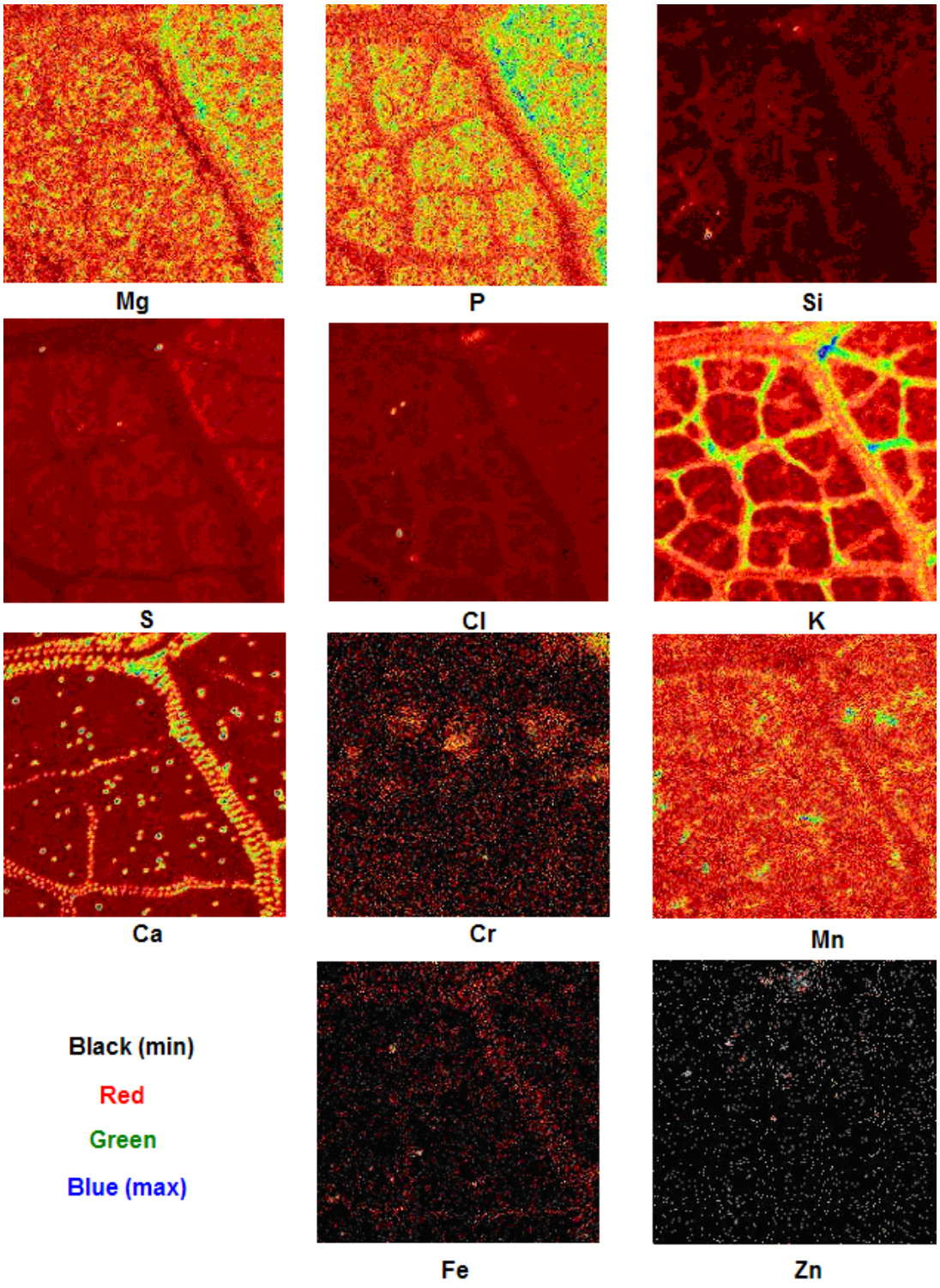


Figure 12. Microbeam topographies of post oak leaf Spot B (images courtesy of Dr. Naab, Ion Beam Modification and Analysis Laboratory, University of North Texas, Denton, Texas). Images are 850 μm x 850 μm.



DISCUSSION

Post oak is a notoriously difficult plant to grow in the greenhouse or nursery environment (Dr. Smith personal communication). The reason for this may be due to its large and extensive root system. Furthermore, the post oak may have a high reliance on its mycorrhizal symbiont. Unfortunately, the plants in this experiment did not live long enough for a more accurate analysis of mycorrhizal influence on element uptake.

Post oak is a common tree of the southwest United States. Therefore, the post oak will be on the defensive front lines of rapid urbanization, which is endemic to most areas of the southwest United States. Mycorrhiza enhances the nutritional status of the host plant, increasing its ability to sustain damage without compromising productivity (Rieske 2001). However, to maintain the health of our urban trees and to reconstruct damaged ecosystems, it is vital that we monitor the tree's nutrient status, especially considering that mycorrhizal community diversity has been found to be negatively correlated with land use intensity (Oehl et al. 2003). Furthermore, research on ecosystems has indicated a strong link between plant tissue chemistry and soil chemistry (Hoffmann et al. 2000). Therefore, the element concentrations in tree leaves may be used to assess the state of pollution levels in the environment and, of course, the nutritional status of the tree.

The use of PIXE as an analytical tool can provide a rapid assessment of a plant's nutrient status. Furthermore, the use of hard tissue such as leaves dramatically reduces sample preparation time, thereby allowing for a relatively quick analysis of elemental concentration. However, it has been well established that the elemental concentration in one organ of the plant is only indicative of partial nutrient assimilation.

Some elements are sequestered in mycorrhizal roots thereby preventing their transport further up the plant. This phenomenon is important for plants since most macro and micronutrients are toxic at high levels. Weiersbye et al. (1999) using micro-PIXE found Mn, Cu, Ni, and U had accumulated in the mycorrhizal vesicles of the grass, *Cynodon dactylon*, growing on uranium mine tailings. Jurkiewicz et al. (2001) detected increased concentrations of Fe, Cd, Ti, Mn, Si, Ca, S, and especially Zn and Pb in the root epidermis and within the mycorrhizal hyphae inside cortical cells of orchids growing on zinc mine tailings. The concentration of Zn and Pb in the cortical cell hyphae was 4 to 5 times higher compared to root epidermis. Using PIXE, they found that the concentration of these elements decreased significantly toward the pith of the root, suggesting that the mycorrhizal fungi had a filtering affect. Analyzing the concentration of elements in the leaves of these plants, while not reported by Jurkiewicz et al. or Weiersbye et al., would certainly give an incomplete picture of element uptake.

A very interesting study was one conducted by Hoffman et al. (2000). They mapped the concentration of various elements over the entire leaf area of

Quercus robur including along the central vascular bundle using Laser ablation inductively coupled plasma mass spectrometry. They found higher concentrations of Mg at the tip of the leaf and lower concentrations toward the petiole. A similar pattern was found with Pb, Al, Mn, Cu and Ni. However, an opposite pattern was found for Ca. The concentration of Ca was highest toward the petiole. While the scope of this thesis project was not as thorough as Hoffman et al., it would be possible to map the element content of an entire leaf using PIXE. In fact, while Hoffman et al. mapped seventy quadratic fields of about 0.5 up to 0.8 cm^2 for a total area of up to 56 cm^2, the technique of macro-PIXE would be able to map about 622 quadrants, theoretically, in the same total area.

The work of Hoffman et al., Weiersbye et al., and Jurkiewicz et al. underscores the important point that the elemental analysis of a few spots on a leaf will not necessarily give a complete picture of element uptake and assimilation. While there may be a strong link between plant tissue chemistry and soil chemistry, elements can have a spatial variation within the same organ. Elements will be distributed in tissue according to their structural and/or physiological function. For example, calcium oxalate crystals are known to accumulate in leaf vascular tissue (Doaigey 1991; Cervantes-Martinez et al. 2005). This would explain the results from the micro-PIXE analysis, see Figure 11-Ca and Figure 12-Ca. A thorough analysis of element concentrations should include an analysis of whole organs.

The work of this thesis is a preliminary analysis of elemental concentrations in post oak leaves using particle-induced X-ray emission and Rutherford backscattering. A literature search has failed to locate any published article concerning element concentration in post oak. A definitive link between mycorrhizal inoculation and element concentration could not be established. However, since this work may be the first to investigate element accumulation in post oak as well as the benefits of mycorrhizal inoculation in this species of oak, it is hoped that with further study we will be able to have a better understanding of the nutrient requirements of this tree as well as element distributions and the importance of mycorrhizae. This knowledge in the long run will help us to manage our hardwoods living in a rapidly changing environment.

APPENDIX

FERTILIZER GUARANTEED ANALYSIS AND MASS CONCENTRATION RAW DATA

Table 10. Plant-tone® plant food guaranteed analysis (The Espoma Co., 6 Espoma Rd., Millville, NJ, www.espoma.com).

Total Nitrogen	5.0
Ammoniacal Nitrogen	0.5
Other Water Soluble Nitrogen	0.5
Water Insoluble Nitrogen	4.0
Available Phosphate (P_2O_5)	3.0
Soluble Potash (K_2O)	3.0
Calcium	3.0
Total Magnesium	0.5
Water Soluble Magnesium	0.3
Sulfur	1.0
Combined Sulfur	1.0
Boron	0.02
Chlorine	0.1
Cobalt	0.0005
Total Copper	0.05
Total Iron	1.0
Total Manganese	0.05
Water Soluble Manganese	0.01
Molybdenum	0.0005
Sodium	0.1
Total Zinc	0.05

Table 11. Mass concentration raw data for NM-NF treatment (weight fraction normalized to 1).

Plant	Mg	Al	Si	P	S	Cl	K	Ca	Ba
S2-M11	0.00389	1.65E-04	0.00786	0.00137	0.00163	9.07E-04	0.01153	0.00769	0
30-3	7.72E-04	9.84E-04	0.07962	7.93E-04	0.00141	0.00204	0.00601	0.01117	0
30-17	0.00256	2.16E-04	0.00539	0.0025	0.00307	6.39E-04	0.01669	0.00244	0
2-11	0.00219	0.00194	0.00172	0.00254	0.00195	6.16E-04	0.01523	0.00508	0
11-3	0.00452	7.02E-04	0.00185	0.00278	0.00221	5.18E-04	0.0126	0.00625	0
24-3	0.00307	0.00157	0.00166	0.00216	0.00322	7.54E-04	0.01523	0.00775	0
24-3B	0.00346	0.00142	0.0035	0.00226	0.00319	5.11E-04	0.01378	0.00865	1
26-9	0.00494	8.22E-04	0.00821	0.00138	0.00116	6.24E-04	0.00871	0.0132	0
31-11	0.00166	0.00107	6.22E-04	0.0032	0.00166	5.28E-04	0.02324	0.0019	0
32-1	0.00544	0.00171	1.77E-04	0.00254	0.00365	0.00172	0.01547	0.006	0
32-1B	0.00631	0.00251	0.00178	0.0031	0.00373	0.00139	0.00917	0.00814	0
34-14	0.00413	3.01E-04	0.00648	0.00117	0.00262	9.14E-04	0.00576	0.00937	0
Plant	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Br**	**Rb**	**Sr**
S2-M11	0	6.61E-04	7.22E-05	1.23E-06	6.64E-06	5.80E-05	0	0	3.52E-05
30-3	0	0.00851	3.81E-04	3.41E-05	2.33E-06	1.39E-05	0	6.62E-06	4.19E-06
30-17	0	3.76E-04	6.83E-05	2.16E-06	1.38E-05	5.88E-05	0	4.37E-05	1.26E-05
2-11	9.97E-06	6.36E-04	1.26E-04	8.01E-06	1.59E-04	6.63E-05	0	0	0
11-3	0	6.48E-04	1.21E-04	0	1.51E-05	7.57E-05	0	0	0
24-3	0	5.82E-04	8.76E-05	0	1.72E-05	7.34E-05	0	1.91E-05	6.20E-05
24-3B	7.72E-06	6.65E-04	1.36E-04	3.57E-06	1.93E-05	6.90E-05	0	8.95E-06	3.56E-05
26-9	0	0.00136	1.02E-04	1.84E-06	2.33E-05	6.01E-05	0	0	4.75E-05
31-11	0	3.84E-04	6.78E-05	3.23E-06	3.04E-05	5.23E-05	0	3.81E-05	0
32-1	0	3.51E-04	8.92E-05	0	1.63E-05	5.02E-05	0	3.46E-05	0
32-1B	0	8.02E-04	2.03E-05	0	2.15E-05	3.58E-05	0	1.70E-05	6.13E-06
34-14	0	0.00108	8.35E-05	0	3.31E-05	8.68E-05	0	0	0

Table 12. Mass concentration raw data for NM-F treatment (weight fraction normalized to 1).

Plant	Mg	Al	Si	P	S	Cl	K	Ca	Ba
11-7	0.00377	0.00176	0.00208	0.00155	0.00277	0.00193	0.00892	0.01234	1
11-11	0.00119	0.00135	0.01979	0.00348	0.00203	0.0015	0.00792	0.02316	1
12-1	0.00319	0.00169	0.00121	0.00292	0.00253	7.57E-	0.00926	0.00965	1
14-11	0.00182	0.00214	0.00104	0.00218	0.002	0.00101	0.0147	0.00679	0
15-3	9.86E-	0.00291	0.02655	0.0025	0.00209	0.00141	0.00982	0.02934	0
19-1	0.00298	0.00214	0.01045	0.00573	0.00239	0.00242	0.01025	0.0151	0
21-4	0.00148	0.00131	7.23E-	0.00215	0.00137	2.83E-	0.00981	0.00544	1
21-18	0.00257	0.00128	0.00269	0.00444	0.00243	0.00112	0.01064	0.01175	0
22-10	0.00194	0.00145	0.01396	0.00407	0.00167	0.00192	0.00803	0.01066	0
S2-M7	0.00228	0.00174	0.01291	0.00214	0.00151	8.43E-	0.00702	0.02136	0
Plant	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Br**	**Rb**	**Sr**
11-7	0	8.69E-	5.74E-	2.73E-	2.41E-	8.84E-	1.35E-	0	5.77E-
11-11	0	0.0011	5.76E-	4.22E-	1.52E-	3.65E-	2.41E-	0	4.50E-
12-1	0	5.13E-	1.76E-	0	1.88E-	5.86E-	0	0	5.88E-
14-11	0	7.13E-	6.22E-	3.31E-	2.58E-	3.58E-	0	0	3.98E-
15-3	0	0.00163	8.76E-	8.15E-	2.60E-	5.26E-	1.00E-	0	3.82E-
19-1	0	4.26E-	8.75E-	0	1.03E-	2.80E-	1.36E-	7.88E-	1.33E-
21-4	0	1.71E-	6.11E-	0	3.56E-	4.16E-	0	0	0
21-18	0	6.26E-	1.10E-	0	2.04E-	8.01E-	0	0	6.13E-
22-10	0	0.00141	1.17E-	8.77E-	1.54E-	4.41E-	1.08E-	0	4.91E-
S2-M7	0	0.00141	1.23E-	2.64E-	1.16E-	2.70E-	1.48E-	7.21E-	3.73E-

Table 13. Mass concentration raw data for M-NF treatment (weight fraction normalized to 1).

Plant	Mg	Al	Si	P	S	Cl	K	Ca	Ba
4-6	0.00554	4.15E-04	0.01092	0.00934	0.00151	9.94E-04	0.0052	0.01684	1
5-2	0.00494	0.00157	0.00676	0.0023	0.00139	0.00141	0.01548	0.01449	1
7-12	0.00608	0.00106	0.02599	0.00315	0.00171	0.00142	0.00627	0.02305	1
10-8	0.00398	7.89E-04	0.03123	0.00301	0.00187	0.00192	0.00487	0.02313	1
11-14	0.00487	0.00107	0.05113	0.00143	0.00174	0.00118	0.00481	0.01978	0
12-10	0.0052	0.00148	0.01009	0.00367	0.00159	7.64E-04	0.004	0.01617	0
12-12	0.00309	7.80E-04	0.00292	0.00278	0.00246	9.35E-04	0.01082	0.01311	0
14-2	0.00209	4.86E-04	8.82E-04	0.00347	0.00164	9.06E-04	0.01474	0.00225	0
14-6	0.00227	5.24E-04	0.01681	0.0029	0.00124	0.00387	0.0077	0.02233	1
15-10	0.00378	6.29E-04	0.00972	0.0041	0.00174	0.00107	0.00781	0.02314	1
Plant	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Br**	**Rb**	**Sr**
4-6	3.90E-05	0.00118	1.78E-04	0	8.41E-06	4.22E-05	8.48E-06	0	4.44E-05
5-2	0	0.0015	9.96E-05	0	1.62E-05	2.51E-05	0	0	6.81E-05
7-12	0	0.00186	5.45E-04	0	2.26E-05	4.21E-05	0	0	6.62E-05
10-8	0	0.00203	2.13E-04	1.02E-05	1.66E-05	4.51E-05	2.84E-05	1.07E-05	4.98E-05
11-14	3.90E-05	3.96E-04	1.15E-04	7.99E-06	9.67E-06	2.29E-05	1.79E-05	0	2.64E-05
12-10	0	0.00147	1.41E-04	0	1.36E-05	4.68E-05	5.14E-06	6.48E-06	7.54E-05
12-12	0	6.62E-04	9.52E-05	7.55E-06	1.34E-05	3.64E-05	0	0	4.18E-05
14-2	2.68E-04	4.63E-04	7.23E-04	7.90E-05	2.47E-05	4.16E-05	0	0	0
14-6	5.46E-05	7.33E-04	2.09E-04	1.38E-05	6.97E-06	3.72E-05	3.58E-05	0	4.54E-05
15-10	0	5.28E-04	7.74E-05	4.81E-06	1.21E-05	3.64E-05	0	0	6.09E-05

Table 14. Mass concentration raw data for M-F treatment (weight fraction normalized to 1).

Plant	Mg	Al	Si	P	S	Cl	K	Ca	Ba
S2-M11	0.00389	1.65E-04	0.00786	0.00137	0.00163	9.07E-04	0.01153	0.00769	0
30-3	7.72E-04	9.84E-04	0.07962	7.93E-04	0.00141	0.00204	0.00601	0.01117	0
30-17	0.00256	2.16E-04	0.00539	0.0025	0.00307	6.39E-04	0.01669	0.00244	0
2-11	0.00219	0.00194	0.00172	0.00254	0.00195	6.16E-04	0.01523	0.00508	0
11-3	0.00452	7.02E-04	0.00185	0.00278	0.00221	5.18E-04	0.0126	0.00625	0
24-3	0.00307	0.00157	0.00166	0.00216	0.00322	7.54E-04	0.01523	0.00775	0
24-3B	0.00346	0.00142	0.0035	0.00226	0.00319	5.11E-04	0.01378	0.00865	1
26-9	0.00494	8.22E-04	0.00821	0.00138	0.00116	6.24E-04	0.00871	0.0132	0
31-11	0.00166	0.00107	6.22E-04	0.0032	0.00166	5.28E-04	0.02324	0.0019	0
32-1	0.00544	0.00171	1.77E-04	0.00254	0.00365	0.00172	0.01547	0.006	0
32-1B	0.00631	0.00251	0.00178	0.0031	0.00373	0.00139	0.00917	0.00814	0
34-14	0.00413	3.01E-04	0.00648	0.00117	0.00262	9.14E-04	0.00576	0.00937	0
Plant	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Br**	**Rb**	**Sr**
S2-M11	0	6.61E-04	7.22E-05	1.23E-06	6.64E-06	5.80E-05	0	0	3.52E-05
30-3	0	0.00851	3.81E-04	3.41E-05	2.33E-06	1.39E-05	0	6.62E-06	4.19E-06
30-17	0	3.76E-04	6.83E-05	2.16E-06	1.38E-05	5.88E-05	0	4.37E-05	1.26E-05
2-11	9.97E-06	6.36E-04	1.26E-04	8.01E-06	1.59E-04	6.63E-05	0	0	0
11-3	0	6.48E-04	1.21E-04	0	1.51E-05	7.57E-05	0	0	0
24-3	0	5.82E-04	8.76E-05	0	1.72E-05	7.34E-05	0	1.91E-05	6.20E-05
24-3B	7.72E-06	6.65E-04	1.36E-04	3.57E-06	1.93E-05	6.90E-05	0	8.95E-06	3.56E-05
26-9	0	0.00136	1.02E-04	1.84E-06	2.33E-05	6.01E-05	0	0	4.75E-05
31-11	0	3.84E-04	6.78E-05	3.23E-06	3.04E-05	5.23E-05	0	3.81E-05	0
32-1	0	3.51E-04	8.92E-05	0	1.63E-05	5.02E-05	0	3.46E-05	0
32-1B	0	8.02E-04	2.03E-05	0	2.15E-05	3.58E-05	0	1.70E-05	6.13E-06
34-14	0	0.00108	8.35E-05	0	3.31E-05	8.68E-05	0	0	0

REFERENCES

Agerer, R. 1987. Colour Atlas of Ectomycorrhizae : with glossary. Einhorn-Verlag, Schwèabisch-Gmèund.

Allen, M. F. 1991. The Ecology of Mycorrhizae. Cambridge University Press, Cambridge ; New York.

Amaranthus, M. P., D. Page-Dumroese, A. Harvey, E. Cazares, and L. F. Bednar. 1996. Soil compaction and organic matter affect conifer seedling nonmycorrhizal and ectomycorrhizal root tip abundance and diversity. Pages 1-12 *in* Research Paper PNW-RP-494. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR.

Anderson, L. M., A. L. Clark, and D. H. Marx. 1983. Growth of oak seedlings with specific ectomycorrhizae in urban stress environments. Journal of Arboriculture **9**:156-159.

Appleton, B., J. Koci, S. French, M. Lestyan, and R. Harris. 2003. Mycorrhizal fungal inoculation of established street trees. Journal of Arboriculture **29**:107-110.

Bagyaraj, D. J. 1991. Ecology of vesicular-arbuscular mycorrhizae. Pages 3-34 *in* D. K. Arora, B. Rai, K. G. Mukerji, and G. R. Knudsen, editors. Handbook of Applied Mycology. Marcel Dekker, New York.

Beckjord, P. R., J. H. Melhuish, Jr., and M. S. McIntosh. 1985. Effects of nitrogen and phosphorus fertilization on growth and formation of ectomycorrhizae of *Quercus alba* and *Q. rubra* seedlings by *Pisolithus tinctorius* and *Scleroderma auranteum*. Canadian Journal of Botany **63**:1677–1680.

Berman, J. T., and C. S. Bledsoe. 1998. Soil transfers from valley oak (*Quercus lobata* Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings. Mycorrhiza **7**:223–235.

Bridges, E. M. 1991. Waste Materials in Urban Soils. Pages 28-46 *in* P. Bullock and P. J. Gregory, editors. Soils in the Urban Environment. Blackwell Scientific Publications, Oxford ; Boston.

Campbell, C. R., and C. O. Plank. 2000. Foundation for Practical Application of Plant Analysis. *in*. Agronomic Division of the N.C. Department of Agriculture and Consumer Services.

Campbell, J. L. 1995. Instrumentation, Fundamentals, and Quantification. *in* S. A. E. Johansson, J. L. Campbell, and K. G. Malmqvist, editors. Particle-Induced X-Ray Emission Spectrometry. John Wiley & Sons, Inc., New York.

Cervantes-Martinez, T., H. Horner, R. Palmer, T. Hymowitz, and A. Brown. 2005. Calcium Oxalate Crystal Macropattern in Leaves of Species from Groups Glycine and Shuteria (Glycininae; Phaseoleae; Papilionoideae; Fabaceae). Canadian Journal of Botany **83**:1410-1421.

Daughtridge, A. T., S. G. Pallardy, H. G. Garrett, and I. L. Sander. 1986. Growth analysis of mycorrhizal and nonmycorrhizal black oak (*Quercus velutina* Lam.) seedlings. New Phytologist **103**:473-480.

Dixon, R. K., H. E. Garrett, G. S. Cox, D. H. Marx, and I. L. Sander. 1984. Inoculation of three *Quercus* species with eleven isolates of ectomycorrhizal fungi. I. Inoculation success and seedling growth relationships. Forestry Science **30**:364–372.

Dixon, R. K., G. M. Wright, H. E. Garrett, G. S. Cox, P. S. Johnson, and I. L. Sander. 1981. Container- and nursery-grown black oak seedlings inoculated with *Pisolithus tinctorius*: growth and ectomycorrhizal development during seedling production period. Canadian journal of forest research = Journal canadien de la recherche forestiere. **11**:487-491.

Doaigey, Abdullah R. 1991. Occurrence, type, and location of calcium oxalate crystals in leaves and stems of 16 species of poisonous plants. American Journal of Botany **78**:1608-1616.

Egerton-Warburton, L., and M. F. Allen. 2001. Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization and effects on seedling growth. Mycorrhiza **11**:283-290.

Firestone, R. B., and V. S. Shirley. 1996. Table of isotopes, 8th edition. Wiley, New York.

Garbaye, J., and J. L. Churin. 1997. Growth stimulation of young oak plantations inoculated with the ectomycorrhizal fungus *Paxillus involutus* with special reference to summer drought. Forest Ecology and Management **98**:221-228.

Harley, J. L., and S. E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press, London ; New York.

Hartman, J. R., T. P. Pirone, M. A. Sall, P. P. Pirone, and P. P. Pirone. 2000. Pirone's tree maintenance, 7th edition. Oxford University Press, Oxford ; New York.

Hauer, R. J., and J. O. Dawson. 1996. Growth and iron sequestring of pin oak (*Quercus palustris*) seedlings inoculated with soil containing ectomycorrhizal fungi. Journal of Arboriculture **22**:122-130.

Herrera, E. 1998. Interpreting Leaf Analysis and Deficiency Symptoms of Pecans. *in*. New Mexico State University, College of Agriculture and Home Economics.

Hoffmann, E., C. Lüdke, J. Skole, H. Stephanowitz , E. Ullrich, and D. Colditz. 2000. Spatial determination of elements in green leaves of oak trees (*Quercus robur*) by laser ablation-ICP-MS. Fresenius' Journal of Analytical Chemistry **367**:579 - 585.

Hubbell, J. H., and S. M. Seltzer. 1996. Tables of X-Ray Mass Attenuation Coefficients and Mass Energy-Absorption Coefficients. http://physics.nist.gov/PhysRefData/XrayMassCoef/cover.html (Accessed: February 15, 2006)

Interpretive nutrient levels for plant analysis. http://www.aasl.psu.edu/plant%20recs/Oak,%20Pin.pdf (Accessed: October 23, 2005)

Johansson, S. A. E., J. L. Campbell, and K. G. Malmqvist, editors. 1995. Particle-Induced X-ray Emission Spectrometry (PIXE). Wiley, New York.

Jurkiewicz, A., K. Turnau, J. Mesjasz-Przybytowicz, W. Przybytowicz, and B. Godzik. 2001. Heavy metal localisation in mycorrhizas of Epipactis atrorubens (Hoffm.) Besser (Orchidaceae) from zinc mine tailings. Protoplasma. **218 (3/4)**:117-124.

Landis, T. D., R. W. Tinus, S. E. McDonald, and J. P. Barnett. 1989a. The Biological Component: Nursery Pests and Mycorrhizae. *in* The Container Tree Nursery Manual. Agric. Handbk. 674. U.S. Dept. of Agriculture, Forest Service, Washington, DC.

Landis, T. D., R. W. Tinus, S. E. McDonald, and J. P. Barnett. 1989b. Containers and Growing Media. *in* The Container Tree Nursery Manual. Agric. Handbk. 674. U.S. Dept. of Agriculture, Forest Service, Washington, DC.

Landis, T. D., R. W. Tinus, S. E. McDonald, and J. P. Barnett. 1989c. Seedling Nutrition and Irrigation. *in* The Container Tree Nursery Manual. Agric. Handbk. 674. U.S. Dept. of Agriculture, Forest Service, Washington, DC.

Legge, A. H., H. C. Haufmann, and J. W. Winchester. 1984. Tree-ring analysis by PIXE for a historical record of soil chemistry response to acidic air pollution. Nuclear Instruments and Methods in Physics Research - B **3**:507-510.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological, and applied aspects. Mycorrhiza **7**:139-153.

Maetz, M., A. Schüßler, A. Wallianos, and K. Traxel. 1999. Subcellular trace element distribution in *Geosiphon pyriforme*. Nuclear Instruments and Methods in Physics Research - B **150**:200-207.

Malmqvist, K. G. 1995. Biological and medical applications. Pages 167-236 *in* S. A. E. Johansson, C. R. Campbell, and K. G. Malmqvist, editors. Particle-Induced X-Ray Emission Spectrometry. John Wiley & Sons, Inc., New York.

Martin, T. P., J. R. Harris, G. K. Eaton, and O. K. Miller. 2003. The efficacy of ectomycorrhizal colonization of pin and scarlet oak in nursery production. Journal of Environmental Horticulture **21**:45-50.

Marx, D. H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorious*. Canadian Journal of Microbiology **23**:217-223.

Marx, D. H., J. L. Ruehle, D. S. Kenney, C. E. Cordell, J. W. Riffle, R. J. Molina, W. H. Pawuk, S. Navratil, R. W. Tinus, and O. C. Goodwin. 1982. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on container-grown tree seedlings. Forest Science **28**:373-400.

McDaniel, F. D., J. L. Duggan, C. Yang, B. N. Guo, M. E. Bouanani, and M. Nigam. 2001. The high-energy heavy ion nuclear microprobe at the University of North Texas. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **181**:99-103.

Mesjasz-Przybylowicz, J. 2001. The nuclear microprobe: a challenging tool in plant sciences. Acta Physiologica Polonica **100**:659-668.

Meyer, F. H. 1973. Distribution of ectomycorrhizae in native and man-made forests. Pages 79-105 *in* G. C. Marks and T. T. Kozlowski, editors. Ectomycorrhizae: their ecology and physiology. Academic Press, New York.

Moore, R., W. D. Clark, and D. S. Vodopich. 1998. Botany, 2nd edition. WCB/McGraw-Hill, New York.

Naab, F., O. W. Holland, J. L. Duggan, and F. D. McDaniel. 2005. Ion Beam Analyses of Carbon Nanotubes. The journal of physical chemistry. B **109**:1415-1419.

Naab, F. U. (2006a) *in* Elemental distribution in mycorrhizal post oak leaves. (Preprint)

Naab, F. U. (2006b) *in* In situ elemental analysis in mycorrhizal post oak leaves. B. C. Boling, D. Smith, J. L. Duggan, and F. D. McDaniel. (Preprint)

Oehl, F., E. Sieverding, K. Ineichen, P. Mader, T. Boller, and A. Wiemken. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. Applied and environmental microbiology. **69**:2816-2824.

Oh, K. I., L. H. Melville, and R. L. Peterson. 1995. Comparative structural study of *Quercus serrata* and *Q. acutissima* formed by *Pisolithus tinctorius* and *Hebeloma cylindrosporum*. Trees(Berl.) **9**:171-179.

Orlic, I., R. Siegele, D. D. Menon, S. J. Markich, D. D. Cohen, R. A. Jeffree, D. C. McPhail, A. Sarbutt, and E. Stelcer. 2002. Heavy metal pathways and archives in biological tissue. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **190**:439-444.

Pelicon, P., J. Simčič, M. Jakšić, Z. Medunić, F. Naab, and F. D. McDaniel. 2005. Spherical chamber – effective solution for multipurpose nuclear microprobe. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms **231**:53-59.

Pirone, P. P. 1978. Tree Maintenance, 5th edition. Oxford University Press, New York.

Quoreshi, A. M., and V. R. Timmer. 1998. Exponential fertilization increases nutrient uptake and ectomycorrhizal development of black spruce seedlings. Canadian journal of forest research. **28**:674-682.

Remy, W., T. N. Taylor, H. Hass, and H. Kerp. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proceedings of the National Academy of Sciences **91**:11841-11843.

Rieske, L. K. 2001. Influence of symbiotic fungal colonization on oak seedling growth and suitability for insect herbivory. Environmental Entomology **30**:849-854.

Simon, L., J. Bousquet, R. C. Lévesque, and M. Lalonde. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular plants. Nature (London) **363**:67-69.

Smith, S. E., D. J. Read, and J. L. Harley. 1997. Mycorrhizal Symbiosis, 2nd edition. Academic Press, San Diego, Calif.

Tinker, P. B., and A. Gildon. 1983. Metals and micronutrients--uptake and utilization by plants. Pages 21-32 *in* D. A. Robb and W. S. Pierpoint, editors. Annual proceedings of the Phytochemical Society of Europe ; no. 21. Academic Press, London ; New York.

Tyler, G. 1992. Tree species affinity of decomposer and ectomycorrhizal macrofungi in beech (*Fagus sylvatica* L.), oak (*Quercus robur* L.) and hornbeam (*Carpinus betulus* L.) forests. Forest Ecology and Management **47**:269-284.

Urban, A. 1998. Identification and characterization of ectomycorrhizas of oak-species. *in*. Vienna University, Institute of Botany, Department of Cryptogamic Botany.

Valentine, L. L., Fielder, T.l., Hart, A.N., Petersen, C.A., Berninghausen, H.K., Southworth, D. 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. Canadian Journal of Botany **82**:123-135.

Wagner, C. A., and T. N. Taylor. 1981. Evidence for endomycorrhizae in Pennsylvanian age plant fossils. Science **212**:562-563.

Walker, R. F. 1990. Ectomycorrhizal formation by *Pisolithus tinctorius* on *Quercus gambelii* X *Quercus turbinella* hybrid in an acidic Sierra Nevada minesoil. Great Basin naturalist. **50**:367-370.

Walker, R. F., and S. B. McLaughlin. 2002a. Ozone, acid deposition, and mycorrhization effects on loblolly pine and white oak. I. Above- and below-ground growth and development. Journal of sustainable forestry. **14 (2/3)**:31-58.

Walker, R. F., and S. B. McLaughlin. 2002b. Ozone, acid deposition, and mycorrhization effects on loblolly pine and white oak. II. Nutrition and water relations. Journal of sustainable forestry. **14 (2/3)**:59-91.

Watt, F., and G. W. Grime. 1995. The High Energy Ion Microprobe. Pages 101-165 *in* S. A. E. Johansson, J. L. Campbell, and K. G. Malmqvist, editors. Particle-Induced X-Ray Emission Spectrometry (PIXE). John Wiley & Sons, Inc., New York.

Weiersbye, I. M., C. J. Straker, and W. J. Przybylowicz. 1999. Micro-PIXE mapping of elemental distribution in arbuscular mycorrhizal roots of the grass, *Cynodon dactylon*, from gold and uranium mine tailings. Nuclear Instruments and Methods in Physics Research - B **158**:335-343.

Whiting, D., C. Wilson, and A. Card. 2006. Soil pH. http://www.ext.colostate.edu/pubs/garden/07727.html (Accessed: February 15, 2006)