EVALUATING PLANT FUNCTIONAL DIVERSITY IN AN ORGANIC INTERCROPPING SYSTEM

A Dissertation

by

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ABSTRACT

Overyielding, a phenomenon whereby plant production in mixture exceeds that of production in monoculture, has been attributed to complementary use of resources by different plant functional types. Few studies have examined the role of plant functional diversity and the concept of overyielding in food production systems. Different combinations of peanut (*Arachis hypogaea* L.), watermelon (*Citrullus lanatus* Thunb.), okra (*Abelmoschus esculentus* Moench.), cowpea (*Vigna unguiculata* L.), and pepper (*Capsicum annuum* L.) planted alone or in various intercropping combinations were investigated over two growing seasons in an organic system in the peak of summer heat in Texas. Results from land equivalent ratio (LER) indicate that the within-row intercropping combination of peanut, watermelon, and okra (W_{pwo}) and the four species combination with the addition of cowpea (W_{pwoc}) consistently overyielded both growing seasons, despite a reversal in dominance patterns exhibited by watermelon and okra between years. There was no effect of intercropping system on changes in total nitrogen (N) or soil organic carbon (SOC). However, soil microbial biomass carbon (SMB-C) was lower in W_{pwo} as compared to okra grown in monoculture. Although there was no difference in root length density (RLD) between cropping system, there was a significant positive linear relationship between RLD and SMB-C. Low leaf area index (LAI) values in cowpea and peanut monocultures resulted in higher daily soil temperatures and an increase in weed biomass. There was a strong inverse relationship between LAI and soil temperature, particularly with daily maximum soil temperature. No differences in physiological parameters were detected. In 2012, when watermelon was a subordinate crop, specific leaf area (SLA) and leaf N content were highest in the multispecies systems, particularly W_{pwoc}. Carbon to nitrogen ratio (C:N) was also lowest in W_{pwo} and W_{pwoc} as compared to watermelon grown in monoculture indicating watermelon underwent morphological changes at the leaf level due to competition for light and allocated less C to leaves when competition was reduced. Overall findings suggest that three and four species intercropping combinations, whereby each crop is selected to perform a specific function within the system, may provide small-scale sustainably-minded producers a model system that can be utilized in the peak of summer in southern climates and allow them to reduce inputs while increasing overall yields.

DEDICATION

This dissertation is dedicated to my friends and family, especially my dear mother and my sisters, for their support and encouragement.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Concerns over the availability of adequate food supplies have been heightened due to an exponential growth in the global human population. The increase in population has led to the encroachment of metropolitan areas into rural, traditionally agricultural areas. This, consequently, has led to a decrease in the land available for agricultural production. Additional concerns over the impacts humans are having on the environment have been raised due to urbanization and increased environmental education programs. As a result, now more than ever, politicians, scientists, and growers are seeking ways in which to meet the high demand for food without compromising its safety and minimizing impacts on the environment.

Biodiversity in natural ecosystems is thought to provide a variety of ecosystem services. In traditional row crop systems, however, monocultures dominate the landscape. Homogenous fields are thought to increase plant susceptibility to pest and disease problems, and decrease ecosystem functioning and stability. Agrobiodiversity is a mix of the ecological concepts that make up biodiversity with traditional agricultural production practices. Despite recent efforts to further combine them, there continues to exist a lack of understanding about how introducing biodiversity in production practices can balance the need for sustainable yields and quality while enhancing ecosystem functioning.

Agrobiodiversity and Ecosystem Functioning

According to the Millennium Ecosystem Assessment (2005), global changes such as land use and land cover have significant effects on the ecological properties of ecosystems and, consequently, on the ecosystem services that humans rely on. Because of these changes, biodiversity is decreasing at a rate never before seen (Balvanera et al., 2006). Plant functional diversity, including the value, range, and plant functional traits in a given ecosystem, is considered to be a manifestation of changes in biodiversity (Diaz et al., 2007). As Koocheki and colleagues (2008) explain, biodiversity has evolved in order to fill the multiplicity of niches that exist in the worlds ecosystems. Biodiversity, in turn, provides the foundations for nutrient cycling efficiency and natural pest and disease control (Altieri, 1999), among other ecological cycles and processes. Thus, the concepts of biodiversity and ecosystem functioning are directly associated and one cannot be discussed without the other.

The concept of agrobiodiversity is a relatively new one (Altieri, 1999; Duffy, 2002). Agrobiodiversity, also called agrodiversity or agricultural biodiversity, has been described as, “the different variety of practices and attributes of the farm systems used by farmers for food production, especially evident in small farmer systems” (Brookfield and Padoch, 1994). Agricultural systems, and in particular intensive systems, are typically based on optimizing productivity in large monotypic stands (Malézieux et al., 2009). As such, the ecosystem is dramatically altered and diversity is reduced to one or few species, creating a homogenous landscape.

In order to manage for biodiversity and provide ecosystem services in these landscapes, an understanding of ecological concepts is necessary (Smukler et al., 2010). Ecosystem services provided by biodiversity in agricultural landscapes include water regulation, pest and disease control, preservation of genetic diversity, nutrient cycling, and soil fertility and erosion control (Swift et al., 2004). In addition, diversification of plant species’ has been shown to increase net primary productivity (Tilman et al., 1996). From an agricultural production standpoint, biodiversity counteracts the deterioration of genetic resources that have been found in many field crops by increasing the gene pool (Baudry, 1989). Furthermore, increasing net primary productivity and nutrient retention result in an increase in soil fertility.

The exact role biodiversity plays in an agricultural ecosystem is still under debate and poorly understood, however. This is especially true at various spatial and temporal scales. According to Swift et al. (2004), agricultural development probably has a more pronounced effect on diversity at larger scales rather than the plot scale. Thus, understanding the mechanisms underlying biodiversity and associated relationships requires multi-scale and long-term research (Smukler et al., 2010). In their California-based farm-level study, Smukler and colleagues (2010) examined biodiversity and ecosystem functioning. They measured above and below ground biodiversity, soil carbon (C), gas emissions, nutrient availability, water infiltration, leaching, and sediment loss and compared their results between production areas, riparian corridors, hedgerows, drainage ditches, and tailwater ponds. They found non-production habitats increased biodiversity and specific ecosystem functions such as water regulation and carbon storage. They concluded that utilizing approaches such as farmscaping could increase biodiversity as well as multiple ecosystem functions without major production losses. In their review on biodiversity and ecosystem services, Swift et al. (2004) argue that landscape- and farm-scale diversity may potentially provide more ecosystem services than plot or field scale applications due to the level of habitat complexity introduced.

Other questions raised by researchers working in the biodiversity field relate to the concepts of functional groups and functional redundancy. As defined by Swift et al. (2004), a functional group is “a set of species that have similar effects on a specific ecosystem-level biogeochemical process”. They are the species that contribute in different ways to the overall functioning of the ecosystem (Vitousek and Hooper, 1993). Functional redundancy is defined as a set of species that have a similar affect and contribute equally to a specific biogeochemical process within an ecosystem (Wohl, 2004). Functional groups may include perennials, annuals, and woody species but may take on any number of meanings. Perennials and woody species, which are deep-rooted may enable more complementary water and nutrient use by plants (Malézieux et al., 2009), thereby minimizing competition. A study conducted by Wohl (2004) and colleagues on microbial populations supported the idea that functionally redundant species play an important role in ecosystem functioning.

In their 12-year Michigan-based study, Snapp et al. (2010) compared two management strategies, integrated conventional and organic, along with an incremental increase in the number of species and their effects on ecosystem services and yields . They concluded that management intensity is responsible enhancing soil health, not agrobiodiversity. However, their study was conducted on grain crops and they did not incorporate the concept of functional diversity into their study. Swift and colleagues (2004) made the point that the relationship between species in space and time in an ecosystem, whether natural or agricultural, is more important than simply the number of species present.

Plant Functional Diversity and Overyielding

In natural ecosystems, increased plant species diversity has been shown to increase net primary productivity (Tilman et al., 1996). Two possible explanations have been proposed to explain this; the sampling effect hypothesis and the complementarity effect hypothesis.

In multispecies systems, complementarity and facilitation can offset the negative effects of competition (Hooper et al., 2005). Complementarity results from niche partitioning and a reduction of interspecies competition (Vandermeer, 1989), while facilitation occurs when neighboring plants have a beneficial effect on each other (Chu et al., 2008). Facilitation can occur during times of suboptimal environmental conditions when one species alleviates those conditions or provides a resource for neighboring species (Hooper et al., 2005). Complementarity occurs when species use different resources or use the same resource but separate its utilization in time or space. This can result in more efficient use of resources by the community as more of the total available resources are being used (Harper, 1977; Vandermeer, 1989). Competition in plants ultimately occurs between individuals (Weiner, 1990) and classic competition theory asserts that intraspecies competition is often more intense than interspecies competition due to greater niche overlap (Bengtsson et al., 1994). Overyielding, a phenomenon where plant production in mixture exceeds that of production in monoculture, has been attributed to complementarity in resource use and minimal niche overlap between species (Vandermeer, 1990). Complementarity, along with facilitation, has been cited as a reason why species are able to coexist in diverse natural communities and it is thought that even partial complementarity may increase system productivity (Hooper, 1998).

Some researchers have emphasized the importance of functional differences between species and the relationship between species in space and time rather than the effect of species richness per se on improving ecosystem functioning (Landis et al., 2000). Species from different functional groups differ significantly in their use and acquisition of resources (Reich et al., 2004). The loss or gain of functional types within a plant community can change the flow of energy and the supply of resources such as N, thereby changing the productivity of the community and functioning of the ecosystem (Reich et al., 2012).

Small farmers in tropical forest areas have long utilized intercropping systems and have incorporated a variety of crops with different growth forms, which creates a complex multi-layered habitat that closely mimics nature (Denevan, 1995). In agroforestry systems of the tropics, it has been observed that deep-rooted trees bring nutrients up from deeper soil layers, thereby increasing nutrient use efficiency and reducing leachate (van Noordwijk et al., 1996). The “three sisters” intercropping system of squash, bean, and corn practiced by Native Americans located in North America is another well documented example of a multi-layered agroecosystem (Mohler and Stoner, 2009). In these types of systems, each crop occupies a functional group niche and contributes differently to the overall ecosystem (Vitousek and Hooper, 1993). In the case of the “three sisters”, squash suppresses weed growth (smother crop), bean as the nitrogen-fixer, and corn as structural support (Mohler and Stoner, 2009).

Diversity Effects on Below-Ground Response

Soils play a major role in nutrient cycling and, specifically, the global C and N cycles (Nair et al., 2009). The C-carrying capacity of a soil will vary based on climactic factors such as temperature and precipitation and on biotic factors such as vegetation type (Guo and Gifford, 2002). The top one meter of soil is estimated to contain 2 to 3 times the amount of total C than the atmosphere and aboveground vegetation (Sommer et al., 2000; Lal, 2003; Nair et al., 2009) and is therefore regarded as a major sink for C in terrestrial ecosystems (Ceccon et al., 2011). There is a key distinction between longer-term (stable) soil C pools that have a turnover rate that range from decades to centuries (Bruun et al., 2008; Rumpel and Kögel-Knabner, 2010) and short-term (labile) soil C pools that have a rapid turnover rate that range from months to years in terrestrial ecosystems (Krull et al., 2003; Jagadamma and Lal, 2010). With regards to labile C pools, C in the form of organic matter is a particularly key component in fertile soils in agricultural systems (Kumar et al., 2006). Soil organic carbon (SOC), along with soil total nitrogen (TN), is considered a good indicator of soil quality (Dilly et al., 2003). Not only can organic C enhance soil fertility and plant nutrient availability, but it can improve soil aggregates and soil biological activity (Zeng et al., 2010). Improvements in soil aggregates result in an increase in specific surface area per unit of soil mass, which contributes to an increase in soil water retention (Blanco-Canqui et al., 2013). On average, a 1% increase in SOC can increase plant available water by 12.5 mm in the top 20 cm of the soil profile of medium-textured soils (Blanco-Canqui et al., 2013). Thus, agricultural practices that manage for enhanced SOC are important for the productivity and long-term stability of agricultural systems (Wang et al., 2011; Bajoriene et al., 2013).

At equilibrium, inflows and outflows to the C pool are equal; a balance that is important to changes in SOC (Lal, 2003; Blanco-Canqui et al., 2013). Sources of organic C can come from above- and below-ground. In agroecosystems, crop residues in form of leaf and crop litter contribute to soil organic matter, providing soil with material that has varying rates of decomposition (Oelbermann et al., 2005; Zeng et al., 2010). Litter quantity and quality (carbon to nitrogen ratio C:N), along with environmental factors, strongly contribute to these differential rates of decomposition (Hooper et al., 2000; Orwin et al., 2010). This, in turn, affects the dynamics of the microbial population and mineralization of nutrients (Mungai and Motavalli, 2006). Above-ground litter material is decomposed and mineralized, increasing the amount of plant available nutrients (Laossi et al., 2008), or immobilized by soil microorganisms depending upon the C:N ratio (Burger and Jackson, 2003). Due to the linked nature of C and N decomposition, factors affecting the rhizosphere (root zone) will also affect N cycling (Dijkstra et al., 2009) as microbes utilize organic material deposited onto the soil as a C source and acquire N from the decomposition of this material (Kuzyakov et al., 2000). Therefore, as a living part of soil organic matter, soil microbes are a critical component in the cycling of C and N in terrestrial ecosystems (Gastine et al., 2003; Laossi et al., 2008; Wang et al., 2011).

Below-ground processes are still not well understood due to both the complexity of interactions in the rhizosphere and variability in measurement techniques (McCormack and Fernandez, 2011). The primary sources of organic C below-ground are root exudates and other organic substances, sloughing of root hairs and fine roots, and root turnover (Kumar et al., 2006; Orwin et al., 2010). It is estimated that up to 33% of global annual net primary production is comprised of fine root production (Gill and Jackson, 2000). Roots differ in their ability to absorb water and nutrients (Volder et al., 2005), with fine roots responsible for most of the nutrient and water uptake from the soil (Brassard et al., 2011). Root turnover contributes a significant portion to soil C pool (Nair et al., 2009) and it is estimated that 15-25% of C allocated to roots is exuded from fine roots back into the soil (Kuzyakov and Cheng, 2001; Kuzyakov, 2002). A greater accumulation of fine roots, in particular, enhances microbial populations due to faster decomposition rates as compared to lignified coarse roots (van Noordwijk et al., 1996; Wang et al., 2010). Additionally, heterotrophic soil microbes utilize plant exudates and decomposing roots as a food source (Jackson et al., 2012) and, consequently, a strong relationship has been found to exist between root density and microbial biomass (Gastine et al., 2003). Due to this rhizosphere priming effect (Kuzyakov, 2002), roots play a significant role in C and N cycling.

Plant community composition and diversity can affect both above- and below-ground inputs that may alter rhizosphere dynamics (Fornara and Tilman, 2008; Jackson et al., 2012; Chanteloup and Bonis, 2013). Results from diversity studies have yielded conflicting results with regards to below-ground processes. On the one hand, above-ground competition for light may alter biomass partitioning to below-ground resources and actually reduce root and microbial biomass (Bloom et al., 1985; Bessler et al., 2009), which will in turn affect C and N dynamics. On the other hand, a higher diversity of litter quality is expected at high levels of specific or functional diversity of plants (Laossi et al., 2008), which may, in turn, increase soil fauna diversity if there is a complementary use of different types of litter by these microorganisms (Hooper et al., 2000). With regards to SOC, Tilman et al. (2006a) found greater soil C accumulation in more species-rich N-limited grassland communities. Species richness can also lead to an increase in below-ground biomass (Reich et al., 2004). While species richness itself may lead to increased SOC and root biomass, others have found that plant functional traits and the presence of key functional groups (legumes in particular) are more important determining factors of below-ground response (Bardgett et al., 1999; Diaz and Cabido, 2001; Gastine et al., 2003). Fornara and Tilman (2008) found that enhanced accumulation of soil C and N in the top 20 cm of the soil profile with higher plant diversity was positively associated with total root biomass. They attributed this increase in total root biomass to complementarity between plant functional types, specifically between C4 grasses and legumes when these two functional groups were present. This complementarity was thought to occur due to both facilitation and niche differentiation between the two functional groups. Species that are complementary in their resource use often differ in rooting depth, plant phenology, or other traits that regulate the timing of resource utilization (Polley et al., 2013). Niche differentiation and complementarity by plant roots in mixed species systems may lead to greater filling of soil pore space by roots (Brassard et al., 2011; Postma and Lynch, 2012). Additionally, legumes, which have a higher litter quality (low C:N), have high litter decomposition rates and have significant effects on N availability and supply in N-limited ecosystems (Chapin et al., 1986; Vitousek and Howarth, 1991; Fornara and Tilman, 2008). Under low N-supply, legumes may also provide a source of N for neighboring species through root and nodular tissue decay and root exudates (Chu et al., 2004; Wichern et al., 2007a).

Species diversity and species interactions may also indirectly affect below-ground processes through the alteration of soil moisture and soil temperature. Higher leaf area index (LAI), often associated with mixed species systems, has been shown to reduce soil temperature and reduce soil moisture losses due to evaporation (Ikeorgu and Ezumah, 1991; Olasantan and Babalola, 2007; Ghanbari et al., 2010; Ossom, 2010). Modifications in soil temperature and moisture can both influence microbial activity and organic C decomposition (Dijkstra et al., 2006; Ceccon et al., 2011). While the importance of the interactive effects of soil moisture and temperature on soil respiration have been demonstrated (Wildung et al., 1975; Bryla et al., 2001; Huang, 2005), soil temperature may be of particular interest as soil respiration has been found to be especially sensitive to fluctuations in temperature (Boone et al., 1998).

Soil respiration is made up of two components: root-derived (autotrophic) respiration and microbial (heterotrophic) respiration (Ceccon et al. 2011). Both components react differently to changes in environmental conditions and slight changes in temperature may affect total soil respiration (Li et al., 2011). Together they represent a significant source of C losses through CO_2 efflux into the atmosphere (Ceccon et al., 2011; Thurgood et al., 2014). Accounting for 33 to 60% of total soil respiration (Bowden et al., 1993), root-derived respiration is of particular interest as fine roots are especially sensitive to fluctuations in temperature (Desrochers et al., 2002; Huang, 2005). Liu et al. (2013) demonstrated there was a linear relationship between soil temperature and soil respiration. However, they did not partition out root and microbial respiration in their study. This root respiration and soil temperature relationship may vary between species (Atkin et al., 2000) but, in general, studies have found that root respiration reaches its peak at midday when temperatures are at their maximum (Lipp and Andersen, 2003; Ceccon et al., 2011; Qin et al., 2013). Therefore, agricultural practices that reduce maximum daytime soil temperatures via increased canopy cover may reduce C losses from soil and contribute to the accumulation of soil C.

In summary, a complex set of above- and below-ground interactions drive rhizosphere processes. These interactions may be affected by plant species diversity and community composition. Integrated cropping systems that can optimize C inputs, enhance long-term SOC accumulation, enhance microbial activity and plant-available N, and reduce C losses to the atmosphere may offer more sustainable approaches to food production.

Diversity Effects on Above-Ground Response

In multispecies systems, positive interactions such as facilitation and complementarity can offset some of the negative interactions associated with plant resource competition (Callaway, 1998; Hooper et al., 2005). Complementarity results from niche partitioning and a reduction of competition between species (Vandermeer, 1989; Hille Ris Lambers et al., 2004; van Ruijven and Berendse, 2005), while facilitation occurs when neighboring plants ameliorate habitat through the moderation of abiotic stress during times of suboptimal conditions (Hooper et al., 2005; Chu et al., 2008; Zhang et al., 2012). The stress-gradient hypothesis, which was formulated at the interspecies competition level, states there is a shift from competition to facilitation in plant communities as abiotic stress is amplified along environmental gradients (Callaway and Walker, 1997; Zhang et al., 2012). Complementarity occurs when functionally different species differ in their acquisition of resources in either time or space (Reich et al., 2004; van Ruijven and Berendse, 2005; Tilman et al., 2006b). Complementary use of resources, along with facilitative interactions between species, has been cited as a reason why species are able to coexist and why plant growth and productivity are maximized in diverse natural plant communities (Hooper, 1998; Hille Ris Lambers et al., 2004; Hauggaard-Nielsen et al., 2009a).

The efficiency of converting resources into biomass depends on the total amount of light intercepted by the canopy (i.e., a function of canopy size and competition for light) and the rate of net photosynthesis per unit leaf area (Lambers et al., 2008). Plants that form part of a more dense canopy, such as that found in diverse ecosystems, will undergo more competition for light and, consequently, a stronger vertical light gradient (Poorter et al., 2006). Since leaves are the primary photosynthetic organs of a plant, plants respond to changes in their light environment by changing leaf morphology and altering resource allocation patterns to leaves (Poorter et al., 2009). Plants can modify specific leaf area (SLA), leaf area per unit dry mass, and the relative investment of nitrogen between leaf photosynthetic machinery (Evans and Poorter, 2001). For instance, shading can result in reduced leaf thickness due to reduced thickness of palisade parenchyma, thereby increasing SLA (Poorter et al., 2006). As an underlying component of relative growth rate (RGR), an increase in SLA maximizes the amount of light interception by increasing RGR (Lambers et al., 2008) and increasing a plants competitive ability. Furthermore, there is a strong linear relationship between SLA and leaf N, with leaf N increasing as SLA increases. Since photosynthetic machinery accounts for more than half of leaf N content (Evans, 1989; Lambers et al., 2008), photosynthetic capacity is tightly associated with N availability and leaf N content (Evans, 1989; Loomis, 1997). Therefore, changes in leaf resource allocation and specific leaf area can be better predictors of plant growth than minor changes in net assimilation rates as photosynthetic capacity per unit leaf area is optimized (Potter and Jones, 1977) .

Intercropping has become an important management strategy for enhancing crop resource use efficiency and maximizing plant productivity through the deliberate manipulation of interspecific species interactions (Vandermeer, 1989; Li et al., 1999; Andersen et al., 2007; Hauggaard-Nielsen et al., 2009a). Intercropping can have both positive and negative effects on net assimilation rates and plant growth (Hooper and

Vitousek, 1998; Andersen et al., 2005). Intercropping with functionally dissimilar species may lead to an increase in leaf area index (LAI) and overall light interception (Bilalis et al., 2010; Salau et al., 2014), thereby capturing more available light but also potentially creating a more dense canopy leading to increased competition for light. Changes in leaf-level traits (LMA: leaf mass per unit area or the reciprocal of SLA), gas exchange and WUE have been found to be associated with growth habit in row crops such as soybean and wheat (Tanaka et al., 2008; Barrios-Masias et al., 2014). The morphological and anatomical changes that occur at the leaf-level can affect chlorophyll content and, consequently, stomatal conductance and photosynthetic rates (Makoi et al., 2010; Barrios-Masias et al., 2014). As previously discussed, photosynthetic activity is linked to efficient plant nitrogen uptake and partitioning within a leaf, which is, in part, influenced by growth habit (Evans and Poorter, 2001). Photosynthetic nitrogen-use efficiency (PNUE, photosynthetic rate per unit N) is associated with a high relative growth rate, thereby increasing the fitness of the plant and its ability to compete with neighbors (Hikosaka, 2004). However, there is a tradeoff between investing in a high-N leaf which has a shorter lifespan versus investing in C compounds associated with leaf longevity (Field and Mooney, 1986). Due to this N investment in leaves with higher SLA, and ultimately greater photosynthetic capacity, a decrease in PNUE is expected as SLA decreases (Harrison et al., 2009). In addition, since leaf N content is correlated with photosynthesis and stomatal conductance (Niinemets and Kull, 1998; Reich et al., 2003; Hikosaka, 2004), PNUE is tightly coupled with water-use efficiency in some species

(Sage and Pearcy, 1987). Moreover, sustained high photosynthetic rates are often correlated with higher crop yields (Ainsworth et al., 2002; Ainsworth and Long, 2005).

Most intercropping studies have limited their approach to simplified two-species systems and occasionally three-species systems (Andersen et al., 2007). In addition, the majority of studies conducted in temperate regions have focused on legume-cereal intercropping systems (Zhang and Long, 2003; Gao et al., 2009; Hauggaard-Nielsen et al., 2009a; Dahmardeh et al., 2010; Gao et al., 2010). Makoi et al. (2010) found that photosynthetic rates and water-use efficiency (as measured by isotopic discrimination) decreased in cowpea when intercropped with sorghum at different planting densities but particularly at high densities. Su et al. (2014) also observed a decrease in photosynthetic rate in soybean seedlings when intercropped with maize. In contrast, Pinheiro and Filho (2000) observed an increase in maize photosynthetic activity when intercropped with cowpea as compared to monocropped maize. There was a decline in cowpea photosynthesis, however. They also observed an improvement in water relations in both crops as measured by leaf water potential. They attributed both the improved water relations and lower net photosynthesis measurements in intercropped cowpea to facilitative environmental modifications from shading by maize. Light interception is important as plants grown under higher irradiances tend have higher photosynthetic rates and stomatal conductance (g_s) values, but lower WUE (Evans and Poorter, 2001; Tanaka et al., 2008; Barrios-Masias et al., 2014). Other studies have also found higher photosynthetic rates associated with two-species intercropping systems (Gomez-Rodriguez et al., 2007; Ahmad et al., 2013).

In summary, there is little agreement on how intercropping may impact sustained crop productivity from a physiological perspective owing to the variability in response. Studies have primarily been limited to two-species systems and there is a gap in our understanding of how component crops will respond to a functionally diverse intercropping system with regards to physiology and resource-use efficiency. In addition, there is little mention of leaf-level acclimation in existing intercropping studies.

Intercropping and Pest and Disease Suppression

Weed management in any cropping system is the single greatest input (Wang et al., 2009; Dayan et al., 2011). Weed control is important to reduce competition with the target crop for moisture, nutrients, and light (Uchino et al., 2009). Thus, weeds, more so than any other pest, have the greatest adverse impact on crop yields (Dayan et al., 2011). Yield losses from 40 to 80% have been cited due to weed populations left unmanaged and outcompeting cash crops for resources (Akobundu, 1987; Karlen et al., 2002). The cost of weed control, along with yield losses due to competition for resources with weeds, has been estimated at more than $15 billion annually in developed nations (Buhler, 2003). Due to their highly effective mode of action and relative low cost, synthetic herbicides have been a reliable tool for weed control in conventional systems (den Hollander et al., 2007; Dayan et al., 2011). However, the increasing demand for organic food and concerns over the potential detrimental effects these herbicides have on human health and on the environment have driven the search for safer alternatives for managing and controlling weed populations (den Hollander et al., 2007; Dayan et al., 2009; Dayan et al., 2011; Webber III et al., 2014).

In organic systems, options for weed control are limited (Peruzzi et al., 2007). This creates a challenge for organic farmers (Barberi, 2002; Anderson, 2010) and is the largest obstacle for producers considering transitioning from conventional production systems (Barberi, 2002), especially for the management of perennial weeds (Wedryk et al., 2012). Weeds are an even bigger problem in low-input systems, and in particular with vegetable crops that are inferior competitors (den Hollander et al., 2007). Organic farmers are primarily restricted to cultural and mechanical techniques, such as mulching, smother cover crops, cultivation, and direct manual weeding, to suppress weeds (Isik et al., 2008; Bilalis et al., 2010; Mulvaney et al., 2011).

Mechanical weed control such as manual weeding is ineffective for long-term management of weeds and can damage soil structure (den Hollander et al., 2007). In addition, this requires high labor input and thus is costly (Karlen et al., 2002; den Hollander et al., 2007). Organic mulching with straw or hay has been found to be an effective barrier to weed establishment by inhibiting light transmittance (Steinmaus et al., 2008) and creating a physical barrier to germination (Rowley et al., 2011), but efficacy varies by mulch type (Bajoriene et al., 2013) and may harbor additional weed seed sources (Rowley et al., 2011). In addition, the effect organic mulches have on soil organic carbon (SOC) and soil nutrient availability is still under debate (Blanco-Canqui and Lal, 2007; Bajoriene et al., 2013). Plastic mulching, another effective method for weed suppression, can be cost prohibitive and may facilitate the spread of certain diseases through splash-dispersal (Coelho et al., 2008). Cover crops such as rye, vetch, and clover behave similarly to mulches and can be effective in suppressing weed growth prior to planting of the main crop, but require elimination by tillage or herbicide (Buhler et al., 2001) and may act as hosts for diseases that can infect the target crop (Jackson, 2004). Cultivation practices such as tillage only serve to shift weed populations depending upon the type of tillage utilized, can destroy soil aggregate structure , and may decrease soil quality (Mulvaney et al., 2011) which is essential in organic and low-input systems. Additionally, tillage can be costly (Madden et al., 2004). The availability of non-synthetic herbicides is very limited (Rowley et al., 2011). Chemical strategies that are effective, such as acetic acid and clove oil, can become cost prohibitive due to the large volumes required to suppress weed growth and do not provide long-term control (Dayan et al., 2009; Webber III et al., 2014). Other non-synthetic herbicide such as pelargonic acid are currently under investigation (Webber III et al., 2014). Further, all commercially available organic herbicides are non-selective and must be applied with caution (Dayan et al., 2009).

Intercropping has been cited as a potentially effective cultural control strategy for weeds in organic production systems. A reduction in weed emergence has been attributed to intercropping with either cover crops or cash crops (Barberi, 2002). The ability of the target crop to outcompete weeds is enhanced in intercropping systems through an increase in resource capture by the desired species which reduces availability of resources such as light, water, and nutrients to weeds (Barberi, 2002; Baumann et al., 2002; Saudy, 2014) as well as through allelopathy (Iqbal et al., 2007). However, this may depend upon factors such as crop geometry, canopy architecture, planting density, and crop growth rate (Keating and Carberry, 1993; Isik et al., 2008). Small farmers in tropical forest areas have long utilized intercropping systems and have incorporated a variety of crops with different growth forms, to create a complex multi-layered habitat (Denevan, 1995). The “three sisters” intercropping system of squash, bean, and corn practiced by the Native Americans is a well-documented example of a multi-layered agroecosystem (Mohler and Stoner, 2009). In these types of systems, each crop occupies a functional group niche and in the case of the “three sisters”, squash acts as a smother crop to suppress weed growth, bean is the nitrogen fixer, and corn provides support for bean vines (Vitousek and Hooper, 1993; Mohler and Stoner, 2009). The ability of a multi-layer intercropping system to suppress weed growth is owed to a reduction of light transmittance due to an increase in canopy density (Baumann et al., 2002). As a result, the enhanced competitive ability of mixed cropping systems makes them a potentially useful tool for weed management in low-input farming systems (Saudy, 2014).

Traditional chemical control methods for pests and diseases such as insecticides, fungicides, and fumigants are harmful to the environment (Ren et al., 2008). The elimination of synthetic chemicals is an important component of organic farming. However, as with weeds, options for pests and diseases management in organic systems are limited. Although not as effective as synthetics, organic pesticides such as azadirachtin and pyrethrin have been found to reduce populations of certain pests (Immaraju, 1998; Moreau et al., 2006; Vassiliou, 2011). However, they do not discriminate against beneficial insects and pollinators. Therefore the timing of spray is critical for minimizing mortality to beneficials and pollinators (Immaraju, 1998). In addition, organic insecticides do not effectively control adult pest insects and multiple applications must be made to control pest populations (Stark and Walter, 1995; Aliakbarpour et al., 2011). The availability of organic naturally-derived fungicides is very limited and the few that are available are not as effective as chemical fungicides (Dayan et al., 2009).

A proven strategy for the effective control of pests and the spread of disease is intercropping (Ren et al., 2008; Narla et al., 2011). There are a number of explanations offered for this. A multi-layered canopy formed by intercropping two or more species can create mechanical barriers for insects harboring and spreading viruses and diseases, and can create olfactory and visual confusion for insects (Ramkat et al., 2008). The physical barriers created by multi-species cropping also prevent direct contact of crop roots or shoots of individuals within the same species, which may reduce the spread of diseases such as bacterial wilt (Kloos et al., 1987). Therefore, decreasing the density of any one crop and increasing the distance between individuals of the same species can reduce or delay the spread of disease, as well as make it more difficult for pests such as aphids and other specialist to move between plants of the same species (Potts, 1990; Noman et al., 2013). There are additional microclimate effects of an architecturally complex intercropping system on temperature, moisture, and light that may reduce the incidence and severity of diseases (Theunissen, 1994; Finckh, 2008). Moreover, adding non-host plants in a mixed cropping system can dilute cues that attract pests to a specific crop (George et al., 2013).

Some crops may act as a repellent or deterrent, likely through chemical or olfactory signals. Asare-Bediako et al. (2010) found that an intercropping of cabbage with tomato reduced the infestation of diamondback moth, a significant pest in cabbage production. It was believed that odor emitted from tomato repelled the diamondback moth or may have had an oviposition deterring effect. Plant extracts such as capsaicin from hot pepper and red pepper have been found to reduce pests such as cabbage looper, onion fly, and spider mite due to this repellent or deterrent effect (Cowles et al., 1989; Antonious et al., 2007). Other crops as well as their extracts, such as onion, garlic, and spices have also been found to reduce the incidence of fungal diseases such as Fusarium wilt in watermelon (Hu et al., 2012) and reduce aphid populations on cabbage and mustard (Baidoo et al., 2012; Noman et al., 2013).

Another explanation for the ability of mixed cropping systems to reduce disease and pest populations is that they increase numbers of natural enemies of pests such as aphids (Rizk, 2011). This increase in natural predators is, at least in part, due to the elimination of synthetic chemicals which reduce predator populations in conventional systems (Kromp, 1999). Additionally, it is believed that mixed cropping systems provide favorable microclimates and conditions for the survival of predators (Risch, 1983; Potts, 1990). Finally, intercropping also may reduce weed populations, as previously discussed. An indirect consequence of this reduction in weeds is that it eliminates or minimizes alternate hosts for the build-up of pest populations and diseases (Potts, 1990).

The numerous benefits of intercropping such as weed and pest control have made intercropping more popular in developed countries where organic practices are on the rise, such as in the United States (Kahn, 2010). Despite the rising popularity of intercropping, the ecology and productivity of multi-layer architecturally complex intercropping systems have not been studied extensively in the Southern United States. There are few studies that have quantitatively evaluated the role of functional diversity on weed, disease, and pest control with the goal of reducing inputs and any potential negative residual impacts on the ecosystem.

CHAPTER II

THE EFFECTS OF FUNCTIONALLY DIVERSE INTERCROPPING ON YIELD AND QUALITY*

Introduction

In natural ecosystems, increased plant species diversity has been shown to increase net primary productivity (Tilman et al., 1996). Two possible explanations have been proposed to explain this; the sampling effect hypothesis and the complementarity effect hypothesis.

In multispecies systems, complementarity and facilitation can offset the negative effects of competition (Hooper et al., 2005). Complementarity results from niche partitioning and a reduction of interspecies competition (Vandermeer, 1989), while facilitation occurs when neighboring plants have a beneficial effect on each other (Chu et al., 2008). Facilitation can occur during times of suboptimal environmental conditions when one species alleviates those conditions or provides a resource for neighboring species (Hooper et al., 2005). Complementarity occurs when species use different resources or use the same resource but separate its utilization in time or space. This can result in more efficient use of resources by the community as more of the total available resources are being used (Harper, 1977; Vandermeer, 1989). Competition in plants

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ultimately occurs between individuals (Weiner, 1990) and classic competition theory asserts that intraspecies competition is often more intense than interspecies competition due to greater niche overlap (Bengtsson et al., 1994). Overyielding, a phenomenon where plant production in mixture exceeds that of production in monoculture, has been attributed to complementarity in resource use and minimal niche overlap between species (Vandermeer, 1990). Complementarity, along with facilitation, has been cited as a reason why species are able to coexist in diverse natural communities and it is thought that even partial complementarity may increase system productivity (Hooper, 1998).

Some researchers have emphasized the importance of functional differences between species and the relationship between species in space and time rather than the effect of species richness per se on improving ecosystem functioning (Landis et al., 2000). Species from different functional groups differ significantly in their use and acquisition of resources (Reich et al., 2004). The loss or gain of functional types within a plant community can change the flow of energy and resource supply, thereby changing the productivity of the community and functioning of the ecosystem (Reich et al., 2012).

Small farmers in tropical forest areas have long utilized intercropping systems and have incorporated a variety of crops with different growth forms, which creates a complex multi-layered habitat that closely mimics nature (Denevan, 1995). In agroforestry systems of the tropics, it has been observed that deep-rooted trees bring nutrients up from deeper soil layers, thereby increasing nutrient use efficiency and reducing leachate (van Noordwijk et al., 1996). The “three sisters” intercropping system of squash, bean, and corn practiced by Native Americans located in North America is another well documented example of a multi-layered agroecosystem (Mohler and Stoner, 2009). In these types of systems, each crop occupies a functional group niche and contributes differently to the overall ecosystem (Vitousek and Hooper, 1993). In the case of the “three sisters”, squash suppresses weed growth (smother crop), bean is the nitrogen-fixer, and corn provides structural support (Mohler and Stoner, 2009).

According to Altieri (1999), Latin American farmers grow 70–90% of their beans in a mixed system with maize, potatoes, and other crops. However, despite the success of intercropping in developing countries and the rising popularity of intercropping in developed ones (Kahn, 2010), multi-layer architecturally complex intercropping systems have not been studied extensively in the United States. Many studies have evaluated the role of biodiversity in agroecosystems. Most have not incorporated the concept of functional diversity, but only the number of species present. What is known is that functionally diverse plant communities can lead to increased total community productivity. What is unknown, however, is if this will translate into an increase in yield and total food production per area and per plant. The objectives of this study were to determine if a multifunctional intercropping system can lead to overyielding of food production and to examine the effects on fruit quality. We hypothesized that a functionally diverse cropping system will lead to overyielding and an increase in total fruit production in an organic system consisting of peanut (*Arachis hypogaea* L.), watermelon (*Citrullus lanatus* Thunb.), okra (*Abelmoschus esculentus* Moench.), cowpea (*Vigna unguiculata* L.), and hot pepper (*Capsicum annuum* L.). We also hypothesized that fruit quality will only be reduced in sub-dominant crops.

Materials and Methods

Study Area

Low input managed plots were established at Texas A&M University's Horticulture Farm (30°37′N, -96°22′W) during the 2011 and 2012 growing seasons. Average monthly air temperatures for this area from May to October when the study was conducted ranged from 28 to 39°C for the maximum and 15 to 26°C for the minimum in 2011 and 27 to 37°C and 15 to 25°C in 2012 (NOAA/NCDC).

Experimental Design

The study design was a randomized complete block with three replicates, five intercropping treatments, and five controls. The controls consisted of monocultures of the five component species; peanut, mini watermelon, okra, cowpea, and hot pepper. The five treatments were a within-row intercropping system of 1) peanut and watermelon (W_{pw}), 2) peanut, watermelon, and okra (W_{pwo}), 3) peanut, watermelon, okra, and cowpea (W_{pwoc}), 4) all five control species (W_{all}), and 5) a strip intercropping system of peanut and watermelon consisting of alternating single rows (S_{pw}). Figure 1.1 illustrates the planting layout for the within-row and strip intercropping schemes. Crops represented four different genera and were selected based on the following criteria: 1) heat tolerance, 2) desired architecture and function (Table 1.1), and 3) no known adverse effects on other component crops. "Tamspan 90" is a small Spanish bunch type peanut which was selected for its small canopy and lack of runners. The "TAMU" mini watermelon variety was developed by the Texas A&M University watermelon breeding program and has a number of advantages for small scale producers including its ability to tolerate narrower spacing and, thus, take up less space, and produces a smaller fruit which is more manageable for small growers. Selected because of its popularity, “clemson spineless” okra is a dwarf variety well suited for this region. “Texas pinkeye” is a purple hull cowpea selected for its erect and bush-type growth form. This taller variety of cowpea can tolerate narrower spacing. Hot pepper varieties were used for this study. Jalapeno was used for year one and Serrano for year two and selection was based upon availability.

Crops were planted in 4 m long double rows on 90 cm wide raised beds with rows spaced approximately 30 cm apart and beds spaced 45 cm apart (total plot size, 4 × 5 m). Using the methodologies recommended by Jeavons (2006) and minimum spacing requirements outlined in the Texas Vegetable Growers Handbook (Masabni and Dainello, 2009), individual plant spacing was based on the mean spacing requirement for all component crops. Densities were kept constant across plots regardless of crop species and individual plants were spaced 30 cm apart in a staggered row pattern so that each plant was neighbored by an individual of a different species and a leguminous species. A 2 m buffer was maintained between plots.

Year 1 Experiment

In year 1, a chisel plow and middle buster were used to prepare the field in May 2011. The field was then irrigated and solarized for 1.5 months with clear greenhouse plastic in order to reduce weed pressure. Upon removal of plastic, a row-maker was used to make three 1.5 m long rows per replicate for a total of nine rows. Drip tape was installed down the center of each row during the row-making process.

Peanut was direct seeded on August 1, 2011 and watermelon was direct seeded approximately one week later on August 7th. Okra and cowpea were direct seeded on August 14 and 15th, respectively. Seven centimeter tall pepper plants were transplanted on August 18th. Peanut and cowpea were inoculated with “Vault” commercial inoculant during planting as this field had not previously been inoculated with *Rhizobia*. Inoculation with *Rhizobia* is considered to benefit leguminous crops by increasing nodulation and N_2 fixation, and maximizing yields (Brockwell and Bottomley, 1995). Although the addition of inoculant may increase costs for the producer in the short-term, the long-term benefits in the form of increased yields outweigh that initial cost (Nyoki and Ndakidemi, 2013). Additionally, re-seeding continued through the end of August in order to maximize the number of plants per species per plot. Weeding was done continuously throughout the growing season from the time of seeding through the conclusion of the study.

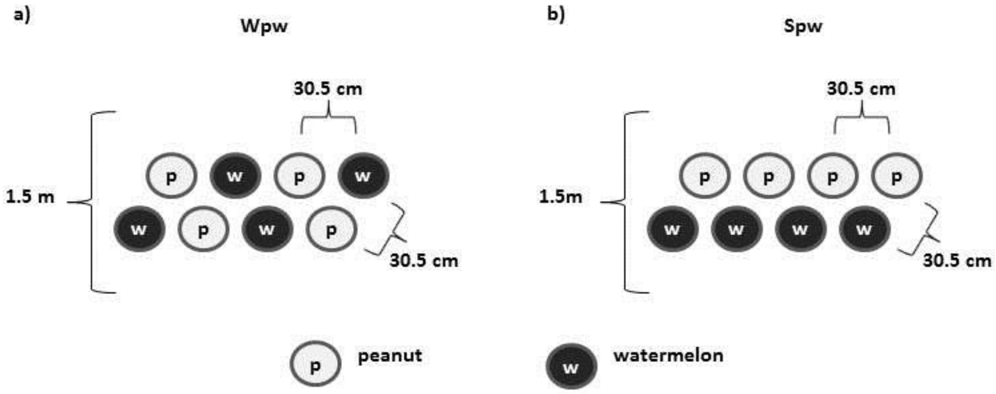


Figure 1.1 Planting layout and spacing for the a) within-row intercropping system of peanut-watermelon (Wpw) and the b) strip intercropping system of peanut-watermelon (Spw). The subsequent within-row intercropping combinations would follow the same spacing and layout as Wpw.

Table 1.1 Component crops and their potential primary and secondary contributions to the system and plant growth habit (architecture)

Crop	Variety	Family	Function	Architecture	Reference
Peanut	Tamspan 90	Fabaceae	nitrogen fixation, smother crop	low/ mid growth form	Baughman et al., 2007
Watermelon	*TAMU mini	Cucurbitaceae	smother crop, shading	low growth form	Masabni and Dainello, 2009
Okra	Clemson spineless	Malvaceae	pollinator attractant, structural support	tall growth form	Masabni and Dainello, 2009
Cowpea	Texas pinkeye	Fabaceae	nitrogen fixation, pollinator attractant	mid growth form	Miller Jr. and Scheuring, 1994
Pepper	Jalapeño/ Serrano	Solanaceae	pest barrier	mid growth form	Masabni and Dainello, 2009

*Unreleased variety

Year 2 Experiment

Based on observations made in year 1, planting dates were altered to allow for peanut harvest and minimize over-competition from watermelon. In year 2, a chisel plow and middle buster were used to prepare the field in April 2012. Clear plastic was laid on the field the second week of May for one month to solarize the soil. A row-maker was then used to make four 1.5 m long planting rows per replicate for a total of twelve rows. Drip tape was again installed down the center of each row during the row-making process. Due to low nutrient levels at the onset of year 2, rows were top dressed with 838 kg ha^{-1} of chicken manure-based organic granular NPK fertilizer (4-2-3) in mid-June (pre-planting).

Peanut and okra were direct seeded on June 21 and 22, 2012, respectively. Cowpea was direct seeded approximately one week later on June 27. Peanut and cowpea were again inoculated with “Vault” commercial inoculant. Seven cm tall pepper plants were transplanted on July 3rd. Watermelon was direct seeded on July 12. Re-seeding of peanut, okra, cowpea and watermelon as well as pepper transplant replacement continued through the last week of July. Weeding was done continuously throughout the growing season from the time of seeding through the conclusion of the study.

Production Analyses

Crops were harvested throughout the season as fruits matured. Total yields (the sum of marketable and unmarketable yields) were expressed per unit area and then used to compute Land Equivalent Ratio (LER, Eq. 1) following Mead and Willey (1980). For comparison, total yields were used rather than marketable yields in order to test the diversity-productivity relationship which takes into account total productivity (Tilman, 2001). To further justify the use of total yields, LERs based on total yields were compared to LERs based on marketable yields to ensure results were not inadvertently biased.

$$\mathrm{LER}_i = \mathrm{Yintercrop}_i / \mathrm{Ymono}_i \text{ (Eq. 1)}$$

Where LER_i is the land equivalent ratio for species *i*, and $\mathrm{Yintercrop}_i$ and Ymono_i are the yields per area for species *i* in an intercropping combination, and when monocropped, respectively. LER for the whole plot can then be calculated by summing up the LER for each individual species in the plot with n species in the plot (Eq. 2).

$$\mathrm{LERplot} = \sum_{i=1}^{n} LER \text{ (Eq. 2)}$$

An LERplot greater than 1 indicates that the intercropping combination overyielded; that an increase in yield with intercropping was observed compared to growing each component crop in monoculture. An LERplot lower than 1 indicates a decrease in yield with intercropping compared to growing each component crop in monoculture. LER can also be thought of as the relative area of land required to produce the same yields in monoculture as those in intercropping mixtures (Mead and Willey, 1980). Production was also expressed on a per plant basis and analyzed using ANOVA in JMP 10.0.2 statistical software (SAS Institute, Cary, NC, USA).

Quality Assessment

Peanut was not harvested in 2011 and harvested at the end of the growing season in 2012. Pods were collected from five randomly selected plants within each plot and allowed to air dry for two weeks at which point quality parameters were measured. Parameters measured were number of pods per plant (pods plant^{-1}) and weight per one hundred seeds (g 100 seed^{-1}).

Watermelon was harvested three times in 2011 and quality parameters were measured at the second harvest. In 2012, watermelon was harvested throughout the growing season and measurements were taken from mid-season harvests. Quality measurements were taken from ten randomly selected fruits in 2011. However, due to low watermelon production in 2012, all fruit was utilized for measurements. Quality measurements included °Brix, flesh firmness (kg$\cdot$cm^{2}), individual fresh fruit weight (kg), and rind width (mm).

Okra harvest was done on a continuous basis in 2011 and 2012. Quality parameters were collected from mid-season harvests and measured from ten randomly selected pods within each plot. Individual fresh pod weight normalized on a pod area basis (g cm^{-2}) and wall width (mm) were measured at the pod midway point.

Cowpea pods were allowed to dry while still attached to plants. They were then harvested and quality parameters measured from five randomly selected plants within each plot, including number of pods per plant (pods plant^{-1}), peas per pod (peas pod^{-1}), and weight per one hundred seeds (g 100 seed^{-1}).

Pepper was harvested on a continuous basis in 2011 and 2012. Quality parameters were collected from mid-season harvests on ten randomly selected fruit. Measurements included individual fresh fruit weight normalized based on area (g cm^{-2}) and wall width (mm).

All quality measurements for each species were averaged within plot, expressed on a per plant and analyzed using ANOVA in JMP 10.0.2 statistical software (SAS Institute, Cary, NC, USA).

Results

Production

Due to the late planting in 2011, peanut plants were not harvested. Therefore, LERs were adjusted to account for un-harvested area. Adjusted plot LERs were greater than 1 with the exception of the strip intercropping system of peanut and watermelon (S_{pw}), indicating that most intercropping combinations resulted in overyielding (Fig. 1.2). LER was highest in the within row intercropping of peanut and watermelon (W_{pw}) at 1.23 followed by W_{pwo} and W_{pwoc} at 1.17 each. In 2012, LERs were highest in the W_{pwo} and W_{pwoc} intercropping combinations at 1.17 and 1.20, respectively. LER was also above 1 when peanut and watermelon were strip intercropped.

Expected LER values for each species were 0.5, 0.33, 0.25 and 0.2 for mixed plots with two, three, four, and five species, respectively. Values above or below these expected ratios indicate up- or down-regulation, respectively. In 2011, watermelon grew vigorously and, therefore, contributed more to plot LERs than the expected values. Watermelon yield was up-regulated in all intercropping combinations indicating the beneficial effect of mixed cropping on watermelon production. All other component crops were down-regulated compared to their expected values in 2011. However, in 2012, with the altered planting dates, watermelon was outcompeted by okra and as a result, okra yield was greater than expected in all intercropping combinations where it was included. Peanut production was also greater than expected in all combinations except for S_{pw} and watermelon production was lower than expected in all combinations except S_{pw}. Just as in 2011, cowpea and pepper had lower yields than expected in intercropping treatments. Since all plots were planted at the same density, per plant production values follow the same statistical pattern as LER. Per plant production data are therefore presented in Table 1.1.

In 2011, watermelon production on a per plant basis was significantly improved in the more complex intercropping combinations of W_{pwo}, W_{pwoc} and W_{all} at 3.7, 5.1 and 5.5 kg plant^{-1} respectively, compared to 2.1 kg plant^{-1} in monoculture (Table 1.2). Due to competition from okra and downy mildew infection in 2012, watermelon production per plant dropped significantly across all intercropping treatments. Although not statistically significant, watermelon yield was lowest in the W_{pwo}, W_{pwoc}, and W_{all} intercropping combinations with 0.3, 0.2, and 0.3 kg plant^{-1} respectively compared to 0.9 kg plant^{-1} in monoculture. A similar but reversed pattern was observed in okra from 2011 to 2012. Okra per plant production was low in 2011 as it was outcompeted by watermelon with no significant differences between treatments. However, in 2012 when okra seemed to have an advantage over watermelon in terms of planting date, production per plant increased overall but was significantly greater in the W_{pwo}, W_{pwoc} and W_{all} treatments at 1.7, 2.5 and 2.3 kg plant^{-1} respectively. These were all significantly greater than the okra monoculture which yielded 1.1 kg plant^{-1}. Cowpea had a reduction in per plant production in both 2011and 2012 in intercropping combinations containing watermelon when compared to cowpea monoculture, suggesting it was also subject to over-competition from watermelon. A similar trend was found in pepper, with the pepper monoculture producing significantly greater amounts per plant. In 2011 production was 0.04 kg plant^{-1} in pepper monoculture versus 0.01 kg plant^{-1} in W_{all} treatment combination. In 2012, pepper monoculture production was 0.16 kg plant^{-1} versus 0.07 in the W_{all} treatment. Increased per plant production in 2012 compared to 2011 for pepper also suggests that the changes in planting dates in 2012 allowed proper pepper establishment and reduced competition from watermelon. Peanut was only harvested in 2012. Peanut production was improved in the W_{pwo} and W_{pwoc} intercropping combinations, 0.10 and 0.11 kg plant^{-1}, respectively, and was lowest in the W_{all} and peanut-watermelon strip intercropping treatment (S_{pw}) at 0.06 and 0.07 kg plant^{-1}, respectively.

Table 1.2 Yield (kg_{fruit} $plant^{-1}$) for each species based on intercropping strategy in 2011 and 2012. Treatments are described in Fig. 1. (a) peanut, (b) watermelon, (c) okra, (d) cowpea, and (e) pepper. Different letters indicate statistically significant (*P* < 0.05) differences between means within years for each crop analyzed separately.

	Peanut	Watermelon		Okra		Cowpea		Pepper	
Treatment/.year	2012	2011	2012	2011	2012	2011	2012	2011	2012
Monoculture	0.07^{b}	2.09^{bc}	0.86	0.15	1.13^{b}	0.02^{a}	0.02	0.05	0.16
W_{pw}	0.08^{ab}	2.44^{bc}	0.67						
S_{pw}	0.06^{b}	1.93^{c}	0.95						
W_{pwo}	0.10^{a}	3.69^{abc}	0.30	0.08	1.66^{ab}				
W_{pwoc}	0.11^{a}	5.13^{ab}	0.25	0.08	2.46^{a}	0.01^{b}	0.02		
W_{all}	0.07^{b}	5.50^{a}	0.30	0.09	2.28^{a}	0.02^{ab}	0.02	0.01	0.07

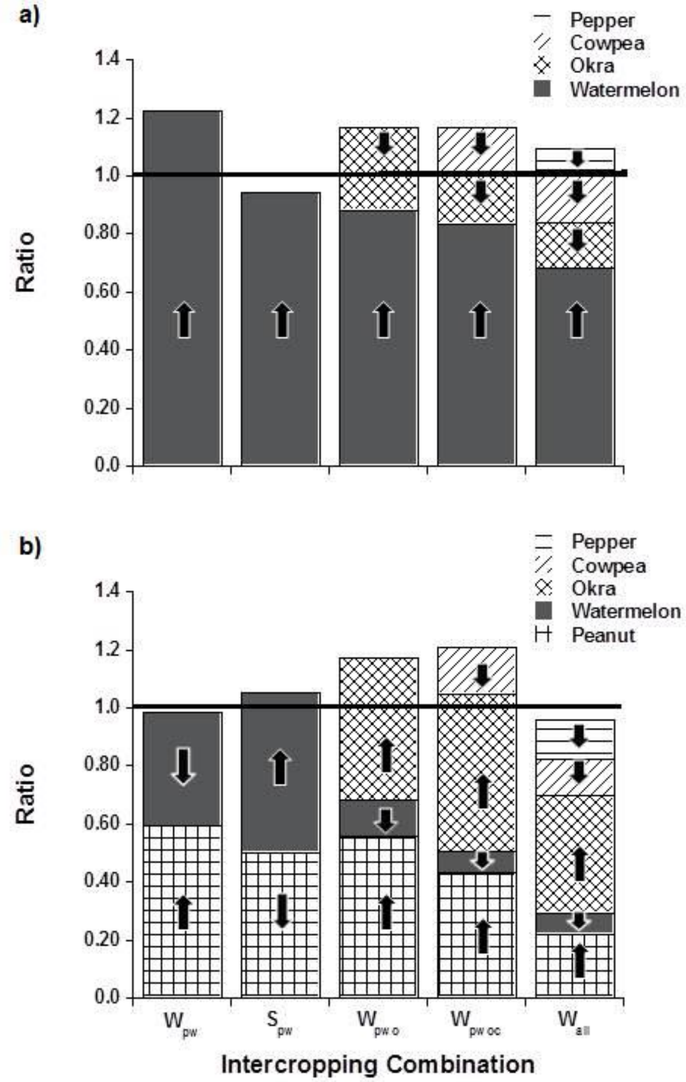


Figure 1.2 Land equivalent ratios (LERs) calculated for (a) 2011 and (b) 2012 and broken down by species (Wpw = within row intercropping of peanut-watermelon, Spw = strip intercropping with peanut-watermelon (e.g. alternating rows), Wpwo = within row intercropping of peanut-watermelon-okra, Wpwoc = within row intercropping of peanut-watermelon-okra-cowpea, Wall = within row intercropping of peanut-watermelon-okra-cowpea-pepper). Cumulative LER values > 1 indicate overyielding at the plot level, while cumulative LER < 1 indicates underyielding. Expected LER values for each individual species are 0.5, 0.33, 0.25 and 0.2 for two, three, four and five mixed species plots, respectively. Values below or above these expected ratios indicate under- and overyielding of individual species, respectively, and are indicated by up or down arrows.

Quality

Although the intercropping combinations of W_{pwo} and W_{pwoc} appear to have more peanut pods per plant, no statistically significant differences were detected for peanut parameters (Appendix Fig. 1.1). There was a 31% reduction in watermelon °Brix in 2012 in the W_{pwoc} treatment combination compared to its corresponding monoculture (Fig. 1.3). However, it is unknown whether this reduction was caused by competition from okra (although no reductions were evident in other intercropping combinations with okra) or due to the downy mildew infestation. No other differences were detected in watermelon quality for both years. However, watermelon fresh fruit weight was lower and flesh firmness was higher in 2012 as compared to 2011. Okra fresh pod weight was statistically different in 2011 ($P < 0.001$), with the monoculture having the lowest fresh pod weight and the W_{pwoc} intercropping combination the highest (Appendix Fig. 1.2). This represents a 15% decrease in pod fresh weight in monoculture as compared to W_{pwoc}. Cowpea pods per plant were significantly reduced in 2011 ($P = 0.010$) in the two intercropping combinations, W_{pwoc} and W_{all} (Fig. 1.4). No other significant differences were detected in 2011 and no differences were detected at all in 2012. There were no significant differences in pepper fresh fruit weight or wall width (Appendix Fig. 1.3). Due to differences in dates of field preparation and planting, as well as differences in precipitation distributions and totals between the two growing seasons, between years analysis was not conducted. Total precipitation for the 2011 growing season (August 1 through October 31) was significantly less than for the 2012 growing season (June 21 through October 31), 98 and 185 mm, respectively (NOAA/NCDC; Fig. 1.5).

Additionally, there were more frequent precipitation events in 2012, particularly at the time of planting.

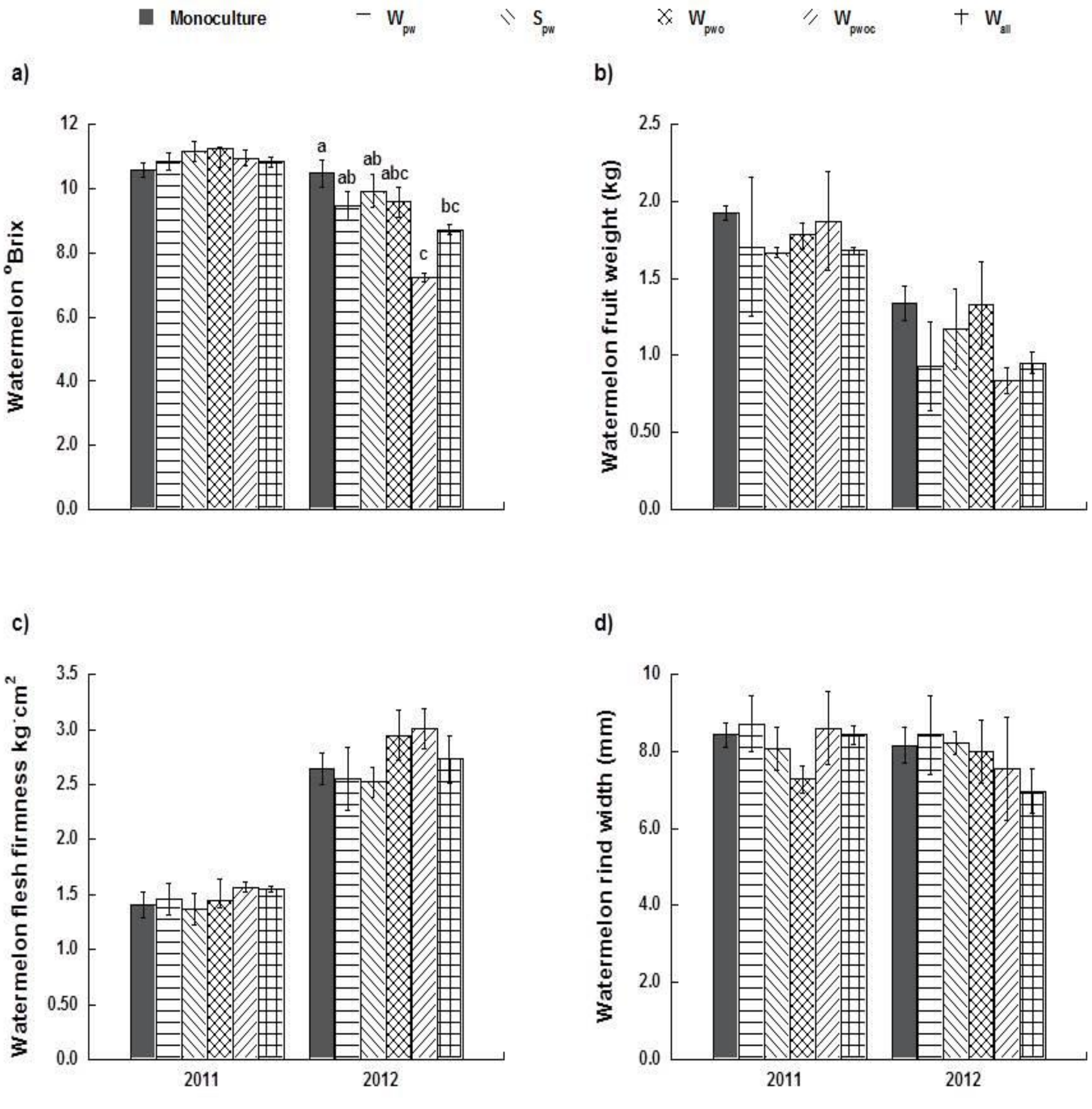


Figure 1.3 Watermelon quality measurements based on intercropping strategy in 2011 and 2012. Treatments are described in Fig. 1. (a) °brix, (b) individual fresh fruit weight (kg), (c) flesh firmness (kg . cm2), (d) rind width (mm). Treatments are described in figure 1.2. Different letters indicate statistically significant ($P < 0.05$) between means within years.

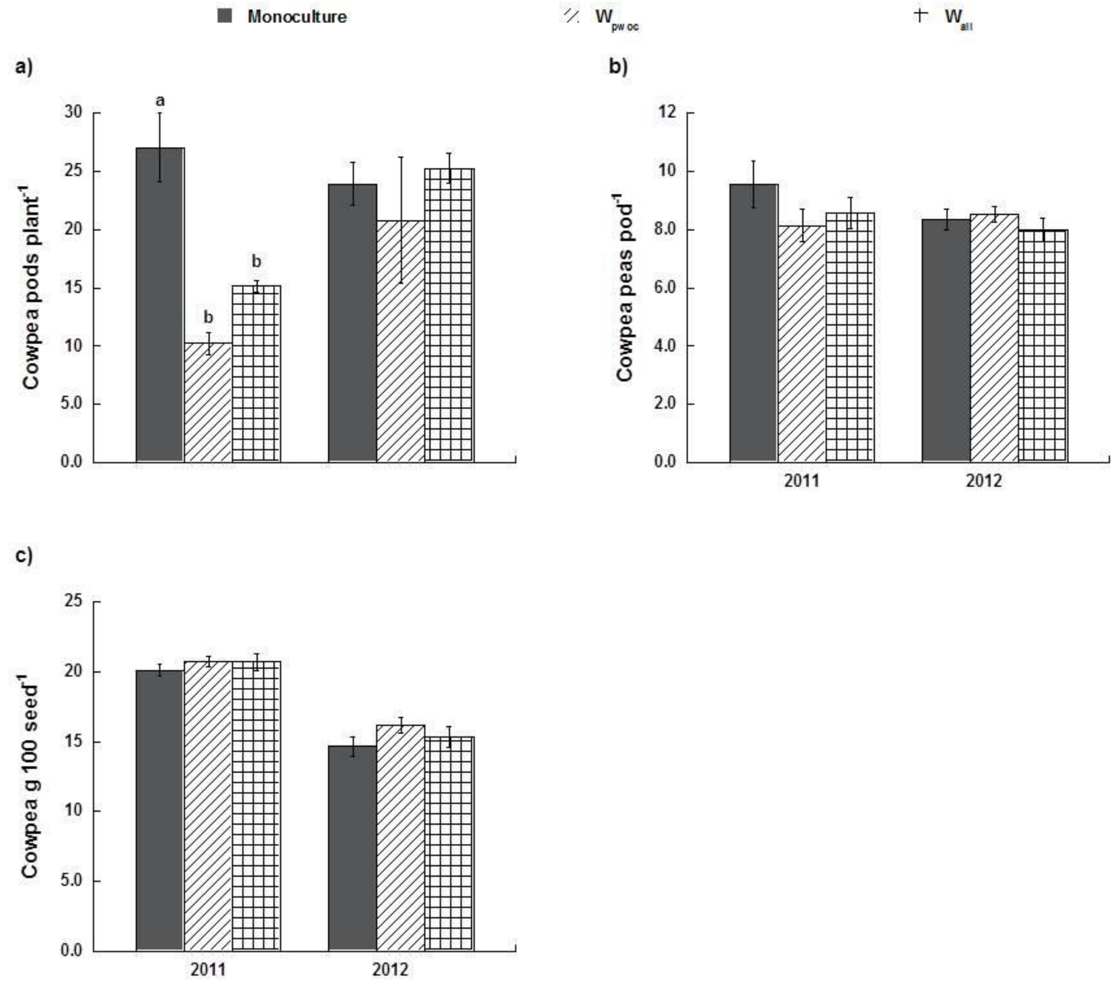


Figure 1.4 Cowpea quality measurements based on intercropping strategy in 2011 and 2012. Treatments are described in Fig. 1. (a) pods plant-1, (b) peas pod-1, (c) g 100 seed-1. Treatments are described in figure 1.2. Different letters indicate statistically significant ($P < 0.05$) between means within years.

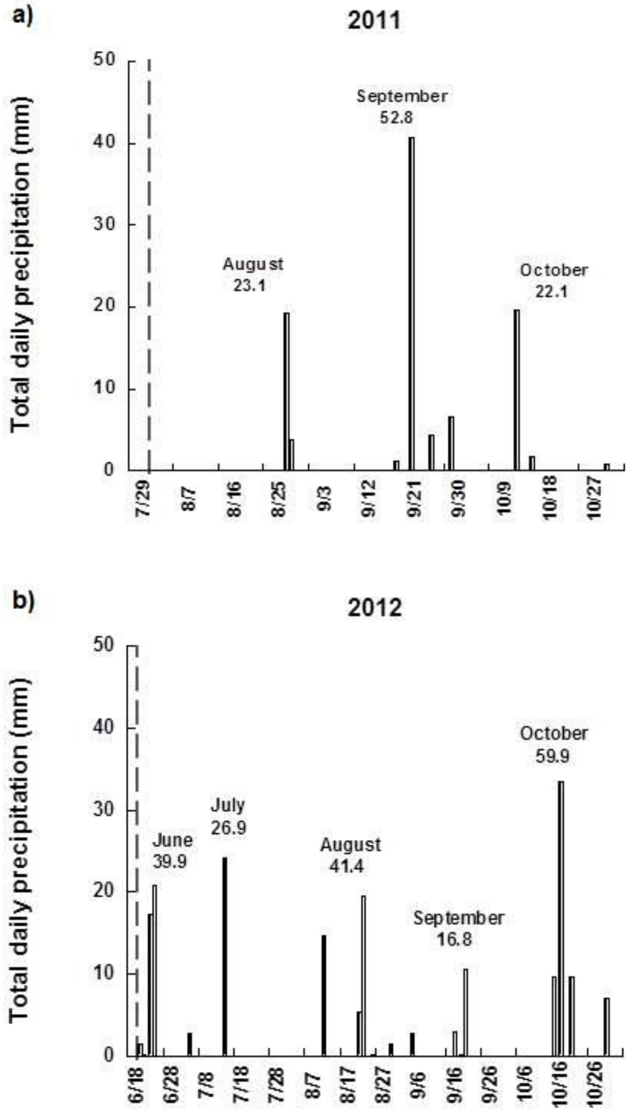


Figure 1.5 Daily precipitation totals (mm) for a) 2011 and b) 2012 with monthly totals displayed. Dashed vertical lines indicate the date of first planting for each growing season.

Discussion

Production data from both years suggest a net benefit of intercropping with functionally diverse species in terms of both overall productivity and per plant productivity. Intercropping combinations incorporating three or four species (W_{pwo} and W_{pwoc}) consistently overyielded, having the highest plot LER in both years and per plant production values for watermelon, okra, and peanut. A decline in plot LER was evident with the addition of pepper (W_{all}). This suggested there may have been a threshold, in other words where a decline in overall yields began to occur, where the benefits of intercropping with this particular combination of species were maximized. It has been suggested that an increase in functional group diversity has the greatest impact on an ecosystem when few functional groups are represented to begin with (Moonen and Barberi, 2008). The diminished positive effects on a community resulting from an increase in species diversity often occur with the introduction of functional redundancy (Wohl, 2004). Thus, it is possible that pepper may have had functionally redundant qualities that did not contribute to an increase in the overall productivity of the system. A more likely explanation for the observed decline in LER with the addition of pepper is that pepper experienced very little intraspecific competition in the monoculture given their relatively small plant size while interspecies competition was much greater in the intercropped combinations, particularly from watermelon and okra due to their much larger plant size.

The difference in competitive/facilitative relationships between the two years, which highlights the importance of planting date as it relates to seedling establishment and competition for resources as well as year-to-year variability in biotic factors such as precipitation, suggests watermelon and okra were the most competitive species in these systems and largely influenced both yield and quality parameters. Given that the sampling effect hypothesis held true in this intercropping system of peanut-watemelon-okra-cowpea-pepper, we would have expected that the species having the largest effect on the community would contribute the greatest to LER and would consistently overyield in mixed cropping systems as compared to monocrop, while all other species would likely underyield. If the complementarity effect hypothesis held true in this scenario, one would expect that all species would contribute relatively equally to LER and that all species would either overyield or there would be a random distribution of under- and overyielding across species in mixed cropping as compared to monocrop. In 2011, watermelon was the dominant species, consistently contributed the greatest to plot LER and consistently overyielded in mixed cropping systems compared to in monocrop, while all other species underyielded. In 2012, okra became the dominant species due to changes in planting dates and generally contributed the greatest to LER. In treatment combinations consisting of okra, all other species (except for peanut) were down-regulated while okra consistently overyielded. Although it is likely that both diversity-productivity theories affected LER and the findings from this study, these patterns suggest that the sampling effect hypothesis, whereby the effects of diversity on productivity are governed by one or few species (Fargione et al., 2007), had a stronger influence in this system.

An important consideration for the application of this type of system in a production setting is finding the optimum plant spacing to meet the needs of all component species. In the past, there has been debate regarding planting density and plant spacing when calculating LER for comparing monocultures with mixed cropping systems (Oyejola and Mead, 1982). Optimum plant densities in monoculture have been suggested for best achievable yields (Huxley and Maingu, 1978), and deviation from these planting densities will reduce the yield of the monocultures and thus bias LER towards the mixed plantings. However, in some instances when certain variables are being isolated it is appropriate to maintain similar densities across treatments (Mead and Willey, 1980). In order to assess the impact of increasing plant functional diversity on yield without the added complication of planting density, planting density was kept stable across the plots and thus sometimes planted the monocultures at slightly larger than optimal planting densities. Monoculture of peanut, pepper and cowpea were planted at less than optimal density. With the exception of number of pods per cowpea plant, these species were the least responsive to mixed cropping with regards to fruit quality and consistently underyielded. It was found that underyielding in cowpea was due to a reduction in the number of pods per plant. Monoculture of okra was planted at optimal density and, although the optimal density of this particular variety of mini watermelon used is not known for certain, we believe it was planted at slightly higher than optimal density. Okra and watermelon were the most responsive to changes in planting densities and multi-cropping combinations with regards to both production and fruit quality. Therefore, there should have been little or no bias on our LER results due to planting densities as suggested by some authors.

Due to overcompetition for resources such as light, water and nutrients, there should have been an expected decline in fruit quality for most component crops. However, in 2011 when watermelon was the dominant species the only decline in quality in mixed cropping compared to monoculture was in the number of pods per cowpea plant. Okra individual pod weight increased in the three species intercropping combination in that year, possibly suggesting they invested more of their resources in fewer pods. However, data on the number of pods per okra plant were not collected and thus we were unable to test this hypothesis. In 2012 when okra was the dominant crop, findings from watermelon ^{o}Brix support our hypothesis that fruit quality of sub-dominant species would decline. In four and five species combinations, watermelon °Brix was lowest. However, this was also the year that watermelon experienced a downy mildew infestation and this may have influenced some of the quality findings.

As distance to nearest neighbor decreases, competition begins to increase (Vandermeer, 1986). Thus, the proper spacing between individuals in a mixed planting should maintain a balance that maximizes facilitative interactions and minimizes competitive interactions. Additionally, small scale producers often are more limited on space and farm more intensively to increase yields on a per area basis. A system incorporating three or four species would be optimal for small scale production systems where manual harvesting is common practice. These findings also suggested that a three or four species system can also be utilized without sacrificing fruit quality, given that optimal planting dates can be found.

Finally, an important finding from this study is that we were able to apply the diversity-productivity relationship from plant community theory, which is based upon plant biomass, to an agricultural ecosystem using fruit yield. These findings that three and four species combinations consistently overyielded with regard to fruit production in this system of peanut-watermelon-okra-cowpea-pepper are consistent with findings of overyielding of plant biomass in natural ecosystems (Tilman et al., 1996; Reich et al., 2004).

Urban gardens, school garden programs, community supported agriculture (CSA's) and other small scale farms, which have gained popularity in recent years, often function on limited space. According to the 2007 Census of Agriculture (USDA/NASS, 2007), small farms account for 91% of all farms in the United States, a 1% increase from 2002. This multispecies design would be ideal for small scale producers and urban farmers who want to optimize per area production on limited space. Such a system also benefits wildlife habitat, water retention, and a lower risk of entire crop failure on the part of the producer. The results from this study suggested that future studies on biodiversity in agricultural landscapes should focus on a methodical selection of species that contribute to a specific function within the agroecosystem rather than on number of species solely.

CHAPTER III

THE EFFECTS OF FUNCTIONALLY DIVERSE INTERCROPPING ON SOIL AND BELOW-GROUND PLANT RESPONSE

Introduction

Soils play a major role in nutrient cycling and, specifically, the global C and N cycles (Nair et al., 2009). The C-carrying capacity of a soil will vary based on climactic factors such as temperature and precipitation and on biotic factors such as vegetation type (Guo and Gifford, 2002). The top one meter of soil is estimated to contain 2 to 3 times the amount of total C than the atmosphere and aboveground vegetation (Sommer et al., 2000; Lal, 2003; Nair et al., 2009) and is therefore regarded as a major sink for C in terrestrial ecosystems (Ceccon et al., 2011). There is a key distinction between longer-term (stable) soil C pools that have a turnover rate that range from decades to centuries (Bruun et al., 2008; Rumpel and Kögel-Knabner, 2010) and short-term (labile) soil C pools that have a rapid turnover rate that range from months to years in terrestrial ecosystems (Krull et al., 2003; Jagadamma and Lal, 2010). With regards to labile C pools, C in the form of organic matter is a particularly key component in fertile soils in agricultural systems (Kumar et al., 2006). Soil organic carbon (SOC), along with soil total nitrogen (TN), is considered a good indicator of soil quality (Dilly et al., 2003). Not only can organic C enhance soil fertility and plant nutrient availability, but it can improve soil aggregates and soil biological activity (Zeng et al., 2010). Improvements in soil aggregates result in an increase in specific surface area per unit of soil mass, which contributes to an increase in soil water retention (Blanco-Canqui et al., 2013). On average, a 1% increase in SOC can increase plant available water by 12.5 mm in the top 20 cm of the soil profile of medium-textured soils (Blanco-Canqui et al., 2013). Thus, agricultural practices that manage for enhanced SOC are important for the productivity and long-term stability of agricultural systems (Wang et al., 2011; Bajoriene et al., 2013).

At equilibrium, inflows and outflows to the C pool are equal; a balance that is important to the accumulation of SOC (Lal, 2003; Blanco-Canqui et al., 2013). Sources of organic C can come from above- and below-ground. In agroecosystems, crop residues in form of leaf and crop litter contribute to soil organic matter, providing soil with material that has varying rates of decomposition (Oelbermann et al., 2005; Zeng et al., 2010). Litter quantity and quality (carbon to nitrogen ratio C:N), along with environmental factors, strongly contribute to these differential rates of decomposition (Hooper et al., 2000; Orwin et al., 2010). This, in turn, affects the dynamics of the microbial population and mineralization of nutrients (Mungai and Motavalli, 2006). Above-ground litter material is decomposed and mineralized, increasing the amount of plant available nutrients (Laossi et al., 2008), or immobilized by soil microorganisms depending upon the C:N ratio (Burger and Jackson, 2003). Due to the linked nature of C and N decomposition, factors affecting the rhizosphere (root zone) will also affect N cycling (Dijkstra et al., 2009) as microbes utilize organic material deposited onto the soil as a C source and acquire N from the decomposition of this material (Kuzyakov et al., 2000). Therefore, as a living part of soil organic matter, soil microbes are a critical component in the cycling of C and N in terrestrial ecosystems (Gastine et al., 2003; Laossi et al., 2008; Wang et al., 2011).

Below-ground processes are still not well understood due to both the complexity of interactions in the rhizosphere and variability in measurement techniques (McCormack and Fernandez, 2011). The primary sources of organic C below-ground are root exudates and other organic substances, sloughing of root hairs and fine roots, and root turnover (Kumar et al., 2006; Orwin et al., 2010). It is estimated that up to 33% of global annual net primary production is comprised of fine root production (Gill and Jackson, 2000). Roots differ in their ability to absorb water and nutrients (Volder et al., 2005), with fine roots responsible for most of the nutrient and water uptake from the soil (Brassard et al., 2011). Root turnover contributes a significant portion to soil C pool (Nair et al., 2009) and it is estimated that 15-25% of C allocated to roots is exuded from fine roots back into the soil (Kuzyakov and Cheng, 2001; Kuzyakov, 2002). A greater accumulation of fine roots, in particular, enhances microbial populations due to faster decomposition rates as compared to lignified coarse roots (van Noordwijk et al., 1996; Wang et al., 2010). Additionally, heterotrophic soil microbes utilize plant exudates and decomposing roots as a food source (Jackson et al., 2012) and, consequently, a strong relationship has been found to exist between root density and microbial biomass (Gastine et al. 2003). Due to this rhizosphere priming effect (Kuzyakov, 2002), roots play a significant role in C and N cycling.

Plant community composition and diversity can affect both above- and below-ground inputs that may alter rhizosphere dynamics (Fornara and Tilman, 2008; Jackson et al., 2012; Chanteloup and Bonis, 2013). Results from diversity studies have yielded conflicting results with regards to below-ground processes. On the one hand, above-ground competition for light may alter biomass partitioning to below-ground resources and actually reduce root and microbial biomass (Bloom et al., 1985; Bessler et al., 2009), which will in turn affect C and N dynamics. On the other hand, a higher diversity of litter quality is expected at high levels of specific or functional diversity of plants (Laossi et al., 2008), which may, in turn, increase soil fauna diversity if there is a complementary use of different types of litter by these microorganisms (Hooper et al., 2000). With regards to SOC, Tilman et al. (2006a) found greater soil C accumulation in more species-rich N-limited grassland communities. Species richness can also lead to an increase in below-ground biomass (Reich et al., 2001). While species richness itself may lead to increased SOC and root biomass, others have found that plant functional traits and the presence of key functional groups (legumes in particular) are more important determining factors of below-ground response (Bardgett et al., 1999; Diaz and Cabido, 2001; Gastine et al., 2003). Fornara and Tilman (2008) found that enhanced accumulation of soil C and N in the top 20 cm of the soil profile with higher plant diversity was positively associated with total root biomass. They attributed this increase in total root biomass to complementarity between plant functional types, specifically between C4 grasses and legumes when these two functional groups were present. This complementarity was thought to occur due to both facilitation and niche differentiation between the two functional groups. Species that are complementary in their resource use often differ in rooting depth, plant phenology, or other traits that regulate the timing of resource utilization (Polley et al., 2013). Niche differentiation and complementarity by plant roots in mixed species systems may lead to greater filling of soil pore space by roots (Brassard et al., 2011; Postma and Lynch, 2012). Additionally, legumes, which have a higher litter quality (low C:N), have high litter decomposition rates and have significant effects on N availability and supply in N-limited ecosystems (Chapin et al., 1986; Vitousek and Howarth, 1991; Fornara and Tilman, 2008). Under low N-supply, legumes may also provide a source of N for neighboring species through root and nodular tissue decay and root exudates (Chu et al., 2004; Wichern et al., 2007a).

Species diversity and species interactions may also indirectly affect below-ground processes through the alteration of soil moisture and soil temperature. Higher leaf area index (LAI), often associated with mixed species systems, has been shown to reduce soil temperature and reduce soil moisture losses due to evaporation (Ikeorgu and Ezumah, 1991; Olasantan and Babalola, 2007; Ghanbari et al., 2010; Ossom, 2010). Modifications in soil temperature and moisture can both influence microbial activity and organic C decomposition (Dijkstra et al., 2006; Ceccon et al., 2011). While the importance of the interactive effects of soil moisture and temperature on soil respiration have been demonstrated (Wildung et al., 1975; Bryla et al., 2001; Huang, 2005), soil temperature may be of particular interest as soil respiration has been found to be especially sensitive to fluctuations in temperature (Boone et al., 1998).

Soil respiration is made up of two components: root-derived (autotrophic) respiration and microbial (heterotrophic) respiration (Ceccon et al. 2011). Both components react differently to changes in environmental conditions and slight changes in temperature may affect total soil respiration (Li et al., 2011). Together they represent a significant source of C losses through CO_2 efflux into the atmosphere (Ceccon et al., 2011; Thurgood et al., 2014). Accounting for 33 to 60% of total soil respiration (Bowden et al., 1993), root-derived respiration is of particular interest as fine roots are especially sensitive to fluctuations in temperature (Desrochers et al., 2002; Huang, 2005). Liu et al. (2013) demonstrated there was a linear relationship between soil temperature and soil respiration. However, they did not partition out root and microbial respiration in their study. This root respiration and soil temperature relationship may vary between species (Atkin et al., 2000) but, in general, studies have found that root respiration reaches its peak at midday when temperatures are at their maximum (Lipp and Andersen, 2003; Ceccon et al., 2011; Qin et al., 2013). Therefore, agricultural practices that reduce maximum daytime soil temperatures via increased canopy cover may reduce C losses from soil and contribute to the accumulation of soil C.

In summary, a complex set of above- and below-ground interactions drive rhizosphere processes. These interactions may be affected by plant species diversity and community composition. Integrated cropping systems that can optimize C inputs, enhance SOC and plant-available N, and reduce C losses to the atmosphere may offer more sustainable approaches to food production. The objectives of this study were to determine if an architecturally complex intercropping system incorporating leguminous species will lead to short-term changes in SOC accumulation, which is associated with long-term processes, microbial biomass C, TN accumulation, root density, and soil temperature and moisture. We hypothesized that a functionally diverse cropping system will lead to an increase in root density, microbial biomass C, and SOC, exhibit less of a reduction in TN, and reduce daytime soil temperatures and increase soil moisture in an organic system consisting of peanut (*Arachis hypogaea* L.), watermelon (*Citrullus lanatus* Thunb.), okra (*Abelmoschus esculentus* Moench.), cowpea (*Vigna unguiculata* L.), and hot pepper (*Capsicum annuum* L.).

Materials and Methods

Study Area

Low input managed plots were established at Texas A&M University's Horticulture Farm (30°37′N, -96°22′W) during the 2011 and 2012 growing seasons. Average monthly air temperatures for this area from May to October when the study was conducted ranged from 28 to 39°C for the maximum and 15 to 26°C for the minimum in 2011 and 27 to 37°C and 15 to 25°C in 2012 (NOAA/NCDC).

Experimental Design

The study design was a randomized complete block with three replicates, five intercropping treatments, and five controls. The controls consisted of monocultures of the five component species; peanut, mini watermelon, okra, cowpea, and hot pepper. The five treatments were a within-row intercropping system of 1) peanut and watermelon

(W_{pw}), 2) peanut, watermelon, and okra (W_{pwo}), 3) peanut, watermelon, okra, and cowpea (W_{pwoc}), 4) all five control species (W_{all}), and 5) a strip intercropping system of peanut and watermelon consisting of alternating single rows (S_{pw}). Figure 1.1 illustrates the planting layout for the within-row and strip intercropping schemes. Crops represented four different genera and were selected based on the following criteria: 1) heat tolerance, 2) desired architecture and function (Table 1.1), and 3) no known adverse effects on other component crops. “Tamspan 90” is a small Spanish bunch type peanut which was selected for its small canopy and lack of runners. The “TAMU” mini watermelon variety was developed by the Texas A&M University watermelon breeding program and has a number of advantages for small scale producers including its ability to tolerate narrower spacing and, thus, take up less space, and produces a smaller fruit which is more manageable for small growers. Selected because of its popularity, “clemson spineless” okra is a dwarf variety well suited for this region. “Texas pinkeye” is a purple hull cowpea selected for its erect and bush-type growth form. This taller variety of cowpea can tolerate narrower spacing. Hot pepper varieties were used for this study. Jalapeno was used for year one and Serrano for year two and selection was based upon availability.

Crops were planted in 4 m long double rows on 90 cm wide raised beds with rows spaced approximately 30 cm apart and beds spaced 45 cm apart (total plot size, 4 × 5 m). Using the methodologies recommended by Jeavons (2006) and minimum spacing requirements outlined in the Texas Vegetable Growers Handbook (Masabni and Dainello, 2009), individual plant spacing was based on the mean spacing requirement for all component crops. Densities were kept constant across plots regardless of crop species and individual plants were spaced 30 cm apart in a staggered row pattern so that each plant was neighbored by an individual of a different species and a leguminous species. A 2 m buffer was maintained between plots.

Soil Carbon and Nitrogen

One composite sample of 3 soil cores (4.8 cm diameter) was taken from 0-5 cm depth from the center 2.5 m of the center row in each plot in 2011, while 3 sets of 3 composite samples were taken from the same depth from the center of the center row in each plot in 2012. Soil was sieved to pass a 2 mm screen, visible pieces of crop residue and roots were removed, and samples were allowed to air dry. Baseline samples were taken 1 day prior to planting and post-harvest samples were taken at the conclusion of harvest for each growing season (late November to early December). A 200 mg subsample was ball-milled to a fine powder and stored for analyses.

Samples were analyzed for soil organic carbon (SOC) using the acid fumigation method (Harris et al., 2001; Ramnarine et al., 2011), whereby the inorganic carbon component is removed. A 30 mg subsample of previously ball-milled soil was oven-dried at 24°C for 24 h and placed in silver-foil capsule (8 × 5 mm). After exposure to hydrochloric acid (HCL) for 7 hr, samples were again over-dried 24°C for 4 hours, placed in tin capsules and analyzed for d^{13}C and total C using a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus isotope ratio mass spectrometer operating in continuous flow mode (Thermo Electron, Waltham, MA).

Total nitrogen (TN) was determined using an NCS 1112 elemental analyzer operating in continuous flow mode (CE Elantech, Lakewood, NJ). A 60 mg subsample of previously ball-milled soil was then oven-dried at 24°C for 24 h and placed in a tin capsule (8 × 5 mm) prior to analysis.

Roots and Microbial Biomass

Soil samples were collected from 0-20 and 20-40 cm depth in mid-November 2012 when it was believed root biomass would be at its maximum. Three soil cores (5.2 cm diameter) were collected from the center 2.5 m of the center row in each plot.

Roots were extracted from soil, washed, and sorted into fine and coarse roots. Roots larger than 0.3 mm in diameter and bearing lateral roots were classified as coarse roots. Roots were rinsed with deionized water and scanned using a flatbed scanner and then analyzed for total root length and oven dried for 48 hours at 24°C. Roots were weighed using a microbalance. Specific root length (ratio of root length to root dry mass) and root length density (total root length per unit volume of soil) were then calculated.

The direct chloroform ($CHCl_3$) fumigation extraction method (Vance et al., 1987) was used to determine soil microbial biomass carbon (SMB-C) and soil microbial biomass nitrogen (SMB-N). The procedure is based on a comparison of the formation of total dissolved organic carbon in chloroform fumigated and non-fumigated soil samples (Brookes *et al.*, 1985; Beck *et al.*, 1997). An 8 g (dry weight equivalent) subsample was taken from each root soil core and fumigated with ethanol-free chloroform for 7 days in the dark. Both fumigated and non-fumigated soils were extracted with 24 mL of 0.5 M K_2SO_4 by shaking for 60 min at 200 rpm and filtered through 2.5 um filters. Filtrates were stored frozen until analyzed for total dissolved C and N with a Shimadzu TOC-VCSH (Kyoto, JP). SMB-C and SMB-N were calculated as the difference between fumigated and non-fumigated samples. To compensate for extraction inefficiencies and to convert the chloroform-labile C pool to soil microbial biomass carbon (SMB-C) and soil microbial biomass nitrogen (SMB-N), a k_{EC} value of 0.45 (Wu et al., 1990; Joergensen and Mueller, 1996) and a k_{EN} value of 0.54 (Brookes et al., 1985; Appuhn and Joergensen, 2006) were applied. A moisture correction factor was applied to each sample based on its gravimetric water content.

Soil Temperature and Moisture

An on-site weather station (EZ Mount GroWeather, Davies Instruments, Hayward, CA) recorded air temperature and relative humidity. At least one soil temperature sensor was installed 2 cm deep in the soil profile toward the center of each plot and connected to a data logger (Hobo U12, Onset Company Corp., Bourne, MA).

In 2011, soil water content was measured instantaneously on September 16th and October 17th during the hours of 1200 and 1400 using a time domain reflectrometry (TDR) probe (Soil Moisture Corp., Santa Barbara, CA). The TDR probe was inserted vertically to give an integrated measure of soil volumetric water content (VWC) in the top 15 cm of the soil profile (± 2%). In 2012, one ECH_20 dielectric aquameter (EC-20; Decagon Devices, Pullman, WA) was installed in one replicate of each treatment to give an integrated measure of soil VWC in the top 20 cm of the soil profile (± 3%) and connected to an EM50 data logger to record soil VWC every hour.

Leaf area index (LAI), or the total leaf area per unit ground area (Schieving and Poorter, 1999), was measured using a ceptometer (AccuPAR; Decagon Scientific, Pullman, WA) in 2012. Measurements were taken on August 14th and 24th and September 13th (33, 43 and 63 days after last planting; DALP) and only when conditions were sunny. As described by Lombardini (2006), the "above canopy" photosynthetic photo flux density was recorded in an open area adjacent to each plot prior to taking measurements. Three readings were consistently taken toward the center of each plot between the hours of 1200 and 1400.

Statistical Analyses

SOC and TN data were analyzed comparing cropping system using ANCOVA with baseline values as the covariates in JMP 11.0.0 statistical software for windows (SAS Institute, Cary, NC, USA). Instantaneous soil moisture, TN reduction, root data, and microbial biomass were analyzed using ANOVA. The Shapiro-Wilk goodness-of-fit test was used to assure assumptions of normality were met. When these assumptions were not met, log transformations were utilized and the model with the highest R^2 value was accepted. Where a significant F-test was observed ($P < 0.05$), mean separations were conducted using Tukey's Least Significant Difference (LSD) test. To examine the relationship between SMB-C and root traits, and soil temperature and leaf area index (LAI) simple linear regressions were also conducted in JMP.

Results

Soil Carbon and Nitrogen

No statistically significant differences were detected in TN for both years or in percent change in TN from the beginning to the end of the growing season in 2011 or in 2012 (Fig. 2.1) TN values ranged from 0.57 to 0.91 g N kg^{-1} soil and 0.67 and 1 g N kg^{-1} soil in 2011 and 2012, respectively. Reduction in TN ranged from 17 to 43% in 2011 and 5 to 24% in 2012.

Similarly, no statistically significant differences were detected in SOC between cropping systems in 2011 and in 2012 (Appendix Fig. A-2.1). SOC values ranged from 6.8 to 10.2 g C kg^{-1} soil in 2011 and 7.4 and 12.2 g C kg^{-1} soil in 2012.

Roots and Microbial Biomass

SMB-C was significantly higher in the okra monoculture as compared to the 3 species intercropping system of peanut-watermelon-okra (W_{pwo}; Fig. 2.2). On average, SMB-C in okra monoculture was 1189 μg C g^{-1} dry soil as compared to 718 μg C g^{-1} dry soil in the mixed cropping system. Peanut and watermelon monoculture SMB-C was 942 and 941 μg C g^{-1} dry soil, respectively. No significant differences in SMB-N were detected between cropping systems.

Due to a small proportion of coarse roots, fine and coarse root data were condensed to represent total roots. SRL was significantly higher in the cowpea monoculture than the peanut monoculture and W_{pwo} intercropping combination at the

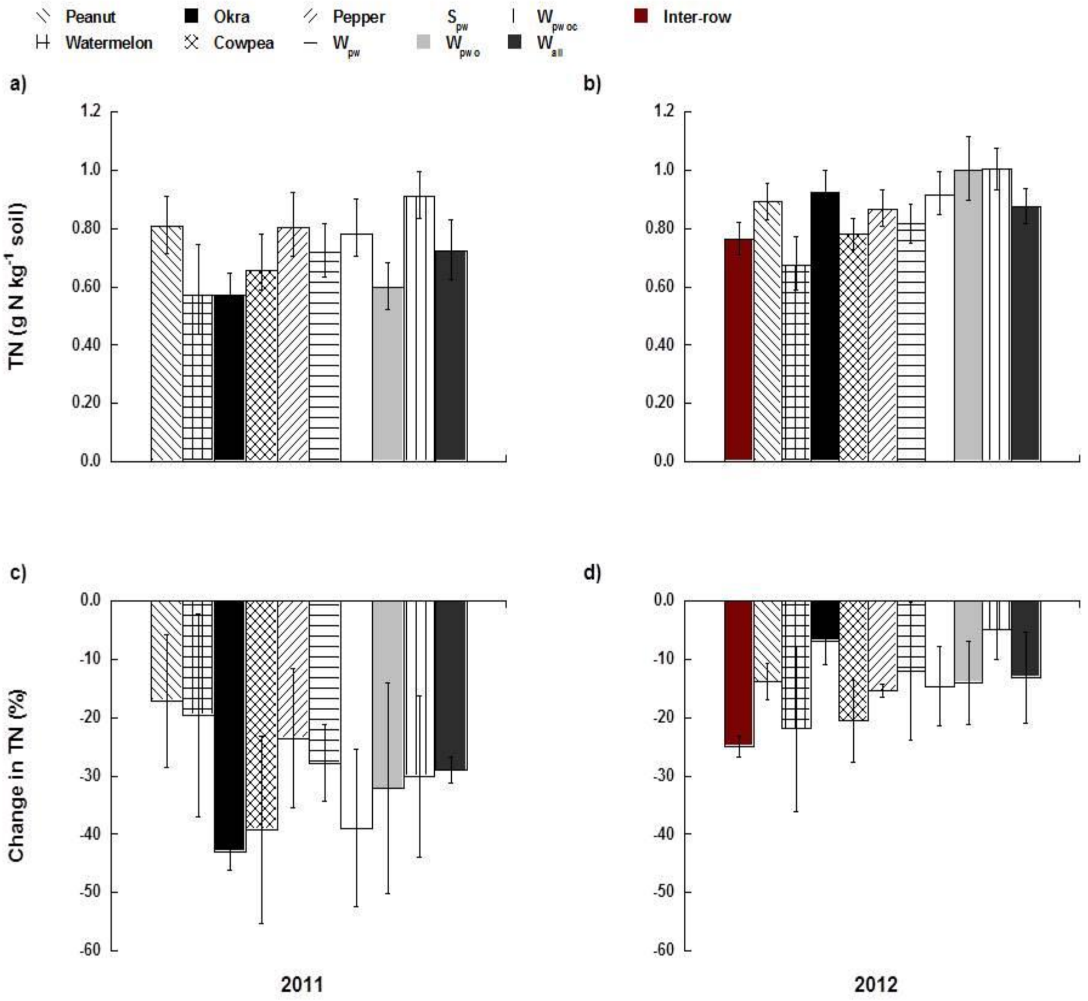


Figure 2.1 Least squares means and standard errors of the mean of soil total nitrogen (TN; g N kg-1 soil) at post-harvest adjusted based on baseline TN values obtained from pre-planting samples in a) 2011 and b) 2012 for each monoculture and intercropping combination and percent change in soil TN (%) from planting to post-harvest in a) 2011 and b) 2012 (Wpw = within row intercropping of peanut-watermelon, Spw = strip intercropping of peanut-watermelon, Wpwo = within row intercropping of peanut-watermelon-okra, Wpwoc = within row intercropping of peanut-watermelon-okra-cowpea, Wall = within row intercropping of peanut-watermelon-okra-cowpea-pepper). Due to fertilizer application in 2012, inter-row samples were taken as reference and highlighted in red. No statistically significant differences were detected.

0-20 cm soil depth (Fig. 2.3a) indicating cowpea had longer and thinner roots when grown in monoculture. SRL was 210 m g^{-1} root dry weight as compared to 84 and 70 m g^{-1} root dry weight for W_{pwo} and peanut, respectively. There were no significant differences in SRL, however, at the 20-40 cm depth. Although there appeared to be fewer total roots per volume of soil (lower RLD) in the top 20 cm of the soil profile in the peanut monoculture, watermelon monoculture, and the W_{pwo} intercropping combination, no statistically significant differences were detected (Fig. 2.3b). Similarly, no differences in RLD were detected in the 20-40 cm soil depth.

Results from simple linear regression analysis indicate a positive linear relationship between RLD and SMB-C ($P < 0.01$) when data for cropping systems were combined (Fig. 2.4). RLD accounted for 25% of the variability in SMB-C. No significant relationship between RLD and SMB-N was evident.

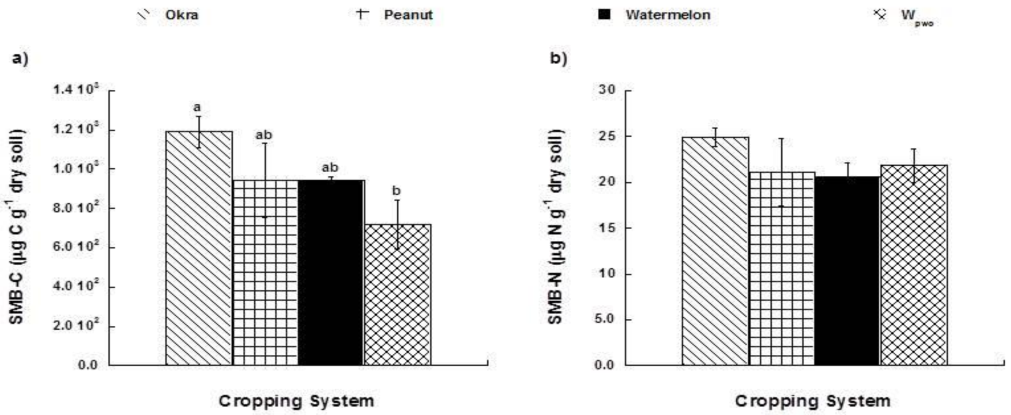


Figure 2.2 Effect of within-row intercropping combination of peanut-watermelon-okra (W_{pwo}) on a) soil microbial biomass carbon (SMB-C; µg C g^{-1} dry soil) and b) microbial biomass nitrogen (SMB-N; µg N g^{-1} dry soil) in 2012. Different letters indicate statistically significant differences ($P < 0.05$) between cropping systems according to Tukey's LSD test.

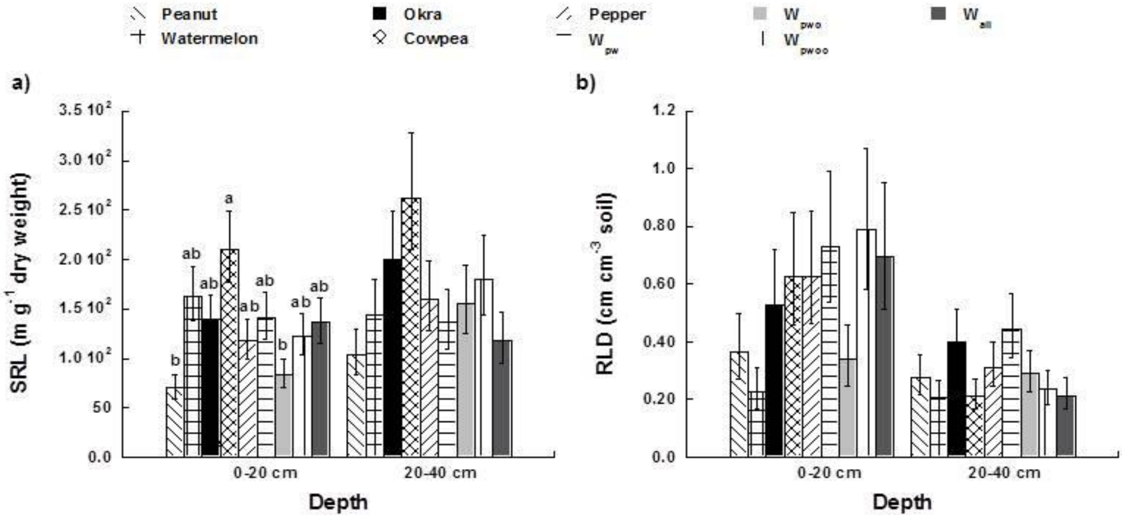


Figure 2.3. Least squares means and standard errors of the mean of a) specific root length based on a weighted average of fine and coarse roots (SRL; m g^{-1} dry weight) and b) root length density based on the sum of fine and coarse roots (RLD; cm cm^{-3} soil) for each monoculture and within-row intercropping combination in 2012. Treatments are described in figure 2.1. Different letters indicate statistically significant differences ($P <$ 0.05) between cropping systems according to Tukey's LSD test.

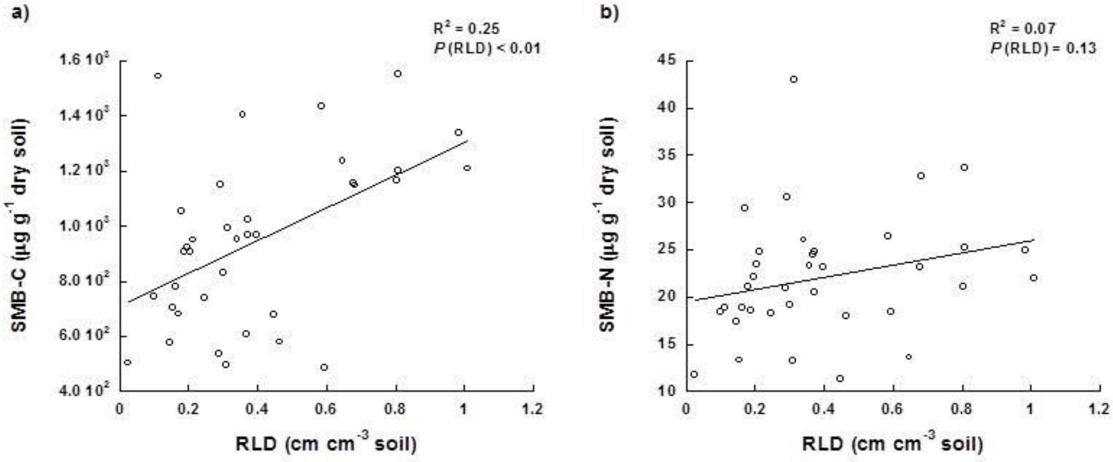


Figure 2.4. Relationship between root length density (RLD; cm cm^{-3} soil) and a) soil microbial biomass carbon (SMB-C; μg C g^{-1} dry soil) and b) soil microbial biomass nitrogen (SMB-N; μg N g^{-1} dry soil) at 0-20 cm soil depth. A positive linear relationship exists between RLD and MB-C, while no significant relationship was detected between RLD and SMB-N.

Soil Temperature and Moisture

Figure 2.5 shows daily air temperatures for both years throughout the duration of the study. In 2011, maximum, average, and daily soil temperature was consistently higher in the pepper monoculture as compared to all other cropping systems (Fig. 2.6a, c, e). Daily soil temperature in peanut monoculture was also higher than watermelon, okra, and cowpea monoculture and W_{pwo} but lower than pepper monoculture. Daily maximum and average soil temperature in W_{pwo} was 2 to 4°C lower than watermelon, okra, and cowpea monocultures and anywhere from 5 to 14°C lower than pepper monoculture. Daily maximum soil temperature peaked at 44°C in pepper monoculture in early September 2011, while the W_{pwo} intercropping combination never reached above 30°C.

In 2012, soil temperature was again higher in pepper monoculture followed by peanut monoculture (Fig. 2.6b, d, f). Daily maximum temperatures were up to 6°C higher in pepper monoculture than all other monoculture and all intercropping combinations. Daily maximum soil temperature peaked at 49°C in pepper monoculture in early August 2012, while intercropping combinations never reached above 43°C. The W_{pwo} intercropping combination never peaked above 41°C. Differences were more notable when looking at daily average temperatures. Daily average temperature was up to 8°C higher in pepper monoculture than all intercropping combinations and up to 4°C higher in peanut monoculture than all intercropping combinations.

There was a significant negative linear relationship ($P < 0.01$) between LAI and daily average, daily maximum, and daily minimum soil temperature in 2012, but this relationship was stronger with daily maximum soil temperature (Fig. 2.7). LAI accounted for 46, 40, and 24% of the variability in daily maximum, daily average, and daily minimum soil temperature, respectively, for the dates that data was collected. As leaf area increased, soil temperature decreased.

Instantaneous VWC was significantly higher in pepper monoculture than all other monocultures and intercropping combinations during the September 2011 measurement period (Fig. 2.8a). VWC was 25% in pepper monoculture and below 18% for all intercropping combinations. Differences were not as notable in October 2011 (Fig. 2.8b). VWC was significantly higher in cowpea, watermelon and the strip intercropping system of peanut-watermelon (S_{pw}) as compared to okra monoculture, pepper, W_{pwoc}, and W_{all}.

Difficulties were had in taking instantaneous VWC measurements in 2012. Continuous VWC in 2012 was measured on only one replicate per treatment. Therefore, values will only be used for reference of water holding capacity (Appendix A-2.3). VWC values ranged from 15 to 37% in 2012. Water holding capacity for this soil type at depths of 0-15 cm and 15-30 cm are between 15-25 and 30-40%, respectively (USDA NRCS Web Soil Survey).

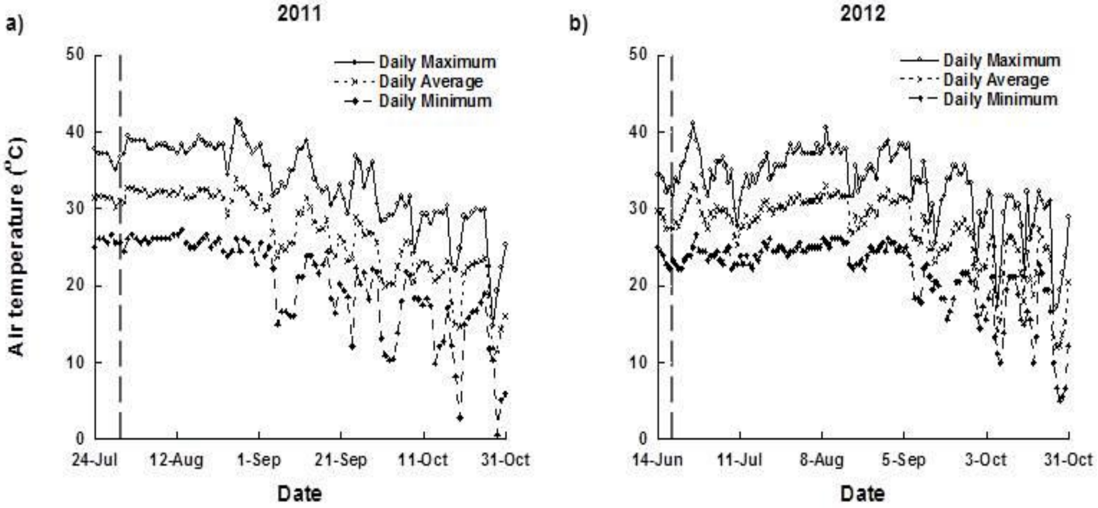


Figure 2.5. Maximum, average, and minimum daily air temperatures (°C) at study site for the duration of the growing season in a) 2011 and b) 2012. Dashed vertical lines indicate the date of first planting for each growing season.

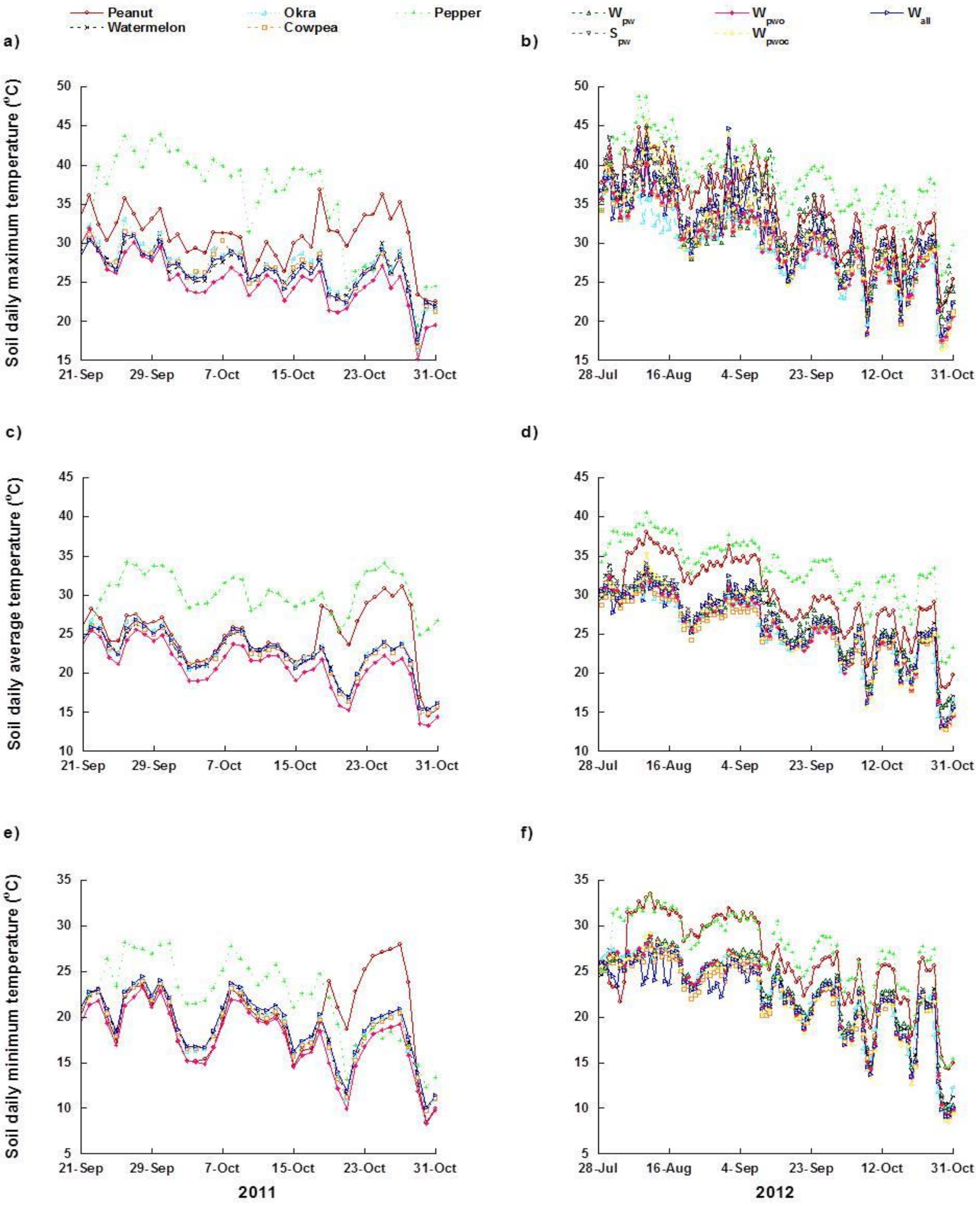


Figure 2.6. Daily soil temperatures measured at 2 cm depth for each monocrop and intercropping combination. Figures a) and b) are daily maximum soil temperature (°C), figures c) and d) are daily average soil temperature, and figures e) and f) are daily minimum soil temperature in 2011 and 2012, respectively. Treatments are described in figure 1. Measurements were only taking for W_{pwo} and W_{all} intercropping combinations in 2011.

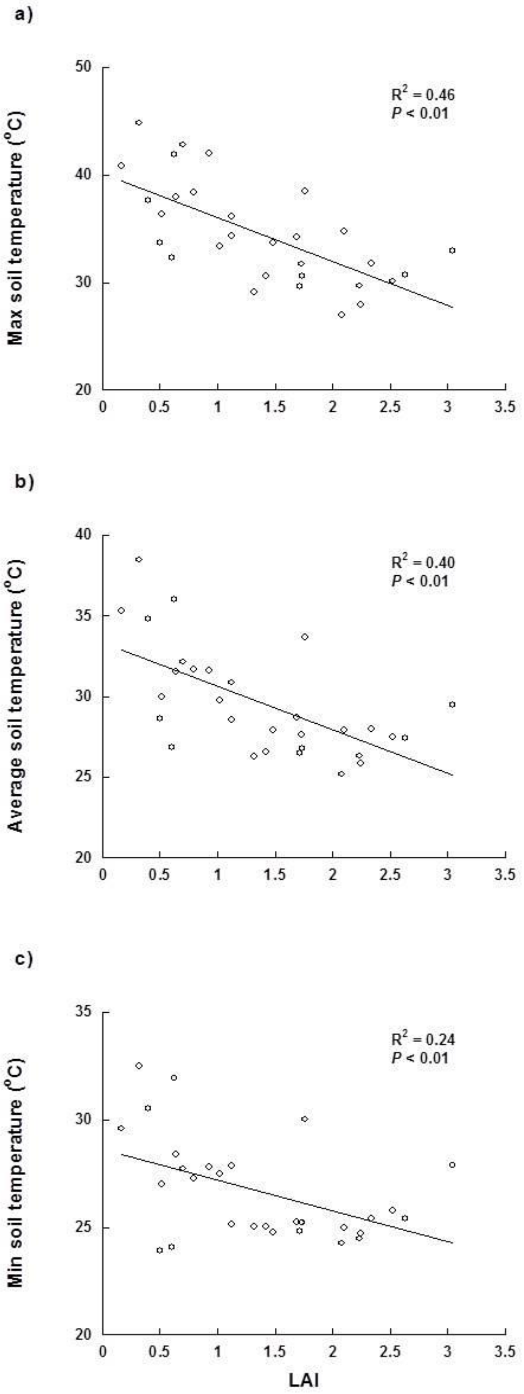


Figure 2.7. Relationship between leaf area index (LAI; measured on Aug 14, Aug 24, and Sep 13, 2012) and a) maximum soil temperature, b) average soil temperature, and c) minimum soil temperature for those same dates. A significant inverse relationship exists between LAI and soil temperature.

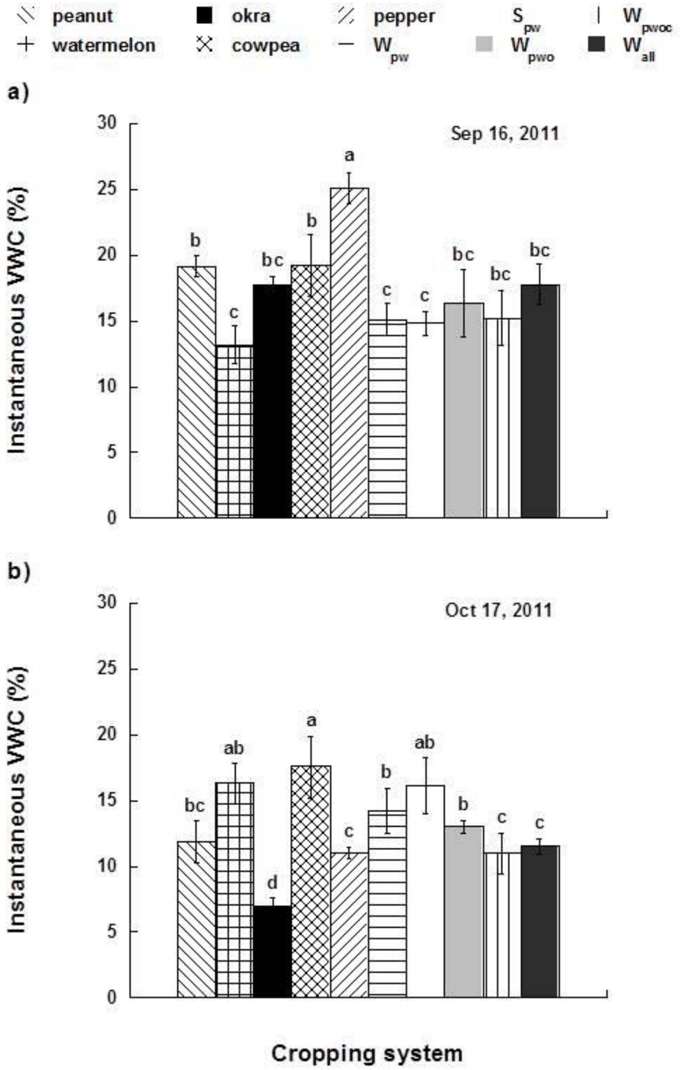


Figure 2.8. Instantaneous soil volumetric water content (VWC; %) measured with time domain reflectometry (TDR) probes at 15 cm soil depth for each monocrop and intercropping combination in 2011; a) measured on September 16 and on b), October 17. Treatments are described in figure 1. Different letters indicate statistically significant differences ($P < 0.05$) between cropping systems according to Tukey's LSD test.

Discussion

Unlike other studies that have found a positive effect of intercropping with legumes on N supply through either N rhizodeposition and transfer (Chu et al., 2004; Wichern et al., 2007a, b) or through above-ground deposition of high quality plant litter associated with legumes (Chapin et al., 1986; Vitousek and Howarth, 1991; Fornara and Tilman, 2008; Rusinamhodzi et al., 2009), there was no significant difference in total soil N nor a significant difference in N reduction at the end of each growing season. Inhibition of N-fixation can occur when N supply is abundant (Zhang et al., 2010). Therefore, while it is possible that the influence of legumes may have been suppressed by pre-planting fertilizer application in 2012, studies have found that this inhibitory affect can be minimized or eliminated by intercropping due to an increase in interspecies specific competition for N, and thus less N available for the legume (Hauggaard-Nielsen et al., 2003; Hauggaard-Nielsen et al., 2009b; Li et al., 2009). Additionally, Dijstra et al. (2010) found that interspecific species competition reduced plant N uptake due to a reduction in N supply from suppressed C decomposition. Thus, it is possible there may not have been a net change in total N supply in the rhizosphere due to a suite of competing processes. The fact that inter-row TN or TN percent reduction were not significantly different than any mono-or intercropping system indicates there may have been N losses due to leaching and, to a smaller extent, uptake by weeds (although weeding was conducted periodically). Hauggaard-Nielsen et al. (2003) found substantial N depletion regardless of cropping system or management in their study of intercropped pea and barley. They attributed these losses to nitrate leaching within the soil profile. Additionally, it is worth noting total N was only measured in this study and not broken down into organic and inorganic components. There may have been changes in the individual N components that were not detected.

Contrary to what was hypothesized, and unlike other intercropping studies that found increases in SOC in mixed species systems (Manna and Singh, 2001; Fornara and Tilman, 2008; Wu et al., 2014), there was no difference in SOC between cropping system in either year. The accumulation of soil C and soil N are slow processes (Fornara and Tilman, 2008). Therefore, the difference between labile short-term C pools more tightly linked with microbial activity that have a quick turnover rate (Krull et al., 2003; Jagadamma and Lal, 2010; Wang et al., 2011) versus stable C pools that have a turnover rate from decades to centuries (Bruun et al., 2008; Rumpel and Kögel-Knabner, 2010) is a key distinction in this study as it was only conducted for 2 growing seasons.

Differences in SMB-C that were found in this study further reinforce the distinction between the two potentially competing pools of C (Orwin et al., 2010). Since microbial community composition is linked with the quality of root organic matter and root exudates (Appuhn and Joergensen, 2006; Dijkstra et al., 2006; Cheng, 2009), as well as litter quality (Laossi et al., 2008), plant community composition should exert a strong influence on soil microbial biomass and composition (Wang et al., 2010). An increase in microbial diversity may be expected if there is a complementary use of organic substrate by the microbial community (Hooper et al., 2000). An increase in soil microbial biomass, which is believed to increase nutrient supply to plant roots, in intercropping versus monocropping systems has been demonstrated (Rivest et al., 2010; Wu et al., 2014). Unlike these other studies, however, there was significantly more SMB-C accumulation in the okra monoculture as compared to the mixed cropping system of peanut-watermelon-okra (W_{pwo}). It has been suggested that a greater diversity of organic compounds may have a negative effect on soil nutrient turnover as the likelihood that some of the compounds may not be consumed by decomposing microbes (Loreau and Hector, 2001). This may offer one explanation for the reduction of SMB-C found in a mixed cropping system in this study. Another possible explanation may be more related to above-ground plant characteristics. Fast-growing species which have a high specific leaf area (SLA) and high leaf nitrogen concentration (Tjoelker et al., 2005; Lavorel and Hutchings, 2013) may contribute to a rapidly decomposing pool of organic material as suggested by Hauggaard-Nielsen et al. (2003). Okra, a slower growing species as compared to watermelon, has a higher C:N ratio than watermelon (Franco unpublished data). Therefore, it may have contributed to a more stabilized (slower initial decomposition) pool of organic material in monoculture, rather than a rapidly decomposing pool of material that watermelon may have contributed to. Sampling at various times throughout the growing season would have given a more complete picture of SMB-C accumulation in this study. Additionally, RLD was found to be positively correlated with SMB-C in this study, which has also been found in other studies (Haynes and Francis, 1993; Gastine et al., 2003). Although the lack of sufficient replication in this study did not allow us to detect a difference in RLD between cropping system, there were more roots per volume of soil in okra monoculture and this may have also influenced the observed pattern in SMB-C. A last consideration is that microbial biomass was only measured and not community composition. Therefore, it is uncertain what effect the mixed cropping system may have had on microbial community diversity.

Above-ground competition for light has been cited as a reason why below-ground biomass may be reduced as plants shift allocation of C to above-ground parts (Bloom et al., 1985; Bessler et al., 2009). Conversely, increased competition for nutrients and root architecture complementarity in mixed species systems may lead to an increase in below-ground biomass partitioning by plants (Postma and Lynch, 2012; Brassard et al., 2013). Li et al. (2006) observed an increase in RLD in intercropped faba bean and maize, supporting the idea that complementarity in root architecture may lead to overall increased root biomass. In this study, however, there were no clear patterns in root biomass as measured by RLD. Although there appears to be a reduction in RLD in W_{pwo}, our statistical analyses did not detect any changes. Cowpea roots had a significantly higher SRL as compared to peanut and W_{pwo}. This suggests cowpea roots were longer and thinner than peanut roots and all roots in the W_{pwo} intercropping combination. Similar patterns were not observed in W_{pwoc} and W_{all}. A possible explanation for these observations is that cowpea may have had a negative selection effect on SRL in these two intercropping systems (Loreau, 2000), whereby the higher SRL for cowpea increased overall SRL in the two intercropping combinations cowpea was planted in. Similarly, high watermelon SRL may have increased overall SRL in W_{pw} and S_{pw}.

High soil temperatures have been associated with high rates of root respiration (Ceccon et al., 2011; Liu et al., 2013). In this study, monocropped pepper, in particular, had the highest soil temperatures throughout both growing seasons. The 3 species intercropping combination, W_{pwo}, had the lowest soil temperatures in 2011. Soil temperature was found to be negatively correlated with LAI, whereby an increase in leaf material intercepting solar radiation resulted in reduced soil temperatures, especially daily maximum soil temperatures. The reduction found in this study has also been demonstrated in other intercropping studies. Salau et al. (2014) found that a Cassava-pumpkin intercrop increased LAI and reduced both soil temperature and soil moisture. Ghanbari et al. (2010) also found a reduction in soil temperature, as well as an increase in soil moisture, in intercropped maize-cowpea. Liu et al. (2013) observed reductions in soil temperature in a wheat-maize intercropping system. They also observed a linear relationship between soil temperature and soil respiration, with respiration decreasing as temperatures decreased. They did not measure root respiration specifically, however. Root respiration is affected by a number of complex interactions including species differences, soil moisture, soil temperature, season, and soil type (Atkin et al., 2000; Bryla et al., 2001; Huang, 2005). As a large component of total soil respiration, root respiration contributes significantly to C losses from soil. Therefore, it would be an important piece of information to measure root respiration directly in the types of cropping systems studied here.

Although results from this study are incongruent, agricultural practices that utilize a multifunctional species approach can at the very least reduce maximum daytime soil temperatures via increased canopy cover and may potentially reduce C losses from soil. Future studies should include measurements of soil respiration, more frequent measurements of SMB-C and roots to evaluate response over the course of a growing season, and should be established on a long-term scale (> 2 years) to more accurately access the effects of multifunctional cropping systems on soil C and N dynamics. This may offer more insight into the dynamics between the stable C pool (SOC) and the labile C pool (SMB-C), as well as the role of interspecific species competition on root dynamics and their combined effect on the microbial community, and on C and N cycling.

CHAPTER IV

THE EFFECTS OF FUNCTIONALLY DIVERSE INTERCROPPING ON ABOVE-GROUND PLANT RESPONSE

Introduction

In multispecies systems, positive interactions such as facilitation and complementarity can offset some of the negative interactions associated with plant resource competition (Callaway, 1998; Hooper et al., 2005). Complementarity results from niche partitioning and a reduction of competition between species (Vandermeer, 1989; Hille Ris Lambers et al., 2004; van Ruijven and Berendse, 2005), while facilitation occurs when neighboring plants ameliorate habitat through the moderation of abiotic stress during times of suboptimal conditions (Hooper et al., 2005; Chu et al., 2008; Zhang et al., 2012). The stress-gradient hypothesis, which was formulated at the interspecies competition level, states there is a shift from competition to facilitation in plant communities as abiotic stress is amplified along environmental gradients (Callaway and Walker, 1997; Zhang et al., 2012). Complementarity occurs when functionally different species differ in their acquisition of resources in either time or space (Reich et al., 2004; van Ruijven and Berendse, 2005; Tilman et al., 2006b). Complementary use of resources, along with facilitative interactions between species, has been cited as a reason why species are able to coexist and why plant growth and productivity are maximized in diverse natural plant communities (Hooper, 1998; Hille Ris Lambers et al., 2004; Hauggaard-Nielsen et al., 2009a).

The efficiency of converting resources into biomass depends on the total amount of light intercepted by the canopy (i.e., a function of canopy size and competition for light) and the rate of net photosynthesis per unit leaf area (Lambers et al., 2008). Plants that form part of a more dense canopy, such as that found in diverse ecosystems, will undergo more competition for light and, consequently, a stronger vertical light gradient (Poorter et al., 2006). Since leaves are the primary photosynthetic organs of a plant, plants respond to changes in their light environment by changing leaf morphology and altering resource allocation patterns to leaves (Poorter et al., 2009). Plants can modify specific leaf area (SLA), leaf area per unit dry mass, and the relative investment of nitrogen between leaf photosynthetic machinery (Evans and Poorter, 2001). For instance, shading can result in reduced leaf thickness due to reduced thickness of palisade parenchyma, thereby increasing SLA (Poorter et al., 2006). As an underlying component of relative growth rate (RGR), an increase in SLA maximizes the amount of light interception by increasing RGR (Lambers et al., 2008) and increasing a plants competitive ability. Furthermore, there is a strong linear relationship between SLA and leaf N concentration with leaf N increasing as SLA increases. Since photosynthetic machinery accounts for more than half of leaf N content (Evans, 1989; Lambers et al., 2008), photosynthetic capacity is tightly associated with N availability and leaf N content (Evans, 1989; Loomis, 1997). Therefore, changes in leaf resource allocation and specific leaf area can be better predictors of plant growth than minor changes in net assimilation rates as photosynthetic capacity per unit leaf area is optimized (Potter and Jones, 1977) .

Intercropping has become an important management strategy for enhancing crop resource use efficiency and maximizing plant productivity through the deliberate manipulation of interspecific species interactions (Vandermeer, 1989; Li et al., 1999; Andersen et al., 2007; Hauggaard-Nielsen et al., 2009a). Intercropping can have both positive and negative effects on net assimilation rates and plant growth (Hooper and Vitousek, 1998; Andersen et al., 2005). Intercropping with functionally dissimilar species may lead to an increase in leaf area index (LAI) and overall light interception (Bilalis et al., 2010; Salau et al., 2014), thereby capturing more available light but also potentially creating a more dense canopy leading to increased competition for light. Changes in leaf-level traits (LMA: leaf mass per unit area or the reciprocal of SLA), gas exchange and WUE have been found to be associated with growth habit in row crops such as soybean and wheat (Tanaka et al., 2008; Barrios-Masias et al., 2014). The morphological and anatomical changes that occur at the leaf-level can affect chlorophyll content and, consequently, stomatal conductance and photosynthetic rates (Makoi et al., 2010; Barrios-Masias et al., 2014). As previously discussed, photosynthetic activity is linked to efficient plant nitrogen uptake and partitioning within a leaf, which is, in part, influenced by growth habit (Evans and Poorter, 2001). Photosynthetic nitrogen-use efficiency (PNUE, photosynthetic rate per unit N) is associated with a high relative growth rate, thereby increasing the fitness of the plant and its ability to compete with neighbors (Hikosaka, 2004). However, there is a tradeoff between investing in a high-N leaf which has a shorter lifespan versus investing in C compounds associated with leaf longevity (Field and Mooney, 1986). Due to this N investment in leaves with higher SLA, and ultimately greater photosynthetic capacity, a decrease in PNUE is expected as SLA decreases (Harrison et al., 2009). In addition, since leaf N content is correlated with photosynthesis and stomatal conductance (Niinemets and Kull, 1998; Reich et al., 2003; Hikosaka, 2004), PNUE is tightly coupled with water-use efficiency in some species (Sage and Pearcy, 1987). Moreover, sustained high photosynthetic rates are often correlated with higher crop yields (Ainsworth et al., 2002; Ainsworth and Long, 2005).

Most intercropping studies have limited their approach to simplified two-species systems and occasionally three-species systems (Andersen et al., 2007). In addition, the majority of studies conducted in temperate regions have focused on legume-cereal intercropping systems (Zhang and Long, 2003; Gao et al., 2009; Hauggaard-Nielsen et al., 2009a; Dahmardeh et al., 2010; Gao et al., 2010). Makoi et al. (2010) found that photosynthetic rates and water-use efficiency (as measured by isotopic discrimination) decreased in cowpea when intercropped with sorghum at different planting densities but particularly at high densities. Su et al. (2014) also observed a decrease in photosynthetic rate in soybean seedlings when intercropped with maize. In contrast, Pinheiro and Filho (2000) observed an increase in maize photosynthetic activity when intercropped with cowpea as compared to monocropped maize. There was a decline in cowpea photosynthesis, however. They also observed an improvement in water relations in both crops as measured by leaf water potential. They attributed both the improved water relations but lower net photosynthesis measurements in intercropped cowpea to facilitative environmental modifications from shading by maize. Light interception is important as plants grown under higher irradiances tend have higher photosynthetic rates and stomatal conductance (g_s) values, but lower WUE (Evans and Poorter, 2001; Tanaka et al.; 2008; Barrios-Masias et al., 2014). Other studies have also found higher photosynthetic rates associated with two-species intercropping systems (Gomez-Rodriguez et al., 2007; Ahmad et al., 2013).

In summary, there is little agreement on how intercropping may impact sustained crop productivity from a physiological perspective owing to the variability in response. Studies have primarily been limited two-species systems and there is a gap in our understanding of how component crops will respond to a functionally diverse intercropping system with regards to physiology and resource-use efficiency. In addition, there is little mention of leaf-level acclimation in existing intercropping studies. We tested the hypothesis that a functionally diverse cropping system would enhance water and nitrogen use efficiency through niche partitioning and complementarity of resource utilization. We also tested the hypothesis that this system would reduce plant stress in crops during the peak of summer through facilitative interactions in a low fertilizer input organic system consisting of peanut, watermelon, okra, cowpea and pepper.

Materials and Methods

Study Area

Low input managed plots were established at Texas A&M University's Horticulture Farm (30°37′N, -96°22′W) during the 2011 and 2012 growing seasons. Average monthly air temperatures for this area from May to October when the study was conducted ranged from 28 to 39°C for the maximum and 15 to 26°C for the minimum in 2011 and 27 to 37°C and 15 to 25°C in 2012 (NOAA/NCDC).

Experimental Design

The study design was a randomized complete block with three replicates, five intercropping treatments, and five controls. The controls consisted of monocultures of the five component species; peanut, mini watermelon, okra, cowpea, and hot pepper. The five treatments were a within-row intercropping system of 1) peanut and watermelon (W_{pw}), 2) peanut, watermelon, and okra (W_{pwo}), 3) peanut, watermelon, okra, and cowpea (W_{pwoc}), 4) all five control species (W_{all}), and 5) a strip intercropping system of peanut and watermelon consisting of alternating single rows (S_{pw}). Figure 1.1 illustrates the planting layout for the within-row and strip intercropping schemes. Crops represented four different genera and were selected based on the following criteria: 1) heat tolerance, 2) desired architecture and function (Table 1.1), and 3) no known adverse effects on other component crops. "Tamspan 90" is a small Spanish bunch type peanut which was selected for its small canopy and lack of runners. The "TAMU" mini watermelon variety was developed by the Texas A&M University watermelon breeding program and has a number of advantages for small scale producers including its ability to tolerate narrower spacing and, thus, take up less space, and produces a smaller fruit which is more manageable for small growers. Selected because of its popularity, “clemson spineless” okra is a dwarf variety well suited for this region. “Texas pinkeye” is a purple hull cowpea selected for its erect and bush-type growth form. This taller variety of cowpea can tolerate narrower spacing. Hot pepper varieties were used for this study. Jalapeno was used for year one and Serrano for year two and selection was based upon availability.

Crops were planted in 4 m long double rows on 90 cm wide raised beds with rows spaced approximately 30 cm apart and beds spaced 45 cm apart (total plot size, 4 × 5 m). Using the methodologies recommended by Jeavons (2006) and minimum spacing requirements outlined in the Texas Vegetable Growers Handbook (Masabni and Dainello, 2009), individual plant spacing was based on the mean spacing requirement for all component crops. Densities were kept constant across plots regardless of crop species and individual plants were spaced 30 cm apart in a staggered row pattern so that each plant was neighbored by an individual of a different species and a leguminous species. A 2 m buffer was maintained between plots.

Gas Exchange

Gas-exchange measurements were conducted on the youngest fully expanded leaf between the hours of 1200 and 1400. One unshaded (when possible) leaf per species per replicate was used. Net CO_2 assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (Ci), and transpiration rate (E) were measured using a portable open-flow infrared gas analyzer (LI-6400XT; LI-COR). In 2011, measurements were taken in the second week of October when most component crops had reached full maturity and full canopy was established (between 54 and 58 days after the last crop was planted; DALP). One replicate of all species in all plots was collected per day for a total of three collection dates, October 11, 13, and 15th. In 2012, measurements were taken on separate days for each species, once at the time of fruit set and a second time when plants had reached full maturity and full canopy was established when competition for resources was at its maximum (50 and 126, 40 and 84, 53 and 104, 41 and 55, and 35 and 114 days after planting (DAP) for peanut, watermelon, okra, cowpea and pepper, respectively).

During 2011 measurements, photosynthetically active radiation, reference CO_2 concentration, air flow rate, and block temperature were maintained constant at 1800 umol m^{-2} s^{-1}, 390 µmol mol^{-1}, 400 µmol s-1, and 28 °C, respectively. Relative humidity in the sample chamber ranged between 38% and 64%. During 2012 measurements, photosynthetically active radiation, reference CO_2 concentration, and air flow rate were maintained at the same rates as 2011, but block temperature ranged from 29 °C to 33 °C depending on the collection date. Relative humidity in the sample chamber ranged between 56% and 70% for all collection dates. Leaves were then scanned using a flatbed scanner in order to derive total leaf area and oven dried at 24°C for 48 hours or to a constant weight. Specific leaf area (SLA) was then calculated as the ratio of leaf area (m^2) to leaf dry mass (kg).

Leaf Water Potential

Predawn and midday water potential (Ψ_p and Ψ_m) were measured on the youngest fully expanded leaf using a Scholander pressure chamber (Scholander et al., 1965). Measurements were taken between the hours of 0400 and 0630 and 1100 and 1400, respectively. Measurements were taken on the same dates as gas exchange measurements. One leaf per species per replicate plot was measured.

Nitrogen-Use Efficiency

Photosynthetic nitrogen use efficiency (PNUE), or the ratio of CO_2 assimilation rate to leaf total nitrogen content (Poorter and Evans, 1998), was calculated as photosynthetic rate (A) per unit leaf area (cm^2) per gram of N per unit leaf area (SLA * grams of N). Dried leaves that were collected during gas exchange measurements were ground to a 1 mm mesh size. C and N content were determined using an elemental analyzer (Carlo Erba EA-1108; CE Elantech, Lakewood, NJ) interfaced with an isotope ratio mass spectrometer (Delta Plus, Thermo Electron, Waltham, MA) operating in continuous flow mode. Leaf N concentration was calculated based on a leaf mass basis (mg N g^{-1}) and on a leaf area basis (g N $[m^2]^{-1}$). Leaf carbon to nitrogen ratio (C:N) was also calculated.

Water-Use Efficiency

Water-use efficiency was derived based on agronomic calculations from both yield and plant biomass (WUE_{yield} and $WUE_{biomass}$), based on gas exchange measurements (A/g_s) and on an integrated approach utilizing carbon (C) stable isotopes (WUE_{leaf}). Agronomic water use efficiency based on yield was calculated on a per plant basis in order to avoid artificial bias toward intercropping treatments that contained more plants and, thus, higher yield per plot. Irrigation duration was closely monitored and recorded throughout both growing seasons. WUE_{yield} was calculated as yield per plant (kg $plant^{-1}$) per mm of total water input (irrigation + precipitation; kg plant $^{-1}$ mm^{-1}).

Plants were harvested from one row of each replicate of each treatment in September 2012 in order to estimate plant biomass. Fruits and pods were omitted from plant biomass estimates so as to include only leaf and stem material. Separated by species, this data was used to calculate agronomic water use efficiency based on plant biomass for 2012. As with yield calculations, $WUE_{biomass}$ was calculated as biomass per plant (kg $plant^{-1}$) per mm of total water input (irrigation + precipitation; kg plant $^{-1}$ mm^{-1}).

Integrated water use efficiency WUE_{leaf} is based on the ratio of $^{13}C/^{12}C$ (denoted as $d^{13}C$) and is used to integrate WUE over the lifespan of the leaf (Funk and Vitousek, 2007). Carbon isotopic composition has been correlated with water-use efficiency in crops such as wheat (Farquhar and Richards, 1984) and is an effective method for understanding water use and physiology of C_3 species (Farquhar et al., 1989). Dried leaves that were collected during gas exchange measurements were ground to a 1 mm mesh size. $d^{13}C$ was determined using an elemental analyzer (Carlo Erba EA-1108; CE Elantech, Lakewood, NJ) interfaced with an isotope ratio mass spectrometer (Delta Plus, Thermo Electron, Waltham, MA) operating in continuous flow mode. $d^{13}C$ is calculated as the relative difference from the Vienna Pee Dee Belemnite (V-PDB) standard in parts per thousand (per mil, ‰) using the following equation:

$$d^{13}C = (R_{sample}/R_{standard} - 1) * 1000 \quad (Eq.1)$$

Where, Rsample and Rstandard are the $^{13}C/^{12}C$ ratios of the samples and the standard, respectively.

Due to small differences in mass between ^{13}C and ^{12}C, plants discriminate against ^{13}C and preferentially take up ^{12}C. During times of water stress, plants close their stomata and discriminate less against ^{13}C, thereby increasing the ratio of ^{13}C to ^{12}C, which is associated with higher WUE (Farquhar and Richards, 1984; Farquhar et al., 1989; Lucero et al., 2000).

Light Interception

Leaf area index (LAI), or the total leaf area per unit ground area (Schieving and Poorter, 1999), was measured using a ceptometer (AccuPAR; Decagon Scientific, Pullman, WA) in 2012. Measurements were taken on August 14th and 24th and September 13th (33, 43 and 63 days after last planting; DALP) and only when conditions were sunny. As described by Lombardini (2006), the “above canopy” photosynthetic photo flux density was recorded in an open area adjacent to each plot prior to taking measurements. Three readings were consistently taken toward the center of each plot between the hours of 1200 and 1400.

Statistical Analyses

All physiology and resource use efficiency data were analyzed by species, comparing intercropping combinations, and by treatments, comparing species within each intercropping combination, using ANOVA in JMP 10.0.2 statistical software for windows (SAS Institute, Cary, NC, USA). The Shapiro-Wilk goodness-of-fit test was used to assure assumptions of normality were met. When these assumptions were not met, log transformations were utilized and the model with the highest R^2 value was accepted. Where a significant *F*-test was observed ($P < 0.05$), mean separations were conducted using Tukey's Least Significant Difference (LSD) test.

Results

Gas Exchange Measurements

No differences were detected in gas exchange measurements in any of the five component species when comparing intercropping combinations within each species (Tbl. 3.1). When comparing species within intercropping combination, peanut in the within-row intercropping combination with watermelon (W_{pw}) was less active than watermelon at full canopy stage (Fig. 3.1). In the strip intercropping combination (S_{pw}), watermelon had higher stomatal conductance and intercellular CO2 concentrations as compared to peanut at flowering stage in 2012 (Fig. 3.2d, f). No differences were detected between species in the three or four species combinations (W_{pwo} and W_{pwoc}; Tbl. 3.2). In the five species combination (W_{all}), pepper had significantly higher net assimilation rates than all other component species in 2012 (Fig. 3.3b).

Table 3.1. *P*-values of the impact of intercropping on physiological parameters

	Impact of species diversity									
Year/ Species	A	gs	ci	E	PD Ψ	MD Ψ	PNUE	Δ13C	A/gs	Yield mm^{-1}
2011										
Peanut	0.264	0.438	0.139	0.183	0.621	0.047	0.890	0.501	0.086	-
Okra	0.665	0.953	0.735	0.603	0.325	0.593	0.411	0.058	0.379	0.059
Watermelon	0.419	0.909	0.731	0.672	0.259	0.073	0.709	0.317	0.295	0.436
Cowpea	0.504	0.730	0.732	0.591	0.205	0.609	0.140	0.353	0.158	0.042
Pepper	0.911	0.996	0.498	0.725	0.584	0.959	0.336	0.301	0.551	0.074
2012 (flowering)										
Peanut	0.452	0.265	0.469	0.254	-	0.799	-	-	0.461	-
Okra	0.438	0.263	0.214	0.242	-	0.288	-	-	0.263	-
Watermelon	0.626	0.425	0.096	0.595	-	-	-	-	0.198	-
Cowpea	0.974	0.961	0.722	0.840	-	0.630	-	-	0.673	-
Pepper	0.566	0.730	0.460	0.856	-	0.554	-	-	0.654	-
2012 (full canopy)										
Peanut	0.104	0.394	0.746	0.560	0.991	0.039	0.081	0.337	0.477	0.213
Okra	0.473	0.495	0.348	0.727	0.495	0.110	0.584	0.341	0.658	0.384
Watermelon	0.112	0.264	0.320	0.162	0.412	0.963	0.087	0.575	0.154	0.221
Cowpea	0.759	0.454	0.091	0.623	0.696	0.219	0.109	0.130	0.062	0.189
Pepper	0.682	0.648	0.493	0.805	0.963	0.939	0.909	0.189	0.304	0.059

P-values < 0.05 are highlighted in red

P-values < 0.10 are highlighted in green

Dashed lines indicate no data was collected during that collection period for a given species and measurement parameter.

Table 3.2. *P*-values of the impact of species within intercropping system on physiological parameters

	Impact of species								
Year/ Species	A	gs	ci	E	PD Ψ	MD Ψ	PNUE	Δ13C	A/gs
2011									
W_{pw}	0.273	0.498	0.782	0.258	0.081	0.012	0.017	0.169	0.389
S_{pw}	0.455	0.270	<0.001	0.319	0.004	0.015	<0.001	0.842	0.038
W_{pwo}	0.459	0.927	0.503	0.452	0.061	0.016	<0.001	0.127	0.442
W_{pwoc}	0.279	0.838	0.775	0.623	0.218	0.018	<0.001	0.695	0.341
W_{all}	0.744	0.880	0.665	0.961	0.004	0.006	<0.001	0.109	0.755
2012 (flowering)									
W_{pw}	0.405	0.243	0.449	0.117	-	-	-	-	0.397
S_{pw}	0.275	0.013	0.017	0.108	-	-	-	-	0.318
W_{pwo}	0.752	0.607	0.683	0.647	-	0.896	-	-	0.706
W_{pwoc}	0.782	0.996	0.795	0.963	-	0.436	-	-	0.507
W_{all}	0.289	0.055	0.183	0.941	-	0.005	-	-	0.660
2012 (full canopy)									
W_{pw}	0.007	0.020	0.011	0.017	0.102	0.127	0.005	0.004	0.005
S_{pw}	0.273	0.055	0.063	0.559	0.039	0.001	0.005	0.010	0.573
W_{pwo}	0.469	0.396	0.163	0.435	0.049	0.335	<0.001	0.049	0.153
W_{pwoc}	0.399	0.963	0.469	0.959	0.005	0.005	<0.001	0.078	0.208
W_{all}	0.048	0.045	0.541	0.003	0.027	0.002	<0.001	0.287	0.038

P-values < 0.05 are highlighted in red
P-values < 0.10 are highlighted in green
Dashed lines indicate no data was collected during that collection period for a given species and measurement parameter.

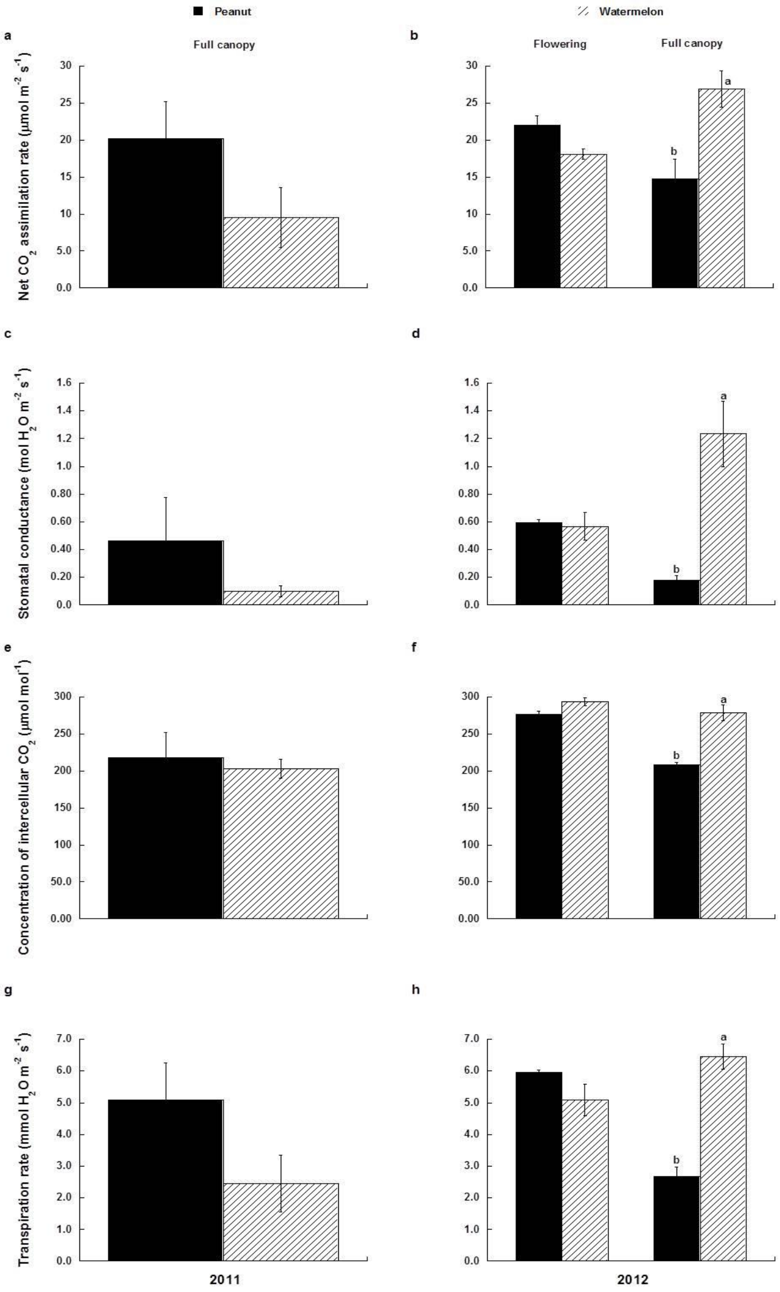


Figure 3.1. Net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 and at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon (W_{pw}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.

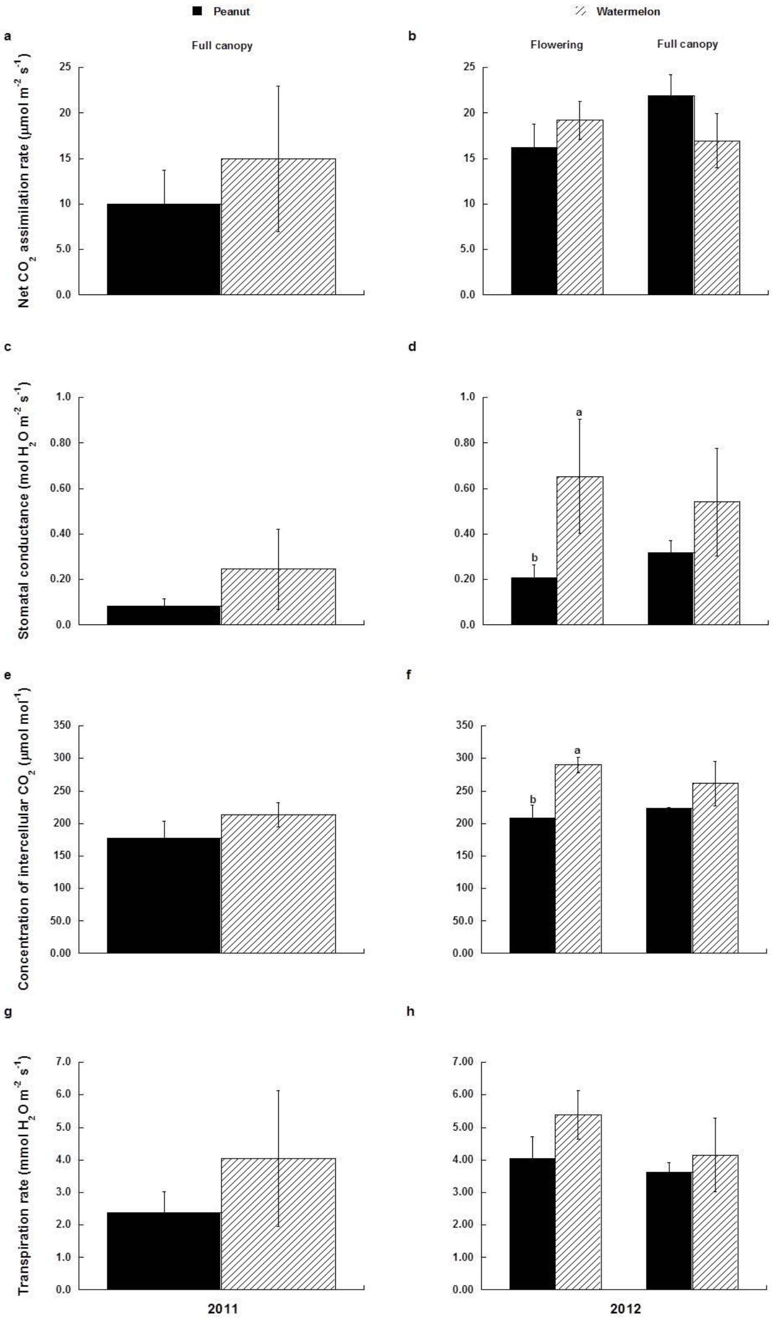


Figure 3.2. Net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 and at flowering stage and full canopy in 2012 for component species in the strip intercropping combination of peanut-watermelon (S_{pw}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.

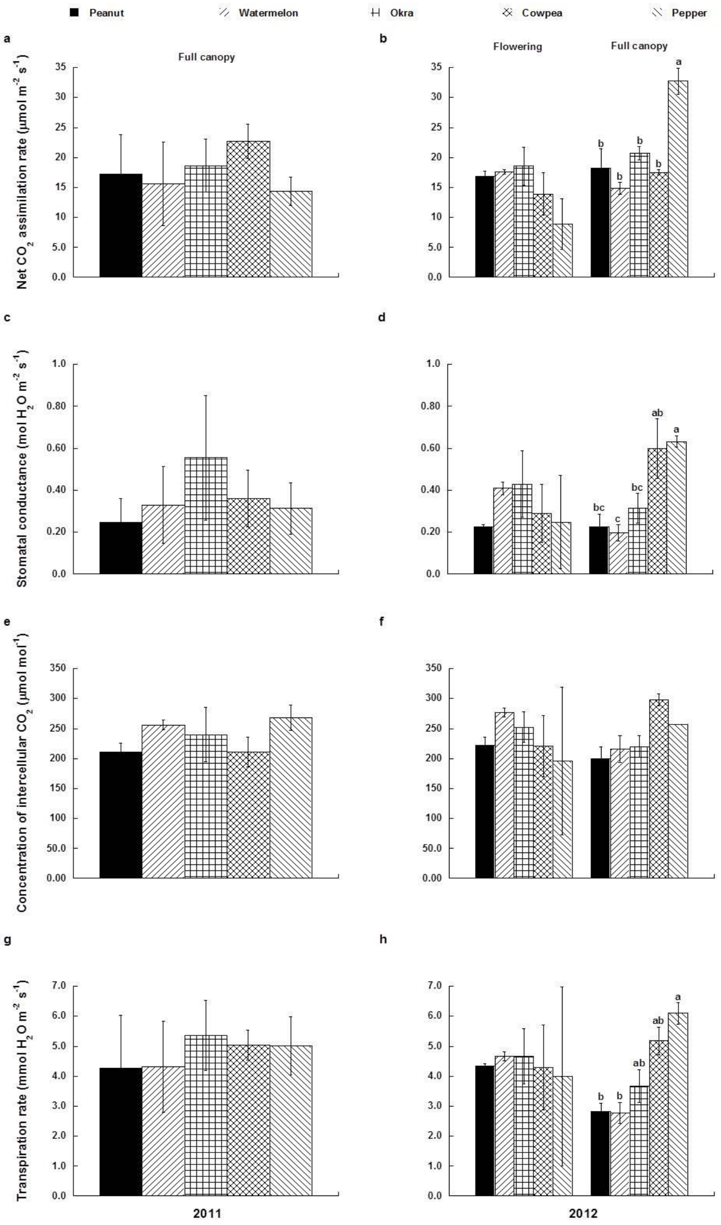


Figure 3.3. Net CO$_2$ assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 and at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra-cowpea-pepper (W_{all}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.

Leaf Water Potential

At full canopy stage in 2011, peanut was significantly less water stressed at midday when grown in the intercropping combination containing all component species (W_{all}), -0.9 MPa as compared to -1.6, -1.5, -1.5, -1.4, and-1.6 MPa in monoculture, W_{pw}, S_{pw}, W_{pwo}, and W_{pwoc}, respectively (Fig. 3.4). In 2012, peanut was significantly less water stressed in W_{pw}, W_{pwo}, and W_{all} at midday (-1.1, -0.6, -1.1 MPa, respectively) as compared to peanut in monoculture, S_{pw}, and W_{pwoc} (-2.2, -2.1, and -1.9 MPa, respectively). Additionally, peanut midday water potential (Ψ_m) values were much more negative at full canopy stage in these treatments as compared to flowering stage. No significant differences were detected in watermelon, okra, cowpea, or pepper between intercropping treatments in 2011 and 2012 (Tbl. 3.2). There were no predawn water potential (Ψ_p) differences for any of the species comparing when intercropping treatments in either year.

Despite the lack of differences in gas exchange measurements between peanut and watermelon at full canopy in 2011 in W_{pw}, peanut was significantly more water stressed than watermelon (-1.5 and -0.1 MPa, respectively; Fig. 3.5). Although not statistically significant, peanut was also more water stressed at full canopy in 2012 when compared to watermelon (-1.1 and -0.1, respectively), which coincides with higher photosynthetic activity measured in watermelon as compared to peanut during this growth stage. Peanut was also significantly more water stressed at predawn and

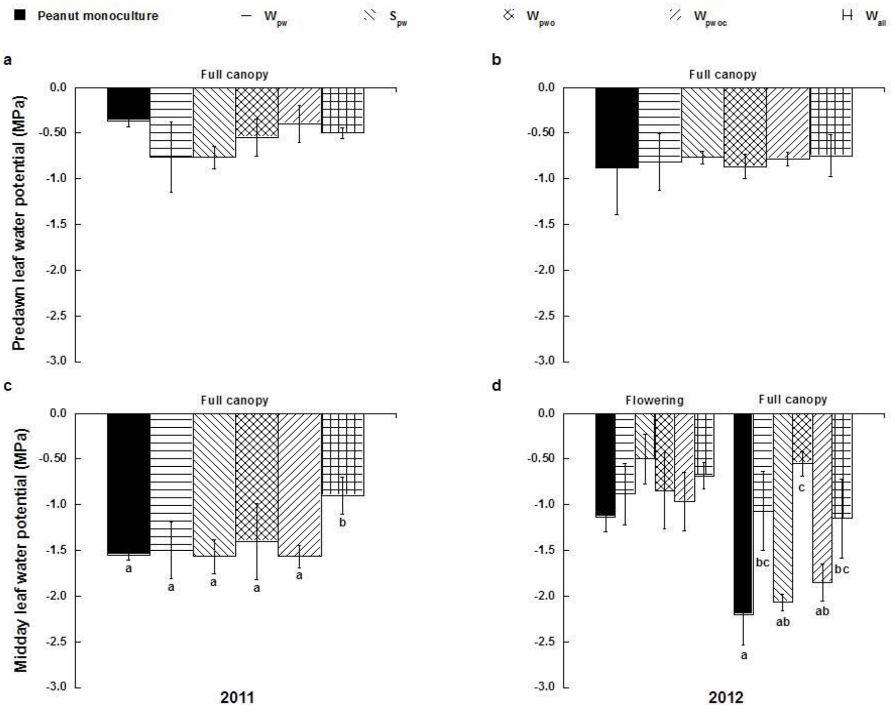
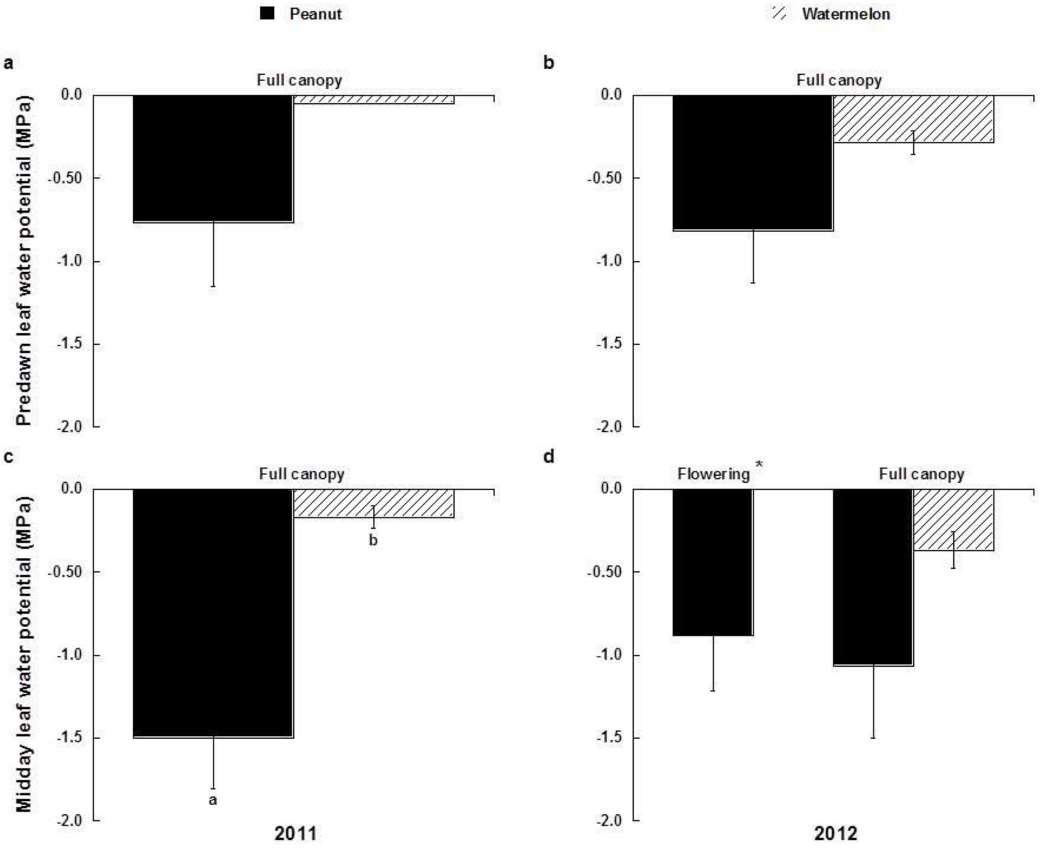


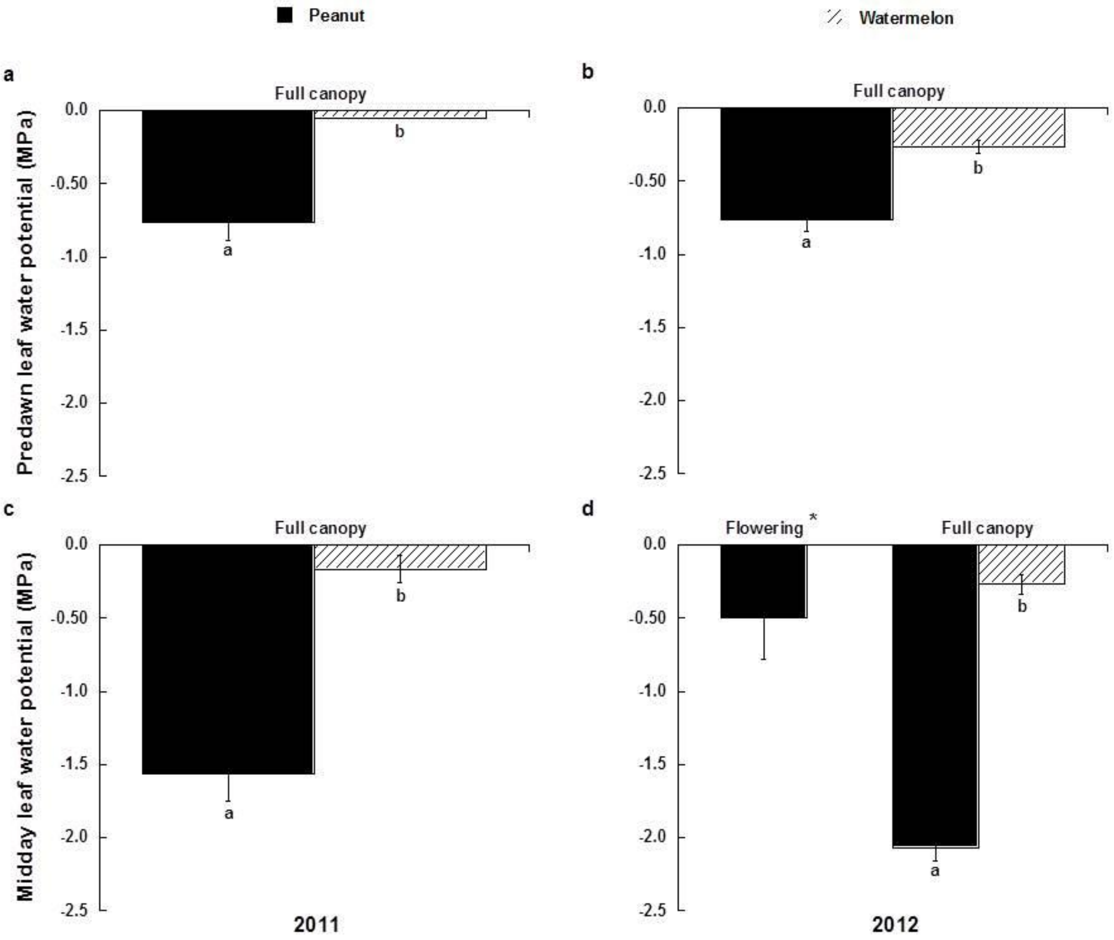
Figure. 3.4. Peanut a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy (71-75 DAP) in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage (76 DAP) and full canopy (126 DAP) in 2012. W_{pw} = within row intercropping of peanut-watermelon, S_{pw} = strip intercropping with peanut-watermelon (e.g. alternating rows), W_{pwo} = within row intercropping of peanut-watermelon-okra, W_{pwoc} = within row intercropping of peanut-watermelon-okra-cowpea, W_{all} = within row intercropping of peanut-watermelon-okra-cowpea-pepper. Different letters indicate statistically significant differences ($P < 0.05$) between intercropping treatments within collection period within years according to Tukey's LSD test.

at midday in 2011 and in 2012 at all full canopy stage in S_{pw} (Fig. 3.6). In the W_{pwo} intercropping treatment, peanut was significantly more water stressed than okra and watermelon at predawn and midday in 2011 (-0.6, -0.1, -0.05 MPa and -1.4, -0.4, -0.3 MPa, respectively; Fig. 3.7). In 2012, peanut again had the lowest Ψ_p at -0.9 MPa as compared to -0.3 and -0.2 MPa for watermelon and okra, respectively. Although not detected by ANOVA, peanut appeared to be more water stressed at midday in 2012 as well (-0.6 as compared to -0.3 and -0.2 MPa for peanut, watermelon and okra, respectively). Peanut was consistently the most water stressed species in the W_{pwoc} intercropping treatment, with significantly lower Ψ_m at full canopy in 2011 as compared to okra, cowpea, and watermelon (-1.6, -0.6, -0.5, and -0.2 MPa, respectively), and significantly lower Ψ_p and Ψ_m at full canopy in 2012 as compared to cowpea, okra, and watermelon (-0.8, -0.5, -0.4, -0.4 MPa and -1.9, -0.6, -0.4, -0.3 MPa, respectively; Fig. 3.8). Biologically, cowpea appeared to be more water stressed as compared to peanut and okra at flowering stage in 2012 (-1.6, -1.0, -0.8 MPa, respectively). These results were not statistically significant, however. In the W_{all} intercropping treatment, peanut was significantly more water stressed at predawn in 2011 while pepper was the most water stressed at midday (Fig. 3.9). Predawn water potential for peanut was -0.5 MPa but well below -0.1 MPa for all other species. Midday water potential for pepper was -1.3 MPa followed by peanut at -0.9 MPa, okra at -0.7 MPa, cowpea at -0.6 MPa, and watermelon at -0.2 MPa. In 2012, similar trends were observed with peanut and pepper having the lowest Ψ_p values at -0.9 and -0.8 MPa, respectively and watermelon having the highest Ψ_p value at -0.2 MPa. At flowering stage, pepper was again the most water stressed species at midday (-1.5 MPa), along with cowpea (-1.2 MPa). Despite being the most photosynthetically active at full canopy in 2012, pepper was significantly more stressed than all other species (-2.6 MPa) with similar trends observed between species as with flowering and predawn measurements.



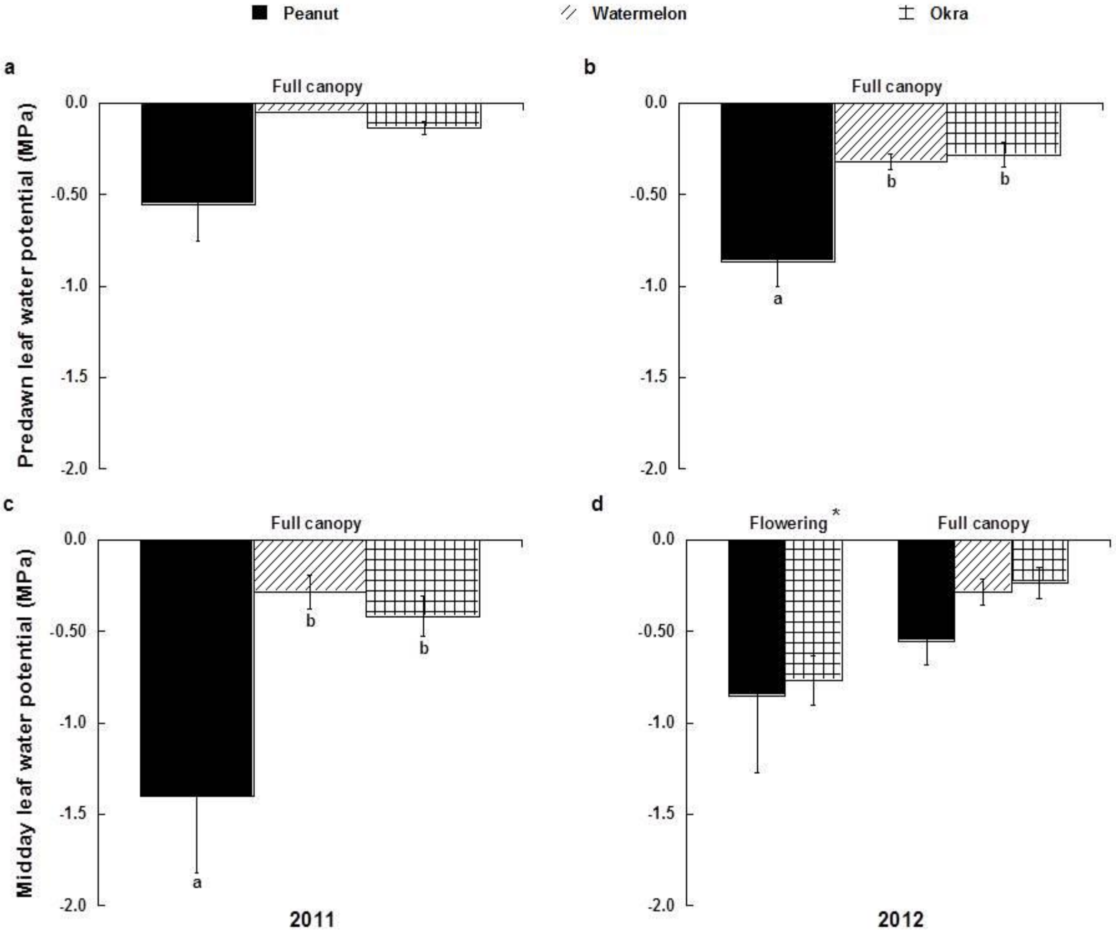
*watermelon measurements only taken at full canopy in 2012 due to downy mildew infestation

Figure 3.5. Effect of species on a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon (W_{pw}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.



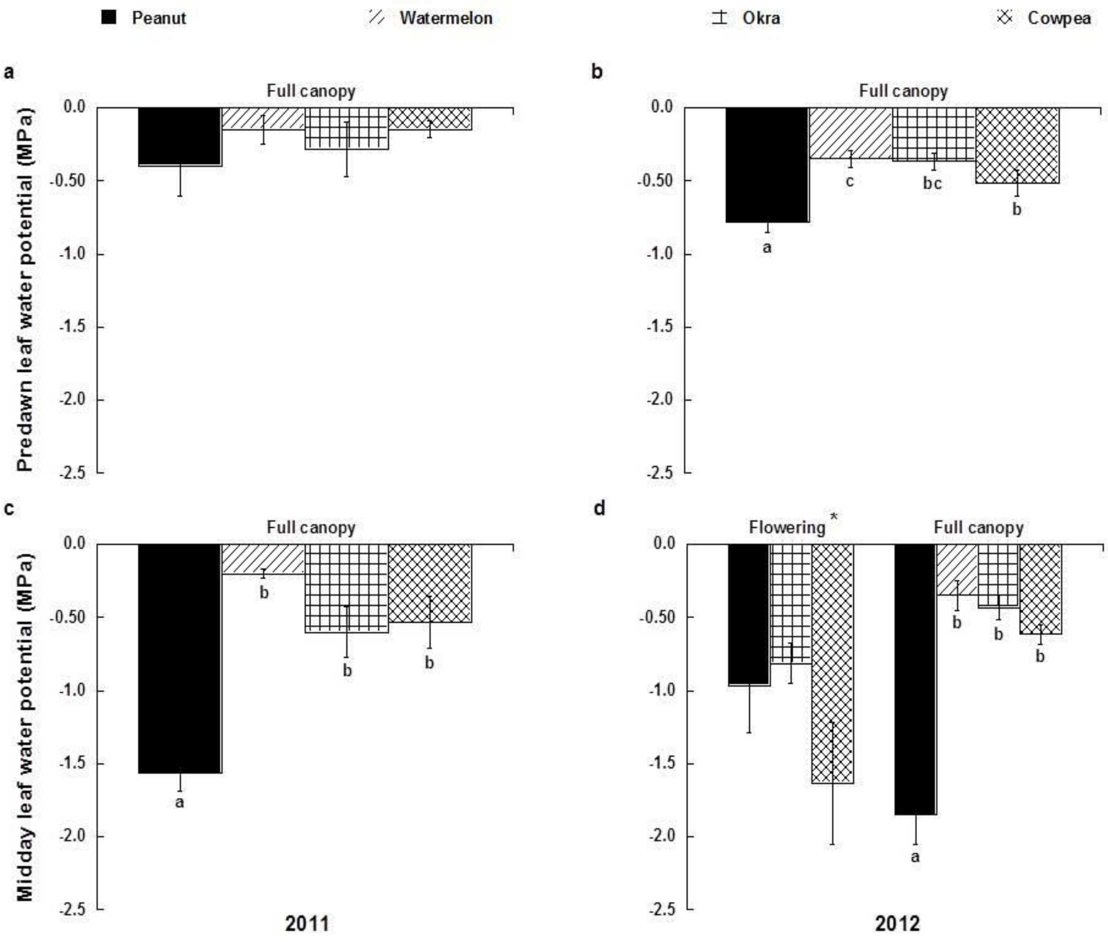
*watermelon measurements only taken at full canopy in 2012 due to downy mildew infestation

Figure 3.6. Effect of species on a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage and full canopy in 2012 for component species in the strip intercropping combination of peanut-watermelon (S_{pw}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.



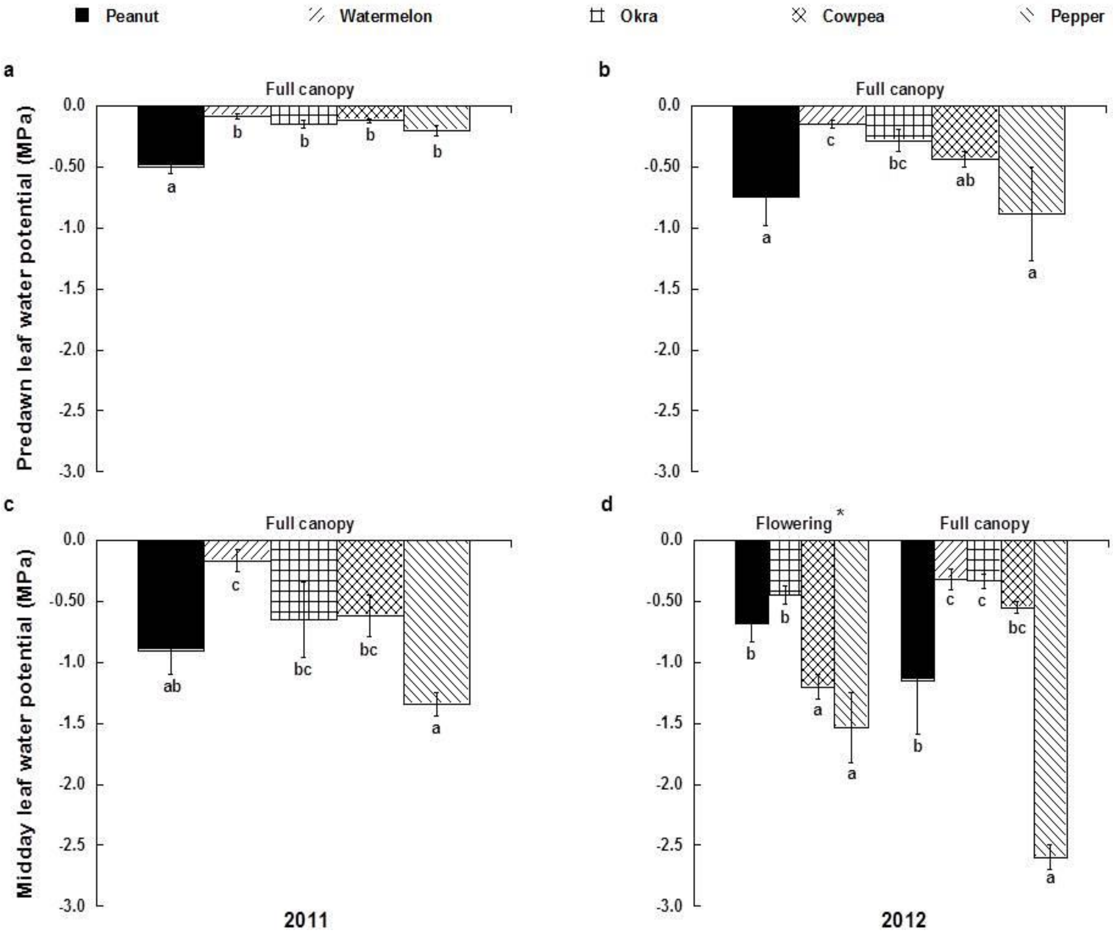
*watermelon measurements only taken at full canopy in 2012 due to downy mildew infestation

Figure 3.7. Effect of species on a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra (W_{pwo}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.



*watermelon measurements only taken at full canopy in 2012 due to downy mildew infestation

Figure 3.8. Effect of species on a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra-cowpea (W_{pwoc}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within year according to Tukey's LSD test.



*watermelon measurements only taken at full canopy in 2012 due to downy mildew infestation

Figure 3.9. Effect of species on a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra-cowpea-pepper (W_{all}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.

Resource-Use Efficiency

Photosynthetic nitrogen use efficiency (PNUE) in peanut did not differ significantly between intercropping treatments in 2011 and 2012 (Tbl. 3.1). Although it appears peanut may have had higher PNUE when grown in the the W_{pwo} intercropping combination (8.0 µmol mol^{-1} s^{-1} in W_{pwo} as compared to 4.6, 4.5, 4.2, 3.3, and 3.0 µmol mol^{-1} s^{-1} in S_{pw}, peanut monoculture, W_{all}, W_{pwoc}, and W_{pw}, respectively), those differences were not detected by statistical analysis. Leaf carbon isotope composition (d^{13}C) did not differ significantly in peanut when comparing intercropping treatments for either year (Tbl. 3.1). Values ranged from -27.1 ‰ to -28.5 ‰ in 2011 and from 26.84 ‰ to 27.7 ‰ in 2012. Peanut water use efficiency calculated based on yield (WUE_{yield}) did not differ significantly between intercropping treatments in 2012 when peanut yield was collected (Tbl. 3.1), but was significantly higher in the W_{pw} intercropping treatment when calculated based on plant biomass ($WUE_{biomass}$) as compared to peanut monoculture, W_{pwo}, S_{pw}, , and W_{pwoc}, 1.8 x 10^{-4}, 1.2 x 10^{-4}, 1.1 x 10^{-4}, 9.8 x 10^{-5}, and 9.1 x 10^{-5} kg plant^{-1} mm^{-1}, respectively (Appendix Fig. 3.1a).

Watermelon PNUE was not significantly different in 2011 and although appearing to be higher in watermelon monoculture, no differences were detected in 2012 (Tbl. 3.1). Leaf carbon isotope composition (d^{13}C) did not differ significantly in watermelon when comparing intercropping treatments for either year (Tbl. 3.1; Appendix Fig. A-3.13). Values ranged from -27.4 ‰ to -28.6 ‰ in 2011 and from 28.3 ‰ to 29.5 ‰ in 2012. Although statistically significant differences were not detected in watermelon WUE_{yield} in 2011, values appeared to be higher in W_{all}, W_{pwoc}, and W_{pwo} as compared to watermelon monoculture, W_{pw}, and S_{pw} (2.0 x 10^{-2}, 1.9 x 10^{-2}, 1.5 x 10^{-2}, 8.2 x 10^{-3}, 8.2 x 10^{-3}, and 7.3 x 10^{-3} kg plant^{-1} m^{-1}, respectively). Differences in watermelon WUE_{yield} were not detected in 2012. Differences in watermelon $WUE_{biomass}$ were detected, however (Fig. 3.10). Watermelon grown in monoculture and in S_{pw} was more water use efficient with respect to plant biomass as compared to other treatments. No differences in cowpea PNUE were detected in either year (Tbl. 3.1; Fig. 3.11). No differences in cowpea leaf carbon isotope composition (d^{13}C) were detected comparing intercropping treatments for either year and values ranged from -27.0 ‰ to -27.9 ‰ in 2011 and from 28.2 ‰ to 28.9 ‰ in 2012. Cowpea WUE_{yield} in 2011 was significantly higher in cowpea grown in monoculture as compared to cowpea grown in the W_{pwoc} intercropping combination, 1.0 x 10^{-4} versus 2.9 x 10^{-5} kg plant^{-1} mm^{-1} (Fig 3.11c). WUE_{yield} for cowpea grown in the five species combination was 7.5 x 10^{-5} kg plant^{-1} mm^{-1}. Although not statistically significant, cowpea grown in monoculture also had the highest WUE_{yield} in 2012. As with WUE_{yield} in 2011, $WUE_{biomass}$ in 2012 showed similar mean separations with cowpea monoculture having the highest $WUE_{biomass}$ and W_{pwoc} the lowest (5.1 x 10^{-5} versus 2.1 x 10^{-5} kg plant^{-1} mm^{-1}; Fig. 3.10d).

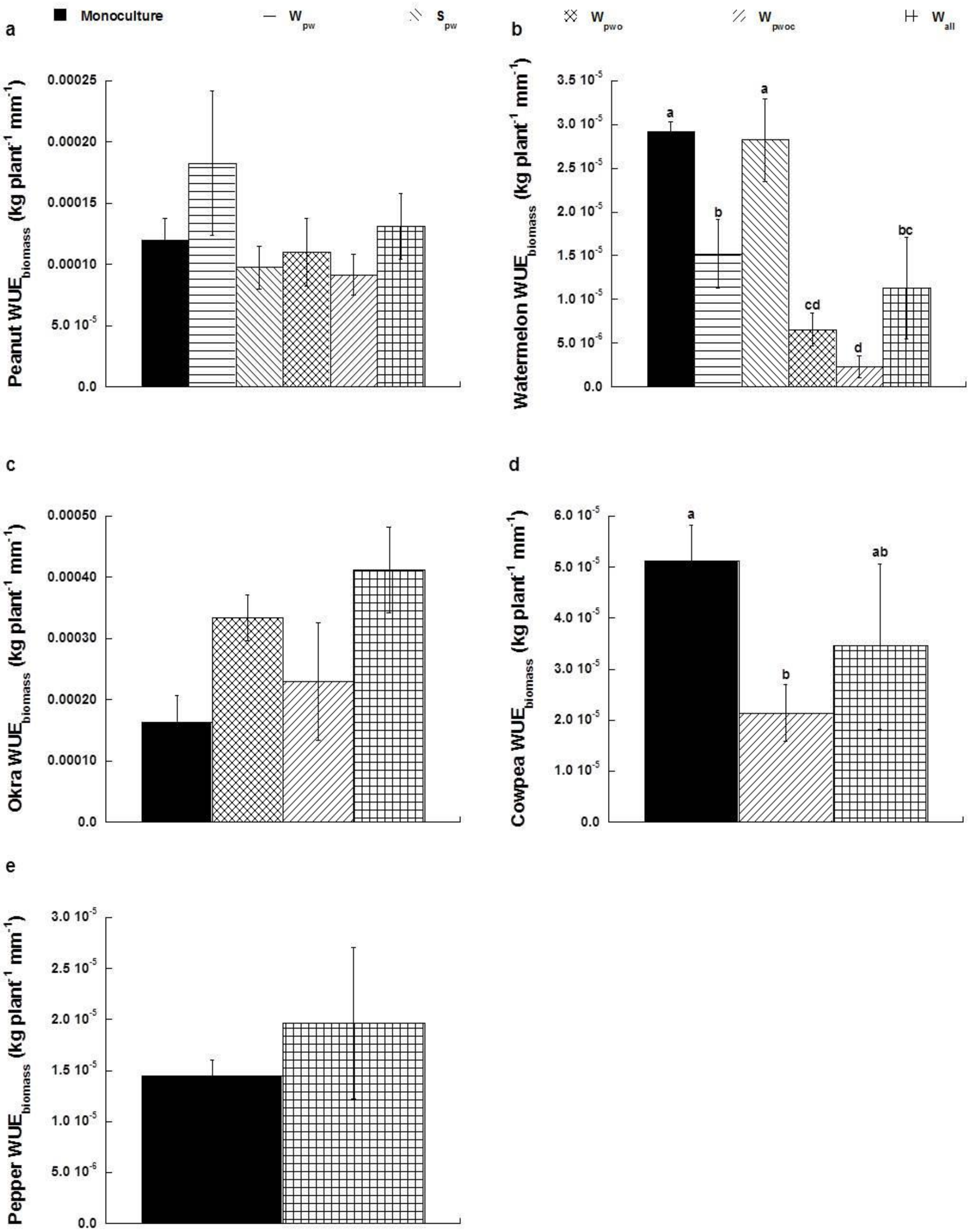


Figure 3.10. Agronomic water use efficiency calculated based on plant biomass (WUE$_{\text{biomass}}$ in kg dry mass plant^{-1} mm^{-1}) in 2012 for a) peanut, b) watermelon, c) okra, d) cowpea, and e) pepper in monoculture and in mixed cropping combinations. Treatments described in figure 3.4. Different letters indicate statistically significant differences ($P < 0.05$) between treatments according to Tukey's LSD test.

Pepper PNUE was not significantly different in 2011 or 2012 (Tbl. 3.1). Leaf carbon isotope composition (d^{13}C) did not differ significantly in pepper when comparing intercropping treatments for either year. Values ranged from -28.7 ‰ to -28.9 ‰ in 2011 and from 26.6 ‰ to 29.0 ‰ in 2012. While it would appear pepper grown in monoculture was more water use efficient with regards to yield in both years, statistical analysis did not detect those differences (Appendix Fig. A-3.15c). WUE_{yield} for pepper grown in monoculture was 1.9 x 10^{-4} and 3.4 x 10^{-4} kg plant^{-1} mm^{-1} in 2011 and 2012, respectively, while it was 5.1 x 10^{-5} and 1.3 x 10^{-4} kg plant^{-1} mm^{-1}, respectively, for pepper grown in the W_{all} intercropping combination. There were no differences in $WUE_{biomass}$ for pepper (Fig. 3.10e).

Peanut was significantly more nitrogen use efficient than watermelon in both years when comparing the two species in the W_{pw} intercropping combination (Fig. 3.12a). Peanut PNUE was 6.5 and 3.0 µmol mol^{-1} s^{-1} in 2011 and 2012, respectively, as compared to 0.03 and 0.1 µmol mol^{-1} s^{-1}, respectively, for watermelon. Additionally, watermelon was significantly less water use efficient (as determined by leaf carbon isotope ratios; WUE_{leaf}) than peanut in 2012, -29.5 ‰ and -27.7 ‰, respectively (Fig. 3.12b). Similar trends were observed with peanut and watermelon in the S_{pw} intercropping combination. Peanut PNUE was significantly higher than watermelon in both years, 5.3 and 4.2 µmol mol^{-1} s^{-1} for peanut in 2011 and 2012, respectively, as compared to 0.01 and 0.3 µmol mol^{-1} s^{-1} for watermelon (Fig. 3.13). Watermelon was, again, less water use efficient than peanut in 2012 (-28.7 ‰ versus -26.8 ‰; Fig. 3.13b). In the three species combination of peanut-watermelon-okra, peanut was the most nitrogen use efficient species in both years, followed by watermelon then okra (Fig. 3.14a). PNUE for 2011 and 2012 was 4.9 and 7.7 μmol mol^{-1} s^{-1}, respectively, for peanut, 0.04 and 0.2 μmol mol^{-1} s^{-1} for watermelon, and 3.9 x 10^{-3} and 0.03 μmol mol^{-1} s^{-1} for okra. WUE$_{leaf}$ was not significantly different between the species in 2011. In 2012, watermelon was significantly less WUE than okra, -28.2 ‰ versus -26.5 ‰, respectively (Fig. 3.14b). In the four species intercropping combination with cowpea, peanut was, again, the most nitrogen use efficient species followed by watermelon and cowpea (Fig. 3.15a). Okra was consistently the least nitrogen use efficient crop. Similar to W$_{pwo}$, watermelon was the least WUE efficient species and significantly less efficient than okra in 2012 (-29.4 ‰ versus -27.1 ‰, respectively; Fig. 3.15). However, with the addition of pepper in the W$_{all}$ intercropping combination, no statistically significant differences were detected in WUE$_{leaf}$ in either year (Fig. 3.16b). Pepper and peanut had the highest PNUE of all five species both years (Fig. 3.16a). Pepper PNUE was 1.9 and 25.2 μmol mol^{-1} s^{-1}, peanut PNUE was 4.8 and 4.1 μmol mol^{-1} s^{-1}, watermelon PNUE was 0.02 and 0.09 μmol mol^{-1} s^{-1}, okra PNUE was 4.5 x 10^{-3} and 0.02 μmol mol^{-1} s^{-1}, and cowpea PNUE was 0.2 and 0.1 μmol mol^{-1} s^{-1} in 2011 and 2012, respectively.

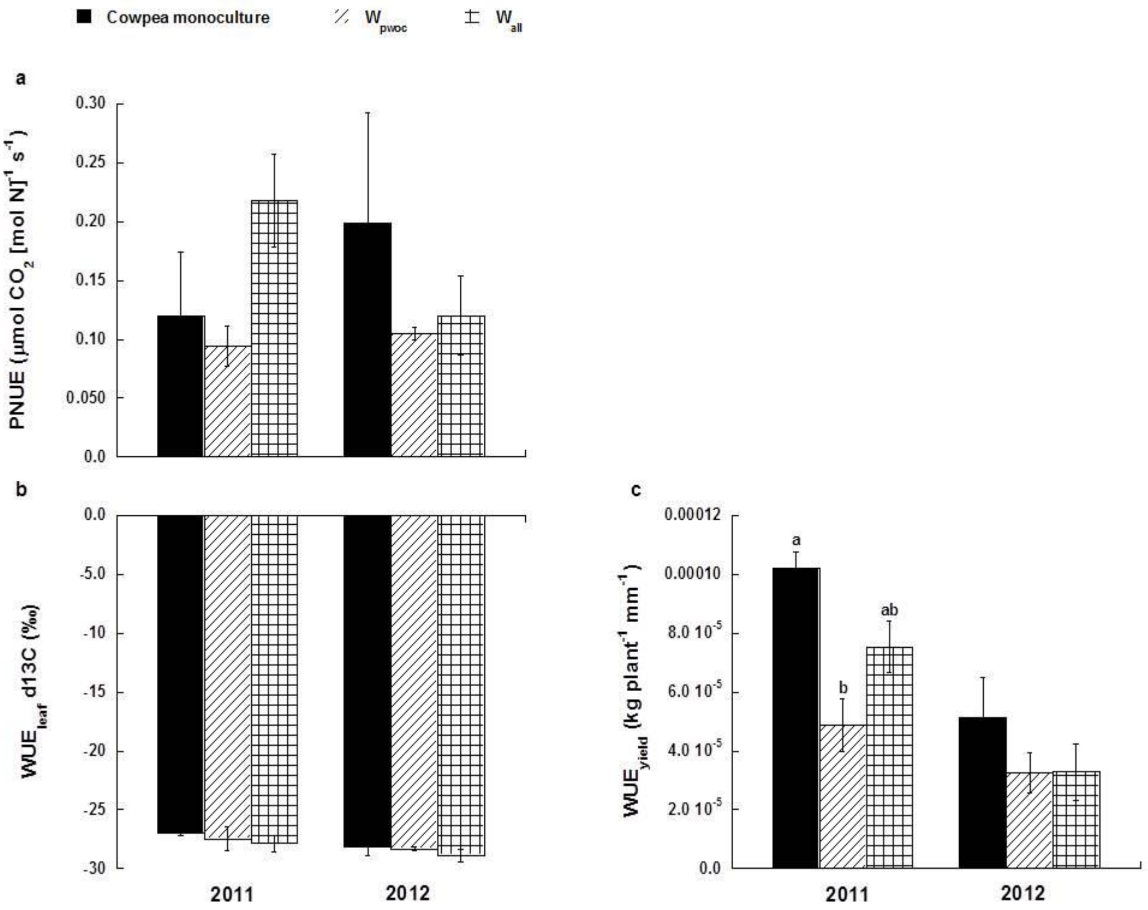


Figure 3.11. Cowpea a) photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}), b) leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰), and c) agronomic water use efficiency calculated on a per plant yield basis per mm water input (WUE$_{yield}$ in kg dry pea plant^{-1} mm^{-1}) in monoculture and mixed cropping combinations in 2011 and 2012. Treatments described in figure 3.4. Different letters indicate statistically significant differences ($P < 0.05$) between intercropping treatments within years according to Tukey's LSD test.

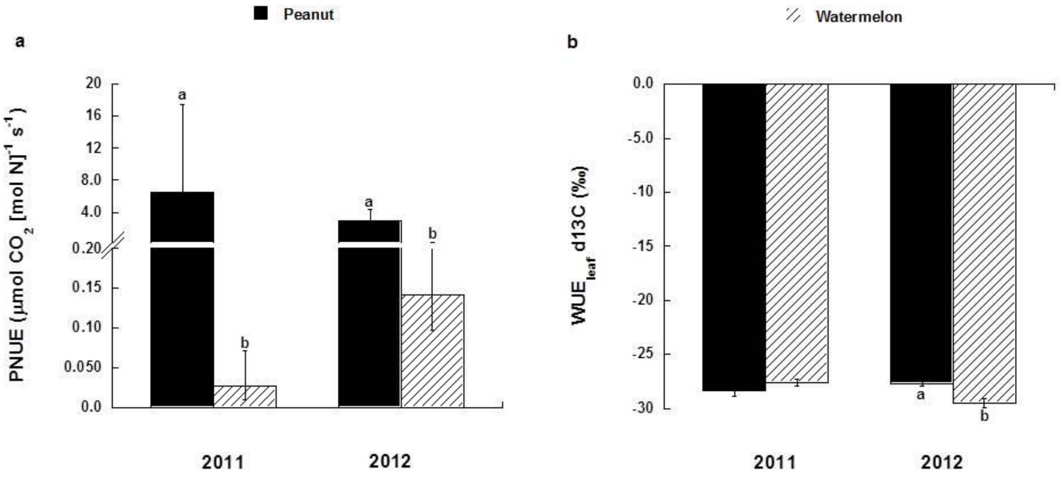


Figure 3.12. Effect of species on a) Photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}) and b) leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰) in the within-row intercropping combination of peanut-watermelon (W$_{pw}$) in 2011 and 2012. Different letters indicate statistically significant differences (*P* < 0.05) between species within years according to Tukey's LSD test.

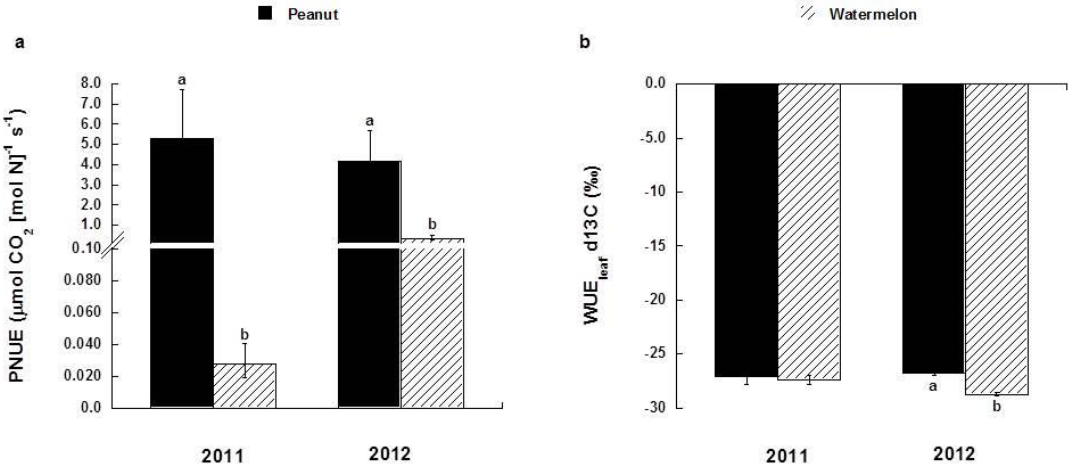


Figure 3.13. Effect of species on a) Photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}) and b) leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰) in the strip intercropping combination of peanut-watermelon (S$_{pw}$) in 2011 and 2012. Different letters indicate statistically significant differences (*P* < 0.05) between species within years according to Tukey's LSD test.

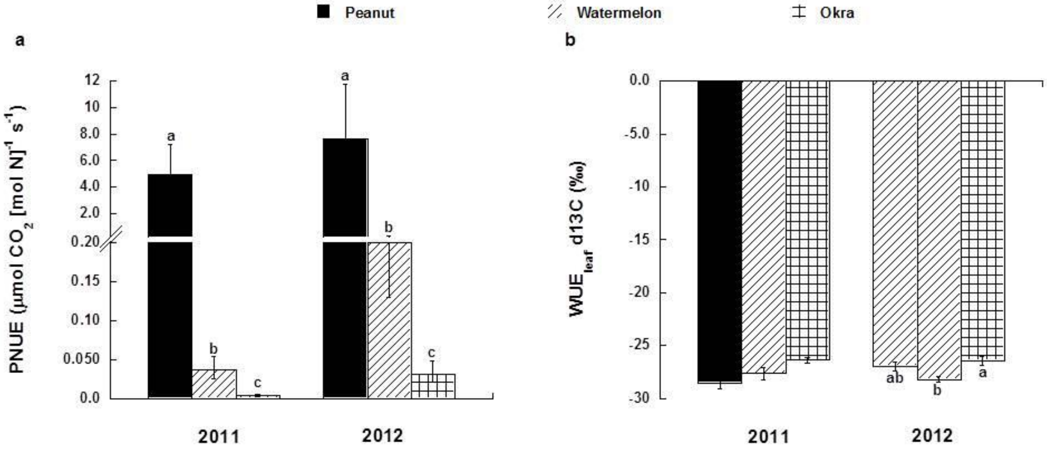


Figure 3.14. Effect of species on a) Photosynthetic nitrogen use efficiency (PNUE in µmol CO_2 [mol N]$^{-1}$ s^{-1}) and b) water use efficiency derived from leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰) in the within-row intercropping combination of peanut-watermelon-okra (W$_{pwo}$) in 2011 and 2012. Different letters indicate statistically significant differences ($P < 0.05$) between species within years according to Tukey's LSD test.

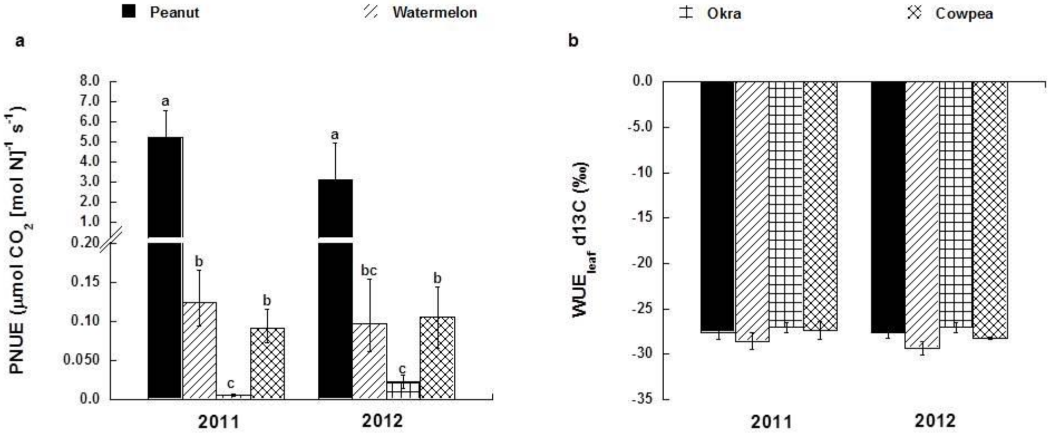


Figure 3.15. Effect of species on a) Photosynthetic nitrogen use efficiency (PNUE in µmol CO_2 [mol N]$^{-1}$ s^{-1}) and b) leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰) in the within-row intercropping combination of peanut-watermelon-okra-cowpea (W$_{pwoc}$) in 2011 and 2012. Different letters indicate statistically significant differences ($P < 0.05$) between species within years according to Tukey's LSD test.

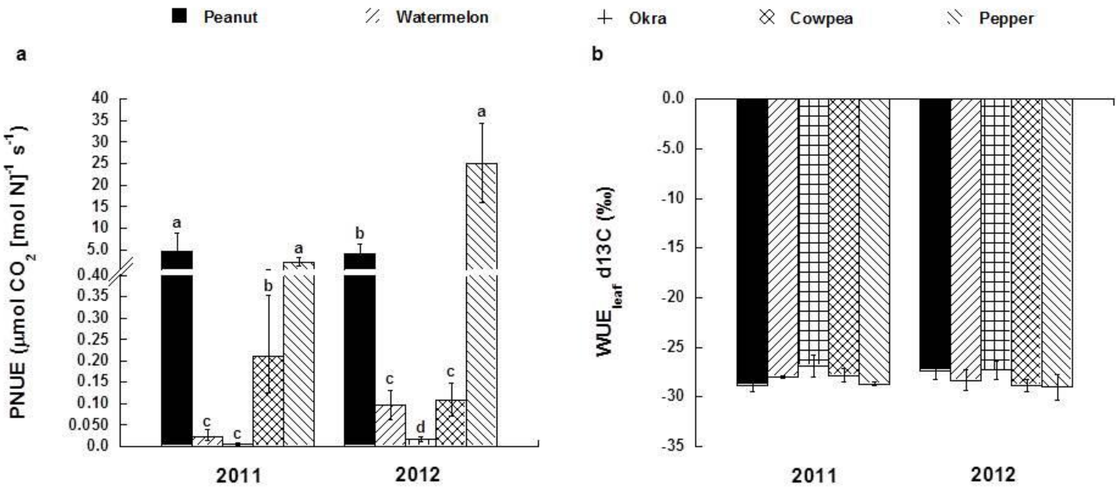


Figure 3.16. Effect of species on a) Photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}) and b) leaf carbon isotope ratios (WUE$_{leaf}$ d^{13}C in ‰) in the within-row intercropping combination of peanut-watermelon-okra-cowpea-pepper (W$_{all}$) in 2011 and 2012. Different letters indicate statistically significant differences ($P < 0.05$) between species within years according to Tukey's LSD test.

Leaf Area Index

Leaf area index (LAI) was significantly higher in okra monoculture and W_{pwo} and significantly lower in pepper monoculture 33 days after last planting (DALP; Fig 31a). LAI was measured at 1.1 in W_{pwo}, 1.0 in okra monoculture, and 0.3 in pepper monoculture. Forty-three DALP, those differences were magnified and cowpea monoculture also had a significantly lower LAI as compare to all intercropping combinations except W_{all} (Fig. 31b). By 63 DALP, peanut had formed a full canopy and had significantly higher LAI than all other monocultures, while pepper continued to have the lowest LAI (3.0 for peanut, 2.1 for okra, 1.7 for watermelon, 1.3 for cowpea, and 0.4 for pepper; fig. 31c). The W_{pw} intercropping combination also increased in canopy with the maturity of peanut, with an LAI of 2.5. The intercropping combinations of W_{pwo} and W_{pwoc} had consistently high LAI values throughout all measurement dates.

Leaf Traits

There were no significant differences in leaf trait parameters when comparing intercropping systems in peanut, cowpea, and pepper (Tbl. 3.3). There were no differences in leaf N concentration on a leaf area basis for any component species. Leaf N on a leaf mass basis was higher in okra when grown in monoculture and in W_{all} as compared to the four species combination of W_{pwoc}. No other traits were significantly different, however. Watermelon SLA was highest in the 3, 4, and 5 species combinations and was significantly higher in W_{pwoc} as compared to watermelon grown in monoculture (Fig. 3.18). Leaf N concentration on a leaf mass basis was significantly higher in W_{pwo} and W_{pwoc} as compared to watermelon monoculture, W_{pw}, and S_{pw}. This corresponded with a higher C:N in watermelon leaves when watermelon was grown in monoculture, W_{pw}, and S_{pw} as compared to watermelon leaves grown in the W_{pwo} and W_{pwoc} intercropping combinations.

There were species differences in SLA within intercropping treatments, particularly in 2012 (Tbl. 3.4). Watermelon consistently had the highest SLA as compared to peanut and okra in all combinations except S_{pw}. Watermelon also had the highest leaf N concentration on both mass and area basis. This was especially true in 2012 in the W_{pwo} and W_{pwoc} intercropping systems. Consequently, watermelon had significantly lower C:N ratio in these intercropping combinations when compared to peanut and okra.

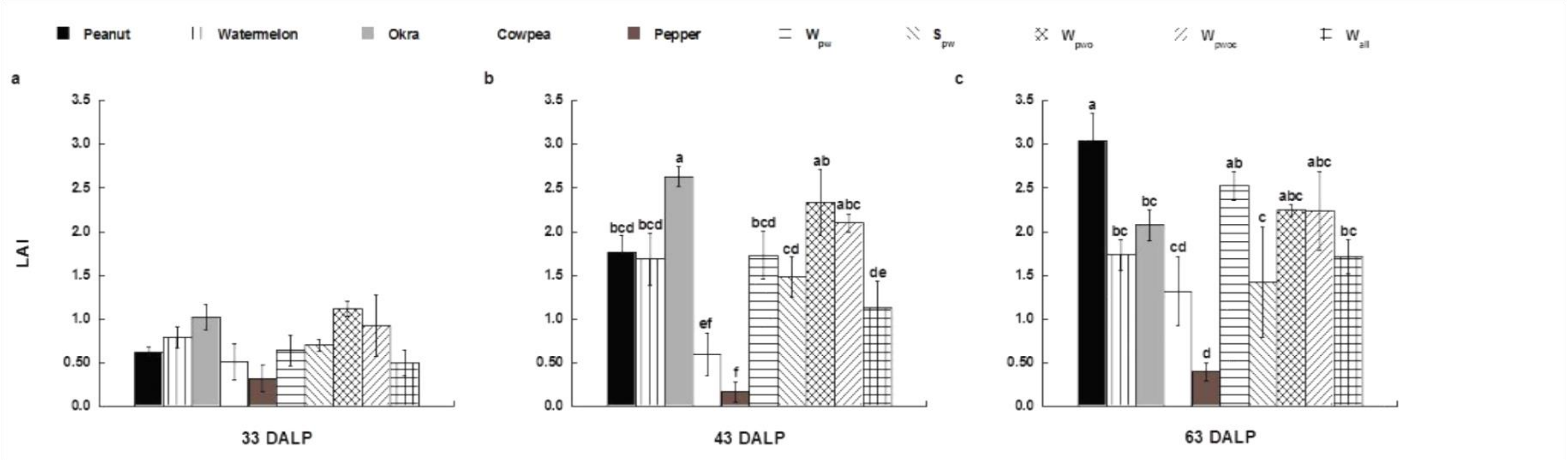


Figure 3.17. Leaf area index (LAI) of monoculture controls and intercropping treatments taken a) 33 days after last planting (DALP), b) 43 DALP, and c) 63 DALP in 2012. Treatments described in figure 3.4. Different letters indicate statistically significant differences (*P* < 0.05) between treatments according to Tukey's LSD test.

Table 3.3. *P*-values of the impact of intercropping on leaf traits

	Impact of species diversity			
Year/ Species	SLA	Leaf N (mg g^{-1})	Leaf N (g $[m^2]^{-1}$)	Leaf C:N
2011				
Peanut	0.330	0.375	0.211	0.193
Okra	0.821	0.047	0.815	0.135
Watermelon	0.909	0.882	0.850	0.705
Cowpea	0.641	0.651	0.919	0.656
Pepper	0.644	0.113	0.313	0.872
2012				
Peanut	0.153	0.209	0.515	0.157
Okra	0.772	0.404	0.630	0.585
Watermelon	0.027	0.006	0.814	0.010
Cowpea	0.456	0.405	0.909	0.355
Pepper	0.214	0.174	0.570	0.253

Table 3.4. *P*-values of the impact of species within intercropping system on leaf traits

	Impact of species			
Year/ Species	SLA	Leaf N (mg g^{-1})	Leaf N (g $[m^2]^{-1}$)	Leaf C:N
2011				
W_{pw}	0.206	0.962	0.047	0.194
S_{pw}	0.139	0.820	0.083	0.002
W_{pwo}	0.084	0.144	0.006	0.660
W_{pwoc}	0.042	0.466	0.132	0.799
W_{all}	0.502	0.104	0.266	0.091
2012				
W_{pw}	0.017	0.078	0.444	0.093
S_{pw}	0.786	0.015	0.134	0.338
W_{pwo}	0.005	0.006	0.016	0.014
W_{pwoc}	<0.001	0.005	0.040	0.006
W_{all}	0.027	0.099	0.347	0.234

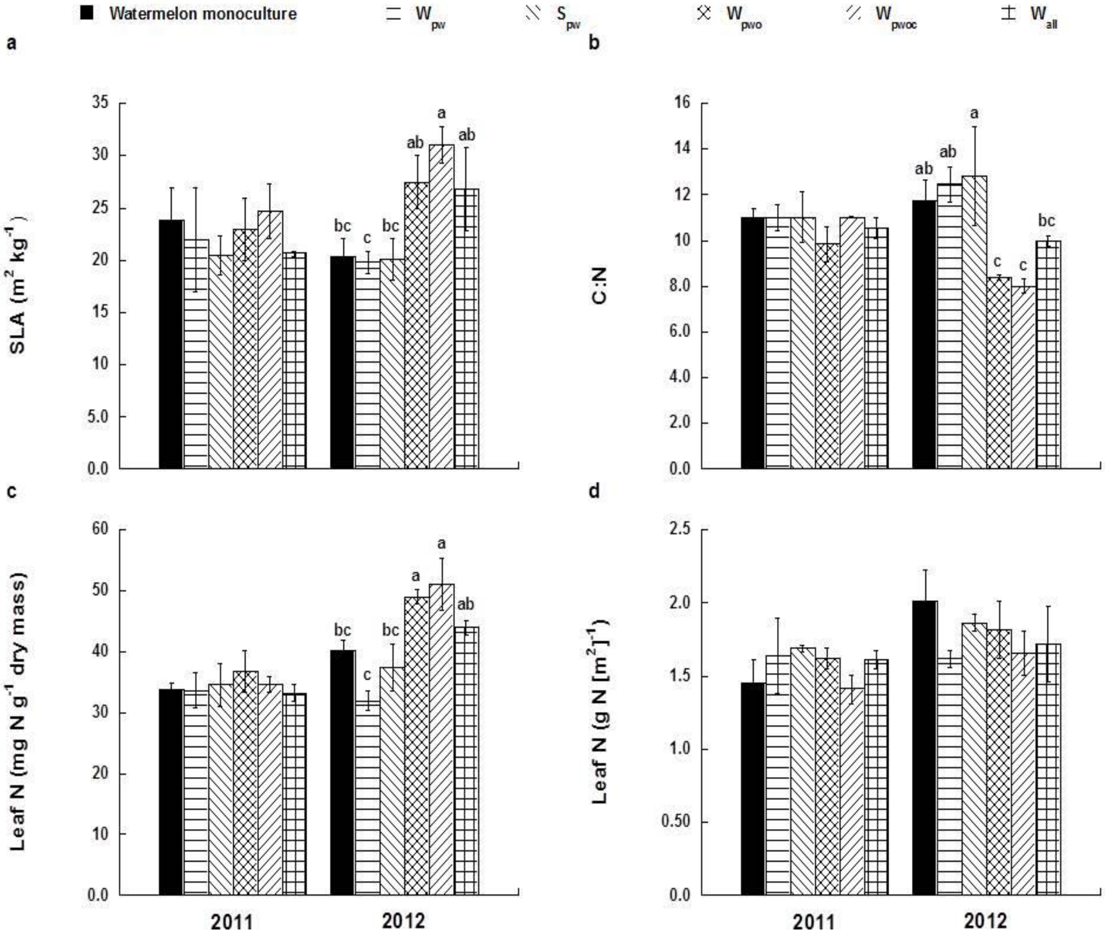


Figure 3.18. Watermelon a) specific leaf area (m^2 kg^{-1}) b) leaf carbon to nitrogen ratio (C:N), c) leaf nitrogen concentration based on leaf dry mass (mg N g^{-1}), and d) leaf nitrogen concentration based on leaf area (g N [m^2]$^{-1}$) in 2011 and 2012. Treatments described in figure 3.4. Different letters indicate statistically significant differences ($P <$ 0.05) between treatments according to Tukey's LSD test.

Discussion

Unlike previous studies, no differences were detected in gas exchange measurements when comparing intercropping treatments and monocultures by species. Pinheiro and Filho (2000) found differences in A, g_s and E between intercropped (maize) and monocropped cowpea. However, these differences varied throughout the day as gas exchange measurements fluctuated diurnally. In their study, the greatest differences between treatments were detected when plants were most photosynthetically active, which was at 1000h. After that time, gas exchange measurements steadily decreased along with treatment differences. It is possible that differences between treatments were not detected due to diurnal fluctuations in photosynthesis that were not detected in this study. Furthermore, leaf-level measurements of photosynthesis may not accurately depict canopy-level processes (Iio et al., 2005; Sprintsin et al., 2012). As was observed in watermelon, changes in plant morphology and leaf traits likely occurred due to variability of within-canopy light availability (Niinemets and Kull, 1998; Niinemets, 2007). Watermelon exhibited a higher SLA, higher leaf N concentration, and lower C:N when grown in the more complex intercropping systems in 2012. Therefore, photosynthetic capacity per unit leaf area may have remained relatively constant in watermelon as it acclimated to its light environment. This phenotypic plasticity is believed to play an important role in optimizing leaf dry mass per area with light availability and, consequently, maximizing canopy-level photosynthesis (Niinemets and Kull, 1998). Although these same patterns were not observed in the other component crops, it’s likely a lack of sufficient sample size reduced our power to detect the effects of these intercropping systems on leaf traits.

In contrast to gas exchange measurements, results from leaf water potential measurements indicated differences in midday leaf water potential for peanut at full canopy stage both years and in watermelon in 2011. Peanut was less water stressed in the intercropping combination containing all 5 species (W_{all}) in 2011 compared to all other cropping systems and in the intercropping combinations of W_{pw}, W_{pwo}, and W_{all} in 2012 as compared to peanut grown in monoculture. Watermelon was less water stressed in the intercropping combinations of W_{pw}, S_{pw}, and W_{pwoc} as compared to watermelon grown in monoculture in 2011. It is believed that canopy density and soil moisture are inversely correlated such that as canopy density increases soil moisture decreases due to increased overall transpiration by the plant canopy (von Arx et al., 2013). However, high temperatures and high solar radiation in the summer in Texas, where this study was conducted, may have had the opposite effect on soil moisture. In this study, LAI measurements taken in 2012 indicated relatively low LAI values for S_{pw}. Thus, it is plausible that more exposed soil in the S_{pw} combination due to less dense canopy led to higher soil moisture losses and, subsequently, more negative leaf water potential values in peanut. However, this would depend upon the rooting depth of peanut as the effect of radiation on soil temperature would be expected only at a shallow soil depth. This may also help explain low leaf water potential in watermelon grown in monoculture in 2011. If the stress-gradient hypothesis held true in this study, as canopy cover increased resulting from the addition of intercrops, a facilitative effect on the community should have played a more important role than competition, whereby canopy shading would have reduced soil moisture losses and increased water availability and, consequently, higher leaf water potential (Callaway and Walker, 1997; Zhang et al., 2012). This does not, however, explain low leaf water potential values in peanut in W_{pwoc} in 2012. It is possible that interspecific competition may have played a more significant role in this case. No differences in predawn leaf water potential suggests plants recovered from water stress overnight (Pinheiro and Filho, 2000).

In 2012, higher peanut PNUE values in W_{pwo} (although not statistically significant) may support the idea that multispecies systems can result in more efficient utilization of resources (Hooper, 1998; Hauggaard-Nielsen et al., 2001). Higher PNUE values in watermelon in monoculture as compared to W_{pw}, W_{pwoc}, and W_{all} appear to contradict that notion. It is worth noting, however, that watermelon in S_{pw} and W_{pwo} had intermediate PNUE values and okra in W_{pwo} and W_{pwoc} had relatively higher PNUE values. A larger sample size may have increased our power to detect differences in PNUE between intercropping systems. WUE_{yield} displayed an increasing trend from watermelon monoculture to watermelon in the 5 species combination (W_{all}) in 2011, which follows the observed trend in watermelon per plant yield (Franco et al., 2015). There was a somewhat similar trend in okra in 2012 when okra was the dominant species (Franco et al., 2015), with highest okra WUE_{yield} values in the W_{pwoc} intercropping system. The opposite was true in 2011 when watermelon was the dominant species, with okra having the highest WUE_{yield} when grown in monoculture. Changes in planting dates likely resulted in a growth advantage for watermelon in 2011 and okra in 2012, leading to more explicit size-asymmetric competition between the two species as the growing season continued (Weiner and Damgaard, 2006; Andersen et al., 2007). WUE_{yield} values for cowpea and pepper in both years suggest these species were inferior competitors as compared to peanut, watermelon, and okra. No differences detected in $d^{13}C$ between intercropping treatments for each species suggests the C composition (that is ^{13}C:^{12}C) of each species was not altered by intercropping scheme. The low $d^{13}C$ values suggest plants were discriminating against ^{13}C and taking up ^{12}C at much higher levels, which indicates that plants were not water stressed throughout the duration of the growing season both years.

Species differences were evident when comparing within intercropping system. Watermelon was more photosynthetically active than peanut in the within-row intercropping system (W_{pw}) in 2012, but only at full canopy stage. Watermelon was also more active in the strip intercropping system (S_{pw}) at flowering stage in 2012. Watermelon also had consistently higher leaf water potential values as compared to peanut in both W_{pw} and S_{pw}. These differences between peanut and watermelon may be more related to leaf functional traits and growth habit that are related to ecological trade-offs, than competitive interactions between the two species (Reich et al., 2003). Interestingly, okra was significantly less active at flowering stage in 2012 than peanut and watermelon (W_{pwo}), although no differences in leaf water potential were measured. On the other hand pepper had the highest photosynthetic rates at full canopy stage in 2012 and the lowest leaf water potential as compared to all other component crops, particularly at full canopy. High gas exchange measurements in pepper suggests it was not being outcompeted for light by taller component crops such as suggested in other studies (Pinheiro and Filho, 2000). However, low leaf water potential values indicate that high gas exchange in pepper came at a cost which was plant water loss. The results from okra and pepper gas exchange measurements seem to contradict the notion that higher photosynthetic rates equate to higher yields (Ainsworth et al., 2002; Ainsworth and Long, 2005) since intercropped okra yields were found to be greater in 2012 as compared to monoculture and intercropped pepper yields decreased that same year (Franco et al., 2015).

When comparing species within intercropping system, peanut had the highest PNUE and lowest $d^{13}C$ values in 2012 as compared to watermelon in W_{pw} and S_{pw}. Peanut also had the highest PNUE values in 2011. This suggests peanut was the more nitrogen-use and water-use efficient species between the two overall, which is expected of nitrogen-fixing crops. Okra was the least nitrogen efficient crop across years and in all intercropping systems and watermelon was the least water-use efficient crop when differences were detected in $d^{13}C$. PNUE values were consistently comparable to watermelon in cowpea. Contrary to what was observed in gas exchange measurements, pepper had PNUE values comparable to peanut in 2011 and significantly higher than all other species in 2012. Pepper varietal differences may have contributed to higher PNUE values in 2012 as compared to 2011 as a Jalapeno variety was used in year 1 and Serrano in year 2. There is believed to be a negative relationship between PNUE and leaf mass per area (LMA, reciprocal of SLA), whereby there is a trade-of between plant productivity and persistence (Hikosaka, 2004). However, higher PNUE is thought to be associated with species that exhibit high growth rates (Schieving and Poorter, 1999; Hikosaka, 2004). Pepper typically exhibits slow growth rates relative to other component species in this study. No differences in SLA were detected in pepper, thus, it may be more likely that there are other processes at work such as root scavenging for nutrients that may help explain the observed high PNUE in pepper.

Although these results do not show any clear trends across systems, this study demonstrates the potential for a multifunctionally diverse cropping system to maximize resource-use efficiency and reduce plant stress through interspecific species interactions such as competition for light and changes in micro-climate due to canopy structure and density. The 3 and 4 species combinations, W_{pwo} and W_{pwoc}, may offer some promise for maximizing these interactions. Future studies should test variations on resource supply such as deficit irrigation and how this interrelates with interspecific species competition in a multispecies intercropping system to determine the most productive systems to increase food production while minimizing inputs.

CHAPTER V

THE EFFECTS OF FUNCTIONALLY DIVERSE INTERCROPPING ON PEST AND DISEASE

Introduction

Intercropping is the practice of growing two or more crops within the same area such that there is biological and agronomic interaction (Vandermeer, 1989; Mohler and Stoner, 2009). Often used as way to introduce biodiversity into agroecosystems (Unlu et al., 2010), intercropping can also be used in conjunction with other cultural practices as a management strategy for managing weed populations, reducing pests pressure, and suppressing the spread of disease, particularly in organic and low-input farming systems.

Weeds

Weed management in any cropping system is the single greatest input (Wang et al., 2009; Dayan et al., 2011). Weed control is important to reduce competition with the target crop for moisture, nutrients, and light (Uchino et al., 2009). Thus, weeds, more so than any other pest, have the greatest adverse impact on crop yields (Dayan et al., 2011). Yield losses from 40 to 80% have been cited due to weed populations left unmanaged and outcompeting cash crops for resources (Akobundu, 1987; Karlen et al., 2002). The cost of weed control, along with yield losses due to weed pressure, has been estimated at more than $15 billion annually in developed nations (Buhler, 2003). Due to their highly effective mode of action and relative low cost, synthetic herbicides have been a reliable tool for weed control in conventional systems (den Hollander et al., 2007; Dayan et al., 2011). However, the increasing demand for organic food and concerns over the potential detrimental effects these herbicides have on human health and on the environment have driven the search for safer alternatives for managing and controlling weed populations (den Hollander et al., 2007; Dayan et al., 2009; Dayan et al., 2011; Webber III et al., 2014).

In organic systems, options for weed control are limited (Peruzzi et al., 2007). This creates a challenge for organic farmers (Barberi, 2002; Anderson, 2010) and is the largest obstacle for producers considering transitioning from conventional production systems (Barberi, 2002), especially for the management of perennial weeds (Wedryk et al., 2012). Weeds are an even bigger problem in low-input systems, and in particular with vegetable crops that are inferior competitors (den Hollander et al., 2007). Organic farmers are primarily restricted to cultural and mechanical techniques, such as mulching, smother cover crops, cultivation, and direct manual weeding, to suppress weeds (Isik et al., 2008; Bilalis et al., 2010; Mulvaney et al., 2011).

Mechanical weed control such as manual weeding is ineffective for long-term management of weeds and can damage soil structure (den Hollander et al., 2007). In addition, this requires high labor input and thus is costly (Karlen et al., 2002; den Hollander et al., 2007). Organic mulching with straw or hay has been found to be an effective barrier to weed establishment by inhibiting light transmittance (Steinmaus et al., 2008) and creating a physical barrier to germination (Rowley et al., 2011), but efficacy varies by mulch type (Bajoriene et al., 2013) and may harbor additional weed seed sources (Rowley et al., 2011). In addition, the effect organic mulches have on soil organic carbon (SOC) and soil nutrient availability is still under debate (Blanco-Canqui and Lal, 2007; Bajoriene et al., 2013). Plastic mulching, another effective method for weed suppression, can be cost prohibitive and may facilitate the spread of certain diseases through splash-dispersal (Coelho et al., 2008). Cover crops such as rye, vetch, and clover behave similarly to mulches and can be effective in suppressing weed growth prior to planting of the main crop, but require elimination by tillage or herbicide (Buhler et al., 2001) and may act as hosts for diseases that can infect the target crop (Jackson, 2004). Cultivation practices such as tillage only serve to shift weed populations depending upon the type of tillage utilized, can destroy soil aggregate structure , and may decrease soil quality (Mulvaney et al., 2011) which is essential in organic and low-input systems. Additionally, tillage can be costly (Madden et al., 2004). The availability of non-synthetic herbicides is very limited (Rowley et al., 2011). Chemical strategies that are effective, such as acetic acid and clove oil, can become cost prohibitive due to the large volumes required to suppress weed growth and do not provide long-term control (Dayan et al., 2009; Webber III et al., 2014). Other non-synthetic herbicide such as pelargonic acid are currently under investigation (Webber III et al., 2014). Further, all commercially available organic herbicides are non-selective and must be applied with caution (Dayan et al., 2009).

Intercropping has been cited as a potentially effective cultural control strategy for weeds in organic production systems. A reduction in weed emergence has been attributed to intercropping with either cover crops or cash crops (Barberi, 2002). The ability of the target crop to outcompete weeds is enhanced in intercropping systems through an increase in resource capture by the desired species which reduces availability of resources such as light, water, and nutrients to weeds (Barberi, 2002; Baumann et al., 2002; Saudy, 2014) as well as through allelopathy (Iqbal et al., 2007). However, this may depend upon factors such as crop geometry, canopy architecture, planting density, and crop growth rate (Keating and Carberry, 1993; Isik et al., 2008). Small farmers in tropical forest areas have long utilized intercropping systems and have incorporated a variety of crops with different growth forms, to create a complex multi-layered habitat (Denevan, 1995). The "three sisters" intercropping system of squash, bean, and corn practiced by the Native Americans is a well-documented example of a multi-layered agroecosystem (Mohler and Stoner, 2009). In these types of systems, each crop occupies a functional group niche and in the case of the "three sisters", squash acts as a smother crop to suppress weed growth, bean is the nitrogen fixer, and corn provides support for bean vines (Vitousek and Hooper, 1993a; Mohler and Stoner, 2009). The ability of a multi-layer intercropping system to suppress weed growth is owed to a reduction of light transmittance due to an increase in canopy density (Baumann et al., 2002). As a result, the enhanced competitive ability of mixed cropping systems makes them a potentially useful tool for weed management in low-input farming systems (Saudy, 2014).

Pests and Disease

Traditional chemical control methods for pests and diseases such as insecticides, fungicides, and fumigants are harmful to the environment (Ren et al., 2008). The elimination of synthetic chemicals is an important component of organic farming. However, as with weeds, options for pests and diseases management in organic systems are limited. Although not as effective as synthetics, organic pesticides such as azadirachtin and pyrethrin have been found to reduce populations of certain pests (Immaraju, 1998; Moreau et al., 2006; Vassiliou, 2011). However, they do not discriminate against beneficial insects and pollinators. Therefore the timing of spray is critical for minimizing mortality to beneficials and pollinators (Immaraju, 1998). In addition, organic insecticides do not effectively control adult pest insects and multiple applications must be made to control pest populations (Stark and Walter, 1995; Aliakbarpour et al., 2011). Furthermore, the availability of organic naturally-derived fungicides is very limited and the few that are available are not as effective as chemical fungicides (Dayan et al., 2009).

A proven strategy for the effective control of pests and the spread of disease is intercropping (Ren et al., 2008; Narla et al., 2011). There are a number of explanations offered for this. A multi-layered canopy formed by intercropping two or more species can create mechanical barriers for insects harboring and spreading viruses and diseases, and can create olfactory and visual confusion for insects (Ramkat et al., 2008). The physical barriers created by multi-species cropping also prevent direct contact of crop roots or shoots of individuals within the same species, which may reduce the spread of diseases such as bacterial wilt (Kloos et al., 1987). Therefore, decreasing the density of any one crop and increasing the distance between individuals of the same species can reduce or delay the spread of disease, as well as make it more difficult for pests such as aphids and other specialist to move between plants of the same species (Potts, 1990; Noman et al., 2013). There are additional microclimate effects of an architecturally complex intercropping system on temperature, moisture, and light that may reduce the incidence and severity of diseases (Theunissen, 1994; Finckh, 2008). Moreover, adding non-host plants in a mixed cropping system can dilute cues that attract pests to a specific crop (George et al., 2013).

Some crops may act as a repellent or deterrent, likely through chemical or olfactory signals. Asare-Bediako et al. (2010) found that an intercropping of cabbage with tomato reduced the infestation of diamondback moth, a significant pest in cabbage production. It was believed that odor emitted from tomato repelled the diamondback moth or may have had an oviposition deterring effect. Plant extracts such as capsaicin from hot pepper and red pepper have been found to reduce pests such as cabbage looper, onion fly, and spider mite due to this repellent or deterrent effect (Cowles et al., 1989; Antonious et al., 2007). Other crops as well as their extracts, such as onion, garlic, and spices have also been found to reduce the incidence of fungal diseases such as *Fusarium* wilt in watermelon (Hu et al., 2012) and reduce aphid populations on cabbage and mustard. (Baidoo et al., 2012; Noman et al., 2013).

Another explanation for the ability of mixed cropping systems to reduce disease and pest populations is that they increase numbers of natural enemies of pests such as aphids (Rizk, 2011). This increase in natural predators is, at least in part, due to the elimination of synthetic chemicals which reduce predator populations in conventional systems (Kromp, 1999). Additionally, it is believed that mixed cropping systems provide favorable microclimates and conditions for the survival of predators (Risch, 1983; Potts, 1990). Finally, intercropping also may reduce weed populations, as previously discussed. An indirect consequence of this reduction in weeds is that it eliminates or minimizes alternate hosts for the build-up of pest populations and diseases (Potts, 1990).

In summary, the numerous benefits of intercropping such as weed and pest control have made intercropping more popular in developed countries where organic practices are on the rise, such as in the United States (Kahn, 2010). Despite the rising popularity of intercropping, the ecology and productivity of multi-layer architecturally complex intercropping systems have not been studied extensively in the Southern United States. There are few studies that have quantitatively evaluated the role of functional diversity on weed, disease, and pest control with the goal of reducing inputs and any potential negative residual impacts on the ecosystem. The objectives of this study were to quantify the effects of an incremental increase in functional diversity on weed suppression, disease incidence and severity, and aphid infestation in a low-input organic system.

Materials and Methods

Study Area

Low input managed plots were established at Texas A&M University's Horticulture Farm (30°37′N, -96°22′W) during the 2011 and 2012 growing seasons. Average monthly air temperatures for this area from May to October when the study was conducted ranged from 28 to 39°C for the maximum and 15 to 26°C for the minimum in 2011 and 27 to 37°C and 15 to 25°C in 2012 (NOAA/NCDC).

Experimental Design

The study design was a randomized complete block with three replicates, five intercropping treatments, and five controls. The controls consisted of monocultures of the five component species; peanut, mini watermelon, okra, cowpea, and hot pepper. The five treatments were a within-row intercropping system of 1) peanut and watermelon (W_{pw}), 2) peanut, watermelon, and okra (W_{pwo}), 3) peanut, watermelon, okra, and cowpea (W_{pwoc}), 4) all five control species (W_{all}), and 5) a strip intercropping system of peanut and watermelon consisting of alternating single rows (S_{pw}). Figure 1.1 illustrates the planting layout for the within-row and strip intercropping schemes. Crops represented four different genera and were selected based on the following criteria: 1) heat tolerance, 2) desired architecture and function (Table 1.1), and 3) no known adverse effects on other component crops. “Tamspan 90” is a small Spanish bunch type peanut which was selected for its small canopy and lack of runners. The “TAMU” mini watermelon variety was developed by the Texas A&M University watermelon breeding program and has a number of advantages for small scale producers including its ability to tolerate narrower spacing and, thus, take up less space, and produces a smaller fruit which is more manageable for small growers. Selected because of its popularity, “clemson spineless” okra is a dwarf variety well suited for this region. “Texas pinkeye” is a purple hull cowpea selected for its erect and bush-type growth form. This taller variety of cowpea can tolerate narrower spacing. Hot pepper varieties were used for this study. Jalapeno was used for year one and Serrano for year two and selection was based upon availability.

Crops were planted in 4 m long double rows on 90 cm wide raised beds with rows spaced approximately 30 cm apart and beds spaced 45 cm apart (total plot size, 4 × 5 m). Using the methodologies recommended by Jeavons (2006) and minimum spacing requirements outlined in the Texas Vegetable Growers Handbook (Masabni and Dainello, 2009), individual plant spacing was based on the mean spacing requirement for all component crops. Densities were kept constant across plots regardless of crop species and individual plants were spaced 30 cm apart in a staggered row pattern so that each plant was neighbored by an individual of a different species and a leguminous species. A 2 m buffer was maintained between plots.

Sampling and Analyses

Treatment effects on weed suppression were assessed by hand harvesting above-ground weed biomass from the top of the center row raised bed and from the 2 m centers of each plot to avoid edge effect. This was done every two to three weeks through the end of harvest in both 2011 and 2012. Weed biomass was separated into three categories: grasses, sedges and broadleaved weeds. Samples were then rinsed to remove excess soil and oven dried for 48 hours or until constant weight at 24°C. Total dry weed biomass was then converted from plot scale to kg ha^{-1}. Methodologies for above-ground plant biomass data collection are described in Franco et al. (unpublished data).

Experimental plots were monitored for disease throughout the growing season. Disease occurrence was verified with the Texas Plant Disease Diagnostic Laboratory

(TPDDL). Visual estimates of disease incidence and severity were made if the presence of disease was confirmed. Incidence was estimated as the presence or absence of disease on each plant within each plot expressed as a percentage of the entire plot. Severity was estimated on a subsample of five plants within each plot and expressed as the percentage of foliage per plant showing signs of disease.

In year 2, data on aphid infestation and aphid density were collected on okra and cowpea to evaluate the efficacy of a multifunctional intercropping system on reducing pest pressure. The presence of aphid mummies indicates parasitic activity on aphids from predatory insects (Singh and Hoy, 2007; Acebes and Messing, 2013). Therefore, data on aphid mummy density was also collected. Aphid infestation was estimated as the presence of aphids on each okra or cowpea plant within each plot expressed as a percentage of the entire plot. Aphid and aphid mummy density were calculated by counting the number of aphids or mummies on each of 3 leaves from each infested plant, averaging over each infested plant, then taking the average of all infested plants within each plot.

All weed, pest, and disease data were analyzed using ANOVA in JMP 10.0.2 statistical software for windows (SAS Institute, Cary, NC, USA). The Shapiro-Wilk goodness-of-fit test was used to assure assumptions of normality were met. When these assumptions were not met, log transformations were utilized and the model with the highest R^2 value was accepted. Where a significant *F*-test was observed ($P < 0.05$), mean separations were conducted using Tukey's Least Significant Difference (LSD) test.

Results

Weeds

Weed pressure in 2011 was significantly reduced in intercropping combinations containing watermelon, suggesting watermelon was an effective smother crop. Weed biomass remained below 130 kg ha^{-1} in watermelon monocrop and any combinations incorporating watermelon while peanut, pepper, okra, and cowpea monocrops had weed biomass values of 500, 564, 808, and 1201 kg ha^{-1}, respectively (Fig. 4.1). When averaged over monocropped and intercropping combinations containing watermelon, total weed biomass was reduced by 81, 83, 88 and 92% in pepper, peanut, okra and cowpea monocultures, respectively.

In 2012, however, with a reduction in watermelon biomass due to okra competition and downy mildew, watermelon effectiveness as a smother crop was minimal. Watermelon monocrop still had the lowest weed biomass at 261 kg ha^{-1}. Weed biomass increased to 666 kg ha^{-1} in the watermelon-peanut strip intercropping combination and was highest in the pepper monocrop at 1225 kg ha^{-1}. Although not statistically significant, this represents a 79% reduction in weed biomass in pepper monocrop compared to the lowest yielding intercropping combination (W_{pwo}), a 46% reduction in biomass as compared to the highest yielding intercropping combination (S_{pw}) and a 70% decrease compared to the intercropping combination containing pepper (W_{all}). There were no significant differences between the cowpea, peanut, and okra monocrops and intercropping combinations that contained watermelon in 2012.

Broadleaf weed biomass was significantly higher in 2011 in okra and peanut monocultures, at 332 and 420 kg ha^{-1}, respectively, compared to the other cropping combinations (Fig. 4.2a). As with total weed biomass, watermelon monocultures had the least amount of broadleaved weeds at 4 kg ha^{-1}. The lowest broadleaf weed biomass was found in the four species combination (W_{pwoc}) and averaged 23 kg ha^{-1}. This represented an 81, 87, 93 and 95% reduction in broadleaved weeds from monocropped pepper, cowpea, peanut, and okra, respectively in 2011. In 2012, the watermelon-peanut strip intercropping combination and monocropped pepper had the most broadleaved weeds, 400 and 405 kg ha^{-1}, respectively (Fig. 4.2b). There was a 46% reduction in broadleaf weed biomass in the intercropping combination containing pepper (W_{all}) compared to monocropped pepper. The most prevalent broadleaf weeds were carpetweed (*Mollugo verticillata* L.), common purslane (*Portulaca oleracea* L.), and spurge (*Euphorbia* spp.).

Sedge weed biomass was statistically higher in monocropped cowpea and pepper in 2011 and in monocropped pepper in 2012 than in the other cropping combinations, at 715, 321, 689 kg ha^{-1}, respectively (Fig. 4.2c, d). As with total weed biomass, sedge biomass was consistently lowest in monocropped or intercropping combinations containing watermelon (S_{pw} = 22, W_{pwo} = 29, Watermelon monocrop = 44, W_{all} = 45, W_{pwoc} = 52, and W_{pw} =68 kg ha^{-1}) in 2011. Although not statistically different from the other intercropping combinations, the three species intercropping combination (W_{pw}) and the strip intercropping of watermelon-peanut (S_{pw}) yielded the lowest sedge biomass in 2012 with 71 kg ha^{-1} each. This is consistent with the results from 2011 where sedge biomass was lowest in these two intercropping combinations. The most prevalent sedges were yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.).

Weedy grass biomass was not statistically different between intercropping treatments and monocrops in both years (Fig. 4.3e, f). In 2011, cowpea, okra, and peanut had the highest biomass with 38, 52, and 70 kg ha^{-1}, respectively. In 2012, okra again had the most weedy grass biomass with 160 kg ha^{-1} followed by the strip intercropping of watermelon-peanut with 158 kg ha^{-1} of grass biomass. Consistent with other findings, grass biomass was lowest when watermelon was monocropped, 19 kg ha^{-1}. The most prevalent weedy grasses were crabgrass (*Digitaria* spp.) and bermudagrass (*Cynodon dactylon* (L.) Pers.).

In 2011, total yield to weed biomass ratio was significantly higher in monocropped watermelon and in intercropping combinations containing watermelon (watermelon monocropped = 1428, S_{pw} = 573, W_{all} = 532, W_{pwo} = 509, W_{pw} = 402, W_{pwoc} = 386; Fig. 4.3). In 2012, total yield to weed biomass ratio was also highest in monocropped watermelon and in all intercropping combinations and was also high in monocropped okra, but was greatly reduced as compared to 2011 (watermelon monocropped 114, okra monocropped = 44 W_{pwo} = 55, W_{all} = 32, W_{pw} = 32, S_{pw} = 26, W_{pwoc} = 25).

In 2011 when total yield to weed biomass ratios were highest, there was a significant negative linear relationship between total weed biomass and total yield (Fig. 4.4a). As total weed biomass increased, total crop yield decreased. Weed biomass was

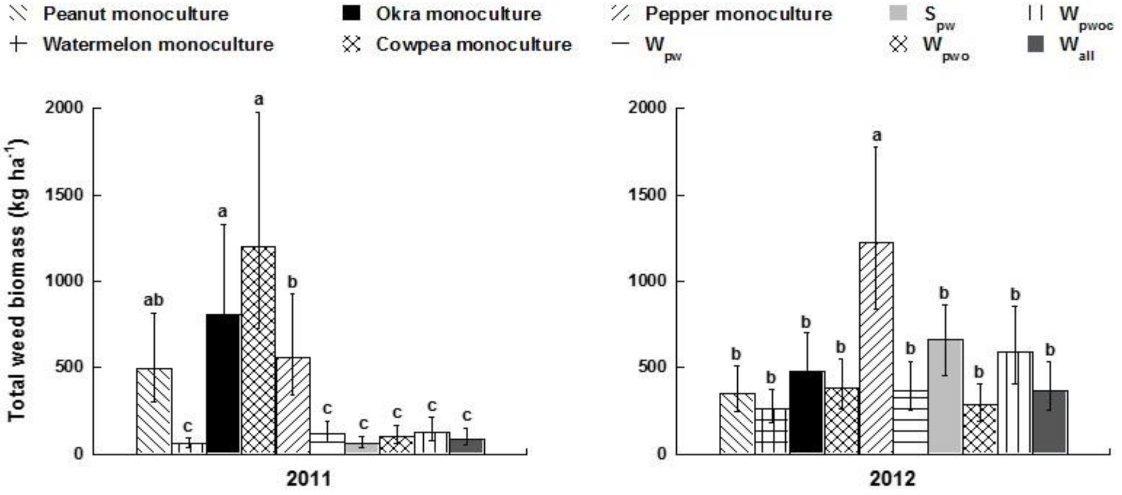


Figure 4.1. Least squares means and standard errors of the mean of total weed biomass (kg ha^{-1}) for each monoculture and intercropping combination in (a) 2011 and (b) 2012 (W_{pw} = within row intercropping of peanut-watermelon, Spw = strip intercropping with peanut-watermelon (e.g. alternating rows), W_{pwo} = within row intercropping of peanut-watermelon-okra, W_{pwoc} = within row intercropping of peanut-watermelon-okra-cowpea, W_{pwocr} = within row intercropping of peanut-watermelon-okra-cowpea-pepper). Different letters indicate statistically significant differences ($P < 0.05$) between means within years according to Tukey’s LSD test.

a significant predictor of total yield and accounted for 39% of the variability in total yield. However, in 2012 there was not a strong relationship between weed biomass and total yield. Weed biomass accounted for only 3% of the variability in total yield, and this relationship was not significant. Similar significant relationships were found when broadleaf weed and sedge biomass were regressed separately against total yield. Broadleaved weeds, sedges, and grasses accounted for 19, 33, and 31% of the variability in total yield in 2011, respectively (Fig. 4.5a, c, e). Despite a slight trend in decreasing weed biomass with increasing total above-ground plant biomass, total above-ground plant biomass was not a significant predictor of weed biomass in 2012 (Appendix Fig A-4.1). The omission of two outlier data points makes this relationship significant. However, this relationship is weak and there is no evidence to suggest those outliers were erroneous.

Pests and Disease

Due to an extraordinarily dry year in 2011, no severe disease issues were observed in any of the component crops. However, in 2012, watermelon experienced a severe downy mildew infestation. Data on disease incidence and severity were recorded 3 days after the infestation was observed. Downy mildew incidence ranged from 73% in the intercropping combination containing all five species (W_{all}) to 96% in watermelon monoculture (Appendix Fig. A-4.7). Severity followed a similar trend and ranged from 55% in the W_{all} intercropping treatment to 83% in watermelon monoculture. Both incidence and severity followed a downward trend between monoculture and the incremental addition of component crops. No statistically significant differences in incidence and severity were observed between intercropping combinations, however.

Aphid infestation and density on cowpea were not found to be statistically different between monocropped cowpea and cowpea in the two intercropping combinations that contained cowpea (Appendix Fig. A-4.8). However, there were no plants infested with aphids in monocropped cowpea and, subsequently, densities were 0. Infestation was as high as 20% in the 4 species intercrop (W_{pwoc}) and just under 5% in the 5 species intercrop (W_{all}). Densities were similar between both intercropping combinations, between an average of about 70 and 100 aphids per infested cowpea plant.

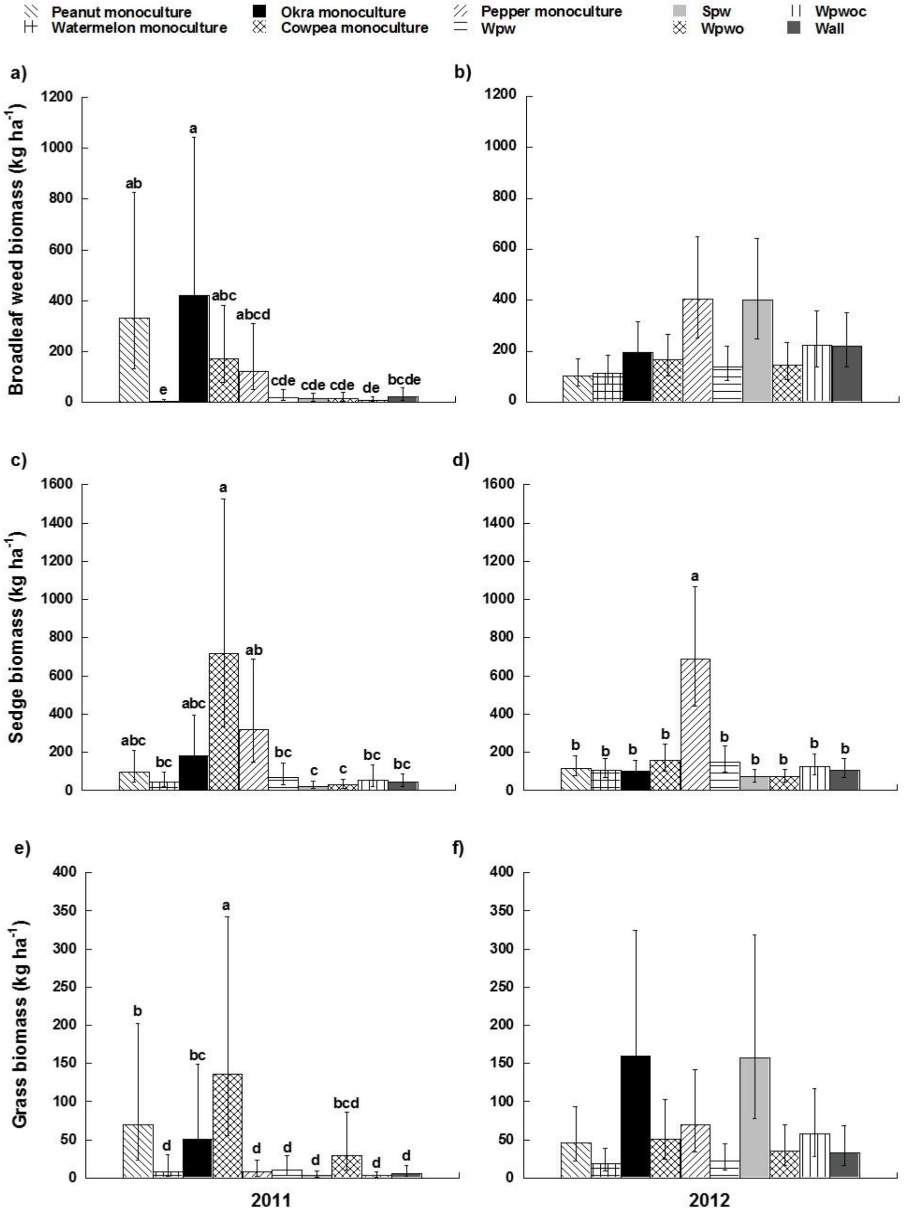


Figure 4.2. Least squares means (LS means) and standard errors of the mean (SEM) of (a)(b) broadleaf, (c) (d) sedge, and (e)(f) grass weed biomass in kg ha^{-1} for each monoculture and intercropping combination in 2011 and 2012, respectively. Treatments are described in Figure 1. The most prevalent broadleaf weeds were carpetweed (*Mollugo verticillata* L.), common purslane (*Portulaca oleracea* L.), and spurge (*Euphorbia* spp.). The most prevalent sedges were yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*Cyperus rotundus* L.). The most prevalent weedy grasses were crabgrass (*Digitaria* spp.) and bermudagrass (*Cynodon dactylon* (L.) Pers.). Treatments are described in figure4.1. Different letters indicate statistically significant differences (*P* ≤ 0.05) between means within years according to Tukey's LSD test.

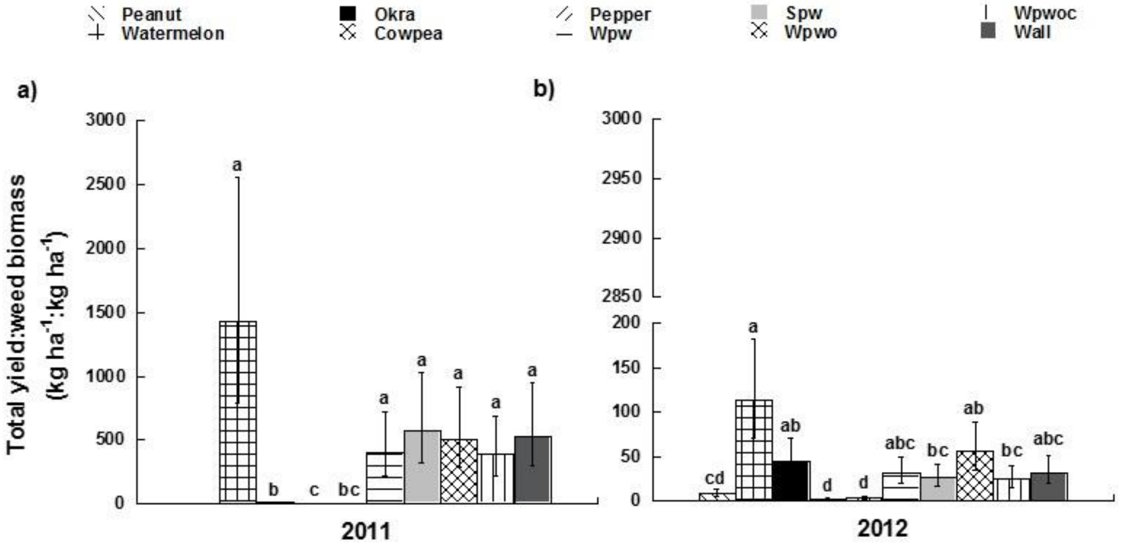


Figure 4.3. Least squares means (LS means) and standard errors of the mean (SEM) of total fruit yield (kg ha^{-1}) to weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in (a) 2011 and (b) 2012. Treatments are described in Figure 4.1. Different letters indicate statistically significant differences ($P < 0.05$) between means within years according to Tukey's LSD test.

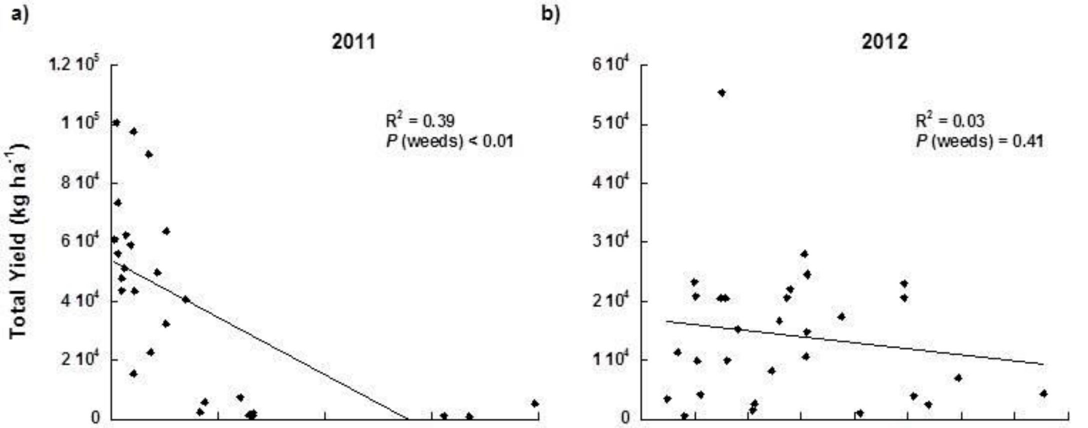


Figure 4.4. Relationship between total weed biomass (kg ha^{-1}) and total plot yield (kg ha^{-1}) in (a) 2011 and (b) 2012 across all intercropping and monocropping systems. There was a significant ($P < 0.05$) negative linear relationship between weed biomass and total plot yield in 2011, but no significant relationship in 2012.

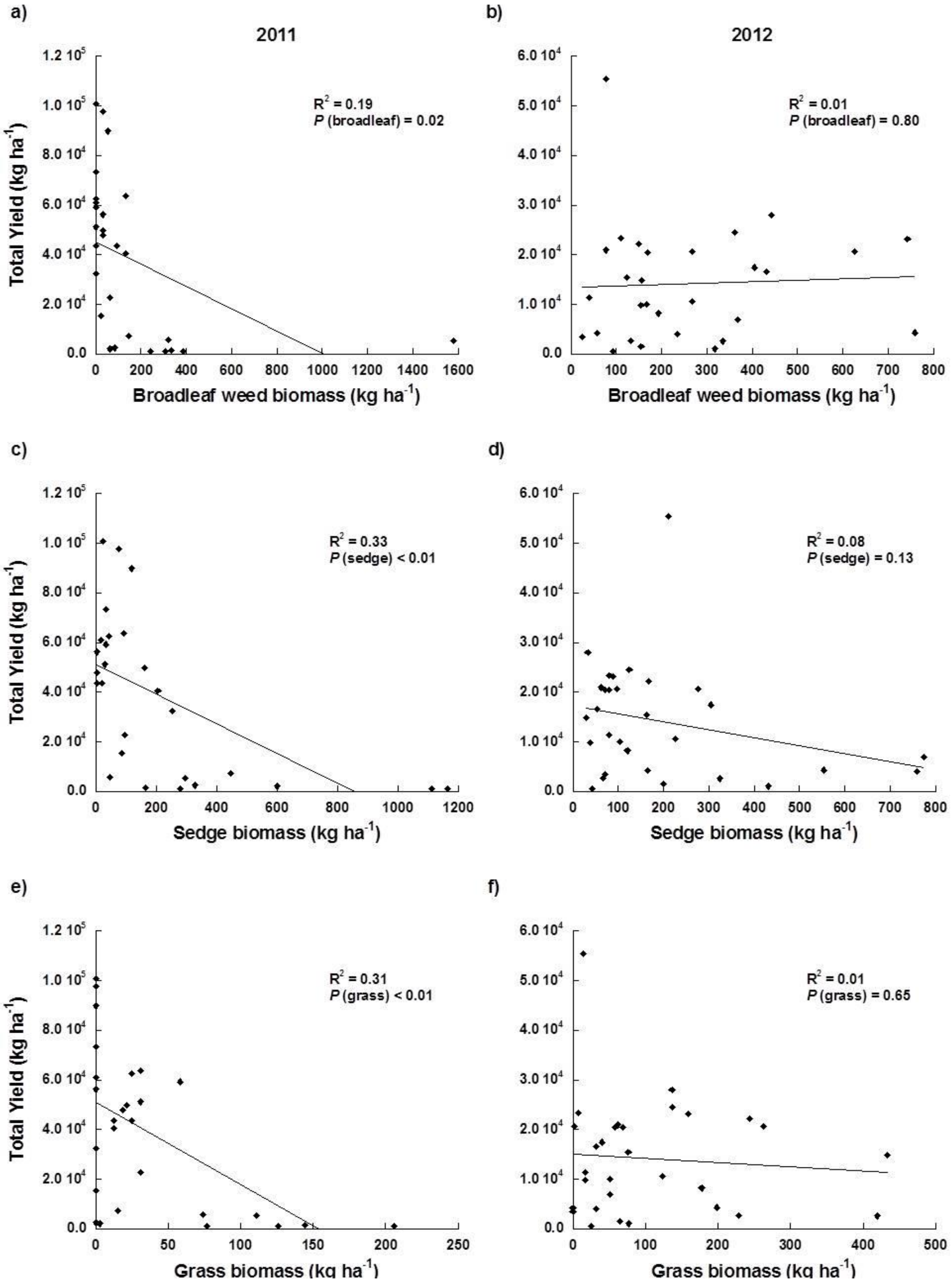


Figure 4.5. Relationships between (a)(b) broadleaf weed, (c)(d) sedge, and (e)(f) grass biomass in kg ha^{-1} and total plot yield in kg ha^{-1} in 2011 and 2012, respectively. There were significant ($P < 0.05$) negative linear relationships between all three weed types and total plot yield in 2011, but no significant relationship in 2012.

Although not statistically significant, aphid infestation on okra was lowest in the 4 species combination (W_{pwoc}) with about 30% infestation and close to 80% infestation in both monocropped okra and okra in the 3 species combination ($W_{pwo;}$; Appendix Fig. A-4.9). This represents a reduction in aphid infestation of approximately 62%. Aphid density and aphid mummy density were not significantly different between intercropping combinations and monocropped okra. Numerically, however, aphid density and infestation were lowest in the 4 species combination. Mummy density was highest in monocropped okra with greater than 20 aphid mummies per infested okra plant and as few as 5 aphid mummies per infested okra plant in the 3 and 4 species combinations.

Discussion

Weeds

Watermelon was an effective smother crop for weed suppression in 2011 but not in 2012 due to competition from okra and an outbreak of downy mildew. The only crop that benefited from the weed-suppressive effects of intercropping when compared to its monocrop was pepper. Sedges and broadleaved weeds were most effectively suppressed in 2011. In 2012, sedges were again effectively suppressed and accounted for the majority of weed biomass in pepper monocultures. Purple and yellow nutsedge pose a particular challenge to organic producers due to their mode of reproduction and limited control options (Wang et al., 2009). Initial results from both years suggest that introducing a functionally diverse cropping system and selecting appropriate planting dates can effectively suppress resilient weed such as nutsedge. This may offer producers, who are hesitant to transitioning to organic production due to the difficulty of managing perennial weeds (Wedryk et al., 2012), another management tool for their control. Additionally, this holistic approach to weed management may offer a higher economic return than other cultural strategies such as cover crops, cultivation, and solarization (Wang et al., 2009) due to the value added production of watermelon. Further value is added with the reduction of manual labor and its associated high cost (Weston and Duke, 2003; den Hollander et al., 2007). It is unknown, however, if the benefits of reducing weed competition outweigh the competitive interactions between component crops.

Yield to weed ratios indicate that high watermelon yields and the ability of watermelon to suppress weed growth led to high yield to weed ratios in 2011. Although still highest in monocropped watermelon, these ratios were drastically reduced in 2012 due to the reduction in watermelon yields in monocropped watermelon from downy mildew and in a reduction in watermelon yields in intercropping combinations due to competition with okra (Franco et al., 2015). The exact mechanisms by which watermelon was able to suppress weed growth are unknown. Weed suppression by cover crops has been partially attributed to a reduction in light transmittance due to high biomass cover (Mulvaney et al., 2011). The relationship between total above-ground plant biomass and weed biomass did not support that idea in this study. However, this data was only collected in 2012 when the weed suppressive effects of watermelon were drastically reduced. It is likely that aboveground competition for space and increased shading caused by watermelon canopy in 2011, as well as by the dense canopy created by all component crops in 2012, led to less available light for weed populations as suggested by other authors (Baumann et al., 2002; Steinmaus et al., 2008; Saudy, 2014). It is also plausible, however, that more efficient use of belowground resources such as water and nutrients (Buhler et al., 2001) due to enhanced niche differentiation by multiple species (Tilman, 2001) and possible allelopathic effects from watermelon root exudates (Yun et al., 2000; Hao et al., 2007) may have further reduced the competitive ability of weeds (Weston and Duke, 2003; Singh et al., 2010).

Pests and Disease

Disease issues with watermelon in 2012 highlight the importance of having a diverse cropping system such as the ones incorporated in this study to assure producers would still have one or more cash crops to harvest when one component crop fails. Although peppers and their close relatives are known to suppress pest populations and disease (Cowles et al., 1989; Dayan et al., 2009), this phenomenon was not observed in this study with regard to downy mildew infestation on watermelon and aphid infestation and density on cowpea and okra. There were no clear trends in the pest suppressive abilities of intercropping treatments, although it is worth noting that no aphid infestation was found in monocropped cowpea. Potting et al. (2005) suggested that pests such as aphids that colonize aerially do not respond as favorably to mixed cropping systems as compared to pests that display host detection behaviors. Further, data on predator species such as the Carabid beetle, which have been found to reduce aphid populations (Kromp, 1999), is currently unavailable in this multifunction intercropping system. It has also been suggested that intercropping may sometimes have adverse effects on target crops by attracting pests (Potts, 1990; Theunissen, 1994).

An important consideration this study did not address is the issue of scale as small-scale plot studies can underestimate the effects of diversity on disease control (Zhu et al., 2000). In addition, future studies should incorporate the use of pathogen inoculation to properly evaluate the effects of this type of system on disease incidence and severity. Additionally, other researchers have found that members of the Brassica and Allium families help reduce soil borne diseases and pests (Fayzalla et al., 2009; Friberg et al., 2009; Baidoo et al., 2012; Hu et al., 2012; Noman et al., 2013). Members of these families may be well suited for inclusion in a multifunctional intercropping system such as this one for their disease suppressive abilities. Finally, intercropping only serves to influence the rate of spread of pests and disease, not the viability of these organisms directly (Potts, 1990). Thus, it is plausible that disease and aphid data were collected too late to effectively measure the effects of this intercropping system on their populations. Future studies should incorporate multiple disease and pest data collection times throughout the growing season to more effectively monitor their rate of spread.

CHAPTER VI

CONCLUSION

Summary of Findings

In this study, we observed overyielding in functionally diverse intercropping systems and an increase in overall food production on a per unit land basis. Land equivalent ratio's (LER) were consistently highest in the three-species within-row combination with peanut, watermelon and okra (W_{pwo}) and the four-species combination of peanut, watermelon, okra and cowpea (W_{pwoc}). In 2011, watermelon was the dominant crop and was up-regulated in all intercropping combinations while all other component crops were down-regulated. Watermelon per plant production was significantly higher in the combination containing all species (W_{all}) when compared to its monoculture, 5.50 and 2.09 kg_{fruit} plant^{-1} respectively. In 2012, okra was the dominant crop and was up-regulated in all intercropping combinations while watermelon, cowpea, and pepper were down-regulated. Okra per plant production was significantly higher in W_{pwoc} and W_{all} than in monoculture, 2.28, 2.46, and 1.13 kg_{fruit} plant^{-1}, respectively.

There was no effect of functionally diverse intercropping on SOC and TN over the two growing seasons this study was conducted. SMB-C was significantly higher in okra monoculture as compared to the 3 species intercropping combination of peanut-watermelon-okra (W_{pwo}), 1189 and 718 µg C g^{-1} dry soil, respectively. No differences were detected in RLD in either sampling depth but differences in SRL in the top 20 cm of the soil profile were detected. SRL was significantly higher in monocropped cowpea as compared to monocropped peanut and W_{pwo}, 11075, 4276, and 4977 m g^{-1} dry weight root, respectively. SMB-C was positively correlated with RLD ($P < 0.01$, $R^2 = 0.25$). Daily average soil temperature was on average 6°C higher in pepper and peanut monoculture both years and 2 to 4°C lower in W_{pwo} in 2011. Daily soil temperature was linearly correlated with leaf area index (LAI) and as LAI increased, soil temperature decreased. This relationship was especially strong with daily maximum soil temperatures ($P < 0.01$, $R^2 = 0.46$).

There were no differences detected in gas exchange measurements and no differences in $d^{13}C$ composition within individual species between cropping system. Peanut grown in strip intercropping with watermelon, and in the 3 and 5 species systems in 2012 and in the 5 species system in 2011 had the highest midday water potential values as compared to peanut grown in monoculture, indicating peanut may have benefited from facilitative interactions with companion crops in the intercropping systems through a reduction in plant water stress. Differences in specific leaf area (SLA), leaf nitrogen, and carbon to nitrogen (C:N) ratios in watermelon may at least partially explain the lack of changes in gas exchange measurements detected. In 2012, when watermelon was a subordinate crop, specific leaf area and leaf N content were highest in the multispecies systems, particularly W_{pwoc}. Carbon to nitrogen ratio (C:N) was also lowest in W_{pwo} and W_{pwoc} as compared to watermelon grown in monoculture indicating watermelon underwent morphological changes at the leaf-level due to competition for light and allocated less C to leaves when competition was reduced. This likely led to optimization of net assimilation rate per unit leaf area. There were species differences, however, when comparing species within cropping system with peanut and pepper consistently having the lowest midday water potentials as well as the highest photosynthetic nitrogen-use efficiency values. d^{13}C values were generally highest for peanut indicating higher water-use efficiency, and lowest for watermelon indicating low water-use efficiency

Watermelon was an effective smother crop and significantly reduced total weed biomass when planted alone and in all intercropping combinations in 2011. Total weed biomass was reduced by 81, 83, 88 and 92% in treatments containing watermelon as compared to pepper, peanut, okra and cowpea grown in monoculture. Pepper grown in monoculture had significantly higher weed biomass than all other treatments in 2012, with 46% more weed biomass than the next highest yielding treatment. Sedges were consistently most effectively reduced ($P < 0.10$) and pepper, cowpea, peanut and okra benefited most from intercropping in both years. Total above-ground plant biomass was not a significant predictor of weed biomass. Weed biomass was a significant predictor of total fruit yield, however, accounting for 39% of yield variability. No significant differences in downy mildew infestation of watermelon or aphid infestation of okra and cowpea were found in 2012.

Conclusion

The concept of overyielding is typically applied to plant biomass in natural ecosystems. In this study we investigated the role of plant functional diversity and overyielding in an organic, low-input agroecosystem. Overyielding occurred consistently in the three-species intercropping system incorporating a smother crop (watermelon), a leguminous species (peanut), and a taller pollinator attractant (okra) and the four-species combination incorporating these same species with an additional legume (cowpea). This translates to an increase in overall food production per unit of land. Of particular importance is that overyielding occurred despite alterations to planting dates and differences in environmental conditions between growing seasons. The dominant crop in each year was always up-regulated and produced more fruit per plant while most subordinate crops (with the exception of peanut in year 2) were down-regulated. This highlights the importance of interspecific species competition in intercropping systems.

In an attempt to reveal some of the underling mechanisms driving the diversity-productivity relationship observed in this study, we found no differences in gas exchange measurements for each species between intercropping systems. However, changes in watermelon leaf traits such as SLA, leaf N concentration, and C:N in year 2 when it was a subordinate species indicate that watermelon responded to intense competition for light from okra by investing in larger but thinner leaves and in more N in photosynthetic machinery per unit leaf area to optimize net assimilation rates over the entire leaf. Peanut benefited most from intercropping with regards to leaf water potential as peanut was more water stressed when grown in monoculture for the dates those measurements were taken. There were species differences in gas exchange, leaf water potential, and water-use efficiency, and leaf traits which is not unexpected in species with such different growth forms and life strategies.

Cowpea and pepper monocultures had the lowest LAI values in year 2. The reduction in crop canopy density led to more overall weed pressure as indicated by weed biomass, particularly in pepper monoculture. Watermelon was an effective smother crop in year 1 when it was the dominant species, reducing weed pressure by as much as 92% as compared to cropping systems that did not incorporate watermelon. No differences were detected in aphid pressure on cowpea or okra and in downy mildew in watermelon in year 2.

LAI measurements were found to be negatively related to soil temperatures. Thus, it is possible that an architecturally complex intercropping system that can reduce soil temperatures as we observed in this study may reduce root respiration and, consequently, soil C losses to the atmosphere (although these were not measured directly in this study). No differences in SOC or TN accumulation were observed. However, we observed a reduction in SMB-C in the three-species combination as compared to okra monoculture. This may have been due to higher C:N in okra tissue, leading to a longer decomposition time and more available C for microbes. Although no differences in RLD were observed, we found a positive linear relationship between RLD and SMB-C in this study. Changes in the soil, particularly with regards to SOC, are expected over a longer period of time than this study was conducted. We hypothesized that the presence of legumes may reduce N losses through two possible mechanisms: 1. by adding plant-available N to the soil from root exudates, thereby increasing the amount of N available to neighboring species and 2. by reducing the amount of N being taken up due to their N-fixing ability, thereby reducing N depletion. A lack of sufficient replication reduced our power to detect differences in N and other measured parameters.

Overall, results from this study suggest that the limiting factor in this type of system was solar radiation. The observed increase in LAI, reduction in weed biomass, changes in watermelon leaf morphology in year 2, and reduction in soil temperatures in mixed cropping, particularly the three- and four-species combinations, indicate competition for light was a major driver of the interactions observed between component species. Therefore, producers interested in utilizing this type of system should select species that will create an architecturally complex and dense canopy that will optimize the utilization of solar radiation but may want to limit the system to only three or four species in order to avoid excessive competition light.

Finally, there is increasing interest in small-scale farming and there is a need to produce more food per area of land. This multispecies design would be ideal for small scale producers and urban farmers who want to optimize per area production on limited space. Such a system may also benefit a producer by eliminating the dependence on a single crop and reducing risk of entire crop failure. The results from this study suggested that future studies on biodiversity in agricultural landscapes should focus on a methodical selection of species that contribute to a specific function within the agroecosystem rather than on number of species solely.

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APPENDIX

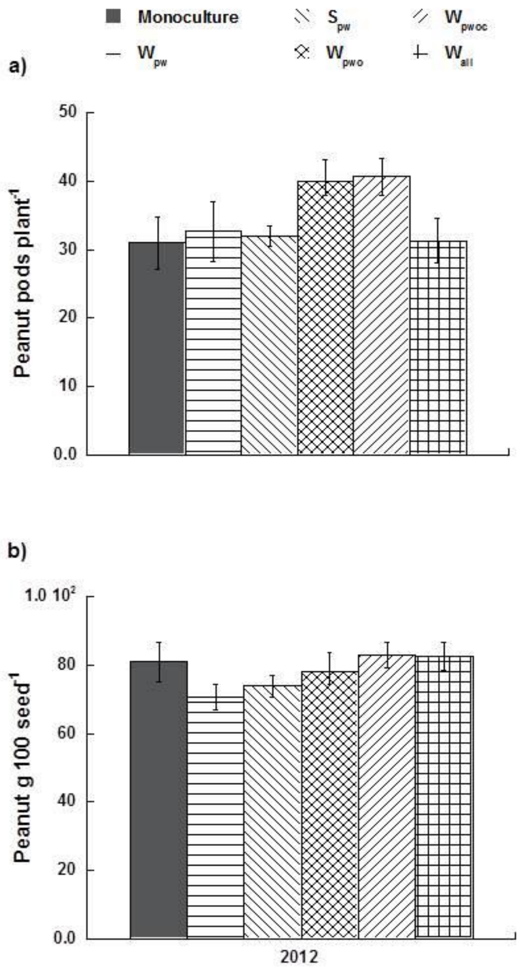

Figure A-1.1. Peanut quality measurements taken in 2011 based on intercropping strategy. Treatments are described in Fig. 1. (a) pods plant^{-1}, (b) g 100 seed^{-1}. No statistical differences were detected ($P < 0.05$).

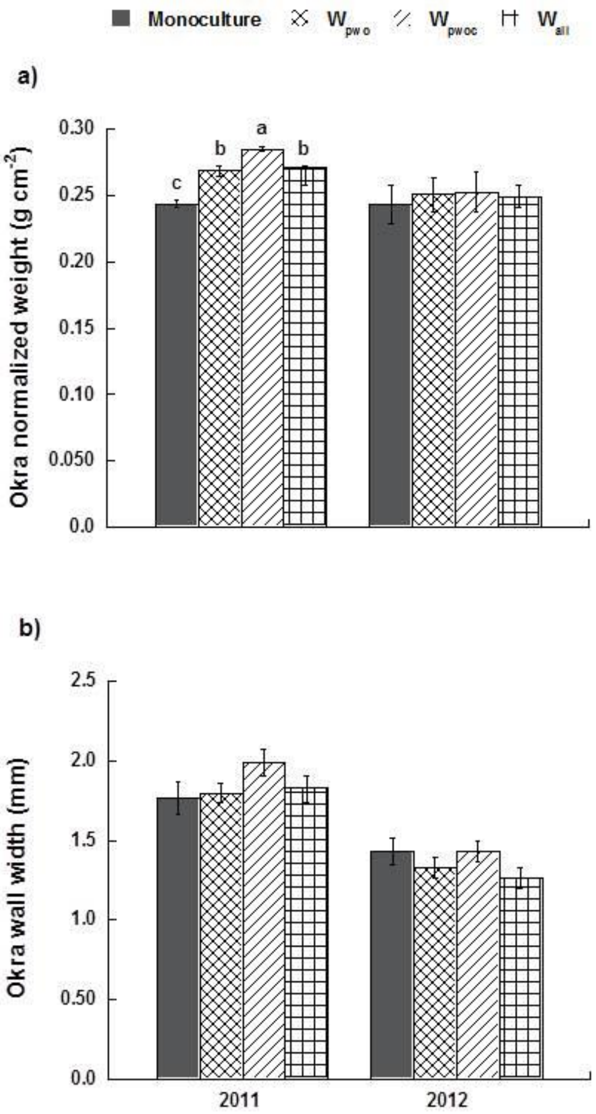

Figure A-1.2. Okra quality measurements based on intercropping strategy in 2011 and 2012. Treatments are described in Fig. 1. (a) normalized fresh pod weight (g cm^{-2}), (b) wall width (mm). Treatments are described in figure 1.2. Different letters indicate statistically significant ($P < 0.05$) between means within years.

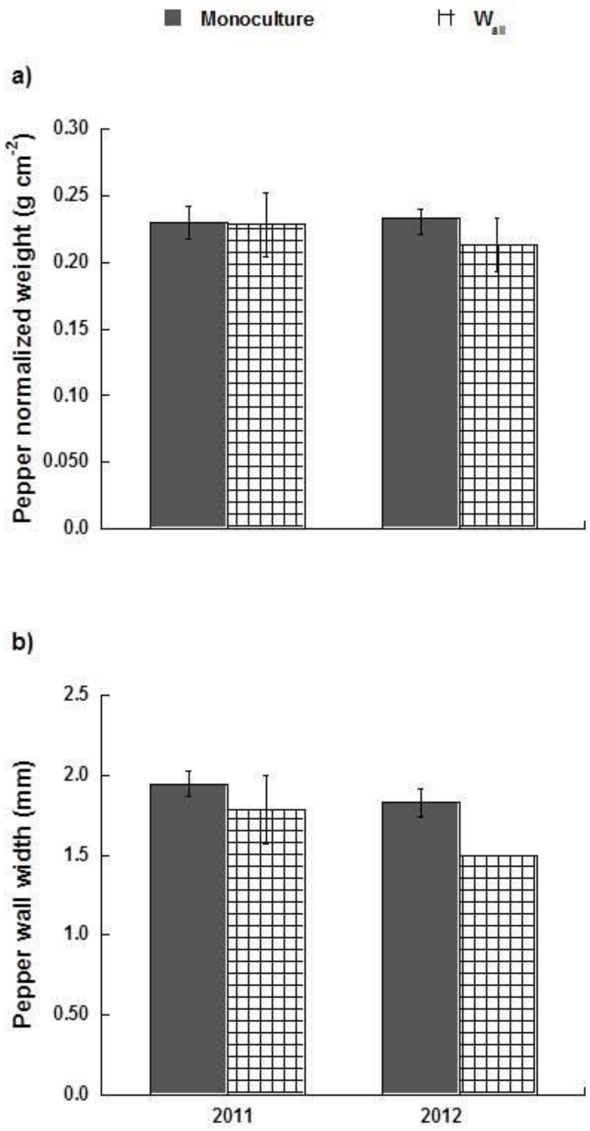

Figure A-1.3. Pepper quality measurements based on intercropping strategy in 2011 and 2012. Treatments are described in Fig. 1. (a) normalized fresh fruit weight (g cm^{-2}), (b) wall width (mm). Treatments are described in figure 1.2. No statistical differences were detected.

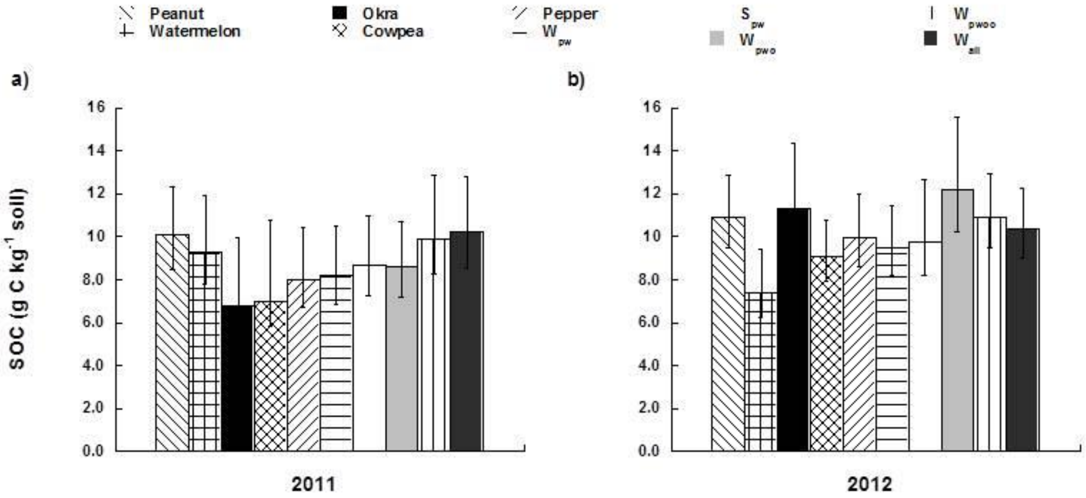

Figure A-2.1. Least squares means and standard errors of the mean of soil organic carbon (SOC; g C kg^{-1} soil) at post-harvest adjusted based on baseline SOC values obtained from pre-planting samples in a) 2011 and b) 2012 for each monoculture and intercropping combination. Treatments are described in figure 1. No statistically significant differences were detected.

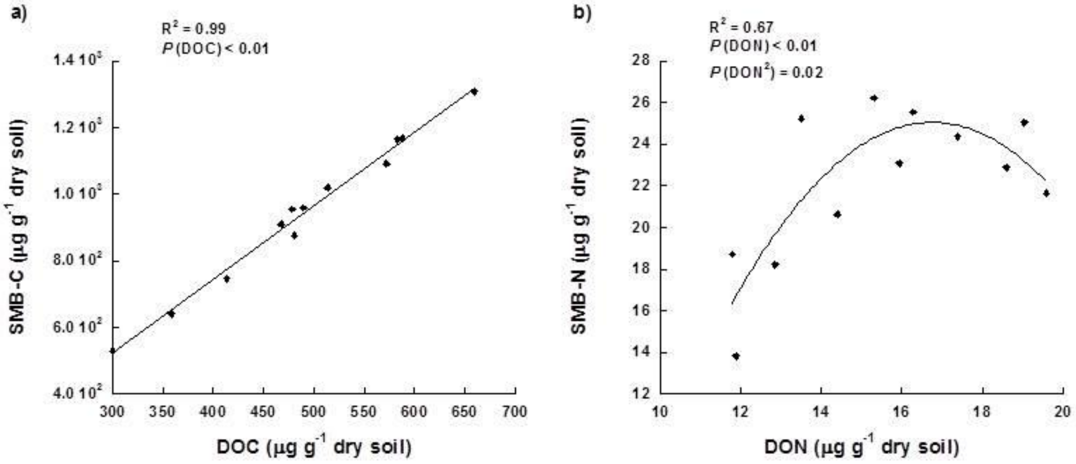

Figure A-2.2. Relationship between a) dissolved organic carbon (DOC; µg g^{-1} dry soil) and soil microbial biomass carbon (SMB-C; µg g^{-1} dry soil) and b) dissolved organic nitrogen (DON; µg g^{-1} dry soil) and soil microbial biomass nitrogen (SMB-N; µg g^{-1} dry soil). A positive linear relationship exists between DOC and SMB-C, while a quadratic relationship exists with DON and SMB-N.

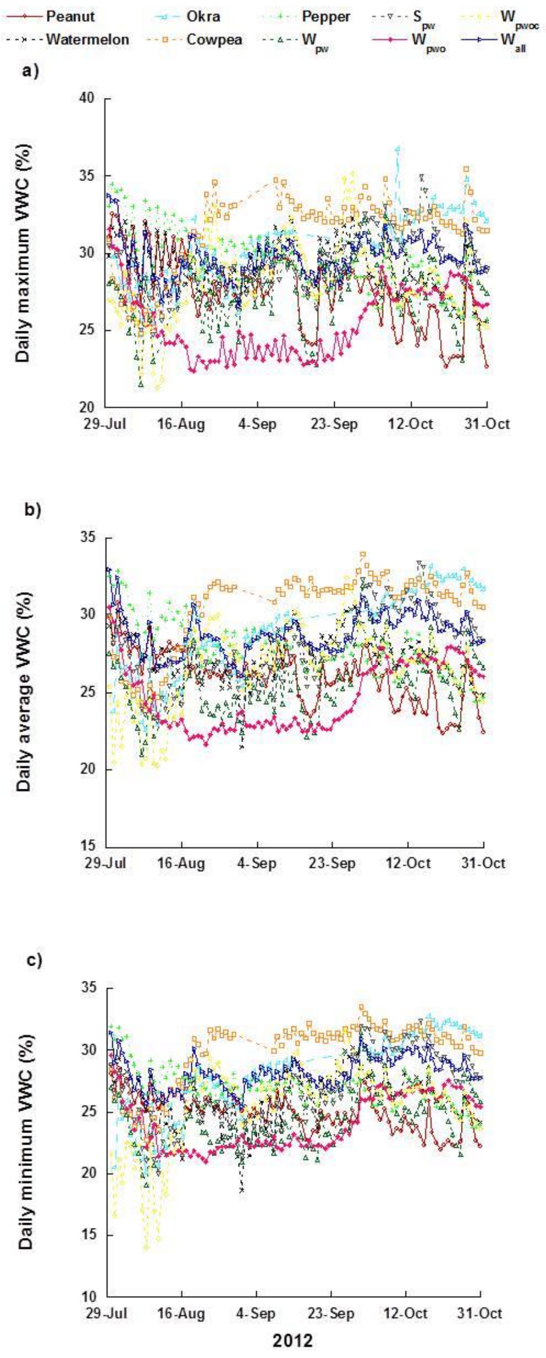

Figure A-2.3. Continuous soil volumetric water content (VWC; %) measured with ECH_20 dielectric aquameter sensors at 20 cm soil depth for each monocrop and intercropping combination in 2012; a) maximum daily VWC b), average daily VWC, and c) minimum daily VWC. Measurements based on a sample size of 1 sensor per treatment.

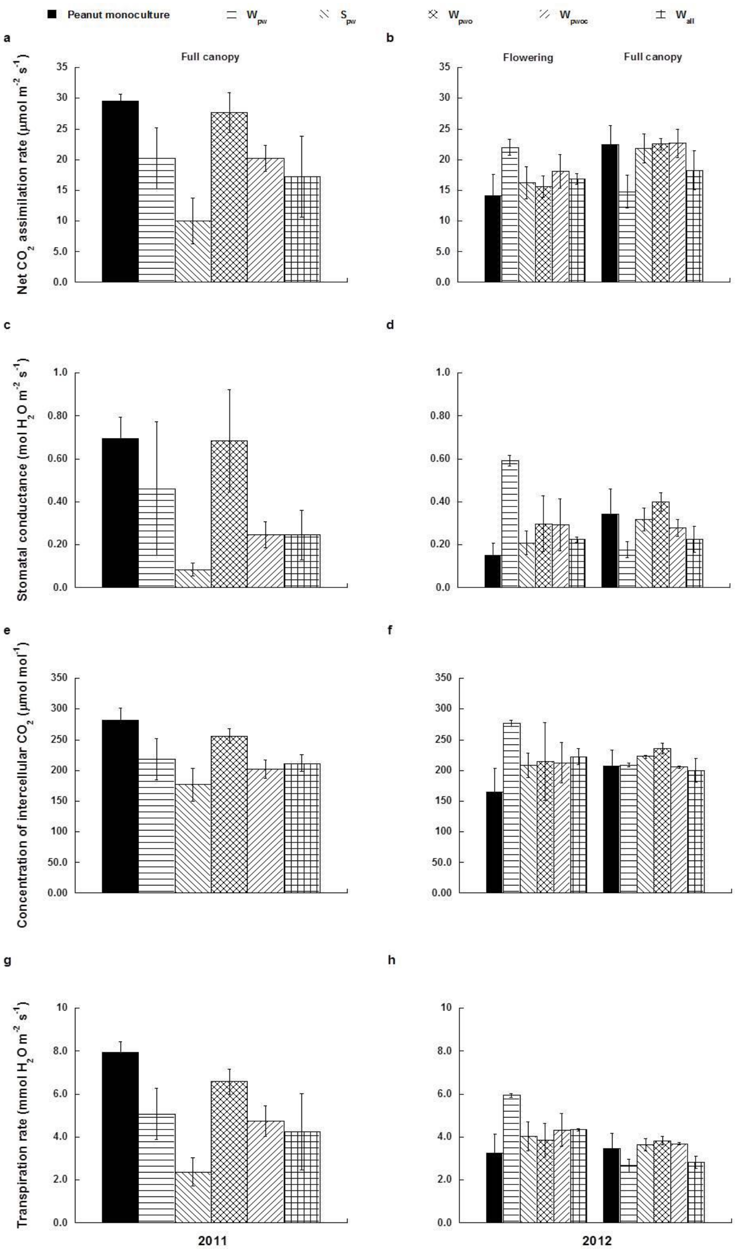

Figure A-3.1. Peanut net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 (71-75 DAP) and at flowering stage and full canopy in 2012 (50 and 126 DAP, respectively). W_{pw} = within row intercropping of peanut-watermelon, S_{pw} = strip intercropping with peanut-watermelon (e.g. alternating rows), W_{pwo} = within row intercropping of peanut-watermelon-okra, W_{pwoc} = within row intercropping of peanut-watermelon-okra-cowpea, W_{all} = within row intercropping of peanut-watermelon-okra-cowpea-pepper. No statistically significant differences ($P < 0.05$) were detected.

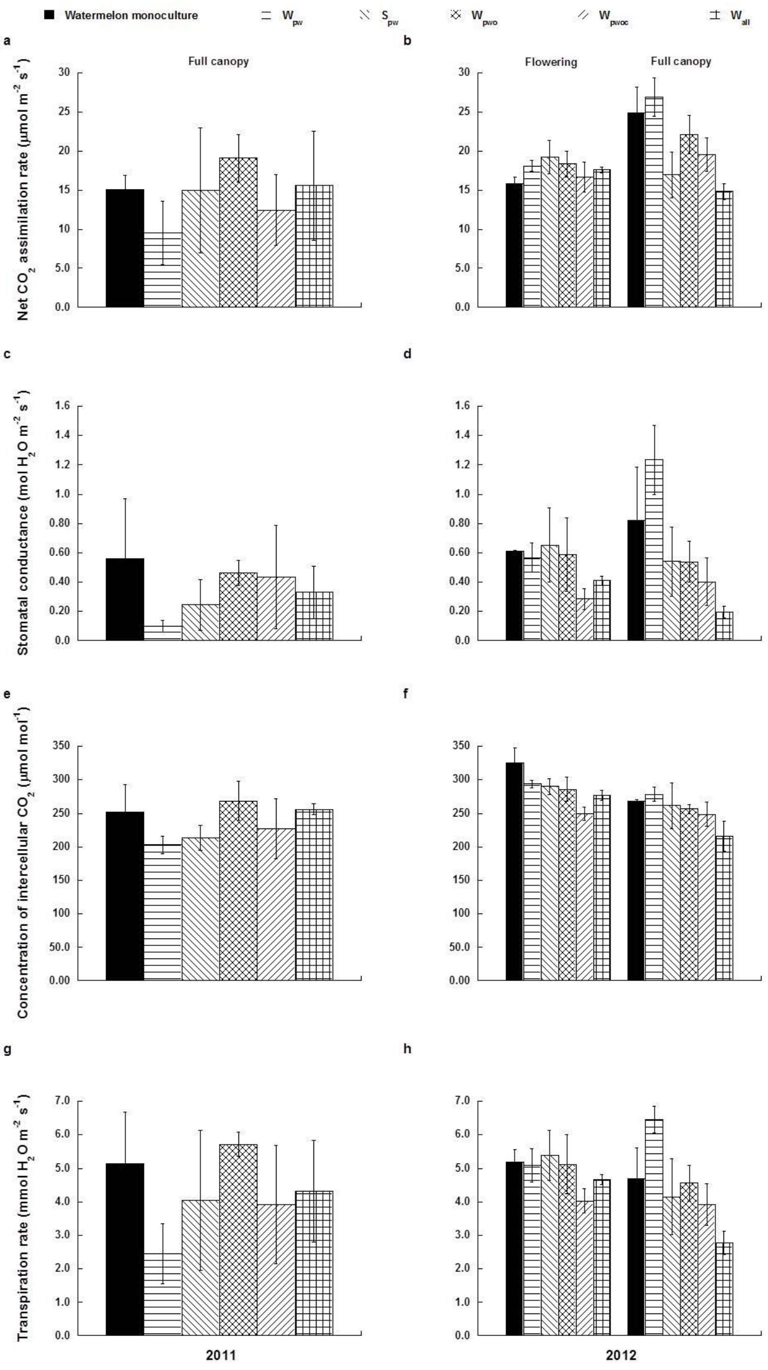

Figure A-3.2. Watermelon net CO$_2$ assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 (65-69 DAP) and at flowering stage and full canopy in 2012 (40 and 84 DAP, respectively). Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

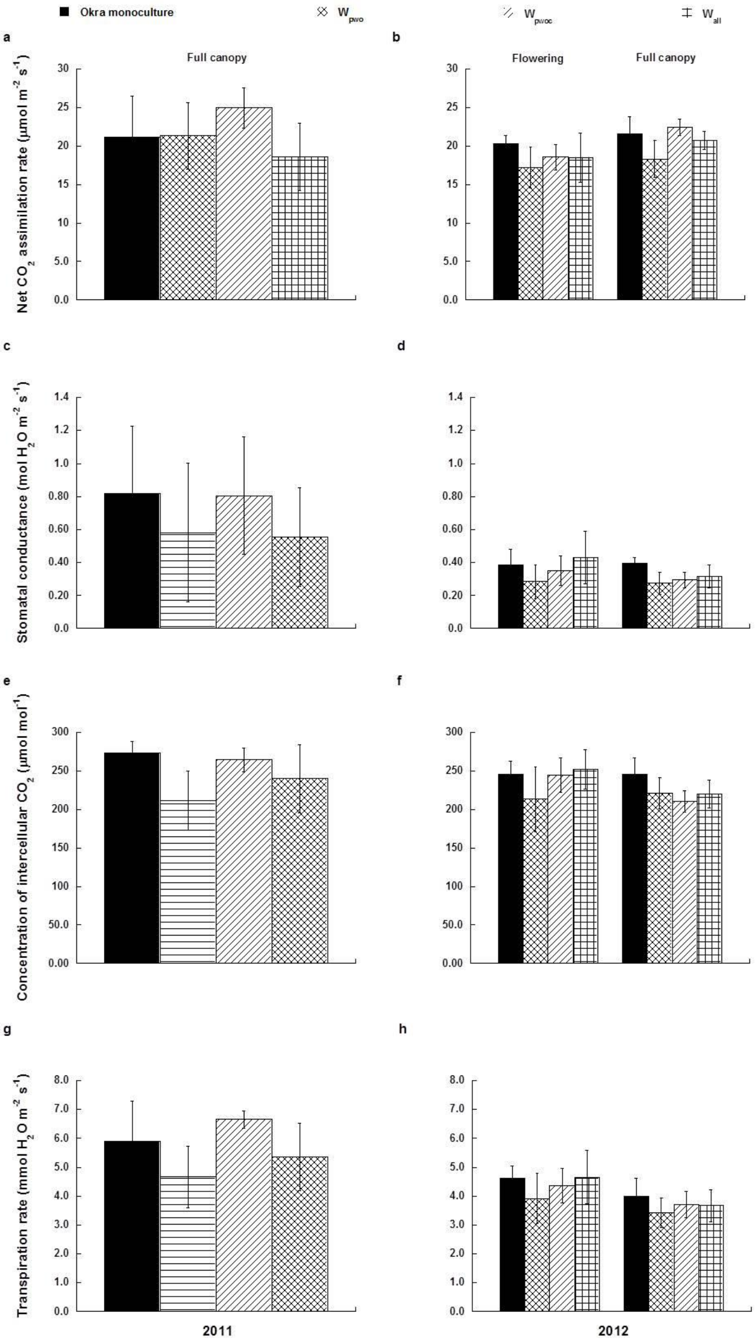

Figure A-3.3. Okra net CO$_2$ assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 (58-62 DAP) and at flowering stage and full canopy in 2012 (53 and 104 DAP, respectively). Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

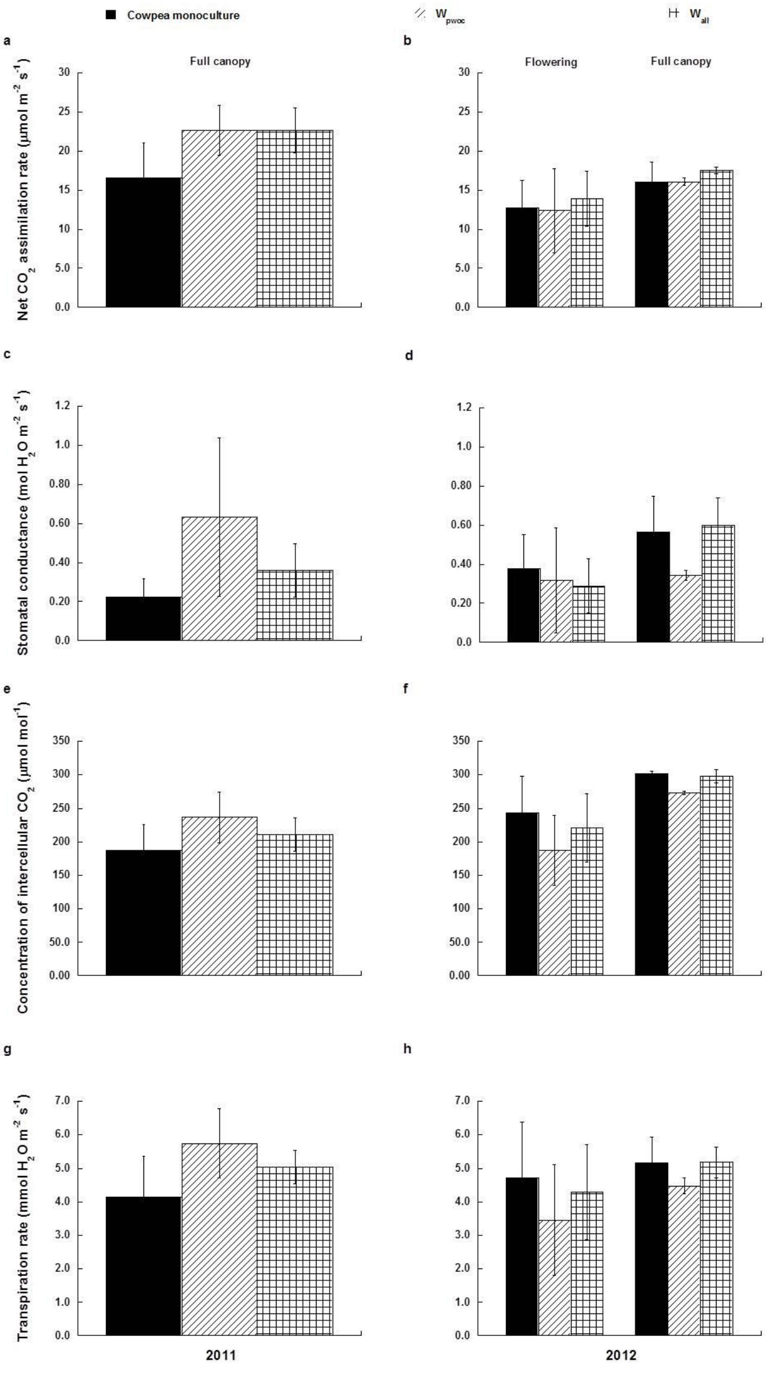

Figure A-3.4. Cowpea net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 (57-61 DAP) and at flowering stage and full canopy in 2012 (41 and 55 DAP, respectively). Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

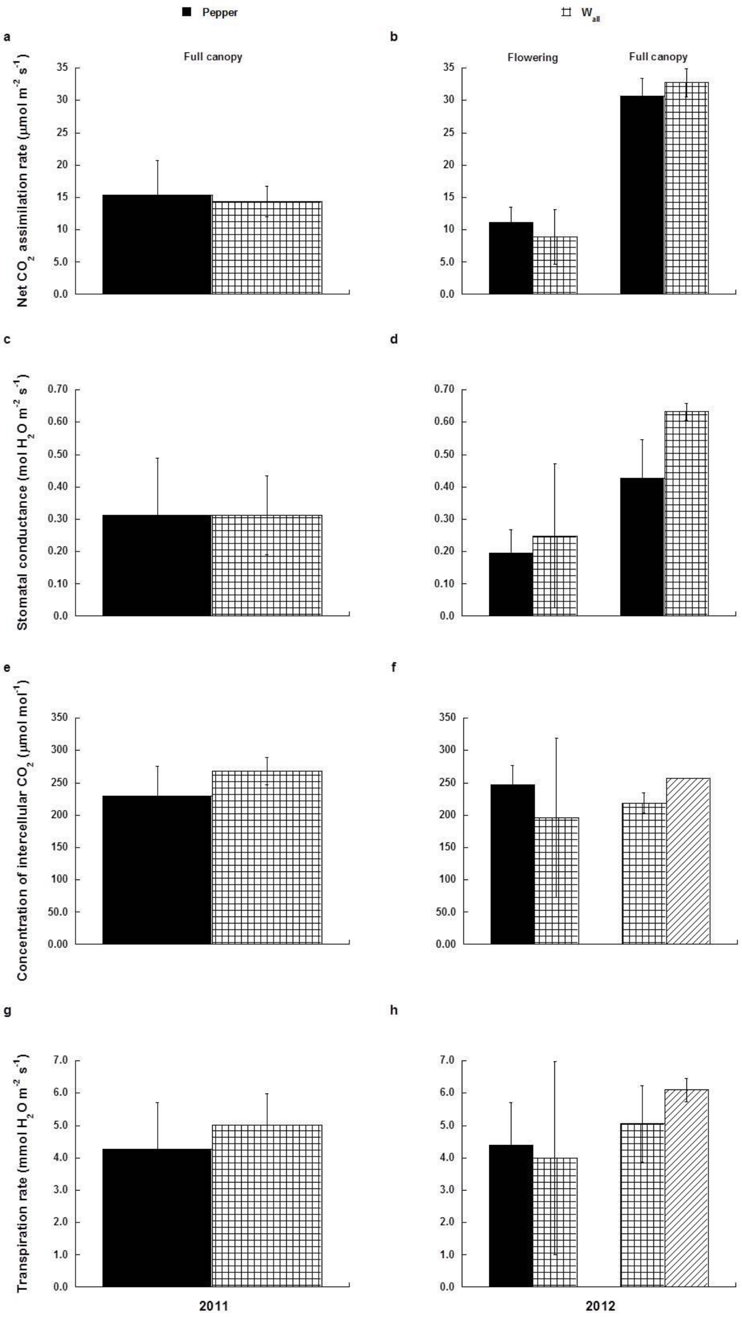

Figure A-3.5. Pepper net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 (54-58 DAP) and at flowering stage and full canopy in 2012 (35 and 114 DAP, respectively). Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

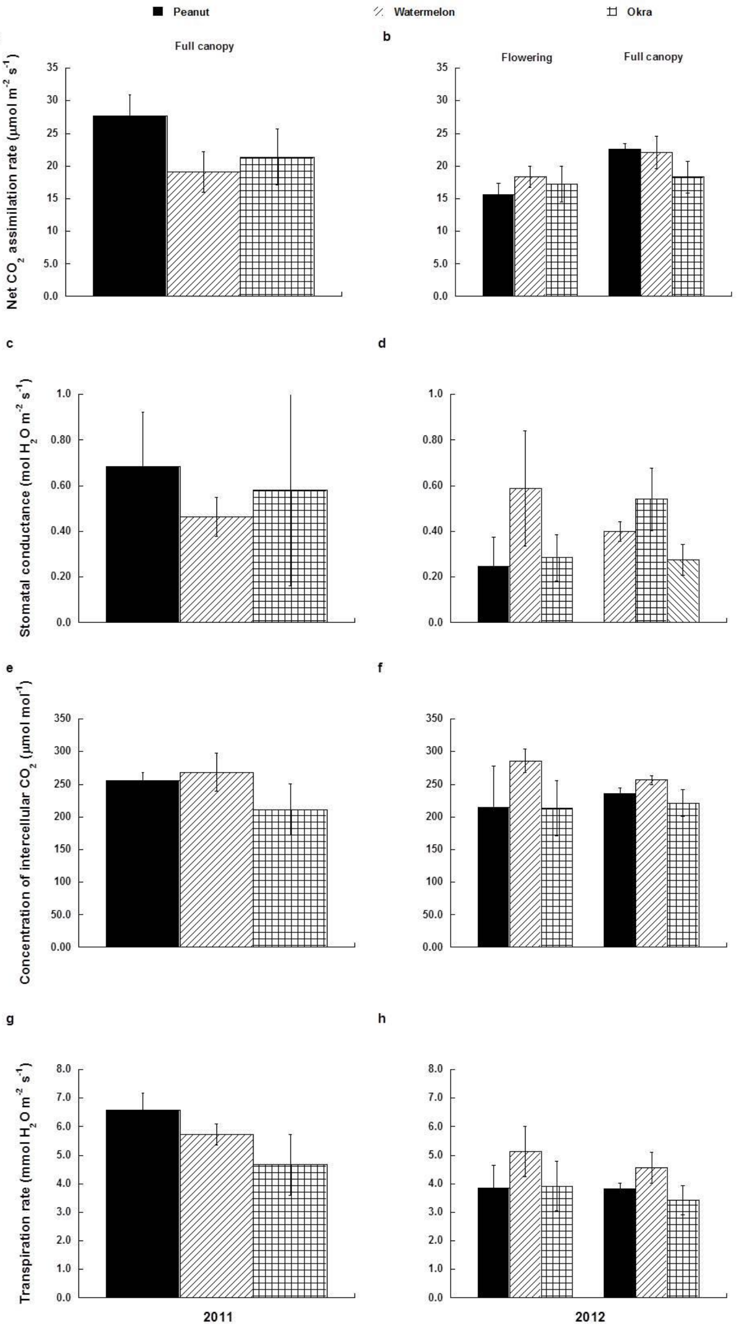

Figure A-3.6. Net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 and at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra (W_{pwo}). Different letters indicate statistically significant differences ($P < 0.05$) between species within collection period within years according to Tukey's LSD test.

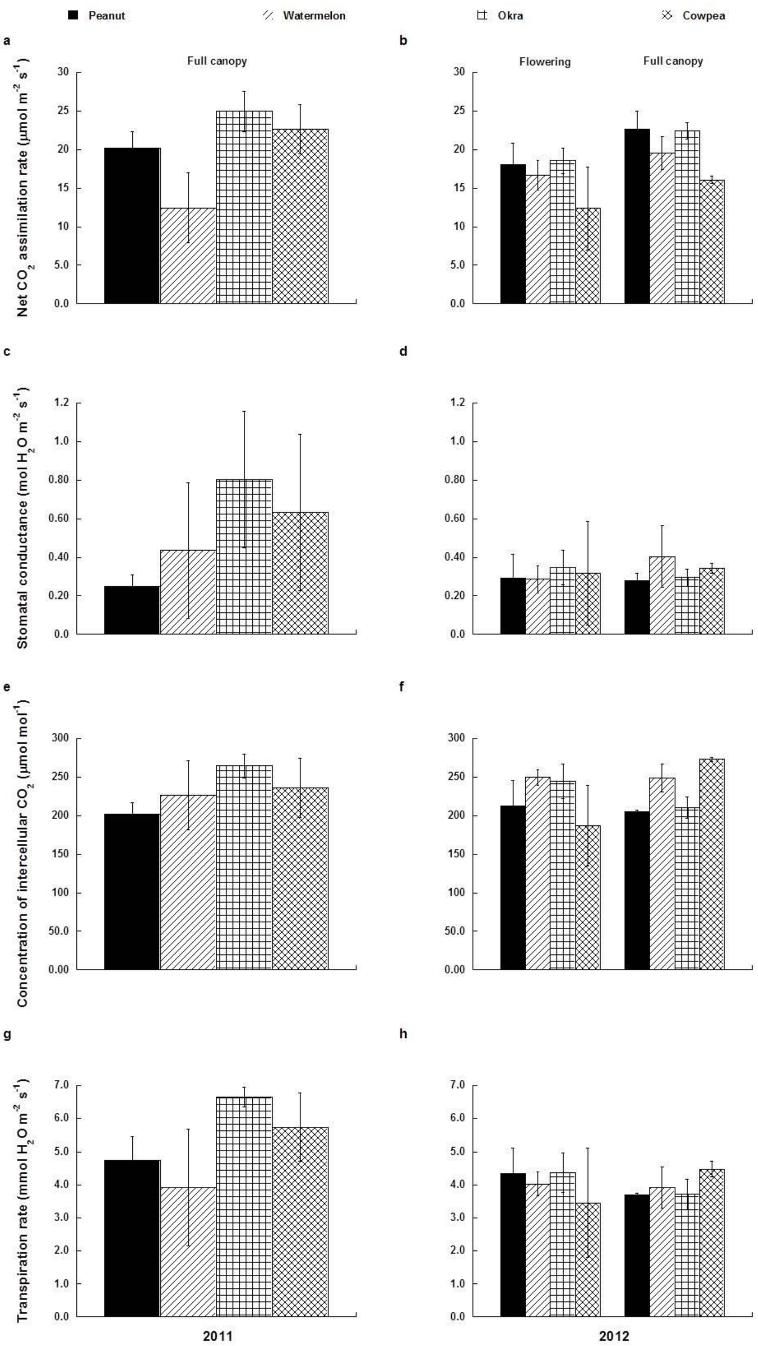

Figure A-3.7. Net CO_2 assimilation rate (A) in a) 2011 and b) 2012, stomatal conductance (g_s) in c) 2011 and d) 2012, intercellular CO2 concentration (C_i) in e) 2011 and f) 2012, and transpiration rate (E) in g) 2011 and h) 2012 measured at full canopy in 2011 and at flowering stage and full canopy in 2012 for component species in the within-row intercropping combination of peanut-watermelon-okra-cowpea (W_{pwoc}). No statistically significant differences ($P < 0.05$) were detected.

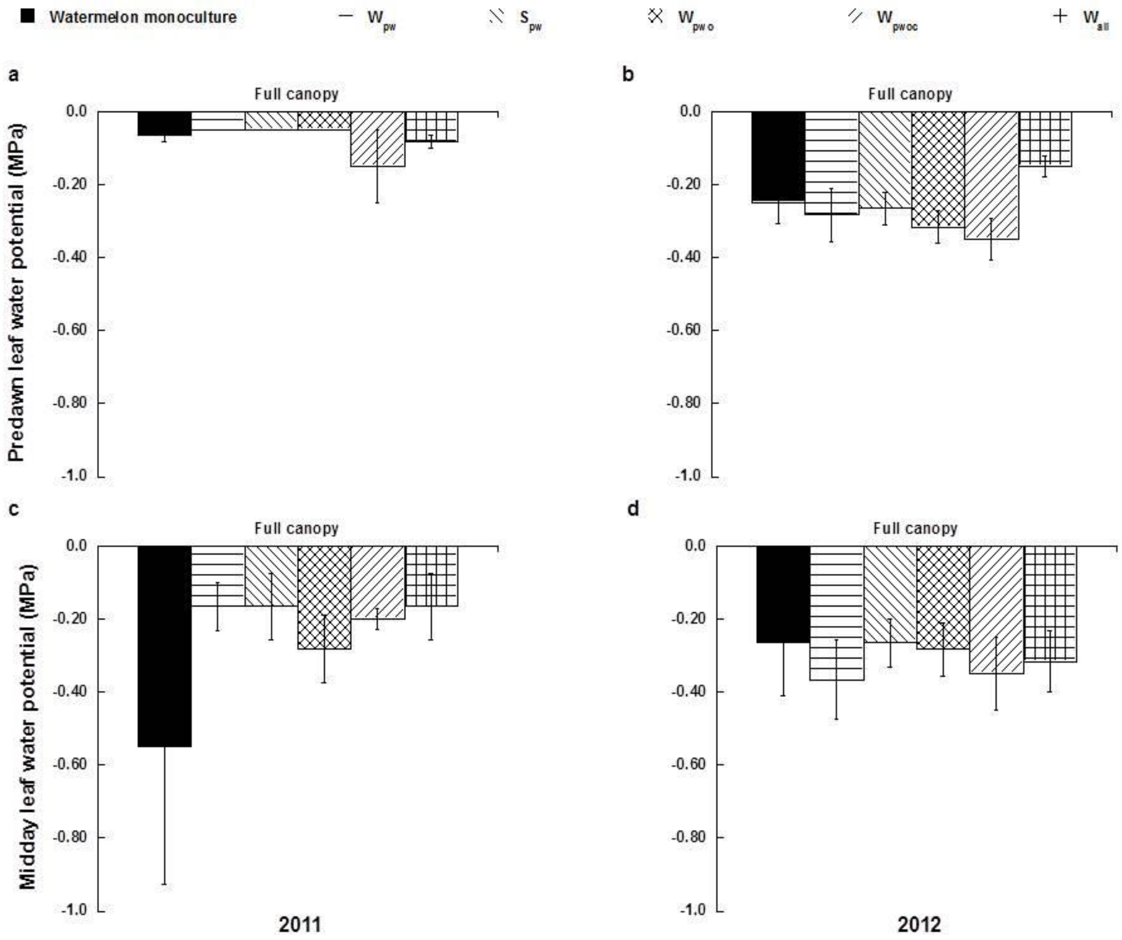

Figure A-3.8. Watermelon a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy (65-69 DAP) in 2011 and b) predawn and d) midday leaf water potential measured at full canopy (84 DAP) in 2012. Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

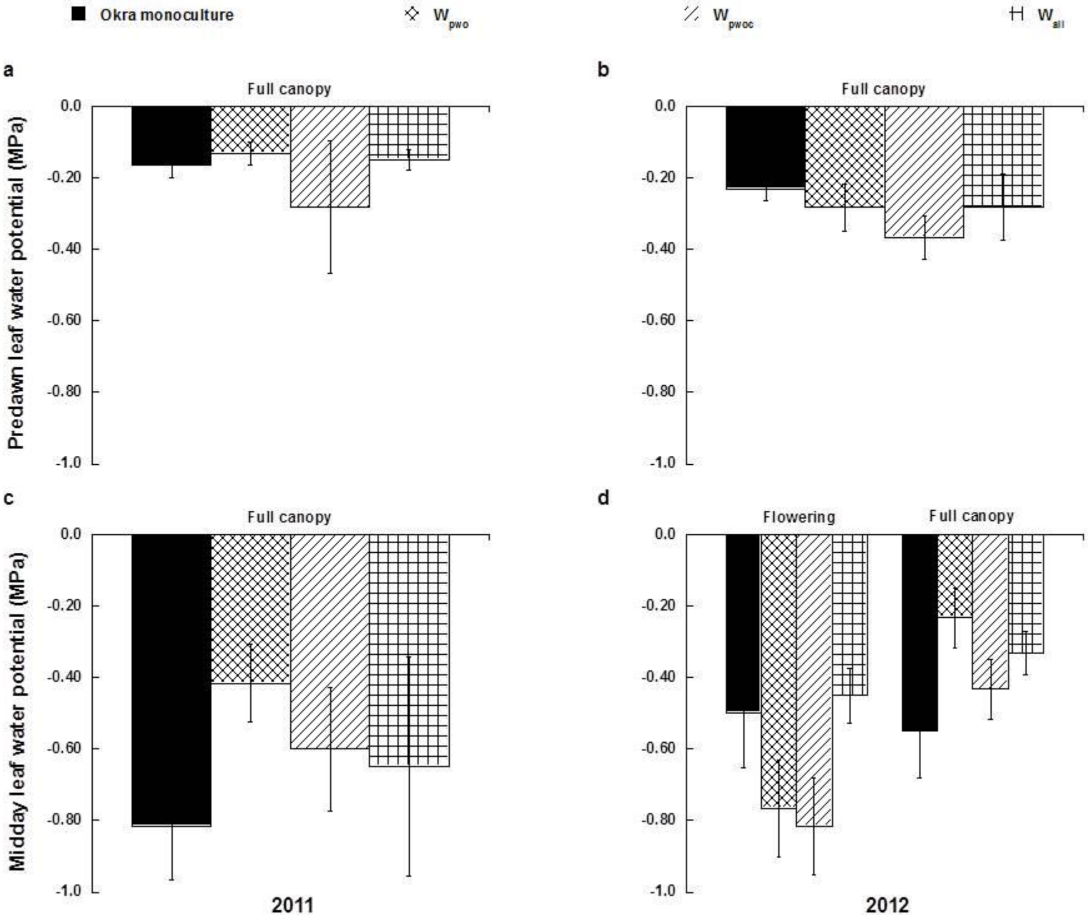

Figure A-3.9. Okra a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy (58-62 DAP) in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage (53 DAP) and full canopy (104 DAP) in 2012. Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

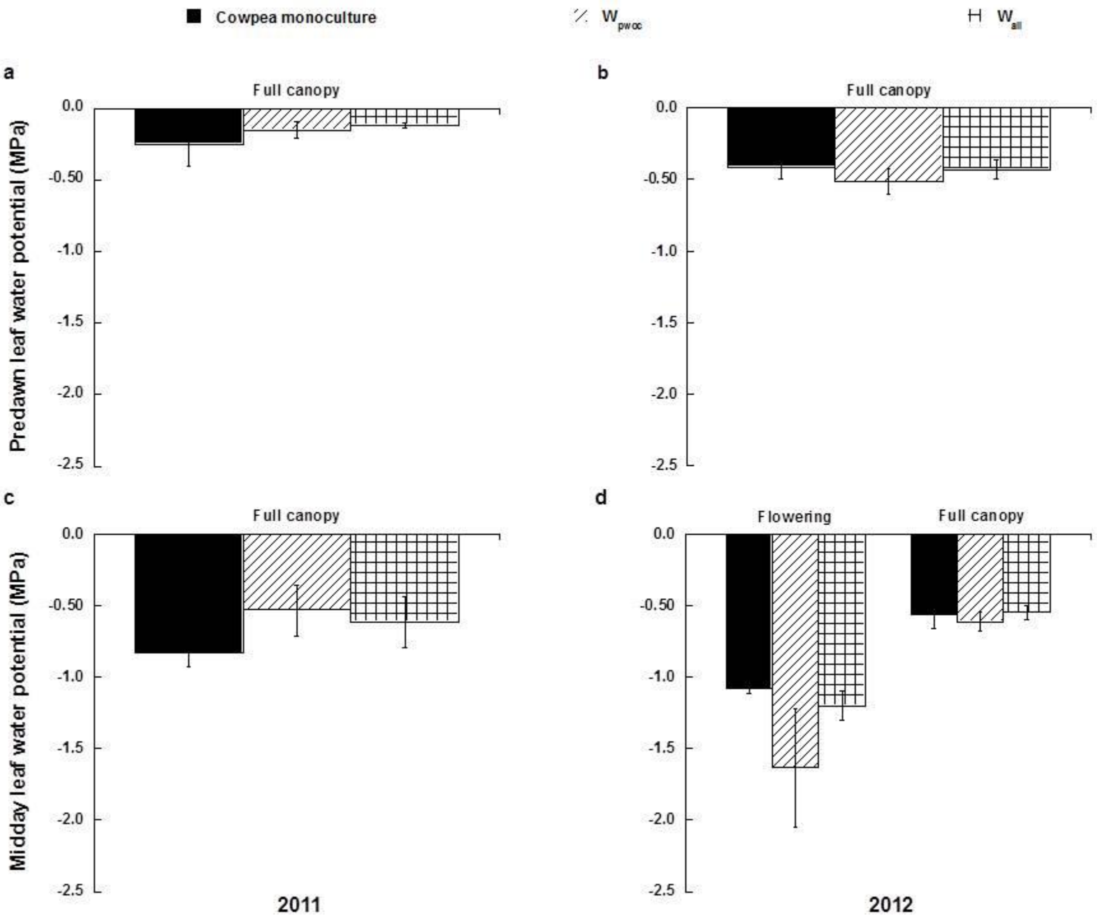

Figure A-3.10. Cowpea a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy (57-61 DAP) in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage (41 DAP) and full canopy (77 DAP) in 2012. Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

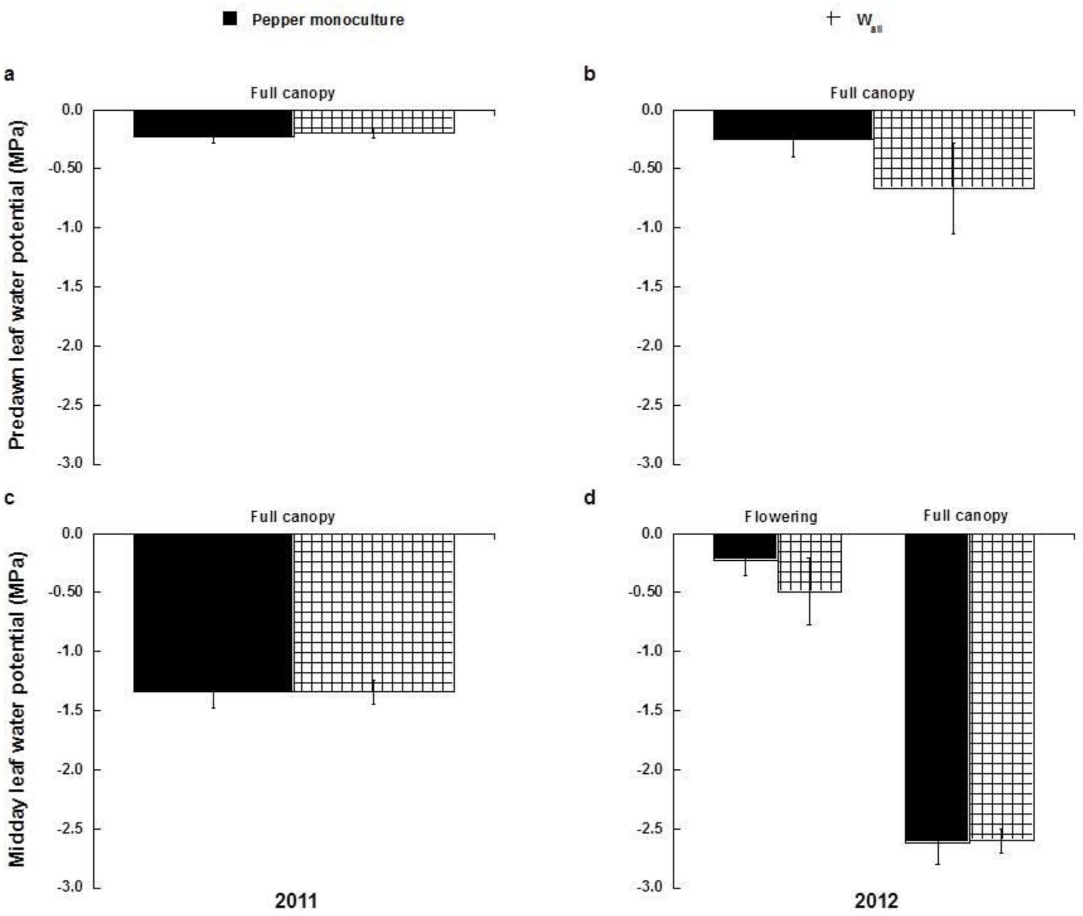

Figure A-3.11. Pepper a) predawn (Ψ_p) and c) midday (Ψ_m) leaf water potential measured at full canopy (54-58 DAP) in 2011 and b) predawn and d) midday leaf water potential measured at flowering stage (35 DAP) and full canopy (114 DAP) in 2012. Treatments described in figure A-3.1. No statistically significant differences ($P < 0.05$) were detected.

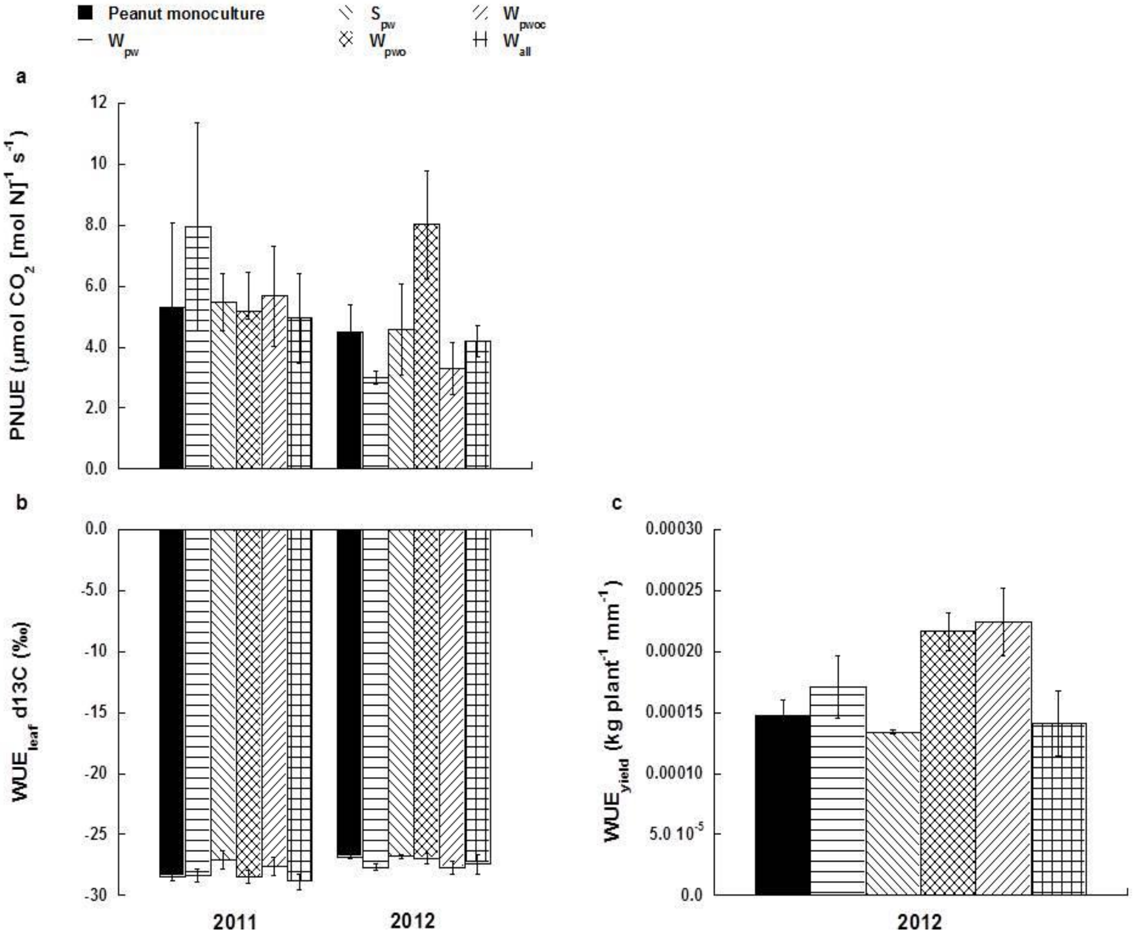

Figure A-3.12. Peanut a) photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}), b) leaf carbon isotope ratios (WUE_{leaf} d^{13}C in ‰), and c) agronomic water use efficiency calculated on a per plant yield basis per mm water input (WUE_{yield} in kg pods plant^{-1} mm^{-1}) in monoculture and mixed cropping combinations in 2011 and 2012 (W_{pw} = within row intercropping of peanut-watermelon, S_{pw} = strip intercropping with peanut-watermelon (e.g. alternating rows), W_{pwo} = within row intercropping of peanut-watermelon-okra, W_{pwoc} = within row intercropping of peanut-watermelon-okra-cowpea, W_{all} = within row intercropping of peanut-watermelon-okra-cowpea-pepper). No statistically significant differences ($P < 0.05$) were detected.

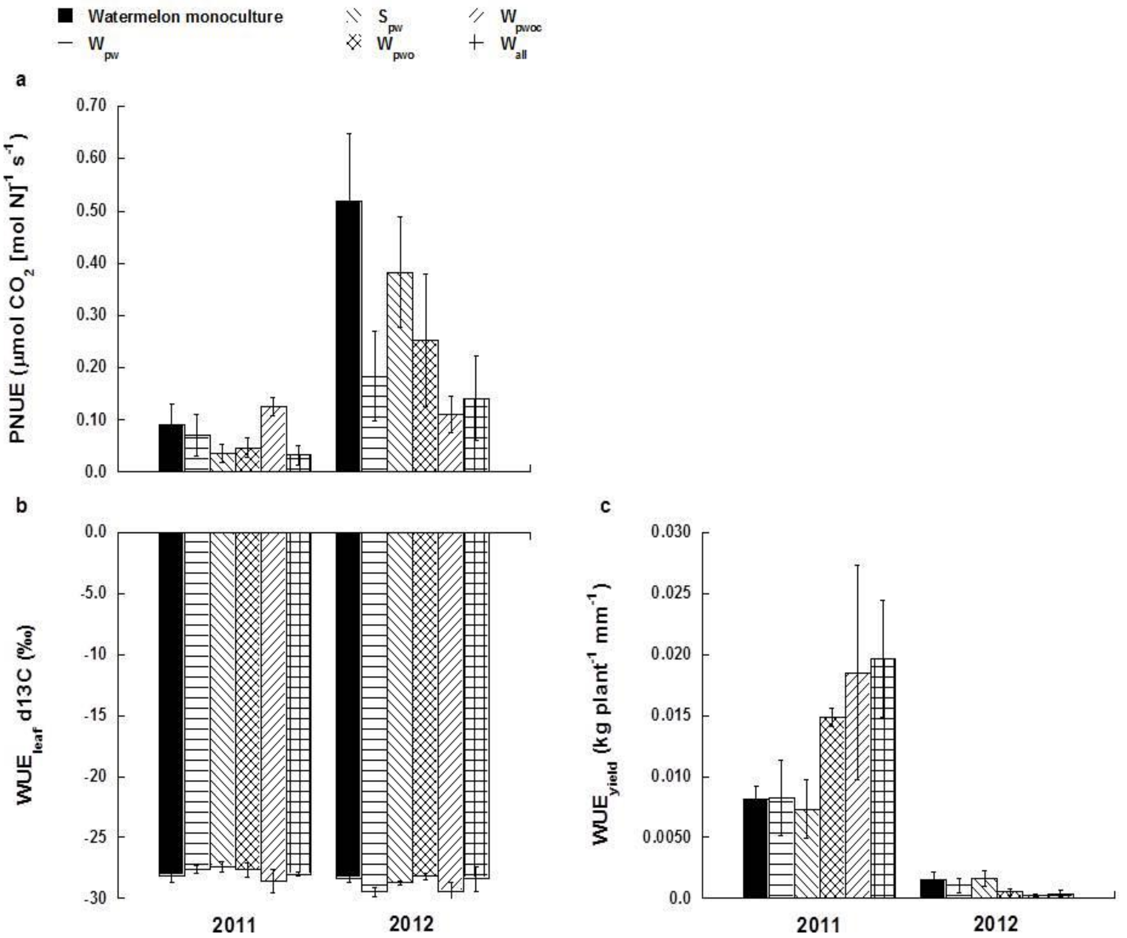

Figure A-3.13. Watermelon a) photosynthetic nitrogen use efficiency (PNUE in µmol CO_2 [mol N]$^{-1}$ s^{-1}), b) leaf carbon isotope ratios (WUE_{leaf} d^{13}C in ‰), and c) agronomic water use efficiency calculated on a per plant yield basis per mm water input (WUE_{yield} in kg fresh fruit plant^{-1} mm^{-1}) in monoculture and mixed cropping combinations in 2011 and 2012. Treatments described in figure A-3.12. No statistically significant differences ($P < 0.05$) were detected.

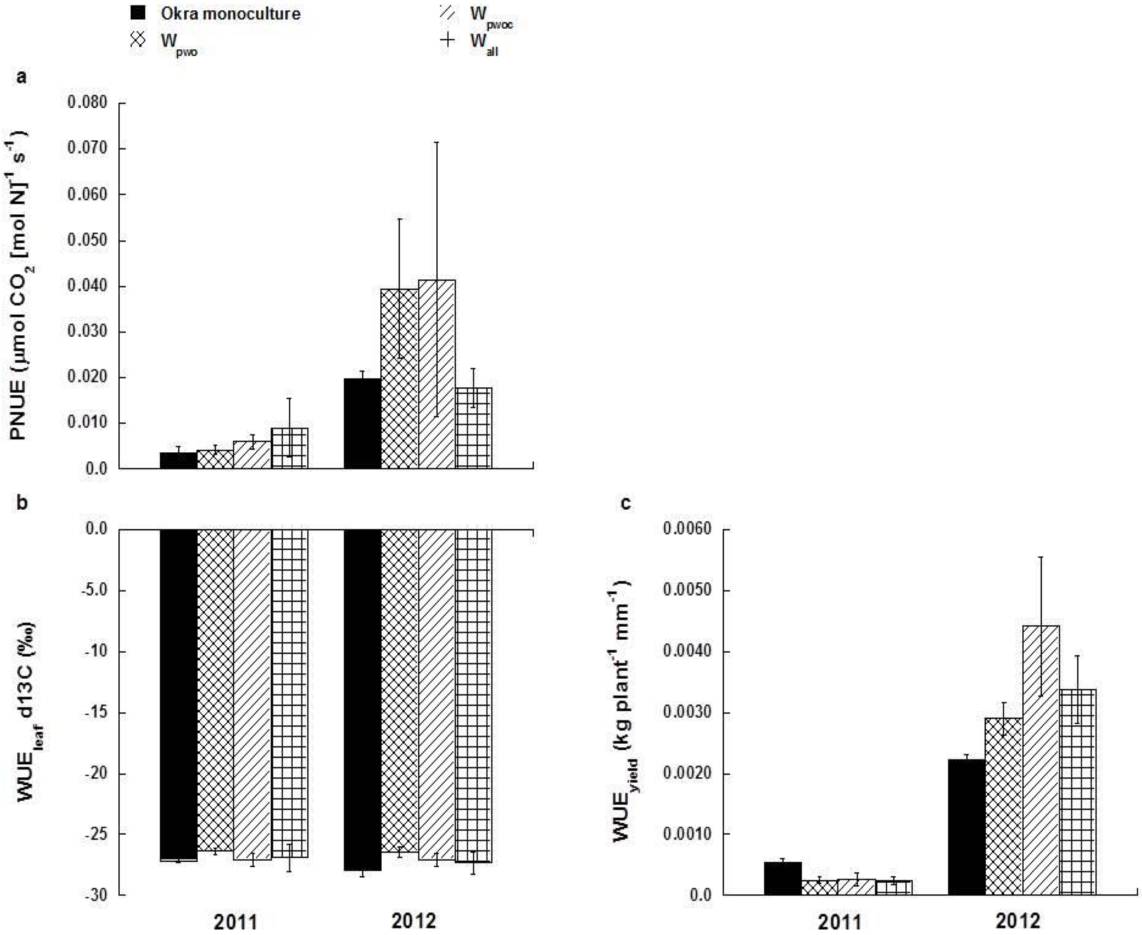

Figure A-3.14. Okra a) photosynthetic nitrogen use efficiency (PNUE in μmol CO_2 [mol N]$^{-1}$ s^{-1}), b) leaf carbon isotope ratios (WUE_{leaf} d^{13}C in ‰), and c) agronomic water use efficiency calculated on a per plant yield basis per mm water input (WUE_{yield} in kg pods plant^{-1} mm^{-1}) in monoculture and mixed cropping combinations in 2011 and 2012. Treatments described in figure A-3.12. No statistically significant differences ($P < 0.05$) were detected.

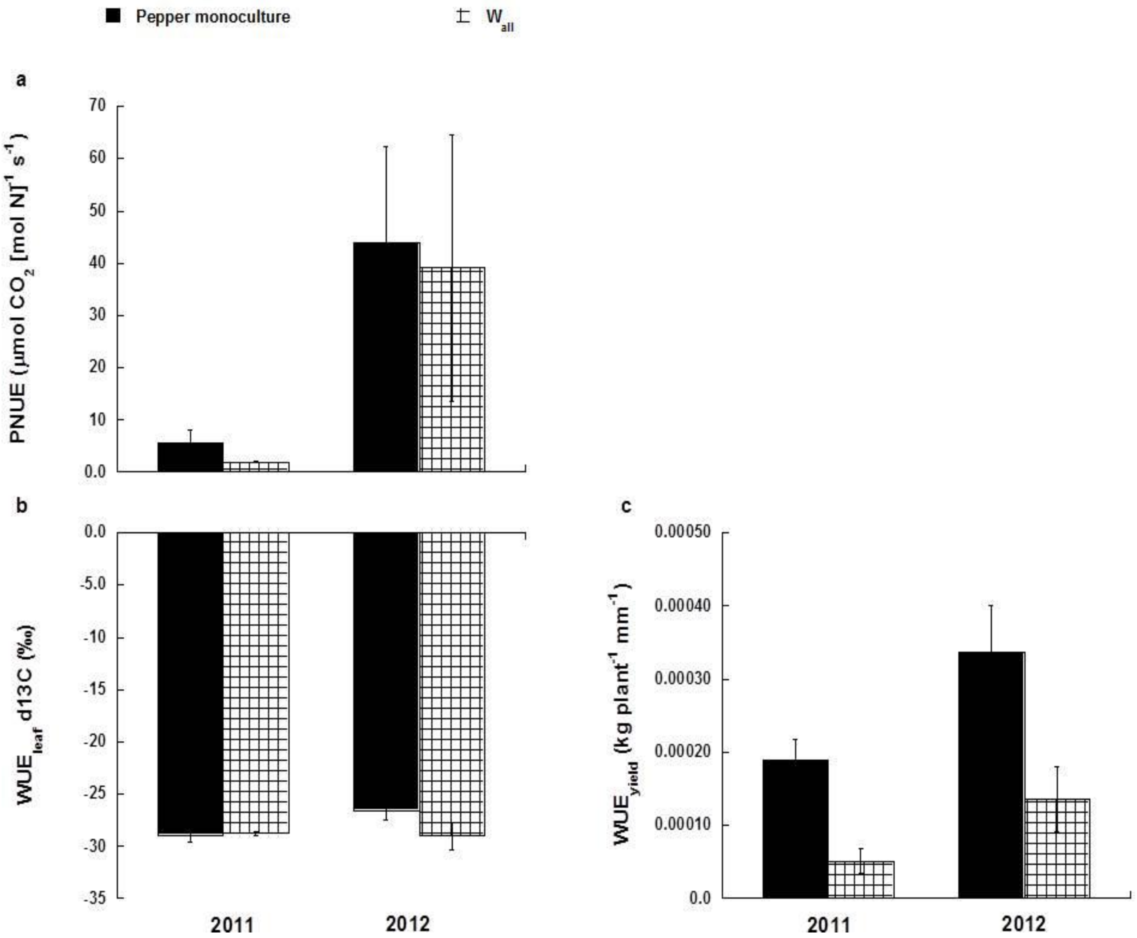

Figure A-3.15. Pepper a) photosynthetic nitrogen use efficiency (PNUE in µmol CO_2 [mol N]$^{-1}$ s^{-1}), b) leaf carbon isotope ratios (WUE_{leaf} d^{13}C in ‰), and c) agronomic water use efficiency calculated on a per plant yield basis per mm water input (WUE_{yield} in kg fresh fruit plant^{-1} mm^{-1}) in monoculture and mixed cropping combinations in 2011 and 2012. Treatments described in figure A-3.12. No statistically significant differences ($P <$ 0.05) were detected.

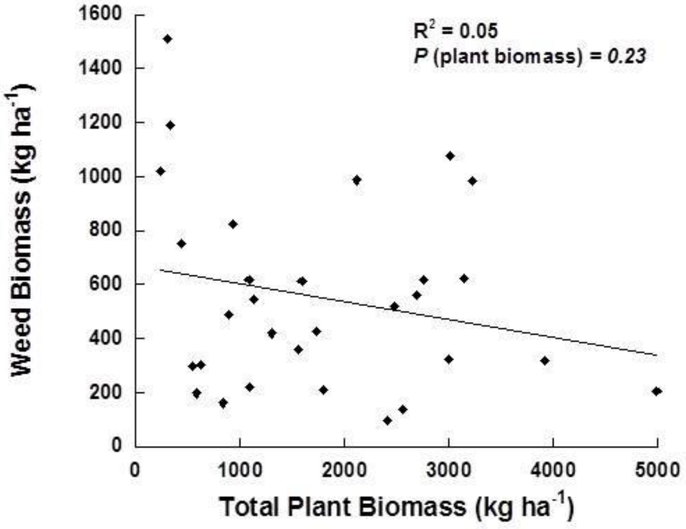

Figure A-4.1. Relationship between total above-ground plant biomass (kg ha^{-1}) and total weed biomass (kg ha^{-1}) in 2012 across all intercropping and monocropping systems. No significant linear relationship was found ($P < 0.05$). The relationship between leaf area index (LAI) and weed biomass follows a similar pattern.

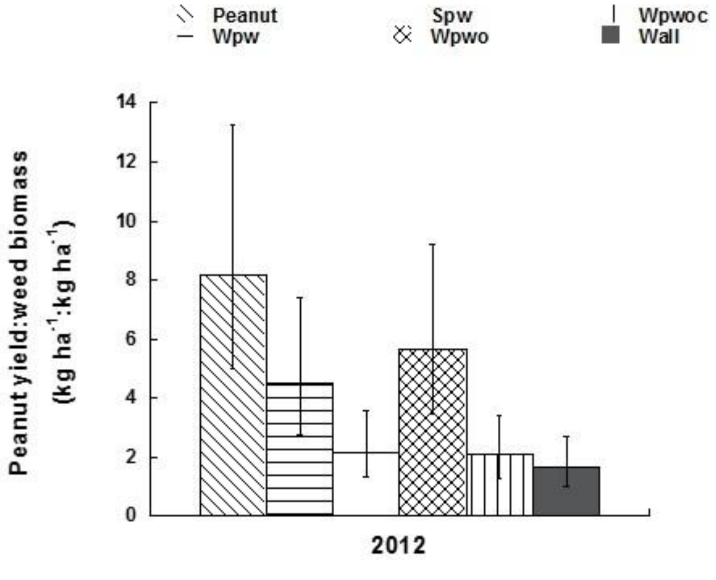

Figure A-4.2. Peanut yield (kg ha^{-1}) to total weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in 2012. There were no statistically significant differences ($P < 0.05$) between treatments.

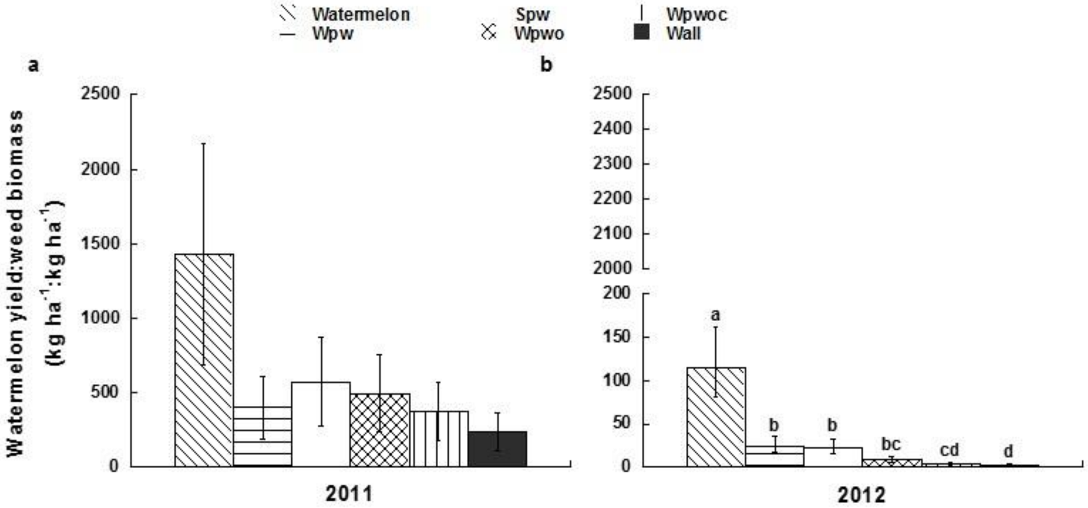

Figure A-4.3. Watermelon fruit yield (kg ha^{-1}) to total weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in (a) 2011 and (b) 2012. Different letters indicate statistically significant differences ($P < 0.05$) between means within years according to Tukey’s LSD test.

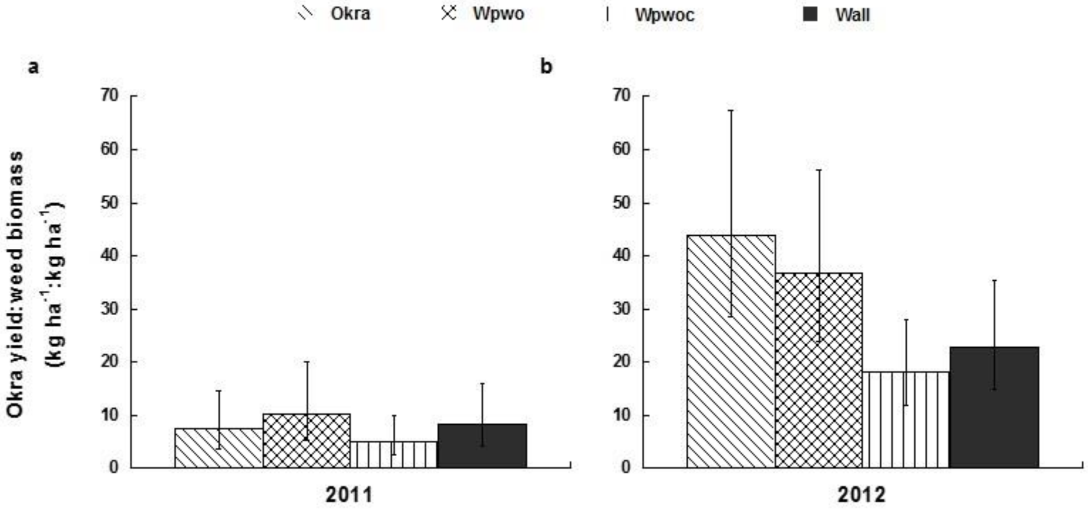

Figure A-4.4. Okra yield (kg ha^{-1}) to total weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in (a) 2011 and (b) 2012. Treatments are described in Figure 1. There were no statistically significant differences ($P < 0.05$) between treatments.

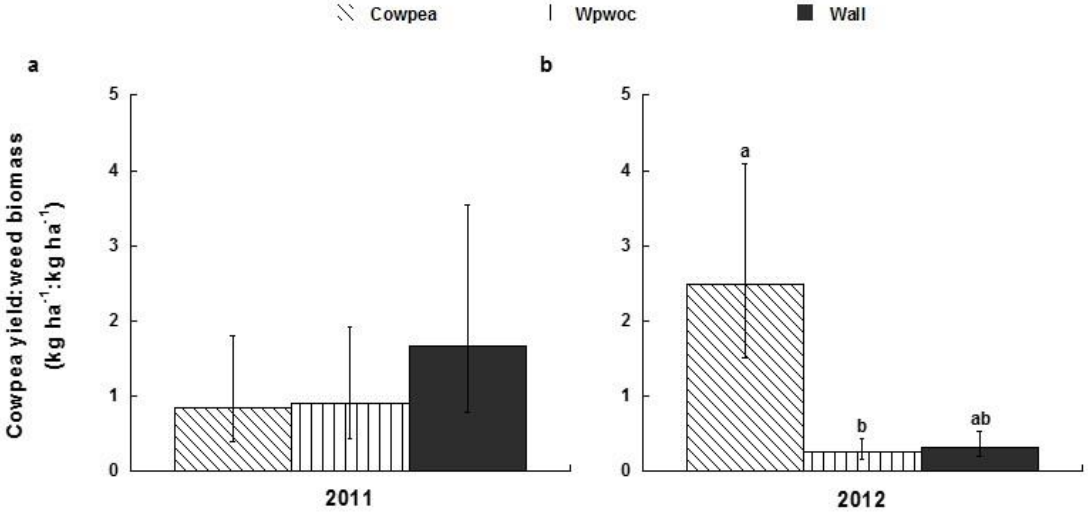

Figure A-4.5. Cowpea yield (kg ha^{-1}) to total weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in (a) 2011 and (b) 2012. Different letters indicate statistically significant differences ($P < 0.05$) between means within years according to Tukey's LSD test.

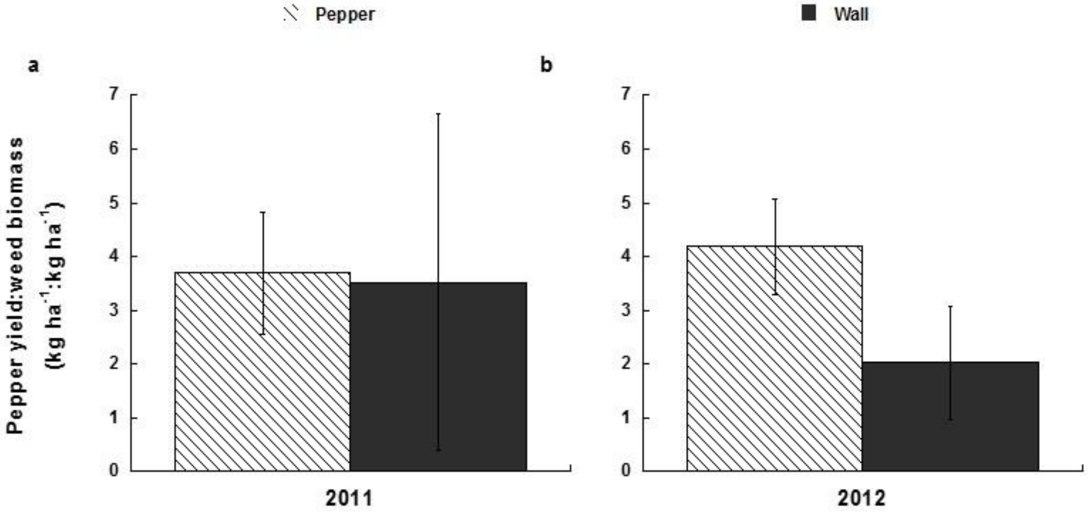

Figure A-4.6. Pepper fruit yield (kg ha^{-1}) to total weed biomass (kg ha^{-1}) ratio for each monoculture and intercropping combination in (a) 2011 and (b) 2012. There were no statistically significant differences ($P < 0.05$) between treatments.

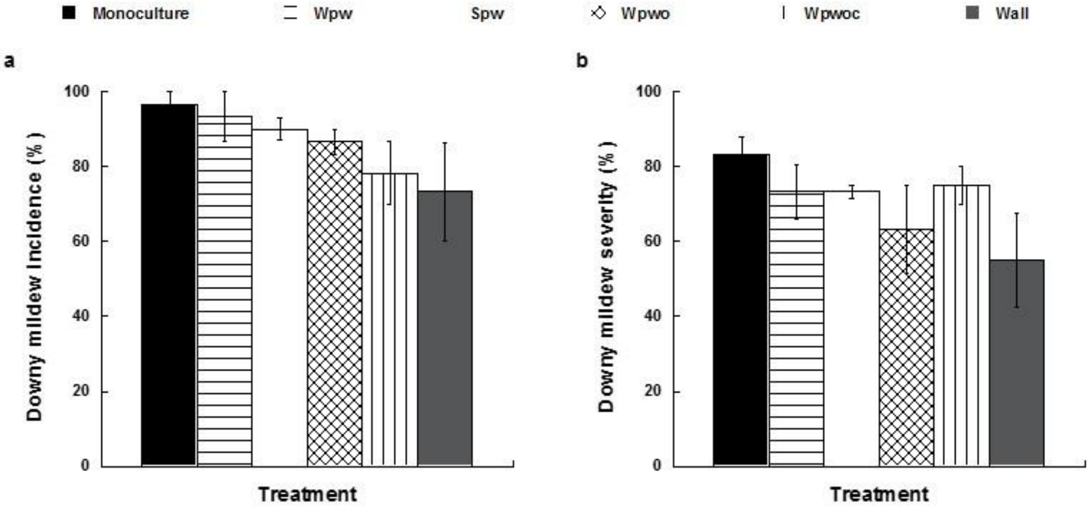

Figure A-4.7. Effect of intercropping on a) incidence and b) severity of downy mildew on watermelon in 2012. There were no statistically significant differences ($P < 0.05$) between treatments.

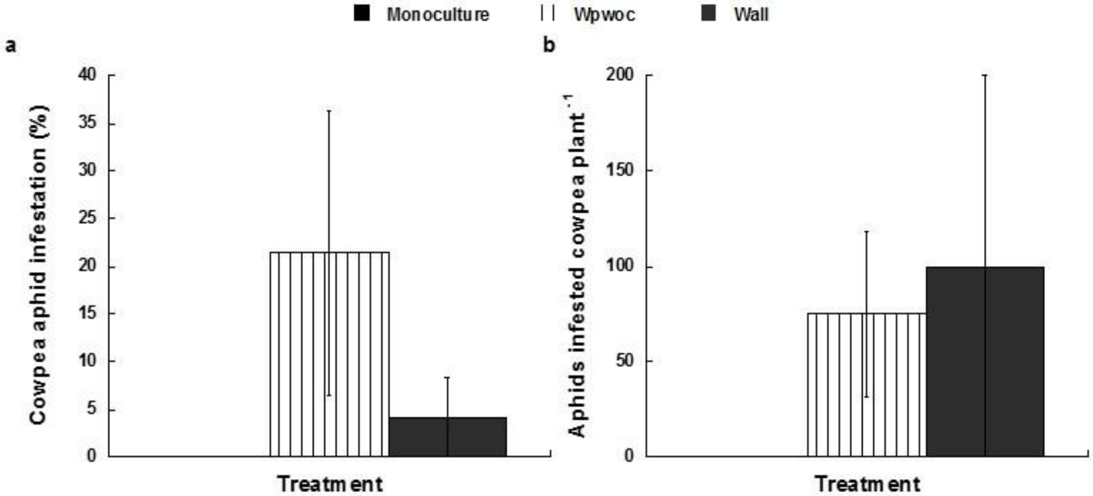

Figure A-4.8. Least squares means (LS means) and standard errors of the mean (SEM) of aphid (a) infestation and (b) density on cowpea in 2012. There were no statistically significant differences ($P < 0.05$) between treatments.

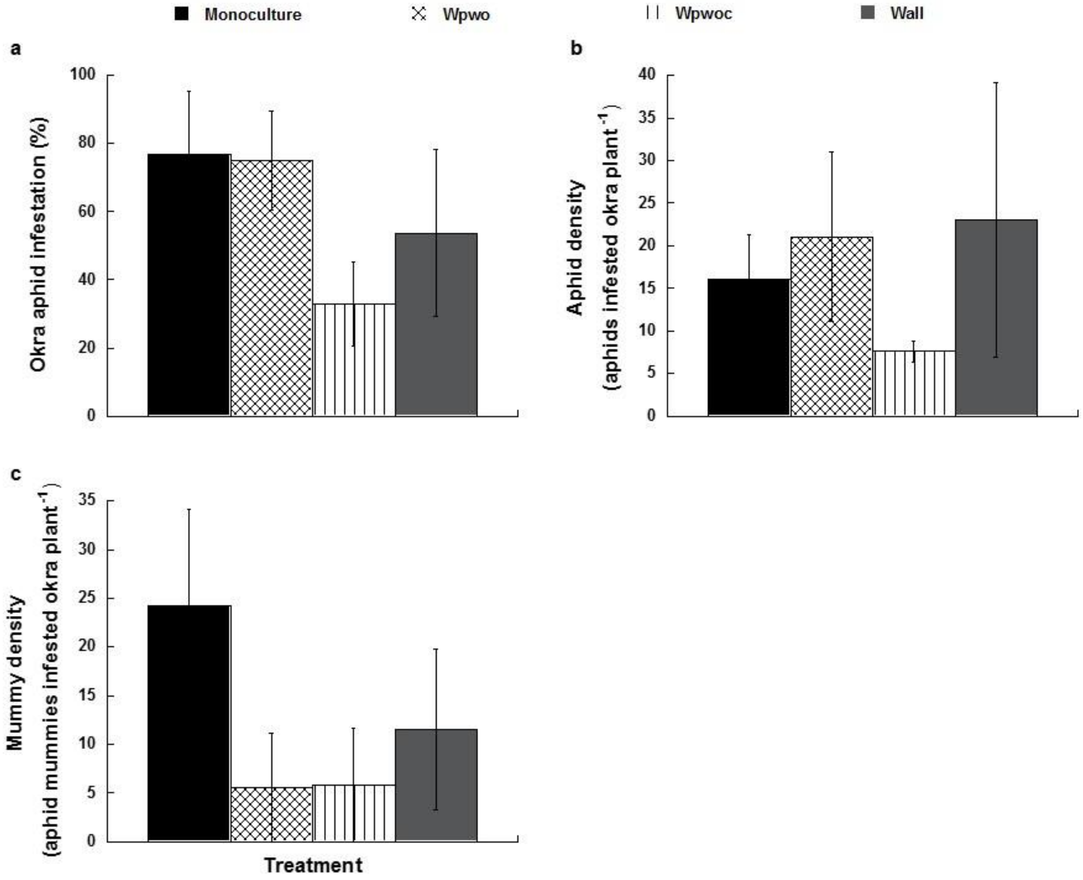

Figure A-4.9. Aphid (a) infestation, (b) density, and (c) mummy density on okra in 2012. There were no statistically significant differences ($P < 0.05$) between treatments.