ESTABLISHMENT OF NATIVE PLANT SPECIES FOR LIVESTOCK FORAGE IN SILVOPASTURE SYSTEMS

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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ABSTRACT

Incorporating sustainable agriculture practices and environmental stewardship into existing forage-livestock systems is growing in popularity. This project takes a novel approach incorporating native rather than introduced forage species. Our objectives were to: determine success of establishment of native cool and warm season grasses, and native forb and legume species in two different existing silvopasture systems – pitch pine (*Pinus rigida* Mill.) x loblolly pine (*Pinus taeda L.*) hybrid and eastern American black walnut (*Juglans nigra L.*) – with open pasture as a control, and determine how differences in environmental factors (Photosynthetically Active Radiation (PAR), ambient temperature, and soil moisture) associated with each treatment affect establishment of native plant species. The experiment was a Randomized Complete Block Design replicated in 2020 and 2021. Treatment establishment (3 treatments, 3 replications each) began each fall preceding the growing season by no-till drilling the cool season grasses. All remaining forages were no-till drilled each winter prior to the growing season. Environmental (PAR, ambient temperature, soil water content), plant community composition, herbage accumulation, and forage quality data were collected for treatments during each growing season. There were significant environmental differences among treatments. Additionally, there were significant differences in forage quality and herbage accumulation among treatments for the cool season grass species. Upon completion of the study, there were no differences in stand counts between treatments for all plant functional groups.

CHAPTER 1: LITERATURE REVIEW

Introduction

While technological developments for improvement and increased productivity are commonly viewed as positive among members of the agricultural community, some strategies such as machinery, plant genetics, and pesticides may not necessarily be the best solutions to ensure long-term productivity or sustainability within these systems (Krueger, 1981; Cameron et al., 1991; Burger, 1994). Conversely, these strategies can be conducive to environmental degradation through soil erosion, pollution from synthetic fertilizers, increased pesticide resistance, and decreased biological diversity (Dangerfield and Harwell, 1990; Workman et al., 2003).

Alternative practices exist that can provide the desired increased production levels with less negative impacts such as those listed in the previous paragraph. Agroforestry, which is defined by the European Agroforestry Federations (EURAF) as “the integration of woody vegetation, crops, and/or livestock on the same area of land,” is a type of agroecological practice that may serve as an alternative.

Agroforestry practices encourage biodiversity and promote positive interactions between system components, rather than focus on a monoculture crop supplemented by a continuous stream of external inputs (Matson et al., 1997; Garrett and McGraw, 2000). Silvopasture is one type of agroforestry practice based on the interactions of livestock, timber, and forage crops in the same land area. Thoughtfully planned silvopastoral practices can be productive, economically viable, and contribute to sustainable livestock production (Paciullo et al., 2017; Pontes et al., 2018, Pontes et al., 2016). Successfully integrating the forage plant species and tree species components, where the forage is capable of persistence and maintaining stable yield levels, leads to sustainable silvopasture practices (Barro et al., 2012).

Utilizing native plant species as livestock forage is a growing research topic within environmental agriculture (Kallah et al., 2000; Licitra et al., 1997; Jones, 1995). Non-native plant species can be threatening to forest ecosystems in several ways if they become invasive. Biodiversity, nutrient cycling, and overall forest structure and composition are all factors that can be affected by invasive plant species (Richardson, 1998; Lugo, 2004; Stinson et al., 2007; Kourtev et al., 1998; Ehrenfeld et al., 2001; Ashton et al., 2005). Replacing introduced species with native plant species could reduce the chance of negative impacts on the previously mentioned factors. Therefore, it is important to understand potential challenges faced with reintroducing native species and how they compare to introduced species in terms of herbage accumulation and forage quality.

Use of silvopastoral practices in livestock systems

Across the temperate regions of North America, grazing of livestock and woodlots are two of the most common uses for land unsuitable for traditional crop production (Pent et al., 2021). Approximately 21% of the 256 million and 396 million hectares of forested land in the United States and Canada, respectively, are grazed (Bigelow and Borchers, 2017; NFI, 2013). It only makes sense then that the potentially most frequently used form of agroforestry across the United States and Canada involves the intensive integration of livestock and tree products (Pent et al., 2021).

Garrett et al. (2004) defines silvopasture more specifically as “an intermixture of trees and herbaceous forages managed for livestock grazing in a way that optimizes environmental protection, production, and economic value of the combined tree-forage-livestock system.” There are two common forms of silvopastoralism practiced in North America: integrated forest grazing and silvopastures, with forest grazing being more of an extensively rather than intensively managed system (Pent et al., 2021). This review is primarily focused on silvopastures.

Historical use of silvopasture

Silvopastoral practices are not a new concept. The dehasas in Spain and montados in Portugal serve as some of the oldest agroforestry practice examples, where animals grazed on the vegetation beneath canopies of scattered oaks (Jose and Dollinger, 2019). Modern-day silvopasture differs from these historical practices. Nowadays, it is essential to understand the hierarchy of ecosystem relationships in addition to the realization that the predominant reason for the existence of defined ecosystem boundaries is for convenient management of the system (Garrett and Buck, 1997).

Silvopasture as an agroforestry practice

Silvopasture is one of the six primary agroforestry practices. It consists of planned and intentional interactions between trees, forage crops, and livestock components (Clason and Sharrow, 2000). As silvopasture is an agroforestry practice, it is necessary to meet the four basic principles of agroforestry systems to be deemed successful. These principles are as follows: biologically possible, ecologically sustainable, economically feasible, and socially acceptable (Pent et al., 2021).

Biologically possible. Incorporating silvopastoral practices into existing livestock production systems can reduce climatic variability (Paciullo et al., 2017). There is also the additional value of producing both livestock and tree products simultaneously without increasing the amount of land area in production (Paciullo et al., 2017). The relationships between the components of a silvopasture system are intricate and dynamic; careful consideration should be taken when choosing forage, tree, and animal species as well as tree arrangement to ensure optimal forage yields are obtained (Carvalho et al., 2019; Gomes et al., 2020).

Silvopasture's elevated levels of biological fecundity make these practices an instrumental tool for carbon sequestration to combat global climate change (Montagnin and Nair, 2004). Most terrestrial carbon is stored in the soil (Pent et al., 2021). Both grasslands and forests have the capacity to store carbon, either underground as soil organic matter or within the wood of the trees; because silvopasture is a combination of these two carbon storage contributors, silvopastures have the potential to store more carbon than either individual system (Corre et al., 2000; de Groot, 1990, Haile et al., 2010; Pent et al., 2021).

A meticulously designed and managed silvopasture provides opportunity for a multitude of positive interactions between components. For instance, trees function as a form of shelter for both livestock and forage plants; grazing helps to control weeds and reduce cover for tree pests such as rodents, and livestock contribute to nutrient recycling through urination and defecation (Pent et al., 2021).

Ecologically sustainable. While grazing ruminant livestock can be an excellent way to reduce competition between undesired herbaceous vegetation and trees, mismanagement of grazing can lead to grave consequences, including but not limited to soil degradation and erosion, reduced tree vigor, reduced biodiversity, and overall reductions in ecosystem health (Pent et al., 2021). Because silvopasture in an intensively managed practice designed to produce multiple high-quality products (timber, forage, non-timber forest products, and livestock) the overall environmental impacts are less degrading and more sustainable (Pent et al., 2021).

Economically feasible. Traditional incorporation of pasture-based livestock shade systems is typically cost prohibitive to the producer (St-Pierre et al., 2003). However, the trees in silvopasture can provide shade to livestock while increasing in value as timber products, which can be seen as a more cost-effective benefit to the producer (Pent et al., 2021). Most of the time silvopasture can be more profitable with reduced economic risks compared to stand-alone forest or livestock systems (Pent et al., 2021). The intensive management of grazing, fertilizing, and thinning reduces the amount of time necessary to produce desired timber products, thus speeding up the producer’s return on investment (Pent et al., 2021). The forest and livestock aspects of silvopasture have different inputs, different markets, and few similarities in diseases and pests, making them a lower economic risk to the producer (Pent et al., 2021). Additionally, the reduction in purchased inputs diminishes operating costs and the necessity of outside suppliers, thereby boosting profit margins for both livestock and tree products (Pent et al., 2021). A separate study demonstrated the economic benefits of silvopastoral practices in the southern United States; financial reports showed steady profitability improvement of silvopastures compared to traditional tree plantations or open pastures (Garrett et al., 2004).

Socially acceptable. While aesthetics may be a key factor for silvopasture adoption near urban areas, there are additional contributing factors (Lawrence et al., 1992; Workman et al., 2003; Orefice et al., 2017). Producers with a goal of making a living off silvopasture may be more interested in its shade and animal welfare benefits; out of a group of 20 silvopasture landowners in the northeastern United States, 80% listed livestock shade and 30% listed animal welfare as their primary reasons for implementation of silvopasture systems (Orefice et al., 2017).

Designing of silvopastoral systems

When designing a silvopasture system, it is important to carefully consider plant and livestock species suitable for the intended site. Choosing an appropriate tree species is especially important because they will be in the same location for lengthy periods of time, which could result in greater likelihood of exposure to abnormal weather events and potential disease or insect outbreaks compared to the forage or livestock components (Pent et al. 2021). Tree species may also have negative interactions with various forage crop species. For instance, the allelochemical juglone is produced by black walnut trees (*Juglans nigra* L.) and prevents plant growth in the Solanaceae family (Pent et al., 2021).

Additional benefits of silvopastoral practices

A report by Jose et al. (2004) showed that while several crop species typically have yield reductions in shaded conditions, forage species demonstrate little to no change or potential yield increases under moderate shade conditions. Conversely, several prior studies (Smith, 1942; Garrett and Kurtz, 1983; Burner and Brauer, 2003) have shown that pastures under trees have increased forage yield and nutritive values as well as changes in plant community composition compared to those in open pastures of the same area. Additional research studies showed that the addition of deciduous trees, such as black walnut (*Julglans nigra* L.) or honey locust (*Gleditisia triacanthos* L.) to pastures in the Appalachian region of the United States have the potential to increase forage yield over that of an open pasture of similar botanical composition, given that the tree spacing is managed correctly (Buergler et al., 2005).

Forage quality and nutritive values can be altered when grown in a silvopasture system. Forages grown in shade environments tend to have increased levels of crude protein and decreased levels of non-structural carbohydrates (Kephart and Buxton, 1993; Buergler et al., 2006; Neel et al., 2008). Another reported benefit of silvopastoral systems is an extended grazing period because forage plants are protected from environmental extremes (Sibbald, 1999).

A more recent study showed the addition of trees to pasture systems did not have negative impacts on forage growth or water availability for forage crops. From a livestock producer standpoint, this along with other benefits of silvopasture such as improved animal health and rate of gain as well as additional revenue from timber and fruit or nut crops, adds to the economic enticement for incorporating silvopastoral practices into existing livestock systems (DeBruyne et al., 2011).

Selection of forage species

Cool and warm season grasses

Cool season forage species are a crucial part of pasture systems in temperate regions of the United States because of their capacity to provide both winter stockpile and early spring forage production; at higher elevations, these species can continue producing into the summer months (Pent et al., 2021).

Both introduced and native warm season grass species, such as big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), Indiangrass (*Sorghastrum nutans* (L.) Nash), and eastern grammagass (*Tripsacum dactyloides* (L.) L.)

have been successfully managed in silvopastures, though studies have shown the greatest success occurring beneath older trees (Burton, 1973; Lewis et al., 1983; Franzluebbers et al., 2017).

Forbs and legumes

The addition of a legume component is also common in silvopasture practices. In addition to being a quality forage for livestock, legumes have a symbiotic relationship with *Rhizobium* bacteria which fix nitrogen for the system (Pent et al., 2021). For example, healthy, mixed pastures of grass and clover can fix more than 100 kg $ha^{-1}year^{-1}$ of atmospheric nitrogen, which is equivalent to more than 200 kg $ha^{-1}year^{-1}$ of ammonium nitrate fertilizer (Heichel, 1983). When legumes begin to senesce and decompose, the nitrogen is then made available to the surrounding trees and grasses; grazing livestock can also speed up this process because an amount of roots equal to the amount of aboveground biomass removed by grazers is senesced to maintain a balanced root/shoot ratio (Pent et al., 2021).

A negative factor to utilizing native forage legumes in silvopasture, is their lack of commercial availability and domestication compared to more commonly used forage species such as clovers. In warmer climates, there is a slew of factors that can impact establishment of legumes such as inadequate rainfall, lengthy dry seasons, extreme temperatures, and competition from aggressive warm season grass species (Muir et al, 2014).

Native plant species and non-native forage species

In places like the semiarid regions of Brazil, the primary food source of herbivores is comprised of native forage plants (Leao et al., 2017). An example of this is the Caatinga, a seasonally dry tropical forest which covers 86.1% of the semiarid region alone, in addition to other parts of the country (Souza et al., 2015). Because these parts of the world experience dry seasons, where the region can go months without rainfall, having forage species capable of producing copious quantities of dry forage mass that can be stored beyond the growing season is important to livestock producers. Leao, et al. (2017) conducted a study on the haying potential of native plant species found in the Caatinga. Their results demonstrated that different forage species – in their case native tropical legumes compared to native forbs – require varying rates of sun exposure to reduce the water content to ideal levels for hayed stored forage. For instance, *Mucuna spp.*, a tropical legume, reached the ideal dehydration point for hay after 12 hours of sun exposure, but species such as spiny amaranth (*Amaranthus spinosus*) and Ervanco (*Froelichia humboldtiana*) took 15.9 hours to reach the same point of dehydration (Leao et al., 2017).

Producing hay from native pastures, like those of the Caatinga, as a means of forage mass preservation would provide another option in addition to silage for farmers. However, when haying a mixed stand of plant species, variance in drying time would be a factor to take into consideration to avoid spoilage from too high of a moisture content if one species took significantly longer to reach the dehydration point compared to the others.

Being able to meet forage requirements for livestock primarily from native pastures is desired in areas that experience some level of isolation (i.e., islands), as there are restrictions in place for the introduction of new species to avoid potentially devasting effects of natural habitats (Arevalo, Mora, and Chinea, 2012). Utilization of native plant species as forage helps to protect these native species by serving as an alternative to conversion of grasslands into high-value cash crops (Arevalo, Mora, and Chinea, 2012; Arevalo and Chinea, 2009).

A primary concern when using introduced plant species is the potential for them to become invasive and expand beyond their area of introduction (Muller, Lopes, and Hermann, 2017). There is an increasing desire for the conversion of grasslands back to their native state, but there are little to no standardized measures in place for seed sourcing, testing, and propagation for native forage species; additionally, when trying to collect regional species for colonization of native species, there is the risk of contamination from non-native species (Muller, Lopes, and Herman, 2017).

A disadvantage of several native forage species used for livestock production, especially in the case of C4 perennial grasses, is that while they can produce consistently high yields of biomass, the nutritional value of that forage is low by comparison (Reid et al., 1988, Bonin and Tracy, 2011). For livestock production to be profitable, it is vital to have consistency in forage production; perennial C4 grasses can contribute this stability when added to a cool-season forage mix due to their improved persistence in periods of extreme drought or elevated temperatures (Bonin and Tracy, 2012).

Mixed and monoculture pastures

Various biodiversity studies in perennial herbaceous systems demonstrate that increasing plant species richness has the potential to increase biomass quantity (Tilman, et al, 1997). There are also several ecosystem services and functions that have the potential to be amplified through biodiversity (Hooper et al., 2005). Both highly diverse polycultures and uncomplicated perennial bicultures have the potential to be more productive and generate greater amounts of biomass bioenergy when compared to monocultures; additionally, it has been shown that the proper selection of a few perennial plant species can produce biomass yields comparable to those of more complex mixtures (Bonin and Tracy, 2012; Tilman et al, 2006; Tracy and Faulkner, 2006; DeHaan et al., 2010). Weed biomass may also be significantly greater in monocultures compared to multi-species systems; a study by Bonin and Tracy (2012) showed that over a two-year period, weed biomass comprised 30% of the total area biomass in individual species monocultures compared to less than 1% in the multi-species systems.

Plant community composition can impact fire characteristics (Much, 1970). For instance, the fuel quantity can vary based on plant composition; this, in turn, would mean that higher fuel quantities boost both fire spread and intensity (Wragg, Mielke, & Tilman, 2018; Byram, 1959; Cheney, Gould, & Catchpole, 1993; Pausas, Keeley, & Schwilk, 2017). Plant communities comprised primarily of grass species tend to increase fire spread, height, and other fire behaviors; this occurs because grass species can create higher quality fuel beds compared to forb species (Wragg, Mielke, & Tilman, 2018).

Species composition in diverse stands can change over time. Because some species have high seed dormancy and/or a reduced above-ground growth rate, it can take multiple years for them to fully establish (McLaughlin and Kszos, 2005). Therefore, while species such as early successional perennial forbs may dominate composition at the beginning of establishment, that dominance can shift to perennial grasses as they reach full establishment, which also contributes to yield stability (Bonin and Tracy, 2012).

Annual ryegrass (*Lolium multiflorum* Lam.) and cereal rye (*Secale cereale* L.) mixtures are desirable in silvopasture systems because 70% of their annual growth occurs when several tree species are dormant or not under severe environmental stressors (Keatinge et al., 1980; Altom et al., 1996; Zhang et al., 1997; Balandier et al., 2000). This pasture mix is also desirable from a livestock producer standpoint. Establishing these species in late summer allows for late winter deferred, or 'stockpile,' grazing (Kallenbach et al., 2003). Extended grazing would reduce the need for stored forage during winter months and may reduce overall livestock production costs.

Environmental factors affecting seed germination and emergence

Light quality and availability

If soil moisture, nutrient availability, and grazing management factors are not limiting growth, solar radiation can serve as a strong determinant of pasture canopy characteristics (Pontes et al., 2017). Economic and environmental value has swayed research in the direction of observing native forage grasses in full sun extensive grazing conditions rather than under shade conditions like those found in silvopasture practices (Valls et al., 2009). Photosynthetically active radiation (PAR) is defined as "radiation in the 400 to 700 nm wavelength which plants use to photosynthesize"; retention of this light in silvopasture systems is related to the type, size, and leaf density of the tree canopy (de Mendonca et al, 2017). Leaf area index (LAI) which refers to the ratio between the total leaf area and the unit of soil cover is also considered to be a critical component when examining ecosystem models (White et al., 2000). Light availability below the tree canopy is related to LAI because tree density, height, and spatial relationships of the canopy affect the amount of light allowed to pass below (Gower et al., 1999).

Shading affects can vary in silvopasture systems based on tree species and spacing in relation to the forage crops (Wilson, 1996; Cruz, 1997; Kohli and Saini, 2003.) Studies in Virginia and West Virginia showed increased levels of forage yield in locations with moderate shading compared to open pasture and heavy shading (Buergler et al., 2005; Belesky, 2005). Wilson (1998) reported reduced forage growth when light was the limiting resource in temperate climates. Silvopastures may have positive effects on the microclimates beneath the tree canopy. For instance, Feldhake (2002) reported in a study from West Virginia that orchard grass grown in a mixed pine silvopasture beneath 77% cover was less likely to suffer damage from radiant frost events. This was because the temperature near the forage beneath the tree cover was up to 10.4 ºC warmer than forage in an open pasture under the same frost risk. An additional study showed that while a mixed pasture of tall fescue (*Lolium arundinaceum*) and orchard grass (*Dactylis glomerata*) performed better in open pasture compared to silvopasture, the orchard grass monoculture experienced increased yields in a loblolly pine (*Pinus taeda*) silvopasture (Burner, 2003).

It is to be expected that forages planted in open pastures would produce greater yields than those same species grown under shade environments. However, a study by Lin et al. (2001) reported that cool season C3 grasses grew just as rapidly under 50% shade conditions as in full sun; C4 warm season grasses, on the other hand only produced 66-75% of their full sun yields. At 80% shade, both C3 and C4 plant species declined in yield, reporting 66-81% and 15-39%, respectively compared to full sun yields (Lin et al., 2001). Additional studies demonstrated yields of C3-C4 mixed grass pastures grown under six- to eight-year-old loblolly pines with 20-32% tree canopy cover experienced

50-60% decreases compared to open pasture production, with the C4 grass declining at a faster rate than the C3 grass (Brauer et al., 2004).

Kallenbach et al. (2006) reported that cool season forages grown beneath conifer tree species begin growth early in the season, possibly due to the heat trapping effects of the conifers. Conversely, Feldhake et al. (2010) reported delayed spring green up for deciduous type silvopastures, thought to be the result of residual leaf cover from autumn senescence. A separate study has shown that allelochemicals and cations present in the leaf litter from deciduous trees may inhibit or slow germination in grass and legume seedlings (Halvorson et al., 2017).

Shading levels can also impact the nitrogen content of forage plant species (Wilson and Wong, 1982; Cruz et al., 1995). A study by Barro et al. (2012) showed that moderate shading (50%) positively affected dry matter yield of native forage grasses; however, dry matter yield was negatively affected at 80% shading. The study also showed that as shading levels increased, nitrogen levels in the grass tended to increase, which in turn improves overall forage quality (Barro et al., 2012). Additional studies (Stur, 1991; Cruz et al., 1995; Soares et al, 2009) also reported increased forage yields of warm-season grasses in both artificial and natural shading.

Temperature

High temperatures and smoke from fires can help facilitate germination by breaking down hard seed coats and allowing water imbibition to occur (Downes et al., 2010; Moreira et al., 2010; Tavsanoglu, 2011, Chou et al., 2012). Multiple studies have demonstrated that application of thermal shocks between 50°C and 150°C at varying intervals of time can influence, or prevent entirely, the rate of germination based on plant species (Shaukat and Burhan, 2000; Williams et al., 2003; Madueno-Molin et al., 2006; Paula & Pausas, 2008; Bolin, 2009; Cruz, Medina, and Orozco-Almanza, 2010; Haider Ali et al., 2011). In terms of fires, those that move along the surface removing the vegetative canopy can function as a germination stimulant for some varieties of tropical legumes; however, severe fires with temperatures greater than 120°C would prevent these species from regenerating due to the destruction of stored seed in the soil seed bank (Martinat and Fuentes, 2016).

Soil moisture

Grasslands in semi-arid regions, like those found in the southwestern United States, do not have steady periods of rainfall, which leads to variability in soil moisture (Humphrey, 1958; McClaran, 1995). Because of this, native warm-season perennial grasses tend to fail to establish compared to their non-native counterparts (Cox et al, 1982, Roundy and Biedenbender, 1995). Warm season grass species typically need several weeks of soil water availability near the surface for the seedlings to develop adventitious roots (Wilson and Briske, 1979; Ries and Svejcar, 1991; Roundy et al., 1993; Abbot, 1999). A slower germinating species can be more advantageous in these instances by delaying germination until there is a significant amount of soil water availability necessary for root development (Abbott and Roundy, 2003). There is significant potential for environmental conditions of a region to vary between years; studies have demonstrated that this variability can have an influence on which plant species are able to successfully establish from year to year (Abbot and Roundy, 2003). Perennial herbaceous species utilize adaptive strategies to persist in periods of severe drought (Ludlow, 1989; Blum, 1996). Studies have shown that native forage species are capable of surviving and recovering from extreme drought conditions; however, the amount of time for recovery can vary significantly among species (Zwicke et al., 2015).

In several regions, seed germination takes place during the winter month and can therefore be influenced by the variable levels of snowfall (Gornish et al., 2014). The amount of snowpack can impact environmental conditions such as temperature, moisture, and nutrient availability (Gornish et al., 2014). Deep snowpack can provide insulation to germinating seeds from cold, harsh ambient temperatures while also suppling the seeds with adequate soil moisture (Mondoni et al., 2012). On the other end of the spectrum, a shallow snowpack is more likely to expose seeds and seedlings to multiple freeze-thaw cycles; these conditions tend to increase seedling stress, leaving them more susceptible to soil pathogens (Aanderud et al., 2011; Hardy et al., 2001).

Under drought conditions, forage plants grown under moderate shade have been shown to maintain greater leaf water potential and photosynthetic rates compared to plants grown in open pastures (Johnson et al., 1994).

Silvopasture microclimate

The addition of trees to existing pasture systems can be beneficial to both reducing plant stress and improving use of resources, especially water. For example, a study in the Texas savanna area showed that removing trees led to an increase in water stress for shallow rooted plants; deep rooted plants remained unaffected (Zou et al, 2005). Another savanna study showed the majority of water uptake by older (greater than two years old) Emory oaks (*Quercus emoryiI*) came from deeper soil horizons that were not being accessed by the various bunchgrasses (*Poaceae* spp.) growing beneath the trees (Weltzin and McPherson, 1997). Fernandez et al. (2008) observed comparable results in

Argentina; grasses grown underneath ponderosa pine (*Pinus ponderosa*) utilized soil water found in the upper 20 cm of the soil horizon, while the pines accessed less than 20% of their total water from that same soil layer. The presence of trees may also supply water from deeper soil horizons to shallow rooted plants in times of drought stress via hydraulic lift. In a study from Spain, the roots of Austrian pine (*Pinus nigra*) were watered with deuterium-labeled water; the increased foliar concentrations of deuterium in the surrounding plants indicated the occurrence of hydraulic lift by the Austrian pine (Penuelas and Filella, 2003).

Establishing forage crops into existing tree stands, however, can be challenging. Proper spacing and thinning of trees to a density suitable for emergence and subsequent growth is required (Pent et al., 2021). In some instances, soil nutrients and pH may need adjustments. Elimination of undesired or existing vegetation may also be needed to reduce competition with newly seeded species, but producers must be cautious with removal methods as to not damage the tree stand (Pent et al., 2021). Compared to an open pasture, forages grown in silvopasture tend to take longer to reach maturity, which in turn impacts forage quality and nutritive value (Neel et al., 2016). As can be expected, trees may compete with forage plants for resources like light, nutrients, and soil moisture. However, factors like species age, morphology, phenology, physiology, and site weather attributes can impact the level of competition that occurs (Pent et al., 2021). A study by Sharrow (1999) showed that competition may be equal for moisture, nutrients, and light availability in open canopy stands.

A study by Neel et al. (2008) suggested that to achieve an ideal herbage dry matter yield and forage nutritive value, the maximum solar radiation (MSR) should not be reduced by more than 20% compared to open pastures. In addition to shading, forages grown in silvopastures are subject to reduced wind speeds and moderated ambient temperatures (Karki and Goodman, 2010, 2015).

The literature shows conflictions between reports on dry matter yields of silvopasture systems compared to open pastures, evidence of which can be seen in previous sections of this review. For instance, mature, closely spaced trees would have a greater negative impact on forage dry matter yields compared to younger, widely spaced trees (Douglas et al, 2001; Guevara-Escobar et al., 2007; Karki and Goodman, 2013, 2015).

It is commonplace for plants grown in low-light conditions to experience reduced root growth due to the allocation of resources to aboveground tissues to encourage photosynthesis (Poorter et al., 2012). This can necessitate lengthier grazing intervals to allow for new leaf regeneration (Mercier et al., 2020). The utilization of shade-tolerant species can help alleviate some of these negative effects (Mercier et al., 2020). Mercier et al. (2020) reported lower dry matter yields of cool season forage species under artificial shade during spring and fall; however, shade during the summer showed yield improvement where full sun conditions would typically reduce cool season photosynthesis efficiency (Mercier et al., 2020; McDonald, 2003). In this case, the negative effects of the “summer slump” typically seen in cool season pastures may be reduced with silvopastoral practices (Karki and Goodman, 2015).

Additional factors

Assorted studies have shown the plant population mortality primarily occurs during the early life stage transitions between seed germination, emergence, and establishment (Fenner, 1987; James et al., 2011). These transitions may also control population growth rates and community composition (Silvertown and Charlesworth, 2001; Oster et al., 2009).

Seedling competition post-germination can impact which species establish and persist in a microsite (File et al., 2012). Earlier germination can be an advantage for species that would traditionally be competitively inferior (Dickson et al., 2012; Porensky et al., 2012; Ulrich and Perkins, 2014; Cleland et al., 2015). Additional competition can occur from non-seeded species already present in the soil seed bank (Callaway and Aschenhoug, 2000; Loydi et a., 2014; Sheley and James, 2014).

Looking beyond environmental factors, the disintegration of Indigenous knowledge of specific regional land management practices and replacement with blanket management practices across all regions can significantly impact establishment and persistence (Muir et al., 2014). Seed costs, the need for specialized harvesting equipment, and limited availability of suitable species ecotypes for each region are also factors that can hinder adoption of native legumes for forage production (Muir et al., 2014).

Concluding thoughts

The possible combinations of tree and forage species are vast; silvopasture is practiced in multiple regions across the globe with potentially vast differences in climate, and plant species availability can change from region to region. While the number of publications relating to silvopasture and native forage plants continues to grow, the literature is by no means comprehensive of the potential interactions that occur within these dynamic systems. With the growing trends to shift towards more sustainable agricultural practices and both incorporation and restoration of native landscapes, additional research of the interactions between these components – like silvopasture and native plants as forage – would prove beneficial.

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CHAPTER 2: ENVIRONMENTAL EFFECTS ON NATIVE PLANT SPECIES ESTABLISHMENT IN DIFFERENT SILVOPASTURE SYSTEMS

Abstract

Incorporating sustainable agriculture practices and environmental stewardship into existing forage-livestock systems is a continually growing concept. Developing grazing systems that include multiple plant species introduces diversity, promoting a plant community that contributes to both livestock forage and wildlife habitat. This project takes a novel approach by utilizing native rather than introduced forage species. Our objectives were to: evaluate establishment of native cool and warm season grasses, and native forb and legume species in two different existing silvopasture systems – pitch pine (*Pinus rigida* Mill.) x loblolly pine (*Pinus taeda L.*) hybrid (PINE) and eastern American black walnut (*Juglans nigra L.*) (WALNUT) – with open pasture as a control (OPEN), and determine how differences in environmental factors (Photosynthetically Active Radiation (PAR), ambient temperature, and soil moisture) associated with each treatment affect establishment of native plant species. The experiment was a Randomized Complete Block Design replicated in 2020 and 2021. Treatment establishment (3 treatments, 3 replications each) began each fall preceding the growing season by no-till drilling cool season grasses. All remaining forages were no-till drilled each winter prior to the growing season. Environmental data were collected using HOBO Data Loggers. Data loggers were rotated through each treatment throughout the growing seasons. Sensor data for each treatment were averaged on a per hour basis over a 24-hour period. Stand counts for plant functional groups were measured monthly throughout the growing seasons. Cool season grass species in each treatment were sampled to calculate herbage

accumulation and conduct forage quality analyses. All data were analyzed using the PROC GLIMMIX procedure in SAS 9.4. Environmental data were considered significant at $P \leq 0.05$; stand count, forage quality, and herbage accumulation data were considered significant at $P \leq 0.1$.

Over a two-year period, OPEN had greater PAR values over a 24-hour period compared to PINE and WALNUT. PINE and WALNUT had greater ambient temperatures in the early morning and evening, while OPEN had the greatest ambient temperatures through mid-day. For much of the day, OPEN had the greatest soil water content compared to PINE and WALNUT. PINE had a greater soil water content than WALNUT at a few points during the early morning and late-night hours.

Cool season grass establishment counts were greater in PINE compared to OPEN; however, there were no differences between PINE and WALNUT. Forb and legume plant species establishment counts were greater in WALNUT and PINE compared to OPEN. Warm season grasses were not present in any of the tree treatments in the initial growing season following planting. There were no other statistical differences among treatments.

Neutral detergent fiber (NDF) concentration was greater in PINE compared to OPEN. Acid detergent fiber (ADF) concentration was greater in PINE and WALNUT compared to OPEN. PINE had the greatest *in vitro* true digestibility (IVTD) concentration. NDF digestibility (NDFd) and indigestible NDF (iNDF) concentrations were both greater in OPEN and WALNUT compared to PINE. WALNUT had a greater digestible (dNDF) concentration compared to PINE. Crude protein (CP) concentration was greater in OPEN and WALNUT compared to PINE. OPEN had a greater biomass accumulation compared to the WALNUT treatment.

Regression analyses suggest the positive relationships between cool season grass biomass accumulation and PAR and soil water content and the negative relationship between biomass accumulation and ambient temperatures were significant. There were also significant negative correlations between NDF and ADF concentrations and PAR as well as positive correlations between NDF and soil water content. There was no relationship between environmental factors and plant establishment counts.

The quick establishment of the cool season grasses makes them ideal for a readily available forage source compared to the warm season grasses, forbs, and legumes. As the legume components of the system continue maturing, they may provide additional CP, improving the overall forage quality of the system and providing a more diverse diet for grazing animals. It should be noted, however, that environmental factors can impact forage quality in pine and black walnut silvopasture systems.

Introduction

Incorporating sustainable agriculture practices and environmental stewardship into existing systems is growing in popularity. Agroforestry is a prime example of these practices that focuses on biodiversity and positive interactions within a system (Matson et al., 1997). Silvopasture is one of the six types of agroforestry practices.

Silvopasture is defined as "an intermixture of trees and herbaceous forages managed for livestock grazing in a way that optimizes environmental protection, production, and economic value of the combined tree-forage-livestock system," (Garrett et al., 2004). While silvopastoralism is not a new concept, modern-day silvopasture differs from its historic counterparts like the dehasas in Spain because today's systems are planned with greater thought and intent (Jose and Dollinger, 2019; Paciullo et al., 2017). Within North America, the two most common forms of silvopastoralism practiced are integrated forest grazing and silvopasture, with silvopasture being the more intensively managed system of the two (Pent et al., 2021).

Because silvopasture is an agroforestry practice, a successful system must meet the four basic principles of agroforestry: biologically possible, ecologically sustainable, economically feasible, and socially acceptable (Pent et al., 2021). Meeting these requirements can provide a multitude of benefits to the producer compared to conventional grazing and tree plantation systems. A silvopasture system has components in several different markets rather than one or two, thus reducing a producer's overall economic risk (Pent et al., 2021). Another benefit of these systems is an extended grazing period due to protection of forage species from environmental extremes (Sibbald, 1999). While intensive management generates several positive aspects, some producers may see this as a drawback. Regardless of beginning from nothing or converting an existing system, a successful silvopasture system requires a great deal of planning and thought before purchasing any materials and planting to ensure the selection of each component in the system will work together cooperatively within their unique microclimate.

While silvopasture is a fairly widespread practice on a global scale, within the southern Midwest there has been limited silvopasture research; these studies primarily focused on the establishment of the tree component rather than the plant species being grown for forage (Walter, 2008). This study takes a novel approach compared to previous silvopasture studies by incorporating native rather than introduced forage species. The objectives of this study were to: evaluate establishment of native cool- and warm-season grasses, and native forb and legume species in two different existing silvopasture systems with open pasture as a control and determine how differences in environmental factors associated with each treatment relate to establishment of native plant species.

Materials and Methods

Study site characteristics

The study was conducted from September 2019 through August 2021 at the Horticultural and Agroforestry Research Center, University of Missouri, New Franklin, Missouri, USA (39°0’59.304” N; 92°46’12.758” W; elevation 195m). Soils at the experimental site were characterized as a Menfro silt loam (Fine-silty, mixed, superactive, mesic Typic Halpudalfs). Average annual precipitation is 1067 mm, and the mean annual temperature is 11.9°C.

Experimental design and treatment establishment

Each treatment was replicated three times in a randomized complete block design (RCBD) over a two-year period (2020 and 2021). Treatment establishment began in Fall 2019. (Image 2.1)

The treatments consisted of (1) a stand of pitch pine (*Pinus rigida* Mill.) X loblolly pine (*Pinus taeda* L.) hybrids (PINE), (2) a stand of black walnut (*Juglans nigra* L.) (WALNUT), and (3) pastures without trees (OPEN). Henceforth, treatment 1 listed above will be referred to as PINE, treatment 2 will be referred to as WALNUT, and treatment 3 will be referred to as OPEN.

The stands of trees in PINE and WALNUT were established in 1995 as described by Kallenbach et al., (2006). The original distance between trees within a row and between rows in double and triple row configurations was 3-m. All row configurations were separated by 9.1-m alleys. Various circumstances (tree death, weather, etc.) required removal of some of the initial trees from the system. At the time of this experiment, PINE consisted of double rows with 3-m between rows separated by a 9.1-m alley while

WALNUT consisted of singular rows of trees separated by a 15.1-m alley. The alley widths were designed according to the original configuration resulting in the present alley widths in WALNUT being wider than that of PINE due to the removal of entire tree rows.

Prior to planting, 8-10 soil samples were randomly collected and composited from each treatment area in each replication. Samples were taken to a depth of 25-cm. The samples were analyzed by the University of Missouri Extension Soil and Plant Testing Laboratory to obtain soil pH and fertility data (Table 2.1). No additional fertilizer inputs were added to the treatments for the duration of the study.

To reduce competition from existing species, all treatment areas were mowed to a height of 10 cm and allowed to regrow during Summer 2019. Approximately 42 heifer calves grazed the treatment site until two weeks prior to experiment preparations. Residue from grazing was sprayed with Roundup® (14111 Scottslawn Road Marysville, OH 43041) according to label instructions mid-July and then mowed to a height of 2-cm. This process was repeated again 45 days prior to planting. Areas that were planted for Year 2 were mowed to a height of 2-cm in September 2020 prior to planting, but no herbicide was used to reduce the chances of damaging the Year 1 plantings via drift. Additionally, no pine straw was removed from the treatment areas during the course of the study.

A mixture of 23 plant species (Table 2.2) native to Missouri was no-till drilled into each treatment using a TRUAX FLXII-88RD Grass Drill (Truax Company, Inc.; New Hope, MN 55428) with 19.8-cm row spacing between planter assemblies. The total seed mix was planted at a rate of 15.7 kg^{-1} ha^{-1}. Warm season grass species comprised

50% of the total seed mix, cool season grass species accounted for 30%, and forb and legume plant species made up the remaining 20% of the total mix. Plant species classified as cool season grasses were no-till drilled in September 2019 and 2020. All other plant species – warm season grasses, forbs, and legumes – were no-till drilled in December 2019 and February 2021.

Data collection

Environmental Data. Environmental factors measured throughout this study included photosynthetically active radiation (PAR), ambient temperature, and soil water content. Photosynthetic Light (Model #S-LIA-M003), 12-Bit Temperature (Model #S-TMB-M002), and 10HS Soil Moisture (Model #S-SMD-M005) Smart Sensors were connected to a HOBO USB Micro Station Data Logger (Model #H21-USB) to measure these environmental factors (Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532). The data logger and wires connecting the sensors were housed in a protective box (Image 2.2) to keep these parts of the system from being exposed to excessive weather and potential animals. There were three total sensor systems so that one system could be placed in each treatment of a replication simultaneously.

Sensor systems were rotated throughout each treatment in each of the three replications during the course of the growing season (April-September). Sensors were rotated through three random locations at varying differences from the tree rows in each treatment for each replication to account for potential environmental differences present from trunk to dripline of the tree rows. Sensor systems in the OPEN treatment for each replication were moved to three different locations within the plot area. Sensor systems were set to log data for two minutes at a time, in five-minute increments; the system would then average these measurements and record a singular reading for each sensor type. Sensor systems logged in each location for 24-48 hours before being rotated to the next location. Soil water content sensors were inserted to a depth of 10-cm. Ambient temperature and PAR sensors collected data 15-cm from ground level.

A 24-hour window (12:00AM-11:59PM) was selected from the logged duration for each location within each treatment. The measurements for each sensor type during this selected time period for each location were then averaged by hour for each treatment. These 24 hourly values were plotted to show the average hourly change over a 24-hour period for each sensor type during the course of the growing season for each treatment.

Plant Stand Counts and Community Composition. Stand counts for plant functional groups were measured monthly throughout the growing season. Due to the uneven emergence of the warm season grass, forb, and legume plant species, stand counts were taken over the entire planted area of each replication for each treatment. The plots were walked back and forth in a snake-like pattern to account for all plants. Since emergence of the cool season grass species was more uniform, stand counts were made as follows. A 0.1-meter square was placed at three different distances (1-, 3-, and 5-m) from the tree line in three random locations within in each treatment in each replication, for a total of nine measurement areas per treatment per replication. The plants within the nine measurement areas were averaged to create an average plant stand count for each treatment in each replication. Averages of plant stand counts were then extrapolated to a plant stand count for the total plot size to compare the cool season grass stand counts to those of other plant functional groups.

Biomass Harvest. Aboveground biomass samples were collected on the Year 1 cool season grass species for each treatment in late May of 2020 and 2021 to determine dry matter (DM) accumulation and forage nutritive value. A composite sample for each replication of each treatment was collected from four, randomly selected 0.1 square meter areas. All forage material was clipped at 10-cm above the ground to simulate grazing height. Fresh weights of all samples were collected immediately after harvest. All samples were dried in a 55 °C forced-air oven for 72 hours and then weighed to determine partial DM (%). Samples were then ground through a 1.0 mm screen in a Wiley cutting mill to determine laboratory and total DM. The procedures outlined by Undersander et al. (1993) were used to determine partial, laboratory, and total DM. Forage DM accumulation (kg DM ha^{1}) was calculated as a product of total DM (%) and fresh weight of samples (kg ha^{-1}). Ground samples were used for successive laboratory analyses.

Laboratory analyses

Approximately two grams of each ground forage sample were dried at 102°C for 24 hours; the samples were then allowed to return to room temperature inside a sealed glass container with desiccant crystals, and then samples were weighed to calculate laboratory DM content (%). All samples were additionally analyzed for nitrogen content (%) using an Elementar vario MACRO cube carbon-nitrogen analyzer (Elementar Americas Inc., 119 Comac Street, Ronkonkoma, NY 11779). Crude protein (CP) content was calculated using a factor of 6.25 on a DM basis using the laboratory DM content. Fiber fractions were determined in an ANKOM200 Fiber analyzer using the neutral detergent fiber (NDF) and acid detergent fiber (ADF) batch procedures as outlined by

ANKOM Technology Corp. (2052 O'Neil Road, Macedon NY 14502). The Daisy II system (ANKOM Technology Corp., 2052 O'Neil Road, Macedon NY 14502) was used to determine forage *in-vitro* true digestibility. Rumen fluid for this analysis was collected from previously cannulated 18-month old Simmental Angus heifers. Animals had ad libitum access to mixed cool season grass haylage during the time of collection.

Statistical analyses

Environmental Data. All data were analyzed using the PROC GLIMMIX procedure in SAS 9.4 (100 SAS Campus Drive Cary, NC 27513-2414, USA). Treatments were considered fixed effects. Year was considered a random effect. All treatment differences were considered significant if $P \leq 0.05$.

Plant Stand Counts and Community Composition. All plant stand count data were analyzed using the PROC GLIMMIX procedure in SAS 9.4 (100 SAS Campus Drive Cary, NC 27513-2414, USA). Treatment differences were considered significant if $P \leq 0.1$.

Biomass Harvest. All data were analyzed using the PROC GLIMMIX procedure in SAS 9.4 (100 SAS Campus Drive Cary, NC 27513-2414, USA). Treatments were considered fixed effects, while year was considered a random effect. All treatment differences were considered significant if $P \leq 0.1$.

Environmental Factor Correlations. Forage quality measures, along with biomass accumulation and plant establishment counts were analyzed to determine if there were significant relationships between them and the environmental factors measured. Relationships between environmental factors, forage quality, and plant establishment counts were determined using a simple linear regression model in GraphPad Prism 9.3.1

(GraphPad Software, 2365 Northside Drive, Suite 560, San Diego, CA 92108). Values for R-squared were considered significant if $P \leq 0.05$.

Results and Discussion

Previous research has been conducted at the University of Missouri in Pine-Walnut silvopasture systems (Kallenbach et al, 2006; Kallenbach, 2009). These studies focused primarily on integration of silvopasture into existing forage-livestock systems and animal performance. In both studies, forage quality and yield data were collected. One study used a mixture of tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire = *Schedonorus phoenix* (Scop.) Holub), alfalfa (*Medicago sativa* L.), and red clover (*Trifolium pretense,* L.) that had been established prior to beginning this project. Another focused on two annual species: annual ryegrass (*Lolium multiflorum* Lam.) and cereal rye (*Secale cereale* L.). While the results of the study using annual grass species demonstrated a difference in forage production between the two silvopasture treatments (pine compared to walnut), no environmental data were collected to determine what factors may have affected the difference in yield.

Environmental data

PAR. Figure 2.1 shows the average daily PAR readings by hour during the growing season for each treatment. OPEN PAR values were greater than PINE and WALNUT for Hours 6-22 (Hour 6 P=0.0026; Hours 7-21 P≤0.0001; Hour 22 P=0.0085). Additionally, PINE PAR values were greater than WALNUT for Hours 7 (P≤0.0001), 14 (P≤0.0001), 17 (P≤0.0001), and 18 (P≤0.0001). PAR values for WALNUT were greater than those of PINE at Hour 15 (P≤0.0001). There were no other differences among treatments. It is to be expected that an open pasture would have the greatest PAR values of the three treatments, as there is no tree canopy cover preventing light from reaching ground level where measurements were taken. The occasional differences in PAR values between PINE and WALNUT could be caused by the variation in leaf architecture and leaf area between the different tree species. Additionally, the orientation of tree rows being north to south may have created an unevenness of light distribution as the day progressed. The position of PINE being west of WALNUT may have also affected the angles of light reaching ground level in WALNUT, especially towards the end of the day.

Ambient temperature. Figure 2.2 shows the average daily ambient temperatures by hour during the growing season for each treatment. Temperatures were higher in PINE and WALNUT compared to OPEN for Hours 1-8 ($P \leq 0.0001$) and Hours 21-24 (Hour 21 $P=0.0022$; Hours 22-24 $P \leq 0.0001$). Temperatures were higher in OPEN during Hours 12-17 ($P \leq 0.0001$). Additionally, PINE ambient temperature was higher than WALNUT at Hour 14 ($P \leq 0.0001$). PINE had a higher temperature compared to OPEN at Hour 9 ($P=0.0163$). PINE and OPEN had higher temperatures compared to WALNUT at Hours 10 ($P=0.0020$), 11 ($P \leq 0.0001$), and 18 ($P=0.0003$). OPEN had a higher temperature compared to WALNUT at Hour 19 ($P=0.01175$). There were no other differences among treatments. Gosme et al. (2016) reported a similar pattern of low day and higher night temperatures in their southern France agroforestry plots. Increased ambient temperatures under tree canopies at night could be beneficial in reducing frost damage to forage crops during early spring and fall when there can be sporadic drops in temperatures, even during stretches of warm weather.

Soil water content. Figure 2.3 shows the average daily soil water content by hour during the growing season for each treatment. Soil water content was greatest in OPEN for all 24 hours (Hours 1-21 $P \leq 0.0001$; Hour 22 $P=0.0006$; Hours 23-34 $P \leq 0.0001$). Soil water content in PINE was greater than WALNUT for Hours 1, 2, and 24 ($P \leq 0.0001$).

There were no other differences among treatments related to soil water content. OPEN treatment areas were located at the bottom of a slope compared to PINE and WALNUT, so excess runoff may have partially accounted for the greater soil water content in OPEN. The absence of trees would also reduce the amount of water uptake by vegetation, thus leaving a greater amount of water available in the soil. Lower soil water content in PINE and WALNUT supports the findings from Karki and Goodman's (2014) silvopasture study which also included mature (18-20 years) trees with extensively developed root systems that require greater water uptake. An Argentinian study using ponderosa pine (*Pinus ponderosa*) and mixed grasses showed that less than 20% of the pines' water uptake came from the same layer as the grasses (Fernandez et al., 2008). The slight differences in soil water content in PINE and WALNUT could be accounted for by the variance in water requirements between the two tree species.

Plant establishment counts and community composition

Figures 2.4a and 2.4b show the average establishment counts during the first growing season following planting in plants per hectare for each treatment by plant functional group. Cool season grass establishment counts (Figure 2.4a) were greater in PINE with 18,680 plants per hectare compared to OPEN with 3,101 plants per hectare (*P*=0.0199). There were no other differences among treatments.

Forb and legume plant species establishment counts (Figure 2.4b) were greater in WALNUT and PINE with 79 and 68 plants per hectare, respectively, compared to OPEN with less than 1 plant per hectare (*P*=0.1055). There were no other statistical differences among treatments.

There are no data for warm season grasses, forbs, or legumes for Fall 2019 because their initial emergence did not occur until the 2020 growing season (GS) due to frost seeding these plant functional groups. Additionally, there was no evidence of warm season grass emergence for the 2020 growing season. During the 2021 growing season, the average warm season grass stand count for OPEN was 10 plants per hectare, while the PINE and WALNUT treatments had stand counts of <1 plant per hectare. There were no statistical differences among the three treatments (*P*=0.2352). It should be noted that of the five warm season grass species initially seeded, only big bluestem (*Andropogon gerardi* Vitman) was observed in any of the treatments. Literature from previous research suggests it can take up to three growing seasons for native grass species in particular to fully mature (Keyser et al., 2015).

Forage quality and biomass accumulation

Neutral detergent fiber. Figure 2.5a shows the average neutral detergent fiber concentration for each treatment. NDF concentration was greater in PINE at 56.77% compared to OPEN with 51.85% (*P*=0.1457). WALNUT (55.75%) was intermediate to PINE and OPEN but not different from either.

Acid detergent fiber. Figure 2.5b shows the average acid detergent fiber concentration for each treatment. ADF concentration was greater in the PINE and WALNUT treatments at 34.96% and 34.92% ADF, respectively compared to OPEN with 32.62% (*P*=0.0920).

Previous studies have shown no impact or slight increases in NDF and ADF concentrations under shady environmental conditions (Lin et al., 2001; Kyriazopoulos et al., 2013). This supports the findings in this study that both silvopasture treatments had greater NDF and ADF concentrations compared to the open pasture. Increases in ambient temperature can also increase NDF concentrations in forage species (Rojas-Downing et al., 2017). Therefore, the increased night temperatures seen in PINE and WALNUT may have influenced fiber concentrations and digestibility.

In-vitro True Digestibility. Figure 2.5c shows the average percent *in-vitro* true digestibility for each treatment. PINE had the greatest IVTD percentage, with 34.65% (*P*=0.0059). There were no statistical differences between WALNUT (29.30%) and OPEN (26.55%).

Digestible and indigestible NDF. Figure 2.5d shows the average percent indigestible NDF for each treatment. OPEN and WALNUT had greater indigestible NDF percentages (73.45% and 70.70%, respectively) compared to PINE with 65.35% (*P*=0.0059). Figure 2.5e shows the averages percent digestible NDF for each treatment. WALNUT had a greater percentage (26.45%) compared to PINE with 22.12% digestible NDF (*P*=0.1876).

NDF digestibility. Figure 2.5f shows the average percent NDF digestibility for each treatment. OPEN and WALNUT had greater percentages (48.64% and 47.65%, respectively) compared to PINE with 38.40% (*P*=0.0284).

Results from previous studies have been conflicting around the impact of shade on digestibility of forage dry matter (Hight et al., 1968; Masuda, 1977; Garrett and Kurtz, 1983). A more recent study (Moyo and Nsahlai, 2021) showed that ambient temperatures can impact the potential degradability of forage dry matter in the rumen. They found that just a 1°C increase in ambient temperature can decrease the potential degradability of forage dry matter by 0.39%.

Crude protein. Figure 2.5g shows the average crude protein concentrations for each treatment. CP concentration was greater in OPEN and WALNUT with 9.95% and 9.60% CP, respectively compared to PINE with 7.81% CP (*P*=0.1100). Typically, plants grown under shade have increased CP levels (Allard et al., 1991; Lin et al., 2001; Kyriazopolous et al., 2013). Conversely, increased ambient temperatures can decrease CP concentrations (Rojas-Downing et al., 2017). The warmer night temperatures in PINE may have contributed to their lower CP levels compared to OPEN and WALNUT.

Biomass accumulation. Figure 2.5h shows the average biomass accumulation on a DM basis for each treatment. OPEN had a greater herbage accumulation with 4510 kg DM ha^{-1} compared to WALNUT, with an herbage accumulation of 2203 kg DM ha^{-1} (*P*=0.0840). PINE (3614 kg DM ha^{-1}) was indifferent from both OPEN and WALNUT. As tree canopies become denser with maturity, cumulative biomass accumulation tends to decrease in silvopasture systems compared to their open pasture counterparts (Sibbald et al, 1991; Silva-Pando et al., 2002). This microclimate from the tree component, however, can add protection against early season forage losses due to frost that the open pastures experience (Kallenbach et al., 2006; Silva-Pando et al., 2002).

Environmental factor correlations

Forage quality and biomass accumulation. Figures 2.6-2.8 show the correlations between forage quality and biomass accumulation of the cool season grass species and environmental factors. Relationships between biomass accumulation and PAR (Fig. 2.6a; *P*=0.0314), ambient temperature (Fig. 2.7a; *P*=0.0395), and soil water content (Fig 2.8a; *P*=0.0417) were significant. In general, as PAR values increased, biomass accumulation increased. This trend was truer for OPEN compared to PINE and WALNUT. Biomass accumulation also tended to increase as soil water content increased. However, biomass accumulation tended to decrease as ambient temperature increased.

There were also significant relationships between NDF and PAR (Fig. 2.6b; P=0.0376) as well as soil water content (Fig. 2.8b; P=0.0215). Percent NDF tended to decrease as PAR values increased. Conversely, percent NDF increased as soil water content increased. Relationships between ADF and PAR (Fig. 2.6c; P=0.0004) were significant as well. As PAR values increased, percent ADF tended to decrease. These relationships are supported by previous studies that have shown forage species grown in shade environments may have reduced fiber concentrations (Buergler et al., 2006).

It should be noted that this study compared a singular harvest (late May) to environmental growing conditions over the course of the entire growing season (April-November). Collecting forage samples at periodic intervals throughout the growing season to coincide with the seasonal environmental changes may demonstrate a more dynamic relationship between forage quality and yield measures and environmental factors.

Plant establishment counts. Figures 2.9a-2.9f show the relationships between environmental factors and plant establishment counts. There were no significant correlations between cool season grass establishment counts and environmental factors. There were also no significant correlations between forb and legume plant species establishment counts and environmental factors.

Conclusions

While native plant species from each functional group were present within each treatment, the rate at which they established varied among the PINE, WALNUT, and OPEN treatments. The variability in environmental conditions among treatments did not influence this rate of establishment. However, there may be other environmental factors beyond the scope of this study such as relative humidity and soil temperature that may contribute to these differing establishment rates.

Variations in environmental conditions also contribute to some of the differences in forage quality and herbage accumulation of cool season grass species among treatments. Increases in PAR values were shown to increase fiber concentrations. If successful in establishment, the legume components can provide additional crude protein, improving the overall forage quality of the system and providing more dietary diversity for grazing animals.

Cool season grasses had the greatest herbage accumulation of all plant functional groups, making them an ideal immediate forage source compared to the warm season grasses, forbs, and legumes. Several of these aforementioned plant species were not present within the time-period in which this study was conducted. This is not to say that these plant species may not establish eventually, but they would not be an optimal forage selection compared to the cool season grasses.

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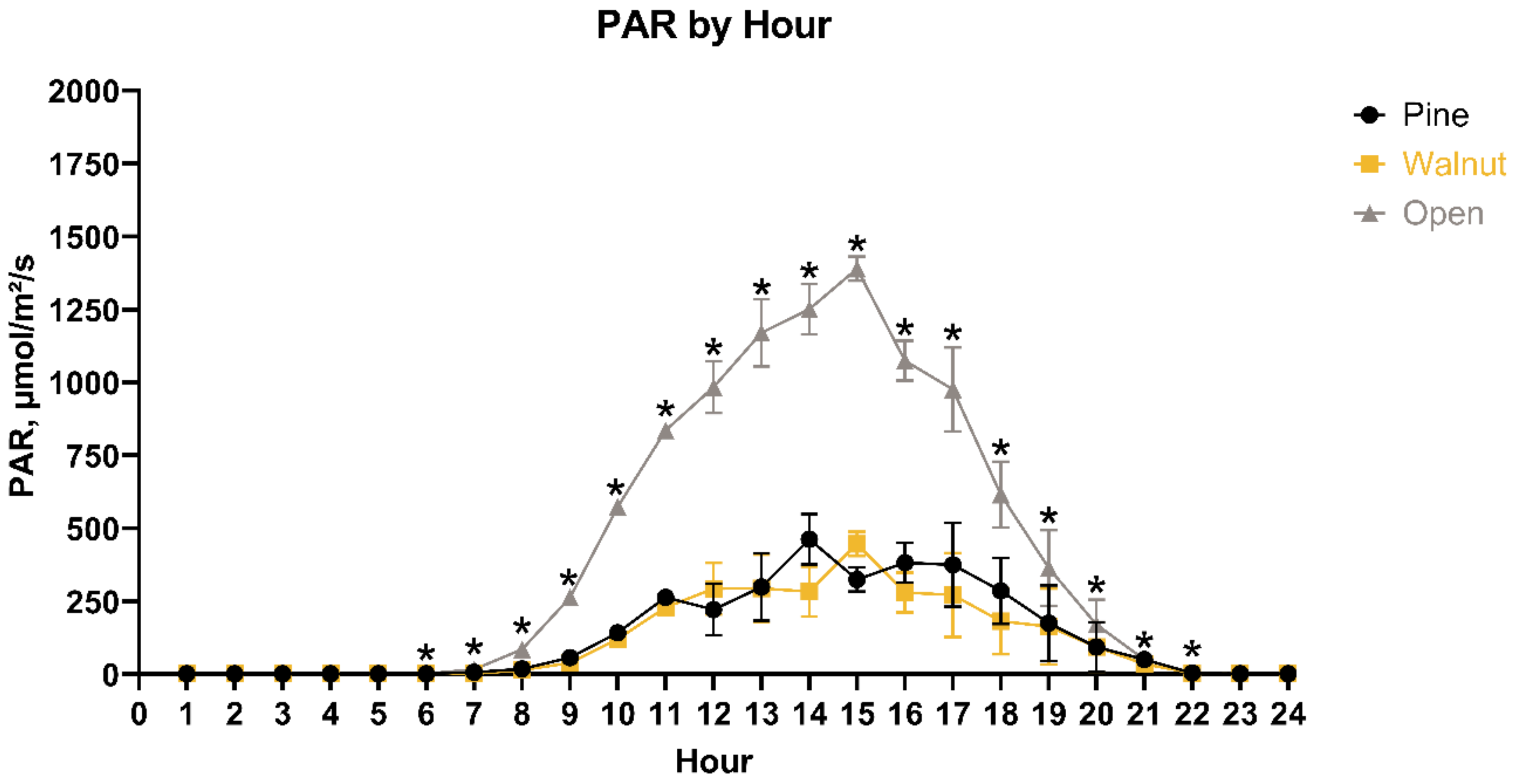


Figure 2.1. Figure 2.1 shows the average daily PAR readings (n=6) by hour during the growing season for each treatment. An asterisk designates a statistically significant difference between treatments at that point in time. Error bars reflect standard error of the mean (SEM Hour 1 = 0; Hour 2 = 0; Hour 3 = 0; Hour 4 = 0; Hour 5 = 0; Hour 6 = 0.0698; Hour 7 = 6.2776; Hour 8 = 17.1329; Hour 9 = 31.4133; Hour 10 = 24.8776; Hour 11 = 33.5066; Hour 12 = 88.9164; Hour 13 = 115.3500; Hour 14 = 85.9079; Hour 15 = 41.7007; Hour 16 = 68.8397; Hour 17 = 143.7400; Hour 18 = 113.3500; Hour 19 = 129.8900; Hour 20 = 85.1365; Hour 21 = 35.7125; Hour 22 = 2.4812; Hour 23 = 0; Hour 24 = 0).

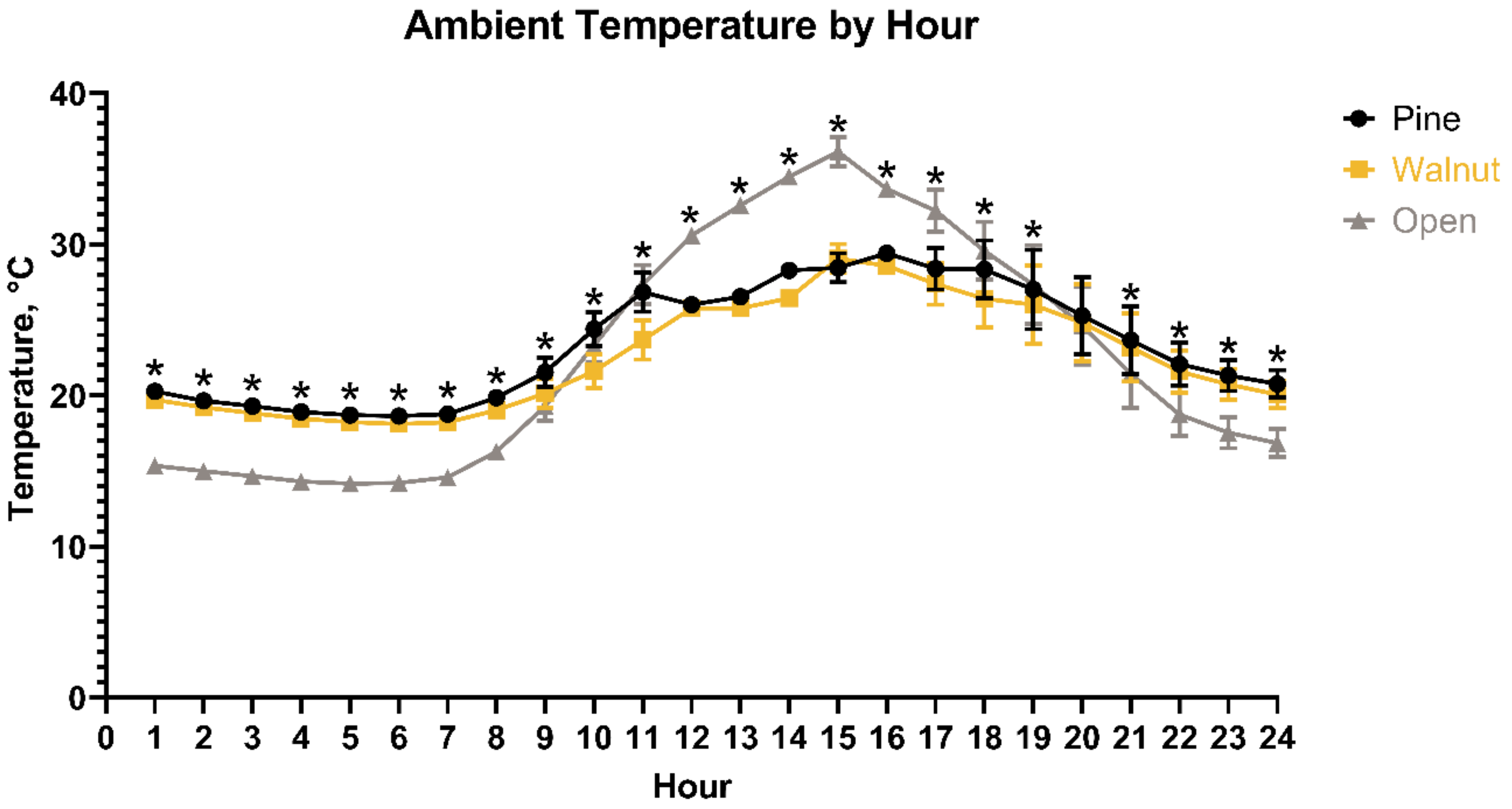
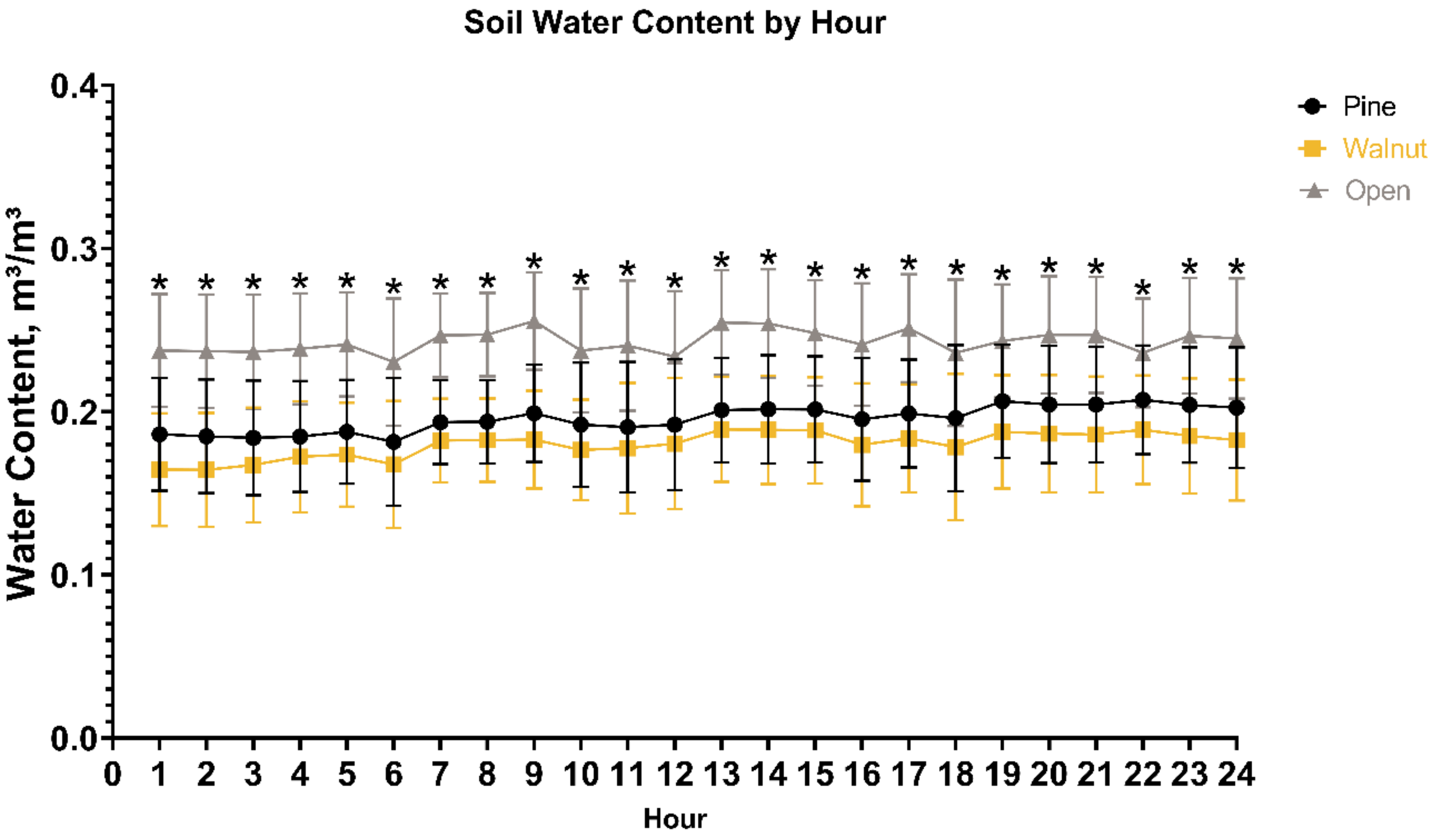


Figure 2.2. Figure 2.2 shows the average daily ambient temperature readings (n=6) by hour during the growing season for each treatment. An asterisk designates a statistically significant difference between treatments at that point in time. Error bars reflect standard error of the mean (SEM Hour 1 = 0.5693; Hour 2 = 0.5758; Hour 3 = 0.6316; Hour 4 = 0.5886; Hour 5 = 0.5885; Hour 6 = 0.5917; Hour 7 = 0.5884; Hour 8 = 0.5710; Hour 9 = 0.9611; Hour 10 = 1.1275; Hour 11 = 1.3026; Hour 12 = 0.6254; Hour 13 = 0.6338; Hour 14 = 0.6455; Hour 15 = 0.9638; Hour 16 = 0.6339; Hour 17 = 1.3786; Hour 18 = 1.9131; Hour 19 = 2.6188; Hour 20 = 2.5571; Hour 21 = 2.2638; Hour 22 = 1.4031; Hour 23 = 1.0203; Hour 24 = 0.9205).



Figures 2.3. Figure 2.3 shows the average daily soil water content readings (n=6) by hour during the growing season for each treatment. An asterisk designates a statistically significant difference between treatments at that point in time. Error bars reflect standard error of the mean (SEM Hour 1 = 0.0346; Hour 2 = 0.0348; Hour 3 = 0.0351; Hour 4 = 0.0341; Hour 5 = 0.0318; Hour 6 = 0.0390; Hour 7 = 0.0256; Hour 8 = 0.0255; Hour 9 = 0.0299; Hour 10 = 0.0381; Hour 11 = 0.0299; Hour 12 = 0.0402; Hour 13 = 0.0321; Hour 14 = 0.0332; Hour 15 = 0.0325; Hour 16 = 0.0377; Hour 17 = 0.0330; Hour 18 = 0.0448; Hour 19 = 0.0348; Hour 20 = 0.0360; Hour 21 = 0.0356; Hour 22 = 0.0333; Hour 23 = 0.0354; Hour 24 = 0.0370).

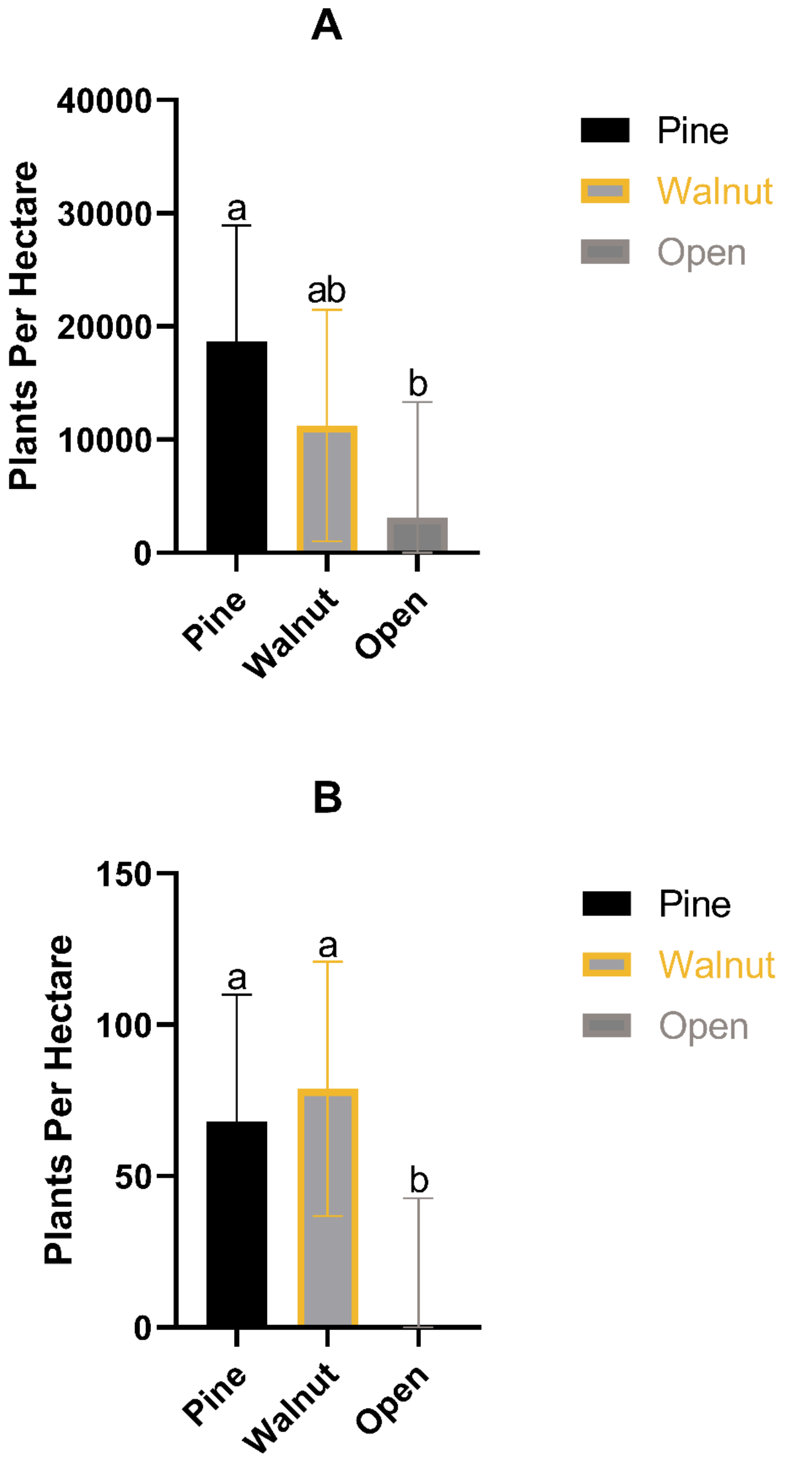


Figure 2.4a-b. Values for plants per hectare were determined by averaging the monthly stand counts April-November in each replication for each treatment with Years 1 and 2 combined. Error bars represent the standard error of the mean. Figure 2.4a shows the average cool season grass stand count by treatment in plants per hectare (SEM = 10,229). Figure 2.4b shows the average forb and legume plant species stand count by treatment in plants per hectare (SEM = 42.0368).

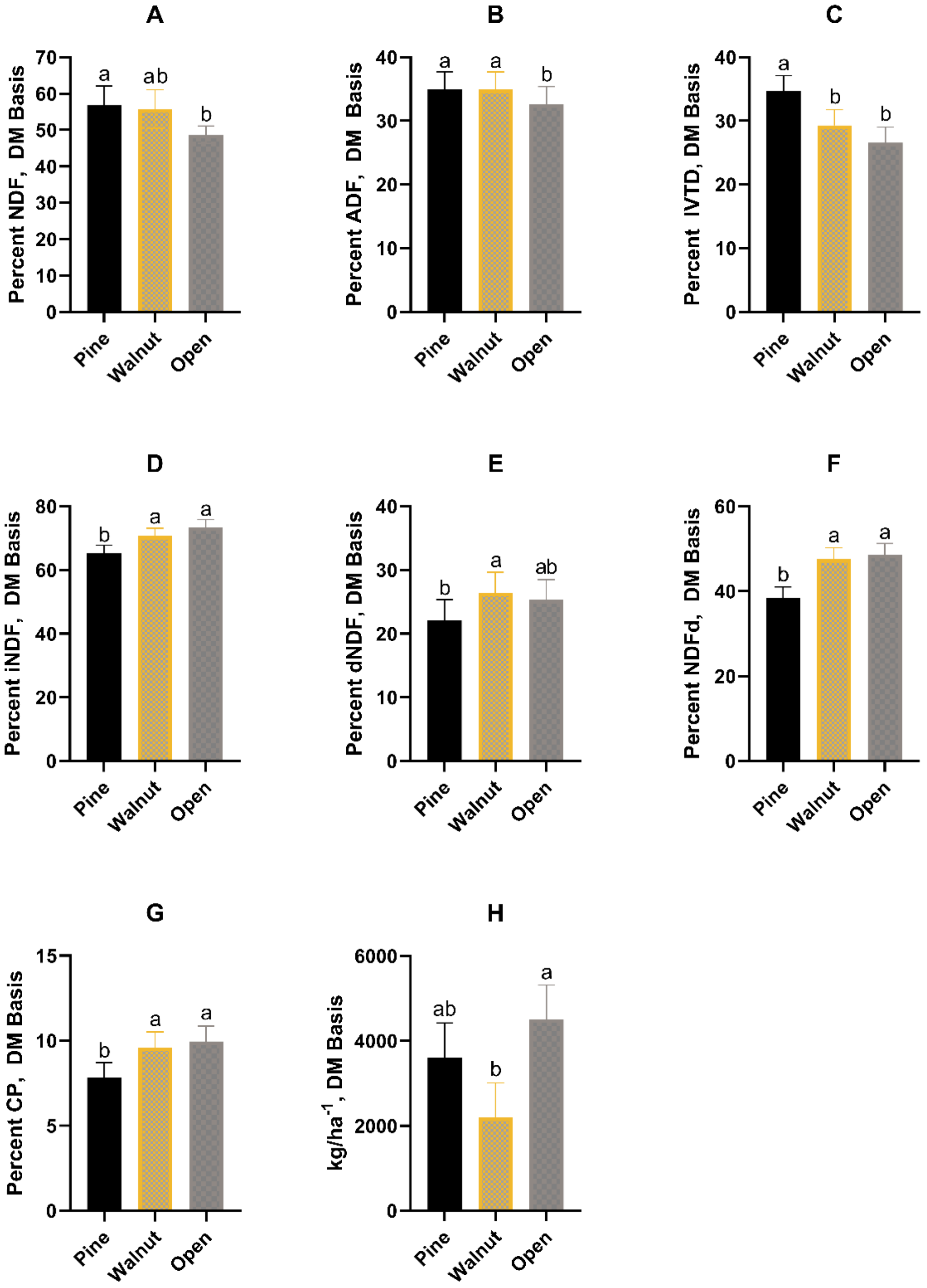


Figure 2.5a-h. Figure 2.5a shows average percent NDF by treatment (SEM = 5.2799). Figure 2.5b shows average percent ADF by treatment(SEM = 2.7622). Figure 2.5c shows average percent IVTD by treatment(SEM = 2.4786). Figure 2.5d shows average percent iNDF by treatment (SEM = 2.4786). Figure 2.5e shows average percent dNDF by treatment(SEM = 3.2156). Figure 2.5f shows average percent NDFd by treatment (SEM = 2.6193). Figure 2.5g shows average percent CP by treatment(SEM = 0.9091). Figure 2.5h shows average biomass accumulation by treatment(SEM = 807.30).

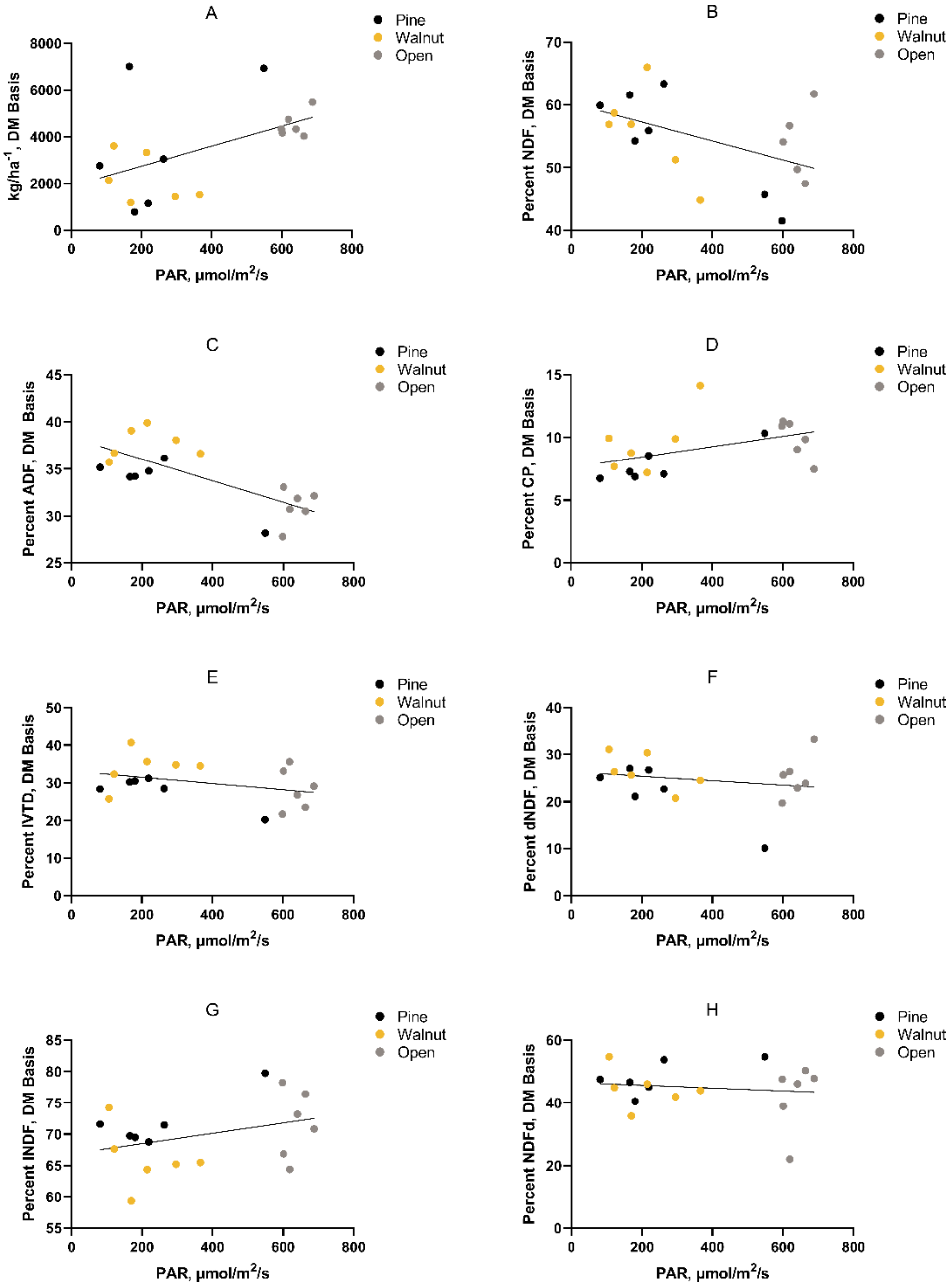


Figure 2.6a-h. PAR values represent PAR over the course of the entire growing season (April-November) for 2020 and 2021; forage quality values are representative of a singular sample taken late May in 2020 and 2021. Figure 2.6a shows the relationship between PAR and biomass accumulation (y=4.280x+1884; R^2=0.2580; P=0.0314). Figure 2.6b shows the relationship between PAR and percent NDF (y=-0.01509x+60.29; R^2=0.2432; P=0.0376). Figure 2.6c shows the relationship between PAR and percent ADF (y=-0.01145x+38.33; R^2=0.5486; P=0.0004) Figure 2.6d shows the relationship between PAR and percent CP (y=0.004118x+7.624; R^2=0.2158; P=0.0521). Figure 2.63 shows the relationship between PAR and percent IVTD (y=-0.008265x+33.17; R^2=0.1231; P=0.1534) Figure 2.6f shows the relationship between PAR and percent dNDF (y=-0.004686x+26.33; R^2=0.0429; P=0.4096). Figure 2.6g shows the relationship between PAR and percent INDF (y=0.008265x+66.83; R^2=0.1231; P=0.1534). Figure 2.6h shows the relationship between PAR and percent NDFd (y=-0.004474x+46.53; R^2=0.1713; P=0.6047).

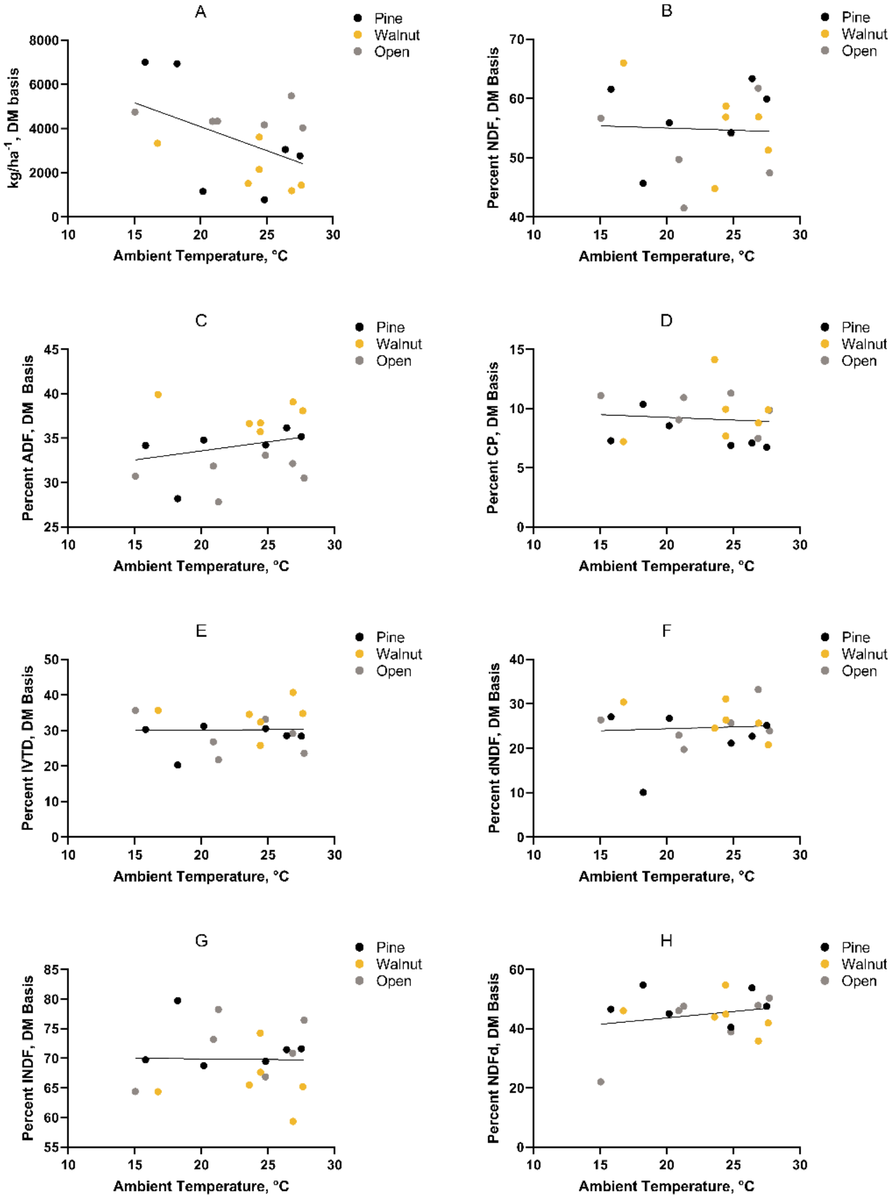


Figure 2.7a-h. Ambient temperature values represent ambient temperature over the course of the entire growing season (April-November) for 2020 and 2021; forage quality values are representative of a singular sample taken late May in 2020 and 2021. Figure 2.7a shows the relationship between ambient temperature and biomass accumulation ($y=-217.0x+8424$; $R^2=0.2345$; $P=0.0395$). Figure 2.7b shows the relationship between ambient temperature and percent NDF ($y=-0.07446x+56.50$; $R^2=0.2887$; $P=0.8556$). Figure 2.7c shows the relations between ambient temperature and percent ADF ($y=0.2034x+29.50$; $R^2=0.6624$; $P=0.3174$). Figure 2.7d shows the relationship between ambient temperature and percent CP ($y=-0.04521x+10.16$; $R^2=0.0843$; $P=0.7023$). Figure 2.7e shows the relationship between ambient temperature and percent IVTD ($y=0.02121x+29.68$; $R^2=0.0003$; $P=0.9463$). Figure 2.7f shows the relationship between ambient temperature and percent dNDF ($y=0.08680x+22.63$; $R^2=0.0786$; $P=0.7739$). Figure 2.7g shows the relationship between ambient temperature and percent INDF ($y=-0.2121x+70.32$; $R^2=0.0003$; $P=0.9463$). Figure 2.7h shows the relationship between ambient temperature and percent NDFd ($y=0.4306x+35.01$; $R^2=0.0572$; $P=0.3392$).

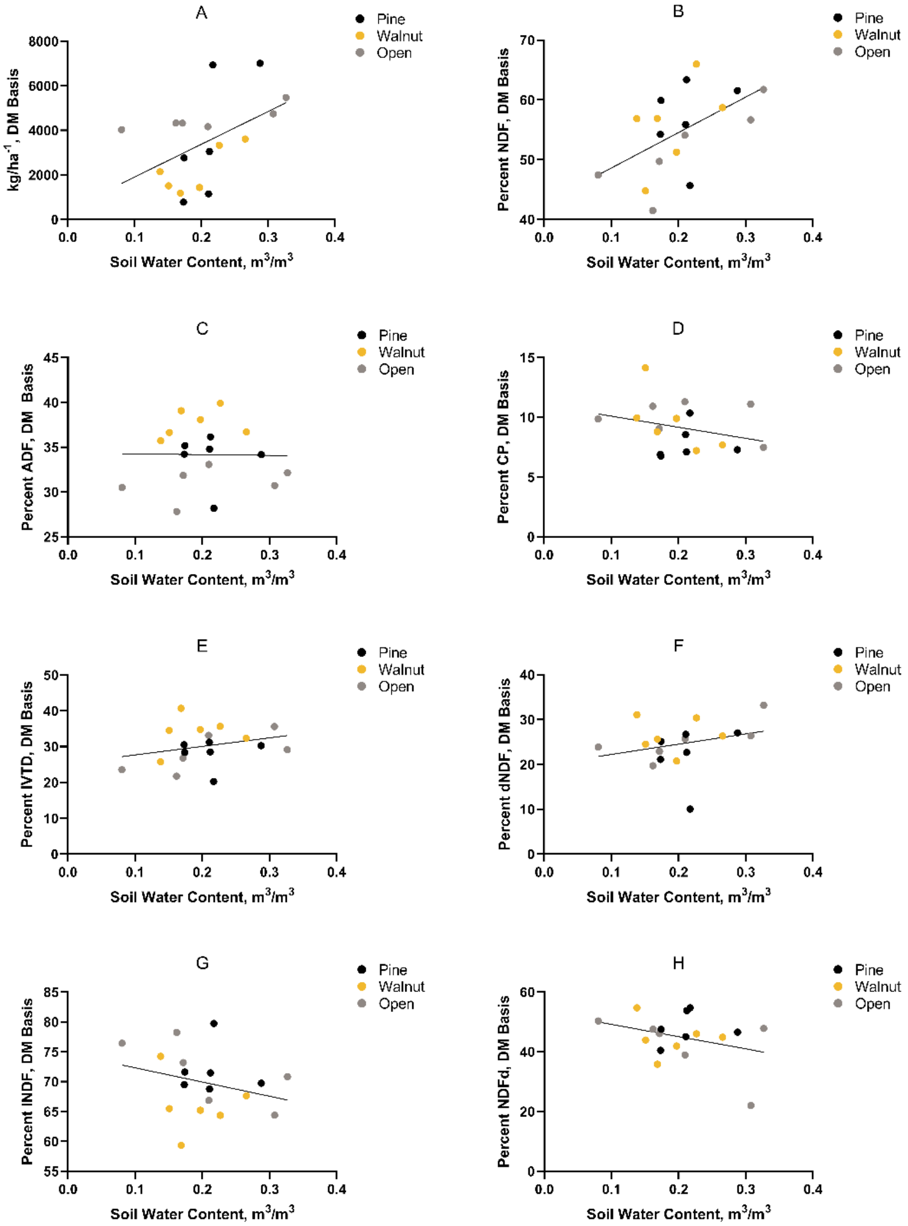


Figure 2.8a-h. Soil water content values represent soil water content over the course of the entire growing season (April-November) for 2020 and 2021; forage quality values are representative of a singular sample taken late May in 2020 and 2021. Figure 2.8a shows the relationship between soil water content and biomass accumulation (y=14713x+433.8; R^2=0.2580; P=0.0417). Figure 2.8b shows the relationship between soil water content and percent NDF (y=59.31x+42.66; R^2=0.2432; P=0.0215). Figure 2.8c shows the relationship between soil water content and percent ADF (y=-0.8574x+34.34; R^2=0.0002; P=0.9517). Figure 2.8d shows the relationship between soil water content and percent CP (y=-9.282x+11.02; R^2=0.2158; P=0.2424). Figure 2.8e shows the relationship between soil water content and percent IVTD (y=23.80x+25.30; R^2=0.0785; P=0.2602). Figure 2.8f shows the relationship between soil water content and percent dNDF (y=22.88x+19.95; R^2=0.0429; P=0.2597). Figure 2.8g shows the relationship between soil water content and percent INDF (y=-23.80x+74.70; R^2=0.0785; P=0.2602). Figure 2.8h shows the relationship between soil water content and percent NDFd (y=-40.94x+53.27; R^2=0.1103; P=0.1781).

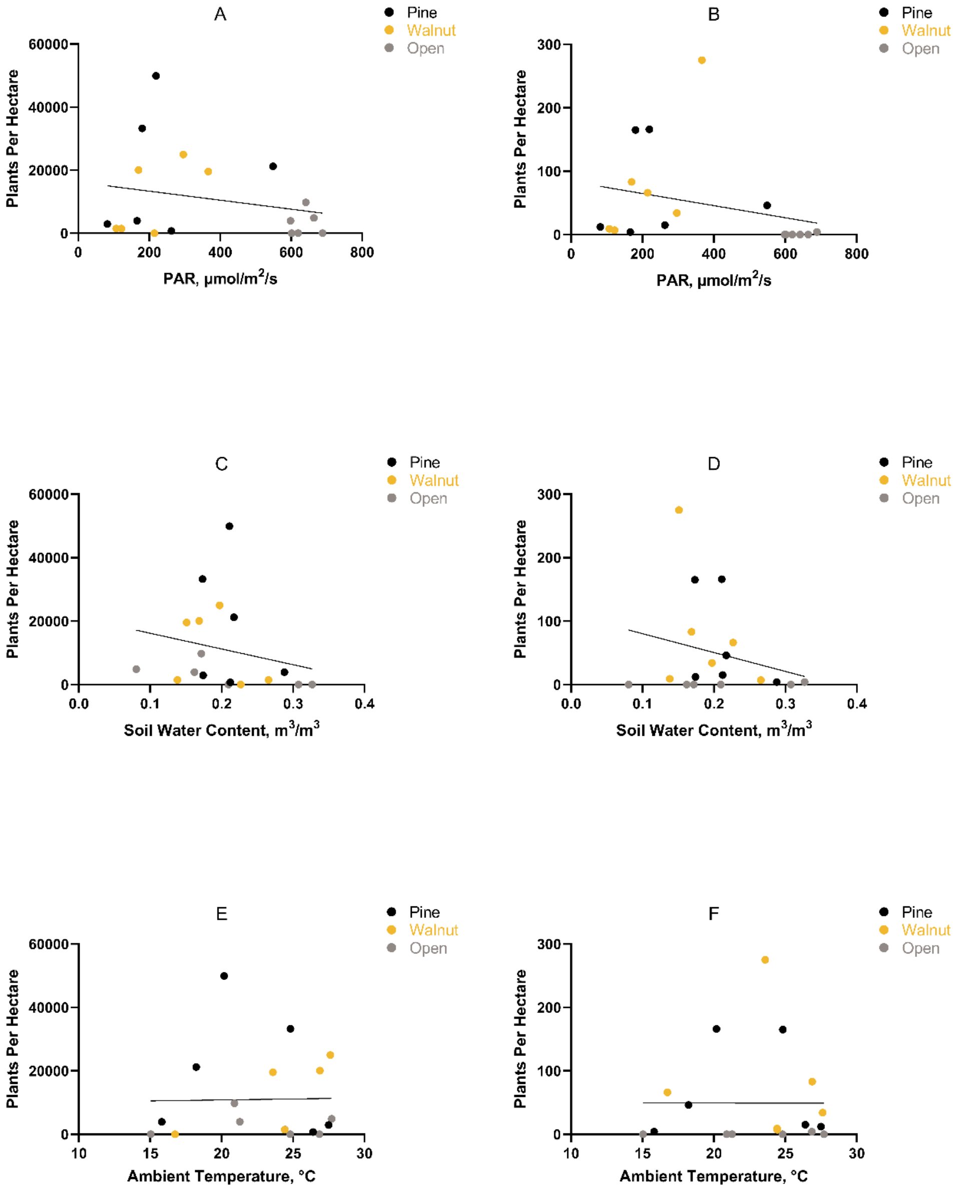


Figure 2.9a-f. PAR, soil water content, and ambient values are representative of the entire growing season (April-November) for 2020 and 2021; values for plants per hectare were determined by averaging the monthly stand counts April-November in each replication for each treatment with Years 1 and 2 combined. Figure 2.9a shows the relationship between PAR and cool season grass establishment counts (*y*=-14.29*x*+16215; R^2=0.0507; *P*=0.3690). Figure 2.9b shows the relationship between PAR and forb and legume plant species establishment counts (*y*=-0.09545*x*+83.96; R^2=0.0766; *P*=0.2664). Figure 2.9c shows the relationship between soil water content and cool season grass establishment counts (*y*=-49551*x*+21147; R^2=0.0469; *P*=0.3881). Figure 2.9d shows the relationship between soil water content and forb and legume plant species establishment counts (*y*=-297.3*x*+110.0; R^2=0.0571; *P*=0.3395). Figure 2.9e shows the relationship between ambient temperature and cool season grass establishment counts (*y*=61.27*x*+9608; R^2=0.0003; *P*=0.9424). Figure 2.9f shows the relationship between ambient temperature and forb and legume plant species establishment counts (*y*=-0.-1869*x*+49.65; R^2=01.058e^{-6}; *P*=0.9968).

Soil Test Results by Treatment

Treatment		pH	P (kg/ha)	K (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Organic Matter (%)	Neutralizable Acidity (meq/100g)	Cation Exchange Capacity (meq/100g)
Latin Binomial	**Common Name**								
Pinus rigida Mill. X *Pinus taeda* L.	Pitch X Loblolly Pine	5.6	47.08	465.9	3890	562.7	2.1	3.0	14.33
Juglans nigra L.	Eastern Black Walnut	5.9	37.74	462.2	4805	732.7	2.5	2.5	16.47
	Open Pasture	6.0	54.17	472.2	5340	881.4	2.9	2.2	17.87

Table 2.1 Soil test results for each treatment taken in 2019 prior to any planting. A composite sample consisting of 8-10 randomly collected cores was taken for each treatment for each replication. The values from each replication were compiled to create one average value for each of the test results for each treatment as shown in the table.

Latin binomial	**Common name**	**Percent of seed mix**
COOL SEASON GRASSES		
Elymus canadensis L.	Canada wild rye	3.25%
Elymus macgregorii R.E. Brooks & J.J. N. Campb.	Early wild rye	10.4%
Elymus virginicus L.	Virginia wild rye	16.2%
WARM SEASON GRASSES		
Andropogon gerardi Vitman	Big bluestem	19.5%
Tripsacum dactyloides (L.) L.	Eastern gamagrass	11.7%
Sorghastrum nutans (L.) Nash	Indian grass	14.9%
Panicum virgatum L.	Switchgrass	2.60%
Sporobolus compositus (Poir.) Merr.	Tall dropseed	1.30%
FORBS		
Silphium perfoliatum L.	Cup plant	1.30%
Helianthus maximiliani Schrad.	Maximillian sunflower	1.95%
Heliopsis helianthoides (L.) Sweet	Oxeye sunflower	1.30%
Echinacea pallida (Nutt.) Nutt.	Pale purple coneflower	0.65%
Coreopsis tinctoria Nutt.	Plains coreopsis	1.30%
Echinacea purpurea (L.) Moench	Purple coneflower	2.60%
Coreopsis lanceolata L.	Tickseed coreopsis	1.30%
Monarda fistulosa L.	Wild bergamot	0.65%
Parthenium integrifolium L.	Wild quinine	0.65%
LEGUMES		
Desmanthus illinoensis (Michx.) MacMill. Ex B.L. Rob. & Fernald	Illinois bundleflower	2.60%
Amorpha canescens Pursh	Lead plant	0.65%
Chamaecrista fasciculata (Michx.) Greene	Partridge pea	1.95%
Dalea purpurea Vent.	Purple prairie clover	1.30%
Lespedeza capitata Michx.	Roundhead lespedeza	0.65%
Desmodium canadense (L.) DC.	Showey tick trefoil	1.30%

Table 2.2. List of 23 native plant species divided by plant functional group, including their common and Latin names, and percent of the planting rate (15.71kg^{-1}ha^{-1}) for the total seed mix.

Image 2.1 Map of study area overlayed with experimental design. Areas labeled with a “1” were measured during the 2020 growing season. Areas labeled with a “2” were measured during the 2021 growing season.

Image 2.2. Image of the box that was used to house and protect the data logger systems throughout the course of the study.