

PRODUCTIVITY, CARBON SEQUESTRATION, NUTRIENT ACCUMULATION,
AND SPECIES INTERACTIONS IN PERENNIAL BIOMASS ALLEY CROPPING
SYSTEMS

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Dedication

This work is dedicated to my grandfather Howard Gamble, whose adventurous spirit and conservation ethic influenced me at an early age; and also to my late grandmother Una Miller, whose love of plants will always inspire me.

Abstract

Perennial biomass production in agroforestry systems has been promoted as a strategy to increase productivity and ecosystem services from marginal agricultural lands. However, little is known about appropriate species combinations and production potential for biomass crops in agroforestry systems. Our objectives were to evaluate the potential for biomass feedstock production, nutrient uptake and accumulation, and carbon sequestration in alley cropping agroforestry systems at two Minnesota sites, and to determine how tree – crop interactions influenced productivity in these systems. Short-rotation woody crops (SRWC) were hybrid poplar (*Populus maximowiczii* x *P. nigra* ‘NM6’) and shrub willow (*Salix purpurea* ‘Fish Creek’). Herbaceous alley crops were switchgrass (*Panicum virgatum* L.), prairie cordgrass (*Spartina pectinata* Bosc ex Link), ‘Rush’ intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey cv. Rush), and an eleven species native polyculture.

After four years of growth, we found that NM6 poplar alley cropping systems maximized biomass yields at Empire (13.5 Mg ha⁻¹ yr⁻¹) and Granada, MN (9.6 Mg ha⁻¹ yr⁻¹), irrespective of herbaceous crop type. NM6 poplar – intermediate wheatgrass systems showed the greatest potential for aboveground N, P, and K uptake (477, 62, and 301 kg ha⁻¹), while NM6 poplar – prairie cordgrass systems had among the highest root biomass, and root C, N, P, and K due to extensive coarse roots. Soil carbon declined slightly over the study period, although alley system roots sequestered up to 7.0 and 6.3 Mg C ha⁻¹ at Empire, and Granada, respectively. At Empire, above- and belowground biomass of herbaceous alley crops declined substantially with proximity to SRWC rows, as did soil

water potential, soil $\text{NO}_3 - \text{N}$, and transmittance of photosynthetically active radiation (PAR). A mixed effects model with predictors for PAR and soil water potential best explained patterns in prairie cordgrass and native polyculture yield, suggesting that competition for light and water limited crop growth at this site.

Our results show that after four years of production, NM6 poplar and prairie cordgrass were among the best SRWC and herbaceous crop choices for biomass production, C sequestration, and nutrient accumulation in alley cropping systems. However, competition may limit the stand longevity of herbaceous crops, which could reduce the utility of these systems for biomass production and ecosystem services over time.

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Chapter 1 : General introduction

1.1 Scope

Increasing the “perennialization” of agriculture in the US Midwest is essential to improving agricultural resilience to climate change and addressing concerns regarding water quality and regulation, decline of pollinators, control of pests and pathogens, lack of diversity, and C emissions (Boody et al., 2005; Jordan and Warner, 2010; Asbjornsen et al., 2013). Perennial biomass production in agroforestry systems has been promoted as one approach to perennialization that could address these pressing environmental concerns, and may also increase productivity from marginal agricultural lands, reduce risk through a more diversified approach to crop production, and support emerging bioenergy, biofuels, and bioproducts markets (Thelemann et al., 2010; Holzmueller and Jose, 2012; Jose and Bardhan, 2012; Bardhan and Jose, 2014). However, little is known about the capacity of agroforestry systems to produce biomass feedstocks and associated ecosystem services, such as C sequestration and nutrient accumulation. Competition between trees and companion crops in agroforestry can reduce productivity to a point where mixed-culture is not economically justified. Understanding tree-crop interactions improves our ability to design systems to reduce competitive effects and increase yield while maintaining the positive benefits of mixed-culture. Research is needed to identify appropriate species combinations and other design characteristics that minimize competition and maximize productivity and ecosystem services in agroforestry systems for the US Midwest (Holzmueller and Jose, 2012; Jose and Bardhan, 2012).

1.2 Objectives

Three studies were conducted to address some of the gaps in knowledge about the potential for biomass feedstock production in agroforestry systems described in the preceding section. All studies were conducted in an alley cropping agroforestry system designed for biomass feedstock production. The system was comprised of short-rotation woody crops (SRWC), hybrid poplar (*Populus maximowiczii* x *P. nigra* 'NM6') and shrub willow (*Salix purpurea* 'Fish Creek'), paired with herbaceous alley crops switchgrass (*Panicum virgatum* L.), prairie cordgrass (*Spartina pectinata* Bosc ex Link), 'Rush' intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey cv. Rush), and an eleven species native polyculture. The objectives of the first study were to determine the best pairing of perennial woody and herbaceous crops to maximize biomass yield and nutrient uptake in alley cropping systems at three Minnesota sites, and to understand how woody and herbaceous biomass crop yields vary with distance from the tree-crop interface. The objectives of the second study were to: 1) quantify root biomass C and root nutrient accumulation of alley cropped SRWC and herbaceous perennial crops; 2) determine the spatial distribution and relative allocation of belowground biomass C within alley systems; and 3) quantify changes in SOC and system C sequestration (root biomass C + SOC) associated with alley cropped SRWC and herbaceous perennial crops. The objectives of the third study were to evaluate the availability of primary growth resources (light, water, N) in alley cropping systems, and to determine which resources best explained observed patterns in herbaceous crop yield at two environments in Minnesota.

1.3 Dissertation organization

This dissertation consists of three research chapters, along with a general introduction, this chapter (Chapter 1) and general conclusions chapter (Chapter 5). One research chapter is devoted to each of the three studies described in the preceding section. Chapter 2 is titled “*Species pairing and edge effects on biomass yield and nutrient uptake in perennial alley cropping systems*” and has been submitted to *Agronomy Journal*. Chapter 3 is titled “*Carbon sequestration and nutrient accumulation potential of perennial biomass alley cropping systems*” and will be submitted to *Agriculture, Ecosystems, and Environment*. Chapter 4 is titled “*Biophysical interactions in shrub willow – grassland alley cropping systems*” and will be submitted to *Agroforestry Systems*.

Authors on the research chapters include Joshua D. Gamble, Craig C. Sheaffer, Gregg A Johnson, Donald L. Wyse, Diomedes S. Zamora, and Dean A. Current. Mr. Gamble designed and implemented the experiments, collected and analyzed the data, and wrote the chapters. Drs. Sheaffer, Wyse, Johnson, and Current provided oversight and input for the design, implementation, analysis, and writing of the research. Dr. Zamora provided input for the analyses described in Chapter 4. Throughout this dissertation I refer to “we” or “our” rather than “I” or “my” in reference to co-authorship.

Chapter 2 : Species pairing and edge effects on biomass yield and nutrient uptake in perennial alley cropping systems

Abstract

We measured biomass yield, nutrient uptake, and edge effects on productivity in alley cropping agroforestry systems consisting of four herbaceous perennial crops and two short-rotation woody crops (SRWC) at three sites in Minnesota. Alley cropping with NM6 poplar (*Populus maximowiczii* x *P. nigra* 'NM6') maximized biomass yields at Empire (13.5 Mg ha⁻¹ yr⁻¹) and Granada (9.6 Mg ha⁻¹ yr⁻¹), irrespective of herbaceous crop. At Fairmont, yields were similar for NM6 poplar and Fish Creek willow (*Salix purpurea* 'Fish Creek') alley systems (average of 7.0 Mg ha⁻¹ yr⁻¹). NM6 poplar – intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey cv. Rush) systems had among the highest N, P, and K uptake at Empire (477, 62, and 301 kg ha⁻¹, respectively). At Granada, NM6 poplar – prairie cordgrass (*Spartina pectinata* Bosc ex Link) and NM6 poplar – native polyculture systems had among the highest nutrient uptake. At Fairmont, N and P uptake were maximized in Fish Creek willow – native polyculture systems (140 and 19 kg ha⁻¹, respectively), while K uptake was similar in willow – and poplar – native polyculture systems (68 kg ha⁻¹). Edge effects on productivity were not consistent across sites, species, and years, though we show that edge effect is an important consideration for accurately quantifying SRWC and herbaceous crop yield in alley cropping systems. Appropriate species combinations for maximizing yield and nutrient uptake in perennial biomass alley cropping systems vary based on site characteristics.

2.1 Introduction

Recently, increasing the “perennialization” of agriculture in the US Midwest has become an issue of national attention due to a variety of concerns such as water quality and regulation, decline of pollinators, control of pests and pathogens, C emissions, and resilience to climate change (Boody et al., 2005; Jordan and Warner, 2010; Asbjornsen et al., 2013). Agroforestry has been promoted as one approach to perennialization that can help address environmental concerns and provide a wide range of goods and services to society, including lignocellulosic feedstocks for bioindustrial applications (Holzmueller and Jose, 2012; Ehret et al., 2014; Bardhan and Jose, 2014). In the US Midwest, agroforestry systems such as alley cropping and riparian buffers show particular promise for biomass feedstock production due to their potential adaptability, both spatially and logistically, to modern farming systems (Holzmueller and Jose, 2012; Jose and Bardhan, 2012). Widely spaced tree rows in alley cropping systems lend well to the high degree of mechanization present in modern farming, while perennial biomass crop-based buffers strips could fit strategically into lowland or floodplain agricultural sites to protect waterways. However, little is known about how spatially integrating woody and herbaceous perennial crops will affect biomass production.

Riparian buffers composed of woody and herbaceous perennial vegetation can sequester substantial nutrient loads (Schultz et al., 1995; Lee et al., 2003; Schoonover et al., 2005). However, nutrients sequestered in riparian vegetation are, over time, mineralized through decomposition and could enter waterways (Vanek, 1991; Jaynes and Isenhardt, 2014). Therefore, the efficiency of nutrient removal could be enhanced through repeated harvest of aboveground vegetation. Certain perennial biomass crops could be well suited to this

niche due to their rapid growth rates, high productivity in lowland or floodplain agricultural sites, and nutrient export offsite with repeated harvesting (Pallardy et al., 2003; Tufekcioglu et al., 2003; Lee et al., 2009; Thelemann et al., 2010; Fortier et al., 2010b; Wilson et al., 2014; Zilverberg et al., 2014).

Short rotation woody crops (SRWC) such as hybrid poplar (*Populus* spp.) and shrub willow (*Salix* spp.) are ideal for biomass production and nutrient sequestration in temperate regions because they are easily propagated, quick to establish, fast growing, high yielding, and can be harvested many times before replanting. Poplars and willows have been used to remediate nutrients in municipal wastewater (Holm and Heinsoo, 2013), biosolids (Heller et al., 2003; Börjesson and Berndes, 2006; Felix et al., 2008), landfill leachate (Zalesny et al., 2008), and have been used in riparian buffers to intercept nutrient flows from upland sources (Tufekcioglu et al., 2003; Lee et al., 2003; Young and Briggs, 2005, 2007; Fortier et al., 2010a; Jaynes and Isenhardt, 2014). Herbaceous perennial crops such as native grasses and native grass-forb-legume polycultures have also been promoted as suitable biomass crops for lowland or marginal sites (Wilson et al., 2014; Zilverberg et al., 2014), though less is known about their nutrient sequestration potential since they are often promoted and utilized as low-input crops. However, data on nutrient removal from harvested plant material is important for effective design and management of any cropping system, including biomass systems. In buffer strips that are used to manage nutrients from agricultural fields, or systems designed to manage wastes like biosolids or manure, crops with low nutrient use efficiency and high nutrient removal are desirable.

Few studies have assessed the potential of perennial biomass crops as nutrient sinks in the riparian zone. Fortier et al. (2010a) assessed nutrient accumulation in a variety of poplar clones in southern Quebec, while Tufekcioglu et al. (2003) assessed N accumulation in poplar and switchgrass buffers in Iowa. In both cases, the buffers were not explicitly managed as biomass production systems, though each showed greater potential for nutrient accumulation in poplar or switchgrass buffers relative to cool-season grass or unmanaged buffers. To our knowledge, no studies have assessed the biomass production and nutrient uptake potential of integrated woody and herbaceous perennial biomass cropping systems. Little is known about appropriate species combinations that optimize productive potential of SRWC and herbaceous biomass crops when grown in agroforestry configurations. The objectives of this research were to determine the best pairing of perennial woody and herbaceous crops to maximize biomass yield and nutrient uptake in alley cropping systems at three Minnesota sites, and to understand how woody and herbaceous biomass crop yields vary with distance from the tree-crop interface.

2.2 Materials and Methods

2.2.1 Study sites and experimental design

The study was established in May and June 2010 at three privately owned farm fields in Minnesota. Sites were located on floodplains near Granada and Fairmont, MN and on a stream terrace near Empire, MN (Figure 2.1). The previous cropping system at Granada was a corn (*Zea mays* L.)—soybean (*Glycine max* [L.] Merr.) rotation; at Empire, continuous corn; and at Fairmont, alfalfa (*Medicago sativa* L.). Annual spring applications of municipal biosolids at Empire from 2000 to 2010 prior to establishment of the experiment resulted in very high levels of P and K at this site (Table 2.1).

The experimental design was a randomized complete block in a split-plot arrangement. Three replicates were established at Empire and Granada, and two replicates were established at Fairmont. Two SRWC were randomly assigned to 38.1 by 36.6 m whole plots and four herbaceous crops were randomly assigned to 12.2 by 9.1 m subplots within each whole plot. Woody crops were established in multi-row strips, with a 15.2 m alleyway between strips and a 1.5 m unsown buffer between trees and herbaceous crops on each side of the alley (Figure 2.2). Woody crops were ‘NM6’ poplar ‘Fish Creek’ willow. Herbaceous crops were a local, lowland-ecotype switchgrass (*Panicum virgatum* L.), prairie cordgrass, a mixture of Pioneer Brand ‘54V48’ alfalfa and ‘Rush’ intermediate wheatgrass, and an eleven species native tallgrass-forb-legume polyculture (Table 2.2). A local ecotype prairie cordgrass was planted at Fairmont and Granada. At Empire, ‘Red River’ prairie cordgrass was planted. Switchgrass and native polyculture seed were grown or collected in Minnesota and were purchased from a commercial seed company (Feder Prairie Seed Company, Blue Earth, MN). Alfalfa and intermediate wheatgrass seed were also purchased from a commercial seed company (Albert Lea Seed House, Albert Lea, MN), and the initial mixture was 64 % alfalfa and 36 % intermediate wheatgrass by seed count.

Willows were established following guidelines of Abrahamson et al., (2002) in three twin rows on either side of the alley, with 75 cm between rows, 60 cm between plants within a row, and 150 cm between twin rows. This resulted in a planting density of 7,596 willows ha⁻¹ when accounting for area occupied by herbaceous crops. Poplars were established in five rows on either side of the alley, at 1.2 m within and between rows (Debell et al., 1996, 1997; Benomar et al., 2012). This resulted in planting density 3,402 poplars ha⁻¹

when accounting for area occupied by herbaceous crops. Following established management guidelines, willows were coppiced following the first growing season, while poplars were not coppiced (Herve and Ceulemans, 1996; Volk, 2002). Pre-coppice willow biomass was not added to first rotation biomass estimates.

By late 2011, alfalfa-intermediate wheatgrass subplots at Empire and Granada contained no alfalfa. Therefore, this treatment will be referred to hereafter as “intermediate wheatgrass”. At Fairmont, no herbaceous treatments were successfully established in 2010. In 2011, the native polyculture was established in all subplots at Fairmont; therefore results from this site do not include switchgrass, prairie cordgrass, or intermediate wheatgrass treatments. Native polyculture biomass harvest was not conducted at Fairmont until 2012 and 2013. In mid-June 2012 and 2013, switchgrass, prairie cordgrass, and intermediate wheatgrass plots at Granada and Empire were sprayed with 0.32 L a.i. ha⁻¹ clopyralid [3,6-dichloro-2-pyridinecarboxylic acid, monoethanolamine salt] to control broadleaf weeds. A detailed description of crop establishment and management in 2010 and 2011 can be found in Gamble et al. (2014).

2.2.2 Field and laboratory methods

In November 2013, willow yield was determined from a 2.8 m² area (1 twin row comprising six trees) along the tree-crop interface (edge rows) and in the center of the hedgerow (interior rows) on each side of the alley for each willow – herbaceous crop combination, for a total of 192 trees in each replicate. Poplar yield was determined by harvesting a 4.5 m² area (3 trees) in each of the first (edge), second, and third (center) rows from the tree-crop interface on each side of the alley for each poplar – herbaceous

crop combination, for a total of 144 trees in each replicate. Trees were chipped and weighed to determine aboveground wet leafless biomass yield. Wood chip subsamples were then dried to a constant weight at 60 °C to calculate biomass yield on a dry weight basis.

Biomass yield of herbaceous alley crops was measured in fall of 2010 - 2013 following a killing frost (0° C). Herbage biomass was harvested from a 2.78 m² area (0.91 x 3.05 m) with a flail-type forage harvester to a 10 cm stubble height in the alley center (7.6 m from the nearest tree) and at both edge positions (2.4 m from tree rows). In 2013, two additional samples were collected; each located between the alley center and alley edge positions at 4.8 m from tree rows. In all years, herbaceous samples were weighed following harvest to obtain fresh weights and 1000 g sub-samples were dried to a constant weight at 60 °C to calculate biomass yield on a dry weight basis. Weed biomass was manually separated from crop biomass in dried sub-samples to obtain weed free biomass yield estimates.

For both woody and herbaceous biomass, dried subsamples were ground with a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) to pass a 1 mm screen, and then reground with a cyclone mill. Nutrient concentrations were determined with inductively coupled plasma mass spectroscopy following digestion with HNO₃ and H₂O₂ (Gavlak et al., 2003), except for N, which was determined via dry combustion and CHNS analyzer (Model 2400 Perkin – Elmer Inc., Waltham, MA) at a commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH).

Herbaceous biomass yield and nutrient uptake are reported on a subplot basis (Mg or kg ha⁻¹) for each year and were then summed over years to obtain cumulative biomass yield and nutrient uptake. Biomass yield and nutrient uptake of SRWC are reported on a per-tree basis, and were then converted to a whole plot basis. These estimates were weighted according to the area that each component occupied within the alley system as follows: 51% of land area in SRWC for poplar systems, 53% of land area in SRWC for willow systems, and 47 to 49% of land area in herbaceous crops depending on SRWC. These weights assume the 1.5 m unsown buffer on either side of the alley as part of the woody crop area. Weighted values were then summed to obtain cumulative alley system yield and nutrient uptake for each tree-crop combination (alley system yield and alley system nutrient uptake, hereafter).

2.2.3 Statistical analysis

Response variables included woody and herbaceous aboveground dry biomass yield, nutrient (N, P, and K) concentrations, nutrient uptake, as well as system (woody + herbaceous) yield and nutrient uptake. For each response variable, mixed effects models were used to test for main effects and interactions between whole plot treatments (SRWC), subplot treatments (herbaceous crop), and sampling distance from the tree-crop interface. All analyses were conducted by site, or year and site where applicable. Effects of replicates were considered random. To account for spatial autocorrelation occurring as a result of sampling at multiple, systematic locations within the same plot or subplot, we included a random effect for sampling distance, nested within subplot or plot. Where a significant effect of distance from the tree row was observed on herbaceous biomass yield, relative yields were calculated as the ratio of yield at a given distance to yield in

the center of the alley. Biomass yield of second and center row poplar was similar at all sites. Therefore, these row positions were not considered separately and are grouped as “interior rows” hereafter. Maximum likelihood ratio tests using nested null and alternative hypothesis models were performed to verify the significance of fixed effects (Pinheiro and Bates, 2000; Johnson and Omland, 2004). Where significant effects were observed, Tukey’s test was used for mean separation at $\alpha = 0.05$. Mixed effects analyses were conducted with the ‘lme4’ package (Bates et al., 2015) in program R (R Core Team, 2013).

2.3 Results

2.3.1 Environments

Rainfall amount and mean daily temperature varied considerably at each site from 2010 to 2013 (Figure 2.3). During the establishment year, growing season (April to October) precipitation was 18% above normal at Empire and 34% above normal at Fairmont and Granada, despite deficits in April and May. Over the following three years (2011 – 2013), growing season precipitation was 18 to 37% below normal at Fairmont and Granada, with the largest deficits generally occurring in August and October. At Empire, growing season precipitation was about normal in 2012, but 27% and 20% below normal in 2011 and 2013, respectively. Mean monthly temperatures at all sites from April to October were 2% to 7% above normal from 2010 to 2012, 4% below normal at Empire in 2013, and about normal at Fairmont and Granada in 2013.

Flooding occurred at Granada in all years of the study, with the most severe in September 2010. At this time, one replicate was fully submerged for 21 days and partially

submerged for an additional 15 days, while the other two replicates were submerged for about 15 days. Other flood events at this site occurred in late May or early June of each year, with the exception of 2012, and lasted from 3 to 7 days. In June 2010, one replicate was submerged for approximately 7 days at Empire, while both replicates at Fairmont were submerged for approximately 14 days.

2.3.2 *Alley system yield and nutrient uptake*

Alley system yield was 35% and 23% greater for poplar than willow alley cropping systems at Empire and Granada, respectively (Table 2.3). Alley system yield did not vary based on herbaceous crop type at either site, and herbaceous crop yields were similar in poplar versus willow alley cropping systems. At Fairmont, alley system yield averaged 28.1 Mg ha⁻¹ (7.0 Mg ha⁻¹ yr⁻¹) and was similar for both poplar – native polyculture and willow– native polyculture alley cropping systems.

At Empire, N uptake in harvested biomass was greater for poplar – intermediate wheatgrass (477 kg N ha⁻¹) and poplar – native polyculture systems (444 kg N ha⁻¹) than all other systems except poplar – prairie cordgrass (396 kg N ha⁻¹, L ratio = 13.13, P = 0.004). The poplar – switchgrass system had the lowest N uptake of all poplar systems (249 kg N ha⁻¹), which was similar to all willow – herbaceous crop combinations (average of 259 kg N ha⁻¹). At Granada, N uptake was 25% higher in poplar than willow alley systems, irrespective of herbaceous crop.

At Empire, P uptake in harvested biomass was greater for poplar – intermediate wheatgrass (61.8 kg P ha⁻¹) and poplar – native polyculture (61.2 kg P ha⁻¹) than all other systems except poplar – prairie cordgrass (52.0 kg P ha⁻¹, L ratio = 11.19, P = 0.011).

The poplar – switchgrass system had the lowest P uptake of all poplar systems (33.1 kg P ha⁻¹), which was similar to the average of all willow – herbaceous crop combinations (38.6 kg P ha⁻¹). At Granada, P uptake was 30% greater in poplar than willow alley systems when averaged across herbaceous crop types. Both poplar – and willow – native polyculture systems had greater P uptake (average of 34.7 kg ha⁻¹) than those with intermediate wheatgrass (average of 25.1 kg ha⁻¹; L ratio = 20.03, $P < 0.001$). This was the only difference observed in alley system P uptake based on herbaceous crop type.

At Empire, K uptake in harvested biomass was 51% greater in poplar than willow alley systems, when averaged across herbaceous crop types. In both poplar and willow alleys, K uptake was greater with intermediate wheatgrass (234.4 kg ha⁻¹), native polyculture (220.2 kg ha⁻¹), and prairie cordgrass (221.5 kg ha⁻¹) than with switchgrass (114.9 kg ha⁻¹, L ratio = 18.48, $P < 0.001$). At Granada, K uptake was 33% higher in poplar than willow alley systems, and was greater in alleys with prairie cordgrass (140.2 kg ha⁻¹) and the native polyculture (126.3 kg ha⁻¹) than with intermediate wheatgrass (97.8 kg ha⁻¹) and switchgrass (101.6 kg ha⁻¹, L ratio = 20.90, $P < 0.001$) for both poplar and willow.

At Fairmont, N and P uptake were 18% and 16% higher in willow – native polyculture than poplar – native polyculture systems, respectively. Potassium uptake averaged 68.8 kg ha⁻¹ and was similar for poplar and willow alley systems.

2.3.3 Woody biomass yield and nutrient uptake

Poplars consistently had greater biomass per tree than willows at all sites and row positions. At Empire, willow and poplar biomass was 48% and 197% greater, respectively, for edge row than interior row trees (Table 2.4). At Granada, poplar

biomass was 82% greater in edge than in interior rows, but there was no difference between row positions for willow. Tree biomass was not influenced by herbaceous alley crop type at either site. At Fairmont, there was no effect of row position on tree biomass and poplar trees had 82% greater biomass than willows.

Nitrogen concentration of woody biomass was similar for poplars and willows and for all row positions at Fairmont and Granada, with averages of 4.3 g kg⁻¹ and 4.1 g kg⁻¹, respectively. At Empire, N concentration was greater in edge row (5.6 g kg⁻¹) than interior row poplar trees (4.7 g kg⁻¹, *L* ratio = 8.87, *P* = 0.003). There was no difference between row positions for willows, with an average N concentration of 4.8 g kg⁻¹.

Phosphorus concentration of harvested woody biomass was similar for willows and poplars at all three sites, with averages of 0.68 g kg⁻¹ at Empire, 0.44 g kg⁻¹ at Fairmont, and 0.61 g kg⁻¹ at Granada. There was no effect of row position on P concentration of aboveground woody biomass at any site. At Empire, K concentration was greater in poplar (2.6 g kg⁻¹) than willow (1.7 g kg⁻¹) biomass. Similarly, poplars had greater K concentration (2.4 g kg⁻¹) than willows (1.6 g kg⁻¹) at Granada. At Fairmont, there was no difference in K concentration of poplar (1.8 g kg⁻¹) and willow (1.4 g kg⁻¹) biomass.

At Empire, nutrient uptake was 197 – 236% greater in edge row than interior row poplars and 43 – 52% greater in edge row than interior row willows. At Granada, nutrient uptake was 75 – 86% greater in edge row than interior row poplars, whereas there was no difference in row positions for willows. At Fairmont, there were no differences in nutrient uptake between row positions for either SRWC, though uptake of N, P, and K were 75%, 73%, and 123% greater for poplar than willow trees.

2.3.4 Herbaceous biomass yield and nutrient uptake

Total 4-year biomass yields at Granada and Empire were highest for the native polyculture and prairie cordgrass (Table 2.5). At Granada, herbaceous biomass yield varied by crop within all years. Prairie cordgrass yielded less than all other crops in 2010, while intermediate wheatgrass did so in all remaining years. After the establishment year, prairie cordgrass and the native polyculture had the highest yields at Granada. Switchgrass yields declined after 2011, and native polyculture yield was similar in all years. Intermediate wheatgrass yield declined substantially after the establishment year due to repeated flooding each year of the study. Herbaceous crop yields did not differ between poplar and willow alleys. From 2012 to 2013, average annual biomass yield of the native polyculture at Fairmont was $5.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$.

At Empire, herbaceous biomass yield also varied by crop within each year, except in 2010. In 2011, prairie cordgrass and the native polyculture yielded more than other crops. Switchgrass yield declined each year, and yielded less than all other crops from 2011 to 2013. Yield was similar among the three other crops in 2012, but in 2013 the native polyculture yielded less than intermediate wheatgrass and the native polyculture. With the exception of switchgrass, all crop yields increased from 2010 to 2011, and then declined from 2011 to 2012. Yield of prairie cordgrass and the native polyculture continued to decline from 2012 to 2013. Switchgrass yield decline at Empire coincided with annual increases in weed biomass (data not shown), despite annual herbicide applications from 2011 – 2013 to control broadleaf weeds. We observed delayed emergence timing of switchgrass compared to other species, which resulted in

opportunities for early emerging weed species to dominate and interfere with switchgrass growth.

Herbaceous crop yields did not differ between poplar and willow alleys at any of the three sites. However, in 2013, biomass yield of all herbaceous crops varied with distance from the tree-crop interface at Empire (L ratio = 32.73, $P < 0.001$). Yields closest to tree rows (2.4 m west and 2.4 m east) were 42% and 34% lower than yield in the center of the alley (7.6 m; Figure 2.4), while yields were similar for the central three distances sampled (4.8 m west, 7.6 m, 4.8 east). Yield was 27% lower at 2.4 m west than at 4.8 m west, while yields at 2.4 m east and 4.8 m east did not differ. No effects of distance from the tree-crop interface were observed on herbaceous crop yield at Granada or Fairmont.

From 2010 to 2013, average concentrations of N and K in herbaceous biomass varied by crop at both Empire and Granada. Nitrogen concentration was greatest for intermediate wheatgrass at both sites (Table 2.6). Potassium concentration was greatest in intermediate wheatgrass at Empire. At Granada, K concentration was lower for switchgrass than for intermediate wheatgrass and prairie cordgrass, while K concentration in the native polyculture was similar to that of all other crops. Phosphorus concentration varied by crop at Empire, and was greater for intermediate wheatgrass and the native polyculture than other crops. Phosphorus concentration was similar for all crops at Granada, with an average of 1.5 g P kg⁻¹. At Fairmont, P and K concentrations in the native polyculture were similar to those of the native polyculture at Granada, but N concentration was higher than at Granada.

Nutrient uptake also varied by crop at both Empire and Granada. At Empire, switchgrass had the lowest N, P, and K uptake of all herbaceous crops. Nitrogen uptake was greatest for intermediate wheatgrass, while P and K uptake were similar for intermediate wheatgrass, the native polyculture and prairie cordgrass. At Granada, N uptake was greater for prairie cordgrass than intermediate wheatgrass, which was the only difference in N uptake among herbaceous crops. Intermediate wheatgrass had lower P uptake than all other crops, and the native polyculture and prairie cordgrass had the highest K uptake. At Fairmont, N, P, and K uptake in the native polyculture were generally low, since herbaceous biomass was only harvested in 2012 and 2013 at this site.

2.4 Discussion

2.4.1 Alley system yield and nutrient uptake

We found that different species pairings maximized yield and nutrient uptake in perennial biomass alley cropping systems at each of three Minnesota sites. Yield and nutrient uptake were maximized by NM6 poplar – intermediate wheatgrass alley cropping systems at Empire. At Granada, yield and N uptake were maximized in NM6 poplar alleys, regardless of herbaceous crop. Uptake of P and K were greatest in alley systems with the native polyculture or prairie cordgrass, regardless of SRWC. At Fairmont, yield and K uptake were similar Fish Creek willow – and NM6 poplar – native polyculture alley cropping systems, though P and K uptake were greater in the former. At each site, woody biomass comprised 71% to 84% of system yield across sites and treatments, suggesting that SRWC selection is important for maximizing yields in similarly designed alley systems. NM6 poplar performed better than Fish Creek willow at two of three sites. However, we suspect that Fish Creek willow will have greater long-term yields due to the

poor coppice ability of NM6 poplar (Volk, 2002). In our analysis, herbaceous crop selection had no effect on system level yield, though differences in herbaceous crop yield were observed on the subplot level. At Empire and Granada, the native polyculture and prairie cordgrass were among the highest yielding crops over the four years of study, suggesting these crops are well suited to alley cropping.

Combined SWRC and herbaceous biomass yields of alley cropping systems ranged from 6.5 to 13.5 Mg ha⁻¹ yr⁻¹ for poplar alley systems and from 7.6 to 10.2 Mg ha⁻¹ yr⁻¹ for willow alley systems. These yields compare well to other biomass production systems. For comparison, shrub willow – grassland alley cropping systems yielded only 4 Mg ha⁻¹ yr⁻¹ in Lower Saxony, Germany. Monocultures yields of herbaceous biomass crops in Minnesota range up to 11.6 Mg ha⁻¹ yr⁻¹ for prairie cordgrass, 9.2 Mg ha⁻¹ yr⁻¹ for switchgrass (*P. virgatum* L. cv. Sunburst), and 7.6 Mg ha⁻¹ yr⁻¹ for native polycultures (Johnson et al., 2013). Woody crop yields range up to 18.3 Mg ha⁻¹ yr⁻¹ for SX67 willow (*S. miyabeana*), 12.5 Mg ha⁻¹ yr⁻¹ for ‘9882-41’ willow (*S. purpurea*), 13.2 Mg ha⁻¹ yr⁻¹ NM6 poplar, and 15.3 Mg ha⁻¹ yr⁻¹ for D125 cottonwood (*P. deltoides*; Thelemann et al., 2010; Johnson et al., 2013). However, most SRWC yields are derived from small plot studies where edge effects on yield were unaccounted for. We demonstrated here that edge effect is an important consideration for accurately quantifying SRWC yield in small plots. While alley system yield at Granada and Fairmont were lower than many previous reports for SRWC monocultures, these sites were repeatedly flooded and were also managed with no fertilizer inputs. At Empire, where biosolids were applied annually for 11 years prior to establishment of the experiment, our alley system yields were similar to monoculture yields of SRWC.

In terms of nutrient uptake, our alley systems compare well to other riparian vegetation. For instance, Peterjohn and Correll (1984) estimated vegetation N uptake of riparian forests in Maryland as $77 \text{ kg N ha}^{-1} \text{ y}^{-1}$. This is more than we observed for any alley system at Granada or Fairmont, but less than our observed maximum N uptake rates of 111 and $119 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for poplar – native polyculture and poplar – intermediate wheatgrass alley systems at Empire. In southern Quebec, Fortier et al. (2010a) found nutrient uptake in hybrid poplar riparian buffers to range from $15 - 128 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $1.7 - 13.7 \text{ kg P ha}^{-1} \text{ yr}^{-1}$, and $8 - 97 \text{ kg K ha}^{-1} \text{ yr}^{-1}$ after 6 years of growth, depending on site and clone. The maximum P uptake we observed was in poplar – native polyculture and poplar – intermediate wheatgrass alley systems at Empire, both at just over $15 \text{ kg P ha}^{-1} \text{ yr}^{-1}$. The maximum K uptake observed in the present study was for alley systems (poplar or willow) that included intermediate wheatgrass, at $59 \text{ kg K ha}^{-1} \text{ yr}^{-1}$, which is lower than values reported by Fortier et al. (2010a). However, they include nutrients in live leaf biomass, which explains higher maximum N and P uptake estimates than in the present study. Tufekcioglu et al. (2003) estimated immobilization of N in riparian poplar (*Populus × euroamericana* ‘Eugenei’) and switchgrass vegetation as 37 and $16 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, respectively, which is lower than our estimates for these crops.

2.4.2 Woody biomass yield and nutrient uptake

Poplar trees had up to 212% greater aboveground woody biomass than willow trees at our sites. Similarly, Thelemann et al. (2010) reported that NM6 poplar had over 250% greater biomass per tree than shrub willow 9882-41 (*Salix purpurea*) when planted at similar densities. On an area basis, poplar yield was greater than willow at Empire and Granada, despite being planted at less than half the planting density. Volk (2002) found that with

identical planting densities, NM6 poplar produced more biomass than a variety of willow clones at three sites in New York by the end of a four year rotation. In contrast, yield on an area basis was greater for willow than poplar at Fairmont, as individual tree biomass only differed by 82%. It's not clear why yield differences between SRWC were reversed at Fairmont compared to Empire and Granada, though this was the only site in which no edge effects on poplar yield were observed.

Woody biomass yield varied with distance from the tree-crop interface for both SRWC at Empire but only for NM6 poplar at Granada. No effect of distance was observed at Fairmont. Quantifying these edge effects is important because they greatly influenced estimates of overall yield. For instance, at Empire, edge row NM6 poplar trees had 197% greater biomass than interior row trees. Accounting for this edge effect resulted in a 79% higher estimate of yield compared to utilizing only the interior rows when extrapolating to an area-basis. At each site, however, we observed different effects of distance from the tree-crop interface on tree yield (large, moderate, or no effect). The lack of edge effect on willow yield at Granada and Fairmont is consistent with the findings of Ehret et al. (2014), who reported that yield of shrub willow 'Tordis' (*Salix schwerinii* x *S. viminalis* x *S. vim.*) did not differ between interior and edge rows in grassland – willow alley cropping systems. We hypothesize that alley orientation plays a role in the presence and magnitude of edge effects on SRWC yield; however, we were unable to test this hypothesis with our experimental design.

Patterns of nutrient uptake in SRWC largely paralleled patterns of productivity, with one notable exception. At Empire, poplar trees in edge rows not only had greater biomass than those in interior rows, but also had 19% higher N concentration. Positive

relationships between the concentration of N in poplar branches and N supply rate have been observed by others (Fortier et al., 2010a). In addition, higher nitrification rates and mineral N availability have been observed within crop alleys compared to tree rows of hybrid poplar alley systems (Rivest et al., 2010). Thus, increased N availability for edge row versus interior row trees may explain this observed difference in N concentration.

2.4.3 Herbaceous biomass yield and nutrient uptake

Average annual herbaceous crop yields in this study were similar to reports of yields of other low-input perennial herbaceous crops in the region, but were lower, in general, than yields of fertilized herbaceous perennial biomass crops. For example, others have reported yields of mature prairie cordgrass to range from 9.1 to 12.7 Mg ha⁻¹ yr⁻¹ in fertilized monocultures (Boe et al., 2009; Johnson et al., 2013), while yield in our alley systems ranged from 6.0 to 7.1 Mg ha⁻¹ yr⁻¹. Reported yields of mature native polyculture bioenergy crops in Minnesota, which are typically unfertilized, vary widely from 0.5 to 7.6 Mg ha⁻¹ yr⁻¹ depending on site characteristics and species composition (Mangan et al., 2011; Gillitzer et al., 2012; Jungers et al., 2013, 2015a; Johnson et al., 2013). At Empire and Granada, native polyculture yield was near the upper extent of this range, at 6.1 to 6.3 Mg ha⁻¹ yr⁻¹. At Empire, intermediate wheatgrass yield was among the highest yielding crops, averaging 5.5 Mg ha⁻¹ yr⁻¹ over the study duration, which is similar to the range of 4.4 to 5.2 Mg ha⁻¹ reported by Lee et al. (2009). At Granada, switchgrass yield averaged 4.5 Mg ha⁻¹ yr⁻¹, which is similar to other reports for low-input switchgrass (Mangan et al., 2011; Jungers et al., 2015a), but is substantially lower than yields of fertilized switchgrass cultivars in Minnesota (Thelemann et al., 2010; Johnson et al., 2013; Jungers et al., 2015c).

Within years, the effect of distance from the tree-crop interface was similar for all herbaceous crops at each site, suggesting that all crops were similarly impacted by the alley cropping environment. In 2013, competition with trees reduced herbaceous crop yield along the tree-crop interface at Empire. Furthermore, overall yields declined at this site from 2011 to 2013. This suggests that trees were outcompeting herbaceous crops for growth resources (light, water, nutrients) at this site. However, the precise mechanism(s) of this decline are unclear. At Granada, yield was consistently similar between the alley center and along the tree-crop interface, and overall yields remained relatively stable. This suggests little or no competition between trees and crops at this site. However, more research is needed to evaluate the role of tree-crop interactions on yield in these systems.

Maintaining vigorous stands of herbaceous crops is important as they contributed substantially to overall nutrient uptake and are an integral part of the potential benefits derived from the agroforestry system. Herbaceous crops only comprised 16% to 29% of alley system biomass yield across sites and treatments, but accounted for 22 – 45% of total N uptake, 39 – 49% of total P uptake, and 46 - 65% of total K uptake. Therefore, in terms of maximizing nutrient uptake, attention should be focused on maximizing herbaceous crop productivity and longevity in alley systems through species and cultivar selection, as well as through alley orientation and other design considerations. For instance, wider alleys would increase the area allocated to herbaceous crops and likely result in greater system nutrient uptake. However, this could reduce system yield due to reduced area allocated to SRWC. Tradeoffs in system design may be required to balance productivity with herbaceous crop longevity.

2.5 Conclusions

Different species pairings maximized alley cropping yield and nutrient at each of three Minnesota sites. At Empire, the NM6 poplar – intermediate wheatgrass system had among the highest yield and nutrient uptake of all alley cropping systems. At Granada, NM6 poplar – prairie cordgrass and NM6 poplar – native polyculture had among the highest yield and nutrient uptake. At Fairmont, Fish Creek willow – native polyculture systems generally outperformed NM6 poplar – native polyculture systems, though no other herbaceous crops were compared at this site. Thus, NM6 poplar was more productive than Fish Creek willow at two of three sites. Intermediate wheatgrass showed the greatest potential for N, P, and K uptake in alley cropping systems, though the native polyculture and prairie cordgrass also performed well and are better choices for sites with occasional flooding.

Our research also provides a basis for understanding the effects of spatially integrating herbaceous and woody biomass crops into an alley cropping system. Effects of distance from the tree-crop interface on SRWC and herbaceous crop productivity were not consistent across sites, species, and years. However, we found that quantifying edge effects on SRWC productivity in agroforestry systems is important for accurately quantifying system yield and nutrient uptake. Yield response to distance from the tree-crop interface was similar for all herbaceous crops at each site, and herbaceous crops yielded similarly in poplar versus willow alleys.

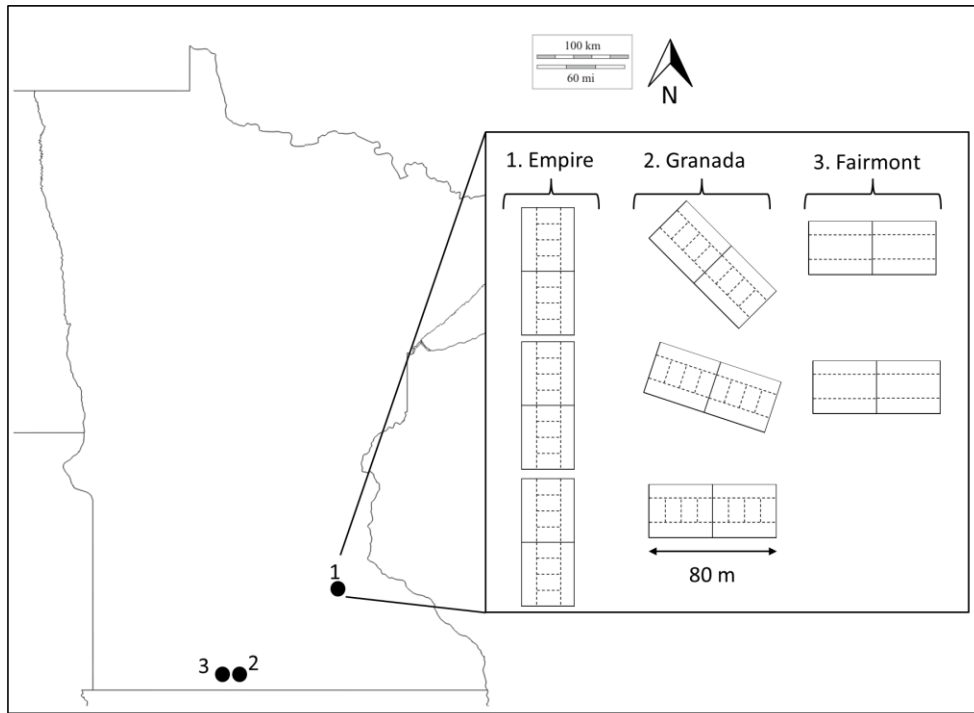


Figure 2.1. Location of study sites within Minnesota. Inset shows number and orientation of replicates at each site. For each replicate, whole plot boundaries are denoted by solids lines and subplot boundaries by dashed lines.

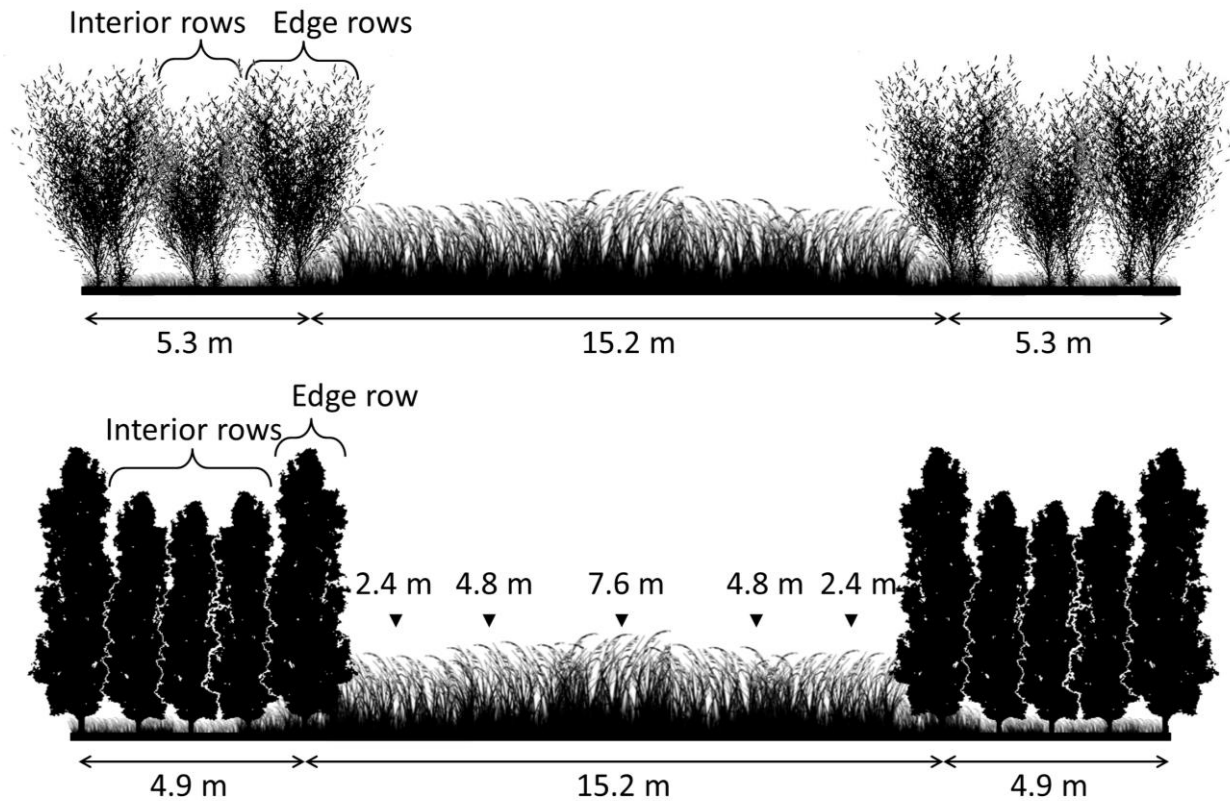


Figure 2.2. Fish Creek willow (top) and NM6 poplar (bottom) alley cropping system configuration and herbaceous crop sample locations with distance from tree rows. At Empire, sampling distances are denoted as either west or east of the center of the alley. At Granada and Fairmont, sampling distances are denoted as either north or south of the center of the alley.

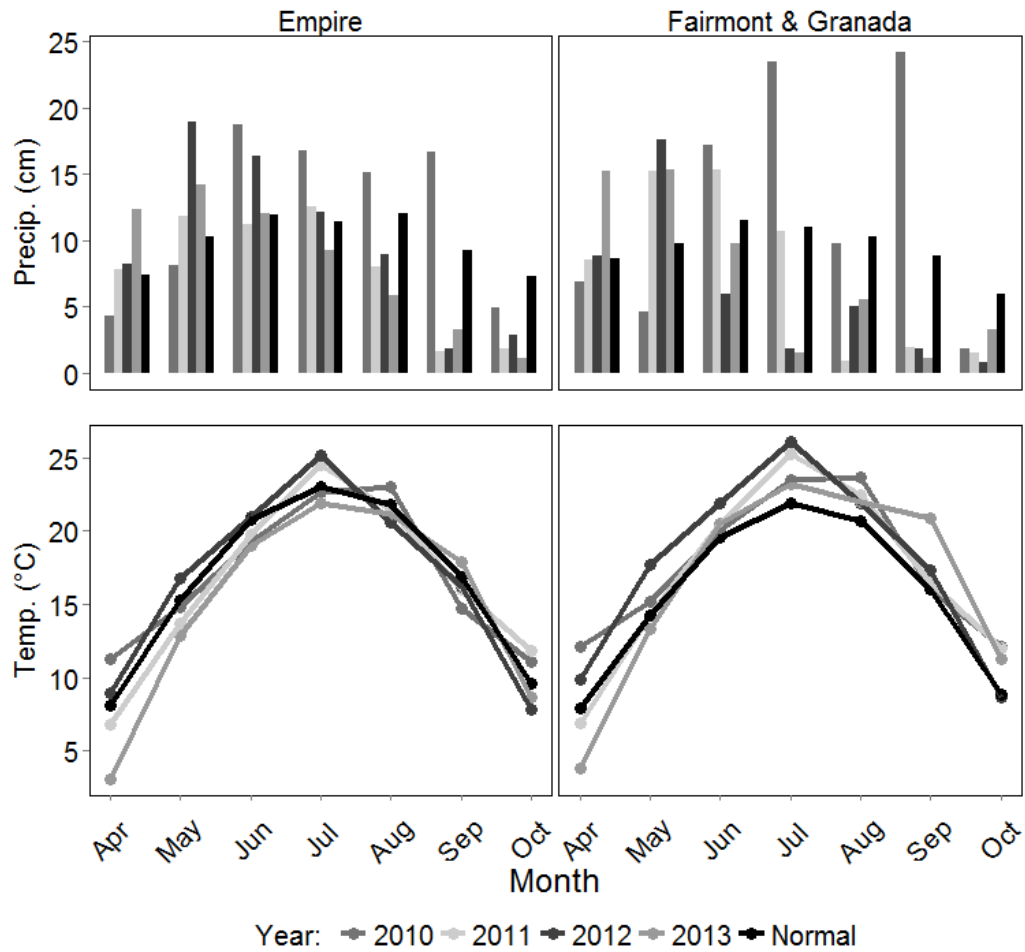


Figure 2.3. April to October monthly precipitation and mean daily temperature from 2010 to 2013, and 30-year climatological means, 1981 – 2010 (<http://www.ncdc.noaa.gov/cdo-web/datatools/normals>) observed for three environments in Minnesota. The nearest weather station was shared by Fairmont and Granada sites.

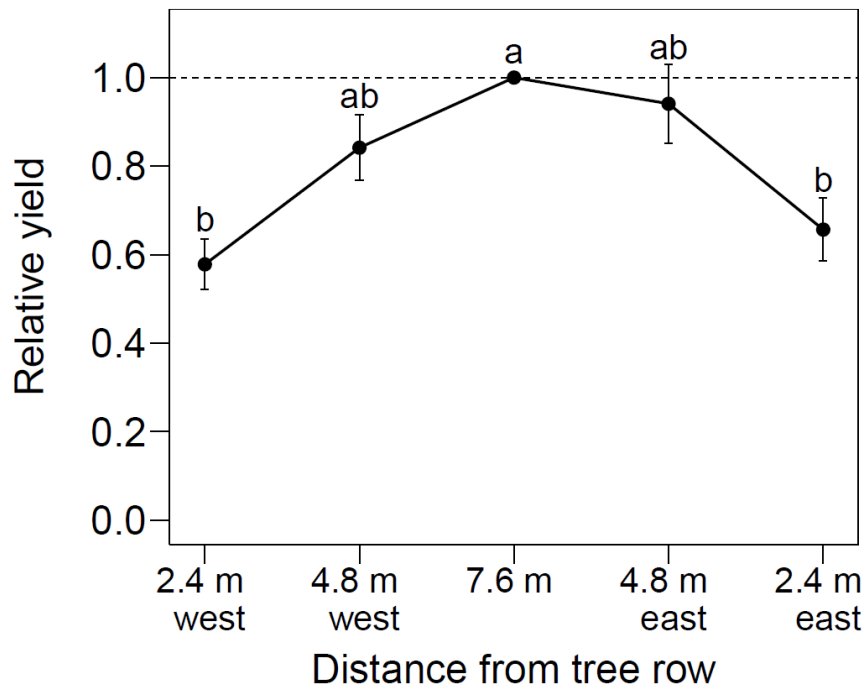


Figure 2.4. Relative yield of herbaceous biomass with distance from the tree-crop interface at Empire, MN in 2013. Yield in the center of the alley (7.6 m from tree row) is the reference (1.0) and is also denoted by the horizontal dashed line. Error bars represent one standard error of the mean. Points that share the same letter are not different, $\alpha = 0.05$.

Table 2.1. Selected site and soil characteristics at three Minnesota sites.

Characteristic	Empire	Fairmont	Granada
Latitude, Longitude	44.7178, -93.0975	43.7528, -94.4851	43.7575, -94.3465
Landform	Stream terrace	Floodplain	Floodplain
Soil classification	Cylinder loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic aquic hapludolls)	Coland loam (fine-loamy, mixed, superactive, mesic cumulic endoaquolls)	Coland loam (fine-loamy, mixed, superactive, mesic cumulic endoaquolls)
Soil organic matter (g kg ⁻¹) [†]	52	74	36
Soil pH	5.5	6.7	6.4
Soil total N (g kg ⁻¹)	2.6	3.3	1.4
Soil C/N	11.35	10.98	12.16
Soil NO ₃ ⁻ (mg kg ⁻¹)	25.4	42.7	11.4
Soil Bray P (mg kg ⁻¹)	964	46	36
Soil NH ₄ OAc-K (mg kg ⁻¹)	236	118	114
Previous management	Continuous corn with biosolids	Alfalfa	Corn- soybean rotation
Alley orientation (rep 1, 2, 3)	0°, 0°, 0°	90°, 90°	135°, 100°, 90°
No. days flooded (rep 1, 2, 3)	0, 7, 0	14, 14	55, 20, 19

[†]Soil samples collected in May 2010

Table 2.2. List of species planted in the native polyculture treatment

Common Name	Latin name	Functional group
Canada wild rye	<i>Elymus Canadensis</i> L.	Grass
Switch grass	<i>Panicum virgatum</i> L.	Grass
Big bluestem	<i>Andropogon girardii</i> Vitman	Grass
Partridge pea	<i>Chamaecrista fasciculata</i> Michx.	Legume
Purple prairie clover	<i>Dalea pupureum</i> Vent.	Legume
Canada milkvetch	<i>Astragalus canadensis</i> L.	Legume
Showy tick-trefoil	<i>Desmodium canadense</i> L.	Legume
Wild bergamot	<i>Monarda fistulosa</i> L.	Forb
Maximilian Sunflower	<i>Helianthus maximiliani</i> Schrad.	Forb
Smooth blue aster	<i>Symphyotrichum leave</i> (L.) Á. Löve & D. Löve var. <i>laeve</i>	Forb
Yellow coneflower	<i>Ratibida pinnata</i> (Vent.) Barnhart	Forb

Table 2.3. Cumulative aboveground biomass yield and nutrient uptake (and 95% CI) by fraction in alley cropping systems at three Minnesota sites from May 2010 to November 2013.

Site	Fraction	Yield (Mg ha ⁻¹)		N uptake (kg ha ⁻¹)		P uptake (kg ha ⁻¹)		K uptake (kg ha ⁻¹)	
		Willow alleys	Poplar alleys	Willow alleys	Poplar alleys	Willow alleys	Poplar alleys	Willow alleys	Poplar alleys
Empire	Tree	30.9 (2.6)	43.4 (4.4)	140.8 (13.1)	232.9 (37.5)	20.3 (1.3)	29.6 (4.7)	56.4 (8.3)	113.1 (14.9)
	Crop	9.7 (2.3)	10.7 (2.5)	117.5 (33.6)	143.6 (39.7)	18.4 (4.5)	21.0 (5.6)	106.4 (32.0)	132.1 (35.8)
	Sum	40.7 b [†]	54.1 a	258.3 (31.3)	376.5 (66.2)	38.7 (4.3)	50.7 (8.9)	162.7 b	245.1 a
Fairmont	Tree	25.6 (3.6)	21.0 (3.5)	109.0 (8.6)	85.9 (4.2)	11.5 (1.3)	8.7 (0.8)	36.5 (3.2)	36.5 (2.4)
	Crop	4.7 (0.4)	4.9 (0.4)	30.7 (2.5)	32.0 (2.6)	7.3 (0.6)	7.6 (0.6)	31.3 (2.6)	32.6 (2.7)
	Sum	30.3 a	25.9 a	139.7 a	117.8 b	18.8 a	16.4 b	67.7 ns [‡]	69.1 ns
Granada	Tree	22.1 (2.8)	28.5 (4.7)	90.6 (17.7)	122.9 (17.8)	14.0 (3.1)	18.2 (3.0)	43.2 (13.3)	68.4 (12.2)
	Crop	9.0 (2.0)	9.8 (2.4)	57.8 (10.2)	62.3 (12.6)	13.8 (3.4)	15.3 (14.3)	59.9 (14.2)	64.4 (18.1)
	Sum	31.1 b	38.3 a	148.4 b	185.2 a	27.7 b	33.5 a	103.1 b	132.8 a

[†] Within sites and responses, means with the same letter are not different, $\alpha = 0.05$. At Empire, statistical comparisons between systems were not made for N and P uptake due to significant SRWC x herbaceous crop interactions.

[‡] ns, not significant, $\alpha = 0.05$

Table 2.4. Aboveground leafless biomass yield and nutrient uptake of alley cropped SRWC after four years at three Minnesota sites.

Site	Woody crop	Row position	kg DM plant ⁻¹	g N plant ⁻¹	g P plant ⁻¹	g K plant ⁻¹
Empire	Fish Creek willow	Edge rows	4.6 c [†]	21.8 c	3.2 c	8.0 c
		Interior rows	3.1 d	15.2 d	2.1 d	5.4 d
	NM6 poplar	Edge rows	21.4 a	116.3 a	15.0 a	55.2 a
		Interior rows	7.2 b	34.6 b	4.8 b	18.6 b
Fairmont	Fish Creek willow	Weighted mean [‡]	3.4 b	14.4 b	1.5 b	4.8 ns [§]
	NM6 poplar	Weighted mean	6.2 a	25.2 a	2.6 a	10.7 ns
Granada	Fish Creek willow	Edge rows	3.0 c	11.2 c	1.7 c	4.7 c
		Interior rows	2.8 c	10.8 c	1.6 c	4.4 c
	NM6 poplar	Edge rows	11.7 a	48.1 a	7.4 a	28.3 a
		Interior rows	6.4 b	27.4 b	4.0 b	15.2 b

[†] Within columns and sites, means with the same letter are not different, $\alpha = 0.05$

[‡] For NM6 poplar, the weighted mean was calculated as 40% edge rows and 60% interior rows; for Fish Creek willow it was calculated as 66.6% edge rows and 33.3% interior rows

[§] ns, not significant, $\alpha = 0.05$

Table 2.5. Yield of alley-cropped herbaceous biomass in each of four years at three Minnesota sites.

Site	Crop	2010 [‡]	2011	2012	2013	Total
		Mg ha ⁻¹				
Empire	Intermediate wheatgrass	4.8 ns	8.0 b [†]	4.7 a	4.6 a	22.1
	Native polyculture	4.3 ns	10.7 a	5.6 a	2.8 b	23.4
	Prairie cordgrass	4.0 ns	10.4 a	6.4 a	5.0 a	25.8
	Switchgrass	5.1 ns	2.3 c	1.1 b	0.1 c	8.6
Fairmont	Native polyculture	- [§]	-	4.2	5.8	10.0
Granada	Intermediate wheatgrass	6.7 a	1.0 c	0.3 c	0.9 c	8.9
	Native polyculture	5.5 a	6.8 a	5.2 a	6.5 a	24.0
	Prairie cordgrass	2.8 b	7.3 a	6.0 a	7.1 a	23.2
	Switchgrass	5.2 a	5.1 b	3.7 b	4.2 b	18.2

[†] Within years and sites, means with the same letter are not different, $\alpha = 0.05$

[‡] 2010 and 2011 yield data from Gamble et al. (2014).

[§] Native polyculture biomass was not harvested at Fairmont in 2010 and 2011

Table 2.6. Mean nutrient concentration and cumulative nutrient uptake of alley-cropped herbaceous biomass at three Minnesota sites.

Site	Crop	N				P				K			
		g kg ⁻¹		kg ha ⁻¹		g kg ⁻¹		kg ha ⁻¹		g kg ⁻¹		kg ha ⁻¹	
Empire	Intermediate wheatgrass	18.0	a [†]	399.1	a	2.4	a	52.7	a	15.3	a	350.6	a
	Native polyculture	14.4	b	345.6	b	2.0	a	50.8	a	11.1	b	287.4	a
	Prairie cordgrass	10.0	c	276.3	b	1.7	b	47.3	a	10.3	b	299.0	a
	Switchgrass	11.8	bc	99.4	c	1.9	b	15.4	b	10.4	b	86.4	b
Fairmont	Native polyculture [‡]	6.5		65.2		1.6		15.6		6.7		66.6	
Granada	Intermediate wheatgrass	8.5	a	91.2	bc	1.6	ns	16.2	b	7.2	a	81.1	b
	Native polyculture	4.9	c	123.2	ab	1.5	ns	38.4	a	6.2	ab	159.1	a
	Prairie cordgrass	6.1	bc	150.0	a	1.4	ns	35.1	a	7.5	a	184.8	a
	Switchgrass	6.7	b	125.9	ab	1.7	ns	30.7	a	4.8	b	93.1	b

[†] Within columns and sites, means with the same letter are not different, $\alpha = 0.05$

[‡] Values at Fairmont represent only two years (2012, 2013), and the native polyculture was the only herbaceous crop established at this site.

Chapter 3 : Belowground carbon sequestration and nutrient accumulation potential of perennial biomass alley cropping systems

Abstract

We quantified root biomass C and root nutrient accumulation, determined the spatial distribution and relative allocation of belowground biomass, and quantified changes in SOC and system C sequestration (root biomass C + SOC) associated with alley cropped herbaceous perennial and short-rotation woody crops (SRWC) over four years at two Minnesota sites. Alley cropping systems consisted of switchgrass (*Panicum virgatum* L.), prairie cordgrass (*Spartina pectinata* Bosc ex Link), ‘Rush’ intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey cv. Rush), and an 11-species native polyculture planted between rows of ‘NM6’ poplar (*Populus maximowiczii* x *P. nigra*) and ‘Fish Creek’ willow (*Salix purpurea*). At Empire, accumulation of C and N was greater in poplar than willow alley cropping systems, and among the highest with prairie cordgrass and intermediate wheatgrass alley crops. At Granada, poplar – prairie cordgrass alley systems had among the highest accumulation of C, N, P, and K. Fine roots accounted for 59 to 65% of root C across sites, though small and coarse root biomass were substantial pools for poplar and prairie cordgrass. Intermediate wheatgrass, switchgrass, and prairie cordgrass fine root biomass was greater in willow than poplar alleys at Empire. Proximity to SRWC substantially reduced herbaceous crop root biomass and C, as up to 25% of SRWC fine root biomass was found within the herbaceous crop alley. SRWC and herbaceous crop selection did not influence SOC, though SOC declined at each site. This study highlights the importance of fine root

biomass as a labile C pool and of plant roots in general for C sequestration in these systems. It also underscores the role that competition has on root spatial distribution, C sequestration, and nutrient accumulation in agroforestry systems.

3.1 Introduction

Anthropogenic greenhouse gas (GHG) emissions are the main cause of shifts in global climate (Ciais et al., 2013; Stocker et al., 2013). Reducing and mitigating current GHG emissions are key steps in slowing shifts in climate and reducing the long-term effects of climate change. The Inter-governmental Panel on Climate Change (IPCC) estimates that land-related mitigation, including forestry, agriculture, and bioenergy could contribute 20 to 60% of total cumulative GHG emissions abatement by 2030, and 15 to 40% by 2100 (Smith et al., 2014). Thus, land-uses that maximize GHG mitigation can play a large role in our ability to cope with long-term climate uncertainty.

Agroforestry, the intentional integration of woody perennials with crops or livestock, has been cited as a key strategy to mitigate GHG emissions and adapt agricultural systems to the impacts of shifting climate (Verchot et al., 2007; Schoeneberger et al., 2012; IPCC, 2014; Mbow et al., 2014). Trees in agroforestry can serve as sinks to offset emissions by increasing C capture and storage in agricultural soils and long-lived stems and roots (Schoeneberger et al., 2012). An addition, agroforestry systems have shown potential for production of C neutral or negative biomass feedstocks for energy and fuel production using perennial herbaceous and short rotation woody crops (SRWC) (Ehret et al., 2014; Gamble et al., 2014; Lamerre et al., 2015). Energy and fuels derived from such systems could displace more C-intensive fossil fuels (Holzmueller and Jose, 2012), and the

combined effects of C sequestration and fuel substitution could substantially improve GHG mitigation and offsets relative to other land use options (Jose and Bardhan, 2012). However, considerable uncertainty exists about the capacity of specific agroforestry configurations to mitigate GHGs in the Midwest U.S.

Roots are an important sink to account for when considering the C sequestration potential of perennial bioenergy systems. In shrub willow bioenergy systems in New York, Pacaldo et al., (2013) found that root C accounted for 69% of total C sequestered. In a switchgrass bioenergy production system, root C accounted for 47% of total C sequestered in Washington (Collins et al., 2010). Likewise, Jungers et al., (2015) reported what in Minnesota, standing root C in switchgrass and perennial polyculture bioenergy systems accounted for 41% of total GHG mitigation on average, which was comparable to the fossil fuel offsets derived from utilizing the aboveground harvested biomass to produce cellulosic ethanol. Roots also play an important role as nutrient sinks in perennial bioenergy systems. Translocation of nutrients from aboveground to belowground plant components during the dormant season influences nutrient harvest, which has implications for nutrient cycling, fertilizer requirements, long term productivity, and C sequestration.

Despite large bodies of literature for agroforestry and bioenergy production systems, little information is available on the long-term effects of intercropping woody and herbaceous bioenergy crops on root biomass and soil C. The potential of a variety of SRWC and herbaceous perennial crops to act as C and nutrient sinks has been well documented in monoculture production. However, it is unclear how combinations of these biomass crops in agroforestry systems will influence C sequestration and nutrient accumulation

potential. The spatial extent of tree and crop root overlap in agroforestry systems influences the extent of belowground competition (Zamora et al., 2007). This, in turn, influences plant productivity, subsequent litter production, and C accumulation. Thus, understanding the spatial distribution and relative allocation of tree and crop root systems is essential for proper C and nutrient accounting in agroforestry systems.

Our goal was quantify the belowground C sequestration and nutrient accumulation potential of perennial biomass alley cropping systems. Therefore, the objectives of this study were to: 1) quantify root biomass C and root nutrient accumulation of alley cropped SRWC and herbaceous perennial crops; 2) determine the spatial distribution and relative allocation of belowground biomass C within alley systems; and 3) quantify changes in SOC and system C sequestration (root biomass C + SOC) associated with alley cropped SRWC and herbaceous perennial crops.

3.2 Materials and Methods

3.2.1 Study sites and experimental design

The study was established in May and June 2010 on a floodplain soil near Granada, MN and on a stream terrace near Empire, MN (Table 3.1). The previous cropping system at Granada was a corn (*Zea mays* L.)—soybean (*Glycine max* [L.] Merr.) rotation, and at Empire it was continuous corn. Annual spring applications of municipal biosolids at Empire from 2000 to 2010 prior to establishment of the experiment resulted in very high organic matter, P, and K at this site. Further soil and climatological detail during the study period are provided in Chapter 2 of this volume.

The experimental design was a randomized complete block in a split-plot arrangement. Three replicates were established at each site. Two SRWC were randomly assigned to 38.1 m by 36.6 m whole plots and four herbaceous crops were randomly assigned to 12.2 m by 9.1 m subplots within each whole plot. Woody crops were established in multi-row strips, with a 15.2 m alleyway between strips and a 1.5 m unsown buffer between trees and herbaceous crops on each side of the alley (Gamble et al., 2014). Woody crops were ‘NM6’ poplar (*Populus maximowiczii* x *P. nigra*) and ‘Fish Creek’ willow (*Salix purpurea*). Herbaceous crops were a local, lowland-ecotype switchgrass (*Panicum virgatum* L.), prairie cordgrass (*Spartina pectinata* Bosc ex Link), a mixture of Pioneer Brand ‘54V48’ alfalfa and ‘Rush’ intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey cv. Rush), and an eleven species native tallgrass-forb-legume polyculture. A local ecotype prairie cordgrass was planted at Granada. At Empire, ‘Red River’ prairie cordgrass was planted. Switchgrass and native polyculture seed were grown or collected in Minnesota and were purchased from Feder Prairie Seed Company (Blue Earth, MN). Alfalfa and intermediate wheatgrass seed were purchased from Albert Lea Seed House (Albert Lea, MN), and the initial mixture was 64 % alfalfa and 36 % intermediate wheatgrass by seed count. By late 2011, alfalfa-intermediate wheatgrass subplots at Empire and Granada contained no alfalfa. Therefore, this treatment will be referred to hereafter as “intermediate wheatgrass”

Willows were established following the guidelines of Abrahamson et al. (2002) in three twin rows on either side of the alley, with 75 cm between rows, 60 cm between plants within a row, and 150 cm between twin rows. This resulted in a planting density of 7,596 willows ha⁻¹ when accounting for area occupied by herbaceous crops. Poplars were

established in five rows on either side of the alley, at 1.2 m within and between rows (Debell et al., 1996, 1997; Benomar et al., 2012). This resulted in planting density 3,402 poplars ha⁻¹ when accounting for area occupied by herbaceous crops. Following established management guidelines, willows were coppiced following the first growing season, while poplars were not coppiced (Herve and Ceulemans, 1996; Volk, 2002). For detailed descriptions of crop establishment and management, see Gamble et al., (2014).

3.2.2 Field and laboratory methods

Soil cores were collected prior to initiation of the study in May 2010, and again in November 2013. Cores were collected in four depth increments (0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 – 90 cm), except at Empire, where a gravel layer prevented collection of samples in the 60 – 90 cm increment. In 2010, soil cores (1.5 cm diameter) were collected by hand near the center of each plot and composited by depth increment. In 2013, soil cores were collected with a hydraulic probe truck equipped with a high relief bit (4.1 cm diameter) to minimize soil compaction. In each subplot, cores were collected at 0 m (between trees within the first row), 1 m, 3.5 m, and 6 m from the tree row in the herbaceous alley. At each sampling location, residue was brushed aside to expose mineral soil, and three cores were collected and composited by depth increment. Subsamples of each composite were passed through a 2 mm sieve to remove roots, which were then returned to the composite sample. The subsamples were air-dried, ground using a mechanical grinder and ball mill, and analyzed for total C content using an elemental analyzer (Model NA 1500 NC, Carlo Erba/Fisons Instruments, Milan, Italy). Carbonates were measured with an acid test consisting of 1 M HCL and subtracted from total C to obtain organic C. In 2013, additional soil cores were collected from the center

of each plot to calculate soil bulk density. The cores were dried to a constant weight at 35°C. Dry weights were determined and soil bulk density was calculated as the ratio of the oven dried mass of soil to the core volume. The SOC stock was determined on an equivalent mass basis and was calculated by multiplying the SOC concentration by soil bulk density and the soil depth of each layer (Ellert and Bettany, 1995).

A hydropneumatic root washer was used to remove soil from plant biomass in composite samples (Gillisons Variety Fabrication; Smucker et al., 1982). The washed biomass was collected on a 0.41 mm sieve, cleaned of any non-organic debris, and then dried to a constant weight at 35°C. Dry biomass was manually sorted into SRWC and herbaceous crop fine root (< 2 mm diameter) and small + coarse root (> 2 mm diameter) size classes, dry weights for each SRWC or herbaceous crop x size class were determined for each sample. Some samples included herbaceous crowns and rhizomes, which were included in the root biomass sample. Fine and small root biomass were then summed over depths and weighted by sampling distance to obtain area based estimates of root biomass for each SRWC and crop. Area weighting was 40% of land area for the 0 m sample (representing the entire SRWC area), 18% for 1 m, 20% for 3.5 m and 22% for the 6 m sample.

Coarse root, small root, and belowground root stool biomass was determined for each SRWC by excavating one representative tree of each species in the center row of each replicate using an air knife in April 2015. For poplar, a 1.2 × 1.2 m square centered on each sample tree was excavated to a depth of 60 cm. For willow, a 0.6 × 1.1 m rectangle centered on each sample tree was excavated to a depth of 60 cm. It was assumed that roots of surrounding trees within the sample area compensated for roots of the measured

tree that were outside the sampled area. The roots were collected, hand washed to remove soil, dried at 60 °C, then divided into belowground root stool, coarse root (> 5 mm) and small root (2 – 5 mm) fractions and weighed. Area based estimates were calculated as the product of per tree biomass in each pool and the planting density for each SRWC. Total root biomass estimates for each SRWC were calculated as the sum of fine roots, coarse roots, small roots, and belowground root stool biomass.

Dried root and stool fractions were ground with a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) to pass a 1 mm screen, and then reground with a cyclone mill. Root mineral concentrations were determined with inductively coupled plasma (ICP) mass spectroscopy following digestion with HNO₃ and H₂O₂ (Gavlak et al., 2003) except for C and N, which were determined via dry combustion and CHNS analyzer (Model 2400 Perkin – Elmer Inc., Waltham, MA, USA) at a commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH, USA). Fine and small root biomass samples were combined by SRWC or herbaceous crop type for mineral and C analysis. Standing C and nutrient accumulation in each root biomass pool was calculated by multiplying the concentration of C, N, P, or K by the total biomass for each pool for each SRWC and herbaceous crop.

Total belowground C stocks were calculated as the sum of SOC, SRWC root, and herbaceous crop root pools on the subplot level, while nutrient accumulation was calculated as the sum of SRWC root and herbaceous crop root pools. Carbon stocks are reported as Mg C ha⁻¹ to 60 cm soil depth and C sequestration potential by roots was calculated as the product of root biomass, root C content, and the CO₂ e constant.

3.2.3 Statistical analysis

We used mixed-effects analysis of variance (ANOVA) to explain variation in SOC; tree and crop fine and small root biomass; C sequestration and nutrient accumulation in root biomass; and system C sequestration and nutrient accumulation. For each site, we fit fixed effects of SRWC and herbaceous crop as categorical variables, and where applicable, distance from the tree-crop interface (“distance”, hereafter) as a continuous variable, and soil depth as a categorical variable. To account for potential spatial autocorrelation occurring as a result of sampling at multiple, systematic locations within the same subplot, we allowed the effects of herbaceous crop and distance to vary by SRWC by nesting subplot and distance within whole plot as random effects. We first analyzed standing fine and small root biomass by type (SRWC or herbaceous crop), distance, soil depth, and all possible interactions among these variables. Area-based estimates of fine and small root biomass, C, and nutrient accumulation were analyzed by SRWC type or herbaceous crop type. We used mixed-effects ANOVA to explain variation in biomass allocation within SRWC, within herbaceous crops, and logistic regression to explain biomass allocation within the alley system (fraction of biomass in SRWC vs. herbaceous crops), treating the fraction of biomass allocated to each pool as a binomial response. We fit categorical fixed effects for each biomass pool and for SRWC or herbaceous crop type. We tested for multicollinearity among predictors using the “vif” function in “usdm” package with program R (Naimi, 2013) to ensure that the fraction of biomass allocated to each pool was not sensitive to changes in allocation to other pools. Predictor variables with a variance inflation factor greater than three were excluded from the analysis. Mixed effects ANOVA and logistic regressions were fit using the ‘nlme’

and ‘lme4’ packages with program R (Pinheiro et al., 2013; R Core Team, 2013; Bates et al., 2015). Where significant ($P < 0.1$) effects were found, Tukey’s Honestly Significant Difference (HSD) test for multiple comparisons was used to determine differences between means.

3.3 Results

3.3.1 SRWC root biomass, C, nutrients, and distribution

There were significant differences in root biomass between Empire and Granada, therefore analysis was conducted by site. Within locations, SWRC root biomass was not affected by the herbaceous alley crop. At Empire, total root biomass, C, N, and K were greater for poplar than willow, while P in root biomass was similar between SRWC (Table 3.2). Fine roots comprised the largest fraction of belowground biomass, and fine root biomass was similar for poplar and willow (Figure 3.1). Coarse root biomass was greater for poplar than willow, though small root and root stool biomass were similar between SRWC. Poplar root stool and coarse root biomass was similar, but greater than the fraction in small roots. For willow, root stool biomass was greater than coarse root and small root biomass.

At Granada, C, P, and K accumulation were similar for poplar and willow, but root biomass and N accumulation were greater for willow. For both SRWC, fine roots comprised the largest fraction of belowground biomass, followed by coarse roots, root stool, and small roots. Biomass within each pool was similar between SRWC.

Carbon and nutrient content was greatly influenced by root biomass as the C, N, P, and K concentration was consistent over SRWC within location. However, C, N, P, and K concentration varied by biomass pool when averaged across SRWC (Table 3.3). At both sites, fine and small roots had lower C and higher N, P, and K concentrations than all other pools, for both SRWC. Coarse root and root stool C and nutrient concentrations were similar.

Fine, small, and coarse root biomass varied by depth and distance from the tree row at both sites. In general, fine root biomass was greatest near trees and declined with distance from the tree row (Figure 3.2). At Empire, fine root biomass was greatest at 0 – 15 cm depth at all sampling distances except at 6 m, where biomass was similar among depths. At Granada, fine root biomass was greatest in the 0 – 15 cm increment at 0 and 1 m, but similar among depths at 3.5 and 6 m. At both sites, small and coarse root biomass generally declined with distance from the tree row at all depths, with virtually no small or coarse roots at 3.5 m and 6 m from the tree (Figure 3.3). Small and coarse root biomass was generally similar at 0 – 15 cm and 15 – 30 cm depths and similar at 30 – 60 cm and 60 – 90 cm depths at Granada.

3.3.2 Herbaceous crop root biomass, C, nutrients, and distribution

Total root biomass varied by herbaceous crop at Empire. Intermediate wheatgrass had greater total root biomass than the native polyculture and switchgrass, but had similar root biomass to prairie cordgrass (Table 3.4). The native polyculture had lower total root biomass than prairie cordgrass, but was similar to switchgrass. Small and coarse root biomass also varied by crop, and was greater for prairie cordgrass ($1,882 \text{ kg ha}^{-1}$) than all

other crops (average of 227 kg ha⁻¹; $F = 9.60$, $P < 0.001$). This corresponded to 31% of the total root biomass for prairie cordgrass, but only of 1 – 8% of total root biomass for the other herbaceous crops, respectively. However, fine root biomass was greater than small and coarse root biomass for all herbaceous alley crops (Figure 3.4).

Fine root biomass of herbaceous crops was affected SWRC (i.e., a herbaceous crop by SRWC interaction), but for small and coarse root biomass, there was no effect of SRWC. Intermediate wheatgrass and prairie cordgrass had greater fine root biomass when grown in willow versus poplar alleys (Table 3.5). Within poplar alleys, intermediate wheatgrass had greater fine root biomass than prairie cordgrass and switchgrass. Within willow alleys, intermediate wheatgrass had greater fine root biomass than all other crops, and the native polyculture had lower fine root biomass than all crops except switchgrass.

Total root; fine root; and small and coarse root biomass all varied by herbaceous crop at Granada. Prairie cordgrass had similar total root biomass as the native polyculture but greater root biomass than the other crops. Fine root biomass was greater for the native polyculture and prairie cordgrass than for intermediate wheatgrass. Small and coarse root biomass was greater for prairie cordgrass (2,851 kg ha⁻¹) than all other crops (average of 722 kg ha⁻¹; $F = 5.64$, $P = 0.002$). Fine root biomass was greater than small and coarse root biomass for all herbaceous alley crops, though small and coarse roots represented 37% of belowground prairie cordgrass biomass.

Fine, small, and coarse root biomass varied by depth and distance from the tree row at both Empire and Granada. In general, herbaceous fine root biomass at both sites increased with distance from the tree row at all depths (Figure 3.2). At Granada, crop

fine root biomass at each depth increased from 0 to 3.5 m from tree rows, but was similar at 3.5 and 6 m for all depth increments. There was a similar pattern at Empire, except that fine root biomass in the 0 – 15 cm increment was similar at 0 and 1 m. At both sites, fine root biomass was greatest in the 0 – 15 cm increment at all sampling distances except a 0m, where there was almost no fine root biomass at any depth. Within sampling distances, crop fine root biomass was similar for all other depth increments at each site. At Empire, herbaceous small and coarse root biomass at 0 – 15 cm depth was lowest adjacent to the SRWC row and similar at all other distances. At Granada, herbaceous small and coarse root biomass at 0 – 15 cm depth was greater at 3.5 m and 6 m than at 0 m, and was similar at 1 m to all other distances. Virtually no herbaceous small roots were detected at other depths.

At Empire, C accumulation and concentrations of C, N, and K varied by herbaceous crop. Prairie cordgrass and intermediate wheatgrass had among the highest C and lowest nutrient concentrations of all crops. Prairie cordgrass and intermediate wheatgrass had greater C accumulation than the other crops. Nitrogen, P, and K accumulation in root biomass was similar for all crops, despite differences in biomass and concentrations of N and K. At Granada, C accumulation, nutrient concentrations, and nutrient accumulation varied by herbaceous crop. The native polyculture had among the lowest concentrations of N, P, and K, while intermediate wheatgrass and prairie cordgrass had among the highest concentrations of these nutrients. Concentration of C in root biomass was similar for all crops. Intermediate wheatgrass had lower C accumulation than prairie cordgrass, which was the only difference in C accumulation among crops. Prairie cordgrass and

switchgrass had greater N accumulation in roots than intermediate wheatgrass, and prairie cordgrass had greater P and K accumulation than all other crops.

3.3.3 Soil organic carbon

In 2013, total mass of SOC to 60 cm depth was similar for all alley cropping systems at each site, averaging 102.1 Mg C ha⁻¹ at Empire and 91.2 Mg C ha⁻¹ at Granada. There was no effect of SRWC, herbaceous crop, or distance from the SRWC row on SOC at either site. However, there were changes in SOC from 2010 to 2013. At Empire, SOC declined 0.41% over soil depths ($t = 16.15$, $P < 0.001$), with declines in each depth increment (Table 3.6). Decline was greatest in the 0 – 15 cm increment and lowest in the 30 – 60 cm depth increment. At Granada, SOC total decline from 2010 to 2013 was 0.07% ($t = 4.76$, $P < 0.001$), primarily due to changes in the 15 – 30 cm and 30 – 60 cm depth increments.

3.3.4 System C and nutrient accumulation

Total C stock (SOC + SRWC root C + herbaceous crop root C) was similar for all alley cropping systems at each site, averaging 107.9 Mg C ha⁻¹ at Empire and 96.4 Mg C ha⁻¹ at Granada. There was no effect of SRWC, herbaceous crop, or their interactions on total C stock at either site. However, C sequestration in root biomass varied by alley system at Granada, and was greater in poplar – prairie cordgrass (23.2 Mg CO₂ eq ha⁻¹) and willow – switchgrass (20.3 Mg CO₂ eq ha⁻¹) systems than in the willow – intermediate wheatgrass (16.8 Mg CO₂ eq ha⁻¹), willow – native polyculture (16.7 Mg CO₂ eq ha⁻¹) and poplar – intermediate wheatgrass alley cropping systems (16.5 Mg CO₂ eq ha⁻¹; Figure 3.5). Carbon sequestration in the willow – prairie cordgrass (20.0 Mg CO₂ eq ha⁻¹)

¹), poplar – native polyculture (20.0 Mg CO₂ eq ha⁻¹), and poplar – switchgrass systems (19.4 Mg CO₂ eq ha⁻¹) was similar to all other systems at this site. The amount of C sequestered was similar between SRWC roots and herbaceous crop roots in all alley systems except poplar – intermediate wheatgrass and willow – intermediate wheatgrass systems, where the larger fraction of C was in SRWC roots (Figure 3.6).

At Empire, C sequestration varied by SRWC and herbaceous crop, but was not affected by their interaction. Carbon sequestration was greater in poplar (23.2 Mg CO₂ – e ha⁻¹) than willow (19.8 Mg CO₂ – e ha⁻¹; $F = 2.86$, $P = 0.09$) alley cropping systems, irrespective of herbaceous alley crop. Prairie cordgrass alleys had higher C sequestration (24.2 Mg CO₂ – e ha⁻¹) than those with the native polyculture (19.1 Mg CO₂ – e ha⁻¹) or switchgrass (20.6 Mg CO₂ – e ha⁻¹; $F = 9.86$, $P = 0.02$). Intermediate wheatgrass alleys sequestered 22.0 Mg CO₂ – e ha⁻¹, which was similar to all other systems at this site. In all poplar alley systems, the majority of C was found in SRWC roots. In contrast, SRWC root and herbaceous crop root C were similar in all willow alley systems except the willow – native polyculture, where the larger fraction of C was in SRWC roots.

Total accumulation of N, P, and K in root biomass varied by alley system at Granada.

The poplar – prairie cordgrass system was consistently among the highest in terms of N, K, and P accumulation in root biomass. At Empire, accumulation of N varied by SRWC, but was not influenced by herbaceous crop or the interaction of SRWC and herbaceous crop. Nitrogen accumulation was greater in poplar (168 kg ha⁻¹) than willow (139 kg ha⁻¹) alley cropping systems, ($t = 2.01$, $P = 0.06$). Accumulation of P and K was similar for all alley cropping systems at Empire, averaging 32, and 78 kg ha⁻¹, respectively.

3.4 Discussion

This study is the first to demonstrate the relative and absolute distribution of root biomass C, root nutrient accumulation, and changes in SOC in alley cropping systems comprised of SRWC and herbaceous perennial crop combinations. Our results show that after four years of production, NM6 poplar – prairie cordgrass systems had among the highest C sequestration and nutrient accumulation in root biomass at two sites of varying fertility in Minnesota. This was largely a result of greater coarse root biomass for poplar and prairie cordgrass than other SRWC and herbaceous crops. However, fine roots accounted for 59 – 65% of root C across sites, and both SRWC and herbaceous crop fine root biomass varied substantially with distance from the tree row. SRWC and herbaceous crops had no measurable effect on SOC, but overall SOC declined at each site over the study period.

At Granada, the less fertile site, a weak SRWC and herbaceous crop interaction demonstrated that both NM6 poplar and prairie cordgrass performed better when paired together than in other pairings. As a result, this system had among the highest belowground productivity at this site, though carbon sequestration and N accumulation were matched by the willow – switchgrass and poplar – switchgrass systems. At Empire, the more fertile site, SRWC and herbaceous crop performance did not depend on species pairing; all systems with NM6 poplar performed similarly well, as did all systems with prairie cordgrass or intermediate wheatgrass. Thus, at Empire, poplar – intermediate wheatgrass systems had similarly high C sequestration and nutrient accumulation to poplar – prairie cordgrass systems.

The highest average values of biomass, C, and nutrient accumulation were found at Empire. Maximum belowground biomass was 16.3 Mg ha⁻¹, C sequestration was 25.7 Mg CO₂ – e ha⁻¹, and N accumulation was 175 kg N ha⁻¹ for the NM6 poplar – prairie cordgrass system. The highest average P accumulation was 39.0 kg P ha⁻¹ in the Fish Creek willow – switchgrass system at Empire, and highest K accumulation was 97.2 kg K ha⁻¹ for the NM6 poplar – native polyculture system.

Relative allocation of biomass C between SRWC and herbaceous crop roots was similar in most alley systems, though there were some exceptions. In the case of both poplar – and willow – intermediate wheatgrass systems at Granada, a larger fraction of belowground biomass was SRWC roots than herbaceous crop roots. This is a result of poor intermediate wheatgrass establishment and yield in some plots at this site (Gamble et al., 2014). In the case of all poplar systems at Empire, a larger fraction of root biomass was SRWC roots than herbaceous crop roots. High poplar root biomass and reduced herbaceous crop fine root biomass in poplar versus willow alleys contributed to this phenomenon. The willow – native polyculture also had a larger fraction of belowground biomass as SRWC roots than herbaceous crop roots, when all other willow systems had similar allocation between components. Although not significant, there was evidence that willow fine and total root biomass were greater when paired with the native polyculture than with other herbaceous crops. Furthermore, the native polyculture had low fine root biomass in willow alleys. These findings are likely a result of declining native polyculture stands at Empire due to competition with weeds (Chapter 2, this volume).

Root biomass observed for NM6 poplar and Fish Creek willow in this study corresponded well with data reported in the literature for similarly aged SRWC bioenergy plantations. Root biomass ranges from 10.4 to 14.1 Mg ha⁻¹ for shrub willows (Rytter, 2012; Pacaldo et al., 2013; Hangs et al., 2014) and 11.9 to 14.2 Mg ha⁻¹ for poplars (Tufekcioglu et al., 2003; Rytter, 2012). While SRWC root biomass in this study ranged from a low of 7.1 Mg ha⁻¹ (willow at Empire) to a high of 11.2 Mg ha⁻¹ (poplar at Empire), these values were weighted to account for area occupied by herbaceous crops within the alley system. Unweighted root biomass values ranged from 12.5 to 15.4 Mg ha⁻¹, which is within or above the aforementioned ranges for these SRWC.

Between SRWC, poplars had 56% greater root biomass than willows at Empire, mainly due to differences in coarse root biomass. While not statistically significant, poplars also tended to have greater fine root and root stool biomass than willows. Willow root biomass was relatively consistent across sites, differing by only 7% between Empire and Granada. In contrast, poplar root biomass at Empire was higher than at Granada, and was the highest SRWC root biomass observed in this study. Similarly, aboveground biomass yield of poplar at this site was highest biomass yield observed in Chapter 2 of this volume.

Fine roots were the largest biomass pool for SRWC, accounting for 50 – 58% of root biomass at Empire and 40 – 45% of root biomass at Granada. This is a greater portion than found by Pacaldo et al., (2013a), who reported that standing fine roots accounted for about 29% of belowground biomass in a 5 year old willow bioenergy system. For poplar, coarse roots were the second largest C sink, accounting for 26% and 27% of root C at Empire and Granada, respectively. For willow, coarse roots were the second largest pool

at Granada, while the root stool was the second largest pool at Empire. Coarse roots accounted for over twice the fraction of biomass at Granada (28%) than Empire (13%). Although not statistically significant, poplars tended toward greater fine root biomass when grown with prairie cordgrass than any other crop (data not shown), which explains why system C sequestration was still high in poplar – prairie cordgrass systems despite lower prairie cordgrass fine root biomass in poplar versus willow alleys.

Coarse roots and root stools are considered long-lived plant parts, where C accrues over the life of the plant. In contrast, C in fine roots is considered a more labile pool. Fine root production and decay in SRWC plantations is generally quite rapid, with rates of turnover ranging from 0.9 – 5.8 year⁻¹ (Rytter, 1999; Stadnyk, 2010; Pacaldo et al., 2013). Assuming an average root turnover of 3 year⁻¹ (Pacaldo et al., 2013), fine root production and litter additions at our sites ranged from 9.0 to 16.8 Mg ha⁻¹ yr⁻¹. While these represent substantial C additions to soil, C emissions in SRWC plantations can offset accrual in fine roots (Pacaldo et al., 2014), depending on root C:N ratios and the extent to which conditions favor microbial activity. However, Rytter, (1999) found that nearly 60% of the total SOC accumulation in poplar (0.52 Mg C ha⁻¹ yr⁻¹) and shrub willow (0.41 Mg C ha⁻¹ yr⁻¹) plantations was due to fine root litter additions. Thus, contribution of fine root litter to SOC accumulation can be substantial over time, if C additions exceed rates of C emission.

Total root biomass for herbaceous crops was generally lower than data reported in the literature for similarly aged stands. However, this was mainly due to competition with SRWC. For instance, (Jungers et al., 2015) recently reported that 7 years after seeding, unfertilized switchgrass and 12-species native polyculture root biomass to 60 cm depth

was 18.9 and 9.1 Mg ha⁻¹, respectively across a range of Minnesota sites. We found biomass of switchgrass to range from 3.9 to 5.2 Mg ha⁻¹ across sites, and our 11-species polyculture root biomass to range from 3.6 to 6.3 Mg ha⁻¹ four years after seeding. However, these estimates account for root biomass decline with proximity to the SRWC row. In the center of the alley, root biomass was 6.9 and 13.0 Mg ha⁻¹ for the native polyculture and 8.1 and 16.7 Mg ha⁻¹ for switchgrass at Granada and Empire, respectively. Intermediate wheatgrass root biomass averaged 3.4 and 6.2 Mg ha⁻¹ at Granada and Empire, but was 8.3 and 9.5 Mg ha⁻¹ in the center of the alley. Alley center yield was comparable to the 7.9 Mg ha⁻¹ observed for intermediate wheatgrass in Michigan (Sprunger et al., 2012).

Small and coarse root biomass was not affected by SRWC and represented a relatively small fraction of total root biomass for all crops except prairie cordgrass. A thick mat of rhizomes in the top 30 cm of soil contributed substantially to total root biomass and C accumulation for prairie cordgrass. Boe et al., (2009) reported that biomass of coarse root biomass of 8 to 10 year old ‘Red River’ prairie cordgrass stands was 20.6 Mg ha⁻¹ to 25 cm soil depth, which is substantially greater than our finding of 1.8– 2.8 Mg ha⁻¹ to 60 cm depth. However our stands were only 4 years old at sampling, and coarse root biomass should increase through time (Baer et al., 2002).

At Empire, intermediate wheatgrass, switchgrass, and prairie cordgrass had greater fine root biomass in willow than poplar alleys. This could be a result of greater competition with poplars than willows, since poplars had greater belowground biomass. Total root biomass showed a similar pattern as fine root biomass, but differences between SRWC alleys were not significant due to large variance in small and coarse root biomass.

Increased crop fine root biomass in willow alleys corresponded to lower SRWC root biomass (willows vs. poplars). On the other hand, low crop fine root biomass in poplar alleys was offset by high poplar root biomass.

As expected, crop root biomass generally increased with distance from the tree row, while tree root biomass generally increased. Poplar and willow roots were detected up to 6 m from tree rows at all depths sampled, though by 3.5 m, crop fine root biomass exceeded that of SRWC. The pattern in herbaceous crop root biomass suggests that trees and crops were competing for belowground resources up to 3.5 m from tree rows.

Interestingly, all herbaceous crops exhibited similar patterns of root distribution within the alley and patterns were similar between SRWC alleys. This suggests that the spatial configuration of trees and crops within the alley was as important as species selection in influencing root biomass and C, at least in the short-term. Proximity to SRWC substantially reduced herbaceous crop root biomass and C.

Quantifying the spatial distribution of tree and crop roots within the alley was important for accurately quantifying nutrient accumulation and C sequestration. At Empire for example, 25 % of willow and poplar fine roots were within the herbaceous crop alley. Therefore, only sampling within the tree rows would result in under estimation of C sequestration. Conversely, only sampling within the center of the herbaceous crop alley would result in over estimation of crop fine roots. For example, at Empire, fine root biomass of all crops averaged 7.0 Mg ha⁻¹ near the center of poplar alleys and 9.0 Mg ha⁻¹ near the center of willow alleys. Assuming these values were representative of the entire alley results in an over estimation of root biomass by 221% and 164%, respectively. The net result of underestimating SRWC roots and overestimating crop roots would be an

over estimation of total fine root biomass and C ranging from 44 – 97%, depending on site and SRWC.

Following conversion from annually tilled to perennial systems, temporal patterns of SOC often show an initial decrease in soil C during the first 5 years, and then an increase to net C gains coincident with greater above- and belowground plant litter inputs (Deng et al., 2014). We observed a similar pattern at Empire over the four year study period. In contrast, at Granada, there was no change in SOC from 0 – 15 cm over the study period. It's possible that SOC declined initially, and then began accruing such that no change was detected from 2010 to 2013. Two years after converting an annual tilled field to perennial grassland, Steinbeiss et al., (2008) found a net C loss, but by year 4, C stocks had exceeded initial values.

The size of the initial SOC pool affects the rate and magnitude of change following land-use conversion. Soils with SOC near their expected maximum may not increase in SOC as quickly or as much as soils that have relatively low SOC (Sartori et al., 2007).

Conversely, soils with relatively high SOC tend to lose SOC more rapidly than those with relatively low SOC following disturbances such as tillage or reduced residue inputs (Sartori et al., 2007). This may explain why we observed greater losses in SOC at Empire versus Granada. Initial SOC pools at Empire were greater at all depths than at Granada. Thus, soils at Empire had more C to lose, while those at Granada were likely well below their maximum and therefore, responded quickly to litter C additions from perennial crops.

No significant differences were detected in SOC among SRWC or crop species or SRWC-crop combinations (alley systems) four years after establishment. Others have observed short-term changes in SOC under perennial bioenergy crops. For instance, Zan et al., (2001) found increased SOC under shrub willow production versus switchgrass and continuous corn after only four years on fine sandy loam soil in southern Quebec. More commonly, however, effects of species on SOC are not observed until 7 - 10 years after bioenergy crop establishment (Ceotto and Di Candilo, 2011). Since bioenergy systems are typically harvested annually, only belowground litter additions contribute substantially to SOC accumulation. This results in slower SOC accumulation relative to unharvested systems. As noted above, the rate of change in SOC also depends on the initial pool size.

3.5 Conclusions

This study is the first to demonstrate the effects of alley cropping herbaceous perennials with short-rotation woody crops on root biomass C, root nutrient accumulation, and changes in SOC. Our results show that after four years of production, NM6 poplar – prairie cordgrass systems had among the highest C sequestration and nutrient accumulation in root biomass, which was largely a result of greater coarse root biomass for poplar and prairie cordgrass than other SRWC and herbaceous crops. Belowground competition reduced C sequestration of herbaceous crops in alley cropping systems and may influence stand longevity. Converting from annual grain crops to perennial biomass alley cropping resulted in short-term SOC losses, but net C gains should be realized over the long-term due to rapid C accrual in belowground biomass. However, this depends largely on the extent to which C in fine roots is converted to recalcitrant SOM versus lost

as C emissions. Finally, this study highlights the importance of evaluating the spatial distribution of tree and crop roots for accurately quantifying C sequestration and nutrient accumulation in agroforestry systems.

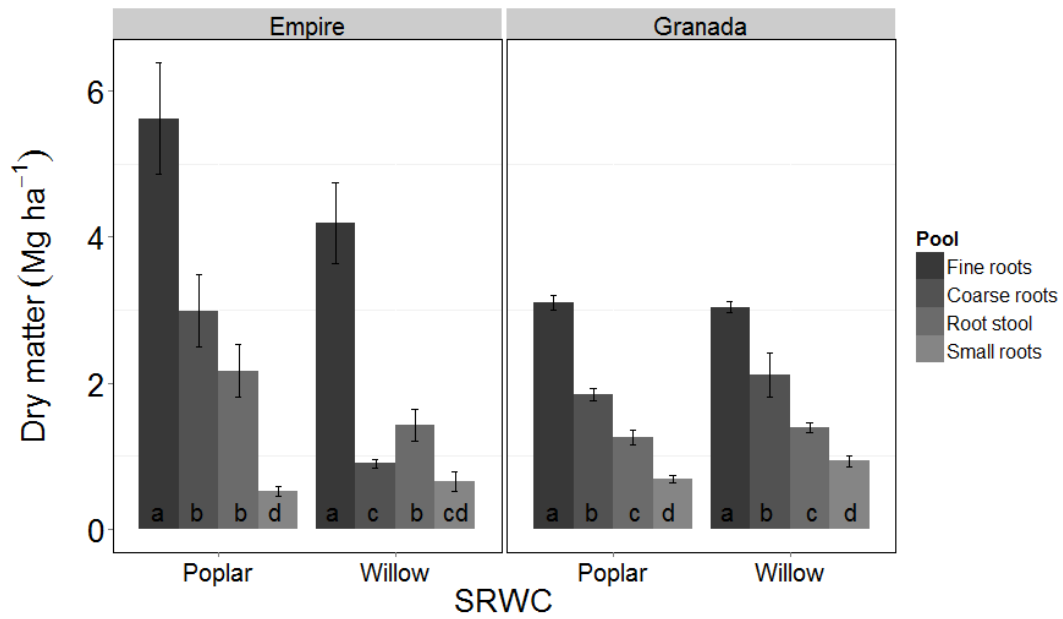


Figure 3.1. NM6 poplar and Fish Creek willow root biomass by pool and site. Error bars represent one standard error of the mean. Within sites, bars with the same letter are not different ($\alpha = 0.1$).

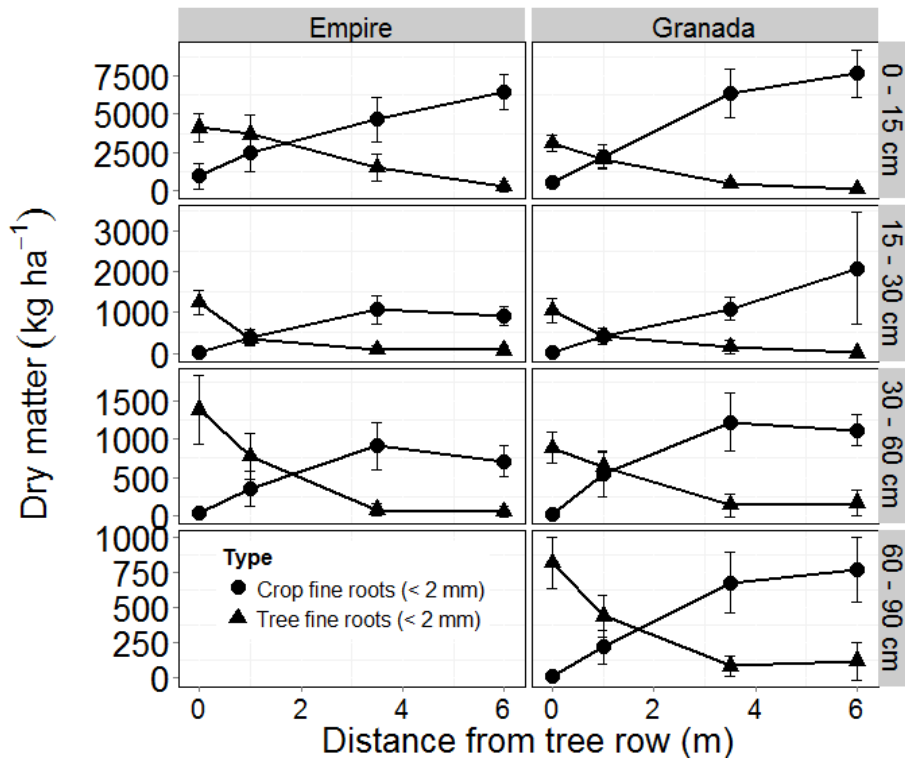


Figure 3.2. Fine root (< 2 mm) biomass with distance from the tree row and soil depth after four years in alley cropping systems at two Minnesota sites. Error bars represent 95% confidence intervals of the mean. Note different y-axis scales for each depth increment.

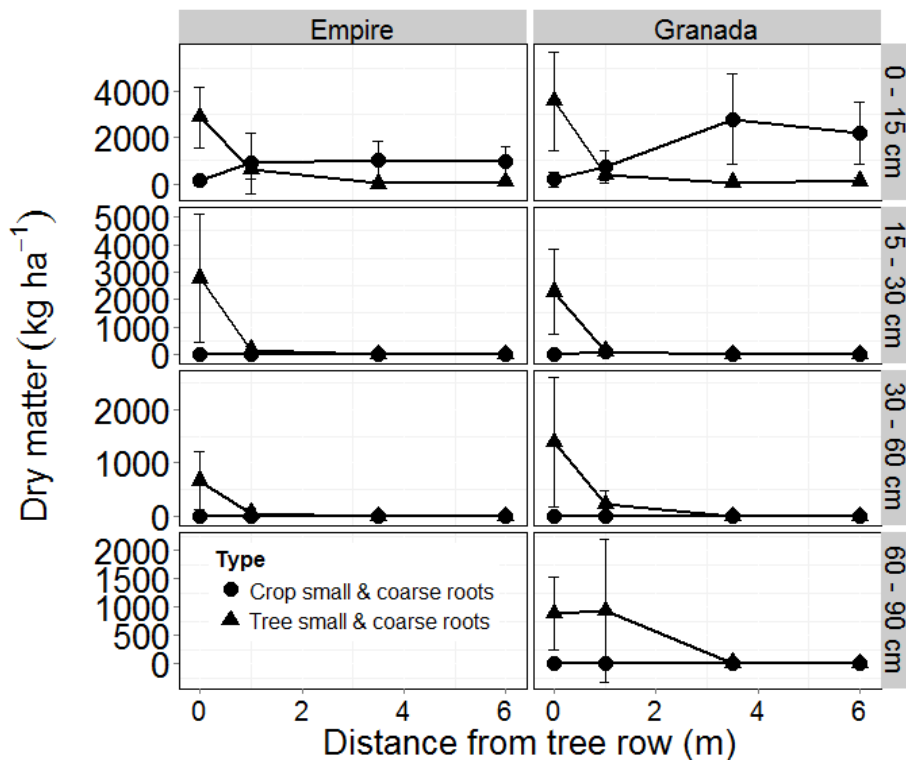


Figure 3.3. Small and coarse root (> 2 mm) biomass with distance from the tree row and soil depth after four years in alley cropping systems at two Minnesota sites. Error bars represent 95% confidence intervals of the mean. Note different y-axis scales for each depth increment.

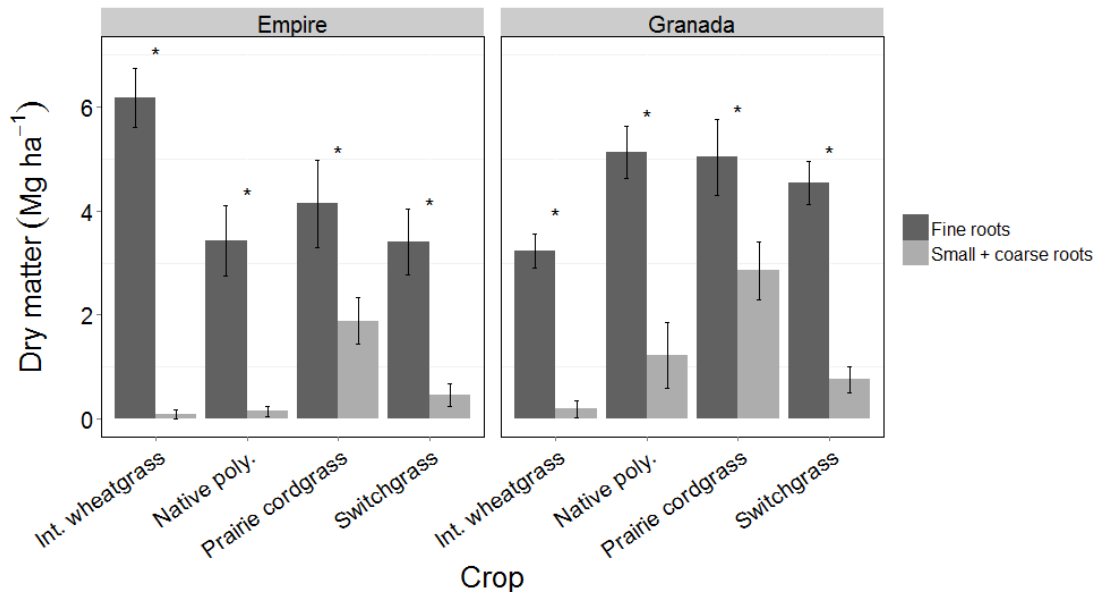


Figure 3.4. Herbaceous crop root biomass by pool and site. Error bars represent one standard error of the mean. Stars indicate significant differences between Fine root and small + coarse root fractions within each crop ($\alpha = 0.1$).

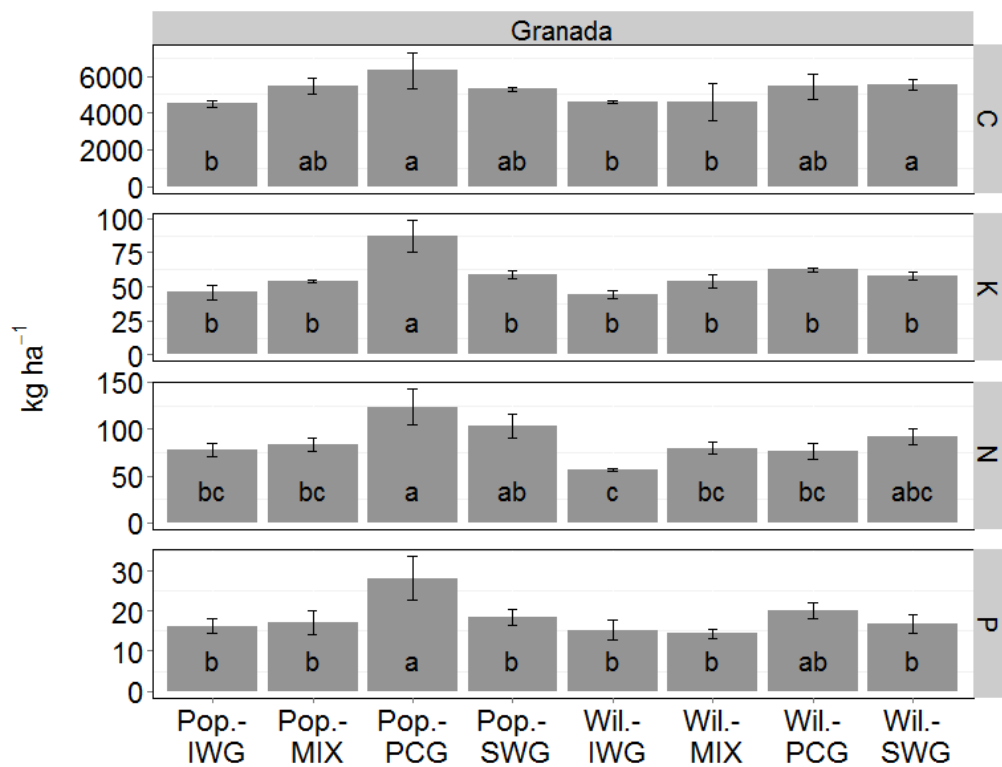


Figure 3.5. C sequestration and nutrient accumulation in root biomass by alley system after four years at Granada, Minnesota. Error bars represent one standard error of the mean. Note different y- axis scales for each panel. Bars with the same letter are not different ($\alpha = 0.1$). “Pop.” = NM6 poplar; “Wil.” = Fish Creek willow; “IWG” = intermediate wheatgrass; “MIX” = native polyculture; “PCG” = prairie cordgrass; “SWG” = switchgrass.

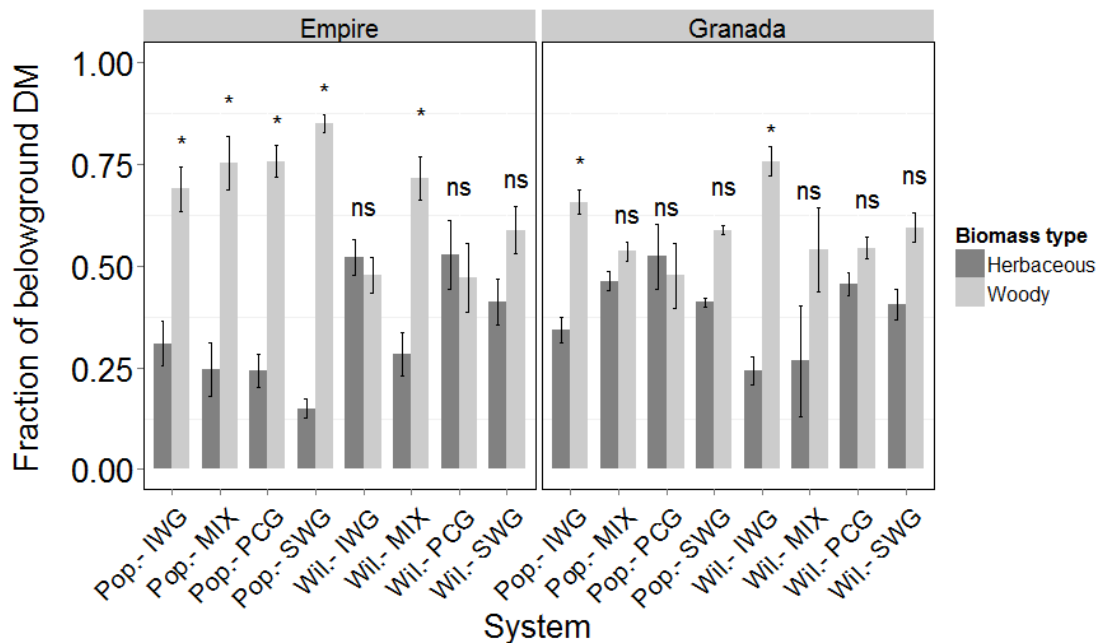


Figure 3.6. Relative allocation of belowground alley system biomass by bioenergy crop type and site. Error bars represent one standard error of the mean. Stars indicate significant differences between Crop and SRWC fractions within each system ($\alpha = 0.1$). “ns” indicates no significant difference between Crop and SRWC fractions. “Pop.” = NM6 poplar; “Wil.” = Fish Creek willow; “IWG” = intermediate wheatgrass; “MIX” = native polyculture; “PCG” = prairie cordgrass; “SWG” = switchgrass.

Table 3.1. Selected site and soil characteristics at Empire and Granada, MN

Characteristic	Empire	Granada
Latitude, Longitude	44.7178, -93.0975	43.7575, -94.3465
Landform	Stream terrace	Floodplain
Soil classification	Cylinder loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic aquic hapludolls)	Coland loam (fine-loamy, mixed, superactive, mesic cumulic endoaquolls)
Soil organic matter (%) ^{†‡}	5.2	3.6
Soil pH ^c	5.5	6.4
Soil total N (%)	0.26	0.14
Soil C/N	11.35	12.16
Fall residual soil NO ₃ ⁻ (ppm)	25.4	11.4
Soil Bray P (ppm)	964	36
Soil NH ₄ OAc-K (ppm)	236	114
Previous management	Continuous corn with biosolids	Corn- soybean rotation

[†]Mass fraction

[‡]Soil samples collected in May 2010, except NO₃⁻, for which samples were collected in November 2010.

Table 3.2. Mean root biomass, C, and nutrient accumulation of SRWC root biomass at Empire and Granada, MN following four growing seasons. Values are area-weighted to represent the proportion of area that herbaceous crops occupy within the alley system (51% for poplar, 53% for willow).

Site	SRWC	Biomass	C	N	P	K
		Mg ha ⁻¹			kg ha ⁻¹	
Empire	Poplar	11.2 a [†]	4.8 a	114.7 a	24.0 a	63.5 a
	Willow	7.1 b	3.0 b	59.1 b	20.5 a	43.6 b
Granada	Poplar	6.8 b	3.0 a	32.5 b	10.1 a	29.9 a
	Willow	7.5 a	3.0 a	40.7 a	8.7 a	28.5 a

[†]Within response variables and sites, means with the same letter are not different based on a two-sample *t*-test, $P > 0.1$.

Table 3.3. Mean C and nutrient concentrations of poplar and willow belowground biomass pools at Empire and Granada, MN

Site	Pool	C	N	P	K
		g kg^{-1}			
Empire	Fine & small roots	401 b	12.3 a	2.9 a	6.8 a
	Coarse roots	455 a	6.8 b	1.7 b	4.9 b
	Root stool	448 a	4.0 b	0.8 c	3.3 c
Granada	Fine & small roots	400 b	8.2 a	1.7 a	5.3 a
	Coarse roots	465 a	3.3 b	0.9 b	2.5 b
	Root stool	467 a	2.2 b	0.5 c	2.5 b

[†]Within response variables and sites, means with the same letter are not different based ($P > 0.1$)

Table 3.4. Mean standing root biomass, C, nutrient content, and nutrient concentration of herbaceous alley crops at two Minnesota sites following the 2013 growing season. Area – based values are weighted to represent the proportion of area that herbaceous crops occupy within the alley system (average of 48%).

Site	Crop	Biomass	C	N	P	K	C	N	P	K
		Mg ha ⁻¹	kg ha ⁻¹			g kg ⁻¹				
Empire	Int. wheatgrass	6.2 a [†]	2.4 a	84.2 ns	13.8 ns	27.6 ns	382 b	13.4 ab	2.3 ns	4.6 b
	Native poly.	3.6 c	1.4 b	54.2 ns	10.6 ns	31.1 ns	390 ab	14.9 a	2.8 ns	8.3 a
	Prairie cordgrass	6.0 ab	2.5 a	74.2 ns	12.5 ns	31.3 ns	422 a	11.9 b	2.1 ns	5.3 b
	Switchgrass	3.9 bc	1.5 b	55.1 ns	9.8 ns	25.0 ns	403 ab	14.1 ab	2.5 ns	6.1 ab
Granada	Int. wheatgrass	3.4 c	1.3 b	33.2 b	6.1 b	16.9 b	392 ns	9.7 ab	1.8 ab	4.9 ab
	Native poly.	6.3 ab	2.0 ab	46.0 ab	6.8 b	25.5 b	392 ns	7.3 b	1.1 c	4.2 b
	Prairie cordgrass	7.9 a	2.9 a	63.7 a	15.3 a	47.2 a	381 ns	8.7 ab	2.1 a	6.3 a
	Switchgrass	5.2 bc	2.2 ab	56.7 a	7.5 b	25.7 b	416 ns	10.8 a	1.4 bc	4.9 ab

[†]Within response variables and sites, means with the same letter are not different ($P > 0.1$). ns = not significantly different

Table 3.5. Herbaceous crop fine root biomass in alley cropping systems at two Minnesota sites following the 2013 growing season. At Granada, biomass is averaged across NM6 poplar and Fish Creek willow alleys.

Crop	Empire		Granada
	NM6 poplar alleys	Fish Creek willow alleys	
	Mg ha ⁻¹		
Intermediate wheatgrass	5.1 ar ^a	7.5 as	3.2 b
Native polyculture	3.7 abr	3.2 cr	5.1 a
Prairie cordgrass	2.4 br	5.8 bs	5.0 a
Switchgrass	2.2 br	4.7 bcs	4.5 ab

^aLetters a – c denote differences (Tukey HSD, $P \leq 0.1$) within columns, while letters r – s denote differences within rows within site

Table 3.6. Soil organic carbon by soil depth and sampling date and percent change in SOC by depth in alley cropping systems at two Minnesota sites.

Soil depth	Empire			Granada		
	2010 SOC	2013 SOC	% Change	2010 SOC	2013 SOC	% Change
0 – 15 cm	2.96 a	2.34 b	-0.65 A	1.66 a	1.59 a	-0.07 ns
15 – 30 cm	1.98 a	1.56 b	-0.42 B	1.46 a	1.36 b	-0.1 ns
30 – 60 cm	0.83 a	0.63 b	-0.20 C	0.91 a	0.83 b	-0.08 ns
60 – 90	NA	NA	NA	0.64 a	0.62 a	-0.02 ns

^aWithin sites, letters a – b denote differences (Tukey HSD, $P \leq 0.05$) within rows (between dates), while letters A – C denotes differences within columns (among depths).

Chapter 4 : Biophysical interactions in shrub willow – grassland alley cropping systems

Abstract

During the 2013 growing season, we measured variation in herbaceous biomass yield, photosynthetically active radiation (PAR), soil water potential, and soil NO₃ in alley cropping systems consisting of prairie cordgrass (*Spartina pectinata* Bosc ex Link) or an 11-species native grass-forb-legume polyculture planted between rows of shrub willow (*Salix purpurea* ‘Fish creek’) at two Minnesota sites. Crop yield at Empire increased with distance from the tree row for both crops, as did PAR and NO₃ availability. At Granada, no spatial pattern in crop yield was evident, despite reduced PAR and NO₃ availability adjacent to tree rows. At both sites, patterns in water potential suggested that trees competed with crops for soil water within 2.4 m of tree rows, but had a facilitative effect on crop water use at 4.8 m. Alleys had differing cardinal orientations at the two sites, and light availability was lower in a north-south alley (Empire) than in west-east and northwest-southeast orientations (Granada). Mixed effects analysis indicated that competition for light and soil water at Empire substantially reduced crop yield 2.4 m from tree rows. At Granada, effects of soil N and soil water availability on crop yield were observed but were inconsistent. Alley cropping systems comprised of shrub willow and herbaceous perennial biomass crops should avoid north-south row orientations to minimize shading. Competition for soil moisture reduced crop yield, but could be mitigated through appropriate management intervention. Spatially, prairie cordgrass and a native polyculture responded similarly to the alley cropping environment, though prairie cordgrass had higher yields.

4.1 Introduction

Polyculture plantings of multiple species are sometimes recommended to increase land productivity over monoculture plantings (Malézieux et al., 2009). Agroforestry, the integration of trees and shrubs into agricultural systems, is one commonly promoted polyculture approach that can add social, economic, and ecological value to agricultural landscapes. However, competition between trees and co-planted crops can also reduce productivity in agroforestry systems to a point where mixed-culture is not economically justified. Competition occurs when coexistent plants reduce the availability of one or more growth resources to the point where growth or survival of either is negatively impacted (García-Barrios and Ong, 2004; Harper, 2010). The nature and degree of competition in multi-species systems is dictated by the extent of niche differentiation versus niche overlap in spatial, temporal, or physical dimensions (Howard et al. 1996). In contrast, weak interspecies competition can be offset by facilitative interactions under certain conditions, resulting in complementarity between trees and crops. For instance, moderate shading by trees can improve crop growth and yield by reducing heat and wind stress, crop evaporative loss, and soil surface temperatures (Jose et al., 2004; Clinch et al., 2009; Quinkenstein et al., 2009).

Cannell et al (1996) assert that “*the benefits of growing trees with crops will occur only when the trees are able to acquire resources of water, light, and nutrients that the crop would not otherwise acquire.*” For coexistent plants, niches could be defined by differences in rooting depth and habit, shoot morphology, growth phenology, or preferences for mineral species (NO_3 vs NH_4 , for example). Niches can also be created spatially by reducing tree and crop planting density. Species selection, plant density,

weather, site edaphic characteristics, and time all influence the extent to which tree and crop niches will overlap. However, species selection and planting density are the main factors that can be influenced by land managers. Understanding the spatiotemporal partitioning of light, water, and nutrients in agroforestry systems is important in identifying where competition is occurring between a given tree-crop pairing, and can help illuminate areas of niche differentiation versus niche overlap. This, in turn, improves our ability to design systems to reduce competitive effects and increase yield while maintaining the positive benefits of mixed-culture.

Agroforestry systems comprised of short-rotation woody crops (SRWC) and herbaceous perennials have shown potential for biomass and bioenergy production (Ehret et al., 2014; Gamble et al., 2014; Lamerre et al., 2015), and nutrient accumulation (Chapter 3, this volume). In these systems, fast growing SRWC such as hybrid poplar (*Populus* spp.) and shrub willows (*Salix* spp.) are planted in high-density strips, with perennial herbaceous biomass crops in inter-row alleys. The SRWC create a substantial amount of shade in alleys adjacent to tree rows, and also form dense root systems which likely extend into the crop alley and result in belowground competition for water and nutrients. For example, in hybrid poplar and shrub willow alleys at one Minnesota site, herbaceous crop yield was reduced along the tree-crop interface relative to the center of the alley for four herbaceous perennial biomass crops (Chapter 2, this volume). However, the authors found no effect of proximity to tree rows in similar alley systems at two other Minnesota sites. This research is still in its infancy; little is known about the long-term effects of interspecies interactions on productivity in such systems.

Here, we aim to better understand how interactions with short rotation shrub willow ‘Fish Creek’ (*Salix purpurea* L.) influence biomass yield of two herbaceous perennial biomass crops, prairie cordgrass (*Spartina pectinata* Bosc ex Link), and an 11-species native polyculture, in an alley cropping configuration. Our objectives were to evaluate the spatiotemporal variation in PAR, soil water potential, and soil NO₃ in these alley cropping systems, and to use these data to explain observed patterns in herbaceous crop yield at two environments in Minnesota.

4.2 Materials & methods

4.2.1 Study sites and experimental design

We collected data in 2013 from an ongoing study that was established in May and June 2010 at two farm sites in Minnesota. Sites were located on a floodplain near Granada, MN (43°45’28” N; 94°20’48” W), and on a stream terrace near Empire, MN (44°39’59” N; 93°06’39” W; Table 4.1). Soils at Granada are very deep, poorly to somewhat poorly drained, formed in alluvium, and consist of the Coland (Fine-loamy, mixed, superactive, mesic Cumulic Endoaquolls) soil series. Soils at Empire are very deep, somewhat poorly drained, formed in loamy alluvium overlying sand and gravel outwash and are of the Cylinder (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aquic Hapludolls) soil series. Average annual temperature at Granada is 7.4 °C and at Empire 6.4 °C. Growing season (April – October) precipitation at Granada is 66 cm and at Empire 70 cm. In 2013, precipitation was 22% and 16% below normal at Granada and Empire, respectively, with the majority of the deficit occurring July – October (Figure 4.1).

The experimental design was a randomized complete block in a split-plot arrangement. Three replicates were established at each site. Two SRWC were randomly assigned to 38.1 m by 36.6 m whole plots and four herbaceous crops were randomly assigned to 12.2 m by 9.1 m subplots within each whole plot. Woody crops were established in multi-row strips, with a 15.2 m alleyway between strips and a 1.5 m unsown buffer between trees and herbaceous crops on each side of the alley. For the present study, a subset of plots was chosen for instrumentation and detailed data collection. Alley cropping systems were prairie cordgrass (*Spartina pectinata* Bosc ex Link), and an eleven species native tallgrass-forb-legume polyculture nested between rows of willow cultivar ‘Fish Creek’ (*Salix purpurea*). Further detail about sites and experimental layout can be found in Chapter 2 of this volume.

4.2.2 Field and laboratory methods

A Decagon AccuPar LP-80 Ceptometer (Decagon Devices Inc., Pullman, WA) was used to measure incident photosynthetically active radiation (PAR, 400-700 nm) and leaf area index (LAI) in the alleys. Diurnal transmission of incident PAR was measured above the herbaceous crop canopy at five locations in a transect across the alley every two hours from 09:00 to 17:00 hours on 18 and 19 July, 2013. Ten random samples were measured and the average recorded in the center of the alley, and at 2.4 and 4.8 m from tree rows on either side of the alley under cloudless conditions. Incident PAR outside the alley system was also measured one meter above the ground before and after measuring for each transect. Data collected outside the alley system displayed a concave shape when plotted against time, and were thus fit to a nonlinear quadratic function (data not shown). This function was used to estimate incident PAR outside the alley system for each minute

throughout the sampling period. Relative PAR was calculated as (I_c/I_o) where I_c is the incident photosynthetically active radiation (PAR) at the top of the herbaceous canopy within the alley and I_o is the modelled PAR in open conditions.

LAI, defined as the projected leaf area per unit ground area was measured for herbaceous crops during late-July 2013. LAI measurements were taken in the center of the alley and at both tree-crop interfaces (2.4 m from tree rows). A measurement consisted of a simultaneous reference measurement in a clearing away from or above the canopy and the average of ten below-canopy readings. All measurements were taken at ground level either early in the morning or late in the afternoon to allow for diffuse light conditions (Tharakan and Volk, 2005)

Soil matric potential and temperature were monitored continuously throughout the 2013 growing season using MPS-2 dielectric water potential sensors paired with EM50 data loggers (Decagon Devices, Pullman, WA). Sensors were buried 15 cm below the soil surface, and data loggers were programmed to record hourly from 31 May to 25 October 2013. In each instrumented plot, five sensors were installed in a transect across the alley. One sensor was installed between willow twin rows on each side of the alley, one sensor was installed in the center of the herbaceous plot (7.6 m from tree rows), and two sensors were placed at intermediate positions within the plot, 2.4 m from tree rows on either side of the alley. Hourly matric potential readings were averaged by day and grouped by season for analysis (Spring, 25 May to 21 June; Summer, 22 June to 22 September; and Fall, 23 September to 25 October).

Soil cores were collected in November 2013 in four depth increments (0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 – 90 cm), except at Empire, where a gravel layer prevented collection of samples in the 60 – 90 cm increment. Cores were collected with a hydraulic probe truck equipped with a high relief bit to minimize soil compaction (40.8 mm ID probe tip). In each sub-plot, cores were collected at four distances from the tree row: 0 m (immediately adjacent to trees), 1 m, 3.5 m, and 6 m from the tree row. At each sampling location, three cores were collected and composited by depth increment to increase sample volume. The samples were sent to a commercial laboratory (Agvise Laboratories Inc., Benson, MN, USA) where they were air-dried, ground using a mechanical grinder and ball mill, and analyzed for $\text{NO}_3 - \text{N}$.

Biomass yield of herbaceous alley crops was measured in Oct 2013 following a killing frost (0°C). Herbage biomass was harvested from a 2.78 m^2 area ($0.91 \times 3.05 \text{ m}$) with a flail-type forage harvester to a 10 cm stubble height in five strips within each alley sub-plot. Strips were located in the alley center (7.6 m from the nearest tree), at both edge positions (2.4 m from tree rows), and two intermediate positions (4.8 m from tree rows). Herbaceous samples were weighed following harvest to obtain fresh weights and 1000 g sub-samples were dried in a 60°C to a constant weight to calculate dry matter yield. Weed biomass was manually separated from crop biomass in dried sub-samples to obtain weed free biomass yield estimates.

4.2.3 Statistical analysis

To evaluate spatial variation in biomass yields, we expressed sampling distance as the ratio of distance from the tree row to tree height (D/H, hereafter) to account for the

potential combined influence of these factors. Initial data exploration included plotting biomass yield against D/H. The herbaceous biomass yield response to D/H showed a concave pattern, suggesting that a nonlinear quadratic function should be fit and tested against other models. We then compared nonlinear models to the linear form and also to a model without D/H (intercept-only) using maximum likelihood ratio tests (Johnson and Omland, 2004). After determining the best-fit model for the effect of D/H on biomass yield, we added the categorical variable “crop type” and an interaction term with D/H to determine if the effect of D/H was the same for both crops. We compared the best-fit model with the crop type covariate to one without using the maximum likelihood ratio test.

To assess the availability of growth resources within alley systems, response variables included relative PAR, water potential, and soil NO₃. Mixed effects ANOVA was used to test the effects of crop, distance from the tree row, and soil depth on fall residual soil nitrate. For water potential and soil temperature, mixed effects ANOVA was used to test for main effects of sub-plot treatments (native polyculture and prairie cordgrass), and distance from the tree-crop interface within Fish Creek willow whole plots for each season. For relative PAR, mixed effects ANOVA was used to for effects of sampling time of day and distance from the tree-crop interface. Each response was analyzed by site, except soil water potential, which was analyzed by site and season. For all analyses, effects of replicates were considered random. To account for spatial autocorrelation occurring as a result of sampling at multiple, systematic locations within the same subplot, we allowed the effect of distance from the tree-crop interface to vary by subplot by nesting distance within subplot as a random effect.

A linear mixed effects model was constructed to evaluate differences in relative PAR between alley orientations. For this analysis, alley orientation was considered a continuous, fixed effect. Alley orientation ranged from 0° to 135°, where 0° is north-south and 90° is west-east. Effects of tree height, tree LAI, time of day, distance, side of the alley, and the ratio of sampling distance to tree height were considered random. A model with only random effects was used to determine the variance associated with each. The proportion of variance was calculated as the ratio of the variation associated with each random effect to the total variation. This approach allows for variation in the dependent variable to be assessed across levels of the random effects. The results can therefore be generalized to a greater hypothetical population of levels (West et al., 2014), in this case, alley orientations.

To assess the effects of PAR, water potential, and soil NO₃ on herbaceous biomass yield, linear mixed effects models were constructed for each site treating the effects of PAR, water potential and soil NO₃ as fixed, and the effects of replicate, tree, crop, and subplot as random. All two- and three-way interactions between explanatory variables were tested. Since soil NO₃ and biomass yield were assessed at different distances, we assumed that the 6 m NO₃ sample corresponded to the 7.6 m biomass sample, while the mean of the 1 m and 3.5 m NO₃ samples corresponded to the 2.4 m biomass sample. We tested for multicollinearity among predictors using the “vif” function in “usdm” package with program R (Naimi, 2013). Linear mixed effects models and mixed effects ANOVA were fit using the “lme4” package with program R (Pinheiro et al., 2013; R Core Team, 2013). The probability for rejection of the null hypothesis was set at $\alpha = 0.10$. Where

significant effects were found, Tukey's Honestly Significant Difference (HSD) test for multiple comparisons was used to compare treatment means.

4.3 Results

4.3.1 Spatial variation in herbaceous crop productivity

At Empire, herbaceous biomass yield response to distance from the tree row to tree height (D/H) showed a concave pattern (Figure 4.2). A mixed effects model that included a quadratic polynomial term for the effect of D/H and a term for crop best predicted herbaceous crop yield (L ratio = 3.08, P = 0.079; L ratio = 3.95, P = 0.047; Table 4.2). Yield of both prairie cordgrass and the native polyculture increased from 0.25 to 1.14 D/H, and then declined slightly from 1.15 to 1.52 D/H. Yield of prairie cordgrass was greater than that of the native polyculture over the range of observed D/H. Leaf area index was similar for prairie cordgrass (5.5 ± 0.8) and the native polyculture (4.7 ± 0.5), but varied by distance from the tree row (L ratio = 12.90, P < 0.001). When averaged across crops, LAI was greater in the center of the alley (5.5 ± 0.6) than adjacent to tree rows (average of 3.8 ± 0.3).

At Granada, no pattern was apparent in the herbaceous biomass yield response to D/H. A mixed effects model that included a term for the effect of crop best predicted herbaceous crop yield (L ratio = 10.55, P = 0.005), though mean yield was similar for prairie cordgrass (7.1 Mg ha^{-1}) and the native polyculture (6.5 Mg ha^{-1}). Including terms for a linear or quadratic polynomial effect of D/H on biomass yield did not improve model fit. At Granada, herbaceous crop LAI averaged 3.2 ± 0.2 , and was similar for all crops and distances from the tree row.

4.3.2 Soil water potential

In spring, average daily water potential was similar for all crops and distances from the tree row at both sites, averaging -9.6 kPa at Empire and -13.2 kPa at Granada (Table 4.3). In summer, average daily water potential varied by crop and distance from the tree row at both Empire and Granada. At Empire, water potential in prairie cordgrass alleys was highest in the center of the alley (7.6 m; Table 4.4). In native polyculture alleys, water potential was lower in the center of the alley than at either 2.4 m east or west. Water potential was higher in the eastern than the western willow rows, but both rows had lower water potential than in prairie cordgrass alleys. In contrast, water potential in the center of the native polyculture alley was similar to that of the west willow row. At Granada, water potential was lower in native polyculture than prairie cordgrass alleys at all distances from the tree row and was lower in willow rows than in either crop alley. In both prairie cordgrass and native polyculture alleys, water potential was lower on the northern edge (2.4 m north) than the southern edge (2.4 m south) of the alley.

In fall, average daily water potential varied by crop and distance from the tree row at both Empire and Granada. Water potential at Empire was similar for all distances from the tree row in prairie cordgrass alleys. In native polyculture alleys, water potential was lower in the center of the alley than at either 2.4 m west or east. Willow rows had lower water potential than in crop alleys, except in the center (7.6 m) of native polyculture alleys. At Granada, water potential was similar for all distances from the tree row in prairie cordgrass alleys. In native polyculture alleys, water potential was lowest on the northern edge of the alley (2.4 m north). Water potential was lower in willow rows than at any distance in the crop alleys, except for at 2.4 m north in native polyculture alleys.

4.3.3 Relative PAR & alley orientation

At Empire, relative PAR varied by distance from the tree row and time of day.

Compared to the center of the alley, relative PAR was reduced along the eastern edge of the alley during the morning and reduced along the western edge of the alley in the afternoon (Figure 4.3). The western half of the alley received most of its PAR during the morning when the eastern half was shaded. This pattern was reversed in the afternoon when the western half was shaded and the eastern half received a greater amount of PAR. During midday, shading was similar between the furthest east and furthest west sampling distances and relative PAR was similar at the central three sampling distances. At

Granada, relative PAR also varied with distance from the tree row and time of day.

Compared to the center of the alley, relative PAR was reduced along the southern edge of the alley during the morning hours and declined further throughout the day. The northern half of the alley received relatively consistent levels of PAR throughout the day, while relative PAR declined slightly in the center of the alley from midday to late afternoon.

A linear mixed effects model that included a term for alley orientation better predicted relative PAR across sites than the intercept-only model (Table 4.5). For every 10° increase in cardinal alley orientation (0 - 180°), model estimated relative PAR increased by approximately 2%. This corresponded to 26% greater relative PAR in 135° alleys than 0° alleys. Random effects for sampling time and distance from the tree explained 44% of model variance. Of the remaining variance, 9% was explained by the ratio of sampling distance to tree height, 20% was explained by tree LAI, and the remaining 27% was residual, within plot variance. Tree height and whole plot accounted for no additional variance in the model.

4.3.4 Soil nitrate

Fall residual $\text{NO}_3\text{-N}$ at Empire varied by soil depth with distance from the tree row (Figure 4.4). At 0 – 15 cm and 15 – 30 cm depths, $\text{NO}_3\text{-N}$ generally increased with distance from the tree row, whereas at 30 – 60 cm depth, no effect of distance was observed. Soil $\text{NO}_3\text{-N}$ decreased with depth, except at 0 m, where it was similar at all depths. At other distances, $\text{NO}_3\text{-N}$ was lower at 30 – 60 cm than at 0 – 15 cm depth. At 6 m from tree rows, $\text{NO}_3\text{-N}$ was also greater at 15 – 30 cm compared to 30 – 60 cm. Soil $\text{NO}_3\text{-N}$ at Granada varied by soil depth with distance from the tree row. At 0 – 15 cm and 15 – 30 cm depths, $\text{NO}_3\text{-N}$ generally increased with distance from the tree row, whereas at 30 – 60 cm and 60 – 90 cm, no effect of distance was observed. Soil $\text{NO}_3\text{-N}$ was similar for all depths at 0 m and 1 m from tree rows. At 3.5 m from tree rows, $\text{NO}_3\text{-N}$ was greatest at 0 – 15 cm and lowest at 30 – 60 cm and 60 – 90 cm. At 6 m from tree rows, $\text{NO}_3\text{-N}$ was greater at 0 – 15 cm and 15 – 30 cm than 30 – 60 cm and 60 – 90 cm depths. At both sites, fall residual soil $\text{NO}_3\text{-N}$ was similar in willow-prairie cordgrass and willow-native polyculture alley systems.

4.3.5 Effects of light, water, and N availability on biomass yield

At Empire, a mixed effects model that included terms for soil water potential and PAR best estimated herbaceous crop yield (Table 4.6). Including terms for soil $\text{NO}_3\text{-N}$ and all two- and three-way interactions between water potential, PAR, and soil $\text{NO}_3\text{-N}$ did not improve model fit. Model estimated herbaceous biomass yield responded positively to increasing PAR and water potential. Over the range of observed PAR, model estimated crop yield varied by $2.8 \text{ Mg DM ha}^{-1}$. For every $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ increase in PAR, model

estimated herbaceous crop yield increased by 0.62 Mg DM ha⁻¹. Over the range of observed water potential, model estimated crop yield varied by 5.2 Mg DM ha⁻¹. For every 20 kPa increase in average daily water potential, model estimated herbaceous crop yield increased by 1.04 Mg DM ha⁻¹.

At Granada, a mixed effects model for herbaceous crop yield that included an interaction term for soil NO₃-N and water potential best estimated herbaceous crop yield. Including terms for PAR and all other two- and three-way interactions between water potential, PAR, and soil NO₃-N did not improve model fit. At the lowest soil NO₃-N concentrations (0.8 – 1.2 ppm), model estimated herbaceous crop yield increased with increasing average daily water potential (Table 4.7). At moderate soil NO₃-N concentration (1.4 ppm), there was no effect of average daily soil water potential on model estimated herbaceous crop yield. At the highest soil NO₃-N concentrations (1.6 – 2 ppm), model estimated herbaceous crop yield decreased with increasing average daily water potential. Conversely, at low mean daily water potential (-80 kPa), model estimated herbaceous crop yield increased with increasing NO₃-N concentrations. Yield was similar across the range of observed NO₃-N concentrations when mean daily water potential was around -60 kPa. At higher mean daily water potentials (-40 to -20 kPa), model estimated herbaceous crop yield decreased with increasing NO₃-N concentration.

4.4 Discussion

Herbaceous alley crop yields and availability of light, water, and N within alley systems were not consistent across sites. At Empire, competition with willows for light and soil water substantially reduced prairie cordgrass and native polyculture biomass yield 2.4 m

from tree rows, and had a moderate effect on yield up to 4.8 m from trees. Predictably, competition for N was not evident at Empire, where prior biosolids additions resulted in high antecedent organic and inorganic N. Similarly, competition for N has been found to be minimal or absent when standard rates of fertilizer N are applied in other temperate alley cropping systems (Jose et al., 2000; Miller and Pallardy, 2001; Zamora et al., 2006).

In contrast to Empire, soil NO₃-N and soil water potential helped explain variation in herbaceous crop yield at Granada. Model predicted herbaceous crop yields were lowest where availability of NO₃-N and water were highest. This is counterintuitive, as high availability of both resources should, in theory, result in the highest yield. However, relatively high availability of NO₃-N and water were likely due to low plant N uptake and water use as a result of low yields at these sample positions. For example, the lowest observed sample yields of 4.5 and 4.6 Mg ha⁻¹ were observed in one native polyculture plot, at the 2.4 m and 7.6 m distances, respectively. Observed NO₃-N concentrations were relatively high at these sample locations, 2.1 and 1.8 ppm, as were average daily water potentials, -20.8 kPa and -15.0 kPa, respectively. It is unclear why yields were low in these instances. However, repeated flooding at Granada since 2010 has resulted in substantial within plot spatial heterogeneity, which could partially explain these results. In addition, data used for this analysis greatly simplified the spatial and temporal variation in resource availability within the alley system and may be too coarse to determine effects on crop yield.

Interestingly, competition for soil water influenced herbaceous crop yield at both sites. This was somewhat surprising given the shallow water tables at each site and higher than average precipitation in 2013. However, hydrophytic species such as willow generally

have high rates of evapotranspiration (ET) and low water use efficiency compared to both C₃ and C₄ perennial grasses (Jørgensen and Schelde, 2001). Thus, we expected water use by willow to reduce soil water availability to herbaceous alley crops in the zone of tree and crop root overlap. We observed evidence of this during the summer at both sites, and also in fall at Granada. In willow-prairie cordgrass alleys at Empire, water potential was lower along the tree-crop interface relative to the center of the alley. Similarly, in willow-native polyculture alleys at Granada, water potential was reduced along the northern edge of the alley relative to the center. These patterns suggest that competition for soil moisture between willows and herbaceous crops will occur up to at least 2.4 m from tree rows in these alley systems, depending on seasonal water availability.

A north-south alley orientation was detrimental to herbaceous crop growth in terms of light availability, whereas other alley orientations appeared to have no effect in this regard. The extent of overall shading throughout the day was greater at Empire than at Granada, which is consistent with our finding that competition for light reduced herbaceous crop yield at the former, but not the latter. This can largely be attributed to alley orientation, though tree LAI was also identified as a significant source of variation in relative PAR. At Empire, there was a 57% reduction in PAR along the tree-crop interface (2.4 m) and a 21% reduction in the center of the alley. Lin et al (1999) found that many C₃ forage species can tolerate up to 50% shade without significant reductions in yield, while yields of C₄ forages were reduced under these conditions. At Empire, yields were reduced where PAR reduction exceeded 50%, even for C₃ intermediate wheatgrass (Chapter 2, this volume). At Granada, reduction in PAR was over 75% at the

southern edge of the alley, but curiously, no reduction in yield was apparent. Gillespie et al (2000) found that maize (*Zea mays* L.) yield was not impacted by up to 41% shade in black walnut (*Juglans nigra* L.) and red oak (*Quercus rubra* L.) alley cropping systems if belowground competition was eliminated. Lin et al (1999) found yield of both C₃ and C₄ forages reduced under 80% shade. While some degree of belowground competition was surely present at Granada, it's clear that competition for light was not influencing yield of herbaceous alley crops at this site since yields were similar across the entire alley.

Shading within the alley also played a facilitative role for herbaceous crops during certain periods at each site. For example, at Empire, higher water potentials adjacent to tree rows relative to the center of native polyculture alleys suggest plant water use was higher in the open than in the shelter of tree rows. Greater leaf area in the center of the alley partially explains these findings, as this likely resulted in greater ET at this position. However, we observed similar patterns in summer moisture availability at Granada with no differences in leaf area or biomass yield. Water potential in both native polyculture and prairie cordgrass alleys was greater along the southern edge of the alley than in the center. Thus, there was higher water use per unit leaf area in the center versus the southern edge of the alley, which suggests that shading played a facilitative role for both herbaceous crops under these circumstances. This is not surprising for the native polyculture, which contained a high proportion of C₃ species such as Canada wild rye (*Elymus canadensis* L.) and wild bergamot (*Monarda fistulosa* L.). Cool season (C₃) species can benefit from moderate shading due to their low light saturation point and low water-use efficiency (Kephart et al., 1992; Lambers et al., 2008). In contrast, C₄ species such as prairie cordgrass have a higher light saturation point, higher optimum

temperature for photosynthesis, and higher water use efficiency than C₃ species (Kephart et al., 1992; Taiz and Zeiger, 2010). This is evident in the fact that across sites, seasons, and alley positions, soil water potential was generally lower under native polyculture than prairie cordgrass plots in this study. However, it appears that even prairie cordgrass, a C₄ species, was the benefactor of facilitative shading during the summer at Granada.

Management interventions and alternative system designs could reduce competition in these systems and further improve productivity. Similar alley systems should be planted to west-east or northwest-southeast alley orientations to minimize competition for light. Alternatively, if a north-south orientation is used, an alley width greater than 15.2 m would also reduce competition for light. However, this would likely result in lower alley system yields since the ratio of woody to herbaceous crop area would decrease.

Additional modifications such as utilizing agricultural subsurface tile drainage water, alternative harvest regimes, or evaluating additional woody and herbaceous crops should be considered to improve yield, on-farm utility, and ecosystem services from these systems.

4.5 Conclusions

We found that competition for light and soil water substantially reduced crop yield up to 2.4 m from tree rows at Empire, while effects of soil N and soil water availability on crop yield were observed but were inconsistent at Granada. Competition for soil moisture between willows and herbaceous crops occurred up to at least 2.4 m from tree rows in these alley systems, depending on seasonal water availability. Light availability was lower in a north-south alley (Empire) than in west-east and northwest-southeast

orientations (Granada), but shading appears to have played a facilitative role during the summer, resulting in reduced crop water use. Spatially, prairie cordgrass and a native polyculture responded similarly to the alley cropping environment, though prairie cordgrass had slightly higher yields at both sites. Alley cropping systems comprised of shrub willow and herbaceous perennial biomass crops should avoid north-south row orientations to minimize shading. Competition for soil moisture reduced crop yield, but could be mitigated through appropriate management intervention.

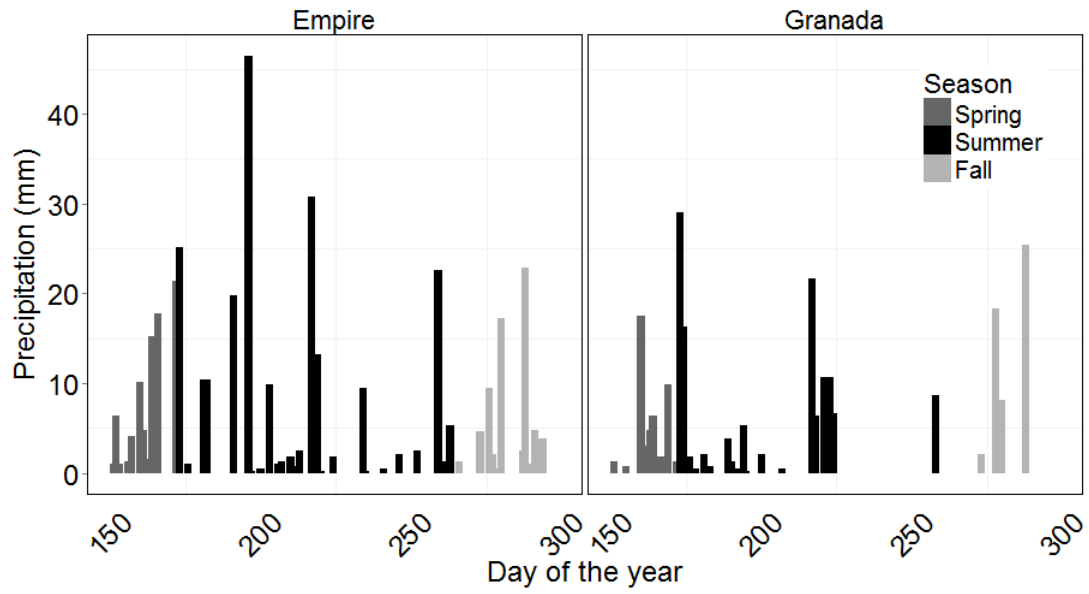


Figure 4.1. Seasonal precipitation at Empire (left) and Granada (right) Minnesota by day of the year from 31 May to 25 October 2013.

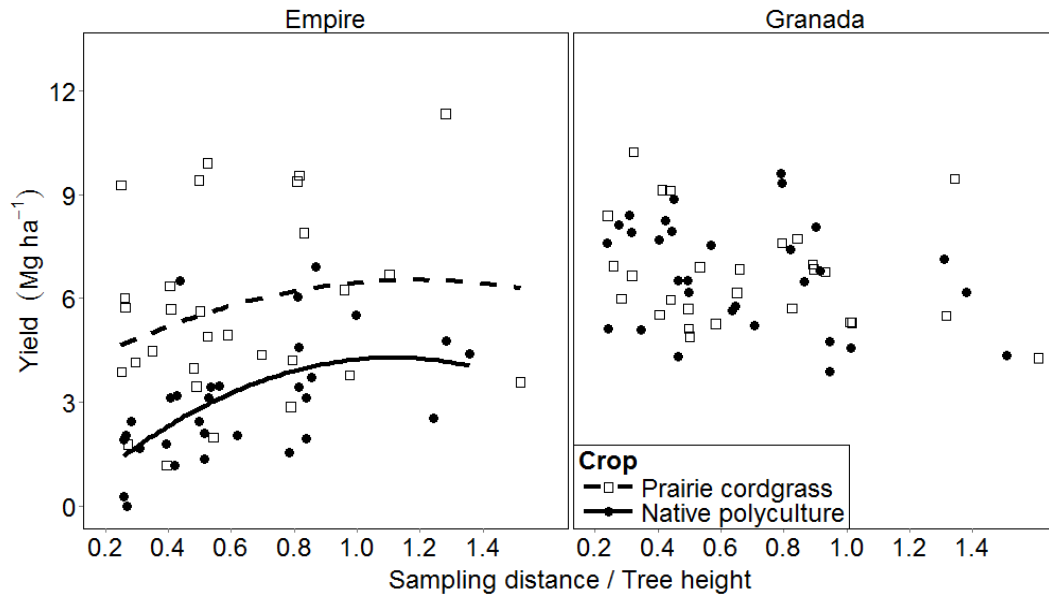


Figure 4.2. 2013 biomass yield of prairie cordgrass and native polyculture crops with respect to distance from the tree and tree height (D/H) at Empire and Granada, MN.

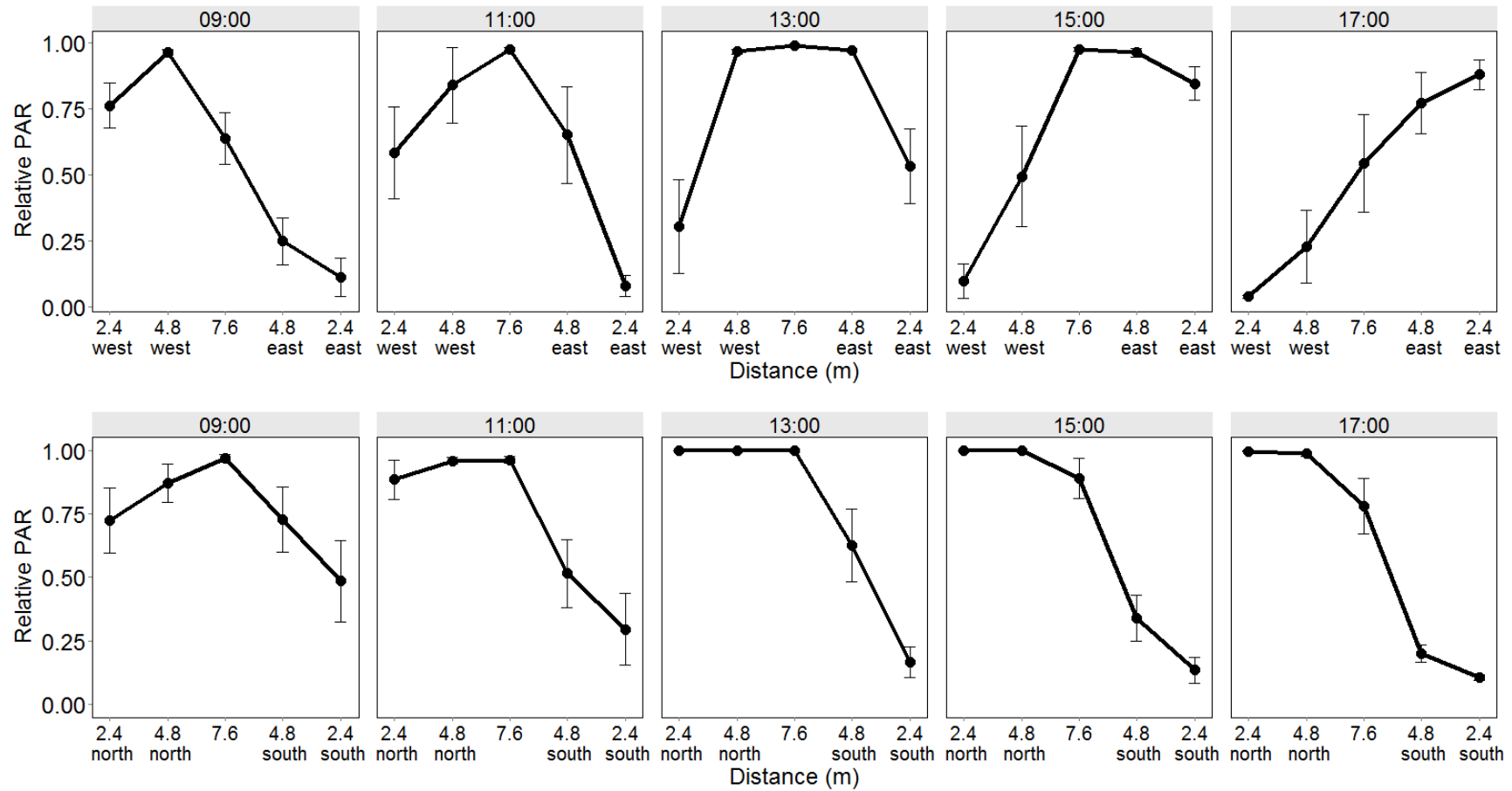


Figure 4.3. Diurnal relative PAR in Empire (top panels) and Granada (bottom panels) alley cropping systems with distance from the tree row on July 18 and 19, 2013. Error bars represent one standard error of the mean.

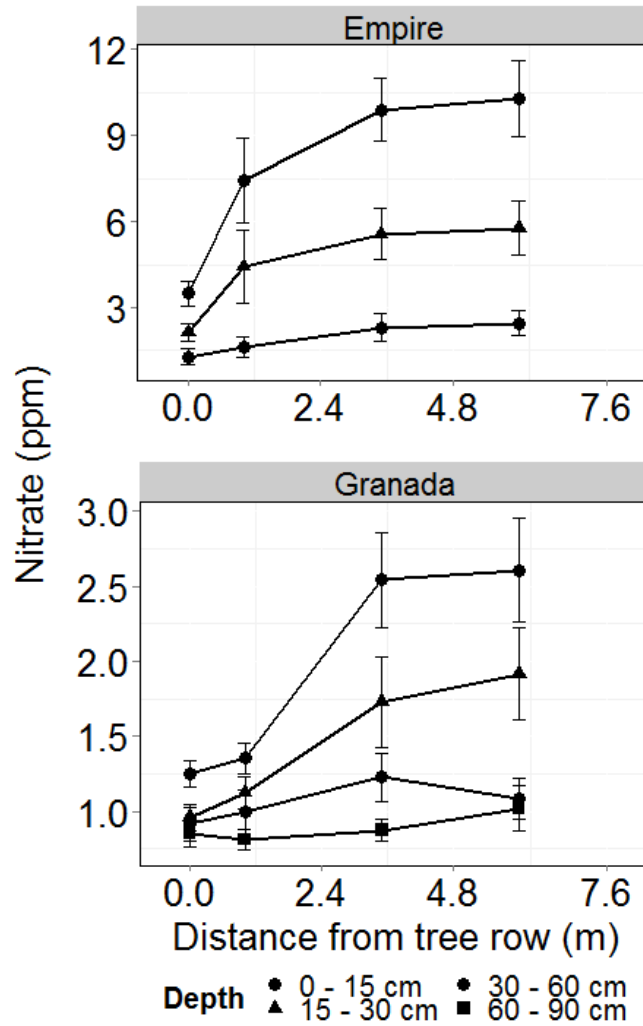


Figure 4.4. 2013 fall residual nitrate with distance from the tree row and soil depth in alley cropping systems at Empire and Granada, MN. Error bars represent one standard error of the mean. Note different x-axis scales for each site.

Table 4.1. Selected site and soil characteristics at Empire and Granada, MN

Characteristic	Empire	Granada
Latitude, Longitude	44.7178, -93.0975	43.7575, -94.3465
Landform	Stream terrace, outwash plain	Floodplain
Soil classification	Cylinder loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic aquic hapludolls)	Coland loam (fine-loamy, mixed, superactive, mesic cumulic endoaquolls)
Depth to water table	Approx. 45 cm	Approx. 15 - 45 cm
Soil organic matter (g kg ⁻¹) [†]	58	33
Soil pH	5.6	6.3
Soil NO ₃ ⁻ (mg kg ⁻¹)	42.3	6.8
Soil Bray P (mg kg ⁻¹)	761	25
Soil NH ₄ OAc-K (mg kg ⁻¹)	229	86
Previous management	Continuous corn with municipal biosolids	Corn- soybean rotation
Alley orientation (rep 1, 2, 3)	0°, 0°, 0°	90°, 100°, 135°

[†]Soil samples collected in November 2012

Table 4.2. Parameter estimates from best fitted mixed effects models for 2013 herbaceous biomass yield at Empire and Granada, MN

Site	Variable	β	SE(β)	t-value	p-value
Empire	Intercept	-0.2768	1.1353	-0.244	0.1389
	D/H	7.9601	2.4915	3.195	< 0.001
	poly(D/H~2)	-3.4990	1.5785	-2.217	0.02929
	Crop PCG	2.7766	1.0725	2.589	0.01286
Granada	Intercept	6.6777	0.8414	7.936	< 0.001
	Crop PCG	0.7907	1.1899	0.664	0.521

Table 4.3. Tests of fixed effects on water potential, NO₃, LAI, and PAR

Relative PAR							LAI						
Effect	Empire			Granada			Effect	Empire			Granada		
	F val.	P (> F)	Sig	F val.	P (> F)	Sig		F val.	P (> F)	Sig	F val.	P (> F)	Sig
Time of day (A)	9.30	< 0.001	***	3.93	0.004	**	Crop (A)	13.36	0.004	**	1.28	0.377	ns
Distance (B)	31.57	< 0.001	***	111.71	< 0.001	***	Distance (B)	8.44	0.003	**	0.12	0.886	ns
A x B	21.95	< 0.001	***	3.39	< 0.001	***	A x B	0.96	0.482	ns	0.17	0.981	ns
NO ₃							Spring water potential						
Effect	Empire			Granada			Effect	Empire			Granada		
	F val.	P (> F)	Sig	F val.	P (> F)	Sig		F val.	P (> F)	Sig	F val.	P (> F)	Sig
Distance (A)	16.29	< 0.001	***	17.85	< 0.001	***	Crop (A)	0.65	0.466	ns	2.04	0.227	ns
Depth (B)	59.57	< 0.001	***	32.58	< 0.001	***	Distance (B)	1.06	0.376	ns	1.06	0.376	ns
A x B	2.93	0.008	**	3.88	< 0.001	***	A x B	1.65	0.159	ns	1.65	0.159	ns
Summer water potential							Fall water potential						
Effect	Empire			Granada			Effect	Empire			Granada		
	F val.	P (> F)	Sig	F val.	P (> F)	Sig		F val.	P (> F)	Sig	F val.	P (> F)	Sig
Crop (A)	0.23	0.656	ns	1.32	0.314	ns	Crop (A)	0.08	0.466	ns	2.09	0.221	ns
Distance (B)	35.52	< 0.001	***	159.33	< 0.001	***	Distance (B)	41.24	< 0.001	***	125.74	< 0.001	***
A x B	69.9	< 0.001	***	43.49	< 0.001	***	A x B	15.09	< 0.001	***	37.63	< 0.001	***

* Significant at 0.1 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.001 level of probability.

† ns, not significant at the 0.1 level of probability.

Table 4.4. Average daily soil water potential at 15 cm depth in alley cropping systems at two Minnesota sites

Site	Season	System	Distance from tree row (m)				
			0 (S/W tree row)	2.4 S/W	7.6	2.4 N/E	0 (N/E tree row)
			kPa				
Empire	Summer	Willow – native polyculture	-119.54 ar [†]	-60.17 br	-202.60 cr	-91.47 dr	-81.00 dr
		Willow – prairie cordgrass	-148.36 ar	-54.74 br	-25.44 cs	-48.123 br	-146.41 ar
	Fall	Willow – native polyculture	-186.31 ar	-39.82 cr	-109.93 br	-65.71 cr	-81.01 br
		Willow – prairie cordgrass	-181.65 ar	-20.67 br	-19.49 bs	-22.73 br	-213.31 ar
Granada	Summer	Willow – native polyculture	-102.54 br	-44.52 er	-67.68 dr	-88.99 cr	-150.23 ar
		Willow – prairie cordgrass	-136.32 ar	-26.44 dr	-47.29 cr	-44.30 cr	-79.94 bs
	Fall	Willow – native polyculture	-140.87 bs	-48.21 dr	-54.58 dr	-112.88 cr	-178.14 ar
		Willow – prairie cordgrass	-215.89 ar	-23.57 cr	-27.78 cr	-25.68 cs	-85.23 br

[†]Means followed by the same letter are not different ($P > 0.1$). Letters a – d are used to denote differences within rows, while letter r – s are used to denote differences within sites and seasons (columns).

Table 4.5. Parameter estimates and variance partitioning from best fitted mixed effects models for 2013 effects of alley orientation on relative PAR at Empire and Granada, Minnesota.

Variable – fixed	β	SE(β)	t-value	p-value
Intercept	0.582118	0.1123	5.182	0.00101
Alley orientation	0.001963	0.0007711	2.546	0.01784

Variable – random	SD	p-value	Proportion of variance
Time of day	0.188	< 0.001	0.21
Distance from tree (D)	0.208	< 0.001	0.23
Tree height (H)	0.0	1.0	0.00
D / H	0.083	0.0140	0.09
Plot	0.0	1.0	0.00
Tree LAI	0.181	<0.001	0.20
Residual variance	0.242	NA	0.27

Table 4.6. Parameter estimates from best fitted mixed effects models for 2013 herbaceous biomass yield at Empire and Granada, MN

Site	Variable	β	SE(β)	t-value	p-value
Empire	Intercept	3.6209	1.7024	2.217	0.0527
	PAR	0.0047	0.0159	3.23	0.0063
	Water potential	0.0514	0.0015	3.168	0.098
Granada	Intercept	4.930	1.669	2.954	0.0147
	Nitrate	-2.988	2.167	-1.378	0.1935
	Water potential	-4.021	1.066	-3.770	0.0041
	Nitrate:water potential	-7.024	1.929	-3.642	0.0046

Table 4.7. 2013 model estimated herbaceous crop yield in alley systems at Granada, MN as a function of average daily water potential and fall residual NO₃.

Mean daily water potential (kPa)	Fall residual soil NO ₃ (mg kg ⁻¹)						
	0.8	1.0	1.2	1.4	1.6	1.8	2.0
	Mg ha ⁻¹						
- 20	8.1	7.5	6.9	6.3	5.7	5.1	4.5
- 40	7.2	6.9	6.6	6.3	6.0	5.7	5.4
- 60	6.3	6.3	6.3	6.3	6.3	6.3	6.4
- 80	5.4	5.7	6.0	6.3	6.7	7.0	7.3

Chapter 5 : General conclusions

The results of the first study demonstrated that different species pairings maximized alley cropping yield and nutrient uptake at each of three Minnesota sites. At Empire, the NM6 poplar – intermediate wheatgrass system had among the highest yield and nutrient uptake of all alley cropping systems. At Granada, NM6 poplar – prairie cordgrass and NM6 poplar – native polyculture had among the highest yield and nutrient uptake. At Fairmont, Fish Creek willow – native polyculture systems generally outperformed NM6 poplar – native polyculture systems, though no other herbaceous crops were compared at this site. Thus, NM6 poplar was more productive than Fish Creek willow at two of three sites. Intermediate wheatgrass showed the greatest potential for N, P, and K uptake in alley cropping systems, though the native polyculture and prairie cordgrass also performed well and are better choices for sites with occasional flooding. Effects of distance from the tree-crop interface on SRWC and herbaceous crop productivity were not consistent across sites, species, and years. However, we found that quantifying edge effects on SRWC productivity in agroforestry systems is important for accurately quantifying system yield and nutrient uptake. Yield response to distance from the tree-crop interface was similar for all herbaceous crops at each site, and herbaceous crops yielded similarly in poplar versus willow alleys.

The second study demonstrated the effects of alley cropping herbaceous perennials with short-rotation woody crops on root biomass C, root nutrient accumulation, and changes in SOC. Our results show that after four years of production, NM6 poplar – prairie cordgrass systems had among the highest C sequestration and nutrient accumulation in root biomass, which was largely a result of greater coarse root biomass for poplar and

prairie cordgrass than other SRWC and herbaceous crops. Belowground competition reduced C sequestration of herbaceous crops in alley cropping systems and may influence stand longevity. Converting from annual grain crops to perennial biomass alley cropping resulted in short-term SOC losses, but net C gains should be realized over the long-term due to rapid C accrual in belowground biomass. However, this depends largely on the extent to which C in fine roots is converted to recalcitrant SOM versus lost as C emissions. Finally, this study highlights the importance of evaluating the spatial distribution of tree and crop roots for accurately quantifying C sequestration and nutrient accumulation in agroforestry systems.

In the third study, we found that competition for light and soil water substantially reduced crop yield up to 2.4 m from tree rows at Empire, while effects of soil N and soil water availability on crop yield were observed but were inconsistent at Granada. Competition for soil moisture between willows and herbaceous crops occurred up to at least 2.4 m from tree rows in these alley systems, depending on seasonal water availability. Light availability was lower in a north-south alley (Empire) than in west-east and northwest-southeast orientations (Granada), but shading appears to have played a facilitative role during the summer, resulting in reduced crop water use. Spatially, prairie cordgrass and a native polyculture responded similarly to the alley cropping environment, though prairie cordgrass had slightly higher yields at both sites.

Collectively, our results show that after four years of production, NM6 poplar and prairie cordgrass were among the best SRWC and herbaceous crop choices for biomass production, C sequestration, and nutrient accumulation in alley cropping systems. However, competition may limit the stand longevity of herbaceous crops, which could

reduce the utility of these systems for biomass production and ecosystem services over time. Further consideration should be given to management interventions and alternative system designs to reduce competition in these systems and improve productivity. Similar alley systems should avoid north-south row orientation to minimize shading. Additional modifications such as utilizing agricultural subsurface tile drainage water for irrigation, alternative harvest regimes, or evaluating additional woody and herbaceous crops should be considered to improve yield, on-farm utility, and ecosystem services from these systems.

Bibliography

- Abrahamson, L.P., T.A. Volk, R.F. Kopp, E.H. White, and J.L. Ballard. 2002. Willow biomass producer's handbook. New York College of Environmental Science and Forestry. Syracuse, NY, USA.
- Asbjornsen, H., V. Hernandez-Santana, M. Liebman, J. Bayala, J. Chen, M. Helmers, C.K. Ong, and L.A. Schulte. 2013. Targeting perennial vegetation in agricultural landscapes for enhancing ecosystem services. *Renew. Agric. Food Syst.* 29(02): 101–125.
- Baer, S.G., D.J. Kitchen, J.M. Blair, and C.W. Rice. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. *Ecol. Appl.* 12(6): 1688–1701.
- Bardhan, S., and S. Jose. 2014. The potential for floodplains to sustain biomass feedstock production systems. *Biofuels* 3(5): 575–588.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. lme4: Linear mixed-effects models using Eigen and S4. version 1.1-9, <https://CRAN.R-project.org/package=lme4>.
- Benomar, L., A. DesRochers, and G.R. Larocque. 2012. The effects of spacing on growth, morphology and biomass production and allocation in two hybrid poplar clones growing in the boreal region of Canada. *Trees* 26: 939–949.
- Boe, A., V. Owens, J. Gonzalez-Hernandez, J. Stein, D.K. Lee, and B.C. Koo. 2009. Morphology and biomass production of prairie cordgrass on marginal lands. *GCB Bioenergy* 1(3): 240–250.
- Boody, G., B. Vondracek, D.A. Andow, M. Krinke, J. Zimmerman, and P. Welle. 2005. Multifunctional agriculture in the United States. *Bioscience* 55(1): 27–38.
- Börjesson, P., and G. Berndes. 2006. The prospects for willow plantations for wastewater treatment in Sweden. *Biomass Bioenergy* 30(5): 428–438.
- Cannell, M.G.R., M. Van Noordwijk, and C.K. Ong. 1996. The central agroforestry hypothesis: the trees must acquire resources that the crop would not otherwise acquire. *Agrofor. Syst.* 34(1): 27–31.
- Ceotto, E., and M. Di Candilo. 2011. Medium-term effect of perennial energy crops on soil organic carbon storage. *Ital. J. Agron.* 6(4): 33.

- Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. Le Quéré, R.B. Myneni, S. Piao, and P. Thornton. 2013. Carbon and Other Biogeochemical Cycles. p. 465 – 570. *In* Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Clinch, R.L., N. V. Thevathasan, A.M. Gordon, T.A. Volk, and D. Sidders. 2009. Biophysical interactions in a short rotation willow intercropping system in southern Ontario, Canada. *Agric. Ecosyst. Environ.* 131: 61–69.
- Collins, H.P., J.L. Smith, S. Fransen, A.K. Alva, C.E. Kruger, and D.M. Granatstein. 2010. Carbon sequestration under irrigated switchgrass (*Panicum virgatum* L.) production. *Soil Sci. Soc. Am. J.* 74(6): 2049.
- Debell, D., G. Clendenen, C. Harrington, and J. Zasada. 1996. Tree growth and stand development in short-rotation *Populus* plantings: 7-year results for two clones at three spacings. *Biomass Bioenerg* 11(4): 253–269.
- Debell, D.S., C.A. Harrington, G.W. Clendenen, and J.C. Zasada. 1997. Tree growth and stand development of four *Populus* clones in large monoclonal plots. *New For.* 14: 1–18.
- Deng, L., G. Bin Liu, and Z.P. Shangguan. 2014. Land-use conversion and changing soil carbon stocks in China's "Grain-for-Green" Program: A synthesis. *Glob. Chang. Biol.*: 3544–3556.
- Ehret, M., L. Bühle, R. Graß, N. Lamersdorf, and M. Wachendorf. 2014. Bioenergy provision by an alley cropping system of grassland and shrub willow hybrids: biomass, fuel characteristics and net energy yields. *Agrofor. Syst.* 89: 365–381.
- Ellert, B.H., and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75(4): 529–538.
- Felix, E., D.R. Tilley, G. Felton, and E. Flamino. 2008. Biomass production of hybrid poplar (*Populus* sp.) grown on deep-trenched municipal biosolids. *Ecol. Eng.* 33(1): 8–14.
- Fortier, J., D. Gagnon, B. Truax, and F. Lambert. 2010a. Nutrient accumulation and carbon sequestration in 6-year-old hybrid poplars in multiclonal agricultural riparian buffer strips. *Agric. Ecosyst. Environ.* 137: 276–287.

- Fortier, J., D. Gagnon, B. Truax, and F. Lambert. 2010b. Biomass and volume yield after 6 years in multiclonal hybrid poplar riparian buffer strips. *Biomass Bioenerg* 34: 1028–1040.
- Gamble, J.D., G. Johnson, C.C. Sheaffer, D.A. Current, and D.L. Wyse. 2014. Establishment and early productivity of perennial biomass alley cropping systems in Minnesota, USA. *Agrofor. Syst.* 88: 75–85.
- Gamble, J.D., D.L. Wyse, G. Johnson, C.C. Sheaffer, and D.A. Current. 2016. Species pairing and edge effects on biomass yield and nutrient uptake in perennial alley cropping systems. *Agron. J.* In press.
- García-Barrios, L., and C.K. Ong. 2004. Ecological interactions, management lessons and design tools in tropical agroforestry systems. *Agrofor. Syst.* 61-62(1-3): 221–236.
- Gavlak, R., D. Horneck, R.O. Miller, and J. Kotuby-Amacher. 2003. Soil, plant and water reference methods for the western region. WREP-125, 2nd Ed. WERA-103 Publ. Color. State Univ. Ft. Collins.
- Gillespie, A., S. Jose, and D. Mengel. 2000. Defining competition vectors in a temperate alley cropping system in the midwestern USA: 1. Production physiology. *Agrofor. Syst.* 48: 25–40.
- Gillitzer, P.A., D.L. Wyse, C.C. Sheaffer, S.J. Taff, and C.C. Lehman. 2012. Biomass production potential of grasslands in the oak savanna region of Minnesota, USA. *BioEnergy Res.* 6(1): 131–141.
- Hangs, R.D., J.J. Schoenau, K.C.J. Van Rees, N. Bélanger, T. Volk, and T. Jensen. 2014. First rotation biomass production and nutrient cycling within short-rotation coppice willow plantations in Saskatchewan, Canada. *BioEnergy Res.* 7: 1091 – 1111.
- Harper, J.L. 2010. Population biology of plants. The Blackburn Press.
- Heller, M.C., G.A. Keoleian, and T.A. Volk. 2003. Life cycle assessment of a willow bioenergy cropping system. *Biomass Bioenerg* 25(2): 147–165.
- Herve, C., and R. Ceulemans. 1996. Short-rotation coppiced vs non-coppiced poplar: A comparative study at two different field sites. *Biomass Bioenerg* 11(2-3): 139–150.
- Holm, B., and K. Heinsoo. 2013. Municipal wastewater application to short rotation coppice of willows – treatment efficiency and clone response in Estonian case study. *Biomass Bioenerg* 57: 126–135.
- Holzmueller, E., and S. Jose. 2012. Biomass production for biofuels using agroforestry: potential for the North Central Region of the United States. *Agrofor. Syst.* 85: 305 – 314.

- IPCC. 2014. IPCC Working Group III, Fifth Assessment Report - Mitigation of Climate Change.
- Jaynes, D.B., and T.M. Isenhardt. 2014. Reconnecting tile drainage to riparian buffer hydrology for enhanced nitrate removal. *J. Environ. Qual.* 43: 631–638.
- Johnson, J.B., and K.S. Omland. 2004. Model selection in ecology and evolution. *Trends Ecol. Evol.* 19(2): 101–8.
- Johnson, G.A., D.L. Wyse, and C.C. Sheaffer. 2013. Yield of perennial herbaceous and woody biomass crops over time across three locations. *Biomass Bioenergy* 58: 267–274.
- Jordan, N., and K.D. Warner. 2010. Enhancing the multifunctionality of US agriculture. *Bioscience* 60(1): 60–66.
- Jørgensen, U., and K. Schelde. 2001. Energy crop water and nutrient use efficiency. Prepared for the International Energy IEA Bioenergy Task 17, Short Rotation Crops.
- Jose, S., and S. Bardhan. 2012. Agroforestry for biomass production and carbon sequestration: An overview. *Agrofor. Syst.* 86: 105–111.
- Jose, S., A.R. Gillespie, and S.G. Pallardy. 2004. Interspecific interactions in temperate agroforestry. *Agrofor. Syst.* 61: 237–255.
- Jose, S., A. Gillespie, and J. Seifert. 2000. Defining competition vectors in a temperate alley cropping system in the midwestern USA: 3. Competition for nitrogen and litter decomposition dynamics. *Agrofor. Syst.* 48: 61–77.
- Jungers, J.M., A.T. Clark, K. Betts, M.E. Mangan, C.C. Sheaffer, and D.L. Wyse. 2015a. Long-term biomass yield and species composition in native perennial bioenergy cropping systems. *Agron. J.* 7(5): 1627 – 1640.
- Jungers, J.M., J.O. Eckberg, K. Betts, M.E. Mangan, D.L. Wyse, and C.C. Sheaffer. 2015b. Plant roots and GHG mitigation in native perennial bioenergy cropping systems. *GCB Bioenergy* In press.
- Jungers, J.M., J.E. Fargione, C.C. Sheaffer, D.L. Wyse, and C. Lehman. 2013. Energy potential of biomass from conservation grasslands in Minnesota, USA. *PLoS One* 8(4): 1 – 11.
- Jungers, J.M., C.C. Sheaffer, and J. a. Lamb. 2015c. The effect of nitrogen, phosphorus, and potassium fertilizers on prairie biomass yield, ethanol yield, and nutrient harvest. *BioEnergy Res.* 8(1): 279–291.

- Kephart, K.D., D.R. Buxton, and S.E. Taylor. 1992. Growth of C3 and C4 perennial grasses under reduced irradiance. *Crop Sci.* 32: 1033 – 1038.
- Lambers, H., F.S. Chapin III, and T.L. Pons. 2008. *Plant Physiological Ecology*. Second Edi. Springer New York.
- Lamerre, J., K.-U. Schwarz, M. Langhof, G. von Wühlisch, and J.-M. Greef. 2015. Productivity of poplar short rotation coppice in an alley-cropping agroforestry system. *Agrofor. Syst.*
- Lee, K.H., T.M. Isenhardt, and R.C. Schultz. 2003. Sediment and nutrient removal in an established multi-species riparian buffer. *J. Soil Water Conserv.* 58(1): 1–8.
- Lee, D.K., V.N. Owens, A. Boe, and B.C. Koo. 2009. Biomass and seed yields of big bluestem, switchgrass, and intermediate wheatgrass in response to manure and harvest timing at two topographic positions. *GCB Bioenergy* 1(2): 171–179.
- Lin, C.H., R.L.M.C. Graw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. : 109–119.
- Malézieux, E., Y. Crozat, C. Dupraz, M. Laurans, D. Makowski, H. Ozier-Lafontaine, B. Rapidel, S. Tourdonnet, and M. Valantin-Morison. 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. *Agron. Sustain. Dev.* 29(1): 43–62.
- Mangan, M.E., C. Sheaffer, D.L. Wyse, N.J. Ehlke, and P.B. Reich. 2011. Native perennial grassland species for bioenergy: establishment and biomass productivity. *Agron. J.* 103(2): 509–519.
- Mbow, C., P. Smith, D. Skole, L. Duguma, and M. Bustamante. 2014. Achieving mitigation and adaptation to climate change through sustainable agroforestry practices in Africa. *Curr. Opin. Environ. Sustain.* 6: 8–14.
- Miller, A.W., and S.G. Pallardy. 2001. Resource competition across the crop-tree interface in a maize-silver maple temperate alley cropping stand in Missouri. *Agrofor. Syst.* 53: 247–259.
- Naimi, B. 2013. usdm: Uncertainty analysis for species distribution models. R package version 1.1-12. <http://CRAN.R-project.org/package=usdm>.
- Pacaldo, R.S., T.A. Volk, and R.D. Briggs. 2013. Greenhouse gas potentials of shrub willow biomass crops based on below-and aboveground biomass inventory along a 19-year chronosequence. *Bioenergy Res.* 6(1): 252–262.

- Pacaldo, R., T. Volk, and R. Briggs. 2014. Carbon sequestration in fine roots and foliage biomass offsets soil CO₂ effluxes along a 19-year chronosequence of shrub willow (*Salix x dasyclados*) biomass. *BioEnergy Res.* 7(3): 769–776.
- Pallardy, S., D. Gibbins, and J. Rhoads. 2003. Biomass production by two-year-old poplar clones on floodplain sites in the Lower Midwest, USA. *Agrofor. Syst.* 59: 21–26.
- Peterjohn, W., and D. Correll. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. *Ecology* 65: 1466 – 1476.
- Pinheiro, J., and D. Bates. 2000. Mixed-effects models in S and S-PLUS. (J Chambers, W Eddy, W Hardle, S Sheather, and L Tierney, Eds.). Springer Verlag New York LLC, New York, NY.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Development Core Team. 2013. nlme: Linear and Nonlinear Mixed Effects Models. R Packag. version 3.1-111. Available at <http://dosen.narotama.ac.id/wp-content/uploads/2012/03/Package-nlme.pdf> (verified 15 August 2014).
- Quinkenstein, A., J. Woellecke, C. Bohm, H. Grunwald, D. Freese, B.U. Schneider, and R.F. Huttel. 2009. Ecological benefits of the alley cropping agroforestry system in sensitive regions of Europe. *Environ. Sci. Policy* 12(8): 1112–1121.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rivest, D., A. Cogliastro, R.L. Bradley, and A. Olivier. 2010. Intercropping hybrid poplar with soybean increases soil microbial biomass, mineral N supply and tree growth. *Agrofor. Syst.* 80(1): 33–40.
- Rytter, R.-M. 1999. Fine-root production and turnover in a willow plantation estimated by different calculation methods. *Scand. J. For. Res.* 14(6): 526–537.
- Rytter, R.-M. 2012. The potential of willow and poplar plantations as carbon sinks in Sweden. *Biomass Bioenerg* 36: 86–95.
- Sartori, F., R. Lal, M.H. Ebinger, and D.J. Parrish. 2007. Potential soil carbon sequestration and CO₂ offset by dedicated energy crops in the USA. *CRC. Crit. Rev. Plant Sci.*
- Schoeneberger, M., G. Bentrup, H. de Gooijer, R. Soolanayakanahally, T. Sauer, J. Brandle, X. Zhou, and D. Current. 2012. Branching out: Agroforestry as a climate change mitigation and adaptation tool for agriculture. *J. Soil Water Conserv.* 67(5): 128A–136A.

- Schoonover, J.E., K.W.J. Williard, J.J. Zaczek, J.C. Mangun, and A.D. Carver. 2005. Nutrient attenuation in agricultural surface runoff by riparian buffer zones in southern Illinois, USA. *Agrofor. Syst.* 64(2): 169–180.
- Schultz, R.C., J.P. Colletti, T.M. Isenhardt, W.W. Simpkins, C.W. Mize, and M.L. Thompson. 1995. Design and placement of a multi-species riparian buffer strip system. *Agrofor. Syst.* 29(3): 201–226.
- Smith, P., M. Bustamante, H. Ahammad, H. Clark, H. Dong, E.A. Elsidig, H. Haberl, R. Harper, J. House, M. Jafari, O. Masera, C. Mbow, N.H. Ravindranath, C.W. Rice, C.R. Abad, A. Romanovskaya, F. Sperling, and F. Tubiello. 2014. Agriculture, Forestry and Other Land Use (AFOLU). In: *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Edenhofer, O., R. Pichs-Madruga, Y.
- Smucker, A.J.M., S.L. Mcburney, and A.K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy* 74: 500 – 503.
- Sprunger, C.D., S.S. Snapp, and S.W. Culman. 2012. Implications for carbon sequestration: management effects on annual versus perennial root production. ASA, CSSA SSSA Annu. Meet. Cincinnati, OH. Oct. 21 - Oct. 24, 2012. Available at <https://scisoc.confex.com/scisoc/2012am/webprogramcd/Paper71449.html> (verified 28 January 2016).
- Stadnyk, C.N. 2010. Root dynamics and carbon accumulation of six willow clones in Saskatchewan. M.S. Thesis. Univ. Saskatchewan. Saskatoon, Canada.
- Steinbeiss, S., H. Beßler, C. Engels, V.M. Temperton, N. Buchmann, C. Roscher, Y. Kreuziger, J. Baade, M. Habekost, and G. Gleixner. 2008. Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Glob. Chang. Biol.* 14(12): 2937–2949.
- Stocker, T.F., D. Qin, G.-K. Plattner, L.V. Alexander, S.K. Allen, N.L. Bindoff, F.-M. Bréon, J.A. Church, U. Cubasch, S. Emori, P. Forster, P. Friedlingstein, N. Gillett, J.M. Gregory, D.L. Hartmann, E. Jansen, B. Kirtman, R. Knutti, K.K.P. Kumar, D.G. Vaughan, and S.-P. Xi. 2013. Technical Summary. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*.
- Taiz, L., and E. Zeiger. 2010. *Plant physiology*. 5th ed. Sinauer Assoc, Sunderland, MA.
- Tharakan, P., and T. Volk. 2005. Morphological traits of 30 willow clones and their relationship to biomass production. *Can. J. For. Res.* 431: 421–431.

- Thelemann, R., G. Johnson, C. Sheaffer, S. Banerjee, H. Cai, and D. Wyse. 2010. The effect of landscape position on biomass crop yield. *Agron. J.* 102(2): 513 – 522.
- Tufekcioglu, A., J.W. Raich, T.M. Isenhardt, and R.C. Schultz. 2003. Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa, USA. *Agrofor. Syst.* 57: 187–198.
- Vanek, V. 1991. Riparian zone as a source of phosphorus for a groundwater-dominated lake. *Water Res.* 25(4): 409–418.
- Verchot, L. V., M. Noordwijk, S. Kandji, T. Tomich, C. Ong, A. Albrecht, J. Mackensen, C. Bantilan, K. V. Anupama, and C. Palm. 2007. Climate change: linking adaptation and mitigation through agroforestry. *Mitig. Adapt. Strateg. Glob. Chang.* 12(5): 901–918.
- Volk, T. 2002. Alternative methods of site preparations and coppice management during the establishment of short-rotation woody crops. PhD dissertation.
- West, B., K. Welch, and A. Galecki. 2014. Linear mixed models: a practical guide using statistical software. CRC Press.
- Wilson, D.M., E.A. Heaton, L.A. Schulte, T.P. Gunther, M.E. Shea, R.B. Hall, W.L. Headlee, K.J. Moore, and N.N. Boersma. 2014. Establishment and short-term productivity of annual and perennial bioenergy crops across a landscape gradient. *BioEnergy Res.* 7(3): 885–898.
- Young, E.O., and R.D. Briggs. 2005. Shallow ground water nitrate-N and ammonium-N in cropland and riparian buffers. *Agric. Ecosyst. Environ.* 109(3-4): 297–309.
- Young, E.O., and R.D. Briggs. 2007. Nitrogen dynamics among cropland and riparian buffers: soil-landscape influences. *J. Environ. Qual.* 36(3): 801–14.
- Zalesny, J.A., R.S. Zalesny, A.H. Wiese, B. Sexton, and R.B. Hall. 2008. Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. *J. Sustain. For.* 27(3): 303 – 327.
- Zamora, D.S., S. Jose, and P. Nair. 2007. Morphological plasticity of cotton roots in response to interspecific competition with pecan in an alleycropping system in the southern United States. *Agrofor. Syst.* 69: 107–116.
- Zamora, D.S., S. Jose, P.K.R. Nair, and C.L. Ramsey. 2006. Interspecific competition in a pecan – cotton alleycropping system in the southern United States : Production physiology. *Can. J. Bot.* (84): 1686–1694.

Zan, C., J. Fyles, P. Girouard, and R. Samson. 2001. Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. *Agric. , Ecosyst. Environ.* 86(2): 135–144.

Zilverberg, C.J., W.C. Johnson, V. Owens, A. Boe, T. Schumacher, K. Reitsma, C.O. Hong, C. Novotny, M. Volke, and B. Werner. 2014. Biomass yield from planted mixtures and monocultures of native prairie vegetation across a heterogeneous farm landscape. *Agric. Ecosyst. Environ.* 186: 148–159.