Comparing the effects of summer cover crop monocultures and mixture on an organic vegetable rotation in Florida

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ACKNOWLEDGMENTS

Throughout the writing process of this paper, I have received countless support and assistance.

First, I would like to thank my advisor, Dr. Maltais-Landry Gabriel, for his precious support in the writing process of this paper. I am incredibly thankful for his patience, support, and the opportunity he has given me to gain some research skills that will benefit me throughout my future career.

I would like to thank Dr. Allan R. Bacon and Dr. Chris H. Wilson for being part of my committee and contributing to my training via their teaching.

I would like to acknowledge the early Scientist Career Seed Grant from UF/IFAS for providing the funding for this project.

I would like to acknowledge Anna Prizzia, James Longanecker, Dina Liebowitz, and all the interns and volunteers at the UF Field and Fork Program for their support and guidance in the field.

Finally, I would like to acknowledge Mica James, Emma Goldberg, Madelene Clark, Kaitlyn Tucker, Ethan Weinrich, Mia Acker, Adnan Fatehi, Roxanne Sandi, John Allar, and Gabriel Pelegrina for their assistance both in the field and in the lab.

ABSTRACT

Cover crops are increasingly used as residual nutrient scavengers and green manures, including in Florida, characterized by coarse-textured soils and a humid and hot climate. Legume cover crop residues (low C:N ratio) can quickly mineralize before subsequent plant uptake and grass cover crop residues (high C:N ratio) can immobilize nitrogen. Cover crop mixtures could potentially offset these tradeoffs, but the extent to which mixtures can optimize such services relative to monocultures is unclear. A two-year experiment was set up at Field and Fork (Gainesville, FL) to compare the effects of four cover crop monocultures - sorghum sudangrass, millet, sunn hemp, and cowpea - and a mixture combining these four species on a Florida vegetable rotation (bok choy-carrot-squash). Cover crops were grown from June to August 2019, followed by bok choy, carrot, a short buckwheat cover crop, and squash. Soil samples were taken before each crop to measure Mehlich-3 extractable nutrients, and incubations measured N release after cover crop termination. Millet produced higher (p =0.073) biomass than cowpea, but similar biomass to sorghum, sunn hemp and the mixture. Cowpea and millet N uptake was significantly higher (p < 0.05) than sorghum. Bok choy, carrot, and squash yields were similar among all cover crop treatments. There were similar N, P, and K concentrations and uptake in all cash crops among all the treatments. Soil N was generally highest in cowpea, sunn hemp and millet treatments at cover crop termination and during the first 28 days of incubation, but there were no significant differences among treatments after 42 days. The first year of this experiment showed that mixture could perform similarly to monocultures, although these results need to be confirmed in future research to determine if mixtures can perform similarly or better than monocultures.

1.0. INTRODUCTION

The green revolution has significantly increased yields (Drinkwater & Snapp, 2007) and enhanced food security worldwide in the past decades. The use of synthetic fertilizers, highyielding varieties resulting from successful breeding programs, irrigation, conventional tillage, and pesticides have been the main pillars underpinning this success (Tilman et al., 2001). However, these accomplishments have resulted in undesirable environmental effects such as greater greenhouse gas fluxes (Robertson et al., 2000), soil organic carbon losses (Luo et al., 2010), soil degradation (Drinkwater & Snapp, 2007), and water quality impairments due to nutrient leaching and subsequent eutrophication (Shelton et al., 2018). These adverse effects, particularly soil degradation and soil organic carbon (SOC) loss, are partly due to the adoption of practices that dissociate carbon (C), nitrogen (N), and phosphorus (P) cycles (Drinkwater & Snapp, 2007). Such practices consist of meeting plant needs with ample amounts of soluble and inorganic fertilizers leading to soil saturation and impeding microbially and plant-mediated processes such as decomposition, assimilation, and nutrient mobilization from organic and mineral soil pools (Asner et al., 1997; Drinkwater & Snapp, 2007; Yuan & Chen, 2015). Accordingly, management practices that recouple these cycles and mitigate adverse environmental impacts are needed for sustainable agriculture.

Cover crops are one beneficial practice that can offset the damaging impacts of conventional practices (Kaye & Quemada, 2017), as it can alleviate SOC loss (Plaza-Bonilla et al., 2018) or increase SOC (Poeplau & Don, 2015), reduce nitrate leaching (Constantin et al., 2010; Thapa et al., 2018) and mitigate greenhouse gas emissions (Bayer et al., 2016; Kaye & Quemada, 2017) in both conventional and organic agriculture. Cover crops are multifunctional (Blanco-Canqui et al., 2015) and they can add temporal diversity to cropping systems. Thus,

integrating cover crops in cropping systems can help reverse negative impacts of certain farming practices on SOC and nutrient leaching, as well as other ecosystem services such as the enhancement of soil physical, chemical, and biological properties.

1.1. Cover crop effects on soil physical and biological properties

A meta-analysis conducted by Poeplau and Don (2015), using data from 139 plots at 37 different sites, showed that cover crop introduction into cropping systems enhance SOC stock by adding 0.32 ± 0.08 Mg SOC ha⁻¹ yr⁻¹ at a mean soil depth of 22 cm. Blanco-Canqui et al. (2015) also found that cover crops increase SOC, although the extent of this increase depends on multiple factors including soil type, biomass production, tillage, climate, and the time since adoption of cover cropping. Due to their positive effects on SOC stock, cover crops lower the carbon footprint of agricultural cropping systems (Plaza-Bonilla et al., 2018). At the Coastal Plain Experiment Station in the southeastern USA (Tifton, GA), Hubbard et al. (2013) found cover crop inputs added 0.3–4.7 mg g⁻¹ C to the soil. The positive impact of cover crops on SOC contributes to enhancing soil aggregation and aggregate stability by increasing mean weight diameter of aggregates and reducing soil compaction (Abdollahi & Munkholm, 2014; Blanco-Canqui et al., 2011; Liu et al., 2005).

In a long-term study conducted at Hesston, KS, Blanco-Canqui et al. (2011) found that cover crops significantly improved soil physical properties in the top soil (0-7.5 cm) of no-till systems. After 15 years of cover cropping, sunn hemp (*Crotalaria juncea* L.) significantly improved SOC (by 30%), resulting in greater water infiltration compared to a control, but late-maturing soybean (*Glycine max* L. Merr.) did not have the same effects. Both sunn hemp and late-maturing soybean increased soil water holding capacity while decreasing the soil temperature in springtime compared to treatments receiving no cover crops. Hubbard et al.

(2013) carried out a similar experiment in Tifton, GA, that involved four rotational systems: sunn hemp/fallow/sweet corn, fallow/crimson clover/sweet corn, fallow/fallow/sweet corn, and fallow/fallow/fallow. Hubbard et al. (2013) found that sunn hemp-based rotations significantly increased soil volumetric water content (0.126 cm³ cm⁻³) and had significantly lower soil bulk density (1.71 Mg m⁻³) compared to rotations where fallow began the rotations (0.113 cm³ cm⁻³ and 1.73 Mg m⁻³, respectively).

Cover crops can also be used as a form of biological tillage to reverse soil compaction in no-till agroecosystems (Chen & Weil, 2010). However, not all cover crop roots are adapted to grow in compacted soils, as Chen and Weil (2010) demonstrated that tap-rooted cover crops such as forage radish (*Raphanus sativus* var. longipinnatus, cv. 'Daikon') and rapeseed (*Brassica napus*, cv. 'Essex') had double the root numbers of cereal rye (*Secale cereale* L., cv. 'Wheeler') in highly compacted soil. Abdollahi and Munkholm (2014) found that fodder radish can reduce soil compaction and root penetration resistance at 20-40 cm depth in a long-term tillage and rotation trial in Denmark. Acuña and Villamil (2014) showed that soil compaction could be alleviated following radish cover crops grown in monoculture or in mixture with rye, triticale (*Triticosecale* 'Presto'), buckwheat (*Fagopyrum esculentum* L. Moench), or hairy vetch (*Vicia villosa* Roth). Due to their positive impact on alleviating soil compaction, cover crops can improve water infiltration, enhance water- and wind-stable aggregates, and reduce water and wind erosion (Blanco-Canqui et al., 2011; De Baets et al., 2011; Krutz et al., 2009; Liu et al., 2005).

By increasing SOC and improving physical properties, cover crops also shape a better environment for soil microbes (Buyer et al., 2010; Mullen et al., 1998) and significantly alter the microbial community structure (Carrera et al., 2007; Wortman et al., 2013). After an experiment

conducted in a no-till system, Mullen et al. (1998) found higher bacterial abundance and enzyme activity (e.g., acid phosphatase, arylsulfatase, β-glucosidase, L-asparaginase) in the vetch treatment without N inputs than fertilized treatments without cover crops. In a 3-year experiment conducted at the USDA-ARS Beltsville Agricultural Research Center (Beltsville, Md), Buyer et al. (2010) found higher microbial biomass and diversity under hairy vetch and rye cover crop treatments than a bare soil, but noticed a decrease in the proportion of gram-positive bacteria compared to gram-negative bacteria. That decrease in gram-positive bacteria proportion can be viewed as an indicator of soil fertility enhancement as gram-negative bacteria (copiotrophs) mostly thrive in condition of high soil nutrient availability such as labile carbon compared to gram-positive bacteria (oligotrophs) that are more competitive in condition of low soil fertility (Bastian et al., 2009; Fanin et al., 2019; Pascault et al., 2013). Among all cover crop treatments, Buyer et al. (2010) also observed that treatments receiving hairy vetch had a greater proportion of gram-negative bacteria, fungi, and arbuscular mycorrhizal fungi in the tomato rhizosphere, which were attributed to higher-quality labile carbon inputs that vetch shoots added to the soil. Cover cropping also increased the number of arbuscular mycorrhizal fungi that are associated with corn roots and in the bulk soil, in an experiment conducted by Ramos-Zapata et al. (2012). These findings highlight the significance of cover crops in enhancing soil biological communities, which plays a crucial role in maintaining agricultural soil quality.

1.2. Cover crops and weed suppression

Cover crops can also provide useful services related to weed suppression compared to fallows (Holmes et al., 2017; Zotarelli et al., 2009). There are several mechanisms by which cover crops control weeds. They can be involved in direct competition with weeds for resources such as nutrients, water, and space (Bicksler & Masiunas, 2009). The decomposition of cover

germination by limiting light exposure and reducing the temperature of the soil surface when used as organic mulch (Sturm et al., 2016). The contribution of cover crops in terms of weed suppression varies among different cover crop species, and the biomass production capacity of a given species is positively correlated to its ability to suppress weed growth (Finney et al., 2016; Holmes et al., 2017; Wayman et al., 2015).

Although a given cover crop's capacity to quickly establish and produce high biomass positively correlates with its ability to suppress weeds, allelopathic effects can be as decisive as biomass production regarding weed-suppressive ability. In laboratory and field studies conducted in Germany, Kunz et al. (2016) found that cover crop monocultures - mustard (*Sinapis alba* L.), fodder radish, and spring vetch - and cover crop mixtures reduced weed biomass by 60% and 66%, respectively between 2013 and 2015 due to their rapid growth and release of allelopathic biochemical compounds. They also found that biochemical extracts from cover crops can delay weed germination by 54%. Similarly, while investigating the effects of cover crops on weed suppression through several laboratory, greenhouse and field studies, Sturm et al. (2016) found that allelopathic compounds embedded in cover crop tissues were responsible for weed suppression following their residue decomposition. Holmes et al. (2017) found that forage radish reduced weed biomass by 45-100% despite having lower biomass production than sudangrass (*Sorghum bicolor* L.ssp. Drummondii), highlighting that allelopathic compound release can be as effective as large biomass production at suppressing weeds.

Similar to cover crop monocultures, cover crop mixtures are capable of providing weed suppression services in agroecosystems. In experiments conducted at PrairiErth Farm near Atlanta, IL, and Kinnikinnick Farm near Caledonia, IL, Holmes et al. (2017) found that mixtures can be as

effective in suppressing weeds as highly weed-suppressive monocultures, although this depends on the identity of the individual species included in the mixture. They found that in monoculture or a 5-species cover crop mixture, mustard (*Brassica juncea* L. Czern) and oat (*Avena sativa* L.) were the most productive and weed-suppressive cover crops sown in spring while sudangrass and buckwheat were among the most weed-suppressive summer-sown cover crops. Bicksler and Masiunas (2009) also found that sudangrass grown in mixtures or monocultures suppressed Canada Thistle (*Cirsium arvense*). Furthermore, Smith et al. (2015) found that weed species number was down by 25% and 40% after introducing sorghum sudangrass (*Sorghum bicolor x S. bicolor* var. sudanese) in 2011 and 2012, respectively, while buckwheat curtailed the number of weed species by 36% and 59% in 2011 and 2012, respectively. In summary, both cover crop monocultures and mixtures provide useful weed suppression services to agroecosystems via their biomass production and exudation of allelopathic compounds, impeding weed germination and growth. However, exudation of allelopathic compounds may also harm the subsequent cash crops, so it is critical that the cover crops be compatible with the subsequent cash crops.

1.3. Cover crops and pest control

Cover crops can reduce pest damage to subsequent vegetable cash crops due to their suppressive effects on weeds that can serve as habitat for pests and pathogens, including in Florida where pest pressure is high (Li et al., 2006). Furthermore, cover crops can provide habitat for various natural enemies that consume pests of subsequent cash crops, but this depends on the availability of secondary hosts and food sources in the lag time between cover crop termination and cash crops establishment (McNeill et al., 2012; Rodríguez et al., 2012). Cover crops such as sorghum sudangrass, sunn hemp (*Crotalaria juncea* L.), and cowpea (*Vigna unguiculata* L. cv. Iron & Clay) can also reduce plant-parasitic nematodes in vegetable systems,

including in Florida organic tomato production (Li et al., 2006; Wang et al., 2003a; Wang et al., 2015). However, cover crops can be both non-hosts for some plant-parasitic nematodes while being hosts for other pests: sorghum sudangrass hosts armyworms (*Spodoptera* sp.) and corn silk flies that might adversely affect subsequent vegetables (Li et al., 2006). Crow et al. (2001) also found sorghum sudangrass to be a host for *Belonolaimus longicaudatus* and Paratrichodorus *minor*, two plant-parasitic nematodes that can be detrimental to potato (*Solanum tuberosum* L.) production in Florida. Thus, carefully choosing which cover crop to grow in rotation is critical to avoid potential damage on the following cash crops (Wang et al., 2005b).

1.4. Cover crops and nutrient cycling

Cover crops play a critical role in nutrient cycling, especially N, through N-fixation and nutrient scavenging, although it is not easy to optimize these two ecosystem services with a single species (Ramírez-García et al., 2015; White et al., 2017). Legume cover crops can fix N₂ biologically, while non-legume cover crops are better at scavenging soil nitrate (Kaspar et al., 2012; Thapa et al., 2018). Combining these two categories of cover crops could enhance N cycling, as legume cover crops produce residues with low C:N ratios that are prone to rapid mineralization, whereas grass cover crop residues have a high C:N ratio that tends to delay N mineralization (Finney et al., 2016; O'Connell et al., 2015; White et al., 2017). As both functions are vital for crop production, i.e., N supply to the subsequent crops via residue decomposition and soil N capture before leaching, growing a legume-grass cover crop mixture could optimize N cycling (Couëdel et al., 2018; Reiss & Drinkwater, 2020; Vann et al., 2017).

The capacity of non-legume cover crops to scavenge N from the soil allows them to mitigate soil nutrient leaching, particularly nitrate. Kaspar et al. (2012) found monocultures of winter rye and fall oat cover crops reduced nitrate leaching by 48% and 26% over five years

compared to a control without cover crops, respectively. In an experiment investigating the effects of no-till, cover cropping, and reduced fertilizers on leaching, Constantin et al. (2010) found that cover cropping reduced nitrate leaching by 36-62% and was the most effective practice at reducing nitrate leaching. In Florida, Wang et al. (2005a) found that sunn hemp reduced leachate amount to a greater extent (90.8%) than sorghum sudangrass (71.3%), although both crops had similar N and P concentrations in leachate. Couëdel et al. (2018) found that crucifer-legume mixtures had similar nitrate retention (59%) than crucifer monocultures but were significantly more effective than legumes, which reduced nitrate leaching by 35%. In contrast to these studies reporting a reduction in nitrate leaching with cover cropping, Campiglia et al. (2011) found higher cumulative nitrate leaching in cover crops such as hairy vetch (102.3 kg N ha⁻¹), subterranean clover (*Trifolium subterraneum* L., 95.3 kg N ha⁻¹), and hairy vetch/oat mixture (94.7 kg N ha⁻¹, respectively) relative to a conventional system without cover crops (48.2 kg N ha⁻¹) in a 2-year experiment on pepper (*Capsicum annuum* L.) carried out in Italy.

A global meta-analysis by Thapa et al. (2018) further confirmed the findings of these individual experiments. Their meta-analysis revealed that non-legume cover crops reduced nitrate leaching by 56% compared to bare fallow, but noted that this ability is highly variable and depends on many factors such as the amount of cover crop biomass production, cover crop planting dates, and precipitation regime. For instance, they found that non-legume cover crops were more effective at reducing nitrate leaching in coarse-textured (by nearly 90%) than in fine-textured soil. Thapa et al. (2018) also found that nitrate leaching negatively correlated with cover crop biomass at termination, meaning that higher cover crop biomass resulted in higher N uptake and, consequently, in lower nitrate leaching. As opposed to non-legume cover crops, Thapa et al. (2018) found that legume cover crops and cover crop mixtures with non-legumes and legumes

did not significantly reduce nitrate leaching relative to fallow. However, when they removed the experiment conducted by Campiglia et al. (2011) mentioned earlier, they found that legume monocultures or legume-non-legume mixtures significantly reduced nitrate leaching compared to fallow.

In addition to biological N fixation and nitrate scavenging, the transfer of N from cover crop residues to subsequent cash crops is another critical service on which farmers, particularly organic farmers, can rely to reduce external N inputs. Nitrogen deficiency is a major driver of the limited productivity observed in organic farming systems worldwide (Berry et al., 2006), and cover crops could provide substantial N amounts to cropping systems. Li et al. (2015), using ¹⁵N tracers, showed that legume-based cover crops (e.g., clover or clover-rye mixture) produced residues containing 153-226 kg N ha⁻¹, which contributed to an increase in the yield and dry matter of subsequent barley similar to what was observed with 50 kg N ha⁻¹ provided with inorganic fertilizers. In contrast, non-legume cover crops (e.g., rye) had little or no effect on subsequent yields, probably due to N immobilization. Wang et al. (2009) also found that legume summer cover crop residues (e.g., sunn hemp) increased the yield of Florida organic tomato compared with non-legume cover crop residues (e.g., sorghum sudangrass). In northern Florida, Cherr et al. (2006) found that sunn hemp can produce nearly 12.2 Mg ha⁻¹ of aboveground biomass and accumulate 172 kg N ha⁻¹ in 14 weeks, highlighting the ability of sunn hemp to provide green manure benefits. However, 45% to 58% of that biomass N was lost in the first four weeks following sunn hemp termination (Cherr et al., 2006), highlighting how challenging it is for a subsequent cash crop to actually benefit from sunn hemp as a green manure. In the coastal plain of the southeastern United States, Schomberg et al. (2007) confirmed the result obtained by Cherr et al. (2006), as sunn hemp biomass production varied between 8.9 to 13.0 Mg ha⁻¹ with

biomass N ranging from 135 to 285 kg N ha⁻¹, providing a good green manure for vegetable producers. In the end, the magnitude of N transfer from cover crop residues to the following cash crop is highly variable and depends on soil type, climate, and cropping system (Doltra & Olesen, 2013).

1.5. Challenges of using cover crops in vegetable production in Florida

Given the sandy nature of Florida soils resulting in low nutrient holding capacity and its hot and humid climate (Munoz-Arboleda et al., 2008), Florida farmers have two additional challenges to synchronize N release from summer cover crop residues with cash crop N uptake. On the one hand, the rapid mineralization of low C:N legume residues should lead to high N availability at a time when the cash crops are not established yet for an adequate N uptake, which Crews and Peoples (2005) called "excess-asynchrony". On the other hand, high C:N non-legume residues may lead to N immobilization when cash crops need N, designated by "insufficient asynchrony" by Crews and Peoples (2005). In both cases, substantial loss via leaching may occur (Weinert et al., 2002) given the low nutrient retention capacity of most Florida soils (Li et al., 2006; Wang et al., 2015), which would affect the sustainability of agroecosystems. Therefore, it is important to develop management practices that will enhance the synchronization between N release and uptake in Florida soils to maximize the recovery of cover crop N. Combining legume and non-legume cover crops could produce residues with intermediate C:N that would slow N release while preventing immobilization (Poffenbarger et al., 2015; Thapa et al., 2018), which could result in better synchronization with cash crop N demand.

Organic and conventional farmers along with researchers are increasingly interested in using cover crop mixtures to optimize cover crop benefits (Holmes et al., 2019; Reiss & Drinkwater, 2020). White et al. (2017) found a lower N transfer to subsequent cash crops in

legume-grass mixtures compared to legume monocultures, despite the grass-legume mixture being as effective in capturing soil nitrate as the grass monoculture. Cherr et al. (2006) noted that the end result of cover crops used as green manure depends on the interaction among many factors, including green manure quality, environmental conditions, and management practices. Given the sandy nature of Florida soils and its hot and humid climate (Munoz-Arboleda et al., 2008), N mineralization patterns could differ relative to the trade-offs observed by White et al. (2017) in cover crop mixtures. Moreover, as most studies focused on the effects of green manures on grain crops, cover crop mixtures could affect vegetables differently. Thus, determining if cover crop mixtures provide more N benefits than cover crop monocultures in organic vegetable production in Florida is critical to optimize N management in these systems.

Using cover crops as a source of N is challenging in organic systems because yields in organic agriculture are typically lower than in conventional systems, with N limitation playing a key role. Meta-analyses by Ponisio et al. (2015) and Seufert et al. (2012) showed that organic agriculture typically had lower yield than conventional agriculture, albeit Ponisio et al. (2015) found a lower yield gap (up to 19%) than Seufert et al. (2012) (up to 34%). Limited N availability, which relates to the nature of N sources and cash crop types, is one of the main reasons explaining lower yields in organic agriculture (Berry et al., 2006). Organic farming mostly relies on organic amendments (manure and compost) with slow N release to meet crop N needs, but mineralization patterns of those amendments often differ from crop N uptake patterns, with several crops (e.g., corn) having a high and narrow peak N of demand (Pang & Letey, 2000). Therefore, one strategy to address this challenge is to apply high amounts of amendments to meet plant N needs. Nonetheless, as with cover crop residues, asynchrony between N release

and crop N uptake may occur (Crews & Peoples, 2005; Pang & Letey, 2000), which can be detrimental to the environment.

Another challenge with using manure and compost to meet plant N needs in organic vegetable systems is the potential to end up with P surpluses (Zikeli et al., 2017), as those amendments often have low N:P ratios while plant outputs have high N:P ratios (Maltais-Landry et al., 2016). Thus, cover crops, especially legume cover crops, can reduce the dependency on external N sources through biological N₂ fixation (Maltais-Landry et al., 2016), reducing P surpluses occurring when using manures and composts by taking up soil excess P and quickly releasing it for subsequent cash crops (Maltais-Landry & Frossard, 2015). However, cover crop residues must be mineralized, which depends on environmental factors (temperature and moisture) and residue quality (C:N ratio and carbon quality). Therefore, they are subject to similar challenges to manures and composts, such as asynchrony with plant uptake (Crews & Peoples, 2005; Pang & Letey, 2000). Given the coarse-textured nature of Florida soil and its humid and hot climate, nutrient release from organic amendments might be quicker than in finetextured soils and cold climates (Hochmuth et al., 2009). Therefore, management that is adapted to local conditions is critical for successful nutrient management in organic agriculture (Maltais-Landry et al., 2016; Zikeli et al., 2017).

Vegetable systems require large amounts of nutrient inputs, especially N, during a short growing season (Zikeli et al., 2017), which adds additional nutrient management constraints to organic production. Low N recovery (less than 50%) in vegetable systems adds to the challenges of managing nutrients, making it harder to limit N leaching in the coarse-textured soils of Florida that have low water and nutrient holding capacity (Jalpa et al., 2020; Marchi et al., 2016). Cover cropping should help with nutrient scavenging, nutrient leaching control and N release in

synchrony with plant N needs. Legumes are well known to be able to biologically fix N and serve as excellent green manure, while grasses are known for their ability to scavenge N and prevent. Given the sandy nature and low organic matter content of Florida soil coupled with Florida vegetable systems' low nutrient use efficiency, cover cropping can be an attractive strategy to face those challenges. Hence, cover cropping strategies that can optimize N fixation and retention as well as adding organic matter via high biomass production could be more desirable in Florida soils. Mixtures of legumes and grasses could combine those ecosystem services, but more research assessing their impact in organic vegetable systems is needed.

1.6. Objectives

This study aims to determine the effects of summer cover crops on N cycling in Florida vegetable production, using a bok choy (*Brassica rapa* subsp. chinensis) - carrot (*Daucus carota* subsp. sativus) - squash (*Cucurbita pepo*) rotation. I will compare how four monocultures – sunn hemp, cowpea, pearl millet (*Pennisetum americanum* L.), sorghum sudangrass – and a mixture containing all of these species affect:

- o Cover crop biomass production and N uptake;
- o Nitrogen release and other soil fertility indicators;
- Yields and nutrient uptake in subsequent vegetables.

1.7. Hypotheses

I expect biomass production to be similar or slightly higher between legume monocultures and the mixture, and I expect grass monocultures to have lower biomass (Table 1). The rationale for lower biomass production with grass monocultures is that the study site was previously occupied by bahiagrass (*Paspalum notatum* Flüggé) with possibly high C:N residues and no residual N from previous fertilization. Hence, millet and sorghum sudangrass could be

deprived of N due to possible N immobilization. In contrast, legumes can biologically fix N and be more competitive at low soil N. I expect the mixture to produce similar or slightly less biomass than legumes, as Finney et al. (2016), Holmes et al. (2017), Thapa et al. (2018), and Poffenbarger et al. (2015) showed that mixture could be as productive as highly productive monocultures.

I expect residue C:N and N mineralization to be respectively lower and higher in legume monocultures, higher and lower in grass monocultures (leading to immobilization), and intermediate and more balanced in the mixture (Table 1). Campiglia et al. (2011), Finney et al. (2016), and Li et al. (2006) found lower C:N in legume, intermediate in the mixture, and higher in grasses, and with a negative correlation between the C:N ratio and N mineralization.

Based on C:N ratio and N mineralization of cover crop residues, I anticipate similar bok choy yield after legumes or mixtures and lower yield after grasses, as bok choy is a fast-growing and short-season vegetable with a high N demand during early growth (Table 1). I hypothesize that carrot yield will follow the order mixture > grasses > legumes, as rapid N mineralization from legumes might lead to a N deficit at the carrot stage due to leaching, whereas the better-balanced C:N ratio of the mixture should result in a more even N mineralization that benefits carrot. Nitrogen immobilization of grass monoculture residues at the bok choy stage should delay mineralization, which should occur at the carrot stage, providing N. Lastly, due to Florida soil's sandy nature, I anticipate no effect from summer cover crops on squash, as most mineralized N not captured at the bok choy and carrot stages would be gone before squash. Therefore, squash would only rely on N fertilizers, with identical rates and yields among all treatments.

Table 1: Summary of expected effects of cover crop treatments on response variables

Response variables	Hypothesis
Cover crop biomass production	Legumes ≥ Mixture > Grasses
Cover crop C:N ratio	Legumes < Mixture < Grasses
Cover crop N release	Legumes = Mixture > Grasses
Bok choy yield	Legumes = Mixture > Grasses
Carrot yield	Mixture > Grasses > Legumes
Squash yield	No expected effects of cover crops

2.0. MATERIALS AND METHODS

2.1. Study site

This study took place at the Field and Fork garden located at the University of Florida in Gainesville, Florida. The experiment was established on soils mapped as Arredondo series (Loamy, Siliceous, Semiactive, Hyperthermic Grossarenic Paleudults) and Lake series (Hyperthermic, coated Typic Quartzipsamments) (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2020). Previously, soils of this study site were covered by a bahiagrass and wildflower meadow. This 2-year experiment was started in June 2019 and will be completed in spring 2021, although this paper focuses on the first year only, i.e., until July 2020.

2.1. Experimental design

The experimental setup is a randomized block design (Figure 1) with five treatments arranged in four blocks. The treatments consist of four cover crop monocultures - sorghum sudangrass, millet, sunn hemp, and cowpea - and a mixture encompassing all the four cover crops in monocultures. Each plot within the study was 7.62 m x 2.74 m (Figure 1).

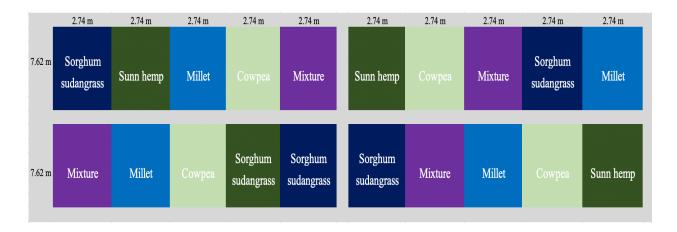


Figure 1:Experimental design

2.2. Crop management

Cover crops were sown on June 25, 2019 and terminated on August 28, 2019, when they were mowed and disked into the soil. In the monocultures, sunn hemp was seeded at 56 kg ha⁻¹, sorghum sudangrass at 56 kg ha⁻¹, millet at 45 kg ha⁻¹, and cowpea at 112 kg ha⁻¹. In the mixture, half of the seeding rate of each cover crop in monoculture was used. Nearly three weeks after cover termination (September 18, 2019), bok choy was transplanted at a density of 47,840 plants ha⁻¹ and harvested on October 17, 2019. Carrot was sown at 956,791 plants ha⁻¹ on October 28, 2019 and harvested on February 10, 2020. A short buckwheat cover crop was seeded on February 11, 2020 and terminated on March 16, 2020. Four days later, squash was seeded at 7,655 ha⁻¹ and harvested in late June and early July during four harvests. Bok choy was fertilized at 196 kg N ha⁻¹ (13-0-0) as a single pre-plant application, the carrot at 112 kg N ha⁻¹ (13-0-0) split into two 56 kg N ha⁻¹ applications, and squash at 168 kg N ha⁻¹ (13-0-0) split into two 84 kg N ha⁻¹ applications. Carrot fertilizer inputs were somewhat lower than recommended, as carrot was assumed to benefit from N carryover from bok choy, which was likely over-fertilized. All crops were drip-irrigated and hay-mulched.

2.2.1. Crop sampling and analysis

Cover crop biomass was sampled using one 0.49 m² quadrat by plot and sorted by species. In the mixture, sorghum sudangrass and millet were combined as grasses due to difficulties in distinguishing between them at sampling time. Cover crop biomass dry weight was determined after being oven-dried at 65°C. Dried biomass was ground with a Wiley-mill for C and N analysis by combustion. In each plot, C and N returned to the soil (in kg ha⁻¹) was determined by multiplying C and N concentration by biomass, and total biomass C and N was obtained by summing cover crop biomass C and weed C and cover crop biomass N and weed N, respectively.

C and N concentration and C:N ratio were determined for cover crops and weeds separately in each monoculture treatment. A similar approach was adopted in the mixture to determine C and N concentration and C:N ratio among its individual components. A weighted average was used to determine C and N concentration and C:N ratio in monocultures, where the contributions of cover crops and weeds were weighed based on their respective biomass weight in each plot. A similar weighted average was used in mixtures.

2.2.2. Cash crop data collection

Aboveground biomass and yield were determined differently depending on the cash crop. Bok choy yields were measured by sampling and weighing 15 contiguous plants in the center of one of the two rows in each plot. A subset of bok choy marketable yield was freeze-dried and weighed to determine its moisture content. Freeze-dried samples were ground using a Wiley-mill and shipped to an external laboratory, Waters Agricultural Labs Inc. (Camilla, GA), for nutrient concentration analyses determined by digestion (N, P, other macronutrients, and micronutrients).

Bok choy uptake for a given nutrient was obtained by multiplying yield by the concentration of this nutrient.

For carrot, aboveground biomass and yields were obtained by collecting all plants in a 3 ft row in the center of one of the two rows in each plot, to avoid edge effects. A subset of 10 representative carrots was randomly taken from each plot, and the shoot and root were weighed separately to determine the harvest index. Shoot samples were oven-dried whereas root samples were freeze-dried separately. Dried samples were weighed for moisture content determination, ground with a Wiley-mill, and analyzed for nutrient concentration (N, P, other macronutrients and micronutrients) by digestion for shoot and root separately by Waters Agricultural Labs Inc. (Camilla, GA). Nutrient uptake was determined for shoot and root separately, and the total nutrient uptake in each plot was calculated by adding shoot and root nutrient uptake.

For squash, all fruits in each plot were harvested and weighed to determine yield. One representative squash fruit was randomly chosen in each plot, and a subsample of its different parts, including seeds, was taken. Subsamples were freeze-dried, weighed, and ground with a Wiley-mill before shipment to Waters Agricultural Labs Inc. (Camilla, GA), for nutrient concentration analyses by digestion (N, P, and other macros- and micronutrients).

2.3. Soil sampling and analyses

Soil was sampled by taking six soil cores at 0-15 cm depth: two cores in the middle of the rows and two at each end of the rows to cover as much as possible the variability existing within the plot. Samples were kept in a cooler in the field and refrigerated upon arrival to the lab, until processing. Twelve grams of wet soil were oven-dried for at least 48 hours at 105 °C to determine its dry weight.

After cover crop termination, N release from cover crop residues was measured by incubating duplicate soil samples (8 g) mixed with cover crop residues for 7, 14, 28, and 42 days, at a temperature of 27 °C to better represent field conditions. Moisture content was adjusted to 15 % of the soil dry weight (0.15 g/g of dry soil) at the beginning of the incubation and adjusted periodically. At each termination time, samples were extracted with 40 ml of 2M KCl and shook in a reciprocal shaker for 30 minutes. Samples were left to settle overnight, before filtration on Fisherbrand Q2 filters and freezing until analysis. Soil N-NH₄⁺ and N-NO₃⁻ were measured as described respectively by Weatherburn (1967) and Doane and Horwáth (2003), and N mineralization was computed according to Robertson et al. (1999). Initial soil N concentrations after termination were determined with a similar procedure.

Soil chemistry was assessed on air-dried soils taken before cover crop sowing, after cover crop termination, and before each cash crop sowing or transplantation. Mehlich-III extractable nutrients (P, macronutrients, and micronutrients), pH, cation exchange capacity (CEC), and percent base saturation were analyzed at Waters Agricultural Labs Inc. (Camilla, GA).

2.4. Data analysis

For each cash crop phase and for the whole rotation, partial N budgets and N recovery were calculated. For bok choy, partial N budgets included either N returned to the soil in cover crop biomass and N fertilizers as inputs, or only fertilizers as the input; bok choy N removal was considered as the only output. For carrot and squash, only N fertilizers added to carrot and squash were considered inputs, even though carrot might have benefitted from residual N remaining after bok choy; carrot and squash N removal were the only outputs. For the whole rotation, partial N budgets were computed similarly as for bok choy, with one scenario including

cover crop N and N fertilizers as inputs, and one scenario ignoring cover crop N and using only fertilizer N as inputs.

The following equations were used to compute partial N budgets, with and without cover crop N inputs included:

The following equations were used to compute N recovery, with and without cover crop N inputs included:

N recovery (%) =
$$\frac{\text{Cash crop N removal}}{\text{Cover crop N+Fertilizer N}} *100$$
 (3)

N recovery (%) =
$$\frac{\text{Cash crop N removal}}{\text{Fertilizer N}} * 100$$
 (4)

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to compare the difference in cover crop and cash crop variables, using R version 3.4.3 (R Core Team, 2017). Cover crop treatments were treated as a fixed factor and block as a random factor. Soil Mehlich-III-extractable nutrients were analyzed with a two-way ANOVA, with cover crop treatments and time as fixed factors. The significance level was set at $\alpha=0.05$ for ANOVAs, and Tukey HSD tests were performed when the ANOVA was significant at $\alpha=0.05$, although Tukey HSD tests were also performed when $\alpha=0.10$ to detect marginally significant differences among treatments. Shapiro's and Levene's tests were used to verify the assumption of normality of ANOVA residuals and homogeneity of variances, respectively. ANOVAs were also performed within treatments to detect differences in N concentration and uptake and C:N ratio between cover crops and weeds, and for different species in monocultures vs. mixture.

3.0. RESULTS

3.1. Cover crops

Cover crop aboveground biomass was marginally greater (F value = 2.93; p-value = 0.073) in millet (8,986 kg ha⁻¹) relative to cowpea (6,712 kg ha⁻¹), with no significant differences among sorghum (8,259 kg ha⁻¹), sunn hemp (7,260 kg ha⁻¹), or the mixture (7,611 kg ha⁻¹) (Figure 2). Cowpea N uptake (186 kg ha⁻¹) was similar to millet N uptake (182 kg ha⁻¹) and both were significantly higher (F value = 3.41; p-value = 0.044) than sorghum (102 kg ha⁻¹); N uptake in sunn hemp (145 kg ha⁻¹) and the mixture (159 kg ha⁻¹) was not statistically different from other cover crops (Table 2).

The C:N ratio of cover crop residues was significantly higher (F value = 5.11; p-value = 0.008) in sorghum sudangrass than in other cover crops, which were not different from one another (Table 2). In monocultures, sorghum residue had a significantly higher C:N ratio (F value = 45.49; p-value = 0.007) than weeds, sunn hemp had a marginally higher C:N ratio (F value = 9.20; p-value = 0.056) compared to weeds, and millet and cowpea (F value = 4.71; p-value = 0.12) had a C:N ratio similar to weeds. In the mixture, sunn hemp had a marginally higher C:N ratio (F value = 3.25; p-value = 0.068) than cowpea but comparable C:N ratio to weeds and grasses. The C:N ratios of sunn hemp and cowpea was similar in the mixture or in the monoculture, and the same was observed for millet in monoculture relative to grasses in the mixture. In contrast, the C:N ratio of sorghum in monoculture was significantly higher (F value =

59.15; p-value = 0.005) than the C:N ratio of grasses that combines sorghum sudangrass and millet in the mixture.

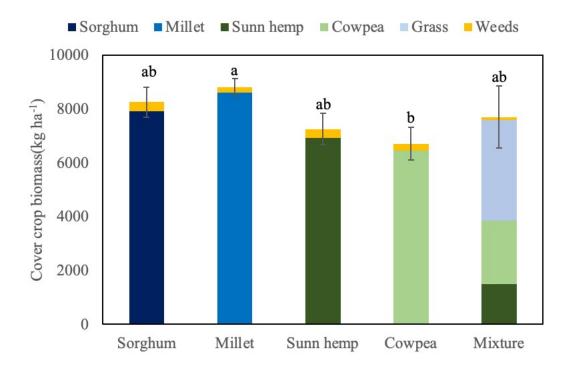


Figure 2: Mean (± standard error of the mean) biomass production (kg ha⁻¹) of four cover crop monocultures and a mixture of the four cover crops. In the mixture, sorghum and millet are combined as grasses.

The weighted average of weed and cover crop N concentration was similar in cowpea and the mixture, and both had a significantly higher N concentration than sorghum (F value = 5.11; p-value = 0.008); N concentration in sunn hemp and millet was not statistically different from the other cover crops (Table 2). In monocultures, N concentration was statistically lower in sunn hemp (F value = 45.14; p-value = 0.007) and sorghum (F value = 30.37; p-value = 0.012) than in weeds; millet and cowpea exhibited similar N concentrations to weeds. In the mixture, cowpea N concentration was marginally greater than sunn hemp N concentration (F value = 3.11; p-value = 0.076); weed and grass N concentrations were not statistically different from the other crops in the mixture. Nitrogen concentrations of cover crop species in monocultures did not significantly

differ from their values in the mixture, except for sorghum, whose N concentration was significantly lower than N concentration for grasses.

Table 2: Mean (± standard error of the mean) N concentration (g kg⁻¹), N uptake (kg ha⁻¹), and C:N ratio of cover crop residues.

Treatments	N concer	ntration		N uptake		C:N ratio		
	g kg ⁻¹			kg ha ⁻¹				
Sorghum monoculture								
Sorghum	*12 ± 1	b		95 ± 10		$*36 \pm 3$	a	
Weeds	22 ± 2	a		8 ± 1		19 ± 2	b	
weighted average	12 ± 1		В	102 ± 11	В	35 ± 3		A
millet monoculture								
Millet	20 ± 3	a		173 ± 24		22 ± 3	a	
Weeds	28 ± 3	a		11 ± 5		16 ± 2	a	
weighted average	20 ± 3		AB	182 ± 21	A	22 ± 3		В
sunn hemp mono	culture							
Sunn hemp	19 ± 3	b		134 ± 21		24 ± 4	a	
Weeds	34 ± 1	a		11 ± 3		13 ± 1	b	
weighted average	20 ± 3		AB	145 ± 21	AB	24 ± 4		В
Cowpea monocul	ture							
Cowpea	28 ± 4	a		179 ± 27		16 ± 2	a	
Weeds 27 ± 2		a		7 ± 3		14 ± 2	a	
weighted average	28 ± 3		A	186 ± 26	A	16 ± 2		В
Mixture								
Grasses	$*20 \pm 2$	ab		75 ± 18		$*22 \pm 2$	ab	
Cowpea 27 ± 3 a		a		57 ± 15 1			b	
Sunn hemp	18 ± 2	b		26 ± 7		26 ± 4	a	
Weeds	25 ± 0	0 ab		1 ± 1		17 ± 0	ab	
weighted average	21 ± 1		A	159 ± 21	AB	22 ± 2		В

Note: Different letters in a given column indicate statistical differences as computed with a one-way ANOVA followed by Tukey HSD tests. In a given treatment, lowercase letters (a, b) indicate differences between cover crops and weeds. Upper case letters (A, B) indicate differences among treatments; asterisks (*) indicate a significant difference between the value of a given cover crop in monoculture and its value in mixture, although sorghum and millet were compared to grasses in the mixture.

3.2. Soil nutrients

Mehlich-III extractable (M3) P was marginally higher in the mixture than in sunn hemp (F value = 2.40; p-value = 0.058), whereas there was no significant difference in M3-P among cowpea, sorghum sudangrass and millet (Table 3). Time affected M3-P (F value = 9.34; p-value < 0.001), being higher after cover crop termination (September 2019) than after harvest of bok choy (October 2019) and carrot (February 2020) but similar than after squash harvest (July 2020) and before cover crop establishment (June 2019). M3-P was also greater after carrot harvest compared to after bok choy harvest. There was no interaction between treatments and sampling time for M3-P (F value = 0.57; p-value = 0.894).

There was no treatment effect (F value =1.69; p-value = 0.163) or interaction between treatment and sampling time (F value =1.05; p-value = 0.421) for M3-K (Table 3). However, M3-K was affected by time (F value =27.39; p-value < 0.001): M3-K was similar before cover crop establishment, after cover crop termination and bok choy harvest, but significantly higher than after carrot and squash harvest.

M3-Ca was greater in sorghum sudangrass than in millet and cowpea treatments (F value = 5.48; p-value < 0.001), and M3-Ca did not differ statistically in the mixture or sunn hemp relative to the other treatments (Table 3). There was a significant sampling time effect (F value = 6.70; p-value < 0.001), with higher M3-Ca following cover crop termination and bok choy harvest than before cover crop establishment and after squash harvest. M3-Ca was also higher after carrot harvest than before cover crop establishment. There was no significant interaction between sampling time and treatment for M3-Ca (F value = 0.74; p-value = 0.743).

The sorghum sudangrass treatment had significantly higher M3-Mg than the cowpea treatment (F value = 3.70; p-value < 0.009), with no significant difference among other

treatments (Table 3). There was no interaction between treatment and sampling date (F value = 0.38; p-value = 0.983), but the effect of sampling time was significant (F value = 13.61; p-value < 0.001). M3-Mg following bok choy harvest was significantly higher than at any other sampling time, and M3-Mg was higher after cover crop termination than after squash harvest.

Base saturation (BS) was significantly higher in sorghum sudangrass than in cowpea (F value = 3.44; P value = 0.012), with no differences among other treatments (Table 3). Sampling time had a significant effect on BS (F value = 12.64; p-value < 0.001), with higher BS before cover cop establishment than after carrot and squash harvest, and greater BS after cover crop termination and bok choy harvest compared to after squash harvest. There was no interaction between treatment and sampling time (F value = 0.75; p-value = 0.734).

Soil pH was higher in sorghum sudangrass than in cowpea (F value = 5.16; p-value < 0.001), with no difference among other treatments (Table 3). No interaction between sampling time and treatment was found (F value = 0.98; p-value < 0.490). Sampling time had a significant effect on soil pH (F value = 24.87; p-value < 0.001), with a general decline in soil pH during the experiment. Soil pH was higher after cover crop termination than at any other sampling times, and higher before cover crop establishment and after bok choy harvest than after squash harvest. Soil pH after carrot harvest was similar to pH after squash.

At cover crop termination, soil N was similar among sunn hemp (23 mg kg⁻¹), cowpea (23 mg kg⁻¹), and millet (19 mg kg⁻¹) treatments and significantly higher than sorghum sudangrass (8 mg kg⁻¹) (F value = 7.51; p-value = 0.002), with the mixture not differing from any other cover crop treatment (Figure 3). After 7, 14, and 28 days of incubation, soil N was similar among legume cover crops (sunn hemp and cowpea) and significantly higher than in sorghum sudangrass (F values = 3.70; p-values = 0.035), with the exception of sunn hemp and millet

being marginally higher than sorghum after 28 days and 14 days, respectively. Millet had marginally higher (14 days) or significantly higher (28 days) soil N than sorghum, whereas the mixture was not statistically different from the other cover crop treatments. After 42 days of incubation, there was no statistical difference in soil N among treatments (Figure 3).

Table 3: Mean (± standard error of the mean) of soil nutrient availability (mg kg⁻¹), soil pH, and percent base saturation before and after each cropping cycle.

	S	Base					
Sampling sequence	Phosphorus (P)	•		Magnesium (Mg)	рН	saturation (%)	
Before cover crops	ABC	A	С	ВС	A	A	
(Jun-2019)	ABC	Α	C	ьс	А	A	
Sorghum	494±10 ab	187±30 a	719±101 a	133±17 a	5.9±0.1 a	60 ±5 a	
Millet	492±10 ab	152±7 a	555±32 bc	103±8 ab	5.9±0.1 ab	$60 \pm 4 \text{ ab}$	
Sunn hemp	475±11 b	168±26 a	714±129 ab	123±23 ab	5.8±0.1 a	62±9 ab	
Cowpea	484±12 ab	174±35 a	591±92 c	101±13 b	5.8±0.1 b	58±5 b	
Mixture	500±9 a	192±31 a	622±90 abc	106±10 ab	5.8±0.0 ab	54±5 ab	
After cover crop termination				D		A.D.	
(Aug-2019)	A	A	A	В	A	AB	
Sorghum	503±10 ab	162±21 a	847±41 a	134±6 a	5.8±0.1 a	57±2 a	
Millet	499±8 ab	163±16 a	819±75 bc	133±11 ab	5.8±0.1 ab	55±3 ab	
Sunn hemp	501±7 b	188±31 a	912±188 ab	138±28 ab	5.8±0.2 a	57±6 ab	
Cowpea	514±8 ab	188±25 a	753±88 c	121±16 b	5.6±0.1 b	52±6 b	
Mixture	512±17 a	176±31 a	826±85 abc	125±10 ab	5.7±0.1 ab	55±3 ab	
After bok choy harvest	С	A		A	D	A.D.	
(Oct-2019)	C	A	A	Α	В	AB	
Sorghum	470±16 ab	184±20 a	902±126 a	185±25 a	5.8±0.0 a	63±3 a	
Millet	475±4 ab	188±26 a	805±105 bc	156±9 ab	5.5±0.1 ab	56±2 ab	
Sunn hemp	472±10 b	130±18 a	1025±52 ab	163±30 ab	5.6±0.3 a	60±2 ab	
Cowpea	471±7 ab	126±16 a	725±54 c	142±8 b	5.5±0.2 b	54±2 b	
Mixture	474±7 a	167±29 a	755±102 abc	156±14 ab	5.5±0.1 ab	56±4 ab	
After carrot harvest	ВС	В	AB	ВС	ВС	ВС	
(Feb-2020)	ьс	Б	AD	ьс	ьс	ьс	
Sorghum	476±10 ab	122±19 a	1060±165a	137±12 a	5.6±0.1 a	59±4 a	
Millet	485±6 ab	101±10 a	622±71 bc	106±13 ab	5.4±0.1 ab	46±4 ab	
Sunn hemp	476±7 b	101±18 a	820±148 ab	129±17 ab	5.6±0.1 a	51±5 ab	
Cowpea	483±12 ab	95±24 a	670±69 c	119±8 b	5.4±0.1 b	49±3 b	
Mixture	490±14 a	139±29 a	879±107 abc	135±19 ab	5.7±0.1 ab	57±5 ab	
After squash harvest	A D	В	ВС	С	С	С	
(Jul-2019)	AB	В	вс	C	C	C	
Sorghum	496±17 ab	94±10 a	740±84 a	124±15 a	5.4±0.1 a	50±4 a	
Millet	483±1 ab	84±3 a	639±45 bc	108±7 ab	5.4±0.1 ab	47±2 ab	
Sunn hemp	486±5 b	84±9 a	749±166 ab	105±15 ab	5.5±0.2 a	48±6 ab	
Cowpea	492±4 ab	81±8 a	544±60 c	83±7 b	5.2±0.1 b	41±2 b	
Mixture	520±18 a	100±17a	617±109 abc	101±14 ab	5.3±0.1 ab	44±5 ab	
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Note: different uppercase letters indicate statistically significant differences among sampling dates, for a given nutrient; different lowercase letters indicate statistically significant differences among cover crop treatments for the whole experiment (time by treatment interactions were not significant).

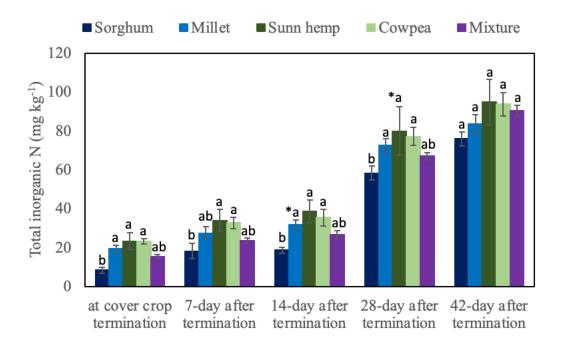


Figure 3: Mean concentration (± standard error of the mean) of soil inorganic nitrogen (N-NH₄⁺ and N-NO₃⁻) at cover crop termination and after 7, 14, 28, and 42 days of incubation. Asterisks indicate marginal significance.

3.3. Cash crops

There was a non-significant trend (F value = 1.76; p value = 0.205) of greater bok choy yield in millet (1,604 kg ha⁻¹) than in cowpea (1,502 kg ha⁻¹), mixture (1,438 kg ha⁻¹), sunn hemp (1,342 kg ha⁻¹), or sorghum (1,305 kg ha⁻¹) (Figure 4). There was no significant difference in bok choy N, P, and K concentrations and bok choy N and P uptake among cover crop treatments (Table 4). In contrast, K uptake was marginally higher in millet than in sorghum sudangrass and cowpea, whereas sunn hemp and the mixture were not statistically different from any other cover crop treatments (Table 4).

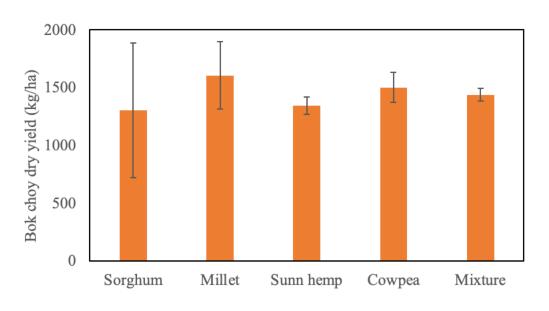


Figure 4: Mean (± standard error of the mean) dry yield (kg ha⁻¹) of bok choy following cover crop residue incorporation. The mean for millet was computed from three plots as the value of one plot (3761 kg ha⁻¹) was twice the value of other plots and considered to be an outlier.

Table 4: Mean (± standard error of the mean) N, P, and K concentration (%) and uptake (kg ha⁻¹) of bok choy following cover crop residue incorporation.

	Nutrient concentration (%)						Nutrient uptake (kg ha ⁻¹)						
Treatments	Nitrogen		Phosphorus		Potassium		Nitrogen	Nitrogen		Phosphorus		Potassium	
	(N)		(P)		(K)		(N)		(P)		(K)		
Sorghum	5.1 ±0.3	a	0.88 ± 0.01	a	5.5 ± 0.8	a	67±6	a	11 ±1	a	71±7	b	
Millet	5.2 ± 0.3	a	$0.85 \pm\! 0.03$	a	6.1 ± 0.3	a	82 ± 11	a	13±1	a	101 ± 8	a	
Sunn hemp	5.1 ± 0.2	a	0.88 ± 0.06	a	5.7 ± 0.5	a	67±2	a	12 ±1	a	77±8	ab	
Cowpea	5.8 ± 0.2	a	$0.86 \pm\! 0.06$	a	4.9 ± 0.6	a	87 ± 9	a	13 ± 1	a	72 ± 10	b	
Mixture	5.3 ± 0.3	a	$0.85 \pm\! 0.04$	a	6.1 ± 0.5	a	76 ± 8	a	12 ± 1	a	87±4	ab	
Statistical tests													
F value	1.86		0.22		1.08		1.57		1.04		2.93		
p-value	0.169		0.923		0.409		0.237		0.420		0.073		

Note: Values in millet for nutrient uptake represent mean for only three plots, because values of N, P, and K uptake (199, 32, 202, kg ha⁻¹, respectively) in one plot were removed as they were considered to be outliers.

Carrot total biomass (F value = 1.52; p-value = 0.261), marketable yield (F value = 1.66; p-value = 0.211), and shoot biomass (F value = 1.15; p-value = 0.379) were not significantly different among cover crop treatments (Figure 5). Similarly, there was no significant difference

in carrot N, P, and K concentration and uptake among all treatments, either in the shoot, root, or total biomass (Table 5).

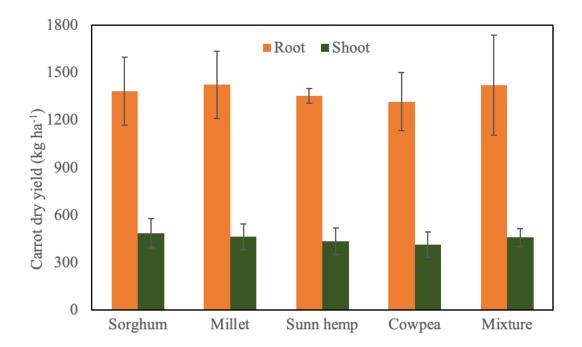


Figure 5:Mean (\pm standard error of the mean) yield (kg ha⁻¹) of carrot following a bok choy cash crop.

Table 5: Mean (± standard error of the mean) of N, P, and K concentration and uptake (kg ha⁻¹) of carrot.

Treatments				Nutrient con	centratio	on (%)			Nutrient uptake (kg ha ⁻¹)									
	Nitrogen			Phosphorus			Potassium			Nitrogen			Phosphorus			Potassiu		m
	(N)			(P)			(K)			(N)			(P)				(K)	
Sorghum Top Root	2.49±0.11 1.32±0.5	a	A	0.28±0.04 0.27±0.02	a	A	2.93±0.56 1.51±0.18	a	A	12±3 25±7	a	A	1±1 4±1	a	A	15±5 21±3	a	A
Total										38±6			5±1			36±8		
Millet Top Root Total	2.74±0.06 1.15±0.07	a	A	0.27±0.02 0.24±0.01	a	A	3.88±0.14 1.88±0.15	a	A	13±2 17±3	a	A	1±0 3±0	a	A	18±3 26±2	a	A
Sunn										29±5			5±1			44±5		
Top Root Total	2.63±0.09 1.43±0.17	a	A	0.30±0.04 0.26±0.03	a	A	3.05±0.15 1.59±0.22	a	A	11±2 19±4 30±6	a	A	1±0 4±1 5±1	a	A	13±3 21±4 34±7	a	A
Cowpea Top Root Total	2.64±0.07 1.33±0.13	a	A	0.28±0.02 0.24±0.01	a	A	3.18±0.16 1.61±0.04	a	A	11±2 18±4 29±6	a	A	1±0 3±0 4±1	a	A	13±2 21±4 34±5	a	A
Mixture Top Root Total	2.66±0.17 1.55±0.09	a	A	0.32±0.04 0.29±0.02	a	A	3.85±0.47 1.96±0.29	a	A	12±2 22±3 34±5	a	A	2±0 4±1 6±1	a	A	18±4 28±7 46±11	a	A
Statistical tes F value	sts	0.76	2.25		2.12	2.19	_	2.33	1.51	* 0.51	0.11	0.71	* 0.58	0.27	0.59	*1.04	0.89	1.00
P value		0.57	0.12		0.14	0.13		0.12	0.26	* 0.73	0.98	0.60	* 0.69	0.89	0.67	* 0.43	0.50	0.45

Note: different lowercase letters, if any, indicate statistically significant differences among treatments for carrot shoots; different uppercase letters, if any, indicate statistically significant difference for carrot roots among treatments; values preceded by (*) indicate F values and p-values for total nutrient uptake (N, P, K).

Squash total (F value = 0.36; p-value = 0.843) and marketable yield (F value = 1.79; p-value = 0.182) was similar among cover crop treatments, despite a non-significant trend of greater yields in sorghum relative to sunn hemp treatments (Figure 6). There was no significant difference in squash N, P, and K concentration and uptake among treatments (Table 6).

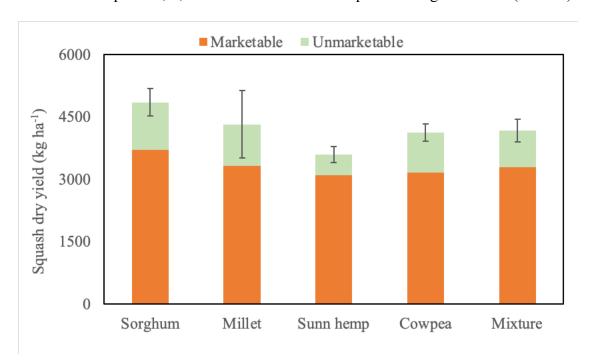


Figure 6: Mean (\pm standard error of the mean) of squash yield (kg ha⁻¹) of squash.

Table 6: Mean (± standard error of the mean), N, P, and K concentrations (%) and uptake (kg ha⁻¹) in squash.

	N	Jutri	ent concentration	on (%	Ď)			ha ⁻¹)				
Treatments	Nitrogen (N)		Phosphorus (P)	Potassium (K)			Nitrogen (N)		Phosphorus (P)	Potassium (K)		
Sorghum	1.89±0.20	a	0.54±0.07	a	2.66±0.24	a	92±10	a	26±3	a	129±14	a
Millet	1.66±0.30	a	0.47 ± 0.04	a	2.60±0.15	a	73±16	a	21±4	a	114±14	a
Sunn hemp	2.24±0.32	a	0.54 ± 0.02	a	2.66±0.23	a	81±13	a	19±2	a	96±13	a
Cowpea	2.16±0.21	a	0.51±0.06	a	2.64±0.14	a	86±4	a	21±3	a	108±11	a
Mixture	2.08 ± 0.17	a	0.58 ± 0.01	a	2.87 ± 0.11	a	87±10	a	24±2	a	120±10	a
Statistical tes	sts											
F value	1.99		0.82		0.39		0.54		1.37		1.09	
p-value	0.161		0.533		0.815		0.712		0.291		0.398	

Note: identical letters indicate that there was no significant difference among treatments.

3.4. N budget and recovery

Regardless of the cash crop or cover crop treatments, and regardless of the inclusion of N credits from cover crop residue mineralization, partial N budgets revealed N surpluses and no significant difference among treatments (Table 7). Partial N budget for the whole rotation also showed a surplus, with no significant difference among treatments. Nitrogen recovery was similar among treatments in all cases (Table 7). When N inputs from cover crops were not considered, N recovery was less than 55% for all cash crops, with a trend for the carrot to exhibit the lowest N recovery (less than 30%, except for sorghum sudangrass, which had 34%). Nitrogen recovery was even lower (less than 30% for bok choy and 40% for the whole rotation) when N inputs from cover crop residues were included in the computations.

Table 7: Mean (± standard error of the mean) N budget (kg ha⁻¹) and N recovery (%) following each cash crop and for the whole cover crop-bok choy-carrot-squash rotation.

			N bı	ıdget	(kg ha ⁻¹)						N re	cover	y (%)			
Treatments	Bok choy	7	Carrot		Squash	wh	ole rotation	_	Bok choy		Carrot		Squash		whole rota	tion
	Inc	ludin	ıg N inpu	ts fro	m cover cr	op re	sidues	_		es						
Sorghum	232±22	a	-		-		383±36	a	22±4	a	-		-		34±6	a
Millet	294±37	a	-		-		441±49	a	22±3	a	-		-		33±6	a
Sunn hemp	274±39	a	-		-		444±28	a	20±2	a	-		-		29±4	a
Cowpea	295±58	a	-		-		460±69	a	23±7	a	-		-		31±6	a
Mixture	259±49	a	-		-		423±54	a	23±6	a	-		-		31±6	a
Statistical tests F value P value	1.62 0.236						1.35 0.309		0.31 0.868						0.41 0.801	_
	Excluding N inputs from cover crop residues								Excluding N inputs from cover crop residue inputs							
Sorghum	130±46	a	74±6	a	77±19	a	281±40	a	34±7	a	34±6	a	54±12	a	41±6	a
Millet	114±19	a	79±2	a	95±32	a	259±46	a	42±10	a	29±2	a	43±19	a	46±10	a
Sunn hemp	129±3	a	82±6	a	88±26	a	298±30	a	34±2	a	27±5	a	48±15	a	37±6	a
Cowpea	109±17	a	83±6	a	82±7	a	274±30	a	45±9	a	26±6	a	51±4	a	42±6	a
Mixture	120±16	a	82±7	a	82±20	a	284±32	a	39±8	a	27±6	a	51±12	a	40±7	a
Statistical tests F value p-value	1.55 0.242		0.58 0.68		0.54 0.712		0.51 0.729		1.56 0.241		0.58 0.68		0.54 0.712		0.51 0.729	

Note: identical letters indicate that there was no significant difference among treatments.

4.0. DISCUSSION

4.1. Cover crops

Grass cover crops tended to produce more biomass than legume cover crops and the mixture, and this could be attributed to adequate soil fertility. Wang et al. (2009) noted that in a condition of low soil fertility, legume cover crops usually performed better than grass cover crops in the first year, but did better with adequate soil fertility in the first year or during the second year as soil fertility was enhanced. For instance, Wang et al. (2009) found higher biomass production for sunn hemp (11,200 kg ha⁻¹) than sorghum sudangrass (4,100 kg ha⁻¹) in the first year of their experiment, but lower biomass in sunn hemp (6,700 kg ha⁻¹) than sorghum sudangrass (12,100 kg ha⁻¹) in the second year of their experiment. Generally, sunn hemp produces higher biomass than sorghum sudangrass or cowpea (Wang, 2003b; 2005b, 2006, 2009), although biomass production varies among studies depending on growing period length, experimental conditions (e.g., pot vs. open field), and the soil fertility status.

Cover crop N concentration was significantly higher in cowpea and the mixture than sorghum sudangrass, which is somewhat consistent with Wang et al. (2006) who found similar results for cowpea and sunn hemp, and greater N concentration compared to sorghum sudangrass. Prior to that study, Wang et al. (2003b) had found a higher N concentration in sunn hemp (2.85%) than in cowpea (2.08%), with a much lower N concentration (0.92%) in sorghum sudangrass. Biomass N was greater in millet and cowpea than in sorghum sudangrass, whereas Wang et al. (2009) found greater N uptake in sunn hemp (190.2 - 319.2 kg ha⁻¹) than cowpea (118.6 - 132.1 kg ha⁻¹) or sorghum sudangrass (37.2 - 109.9 kg ha⁻¹). Cherr et al. (2006) found higher sunn hemp biomass (8,000 kg ha⁻¹ and 12,000 kg ha⁻¹, respectively in 2005 and 2006) than what was found in this study, but the growth period (12 weeks and 14 weeks) was longer in

their study. Other studies also found sunn hemp accumulated higher biomass N than other cover crops (Wang et al., 2003b; 2005b, 2006). Lower N uptake in sunn hemp in this study could be due to lower biomass production and lower N concentration, as well as a shorter growth period compared to other studies.

Sorghum sudangrass had a significantly higher C:N ratio than any other cover crop, similar to previous studies that found higher C: N ratio in grass residues than legume residues (Finney et al., 2016; O'Connell et al., 2015; White et al., 2017). As stated in the hypotheses, the mixture of grass and legume cover crops led to an intermediate C:N ratio, as legume cover crops "diluted" the sorghum sudangrass C:N ratio. However, the hypothesis regarding legume cover crops having a lower C:N ratio than grass-based cover crop was only partially confirmed, as sunn hemp and millet had similar C:N ratios. The relatively higher C:N ratio of sunn hemp residues may be explained by its tendency to produce a lot of stem biomass (Cherr et al., 2006). Along with other environmental factors, the C:N ratio of cover crop residues is critical in determining how quickly residue N can be mineralized and transferred to subsequent cash crops. The high C:N ratio of sorghum likely induced N immobilization and temporarily deprived plants of N (Finney et al., 2016; O'Connell et al., 2015; White et al., 2017), as soil microbes will tend to use soil N to decompose residues with high C:N ratios.

4.2. Soil fertility

M3-P was similar among cover crop treatments, except that the mixture was marginally higher than sunn hemp. However, this difference may not be due to the cover crop treatments, as M3-P was higher in plots planted with the mixture relative to those planted with sunn hemp before cover crop establishment. Therefore, this difference might be due to pre-existing heterogeneity in this field. A lack of cover crop effect on soil P would be consistent with Wang

et al. (2005b), who found no difference among cover crops and fallow treatments regarding soil P. Temporal changes in M3-P suggests that cash crop took up P from the soil pool measured by M3-P, as it decreased after bok choy harvest. However, there was an increase in M3-P following carrot and squash harvests. That might be indicative of P mineralization from organic P pools and P mobilization from mineral pools mediated by soil microbes affected by plant root exudates (Jonasson et al., 2006; Spohn et al., 2013). As there was no statistically significant increase of M3-P after legume cover crops relative to grass cover crops, there was no indication that legume cover crops increased P mobilization in this study (Maltais-Landry, 2015; Nuruzzaman et al., 2005; Tang et al., 1999).

There was no strong and consistent cover crop effect on M3-K, M3-Ca and M3-Mg, except for an increase in M3-Ca and M3-Mg with sorghum sudangrass relative to cowpea that can be attributed to pre-existing field conditions. There was no interaction effect between cover crop treatment and sampling date, but there were significant temporal changes in M3-K, which significantly decreased following carrot and squash harvest. This decrease could stem both from crop K uptake and K leaching (Aronsson et al., 2007; Rosolem et al., 2010). In contrast, there was an early peak in M3-Ca (after cover crop termination) and M3-Mg (after bok choy harvest) that then declined with time, most likely the result of cash crop uptake. These results are consistent with the findings by Wang et al. (2005b), who found no difference in soil K, Ca and Mg among cover crops treatments. The failure to detect significant time by treatment interactions could be due to the short duration of this study, as changes in soil quality take time to occur (Abdollahi & Munkholm, 2014), or the low sample size.

Soil pH was highest early in the experiment and declined with time, most likely because of mineralization of cover crop residue and organic N from fertilizer and subsequent nitrification

that acidifies the soil (Abdollahi & Munkholm, 2014; Vanzolini et al., 2017). There was significantly higher soil pH in the sorghum treatment relative to the cowpea treatment, which could be due to N-fixation in cowpea, as legumes tend to have a lower soil pH than grass cover crops (Maltais-Landry, 2015; Tang et al., 1999).

Overall, as the number of cropping cycles increased, base saturation decreased, which could be driven by plant nutrient uptake, nutrient leaching, and cover crop residue decomposition that can all increase soil acidity (Tang et al., 1999; Vanzolini et al., 2017). There was also a treatment effect on base saturation, as the sorghum sudangrass treatment had significantly higher base saturation than cowpea, which could be due to the greater soil acidification observed with N-fixing legumes (Maltais-Landry, 2015; Nuruzzaman et al., 2005; Tang et al., 1999) or pre-existing field conditions.

Soil N was higher in legumes and millet relative to the sorghum treatment at cover crop termination and during the first 28 days of the incubation, whereas soil N in the mixture was intermediate. Holmes et al. (2019) and Couëdel et al. (2018) also found higher N availability in legume monocultures, intermediate in a mixture of legumes and non-legumes, and lower N availability in non-legume monocultures. Similarly, other studies found higher N availability in legume cover crops than grass cover crops (Finney et al., 2016; Holmes et al., 2017; Wang et al., 2005b; White et al., 2017). However, differences among treatments disappeared after 42 days. These findings partially support the hypothesis that soil N in the mixture would be intermediate between legume and grass cover crops, as millet had a soil N release that was similar to legume cover crops throughout the incubation.

Lower soil N availability in the sorghum sudangrass treatment suggests that bok choy could experience N deficiency due to N immobilization (Couëdel et al., 2018; Finney et al.,

2016) while N availability could recover for the next cash crop (carrot). Yet, there was no evidence of N deficiency at the bok choy stage in the sorghum treatment, most likely because of high N fertilizer inputs provided to all treatments at the bok choy stage. Given the hot and humid climate and the coarse-textured soils found in Florida, this temporary N retention with sorghum sudangrass could prevent N loss from leaching (Couëdel et al., 2018; Kaspar et al., 2012; Thapa et al., 2018). In contrast, high N availability in the legume cover crop could be beneficial to the following cash crop (bok choy) but might increase leaching losses (Campiglia et al., 2011; Cherr et al., 2006), potentially reducing availability for carrot (Weinert et al., 2002). Intermediate N mineralization with the mixture could offset the drawbacks of different monocultures by providing benefits to both bok choy and the carrot as well as reducing N leaching (Treadwell, 2006). In the end, N release from the different treatments followed their respective C:N ratio, consistent with previous findings by Campiglia et al. (2011), Finney et al. (2016), and Li et al. (2006), finding higher N mineralization with legume residues (lower C:N ratio).

4.3. Cash crops

There was no significant difference for bok choy yield among treatments, although the trend in yields followed the same pattern as cover crop biomass N and C:N ratios. One explanation for this lack of significant difference is that all treatments were likely overfertilized, which could mask cover crop effects. Previous experiments looking at cover crop effects on vegetable yields in Florida found consistently higher yield in sunn hemp treatments than in sorghum sudangrass treatments (Wang et al., 2003, 2005b, 2006, 2007, 2009), although these experiments reported higher sunn hemp N uptake than in this experiment, with tomato and okra cash crops.

Carrot yields were not different among treatments, and did not support the hypothesis of higher yield in the mixture and the grass treatments compared to legume-based treatments. This hypothesis was made thinking that the higher C:N ratio for grass residues and intermediate C:N ratio for the mixture would allow for a more even N mineralization through time, whereas the rapid mineralization of legume residues could result in N loss by leaching (Cherr et al., 2006; Weinert et al., 2002), which would result in low N availability from residues at the carrot stage. The lack of effect could be due to low yields in this experiment, as an experiment conducted by Lynch et al. (2012) found a much higher total yield (24,134 - 51,878 kg ha⁻¹), using higher N inputs (168 kg ha⁻¹) than in this experiment. The N rate applied to carrot in this study was based on the assumption that bok choy was overfertilized and that carrot might benefit from N carryover from bok choy, although the low yields observed do not support an important N carryover from bok choy benefitting carrots, which raises concerns about N leaching below the rooting zone.

Sorghum sudangrass tended to have higher squash yields compared to legume cover crops, while the mixture had an intermediate yield, albeit there was no significant difference among treatments. Although it was hypothesized that there would not be any cover crop effect on squash, this result suggests that grass cover crop effects may have lasted longer than expected, potentially through a lower mineralization rate that facilitated nutrient retention. The incubation results indicated that N mineralization was slower with sorghum sudangrass residues, although the time between cover crop termination and squash seeding (over 6 months) would suggest that cover crops had a minor contribution to squash nutrition. Overall, yields in the mixture were consistently among the highest or intermediate for all cash crops, partially supporting the

hypothesis that the mixture could optimize benefits for vegetables growers by combining the effects of different cover crops.

There was no difference among treatments regarding nutrient concentrations and uptake, regardless of the cash crop considered. This could be due to over-fertilization of the bok choy crop, which would mask the effect of cover crops on bok choy N uptake. Partial N budgets for different cash crops revealed similar N surpluses among all the treatments, which could be explained by high N inputs and low N recovery from cash crops (Jalpa et al., 2020; Marchi et al., 2016). Given the hot and humid climate of Florida as well as its coarse-textured soil, those N surpluses do not necessarily mean potential N credits for the following cash crop because of Florida's low soil nutrient holding capacity (Li et al., 2006; Treadwell, 2006). Instead, this could indicate a high potential for nitrate leaching.

5.0. CONCLUSIONS

This study showed that a mixture of legume and grass cover crops can produce similar biomass and accumulate similar amounts of N in their biomass to grass monocultures (millet and sorghum sudangrass) and legume monocultures (cowpea and sunn hemp). In the coarse-textured soil and hot/humid climate of Florida, the mixture had an intermediate release of N relative to monocultures, which could allow for a better synchronization of N mineralization with vegetable N needs, potentially mitigating nitrate leaching and potential N immobilization concerns. However, this remains speculative and would need to be confirmed with additional years of study and direct measurements of N leaching. Even though much emphasis was put on N in this experiment, it is important to consider other nutrients such as P and K, whose cycling can be affected as well. For instance, K significantly decrease with time, indicating that this nutrient

may not be tightly cycled in this system. Likewise, pH is another soil variable that needs to be managed, as pH (and base saturation) decreased with time.

An important takeaway from the first year of this experiment is that the mixture can behave similarly to cover crop monocultures in terms of yields of subsequent cash crops, as it yields were consistently similar to cover crop monocultures. Higher N availability in the legume treatments (cowpea and sunn hemp) did not translate into higher yields and N uptake in the cash crops, suggesting that N inputs might be excessive which could result in significant N leaching. Subsequent studies should consider lowering N inputs or investigating other aspects of N fertilization (e.g., splitting applications) combined with cover crops to better understand the benefits of cover crop monocultures and mixtures.

6.0. REFERENCES

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