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I am submitting herewith a thesis written by Christopher Ryan Miller entitled "Comparative ecophysiology of American chestnut under different planting treatments on reclaimed mine sites." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

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(Original signatures are on file with official student records.)

COMPARATIVE ECOPHYSIOLOGY OF AMERICAN CHESTNUT UNDER DIFFERENT PLANTING TREATMENTS ON RECLAIMED MINE SITES

A Thesis Presented for the Master of Science Degree
The University of Tennessee, Knoxville

Christopher R. Miller
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DEDICATION

This thesis is dedicated to my parents without whose support I couldn't have come this far. Thanks for helping me along the way when times were tough and for always encouraging me to pursue my interests.

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ABSTRACT

American chestnut was once an abundant species that dominated the Eastern U.S. deciduous forests. Although this species is currently functionally extinct due to the chestnut blight, researchers are working on blight-resistant hybrids in hopes of restoring the species. As one potential vector for chestnut reintroduction and dispersal, the reclamation of mine sites are being considered. Recent research has found that reforestation efforts on these reclaimed mine sites provide productive tree growth while also complying with mine-reclamation laws. Understanding how American chestnut performs physiologically on mine sites will aid in the restoration of this species and reclamation of mine sites.

The objective of this study was to determine the effect planting treatments have on survival, physiology, and performance of American chestnut. The response of American chestnuts under planting treatments varying in planting method, slow-release fertilizer, a hydrophilic root polymer (Terra-Sorb), and the addition of native forest soil were examined at three sites: a mine site, a quarry, and a greenhouse. Results from this study suggest that fertilizer, hydrophilic root polymers and soil microorganisms produce varying effects on dissimilar sites. Greatest overall survival was found in greenhouse-grown bare-root seedlings. The introduction of fertilizer to the soil substrate lowered emergence and survival of directly-seeded trees at the mine site and quarry, but increased tree growth at all sites and photosynthetic rate at the quarry. However, use of fertilizer resulted in a more negative water potential at the mine site and higher transpiration rate, potentially increasing moisture stress and demand. Increased seed survival, growth, and water status can be accomplished through the use of Terra-Sorb, but only in direct seeded trees on sites with potentially deficient plant-available water.

Native forest soil can increase survival in both direct seeded and bare-root planted trees. However, more research is needed on the benefits of native forest soil, as growth and physiological results conflict. It is recommended that site characterization be performed prior to selection of planting treatments. Results of this study can prove beneficial to reclamation specialists with an interest in using mine site reclamation in conjunction with American chestnut restoration.

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INTRODUCTION

Mining Background

Before 1977, surface mining throughout the Appalachian coalfield largely resulted in erosion and landslides which contributed to floods, impairment of fish and wildlife habitat, diminished utility of the land and a hazardous environment within and among surrounding communities. In response, the Surface Mining Control and Reclamation Act (SMCRA) of 1977 was formed with the general purpose of creating standardized surface mining methods which would ensure that operations would not impact the environment while prohibiting any mining operations when reclamation would not be feasible ("Surface Mining Control and Reclamation Act," 1977). As a result, mine site reclamation practices have since utilized compaction techniques and aggressive grass seeding to alleviate erosion, sedimentation, landslides and overall mass instability. Compacted soil helped to alleviate the mass instability many mine sites were facing. However, as soil is compacted, the air space between soil particles or porosity decreases. Porosity in soil is vital to plant growth because pore space influences available water, and gas exchange (Sweigard, et al., 2007). Lower porosity means there is less available water because the soil particles are so tightly packed that the necessary capillary action and osmotic potential needed to withdraw the water is raised. Further, lower porosity decreases air space, and low oxygen levels can result in reduced autotrophic respiration in roots. Rodrigue and Burger (2004) found that porosity was the fourth most influential variable on mine soil quality (behind base saturation, coarse fragments, and available water holding capacity). Therefore, to a point, higher porosity leads to higher productivity on mine sites. Further, infiltration decreases as porosity decreases (Sweigard, et al., 2007). Infiltration is the movement of water from a soil's surface to groundwater. During rains, a densely packed soil will resist infiltration, and shed most of the water on the soil's surface (Sweigard, et al., 2007). The resulting sheet and rill erosion removes soil from the site that is vital for rooting. As infiltration increases with higher porosity, more water will be available for plant uptake. Roots can also grow more freely when there is higher porosity. A free growing root system that is not limited through physical constraints such as compaction will have a larger root mass allowing for increased water and nutrient uptake (Sweigard, et al., 2007).

Forest reclamation was often overlooked on mine sites due to previous reforestation failures and belief that grass fields were cheaper to achieve (Angel, Davis, Burger, Graves, & Zipper, 2005). However, recent research shows mine site reforestation can allow cost efficient, productive forests if performed under reclamation guidelines (Burger, Graves, Angel, Davis, & Zipper, 2005; Rodrigue & Burger, 2004). Therefore, the Forest Reclamation Approach (FRA) was developed as a systematic method of converting mine land to forests while also complying with the SMCRA. The FRA is outlined in five steps:

> "1. Create a suitable rooting medium for good tree growth that is no less than 4 feet deep and comprised of topsoil, weathered sandstone and/or the best available material.
>
> 2. Loosely grade the topsoil or topsoil substitute established in step one to create a noncompacted growth medium.
>
> 3. Use ground covers that are compatible with growing trees.
>
> 4. Plant two types of trees--early successional species for wildlife and soil stability, and commercially valuable crop trees.

5. Use proper tree planting techniques." (Burger, et al., 2005).

In response, research projects in mine site reforestation are increasingly utilizing the FRA guidelines (Groninger, et al., 2007). As research advances, benefits are being realized. Reforestation benefits include equal or greater productivity compared with agronomic reclamation and undisturbed forest soils (Burger, et al., 2005; Rathfon, Fillmore, & Groninger, 2004), and erosion control (Torbert & Burger, 1994). However productive the site may be, the selected trees for reforestation must be adapted to the high light intensity and quickly draining soils characterized by mine sites. Consequently, many projects utilize degradation-tolerant but potentially low value species such as black locust (*Robinia pseudoacacia* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) (Rathfon, et al., 2004). Therefore, a species is needed that is potentially valuable with adaptation to xeric sites which are often nutrient limited and under a high light intensity. Pine species (*Pinus* spp.) have often been used that meet these conditions. However, pine plantations don't represent the native mixed hardwood forest type of the southern Appalachians. For this reason, the American chestnut (*Castanea dentata* (Marsh.) Borkh.) is being considered for mine reclamation.

American Chestnut Ecology

American chestnut was once an abundant species that dominated Eastern U.S. deciduous forests (Russell, 1987). Although very plastic in site requirements, American chestnut populations were historically highest on mid-slope sites and ridges with quickly draining soils (Ashe, 1912; Russell, 1987) and enough soil depth to provide for deep tap-root penetration (Ashe, 1912). Therefore, chestnut could out-compete other tree species that were more moisture and nutrient demanding on these harsh sites (Ashe, 1912). As a result, American chestnut gained a foothold on the Appalachians acting as a foundation species (Ellison, et al., 2005). Chestnut provided important resources for both humans and wildlife, as mast and timber were two of this species' favored traits. Further, chestnut provided a strong influence on forest structure and function (Paillet, 2002). In the early 20th century, the chestnut blight fungus (*Cryphonectria parasitica*) struck the eastern U.S. The chestnut blight fungus forms a canker which effectively girdles the vascular cambium (Griffin, 2000) and creates a localized decrease in hydraulic conductivity (McManus & Ewers, 1990). After being killed by the blight, chestnuts release "vigorous basal sprouts" (Paillet, 2002) that have little to no chance of reaching maturity without being suppressed and coppiced by the blight. Within 40 years after introduction, the chestnut blight had spread throughout the entire range of the American chestnut reducing the species from its original population (Russell, 1987) and changing its status from a foundation species to one being functionally defunct (Ellison, et al., 2005).

The American chestnut has not been available in the past as a candidate for mine reclamation because of the chestnut blight. Since the blight is assumed to be more widely spread than the historic native range of the chestnut itself, there was no possibility of reclamation without high chestnut mortality. Over the past 30 years, the American Chestnut Foundation has developed American-Chinese hybrids (*Castanea dentata* x *mollissima*) which show resistance to the blight (Hebard, 2005) while maintaining the desired morphological characteristics of pure American chestnut (Diskin, Steiner, & Hebard, 2006). Further, research has shown that the increased light levels characterized by mine sites can provide rapid growth in American chestnuts (McCament & McCarthy, 2005). The Office of Surface Mining (OSM), Appalachian Regional Reforestation Initiative (ARRI), and The American Chestnut Foundation (TACF) suggest restoration of the American chestnut could coincide with the reclamation of mine sites. Particular interest is paid by these organizations to American chestnut on mine sites due to the overlapping range of American chestnut with the Appalachian coal seam, the wide range of suitable habitat in which American chestnut grows, and its tolerance to slightly acidic soils. Further, the rapid initial growth exhibited by American chestnut will provide aesthetic values in conjunction with wildlife habitat and timber resources (Jacobs & Selig, 2005).

Mine sites reclaimed using the FRA have shown a higher site index for some trees when compared to an undisturbed forest sites (Burger, Keltig, & Zipper, 1998). However, the conditions on mine sites are unlike forested sites and therefore may not be suitable for all Appalachian Forest species. Mine sites often have compacted overburden, which can lead to decreased growth in trees (Sweigard, et al., 2007). Further, water availability is often low on mine sites due to high coarse fragment content and lack of topsoil (Rodrigue & Burger, 2004). Nutritional content is also a limiting factor in plant performance and mine site productivity (Kozlowski, Kramer, & Pallardy, 1991; Rodrigue & Burger, 2004; Walker, 2002). Lastly, the increased light levels on mine sites will influence above ground growth through photosynthetic response. Physiologic responses of American chestnut to mine spoil and site characteristics must be understood in order to successfully restore this species on mine sites.

Soil Biota

Shortly after overburden removal and reclamation, microfauna, macrofauna, and microbial communities are absent on relatively sterile mine sites (Marx, 1975). The use of local native topsoil when planting seedlings can introduce mycorrhizae and earthworms to the system. The presence of mycorrhizae and earthworms can increase soil development, amplifying the humus layer (Frouz, Pizl, & Tajovsky, 2007). Studies have shown that presence or introduction of mycorrhizae onto a mine site can enhance above-ground growth in inoculated seedlings (Herendeen, 2007; Preve, Burger, & Kreh, 1984; Walker, 1982), particularly when combined with organic matter addition (Lunt & Hedger, 2003). Mycorrhizae are vital to a forest ecosystem because they provide a large root surface area, allowing for increased water uptake. Further, mycorrhizae provide nutrients to the roots in return for carbon needed for energy. Physical restraints such as compaction will lower air space, water space, and root hairs needed for mycorrhizal interactions. If a mine soil is compacted, potential for phytophthora root rot (*Phytophthora cinnamomi*) increases. Phytophthora root rot is an oomycete pathogen that occurs in the same native soils that American chestnut once dominated. *Phytophthora* infects a host's roots producing root rot, collar rot, branch dieback, defoliation, and seedling mortality within 4 months (Rhoades, Brosi, Datillo, & Vincelli, 2003). However, certain soil conditions must be met for *Phytophthora* to be present. Rhoades et al. in 2003 found that compacted wet soils are vital to *Phytophthora* populations. *Phytophthora* incidence could be lowered by increasing mycorrhizal populations and using the FRA. Mycorrhizal increase can potentially protect against *Phytophthora* by forming a fungal mantle around root hairs, protecting the root from *Phytophthora* infection (Marx, 1970, 1975). Therefore, a trade-off exists when sterile soil is a factor. Using native soil when planting will introduce mycorrhizae and, potentially, earthworms into the system while risking chances of *Phytophthora* contamination. Likewise, sterile soil will be absent of *Phytophthora*, but will provide no benefit of other soil biota.

Water Relations

Historically, American chestnut grew on a variety of sites (Ashe, 1912). Chestnuts have also been abundant on xeric sites, which indicates a preference to dry conditions (Russell, 1987). Therefore, it appears that for a high chestnut performance, moisture conditions should be mesic to xeric. According to Ashe (1912), soils should be "of considerable depth, to permit the penetration of its ample, deeply ramifying roots, and must be moderately supplied with moisture in the subsoil, but well drained on the surface." Although water use efficiency (WUE) of chestnut is not well documented, some information is known. WUE is known to peak at 32% full sunlight (Wang, Bauerle, & Mudder, 2006). Further, chestnut requires less moisture than yellow poplar, ash and walnut, but more than oak where it is replaced on dry sites regardless of soil depth (Ashe, 1912).

Mine sites may therefore be suitable sites for American chestnut. The high infiltration rates of non-compacted sites reflect the parameters for chestnut growth outlined by Ashe (1912). However, these well drained soils have high coarse fragments, which lower water retention. Therefore, xeric conditions are often faced on mine sites. These xeric conditions are recommended by Herendeen (2007) and supported by Ashe (1912). Under drought situations, however, trees have the ability acclimate to low-moisture conditions by accumulating solutes in the roots, reducing osmotic and water potential (Lambers, Chapin, & Pons, 2008). If conditions get too dry, necrosis can occur. Necrosis is caused when there are higher rates of water lost than water absorbed. Plant growth ceases because there is no water available for cell expansion (Kozlowski, et al., 1991). Further, net water gain can become negative when the surrounding soil is drier than the roots and water will move from the roots to the surrounding areas of higher solutes. According to Kozlowski et al. (1991), consequences of reduced water absorption may include "…leaf dehydration, stomatal closure, reduced photosynthesis, leaf injury, and plasmolysis of root cells." Water stress for plants can be measured directly in situ from the leaf petiole using a Scholander pressure chamber equipped with a N_2 gas chamber (PMS Instrument Company, Albany). This equipment measures water potential as a sum of osmotic potential, turgor potential, and matric potential.

Drought conditions can be mitigated with a gel-based hydrophilic polymer soil amendment that allows for slow release of water (Al-Humaid & Moftah, 2007; Apostol, Jacobs, & Dumroese, 2009; Shi, et al., 2010; Terra-Sorb, 2007). The gel readily absorbs water, and holds the water for long-term availability. However, use of these root polymers may prove to be detrimental to root systems. Recent research shows that when electrolyte concentration and electrical conductivity is raised in the soil (as seen in fertilizer application), the gel may dehydrate at water potentials greater than field capacity, representing a loss in water availability (Taban & Naeini, 2006). Further, as stated before, a hydrated American chestnut rhizosphere may potentially increase risk of *Phytophthora* root rot, leading to mortality (Rhoades, et al., 2003). Lastly, increasing water availability may have a negligible effect due to American chestnut's adaptation to xeric conditions.

Nutrition

Growth limiting properties on mine sites often include N, organic C, and P (Ashby & Baker, 1968; Czapowskyj, 1978; Schoenholtz, Burger, & Kreh, 1992). Increased nutrient levels can increase plant survival and performance (Kozlowski, et al., 1991; Walker, 2002). As the land is mined, nutrient containing topsoil is removed. In the overburden used for reclamation, organic matter is scarce. However, organic matter will increase over time as the forest develops. The importance of organic matter in a soil is evidenced by increased nutrient cycling, and long-term nutrient availability. Nitrogen (N) is most limiting after mining because N fixing organisms aren't yet established on the site. Nitrogen is important to plant growth because it increases shoot growth, and is used in photosynthesis. On the other hand, Phosphorus (P) is available after mining. With time, however, P decreases as the soil weathers and is adsorbed by Iron-Aluminum oxides in acidic soils. With P available early, saplings are able to acquire P and cycle it internally (Rodrigue & Burger, 2004). P is important because it stimulates root growth. Herendeen (2007) reports that American chestnut growth is strongest on nutrient-poor sites. However, Herendeen showed Mg was shown to influence greenness or how "vibrant" the chestnut leaves are. Mg is used in chlorophyll construction, and a dark shade of green is related to high photosynthetic capacity.

Increasing nutrients on mine sites can be accomplished through fertilization. Increased nutrient levels can increase plant survival, performance, stem mass, and specific leaf area (SLA) (Kozlowski, et al., 1991; McCament & McCarthy, 2005; Walker, 2002). However, because of the high coarse fragment content, a slow release fertilizer should be used. If a slow release fertilizer is not used, there is a risk of losses through volatilization of N and leaching from the system. Currently, fertilizer is mainly applied in water soluble form when applied through hydroseeding due to the efficient combination with grass seeding and the management intensive nature of burying slow release tablets. However, it is important to note that over-fertilization of soils can be damaging, especially for American chestnut which is adapted to nutrient limited systems. Nutrient rich soils may initiate luxury consumption of nitrogen, increasing the chance of root rot from *Phytophthora* (Herendeen, 2007; Menge & Marais, 2009). Over-fertilization of soils may also increase salt concentrations in the soil during dry periods. Increased salt concentrations can potentially reduce osmotic potential and decrease water absorption. As previously stated, consequences of reduced water absorption include leaf dehydration, stomatal closure, reduced photosynthesis, leaf injury, and plasmolysis of root cells. Lastly, fertilizer application can stimulate aggressive growth by grasses and other groundcovers, which can cause tree mortality or suppression from reduced site resources (Burger & Zipper, 2002).

Light

Tree species used for mine restoration must be adapted to the high light conditions which often occur on xeric mine sites. American chestnut is a very plastic species, which was historically dominant throughout many environmental conditions. Optimal growth occurred on "moderately cool sites, such as shady slopes and coves" (Ashe, 1912). Reproduction is mainly through sprouts, which respond quickly to high light conditions following a disturbance (Ashe, 1912; McCament & McCarthy, 2005; Wang, et al., 2006). The light compensation point of American chestnut is 29.5 μmol $m^{-2}s^{-1}$, which is similar in shade tolerance to red maple (*Acer rubrum*), and more shade tolerant than oaks (*Quercus* spp.) (Wang, et al., 2006). Therefore, American chestnut is moderately shade tolerant when compared to surrounding native hardwoods. However, maximum growth can be expected in full sunlight. Under near full shade (4% sunlight), American chestnut persists above the light compensation level. These conclusions by Wang et al. (2006) are supported by the numerous overtopped root sprouts found in remnant chestnut forests throughout the Appalachians that are persisting in nearly full shade. Upon disturbance, chestnut will respond readily, experiencing high growth rates (Ashe, 1912;

McCament & McCarthy, 2005; Wang, et al., 2006). After stand initiation, height and diameter growth will decrease with decreasing irradiation (Wang, et al., 2006). As light decreases, SLA will increase (Wang, et al., 2006). Specific leaf area is the ratio of leaf blade area over dry weight and can be used to determine leaf thickness and infer the physiological cost of maintaining that leaf. Therefore, a leaf with a high specific leaf area is a thinner leaf which demands fewer resources. The increase in SLA following a decrease in light intensity is an acclimation used by American chestnut to maintain photosynthetic rate in low light conditions (McCament & McCarthy, 2005). Further, chestnut can increase its light use efficiency by decreasing its height to diameter ratio in shaded environments. Therefore, it is clear that historically, disturbances were vital to chestnut establishment. High growth rates by American chestnut on high light intensity sites will prove beneficial to mine site reclamation.

Objective and Hypotheses

The objective of this study was to determine the effect planting treatments have on physiology and performance of American chestnut. It is hypothesized that: (1) Planting treatments will have an effect on seedling survival and performance. Performance is identified through height, apical meristem elongation, root collar diameter, photosynthetic rate, transpiration rate, specific leaf area and water stress. (2) Treatments with the Terra-Sorb (Plant Health Care Inc., Pittsburgh, PA) amendment will differ from treatments without Terra-Sorb. Terra-Sorb will increase water availability to the saplings, resulting in greater water and nutrient transport, increasing seedling growth. Water stressed saplings will be identified as having more negative pressure potentials. However, this may not always indicate moisture availability, as seedlings can osmotically adjust to lower their osmotic potential. Water potential will be directly measured as total pressure potential using a Scholander pressure chamber. (3) Treatments with the Scott's Agriform 20-10-5 fertilizer pellet (The Scotts Company, Columbus, OH) amendment will differ from treatments without the fertilizer amendment. The addition of fertilizer to the planting medium will increase nutrient levels resulting in increased photosynthesis and seedling performance. Fertilizer amendment effects will be indirectly measured as photosynthetic rate with a LI-6400 Portable Photosynthesis System, total height, root collar diameter and SLA. (4) Treatments with sterilized soil will differ from treatments with unsterilized soil. The effects of sterilized and unsterilized soil amendments will be addressed through assessing overall seedling performance. Soil sterilization effects will be indirectly measured with a LI-6400 Portable Photosynthesis System, total height, and root collar diameter.

METHODS

The physiological response of American chestnut under differing planting treatments was tested at three planting locations: A surface mine, a quarry and at a greenhouse. Methods for each site differ and are as follows.

Mine Site

Site Description

This study site is located at Zeb Mountain, Tennessee (N 36.48972, W -84.27361) in an area mined using the cross ridge mining technique by National Coal Corporation and at an elevation of around 700 m (Figure 1). At this location, two distinct sites, flat and sloped, were used with two plots within each. The flat site (plots A and B) conditions are characterized by a gentle slope with a northeastern aspect and compacted subsurface material covered with uncompacted spoil piles, or end-dumps of overburden. The FRA recommends planting substrate be of the "best available material" (Burger, et al., 2005). Because this was a demonstration site, the substrate material was selected to contain a relatively large proportion of brown sandstone (~50%) and small amounts of residual topsoil. Sloped site (plots C and D) conditions are characterized by a steep slope with a southeastern aspect comprised of compacted backfill with uncompacted overburden contoured to the slope. In both cases, the uncompacted substrate was not less than 4 feet in depth. The substrate consists of a mixture of topsoil, brown weathered sandstone, and shale in proportions of approximately 30:70. Surface layers of substrate on plot C appeared to have larger proportion of sandstone, while plot D had a larger proportion of shale. Soil pH ranged from 6.8 to 7.2.

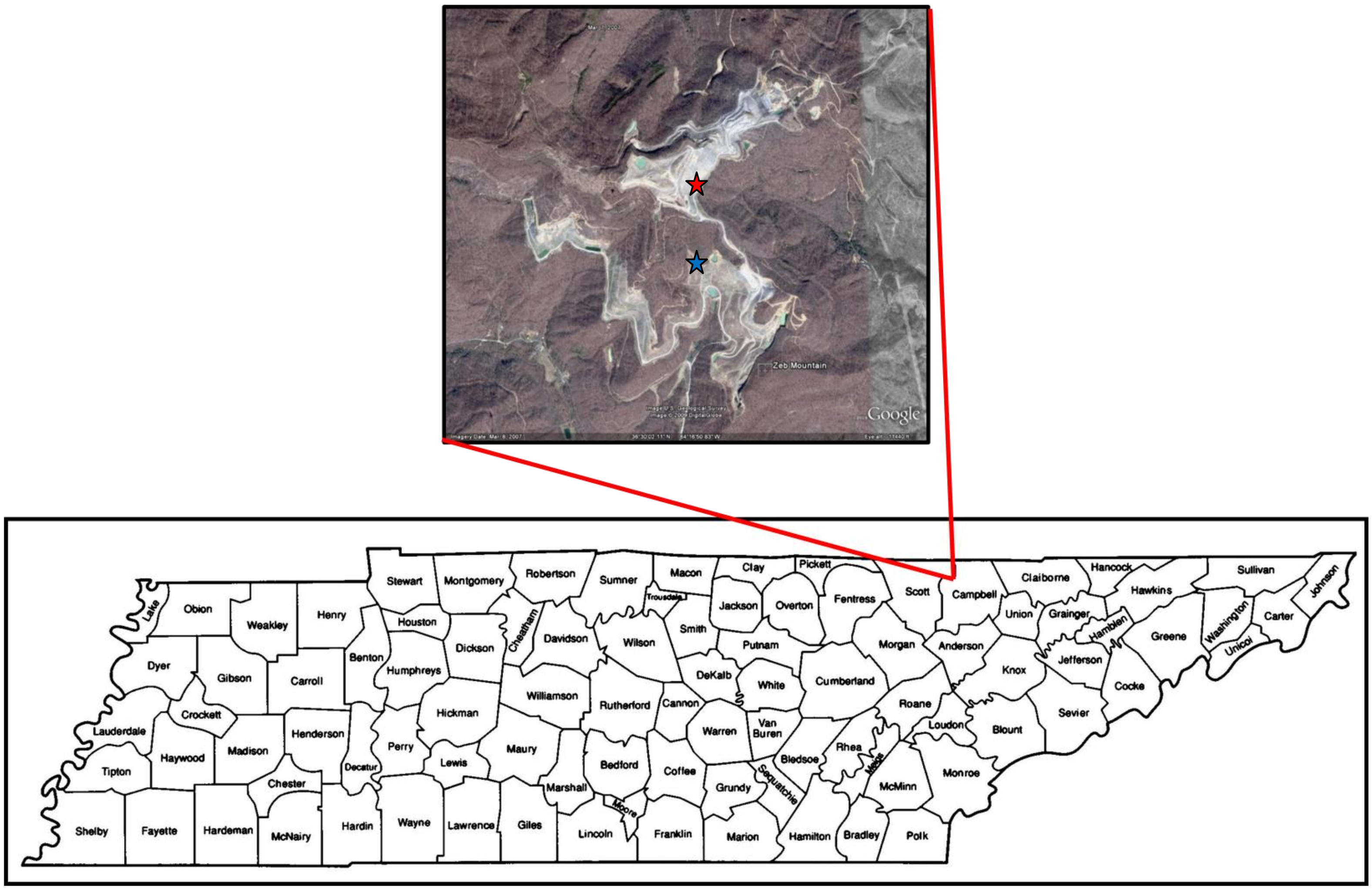

Figure 1: Location of the mine site experiment, at Zeb Mountain, Tennessee (N 36.48972, W -84.27361). The blue star indicates plots A and B, the red star indicates plots C and D.

Experimental Design

The experimental design applied was a randomized complete block design. Four plots were used across the site, with plots A and B on a relatively flat site and plots C and D on a sloped site. Within the flat site, plot A was separated from plot B due to the presence of a sedimentation pond down-slope from both sites (Figure 2). The pond posed potential soil moisture and hydraulic differences between the sites due to a raised water table. On the sloped site, plot C was separated from plot D due to varying proportions of sandstone and shale between the two. These sites represent the varied conditions existing on the site. Treatments were arranged in a 2x2x2 factorial

Planting

Ten treatments were applied with variations in planting medium. Treatments were in a factorial arrangement with factors of forest topsoil which was both sterilized and unsterilized, Terra-Sorb which was applied and not applied, and fertilizer pellets which were applied and not applied (Table 1). Treatment design was created by the American Chestnut Foundation to aid in determining best planting methods for chestnut restoration on mine sites. Four-hundred fifty-five pure American Chestnuts were randomly direct seeded May 2008 with a 46 cm Blue-X tree shelter surrounded at the base by native rocks to deter rodent herbivory.

Topsoil was collected from a forested stand in the Cherokee National Forest, within the native range of the American chestnut. The stand was typical of an upland hardwood forest, dominated by chestnut oak and northern red oak. Historically, this site was host to American chestnut as evidenced by several chestnut sprouts and stumps. Topsoil, or soil from the top 3 inches of the A horizon, was removed from a 10’x10’ plot. Soil was stored in large plastic bins

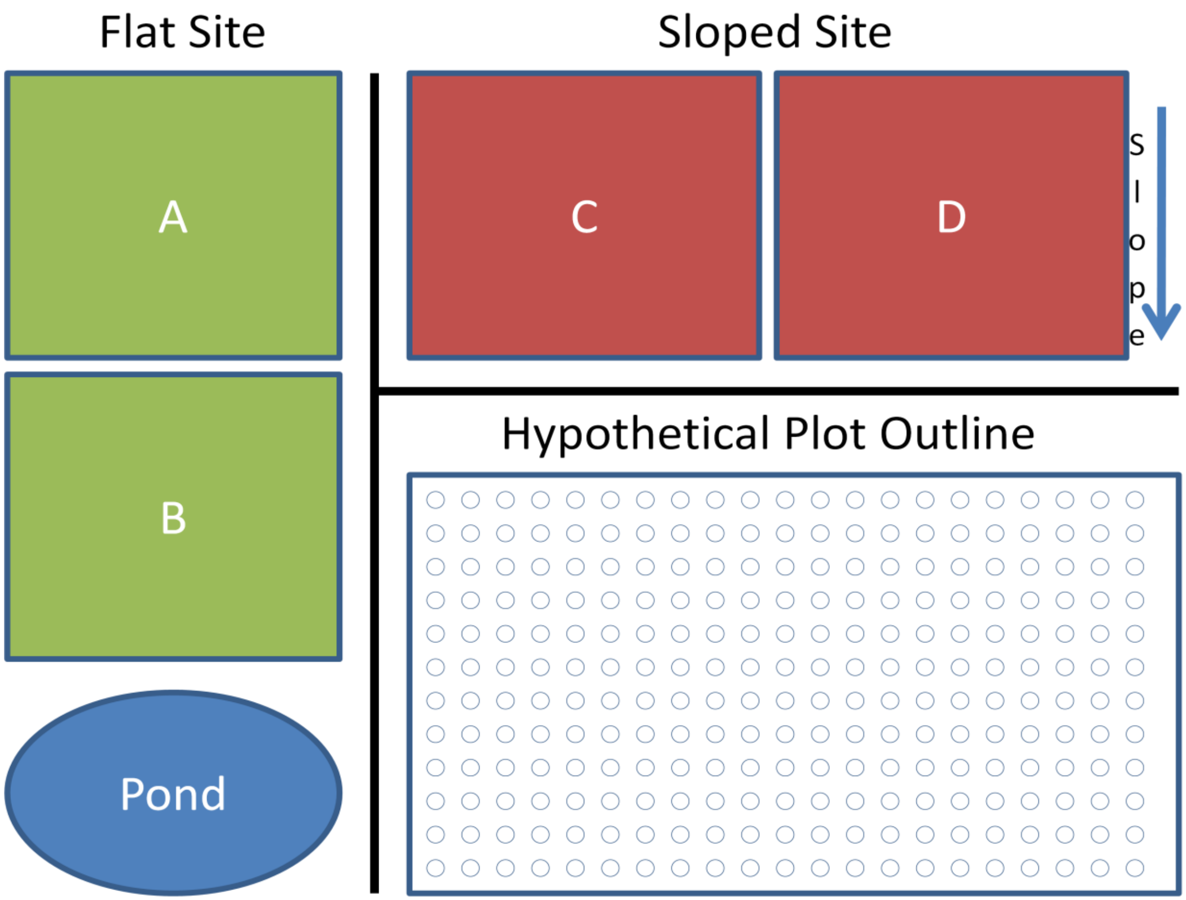

Figure 2: Mine site plot layouts for the flat site (A and B), sloped site (C and D) and a hypothetical plot outline. Plot row and column number varies between each plot. Within the hypothetical plot outline, seeds were planted at 1.5 m intervals.

Table 1: Planting treatments for American chestnut direct seeded at the mine site in May, 2008.

Treatment	½ cup Pro-Mix	½ cup Non-Sterilized Forest Topsoil	½ cup Sterilized Forest Topsoil	12 oz. hydrated Terra-Sorb	Two 10 oz. Scott's Agriform 20-10-5 fertilizer pellets
1	✓	✓		✓	✓
2	✓	✓			✓
3	✓		✓	✓	✓
4	✓	✓		✓	
5	✓		✓		✓
6	✓	✓			
7	✓		✓	✓	
8	✓		✓		

at 4°C in a walk-in cooler until it was ready for use. Sterilization was performed through moist heat autoclaving for 25 minutes at 121°C.

Chestnuts with emergent radicles were direct-seeded in rows on sites A and B on March, 2008. Sites C and D were direct-seeded in rows in May, 2008. Treatments were randomly assigned a planting location along rows, spaced at 1.5 meter intervals (Figure 2). Terra-Sorb was hydrated, and mixed with Pro-mix and topsoil in a 20L pail. One-half cup of this mixture was placed in a hole approximately 4 inches deep, the seed was placed on top of the mixture, and one-half cup of soil was placed on top. Where fertilizer tablets were used, they were placed at the bottom of the hole. In addition to the 20-10-5 concentrations of N, P, and K, the tablets also contained 2% sulfur, 2.8% calcium, 0.5% iron, 0.5% magnesium, 0.05% manganese, 0.05% zinc, 0.05% copper, and 0.05% boron. After treatments were applied to the planting area, seeds were covered with 1 to 2 inches of native mine spoil.

Measurements

Survival, natural height, root collar diameter (RCD), photosynthetic rate, transpiration rate and water potential were recorded for each surviving seedling to assess growth and physiological response to treatments. Growth was monitored using natural height and RCD. Physiological responses of seedlings were measured through photosynthetic rate, transpiration rate and water potential.

Natural heights were recorded from root collar to the highest live bud with a Mayes aluminum yardstick (Great Neck Saw Manufacturers, Inc., Mineola, NY). Natural height, which differs from total height, is a measure of crown height from the soil surface. Root collar diameters were measured with both Fisher Scientific digital calipers (Thermo Fisher Scientific

Inc., Waltham, MA) and Swiss Precision Instruments analog calipers (Swiss Precision Instruments, Inc., Garden Grove, CA). Growth measurements and survival were recorded at the end of growing periods in October 2008 and October 2009.

Photosynthetic rate (μmol CO2 m^{-2} s^{-1}) and transpiration rate (mmol H2O m^{-2} s^{-1}) were recorded using a LI-COR LI-6400 Portable Photosynthesis System with Leaf Chamber Fluorometer and CO_2 Injector System attachments (LI-COR Biosciences, Lincoln, NE). These attachments allow for regulation of photosynthetically active radiation (PAR) and CO_2 concentration such that ambient conditions are extraneous from conditions within the leaf chamber. Within the leaf chamber, PAR was maintained at 1500 nm, 10% of which was blue light. Further, flow rate was 500 μmol, reference CO_2 was 350 μmol, and temperature was within 2 °C of ambient conditions. Photosynthetic measurements were recorded between 8:00 a.m. and 12:00 p.m., at peak photosynthetic activity for American chestnut (unpublished data) in October, 2009. All seedlings with leaves were measured. Leaves selected for photosynthetic measurements were the uppermost undamaged leaf on each seedling that was greater than 2 cm^2.

Stem water potential was used as a proxy for total plant water potential and was recorded using a PMS Scholander Pressure Chamber (PMS Instrument Company, Albany, OR) with nitrogen gas used for chamber pressurization. All seedlings with at least one lateral stem bearing at least one undamaged leaf were selected for measurements. One lateral stem was excised with shears that was at least 8 cm long, in order to fit properly within the pressure chamber. Measurements occurred as soon as stems were removed from the seedling in September, 2009.

Quarry

Site Description

This site is located in Oak Ridge, Tennessee within the domain of the University of Tennessee Arboretum, adjacent to an open pit rock quarry owned by Rogers Group Incorporated (N 36.01526, W 84.18993) (Figure 3). Overburden from the quarry was placed in uncompacted spoil piles bordering a typical upland hardwood forest. Groundcovers planted at this site include buckwheat and alfalfa. Soils are finely textured silts with a pH ranging from 6.5-7.0, with inclusions of clay and calcareous rock.

Experimental Design

This site is a randomized complete block design. Variation is expected between spoil piles due to differing layers of overburden being dumped successively. Further, spoil piles near or bordering the forest will be expected to receive different amounts of sunlight from those further away from the forest (Figure 4). Therefore, three blocks are used to account for variation among mounds. Treatments were arranged in a 2x2x2 factorial.

Planting

Nine treatments were applied with variations in planting medium and genetic stock. Treatments were in a factorial arrangement with factors of forest topsoil which was sterilized and unsterilized, Terra-Sorb which was both applied and not-applied, and fertilizer pellets which were both applied and not applied. Treatment 9 was not included in the factorial, as it was planted with no soil amendments. Seeds and bare-root nursery-grown seedlings were obtained from the American Chestnut Foundation. A total of 375 pure year-old American chestnut nursery-grown seedlings and 375 seeds of 5 different genetic stocks were randomly planted June

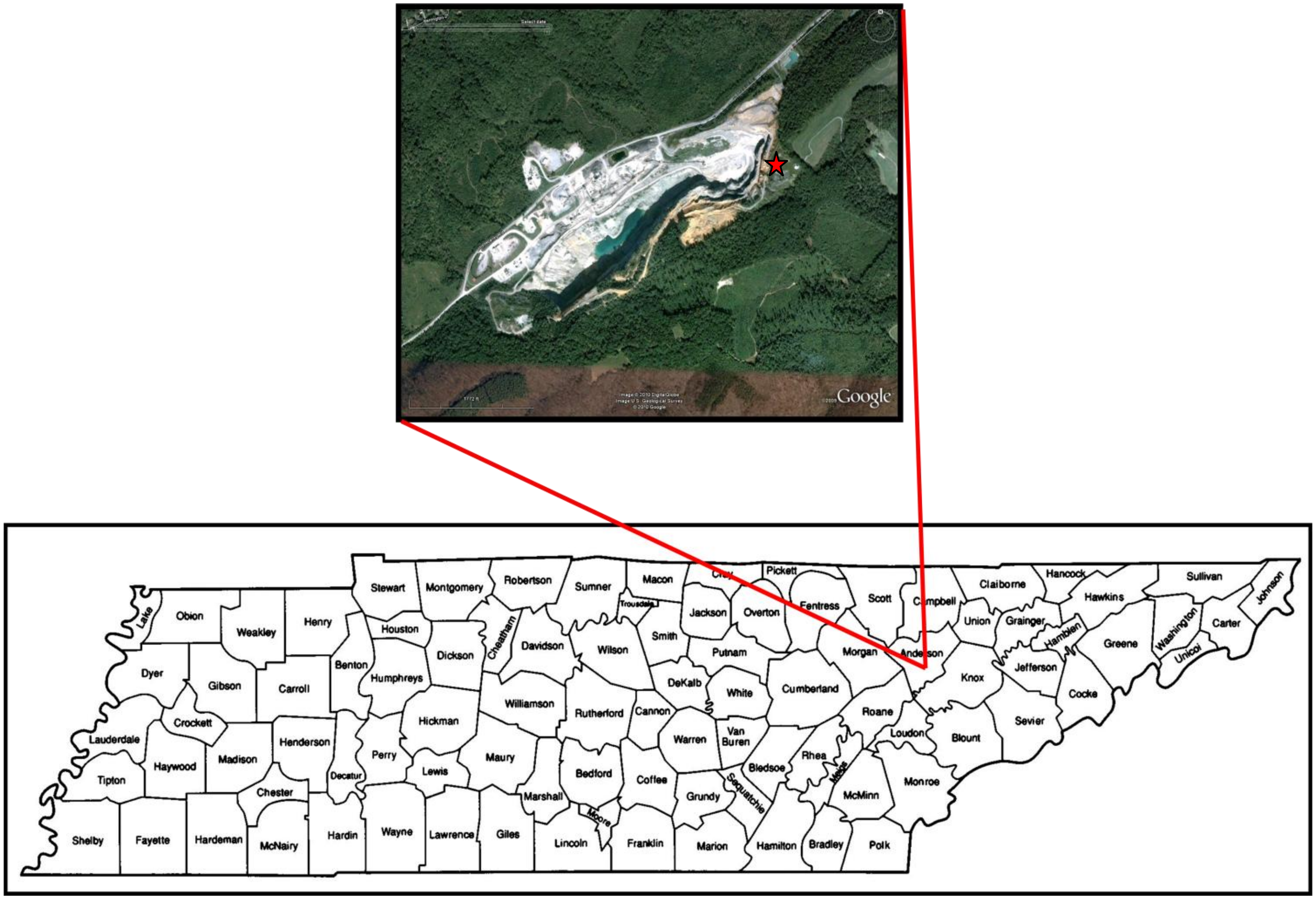

Figure 3: Location of the Quarry site, adjacent to Roger's quarry (N 36.01526, W 84.18993). The star indicates the site location.

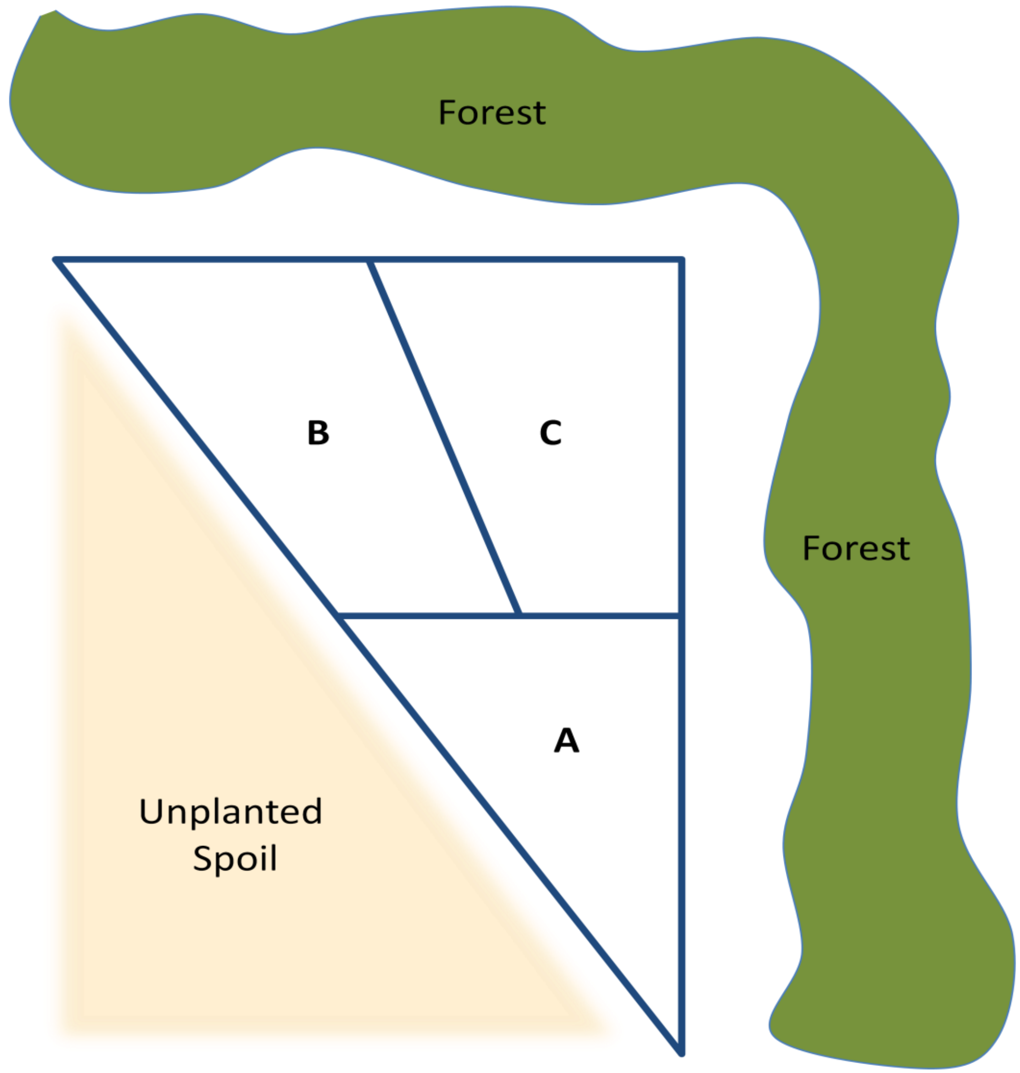

Figure 4: Quarry site plot layout. All plots are equal in size at the site. The distance from all plot exterior edges to the forest is ~4.5 m.

2009 (Table 2). The genetic families were: Ill moss lake (American), Chinese, NB35xOP (hybridB1F3), SA33xOP (hybrid B2F3), and CH283xOP (hybrid B3F2). Trees were planted with a mesh tree shelter and surrounded by native spoil to deter rodent herbivory.

Topsoil was collected from the University of Tennessee Arboretum, within the native range of the American chestnut. The stand was typical of an upland hardwood forest, dominated by chestnut oak and northern red oak. Historically, this site was host to American chestnut as evidenced by several chestnut sprouts and stumps. Soil from the top 3 inches of the A horizon was removed and stored at 4°C until it was ready for use. Sterilization was performed through autoclaving.

Chestnut seeds were direct seeded with emergent radicles facing down. Year-old nursery-grown seedlings were planted to root collar depth. Trees were planted with random treatments at intervals of 1 meter, alternating seeds and seedlings. For the seeds, family type was randomly placed within each planting site. Treatments were assigned at random. Terra-Sorb was hydrated, and added during planting along with fertilizer pellets and topsoil. Seeds were covered with 1 to 2 inches of native mine spoil.

Measurements

Survival, natural height, RCD, apical meristem new growth, photosynthetic rate, transpiration rate and water potential were recorded for each seedling to assess growth and physiological response. Growth was tracked using natural height, RCD and apical meristem new growth. Physiological responses of the nursery-grown seedlings were measured through photosynthetic rate, transpiration rate and water potential.

Table 2: Planting treatments for American chestnuts planted at the quarry site in June, 2009

Treatment	½ cup Pro-Mix	½ cup Non-Sterilized Forest Topsoil	½ cup Sterilized Forest Topsoil	12 oz. hydrated Terra-Sorb	One 21 oz. Scott's Agriform 20-10-5 fertilizer pellets
1	✓	✓		✓	✓
2	✓	✓			✓
3	✓		✓	✓	✓
4	✓	✓		✓	
5	✓		✓		✓
6	✓	✓			
7	✓		✓	✓	
8	✓		✓		

Natural heights were recorded from root collar diameter to the highest live bud with a Mayes yardstick. Root collar diameters were measured with both Fisher Scientific digital calipers and Swiss Precision Instruments analog calipers. New growth from the apical meristem was determined by measuring the length of the apical meristem from the terminal node of the previous year to the terminal bud of the present year. New growth was also measured with a Mayes yardstick. Survival was recorded August 2009. Height and RCD measurements were recorded June and August, 2009. New growth was measured in September, 2009. Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), and water potential were recorded as described above, in August of 2009.

<u>Greenhouse</u>

Site Description

The greenhouse study is located at the University of Tennessee in Knoxville, Tennessee (N 35.94442, W 83.93689). The greenhouse bay was subject to ambient light conditions with 12 hour days and kept at 22.2° C in the day and 15.5 °C at night. Soil used for planting consisted of native mine spoil found near plot C of the mine site, which was a mixture of topsoil, brown weathered sandstone, and shale in proportions of approximately 30:70.

Experimental Design

This study is a randomized complete block design. Variation in light intensity and humidity is expected along both bay length and width gradients. Therefore, 4 blocks were used to account for variation within the bay. Treatments were arranged in a 2x2 factorial.

Planting

Four treatments were applied with variations in planting medium. Treatments were in a factorial arrangement with factors of Terra-Sorb which was both applied and not applied, and fertilizer pellets which was both applied and not applied. Forty bare-root, nursery-grown seedlings of the genetic family NB3xOP which were a year old were obtained from Dr. Stacy Clark of the U.S. Forest Service, and randomly planted June 2009 (Table 3). Since these seedling's roots were not sterilized prior to planting, it is possible they were already exposed to soil biota such as mycorrhizae. Year-old nursery-grown seedlings were planted in large PVC tubes (1'4" tall, 7 7/8" inside diameter) with an adjustable, double layered mesh bottom to allow for drainage. Spoil was added to the bottom of the pots with the seedling set in place with the treatment medium surrounding it. Spoil was then added until it reached the root collar of the seedling and the pot was half-full. The seedlings were randomly located among 4 tables throughout the greenhouse bay. Each treatment was represented within each block, but numbers of treatments within each block differed. Pots were placed at intervals of every half meter, in two rows on each table (Figure 5). Pots were watered to field capacity at the time of planting, and were watered to field capacity twice weekly until bud break. After leaf emergence, seedlings were watered to field capacity each weekday. Due to time constraints, seedlings were not watered on weekends.

Measurements

Survival, natural height, RCD, apical meristem new growth, photosynthetic rate, transpiration rate, and water potential and were recorded using methods previously described for

Table 3: Planting treatments for American chestnuts planted in the greenhouse in June, 2009.

Treatment	1 cup Sterilized Forest Soil	12 oz. hydrated Terra-Sorb	One 21 oz. Scott's Agriform 20-10-5 fertilizer pellets
1	✓		✓
2	✓		
3	✓	✓	✓
4	✓	✓	

Figure 5: Greenhouse layout of seedlings. Pots were placed at 0.5 m intervals and seedlings were arranged such that there was no crown overlap.

each surviving seedling to assess growth and physiological response. Photosynthetic rate, transpiration rate, and water potential were measured in August of 2009. Survival was recorded September, 2009. Height and RCD measurements were recorded July and September, 2009. New growth was measured in September, 2009.

Specific Leaf Area was calculated to determine each seedling's physiological investment in leaves. All leaves were excised from their respective seedlings in October, 2009. Immediately after defoliation, total weight and average leaf area were determined. Fresh leaf weight was determined on a Mettler Toledo digital scale (Mettler-Toledo International Incorporated, Columbus, OH). Average leaf area was calculated using a LI-COR LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE). After initial measurements, leaves were then immediately stored in a freezer. To obtain dry weights, leaves were freeze-dried using a Labconco Freezone 4.5 Freeze Dry System (Labconco Corporation, Kansas City, MO).

Data Analysis

A univariate analysis of variance general linear model was performed for all site experiments using SPSS 17.0 (SPSS Inc., Chicago, IL). Outliers were identified as ± 2 from mean standard error and were not included in the model. Differences in percent survival were calculated using t-tests. For all other dependent variables, contrasts of between-subject effects were used to determine significance at an alpha level of 0.05. If found to be significant at a site, time was used as a covariate when determining treatment main effects of photosynthetic rate and transpiration rate.

RESULTS

Mine Site

Mean overall survival was similar in the first year (2008) (29% ± 5.4 standard error) when compared to the two-year survival (2009) (28% ± 3.7) (Figures 6 and 7). However, in both years, n = 100. Differences in percent survival are due to plot-based means being used for the t-test and possible rounding errors. In 2008, addition of fertilizer, Terra-Sorb, and the sterile soil treatment reduced survival. The highest survival was observed for seedlings planted without fertilizer. The lowest survival was observed in seedlings planted with fertilizer. Fertilizer presence decreased survival by about 11% compared with no fertilizer; Terra-Sorb decreased survival by 3% compared with no Terra-Sorb; and sterilized soil reduced survival by 7% compared with non-sterilized soil (Figure 6). This pattern extended into 2009 where fertilizer presence decreased survival by 8%, Terra-Sorb decreased survival by 1%, and sterilized soil decreased survival by 3% (Figure 7). However, addition of Terra-Sorb and fertilizer to the soil substrate increased natural height in 2008 by 10 cm ($p = 0.002$) and 21.9 cm ($p<0.0001$), respectively (Figure 8). Accordingly, RCD increased by 1.5 mm with Terra-Sorb ($p = 0.001$) and 2.7 mm with fertilizer presence ($p < 0.0001$) (Figure 9). The tallest seedlings observed were those planted with fertilizer, the shortest were those without fertilizer (Figure 8). A similar pattern was found for RCD (Figure 9).

In 2009, Terra-Sorb and fertilizer main effects were significant ($p = 0.012$, $p = 0.015$, respectively), with both increasing natural height over 25% (Figure 10). Again, greatest height occurred in seedlings planted with fertilizer. Interestingly, significant main effects on RCD were absent in this second year of growth.

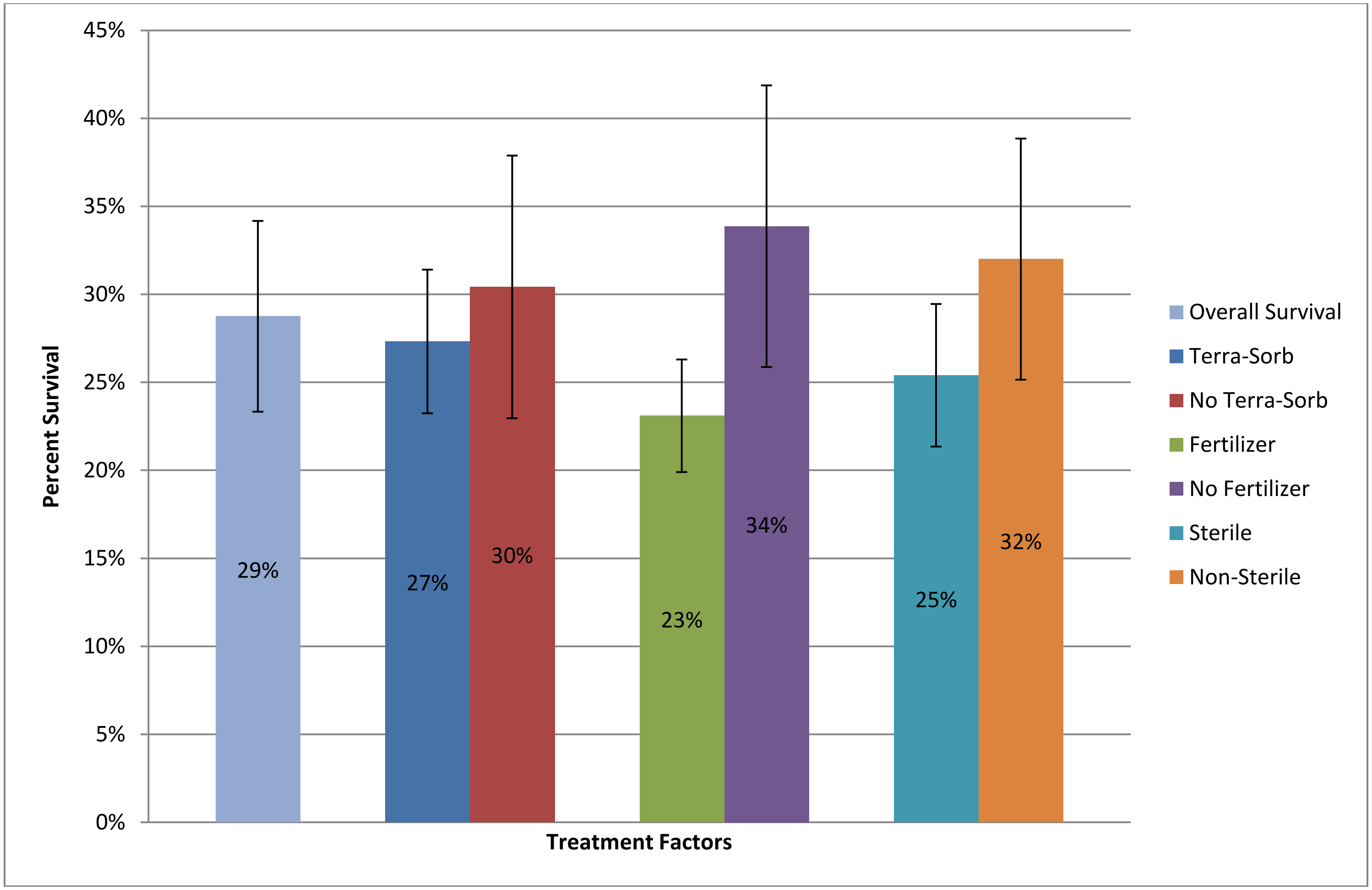

Figure 6: October 2008 survival for chestnut planted on the mine site in the spring of 2008. Means ± standard errors are shown for treatment main effects. Overall survival n = 100. In the order of treatment main effects as listed on the figure, n = 46, 54, 39, 61, 45, 55, respectively.

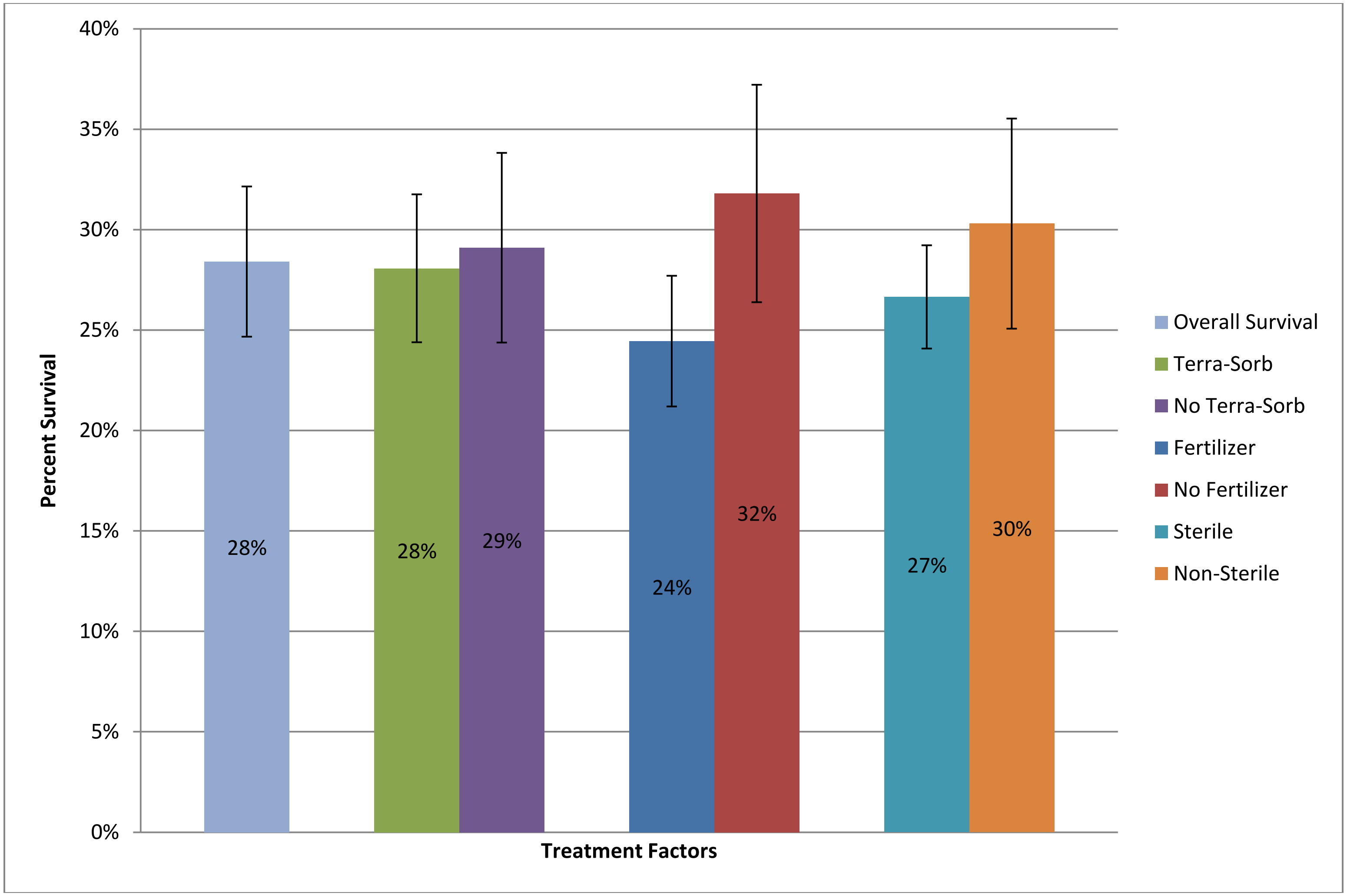

Figure 7: October 2009 survival for chestnut planted on the mine site in the spring of 2008. Means ± standard errors are shown for treatment main effects. Overall Survival n = 100. In the order of treatment main effects as listed on the figure, n = 47, 53, 42, 58, 48, 52, respectively.

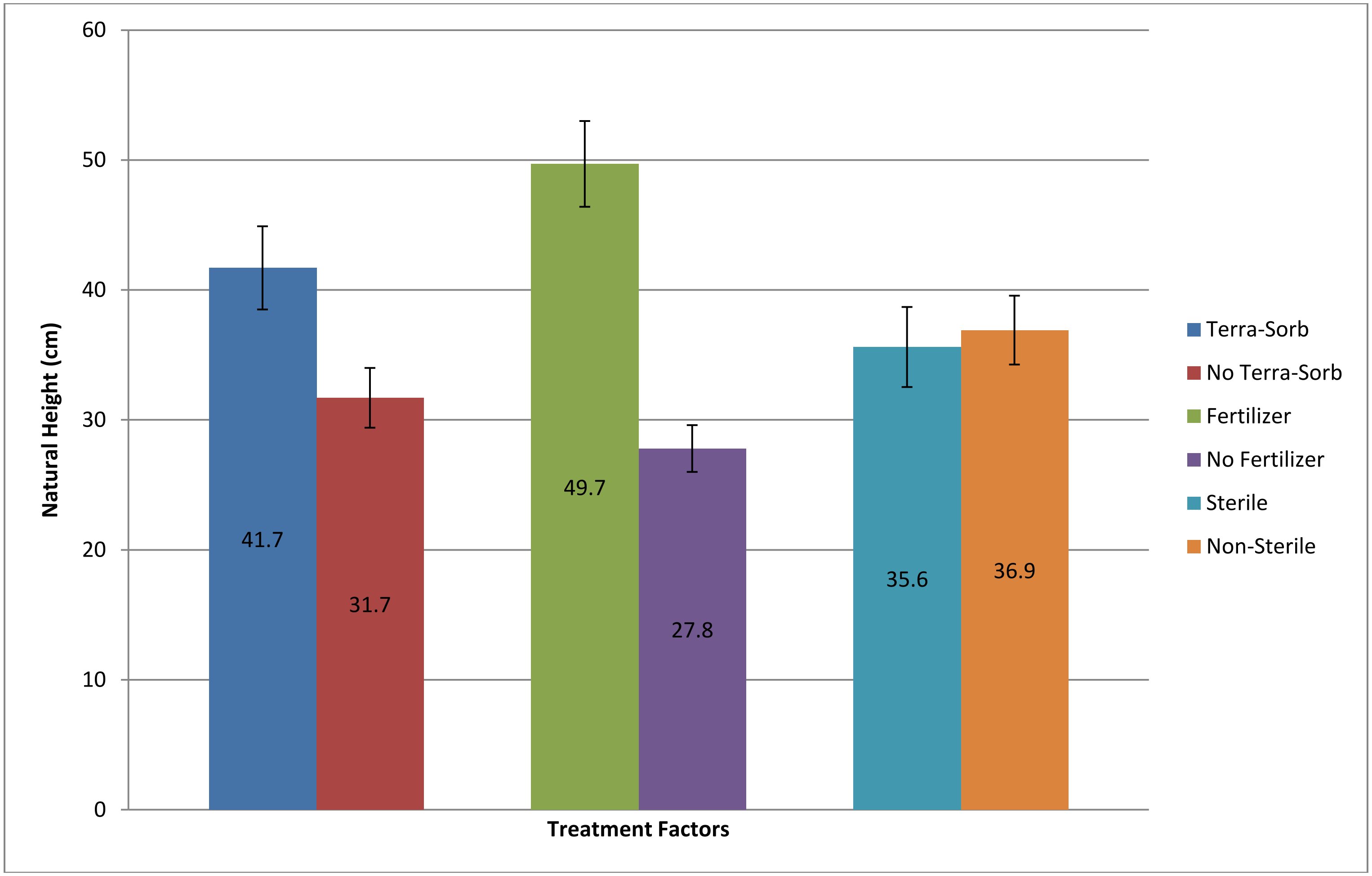

Figure 8: October 2008 natural height for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant ($p = 0.012$, $p=0.005$, respectively). In the order of treatment main effects as listed on the figure, n = 46, 53, 39, 60, 45, 54, respectively.

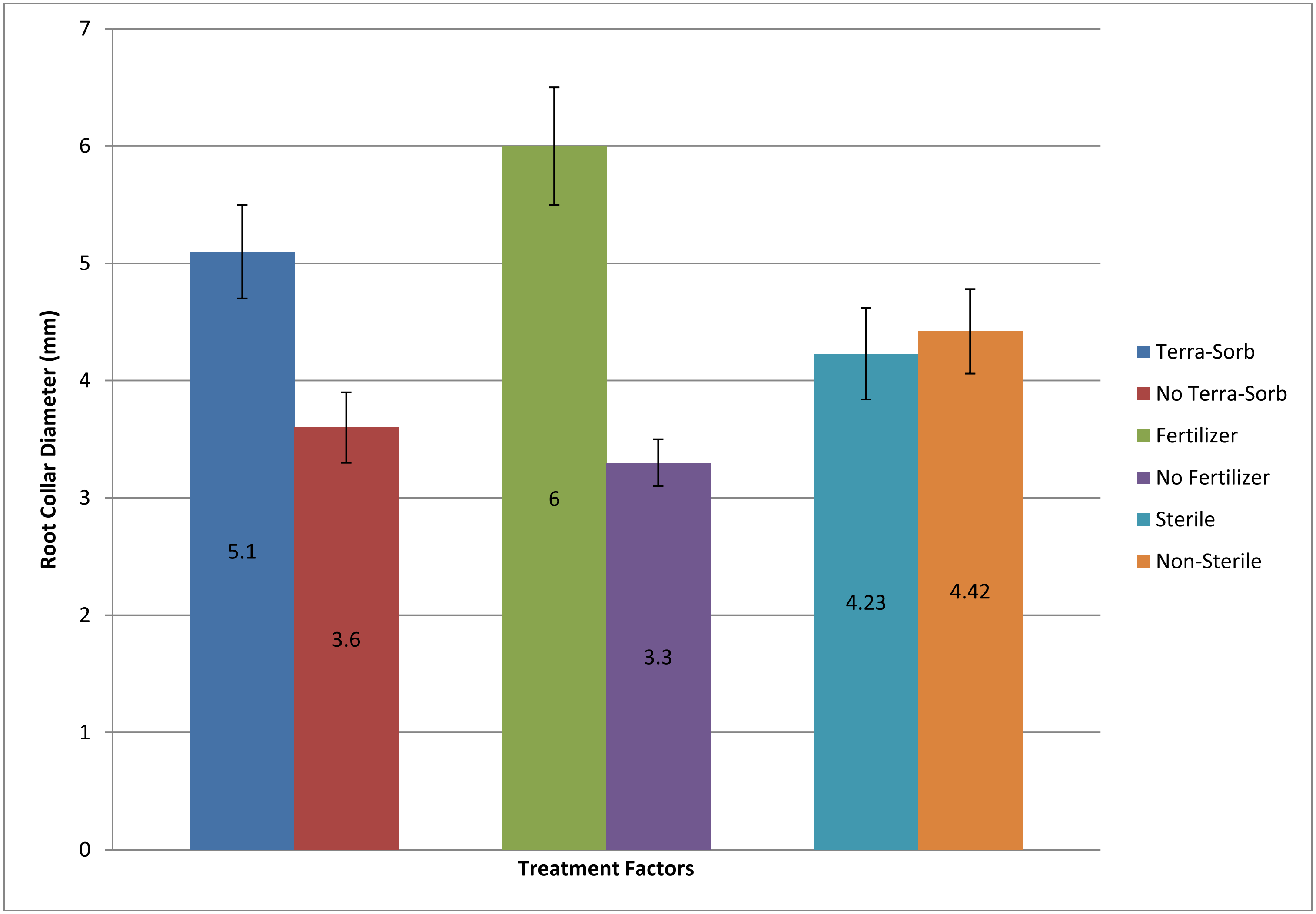

Figure 9: October 2008 RCD for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant ($p = 0.001$, $p < 0.0001$, respectively). In the order of treatment main effects as listed on the figure, n = 46, 53, 39, 60, 45, 54, respectively.

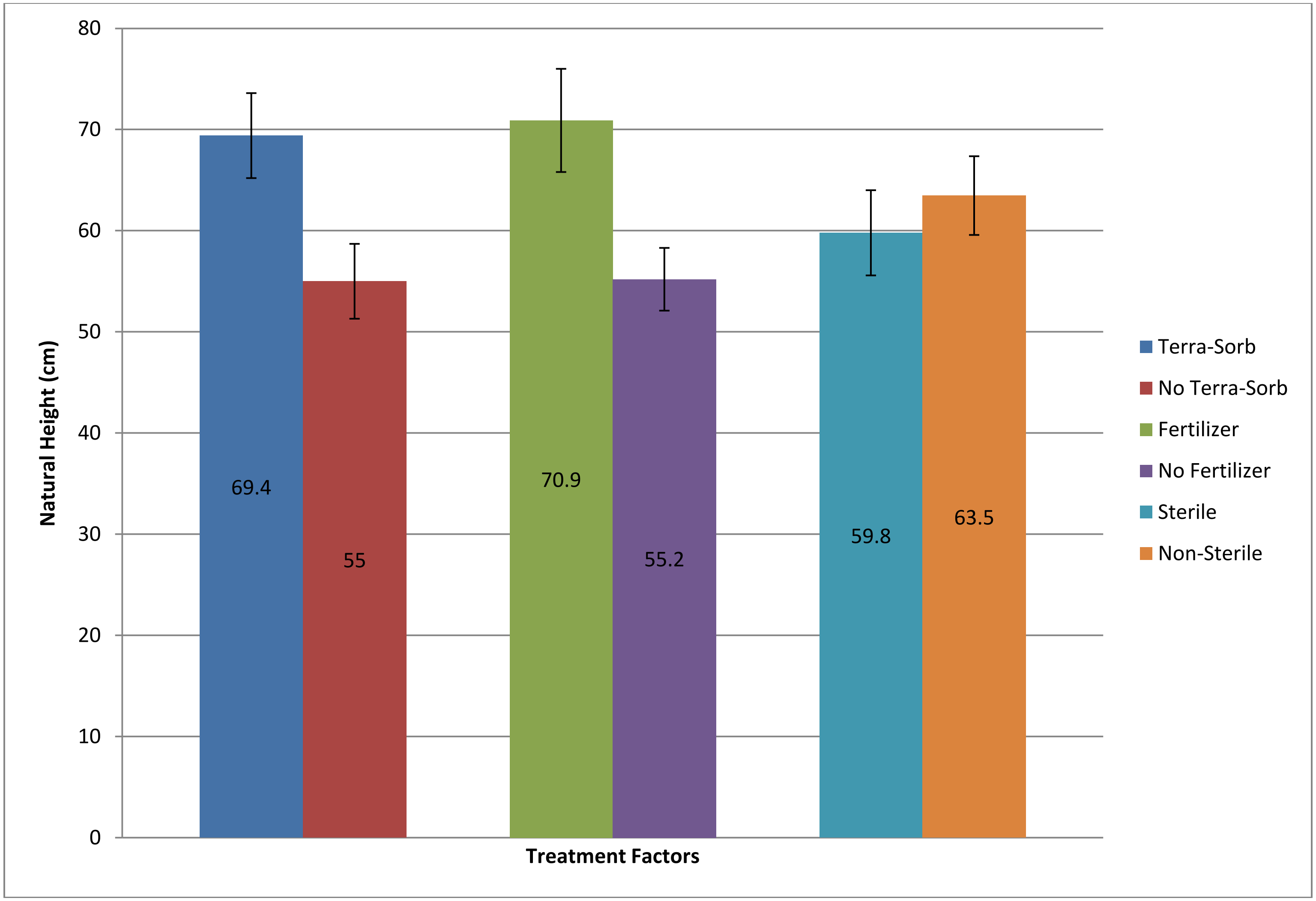

Figure 10: October 2009 natural height for chestnut planted on the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Differences in Terra-Sorb and fertilizer main effects were significant ($p = 0.001$, $p < 0.0001$). In the order of treatment main effects as listed on the figure, n = 48, 55, 43, 60, 49, 54, respectively.

Presence of the sterile soil treatment increased photosynthetic rate by 1.24 μmol CO_2 m^{-2} s^{-1} (p = 0.017), producing the highest rate when compared to other treatment main effects (Figure 11). However, this was not reflected in transpiration rate, with fertilizer being its only significant main effect by increasing 0.54 mmol H_2O m^{-2} s^{-1} ($p < 0.0001$) (Figure 12).

Water potential differences were significant within all three treatment main effects. The presence of Terra-Sorb and the sterile soil treatment provided less negative pre-dawn water potentials (p = 0.021, p = 0.023, respectively). However, fertilizer presence produced a more negative pre-dawn stem water potential (p = 0.015) (Figure 13). Further, there was a significant three-way interaction between all treatment levels, indicating that the differences between each factor differ. The combination of the sterile soil treatment, fertilizer, and Terra-Sorb provided the most negative water potential at -0.93 MPa (p = 0.007). Interestingly, the treatment with the least negative water potential was not the antithesis of the previously mentioned, but the combination of the sterile soil treatment, no fertilizer, and no Terra-Sorb with a water potential of -0.45 MPa (p = 0.007) (Figure 14).

Quarry

Nursery-grown seedlings experienced a higher overall 2-month survival rate of 71%, when compared to the direct seeded trees with a survival rate of 40%. In the nursery seedlings lot, main effects of fertilizer, Terra-Sorb, and the sterile soil treatment reduced survival. The highest survival occurred with seedlings planted without Terra-Sorb. Accordingly, the lowest survival occurred with seedlings planted with Terra-Sorb. Fertilizer presence decreased survival by 1%, Terra-Sorb presence decreased survival by 16%, and the sterile soil treatment decreased survival by 8% (Figure 15). This pattern was mirrored by the direct-seeded trees in fertilizer

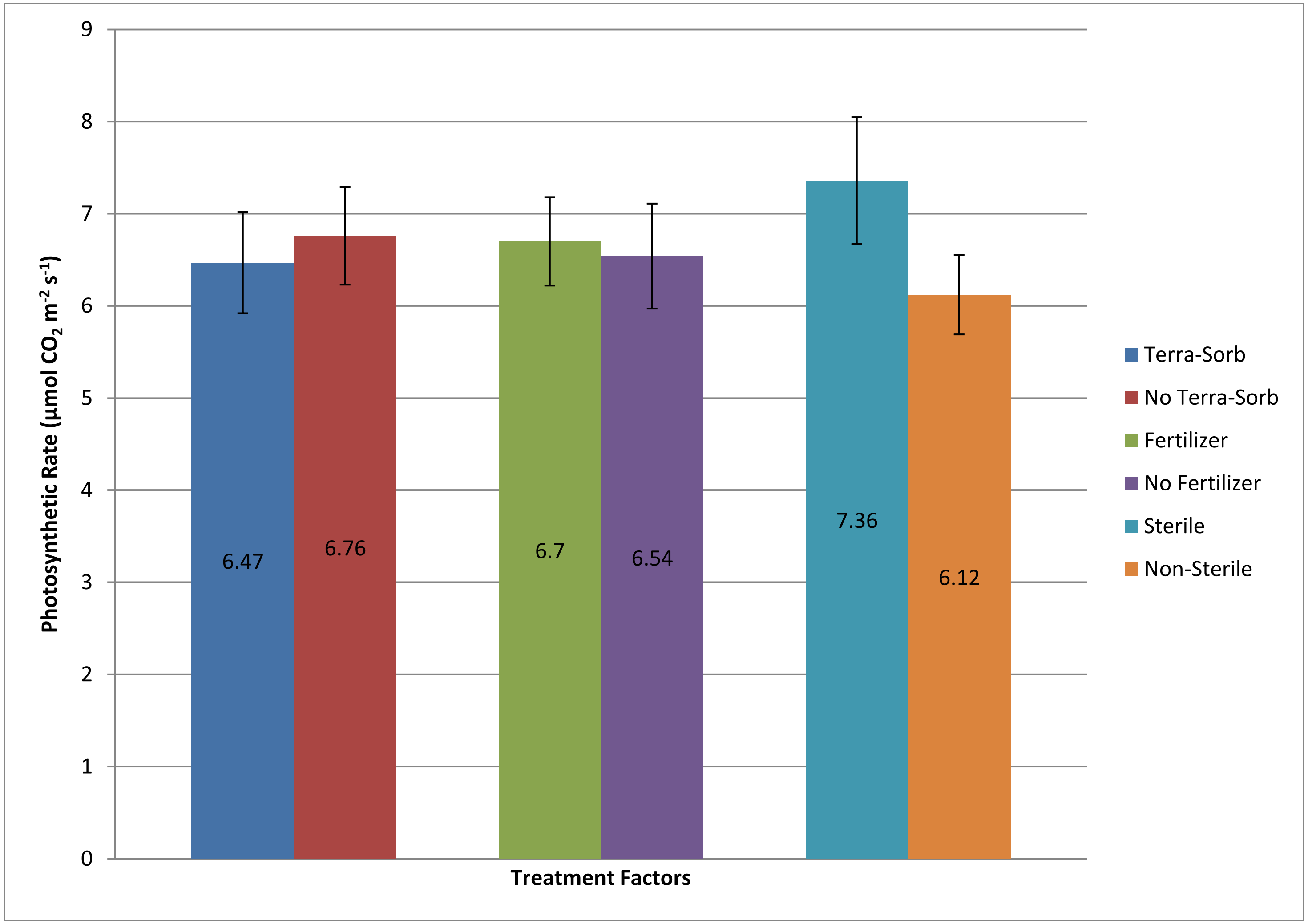

Figure 11: October 2009 photosynthetic rate for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Presence of the sterile soil treatment was the only significant treatment main effect ($p = 0.017$). In the order of treatment main effects as listed on the figure, n = 48, 46, 41, 53, 37, 57, respectively.

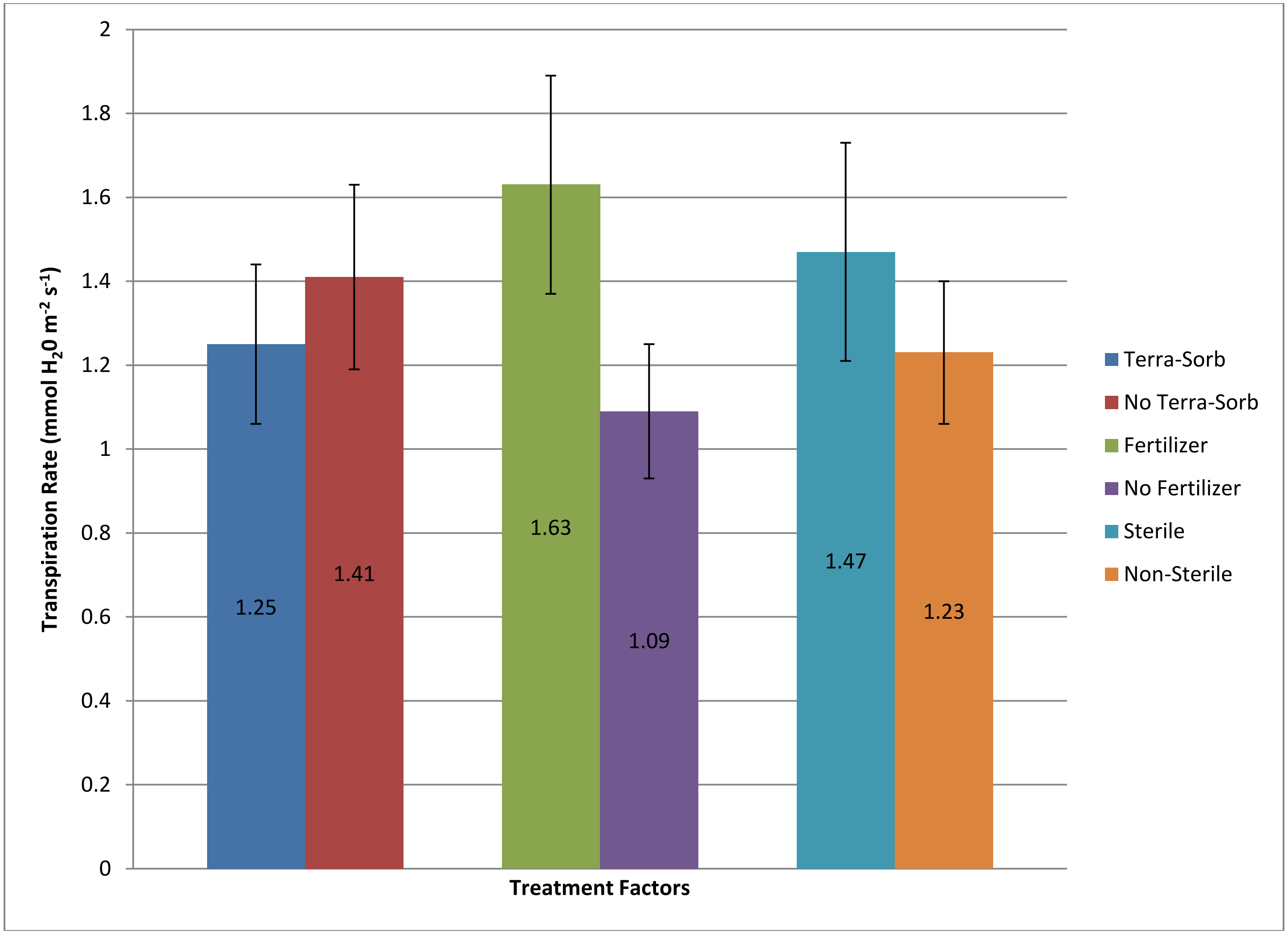

Figure 12: October 2009 transpiration rate for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Presence of fertilizer was the only significant treatment main effect ($p < 0.0001$). In the order of treatment main effects as listed on the figure, n = 48, 46, 41, 53, 37, 57, respectively.

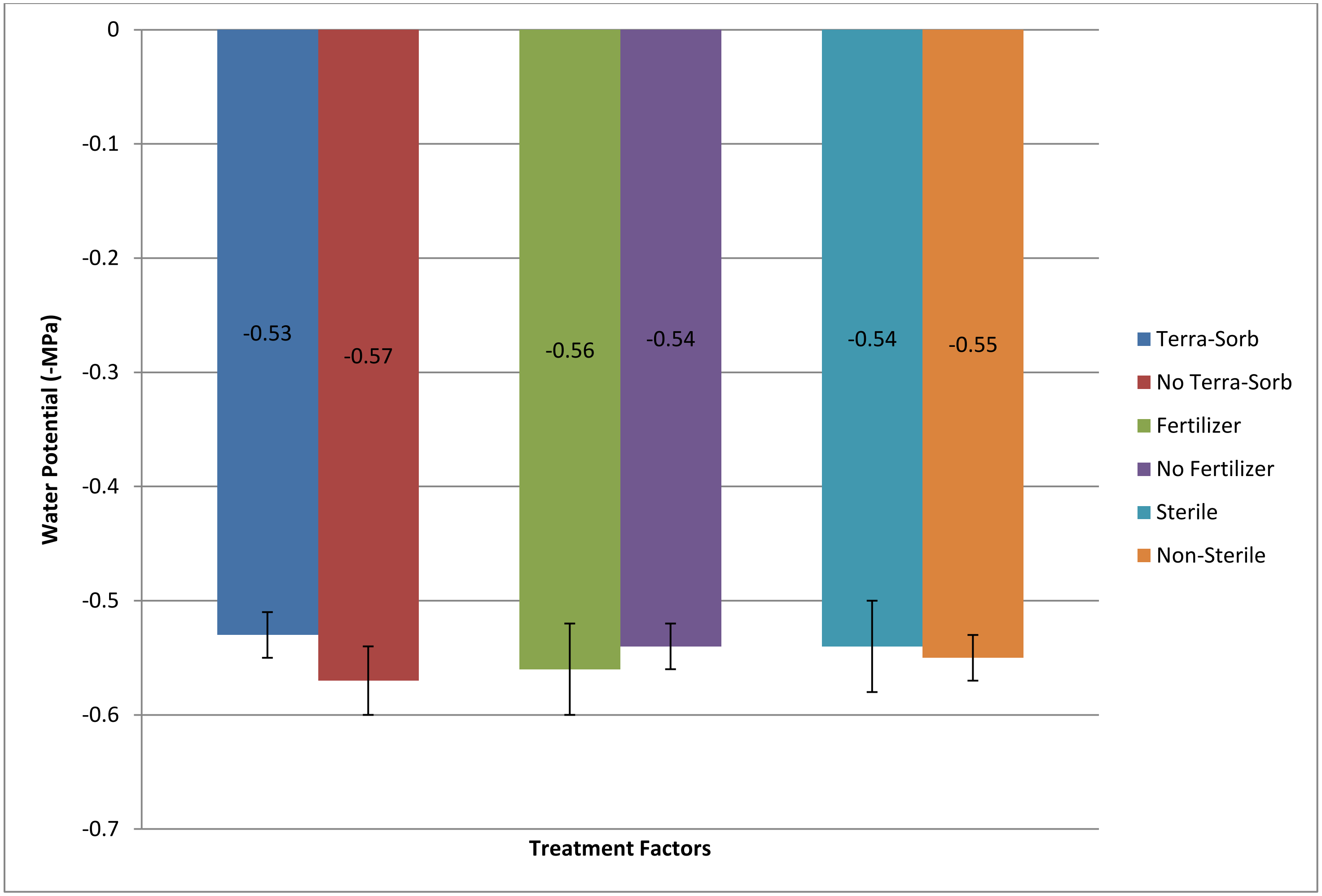

Figure 13: September 2009 pre-dawn water potential for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for treatment main effects are shown. Presence of Terra-Sorb, fertilizer, and the sterile soil treatment were all significant ($p = 0.021$, $p = 0.015$, $p = 0.023$, respectively). In the order of treatment main effects as listed on the figure, n = 42, 36, 32, 46, 31, 47, respectively.

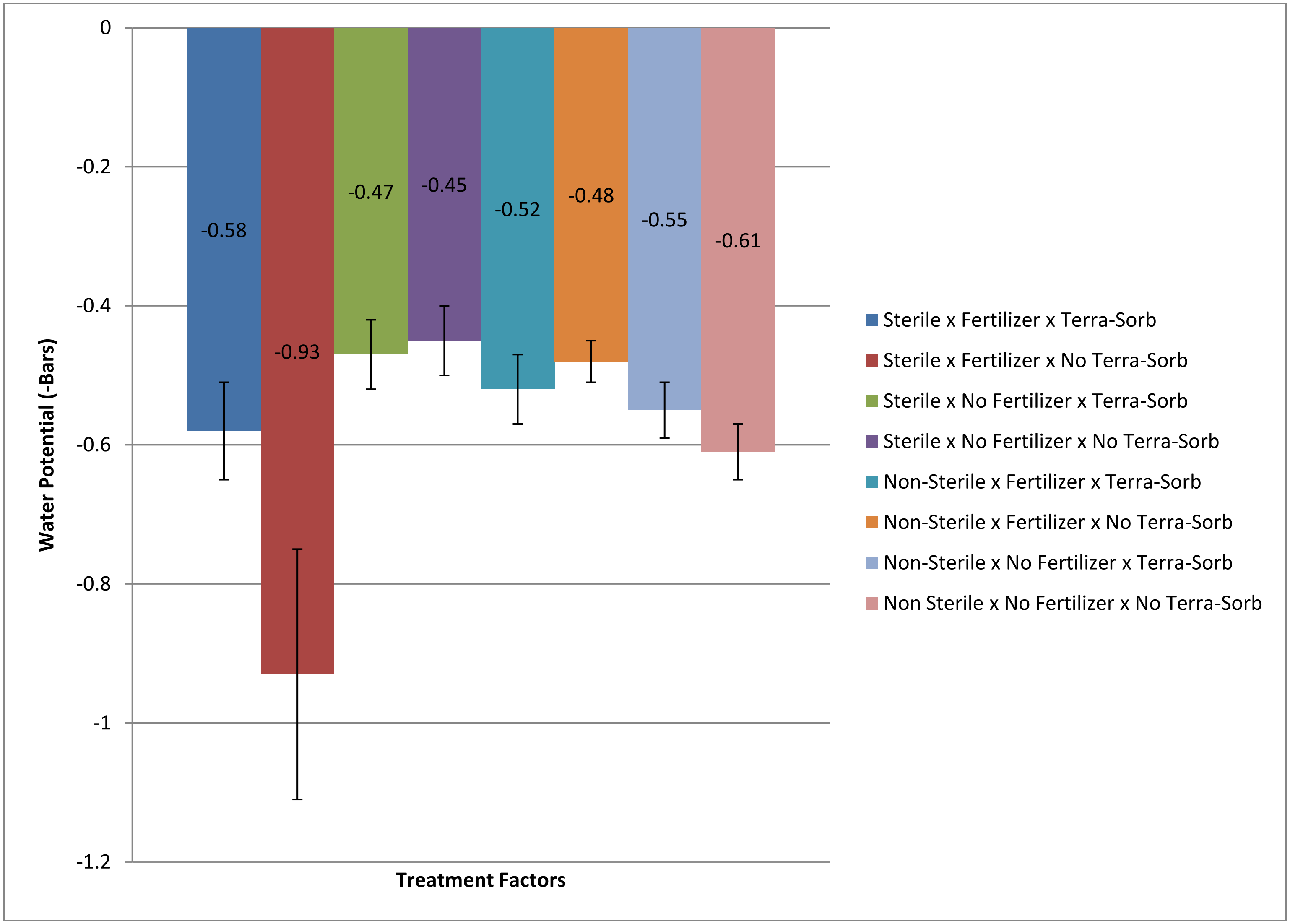

Figure 14: September 2009 pre-dawn water potential for chestnut planted at the mine site in the spring of 2008. Means ± standard errors for a three-way treatment interaction are shown.

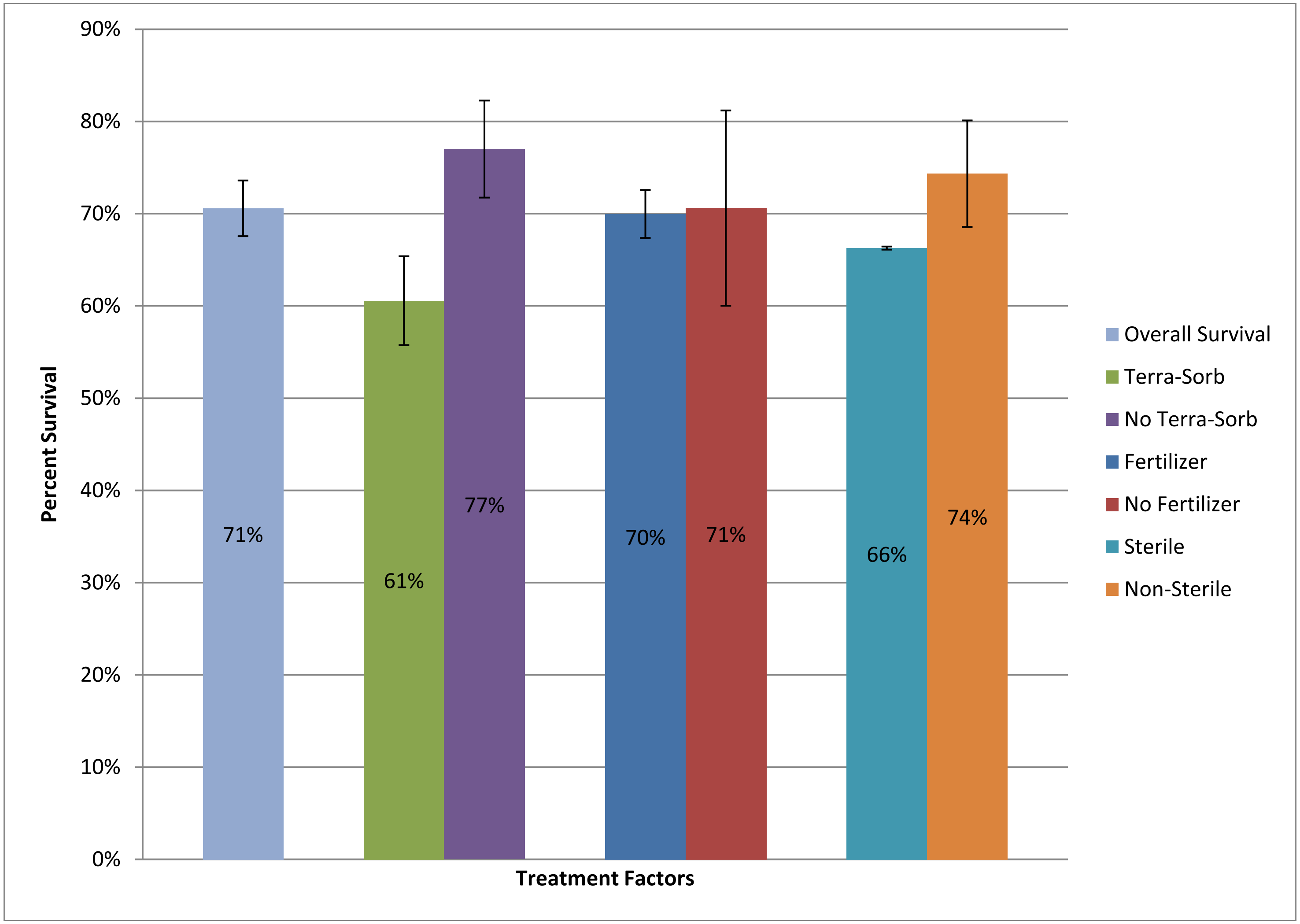

Figure 15: August 2009 seedling survival for chestnut planted at the quarry in June 2009. Means ± standard error for overall survival and treatment main effects are shown. Overall Survival n = 236. In the order of treatment main effects as listed on the figure, n = 87, 149, 119, 117, 114, 122, respectively.

and the sterile soil treatment, which reduced survival by 29% and 1%, respectively. The exception to the pattern was Terra-Sorb presence, which increased survival by 5% (Figure 16).

No significant differences were detected in natural height and RCD growth within the seedling lot. However, fertilizer presence increased apical meristem elongation in two months by over 50% ($p < 0.0001$) (Figure 17). Within the direct-seeded lot, presence of the sterile soil treatment increased natural height by 4 cm, producing the tallest trees when compared to other treatment main effects ($p = 0.004$) (Figure 18). Further, the sterile soil treatment increased RCD in the direct-seeded lot by 0.2 mm, but only within a confidence interval of 90% ($p = 0.057$).

When fertilizer was present in the soil substrate, photosynthetic rate was highest at 10.08 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$, which is a 55% increase from the lowest rate in nursery-grown seedlings without fertilizer ($p < 0.0001$). Further, native forest topsoil increased seedling photosynthesis at a higher rate when compared to sterilized soil ($p = 0.021$) (Figure 19). Water potential was least negative in trees planted with fertilizer at -0.38 MPa, differing significantly from trees planted without fertilizer at -0.44 MPa ($p < 0.0001$) (Figure 20).

Greenhouse

Survival was 100% within this experiment. Differences in natural height were evident in trees planted with Terra-Sorb, growing over 4 times taller within two months ($p = 0.007$) (Figure 21). New growth increased by 1.3 cm with fertilizer presence ($p = 0.012$) (Figure 22). No significant differences in RCD were found. The presence of Terra-Sorb reduced photosynthetic rate by nearly 22% ($p = 0.031$) (Figure 23). No differences in water potential were found. Also, no differences were found in specific leaf area among treatments. However, average leaf dry

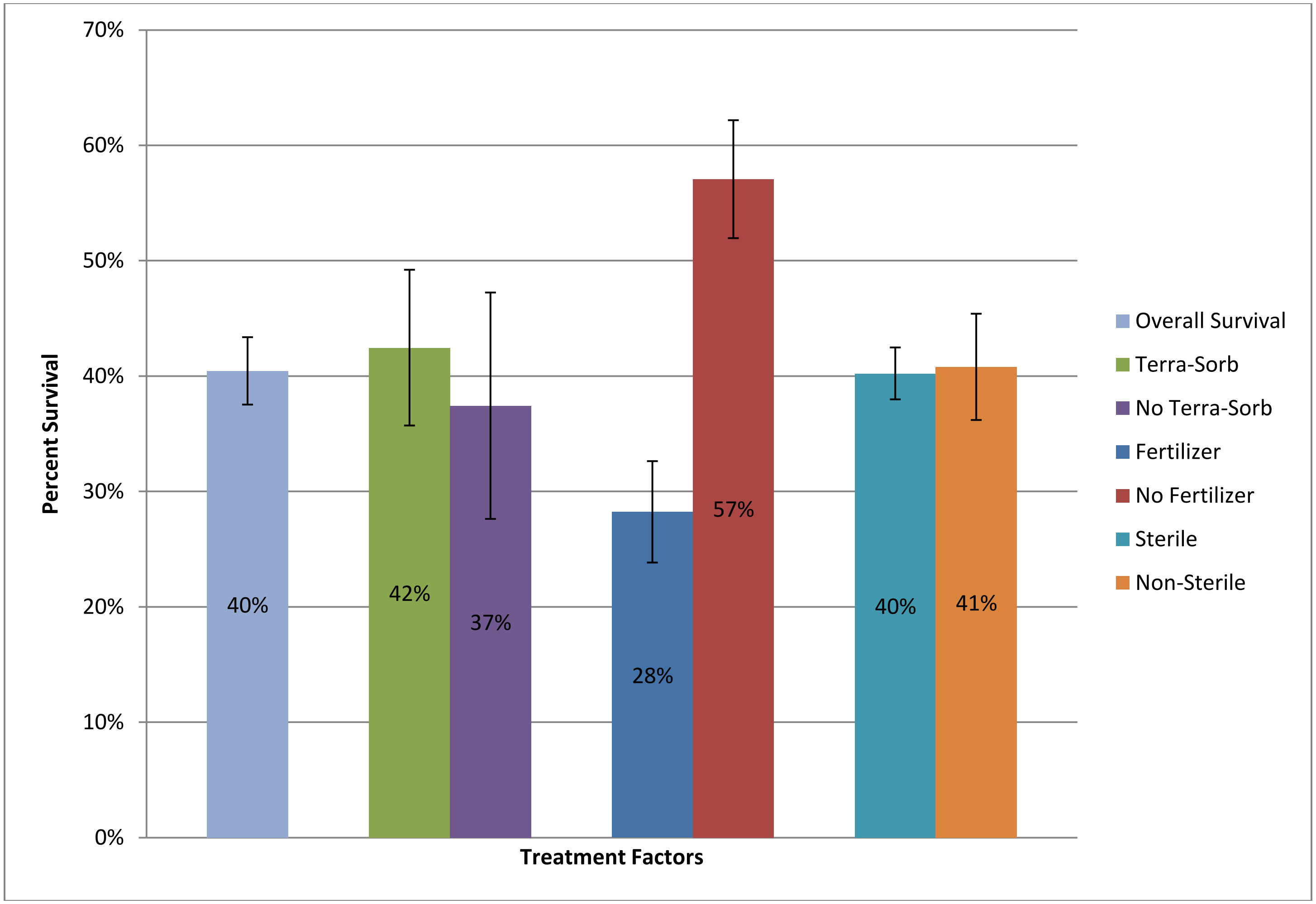

Figure 16: August 2009 directly-seeded tree survival for chestnut planted at the quarry in June 2009. Means ± standard error for overall survival and treatment main effects are shown. Overall survival n = 132. In the order of treatment main effects as listed on the figure, n = 61, 71, 55, 77, 64, 68 respectively.

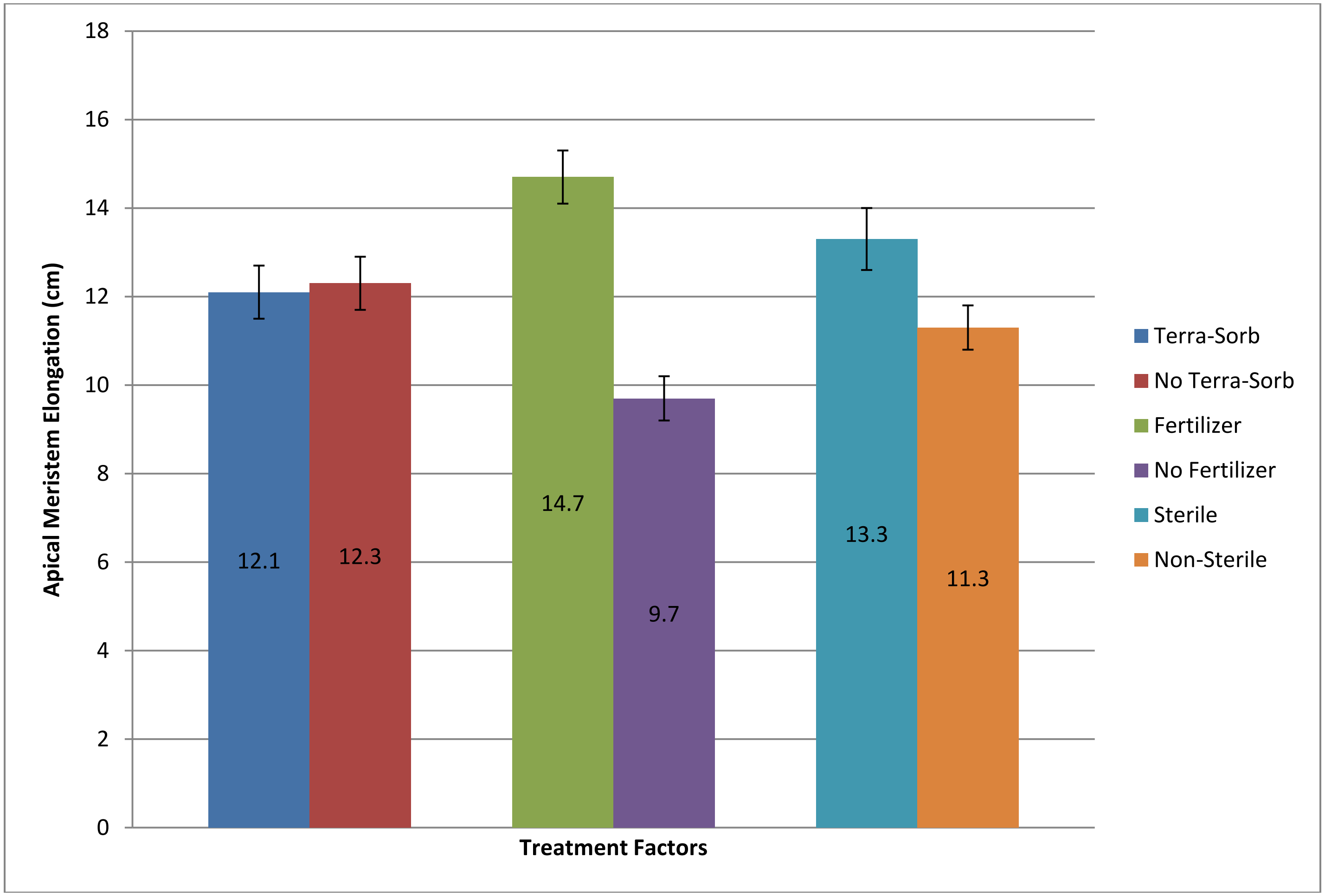

Figure 17: September 2009 seedling apical meristem elongation for chestnut planted at the quarry in June 2009. Means ± standard error for treatment main effects are shown. Presence of fertilizer was the only significant treatment main effect ($p < 0.0001$). In the order of treatment main effects as listed on the figure, n = 74, 137, 108, 103, 100, 111, respectively.

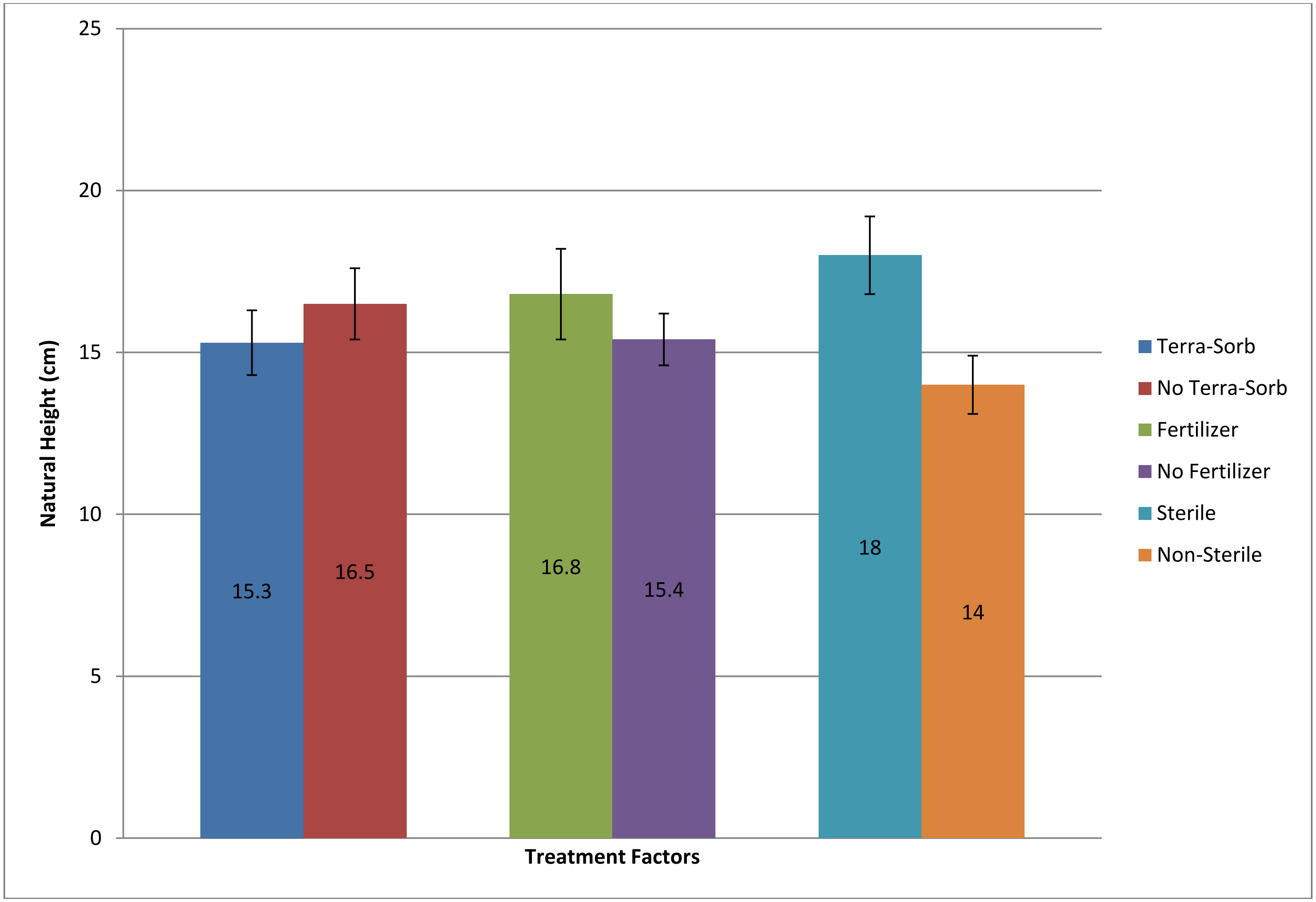

Figure 18: August 2009 directly-seeded tree natural height for chestnut planted at the quarry in June 2009. Means ± standard error for treatment main effects are shown. Presence of the sterile soil treatment was the only significant treatment main effect ($p = 0.004$). In the order of treatment main effects as listed on the figure, n = 61, 71, 55, 77, 64, 68 respectively.

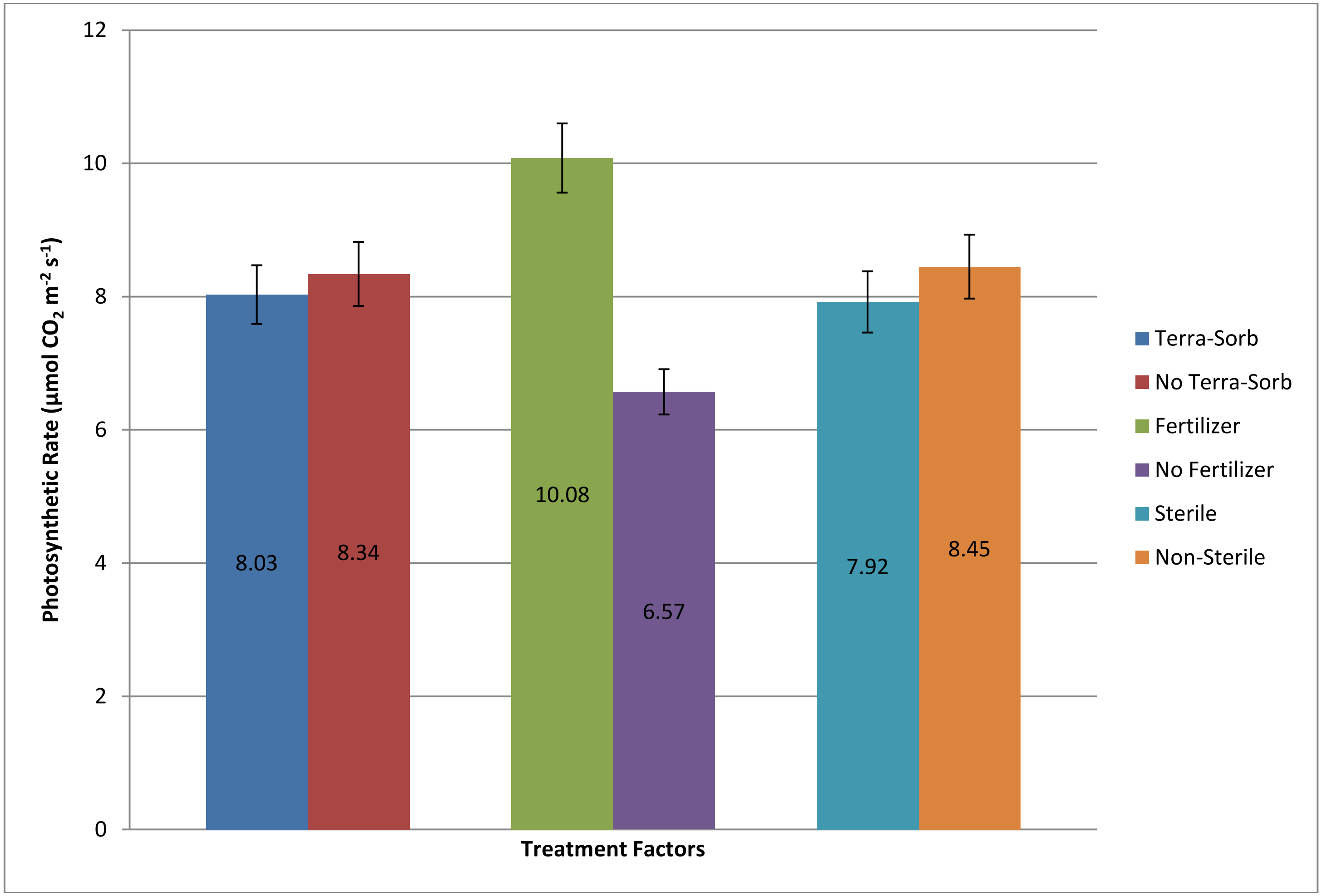

Figure 19: August 2009 seedling photosynthetic rate for chestnut planted at the quarry in June 2009. Means ± standard error for treatment main effects are shown. Presence of fertilizer and the sterile soil treatment were both significant ($p < 0.0001$, $p = 0.021$, respectively). In the order of treatment main effects as listed on the figure, n = 75, 93, 78, 90, 79, 89, respectively.

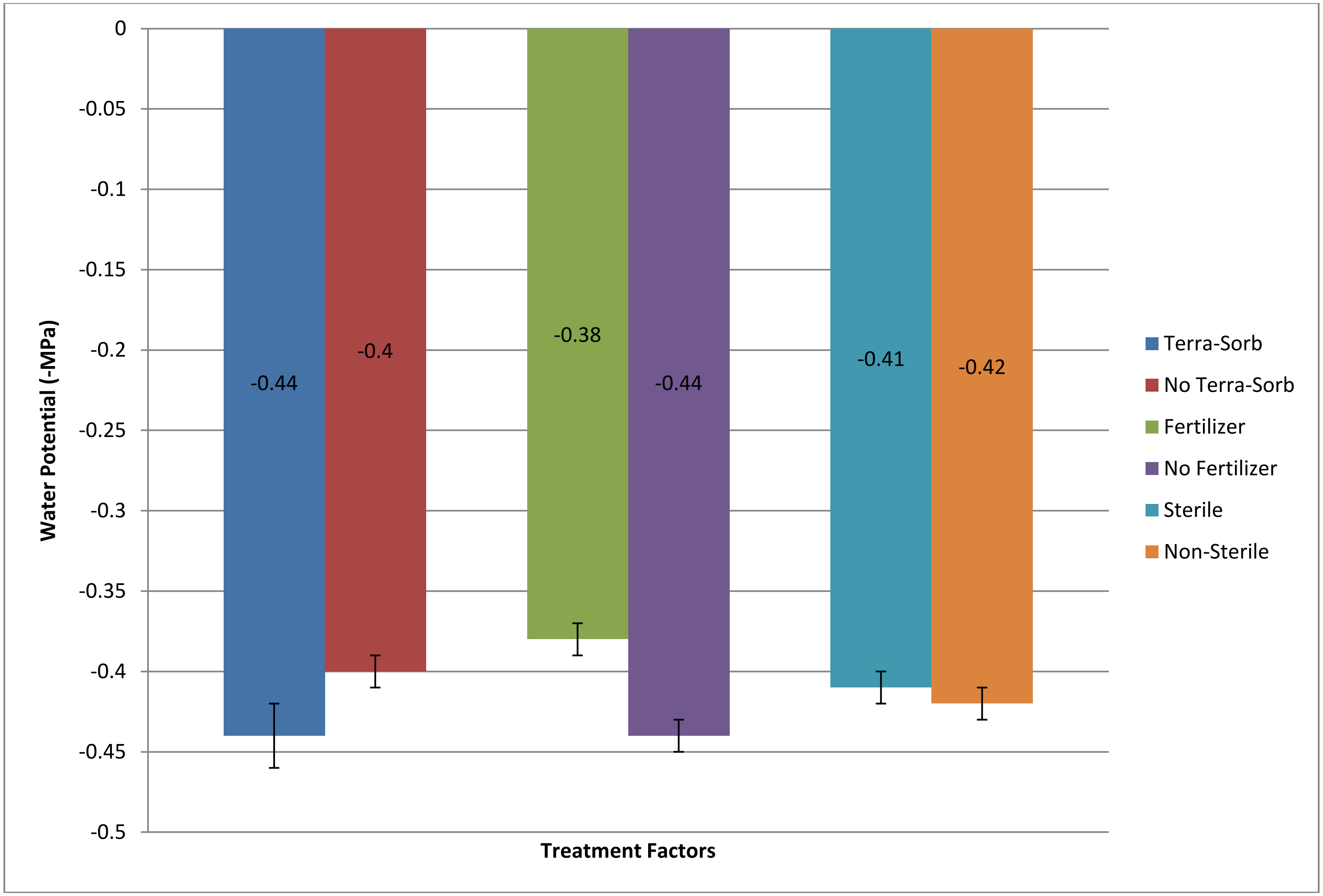

Figure 20: August 2009 seedling water potential for chestnut planted at the quarry in June 2009. Means ± standard error for treatment main effects are shown. Fertilizer main effects were significant ($p < 0.0001$). In the order of treatment main effects as listed on the figure, n = 82, 136, 91, 127, 98, 120, respectively.

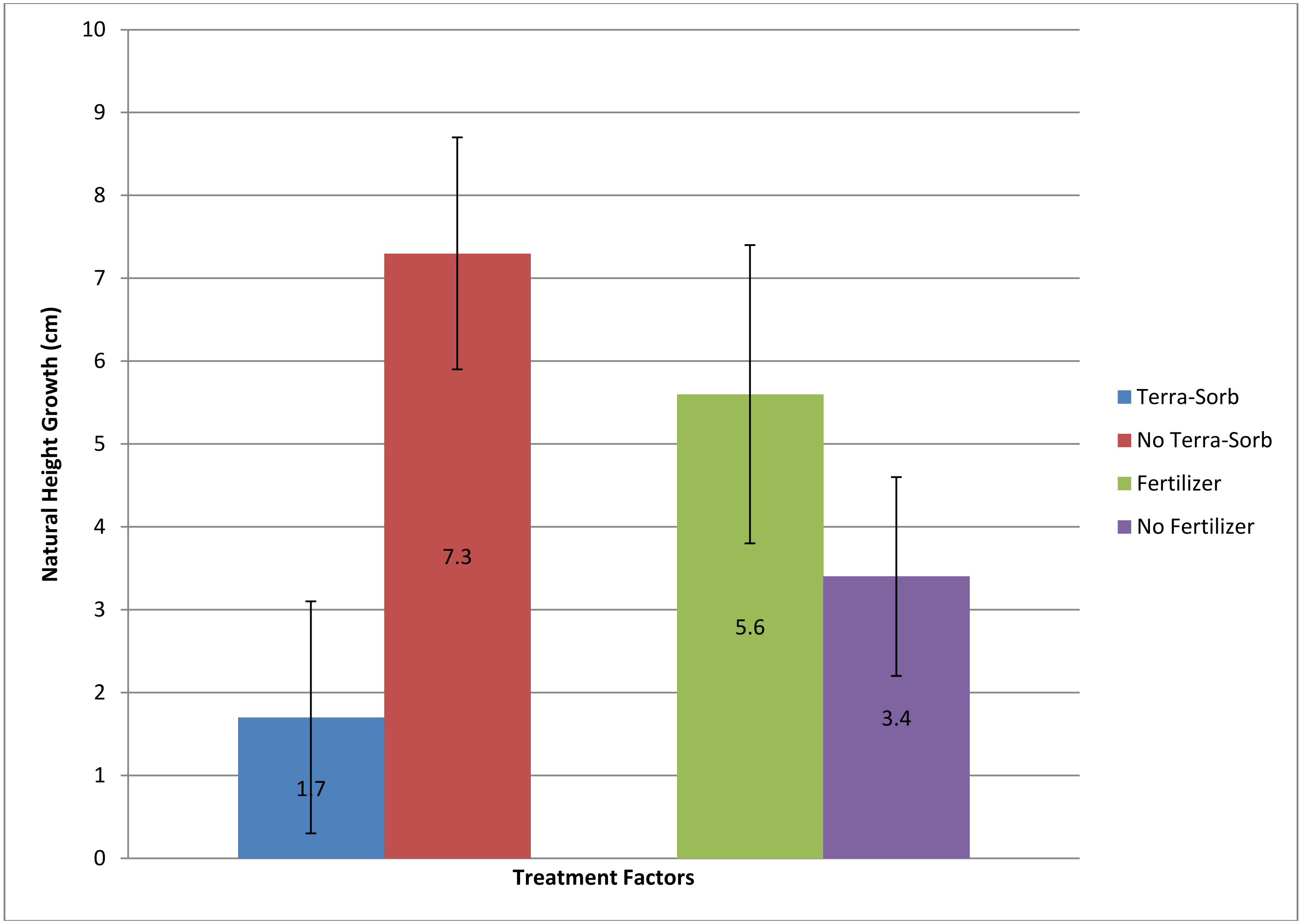

Figure 21: September 2009 natural height growth for chestnut planted in the greenhouse in June 2009. Means ± standard error for treatment main effects are shown. Terra-Sorb main effects were significant ($p = 0.007$). In the order of treatment main effects as listed on the figure, n = 20, 20, 20, 20, respectively.

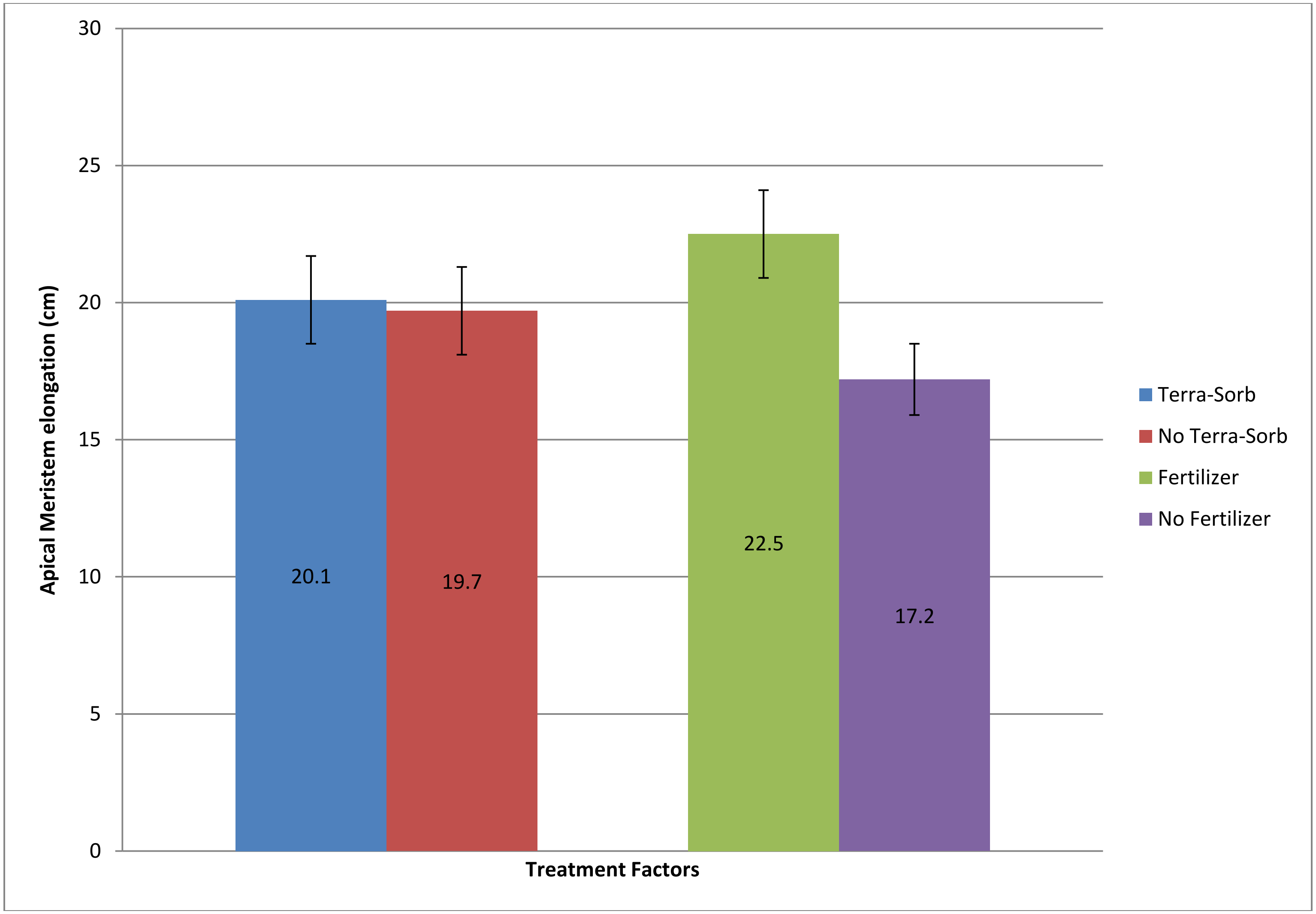

Figure 22: September 2009 apical meristem elongation for chestnut planted in the greenhouse in June 2009. Means ± standard error for treatment main effects are shown. Fertilizer main effects were significant ($p = 0.012$). In the order of treatment main effects as listed on the figure, n = 20, 20, 20, 20, respectively.

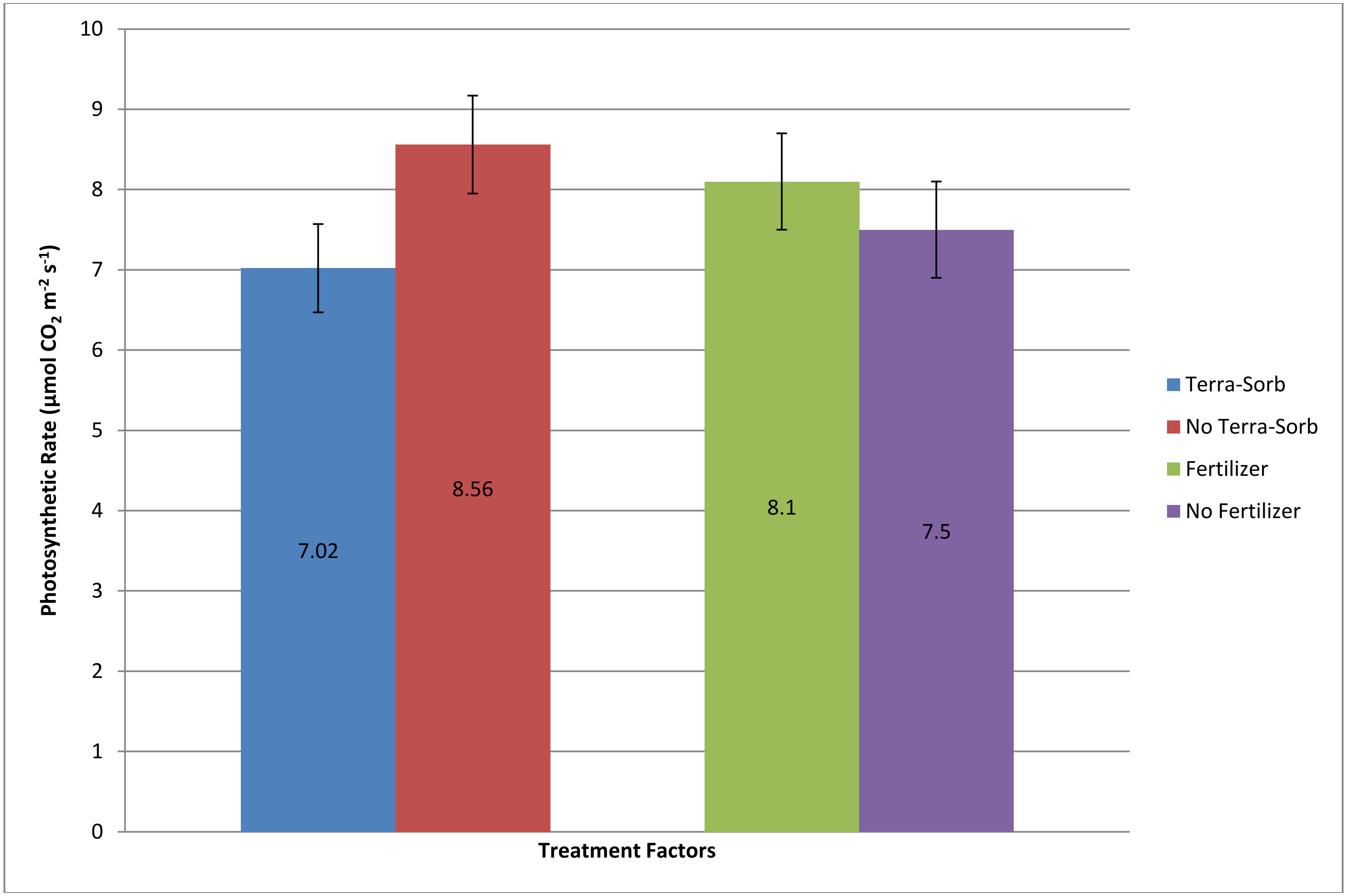

Figure 23: August 2009 photosynthetic rate for chestnut planted in the greenhouse in June 2009. Means ± standard error for treatment main effects are shown. Terra-Sorb main effects were significant (p = 0.031). In the order of treatment main effects as listed on the figure, n = 20, 20, 20, 20, respectively.

matter content, and average leaf area were significantly higher in the presence of fertilizer ($p < 0.0001$, $p=0.004$, respectively) (Figures 24, 25).

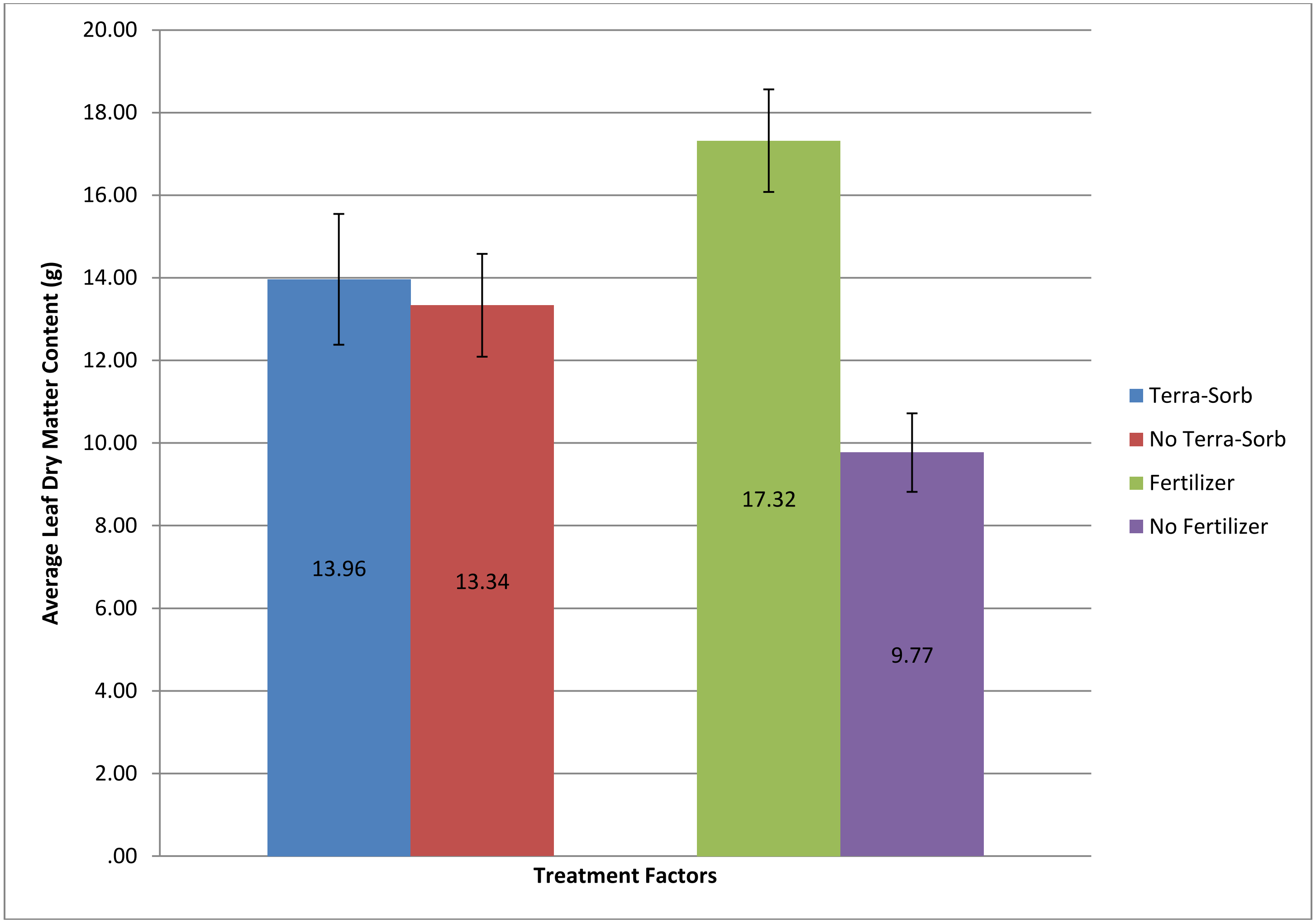

Figure 24: October 2009 average leaf dry matter content for chestnut planted in the greenhouse in June 2009. Means ± standard error for treatment main effects are shown. Fertilizer main effects were significant ($p < 0.0001$). In the order of treatment main effects as listed on the figure, n = 20, 20, 20, 20, respectively.

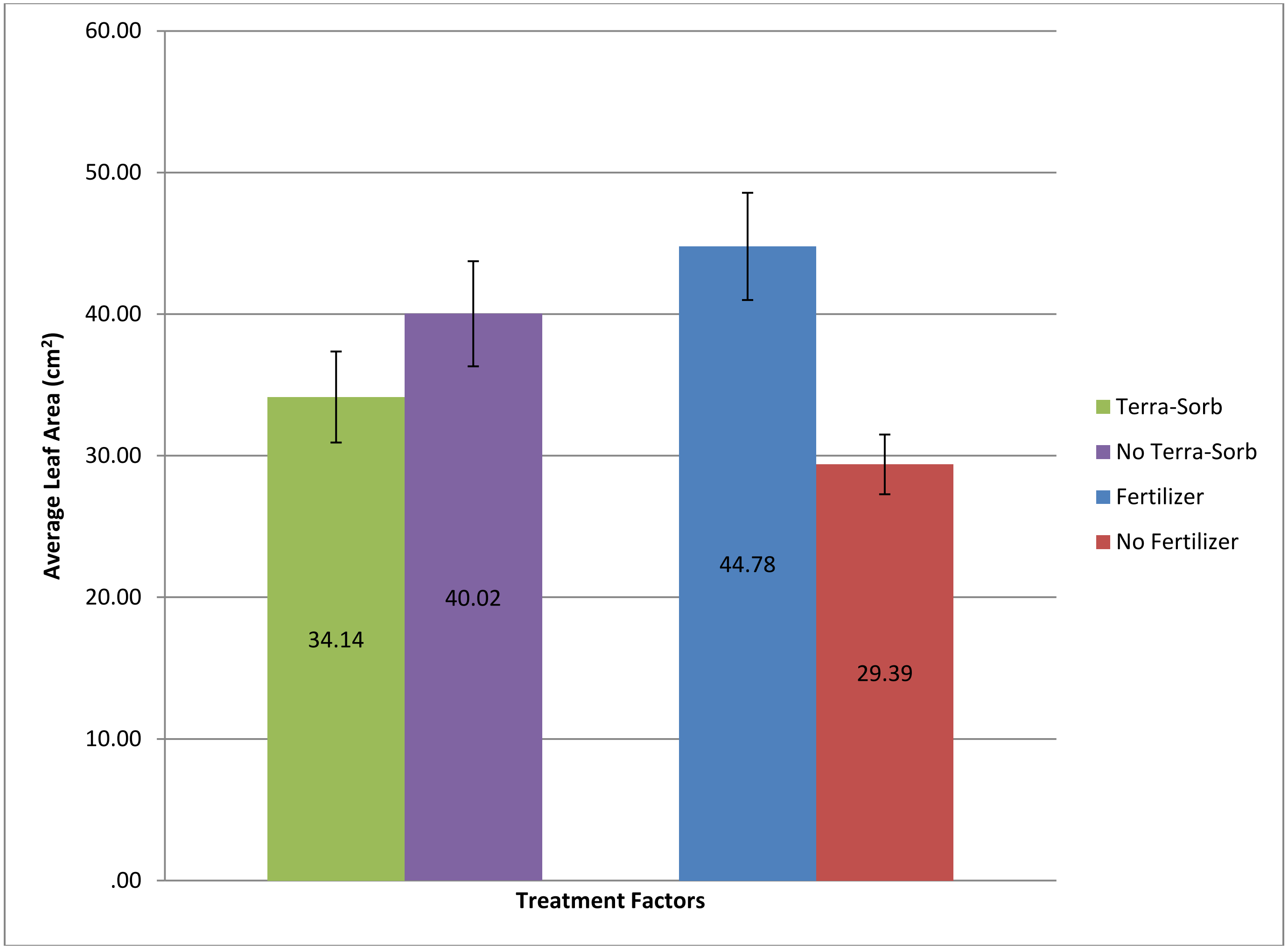

Figure 25: October average leaf area for chestnut planted in the greenhouse in June 2009. Means ± standard error for treatment main effects are shown. Fertilizer main effects were significant ($p = 0.004$). In the order of treatment main effects as listed on the figure, n = 20, 20, 20, 20, respectively.

DISCUSSION

Overall Survival

Mine Site

Overall, the first and second year survival rates of 29 and 28% are unacceptably low for successful reforestation. Similar studies report direct seeding survival rates ranging from 48-84% (Fields-Johnson, Burger, Evans, & Zipper, 2010; French, Barton, & Graves, 2008). In a side study, seeds of various genotypes were inter-planted with the pure American seeds in this experiment and survived at an average rate of 70% (Miller, Franklin, & Buckley, 2009). Further, seed survival at the quarry site was over 10% higher than that of this experiment. However, rodent herbivory was prevalent although un-quantified on our site. It is expected that mortality due to herbivory was high. Further, the shelters used in this experiment were not vented, as those used in Miller et al. (2009) and at the quarry site. Lastly, the growing season in 2008 experienced nearly half the amount of rainfall as 2009, the year in which survival was high at the quarry (NOAA, 2009).

Quarry

Overall seedling survival was 31% higher than direct-seeded trees, differing from previous studies (French, et al., 2008). Seedling survival was similar to that of other studies (Fields-Johnson, et al., 2010; French, et al., 2008). Lower survival in seeded trees at the quarry site can be attributed to initial mass instability and sinking of spoil mounds. Planting occurred soon after mounds were end-dumped. Contrasting from the mine site, the mounds were high in clay content with a lower amount of coarse fragment. Further, there was much precipitation in 2009 (NOAA, 2009). As a result, the mounds settled substantially, reducing their slope and filling in-between slopes. Seeds planted on mound tops were uncovered and those between mounds were buried further. Therefore, emergence and survival were likely lower in direct-seeded trees due to the sinking effect of the mounds.

Greenhouse

Survival was 100% in this experiment due to well controlled environmental conditions.

<u>Fertilizer</u>

Survival

Mine Site

Generally, fertilizer is known to increase survival in seedling transplants (Jacobs & Timmer, 2005; Kozlowski, et al., 1991). However, slow-release fertilizers have shown adverse effects on survival in chestnut seedlings (Herendeen, 2007). The effect of fertilizer tablets on survival of direct-seeded chestnuts has not been clearly quantified. In this experiment, in both 2008 and 2009, fertilizer reduced survival the most when compared to other treatments. Although low-sodium slow release tablets were used, it is possible that since the seeds were planted quite near the fertilizer tablet, excessive sodium concentrations were initially reached within the rhizosphere. High levels of sodium can create Na^+ toxicity (Jacobs & Timmer, 2005), potentially reducing fertilizer uptake (Grattan & Grieve, 1999). Increased solutes in the rhizosphere can also increase osmotic potential of the soil, potentially lowering water and nutrient uptake which was supported by the observation of a more negative water potential in this experiment.

Quarry

Treatment main effects on survival in seedlings mirrored that of the mine site, but to a lesser extent with respect to fertilizer presence. Soils with greater amounts of clay provide greater nutrient retention than coarse soils, and have a higher cation exchange capacity. Sites with a high coarse fragment content limit the site index, and nutrient availability (Rodrigue & Burger, 2004). Further, seedling roots were not sterilized before planting. It is possible that soil attached to the roots pre-planting could have aided survival and buffered nutrient demand. Therefore, the fertilizer effect on survival in seedlings was likely marginalized by the fine soil texture on this site. Survival in direct-seeded trees was highly dependent on absence of fertilizer.

Greenhouse

Survival was 100% at the greenhouse due to well controlled environmental conditions.

Growth

Mine Site

Primary growth was increased both years and secondary growth in the first year with the inclusion of fertilizer. Conversely, growth was most restricted when fertilizer was absent in both years. Therefore, it appears that if survival occurs past the emergence stage, fertilizer will significantly increase primary and secondary growth in at least the first year on similar mine sites. It is probable that with an increased importance of fertilizer in the soil, nutrition benefits from native soil were made negligible to growth after initial emergence and survival.

Quarry

Due to the dramatic changes in the quarry mounds within the first month, many seedlings were partially buried. Therefore, initial natural height and RCD changed dramatically after planting, and most measurements were negative. However, the terminal node of the previous year's growth was evident, and accurate measurement of apical meristem elongation was possible. Results show the primary factor influencing meristem elongation was fertilizer. Similar studies show fertilizer benefits on stem biomass accumulation (Preve, et al., 1984; Walker, 2002).

Greenhouse

There was much settling in the soil substrate within the pots, at times exposing roots. Further, seedlings did not undergo thigmomorphogenesis and were often spindly with drooping tops. Therefore, accurate measurements of natural height and RCD were difficult. However, results show apical meristem elongation was influenced by presence of fertilizer, increasing growth.

Physiological responses

Mine Site

Fertilizer had no effect on photosynthetic rate at the mine site. However, there was an increase in rate of transpiration in fertilizer presence. This can be attributable to the flow of nutrients from the bulk soil to the root surface with water uptake. As this mass flow of nutrients reaches the root surface, the plant responds through rapid transpiration (Lambers, et al., 2008). However, fertilizer presence increased water stress with a more negative water potential, likely as a result of increased osmotic solutes within the soil, reducing water uptake.

Quarry

Photosynthetic rate was significantly increased by fertilization, evidenced by the apical meristem elongation in the nursery-grown seedlings. As the trees used at this site were seedlings when planted, they were likely acclimated to the nutrient rich soils in which they were grown in a greenhouse, resulting in a greater nutrient demand at the quarry when compared to the mine site. Further, the combination of fertilizer with the sterile soil treatment produced an interaction in which fertilizer main effects were found to differ by presence of the sterile soil treatment. No effects of fertilizer were found on transpiration.

Curiously, fertilizer was found to produce a significantly less negative water potential, contrasting with the mine site, where fertilizer produced more negative direct-seeded tree water potential. However, water potential measurements at the quarry were taken from bare-root planted seedlings rather than from direct-seeded trees at the mine site. Bare-root trees at this site were grown in a nursery, under high nutrient conditions. Therefore, addition of fertilizer to the planting site likely cushioned transplant shock. Further, the site received nearly twice as much summer rain in 2009 when compared to 2008 (NOAA, 2009) which was often held between mounds, due to clay saturation. These conditions contrast with the mine site which experienced drier conditions, in a soil that is quickly draining. Therefore, lowered osmotic potential in the soil due to the solutes from the fertilizer tablet (Lambers, et al., 2008) at the mine site was possibly negated at the quarry, which experienced high moisture conditions throughout the summer and at the time of measurement. It is also possible that the addition of fertilizer stimulated osmotic adjustment of the direct-seeded trees at the mine site, causing them to increase solutes within their roots to encourage more flow into their roots, resulting in a more negative water potential.

Greenhouse

Fertilizer did not have any effect on seedling physiology at the greenhouse. The absence of an effect on water potential is likely due to well watered conditions which might have negated potential changes.

Terra-Sorb

Survival

Mine Site

Seedling survival was lowered in the presence of Terra-Sorb, differing from research claiming benefits from similar hydrophilic root polymers (Al-Humaid & Moftah, 2007; Apostol, et al., 2009; Terra-Sorb, 2007). However, during survival monitoring, it was noticed that after rain events the Terra-Sorb gel expanded out of the ground. At times, this uplifted the nut (often with primordial growth) out of the ground. This was likely due to the Terra-Sorb not being fully hydrated during planting.

Quarry

In presence of Terra-Sorb, survival in nursery-grown seedlings was opposite to that of seeds. Terra-Sorb presence lowered seedling survival, while increasing seed survival. Both nursery-grown seedlings and seeds were planted with the same amount of Terra-Sorb. As leaves emerged in the nursery-grown seedlings, moisture demand would be greater than that of direct-seeded trees which have not yet emerged. It has been shown that in seedlings, Terra-Sorb can withhold moisture from root availability (Al-Humaid & Moftah, 2007). The amount of Terra-Sorb relative to root mass in seeds was greater than that in the year-old nursery-grown seedlings, possibly aiding emergence and survival rather than hindering it. The difference in direct-seed survival at the quarry and the mine site is likely due to the Terra-Sorb not being adequately hydrated prior to planting at the mine site.

Greenhouse

Survival was 100% at the greenhouse due to well controlled environmental conditions.

Growth

Mine Site

Throughout the growing seasons of 2008 and 2009, inclusion of Terra-Sorb in the soil medium played a significant role. In 2008, Terra-Sorb inclusion increased natural height and RCD growth. As Terra-Sorb can increase soil moisture and plant water availability (Al-Humaid & Moftah, 2007), enhanced growth can be expected through increased photosynthesis and cell elongation (Lambers, et al., 2008). In 2009, natural height continued to be significantly influenced by Terra-Sorb.

Quarry

No effect of Terra-Sorb was found in seed or seedling growth, contrasting with the increased survival in seeds and lowered survival in nursery-grown seedlings while in the presence of Terra-Sorb. It is possible that at the quarry, Terra-Sorb only played an initial role in seedling emergence and survival before being abated by the heavy summer rains combined with high clay percentage at this site which often left the ground saturated.

Greenhouse

Differences in natural height were significant with trees planted with Terra-Sorb. However, as stated before, this was not a result of apical meristem elongation, but likely resulted from drooping, spindly seedlings.

Physiological Responses

Mine Site

Terra-Sorb was not found to significantly influence photosynthetic rate or transpiration rate. However, presence of Terra-Sorb was beneficial to the seedlings, decreasing water stress as measured by a less negative water potential. The coarse texture of the soil in this experiment makes it particularly susceptible to low water-holding capacity. The advantage of Terra-Sorb in soil is its ability to hold many times its weight in water. As plant-available water increases with Terra-Sorb, water potential becomes less negative, as evidenced and supported by others (Al-Humaid & Moftah, 2007; Apostol, et al., 2009; Lambers, et al., 2008).

Quarry

Terra-Sorb was not found to significantly influence photosynthetic rate, transpiration rate, or water potential at the quarry. As stated previously, heavy summer rains combined with a relatively low infiltration rate likely buffered any potential Terra-Sorb effects.

Greenhouse

Although Terra-Sorb was unlikely responsible for natural height, it did negatively influence photosynthetic rate when present. Seedlings were watered to field capacity every day, but soil volume was low and drained quickly. Higher photosynthetic rate for seedlings planted without Terra-Sorb could be American chestnut's adaptation to and preference for xeric sites.

Soil Sterility

Survival

Mine Site

The inclusion of the non-sterilized native forest soil treatment increased survival in comparison with sterilized forest soil treatment. With the detrimental effects of fertilizer early in seedling establishment and survival, it is likely that when available, seedlings could have relied on non-sterilized soil for increased nutrient availability through mycorrhizae. Since mine sites are often characterized as being deficient in growth-limiting nutrients (Rodrigue & Burger, 2004), adding the sterile soil treatment provides less long-term nourishment. The inclusion of native forest non-sterilized topsoil to the planting substrate could inoculate the substrate with mycorrhizae, earthworms, organic matter, and micro-organisms with which to break down that organic matter, stimulating growth (Lunt & Hedger, 2003; Preve, et al., 1984). With presence of these organisms that break down organic nutrients and build a humus layer, greater survival (Herendeen, 2007) can be expected.

Quarry

The sterile soil treatment greatly reduced seedling survival, while marginally reducing seed survival. When nursery seedlings are directly planted into a substrate differing from that of the nursery, transplant shock can occur (Close, Beadle, & Brown, 2005). Direct seeded trees were not greenhouse-grown so their survival was less affected when compared with the bare-root seedlings.

Growth

Mine Site

No effects of soil sterility were found in first or second year primary or secondary growth. Therefore, it seems that non-sterilized native forest soil is more important for direct-seeded tree emergence and survival than it is for tree growth on mine sites.

Quarry

Only seed growth was influenced by the main effect of the sterile soil treatment. When present, the non-sterilized soil treatment reduced natural height of trees. American chestnut shows high growth on xeric, nutrient poor sites (Ashe, 1912; Herendeen, 2007), and may not be able to make use of abundant nitrogen, similar to other species of Fagaceae (Decker & Boerner, 1997). Therefore, the addition of forest soil may be less advantageous when nutrient availability is likely greater than that of a mine site and when fine particles are present.

Physiological Response

Mine Site

The increase in photosynthetic rate by presence of the sterile soil treatment may relate to American chestnut's adaptation to lowered nutrient availability on xeric sites. This finding is supported by Herendeen (2007), in which chestnut growth performance was best on nutrient poor sites. However, growth parameter results in this experiment were not supported by increased photosynthetic rate.

Quarry

Native forest soil increased photosynthetic rate, likely due to higher nutrient availabilities provided by the forest soil, and the nursery-grown tree’s acclimation to high nutrient availability.

CONCLUSION

In order for successful reforestation on mine sites, potential site characteristics must first be understood. As the results of this study suggest, fertilizer, hydrophilic root polymers and soil sterility produce varying effects on dissimilar sites. Further, seeds and nursery-grown seedlings perform differently under different conditions.

In this study, it appears that American chestnut survival is greatest on sites in which rodent herbivory can be avoided and excessive temperatures are not reached due to unvented tree shelters. Further, greater survival rates in American chestnut can be expected in trees under bare-root plantings rather than those direct-seeded in sites with low coarse fragment content. However, this trend which was experienced at the quarry will likely extend into mine site applications.

Overall, slow-release fertilizer application showed positive and negative effects on seedlings. Fertilizer presence reduced survival in directly-seeded trees on both the mine site and quarry. Nursery-grown seedlings at the quarry were relatively uninfluenced by fertilizer, likely as a result of being grown in fertile soil before being out-planted. However, after emergence and survival, trees planted with fertilizer generally accumulated more biomass than those without. Fertilizer amendments showed no effect on direct-seeded tree photosynthetic rate at the mine site and quarry. However, there was an increase in rate of transpiration in fertilizer presence at the mine site, likely due to nutrient mass flow from soil to roots which can regulate transpiration rate. Bare-root seedlings experienced a higher photosynthetic rate at the quarry, likely due to acclimation to the nutrient-rich soils in which they were grown. Curiously, this same effect was not reproduced at the greenhouse site.

Similarly with fertilizer, the gel-based hydrophilic polymer Terra-Sorb used in this study produced both positive and negative effects on American chestnut performance. Survival in seeds planted at the mine site was reduced in presence of Terra-Sorb. However, this was likely due to inadequate hydration of the gel before planting, which uplifted many seeds out of the ground when hydration was achieved in the soil. At the quarry, seeds experienced much higher survival rates when in the presence of Terra-Sorb. This trend was not reflected in the nursery-grown seedlings, which may be a result of a higher initial moisture demand at the time of leaf expansion, and higher root surface to gel volume ratio than the seeds. Growth responses to Terra-Sorb also varied among sites. At the mine site, both height and RCD were significantly higher in the first year with the presence of Terra-Sorb. In contrast to the mine site, chestnut growth at the quarry was insignificantly affected by Terra-Sorb presence. It is likely that after the initial role Terra-Sorb played on emergence and survival at the quarry, the high amount of rainfall combined with the probable low infiltration rates and high clay content kept the ground relatively saturated, evidenced by standing water which was often present on the site. Therefore, saturated conditions likely negated any effect Terra-Sorb might have had. The insignificant effect of Terra-Sorb on tree growth at the greenhouse is likely also due to the previously stated high root to gel volume. Differences in photosynthetic and transpiration rate were absent with Terra-Sorb main effects at the mine site and quarry, but present at the greenhouse where Terra-Sorb lowered photosynthetic rate. This was likely due the low volume of soil for moisture retention, and American chestnut's adaptation to xeric sites. Terra-Sorb produced a less negative water potential at the mine site, likely correlating with the increased growth. Absence of physiological differences from Terra-Sorb at the quarry could also be due to the high rainfall experienced during the growing season.

At both the mine site and quarry, the non-sterile native forest soil increased survival when compared to the sterile soil treatment. In a system already described as nutrient limiting, inclusion of sterilized soil on a mine site will not provide support for seedling establishment shown by non-sterilized soil. However, no effects of soil sterility were found on growth at the mine site. Therefore, it seems that on mine sites, non-sterilized native forest soil is more important for direct-seeded tree emergence and survival than it is on growth. This trend was followed by bare-root seedlings at the quarry, but was not followed by directly-seeded trees at the quarry. At the quarry, seed growth was positively influenced by presence of the sterile soil treatment. The quarry had an uncharacteristically high amount of clay for “spoil”, and therefore likely had a higher cation exchange capacity which leads to increased nutrient retention and fertility. Since American chestnut is adapted to nutrient poor sites, the addition of sterilized soil to the planting medium may be a viable amendment when nutrient availability is likely higher than that of a mine site and when amount of clay is substantially larger. In accordance, photosynthetic rate was higher in the presence of the sterile soil treatment at the mine site. At the quarry, however, non-sterilized forest soil was more important for a higher photosynthetic rate, likely due to the tree’s adaptation to the high nutrient availability in the greenhouse conditions in which they were grown.

IMPLICATIONS

Results of this study can prove beneficial to reclamation specialists with an interest in using mine site reclamation in conjunction with American chestnut restoration. Understanding how American chestnut performs physiologically on mine sites and similar sites will aid in the restoration of this species. These sites have shown particular promise in providing suitable habitat for this species and may be potential vectors for chestnut dispersal in the future. It is recommended that when planting American chestnuts on mine sites, importance should be placed on sites which are well drained but receive no less than moderate amounts of rainfall. Survival in trees planted as bare-root nursery-grown seedlings is greater than in those direct-seeded. Further, a slow-release fertilizer similar to the one used in this experiment is likely to decrease initial survival in direct-seeded trees, but increase growth in both bare-root and directly-seeded trees. On especially dry sites such as mine sites, fertilizer can create a more negative water potential leading to water stress in direct-seeded trees. Increased seed survival, growth, and water status can be accomplished through the use of Terra-Sorb, but only in direct-seeded trees on sites with potentially deficient plant-available water. Native forest soil can increase survival in both directly-seeded and bare-root planted trees. However, more research is needed on the benefits of native forest soil, as growth and physiological results conflict. In order to better understand the effects of native forest topsoil, the factors which potentially influence these results such as benefits and components of soil biota must be further explored in order to verify which component of soil is more important. Lastly, this study suggests that site characterization should be performed prior to selection of planting treatments. Knowledge of micro-site components such as infiltration rate, moisture and nutrient retention, coarse fragment content and soil chemistry would be helpful in selection of potential sites to be used in American chestnut plantings.

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APPENDIX

Zeb Survival 2008 (T-Test)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fert	4	.2310	.06395	.03198
No Fert	4	.3387	.16004	.08002

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fert	7.224	3	.005	.23100	.1292	.3328
No Fert	4.233	3	.024	.33875	.0841	.5934

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Terra	4	.2732	.08162	.04081
No Terra	4	.3042	.14927	.07464

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Terra	6.695	3	.007	.27325	.1434	.4031
No Terra	4.076	3	.027	.30425	.0667	.5418

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Sterile	4	.254	.0810	.0405
Non-Sterile	4	.320	.1370	.0685

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper

Sterile	6.273	3	.008	.2540	.125	.383
Non-Sterile	4.668	3	.019	.3198	.102	.538

Zeb Survival 2009 (T-Test)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fert	4	.24450	.065067	.032533
No Fert	4	.3180	.10827	.05413

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fert	7.515	3	.005	.244500	.14096	.34804
No Fert	5.874	3	.010	.31800	.1457	.4903

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Terra	4	.28075	.073622	.036811
No Terra	4	.29100	.094435	.047218

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Terra	7.627	3	.005	.280750	.16360	.39790
No Terra	6.163	3	.009	.291000	.14073	.44127

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Sterile	4	.26650	.051410	.025705
Non-Sterile	4	.3030	.10462	.05231

One-Sample Test

Test Value = 0

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Sterile	10.368	3	.002	.266500	.18470	.34830
Non-Sterile	5.793	3	.010	.30300	.1365	.4695

Zeb Height Growth 2008

Between-Subjects Factors

		N
Sterile	1	45
	2	54
Terrasorb	1	46
	2	53
Fert	1	39
	2	60
Block	1	20
	2	21
	3	35
	4	23

Tests of Between-Subjects Effects

Dependent Variable:Height

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	129416.603	1	129416.603	543.325	.000
	Error	862.707	3.622	238.194[a]		
Sterile	Hypothesis	61.706	1	61.706	.236	.629
	Error	23036.539	88	261.779[b]		
Terrasorb	Hypothesis	2587.303	1	2587.303	9.884	.002
	Error	23036.539	88	261.779[b]		
Fert	Hypothesis	9428.858	1	9428.858	36.018	.000
	Error	23036.539	88	261.779[b]		
Block	Hypothesis	708.267	3	236.089	.902	.444

	Error	23036.539	88	261.779[b]		
Sterile * Terrasorb * Fert	Hypothesis	70.089	1	70.089	.268	.606
	Error	23036.539	88	261.779[b]		
Sterile * Terrasorb	Hypothesis	360.962	1	360.962	1.379	.243
	Error	23036.539	88	261.779[b]		
Sterile * Fert	Hypothesis	89.599	1	89.599	.342	.560
	Error	23036.539	88	261.779[b]		
Terrasorb * Fert	Hypothesis	634.333	1	634.333	2.423	.123
	Error	23036.539	88	261.779[b]		

a. .918 MS(Block) + .082 MS(Error)

b. MS(Error)

Zeb Natural Height Growth 2009

Between-Subjects Factors

		N
Fert	1	43
	2	60
Terrasorb	1	48
	2	55
Sterile	1	49
	2	54
Block	1	19
	2	28
	3	31
	4	25

Tests of Between-Subjects Effects

Dependent Variable:Height

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	382498.704	1	382498.704	293.921	.000
	Error	4037.994	3.103	1301.368[a]		
Fert	Hypothesis	6075.028	1	6075.028	8.243	.005

	Error	67801.252	92	736.970[b]		
Terrasorb	Hypothesis	4856.328	1	4856.328	6.590	.012
	Error	67801.252	92	736.970[b]		
Sterile	Hypothesis	618.767	1	618.767	.840	.362
	Error	67801.252	92	736.970[b]		
Block	Hypothesis	3955.626	3	1318.542	1.789	.155
	Error	67801.252	92	736.970[b]		
Fert * Terrasorb * Sterile	Hypothesis	841.385	1	841.385	1.142	.288
	Error	67801.252	92	736.970[b]		
Fert * Terrasorb	Hypothesis	952.659	1	952.659	1.293	.259
	Error	67801.252	92	736.970[b]		
Fert * Sterile	Hypothesis	92.036	1	92.036	.125	.725
	Error	67801.252	92	736.970[b]		
Terrasorb * Sterile	Hypothesis	3.222	1	3.222	.004	.947
	Error	67801.252	92	736.970[b]		

a. .970 MS(Block) + .030 MS(Error)

b. MS(Error)

Zeb RCD Growth 2008

Between-Subjects Factors

		N
Sterile	1	45
	2	54
Terrasorb	1	46
	2	53
Fert	1	39
	2	60
Block	1	20
	2	21
	3	35
	4	23

Tests of Between-Subjects Effects

Dependent Variable:RCD

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1860.305	1	1860.305	370.802	.000
	Error	17.699	3.528	5.017[a]		
Sterile	Hypothesis	1.244	1	1.244	.261	.611
	Error	420.098	88	4.774[b]		
Terrasorb	Hypothesis	56.168	1	56.168	11.766	.001
	Error	420.098	88	4.774[b]		
Fert	Hypothesis	140.499	1	140.499	29.431	.000
	Error	420.098	88	4.774[b]		
Block	Hypothesis	15.116	3	5.039	1.055	.372
	Error	420.098	88	4.774[b]		
Sterile * Terrasorb * Fert	Hypothesis	4.187	1	4.187	.877	.352
	Error	420.098	88	4.774[b]		
Sterile * Terrasorb	Hypothesis	7.571	1	7.571	1.586	.211
	Error	420.098	88	4.774[b]		
Sterile * Fert	Hypothesis	4.030	1	4.030	.844	.361
	Error	420.098	88	4.774[b]		
Terrasorb * Fert	Hypothesis	2.795	1	2.795	.585	.446
	Error	420.098	88	4.774[b]		

a. .918 MS(Block) + .082 MS(Error)

b. MS(Error)

Zeb RCD Growth 2009

Between-Subjects Factors

		N
Fert	1	42
	2	58
Terrasorb	1	47
	2	53
Sterile	1	48

	2	52
Block	1	19
	2	28
	3	30
	4	23

Tests of Between-Subjects Effects

Dependent Variable:RCD

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	6047.686	1	6047.686	105.596	.002
	Error	173.544	3.030	57.272[a]		
Fert	Hypothesis	46.557	1	46.557	3.088	.082
	Error	1341.976	89	15.078[b]		
Terrasorb	Hypothesis	.049	1	.049	.003	.955
	Error	1341.976	89	15.078[b]		
Sterile	Hypothesis	17.489	1	17.489	1.160	.284
	Error	1341.976	89	15.078[b]		
Block	Hypothesis	174.262	3	58.087	3.852	.012
	Error	1341.976	89	15.078[b]		
Fert * Terrasorb * Sterile	Hypothesis	19.546	1	19.546	1.296	.258
	Error	1341.976	89	15.078[b]		
Fert * Terrasorb	Hypothesis	2.603	1	2.603	.173	.679
	Error	1341.976	89	15.078[b]		
Fert * Sterile	Hypothesis	21.851	1	21.851	1.449	.232
	Error	1341.976	89	15.078[b]		
Terrasorb * Sterile	Hypothesis	.474	1	.474	.031	.860
	Error	1341.976	89	15.078[b]		

a. .981 MS(Block) + .019 MS(Error)

b. MS(Error)

Zeb Photosynthetic Rate

Between-Subjects Factors

		N
Sterile	1	37
	2	57
Terrasorb	1	48
	2	46
Fert	1	41
	2	53
Block	1	18
	2	27
	3	28
	4	21

Tests of Between-Subjects Effects

Dependent Variable:Photo

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3704.314	1	3704.314	90.160	.002
	Error	129.858	3.161	41.086[a]		
Sterile	Hypothesis	72.095	1	72.095	5.929	.017
	Error	1009.186	83	12.159[b]		
Terrasorb	Hypothesis	1.701	1	1.701	.140	.709
	Error	1009.186	83	12.159[b]		
Fert	Hypothesis	9.359	1	9.359	.770	.383
	Error	1009.186	83	12.159[b]		
Block	Hypothesis	131.530	3	43.843	3.606	.017
	Error	1009.186	83	12.159[b]		
Sterile * Terrasorb * Fert	Hypothesis	14.351	1	14.351	1.180	.280
	Error	1009.186	83	12.159[b]		
Sterile * Terrasorb	Hypothesis	83.572	1	83.572	6.873	.010
	Error	1009.186	83	12.159[b]		
Sterile * Fert	Hypothesis	16.584	1	16.584	1.364	.246
	Error	1009.186	83	12.159[b]		

Terrasorb * Fert	Hypothesis	28.159	1	28.159	2.316	.132
	Error	1009.186	83	12.159[b]		

a. .913 MS(Block) + .087 MS(Error)

b. MS(Error)

Zeb Transpiration Rate

Between-Subjects Factors

		N
Sterile	1	37
	2	57
Terrasorb	1	48
	2	46
Fert	1	41
	2	53
Block	1	18
	2	27
	3	28
	4	21

Tests of Between-Subjects Effects

Dependent Variable:TR

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	172.923	1	172.923	5.202	.107
	Error	100.127	3.012	33.243[a]		
Sterile	Hypothesis	.152	1	.152	.200	.656
	Error	62.990	83	.759[b]		
Terrasorb	Hypothesis	.594	1	.594	.782	.379
	Error	62.990	83	.759[b]		
Fert	Hypothesis	12.670	1	12.670	16.695	.000
	Error	62.990	83	.759[b]		
Block	Hypothesis	109.019	3	36.340	47.883	.000
	Error	62.990	83	.759[b]		

Sterile * Terrasorb * Fert	Hypothesis	.505	1	.505	.665	.417
	Error	62.990	83	.759[b]		
Sterile * Terrasorb	Hypothesis	.096	1	.096	.126	.723
	Error	62.990	83	.759[b]		
Sterile * Fert	Hypothesis	4.011	1	4.011	5.285	.024
	Error	62.990	83	.759[b]		
Terrasorb * Fert	Hypothesis	1.446	1	1.446	1.905	.171
	Error	62.990	83	.759[b]		

a. .913 MS(Block) + .087 MS(Error)

Zeb Water Potential

Between-Subjects Factors

		N
Sterile	1	31
	2	47
Fert	1	32
	2	46
Terrasorb	1	42
	2	36
Block	1	15
	2	20
	3	25
	4	18

Tests of Between-Subjects Effects

Dependent Variable:Bars

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1926.657	1	1926.657	160.485	.001
	Error	37.491	3.123	12.005[a]		
Sterile	Hypothesis	10.648	1	10.648	5.431	.023
	Error	131.361	67	1.961[b]		
Fert	Hypothesis	12.163	1	12.163	6.204	.015

	Error	131.361	67	1.961[b]		
Terrasorb	Hypothesis	10.898	1	10.898	5.558	.021
	Error	131.361	67	1.961[b]		
Block	Hypothesis	40.193	3	13.398	6.833	.000
	Error	131.361	67	1.961[b]		
Sterile * Fert * Terrasorb	Hypothesis	15.341	1	15.341	7.824	.007
	Error	131.361	67	1.961[b]		
Sterile * Fert	Hypothesis	38.532	1	38.532	19.653	.000
	Error	131.361	67	1.961[b]		
Sterile * Terrasorb	Hypothesis	6.464	1	6.464	3.297	.074
	Error	131.361	67	1.961[b]		
Fert * Terrasorb	Hypothesis	.823	1	.823	.420	.519
	Error	131.361	67	1.961[b]		

a. .878 MS(Block) + .122 MS(Error)

b. MS(Error)

Quarry Seedling Survival (T-Test)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fert	3	.69967	.045059	.026015
No Fert	3	.70600	.183388	.105879

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fert	26.895	2	.001	.699667	.58773	.81160
No Fert	6.668	2	.022	.706000	.25044	1.16156

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Terra	3	.60567	.083345	.048119
No Terra	3	.77000	.091099	.052596

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Terra	12.587	2	.006	.605667	.39863	.81271
No Terra	14.640	2	.005	.770000	.54370	.99630

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Sterile	3	.66267	.002887	.001667
Non-Sterile	3	.74333	.099972	.057719

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Sterile	397.600	2	.000	.662667	.65550	.66984
Non-Sterile	12.879	2	.006	.743333	.49499	.99168

Quarry directly-seeded tree survival (T-Test)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Fert	3	.28233	.076107	.043941
No Fert	3	.57067	.088546	.051122

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Fert	6.425	2	.023	.282333	.09327	.47139
No Fert	11.163	2	.008	.570667	.35071	.79063

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean

Terra	3	.42467	.116895	.067489
No Terra	3	.37433	.169924	.098106

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Terra	6.292	2	.024	.424667	.13428	.71505
No Terra	3.816	2	.062	.374333	-.04778	.79645

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Sterile	3	.40233	.038940	.022482
Non-Sterile	3	.40800	.079775	.046058

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Sterile	17.896	2	.003	.402333	.30560	.49907
Non-Sterile	8.858	2	.013	.408000	.20983	.60617

Quarry Seedling Natural Height

Between-Subjects Factors

		N
Sterile	1	112
	2	112
Terrasorb	1	86
	2	138
Fert	1	113
	2	111
Block	1	75
	2	80
	3	69

Tests of Between-Subjects Effects

Dependent Variable:Height Growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	481.147	1	481.147	29.960	.000
	Error	1127.942	70.234	16.060[a]		
Sterile	Hypothesis	113.083	1	113.083	1.139	.287
	Error	21255.301	214	99.324[b]		
Terrasorb	Hypothesis	227.575	1	227.575	2.291	.132
	Error	21255.301	214	99.324[b]		
Fert	Hypothesis	195.444	1	195.444	1.968	.162
	Error	21255.301	214	99.324[b]		
Block	Hypothesis	5.486	2	2.743	.028	.973
	Error	21255.301	214	99.324[b]		
Sterile * Terrasorb * Fert	Hypothesis	18.665	1	18.665	.188	.665
	Error	21255.301	214	99.324[b]		
Sterile * Terrasorb	Hypothesis	110.381	1	110.381	1.111	.293
	Error	21255.301	214	99.324[b]		
Sterile * Fert	Hypothesis	8.622	1	8.622	.087	.769
	Error	21255.301	214	99.324[b]		
Terrasorb * Fert	Hypothesis	.055	1	.055	.001	.981
	Error	21255.301	214	99.324[b]		

a. .862 MS(Block) + .138 MS(Error)

b. MS(Error)

Quarry Seedling RCD

Between-Subjects Factors

		N
Sterile	1	114
	2	118
Terrasorb	1	85
	2	147

Fert	1	117
	2	115
Block	1	83
	2	80
	3	69

Tests of Between-Subjects Effects

Dependent Variable:RCD Growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	2.970	1	2.970	.711	.475
	Error	9.964	2.386	4.177[a]		
Sterile	Hypothesis	.859	1	.859	.353	.553
	Error	541.021	222	2.437[b]		
Terrasorb	Hypothesis	4.346E-5	1	4.346E-5	.000	.997
	Error	541.021	222	2.437[b]		
Fert	Hypothesis	.219	1	.219	.090	.764
	Error	541.021	222	2.437[b]		
Block	Hypothesis	8.942	2	4.471	1.835	.162
	Error	541.021	222	2.437[b]		
Sterile * Terrasorb * Fert	Hypothesis	3.575	1	3.575	1.467	.227
	Error	541.021	222	2.437[b]		
Sterile * Terrasorb	Hypothesis	5.180	1	5.180	2.125	.146
	Error	541.021	222	2.437[b]		
Sterile * Fert	Hypothesis	.079	1	.079	.033	.857
	Error	541.021	222	2.437[b]		
Terrasorb * Fert	Hypothesis	7.079	1	7.079	2.905	.090
	Error	541.021	222	2.437[b]		

a. .855 MS(Block) + .145 MS(Error)

b. MS(Error)

Quarry Seedling Apical Meristem Elongation

Between-Subjects Factors

		N

Sterile	1	100
	2	111
Terrasorb	1	74
	2	137
Fert	1	108
	2	103
Block	1	81
	2	71
	3	59

Tests of Between-Subjects Effects

Dependent Variable:Apical meristem length

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	23330.232	1	23330.232	3061.667	.000
	Error	386.260	50.690	7.620[a]		
Sterile	Hypothesis	50.093	1	50.093	1.467	.227
	Error	6861.812	201	34.138[b]		
Terrasorb	Hypothesis	4.531	1	4.531	.133	.716
	Error	6861.812	201	34.138[b]		
Fert	Hypothesis	838.750	1	838.750	24.569	.000
	Error	6861.812	201	34.138[b]		
Block	Hypothesis	3.377	2	1.688	.049	.952
	Error	6861.812	201	34.138[b]		
Sterile * Terrasorb * Fert	Hypothesis	1.334	1	1.334	.039	.843
	Error	6861.812	201	34.138[b]		
Sterile * Terrasorb	Hypothesis	20.429	1	20.429	.598	.440
	Error	6861.812	201	34.138[b]		
Sterile * Fert	Hypothesis	12.245	1	12.245	.359	.550
	Error	6861.812	201	34.138[b]		
Terrasorb * Fert	Hypothesis	4.642	1	4.642	.136	.713
	Error	6861.812	201	34.138[b]		

Between-Subjects Factors

		N
Sterile	1	100
	2	111
Terrasorb	1	74
	2	137
Fert	1	108
	2	103
Block	1	81
	2	71

a. .817 MS(Block) + .183 MS(Error)

b. MS(Error)

Quarry Directly-Seeded Tree Height

Between-Subjects Factors

		N
Sterile	1	64
	2	68
Terrasorb	1	61
	2	71
Fert	1	55
	2	77
Block	1	44
	2	38
	3	50

Tests of Between-Subjects Effects

Dependent Variable:Natural Height

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	24387.922	1	24387.922	106.571	.008
	Error	477.035	2.085	228.843[a]		

Sterile	Hypothesis	588.593	1	588.593	8.848	.004
	Error	8116.215	122	66.526[b]		
Terrasorb	Hypothesis	33.024	1	33.024	.496	.482
	Error	8116.215	122	66.526[b]		
Fert	Hypothesis	5.095	1	5.095	.077	.782
	Error	8116.215	122	66.526[b]		
Block	Hypothesis	482.308	2	241.154	3.625	.030
	Error	8116.215	122	66.526[b]		
Sterile * Terrasorb * Fert	Hypothesis	62.861	1	62.861	.945	.333
	Error	8116.215	122	66.526[b]		
Sterile * Terrasorb	Hypothesis	13.243	1	13.243	.199	.656
	Error	8116.215	122	66.526[b]		
Sterile * Fert	Hypothesis	525.651	1	525.651	7.901	.006
	Error	8116.215	122	66.526[b]		
Terrasorb * Fert	Hypothesis	2.415	1	2.415	.036	.849
	Error	8116.215	122	66.526[b]		

a. .929 MS(Block) + .071 MS(Error)

b. MS(Error)

Quarry Directly-Seeded Tree RCD

Between-Subjects Factors

		N
Sterile	1	63
	2	68
Terrasorb	1	61
	2	70
Fert	1	54
	2	77
Block	1	43
	2	38
	3	50

Tests of Between-Subjects Effects

Dependent Variable:RCD

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	257.278	1	257.278	252.673	.003
	Error	2.119	2.082	1.018[a]		
Sterile	Hypothesis	1.173	1	1.173	3.691	.057
	Error	38.465	121	.318[b]		
Terrasorb	Hypothesis	.801	1	.801	2.518	.115
	Error	38.465	121	.318[b]		
Fert	Hypothesis	.052	1	.052	.164	.687
	Error	38.465	121	.318[b]		
Block	Hypothesis	2.131	2	1.066	3.352	.038
	Error	38.465	121	.318[b]		
Sterile * Terrasorb * Fert	Hypothesis	1.876	1	1.876	5.901	.017
	Error	38.465	121	.318[b]		
Sterile * Terrasorb	Hypothesis	.021	1	.021	.066	.797
	Error	38.465	121	.318[b]		
Sterile * Fert	Hypothesis	1.559	1	1.559	4.904	.029
	Error	38.465	121	.318[b]		
Terrasorb * Fert	Hypothesis	.046	1	.046	.146	.703
	Error	38.465	121	.318[b]		

a. .937 MS(Block) + .063 MS(Error)

b. MS(Error)

Quarry Photosynthetic Rate (time covariate)

Between-Subjects Factors

		N
Sterile	1	79
	2	89
Terrasorb	1	75
	2	93
Fert	1	78
	2	90

Block	1	65
	2	63
	3	40

Tests of Between-Subjects Effects

Dependent Variable:Photo

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	72.729	1	72.729	5.708	.018
	Error	2023.703	158.825	12.742[a]		
Sterile	Hypothesis	68.136	1	68.136	5.396	.021
	Error	1982.321	157	12.626[b]		
Terrasorb	Hypothesis	3.544	1	3.544	.281	.597
	Error	1982.321	157	12.626[b]		
Fert	Hypothesis	300.171	1	300.171	23.774	.000
	Error	1982.321	157	12.626[b]		
Block	Hypothesis	56.966	2	28.483	2.256	.108
	Error	1982.321	157	12.626[b]		
Time	Hypothesis	302.108	1	302.108	23.927	.000
	Error	1982.321	157	12.626[b]		
Sterile * Terrasorb * Fert	Hypothesis	.398	1	.398	.032	.859
	Error	1982.321	157	12.626[b]		
Sterile * Terrasorb	Hypothesis	3.659	1	3.659	.290	.591
	Error	1982.321	157	12.626[b]		
Sterile * Fert	Hypothesis	46.799	1	46.799	3.706	.056
	Error	1982.321	157	12.626[b]		
Terrasorb * Fert	Hypothesis	193.648	1	193.648	15.337	.000
	Error	1982.321	157	12.626[b]		

a. .007 MS(Block) + .993 MS(Error)

b. MS(Error)

Quarry Transpiration Rate (time covariate)

Between-Subjects Factors

		N
Sterile	1	77
	2	86
Terrasorb	1	74
	2	89
Fert	1	77
	2	86
Block	1	60
	2	63
	3	40

Tests of Between-Subjects Effects

Dependent Variable:TR

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	117.806	1	117.806	81.120	.000
	Error	25.779	17.751	1.452[a]		
Sterile	Hypothesis	1.178	1	1.178	1.197	.276
	Error	149.635	152	.984[b]		
Terrasorb	Hypothesis	.280	1	.280	.285	.594
	Error	149.635	152	.984[b]		
Fert	Hypothesis	.121	1	.121	.123	.726
	Error	149.635	152	.984[b]		
Block	Hypothesis	141.812	2	70.906	72.027	.000
	Error	149.635	152	.984[b]		
Time	Hypothesis	79.411	1	79.411	80.666	.000
	Error	149.635	152	.984[b]		
Sterile * Terrasorb * Fert	Hypothesis	.404	1	.404	.410	.523
	Error	149.635	152	.984[b]		
Sterile * Terrasorb	Hypothesis	.020	1	.020	.020	.887
	Error	149.635	152	.984[b]		
Sterile * Fert	Hypothesis	1.774	1	1.774	1.802	.181

	Error	149.635	152	.984[b]		
Terrasorb * Fert	Hypothesis	1.121	1	1.121	1.139	.288
	Error	149.635	152	.984[b]		

a. .007 MS(Block) + .993 MS(Error)

b. MS(Error)

Quarry Water Potential

Between-Subjects Factors

		N
Sterile	1	98
	2	120
Terrasorb	1	82
	2	136
Fert	1	91
	2	127
Block	1	81
	2	70
	3	67

Tests of Between-Subjects Effects

Dependent Variable:WP

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3301.769	1	3301.769	78.570	.012
	Error	84.528	2.011	42.023[a]		
Sterile	Hypothesis	1.373	1	1.373	1.161	.282
	Error	245.923	208	1.182[b]		
Terrasorb	Hypothesis	.759	1	.759	.642	.424
	Error	245.923	208	1.182[b]		
Fert	Hypothesis	22.824	1	22.824	19.304	.000
	Error	245.923	208	1.182[b]		
Block	Hypothesis	93.265	2	46.633	39.442	.000
	Error	245.923	208	1.182[b]		

Sterile * Terrasorb * Fert	Hypothesis	8.798	1	8.798	7.441	.007
	Error	245.923	208	1.182[b]		
Sterile * Terrasorb	Hypothesis	2.014	1	2.014	1.704	.193
	Error	245.923	208	1.182[b]		
Sterile * Fert	Hypothesis	2.686	1	2.686	2.272	.133
	Error	245.923	208	1.182[b]		
Terrasorb * Fert	Hypothesis	4.000	1	4.000	3.383	.067
	Error	245.923	208	1.182[b]		

a. .899 MS(Block) + .101 MS(Error)

b. MS(Error)

Greenhouse Natural Height

Between-Subjects Factors

		N
Fert	1	20
	2	20
Terrasorb	1	20
	2	20
Table	1	10
	2	10
	3	10
	4	10

Tests of Between-Subjects Effects

Dependent Variable:Natural height growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	814.506	1	814.506	17.897	.030
	Error	122.999	2.703	45.510[a]		
Fert	Hypothesis	20.089	1	20.089	.590	.448
	Error	1123.786	33	34.054[b]		
Terrasorb	Hypothesis	281.042	1	281.042	8.253	.007
	Error	1123.786	33	34.054[b]		

Table	Hypothesis	134.239	3	44.746	1.314	.286
	Error	1123.786	33	34.054[b]		
Fert * Terrasorb	Hypothesis	107.336	1	107.336	3.152	.085
	Error	1123.786	33	34.054[b]		

a. 1.071 MS(Table) - .071 MS(Error)

b. MS(Error)

Greenhouse RCD

Between-Subjects Factors

		N
Terrasorb	1	19
	2	20
Fert	1	19
	2	20
Table	1	10
	2	9
	3	10
	4	10

Tests of Between-Subjects Effects

Dependent Variable:Root collar growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	42.304	1	42.304	7.599	.074
	Error	15.972	2.869	5.567[a]		
Terrasorb	Hypothesis	2.667	1	2.667	1.621	.212
	Error	52.657	32	1.646[b]		
Fert	Hypothesis	.230	1	.230	.140	.711
	Error	52.657	32	1.646[b]		
Table	Hypothesis	15.867	3	5.289	3.214	.036
	Error	52.657	32	1.646[b]		
Terrasorb * Fert	Hypothesis	.049	1	.049	.030	.864

	Error	52.657	32	1.646[b]		

a. 1.076 MS(Table) - .076 MS(Error)

b. MS(Error)

Greenhouse Apical Meristem Elongation

Between-Subjects Factors

		N
Fert	1	20
	2	20
Terrasorb	1	20
	2	20
Table	1	10
	2	10
	3	10
	4	10

Tests of Between-Subjects Effects

Dependent Variable:New Growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	15820.506	1	15820.506	571.451	.001
	Error	67.950	2.454	27.685[a]		
Fert	Hypothesis	291.512	1	291.512	7.155	.012
	Error	1344.487	33	40.742[b]		
Terrasorb	Hypothesis	.017	1	.017	.000	.984
	Error	1344.487	33	40.742[b]		
Table	Hypothesis	85.666	3	28.555	.701	.558
	Error	1344.487	33	40.742[b]		
Fert * Terrasorb	Hypothesis	170.249	1	170.249	4.179	.049
	Error	1344.487	33	40.742[b]		

a. 1.071 MS(Table) - .071 MS(Error)

Greenhouse Photosynthetic Rate (time covariate)

Between-Subjects Factors

		N
Terrasorb	1	18
	2	20
Fert	1	20
	2	18
Table	1	10
	2	10
	3	10
	4	8

Tests of Between-Subjects Effects

Dependent Variable:Photo

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	50.637	1	50.637	16.037	.000
	Error	95.159	30.137	3.158[a]		
Terrasorb	Hypothesis	16.190	1	16.190	5.137	.031
	Error	94.541	30	3.151[b]		
Fert	Hypothesis	10.866	1	10.866	3.448	.073
	Error	94.541	30	3.151[b]		
Table	Hypothesis	65.736	3	21.912	6.953	.001
	Error	94.541	30	3.151[b]		
HHMMSS	Hypothesis	61.316	1	61.316	19.457	.000
	Error	94.541	30	3.151[b]		
Terrasorb * Fert	Hypothesis	2.098	1	2.098	.666	.421
	Error	94.541	30	3.151[b]		

a. .000 MS(Table) + 1.000 MS(Error)

b. MS(Error)

Greenhouse Transpiration Rate (time covariate)

Between-Subjects Factors

		N

Terrasorb	1	20
	2	20
Fert	1	20
	2	20
Table	1	10
	2	10
	3	10
	4	10

Tests of Between-Subjects Effects

Dependent Variable:TR

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	91.492	1	91.492	7.178	.012
	Error	409.504	32.130	12.745[a]		
Terrasorb	Hypothesis	18.634	1	18.634	1.464	.235
	Error	407.188	32	12.725[b]		
Fert	Hypothesis	6.992	1	6.992	.549	.464
	Error	407.188	32	12.725[b]		
Table	Hypothesis	186.954	3	62.318	4.897	.007
	Error	407.188	32	12.725[b]		
HHMMSS	Hypothesis	109.017	1	109.017	8.567	.006
	Error	407.188	32	12.725[b]		
Terrasorb * Fert	Hypothesis	.334	1	.334	.026	.872
	Error	407.188	32	12.725[b]		

a. .000 MS(Table) + 1.000 MS(Error)

b. MS(Error)

Greenhouse Water Potential

Between-Subjects Factors

		N
Fert	1	20

	2	20
Terrasorb	1	20
	2	20
Table	1	10
	2	10
	3	10
	4	10

Tests of Between-Subjects Effects

Dependent Variable:bars

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	197.580	1	197.580	76.451	.003
	Error	7.654	2.962	2.584[a]		
Fert	Hypothesis	.837	1	.837	3.582	.067
	Error	7.714	33	.234[b]		
Terrasorb	Hypothesis	.405	1	.405	1.732	.197
	Error	7.714	33	.234[b]		
Table	Hypothesis	7.283	3	2.428	10.386	.000
	Error	7.714	33	.234[b]		
Fert * Terrasorb	Hypothesis	.357	1	.357	1.528	.225
	Error	7.714	33	.234[b]		

a. 1.071 MS(Table) - .071 MS(Error)

b. MS(Error)

VITA

Christopher Miller was born in Hendersonville, North Carolina. He attended Western Carolina University, where he received his Bachelor of Science degree in Natural Resource Conservation and Management in May, 2008. Chris' previous work experience include technician jobs with the Fish and Wildlife Service in Texas, Bureau of Land Management in Oregon, Coweeta Hydrologic Laboratory in North Carolina, and the Forest Service in Colorado.

In August 2008 he accepted a graduate research assistantship at the University of Tennessee under the advising of Dr. Jennifer Franklin. While at the University of Tennessee, Chris was an active member of the Society of American Foresters and was inducted into the Xi Sigma Pi forestry honor society and the Gamma Sigma Delta agriculture honor society. He received the Master of Science degree in Forestry in May, 2010.