USING ANNUAL SUMMER COVER CROPS TO MANAGE NITROGEN

FIXATION AND WEED SUPPRESSION IN AGRO-ECOSYSTEMS

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ABSTRACT

Cover crops perform multiple functions in agro-ecosystems, such as nitrogen (N) fixation, nutrient retention and weed suppression. However, there is often a trade-off between N fixation and weed suppression – legumes fix N but are not very weed suppressive, while non-legumes suppress weeds but do not fix N. Legume-based mixtures, consisting of species with spatially and/or temporally complementary traits, can be a strategy to effectively manage N fixation and weed suppression. The aim of this study was to: (1) evaluate the N fixation and weed suppression characteristics of eight legume and four non-legume, annual summer species that can be used in the Northeastern U.S.A, (2) evaluate the performance of legume-based mixtures, in terms of biomass production, N fixation and weed suppression, (3) evaluate the competitive ability of the different legume and non-legume species in mixtures by using a replacement series design and (4) evaluate different management strategies to effectively manage mixtures for N fixation and weed suppression. Experiments were conducted over two years, and mixtures were designed using a replacement series. In monoculture, Crimson Clover fixed the most N (111kgN.Ha-1 in 2008 and 71kgN.Ha-1 in 2009) and it was the most weed suppressive legume (12g.m^{-2} in 2008 and 20g.m^{-2} in 2009). Cowpea fixed the lowest amount of N in both years (12 kg N.Ha^{-1} in 2008 and 13 kg N.Ha^{-1} in 2009), but accessed more soil N (50 kg N.Ha^{-1} in 2008 and 58 kg N.Ha^{-1}) than any of the legumes and it was the least weed suppressive legume in 2009 (250g.m^{-2} in 2009). Regarding the non-legumes, Sorghum Sudan (938g.m^{-2} and 632g.m^{-2}) had the greatest biomass production in monoculture at high and low seeding densities (Tukey's HSD, p<0.0001), while Buckwheat was the most weed suppressive (15 and 30g.m^{-2}) species in monoculture (Tukey's HSD, p<0.0001). Buckwheat took up similar amounts of soil N than Sorghum Sudan, even though it had lower above ground biomass. In all the mixtures, except Buckwheat, the LER was generally greater

than one. The legumes in all the mixtures, except in the Buckwheat mixture, relied slightly more on N fixation than in monoculture. For non-viny legumes, the total N fixed was significantly greater in monocultures than in all the mixtures (Tukey's HSD, $p<0.05$). For the viny legumes, total N fixed in mixtures with C-4 grass species was not significantly different from the monocultures, but in Buckwheat mixtures significantly less N than monoculture was fixed (Tukey's HSD, $p<0.05$). The weed suppressive capacity of the mixtures depended on the species involved, and there was no consistent improvement in mixture weed suppression compared to the monocultures (Tukey's HSD, $p<0.05$). The competitive ability of the non-legumes can be ranked as follows: Buckwheat > Sorghum Sudan > Japanese Millet > Flax. Within non-viny legumes, the Berseem Clover was more competitive than Crimson Clover, and within the viny species Cowpea was more competitive than Soybean Tyrone and Chickling Vetch. There was a functional trade-off between N fixation and weed suppression: mixtures that are effective at suppressing weeds (Buckwheat mixtures) also suppress legumes and legumes that are competitive (Cowpea) in mixture do not fix a lot of nitrogen. In mixtures containing species with complementary growth times (Clovers and Sorghum Sudan / Buckwheat), mowing the competitive non-legumes increased legume biomass five-fold in Buckwheat mixtures and two-fold in Sorghum Sudan mixtures, while weed suppression was maintained. Nitrogen fixation increased eight- to ten-fold in mowed Buckwheat mixtures and two-to four-fold in mowed Sorghum Sudan mixtures. Mowing competitive species in temporally complementary mixtures can avoid the trade-offs in N fixation and weed suppression.

BIOGRAPHICAL SKETCH

Christiaan Burger (Burtie) van Zyl grew up on the ZZ2 family farm in the Mooketsi Valley in the Limpopo Province of South Africa. He attended Duiwelskloof Primary School from 1991-1997, after which he attended Merensky High School from 1998-2002. At Merensky his studies focused on physical science (chemistry and physics) and biology. During this time he had many conversations with two family friends, Prof Erik Holm and Prof Frederick Engelbrecht, which sparked his interest in the sciences, especially ecology. Over holidays he worked at ZZ2, where he helped farmers and agronomists with soil fertility and pest management strategies. This sparked a keen interest in agriculture, which led him to study for a B.Sc. degree at the University of Stellenbosch with Agronomy and Plant Pathology as his major subjects. During his time at the University of Stellenbosch he became increasingly interested in agricultural systems that rely on ecologically-based processes to attain agronomic outcomes. Burtie decided that doing graduate studies in the USA would be the best way to pursue his long-term career goals. Burtie got accepted in the lab of Dr Laurie Drinkwater at Cornell University, who is a world-class scientist in the ecological management of agricultural systems. He moved to Ithaca, NY in August 2007 to begin with his Masters. After the completion of his graduate studies he plans to return to ZZ2, the family farming enterprise.

I dedicate this thesis to my mother and father. Baie dankie vir mamma en pappa se raad en ondersteuning.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: LITERATURE REVIEW

1.1 Intensification of Agriculture

Increased energy inputs through fertilizer, herbicides and pesticides, has enabled agricultural systems in the twentieth century to become intensified (Auclair 1976). A consequence of agricultural intensification has been increased crop specialization (Firbank et al. 2008), where crops are mainly selected for yield (Auclair 1976). This has led to the cultivation of large-scale monocultures, that are both spatially and temporally homogeneous (Liebman and Dyck 1993), and do not rely on natural ecosystem processes for their productivity.

Although intensified agro-ecosystems have been very productive, there have also been unintended environmental consequences. For example in the past century, agricultural intensification has significantly altered the vegetation on landscapes due to a major transition from native vegetation to cropland, where the amount of crop land worldwide increased by 50% (Auclair 1976; Hartemink et al. 2008). This has led to a loss of biodiversity on agricultural landscapes. The alteration of biogeochemical cycles causes modern agricultural systems to lose large amounts of nutrients to surrounding ecosystems and there is an increase in erosion due to long fallow periods.

Crop specialization has led to reduced plant diversity in modern agricultural systems (Altieri 1999), and the low plant genetic diversity can influence herbivore, pest and microbial communities in ways that make modern agricultural systems vulnerable to changes in the biotic and abiotic environment. Cover crops, also called auxiliary crops, are non-cash crops that are grown in agricultural systems to perform agro-ecosystem functions, such as nitrogen (N) fixation, weed management, nutrient cycling and pest management. Cover crops use energy derived from sunlight through photosynthesis to perform functions that would otherwise require heavy energy expenditures (Firbank et al. 2008). Cover crops are usually grown in specific cropping

1

windows when cash crops are not grown, this is referred to as crop rotation, or they can be grown next to cash crops, this is referred to as intercropping.

1.2 How do plants affect ecosystem functioning – the importance of functional traits

A good understanding of how species and communities regulate and affect ecosystem processes is required to understand what the implications of biodiversity loss will be on ecosystem functioning (Hooper et al. 2005). Due to their key role as autotrophs with cascading effects on various trophic and spatial scales, most of this work has focused on plant diversity. There is an increasing body of evidence that suggests that the specific traits and characteristics of plant species are more important than species richness *per se* in determining ecosystem functional traits (Hooper and Vitousek 1997; Wardle et al. 1997; Hooper 1998; Diaz and Cabido 2001).

Plant species respond differently to their environments and also affect their environments in different ways. This response-effect mechanism is known as functional traits (Hooper et al. 2005). Most of the research, especially in agricultural systems, has focused on the response of plants to their environment, with little research focusing on how plants affect their environment. Plants, as ecosystem engineers, can modulate the availability of resources to other organisms by altering their biotic and abioitc environment (Jones et al. 1994; Alper 1998; Eviner and Chapin 2003; Eviner 2004).

In order to find the connection between plant traits, and ecosystem functioning, species with similar functional traits have been categorized into functional groups. Functional groups have been established using single traits, or very closely related suites of traits (Eviner and Chapin 2003). Due to the multiple traits involved in plants affecting ecosystem processes, and the fact that these traits vary independently, the relationship between functional groups and ecosystem processes has not been clear

(Keddy 1992; Lavorel et al. 1997; Eviner and Chapin 2003). This has led Eviner and Chapin (2003) to develop a functional matrix, which accounts for the multiple traits that influence and regulate ecosystem processes. By using multiple traits to construct functional groups, predictions of species effects on ecosystem processes are improved. This may be useful in applied settings, such as cover cropping in agriculture, where plants are grown to influence specific ecosystem processes.

1.3 The use of cover crops to perform specific agro-ecosystem functions

Matching cover crop traits with the functions they need to perform, and selecting cover crop populations adapted for specific regional conditions will allow them to perform functions more reliably (Wilke and Snapp 2008) and will improve the ability of managers to use cover crops as tools to perform specific functions in agro-ecosystems (Eviner and Chapin 2001).

1.3.1 The role of cover crops in nutrient cycling

Agricultural intensification has lead to a nutrient management paradigm that relies on surplus additions of N leading to increases losses of N to the environment and problems such as ground water pollution, and eutrophication of fresh and ocean water bodies (Rabalais et al. 2002; Drinkwater and Snapp 2007) An ecological management approach that aims to manipulate plant species, soil organic matter and soil microbes to improve the internal nutrient cycling capacity of agricultural systems has been proposed as an alternative nutrient management strategy.

Plants, through direct and indirect mechanisms, use plant and microbially mediated processes to cycle nutrients. Cover crops, through diverse rotations and permanent soil cover, have been shown to reduce nutrient losses in agricultural systems, when compared to the application of inorganic fertilizers or when fields were left fallow (Drinkwater et al. 1998; Tonitto et al. 2006; Drinkwater and Snapp 2007).

1.3.1.1 Indirect mechanisms of nutrient cycling

The indirect mechanisms of managing nutrients involve the interplay between plants and soil microbial communities. Plants fix light energy through photosynthesis, and some of this energy is made available to the soil ecosystems through root exudates (El-Shatnawi and Makhadmeh 2001; Clayton et al. 2008), rhizo-deposition (Paterson 2003; Paterson et al. 2006; Paterson et al. 2007) or when the plants are incorporated into the soil. The rhizosphere is consequently a region of enriched microbial activity (Drinkwater 2006). The release of energy into the soil from plant roots has a "priming" effect on the metabolism of soil microbes, and since they control numerous soil processes, such as litter decomposition, mineralization, immobilization, nitrification, and de-nitrification, the increased microbial activity makes nutrients available to the plant. The interplay between nutrient release due to increased microbial activity and, plant uptake and microbial immobilization allows the nutrients to be cycled very tightly.

Variation in plant traits, reflected in differences in the quality and quantity of organic matter and the chemical composition of root exudates, allows plant species to affect the soil microbial community in different ways (Meier and Bowman 2008). Organic matter quality is determined by the C: N ratio and the specific chemical compounds that are present. Plant biomass with high C: N ratios and greater concentrations of recalcitrant nutrients, such as woody plants, mineralize at a slower rate than plants with low C: N ratios, such as herbaceous legumes (Sall et al. 2007). Organic material with a low polyphenol and lignin content mineralizes at a faster rate than organic matter with low levels of these compounds (Sall et al. 2007).

1.3.1.2 Direct mechanisms of nutrient cycling

Some plants have developed direct mechanisms for nutrient acquisition, such as biological N fixation (BNF). Leguminous plants form a symbiotic relationship with

Rhizobia bacteria to supply the plant with N in exchange for energy in the form of photosynthetically fixed carbon. The relationship between *Rhizobia* and the plant species can become parasitic if there is high available N in the soil system (Johnson et al. 1997), in which case the legumes will limit oxygen diffusion to non-mutualistic symbionts (Kiers et al. 2003). This mechanism allows the plant to regulate its nutrient acquisition strategies. In cases where the soil N is low, plants will use direct mechanisms, such as BNF, to acquire N.

Cropping systems that are dependent on legumes for their primary source of nitrogen therefore have a built-in internal feedback mechanism, because as the availability of soil nitrogen increases, symbiotic nitrogen fixation is reduced. This mechanism prevents soils from becoming nitrogen saturated which will reduce the nitrogen losses from the system. An additional advantage of BNF is that it uses sunlight energy through photosynthesis to fix atmospheric nitrogen, a process that is very energy intensive to perform industrially and which generates significant greenhouse gas emissions.

Despite the advantages of BNF, there remain many challenges to its effective management in agricultural systems: (1) There is little information on the amount of N that gets fixed from legumes and only broad estimations are used in extension sources (Peoples and Craswell 1992; Clark 2007), (2) Little research has been done on the effect of management strategies on BNF, (3) Many of the legume cover crops that are used in agricultural systems have not been characterized in terms of their nitrogen fixation traits, (4) Nitrogen fixation in agricultural systems is complex and involves interactions in the soil environment, the plant species and the rhizobium strains (Hardarson 1993), this complexity is reflected in the variability in field measurements of biologically fixed nitrogen (Carlsson and Huss-Danell 2003).

1.3.2 The role of cover crops in weed management

Temporal (crop rotations) and spatial (intercropping) cover crop diversification has been shown to suppress weeds (Liebman and Dyck 1993a). Weeds can be suppressed by cover crops through competition for resources (i.e. sunlight, space, nutrients and water (Brainard et al. 2005)) and allelopathy (Liebman and Dyck 1993a; Weston 1996).

1.3.2.1   Weed control through resource competition

Soils with high plant available nutrients, especially N, tend to favor weed populations and often support more abundant and diverse weed communites (Qasem 1992; Blackshaw et al. 2003; Blackshaw and Brandt 2008). Cover crops that rapidly take up resources (especially soil nutrients, water and light) and make them unavailable for weeds, can effectively suppress weeds (Yin et al. 2006). For example, buckwheat controls weeds through rapid soil nitrogen uptake during growth or by temporarily immobilizing soil N when it gets incorporated (Kumar et al. 2008). Sorghum Sudan and *Brassicas*, have been known to have deep roots that develop rapidly, which allows them to out-compete weeds for limited soil resources (Wang et al. 2008).

Competition for light, especially at weed emergence, is an important way of controlling weed populations (Creamer and Baldwin 2000). Soil shaded by a cover crop, receives reduced levels of light, particularly in the red: far-red range, which affects the morphology, phenology and germination of weeds. Reductions in available light for Powell Amaranth lead to stem elongation, while the germination rates of viable seed were reduced by 50% (Brainard et al. 2005). This is important, since even an agronomically insignificant number of weeds, can produce large amount of seed (Jordan 1996). The ability of cover crops to suppress weeds through light competition largely depends on the factors, such as temperature and moisture and seed size, that

affect the emergence of the cover crop and the weeds (Mohler 1996; Brainard and Bellinder 2004). For example, low temperatures at germination improved winter rye suppression of Powell Amaranth (Brainard and Bellinder 2004). Biomass production *per se*, is not a reliable indicator of the shading capacity of a crop. Den Hollander et al. (2007) found that although Persian clover, Berseem Clover and Crimson Clover had the same biomass, Persian Clover covered the soil surface fastest and was able to suppress weeds most effectively.

1.3.2.2   Weed control through allelopathy

Concerns regarding the environmental impacts of synthetic chemicals and fewer available herbicide products have caused an increased interest in allelopathy as a biorational way to control weeds (Weston 1996). Although allelopathy has long been identified as a mechanism plants use to interfere with neighboring plants, the value of this mechanism for weed control in agricultural systems has received attention relatively recently (Belz 2007a).

Cover crops exude a diverse range of allelochemicals that can affect the growth of neighboring weeds. Allelochemicals that have been isolated from a number of cover crops species are summarized by (Weston 1996). Some examples include: the fatty acids associated with buckwheat (Tsuzuki et al. 1987); the phenolic acids, dhurrin, sorgoleone, ρ-hydroxy benzaldehyde, and ρ-hydroxy benzoic acid associated with sorghum-sudan grass (Weston 1996; Belz 2007b) and the isoflavanoids and phenolics associated with *Trifolium spp*. Cover crops can be managed in two ways to utilize allelopathy: through living cover crops / green manures that actively obstruct the growth of immediate weeds and release allelopathic substances, and through crop residue and decomposing organic matter that release allelochemicals over time (Kruidhof et al. 2009).

There are a number of factors that need to be taken into account when using allelopathy to suppress weeds: (1) Allelopathy biosynthesis and release is a dynamic process, so there are limited time frames when allelochemicals are synthesized and detectable (Belz 2007a). (2) Allelopathy is an inducible phenomenon and can be induced by both biotic and abiotic factors (Belz 2007a). Biotically induced allelopathy has been found in *O. sativa* where allelochemical production was induced by the presence of *E.crusgalli*. (3) The effectiveness of allelochemicals in the soil depends on the response of the allelochemicals to the soil conditions. The interaction of the allelochemicals with soil organic matter, clay particles, microbes and soil moisture affects the turnover rate and toxicity of allelochemicals (Weston 2005a). (4) Knowledge about the target species is important (Kruidhof et al. 2009), small-seeded annual weeds are especially susceptible to suppression by decomposing cover crop residues, especially in the presence of phenolic substances (Weston 2005a)

1.4 Legume-based mixtures - A strategy to control weeds and manage N fixation

Cover crop mixtures, the practice of growing two or more plant species on the same piece of land at the same time, are increasingly being recognized as a practice that can contribute to the development of sustainable agricultural systems. In both the agronomic and ecological literature, mixtures involving legumes have been shown to be very productive when N was the limiting resource, especially mixtures that also contain C-4 grass species (Jensen 1996; Hooper and Vitousek 1997; Hauggaard-Nielsen and Jensen 2001a; Li et al. 2001b; Temperton et al. 2007; Fornara and Tilman 2008). Legume-non-legume mixtures are more productive because of the greater exploitation of the soil profile (Schmidtke et al. 2004) and the reduced competition for resources, especially for soil N.

Plants in mixtures develop differently to plants grown in sole crops. Competition between plants cause both plants' roots to go deeper and more laterally into the soil profile (Hauggaard-Nielsen et al. 2001). Both plants consequently occupy a greater soil volume which leads to the more efficient use of the available soil resources. The intermingling of roots in crop mixtures has led to optimal resource utilization and improved plant nutrition in various mixtures (Thorsted et al. 2006), such as maize / faba bean (Li et al. 1999; Li et al. 2003) and wheat / maize mixtures (Li et al. 2006). Through complementarity, legume-based mixtures are able to utilize soil resources (especially soil N) and sunlight (when component crops occupy different canopy layers) efficiently, which also improves resource use efficiency (Hauggaard-Nielsen and Jensen 2001a; Malezieux et al. 2009) . The improved resource use efficiency in mixtures has two agro-ecological advantages: (1)less nutrients will get leaked out of the system since they are taken up in plant biomass and (2) the efficient utilization of resources limits the resources available to weeds (Liebman and Dyck 1993b; Szumigalski and Van Acker 2005; Saucke and Ackermann 2006). Therefore, crop mixtures are able improve nutrient retention and reduce weed species.

Legume-based mixtures can also be used as a strategy to manage N fixation. Non-legumes, such as cereals and grasses, are more competitive in acquiring soil nitrogen than most legumes (Hauggaard-Nielsen et al. 2001). Sole cropped legumes can be inefficient at recovering soil inorganic N, a phenomenon known as the spared N effect (Evans et al. 1989; Evans et al. 1991; Herridge et al. 1995; Hauggaard-Nielsen et al. 2001b; Reiter et al. 2002; Hauggaard-Nielsen et al. 2003; Schmidtke et al. 2004). Competition for soil nitrogen, increases the legume's reliance on atmospheric nitrogen, and a greater proportion of total legume N is consequently obtained from the atmosphere (Anil et al. 1998; Carr et al. 1998; Corre-Hellou et al.

2006). The absolute amount of nitrogen that is fixed (kg N.ha^{-1}) depends to some extent on the biomass of the legume species in the mixture (van Kessel and Hartley 2000; Corre-Hellou et al. 2006). Mixtures dominated by non-legumes will have less total N fixed due to the reduction in legume biomass (Corre-Hellou et al. 2006). Legume-based mixtures have been found to increase the internal retention of N while maintaining high rates of N fixation, relative to monocultures (Palmborg et al. 2005; Corre-Hellou et al. 2006). Therefore, crop mixtures can be used to successfully manage N fixation and N retention.

Non-legumes perform certain functions more effectively than legumes. For example, grasses are effective at weed suppression and erosion control (Gallandt et al. 1999) and *Brassicas* excel at capturing nitrate to reduce leaching (Kristensen and Thorup-Kristensen 2004). Cover crop mixtures are used to perform multiple functions simultaneously. Drinkwater and Brainard (*in preparation*) found that there is a trade-off between the weed suppressive capacity and N fixation capacity of legume-based mixtures. Mixtures dominated by aggressive non-legumes, such as Buckwheat and Sorghum Sudan, suppress weeds effectively but fix very little N (Kumar et al. 2008). Legume dominated mixtures fix more N, but are often not able to effectively suppress weeds. Legume species also differ in their responses to mixtures, and the responses also vary depending on the species they are mixed with. Cowpea fixed more nitrogen in mixtures with Japanese Millet than it did in monoculture and in mixtures with Sorghum Sudan, while Forage Soybean fixed more N in monoculture than in mixtures with either Japanese Millet or Sorghum Sudan (Drinkwater and Brainard, *in preparation*). Designing mixture combinations that have optimal N fixation and weed suppressive capabilities would improve cover crop effectiveness in agro-ecosystems.

1.5 Interactions in crop mixtures

1.5.1 Interference

Interference is defined as an interaction between two plants that reduces the fitness of one or both species (Vandermeer 1989), or the reduction in plant growth over time due to the presence of another plant (Weston 2005a). The two major ways in which plants interfere with each other is through competition for limiting resources and allelopathy.

1.5.1.1 Resource competition

Since all plant species require water, nutrients, light and space for growth and these resources are required at roughly similar amounts, basic physiological principles suggest that plants will compete when grown close to each other and that would lead to reduced biomass production (Vandermeer 1989). Plants that rapidly acquire resources tend to grow more rapidly and dominate mixtures and as a result, the total biomass produced in mixture is most strongly influenced by the species that is most productive as a sole crop (Hauggaard-Nielsen and Jensen 2001a; Andersen et al. 2004; Andersen et al. 2007). The intensity of competition between plant species for resources is influenced by three factors (Corre-Hellou et al. 2006; Malezieux et al. 2009): (1) The plants' resource acquisition capacity, (2) The plants' resource use efficiency, and (3) The plants' demand for the resource and the availability of the resource.

A plant's below-ground resource acquisition capacity is largely determined by root system characteristics such as morphological plasticity (root location in space and time), root length density, root depth, production of enzymes, surface area and physiological plasticity (rate of nutrient uptake in relation to enzyme functioning) (Casper and Jackson 1997; Li et al. 2006; Malezieux et al. 2009). Species that have fast root growth become more competitive for soil resources. Fast root growth by

barley allowed it to outcompete pea for phosphorous (P) (Hauggaard-Nielsen et al. 2001), and N (Li et al. 2006; Corre-Hellou et al. 2007).

A plant's competitive abilities can be assessed based on the trade-offs that occur between the energy investment in different fitness traits, such as vegetative biomass production or reproductive capacities (Aarssen and Keogh 2002). Resource use efficiency, the ability of a plant to transform resources into biomass, tends to be important for a plant's immediate competitive ability (Nijs and Impens 2000). For example, differences in barley and pea's resource use efficiency lead barley to be dominant (Andersen et al. 2007). There are trade-offs between a plant's ability to take up nutrients efficiently and a plant's ability to conserve nutrients and use them efficiently. In some environments species with higher nutrient uptake abilities have been out-competed by species that have lower nutrient loss rates (Berendse 1994).

The supply and demand for resources also impacts competition between plants. When there is an excess demand for resources relative to supply, the intensity of the competition increases (Aarssen and Keogh 2002). Competition for soil N increases when both crops have increased vegetative growth, which leads to a increase in N demand (Corre-Hellou et al. 2006). It has been hypothesized that mobile soil nutrients, such as nitrate, will be subject to earlier competition than the more immobile nutrient such as P (Bray 1954). Due to the intense competition for mobile nutrients, a fast growing root system can have a competitive advantage since it can access nitrate-rich zones sooner.

1.5.1.2 Allelopathy

Allelopathy is another mechanism that plants use to interfere with each other when grown together. Allelopathy is defined as a mechanism of interference that acts through the release of plant-produced secondary metabolites (Weston 2005a). Plants can also release nutrients to outcompete neighboring plants, through elemental

allelopathy (Morris et al. 2009). This occurs when a specific plant can tolerate higher levels of a particular nutrient and then release the nutrient into the rhizosphere and consequently have an allelopathic effect on its neighbor. The following nutrients have been found to be released during elemental allelopathy– heavy metals, soluble salts and elemental sulphur (S) (only in aquatic systems) (Morris et al. 2009).

All plants contain allelochemicals in their roots, shoots, leaves and seeds. Plants release allelopathic chemicals through the decomposition of residues, volatilization, root exudation and also from pollen (Khanh et al. 2005; Weston 2005b). When allelochemicals get released at sufficient quantities into the rhizosphere, they can suppress neighbouring plants directly, while some allelochemicals need to be modified through microbial activity before they are effective (Weston 1996).

Allelopathy is used by invasive species, such as *Centaurea spp.* (Fortuna et al. 2002) and *Artemisia vulgaris* (Barney and DiTommaso 2003), to outcompete native plant species (Hierro and Callaway 2003; Ens et al. 2009; Jarchow and Cook 2009). Allelopathy therefore has profound consequences for plant community composition and it can affect ecosystem functioning, in that it displaces the native plant community and establishes monocultures of the invading plant (Weston 2005b).

1.5.2 Positive interactions – complementarity and facilitation

Even though competition often occurs between plants grown close together, differences in plant functional traits, the plasticity of plants (Hodge 2004) and a heterogenous soil environment often lead to interactions other than competition (Casper and Jackson 1997; Eviner and Chapin 2003; Thorsted et al. 2006; Brooker et al. 2008). In crop mixtures, over-yielding, can be explained by complementarity and facilitation (Vandermeer 1989; Gross and Cardinale 2007). Complementarity refers to situations where two species use the same resource in a different space or time whereas facilitation occurs when the presence of one species alleviates an

environmental constraint that limits the growth of the other species (Hooper et al. 2005).

1.5.2.1 Complementarity

The traits of plants grown close together are important in determining the type of interactions that dominate. There are many examples where the compatibility in species traits has led to complementary resource use. Legumes and non-legume mixtures are complementary when N is the limiting resource (Hooper et al. 2005). The greater reliance of legumes on N fixation when grown in mixtures leads to reduced competition for soil N, which allows the mixture to be more productive. Combining plant species with distinct rooting patterns which exploit different parts of the soil profile also tend to result in greater productivity compared to monoculture. Cases of horizontal root differentiation (von Felten and Schmid 2008) or vertical root differentiation (Berendse 1982; Fargione and Tilman 2005) have been reported. Species with different growth types, which occupy different canopy heights, have been shown to use light complementarily in space while species with different growth patterns, which grow at different times of the year, use the same resources at different times (Anten and Hirose 1999; Werger et al. 2002).

1.5.2.2 Facilitation

In plant mixtures, it is possible for one plant species to alter the soil characteristics in ways that facilitate the growth of one or more of the component species (Eviner and Chapin 2003). A number of mechanisms that enable one species to facilitate the growth of another plant species have been identified. For example, through the release of phyto-siderophores, maize is able to mobilize Fe (III) and improve the Fe nutrition of peas (Zuo et al. 2000; Zhang and Li 2003; Inal et al. 2007). In pea-barley mixtures, pea lowered the rhizosphere pH and made S more available to barley (Aarssen and Keogh 2002; Andersen et al. 2007). Mobilization of organic P by

maize improved P uptake by faba bean (Li et al. 2003; Li et al. 2003). The release of acid phosphatase by chickpea and peanut increased the P uptake of wheat (Li et al. 2003) and barley (Gunes et al. 2007).

1.5.3 Summary of mixture interactions

The various positive and negative interactions in mixtures occur simultaneously (Li et al. 2001b). Complementarity and facilitation for some resources, and competition for others can occur in the same mixture (Zhang and Li 2003; Ghosh et al. 2006). The success of a specific crop mixture depends on which interactions, positive (complementarity and facilitation) or negative (competition) dominate and this is determined by which resources are limiting.

Increased productivity in a mixture compared to the component monocultures could be due to either low competition or a high degree of complementarity or facilitation. For example, over-yielding occurs in wheat and maize/soybean mixtures, because the intra-specific competitive interactions between the more competitive wheat plants in the sole crop are greater than the inter-specific interactions that occur in the intercrops where the wheat is grown with the less competitive maize and/or soybean (Li et al. 2001). Similar results were obtained for pea and barley mixtures, where pea had relatively low intra-specific competition and barley had high intra-specific competition, this caused barley to dominate the intercrops (Corre-Hellou et al. 2006).

Complementarity in the peak growth times of the component species in a mixture can also increase productivity. The competition between species with different growth times tends to be low. The competitive recovery principle, which is often used in agriculture, relies on this principle. This occurs when a dominant species in an intercrop (wheat) gets harvested sooner than the sub-ordinate species of the intercrop (soybean or maize), the sub-ordinate species then starts to recover by taking up more

15

nutrients and increasing its biomass production (Li et al. 2001a; Zhang and Li 2003). The increase in biomass production by the sub-ordinate species is the result of reduced competition in the mixture through successional complementarity.

1.6 How do we investigate and evaluate mixtures?

1.6.1 Paradigm shift in mixture studies

The questions asked about mixtures occur at different scales, such as yield and biomass production, plant species' interactions, and agro-ecosystem function / sustainability issues. To investigate these questions, a variety of experimental designs are required. A lack of cohesion between questions posed, experimental structure used (design, and indicators), the definitions of species interactions and selection of response variables, has hindered the progress in understanding crop mixtures (Connolly et al. 2001).

(Connolly et al. 2001) found that recent intercropping studies have the following attributes: (1) The experimental structure consists of indicators that measure the performance of an intercrop only once, usually at final harvest, (2) the measurements in these experiments tend to focus on yield related measures and economic parameters such as, biomass production, yield and forage quality, and (3) these experiments are mostly concerned about the way management practices impact the intercrop and do not investigate the dynamic nature of intercropping systems.

Connolly et al. (2001) recommend that studies of mixtures should be broadened to accomplish multiple outcomes. Some of the outcomes of these studies could be to elucidate the interactions between plants grown in close proximity, to measure the performance of mixtures over time to gain insights into the dynamism of the inter-specific interactions, and to use multiple indicators to gain a full understanding of the performance of the intercrops. Static measurements of intercrop output would not be able to give enough information about the dynamism in mixtures.

Multiple measurements, such as sequential harvests, are required in order to investigate the interactions between plants (Connolly et al. 1990; Andersen et al. 2004; Andersen et al. 2007).

1.6.2 Experimental designs used in studying mixtures

A challenge associated with developing productive mixtures is the determination of appropriate seeding rates/densities and seeding proportions for the component crops. The seeding densities for cover crops grown in monoculture have been well established (Bulson et al. 1997; Hauggaard-Nielsen et al. 2006), while mixture rates have not. Changes in the relative frequency of the component species and the total plant density affect the competitive dynamics, biomass production, weed suppression, yield and the percentage and amount of nitrogen obtained from the atmosphere (Willey and Osiru 1972; Weigelt and Jolliffe 2003; Hauggaard-Nielsen et al. 2006). Different experimental designs are used to establish the seeding rates for the mixtures, the most commonly used designs include the pairwise design, replacement series (substitution series), additive series and the response-model design (Connolly et al. 2001).

The simplest design is the pairwise design which consists of a single mixture that is repeated across different levels of a specific factor (Connolly et al. 2001). This design can be used to evaluate the performance of a specific mixture across an abiotic gradient, at different sites or at different management practices. It does not provide sufficient information to evaluate the dynamic interactions in mixtures.

In additive designs the density of one species, the target species, is kept the same while the density of the other species is varied (Park et al. 2002). This design has been used in studying intercropping on smallholder farms, where the yield of the target species (the cash crop) is very important. The additive design leads to a total

plant density that is higher than either of the crop monocultures. The additive design is limited in the inference that can be made about plant interactions (Inouye 2001).

In replacement series designs the total plant density for the mixtures and monocultures stay the same, but the relative frequency of the component crops in the mixtures is varied. The advantage of using the replacement design is that it can be used to determine the relative aggressivity of the component species in the mixture. Although the replacement design is the most used design, there have been a number of criticisms leveled against it. Although total plant density of the plots remain constant, the densities of the component crops get confounded (Park et al. 2002), this limits inferences that can be made about species interactions. Furthermore, estimated coefficients may depend on the total stand density (Vanclay 2006) and it is difficult to distinguish between intra-and inter-specific competition when using a replacement design (Vanclay 2006). Finally, if plants die, it compromises the interpretation of both the individual plant and individual plot performance. This is especially important in forestry systems (Vanclay 2006).

Since all the designs mentioned are limited in the inference that can be made about inter-specific interactions, the response-surface design has been proposed (Inouye 2001). The response-model design is an experimental series where the density of the two species is varied independently, which gives a broad range of random seeding densities (Vanclay 2006). In order to understand the interactions between plant species, both the total plant density and the relative frequency of plant species need to be varied, the response-model does both. The major disadvantage of the response-model is that it is both resource and energy intensitve.

1.6.3 The role of indicators in studying mixtures

Indicators are useful for studying crop mixtures (Joliffe 2000; Weigelt and Jolliffe 2003). They express characteristics clearly and help researchers understand

complex data by condensing experimental results and multiple variables. Indicators also effectively quantify composite ideas, and they allow comparisons across different studies if standardized indices are used. However, there are several limitations to using indicators (Joliffe 2000; Weigelt and Jolliffe 2003). Condensing several variables into a single indicator results in a loss of detail and statistical precision, and it can obscure relationships between variables, especially where ratios are used. Some indicators tend to have size bias, where initial differences between plants favor larger plants in competition studies. The different indicators used in studying crop mixtures are summarized by (Joliffe 2000; Weigelt and Jolliffe 2003).

An indicator that is often used to evaluate mixtures is the land equivalent ratio (LER). The LER is a measure of the amount of land needed for an intercrop to be as productive as the same crop grown in a sole crop (Vandermeer 1989; Joliffe 2000; Weigelt and Jolliffe 2003), LER is also a measure of the efficiency with which resources are used. If the Land Equivalent Ratio (LER) is greater than one, there is an intercrop advantage and resources are used efficiently, if it is less than one there is an intercrop disadvantage and resources are used inefficiently (Dhima et al. 2007).

Although the LER has been widely used (Hauggaard-Nielsen et al. 2001), there have been some criticisms against it (Park et al. 2002). Some of the criticisms include: (1) It is dependent on the seeding densities of the crops planted together and does not give any information about the seeding densities needed for optimal biological output, (2) it does not remain constant for a range of plant densities, (3) the LER does not show similar yield-density relationships under varying environmental conditions and it does not dissociate between intra-and inter-specific competition, (4) the LER aims to give information about the nature of competition by only measuring the net effect of competition and (5) when the mixture is dominated by a crop with high yield potential, the LER tends to be biased towards one.

1.7 Conclusions

Many of the adverse unintended consequences in intensified agricultural systems can be ascribed to a reduction in biodiversity, specifically plant diversity. Plants are important species in ecological systems and play a vital role as ecosystem engineers which affect nutrient, water and energy flows. In order to reduce the negative impacts of intensified agriculture it is important to increase the plant functional diversity in agricultural systems, this can be done through cover cropping.

Plant species, as cover crops, can perform many functions in agricultural systems such as N fixation and weed suppression. There are trade-offs in the functions that specific plant species perform well: legumes are able to fix atmospheric N but are generally not able to suppress weeds effectively, while C4-grasses suppress weeds well but do not fix any N. A strategy that can be followed to avoid these trade-offs is to use plant mixtures.

In order to develop effective plant mixtures, the component species in the mixtures need to have traits that are complementary or the plants need to facilitate each other. The complementarity in traits can be functional (such as using different sources of the same resource-N fixation), spatial (occupying different soil depths or canopy heights) or temporal (species have different growth times). Facilitation occurs when the presence of one species alleviates an environmental constraint which improves the growth of the other species in the mixture. By using plant mixtures, agro-ecosystem functions can be performed more effectively than using sole crops, and multiple agro-ecosystems can be performed simultaneously.

In organic farming systems, N fertility management and weed control are very important agro-ecosystem functions. It is therefore important to identify cover crop species and mixtures that can perform these functions effectively within an available cover cropping window, such as the annual summer cover cropping niche.

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CHAPTER 2: SCREENING ANNUAL SUMMER LEGUMES AND NON-LEGUMES IN MONOCULTURES AND MIXTURES

2.1 INTRODUCTION

The intensification of modern agricultural systems through the use of fertilizers and pesticides has reduced the reliance on cover crops to perform agro-ecosystem functions. For example, in the Midwestern U.S.A the length of the growing season for corn has increased by two weeks since the 1970s, which reduces the time available for cover crops to be grown (Kucharik 2008). Although this intensification has increased agricultural productivity and efficiency, there have been many unintended environmental effects, such as increased nutrient leaching (Nikolaidis et al. 2008).

The reduction in crop diversity, and the resulting reduction in functional diversity, could lower the resilience of these systems given the potential fluctuations in the biotic and abiotic environment. Plants as dominant (Polley et al. 2007) and keystone species (Peres 2000; del Castillo et al. 2009) play important roles in influencing and regulating numerous ecosystem processes. In agricultural systems, cover crops can be used to increase the functional diversity and perform specific agro-ecosystem functions, such as nitrogen fixation, nutrient retention and weed control.

Agro-ecosystems that rely on legume cover crops for N tend to retain a greater proportion of N (Drinkwater et al. 1998; Drinkwater and Snapp 2007). This is because cover crops use plant-microbe interactions to limit the amount of nutrients that gets lost out of the system, since the nutrients get recycled and stored in plant and microbial biomass. Cover crops increase soil microbial activity by supplying the soil with organic material through rhizo-deposition (Paterson 2003; Paterson et al. 2006; Paterson et al. 2007), root exudates (El-Shatnawi and Makhadmeh 2001; Clayton et al. 2008) or when plants are incorporated into the soil. The improved microbial activity improves nutrient cycling.

In addition to improved nutrient cycling, cover crops have been shown to be weed suppressive. Cover crops suppress weeds through two mechanisms, resource competition and allelopathy. The temporal and spatial diversification of plants in agro-ecosystems (through cover crop rotations and intercropping) alter the patterns of resource acquisition, allelopathy and soil disturbance, which changes the soil environment in such a way that it limits the presence of potential dominant weed species (Liebman and Dyck 1993). Soils with high plant available nutrients, especially nitrogen, favor weed populations and it can lead to increased weed community diversity and weed abundance (Qasem 1992; Blackshaw et al. 2003; Blackshaw and Brandt 2008). Cover crops, such as buckwheat (*Fagopyrum esculentum*) and sorghum sudan (*Sorghum bicolor*), that rapidly take up resources (especially soil nutrients, water, and light) and making it unavailable for weeds, have been shown to effectively suppress weeds (Kumar et al. 2008) (Wang et al. 2008). Cover crops' role in tightly recycling nutrients and other resources could therefore also suppress weeds. Allelopathic substances secreted from living cover crops / green manures and crop residues are used to suppress weeds (Kruidhof et al. 2009). Annual summer cover crops that have been found to release allelopathic chemicals include, buckwheat, sorghum sudan and *Trifolium spp.* (Weston 1996).

Growing cover crops in mixtures is a strategy that can be used to simultaneously manage N fixation and weeds. Mixtures that include legumes have been found to be very productive (when nitrogen is the limiting resource) (Trenbath 1974; Vandermeer 1989), and to effectively cycle C and N (Fornara and Tilman 2008). Legume-based mixtures can be very productive and enhance weed suppression through several mechanisms. First, roots of species grown together tend to occupy a larger soil volume resulting in greater exploitation of the soil profile (Schmidtke et al. 2004). Second, when nitrogen is the limiting resource, reduced competition for soil

nitrogen can lead to greater biomass production since legumes can fix nitrogen from the atmosphere. Furthermore, effective resource use in mixtures can limit weed growth and development.

There are trade-offs in the functions that mixtures perform well. Since non-legumes are better able to scavenge for soil nitrogen than legumes, the competition for soil nitrogen in mixtures is expected to increase the legumes' reliance on nitrogen fixation. However, the absolute amount of nitrogen fixed depends on the nitrogen fixation rates and biomass production of the legume in mixture. In mixtures where legume biomass is suppressed due to severe competition nitrogen fixation is reduced compared to the legume monoculture. Non-legumes tend to be better at suppressing weeds than legumes, therefore, mixtures dominated by non-legumes are generally better able to suppress weeds than legume-dominated mixtures. However, non-legume dominated mixtures suppress both weeds and legume biomass. As a result, mixtures that effectively suppress weeds may have reduced N fixation rates (Haugaard-Nielsen et al. 2001b).

In organic cropping systems, biological nitrogen fixation through legume cover crops is an important source of new nitrogen. Major constraints to effectively using legume cover crops as a source of nitrogen, include the limited availability of niches in which to grow legumes without foregoing a cash crop, and uncertainty about the amount of nitrogen that gets fixed. Integrating legumes into organic vegetable production systems has been particularly challenging for growers. The two month period between early planted spring crops and the planting of fall crops provides an opportunity to use fast-growing annual summer cover crops. To our knowledge there has not been an in-depth evaluation of annual summer cover crops' nitrogen fixation and weed suppression capacity in the Northeastern U.S.A.

In order to design effective cover crop mixtures that optimize weed

41

suppression and N fixation, the relative competitive ability of the legume and non-legume species needs to be understood. To do this, knowledge about the relative intensity of the inter-specific competition between the component crops in mixtures is needed. A replacement series design is an effective experimental design that can be used to obtain information about the relative competitive ability of the different cover crops in mixture.

This study is a cover crop screening experiment, with the following aims:

1. To determine whether there are differences in annual summer, legume and non-legume cover crop species' biomass production, weed suppression and nitrogen fixation and uptake traits.

2. To determine the competitive ability of the legume and non-legumes in mixtures.

3. To investigate the trade-offs in legume-based mixtures between weed suppression and nitrogen fixation.

4. To compare the effectiveness of mixtures and monocultures to produce biomass production, suppress weeds and to fix nitrogen.

2.2 MATERIALS AND METOHDS

2.2.1 Site and soil

Experiments were conducted at two different sites over two years at Cornell University's Homer C. Thompson Organic Research Farm in Freeville, NY, USA (42° 30′ 45″ N, 76° 20′ 45″ W). The experiments were conducted from July 7th until October 15th in 2008 and July 10th until October 18th in 2009. The soil in 2008 was a Rhinebeck (Fine, illitic, mesic Aeric Endoaqualfs) and the soil in 2009 was a Howard gravel loam (Loamy-skeletal mixed mesic Glosoboric Hapludalf). The climate is characterized as humid temperate, the mean annual precipitation is 880mm and the

mean annual minimum and maximum air termperature is $3\,^{o}C$ and $14\,^{o}C$, respectively. Weather data from the Freeville research station were taken for the time period covering the experiments, as well as the 10 year historical average (National Climate Data Center) (Figure 2.3). The fields in both 2008 and 2009 had rye on them the year prior to the study, the rye was mowed down and removed from the field to reduce the soil nitrogen levels.

Seven soil samples were taken and composited from each replicate block using a one-piece Dutch auger (Eijkelkamp, Giesbeek, The Netherlands). Soil inorganic nitrogen (NH_4^+ and NO_3^-) levels were determined by extracting a sieved sub-sample of the soil with 2.67KCL and then shaking the samples at 150RPM on the day of sampling. To determine potentially mineralizable nitrogen (N), a sub-sample was incubated at $30\,^{o}C$ for seven days using a 2M KCL extraction. The samples were then centrifuged and NO_3^- and NH_4^+ was extracted. Total NH_4^+ and NO_3^- for both the incubation and extraction samples, was analyzed using an Automated Discrete Analyzer (AQ2) (Seal Analytical Inc., Mequon, Wisconsin).

Replicated composite samples of air-dried soils were sieved to 2mm and were then analyzed for Mehlich 3-extractable P, K, Ca, Mg, Cu, Zn, Fe, Al, and Mn, as well as particle size (Agricultural Services Laboratory, The Pennsylvania State University, University Park, PA)(Table 2.1).

There were differences in the textural and chemical properties at the two sites (Table 2.1). The soil in 2009 was a loam and in 2008 a clay loam, the difference being that the 2009 soil had a higher sand percentage. The soil pH (6.0) in 2009 was lower than the 2008 pH (6.7). The soil in 2009 had greater inorganic nitrogen (3.7 mgN.kg^{-1}) pools than the soil in 2008 (3.1mg N.kg^{-1}), but the nitrogen mineralization rates were similar. All the other soil variables were within ranges that would not adversely affect plant growth and nitrogen fixation rates.

Table 2.1The soil variables that were taken in each replicate block in the fields in 2008 and 2009

Field	Textural Class	Sand	Silt	Clay	Soil pH[a]	Total N	Total C	Inorganic N[b]	Nmin[c]
		-------------g.kg^{-1}-------------				------------g.kg^{-1}---------		mg.kg^{-1}	mg N.Kg Soil^{-1}.Week^{-1}
2008 Field	Clay Loam	310	380	320	6.7	-	-	3.1	16.2
2009 Field	Loam	380	350	270	6	2.4	31	3.7	16.1

Field	P	K	Mg	Ca	Zn	Cu	S	CEC[d]
	mg.kg^{-1}							meq.100g^{-1}
2008 Field	90.9	120	341	1610	1.7	2.9	17.7	12.75
2009 Field	59.3	133	278	1981	2.2	2.2	18.1	17.2

[a] pH was measured in water.
[b] Extractable NO_3^- and NH_4^+
[c] Potentially mineralizable nitrogen
[d] Indicates the cation exchange capacity of the soil

2.2.1.1 Experimental outline and plot establishment

The experimental design in both years was a split-plot design, with four replicate blocks per field. The main treatment was legume species and the sub-treatments were monocultures of the legumes and non-legumes and a replacement series of legume-non legume mixtures at different seeding ratios.

Eight novel, annual summer legume species were identified that could be used in the Northeastern U.S.A. The legume species were divided into two growth types – viny and non-viny - in order to design mixture combinations that are complementary in their use of light. The viny legumes included: Forage Soybean (*Glycine max*) varieties – Tara and Tyrone, Cowpea (*Vigna unguiculata*), Lablab (*Lablab purperea*) and Chickling vetch (*Lathyrus sativus*). The non-viny legume species were Berseem clover (*Trifolium alexandrinum*), Crimson clover (*Trifolium incarnatum*) and Sunnhemp (*Crotalaria juncea*).

Buckwheat (*Fagopyrum esculentum*) and Japanese Millet (*Echinochloa frumentacea*) was grown in mixtures with both the viny and the non-viny legumes. Because these species have moderate growth, stature and biomass production it was expected that these two species would not out-compete the non-viny legumes, while providing sufficient structure for the viny legumes to grow up against. The viny species were also grown in mixtures with Sorghum-Sudan (*Sorghum bicolor*), it was expected that the high biomass production and tall stature of Sorghum Sudan (*Sorghum bicolor*) could provide structure for the viny species to grow up against. The non-viny legumes were also grown with Flax (*Linum usitatissimum*) and Phacelia (*Phacelia tanacetifola*). Flax and Phacelia are cold tolerant and they could extend the growing season into the fall, especially when grown with the two clovers. They could also provide an under story when grown with Sunnhemp.

In 2009, Soybean Tara, Sunnhemp, Lablab and Phacelia were not included in the experiment, due to the relatively poor performance of these species in 2008. Due to farmer interest, Sorghum Sudan was mixed with the two clover legumes in 2009. Clovers are relatively shade-tolerant and could therefore provide a understory for the Sorghum Sudan. Since Japanese Millet had poor germination rates in the 2008 experiment, additional mixtures of Japanese Millet were included in 2009. Japanese Millet was grown in mixtures at half the recommended Japanese Millet monoculture seeding rate ($424plants.m^{-2}$). A summary of all the treatments for viny and non-viny legume main treatments for both the 2008 and 2009 experiments is given in (Table 2.2).

2.2.2 Replacement Design and Seeding Rates

In order to evaluate the competitive ability of the different cover crops species, a replacement series design was used (Figure 2.1). The main advantage of using a replacement design is that the relative aggressivity of the species in mixture can be investigated. One of the limitations of the replacement design is that the total mixture density has to be determined subjectively. Since we were mainly interested in nitrogen fixation, it was decided to use legume density in monoculture as the total seeding density for the mixtures. The additive design, which was the major other option to use, is mainly used in agronomic crops to determine the effect of varying densities of an auxiliary crop on the yield of the major agronomic crop.

The legume monoculture seeding rates were determined by reviewing extension sources and cover cropping handbooks. The seeding rates (usually in $kg.ha^{-1}$ or $lbs.acre^{-1}$) were converted to seeding densities ($\#seed.ha^{-1}$). In order to compensate for differences between seed and plant size, and growth type, legume species with similar traits were grown at similar densities. This led to two broad group of species –

46

high seeding density (HS) and low seeding density (LS). The two clover species had a higher seeding density (HS) than the rest of the species. In 2009, the difference in seeding density corresponded with the viny and non-viny categories. Where the high seeding density species were the non-viny species (Crimson Clover, and Berseem Clover) and where the low seeding density species were the viny species (Cowpea, Chickling Vetch and Soybean Tyrone).

Each of the sub-plots within a legume main treatment contained the same amount of seed as the legume monoculture, therefore, the total mixture and non-legume monoculture seeding densities was similar to the monoculture seeding rate for a specific legume main treatment. The relative frequency of the different legumes and non-legumes was varied in the mixture. The seeding rates for the different legume main treatments are given in (Table 2.3).
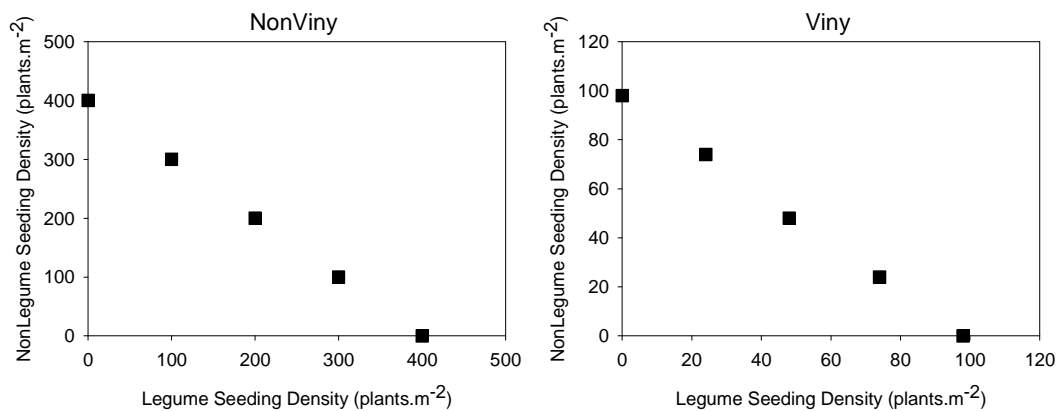
Figure 2.1 The replacement series used for viny and non-viny main treatments. The figures were taken from (Connolly et al. 2001). In the replacement mixtures both species seeding densities are varied to maintain a constant mixture density. In the additive design, the density of one species is kept constant while the other species' density is varied.

2.2.3 Plot Establishment and Plant Counts

The 2008 experiment was planted on 7 July 2008 using a 0.91m wide walk-behind plot drill manufactured by Carter Manufacturing (Brookston, Indiana). Six rows were planted at 15.24 cm spacing. Plant counts were done on July 25th 2008 in the legume and non-legume monoculture's highest and lowest seeding densities to determine the germination rates for the different plant species (Table 2.4). The 2009 experiment was planted on 10 July 2009, using a 6' 3-point no-till drill, manufactured by Great Plains (Great Plains Mfg, Salina, Kansas). The drill was 1.83m wide and rows were spaced 19.5 cm apart. Plant counts were done on July 27th 2009 (Table 2.3).

In both years the plant counts were done in the middle two rows of the plot. For the Berseem Clover and Crimson Clover main treatments the middle two rows were counted for a length of 0.4 m, for all the other main treatments the middle two rows were counted for a length of 1m. The reason for this was that the high seeding densities of the two clover main treatments made counting a full meter impractical.

In 2008 there were problems with the seeding. The cover crops did not establish uniformly across the plots, and the Sorghum Sudan, JapaneseMillet and Phacelia had very variable germination rates. Due to the non-uniformity of cover crop establishment in the plots and problems with germination rates, mixture data from 2008 was not included in this study. The legume monoculture data for 2008 was included in the study.

Table 2.2. Summary of all the mixtures and non-legme monocultures, within the viny and non-viny legume main treatments for 2008 and 2009. All the legumes were grown in monoculture at recommended seeding rates. The seeding rates 100, 75 and 50 represents 100%, 75% and 50% of the recommended seeding rate of the legume main treatment.

2008		2009	
Non Viny Legumes[a]	**Viny Legumes[b]**	**Non Viny Legumes[a]**	**Viny Legumes[a]**
Buckwheat (BW)	*Buckwheat (BW)*	*Buckwheat (BW)*	*Buckwheat (BW)*
BW100	BW100	BW100	BW100
L75:BW25	L75:BW25	L75:BW25	L75:BW25
L50:BW50	L50:BW50	L50:BW50	L50:BW50
L25:BW75	L25:BW75		
		Sorghum Sudan (SS)	*Sorghum Sudan (SS)*
Flax (F)	*Sorghum Sudan (SS)*	SS100	SS100
F100	SS100	L75:SS25	L75:SS25
L75: F25	L75:SS25	L50:SS50	L50:SS50
L50: F50	L50:SS50		
L25: F75	L25:SS75	*Japenese Millet (JM)*	*Japenese Millet (JM)*
		JM 100	JM 100
Phacelia (P)	*Japenese Millet (JM)*	L75: JM25	L75: JM25
F100	JM 100	L50: JM50	L50: JM50
L75: P25	L75: JM25	L75:JM(HIGH)[c]	L75:JM(HIGH)[c]
L50: P50	L50: JM50	L50:JM(HIGH)[c]	L50:JM(HIGH)[c]
L25: P75			
		Flax (F)	
Japenese Millet (JM)		F100	
JM 100		L75: F25	
L75: JM25		L50: F50	
L50: JM50			

[a] Viny legumes include Cowpea, Chickling Vetch, Soybean varieties Tara and Tyrone, Lablab.
[b]Non-Viny legumes include Crimson Clover, Berseem Clover and Sunnhemp.
[c]JM(HIGH) represents half the monoculture seeding rate recommended for Japanese Millet (424 seed sown.m^{-2}).

2.2.4 Biomass sampling and analytical methods

Plots were sampled when the cover crops started to flower. The Buckwheat treatments – monocultures and mixtures – were sampled from 22 – 26 August in 2008 and 2009, the remaining treatments were sampled from 15 – 20 September in 2008 and 2009. On 28 August 2008 all the Buckwheat mixtures for the Crimson Clover and Berseem Clover mixtures were mowed down to a height of 20cm using a Weed Trimmer (Stihl FS110R and Stihl FS85) (Stihl, Norfolk, VA, USA). The mowed treatments were sampled on 15th October 2008. In 2009, the Buckwheat and Sorghum Sudan mixtures with the two clover species were mowed to a height of 20cm on 1 September 2009. The mowed treatments were harvested on October 18th 2009.

During sampling, the middle two rows were sampled for a length of 0.4 m for the Berseem and Crimson Clover main treatments and 1m for the rest of the main treatments. When plants were sampled, the legume, non-legume and weed biomass was separated. The fresh weights of the samples were taken and the samples were stored at 60°C until a constant weight was reached, the dried plant material was weighed. The legume, non-legume and weed dry material was coarsely ground using a hammer mill and a Christy grinder. The legume material in monoculture and mixture and the non-legume plant material (only in monoculture), was further pulverized using a roller-grinder for 48 hours. These samples were micro-balanced and sent to the UC Davis Stable Isotope Facility in Davis, California, U.S.A. to be analyzed for ^{15}N natural abundance and total N content using the PDZ Europa 20-20 continuous flow Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK). The non-legume plant material in the mixtures and all the weed plant material was analyzed for total C and N content using a LECO 2000 CN Analyzer (Leco Corporation, St. Joseph, MO).

2.2.5 N-fixation calculation

The ^{15}N natural abundance method was used to estimate biological nitrogen fixation (BNF) (Shearer and Kohl 1986). The percentage of nitrogen derived from the atmosphere (%Ndfa) for all the legumes in monoculture and mixture was determined using all the non-legume monocultures averaged across replicates as reference plants. The following equation was used to determine the percentage of nitrogen derived from the atmosphere:

%N from fixation = 100 x [(δ^{15}N Reference Plants - δ^{15}N Legume Plants) / [(δ^{15}N Reference Plants – B)]   (2.1)

B is the δ^{15}N value for a legume when atmospheric N_2 is the only source of nitrogen after accounting for seed nitrogen. The total amount of above ground atmospheric nitrogen that was fixed was calculated using the biomass nitrogen concentration and the percentage of nitrogen from fixation.

In order to obtain the B-value for all the legumes, a growth chamber study was conducted where the legumes were grown in N-free, washed and autoclaved sand, mixed with perlite at a ratio of 1:1. Legume seeds were sterelized using 70% ethanol (v/v) for three minutes, and 3% bleach solution for two minutes, and then rinsed in deionized water for three minutes. The seed was inoculated with the same inoculant as the field plots. The plants were fertilized using an N-free Hoagland's solution (Greencare Fertilizers, Chicago, IL) and a gypsum solution. Plants were sampled at the same maturity stage as the plants in the field. The plants were coarse ground using the hammer mill and Christy mill, and finely pulverized using the roller grinder. Samples were then sent to UC Davis where they were analyzed for δ^{15}N using the PDZ Europa 20-20 continous flow Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK).

The *B*-values used for the different legumes were: Crimson Clover (-0.74‰), Berseem Clover (-1.04‰), Sunnhemp (-1.28‰), Chickling Vetch (-0.80‰), Cowpea (-2.56‰), Soybean Tyrone (-0.76‰), Soybean Tara (-0.65‰) and Lablab (-1.08‰).

2.2.6 Inter- and Intra-specific species interactions

In order to investigate relative intensity of the inter- and intra-competition of species in mixtures, a replacement series design was used. The mixture biomass results were analyzed graphically using replacement diagrams (Jolliffe 2000). By using replacement diagrams, the relative intensity of inter- and intra-specific competition can be determined. If the intra- and inter-specific competition experienced by a species is equal, the biomass trend across the mixtures will be linear (Figure 2.2). If the intra-specific competition that a species experience is greater than the inter-specific competition, then the biomass trend will curve upwards. If the inter-specific competition in the mixture is greater than the intra-specific competition, then the trend will tend downward

2.2.7 Land Equivalent Ratio

The Land Equivalent Ratio (LER) was calculated for all the mixtures as an indicator of the biomass yield benefits of the mixtures relative to the monocultures. The LER is a measure of the amount of land needed for an intercrop to be as productive as the same crop grown in a sole crop (Vandermeer 1989)(Weigelt and Jolliffe 2003), LER is also a measure of the efficiency with which resources are used. If the Land Equivalent Ratio (LER) is greater than one, there is an intercrop advantage and resources are used efficiently, if it is less than one there is an intercrop disadvantage and resources are used inefficiently (Dhima et al. 2007).
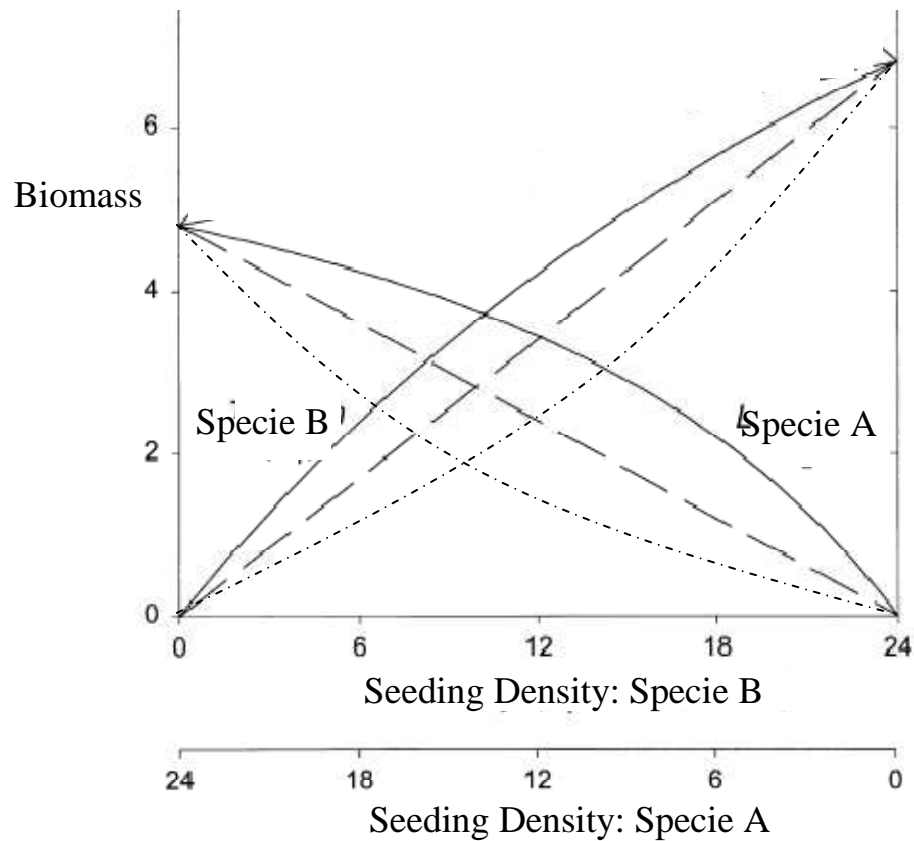
Figure 2.2 Replacement Diagram adapted from (Jolliffe 2000), showing trends in the above ground biomass for component species (A and B) in a mixture. When inter- and intra-specific competition is equal, the trends will be linear (broken lines). When intra-specific competition is greater than inter-specific competition, the trends will curve downward (solid lines). When intra-specific competition is less intense than the inter-specific competition, the trends will curve downward (dotted line).

The LER was calculated using the following equation:

$$LER = M_a/S_a + M_b/S_b \qquad (2.2)$$

Where M is the mixture yield and S is the sole (monoculture) crop yield, and a and b is the component crops of the mixture.

To determine whether cover crop mixtures increased the total biomass nitrogen accumulation relative to monocultures, the following equation was used:

$$N_{LER} = N_{Ma}/N_{Sa} + N_{Mb}/N_{Sb} \qquad (2.3)$$

Where N_{Ma} is the nitrogen content for species a in mixture (M), N_{Sa} is the nitrogen content for species a in sole crop / monoculture (S), N_{Mb} is the nitrogen content for species b in mixture (M) and N_{Sb} is the nitrogen content of species b in sole crop / monoculture (S).

2.2.8 Statistical Analysis

For all the experiments, statistical analysis was performed using the JMP 8.0 (2007 SAS Insititute Inc., Cary, North Carolina). A mixed model was fit to the data with replicate block as a random variable, and legume type (viny/non-viny legumes), main treatment (nested within legume type) and treatment (nested within legume type) were included as fixed effects. All model assumptions were met. In cases where the data did not fit the model assumptions, the count data was transformed either by taking the natural logarithm or by taking the square root. Tukey's honestly sigificant difference (HSD) was performed for multiple means comparisons.

2.3 RESULTS

2.3.1 Weather

In general, both years were cooler than the historical average (Figure 2.3). In 2009, August had more rain than the historical average and in 2008 July there had more rainfall than the historical average.

2.3.2 Germination Rates

Problems with the seeder in 2008 led to an uneven seed distribution within the experimental plots. This made it difficult to sample randomly and to obtain seeding rates that were close to the target values. In 2008 Sorghum Sudan, Japanese Millet and Phacelia had low and variable germination rates. Data from mixtures and the non-legume monocultures in 2008 were not included in this study due to the poor germination rates and uneven seeding. The legume monoculture data was used (Table 2.3). In 2009 the cover crops were evenly distributed throughout the plots. The germination rates for all legumes were acceptable and were from 85% for Crimson Clover to 96% for Berseem Clover. Soybean Tyrone had a germination rate of 72%, this was acceptable since the final Soybean plant density (72 plants.m^{-2}) was still within a range that is commonly used for forage Soybean as a cover crop. The seeding density for Chickling Vetch in 2008 was greater (92 plants.m^{-2}) than that of 2009 (68 plants.m^{-2}) due to the non-uniformity of the cover crop within the plots in 2008. There is a strong correlation for Chickling Vecth and Soybean Tyrone seeding density and biomass production. These seeding issues make year to year comparisons for these two species problematic, since differences in biomass are mostly driven by differences in plant density.
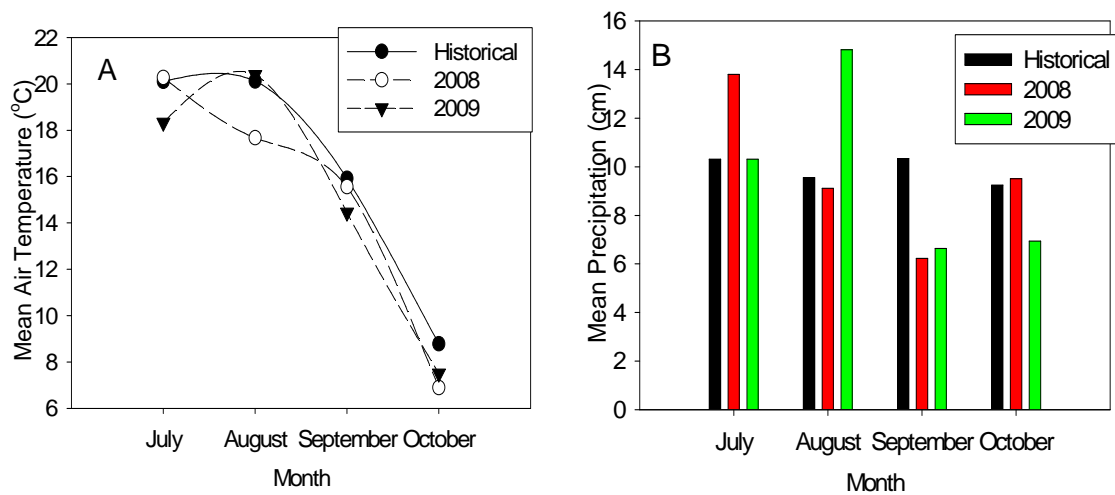
Figure 2.3. Average monthly a) temperature (^{o}C) and b) precipitation (cm) sums for 2008, 2009 and the 10-year historical average for Freeville, NY.

Table 2.3 The amount of seed sown per square meter, the average amount of plants counted per square meter and the average germination rate (in %) for the legume monocultures at recommended seeding rates (Legume 100); and the Buckwheat (BW), Sorghum Sudan)SS0), Phacelia (P) and Flax monoculture (F) at their highest and lowest seeding rates. The different main treatments are Cowpea(CP), Crimson Clover (CC), Berseem Clover), Sunnhemp (SH), Lablab (Lb), Chickling Vetch (CV), Soybean Tyrone (SY) and Soybean Tara (SA).

Main Treat	Species	2008 Seed Sown seed.m-2	kg.Ha-1	Plants.m-2	%Germ	Main Treat	Species	2009 Seed Sown seed.m-2	kg.Ha-1	Plants.m-2	%Germ
CC	CC	449	21	465 (219)	100 (24)	CC	CC	420	20	356 (15)	85 (4)
BC	BC	549	17	470 (74)	88 (14)	BC	BC	476	15	456 (76)	96 (16)
CP	CP	98	92	108 (15)	110 (8)	CP	CP	98	92	92 (6)	94 (7)
CV	CV	75	114	92 (6)	123 (7)	CV	CV	75	114	68 (13)	90 (17)
SY	SY	98	110	92 (17)	94 (18)	SY	SY	98	110	72 (1)	72 (4)
SA	SA	99	110	78 (13)	79 (13)						
LB	LB	50	123	53 (13)	105 (13)						
SH	SH	99	38	109 (25)	110 (26)						
BC	BW	549	189	692 (28)	126 (10)	BC	BW	476	164	429 (15)	90 (3)
LB	BW	50	17	53 (6)	105 (25)	CV	BW	75	26	72 (5)	96 (6)
SY	SS	98	12	100 (26)	102 (20)	BC	SS	476	56	381 (42)	80 (9)
LB	SS	50	7	48 (5)	95 (10)	CV	SS	75	9	62 (5)	82 (7)
BC	P	549	9	219 (35)	40 (6)	BC	JM	476	14	294 (24)	62 (5)
SH	P	99	2	58 (14)	59 (14)	CV	JM	75	2	60 (7)	67 (7)
BC	F	549	23	485 (74)	88 (13)	BC	F	476	20	372 (81)	78 (12)
SH	F	99	4	104 (24)	104 (23)						

2.3.3 Species Performance in Monoculture

2.3.3.1 Legume Species' Traits

Crimson Clover had the greatest biomass production in both years (Figure 2.4). In 2008, from the eight legumes that were evaluated in monoculture Crimson Clover had significantly greater biomass production than Soybean Tara, Lablab, and Sunnhemp (p = 0.0008, Tukey's HSD). Cowpea, Chickling Vetch, Soybean Tyrone and Berseem Clover were not significantly different from Crimson Clover. Due to their low biomass production in 2008, Lablab, Sunnhemp and Soybean Tara were not included in the 2009 experiment.

In 2009, Crimson Clover and Cowpea had significantly greater biomass production compared to Soybean Tyrone and Chickling Vetch with Berseem Clover being intermediate to these species (p = 0.0006, Tukey's HSD). The differences between 2008 and 2009 in Chickling Vetch and Soybean Tyrone biomass production is mainly due to differences in seeding densities across the two years.

Cowpea was less dependent on nitrogen fixation in both years, and managed to access more soil nitrogen than the other legume species (Figure 2.4). In 2008, Cowpea and Lablab had the lowest nitrogen fixation rates (34% and 32%, respectively) compared to Berseem Clover, Crimson Clover, Chickling Vetch and Soybean Tyrone (Tukey's HSD, p < 0.0001). Soybean Tara and Sunnhemp were intermediate to these species in their reliance on nitrogen fixation. In 2009, Cowpea relied significantly less on nitrogen fixation (18%) than all the other legumes (Tukey's HSD< p=0.0092). Crimson Clover had the highest nitrogen fixation rate (71%), followed by Chickling Vetch (59%), Soybean Tyrone (55%) and Berseem Clover (50%).

Crimson Clover fixed more N than any of the legumes due to the combination of greater biomass production and high N fixation rates in both years. In 2008 Crimson Clover and Chickling Vetch fixed significantly more nitrogen (111 kg N.Ha^{-1}

and 98 kg N.Ha^{-1}, respectively) than Cowpea, Soybean Tara, Soybean Tyrone, Lablab and Sunnhemp (Tukey's HSD, p=0.0002). Cowpea fixed the lowest amount of nitrogen (12 kg N.Ha^{-1} in 2008 and 13 kg N.Ha^{-1} in 2009) and relied significantly more on soil nitrogen uptake (50 kg N.Ha^{-1} in 2008 and 58 kg N.Ha^{-1}) than the other legumes (Tukey's HSD, p<0.00021). Berseem Clover was not significantly different from Crimson Clover in the amount of nitrogen fixed. In 2009, Crimson Clover fixed significantly more nitrogen (71kg N.Ha^{-1}) than any of the other legume species (Tukey's HSD, p<0.0001).

The experiment in 2009 had greater weed pressure than in 2008. Crimson Clover was the most effective at suppressing weeds in both years (12 g.m^{-2} in 2008 and 20g.m^{-2} in 2009). In 2009 Crimson Clover had significantly greater weed suppression than Cowpea (250g.m^{-2}). The Cowpea monoculture had ten-fold greater weed biomass than the Crimson Clover monoculture.

Compared to the other legume species, Crimson Clover monocultures fixed the greatest amount of N and also suppressed weeds most effectively (Figure 2.5). Crimson Clover was the only legume species that consistently performed both functions in monoculture. Cowpea fixed small amounts of N and was the most variable in terms of weed control. Chickling Vetch had rather high levels of weeds, and total N fixed was variable.

Figure 2.4 The legume and weed biomass production, percentage of nitrogen derived from the atmosphere (%Ndfa) and the various sources of nitrogen – nitrogen fixation, soil N uptake and weed N uptake for a) 2009 and b) 08.Significance levels were determined by Tukey' s HSD where p<0.05.

2008 Legume Monocultures
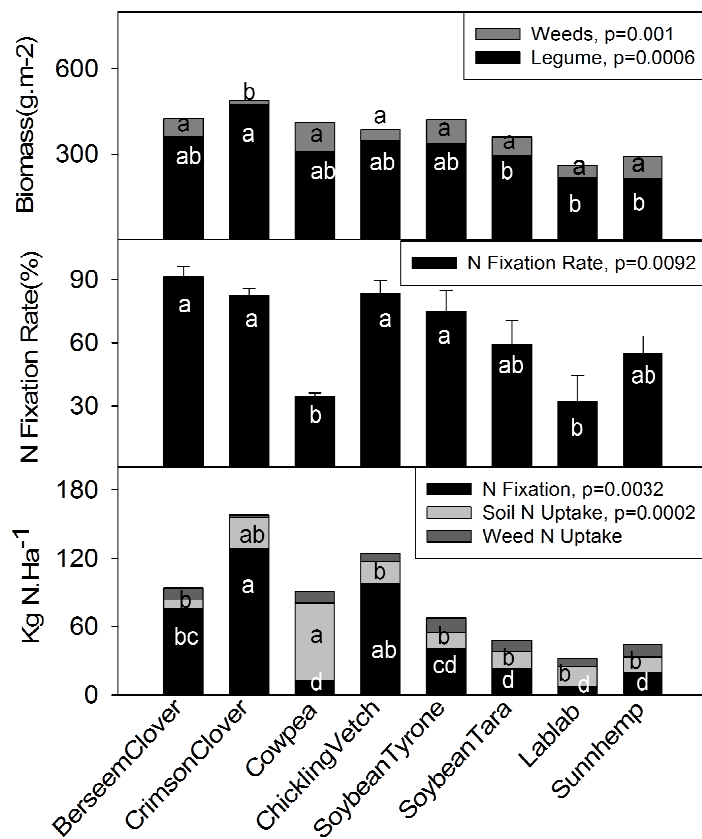
2009 Legume Monocultures
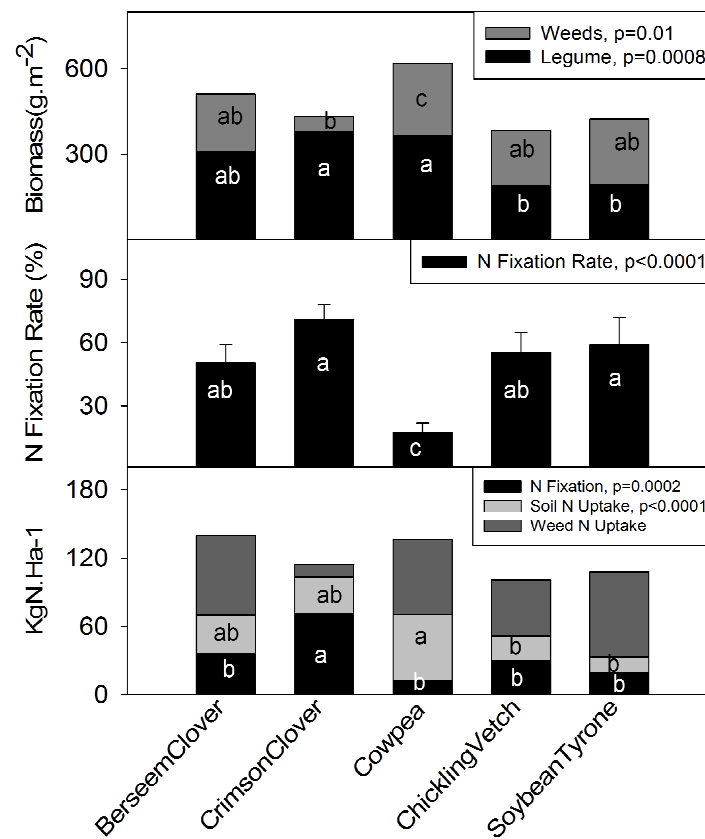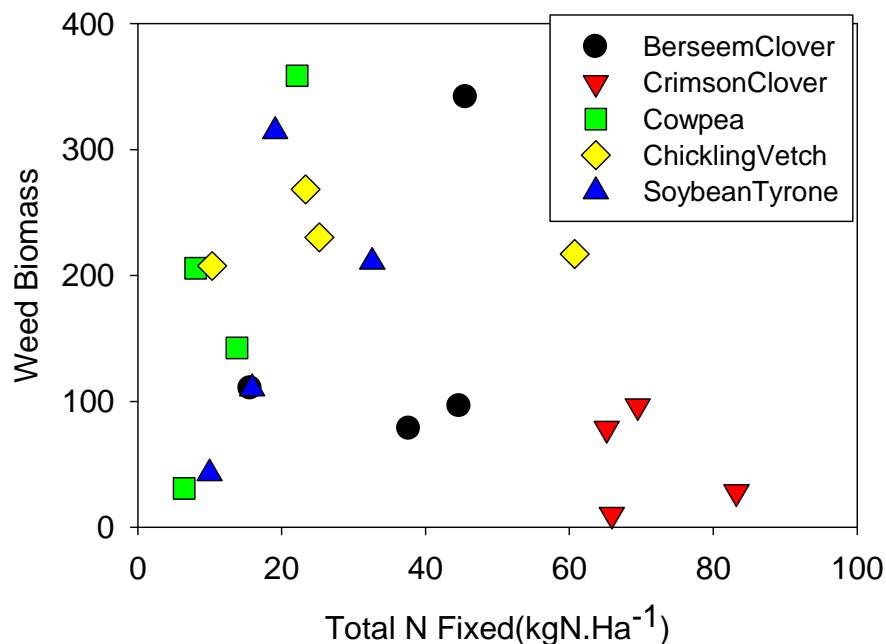
Figure 2.5 Total nitrogen fixed versus weed biomass. Crimson Clover is able to fixed large amounts of nitrogen while suppressing weed biomass.

2.3.3.2 NonLegume Species' Traits

Only the data in 2009 was used for the non-legume monocultures. The four-fold greater seeding densities in non-viny treatments led to significantly greater biomass production across all the non-legume monocultures planted at these densities (Tukey's HSD, $p<0.05$; Figure 2.6). Compared to the other non-legume species, Sorghum Sudan produced the greatest amount of biomass ($909g.m^{-2}$ at high seeding densities and $632g.m^{-2}$ at low seeding densities). Biomass production in Buckwheat ($603g.m-2$) and Japanese Millet were not significantly different.
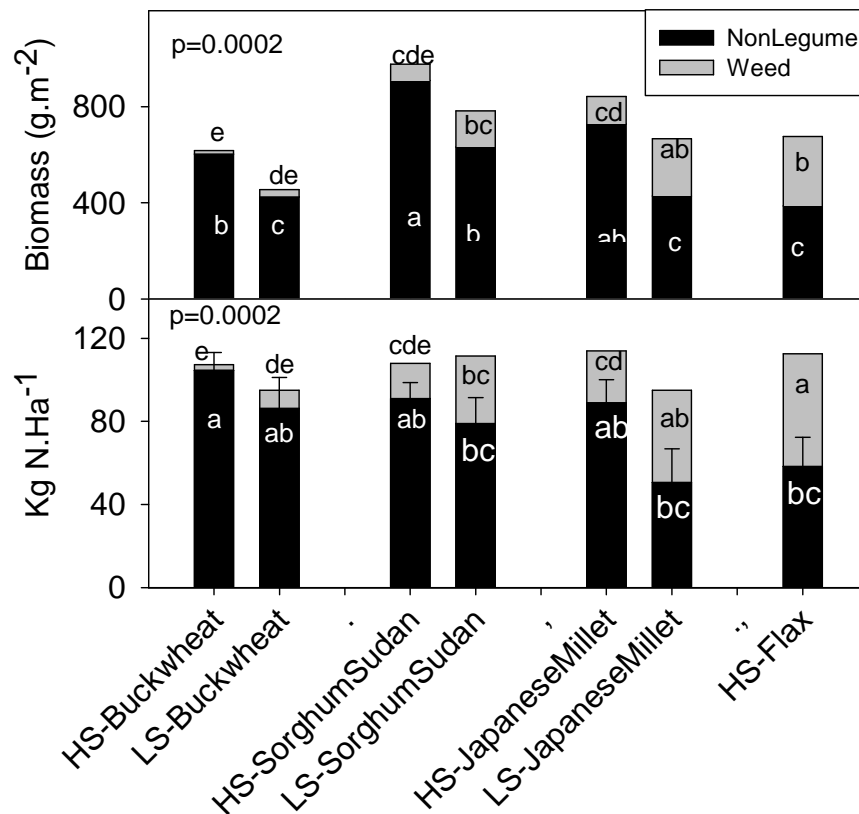
Figure 2.6 The non-legume and weed biomass production and soil N uptake for the for the weeds and non-legume species monocultures sown at high seeding densities (HS) and low seeding densities (LS). Significant levels were determined by Tukey's HSD, p<0.05

Increased biomass production across species did not improve weed suppression. Despite having less biomass production than Sorghum Sudan, Buckwheat was able to suppress weeds more effectively than the other non-legume species (Tukey's HSD, p<0.05), it was followed by Sorghum Sudan, Japanese Millet and Flax. Sorghum Sudan had a biomass of 909 g.m^{-2} with an associated weed biomass of 67g.m^{-2}, while Buckwheat had a biomass of 603 g.m^{-2} with an associated weed biomass of 15g.m^{-2}. Species identity is therefore important for weed suppression.

Increased biomass production within a species increased weed suppression, this was observed for all the non-legume species. Where biomass of the non-legume

64

increases (from 424 to 603g.m^{-2} for Buckwheat, from 623 to 909g.m^{-2} for Sorghum Sudan) the weed biomass gets reduced (from 30 to 15g.m^{-2} for Buckwheat and 151 to 67g.m^{-2} for Sorghum Sudan).

The non-legumes differed in their capacity to capture soil N. Buckwheat assimilated greater amounts of total soil N and soil N per kilogram of above ground biomass compared to the other non-legumes (Figure 2.6 and Figure Figure 2.7). For a given amount of biomass Buckwheat took up more nitrogen than the other non-legumes. For example, for 4000 kg.Ha^{-1} of biomass Buckwheat contains 76 kg N.Ha^{-1}, Sorghum Sudan contains 53 kg N.Ha^{-1}, Japanese Millet contains 45 kg N .Ha^{-1}and Flax contains 60 kg N .Ha^{-1}, on average. This difference may be explained by the fact that the different non-legume species had different N levels in their biomass. Buckwheat had a significantly greater plant nitrogen content (1.92%) than Flax (1.49%) and the two C-4 grass species, Japanese Millet (1.20%) and Sorghum Sudan.(1.17%) (Figure 2.8).
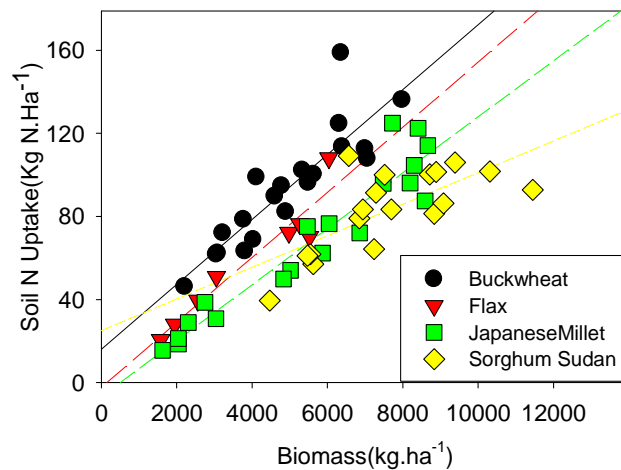
Buckwheat: R^2=0.77, $p < 0.0001$; y = 16+0.15x
Sorghum Sudan: R^2=0.50,$p = 0.0095$; y = 25+0.07x
Japanese Millet: R^2=0.91,$p < 0.0001$; y = -7+ 0.13x
Flax = R^2=0.92,$p = 0.0007$; y = -4+ 0.16x

Figure 2.7 The relationship between soil nitrogen uptake and biomass production for the four non-legume monocultures
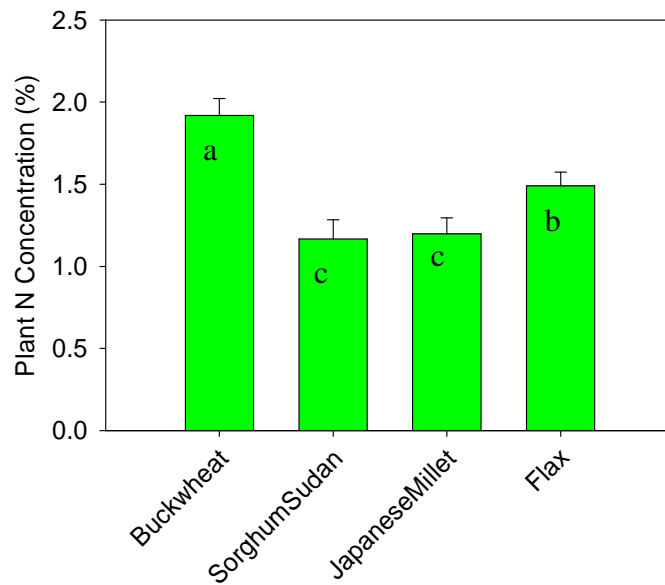
Figure 2.8 The plant nitrogen content (%) in the biomass of the non-legume monocultures. The significance levels are indicated using Tukey's HSD, p<0.05.

2.3.4 Mixture outcomes: Biomass, Nitrogen Fixation and Weed Biomass

Biomass of legumes was consistently reduced in mixtures compared to monocultures, however these reductions were greater for non-viny compared to viny species. Non-viny legumes had significantly greater biomass in monoculture than the viny legumes (343g.m^{-2} and 250g.m^{-2}, respectively)(Figure 2.9). The non-viny legumes' biomass was significantly reduced in the Buckwheat (44g.m^{-2}), Sorghum Sudan (117g.m^{-2}), Japanese Millet (129g.m^{-2}) and Flax (176g.m^{-2}) mixtures compared to monocultures (Tukey's HSD, P<0.0001; Figure 2.9). In the viny mixtures, there was only a significant difference between the legume biomass in the monocultures and in the Buckwheat mixtures (Tukey's HSD, p<0.0001). The viny legume biomass in Sorghum Sudan (170g.m^{-2}) and Japanese Millet (192g.m^{-2}) mixtures was not significantly different from monoculture biomass (Figure 2.9).

The biomass differences between viny and non-viny treatments for the non-legumes followed the same pattern observed for the monocultures with greater biomass associated with the greater seeding rates used in non-viny mixtures (Tukey's

HSD, p<0.0001; Figure 2.9). Only the non-viny mixtures had significant differences in biomass production across non-legume species. Sorghum Sudan had the greatest non-legume biomass production (742g.m^{-2}), followed by Japanese Millet (556g.m^{-2}), Buckwheat (483g.m^{-2}) and Flax (288g.m^{-2}). In the viny mixtures, there was no significant difference in the non-legume biomass production across the mixtures (Figure 2.9).
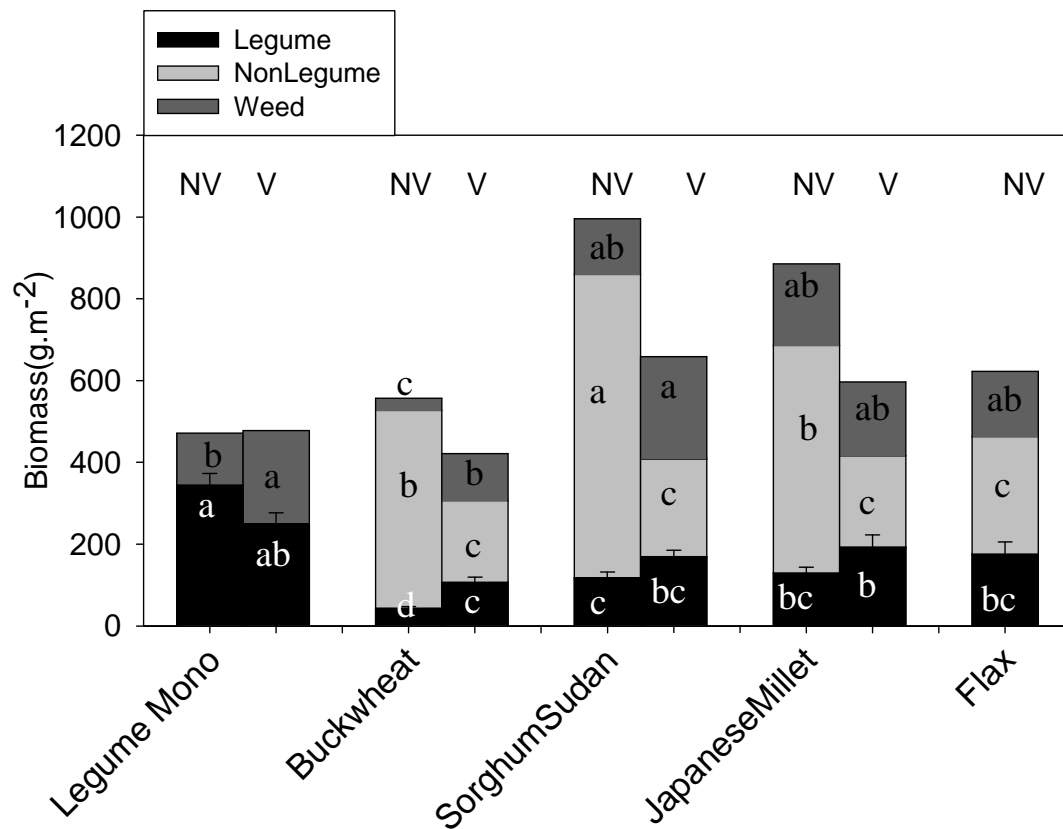
Figure 2.9 The above ground biomass for the legume, non legume and weed component in mixtures consisting of viny (V) and non-viny (NV) legumes and Buckwheat, Sorghum Sudan, JapaneseMillet and Flax. The letters indicate significant differences between the legume, non legume and weed biomass for the different mixtures using Tukey's HSD, with p<0.0001.

The weed biomass in the non-viny mixtures was significantly lower in the Buckwheat (30 g.m^{-2}) mixtures, followed by Sorghum Sudan (136g.m^{-2}), Flax (159g.m^{-2}) and Japanese Millet (200g.m^{-2})(Tukey's HSD, p<0.0001; Figure 2.9). Similar trends were observed in the viny mixtures, where the Buckwheat mixtures had significantly lower weed biomass (116g.m^{-2}) than the Sorghum Sudan (251g.m^{-2}) and Japanese Millet (181g.m^{-2}) mixtures (Tuley's HSD, p<0.0001). Mixtures that are effective at suppressing weed biomass, like Buckwheat mixtures, also suppressed the legume biomass in the mixture (Figure 2.12). In cases where the legume biomass was not suppressed, such as in the viny legume mixtures, the weed biomass was high.

Both the viny and non-viny legumes had significantly reduced N fixation rates in the Buckwheat mixtures, compared to the other treatments (Tukey's HSD, p<0.05; Figure 2.10). Viny legumes in mixtures with the Sorghum Sudan and Japanese Millet had greater reliance on N fixation (52 and 59 % respectively) than the legume monocultures (44%). In the non-viny treatments, there were no significant difference between the N fixation rates in the monoculture (61%) and the Sorghum Sudan (55%), Japanese Millet (60%) and Flax (64%) mixtures.

The total amount of nitrogen fixed for the non-viny legumes was significantly reduced in the mixture compared to the monoculture, across all the mixtures (Tukey's HSD, P<0.05; Figure 2.11). For viny legumes, there were no significant difference between the nitrogen fixed in monoculture and the Sorghum Sudan and Japanese Millet mixtures (Tukey's HSD, p<0.05; Figure 2.11). Both the viny and non-viny legumes in the Buckwheat mixtures, fixed significantly less nitrogen than the other mixtures and monocultures (Tukey's HSD, p<0.0001). The two non-viny legume species fixed more nitrogen (53 kgN.Ha^{-1}) than the viny legumes (21kgN.Ha^{-1}) in monoculture and mixture (Tukey's HSD, p<0.0001).
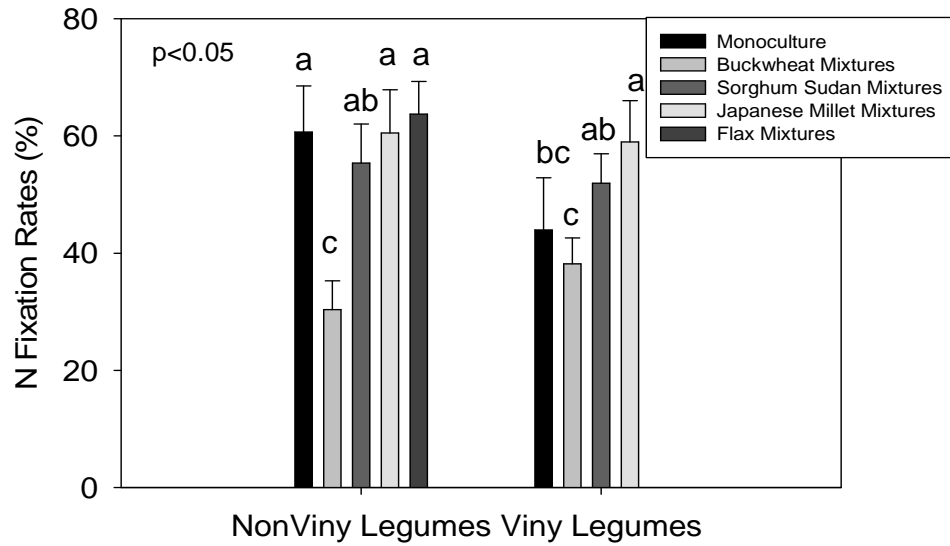
Figure 2.10 The N fixation rates (%) for the non-viny and viny legumes in monoculture and Buckwheat, Sorghum Sudan, Japanese Millet and Flax mixtures.
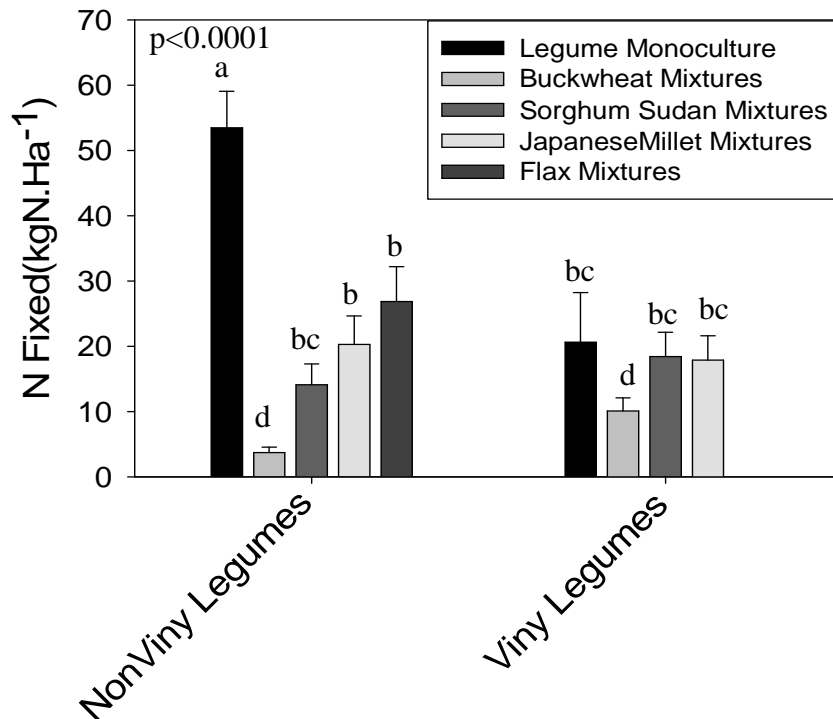
Figure 2.11 The amount if nitrogen fixed for viny (V) and non-viny (NV) legume monocultures (L100) and mixtures with Buckwheat (BW), Sorghum Sudan (SS), Japanese Millet (JM) and Flax (F). Letters indicate significant differences (Tukey's HSD, p<0.05).

2.3.5 Inter- and intra-specific interactions in mixtures

The replacement design permits analysis of the relative importance of intra- and inter-specific competition. In general, for the non-legumes, intra-specific competition was more significant than inter-specific competition indicating that legumes were generally less competitive than the non-legumes species used in these mixtures (Figure 2.12; Figure 2.13). For the smaller statured non-viny legumes, inter-specific competition was greater than the intra-specific competition, with the exception of the Flax mixtures where there was no difference between the inter- and intra-specific competition (Figure 2.12).

In the viny mixtures, there were no consistent trends. Instead, species differences resulted from differences in the competitive ability of both the legume and non-legume species (Figure 2.13). In the Buckwheat mixtures, there was no difference between inter-and intra-specific competition. Similar trends were observed in the Sorghum Sudan with Chickling Vetch and Soybean Tyrone, but Cowpea experienced reduced inter-specific competition and greater intra-specific competition. In the Japanese Millet mixtures, intra-specific competition was greater than inter-specific competition for all three the legumes. In all the mixtures with non-legumes, Cowpea ($153g.m^{-2}$ in Buckwheat, $210g.m^{-2}$ in Sorghum Sudan and $393g.m^{-2}$ in Japanese Millet) had greater biomass than the Chickling Vetch and Soybean Tyrone. Cowpea has been identified as more competitive than the other legume species.

The intensity of the inter-specific competition on the legumes depended largely on the non-legume species (Figure 2.12). The competitive ability of the different non-legumes can be summarized as follows: Buckwheat > Sorghum Sudan> Japanese Millet> Flax.

2.3.6 Land Equivalent Ratios (LER)

The differences in impact of mixtures on total biomass production were largely determined by the non-legume component. In Figure 2.14, mixtures are arranged in the order of competitive ability of the non-legume, which was determined by the analysis of inter- and intrea-specific competition. In keeping with the greater tendency for Buckwheat to out-compete other species, including weeds, Buckwheat mixtures had the lower biomass compared to the combined biomass of the two species in monoculture. The LER for Buckwheat mixtures was consistently less than one with the exception of the Cowpea mixture. Buckwheat biomass was reduced in the Cowpea mixture. All other mixtures, Japanese Millet, Sorghum Sudan and Flax had LERs greater than one, indicating that mixtures over-yielded. The total N contained in the cover crops paralleled biomass, with greater total N in the mixtures compared to the monocultures, with the exception of the Buckwheat mixtures.

2.3.7 Trade-Off – N Fixation vs Weed Suppression

In the mixtures that suppressed weeds effectively, such as the Buckwheat and Sorghum Sudan, the legume biomass was also suppressed (Figure 2.9). Cowpea, the most competitive legume in the mixtures relied more on soil N and fixed the lowest amount of nitrogen (Figure 2.15). For example, the nitrogen fixation rate of Crimson Clover averaged 54% compared to Cowpea's nitrogen fixation rate of 27%. As a result, N fixed.kg^{-1} of legume biomass was 0.178 kgN.kg^{-1} biomass for Crimson Clover while Cowpea fixed only 0.056 kg N.kg^{-1}. Cowpea relied more on taking up soil N than the other legume species (Figure 2.16). Soil nitrogen uptake for Cowpea was 0.133kg N. kg^{-1} of legume biomass while for Crimson Clover it was 0.093 kg N.kg^{-1}. The legume species that are very effective at fixing nitrogen, such as Crimson Clover, were suppressed by competitive non-legumes.

Figure 2.12 Replacement diagrams that illustrate the relative effects of intra-and inter specific competition for the non-viny legumes in mixtures with Buckwheat, Sorghum Sudan, Japanese Millet and Flax.

Figure 2.13 Replacement diagrams that illustrate the relative effects of intra-and inter specific competition for the viny legumes in mixtures with Buckwheat, Sorghum Sudan, Japanese Millet and Flax.
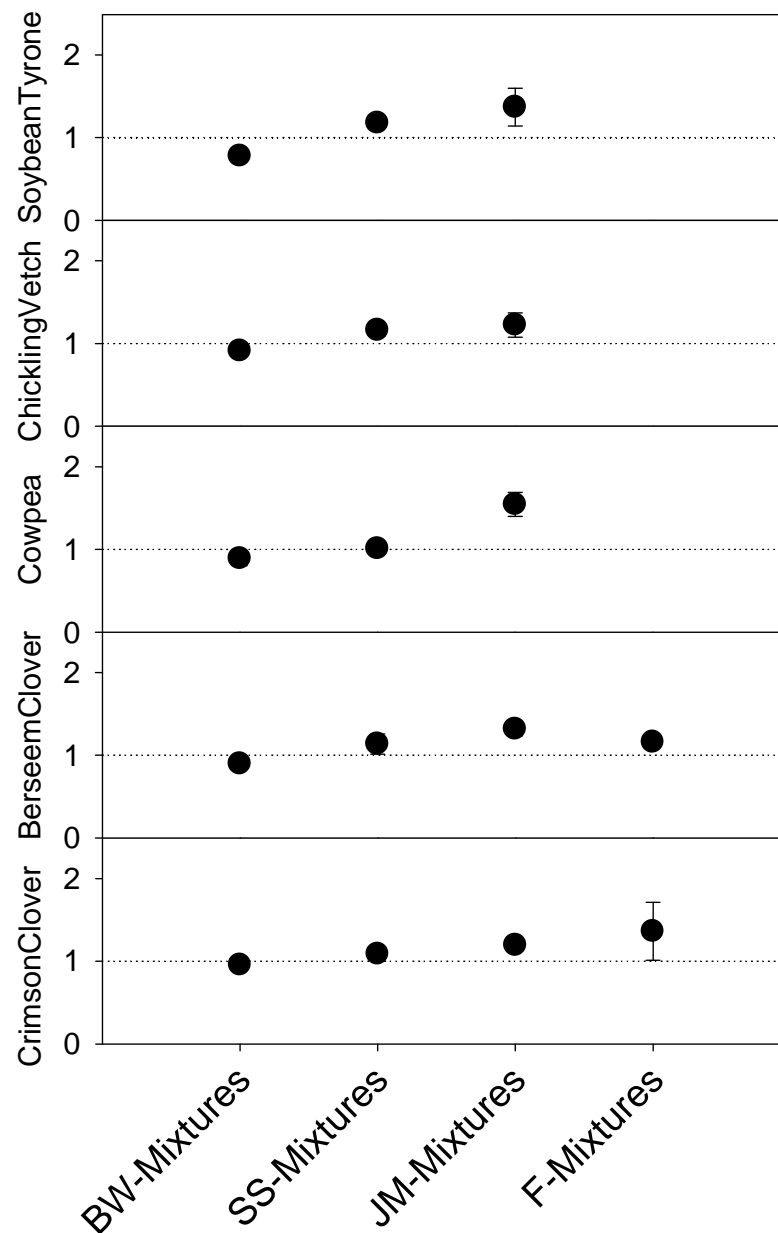
Figure 2.14 The Land Equivalent Ratios (LER) for all the legumes in mixtures with Buckwheat (BW), Sorghum Sudan (SS), Japanese Millet (JM) and Flax(F). The mixtures are organized, from left to right, from the most competitive to the least competitive non-legumes.

In contrast, competitive legumes, like Cowpea, did not fix a lot of nitrogen and were more competitive against non-legumes. Although Crimson Clover suppressed weeds effectively in monoculture, in mixtures the lower seeding densities did not allow it to close the canopy effectively, which reduced its weed suppressive ability. Thus, from an agro-ecological perspective, there is a trade-off between nitrogen fixation and weed suppression in mixtures.

2.3.8 Mowed Treatments – Sorghum Sudan and Buckwheat

A potential strategy that could be used to avoid the trade-off between nitrogen fixation and weed suppression is to mow down the dominant non-legume species in the mixture where there is complementarity in the timing of growth between the species that constitute the mixture. Both the Berseem and Crimson Clover in Buckwheat and Sorghum Sudan mixtures experienced intense inter-specific competition at the time of the August harvest, while the intra-specific competition was not as severe (Figure 2.17a and b). The two clover species did not grow rapidly in the first weeks after planting, and only started to produce large amounts of biomass six to eight weeks after planting. Buckwheat and Sorghum Sudan grew rapidly after planting, and the Buckwheat started to flower at six weeks after planting.

Buckwheat was mowed down when it started to flower. This reduced the inter-specific competition and the shading experienced by the clovers (Figure 2.17). This allowed a significant increase in legume biomass production from August to October - from $42g.m^{-2}$ to $190g.m^{-2}$ for Berseem Clover and $46g.m^{-2}$ to $250g.m^{-2}$ for Crimson Clover (Figure 2.18; Tukey's HSD, p<0.0001). Similar trends were observed in Sorghum Sudan mixtures that were mowed down eight weeks after planting (Figure 2.18).

Crimson Clover: $R^2 = 0.79$; y = 0.18X - 2.18
Cowpea: $R^2 = 0.51$, y = 0.056X - 0.06
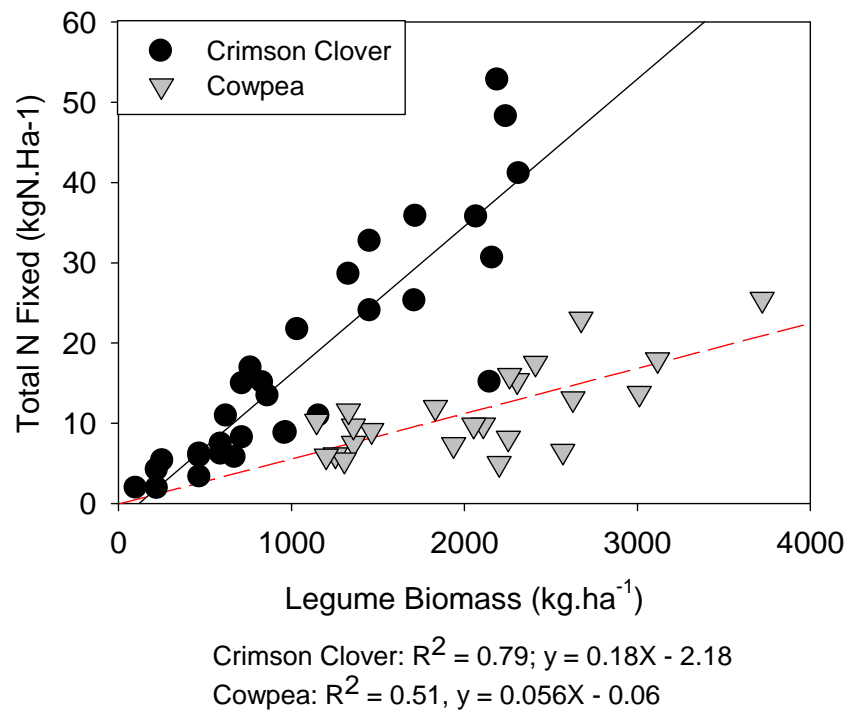
Figure 2.15 Regression of legume biomass (g.m^{-2}) and total nitrogen fixed (kg N.Ha^{-1}) for Cowpea, and Crimson Clover.

All Legumes: $R^2 = 0.54$; y = 0.09X + 2.83
Cowpea: $R^2 = 0.65$, y = 0.13X + 3.05
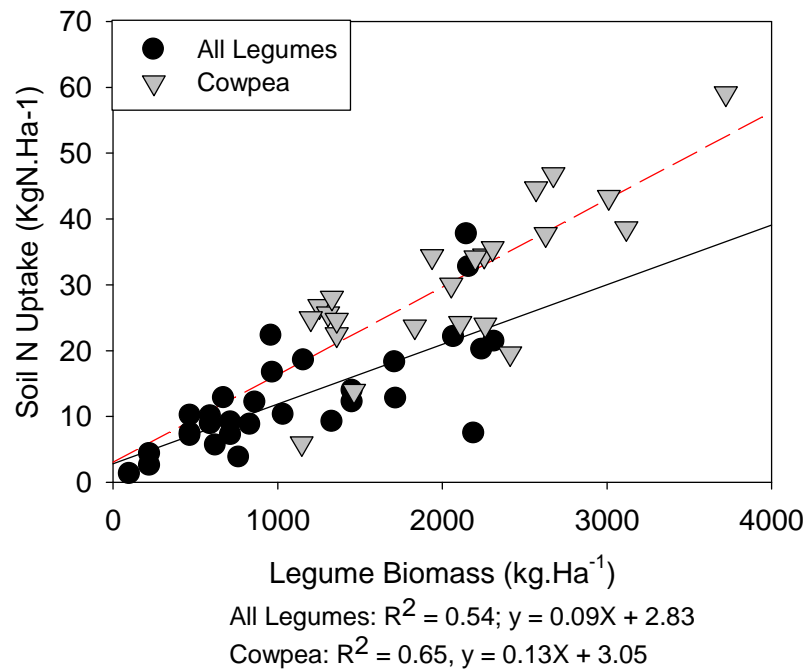
Figure 2.16 The regression of legume biomass (g.m^{-2}) and total soil nitrogen uptake (kg N.Ha^{-1}) for Cowpea and Crimson Clover.

The reduced inter-specific competition due to the mowing, allowed the two clover species to significantly increase their biomass from August to October - for Berseem Clover from 112g.m^{-2} to 233g.m^{-2} and for Crimson Clover from 92g.m^{-2} to 268g.m^{-2} (Tukey's HSD, p<0.0001). Half the Sorghum Sudan plots were left unmowed, these sections maintained some inter-specific competition on the two clover species, and consequently had lower clover biomass than the mowed species – Berseem Clover unmowed had 175g.m^{-2} and Crimson Clover unmowed had 207g.m^{-2} at October (Data not shown).

The reduced inter-specific competition on the legumes and the consequent increase in biomass production resulted in a significant increase in nitrogen fixation (Figure 2.18; Tukey's HSD, p<0.05). In the Buckwheat mixtures, the amount of nitrogen fixed significantly increased from 3 to 23 kg N.Ha^{-1} for Berseem Clover and from 5 to 45 kg N.Ha^{-1} for Crimson Clover from August to October (Figure 2.18). In the mowed Sorghum Sudan mixtures the amount of nitrogen fixed increased from 15 to 29 kg N.Ha^{-1} for Berseem Clover and from 14 to 51 kgN.Ha^{-1} for Crimson Clover from August to October (Figure 2.18).

2.3.9 Japanese Millet mixtures – Regular vs High Seeding rates

Japanese Millet was less competitive than the other non-legume species in both viny and non-viny legume mixtures. Mixtures containing Japanese Millet had greater legume biomass, but the weed biomass was also very high in these mixtures. Increasing the seeding rate of Japanese Millet in the mixture from the original seeding density, 20 to 80% for non-viny mixtures; and 400 to 700% for viny mixtures was a potential solution to the weed problems in the mixtures. The higher seeding rates suppressed both the weed and legume biomass, but the weeds were more severely suppressed than the legumes (Figure 2.19). Increasing the seeding density of Japanese Millet in mixtures improved weed suppression without suppressing the legumes.

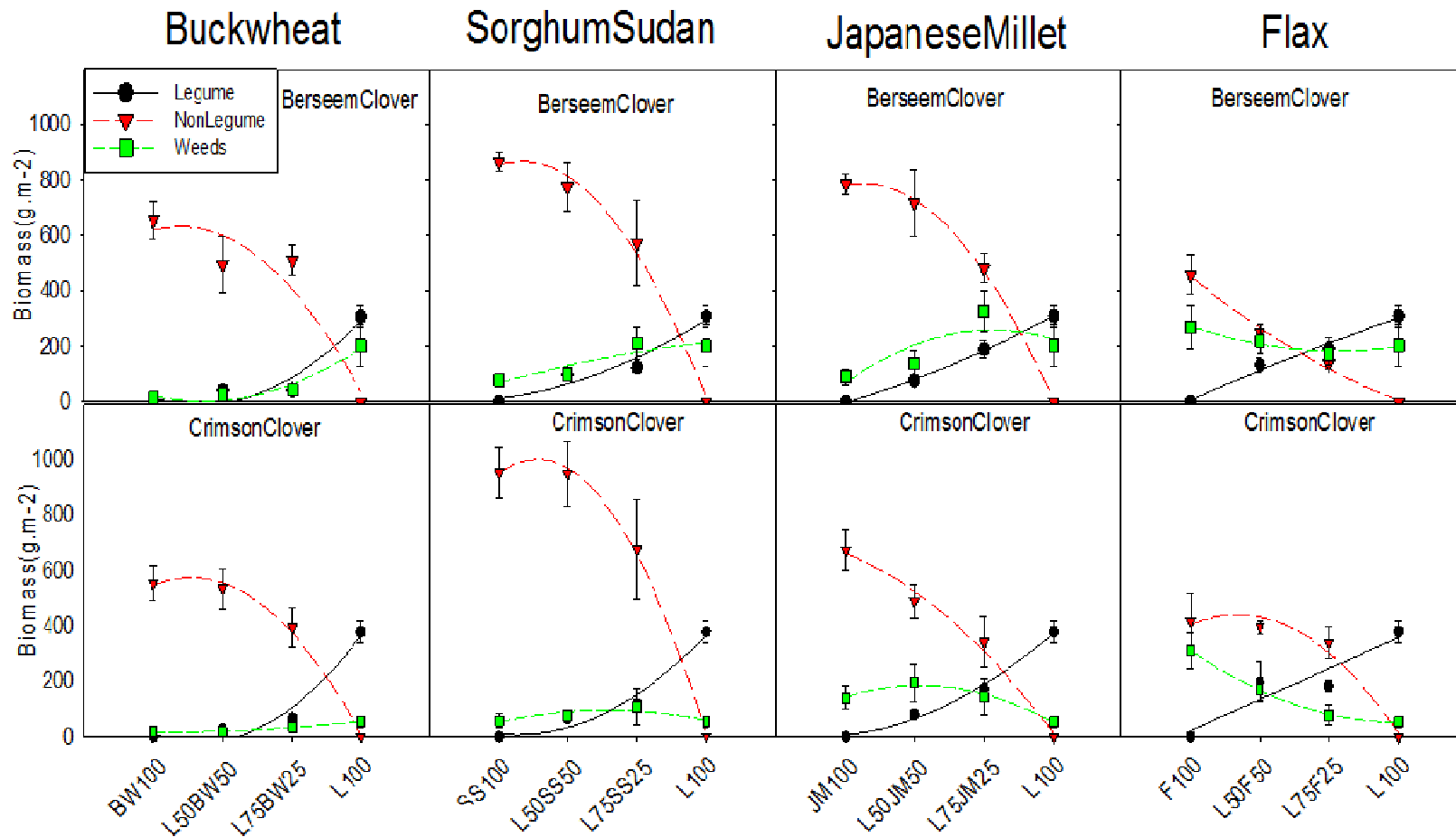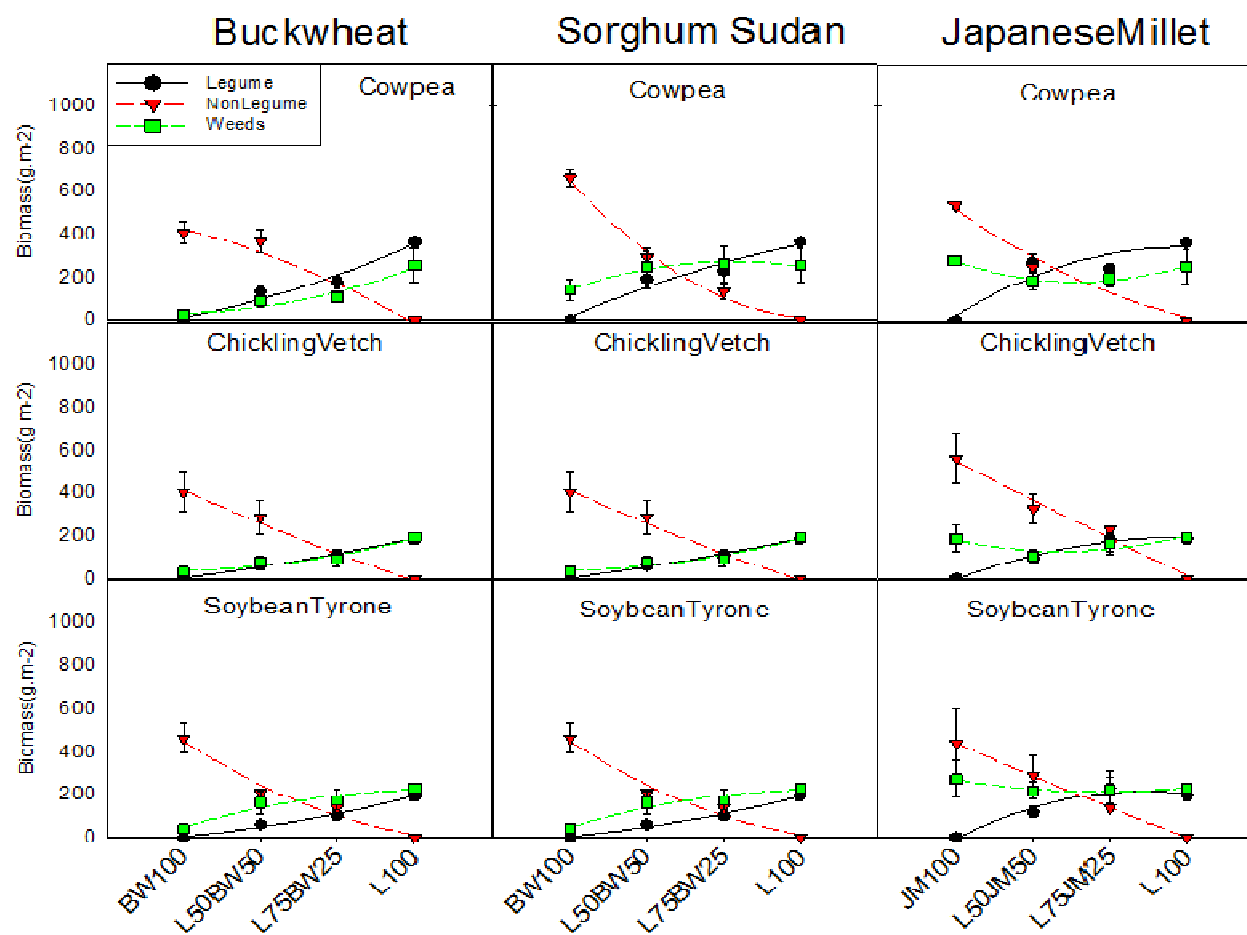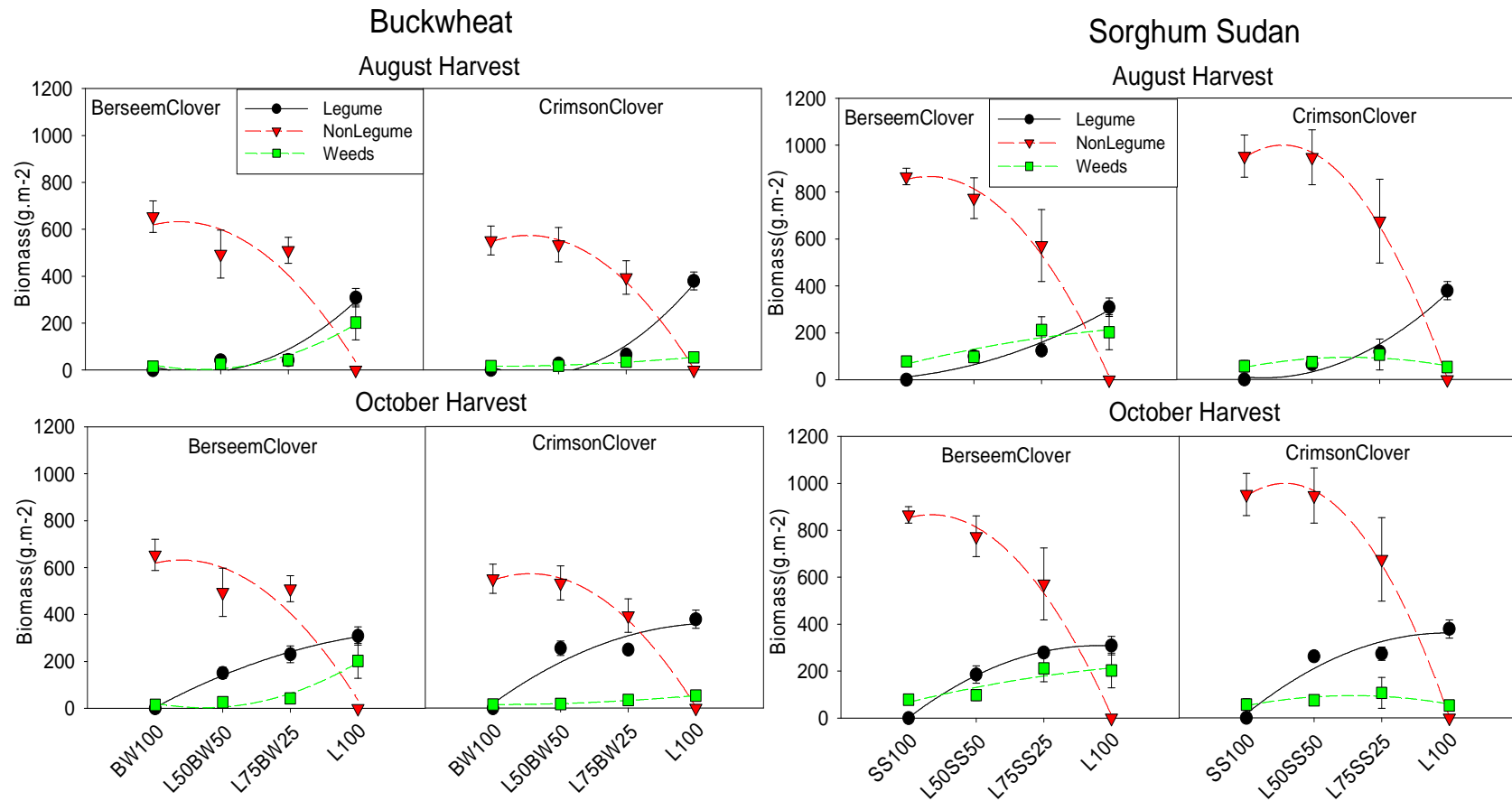**Figure 2.17 Replacement diagrams that illustrate the relative effects of intra-and inter specific competition for the a) Berseem and Crimson Clover mixture with Buckwheat at August harvest and October harvest and b) the Berseem and Crimson Clover mixture with Sorghum Sudan at August harvest and October harvest (mowed and unmowed)**
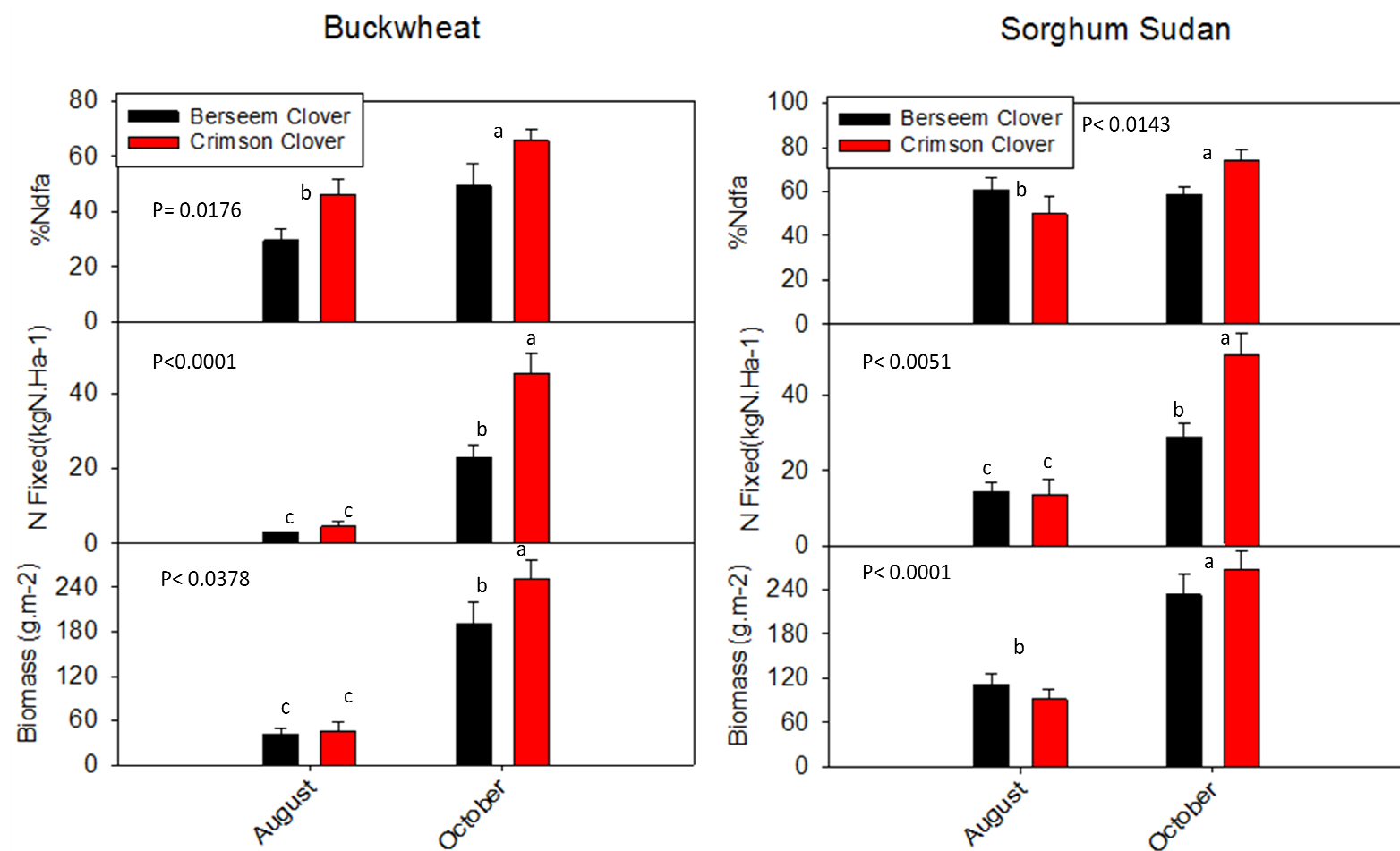
Figure 2.18 Changes in biomass production, nitrogen fixation and nitrogen derived from the atmosphere (%Ndfa) for August and October harvest, for Berseem Clover and Crimson Clover in mixtures with Buckwheat and Sorghum Sudan. Significant differences were obtained using Tukey's HSD, p<0.05.
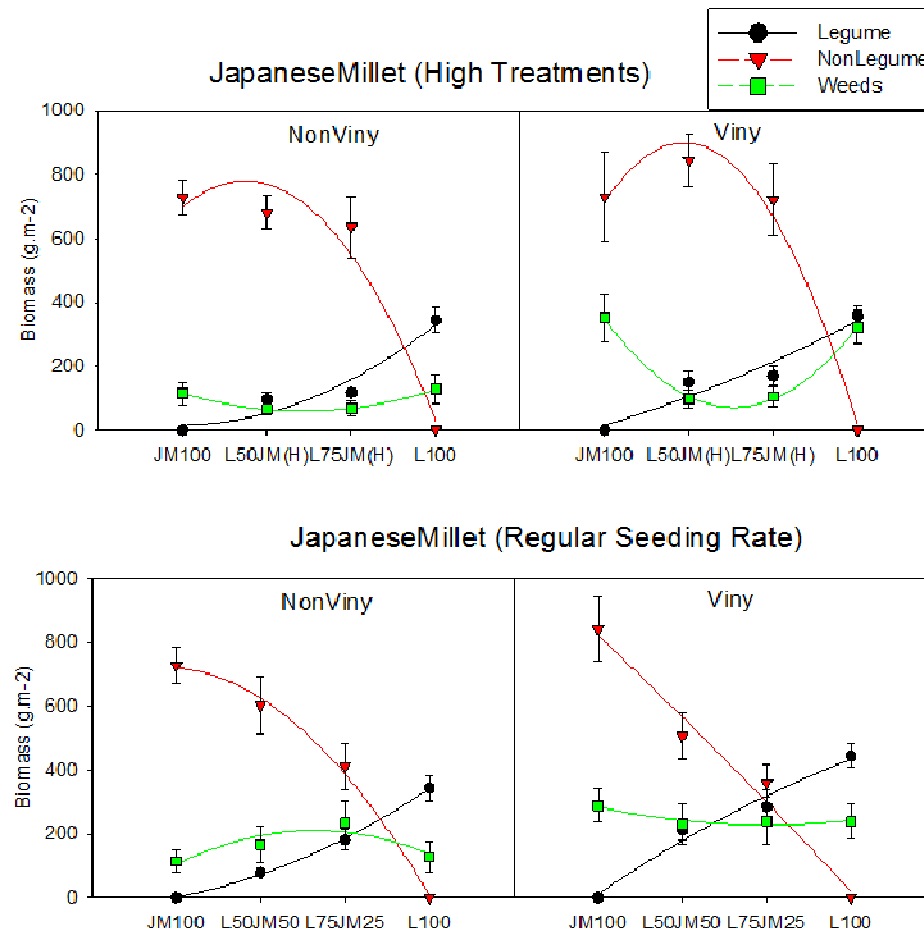
Figure 2.19 Replacement diagrams that illustrate the relative effects of intra-and inter specific competition for the viny and non-viny legumes in mixtures with Japanese Millet at regular seeding rates (see Table 2.3) and at high seeding rates (424g.m⁻²).

2.4 DISCUSSION

2.4.1 Mixtures vs Monocultures: Biomass Production, N fixation and weed suppression

In the ecological literature, studies investigating the relationship between plant diversity and ecosystem functioning have found that plant assemblages with diverse functional traits tend to be more productive than less diverse assemblages (Hooper et al. 2005). The increased productivity that corresponds with greater plant diversity occurs through two distinct mechanisms: complementarity and facilitation (Vandermeer 1989; Gross and Cardinale 2007). Complementarity occurs where different plants use the same resource at a different space or time and facilitation occurs when the presence of one species alleviates a constraint that limits the growth of the other species (Hooper et al. 2005). The greater exploitation of the soil volume, through complementarity between species, increases the access to soil resources and leads to increased productivity. Given the increases in productivity in functionally diverse plant assemblages it is expected that cover crop mixtures will be more productive than sole crops. In this study, most of the mixtures were found to be more productive than the sole crops (with a LER > 1), the only exception being the Buckwheat mixtures that were slightly less productive than the sole crops (LER<1).

Growing legumes and non-legumes together in mixtures frequently increases the proportion of the legume N derived from fixation, because the non-legumes out compete the legume for soil N (Anil et al. 1998; Carr et al. 1998; Corre-Hellou et al. 2006). The absolute amount of N that is fixed depends on the biomass composition of the mixtures and the proportion of fixed N contained in the legume biomass (van Kessel and Hartley 2000; Corre-Hellou et al. 2006). If mixtures are dominated by non-legumes the total N fixed will be reduced despite greater N fixation rates due to the reduction in legume biomass (Corre-Hellou et al. 2006). In this study we found that

the N fixation rates increased in most mixtures (Sorghum Sudan, Japanese Millet and Flax), however legumes growing with Buckwheat had reduced rates of N fixation.

Although both viny and non-viny legumes increased the proportion of N fixed, the impact on total N fixed varied. All the mixtures of non-viny legumes fixed less N compared to the monocultures due to reduced fixation rates and lower legume biomass. For the viny species, mixtures with C-4 grasses fixed the same amount of N as the monocultures. In these mixtures, increased N fixation rates compensated for reductions in legume biomass. Viny species in Buckwheat mixtures fixed less N than the monocultures, again due to reduced N fixation rates and lower legume biomass.

We also expected mixtures to more effectively suppress weeds compared to legume monocultures, in part, due to greater productivity in mixtures and more complete usage of soil (especially N) and above-ground resources (light) making these resources unavailable for weeds. We found that this was the case for most legume species included in our study. Crimson Clover was the exception. Crimson Clover showed extremely low weed biomass due to its ability to rapidly cover the soil surface and through allelopathy (Weston 1996). The weed suppressive ability of the non-legumes can be organized as follows: Buckwheat > Sorghum Sudan > Japanese Millet > Flax. Buckwheat and Sorghum Sudan are weed suppressive through smothering weeds, rapid resource uptake and allelopathy (Weston 1996; Belz 2007; Kumar et al. 2008).

The most effective mixtures for weed control were those consisting of two weed suppressive species (such as Sorghum Sudan or Buckwheat + Crimson Clover). In these mixtures, weed biomass was very low and similar in mixtures and monocultures of the corresponding species. Mixtures consisting of one effective plus one ineffective weed suppressing species, weed biomass was intermediate in the mixture compared to the corresponding monocultures: non-suppressive species

monoculture > mixture > suppressive species monoculture. Mixtures consisting of species that did not suppress weeds as monocultures also had high weed biomass levels, similar to the biomass of their constituent species.

We cannot make broad generalizations about the success of these mixtures in achieving multiple functions compared to monocultures. Instead, the outcomes of mixtures in terms of biomass production, N fixation, and weed suppression depended on the species composition of the mixtures and their competitive ability.

2.4.2 Plant Species Differences: Inter- and Intra-specific competition in mixtures

The replacement series design used in this study has the advantage of being able to determine the relative intensity of the inter- and intra-specific competition in the mixtures, albeit in a non-quantitative way. This knowledge is important for designing future cover crop mixtures that optimize biomass production and agro-ecosystem functions that they are required to perform. One of the main limitations of using the replacement design is that the total density of the mixtures is largely determined by subjective criteria. Since a key focus of this study was the optimal management of N fixation in mixed cover crop stands, the legume monoculture seeding densities were used as the target density for the replacement series.

There were two groups of legumes with different seeding densities, the non-viny legumes (Crimson and Berseem Clover) had about a four-fold greater seeding density than the viny legumes (Soybean Tyrone, Chickling Vetch and Cowpea). The differences in seeding densities were used to compensate for differences in seed and plant stature (non-viny legumes have smaller seeds, are generally smaller than the viny species and are generally seeded at higher rates in agricultural settings).

Based on the trends in relative biomass production in both the non-viny and viny mixtures, the relative aggrssivity of the non-legumes can be ranked as follows: Buckwheat > Sorghum Sudan > Japanese Millet > Flax. When growing an aggressive

and non-aggressive species in a mixture, the aggressive species experiences lower competition in mixture than in monoculture (intra-specific competition is more intense than inter-specific competition). The reduced competition in mixture leads to greater productivity for the more aggressive species. This was the case for Buckwheat and Sorghum Sudan mixtures. Earlier studies have reported similar trends, such as pea-barley and wheat-maize/soybean (Hauggaard-Nielsen and Jensen 2001a; Andersen et al. 2004; Andersen et al. 2007). The dominance of the aggressive species leads to the suppression of both the legume and weed biomass.

The viny legumes differed in their competitive ability. Cowpea was more competitive in all the mixtures compared to Soybean Tyrone and Chickling Vetch. Cowpea's greater competitive ability may be due to its greater capacity to access soil N compared to the other two legume species. Other studies have concluded that Cowpea is an effective competitor for soil resources (Randall et al. 2006), and can grow effectively in a wide range of soil conditions, such as low pH (Rohyadi et al. 2004) or heavy metal contamination (Al-Garni 2006).

The non-viny species also differed in their competitive ability. Berseem Clover was slightly more competitive than Crimson Clover. Berseem Clover has deeper roots and a more upright growth type which makes it a more effective competitor than Crimson Clover. Crimson Clover relied more on N fixation than Berseem Clover, due to its greater tolerance to lower temperatures and acid soils (Evers 2003). Crimson Clover's greater N fixation rates did not make it more competitive in the mixtures. Both clover species were generally not very competitive in mixtures against the non-legumes. Other studies have found that clovers are not very competitive species (Kruidhof et al. 2009). The high soil fertility may have given plants with greater resource acquisition traits a competitive advantage.

In a mixture that contains an intensely competitive species, such as Buckwheat or Sorghum Sudan, it seems that temporal complementarity is a more effective way to maintain mixture productivity than spatial complementarity. This suggests that the competition from the aggressive crop is so intense that it overcomes subtle complementary differences in plant traits. However, in mixtures composed of two species with relatively low aggressivity (i.e. Japanese Millet and the viny legumes), spatial complementarity is an important mechanism that leads to high mixture productivity.

2.4.3 Trade-offs in Weed control and N fixation: The role of mowing

There was a clear trade-off in N fixation and weed suppression in this study. The trade-off occurs because mixtures that are especially effective at suppressing weeds, also suppressed legume biomass and legumes that are competitive in the mixtures did not fix a lot of N and they do not suppress weeds.

A potential way to manage mixtures to optimize both weed suppression and N fixation is to mow the competitive non-legumes once the legumes are established in the understory. Fast-growing non-legumes that put intense inter-specific competition on the legumes in mixtures, reduces both legume and weed biomass. By mowing down the non-legumes the inter-specific competition experienced by the legumes is reduced, and since the intra-specific competition for legumes is relatively low, it allows the legumes to significantly increase their biomass production. The increase in biomass production also leads to a significant increase in N fixation.

Therefore, the temporal complementarity of the legume and non-legume in these mixtures leads to effective N fixation and good weed suppression. The early growth of the non-legume suppresses weeds and the later growth of the legume leads to N fixation. Similar successional complementarity has been obtained in mixtures with wheat and soybean/maize, where the wheat/maize was mowed down early and

this allowed the soybean to grow effectively (Li et al. 2001; Evers 2003). It is important to remember that the mowing strategy for managing mixtures will only work under the following conditions: (1) If the non-legume is an effective weed suppressor and has very fast growth rates soon and after planting and (2) The legumes have slow growth rates soon after planting and produce most biomass after the non-legume has been mowed.

Another strategy that could be used to optimize N fixation and weed suppression is to increase the seeding density of a non-legume with low intra-specific competition (low aggressivity). In this study it was found that increasing the seeding density of Japanese Millet reduced both the weed and legume biomass in the mixture but the legume biomass was reduced less severely than the weed biomass. Although this strategy is not as effective as mowing to avoid weed and N fixation trade-offs, it is still reduces the trade-off.

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CHAPTER 3: CHANGES IN N-FIXATION RATES ACROSS DIFFERENT SITES

3.1 INTRODUCTION

In organic cropping systems, weed suppression and N fertility are two of the most important agro-ecosystem functions that can be achieved through cover cropping. N fixation through the use of legume cover crops is an important source of new atmospheric N into these systems. There are a number of challenges in using legume N fixation as a source of N: (1) N fixation is highly variable and depends on a complex interaction between soil, plant and environmental factors. (2) There is a negative feedback between soil N and N fixation, therefore farmers that add compost and manure onto their soils over an extended period of time are likely to have reduced levels of N fixation. (3) There is a limited niche within which farmers can grow legume cover crops to fix N without foregoing the opportunity of growing a cash crop.

The symbiotic relationship between *Rhizobia* bacteria and the host legume plant, is sensitive to soil and environmental factors. Every step in the process requires optimal ranges of soil pH and plant macro and micro-nutrients. A shortage of Ca, P , Zn, Fe and Mo can limit N fixation whereas high concentrations of Al and Mn in the soil can be toxic to both the *Rhizobia* bacteria and the host plant. Since N fixation requires large energy expenditures from the plant, the plant will preferentially take up soil N if it is available and will consequently not fix as much atmospheric N at high soil N levels. Soil physical factors, such as soil aeration and water content are also important factors that drive N fixation and nodulation – in anaerobic soil conditions nodulation and N fixation get inhibited.

In legume-based mixtures non-legumes out-compete legumes for soil N and consequently increase the legumes' N fixation rates. These higher N fixation rates do

not necessarily increase the total N fixed because in some cases the non-legume also out-competes the legume for light and other resources resulting in reduced legume biomass. Legume-based mixtures are also expected to suppress weeds better than legume monocultures since the non-legumes in the mixtures are better able to compete with weeds. Furthermore, mixtures tend to use resources more effectively, reducing the resources available to weeds.

This study has the following aims:

1. To evaluate the biomass production, N fixation and weed suppression of different legume-based mixtures.

2. To determine whether the biomass production, N fixation rates and total N fixed varies depending on the soil conditions.

3.2 MATERIALS AND METOHDS

3.2.1 Site and soil

Research plots were established at three sites during 2009. The sites were located at Penn Yann, NY (42°45′16″N 76°51′57″W); Lodi, NY (42° 36′ 48″ N, 76° 49′ 27″ W) and Freeville, NY, USA (42° 30′ 45″ N, 76° 20′ 45″ W). The site at Penn Yan was a field of about 5 hectares with a cropping history that consisted mostly of grains, such as spelt, wheat and barley. Relatively low amounts of compost and manure were added to this site. The sites at Freeville and Lodi were slightly smaller (1 hectare at Freeville and 0.75 hectare at Lodi) and had a cropping history of vegetables. The sites at Freeville and Lodi received greater additions of compost and manure than the site at Penn Yan. The experiments were conducted from July 10th until October 18th in 2009. The climate is characterized as humid temperate, the mean annual precipitation is 880mm and the mean annual minimum and maximum air temperature is 3 °C and 14°C, respectively.

3.2.2 Description of soil N fractions

In order to quantify the range of soil N pools that contribute to plant available N, all the soil N fractions spanning the continuum from labile to recalcitrant, were quantified. The fractions that were quantified were, extractable NO_3^- and NH_4^+, N mineralization potential, free particulate organic matter N (fPOM), occluded particulate organic matter N (oPOM) and total C and N. Seven soil samples were taken in mid-October and composited from each replicate block in each field using a one-piece Dutch auger (Eijkelkamp, Giesbeek, The Netherlands). Soil inorganic N (NH_4^+ and NO_3^-) levels were determined by extracting a sieved sub-sample of the soil with 2.67KCL and then shaking the samples at 150RPM on the day of sampling. To determine potentially mineralizable N a sub-sample was anaerobically incubated at 30^oC for seven days using a 2M KCL extraction. The samples were then centrifuged and NO_3^- and NH_4^+ was extracted from the sample. Total NH_4^+ and NO_3^- for both the incubation and extraction samples, was analyzed using an Automated Discrete Analyzer (AQ2) (Seal Analytical Inc., Mequon, Wisconsin). Gravimetric water content was recorded for samples and the remaining soil was air-dried.

Free particulate organic matter (fPOM) and oPOM was separated using size and density separation as in (Marriott and Wander 2006). Briefly, fPOM was separated by floating on sodium polytungstate (1.7 g cm^{-3}). The remaining soil sample was shaken with 10% sodium hexametaphosphate to disperse soil aggregates and then rinsed through a 53 μm filter. The fraction larger than 53 μm, which included sand and particulate organic matter, was retained. Total C and N of POM fractions and total soil were measured using a LECO 2000 CN Analyzer (Leco Corporation, St. Joseph, MO).

Replicated composite samples of air-dried soils were sieved to 2mm and were then analyzed for Mehlich 3-extractable P, K, Ca, Mg, Cu, Zn, Fe, Al, and Mn, as well as

particle size (Agricultural Services Laboratory, The Pennsylvania State University, University Park, PA). The data is shown in (

Table **3.3**).

3.2.3 Experimental outline and plot establishment

The experimental design in each field was a split-plot design, with four replicate blocks per field. The main treatment was the legume species, which determined the total seeding density of all the sub-treatments. The sub-treatments consisted of two monocultures of legume and non-legume species and then a single mixture of the two species.The legume monoculture seeding rates were determined by reviewing extension sources and cover cropping handbooks.

At Freeville and Lodi a replacement series design was used, which means that the total seeding density for the mixtures and monocultures was the same. The monocultures, for legumes and non-legumes, were sown at the recommended seeding density (plants.m^{-2}) of the legume species and the mixtures were seeded to contain 50% legume and 50% non-legume seed. At Penn Yan, the mixtures were sown in an additive design. The legume and non-legume monocultures were sown at half the recommended legume seeding rate. For the mixture at Penn Yan, the legume and non-legume was sown at the same densities that they were sown in monoculture. The mixture at Penn Yan therefore had twice the seeding density of the monocultures. The mixtures at Penn Yan, Lodi and Freeville had the same seeding rates, but the monocultures at Penn Yan had half the seeding density of the monocultures at Lodi and Freeville. For this reason, the monocultures cannot be compared across all three sites due to the differences in seeding density, but the different mixtures can be compared. The seeding rates for the different species are given in (Table 3.2).

The following legume species were included in this study: Forage Soybean

(*Glycine max*) variety Tyrone, Cowpea (*Vigna unguiculata*), Berseem Clover (*Trifolium alexandrinum*), and Crimson clover (*Trifolium incarnatum*). The different non-legumes that were included in the study were Buckwheat (*Fagopyrum esculentum*), Sorghum-Sudan (*Sorghum bicolor*) and Japanese Millet (*Echinochloa frumentacea*) . A summary of all the mixtures that were used in this study can be seen in (Table 3.1).

The field at Freeville was seeded on July 10th and the fields at Penn Yan and Lodi were seeded on July 17th. The seeder used at Freeville was a using a 6' 3-point no-till drill and the seeder used at Penn Yann was a 13' end wheel drill, both drills were manufactured by Great Plains (Great Plains Mfg, Salina, Kansas). The seed at Lodi was broadcast, and the seeding rate was increased by 20% to compensate for the lower germination rates associated with broadcasting. Plant counts were done for every legume and non-legume in monoculture, 2 – 3 weeks after seeding. The plant counts were done in the middle two rows of the plot. For the two Berseem Clover and Crimson Clover main treatments the middle two rows were counted for a length of 0.4 m, for all the other main treatments the middle two rows were counted for a length of 1m. Because of the high seeding densities of the two clover main treatments, counting a full meter would have been impractical.

3.2.4 Biomass sampling and analytical methods

The Buckwheat treatments – monocultures and mixtures – were sampled from 22 – 26 August, the remaining treatments were sampled from 15 – 20 September. On 28 August all the Buckwheat mixtures and on 10 September all the Sorghum Sudan mixtures, for the Crimson Clover and Berseem Clover mixtures were mowed down to a height of 20cm using a Weed Trimmer (Stihl FS110R and Stihl FS85) (Stihl, Norfolk, VA, USA). The mowed treatments were sampled on 18th October.

97

Table 3.1 Summary of all the mixtures planted at each of the farm sites.

Freeville	Penn Yan	Lodi
Cowpea – JapaneseMillet	Cowpea – JapaneseMillet	Cowpea – JapaneseMillet
Soybean – JapaneseMillet		Soybean-JapaneseMillet
Cowpea-SorghumSudan	Cowpea-SorghumSudan	
BerseemClover-Buckwheat	BerseemClover-Buckwheat	
CrimsonClover-Flax	CrimsonClover-Flax	
CrimsonClover-SorghumSudan	CrimsonClover-SorghumSudan	
CrimsonClover-Buckwheat	CrimsonClover-Buckwheat	

During sampling, the middle two rows were sampled for a length of 0.4 m for the Berseem and Crimson Clover main treatments and 1m for the other legume species. When plants were sampled, the legume, non-legume and weed biomass was separated. The fresh weight biomass of the samples was weighed and the samples were stored at 60°C until a constant weight was reached, the dry weight was recorded. The legume, non-legume and weed dry material was coarsely ground using a hammer mill and a Christy grinder. The legume material (in monoculture and mixture) and the non-legume plant material (only in monoculture) were further pulverized using a roller grinder for 48 hours. These samples were micro-balanced and sent to the UC Davis Stable Isotope Facility in Davis, California, U.S.A to be analyzed for ^{15}N natural abundance and total N content using the PDZ Europa 20-20 continous flow Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK). The non-legume plant material in the mixtures and all the weed plant material was analyzed for total C and N content using a LECO 2000 CN Analyzer (Leco Corporation, St. Joseph, MO).

3.2.5 N-fixation calculation

The ^{15}N natural abundance method was used to estimate biological nitrogen fixation (BNF) (Shearer and Kohl 1986). The percentage of nitrogen derived from the

atmosphere for all the legumes in monoculture and mixture was determined using the Sorghum Sudan, Flax and Buckwheat as reference plants. The following equation was used to determine the percentage of nitrogen derived from the atmosphere:

%N from fixation = 100 x [(δ^{15}N Reference Plants - δ^{15}N Legume Plants)

$$/ [(\delta^{15}\text{N Reference Plants} – B)] \qquad (3.1)$$

B is the δ^{15}N value for a legume when atmospheric N_2 is the only source of nitrogen after accounting for seed nitrogen. The total amount of above ground atmospheric nitrogen that was fixed was calculated using the biomass nitrogen concentration and the percentage of nitrogen from fixation.

In order to obtain the *B* value for all the legumes, a growth chamber study was conducted where the legumes were grown in N-free, washed, and autoclaved sand mixed with perlite at a ratio of 1:1. Legume seeds were sterelized using 70% ethanol (v/v) for three minutes, and 3% bleach solution for two minutes, and then rinsed in deionized water for three minutes. The seed was inoculated with the same inoculant as the field plots. The plants were ferrtilized using a N-free Hoagland's solution (Greencare Fertilizers, Chicago, IL) and a gypsum solution. Plants were sampled at the same maturity stage as the plants in the field. The plants were coarse ground using the hammer mill and christy mill, and finely pulverized using the roller grinder. Samples were then sent to UC Davis where they were analyzed for δ^{15}N using the PDZ Europa 20-20 continous flow Isotope Ratio Mass Spectrometer (Sercon Ltd., Cheshire, UK).

The *B*-values used for the different legumes were: Crimson Clover (-0.74‰), Berseem Clover (-1.04‰), Cowpea (-2.56‰), and Soybean Tyrone (-0.76‰).

3.2.6 Statistical Analysis

Statistics was computed using the JMP 8 statistical package. Variables were assessed for normal distribution and were log-transformed when it was not the case. Data was analyzed using mixed models with site as a random factor, and main

treatment and mixture nested within main treatment. Multiple comparisons were calculated using Tukey's HSD. For mixtures occurring at each site correlations between Nfixation rates and total N fixed and the different soil variables were calculated.

3.3 RESULTS

3.3.1 Germination Rates

All the germination rates were within acceptable ranges at all three fields (73 – 90%). The final seeding densities were reasonable close to the target seeding densities (Table 3.2).

3.3.2 Soil Characteristics

There were differences in the soil textural and chemical characteristics across the three sites. The soils at Penn Yan and Lodi contained more sand than the soil at Freeville (Table 3.3). The soil at Freeville had a lower pH (5.95) than Penn Yan (6.1) and Lodi (6.5). All the mineral nutrients were within acceptable ranges to support good plant growth.

There were differences across the three sites regarding the different soil C and N pools, with Penn Yan consistently having C and N levels at the lower end compared to Lodi and Freeville (Table 3.4). Both Freeville and Lodi had a significantly greater inorganic soil N pool (3.7mg.kg^{-1} and 4.2mg.kg^{-1}, respectively) than Penn Yan (1.3mg.kg^{-1}). The N mineralization rate at Lodi was greater than that at Freeville and Penn Yan. Lodi also had greater fPOM C and N levels than the other fields. The fields at Lodi and Freeville had greater oPOM C and N levels compared to Penn Yan. The soil at Lodi had a greater C: N ratio than the other two soils for both oPOM and fPOM. Freeville had the greatest total soil N (2.4g.kg^{-1}) followed by Lodi (1.7 g.kg^{-1})

and Penn Yan (1.0 g.kg^{-1}), while Lodi had the greatest total C followed by Freeville and Penn Yan.

3.3.3 Biomass Production, and Weed Suppression

The differences in legume biomass production across the three sites were less pronounced than differences between the different mixtures within a site (Table 3.5). At all three sites, Cowpea consistently had the greatest biomass production, followed by Soybean Tyrone, Crimson Clover and Berseem Clover. The Crimson Clover biomass was reduced more in the Buckwheat mixtures than the Sorghum Sudan mixtures, which suggests that Buckwheat was more competitive than Sorghum Sudan. The non-legumes generally had greater biomass at Lodi and Freeville fields than Penn Yan, which reflects the N fertility differences across these sites.

The weed biomass was generally higher in Lodi followed by Freeville and Penn Yan (Table 3.5). The weed biomass across different sites was the highest for the Crimson Clover-Flax and Cowpea-SorghumSudan mixtures. The Japanese Millet and the Buckwheat treatments were the most weed suppressive of the mixtures. The effective weed suppression by Japanese Millet is probably due to the higher seeding rates.

3.3.4 Nitrogen Fixation

There were differences in the N fixation rates and the total N fixed across the different sites (Table 3.6). For all the legume species the N fixation rates were lower in Freeville than at Penn Yan and Lodi. The lower N fixation rates also led to lower rates of total N fixed at Freeville compared to Lodi and Penn Yan because the biomass production across the sites were comparable. There were differences in the N fixation traits of the different legume species with Crimson Clover and Soybean Tyrone having

greater N fixation rates than Cowpea and Berseem Clover. This pattern occurred at all three sites. Legumes in Buckwheat mixtures fixed the least total N. This is probably due to the shading and reduced growth of Crimson and Berseem Clover species.

Crimson Clover-Flax fixed the most N at Freeville and Penn Yan it was followed by Soybean-Japanese Millet at Freeville and the Cowpea-Sorghum Sudan treatment at Penn Yan. Although Cowpea had the lowest N fixation rates, in certain circumstances its high biomass production compensated for this and resulted in great amounts of total N fixed.

3.3.5 Mowed Treatments

Mowing Buckwheat and Sorghum Sudan grown in mixture with Crimson Clover and Berseem Clover was an effective way to increase N fixation (Table 3.7). The Crimson Clover biomass increased four to ten-fold at Freeville and four to six-fold at Penn Yan in the mowed Sorghum Sudan and Buckwheat mixtures. As a result, total N fixed increased nine to ten-fold and two to four-fold by the time of the October harvest. The Berseem Clover biomass increased four to seven fold in the mowed Sorghum Sudan mixture at the October harvest. The increases in biomass led to an increase five-fold and a four-fold increase in N fixed for the fields at Freeville and Penn Yan after mowing (Table 3.7).

The weeds in the mowed treatments were suppressed to the same level as the unmowed treatments. Therefore, mowing treatments is an effective strategy to obtain both N fixation and weed suppression.

Table 3.2 The amount of seed sown (seed.m^{-2} and kg.ha^{-1}), plants counted (plants.m^{-2}) and germination rates (%Germ) for the legume and non-legume monocultures at the three sites. The monocultures at Freeville and Lodi were sown at the recommended seeding density of the legume main treatment (Main Tmt) while at Penn Yan the seeding density was half of the recommended rate. Japanese Millet was sown at a higher seeding density (H) than the other non-legume species. The legumes include Crimson Clover (CC), Berseem Clover (BC), Cowpea (CP), and Soybean Tyrone (SY); and the non-legumes include Buckwheat (BW), Sorghum Sudan (SS) and Japanese Millet (JM).

	Freeville					Penn Yan					Lodi			
Spp	Seed	kg. ha^{-1}	Plants. m^{-2}	% Germ	Spp	Seed	kg. ha^{-1}	Plants. m^{-2}	% Germ	Spp	Seed	kg. ha^{-1}	Plants. m^{-2}	% Germ
CC	420	21	356 (15)	85(4)	CC	315	16	303(25)	96(16)	CP	74	69	71 (5)	96(14)
BC	476	17	456(76)	96(16)	BC	357	13	294(35)	82(24)	SY	74	83	62(3)	84(10)
CP	98	92	92(6)	94(7)	CP	45	42	47(4)	94(7)					
SY	98	110	72(1)	72(4)										
BW	476	164	429(15)	90(3)	BW	314	108	285(14)	91(10)					
SS	476	56	381(42)	80(9)	SS	315	37	231(16)	73(14)					
JM	476	14	294(24)	62(5)	JM	424	12	344(18)	81(10)	JM(H)	424	12	379(11)	89(6)
F	476	20	372(81)	78(12)	F	212	9	182(8)	86(9)					

Table 3.3 Soil chemical and textural variables taken at the three sites

Field	pH	P	K	Mg	Ca	CEC	Clay	Sand	Silt
		Ppm				meq.100g-1		g.kg-1	
Freeville	5.95(0.0)	59(5)	133(10)	278(7)	1981(44)	17(0.2)	271(23)	376(39)	254(18)
PennYan	6.1(0.2)	23(4)	95(11)	132(18)	1478(249)	12.7(1.1)	238(14)	496(25)	266.5
Lodi	6.5(0.1)	1.09(3)	163(13)	166(6)	2043(73)	14.9(0.3)	249(4)	415(9)	337(7)

Table 3.4 The different soil C and N fractions for the soils at the three sites

Field	T0 NO$_3^-$	T0 NH$_4^+$	Inorg	NMin	opom N	opom C	lfpom N	opom C:N	lfpom C:N	Tot C	Tot N
		mg.kg-1		(mg.kg-1.wk-1)		mg.kg-1				g.kg-1	
Freeville	3(0.5)	0.8(0.02)	3.7(0.5)	16.3(1.3)	152(20)	3443(628)	31(4)	22.3(1.3)	33.3(3.6)	31.4(0.1)	2.4(0.1)
PennYan	0.7(0.1)	0.7(0.1)	1.3(0.1)	16.8(2.1)	53.8(7.3)	1571(131)	32.3(1.4)	30.1(2.5)	24.3(0.8)	15.7(0.6)	1(0)
Lodi	2.4(0.3)	1.8(0.2)	4.2(0.5)	39(1.6)	189(33)	7755(1394)	135(29)	40.7(1.9)	37.1(1.2)	43.3(6.5)	1.7(0.2)

3.3.6 Soil Factors influencing N fixation rates and total N fixed

The only mixture that was grown at all three sites was the Cowpea-Japanese Millet mixture. The different soil variables were correlated with N fixation rates and the total N fixed across the three sites for this mixture. Nitrogen fixation rates and total N fixed were negatively correlated with total soil N ($R^2 = 0.82$, p<0.0001 and $R^2 = 0.72$, p= 0.0003, respectively). Total N fixed was also negatively correlated with oPOM N ($R^2 = 0.39$, p=0.0233). Texture influenced total N fixed, total N fixed being positively correlated with sand content ($R^2 = 0.44$, p = 0.0132) and negatively with clay ($R^2 = 0.32$, p = 0.042).

Table 3.5 The legume, non-legume and weed biomass (g.m^{-2}) for the different mixtures across the three sites.

Mixtures	Freeville						Penn Yan						Lodi					
	Legume		NonLegume		Weed		Legume		NonLegume		Weed		Legume		NonLegume		Weed	
									g.m^{-2}									
Cowpea - JapaneseMillet	139	(31)	572	(40)	99	(16)	136	(18)	535	(36)	9	(4)	173	(13)	644	(104)	149	(14)
Soybean - JapaneseMillet	134	(36)	588	(47)	67	(30)							156	(8)	815	(81)	160	(21)
Cowpea-SorghumSudan	192	(43)	291	(42)	267	(73)	166	(17)	158	(27)	76	(22)						
BerseemClover-Buckwheat	42	(7)	494	(103)	26	(11)	37	(6)	446	(21)	16	(6)						
CrimsonClover-Flax	196	(74)	405	(24)	170	(38)	178	(14)	325	(26)	200	(100)						
CrimsonClover-SorghumSudan	65	(8)	947	(117)	75	(18)	50	(9)	500	(54)	29	(17)						
CrimsonClover-Buckwheat	26	(8)	534	(73)	17	(4)	41	(5)	400	(21)	8	(5)						

Table 3.6 The N fixation rates as the proportion of legume N fixed (%Ndfa = % N derived from the atmosphere), total N fixed and total soil N uptake (soil N) for all the legumes in the mixtures across the three sites. Means and standard errors are given.

Mixtures	Freeville						Penn Yan						Lodi					
	N Fixed		%Ndfa		Soil N		N Fixed		%Ndfa		Soil N		N Fixed		%Ndfa		Soil N	
	kg N.Ha^{-1}		%		kg N.Ha^{-1}		kg N.Ha^{-1}		%		kg N.Ha^{-1}		kg N.Ha^{-1}		%		kg N.Ha^{-1}	
Cowpea – JapaneseMillet	6	(1)	22	(2)	21	(4)	27	(5)	92	(5)	2	(2)	16	(5)	59	(17)	13	(6)
Soybean – JapaneseMillet	15	(3)	65	(2)	8	(2)							36	(4)	100	(0)	0	(0)
Cowpea-SorghumSudan	11	(2)	38	(10)	23	(8)	34	(4)	90	(4)	4	(1)						
BerseemClover-Buckwheat	3	(0)	33	(6)	7	(2)	8	(1)	97	(3)	0	(0)						
CrimsonClover-Flax	32	(10)	64	(7)	21	(9)	48	(8)	100	(0)	0	(0)						
CrimsonClover-SorghumSudan	9	(2)	49	(5)	9	(1)	20	(6)	100	(0)	0	(0)						
CrimsonClover-Buckwheat	4	(1)	64	(10)	3	(2)	12	(3)	100	(0)	0	(0)						

Table 3.7 Summary of the legume biomass, total N fixed (kg N.Ha-1), N fixation rate (%Ndfa) and legume soil N uptake for the mowed Buckwheat treatments at Freeville and Penn Yann

Mowed Treatment	Freeville								Penn Yan							
	Biomass		N Fixed		%Ndfa		Soil N		Biomass		N Fixed		%Ndfa		Soil N	
	g.m-2		kgN.Ha-1		%		kgN.Ha-1		g.m-2		kgN.Ha-1		%		kgN.Ha-1	
CrimsonClover-Buckwheat-Mowed	273	(15)	41	(2)	60	(3)	21	9	249	(15)	30	(8)	63	(16)	19	(7)
BerseemClover-Buckwheat-Mowed	151	(24)	16	(3)	46	(11)	20	8	217	(11)	42	(1)	96	(1)	2	(0)
CrimsonClover-SorghumSudan-Mowed	273	(28)	53	(7)	72	(5)	20	2	246	(19)	46	(8)	79	(13)	18	(11)

3.4 DISCUSSION

The results indicate that N fixation rates and total N fixed are more sensitive to variation in soil conditions than legume biomass production. The N fixation rates across the three sites were very variable, with the Freeville site fixing the lowest amounts of N. The lower N fixation rates at Freeville can be explained by the relatively high total and inorganic soil N and the heavier soils. Schipanski et al. (2010) also found that soil texture had a strong influence on legume performance and N fixation rates. The lower sand at Freeville means that the soil is more prone to anaearobic conditions, especially during periods of rain. Since N fixation requires well aerated soils, the anaerobic conditions in the heavy soils during periods of rain leads to low N fixation rates as was the case at the Freeville site.

Biomass production and N fixation rates varied with legume species. Crimson Clover and Soybean Tyrone had greater reliance on N fixation than Berseem Clover and Cowpea across the different sites. Especially Cowpea, had much lower N fixation rates, and accessed more soil N. Cowpea is more sensitive to soil conditions than the other legumes, and its N fixation rates varied the most across the three sites. Cowpea's ability to take up soil resources makes it a good competitor in the mixtures.

There were differences in the weed suppression capacity of the different mixtures, with the Buckwheat and Japanese Millet mixtures being more weed suppressive than the other mixtures. Buckwheat is known to be very competitive and being able to take up soil resources very rapidly and suppressing weeds through allelopathy.

The legume species responded in a similar way to environmental variation across the three sites. The N fixation rates and total N fixed for all the legume species were consistently higher in the Penn Yan and Lodi sites and lower at the Freeville site. Although there were differences in the absolute N fixation rates and N total fixed, the

trends in N fixation and N fixed by each species were the same across the three sites. The soil factors that affected N fixation rates include: oPOM N and total soil N had a negative relationship with N fixation rate and the texture played an important role where sand content had a positive correlation and clay a negative correlation with N fixation. Similar results were found by (Riffkin et al. 1999), where lighter textured soils supported improved N fixation and (Schipanski et al. 2010) where higher total soil N lead to reduced N fixation rates.

Since N fixation is a process that is very sensitive to environmental variation, doing studies across various sites allows us to identify the key soil parameters that drive N fixation. Since we were unable to use the Crimson Clover and Berseem Clover species at Lodi due to seeding and germination problems, our analysis of soil parameters across three sites was limited to the Cowpea-Japanese Millet mixture. This limited our ability to tease apart how soil characteristics affect N fixation in various species across sites. Further studies on how the abiotic conditions affect N fixation in different species will be very insightful.

This study showed that the most promising cover crop mixtures for the annual summer niche are those that include Berseem Clover or Crimson Clover species in mixtures with Sorghum Sudan or Buckwheat. These mixtures, when appropriately managed through mowing, are very effective at suppressing weeds and fixing atmospheric N. Another potential mixture is Cowpea and any of the C4-grass species, such as Japanese Millet and Sorghum Sudan. Cowpea is very competitive and can fix large amounts of N due to its great biomass production, while the C4-grass species are more effective at suppressing weeds.

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