Assessment of a novel soil nutrient imaging technique, forage productivity and the soil health status of silage corn and forage soybean cultivated as mono or intercrop under cool climatic conditions

By

Muhammad Zaeem

A thesis submitted to the School of Graduate Studies

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Approved:

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Abstract

Inadequate supply and inferior forage quality are major challenges facing Newfoundland and Labrador dairy industry. Therefore, dairy farmers have to depend on substantial forage imports from mainland. To overcome forage shortage, there is an increasing trend to add silage corn as a high biomass producing crop in existing forage production systems in the Province by using different nutrient management practices including the application of nutrient laden dairy manure (DM) or inorganic fertilizers (IF) for sustainable forage production. Therefore, I investigated the effects of silage corn and forage soybean cultivated as monocropping (MC) or intercropping (IC) on total forage production, forage nutritional quality, and soil health status. I further evaluated the effects of different DM and IF applications on the spatial distribution and quantification of essential nutrients in the root rhizospheres by employing a novel approach consisting of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Three forage soybean varieties (Big Fellow RR, Game Keeper RR, Kester's Bob White Trailing Soybean) were IC with two silage corn genotypes (Yukon-R and DKC26–28RIB) using a random complete block design (RCBD). Study results revealed that corn-soybean IC enhanced the agronomic performance, forage production and nutritional quality compared to MC. The land equivalent ratio surpassed 1, expressing IC had advantages over MC. IC reduced the RS-pH with a concomitant increase in RS-APase activity, that was affiliated with an increase in RS-$P_{available}$ compared to corn and soybean MC. The soil active microbial community composition was also improved in IC systems compared to MC. Superior quality forage nutritional quality was observed in the IC treatment and included higher crude proteins,

essential minerals, omega-3 and omega-6 fatty acids and reduced fiber contents compared to MC. Furthermore, LA-ICP-MS imaging revealed major variation in the spatial distribution of essential minerals in the soil A horizon, and quantitative differences in amounts of nutrients present in the root rhizospheres following different nutrient management practices. Collectively, these finding suggested that silage corn and forage soybean IC could be a viable approach to increase forage production with improved nutritional quality, enhanced the soil chemical or biological activities with better nutrient availability in the plant root zone following cultivation under cool climates in boreal ecosystem characteristic of Newfoundland and Labrador, Canada.

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Muhammad Zaeem

Table of Contents

List of tables

List of figures

List of Abbreviations

ADF – Acid detergent fiber

AP – Available protein

BAME – Bacterial acid methyl ester

C – Corn

CP – Crude protein

DDM – Digestible dry matter

DM – Dairy manure

DM+B – Dairy manure + biochar

DMI – Dry matter intake

FA – Fatty acid

FAME – Fatty Acid Methyl Ester

FID – Flame ionization detector

FP – Forage production

GC-FID – Gas chromatography– flame ionization detector

GC-MS – Gas chromatography–mass spectrometry

IC – Intercropping

ICP-MS – Inductively Coupled Plasma Mass Spectrometry

LA-ICP-MS – Laser Ablation Inductively Coupled Plasma Mass Spectrometry

LER – Land equivalent ratio

MC – Monocropping

NDF – Neutral detergent fiber

NE_G – Net energy for gain

NE_L – Net energy for lactation

NE_M – Net energy for maintenance

ON – Ontario

PLFA – Phospholipid fatty acid

RCBD – Randomized complete block design

RFV – Relative feed value

RS-APase – Rhizosphere soil acid phosphatase activity

RS-$P_{available}$– Rhizosphere soil available phosphorus

RS-pH – Rhizosphere soil pH

S – Soybean

SP – Soluble protein

TDN –Total digestible nutrients

WSC –Water-soluble carbohydrates

Chapter 1

1. Introduction and overview

1.1. Introduction

Feed self-reliance is the major goal of the dairy industry in Newfoundland (NL) Canada, because feed importation is one of the most expensive farm inputs in the dairy production system. (Statistics Canada, 2010). Additionally, the transportation cost is high in the province further increasing the cost of the fodder. The climatic uncertainty is another main hurdle that limits the production of nutritive and high yielding fodder crops. Silage corn *(Zea mays L.*) is a leading fodder crop in NL Canada (Kwabiah, 2003; Statistics Canada, 2016). However, a decline in production is observed starting from 2011 to present coinciding with the replacement of corn with spring wheat. Silage corn production decreased from 803 to 509 acres and spring wheat area increased from 10 to 254 acres from 2011 to 2016 (Statistics Canada, 2016). Overall, the availability of agricultural land is also decreasing in the world due to increasing growth of the human population. So, there is a need to find a suitable and sustainable way to increase the per unit area forage production (Eslamizadeh et al., 2015). Intercropping could be a possible approach to enhance per unit area fodder production (Bedoussac et al., 2015; Hauggaard-Nielsen et al., 2008) with superior quality forage. Intercropping is the practice of planting two or more crops during the same growing season on the same piece of land (Costa et al., 2012; Ijoyah et al., 2012). There are four types of intercropping (Mousavi and Eskandari, 2011);

a) Mixed intercropping: The planting of two or more crops without any plant or row arrangement
b) Row intercropping: The planting system where two or more components crops are planted in alternative rows.
c) Relay intercropping: The planting of a second crop before the completion of the lifecycle of the first crop. As the first crop reached maturity and harvested it makes room for the second crop.
d) Strip intercropping: This is different from row intercropping, as it involves the growing of two or more components crops in alternate strips. Strip may be comprised of one or multiple rows.

Concomitant with the increasing world population, the area under agricultural crop production is decreasing. Thus, the only way to increase the production is to increase the productivity per unit area (Odedina et al., 2014).

Corn is the oldest cereal crop grown both in temperate and tropical regions of the world and is known as the queen of cereals because of the high yield potential (Ananthi et al., 2017), energy, relative feed nutrition value (Eslamizadeh et al., 2015) and palatability (Masoero et al., 2006). Corn is commonly cultivated in over 165 countries, with total area of cultivation equivalent to approximately 179.9 m and producing 1013.6 m.t (Zorya et al., 2011). However, due to high nutrient uptake it is known as an exhaustive crop that depletes the soil nutrient quickly (Kannan et al., 2013), and the fodder produced usually have low crude protein content (Armstrong et al., 2008). As such, extra supplements are required to fulfil this protein deficiency (Stoltz et al., 2013). Protein is a crucial nutrient required in

animal feed not only for improved growth, production and milk yield (Jayanegara et al., 2016), but also required to enhance rumen bacteria to help in feed digestion (Ghanbari-Bonjar, 2000). Corn intercropping with legume is another approach to increase fodder protein contents (Dahmardeh et al., 2009; Zhu et al., 2011).

In contrast to corn, soybean (*Glycine max*) is an important annual legume crop belonging to the family *Fabaceae* (Tefera, 2011). Soybean is a protein rich legume crop that provide 2/3rd of the world protein concentrate for the dairy industry (Agarwal et al., 2013). Fodder obtained from forage soybeans also contain high concentrations of essential minerals and unsaturated fatty acids (Bachlava et al., 2008; Yang et al., 2017) important in the formulation of animal feed. Soybean is a restorative crop and can restock the soil nutrient pool. Taking these points into consideration, corn-soybean intercropping could be a suitable approach to increase the forage yield per unit area, as well as improving the soil health status (fertility status and soil ecology) (He et al., 2006; Tang et al., 2005) during forage production, particularly under cool climatic conditions.

There are a lot of advantages associated with cereal legume intercropping compared to monocropping. These include superior yield with "Land Equivalent Ratio (LER) >1 (Bedoussac et al., 2015; Hauggaard-Nielsen et al., 2008), higher quality fodder with enhanced protein contents (Bedoussac et al., 2015, 2011; Pelzer et al., 2012), reduce soil erosion (Bhatti et al., 2013), minimize the risk of crop failure, better utilization of soil resources because of different root depth, (Muoneke and Asiegbu, 1997; Sanginga and Woomer, 2009) and improved resistance against lodging (Lithourgidis et al., 2006). Other

advantages includes better utilization of plant growth factors such as nutrients, light and water; reduced weed and insect pest indices, (Addo-Quaye et al., 2011; Amossé et al., 2013b, 2013a; Ratnadass et al., 2012; Valantin-Morison et al., 2014), maintain and improve the soil physical condition or fertility status (Akande et al., 2008) and increase the crop and environment quality through better land utilization (Bedoussac and Justes, 2010a, 2010b; Brooker et al., 2015; Matusso et al., 2014). Some studies have demonstrated that intercropping can be more successful and productive than monocropping (Amanullah et al., 2006; Bhatti et al., 2013) during forage production. In severe environmental conditions, intercropping can facilitate the utilization of inaccessible or limited soil nutrients such as phosphorus (Betencourt, 2012; Hauggaard-Nielsen and Jensen, 2005; Latati et al., 2016, 2014; Li et al., 2014) as well as, enhance the soil micro-organism diversity in the root rhizosphere (Hinsinger et al., 2011b; Tang et al., 2014). Intercropping not only enhance soil stability, permeability and aggregation (Carof, 2006; Wezel et al., 2014), but also soil microbial biomass, diversity and activity in the soil (Latati et al., 2016; Song et al., 2007; Tang et al., 2014).

After nitrogen, the second most limiting nutrient required for plant growth is phosphorus (Vance et al., 2000). Most soils are enriched with P, but this P is organic in nature (Tarafdar and Claassen, 1988) and is unavailable to the plants (Schachtman et al., 1998). Intercropping of cereals with legume can increase the available P in the root rhizosphere, as well as the acquisition of P in the plant (Cu et al., 2005; Hinsinger et al., 2011a, 2011b). Legumes have the ability to acidify the plant root rhizosphere by releasing protons, and this can increase the root rhizosphere soil acid phosphatase (RS–APase)

enzyme activity (Houlton et al., 2008), and nutrient release in the soil solution (Li et al., 2008). Phosphate enzyme has the ability to release organic P in the soil, thus increasing the available P to the plants (Li et al., 2007, 2003, 2004; Wang et al., 2014).

A number of studies have demonstrated that corn-soybean intercropping can have positive effects on forage yield, quality (Baghdadi et al., 2016; Htet et al., 2017, 2016; Jahanzad et al., 2014; Reta Sánchez et al., 2010; Serbester et al., 2015; Yucel et al., 2017) and soil health (soil chemical and biological properties) (Latati et al., 2014; Li et al., 2013, 2016, 2004). These studies were performed in warm-temperate, tropical and sub-tropical regions and under controlled conditions. To the best of our knowledge, there is very limited information available about the effects of silage corn intercropped with upright and vine soybean varieties on forage production, soil health and forage quality following field cultivations under cool climatic conditions in boreal ecosystems or northern climates. There work presented in this these attempts to address this issue.

Furthermore, I also investigated the applications of novel LA-ICP-MS technique to quantify and image the spatial distribution of essential nutrients in soil core obtained from fields undergoing different crop and nutrient management practices. This mass spectrometry base technique will not only help to map the nutrient distributions in plant root zone but will also help to identify the potential risks of heavy metal accumulation in soil through different nutrient management practices for sustainable agriculture.

1.2. Hypotheses

a) Corn-soybean intercropping can improve forage yield and nutritional quality due to enhancement of the soil health status following forage cultivation under cool climatic conditions.

b) LA-ICP-MS could be used to develop a novel technique to image the spatial distribution of elements in soil core by optimizing the instrument conditions and the quality of the soil core sample for imaging.

1.3. Purpose of the thesis and objectives

The purpose of thesis was to evaluate the effect of two silage corn varieties intercropped with three forage soybean (upright and vine soybean) varieties on FP, forage quality and the soil health status following field cultivation under cool climatic conditions. In addition, I attempted to use LA-ICP-MS to develop a novel imaging technique to visualize the spatial distribution of elements in soil cores, as well as, use the new technique to discern how micro and macro nutrients vary spatially in the root zone in response to different land use and crop management systems.

The following objectives were investigated to test the proposed hypotheses:

i. Determine the effect of corn-soybean intercropping on the agronomic performance (forage production, plant height and chlorophyll contents) and soil health status (RS-pH, RS-APase, RS-$P_{available}$ and rhizosphere soil microbial community composition).

ii. Study the effects of corn-soybean intercropping system on forage nutritional quality (protein, fiber, mineral, energy and fatty acids contents) when cultivated under cool climatic conditions.

iii. Investigate the relationships between soil health status (rhizosphere soil microbial community and soil chemical properties) and agronomic performances following corn-soybean intercropping.

iv. Determine the associations between forage nutritional quality, fatty acid composition and the active soil microbial community structure.

v. To develop a novel method to image the spatial distribution of plant essential nutrients in the soil, and its applications to evaluate the spatial distribution of plant nutrients in response to different crop management systems.

1.4. Thesis organization

This thesis is divided into four chapters, and the start of each chapter contained related literature germane to the experiments conducted.

Chapter One: Offers a brief overview of forage production scenarios in Newfoundland Canada, intercropping and its types, corn and soybean intercropping and advantages of intercropping on the yield, forage quality and soil health status under cool climatic conditions.

Chapter Two: Describes a comparative study between different cropping systems. In this chapter the effects of corn-soybean monocropping, as well as intercropping system on forage production and soil health (RS-pH, RS-$P_{available,}$ RS-APase, rhizosphere soil

microbial community structure (PLFA's) and their relationships with each other under cool climatic conditions were evaluated and discussed.

Chapter Three: This chapter describes the effects of mono or intercropping systems on forage nutritional quality, mineral content and fatty acid composition; and their relationships with nutritional quality, FAs and the active soil microbial community structure (Chapter 2).

Chapter Four: This chapter describes a novel method to image the spatial distribution of plant nutrients in the soil using soil cores, and its applications to evaluate nutrient distribution and accumulation in different land use or crop management systems.

Chapter Five: General discussion, conclusions and recommendations for future studies.

1.5. Definitions

All the possible definitions are given below (Alberta Agriculture and Rural Development, 2006; Allen et al., 2011; Monsanto Company, 2016; Filley et al., 2002; Shewmaker et al., 2009)

a. **Acid detergent fiber (ADF)** refers to the undigestible or slowly digestible portion of the fodder comprised of cell wall that is made up of cellulose and lignin.
b. **Ash** is the sum of all mineral contents in the fodder.
c. **Available protein (AP)** is the difference between crude protein and unavailable protein and is the naturally bound protein in the forages.

d. **Crude protein** is the sum of true and non-true protein nitrogen and is calculated by multiplying nitrogen with 6.25.

e. **Digestible dry matter (DDM)** is the total digestible fiber present in a forage sample and depends on ADF values in the forage.

f. **Dry matter intake (DMI)** is the consumed quantity of the dry forage by an animal and is dependent on the NDF values of the forage.

g. **Land equivalent ratio (LER)**: is the total land area required for monocropping to produce the same yield as obtained from intercropping (Amanullah et al., 2016).

h. **Net energy for gain (NE_G)** is the form of energy that is used by animals to gain body weight above the energy required for body maintenance.

i. **Net energy for lactation (NE_L)** is that form of energy that is used by animals for body maintenance and milk production.

j. **Net energy for maintenance (NE_M)** is the form of energy that keep animals in an equilibrium position with no gain or loss in weight.

k. **Neutral detergent fiber (NDF)** refers to the percentage of cell wall that is made up of cellulose, lignin and hemicellulose and digested in a specific time.

l. **Relative feed value (RFV)** is an important index that is used to grade the forage on the basis of ADF and NDF values, because ADF related to digestibility and NDF is correlated with forage intake.

m. **Soluble protein (SP)** is the form of protein that degraded rapidly to ammonia in the animal rumen and is used by rumen bacteria during high growth period.

n. **Total digestible nutrients (TDN)** is a sum of protein, lipids, carbohydrates and digestible fiber in an animal feed. There are three forms of feed energy and often calculated from TDN values. These include:

o. **Water-soluble carbohydrates (WSC)** involves numerous kinds of sugars that are soluble in water, including fructan and does not include starch.

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1.7. Co-authorship statement

Manuscripts based on chapter 2, entitled "*The potential of cereal-legume intercropping to improve the soil health and forage productivity under cool climatic conditions*" will be submitted to Plant, Cell and Environment (Muhammad Zaeem, Muhammad Nadeem, Thu Huong Pham, Waqar Ashiq, Waqas Ali, Syed Shah Mohioudin Gilani, Sathya Elavarthi, Vanessa Kavanagh, Mumtaz Cheema, Lakshman Galagedara, Raymond Thomas 2018). Muhammad Zaeem the thesis author will be the primary author and Dr. Raymond Thomas (supervisor), will be the corresponding and the eleventh author. Dr. Mumtaz Cheema (co-supervisor) and Dr. Galagedara (committee member) will be the ninth and tenth authors, respectively. Dr. Vanessa Kavanagh, research collaborator, Department of Fisheries and Land Resources will be the eighth author. Dr. Sathya Elavarthi will be the seventh author as he provided the unique forage soybean varieties and experimental design. Chapter 3 "*Effect of corn-soybean intercropping on forage production and forage quality under cool climatic conditions*" will be submitted to Frontier in plant Science (Muhammad Zaeem, Muhammad Nadeem, Thu Huong Pham, Waqar Ashiq, Waqas Ali, Syed Shah Mohioudin Gilani, Eric Moise, Sathya Elavarthi, Vanessa Kavanagh, Mumtaz Cheema, Lakshman Galagedara, Raymond Thomas 2018). Muhammad Zaeem the thesis author will be the primary author and Dr. Raymond Thomas (supervisor), will be the corresponding and the twelfth author. Dr. Mumtaz Cheema (co-supervisor) and Dr. Galagedara (committee member) will be the tenth and eleventh authors, respectively. Dr. Vanessa Kavanagh, research collaborator, Department of Fisheries and Land Resources will be the ninth author. Chapter 4 "*Development of a novel imaging technique using LA-ICP-MS to show*

the spatial distribution of elements in soil" will be submitted to Nature Methods. (Muhammad Zaeem, Muhammad Nadeem, Thu Huong Pham, Waqar Ashiq, Waqas Ali, Syed Shah Mohioudin Gilani, Eric Moise, Heather Leier, Vanessa Kavanagh, Mumtaz Cheema, Lakshman Galagedara, Raymond Thomas 2018). Muhammad Zaeem the thesis author will be the primary author and Dr. Raymond Thomas (supervisor), will be the corresponding and the twelfth author. Dr. Mumtaz Cheema (co-supervisor) and Dr. Galagedara (committee member) will be the tenth and eleventh authors, respectively. Dr. Vanessa Kavanagh, research collaborator, Department of Fisheries and Land Resources will be the ninth author. Dr. Heather Leier will be the eighth author as she helped with editing and arrangement of the soil core images. For the work in Chapter 2, 3 and 4, Dr. Raymond Thomas wrote the research grants, developed the layout of this research field trial and helped in results interpretation. Mr. Zaeem was involved in data collection, analysis, and writing of the manuscript. Dr. Nadeem and Dr. Pham contributed in field and lab work respectively. Dr. Lakshman provided specific guidance on soil core sample collection. Dr. Nadeem guided in statistical analyses and manuscript editing. Mr. Waqas Ali, Mr. Waqar Ashiq helped in filed and lab analysis. Mr. Gillani helped in field sampling. Dr. Eric assisted with soil sample digestion and analysis.

Chapter 2

2. The potential of cereal-legume intercropping to improve the soil health and forage productivity under cool climatic conditions

2.1. Abstract

Continuous monoculture on agricultural lands can lead to decreased soil fertility and crop productivity. To overcome such issues, cereal–legume intercropping (IC) is a promising approach to promote sustainable crop production. A two-year field study was conducted to evaluate the potential of silage corn and forage soybean IC in enhancing forage productivity and soil health in cool climate production systems. Two-silage corn (Yukon-R and DKC26–28RIB) and three forage soybean genotypes (Big Fellow RR, Game Keeper RR and Kester's Bob White Trailing Soybean) were planted using a randomized complete block design with three replications for each IC and monocropping (MC) treatment. Forage production (FP), soil chemical and biological properties were examined at the end of both growing seasons. IC enhanced the corn plant height (4%), chlorophyll content (5–13%) and the FP (18–42%) compared to MC. The land equivalent ratio was higher than 1 indicating advantage of IC over MC in term of FP. Furthermore, IC resulted in lowered rhizosphere soil pH (RS-pH) compared to MC. Conversely, the IC rhizosphere soil acid phosphatase (RS-APase) activity was 40%–54% higher compared to corn MC and 26%–28% to soybean MC, concomitant with a significant increase of 74%–76% and 21%–26% in rhizosphere soil available phosphorus compared to MC of both corn and soybean respectively. Soil phospholipid fatty acids (PLFA's) analyses showed that IC enhanced the

bacterial PLFA's, fungi, protozoa, and total PLFA's 7%–17% compared to MC. As such, a strong positive correlation was observed between RS–APase, RS-$P_{available,}$ PLFA's and FP; while RS-pH was negatively correlated with FP, RS–APase and RS–$P_{available}$ during both study years. These findings suggested silage corn and forage soybean IC could be a viable approach to enhance FP through improved active microbial community, RS–APase activity and RS–$P_{available}$ in cool climatic production systems**.**

Keywords: Corn, cool climate, forage, intercropping, phospholipid fatty acids, soybean, soil health,

2.2. Introduction

Intercropping (IC) is defined as the growing of two or more crops simultaneously on the same piece of land (Zhou et al. 2011; Li et al. 2013b; Brooker et al. 2015), and is increasingly being adopted as a sustainable approach in modern agricultural production throughout the world (Wang et al. 2014; Zhu et al. 2015; Owusu and Sadick 2016). On the other hand, monocropping (MC); growing of one crop on the same piece of land, decreases farm biodiversity and ability to self-adjust, thereby increasing disease susceptibility and severity (Altieri 2009). Conversely, IC is more sustainable approach that can be used not only to enhance farm biodiversity, but also to increase forage production (FP) and maintain the soil health for sustainable crop production (Zhang et al. 2013; He et al. 2013; Brooker et al. 2016). Additional advantages of cereal–legume IC include increased yield and land utilization (Dhima et al. 2007), improved yield stability of the cropping systems

(Lithourgidis et al. 2006, 2007), land conservation (Anil et al. 1998), enhanced pest or weed control (Banik et al. 2006; Vasilakoglou et al. 2008), increased level of plant available P in the root rhizosphere (Hinsinger et al. 2011), as well as enhanced the soil resource utilization by the component crops (Javanmard et al. 2009). Intercropping can further enhance the root rhizosphere soil acid phosphatase (RS–APase) enzyme activity due to rhizosphere acidification by leguminous crops used in the IC (Houlton et al. 2008). Generally, to quantify the impact of IC over MC is a land equivalent ratio (LER) index, which is a most common and popular index that can be used to explain the IC agronomic performance or yield advantage over MC (Ghosh 2004). The LER is defined as the relative land area required in a MC to produce the same yield as in IC production system under diverse environmental conditions.

Phosphorus (P) is a mandatory mineral nutrient required for plant growth, metabolism, development and improved crop productivity (Kizilkaya et al. 2007). Therefore, the lower plant available P can be an issue due to poor mobility, and the inherent low solubility of P containing compounds in the soil (Ciereszko et al. 2017). The major form of P in agricultural soils is organic P (Tarafdar and Claassen 1988) and can only be used after hydrolyzation by phosphatase enzymes (Gilbert et al. 1999). Soil phosphatase enzyme can convert organic P to plant utilizable forms, potentially increasing P availability in the rhizosphere, thereby improving the crop productivity (Li et al. 2003, 2004, 2007; Wang et al. 2014). Therefore, IC can enhance not only the utilization of above ground resources (sunlight and land area), but also results in efficient acquisition of mineral nutrients and water in underground soil parts (Javanmard et al. 2009; Zhu et al. 2015). The

improved soil enzyme activities and richness of the active microbial community resulted in superior mobilization of nutrients in the root rhizosphere (Wang et al. 2014; Owusu and Sadick 2016; Lasater et al. 2017). Such improvements in the soil enzyme activity was reported to be associated with increase active microbial biomass in IC production systems (Chai et al. 2005). Therefore, there is a need to investigate the composition of active microbial community structure in cereal–legume IC production systems.

Phospholipid fatty acids (PLFA's) including gram positive (G+) or gram negative (G-) bacteria, fungi (F) and protozoa (P) can be used as biomarkers to assess the active microbial community composition in the root rhizosphere (White et al. 1996; He et al. 2007, 2009). Soil PLFA profiles and microbial community are very sensitive to minor changes in soil environment, and thus have been extensively used to compare different crop management practices and land use systems (Bossio et al. 1998), as well as to assess any nutrient stresses in the root rhizosphere (Frostegård et al. 1993; Pennanen et al. 1996). Therefore, PLFA profiling is an efficient way to assess the active microbial community in the root rhizosphere and can be used as a proxy to assess the soil health and soil quality (Sharma et al. 2010). Previous studies have demonstrated that IC can modify the dominant microbial species' composition and their communities in the root rhizosphere (Song et al. 2007; He et al. 2013). Soil microbes are also known to secrete extracellular enzymes that is used in mineralization of soil nutrient sources. Consequently, extracellular enzymes such as RS–APase activity is also used to assess soil quality, because they are easy to measure and very sensitive to environmental stresses and changes in land usages (Yakovchenko et al. 1996; Dick et al. 1997).

Corn (*Zea mays*) is the most important forage crop due to its potential to provide large quantities of forage for animal usage (Eskandari and Ghanbari 2009). It is an exhaustive crop and depletes the soil nutrients compared to other forage crops (Kannan et al. 2013). Due to its tropical and subtropical origin, corn is a warm season crop and requires 19-32^{o}C temperature for better germination and optimum growth (du Plessiss 2003). Soybean on the other hand, is a restorative crop and can replenish the soil with nutrient. Therefore, silage corn and forage soybean IC could result in efficient utilization of nutrients, increase in land productivity, improved soil ecological conditions and fertility status (Tang et al. 2005; He et al. 2006). A number of studies have evaluated the effects of IC of corn with peanut (Li et al. 2016), chickpea (Li et al. 2004), cowpea (Latati et al. 2014), and faba bean (Li et al., 2013) on soil chemical and biological properties. However, there is lack of information on the effects of silage corn and forage soybean IC on soil chemical and biological properties under field conditions, especially in cool climate production systems. In particular, there is no information available in the literature to the best of our knowledge on the effects of silage corn intercropped with vine type forage soybeans on the soil heath and forage production under cool climatic conditions. We hypothesized that silage corn and forage soybean IC could enhance not only forage yield but could also improve soil health by affecting soil microbial community under cool climatic conditions. Therefore, the objectives of the current study were to evaluate: 1) the potential of silage corn and forage soybean IC to enhance forage production, 2) the effects of IC on soil nutrient status and the active microbial communities, and 3) the relationship between agronomic performance, active microbial community, and soil health under cool

climatic conditions by seeding two corn and three soybean genotypes either monocropped or intercropped in a randomized complete block arrangement.

2.3. Materials and Methods

A two-year field research trial was conducted at Pynn's Brook Agricultural Research Station, Pasadena, NL (49.0130° N, 57.5894° W), managed by the Department of Fisheries, and Land Resources, Government of Newfoundland and Labrador (NL), Canada. Two silage corn (C1: Yukon-R, C2: DKC26-28RIB), and three forage soybean genotypes (S1: Big Fellow RR, S2: Game Keeper RR, S3: Kester's Bob White Trailing Soybean-vine type) were sown on June 20th and May 30th during 2016 and 2017 using a SAMCO seeding machine (SAMCO Agricultural Manufacturing, Limerick Ireland), whereas the sowing of vine type soybean (S3) was carried out with a hand drill owing to the small seed size. Silage corn genotypes were selected based on low corn heating units requirements (Kwabiah et al. 2003). Seeding rate used for MC (corn: 77,100 seeds ha^{-1}; soybean: 129,200 seeds ha^{-1}) and IC (60% corn + 40% soybean; total 129,200 seeds ha^{-1}) during both study years. Crop nutrient requirements were fulfilled through inorganic fertilizers using the regional recommended rates for MC or IC based on the soil nutrient status prior to planting (Table 2.2). Soybean seeds were inoculated with *Bradyrhizobium japonicum* @ 10 g kg^{-1} seeds (Egamberdiyeva et al. 2004) before seeding. Roundup WeatherMax® (Monsanto Canada Inc) herbicide application was carried out to keep experimental area weed free during both growing seasons. Crop was harvested on October 25th and 13th during 2016 and 2017, respectively. There were a total eleven treatments for IC and MC of two corn and three

soybean genotypes (Table 2.1). The experiment was laid out in a randomized complete block design with three replications. Each experimental treatment plot was 5 m × 6 m dimensions. The weather data and soil physio-chemical properties for both growing seasons are given in Figure 2.1 and Table 2.2. Rainfall was significantly higher (731 mm) during the 2016 than 2017 (490 mm), however the average minimum and maximum temperatures were almost same during both study years (Figure 2.1).

Table 2.1: The description of experimental treatments during both growing seasons.

Treatment	**Cropping Systems**	**Genotypes**
C1	Corn-MC	Yukon-R
C2	Corn-MC	DKC26-28
S1	Soybean- MC	Big Fellow RR (upright)
S2	Soybean- MC	Game Keeper (upright)
S3	Soybean- MC	Kester's Bob White Trailing Soybeans (vine type)
S1C1	IC	Big Fellow RR + Yukon-R
S2C1	IC	Game Keeper RR + Yukon-R
S3C1	IC	Kester's Bob White Trailing Soybeans + Yukon-R
S1C2	IC	Big Fellow RR + DKC26-28
S2C2	IC	Game Keeper RR + DKC26-28
S3C2	IC	Kester's Bob White Trailing Soybeans + DKC26-28

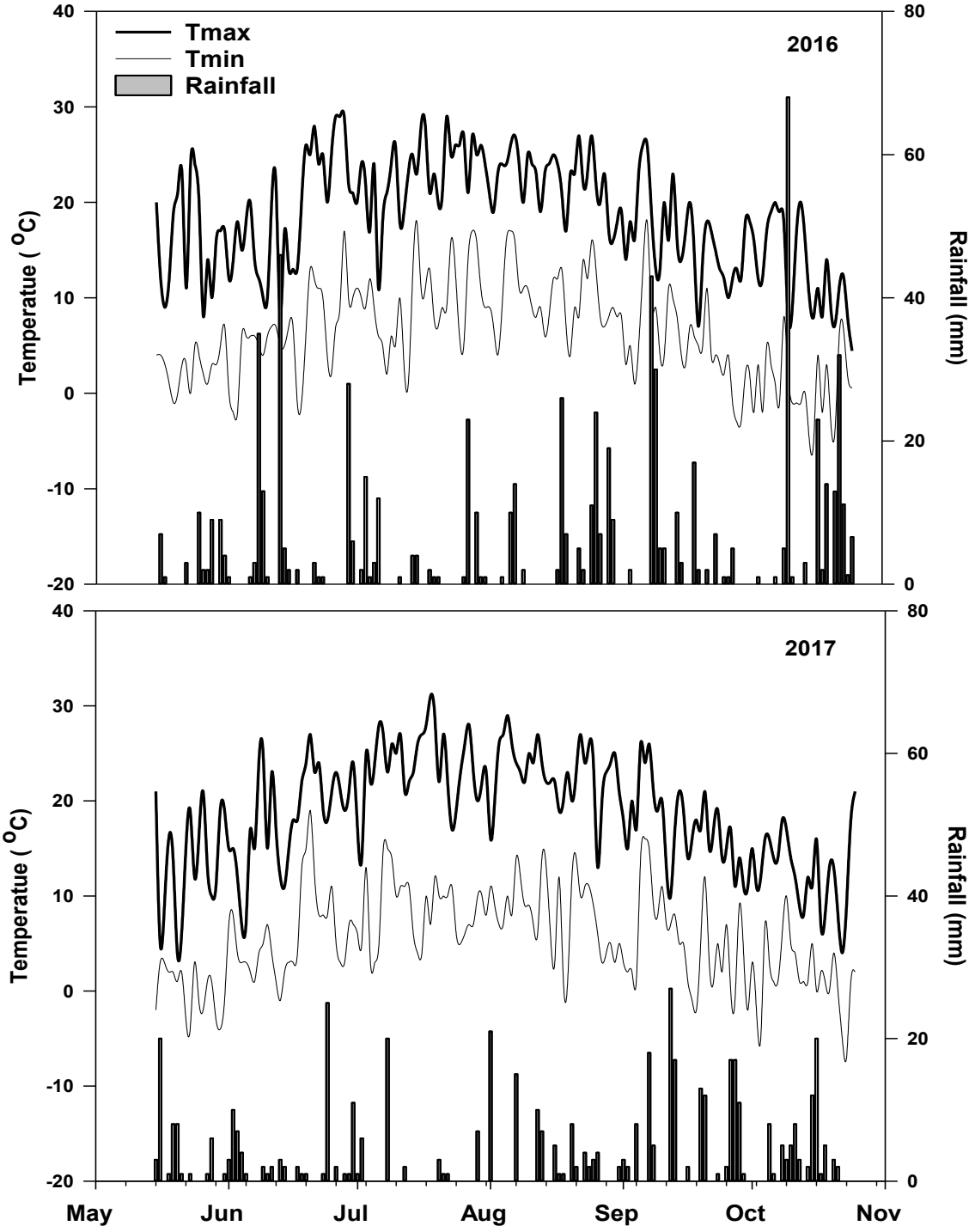


Figure 2.1: The average maximum and minimum temperature and total rainfall during both 2016 and 2017 growing seasons.

Table 2.2: Soil properties at the beginning of the growing season just prior to planting.

Soil Property	2016	2017	Soil Property	2016	2017
pH	6.4	6.8	Zn (mg kg^{-1})	0.6	1.0
Organic matter (%)	3.0	3.4	Mn (mg kg^{-1})	18.0	10.0
N (%)	81.0	68.0	S (mg kg^{-1})	14.0	17.0
P (mg kg^{-1})	38.0	35.0	Fe (mg kg^{-1})	150.0	233.0
K (mg kg^{-1})	38.0	35.0	Ca (mg kg^{-1})	1256.0	1426.0
Na (mg kg^{-1})	7.0	5.0	Mg (mg kg^{-1})	265.0	322.0

2.3.1. Crop agronomic performance: Chlorophyll contents, final plant height, and forage production

Chlorophyll contents were measured by a portable chlorophyll meter (SPAD-502 Konica-Minolta, Japan) from the top three leaves of corn and soybean plants at 65 and 77 days after sowing during 2016 and 2017, respectively. At physiological corn maturity (R6), four plants were selected from each plot, and the plant height was measured from ground to top level of each selected plant. The same plants were then uprooted gently and separated into roots and stem to record the forage production. Briefly, plants fresh weight was recorded, and a subsample was taken from each treatment to measure the dry matter percentage by drying in a forced air oven (Shel Lab®) at 65 ^{o}C for 72 h. Thereafter, the total forage production was calculated considering the dry matter percentage and total fresh biomass per treatment.

2.3.2. Soil health analyses

To quantify the effects of IC and MC on soil health, soil samples were collected from the root rhizosphere to measure the RS-APase, RS-pH, RS-$P_{available}$ and active microbial community by analyzing PLFA's. At R6, the four selected plants were uprooted gently,

and soil samples from root rhizosphere were collected, additionally roots were shaken gently to collect all soil attached to the root surface. The collected soil samples were then sieved through 2 mm mesh to remove plant roots, small stones, gravels, and samples were either analyzed directly or stored at –20 ^{o}C for different soil health parameters.

2.3.3. Rhizosphere soil acid phosphatase activity

Rhizosphere soil acid phosphatase (RS–APase) activity was measured using the modified methods of Tabatabai and Bremner (1969). Briefly, 1 g of 2 mm sieved soil was weighed and extracted in 1 mL of 0.09 M (pH 4.8) citrate buffer. Polypropylene centrifuge tubes containing soil and citrate buffer were then centrifuged (Heraeus™ Megafuge™ 16 Centrifuge Series) at 5000 rpm for 10 min. Supernatant aliquot (50 µL) was collected and RS-APase activity was measured after incubating for 30 min in the oven at 37 ^{o}C with 1 mM of 4-nitrophenyl phosphate (pNP), and 50 µL citrate buffer. Finally, the reaction was terminated immediately after incubation with 20 µL of 0.5 N sodium hydroxide, and the absorbance was recorded at 405 nm (BioTek™ Cytation™ 3 Imaging Reader, USA.), and the RS-APase activity presented in µmol pNP g^{-1} soil min^{-30}.

2.3.4. Rhizosphere soil pH

Rhizosphere soil pH (RS–pH) was measured in a 1:2 (w/v) ratio of soil and $CaCl_2$ solution by using a Mettler Toledo soil pH meter (Hendershot et al. 2006). Briefly, 10 g of 2 mm air-dried soil was weighed in 50 mL polypropylene centrifuge tubes, and 20 mL of 0.01 M $CaCl_2$ was added to soil. Soil solution then shook for 30 min on an orbital shaker (Innova™

2300 Platform Shaker, New Brunswick Scientific, USA) at 120 rpm, and allowed to stand for 1 h before measuring the RS–pH.

2.3.5. Rhizosphere soil available phosphorus

Rhizosphere soil available phosphorus (RS–$P_{available}$) was analyzed using the Mehlich–3 extraction method (Mehlich 1984). Briefly, 2 g air dried soil was weighed in 50 mL Erlenmeyer flasks, and 20 mL of Mehlich–3 extractant solution was added keeping a 1:10 soil–extractant ratio. Flasks were shaken for 5 min on an orbital shaker at 120 rpm, and the filtrate was recovered following filtration using Whatman–42 filter papers (Sigma Aldrich, ON Canada). The aliquot of filtrate was then analyzed using an AA3 Continuous Flow Analytical System (AA3HR, SEAL Analytical USA) to measure the RS–$P_{available}$, which was then converted to total phosphate in the soil sample based on soil weight by following formula;

$$Melich\text{–}3\ P\ (mg\ kg^{-1}) = \ [P\ con.\ Melich\text{–}3\ extrant\ (mg\ L^{-1})] \times \left[\left(\frac{0.002\ L\ extract\ vol.}{0.002\ kg\ soil}\right)\right]$$

2.3.6. Phospholipid fatty acids (PLFA's) analyses

A modified version of the Folch method (1957) was adopted to extract the soil PLFA's. Briefly, the total soil microbial fatty acids were extracted using 4 g sieved (2 mm) soil with 10 mL of chloroform-methanol (2:1 v/v). Sample mixture was sonicated for 5 min (Amplitude 50; Pulse on time: 5 sec; and pulse off time:10 sec) in an ice bath. Sample mixture was then incubated at room temperature for 24 h. Supernatant was filtered with Whatman 42 filter paper, then dried under a gentle stream of nitrogen. The total lipids extracted were resuspended in 2 mL chloroform and fractionated with a Visiprep™ SPE

Vacuum Manifold and Discovery® DSC-Si SPE columns (50 μm, 70 Å, 100 mg 1 mL^{-1}) (Sigma-Aldrich, ON Canada) into neutral lipids, glycolipids, and phospholipids by using 2.5 mL chloroform, 4 mL acetone and 2.5 mL methanol, respectively. Phospholipid fractions were re-dissolved into 500 μL of methyl tert-butyl ether, and aliquots (100 μL) of the phospholipid fractions were derivatized using 50 μL trimethyl sulfonium hydroxide (TMSH) in 2 mL GC vials (Batista et al. 2001). The mixture was vortexed and incubated for 30 min at room temperature. After incubation, 10 μL of the internal standard methyl nonadecanoate (C19:0 @ 160 μgmL^{-1}) was added to the sample vials and analyzed via gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID).

Table 2.3: Phospholipid fatty acids (PLFA) biomarkers used to characterize the active microbial community structure.

Fatty Acids	Organisms	Reference
2OH-C10:0	G-	(Lasater et al. 2017)
C14:0	G+	(Sheng et al. 2012)
C14:1n_5	G-	(Zhang et al. 2016)
i-C15:0	G+	(Wang et al. 2016; Zhang et al. 2016)
a-C15:0	G+	(Wang et al. 2016; Zhang et al. 2016)
C15:0	G+	(Huygens et al. 2011; Papatheodorou et al. 2012)
i-C16:0	G+	(Wang et al. 2016; Zhang et al. 2016)
2OH-C12:0	G-	(Lasater et al. 2017)
C16:0	G+ & G-	(Kujur and Patel 2014; Wu et al. 2015)
C16:1n_7	G+ & G-	(Brockett et al. 2012; Wang et al. 2016)
i-C17:0	G+	(Wang et al. 2016; Zhang et al. 2016)
3OH-C12_0	G-	(Kaur et al. 2005)
C17:0	G+	(Huygens et al. 2011; Papatheodorou et al. 2012)
C17:1n_7	G-	(Gómez-brandón and Domínguez 2010)
cyclo-C17:0	G-	(Wang et al. 2016; Zhang et al. 2016)
C18:0	G+ & G-	(Brockett et al. 2012; Wu et al. 2015)
C18:1n_9trans	G-	(Moreno et al. 2017)
C18:1n_9cis	G+ & G- & F	(Brockett et al. 2012; Zhang et al. 2016)
3OH-C14_0	G-	(Papatheodorou et al. 2012)
C18_2n-6cis	F	(Joergensen and Potthoff 2005; Zhang et al. 2016)
C18:3n-3	F	(Ringelberg et al. 1997; McKinley et al. 2005; Wu et al. 2015; Li et al. 2016)
Cyclo-C19:0	G-	(Wang et al. 2016)
2OH-C16:0	G-	(Sheng et al. 2012)
C20:0	P	(Schindlbacher et al. 2011)
20:1ω9c	F	(McKinley et al. 2005; Li et al. 2016)
20:3n6	P	(Buyer and Sasser 2012)
C20:4n_6	P	(Wu et al. 2013)

G+: gram positive bacteria; G-: gram negative bacteria; F: fungi; P: protozoa

2.3.6.1. GC-MS/FID analysis of soil microbial PLFA's

GC-MS/FID analysis was conducted on a Thermo Scientific Trace-1300 gas chromatography (GC) coupled to a Thermo Scientific TSQ 8000 Triple Quadrupole mass spectrometer (MS) and a flame ionization detector (FID). GC-MS was used for peak identification while GC-FID was used for quantification of detected fatty acids. Methylated fatty acids were separated with a DB23 high resolution column (30 m × 0.25 mm × 0.2 μm; Agilent Technology, Mississauga, Canada) using helium as the carrier gas at a flow rate of 1 mL min^{-1}. One μL of each sample was injected in split less mode using a Tri-plus auto-sampler. The oven temperature was programed as follows: the initial oven temperature of 50 °C was held for 1 min, then programmed to increase at 20 °C min^{-1} to 175 °C, held for 1 min at 175 °C, then increased at 4 °C min^{-1} to 230 °C, where it was held for 5 min. The methylated PLFA's were identified through retention times comparison and mass spectra obtained from commercial standards of NIST database (Thermo Scientific, ON Canada, Supelco 37 Component FAME Mix, and Bacterial Acid Methyl Ester (BAME) Mix obtained from, Sigma Aldrich, ON Canada). Quantification of individual PLFA's was done using standard curves prepared from the standard mixtures, and values presented as nmol g^{-1} soil. Total 37 PLFA's were identified as depicted in (Table 2.3), and 27 of them used as biomarkers to assess different active microbial groups in the plant root rhizosphere soil environment at the time of soil sampling.

2.3.7. Calculations and statistical analyses

The LER is the relative land area needed for MC to produce the same yield attained by IC (Willey 1979). LER was measured to evaluate the effect of IC verses MC (Willey 1979; Hauggaard-Nielsen et al. 2009; He et al. 2013) as given in following Equations:

$$L_C = \frac{\text{Yield}_{\text{corn IC}}}{\text{Yield}_{\text{corn MC}}} \quad \ldots\ldots 1$$

$$L_S = \frac{\text{Yield}_{\text{soybean IC}}}{\text{Yield}_{\text{soybean MC}}} \quad \ldots\ldots 2$$

$$LER = L_C + L_S \quad \ldots\ldots 3$$

Where, L_C and L_S are the partial LER for intercropped corn and soybean, respectively. When the LER value is greater than 1, it indicates that advantage is gained from IC compared to monocrop cropping in terms of the use of environmental resources for plant growth and the combined yield. When the LER is equal to 1, it means IC has no advantage over MC in the use of environmental resources; and when the LER is less than 1, it means MC use resources more efficiently than IC for plant growth and yield (Hauggaard-Nielsen et al. 2009; Eskandari 2012; He et al. 2013).

The mean values are resulted from the measurement of three biological replications of corn and soybean plants for chlorophyll contents, final plant height and forage production. To evaluate the effects of IC and MC on agronomic performance, one-way analysis of variance (ANOVA) was performed by using Statistix-10 software package (Analytical Software, FL, USA). All chemical parameter measurements including RS–pH, RS-APase, RS-$P_{available}$, and PLFA/PLFAs/PLFA's were made in quadruplet to evaluate the soil health in IC and MC. Where the treatment effects were significant, the means were compared using

Fisher's LSD test at alpha 0.05 among IC or MC. XLSTATS (Addinsoft Inc, Paris, France) program was used to carry out the redundancy analysis (RDA), principal component analysis (PCA) and Pearson's correlation coefficient analysis to test the relationships between agronomic performance and soil health status in IC and MC systems. Graphs were created by using Sigma Plot 13.0 software program (Systat Software Inc., San Jose, CA).

2.4. Results

2.4.1. Crop agronomic performance

Plant chlorophyll content, final plant height and forage production was used as indicators of agronomic performance of IC and MC cropping systems in current study. Results revealed that all the agronomic parameters were significantly affected by IC except the corn chlorophyll contents during 2016 (Table 2.4–2.6). In general, averaged IC significantly increased the chlorophyll content compared to corn MC weighted means, whereas a reduction was observed in the soybean plant chlorophyll when IC with corn (Table 2.4). A decrease was observed in upright soybean (US) genotypes when IC with either types of corn, whereas chlorophyll contents remained unchanged in IC vine type soybean (VS).

Corn plant chlorophyll contents were significantly higher in IC compared to MC during 2017, but non–significant differences were observed during 2016, though the trend was similar to 2017 (Table 2.4). High corn chlorophyll contents were measured in S1C1 (47.67) and S2C2 (46.67), whereas the lowest were noticed in C2 (44.4) during 2016 and (38.0) during 2017. For soybean, higher chlorophyll contents were observed in S2 (35.33 and

28.333), while lowest was observed in S3C2 (30.67) and S2C1 (23.67) during 2016 and 2017 growing seasons, respectively.

Table 2.4: Chlorophyll contents of silage corn and soybean plants cultivated either monocropped or intercropped during the 2016 and 2017 growing season.

Treatments	Growing Season (2016)		Growing Season (2017)	
	Corn	Soybean	Corn	Soybean
C1	44.9±1.2	_	40.0±0.5 ^{cd}	_
C2	44.4±1.0	_	38.0±0.8 ^{d}	_
S1	_	35.0±0.7 ^{a}	_	27.0±0.1 ^{ab}
S2	_	35.2±1.0 ^{a}	_	28.3±0.5 ^{a}
S3	_	32.8±0.5 ^{b}	_	27.0±0.6 ^{ab}
S1C1	47.9±0.8	30.8±0.3 ^{c}	42.3±1.1 ^{bc}	24.3±1.2 ^{c}
S2C1	47.8±0.8	31.2±0.7 ^{bc}	45.0±2.1 ^{ab}	23.7±1.3 ^{c}
S3C1	46.7±0.7	31.7±0.4 ^{bc}	42.7±0.3 ^{bc}	26.0±0.6 ^{abc}
S1C2	47.3±0.2	31.9±0.4 ^{bc}	45.0±1.0 ^{ab}	24.0±1.1 ^{c}
S2C2	47.0±0.7	31.4±0.5 ^{bc}	46.7±0.4 ^{a}	25.7 ±0.6 ^{bc}
S3C2	46.0±0.6	30.6±0.3 ^{c}	41.7±0.3 ^{c}	25.0±0.5 ^{bc}
Mono-(US)	35.2±0.6 ^{A}		27.7±0.3 ^{A}	
Inter -(C+US)	31.3±0.3 ^{B}		24.4±0.5 ^{B}	
Mono-(VS)	33.0±0.5		27.0±0.6	
Inter-(C+VS)	31.2±0.3		25.5±0.4	
Mono-C	44.8±0.7^{B}		39.0±0.5 ^{B}	
Inter-C	47.1±0.3 ^{A}		43.9±0.7 ^{A}	
Mono-S	34.3±0.6 ^{A}		27.4±0.3 ^{A}	
Inter-S	31.3±0.2 ^{B}		24.6±0.4 ^{B}	

Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-S: intercropped soybean; Inter-C: corn intercropped with soybean; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

In general corn plant height was not significant between the IC and MC treatments. However, we observed a reduction in the soybean plant height when both upright and vine type soybeans were IC with corn (Table 2.5). In 2016, plant height was 57 cm in upright soybeans MC compared to 52 cm in the IC, whereas it was 51 cm in the MC compared to 47 cm in 2017. On the other hand, the plant height of S3 in MC was 127 cm and 123 cm compared to 115 cm and 109 cm in the IC treatments during 2016 and 2017, respectively (Table 2.5). The plant height trends were opposite for corn plants as compared to soybean. IC increased the corn plant height as compared to MC. Corn plant height increase was significant during 2016 and 2017 growing seasons (Table 2.5). This trend is similar as discussed earlier for FP and chlorophyll content. The maximum and minimum corn plant height were recorded in S1C1 (210 cm) and C2 (185.6 cm) during 2016, and in S3C1 (205.8 cm) and C2 (178 cm) during 2017.

Table 2.5: Plant height (cm) of silage corn and forage soybean either monocropped or intercropped during the 2016 and 2017 growing seasons

Treatments	First Growing Season (2016)		Second Growing Season (2017)	
	Corn	Soybean	Corn	Soybean
C1	202.1±1.8 ^{ab}	–	197 ± 1.86 ^{abc}	–
C2	185.6±4.1 ^{d}	–	178 ± 3.63 ^{d}	–
S1	–	58.3±0.8 ^{c}	–	51 ± 1.23 ^{c}
S2	–	55.4±2.0 ^{cd}	–	50 ± 1.56 ^{bc}
S3	–	127.4±1.0 ^{a}	–	123 ± 3.01 ^{a}
S1C1	209.6±3.2 ^{a}	53.5±2.0 ^{cd}	204 ± 1.97 ^{ab}	48 ± 2.04 ^{cd}
S2C1	208.3±2.2 ^{a}	50.0±2.6 ^{de}	201 ± 4.61^{ab}	47 ± 2.47 ^{cd}
S3C1	206.2±4.0 ^{a}	116.7±1.1 ^{b}	206 ± 3.06 ^{a}	107 ± 1.91 ^{b}
S1C2	196.6±2.1 ^{bc}	55.1±2.6 ^{cd}	192 ± 8.54 ^{bc}	49 ± 1.48 ^{cd}
S2C2	190.5±1.8^{cd}	47.0±3.2 ^{e}	187 ± 2.31 ^{cd}	45 ± 1.20 ^{d}
S3C2	193.6±6.6 ^{bcd}	112.9±2.6 ^{b}	186 ± 0.81 ^{cd}	110 ± 3.26 ^{b}
Mono-(US)	56.8±1.2 ^{A}		51±0.91 ^{A}	
Inter-(C+US)	51.4±1.3 ^{B}		47±0.90 ^{B}	
Mono-(VS)	127.4±1.0 ^{A}		123±3.01 ^{A}	
Inter-(C+VS)	114.8±1.7 ^{B}		109±1.52 ^{B}	
Mono-S	80.4±11.8		75±11.99	
Inter-S	72.5±7.3		68±7.05	
Mono-C	193.8±4.2		188±4.68	
Inter-C	200.8±2.2		196±2.44	

Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2 = DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2 Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-S: intercropped soybean; Inter-C: corn intercropped with soybean; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

Overall, average of IC treatments significantly increased the FP as compared to corresponding MC (Table 2.6). In general, IC treatments FP ranged from averaged 13.20-16.32 Mg ha^{-1} compared to corn MC weighted means 9.86-13.86 Mg ha^{-1} (Table 2.6) during both years. It is important to note that the S3 treatment is a vine soybean that was IC with corn. Although IC increased the corn FP, however, decreased the forage soybean FP during both growing seasons (Table 2.6). Higher FP was observed in the S1C1 (16.99 Mg ha^{-1}), whereas the lowest MC yields were observed in C2 (13.59 Mg ha^{-1}) and S3 (1.36 Mg ha^{-1}) during the 2016 growing season. A similar trend was observed in crops cultivated during 2017 growing season, where higher FP was recorded in S2C1 (13.91 Mg ha^{-1}), and the lowest was noticed in C2 (8.60 Mg ha^{-1}) and S3 (0.74 Mg ha^{-1}). The second growing season resulted in higher FP increase than the 2016 growth season, but the overall trend of increase FP in the IC compared to the MC was the same.

Table 2.6: Dry matter yield (Mg ha^{-1}) of corn and soybean either monocropped or intercropped during the 2016 and 2017 growing seasons.

	Growing Season (2016)			Growing Season (2017)		
Treatments	**C**	**S**	**C+S**	**C**	**S**	**C+S**
C1	14.14±0.82	—	14.14±0.82 ^{bc}	9.95±0.49 ^{b}	—	9.95±0.21^{b}
C2	13.59±0.05	—	13.59±0.05 ^{c}	8.60±0.23 ^{b}	—	8.60±0.02 ^{b}
S1	—	3.63±0.14 ^{a}	3.63±0.14 ^{d}	—	3.50±0.21 ^{a}	3.50±0.03 ^{c}
S2	—	3.72±0.18 ^{a}	3.72±0.18 ^{d}	—	3.00±0.02 ^{b}	3.00±0.49 ^{c}
S3	—	1.36±0.16 ^{b}	1.36±0.16 ^{e}	—	0.74±0.03 ^{d}	0.74±0.23 ^{d}
S1C1	15.86±0.29	1.12±0.07 ^{b}	16.99±0.33 ^{a}	12.59±0.17 ^{a}	1.12±0.07 ^{c}	13.71±0.15 ^{a}
S2C1	15.79±0.93	0.75±0.11 ^{b}	16.95±1.05 ^{a}	12.82±0.91 ^{a}	0.75±0.08 ^{c}	13.91±1.05 ^{a}
S3C1	14.87±0.49	1.09±0.17 ^{b}	15.47±0.50 ^{ab}	12.84±0.10 ^{a}	1.09±0.15 ^{e}	13.10±0.11 ^{a}
S1C2	15.05±0.53	1.03±0.13 ^{b}	16.22±0.59 ^{a}	12.11±0.16 ^{a}	1.03±0.21 ^{d}	12.86±0.13 ^{a}
S2C2	15.87±1.49	0.26±0.01 ^{c}	16.98±1.39 ^{a}	12.24±1.46 ^{a}	0.26±0.01 ^{cd}	13.27±1.50 ^{a}
S3C2	14.78±0.36	0.20±0.01 ^{c}	15.29±0.36 ^{abc}	12.16±0.18 ^{a}	0.20±0.01 ^{e}	12.36±0.18 ^{a}
Mono-(US)		3.68±0.10 ^{A}			3.25±0.15 ^{A}	
Inter -(C+US)		1.14±0.05 ^{B}			0.99±0.07 ^{B}	
Mono-(VS)		1.36±0.16 ^{A}			0.74±0.03 ^{A}	
Inter-(C+VS)		0.55±0.02 ^{B}			0.23±0.01 ^{B}	
Mono-C		13.86±0.39 ^{B}			9.27±0.39 ^{B}	
Inter-C		15.37±0.30 ^{A}			12.46±0.26 ^{A}	
Mono-S		2.90± 0.40 ^{C}			2.41±0.43 ^{C}	
Mono-C		13.86±0.39 ^{B}			9.27±0.39 ^{B}	
Inter-(C+S)		16.32±0.33^{A}			13.20±0.29 ^{A}	

Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2 Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-S: intercropped soybean; Inter-C: corn intercropped with soybean; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

The LER values were higher than 1 for all IC treatments compared to MC during both study years (Table 2.7). The observed LER values ranged from 1.43–1.51 and 1.59–1.75 during 2016 and 2017, respectively. However, non–significant differences were observed in LER among different IC treatments (Table 2.7). Higher LER was recorded during 2017 compared to 2016 which indicates comparatively higher advantage to corn in the form of FP for the second year due to IC as compared to MC. Hence, more land area is required in MC to produce the similar FP as in IC.

Table 2.7: Land Equivalent Ratio (LER) for intercropping treatments of during 2016 and 2017 growing season.

	2016 Growing Season			2017 Growing Season		
Treatments	**C**	**S**	**C+S**	**C**	**S**	**C+S**
S1C1	1.13±0.08	0.31±0.03	1.44±0.09	1.27±0.06	0.32±0.03	1.59±0.05
S2C1	1.11±0.04	0.32±0.04	1.43±0.07	1.41±0.03	0.22±0.03	1.63±0.01
S3C1	1.13±0.10	0.32±0.06	1.44±0.10	1.29±0.08	0.36±0.05	1.65±0.13
S1C2	1.09±0.02	0.38±0.03	1.47±0.05	1.41±0.03	0.27±0.01	1.69±0.04
S2C2	1.17±0.11	0.30±0.04	1.47±0.09	1.40±0.22	0.34±0.07	1.75±0.25
S3C2	1.06±0.09	0.45±0.04	1.51±0.06	1.30±0.06	0.36±0.07	1.65±0.04
Average	1.12±0.03	0.35±0.02	1.45±0.03	1.35±0.04	0.31±0.02	1.66±0.04
LSD $_{0.05}$	ns	ns	ns	ns	ns	ns

ns= non-significant; Values are means ± standard errors. LER: Land Equivalent Ratio; C: corn; S: soybean; and C+S: corn + soybean. S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB.

2.4.2. Rhizosphere soil acid phosphatase activity

RS-APase activity was significantly higher in IC treatments compared to MC during both study years (Figure 2.2). However, no significant differences were observed in RS-APase activity between upright (S1 and S2) and vine (S3) soybean genotypes during 2016, whereas during the 2017 the upright genotypes showed higher RS-APase compared to MC vine type genotypes (Figure 2.3). Generally, IC increased RS-APase activity compared to both soybean and corn MC (Figure 2.2). Individually, higher RS-APase was observed in S2C1 during 2016 (65.13 µmol pNP g^{-1}soil min^{-30}) and 2017 (75.14 µmol pNP g^{-1}soil min^{-30}) growing season. Conversely, the lowest RS-APase was recorded in C2 during 2016 (30.08 µmol pNP g^{-1}soil min^{-30}), and 2017 (39.72 µmol pNP g^{-1}soil min^{-30}) as depicted in Figure 2.2. RS-APase activity was higher during second growing year compared to the first growing season (Figure 2.2). However, the overall RS-APase increase trends in IC compared to MC system was similar during both study years.

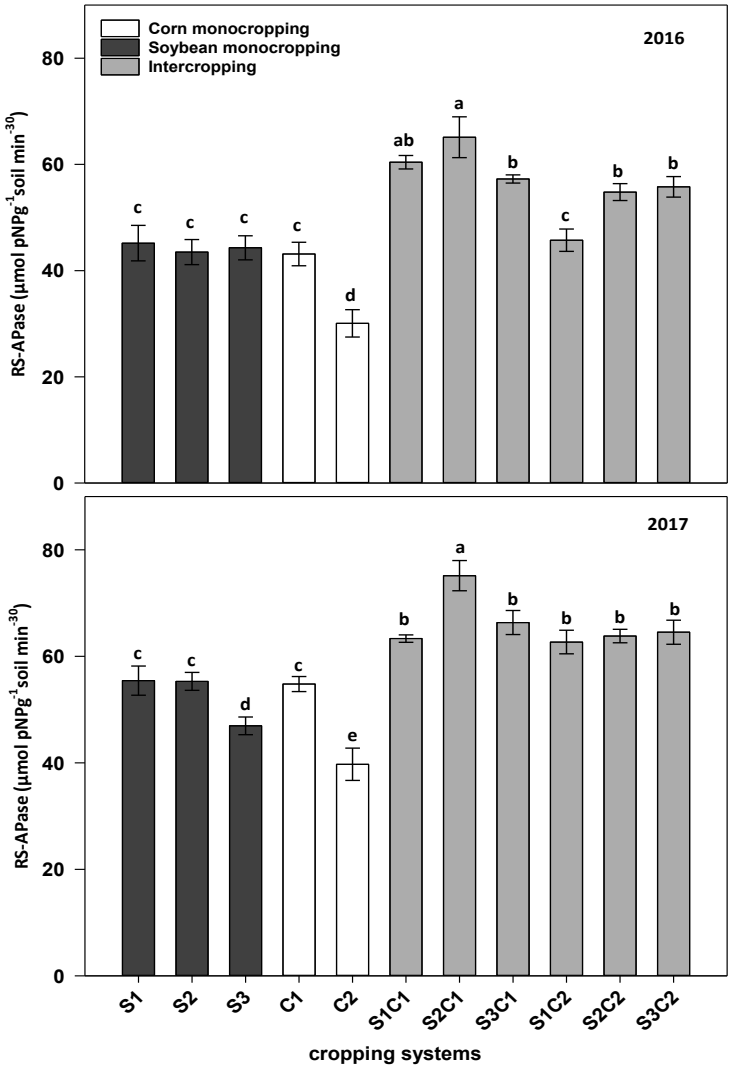


Figure 2.2: Rhizosphere soil acid phosphates (RS-APase) activity of corn and soybean sown as monocrops and as intercropping during the growing season of 2016 and 2017.

(n=33); The error bar represents ± SE. Different letters indicate significant differences at alpha 0.05 between the monocropping as well as intercropping treatments. C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB.

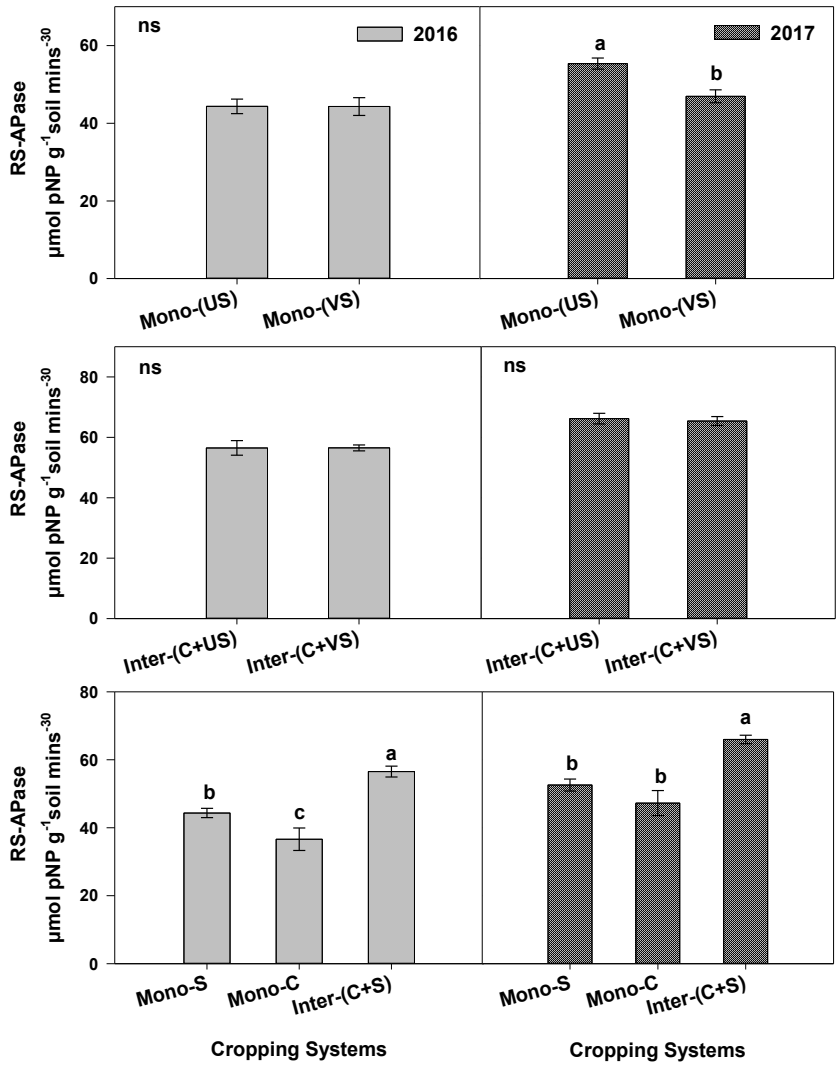


Figure 2.3: Rhizosphere soil acid phosphatase (µmole pNP g^{-1}soil min^{-30}) activity of corn and soybean cropping systems during the 2016 and 2017 growing season.

The error bar represents ±SE. Different letters indicate significant differences between the cropping systems at alpha 0.05. Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-(C+S): corn-soybean intercropping; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

2.4.3. Rhizosphere soil pH

The RS-pH varied significantly during both study years (Table 2.8). In general corn and soybean IC caused a significant decrease in RS-pH compared to MC, except during 2016, where the RS–pH differences were not significant between corn MC and IC treatments. Similarly, there was no significant difference observed between RS-pH of US or VS soybean cultivated as MC. Conversely, corn MC had significantly higher RS–pH values compared to IC during 2017. The IC RS–pH decrease was significant in S1C1 and S3C2 compared to corn MC, and in S1C1, S2C1, S2C2 and S3C2 compared to soybean MC during 2016. Additionally, higher RS–pH was measured in S2 (6.5), and the lowest in S1C1 (5.4) during 2016. During 2017, the RS–pH decrease was also significant in IC compared to corresponding corn (C1, C2) and soybean (S1, S2, and S3) MC with exceptions of S2C1 when compared to corn MC, and in S1C1 and S2C1 compared to soybean MC (Table 2.8). The S1 treatment had the higher RS-pH (5.5), whereas S2C1 rhizosphere had the lowest RS–pH of all treatments evaluated in this study (Table 2.8). Overall, RS–pH was higher during 2016 compared to 2017 (Table 2.8).

2.4.4. Rhizosphere available phosphorus

RS-$P_{available}$ changes were non-significantly among the MC treatments of VS and US (Table 2.8). However, when both the US and VS soybeans were IC with corn, there was a significant increase in RS-$P_{available}$ accompanied by a concomitant decrease in the RS-pH (Table 2.8). The increase in RS-$P_{available}$ was significantly greater in case of US intercropped with corn compared to IC with VS during both growing seasons. The increase in RS–$P_{available}$ was significant for the IC treatments compared to the corn and soybean MC

treatments, except for S3C1 and S2C2, where no statistical differences were observed during 2016 growing season compared to corresponding soybean MC (Table 2.8). Higher RS–$P_{available}$ was noticed in S1C1 (80.68 mg kg^{-1}), whereas the lowest was recorded in C2 (40.21 mg kg^{-1}) during 2016. The RS–$P_{available}$ trend was similar during both study years (Table 2.8). IC treatments showed significant RS-$P_{available}$ increase compared to corn and soybean MC, except in S1C1, S3C1, and S3C2 where the increase was non–significant compared to their corresponding soybean MC (Table 2.8). Higher and the lowest RS–$P_{available}$ was observed in S1C2 (102.71 mg kg^{-1}) and S2 (37.66 mg kg^{-1}), respectively.

Table 2.8: Rhizosphere soil available phosphorus (RS-Pavailable: mg kg^{-1}) and RS-pH of silage corn and forage soybean cultivated as monocrop and intercrop during the 2016 and 2017 growing season.

	Growing Season (2016)		Growing Season (2017)	
Treatments	**RS-$P_{available}$**	**RS-pH**	**RS-$P_{available}$**	**RS-pH**
C1	43.29±0.27 ^{e}	5.72±0.04 ^{c}	50.12±2.64 ^{d}	5.45±0.05 ^{ab}
C2	40.21±1.15 ^{e}	6.02±0.02 ^{b}	42.33±2.34 ^{de}	5.23±0.04 ^{abc}
S1	63.77±2.24 ^{bc}	6.06±0.02 ^{b}	88.55±2.58 ^{b}	5.51±0.12 ^{a}
S2	53.39±1.38 ^{d}	6.50±0.08 ^{a}	37.66±4.08 ^{e}	5.28±0.02 ^{abc}
S3	63.36±1.22 ^{bc}	6.16±0.01 ^{b}	67.72±8.54 ^{c}	5.40±0.35 ^{ab}
S1C1	80.68±0.98 ^{a}	5.4 ±0.06 ^{d}	82.88±3.78 ^{b}	5.13±0.03 ^{bcd}
S2C1	75.47±3.31 ^{a}	6.01 ±0.02 ^{b}	88.28±0.49 ^{b}	4.86±0.04 ^{d}
S3C1	58.54±2.76 ^{cd}	6.13 ±0.05 ^{b}	61.71±4.08 ^{c}	5.20±0.07 ^{abcd}
S1C2	80.17±1.76 ^{a}	6.04 ±0.10 ^{b}	102.71±3.43 ^{a}	5.17±0.06 ^{abcd}
S2C2	66.69±2.67 ^{b}	6.05 ±0.03 ^{b}	82.62±8.54 ^{b}	4.93±0.07 ^{cd}
S3C2	75.22±2.43 ^{a}	5.86 ±0.05 ^{c}	70.54±4.73 ^{c}	5.10±0.04 ^{bcd}
Mono-(US)	58.58±2.60	6.28±0.10	63.11±11.61	5.40±0.08
Mono-(VS)	63.36±1.22	6.16±0.02	67.76±2.58	5.40±0.35
Inter-(C+US)	75.75±1.97 ^{A}	5.88±0.09	89.12±3.25 ^{A}	5.02±0.05
Inter-(C+VS)	66.88±4.08 ^{B}	6.00±0.07	66.12±3.42 ^{B}	5.15±0.04
Mono-S	60.17±1.89 ^{B}	6.24±0.07 ^{A}	64.65±7.57 ^{B}	5.40±0.11 ^{A}
Mono-C	41.75±0.87 ^{C}	5.87±0.08 ^{B}	46.23±2.35 ^{C}	5.34±0.06 ^{A}
Inter-(C+S)	72.80±2.08 ^{A}	5.92±0.06 ^{B}	81.46±3.55 ^{A}	5.07±0.04 ^{B}

Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter(C+S): corn-soybean intercropping; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

2.4.5. Soil microbial community composition

The effect of IC on rhizosphere active soil microbial community structure including Gram positive (G+), Gram-negative (G–), bacteria, fungi, and protozoa, was assessed by PLFA's analysis and is depicted in Table 2.9-2.10. The total bacterial population was significantly higher compared to fungi and protozoa in all experimental treatments during both study years (Table 2.9-2.10). Both G+ (up to 48%) and G– (up to 52%) bacteria contributed to the overall observed total bacterial population. IC treatments expressed significant effects on the active microbial community during 2016. In general, the active microbial population (G+, G– and protozoa) was higher in IC compared to the soybean MC during 2016, whereas to corn and soybean MC during 2017, except for the fungal population which was not significantly different among different treatments (Table 2.9-2.10). The overall trend expressed higher averaged microbial community biomass when corn was IC with US genotypes compared to IC with VS during both study years (Table 2.9-2.10). Higher G+ and G- bacterial populations were found in S1C1 (30.91 and 32.52 nmol g^{-1}), whereas the minimum was found in S3 (20.24 nmol g^{-1}) and S2 (22.63 nmol g^{-1}) MC during 2016. The fungi and protozoa populations also followed the similar trends (Table 2.9). Higher fungi and protozoa populations were observed in S1C1 (9.08 and 1.97 nmol g^{-1}), whereas the lowest were observed in S2 (4.72) and S3 (1.52) treatments (Table 2.9). Similarly, the G+, G–, fungi and bacterial populations were also higher in the S1C1 treatment. A similar trend was observed during 2017, where the active microbial population was increased in IC compared to MC (Table 2.10). Higher G+ and G– populations were observed in S1C1

(24.76 and 26.64 nmol g^{-1}), and the lowest populations were recorded in C2 (19.23 and 19.71 nmol g^{-1}) treatment (Table 2.10). Similarly, higher fungal and protozoan populations were observed in S1C1 (5.82 and 1.75 nmol g^{-1}). Conversely, the G+: G– bacteria showed no significant differences between experimental treatments, however, the fungi: bacteria ratio was significant between the IC and MC. The highest ratio was recorded in S2 (0.12) whereas the lowest was noticed in S3 (0.10). The average of total PLFA's content indicate the overall higher active microbial population in IC treatments compared to the corresponding corn MC, except in the S3C1.

Table 2.9: The sum of selected PLFA's (nmol g^{-1}) from the rhizosphere of corn and soybean sown as monocropping and as intercropping during the 2016 growing season.

Treatments	G+	G-	B	F	P	T (PLFA's)	G+: G-	F: B
C1	21.63±0.15 ^{def}	23.44±0.22 ^{fg}	45.07±0.37 ^{ef}	5.06±0.10 ^{e}	1.66±0.02 ^{def}	51.79±0.26 ^{ef}	0.92±0.00 ^{b}	0.11±0.00 ^{de}
C2	23.60±0.23 ^{c}	25.92±0.26 ^{d}	49.51±0.48 ^{d}	7.03±0.30 ^{b}	1.74±0.04 ^{cd}	58.29±0.68 ^{c}	0.91±0.00 ^{bcde}	0.14±0.01 ^{ab}
S1	22.29±0.14 ^{d}	24.81±0.23 ^{def}	47.10±0.37 ^{de}	5.97±0.08 ^{d}	1.74±0.02 ^{cd}	54.80±0.41 ^{d}	0.90±0.00 ^{defg}	0.13±0.00 ^{bc}
S2	20.46±0.07 ^{fg}	22.63±0.08 ^{g}	43.08±0.08 ^{f}	4.72±0.04 ^{e}	1.58±0.02 ^{ef}	49.39±0.10 ^{f}	0.90±0.01 ^{cdef}	0.11±0.00 ^{e}
S3	20.24±0.07 ^{g}	22.68±0.12 ^{g}	42.92±0.18 ^{f}	4.89±0.10 ^{e}	1.52±0.04 ^{f}	49.33±0.15 ^{f}	0.89±0.00 ^{efg}	0.11±0.00 ^{de}
S1C1	30.91±0.43 ^{a}	32.52±0.42 ^{a}	63.43±0.82 ^{a}	9.08±0.07 ^{a}	1.97±0.01 ^{a}	74.47±0.88 ^{a}	0.95±0.01 ^{a}	0.14±0.00 ^{a}
S2C1	24.72±0.69 ^{c}	28.03±0.94 ^{c}	52.74±1.62 ^{c}	6.40±0.10 ^{c}	1.81±0.12 ^{bc}	60.95±1.67 ^{c}	0.88±0.01 ^{g}	0.12±0.00 ^{cd}
S3C1	21.89±0.47 ^{de}	23.93±0.56 ^{efg}	45.81±1.03 ^{e}	5.00±0.18 ^{e}	1.64±0.01 ^{def}	52.46±1.21 ^{de}	0.91±0.00 ^{bcd}	0.11±0.00 ^{e}
S1C2	27.11±0.68 ^{b}	29.43±0.60 ^{b}	56.54±1.26 ^{b}	6.65±0.06 ^{bc}	1.91±0.06 ^{ab}	65.09±1.30 ^{b}	0.92±0.01 ^{bc}	0.12±0.00 ^{ab}
S2C2	21.00±0.65 ^{efg}	23.64±0.66 ^{fg}	44.64±1.30 ^{ef}	4.95±0.10 ^{e}	1.66±0.02 ^{def}	51.25±1.26 ^{ef}	0.89±0.01 ^{g}	0.11±0.00 ^{de}
S3C2	22.08±0.44 ^{de}	25.05±0.41 ^{de}	47.13±0.85 ^{de}	5.92±0.27 ^{d}	1.69±0.02 ^{cde}	54.74±0.87 ^{d}	0.88±0.00 ^{g}	0.13±0.01 ^{cd}
Average	23.27±0.56	25.64±0.54	48.91±1.09	5.97±0.22	1.72±0.03	56.60±1.32	0.91±0.00	0.12±0.00
Mono-(US)	21.37±0.42	23.72±0.50	45.09±0.91	5.35±0.28	1.66±0.04	52.09±1.23	0.90±0.00	0.12±0.00
Mono-(VS)	20.24±0.07	22.68±0.12	42.92±0.18	4.89±0.10	1.52±0.02	49.33±0.15	0.89±0.00	0.11±0.00
Inter-(C+US)	25.93±1.12 ^{A}	28.41±1.01 ^{A}	54.34±2.12 ^{A}	6.77±0.45	1.84±0.05 ^{A}	62.94±2.58 ^{A}	0.91±0.01	0.12±0.00
Inter-(C+VS)	21.98±0.29 ^{B}	24.49±0.40 ^{B}	46.47±0.66 ^{B}	5.46±0.25	1.67±0.02 ^{B}	53.60±0.84 ^{B}	0.90±0.01	0.12±0.00
Mono-S	21.00±0.33 ^{B}	23.37±0.37 ^{B}	44.37±0.69 ^{B}	5.19±0.20	1.61±0.03 ^{B}	51.17±0.92 ^{B}	0.90±0.00	0.12±0.00
Mono-C	22.62±0.46 ^{AB}	24.68±0.57 ^{AB}	47.29±1.03 ^{AB}	6.05±0.46	1.70±0.03 ^{AB}	55.04±1.49 ^{AB}	0.92±0.00	0.13±0.01
Inter (C+S)	24.62±0.87 ^{A}	27.10±0.81 ^{A}	51.72±1.67 ^{A}	6.33±0.34	1.78±0.04 ^{A}	59.83±2.02 ^{A}	0.91±0.01	0.12±0.00

Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-(C+S): corn-soybean intercropping; Mono-(US): monocropping upright soybean; Mono-(VS):

monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean. G+: gram positive; G-: gram negative; B: bacteria; T (PLFA's): total phospholipid fatty acids; F: fungi; P: protozoa.

Table 2.10: The sum of selected PLFA's (nmol g^{-1}) from the rhizosphere of corn and soybean sown as monocropping and as intercropping during the 2017 growing season.

Treatments	G+	G-	B	F	P	T (PLFA's)	G+: G-	F: B
C1	20.41±0.22 ^{cd}	21.55±0.32 ^{cd}	41.97±0.54 ^{cd}	4.46±0.02 ^{cde}	1.59±0.05 ^{bcde}	48.02±0.53 ^{cd}	0.95±0.01	0.11±0.00 ^{bcd}
C2	19.23±0.67 ^{d}	19.71±0.72 ^{d}	38.94±1.26 ^{d}	4.54±0.30 ^{cde}	1.49±0.05 ^{e}	44.97±1.52 ^{d}	0.98±0.03	0.12±0.01 ^{ab}
S1	20.43±0.34 ^{cd}	21.86±0.30 ^{cd}	42.29±0.62 ^{cd}	4.35±0.22 ^{cde}	1.53±0.08 ^{de}	50.64±0.77 ^{cd}	0.93±0.01	0.10±0.01 ^{cd}
S2	20.86±0.23 ^{bcd}	22.01±0.39 ^{bcd}	42.88±0.60^{bcd}	5.19±0.07 ^{b}	1.52±0.03 ^{de}	48.17±0.68 ^{bcd}	0.95±0.01	0.12±0.00 ^{a}
S3	21.64±1.04 ^{bc}	22.60±1.04 ^{bc}	44.24±2.08 ^{bc}	4.26±0.03 ^{e}	1.53±0.02 ^{de}	49.59±2.09 ^{bcd}	0.96±0.00	0.10±0.00 ^{d}
S1C1	24.76±1.08 ^{a}	26.64±1.11 ^{a}	51.40±2.19 ^{a}	5.82±0.20 ^{a}	1.75±0.07 ^{a}	50.03±2.49 ^{a}	0.93±0.00	0.11±0.00 ^{abc}
S2C1	22.90±0.87 ^{ab}	24.40±1.07 ^{ab}	47.30±1.93 ^{ab}	4.81±0.12 ^{bcd}	1.67±0.02 ^{abc}	53.79±2.05 ^{b}	0.94±0.01	0.10±0.0 ^{cd}
S3C1	22.04±1.60 ^{bc}	23.07±1.62 ^{bc}	45.11±3.22 ^{bc}	4.84±0.01 ^{de}	1.55±0.01 ^{cde}	51.00±3.25 ^{bc}	0.95±0.00	0.10±0.01 ^{d}
S1C2	21.72±0.28 ^{bc}	22.99±0.45 ^{bc}	44.71±0.72 ^{bc}	7.39±0.10 ^{bc}	1.68±0.10 ^{abc}	51.24±0.94 ^{bc}	0.94±0.01	0.11±0.00 ^{bcd}
S2C2	21.73±0.78 ^{bc}	23.15±0.79 ^{bc}	44.88±1.57 ^{bc}	4.41±0.02 ^{cde}	1.66±0.02 ^{abcd}	50.94±1.57 ^{bc}	0.94±0.00	0.10±0.00 ^{d}
S3C2	22.17±0.88 ^{bc}	23.15±0.86 ^{bc}	45.31±1.73 ^{bc}	4.63±0.15 ^{cde}	1.72±0.06 ^{ab}	51.67±1.80 ^{bc}	0.96±0.01	0.10± 0.01 ^{cd}
Average	21.63±0.32	22.83±0.37	44.46±0.69	4.70±0.09	1.61±0.02	50.76±0.75	0.95±0.00	0.11±0.00
Mono-(US)	20.65±0.21	21.94±0.22	42.58±0.41	4.77±0.21	1.53±0.04	48.88±0.56	0.94±0.01	0.11±0.00
Mono-(VS)	21.64±1.04	22.60±1.04	44.24±2.08	4.26±0.04	1.53±0.02	50.03±2.09	0.96±0.00	0.10±0.00
Inter-(C+US)	22.78±0.51	24.30±0.58	47.07±1.09 ^{A}	4.97±0.17	1.69±0.03 ^{A}	53.73±1.25 ^{A}	0.94±0.00	0.11±0.00
Inter-(C+VS)	22.10±0.82	23.11±0.82	45.21±1.64 ^{B}	4.48±0.10	1.64±0.05 ^{B}	51.34±1.67 ^{B}	0.96±0.00	0.10±0.00
Mono-S	20.98±0.37 ^{B}	22.16±0.35 ^{B}	43.14±0.71 ^{B}	4.60±0.16	1.53±0.02 ^{B}	49.27±0.73 ^{B}	0.95±0.01	0.11± 0.00
Mono-C	19.82±0.41 ^{B}	20.63±0.54 ^{B}	40.45±0.91 ^{B}	4.50±0.16	1.54±0.04 ^{B}	46.49±0.99 ^{B}	0.96±0.02	0.11±0.00
Inter-(C+S)	22.55±0.43 ^{A}	23.90±0.48 ^{A}	46.45±0.90 ^{A}	4.81±0.13	1.67±0.02 ^{A}	52.94±1.01 ^{A}	0.95±0.00	0.10±0.00

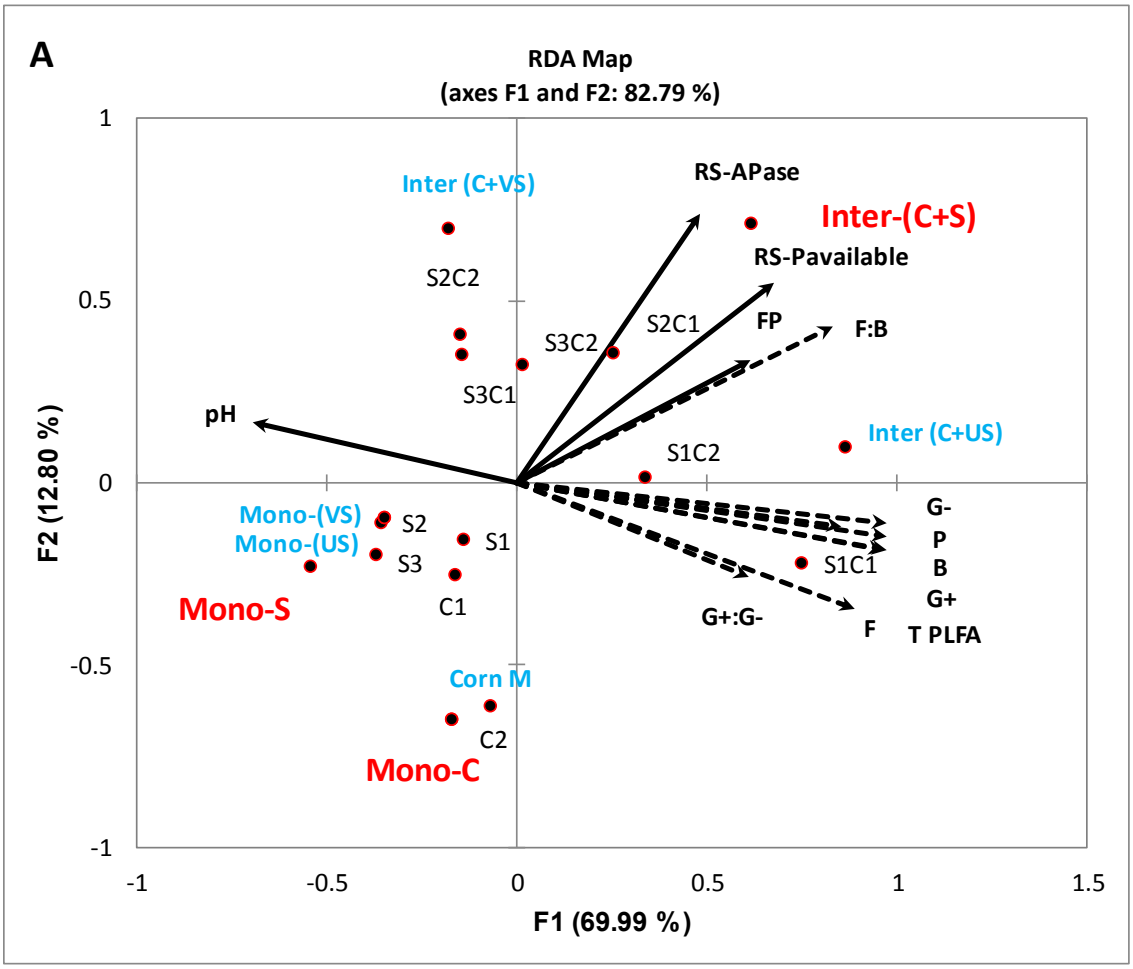
Values are means ± standard errors. Mean values in each column followed by the same superscripts are not significantly different at alpha 0.05. C: corn; S: soybean; C+S: corn + soybean; C1: Yukon-R; C2: DKC26-28RIB; S1: Big Fellow RR; S2: Game Keeper RR; S3: Kester's Bob White Trailing Soybean; S1C1: Big Fellow RR + Yukon-R; S2C1: Game Keeper RR + Yukon-R; S3C1: Kester's Bob White Trailing Soybean + Yukon-R; S1C2: Big Fellow RR + DKC26-28RIB; S2C2: Game Keeper RR + DKC26-28RIB; S3C2: Kester's Bob White Trailing Soybean + DKC26-28RIB; Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-(C+S): corn-soybean intercropping; Mono-(US): monocropping upright soybean; Mono-(VS):

monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean. G+: gram positive; G-: gram negative; B: bacteria; T (PLFA's): total phospholipid fatty acids; F: fungi; P: protozoa.

2.4.6. Relationship between agronomic performance and soil health

The active microbial community structure, RS-pH, RS-APase activity, and RS-$P_{available}$ were used as indicators to assess soil health status; while the chlorophyll contents, final plant height and FP were used as indicators of agronomic performance (Figure 2.2–2.7; Tables 2.4-2.10). Several significant associations were observed between the active microbial community structure, soil biochemical properties and agronomic performance (FP); and these associations were very influential in clustering the MC treatments together, however, well separated in different quadrants of the biplot from the IC following redundancy analysis (Figure 2.4A & 2.4B). For example, RS-APase, RS-$P_{available}$ and FP were the most influential factors clustering the IC together regardless of the growing season (Figure 2.4A & 2.4B). We also have observed that inter (C+VS) clustered in separate quadrants of the biplots compared to inter (C+US) during both growing seasons. The RDA analysis output revealed that axis 1 and axis 2 explained 69.99% and 12.80% of the total variance during 2016, and 59.89% and 17.04% during 2017 (Figure 2.4A & 2.4B). During 2016, a positive correlation was observed between soil microbial community (G+, G–, P, F, T PLFA's, G+: G– ratio, F: B ratio), RS-$P_{available}$, RS-APase activity and FP, however, all these parameters were negatively correlated with RS-pH (Figure 2.4A & 2.4B). During 2017, soil microbial community (G+, G–, P, F, T PLFA's, G+: G– ratio, F: B ratio) showed a positive correlation with FP, RS-$P_{available}$ and RS-APase, whereas a negative correlation was observed with RS-pH, F: B ratio and G+: G– ratio. Conversely, the G+: G– ratio showed a positive correlation with RS–pH. Further analysis by Pearson correlation showed

a significant positive association between soil microbial PLFA's with RS-$P_{available}$, RS-APase activity and FP (Table 2.11, Figure 2.6), which are indicators of the soil health status and agronomic performance respectively during 2016. A similar trend was observed for crop cultivated during2017 growing season (Figure 2.6; Table 2.11). Agronomic parameters were also observed to be significantly positivity correlated with each other during both growing seasons as depicted in Figure 2.7.



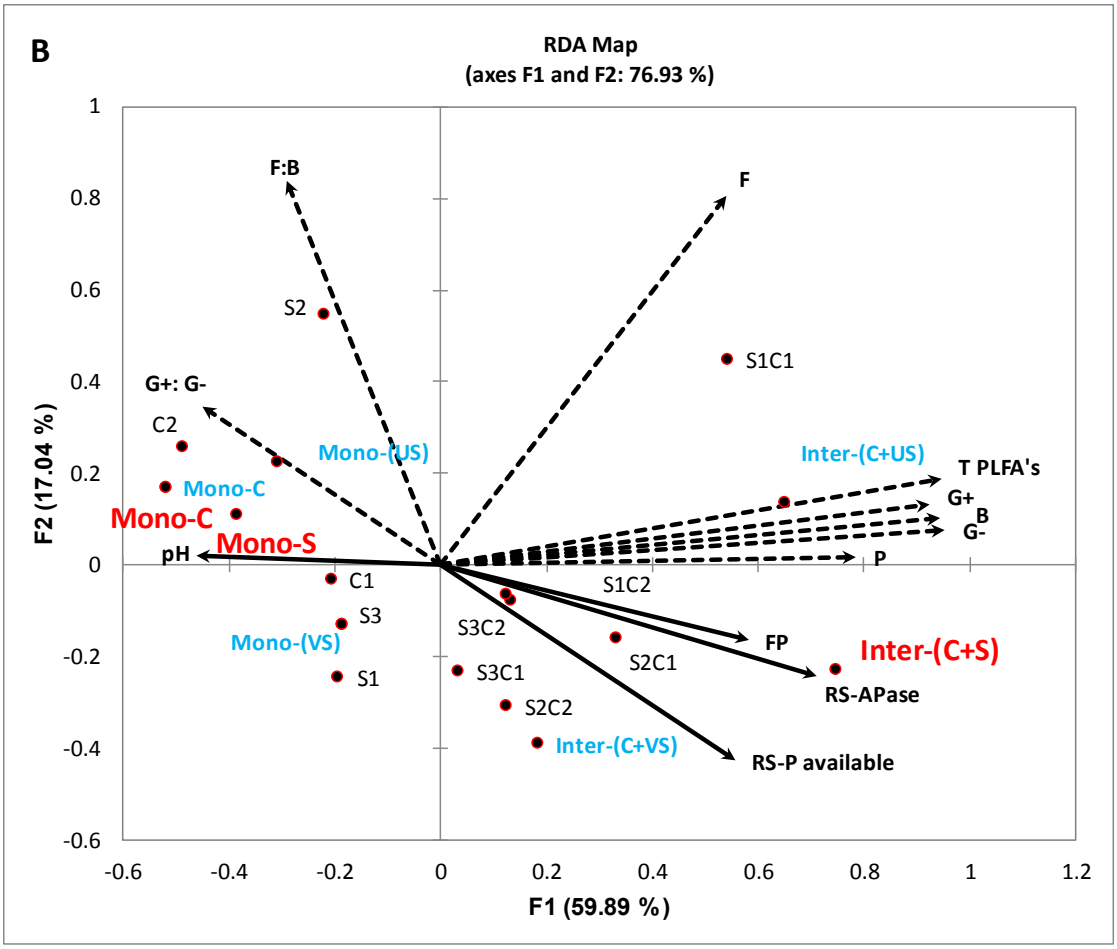


Figure 2.4 A & B: Redundancy analysis (RDA) of the active soil microbial community (PLFA), soil chemical properties and plant FP in corn-soybean MC and IC treatments during 2016, and 2017 growing seasons.

2016 (A), and 2017 (B) growing seasons. RS-APase, RS-$P_{available}$, and FP represent rhizosphere soil acid phosphatase activity, rhizosphere soil available phosphorus and forage production respectively. G+, G-, B, T PLFA's, F, P represent gram positive, gram negative, bacteria, total phospholipid fatty acids, fungi, and protozoa respectively. Mono-C: monocropping corn; Mono-S: monocropping soybean; Inter-(C+S): corn-soybean intercropping; Mono-(US): monocropping upright soybean; Mono-(VS): monocropping vine soybean; Inter-(C+US): corn intercropped with upright soybean; Inter-(C+VS): corn intercropped with vine soybean.

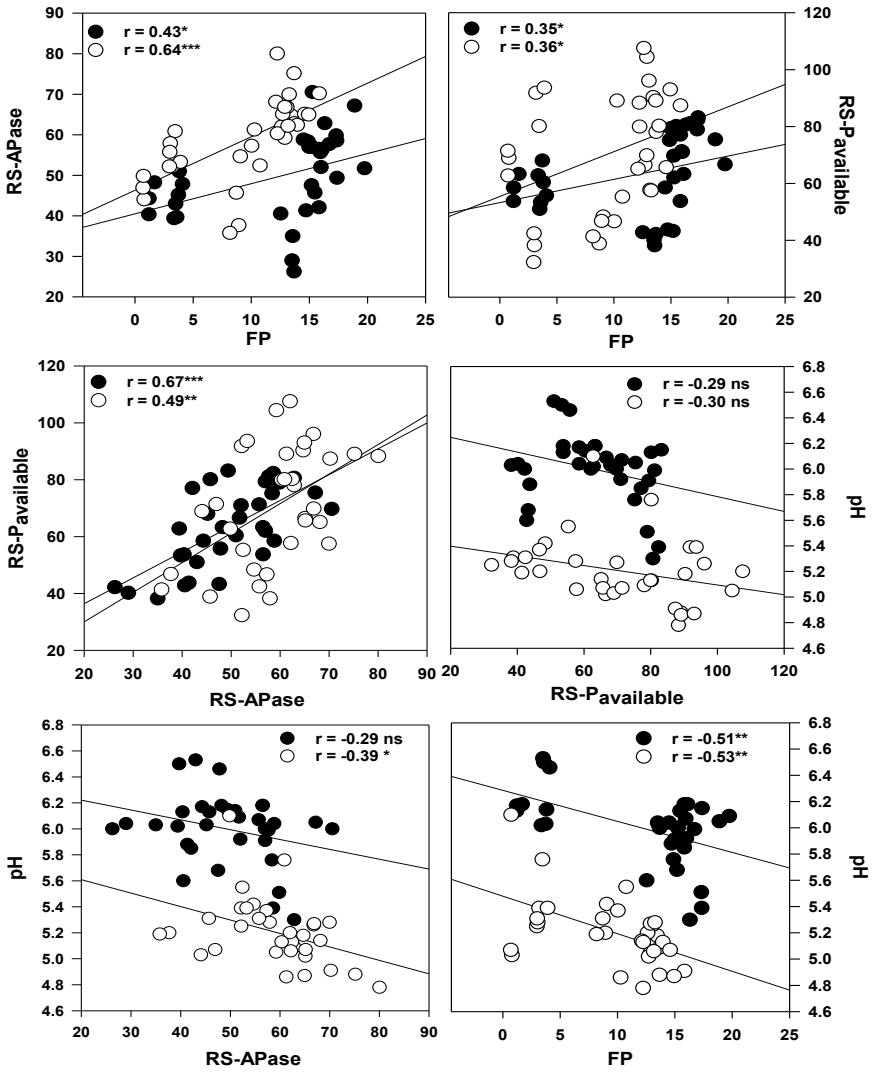


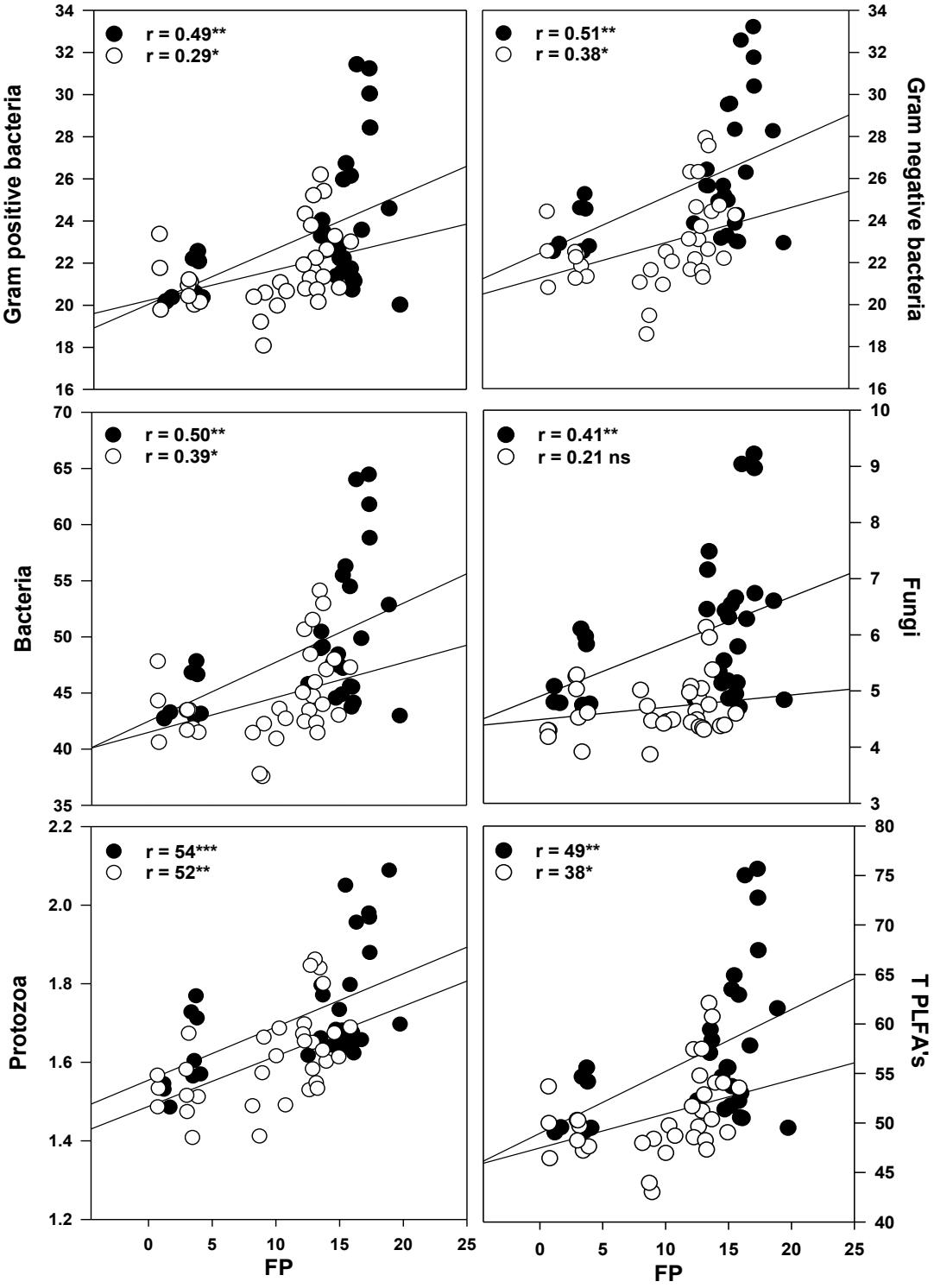
Figure 2.5: Pearson correlation between rhizosphere acid phosphatase activity, rhizosphere available phosphorus, soil pH and forage production for corn-soybean monocropping and intercropping treatments during 2016 and 2017 growing seasons.

Rhizosphere acid phosphatase activity (RS-APase: μmol g^{-1}soil min^{-30}), rhizosphere available phosphorus ($RS\text{-}P_{available}$: mg kg^{-1}), forage production (FP: Mg ha^{-1}). 2016 (filled circles) and 2017 (Empty circles). ns = correlation is non-significant; *correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) and *n* is 33 for all parameters.

Table 2.11: Pearson correlation coefficients between phospholipid fatty acids (PLFA's) and rhizosphere soil acid phosphates activity (RS-APase) and rhizosphere soil available phosphorus ($RS\text{-}P_{available}$) during the 2016 and 2017 seasons.

	G+	G-	B	F	P	T PLFA's	G+: G-	F: B
				2016				
RS-APase	0.33^{ns}	0.39*	0.36*	0.17^{ns}	0.27^{ns}	0.33^{ns}	0.26^{ns}	0.57***
$RS\text{-}P_{availlable}$	0.55***	0.61***	0.58***	0.44*	0.52**	0.57***	0.27^{ns}	0.80***
				2017				
RS-APase	0.54**	0.57***	0.56***	0.23^{ns}	0.49**	0.55***	-0.32^{ns}	-0.28^{ns}
$RS\text{-}P_{availlable}$	0.37*	0.42*	0.40*	0.07^{ns}	0.43*	0.39*	-0.41*	-0.32^{ns}

$n = 33$. ns: correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$).


r = 0.49**
r = 0.29*
Gram positive bacteria
r = 0.51**
r = 0.38*
Gram negative bacteria
r = 0.50**
r = 0.39*
Bacteria
r = 0.41**
r = 0.21 ns
Fungi
r = 54***
r = 52**
Protozoa
r = 49**
r = 38*
T PLFA's
FP
FP

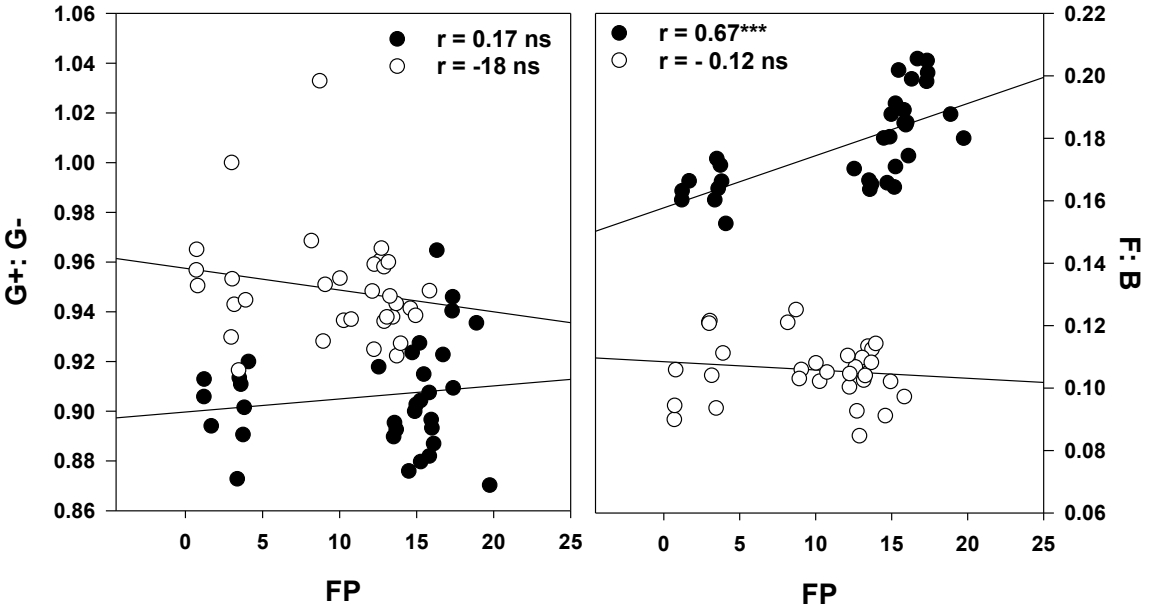


Figure 2.6: Pearson correlation between forage production (FP: Mg ha^{-1}) and rhizosphere soil microbial PLFA's (nmol g^{-1} soil) community for different corn-soybean monocropping and intercropping treatments during 2016 and 2017 seasons.

2016 (filled circles) and 2017 (empty circles). T PLFA's = total phospholipid fatty acids; G+: G- = gram positive to gram negative bacteria ratio; F: B = fungi to bacteria ratio; FP = forage production; ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) and *n* is 33 for all parameters.

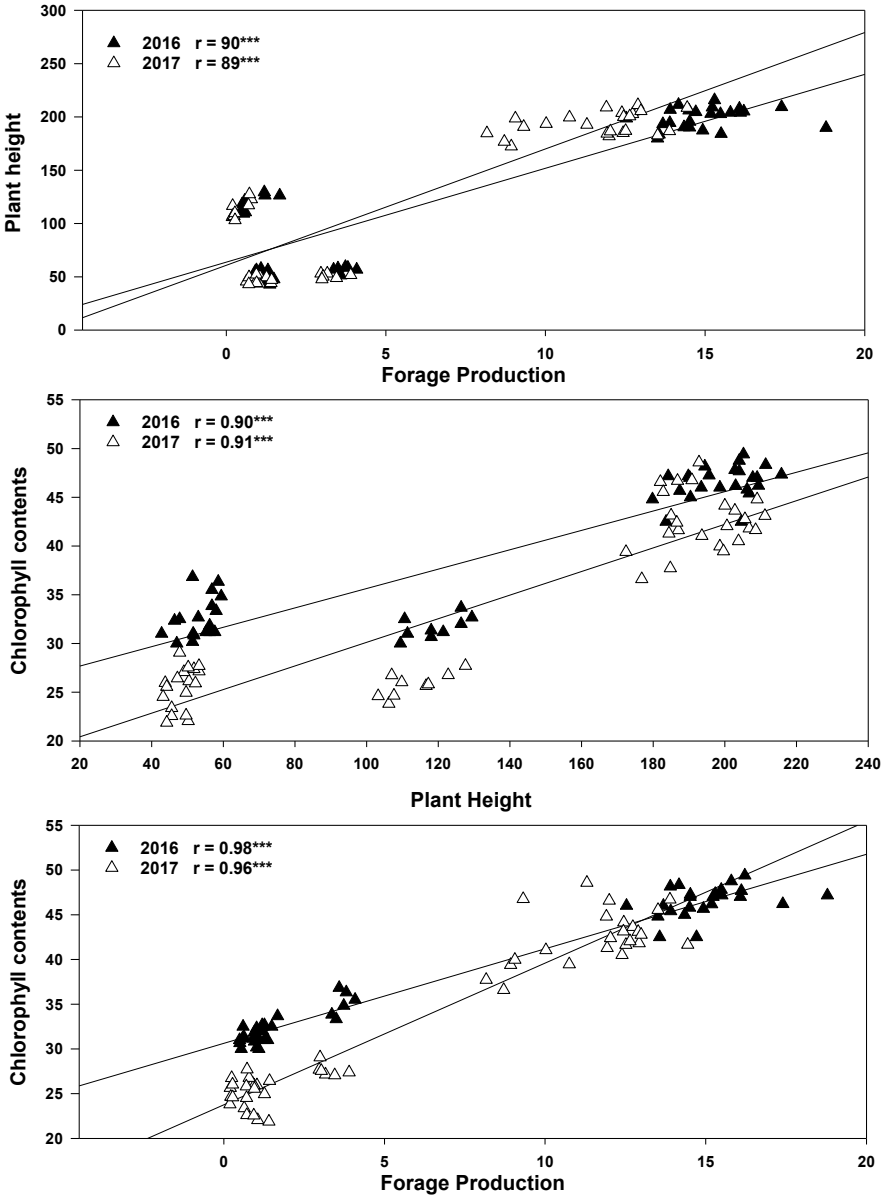


Figure 2.7: Correlation between plant height (cm), chlorophyll contents and forage production (Mg ha^{-1}) for different corn-soybean monocropping and intercropping treatments during 2016 and 2017 growing seasons.

2016 (filled triangle) and 2017 growing seasons (empty triangle). ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) n=33 for all parameter

2.5. Discussion

2.5.1. Crop agronomic performance

Intercropping (IC) can enhance crop growth and crop productivity due to superior utilization of above and below ground resources (Li et al. 2001; Zhou et al. 2011). Plant chlorophyll content is an important indicator of agronomic performance (enhanced crop growth and productivity) and plays a key role in plant photosynthesis (Anten 2005). Chlorophyll content and plant height of corn plants was higher in the IC treatments compared to MC (Table 2.4–2.5). Conversely, the soybean chlorophyll content and plant height decreased in the IC treatments compared to the MC treatments. This translates to enhancing the IC corn chlorophyll content from 5 % to 12.5 % accompanied by a concomitant decrease in the intercropped soybean chlorophyll content between 9.2% to 9.7 % (Table 2.4). Similarly, the plant height increased by 4%, whereas the soybean height decreased between 9%–10% in the IC compared to the MC (Table 2.5). These findings are consistent with previous studies in the literature (Inal et al. 2007; Xiong et al. 2013; Ahmad et al. 2015) and suggest that both the chlorophyll content and plant height could be the possible drivers for the observed changes in forage production.

IC could result in either increased forage production (LER >1), decreased (LER <1), or no effects (LER = 1) (Eskandari 2012; He et al. 2013). In our experiments, IC increased the FP compared to corn or soybean MC, however, individually the yield of dominant specie (corn) increased compared to dominated species (forage soybean) due to IC (Table 2.6). Significant increase in FP was observed in IC compared to corn MC during both study

years. 18 % and 42 % as compared to corn MC during 2016 and 2017, respectively. Similar results have been reported previously, where corn–soybean IC produced higher FP as compared to MC (Eskandari 2012; Eslamizadeh et al. 2015); however, such increases were observed in dominant crops compared to the dominated crop (Li et al. 2013a). Consistent with the findings of Wu et al., (2016), we observed that the dominant corn crop in the IC system suppressed the growth of the less dominant companion soybean. However, the dominant corn displayed superior FP when cultivated in an IC production system as compared to MC system; resulting in an increase in FP in the IC system (Table 2.6). The FP increase suggested that IC has an advantage over MC in terms of plant growth, which is also supported by the LER values which were greater than 1 in all IC treatments further confirming that corn and soybean IC in cool climate production systems was superior compared to MC in regard to increasing FP (Table 2.7). Our LER findings are consistent with the results obtained by several other researchers who reported LER values greater than 1 (Ghosh 2004; Yilmaz et al. 2008; Javanmard et al. 2009). The LER values in our study suggesting that 43% - 75% more land was required for the MC production system to produce a crop yield equal to that of the IC production system. These findings suggest that the IC system appears to use environmental resources more efficiently than the MC system (Dhima et al. 2007), and this may account for its superior agronomic performance (enhanced forage production).

2.5.2. Rhizosphere soil acid phosphatase activity

Study results demonstrated that IC increased the RS-APase activity between 40%–54% and 26%–28% compared to corn and soybean MC treatments, respectively (Figure 2.2). These findings suggest IC may utilize organic P more efficiently than MC corn. Organic P comprised 30%–80% of total P in most agricultural soils and can be converted into RS–$P_{available}$ forms after hydrolyzation by phosphate enzymes (Tarafdar and Claassen 1988; Gilbert et al. 1999). The higher RS-APase activity in IC could also be attributed to compatibility or suitability of the silage corn and soybean combinations as companion plants in an IC production system. In arid soils, RS–$P_{available}$ was significantly correlated with RS–APase activity (Sardans et al. 2008) because of an association between mobilization of organic P and RS–APase activity (Conn and Dighton 2000; Dick et al. 2000). Our results are in-line with other studies, which reported that cereal-legume IC increased the RS-APase activity compared to either sole cultivation of silage corn or soybeans (Inal et al. 2007; Wang et al. 2014). The legume species have been considered as the major contributor to the increase of RS–APase activity observed in IC due to fact that large amounts of acid phosphatase are known to be released from their roots into the root rhizosphere (Li et al. 2004; Gunes et al. 2007).

2.5.3. Rhizosphere soil pH

In these studies, a reduction in RS–pH, as well as, rhizosphere acidification was observed to be associated with the evaluated IC. Similar to these findings, we observed that IC decreased the RS-pH between 5%–6% compared to when corn and soybean were cultivated

as monocrops (Table 2.8). The reduced rhizosphere acidification and lower RS–pH was shown to be due to the release of a large quantities of protons or organic acids in the root rhizosphere (Tang et al. 1997; Li et al. 2007). Similar trend of RS–pH reduction was observed under maize–soybean and maize–turnip IC (Wang et al. 2014) and teak – Leucaena compared to their corresponding monocultures (Kumar et al. 1998).

2.5.4. Changes in rhizosphere soil available P

Intercropping (IC) had positive effects on RS-$P_{available}$ when corn and soybeans are cultivated as the companion crops. Our results demonstrated that IC significantly increased the availability of P between 74%–76% and 21%–26% in the plant root rhizosphere compared to when corn and soybeans were cultivated as monocrops (Table 2.8). Consistent with our findings, increased P availability in the rhizosphere have also been reported in garlic-cucumber (Xiao et al., 2013); and maize-chickpea IC (Li et al. 2004). Increase in RS-APase activity and acidification of the rhizosphere via release of protons and organic acids have been suggested to be responsible for the enhanced RS–$P_{available}$ observed in maize-faba bean IC system (Li et al. 2007). Additionally, a close relationship was observed between modified and dominant microbial communities, RS-$P_{available}$ and RS-pH comparing maize-chickpea, maize-soybean, and maize-wheat IC production systems (He et al. 2013; Wang et al. 2014).

2.5.5. Rhizosphere soil microbial community composition

The analyses of PLFA's were performed to investigate the active microbial community present in the root rhizosphere. PLFA's are present in the membranes of living cells, but

not in dead cells because of rapid degradation during cell death. As such, they can give an accurate estimate of the present living microbial community in the root rhizosphere, and how these community composition change in response to factors such as crop management systems, environmental conditions, and production inputs (Gómez-brandón and Domínguez 2010). Different microbial groups present in the soil are comprised of fatty acids that are diagnostics of their presence and rate of change in the soil habitat (Zelles 1999). Consequently, the diversity of the active microbial community is referred to as an imperative indicator of soil quality or health status of the soil (Kong et al. 2011). Our results showed that IC, in general, increased the total microbial PLFA's population (G+, G–, F and P) in the root rhizosphere as compared to soybean and corn MC. This increase was 16.9% and 8.7% during the 2016, while during 2017, it was 7.5 % and 13.9 % respectively (Table 2.9-2.10). Our results corroborate the findings of Li et al., (2016) and Zhou et al., (2011) who demonstrated that IC can enhance both bacterial and fungal populations in the plant root rhizosphere. We observed that the bacterial population was the highest of the total microbial populations present in corn and soybeans cultivated as MC or IC under cool climatic conditions (Table 2.9-2.10). However, the G– population was 4% higher than the G+ bacterial population (Table 2.9-2.10). Higher proportion of G+ bacterial population compared to G– suggested a deficiency of organic carbon in the soil (Bossio et al. 2005; Herman et al. 2012). In contrast, the dominance of G– bacteria over G+ bacteria in the soil as well as a high fungal population is characteristic of the presence of higher amount of complex organic matter in the soil (Herman et al. 2012; Mathew et al. 2012). The findings from this work demonstrate that corn-soybean IC promoted the growth and diversity of the

active microbial community, and as such enhance the soil health status under cool climatic production system.

2.5.6. Correlations between agronomic performance indicators and soil health related parameters

The significant positive correlation between the agronomic and soil chemical properties demonstrated that these parameters are associated with the superior forage production observed when corn was IC with soybean and cultivated under cool climatic conditions (Figure 2.6–2.7; Table 2.11). The FP revealed a significant positive correlation with G+ bacteria and the total bacterial population (Figure 2.7) consistent with observations in earlier findings (He et al. 2013). The increase in above ground biomass is known to have a strong positive connection with the plant roots located under the soil. Collectively, the RS-APase activity, RS-$P_{available}$ and the fungal: bacterial ratio appears to be the most important determinants of forage production (Figure 2.4 A & B), when corn and soybeans are cultivated as IC systems under cool climatic conditions. Similar relationships have been reported between agronomic performance and soil health indicators in various IC production systems under different climatic conditions (Kizilkaya et al. 2007; Zornoza et al. 2009; Brockett et al. 2012; Li et al. 2013c; He et al. 2013; Zhang et al. 2016). This is the first study demonstrating that similar relationships exist when corn is IC with soybeans (vine or upright varieties) under cool climatic conditions. Soil microbes are known to mineralize organic matter and other sources of plant nutrients located in the soil, thus making them available to the plant for uptake, growth and productivity (He et al. 2013).

Similarly, IC can stimulate the enrichment of P solubilizing soil microbes or microbial species with enhanced soil phosphatase activities, thereby increasing the RS–$P_{available}$ during IC (Brockett et al. 2012; Li et al. 2013c; He et al. 2013). Thus, it appears, under cool climatic conditions, that the increased forage production in IC production system was highly dependent on RS–$P_{available}$ and RS-APase activity, presumably through stimulation or modification of the active microbial community structure. The enhanced microbial population observed in IC might be more efficient in mineralizing and mobilizing P in the root rhizosphere, as well as, have superior RS-APase under cool climatic conditions. This could be the mechanism through which the improved agronomic performance observed in the tested IC system related to the enhanced soil health status. Further experimentation at the molecular genetics and cellular levels are needed to confirm this mechanism and are the subject of future work in our research program.

2.6. Concluding Remarks

IC of silage corn with forage soybean was more effective than MC of both as sole crops in enhancing the agronomic performance and forage production. Additionally, the LER values were higher than 1 for the IC system indicating a higher productivity in the form of plant FP. An increase was also observed in RS-$P_{available}$ to the plants due to increase of RS-APase activity and decrease RS-pH. IC also improved rhizosphere total active microbial community composition. Higher PLFA's (G+, G–, F, and P) were observed in the rhizosphere of corn-soybean IC as compared to MC. In general, the corn IC with upright forage soybean genotypes displayed superior agronomic performance compared to IC with

the vine soybean. Collectively, the RS-APase activity, RS-$P_{available}$ and the fungal: bacterial ratio appears to be the most important determinants of soil health status and increased FP when silage corn and forage soybeans are cultivated as IC under cool climatic conditions. This study is the first to demonstrate that IC silage corn with forage (upright) soybeans is a suitable approach to increase the forage production and enhanced soil health status under cool climate production systems.

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Chapter 3

3. Effect of corn-soybean intercropping on forage production and forage quality under cool climatic conditions

3.1. Abstract

Cereal based cropping systems could be considered as a potential source of energy rich forage for the dairy industry. However, due to low quality of the forage farmers need to add concentrates to fulfill the feeding requirements. An experiment was conducted to evaluate the effect of corn-soybean intercropping (IC) on forage yield, quality, mineral contents and functional lipid composition (omega 3 and 6). Two silage corn varieties (Yukon R and DKC26-28) were cultivated with three soybean varieties (Big Fellow RR, Game Keeper RR, Kester's Bob White Trailing Soybean) both in monocropping (MC) and IC treatments. The experiment was laid out in a randomized complete block design with three replications per treatment. The forage production (FP) was recorded at harvesting and the forage quality was determined. IC significantly increased the crude protein (CP) (22%) and decreased the acid detergent fiber (ADF) contents (14% to 15%) and neutral detergent fiber (NDF) contents (9% to 11%). Forage net energies (net energy lactation = NE_L, net energy maintenance = NE_M, net energy gain = NE_G), total digestible nutrients (TDN), ash, dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were also significantly increased ($p \leq 0.05$) in the IC treatments compared to corn MC. The macro (Ca, K, Mg, P, Na) and micro (Cu, Co, Fe, Zn, B and Mn) mineral uptake was higher in IC

than corn MC. The forage FA composition especially (omega 3 and omega 6) was intermediate between both MC treatments. For example, intercropping increased the omega 3 FA contents (40% to 100%) and decreased the omega 6 FA contents (14% to 15%) compared to corn MC. A significant ($p \leq 0.05$) increase in omega 3/omega 6 ratio was observed because of corn-soybean IC. The results emphasize that corn-soybean IC could be a suitable cropping system to increase forage quality, mineral contents and functional fatty acids following cultivation under cool climatic conditions. The resultant forage has the potential to be a source of high value animal feed for livestock production in cool climate regions of the world.

3.2. Introduction

Corn (*Zea mays*) is the third most important cereal crop after rice and wheat cultivated for human and animal consumption. As a source of animal feed, silage corn has the ability to provide energy rich forage with relatively high nutritional value especially for the dairy industry (Eskandari and Ghanbari, 2009; Eslamizadeh et al., 2015; Geren et al., 2008). Corn is grown across all the crop production zones globally and is one of the most versatile crops because of adaptability under various climatic conditions (Ananthi et al., 2017). The crop growth climatic conditions and cropping system can have significant influence on the nutrient composition of the forage produced for animal feed. Silage corn is enriched with starch, water soluble carbohydrates (WSC) and fiber (Masoero et al., 2006; Nadeau et al., 2010), and can be safely fed to animals at all growth stages; because there is no danger of oxalic or prussic acid toxicity as is the case with sorghum (Dahmardeh et al., 2009). These characteristics made corn forage a popular feed for ruminant animals, especially for dairy cows. In fact, the usage of silage corn as dairy cattle feed has been associated with increased forage uptake, superior animal performance and reduced production cost in dairy production systems (Anil et al., 2000). Even though silage corn is a source of high yielding, high energy cereal forage (Ananthi et al., 2017), the forage produced is low in protein contents (Armstrong et al., 2008; Filya et al., 2006; Tau, 2005). Protein is an important nutrient required for animal growth, reproduction and maintenance (Jayanegara et al., 2016). Protein is not only required in animal feed for better growth and milk production but is also essential for those rumen bacteria that takes part in feed digestion in ruminant

animals (Ghanbari-Bonjar, 2000). Because of low protein contents in forages such as silage corn used as forage for animal feed, additional supplements must be added to balance the diets of both dairy and meat producing animals. This is one of the main reasons for high feed cost (Eskandari and Ghanbari, 2009; Stoltz et al., 2013), and this can be a significant input cost for livestock production in cool or boreal climatic regions. The use of legumes intercropped with corn or other forage species from the Poaceae (cereal or grass) family have been proposed as a suitable alternative to enhance the nutrient composition of forage produced for animal feed. Legumes are high in protein, macro and micro minerals, as well as ash compared to grasses/cereals (Blount et al., 2009; Camacho Barrón and González De Mejía, 1998; Eskandari and Ghanbari, 2009; Paulson et al., 2008). As such are ideal for improving the forage quality without compromising on forage yield. Soybean (*Glycine max*) is a fast growing, protein-rich annual legume, a member of the Fabaceae family that can be intercropped with corn to improve the protein and mineral contents in the forage (Baghdadi et al., 2016). Currently, 2/3rd of the world's protein concentrates use for feed in the livestock industry is obtained from soybean (Agarwal et al., 2013). In addition to protein, soybean forage has high levels of essential minerals and functional lipids (Bachlava et al., 2008; Yang et al., 2017) important in animal nutrition. One negative factor associated with legume-based forage is that the dry matter content is low (Lithourgidis et al., 2006; Ross et al., 2004). Consequently, corn-legume IC could be a good approach to increase the forage production, as well the overall feed quality compared to forage obtained from either corn or soybeans cultivated as monocrops (Baghdadi et al., 2016; Javanmard et al., 2009), particularly under cool climatic conditions. The growing of two or more crops

in the same field during same growing season simultaneously is called intercropping (Brooker et al., 2015; Li et al., 2013; Zhou et al., 2011). Previous studies have demonstrated that cereal intercropped with legumes produce higher DDM (Bingol et al., 2007), more CP per hectare as compared to corn MC (Baghdadi et al., 2014; Javanmard et al., 2009; Reta Sánchez et al., 2010). Similarly, decreased concentrations of NDF and ADF and an increase in forage nutritive value (Baghdadi et al., 2016; Dahmardeh et al., 2009; Eskandari, 2012) was also observed in the cereal legume IC. Other benefits that can be accomplished by cereal legume IC include enhanced crop productivity per unit area, improved soil fertility, decreased soil erosion, soil surface evaporation, weed infestation and land area required for crop production (Eskandari and Ghanbari, 2009; Javanmard et al., 2009; Makgoga, 2013; Matusso et al., 2014; Sullivan, 2003). IC not only improves the productivity but can also help to encourage judicious and equitable utilization of land resources and farm inputs including labor (Marer et al., 2007). Furthermore, legume corn IC can minimize the crop failure risk due to enhanced crop diversity and improved nutrient uptake under field conditions (Li et al., 2003; Mthembu et al., 2018). Geren et al., (2008) reported an increase forage biomass (yield), and crude protein due to corn-cowpea intercropping. Effect of corn-soybean intercropping on forage production and quality have also been documented earlier by other researchers (Baghdadi et al., 2016; Htet et al., 2017, 2016b; Jahanzad et al., 2014; Reta Sánchez et al., 2010; Serbester et al., 2015; Yucel et al., 2017). However, almost all these studies have been conducted in warm-temperate, tropical and sub-tropical regions having long crop growing seasons. The present study was conducted in Newfoundland Canada that comes under cool climatic regions (Harris and Hiller, 2018). Current research

on IC has focused primarily on the effect of cereal legume IC on forage quality (e.g. CP, ADF, NDF and some macronutrients). However, there is limited information regarding how silage corn intercropped with upright (US) or vine type (VS) forage soybean will affect the nutritional quality, net energies, relative feed value (RFV), macro and micro mineral contents and fatty acid composition (omega 3 and omega 6) following cultivation under cool climatic condition. Also, very little is known whether there are any relationships between the active soil microbial composition (Chapter 2) and forage nutrient quality under cool climate forage production. Soil microbial community can enhance the availability of soil-born nutrients (Van Der Heijden et al., 2008). Most of the nutrients in the soil are present in bound forms and they can be mineralize into plant useable form with the help of soil microbial community such as bacteria and fungi (Bonkowski, 2004; Richardson et al., 2009). This nutrient mineralization may lead towards high nutrient uptake. Due to high intake, forages are the main source of polyunsaturated fatty acids (PUFA), for example, linoleic (C18:2n-6) and α-linolenic acids (C18:3n-3) in the dairy animal feed, and they have the ability to transform the milk fatty acid (FA) composition (Dewhurst et al., 2006; Elgersma et al., 2006). In addition, both linoleic and linolenic FAs are of prime significance in producing beef with enhanced levels of omega 3 and conjugated linoleic acids (CLA) (Scollan et al., 2001). CLA and omega 3 fatty acids play significant roles in reducing cardiovascular disease, cancer, diabetes and obesity in human (Pariza, 2004). As such, we hypothesize that corn-soybean IC will increase the nutritional quality of forage cultivated under cool climatic condition via enhancement in the protein, essential FAs and mineral contents and decline in the fiber contents compared to forage (soybean or corn)

cultivated as monocultures; and that the soil active microbial composition (Chapter 2) may play a role in the overall forage quality. The following objectives were used to test this hypothesis

1. To investigate the effects of vine or upright soybean intercropped with silage corn on the forage quality (proteins, fibers, energy contents etc.), minerals and FA contents following field cultivation under cool climatic conditions
2. Determine if there are any relationships of forage quality with forage FA contents and with the active microbial community structure following field cultivation under cool climatic conditions

3.3. Materials and Methods

A two-year field-based study was carried out at Pynn's Brook research station Pasadena "49.0936° N, 57.5359° W", (Department of Fisheries, and Land Resources, Government of Newfoundland and Labrador Canada) during the year 2016 and 2017. Experiment was laid out in a randomized complete block design (RCBD) with total 11 treatments and three replications. The experiment plot size was 5m X 6 m. The experimental treatments are given in (Table 2.1). The experiment was planted on June 20th and May 30th during 2016 and 2017 respectively. The seeding rate for MC was (corn= 77100 seeds ha^{-1}, soybean=129200 seeds ha^{-1}) and for IC (60% corn: 40% soybean = 129200 seeds ha^{-1}). The fertilizer application was done according to the regional recommendations for both inter and MC treatments. Soybean seed was inoculated before sowing with *Bradyrhizobium japonicum* @10 g/kg seeds (Egamberdiyeva et al., 2004) to increase its nitrogen fixation

ability. Weed control was done during both cropping seasons using Weather Max @1.3 liter/acre. Crop harvesting was done at corn black layer stage on October 25th and 13th during 2016 and 2017 growing seasons. The weather data and soil chemical properties for both 2016 and 2017 growing seasons are given in (Figure 2.1 & Table 2.2).

3.3.1. Determination of chlorophyll contents, plant height and forage production:

Plant height and chlorophyll contents were recorded as described in chapter 2. Forage production was recorded at harvesting after oven dry the plant as mentioned earlier in the chapter 2.

3.3.2. Corn-soybean forage quality analysis:

Three plants were selected randomly at harvesting from each treatment and cut into small pieces pooled and dried in an oven at 60^{o}C (Shel Lab FX14-2, Sheldon Manufacturing Inc. USA) till constant plant dry weight was achieved. The samples were then ground using a CryoMill (Retsch® GmbH Germany) and the powdered samples sent for forage quality analysis at Actlabs or Activation Laboratories Ltd. (Ancaster ON, Canada), a member laboratory of Dairy One Feed and Forage Analyses Laboratory (Ithaca, New York USA). Near Infrared Reflectance Spectroscopy technique (Foss NIR System Model 6500 Win ISI II v1.5) was used to determine the forage quality including forage proteins [CP, AP, soluble proteins (SP)], fibers [ADF, NDF], TDN, ash contents, simple sugars (SS), water soluble carbohydrates (WSC), and forage energy (NE_L, NE_M, NE_G), DDM, DMI and RFV.

3.3.3. Determination of mineral nutrients from forage:

250 mg dried and sieved (450 um) forage sample was weighed into pre-cleaned digestion vessels. Aliquots (10 mL) of concentrated (67% –70%) trace metal grade nitric acid (HNO_3) Catalog No. A509P212, Fisher Scientific, Ottawa, Ontario, Canada) was added to each sample vessel. All digestion vessels were tightly caped, and samples digested using a microwave digestion system (Multiwave Go Microwave Digestion System; Anton Paar, United States) for 20 min to ensure complete sample digestion. The microwave digestion process comprised of two steps as given in Table 3.1.

Table 3.1: Steps involved in plant digestion

Steps	Ramp (mm: ss)	Temperature (^oC)	Hold (mm: ss)
1	10:00	140	5:00
2	1:00	160	15:00

After the completion of sample digestion, the vessels were cooled to room temperature, carefully opened in the fume hood and samples filtered in 50 mL Nalgene plastic bottles, then stored at ± 4^{o}C. The sample were later diluted by 1:100 ratio (1uL sample:900uL high-purity deionized distilled water (DDW) 17-18 MOMEGA cm−1). The determination of mineral nutrients composition was performed using an Inductively Coupled Plasma Mass Spectrometry (Thermo Scientific iCAP Q ICP-MS). The instrument working conditions were as auxiliary gas flow (L min−1) 0.79, nebulizer gas flow (L min−1) 1.01, plasma gas flow (L min−1) 14, RF power (W) 1548 and dwell time (s) 0.01. A highly pure multi element (43) ICP–MS standard solution IV–ICPMS–71A obtained from Inorganic™

Ventures, Inc. (Christiansburg, VA 24073, USA) was used for external calibration with standard calibration values ranged from 0.971-1.000.

3.3.4. Plant fatty acids extraction and sample preparation:

Plant samples were extracted by Folch (1957) method with some modifications. Briefly, 250 mg of the ground, dried plant samples were transferred to glass centrifuge tubes containing 2.5 mL of (2:1: 0.0003; v/v/wt.) chloroform methanol and 0.01% butylated hydroxytoluene (BHT). The sample mixture was vortexed then centrifuged at 5000 rpm for 15 mins. The supernatant was collected after centrifugation and 1 mL (0.25 % KCl) added to the supernatant. The samples were incubated in the oven for 10 mins at 70^0C. After incubation, the samples were cooled and the organic layer (bottom layer) transferred with a glass Pasteur pipette into pre-weighed 4 mL glass sample vials having a PTFE lined caps. The samples were then dried under a gentle stream of N_2 and the vials re-weighed to determine the amount of lipids recovered (Folch et al., 1957; Fried et al., 2003). The recovered samples were resuspended in 2 mL chloroform: methanol (2:1 v/v) and used for FA methyl ester analysis.

3.3.4.1. Fatty acid methyl esters (FAME's) analysis:

Aliquots of extracted lipids (200 µL) was transferred to 2mL vials along with 50 µl of C19:0 (1mg/mL 2:1 chloroform: methanol) as an internal standard. The samples were then dried under the gentle stream of N_2 and 200 µL methanolic HCl-3N (Sigma-Aldrich ON Canada) added to the samples, and the sample mixture vortexed then incubated at 90^0C for 30 mins. After incubation, 0.8 mL distilled water was added in each sample, and the

samples extracted three times using 500 µL hexane each time. The hexane fractions were pooled and dried under N_2 then resuspended into 100 µl hexane, then transferred into GC vials fitted with inserts and analyzed with gas chromatography-flame ionization detection (GC-FID). The FA peaks were identified through comparison of retention times obtained from commercial standards (Supelco 37 Component FAME Mix, obtained from, Sigma Aldrich, ON, Canada). The quantification of individual FA was done with the comparison of standard curve prepared from the standard mixture and values presented as g/kg of forage dry matter.

3.4. Results

3.4.1. Forage nutritional quality

The results showed the significant effects of corn-soybean IC treatments compared to MC treatments on forage quality over both (2016 and 2017) growing seasons (Table 3.2 & 3.3). Overall, the corn-soybean IC treatments results in significantly $p<0.05$ higher CP than corn MC; however, the CP content was significantly lower in the IC treatments compared to soybean MC during both years (Table 3.2 & 3.3). The trend for both CP and AP was similar during both years (soybean MC > corn-soybean IC > corn MC). Conversely, the SP content was significantly higher in the IC treatment compared to either corn or soybean cultivated as MC. The highest SP was observed in the S3C1 (49.50%) and S1C2(46.33%) IC treatments, while the lowest was recorded in the S3 (28.33%) and C1 (36.50%) MC treatments during 2016 and 2017 respectively. The vine type soybean (V) cultivated as MC had significantly lower crude and available protein compared to the upright soybeans (U) cultivated as MC in the 2017 growing season (Table 3.3). In 2016, there was no difference in CP and AP in both upright and vine type soybeans cultivated as MC (Table 3.2). The highest CP was observed in S3 (17.85%) and the lowest in C2 (9.95%) during the 2016 growing season (Table 3.2). However, in the 2017 growing season, S1 produced the forage with the highest (21.67%) CP content, while C1 had the lowest (10.40%) CP content (Table 3.3). Conversely, when the vine type soybean was intercropped with silage corn, the forage protein content (CP, AP, SP) was similar in both IC treatments regardless of growing seasons (Table 3.2 and 3.3).

Table 3.2: The effects of corn and soybean intercropping or monocropping treatments on forage protein, fiber, sugar and ash contents during the 2016 growing season.

	Growing Season 2016							
Treatments	**CP[1]**	**AP[1]**	**SP[2]**	**ADF[1]**	**NDF[1]**	**WSC[1]**	**SS[1]**	**Ash[1]**
C1	11.70±0.12 ^{d}	10.90±0.12 ^{cd}	43.0±0.6 ^{c}	41.13±1.83 ^{a}	58.7±0.6 ^{a}	15.20±0.56 ^{b}	8.37±0.50 ^{c}	9.91±0.71 ^{e}
C2	9.95±0.03 ^{e}	9.45±0.03 ^{d}	44.5±0.9 ^{bc}	39.35±0.84 ^{ab}	57.5±0.3 ^{ab}	20.60±0.40 ^{a}	10.15±1.18 ^{abc}	6.90±0.57 ^{f}
S1	17.10±0.95 ^{a}	15.53±0.88 ^{a}	33.0±1.2 ^{d}	37.07±1.82 ^{bcd}	47.2±1.3 ^{c}	5.90±0.35 ^{d}	5.15±0.66 ^{d}	17.23±0.36 ^{b}
S2	16.73±0.17 ^{a}	15.23±0.15 ^{a}	35.5± 0.3 ^{d}	37.05±0.72 ^{bcd}	47.3±1.5 ^{c}	6.95±0.03 ^{d}	4.00±0.35 ^{d}	15.23±0.91 ^{bc}
S3	17.85±1.07 ^{a}	15.20±1.04 ^{a}	28.3±0.9 ^{e}	37.30±1.30 ^{bc}	48.8±2.0 ^{c}	3.60±0.75 ^{e}	3.35±0.09 ^{d}	23.46±1.68 ^{a}
S1C1	12.55±0.32 ^{cd}	10.95±0.14 ^{cd}	48.7±1.5 ^{a}	35.90±0.23 ^{cde}	57.5±0.7 ^{ab}	12.53±1.03 ^{c}	9.55±0.49 ^{bc}	13.16±0.89 ^{cd}
S2C1	12.85±0.20 ^{cd}	11.60±0.23 ^{c}	45.7±0.3 ^{abc}	35.15±0.09 ^{cde}	57.0±1.5 ^{ab}	13.70±0.97 ^{bc}	10.95±0.09 ^{ab}	10.82±0.03 ^{de}
S3C1	11.85±0.32 ^{d}	11.30±0.12 ^{c}	49.5±1.4 ^{a}	34.20±0.25 ^{de}	57.5±1.9 ^{ab}	14.20±1.18 ^{bc}	11.87±1.07 ^{a}	11.50±0.34 ^{de}
S1C2	14.60±0.12 ^{b}	13.50±0.06 ^{b}	48.5±2.0 ^{ab}	33.90±0.51 ^{e}	54.2±0.3 ^{b}	12.43±1.09 ^{c}	10.55±0.72 ^{ab}	11.32±1.10 ^{de}
S2C2	14.00±0.58 ^{bc}	13.30±0.12 ^{b}	42.7±2.7 ^{c}	33.50±0.29 ^{e}	55.9±1.0 ^{ab}	12.50±0.58 ^{c}	10.25±0.72 ^{abc}	14.26±0.05 ^{c}
S3C2	13.13±0.59 ^{bcd}	12.07±0.68 ^{bc}	47.5±0.3 ^{ab}	33.75±0.03 ^{e}	56.4±0.2 ^{ab}	12.90±0.40 ^{c}	10.80±0.23 ^{ab}	11.67±1.48 ^{de}
Average	13.85±0.44	12.64±0.37	42.44±1.24	36.21±0.48	54.3±0.8	11.87±0.82	8.63±0.53	13.22±0.77
Mono (U)	16.92±0.44	15.38±0.41	34.3±0.8	37.06±0.88	47.2±0.9	6.43±0.28 ^{A}	4.58±0.42	16.23±0.62 ^{B}
Mono (V)	17.85±1.07	15.20±1.04	28.3±0.9	37.30±1.30	48.8±2.0	3.60±0.75 ^{B}	3.35±0.09	23.46±1.68 ^{A}
Inter-(U+C)	13.50±0.29	12.34±0.38	46.4±1.1	34.61±0.32	56.1±0.6	12.79±0.43	10.33±0.26	12.39±0.52
Inter-(V+C)	12.49±0.41	11.68±0.35	44.3±0.6	33.98±0.15	56.9±0.9	13.55±0.63	11.33±0.54	11.58±0.68
Mono-S	17.23±0.45 ^{A}	15.32±0.40 ^{A}	32.3±1.1 ^{C}	37.14±0.68 ^{B}	47.8±0.9 ^{B}	5.48±0.55 ^{C}	4.17±0.34 ^{C}	18.64±1.36 ^{A}
Mono-C	10.83±0.39 ^{C}	10.18±0.33 ^{C}	43.8±0.6 ^{B}	40.24±0.98 ^{A}	58.1±0.4 ^{A}	17.90±1.25 ^{A}	9.26±0.70 ^{B}	8.40±0.79 ^{C}
Inter-(C+S)	13.16±0.26 ^{B}	12.12±0.28 ^{B}	47.1±0.8 ^{A}	34.40±0.23 ^{C}	56.4±0.5 ^{A}	13.04±0.35 ^{B}	10.66±0.27 ^{A}	12.12±0.41 ^{B}

[1]= % DM; [2]= % of CP; Crude protein= CP; Available Protein=AP; soluble protein= SP; Acid Detergent Fiber =ADF; Neutral Detergent Fiber=NDF; Water Soluble Carbs= WSC; Simple Sugars (ESC) (1); Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC-2628; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC-2628; S2C2 = Game Keeper RR + DKC-2628; S3C2 = Kester's Bob White Trailing Soybean + DKC-2628. Mono C =

monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean.

Table 3.3: The effects of corn and soybean intercropping or monocropping treatments on forage protein, fiber, sugar and ash contents during the 2017 growing season.

	Growing Season (2017)							
Treatments	**CP[1]**	**AP[1]**	**SP[2]**	**ADF[1]**	**NDF[1]**	**WSC[1]**	**SS[1]**	**Ash[1]**
C1	10.40±0.06 ^{e}	9.65±0.09 ^{e}	36.5±0.3 ^{f}	34.25±0.43 ^{a}	56.8±0.8 ^{ab}	12.65±0.49 ^{b}	3.85±0.26 ^{h}	3.78±0.18 ^{f}
C2	10.73±0.27 ^{e}	10.00±0.25 ^{e}	37.0±0.6 ^{ef}	34.33±0.09 ^{a}	57.9±0.1 ^{a}	14.07±0.56 ^{a}	4.90±0.06 ^{g}	5.06±0.12 ^{e}
S1	21.67±0.79 ^{a}	19.07±0.59 ^{a}	38.3±1.9 ^{def}	32.35±0.55 ^{ab}	35.7±0.3 ^{h}	8.03±0.37 ^{fg}	6.80±0.06 ^{f}	10.69±0.38 ^{b}
S2	19.53±0.85 ^{b}	16.60±0.64 ^{b}	41.5±2.6 ^{bcd}	33.07±0.58 ^{ab}	38.9±1.3 ^{g}	8.35±0.26 ^{ef}	7.45±0.38 ^{ef}	10.50±0.31 ^{b}
S3	17.30±0.29 ^{c}	14.50±0.23 ^{c}	44.0±0.6 ^{abc}	33.90±0.70 ^{a}	45.8±1.8 ^{f}	7.10±0.29 ^{g}	8.40±0.40 ^{d}	12.34±0.34 ^{a}
S1C1	12.40±0.35 ^{d}	11.70±0.23 ^{d}	45.0±2.3 ^{ab}	31.25±0.84 ^{bc}	53.4±0.3 ^{cde}	10.10±0.58 ^{d}	10.80±0.06 ^{bc}	7.10±0.61 ^{c}
S2C1	13.30±0.44 ^{d}	12.23±0.15 ^{d}	43.0±1.2 ^{abc}	31.00±0.29 ^{bc}	53.8±0.4 ^{cd}	9.45±0.09 ^{de}	9.95±0.20 ^{c}	6.80±0.28 ^{cd}
S3C1	12.90±0.29 ^{d}	12.05±0.26 ^{d}	46.0±0.0 ^{a}	30.77±0.29 ^{bcd}	55.1±0.3 ^{bc}	10.47±0.27 ^{cd}	10.85±0.09 ^{b}	6.35±0.03 ^{cd}
S1C2	12.95±0.14 ^{d}	11.85±0.14 ^{d}	46.3±0.3 ^{a}	28.47±1.01 ^{de}	51.1±1.6 ^{e}	11.60±0.47 ^{bc}	11.35±0.66 ^{ab}	5.97±0.17 ^{d}
S2C2	12.50±0.49 ^{d}	11.63±0.47 ^{d}	40.7±0.9 ^{cde}	26.35±1.93 ^{e}	45.9±0.4 ^{f}	11.50±0.23 ^{bc}	7.80±0.06 ^{de}	6.54±0.16 ^{cd}
S3C2	13.37±0.20 ^{d}	11.77±0.09 ^{d}	42.7±0.7 ^{abc}	29.63±0.74 ^{cd}	52.1±0.7 ^{de}	11.85±0.55 ^{b}	11.87±0.32 ^{a}	7.03±0.39 ^{c}
Average	14.28±0.62	12.82±0.48	41.9±0.7	31.40± 0.48	49.7±1.2	10.47±0.37	8.55±0.46	7.47±0.45
Mono (U)	20.60±0.70 ^{A}	17.83±0.68 ^{A}	39.9±1.6	32.71±0.39	37.3±0.9 ^{B}	8.19±0.21 ^{A}	7.13±0.22	10.60±0.22 ^{B}
Mono (V)	17.30±0.29 ^{B}	14.50±0.23 ^{B}	44.0±0.6	33.90±0.70	45.8±1.8 ^{A}	7.10±0.29 ^{B}	8.40±0.40	12.34±0.34 ^{A}
Inter-(U+C)	12.79±0.19	11.85±0.14	43.8±0.9	29.27±0.79	51.0±1.0	10.66±0.32	9.98±0.43	6.60±0.20
Inter-(V+C)	13.13±0.19	11.91±0.14	44.3±0.8	30.20±0.44	53.6±0.7	11.16±0.41	11.36±0.27	6.69±0.23
Mono-S	19.50±0.72 ^{A}	16.72±0.71 ^{A}	41.3±1.2 ^{B}	33.11±0.38 ^{A}	40.1±1.6 ^{C}	7.83±0.24 ^{C}	7.55±0.28 ^{B}	11.18±0.34 ^{A}
Mono-C	10.57±0.14 ^{C}	9.83±0.14 ^{C}	36.8±0.3 ^{C}	34.29±0.20 ^{A}	57.3±0.4 ^{A}	13.36±0.46 ^{A}	4.38±0.26 ^{C}	4.42±0.30 ^{C}
Inter-(C+S)	12.90±0.15 ^{B}	11.87±0.10 ^{B}	43.9±0.6 ^{A}	29.58±0.54 ^{B}	51.9±0.8 ^{B}	10.83±0.25 ^{B}	10.44±0.34 ^{A}	6.63±0.15 ^{B}

[1]= % DM; [2]= % of CP; Crude protein= CP; Available Protein=AP; soluble protein= SP; Acid Detergent Fiber =ADF; Neutral Detergent Fiber=NDF; Lignin= L; Water Soluble Carbs= WSC; Simple Sugars (ESC) (1); Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC26-28; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC-2628; S2C2 = Game Keeper RR + DKC-2628; S3C2 = Kester's Bob White Trailing Soybean + DKC-2628.

Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean

Similar to the protein content, intercropping of silage corn with forage soybean had significant effects on the forage fiber content (ADF and NDF) following field cultivation under cool climatic conditions (Table 3.2 & 3.3). Overall, intercropping produced forage with significantly lower ADF compared to forage produced from corn or soybean cultivated as monocrops, regardless of the growing seasons. On the other hand, the NDF content was significantly lower in the forage obtained from soybean cultivated as monocrops regardless of growing seasons (Table 3.2 & 3.3). This was followed by the intercropping treatments, while the corn MC produced the forage with the highest NDF content. The C1 and C2 monocrop treatments showed the higher ADF contents (41.13%) and (34.33%) compared to all other treatments respectively during 2016 and 2017, while the lowest contents were observed in the S2C2 intercropping treatments (33.50% and 26.35%) during both years. The highest NDF concentration was observed in C1 (58.65%) during 2016 and C2 (57.85%) during 2017, while the lowest concentrations were recorded in S1 (47.17% and 35.65%) for both 2016 and 2017 respectively. The fiber content was similar in forage obtained from vine or upright soybeans cultivated as monocrops or intercropped with silage corn over the duration of the study (Table 3.2 & 3.3).

Different cropping systems also had a significant effect on the WSC and SS contents (Table 3.2 & 3.3). Overall the mean values for WSC were significantly higher in the forage of corn cultivated as monocrops compared to forage obtained from soybean cultivated as monocrop or intercropped with silage corn. All the intercropping treatments showed significantly $p<0.05$ higher WSC contents than soybean MC treatments except S2C1 during 2017. When soybean was grown as monocrops higher levels of WSC were present

in the forage obtained from upright soybean varieties than from the vine type variety. The WSC level was highest in C2 (20.60% and 14.07%), and lowest in S3 (3.60% and 7.10%) during 2016 and 2017 respectively. Conversely, the SS concentration were significantly higher in intercropping compared to corn and soybean MC treatments during both seasons. Higher SS contents were measured in S3C1 (11.87%) in 2016 and S3C2 (11.87%) in 2017, although lower values were recorded in S3 (3.60%) in 2016 and C1 (3.85%) in 2017. Similarly, the different cropping systems also significantly affected the forage ash contents. The ash concentration was significantly higher in soybean MC treatments compared to both corn-soybean IC and corn MC treatments. The VS variety (S3) showed significantly higher ash contents compared to US (S1, S2) varieties when cultivated as monocrops. The lowest ash content was observed in the corn MC treatments, while the IC treatments were intermediate between the soybean and corn MC treatments.

The TDN, NE_L, NE_M and NE_G were used as measures of the energy content of the forages produced under the different cropping systems. Forage produced in the IC treatments had significantly higher TDN, NE_L, NE_M, and NE_G compared to the MC treatments (Table 3.5 & 3.4). The US varieties MC showed higher values of TDN, NE_L, NE_M, and NE_G than vine soybean MC treatments. The highest TDN was observed in the S3C1 (57.0%) and S2C2 (66.3%) treatments, and the lowest S3 (44.0% and 51.7%) during 2016 and 2017 respectively. The TDN levels were higher in the upright soybean MC compared with vine soybean MC but was similar when both soybean types were intercropped with silage corn. The maximum NE_L was record in S2 (1.23 Mcal/kg), while the lowest was recorded in S3 (0.97 Mcal/kg) during 2016. Similarly, the highest NE_M and NE_G were observed in the

S3C1 (1.11 Mcal/kg and 0.56 Mcal/kg) treatment, while the lowest was recorded in S3 (0.70 Mcal/kg and 0.17 Mcal/kg) respectively for the 2016 growing season. Conversely, during the 2017 growing season, the maximum $NE_{L,}$ NE_M and NE_G was recorded in S1C2 (1.38, 1.39 and 0.83) Mcal/kg and lowest values recorded in S3 (1.18, 1.02 and 0.47) Mcal/kg. Soybean MC produced forage with the highest DMI followed by the IC treatment, with the lowest DMI recorded in the forage obtained from corn MC. The US cultivated as monocrops produced forage with higher DMI compared to the VS/ cultivated as monocrops following the 2016 growing season. However, this was unremarkable during the 2017 growing season. The DDM on the other hand, was significantly higher in the IC treatments. As such, the highest DDM was recorded in S2C2 (62.8% and 68.4%) during the 2016 and 2017 growing seasons. Conversely, the lowest DDM was recorded in the S3 (56.9% and 62.2%) treatments over both growing seasons (Table 3.4 & 3.5). As expected, the RFV % was higher for soybean MC than both IC and corn MC treatments (Table 3.4 & 3.5). The trend was similar as we observed for DMI (soybean MC > corn-soybean IC > corn IC) during both years. The highest values were recorded for S2 (122 %) and S1 (166 %) and lowest values recorded in C1 (91 %) and C2 (100 %) during 2016 and 2017 respectively. All the IC mixtures were higher in RFV compared with corresponding corn MC during each year.

Table 3.4: Total digestible nutrients (TDN), net energy for lactation (NEL), net energy for maintenance (NEM), net energy of gain (NEG), digestible dry matter (DDM), dry matter intake (DMI), and relative feed value (RFV) of forage during the 2016 growing season.

	Growing Season (2016)						
Treatments	**TDN %**	**NE_L (Mcal/kg)**	**NE_M (Mcal/kg)**	**NE_G (Mcal/kg)**	**DDM %**	**DMI[3]**	**RFV %**
C1	51.5±0.9 ^{de}	1.07±0.01 ^{c}	0.97±0.00 ^{bc}	0.43±0.00 ^{bc}	58.23±0.66 ^{de}	2.03±0.01 ^{e}	91.3±1.2 ^{f}
C2	51.0±0.6 ^{e}	1.05±0.01 ^{c}	0.93±0.02 ^{c}	0.39±0.02 ^{c}	60.03±0.55 ^{bcd}	2.08±0.01 ^{de}	97.0±1.2 ^{e}
S1	53.0±1.5 ^{cde}	1.23±0.00 ^{a}	1.08±0.01 ^{a}	0.53±0.00 ^{a}	59.83±1.01 ^{cd}	2.61±0.01 ^{b}	121.3±2.6 ^{a}
S2	51.7±0.7 ^{de}	1.23±0.03 ^{a}	1.08±0.05 ^{a}	0.53±0.05 ^{a}	60.00±1.40 ^{bcd}	2.75±0.13 ^{a}	122.0±3.0 ^{a}
S3	44.0±1.5 ^{f}	0.97±0.02 ^{d}	0.70±0.05 ^{d}	0.17±0.05 ^{d}	56.87±1.45 ^{e}	2.56±0.00 ^{b}	112.7±2.9 ^{b}
S1C1	56.0±0.6 ^{ab}	1.17±0.01 ^{b}	1.07±0.04 ^{a}	0.51±0.03 ^{a}	60.90±0.17 ^{abc}	2.11±0.01^{cde}	99.7±0.7 ^{de}
S2C1	55.0±1.2 ^{abc}	1.16±0.01 ^{b}	1.10±0.01 ^{a}	0.54±0.01 ^{a}	61.50±0.06 ^{abc}	2.14±0.04 ^{cde}	102.0± 2.3 ^{cde}
S3C1	57.0±1.0 ^{a}	1.17±0.04 ^{b}	1.11±0.04 ^{a}	0.56±0.05 ^{a}	62.23±0.19 ^{ab}	2.02±0.01 ^{e}	97.7±0.3 ^{e}
S1C2	55.0±0.6 ^{abc}	1.14±0.02 ^{b}	1.05±0.02 ^{a}	0.49±0.02 ^{ab}	62.47±0.39 ^{a}	2.22±0.01 ^{c}	107.3± 1.2 ^{bc}
S2C2	54.0±0.6 ^{bcd}	1.16±0.01 ^{b}	1.07±0.04 ^{a}	0.51±0.03 ^{a}	62.80±0.23 ^{a}	2.18±0.03 ^{cd}	106.0±1.2 ^{c}
S3C2	54.7±0.3 ^{abc}	1.13±0.00 ^{b}	1.04±0.00 ^{ab}	0.49±0.00 ^{ab}	62.60±0.00 ^{a}	2.13±0.01 ^{cde}	103.3±0.7 ^{cd}
Average	53.0±0.6	1.13±0.01	1.02±0.02	0.47±0.02	60.68±0.37	2.26±0.04	105.5±1.7
Mono (U)	52.3±0.8 ^{A}	1.23±0.02 ^{A}	1.08±0.02 ^{A}	0.53±0.02 ^{A}	59.93±0.78	2.62±0.01 ^{A}	121.6±1.8
Mono (V)	44.0±1.5 ^{B}	0.97±0.02 ^{B}	0.70±0.05 ^{B}	0.17±0.05 ^{B}	56.86±1.42	2.56±0.00 ^{B}	113.0±2.9
Inter-(U+C)	55.0±0.4	1.16±0.01	1.07±0.01	0.51±0.01	61.94±0.25	2.16±0.02 ^{A}	103.8±1.1
Inter-(V+C)	55.8±0.7	1.15±0.02	1.08±0.02	0.52±0.02	62.43±0.12	2.08±0.02 ^{B}	100.5±1.3
Mono-S	49.6±1.5 ^{B}	1.14±0.04^{A}	0.95±0.07^{B}	0.41±0.06^{B}	58.91±0.83 ^{B}	2.60±0.01 ^{A}	118.7±2.0 ^{A}
Mono-C	51.3±0.5 ^{B}	1.06±0.01^{B}	0.95±0.01^{B}	0.41±0.01^{B}	59.14±0.56 ^{B}	2.05±0.01 ^{C}	94.2±1.4 ^{C}
Inter-(C+S)	55.3±0.4 ^{A}	1.15±0.01^{A}	1.07±0.01^{A}	0.52±0.01^{A}	62.10±0.18 ^{A}	2.13±0.02 ^{B}	102.7±0.9 ^{B}

[3]=%BW, Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC-2628; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28; S2C2 = Game

Keeper RR + DKC26-28; S3C2 = Kester's Bob White Trailing Soybean + DKC26-28. Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean.

Table 3.5: Total digestible nutrients (TDN), net energy for lactation (NEL), net energy for maintenance (NEM), net energy of gain (NEG), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) of forage during the 2017 growing season.

	Growing Season (2017)						
Treatments	**TDN %**	**NE_L (Mcal/kg)**	**NE_M (Mcal/kg)**	**NE_G (Mcal/kg)**	**DDM %**	**DMI[3]**	**RFV %**
C1	59.7±1.2 ^{de}	1.27±0.01 ^{b}	1.20±0.01 ^{de}	0.63±0.02 ^{de}	62.5±0.5 ^{e}	2.09±0.01 ^{h}	101.3±1.2 ^{f}
C2	57.3±0.9 ^{e}	1.21±0.03 ^{c}	1.14±0.03 ^{e}	0.58±0.03 ^{e}	62.2±0.3 ^{e}	2.07±0.01 ^{h}	100.0±0.6 ^{e}
S1	60.3±1.3 ^{cde}	1.37±0.01 ^{a}	1.25±0.01 ^{cd}	0.68±0.01 ^{cd}	63.7±0.4 ^{de}	3.37±0.03 ^{a}	166.3±1.5 ^{a}
S2	57.7±0.9 ^{e}	1.36±0.01 ^{a}	1.20±0.03 ^{de}	0.64±0.03 ^{de}	63.1±0.5 ^{de}	2.99±0.03 ^{b}	146.0±2.0 ^{a}
S3	51.7±1.5 ^{f}	1.18±0.01 ^{c}	1.02±0.02 ^{f}	0.47±0.02 ^{f}	62.2±0.1 ^{e}	2.72±0.06 ^{c}	131.0±2.9 ^{b}
S1C1	63.7±0.3 ^{ab}	1.36±0.01 ^{a}	1.37±0.00 ^{a}	0.77±0.01 ^{ab}	64.6±0.7 ^{cd}	2.25±0.01 ^{fg}	112.3±1.9 ^{de}
S2C1	63.3±0.7 ^{abc}	1.36±0.01 ^{a}	1.35±0.02 ^{ab}	0.77±0.02 ^{ab}	64.8±0.2 ^{cd}	2.25±0.01 ^{fg}	112.7±0.7 ^{cde}
S3C1	62.3±0.3 ^{bcd}	1.28±0.03 ^{b}	1.30±0.02 ^{bc}	0.73±0.02 ^{bc}	64.9±0.2 ^{bcd}	2.18±0.01 ^{g}	109.7±0.9 ^{e}
S1C2	65.3±0.9 ^{ab}	1.38±0.01 ^{a}	1.39±0.01 ^{a}	0.83±0.02 ^{a}	66.7±0.8 ^{ab}	2.28±0.01 ^{ef}	117.7± 1.9 ^{bc}
S2C2	66.3±2.0 ^{a}	1.38±0.03 ^{a}	1.39±0.03 ^{a}	0.80±0.03 ^{a}	68.4±1.5 ^{a}	2.63±0.01 ^{d}	139.3±3.2 ^{c}
S3C2	63.7±0.9 ^{ab}	1.35±0.00 ^{a}	1.33±0.01 ^{ab}	0.79±0.03 ^{ab}	65.8±0.6 ^{bc}	2.33±0.02 ^{e}	119.0±0.6 ^{cd}
Average	61.0±0.8	1.32±0.01	1.27±0.02	0.70±0.02	64.4±0.4	2.47±0.07	123.2±3.5
Mono (U)	59.0± 0.9 ^{A}	1.37±0.00 ^{A}	1.23±0.02 ^{A}	0.66±0.02 ^{A}	63.42±0.30	3.18±0.09 ^{B}	156.3±4.6 ^{A}
Mono (V)	51.7± 1.5 ^{B}	1.18±0.01 ^{B}	1.02±0.02 ^{B}	0.47±0.02 ^{B}	62.15±0.07	2.72±0.06 ^{A}	131.8±3.8 ^{B}
Inter-(U+C)	64.7±0.6	1.37±0.01 ^{A}	1.37±0.01 ^{A}	0.79±0.01	66.10±0.61	2.35±0.05	120.7±3.5
Inter-(V+C)	63.0±0.5	1.31±0.02 ^{B}	1.32±0.01 ^{B}	0.76±0.02	65.37±0.34	2.26±0.03	114.3±2.1
Mono-S	56.6±1.4 ^{B}	1.30±0.03 ^{AB}	1.16±0.04 ^{B}	0.60±0.03 ^{B}	63.00±0.29 ^{B}	3.03±0.10 ^{A}	148.1±5.2 ^{A}
Mono-C	58.5±0.8 ^{B}	1.24±0.02 ^{B}	1.17±0.02 ^{B}	0.61±0.02 ^{B}	62.36±0.29 ^{B}	2.08±0.01 ^{C}	100.4±0.2 ^{C}
Inter-(C+S)	64.1±0.5 ^{A}	1.35±0.01 ^{A}	1.35±0.01 ^{A}	0.78±0.01 ^{A}	65.86±0.42 ^{A}	2.32±0.04 ^{B}	118.6±2.5 ^{B}

[3]=%BW, Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC-2628; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28; S2C2 = Game

Keeper RR + DKC26-28; S3C2 = Kester's Bob White Trailing Soybean + DKC26-28. Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean

3.4.2. Fodder mineral composition

The macro nutrient levels (Ca, P, Mg, K, and Na) of corn and soybean forage varied significantly with the cropping systems during both crop growing seasons (Table 3.6). Forage obtained from soybeans cultivated as monocrops contained the highest macronutrient content, while the foraged obtained from corn cultivated as a monocrop had the lowest macro nutrients. When corn and soybeans were cultivated as intercrop, the macronutrient level in the forage produced was intermediate between that of the forage produced from corn and soybean MC treatments. Generally, the upright soybean produced forage with significantly higher or similar macronutrients levels compared to the vine type soybeans. During 2016, the highest bioaccumulation of Ca was observed in S3 (16.30 g/kg), while the highest level of K and P was observed in S2 (19.99 and 3.62 g/kg) respectively (Table 3.6). Similarly, the highest Mg uptake was observed in S1 (7.15 g/kg). Conversely, the lowest levels of Ca, K, Mg and P were recorded in C2 (1.87 g/kg), S3 (10.16 g/kg), C2 (2.92 g/kg) and C1 (1.76 g/kg) respectively during 2016. The upright variety S2 produced forage with the highest Mg (7.55 g/kg), K (14.93 g/kg) and Ca (17.33 g/kg), while S1 (the other upright soybean variety), produced forage with the highest P (3.85 g/kg) level during 2017. Similar to the macro nutrients, the cropping system significantly altered the levels of micronutrients (Zn, Fe, B, Mn, Cu, Co) in the forage produced under cool climatic conditions (Table 3.7-3.8). Soybean cultivated as monocrop generally produced forage with the highest micronutrient contents followed by the IC treatments, with corn MC producing forage with the lowest micronutrient contents.

Table 3.6: Macro-nutrient content of forage obtained from corn and soybean cultivated as mono and intercrops during the 2016 and 2017 growing seasons.

	Growing Season 2016					Growing Season 2017				
Treatments	**Ca (g/kg)**	**P (g/kg)**	**Mg (g/kg)**	**K (g/kg)**	**Na (mg/kg)**	**Ca (g/kg)**	**P (g/kg)**	**Mg (g/kg)**	**K (g/kg)**	**Na (mg/kg)**
C1	2.45±0.14 ^{f}	1.76±0.02 ^{g}	3.30±0.13 ^{ef}	15.39±0.52 ^{c}	7.1±0.8 ^{f}	1.55±0.14 ^{g}	2.44±0.12 ^{gh}	2.56±0.13 ^{gh}	10.50±0.09 ^{d}	45.8±1.6 ^{a}
C2	1.87±0.09 ^{f}	2.01±0.08 ^{fg}	2.92±0.16 ^{f}	11.34±0.25 ^{de}	6.7±1.3 ^{f}	1.65±0.09 ^{g}	2.28±0.30 ^{h}	2.37±0.06 ^{h}	7.42±0.01 ^{f}	20.2±1.2 ^{a}
S1	11.75±0.20 ^{c}	3.38±0.07 ^{a}	7.15±0.18 ^{a}	19.21±0.72 ^{a}	33.2±1.8 ^{d}	16.67±0.12 ^{b}	3.85±0.01 ^{a}	6.99±0.13 ^{b}	14.47±0.11 ^{a}	70.2±4.2 ^{a}
S2	13.40±0.64 ^{b}	3.62±0.09 ^{ab}	6.50±0.22 ^{b}	19.98±0.44 ^{a}	50.8±3.9 ^{b}	17.33±0.29^{a}	3.47±0.11 ^{abc}	7.55±0.24 ^{a}	14.93±0.69 ^{a}	67.6±1.3 ^{bc}
S3	16.30±0.52 ^{a}	1.99±0.04 ^{fg}	6.54±0.13 ^{ab}	10.16±0.33 ^{e}	40.2±1.2 ^{c}	12.20±0.32 ^{c}	3.71±0.06 ^{ab}	6.00±0.07 ^{c}	14.33±0.29 ^{a}	78.4±1.3 ^{e}
S1C1	5.03±0.43 ^{d}	2.25±0.16 ^{ef}	3.93±0.18 ^{cde}	16.66±0.31 ^{b}	57.3±2.5 ^{a}	2.35±0.26 ^{f}	2.51±0.29 ^{fgh}	2.65±0.19 ^{gh}	11.68±0.08 ^{c}	26.4±0.6 ^{de}
S2C1	4.50±0.31 ^{def}	2.23±0.10 ^{ef}	3.76±0.13 ^{de}	15.58±0.57 ^{bc}	35.9±2.2 ^{cd}	2.25±0.03 ^{f}	3.28±0.18 ^{bcd}	3.33±0.14 ^{ef}	11.75±0.01 ^{c}	66.8±0.9 ^{a}
S3C1	3.53±0.32 ^{f}	2.38±0.16 ^{de}	4.38±0.33 ^{cd}	16.29±0.06 ^{bc}	32.6±2.2 ^{d}	3.00±0.06 ^{e}	2.85±0.10 ^{defg}	4.17±0.12 ^{d}	12.93±0.39 ^{b}	49.7±3.3 ^{b}
S1C2	3.95±0.20 ^{ef}	2.87±0.19 ^{c}	4.52±0.37 ^{c}	18.75±0.46 ^{a}	26.5±1.4 ^{e}	2.35±0.09 ^{f}	2.55±0.30 ^{efgh}	2.80±0.24 ^{gh}	9.25±0.33 ^{e}	35.7±6.0 ^{cd}
S2C2	4.87±0.37 ^{de}	3.05±0.15 ^{bc}	4.40±0.14 ^{c}	15.46±0.26 ^{bc}	47.4±0.8 ^{b}	2.15±0.03 ^{f}	3.02±0.11 ^{cde}	3.04±0.03 ^{fg}	11.99±0.87 ^{bc}	46.2±6.4 ^{b}
S3C2	4.53±0.46 ^{def}	2.69±0.14 ^{cd}	3.37±0.25 ^{ef}	12.37±0.14 ^{d}	32.0±0.8 ^{de}	3.60±0.06 ^{d}	2.95±0.21 ^{def}	3.65±0.30 ^{e}	8.64±0.17 ^{e}	49.5±3.7 ^{b}
Average	6.56±0.83	2.57±0.11	4.62±0.25	15.56±0.55	33.62±2.75	5.92±1.05	4.10±0.32	2.99± 0.10	11.63± 0.43	49.7±3.05
Mono (U)	12.58±0.48 ^{B}	3.50±0.07 ^{A}	6.82±0.19	19.60±0.42 ^{A}	42.0±4.4	17.00±0.03 ^{A}	3.66±0.10	7.27±0.17 ^{A}	14.70±0.33	68.9±2.0
Mono (V)	16.30±0.52 ^{A}	1.99±0.04 ^{B}	6.54±0.13	10.16±0.33 ^{B}	40.2±1.2	12.20±0.02 ^{B}	3.71±0.06	6.00±0.07 ^{B}	14.33±0.29	68.4±1.3
Inter-(U+C)	4.59±0.19	2.60±0.13	4.15±0.14	16.61±0.44 ^{A}	41.8±3.6	2.28±0.03 ^{B}	2.84±0.14	2.96±0.11 ^{B}	11.17±0.39	43.8±4.9
Inter-(V+C)	4.03±0.34	2.54±0.12	3.88±0.29	14.33±0.88 ^{B}	32.3±1.1	3.30±0.03 ^{A}	2.90±0.11	3.91±0.18 ^{A}	10.79±0.98	49.6±2.2
Mono-S	13.82±0.71^{A}	2.99±0.26 ^{A}	6.73±0.14 ^{A}	16.45±1.60	41.4±2.9 ^{A}	15.40±0.06 ^{A}	3.68±0.07 ^{A}	6.84±0.24 ^{A}	14.58±0.24 ^{A}	68.7±1.4 ^{A}
Mono-C	2.16±0.15 ^{C}	1.88±0.07 ^{B}	3.11±0.13 ^{C}	13.37±0.94	6.9±0.7 ^{B}	1.60±0.01 ^{B}	2.36±0.15 ^{C}	2.46±0.08 ^{C}	8.96±0.69 ^{C}	33.0±5.8 ^{C}
Inter-(C+S)	4.40±0.18 ^{B}	2.58±0.09 ^{A}	4.06±0.13 ^{B}	15.85±0.48	38.6±2.6 ^{A}	2.62±0.02 ^{B}	2.86±0.10 ^{B}	3.28±0.14 ^{B}	11.04±0.40 ^{B}	45.7±3.4 ^{B}

Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC26-28; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28; S2C2= Game Keeper RR + DKC26-28; S3C2 = Kester's Bob White Trailing Soybean + DKC26-28. Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean

The US tend to produce forage with higher micronutrients compared to the VS, when cultivated as monocrops, except for Fe in 2016 (Table 3.7). However, forage produced by the upright soybean during the 2017 growing season had higher Fe content than the vine type soybean, consistent with the trends observed for the other micronutrients (Table 3.8). Surprisingly, when the VS and US varieties were intercropped with silage corn, the micronutrient content of the forage produced was similar between the IC treatments regardless of the soybean variety was intercropped, except for B during the 2016 growing season (Table 3.7-3.8). The concentration ranges of the micro-minerals Zn, Fe, B, Mn, Cu and Co were 13.15-41.79 mg/kg, 365-1166 mg/kg, 5.66- 33.56 mg/kg, 77-303 mg/kg, 10.22-21.96 mg/kg and 0.66-3.62 mg/kg respectively during 2016 (Table 3.7). During 2017 ranges of micro-nutrients Zn, Fe, B, Mn, Cu and Co were 8.36-48.04 mg/kg, 129-1955 mg/kg, 4.07-33.25 mg/kg, 27.16-122.41 mg/kg, 4.32-15.02 mg/kg, 0.05-0.72 mg/kg respectively (Table 3.8). Overall, the soybean MC produced forage with superior micronutrient contents compared to the IC treatments.

Principal component analysis was used to discern the relationships between the mineral nutrient composition of the forage produced across the different cropping systems. The PCA output indicate that 75 % of the variance present in the data could be explained by PCA 1 and PCA 2 (Figure 3.1). Specifically, the first PC-axis explained 54.16% of the variation in the mineral data, while the second PC-axis explained 21.41% of the variation. The PCA output demonstrated that the macronutrients clustered with the upright soybeans, while the micronutrients clustered with the vine type soybeans cultivated as monocrops. Forage obtained from corn MC clustered in a separate quadrant, so too was the IC

treatments. These groupings are consistent with the levels of micronutrients observed in the forage obtained from the different cropping systems, and further support the earlier observations that the cropping systems were effective in altering the macro and macronutrient composition of the forage produced. I further seek to determine if the active microbial population present in the soil played a role in the mineral nutrient composition observed in forage obtained from the different cropping systems. Pearson's correlation was applied to assess this relationship using the soil microbial phospholipid FAs (Chapter 2) and the mineral nutrients observed in the forage. The potassium content of the forage was the only mineral nutrient observed to be significantly correlated with the active soil microbial population. Consistently, significant correlations were observed between the fungal (r=0.37), protozoan (r=0.38), the ratio of the fungal: bacteria populations (r=0.56), the gram + (r=0.33) or gram- (r=0.43) bacteria and the forage potassium content (Figure 3.2).

Table 3.7: Micro-nutrient content of forage obtained from corn and soybean cultivated as mono and intercropping during the 2016 and 2017 growing seasons.

Growing Season (2016)						
Treatments	**Zn (mg/kg)**	**Fe (mg/kg)**	**B (mg/kg)**	**Mn (mg/kg)**	**Cu (mg/kg)**	**Co (mg/kg)**
C1	17.10±0.62 ^{f}	417±7 ^{fg}	6.37±0.25 ^{hi}	119.8±3.3 ^{e}	14.05±1.39 ^{de}	1.75±0.03 ^{e}
C2	13.15±1.29 ^{g}	365±28 ^{g}	6.80±0.85 ^{ghi}	86.6±3.8 ^{f}	10.22±1.53 ^{f}	0.66±0.12 ^{g}
S1	39.04±0.99 ^{a}	616±36 ^{bc}	33.56±0.25 ^{a}	303.1±9.7 ^{a}	17.87±1.61 ^{bc}	3.04±0.14 ^{b}
S2	21.11±1.34 ^{d}	533±24 ^{de}	31.01±0.25 ^{b}	162.3±8.9 ^{d}	18.58±1.81 ^{ab}	1.43±0.04 ^{f}
S3	35.09±1.77 ^{b}	1166±24 ^{a}	19.54±0.98 ^{c}	252.6±2.6 ^{b}	21.96±1.39 ^{a}	3.62±0.02 ^{a}
S1C1	41.79±0.24 ^{a}	637±33 ^{b}	13.99±0.23 ^{d}	124.8±6.2 ^{e}	21.56±1.04 ^{a}	1.84±0.12 ^{e}
S2C1	20.14±0.17 ^{def}	466±21 ^{ef}	8.78±0.75 ^{fg}	115.9±7.2 ^{e}	14.80±0.03 ^{cde}	1.30±0.02 ^{f}
S3C1	21.02±0.49 ^{de}	454±26 ^{f}	5.66±0.57 ^{i}	207.3±9.7 ^{c}	15.36±0.79 ^{bcd}	2.35±0.13 ^{d}
S1C2	17.77±0.69 ^{ef}	463±19 ^{ef}	7.93±0.75 ^{fgh}	76.6±3.6 ^{f}	12.21±0.83 ^{def}	0.90±0.04 ^{g}
S2C2	31.12±1.50 ^{c}	654±20 ^{b}	11.89±0.49 ^{e}	175.7±3.1 ^{c}	11.84±0.23 ^{ef}	2.64±0.08 ^{c}
S3C2	19.07±1.52 ^{def}	551±13 ^{cd}	8.92±1.23 ^{f}	117.8±5.2 ^{e}	12.67±0.21 ^{def}	1.29±0.10 ^{f}
Average	25.13±1.68	575±37	14.04±1.67	158.4±12.1	15.56±0.73	1.90±0.16
Mono (U)	30.07±4.08	574±27 ^{B}	32.28±0.59 ^{A}	232.7±32.0	18.23±1.10 ^{B}	2.23±0.36
Mono (V)	35.09±1.77	1166±24 ^{A}	19.54±0.98 ^{B}	252.6±2.6	21.96±1.39 ^{A}	3.62±0.02
Inter-(U+C)	27.70±2.91	555±29	10.65±0.78 ^{A}	123.2±10.9	15.10±1.21	1.67±0.20
Inter-(V+C)	20.04±0.84	503±25	7.29±0.95 ^{B}	162.6±20.6	14.01±0.71	1.82±0.25
Mono-S	31.75±2.81 ^{A}	772±100 ^{A}	28.04±2.18 ^{A}	239.4±20.9 ^{A}	19.47±1.02 ^{A}	2.69±0.33 ^{A}
Mono-C	15.12±1.09 ^{B}	391±17 ^{B}	6.58±0.41 ^{B}	103.2±7.7 ^{B}	12.13±1.26 ^{B}	1.20±0.25 ^{B}
Inter-(C+S)	25.15±2.12 ^{A}	537±22 ^{B}	9.53±0.70 ^{B}	136.3±10.6 ^{B}	14.74±0.83 ^{B}	1.72±0.15 ^{B}

Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC26-28; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28; S2C2 = Game Keeper RR + DKC26-28; S3C2 = Kester's Bob White Trailing Soybean + DKC26-28. Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean.

Table 3.8: Micro-mineral nutrient composition of corn and soybean forage sown in mono and intercropping systems during 2017.

	First Growing Season (2017)					
Treatments	**Zn (mg/kg)**	**Fe (mg/kg)**	**B (mg/kg)**	**Mn (mg/kg)**	**Cu (mg/kg)**	**Co (mg/kg)**
C1	17.97±1.03 ^{d}	197±32 ^{f}	4.07±0.46 ^{fg}	29.3±0.3 ^{g}	6.23±0.12 ^{gh}	0.09±0.00 ^{cd}
C2	8.36±0.40 ^{f}	129±8 ^{g}	4.17±0.18 ^{g}	27.2±3.3 ^{g}	4.32±0.15 ^{i}	0.05±0.01 ^{d}
S1	31.74±0.53 ^{b}	1568±14 ^{b}	25.41±0.73 ^{b}	108.8±1.2 ^{b}	11.59±0.12 ^{b}	0.66±0.04 ^{a}
S2	13.02±1.11 ^{e}	1954± 26 ^{a}	33.25±2.24 ^{a}	122.4±1.4 ^{a}	15.02±0.05 ^{a}	0.72±0.01 ^{a}
S3	27.67±1.09 ^{c}	759±28 ^{c}	26.13±0.55 ^{b}	77.2±1.5 ^{c}	10.80±0.43 ^{c}	0.25±0.01 ^{b}
S1C1	11.14±0.30 ^{ef}	311±8 ^{e}	6.46±0.21 ^{ef}	41.9±2.3 ^{f}	6.61±0.18 ^{fg}	0.10±0.01 ^{cd}
S2C1	33.08±1.44 ^{b}	307±31 ^{e}	9.31±0.96 ^{cd}	55.3±2.9 ^{e}	9.22±0.08 ^{d}	0.25±0.06 ^{b}
S3C1	18.20±1.87 ^{d}	300±7 ^{e}	8.69±0.31 ^{cde}	50.4±2.9 ^{e}	7.62±0.25 ^{e}	0.16±0.02 ^{c}
S1C2	19.52±0.53 ^{d}	281±18 ^{e}	7.21±0.12 ^{de}	40.2±0.2 ^{f}	5.73±0.06 ^{h}	0.11±0.00 ^{cd}
S2C2	19.73±1.17 ^{d}	393±28 ^{d}	10.03±0.36 ^{c}	63.9±1.7 ^{d}	6.95±0.10 ^{f}	0.29±0.01 ^{b}
S3C2	48.04±2.29 ^{a}	439± 16 ^{d}	9.52±0.89 ^{c}	69.7±2.2 ^{d}	9.04±0.33 ^{d}	0.25±0.02 ^{b}
Average	22.59±1.98	604±101	13.11±1.72	62.4±5.2	8.47±0.52	0.27±0.04
Mono (U)	22.38±4.22	1761±88 ^{A}	29.33±2.05	115.6±3.2 ^{A}	13.31±0.77	0.69±0.02 ^{A}
Mono (V)	27.67±1.09	759±28 ^{B}	26.13±0.55	77.2±1.5 ^{B}	10.80±0.43	0.25±0.01 ^{B}
Inter-(U+C)	20.87±2.40	323±16	8.25±0.50	50.3±3.1	7.13±0.39	0.19±0.03
Inter-(V+C)	33.12±6.80	369±32	9.10±0.46	60.0±4.6	8.33±0.37	0.20±0.02
Mono-S	24.14±2.88 ^{AB}	1427±176 ^{A}	28.27±1.43 ^{A}	102.8±6.7 ^{A}	12.47±0.66 ^{A}	0.54±0.07 ^{A}
Mono-C	13.17±2.21 ^{B}	163±21 ^{B}	4.12±0.22 ^{C}	28.2±1.6 ^{C}	5.28±0.43 ^{C}	0.07±0.01 ^{B}
Inter-(C+S)	24.95±3.00 ^{A}	338±15 ^{B}	8.54±0.37 ^{B}	53.6±2.7 ^{B}	7.53±0.31 ^{B}	0.19±0.02 ^{B}

Micro-mineral nutrient composition of corn and soybean forage sown in mono and IC systems during 2017. Values are means ± standard errors. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05), C1 = Yukon-R; C2 = DKC26-28; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC26-28; S2C2 = Game Keeper RR + DKC26-28; S3C2 = Kester's Bob White Trailing Soybean + DKC26-28. Mono C = monocropping corn; Mono S = monocropping soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean

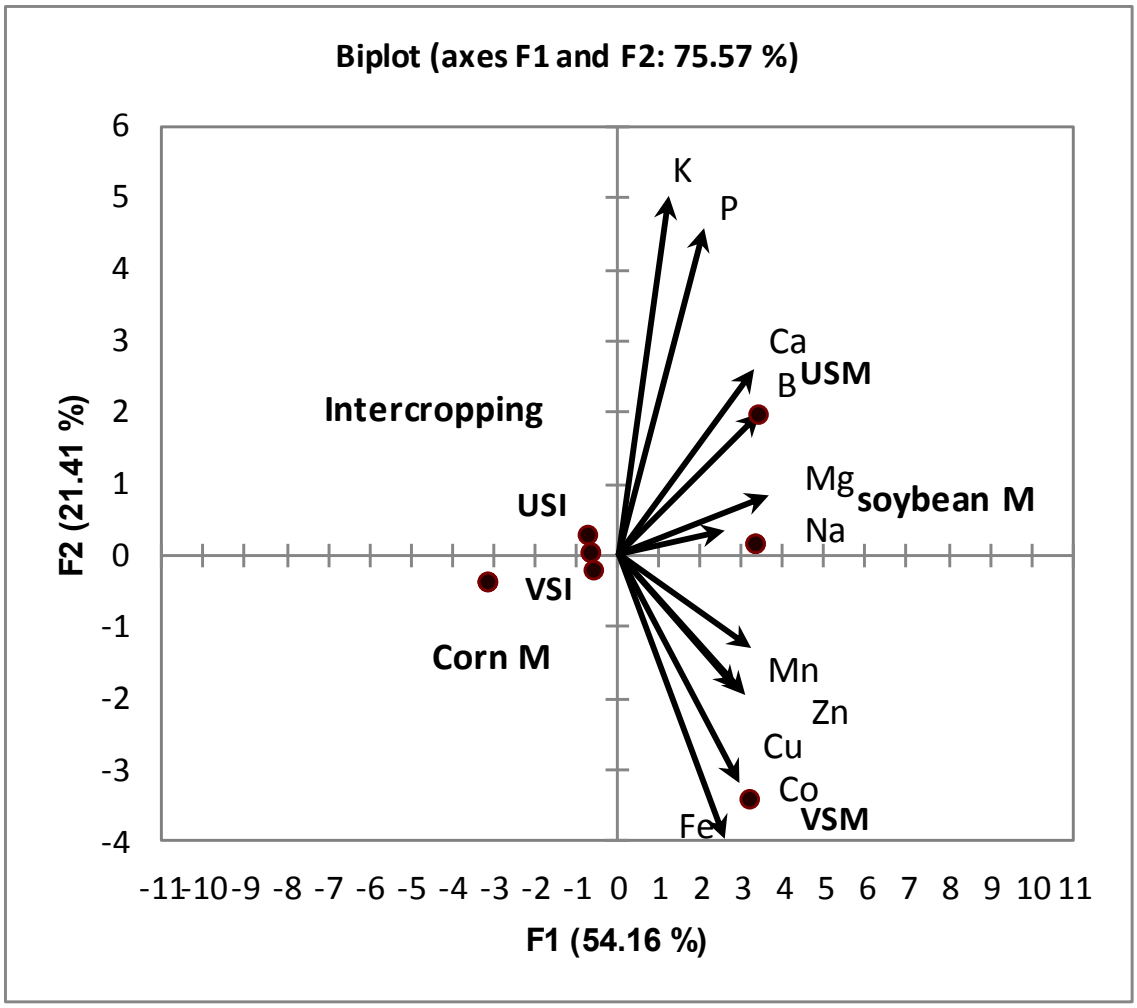

Figure 3.1: Principal component analysis of the macro and micronutrients content of forage obtained from corn and soybeans cultivated as mono or intercrops under cool climatic conditions.

Corn M = corn monocropping; Soybean M = soybean monocropping; VSM = vine soybean monocropping; USM = upright soybean monocropping; I (C+US) = corn intercropped with upright soybean; I (C+VS) = corn intercropped with vine soybean. Data represents mineral nutrients of the forage from two growing seasons (2016 and 2017)

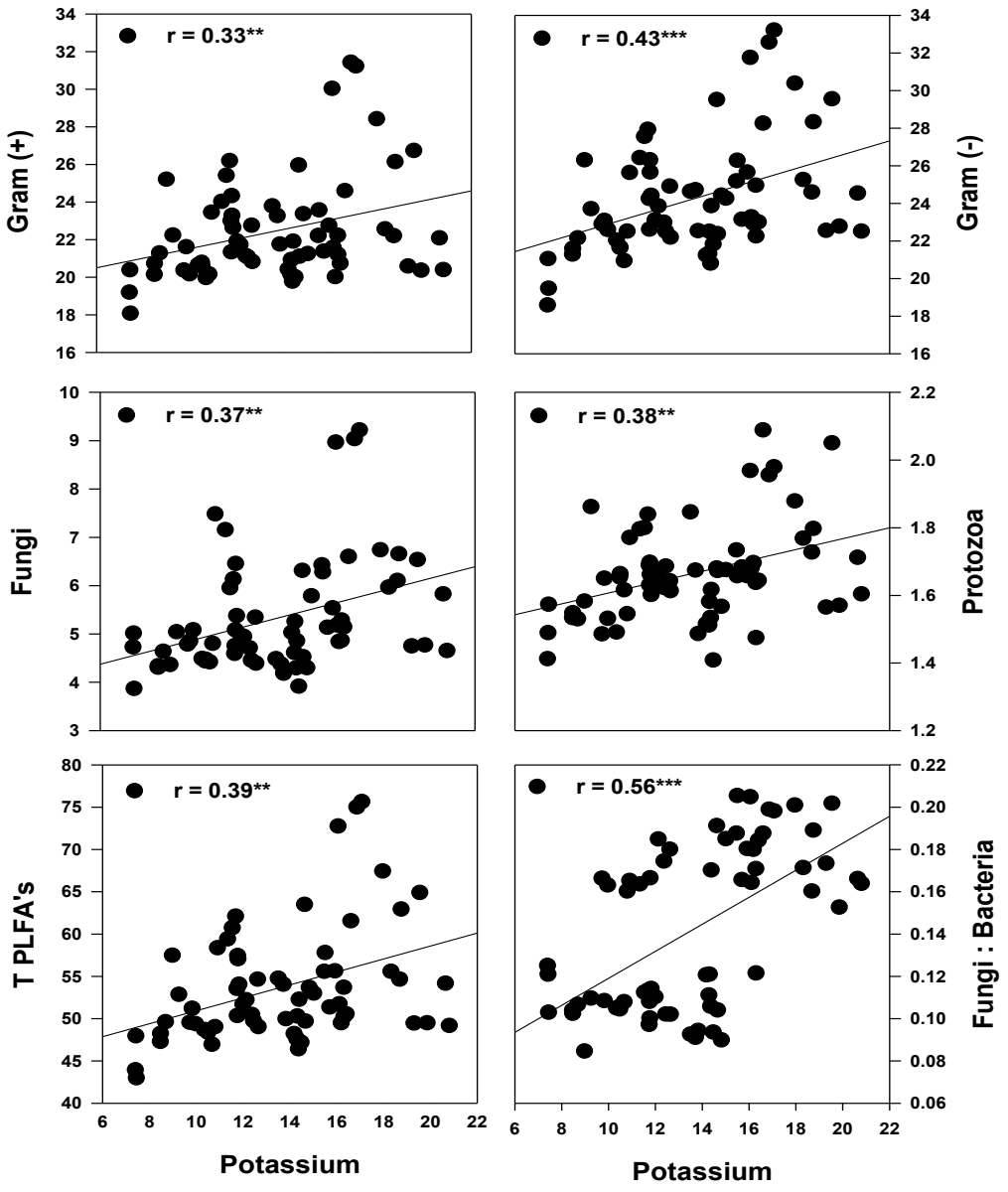
r = 0.33**
r = 0.43***
r = 0.37**
r = 0.38**
r = 0.39**
r = 0.56***
Gram (+)
Gram (-)
Fungi
Protozoa
T PLFA's
Fungi : Bacteria
Potassium
Potassium

Figure 3.2: Pearson correlation showing the association between the K content and the active soil microbial community of forage produced from mono or intercropping corn and soybeans under cool climatic conditions.

T PLFA = total phospholipid fatty acids. ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) n=33 for all parameters

3.4.3. Fodder fatty acid (FA) profiles

Results on the variation of the FA contents of corn and soybean cultivated as either monocrops or intercrops under cool climatic conditions are presented in Table 3.9-3.10 for two growing seasons (years 2016 and 2017). Overall during 2016, total FAs contents were higher in IC compared with corn MC treatments. Means of saturated FAs contents were higher in soybean MC than corn MC and IC treatments, while monounsaturated means were significantly ($p \leq 0.05$) higher in IC than both corn and soybean MC. Briefly, during 2016-2017 the highest C16:0 (g/kg dry matter) was observed in S1C2 (2.15) and S1 (2.47), while the lowest contents were recorded in C1 (1.66) and S3 (1.75) respectively. C18:1n-9cis (g/kg dry matter) was ranges from S2 (0.65 & 0.35) to S1C2 (2.59 & 1.11) and C18:2n-6 (g/kg dry matter) was ranged from S3 (0.92) to C2 (2.08) and S2 (1.82) to C2 (3.73) during 2016 and 2017 respectively. While maximum C18:3n-3 (g/kg dry matter) was maximum depicted in S1 (1.36 & 2.32) and minimum in C1 (0.51 & 0.41). IC of corn with soybean increased the C18:3n-3 (g/kg dry matter) contents while a decrease was observed in C18:2n-6 in the forage as compared to corn MC treatments. Total FA's (g/kg dry matter) contents maximum recorded in S1C2 (8.07) and S1 (8.33) and minimum in C1 (5.26) and S3C1 (6.64) during first and second growing seasons respectively. US varieties MC contain higher omega 3 FA contents than VS variety MC. Omega 3 / omega 6 ratio was significantly higher in IC than corn MC treatments

while omega 6 / omega 3 ratio was behaved oppositely (Figure 3.3-3.4). The PUFA's were the major contributor (38% & 57%) of the total FA profile during 2016 and 2017 respectively. Saturated and MUFA's contribute (35% & 28%) and (31% & 12%) during 2016 and 2017 respectively of the total FAs. Over all saturated FAs (SFA) were lower (31% to 35%) compared to unsaturated FAs (UFA) (65% to 69%) during both years. In all type of monocropped or intercropped forage C16:0 and C18:2n-6 were the major FA with an average contribution of 29 % and 33 % of total measured FA during 2016 and 2017 respectively. During 2016 the contribution sequence followed the trend as C16:0 (30 %) > C18:2n-6 (24 %) > C18:1n-9cis (23 %) > C18-3n-3 (13 %) > C16-1n-7 (5 %) > C18:0 (4 %). The contribution of first four FA's (C16:0, C18:2n-6, C18:1n-9cis, C18-3n-3) was 90 % of the total analyzed FAs in the forage. The rest of the FA's contributed 4-5 %. The trend was different during 2017 as discussed above; where C18:2n-6 was the major contributor during 2017. As such the contribution sequence was C18:2n-6 (41 %) > C16:0 (27 %) > C18-3n-3 (16 %) > C18:1n-9cis (10 %) > C18:0 (3 %) > C16-1n-7 (2 %). Here the first four major FA's contributions were almost 95 % of the total investigated FA's in the forage. The minor FA's contributed only 2-3 %.

Table 3.9: Plant FA profile (g/kg dry matter) of corn and soybean sown as mono and as intercropping during the growing Season of 2016.

Treatments	C16-0	C16-1n-7	C18-0	C18-1n-9cis	C18-2n-6 (omega 6)	C18:3n-3 (omega 3)	Total FA	SFA%	MUFA%	PUFA%
C1	1.66±0.01 ^{f}	0.18±0.00 ^{g}	0.20±0.02 ^{f}	1.22±0.08 ^{e}	1.48±0.01 ^{cde}	0.54±0.03 ^{f}	5.26±0.08 ^{g}	35.33±0.33 ^{cd}	26.59±1.16 ^{de}	38.00±0.58 ^{c}
C2	1.67±0.05 ^{ef}	0.18±0.01 ^{g}	0.23±0.00 ^{ef}	1.76±0.12 ^{e}	2.08±0.02 ^{a}	0.55±0.01 ^{ef}	5.84±0.03 ^{de}	32.67±0.88 ^{ef}	33.21±1.79 ^{ab}	45.00±0.58 ^{a}
S1	2.12±0.10 ^{a}	0.50±0.00 ^{b}	0.39±0.00 ^{a}	0.92±0.08 ^{f}	1.44±0.01 ^{def}	1.27±0.09 ^{a}	6.73±0.18 ^{c}	37.33±0.67 ^{b}	21.00±0.49 ^{fg}	41.67±1.20 ^{b}
S2	1.84±0.03 ^{cd}	0.46±0.01 ^{a}	0.30±0.00 ^{bc}	0.65±0.04 ^{g}	1.36±0.03 ^{ef}	1.16±0.04 ^{a}	5.77±0.08 ^{e}	37.00±0.58 ^{bc}	19.04±0.67 ^{g}	43.67±0.67 ^{ab}
S3	1.87±0.06 ^{bc}	0.57±0.01 ^{c}	0.32± 0.03 ^{b}	0.66±0.01 ^{g}	0.92±0.07 ^{g}	0.97±0.05 ^{b}	5.32±0.10 ^{fg}	41.33±0.88 ^{a}	22.70±0.19 ^{f}	35.67±1.20 ^{cde}
S1C1	1.69±0.00 ^{def}	0.25±0.01 ^{ef}	0.23±0.01 ^{ef}	1.55±0.05 ^{d}	1.40±0.03 ^{def}	0.66±0.01^{de}	5.72±0.03 ^{e}	33.67±0.33 ^{de}	30.43±0.60 ^{c}	36.00±0.58 ^{cd}
S2C1	1.83±0.08 ^{cde}	0.25±0.01 ^{e}	0.29±0.02 ^{bcd}	1.86±0.02 ^{c}	1.62±0.03 ^{c}	0.79±0.03 ^{c}	6.64±0.03 ^{c}	31.67±0.88 ^{fg}	31.06±0.47 ^{bc}	36.33±0.88 ^{cd}
S3C1	1.71±0.01 ^{cdef}	0.21±0.00 ^{fg}	0.25±0.00 ^{de}	1.28±0.05 ^{e}	1.39±0.12 ^{def}	0.72±0.01 ^{cd}	5.56±0.14 ^{ef}	35.33±0.88 ^{cd}	26.23±0.60 ^{e}	38.00±1.53 ^{c}
S1C2	2.15±0.00 ^{a}	0.32±0.01 ^{d}	0.29±0.02 ^{bc}	2.59±0.08 ^{a}	1.90±0.01 ^{b}	0.82±0.03 ^{c}	8.07±0.09 ^{a}	30.33±0.33 ^{g}	34.85±0.42 ^{a}	34.00±0.58 ^{def}
S2C2	1.87±0.06 ^{bc}	0.19±0.02^{g}	0.24±0.01 ^{e}	1.76±0.12 ^{c}	1.30±0.09 ^{f}	0.71 ±0.01^{cd}	6.06±0.13 ^{d}	34.67±0.33 ^{d}	29.03±1.36 ^{cd}	33.00±1.53 ^{ef}
S3C2	2.02±0.06 ^{ab}	0.25±0.02 ^{e}	0.28±0.01 ^{cd}	2.25±0.05 ^{b}	1.53±0.03 ^{cd}	0.76±0.01 ^{cd}	7.10±0.08 ^{b}	32.33±0.33 ^{ef}	33.39±0.28 ^{ab}	32.33±0.67 ^{f}
Average	1.86±0.03	0.31±0.02	0.27±0.01	1.50±0.11	1.49±0.05	0.82±0.04	6.19±0.15	34.70±0.55	27.70±0.99	37.61±0.75
Mono (U)	1.98±0.08	0.48±0.01^{B}	0.35±0.02	0.78±0.07	1.40±0.02^{A}	1.22±0.05^{A}	6.25±0.23	37.19±0.34^{B}	20.15±0.59	42.66±0.71^{A}
Mono (V)	1.87±0.06	0.57±0.01^{A}	0.32±0.03	0.66 ±0.01	0.92±0.07^{B}	0.97±0.05^{B}	5.32±0.10	41.20±0.80^{A}	23.28±0.85	35.52±1.21^{B}
Inter-(U+C)	1.88±0.05	0.25±0.01	0.26±0.01	1.93±0.12	1.55±0.07	0.75±0.02	6.62±0.27	32.58±0.55	32.63±0.75	34.79±0.56
Inter-(V+C)	1.86±0.07	0.23±0.01	0.27±0.01	1.77±0.22	1.46±0.06	0.74±0.01	6.33±0.35	33.84±0.81	31.04±1.92	35.12±1.42
Mono-S	1.94±0.06^{A}	0.51±0.02^{A}	0.34±0.02^{A}	0.74±0.05^{C}	1.24±0.08^{C}	1.14±0.05^{A}	5.94±0.22^{AB}	38.53±0.74^{A}	21.19±0.69^{B}	40.28±1.32^{A}
Mono-C	1.67±0.02^{B}	0.18±0.01^{C}	0.21±0.01^{C}	1.17±0.13^{B}	1.78±0.13^{A}	0.54±0.01^{C}	5.55±0.13^{B}	33.93±0.69^{B}	24.58±1.10^{B}	41.49±1.62^{A}
Inter-(C+S)	1.88±0.05^{A}	0.25±0.01^{B}	0.26±0.01^{B}	1.87±0.11^{A}	1.52±0.05^{B}	0.74±0.01^{B}	6.53±0.21^{A}	33.00±0.46^{B}	32.10±0.80^{A}	34.90±0.58^{B}

Plant FA profile (g/kg dry matter) of corn and soybean sown as monocrops and as intercropping during the growing Season of 2016

FA = Fatty acid; SFA% = Saturated fatty acid; MUFA% = Monounsaturated fatty acid; PUFA% Polyunsaturated fatty acid; C1 = Yukon-R; C2 = DKC-2628; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's

Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC-2628; S2C2 = Game Keeper RR + DKC-2628; S3C2 = Kester's Bob White Trailing Soybean + DKC-2628. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05)

Table 3.10: Plant FA profile (g/kg dry matter) of corn and soybean sown as mono and as intercropping during the growing Season of 2017.

Treatments	C16-0	C16-1n-7	C18-0	C18-1n-9cis	C18-2n-6 (omega 6)	C18:3n-3 (omega 3)	Total FA	SFA%	MUFA%	PUFA%
C1	1.79±0.02 ^{cd}	0.05±0.00 ^{e}	0.10±0.01^{f}	0.74±0.00 ^{de}	3.60±0.10 ^{ab}	0.41±0.01 ^{g}	6.70±0.12 ^{f}	28.33±0.33 ^{cde}	11.67±0.33 ^{bc}	60.00±0.58 ^{a}
C2	1.82±0.16 ^{cd}	0.05±0.00 ^{e}	0.11±0.01 ^{ef}	0.73±0.02 ^{de}	3.73±0.04 ^{a}	0.46±0.02 ^{g}	6.90±0.17 ^{def}	28.00±2.00 ^{de}	11.00±0.00 ^{c}	60.67±1.67 ^{a}
S1	2.47±0.10 ^{a}	0.34±0.01 ^{a}	0.55±0.02 ^{a}	0.40±0.02 ^{f}	2.25±0.11 ^{f}	2.32±0.03 ^{a}	8.33±0.16 ^{a}	36.33±1.33 ^{a}	8.67±0.33 ^{d}	55.00±1.00 ^{bcd}
S2	2.12±0.00 ^{b}	0.29±0.02 ^{b}	0.51±0.02 ^{a}	0.35±0.01 ^{f}	1.82±0.05 ^{g}	2.00±0.05 ^{c}	7.09±0.04 ^{cd}	37.00±0.00 ^{a}	9.00±0.00 ^{d}	54.00±0.00 ^{d}
S3	1.75±0.01 ^{d}	0.19±0.01 ^{c}	0.39±0.03 ^{b}	0.43±0.01 ^{f}	2.34±0.03 ^{f}	2.14±0.05 ^{b}	7.22±0.03 ^{c}	29.67±0.33 ^{cd}	8.67±0.33 ^{d}	62.00±0.58 ^{a}
S1C1	1.87±0.04 ^{cd}	0.08±0.00 ^{d}	0.15±0.01 ^{d}	1.02±0.01 ^{ab}	3.31±0.05 ^{cd}	0.61±0.01 ^{f}	7.06±0.04 ^{cd}	29.00±0.58 ^{cde}	15.67±0.33 ^{a}	57.67±0.67 ^{bcd}
S2C1	1.90±0.01 ^{cd}	0.09±0.00 ^{d}	0.16±0.01 ^{d}	0.85±0.02 ^{cd}	3.33±0.11 ^{cd}	0.71±0.04 ^{ef}	7.04±0.06 ^{cde}	29.33±0.33 ^{cd}	13.33±0.33 ^{b}	57.33±0.67 ^{b}
S3C1	1.94±0.04 ^{c}	0.08±0.00 ^{d}	0.21±0.01 ^{c}	0.64±0.01 ^{e}	2.67±0.09 ^{e}	1.10±0.08 ^{d}	6.64±0.05 ^{f}	32.33±0.33 ^{b}	11.00±0.00 ^{c}	56.67±0.33 ^{bc}
S1C2	1.96±0.03 ^{bc}	0.08±0.00 ^{d}	0.17±0.01 ^{d}	1.11±0.09 ^{a}	3.22±0.06 ^{d}	0.74±0.02 ^{e}	7.27±0.03 ^{c}	29.33±0.33 ^{cd}	16.33±0.88 ^{a}	54.67±1.45 ^{cd}
S2C2	1.92±0.02 ^{cd}	0.09±0.01 ^{d}	0.16±0.00 ^{d}	1.00±0.09 ^{ab}	2.62±0.05 ^{e}	0.98±0.04 ^{d}	6.77±0.04 ^{ef}	30.67±0.67 ^{bc}	16.00±1.15 ^{a}	53.33±0.67 ^{d}
S3C2	1.86±0.01 ^{cd}	0.08±0.00 ^{d}	0.15±0.01 ^{de}	0.89±0.09 ^{bc}	3.48±0.04 ^{bc}	1.09±0.04 ^{d}	7.55±0.09 ^{b}	26.67±0.33 ^{e}	13.00±1.00 ^{b}	60.33±0.67 ^{a}
Average	1.95±0.04	0.13±0.02	0.24±0.03	0.74±0.05	2.94±0.11	1.14±0.12	7.14±0.08	30.61±0.60	12.21±0.51	57.24±0.56
Mono (U)	2.30±0.09^{A}	0.31±0.02^{A}	0.52±0.02^{A}	0.38±0.02	2.04±0.11	2.16±0.08	7.71±0.29	36.63±0.62^{A}	8.99±0.16	54.38±0.51^{B}
Mono (V)	1.75±0.01^{B}	0.19±0.01^{B}	0.38±0.03^{B}	0.43±0.01	2.34±0.03	2.13±0.05	7.22±0.03	29.53±0.41^{B}	8.58±0.06	61.89±0.35^{A}
Inter-(U+C)	1.91±0.01	0.09±0.01	0.16±0.00	1.00±0.04^{A}	3.12±0.09	0.76±0.04^{B}	7.04±0.06	29.49±0.29	15.37±0.49^{A}	55.14±0.59^{B}
Inter-(V+C)	1.90±0.02	0.08±0.01	0.18±0.02	0.77±0.07^{B}	3.07±0.19	1.09±0.04^{A}	7.10±0.21	29.51±1.31	11.89±0.66^{B}	58.59±0.94^{A}
Mono-S	2.11±0.11^{A}	0.27±0.02^{A}	0.48±0.03^{A}	0.40±0.01^{C}	2.14±0.09^{C}	2.15±0.05^{A}	7.55±0.20^{A}	34.26±1.25^{A}	8.85±0.13^{C}	56.88±1.30^{B}
Mono-C	1.81±0.07^{B}	0.05±0.00^{C}	0.11±0.01^{C}	0.73±0.01^{B}	3.67±0.06^{A}	0.43±0.01^{C}	6.80±0.10^{B}	28.12±0.83^{B}	11.51±0.13^{B}	60.37±0.86^{A}
Inter-(C+S)	1.91±0.01^{B}	0.09±0.00^{B}	0.17±0.01^{B}	0.92±0.04^{A}	3.10±0.08^{B}	0.87±0.05^{B}	7.06±0.08^{B}	29.50±0.45^{B}	14.21±0.55^{A}	56.29±0.63^{B}

FA = Fatty acid; SFA% = Saturated fatty acid; MUFA% = Monounsaturated fatty acid; PUFA% Polyunsaturated fatty acid; C1 = Yukon-R; C2 = DKC-2628; S1 = Big Fellow RR; S2 = Game Keeper RR; S3 = Kester's Bob White Trailing Soybean; S1C1 = Big Fellow RR + Yukon-R; S2C1 = Game Keeper RR + Yukon-R; S3C1 = Kester's

Bob White Trailing Soybean + Yukon-R; S1C2 = Big Fellow RR + DKC-2628; S2C2 = Game Keeper RR + DKC-2628; S3C2 = Kester's Bob White Trailing Soybean + DKC-2628. Mean values in each column followed by the same letter are not significantly different at (LSD, 0.05)

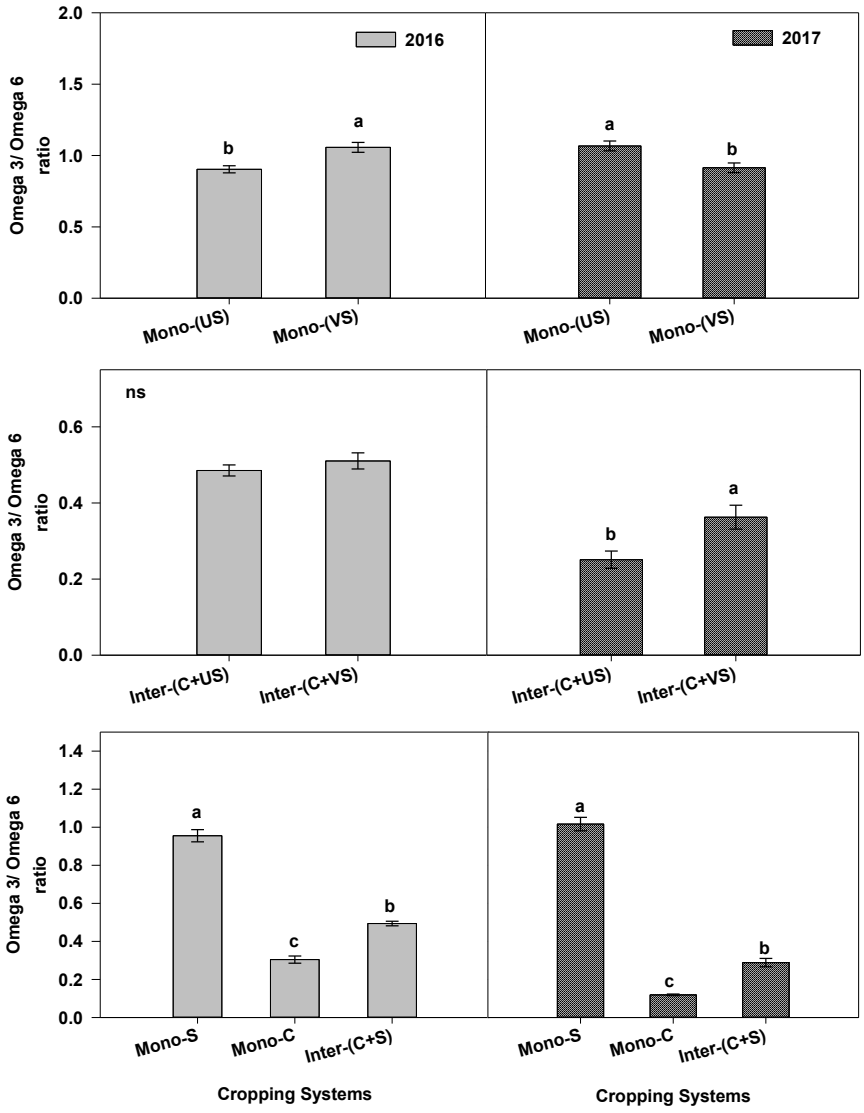

Figure 3.3: Omega 3/omega 6 FA's ratio under corn and soybean cropping systems during the growing season of 2016 and 2017.

ns= non-significant; The error bar represents SE. Different letters indicate differences between the cropping systems at LSD = 0.05, Mono C = monocropping corn; Mono-S = monocropping soybean; Inter (C+S) = corn-soybean intercropping; Mono-(US) = monocropping upright soybean; Mono-(VS) = monocropping vine soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean.

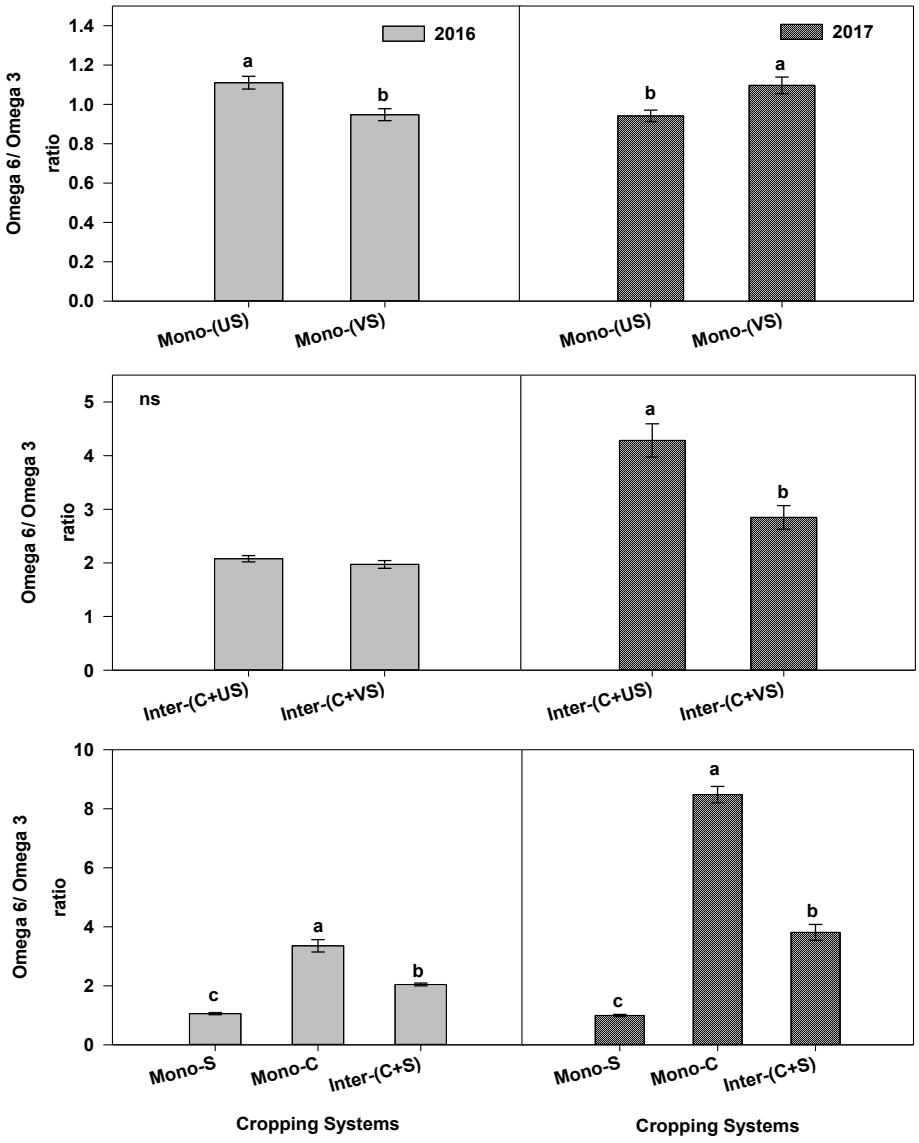

Figure 3.4: Omega 6/omega 3 FA's ratio under corn and soybean cropping systems during the growing season of 2016 and 2017.

ns= non-significant; The error bar represents SE. Different letters indicate differences between the cropping systems at LSD = 0.05, Mono C = monocropping corn; Mono-S = monocropping soybean; Inter (C+S) = corn-soybean intercropping; Mono-(US) = monocropping upright soybean; Mono-(VS) = monocropping vine soybean; Inter-(C+US) = corn intercropped with upright soybean; Inter-(C+VS) = corn intercropped with vine soybean.

3.4.4. Relationship between forage quality and forage FA contents:

The RDA bi-plot (Figure 3.5) showed the relationship between the forage quality and the FA contents of forage obtained from the cropping systems evaluated under cool climatic conditions. The first axis (F1) of the RDA bi-plot explained 38.47% and the second axis (F2) explained 35.07% of the total variation in the data. The first axis (F1) correlated positively with ash, CP, AP, DMI and RFV, but was negatively correlated with SS, SP, WSC and NDF. The second axis (F2) was positively correlated with net forage energies, DDM and TDN, while a negative correlation was observed with ADF contents. The corn and soybean MC, as well as IC treatments clustered in separate quadrants with specific quality parameters and FA contents. For example, DDM, NE_M, NE_G, TDN, SS, SP and C18:2n-6 were the factors most influenced by IC treatments and as such they cluster with the IC treatments in separate quadrant of the biplot compared to corn and soybean MC treatments (Figure 3.5). A positive correlation was observed between the forage quality and the major FA's. Conversely, ADF, NDF, ash, WSC and SS showed a negative correlation with major forage FA's. Further confirmation of these significant relationships was done by running a Pearson correlation between forage FA's and quality parameters (Table 3.11).

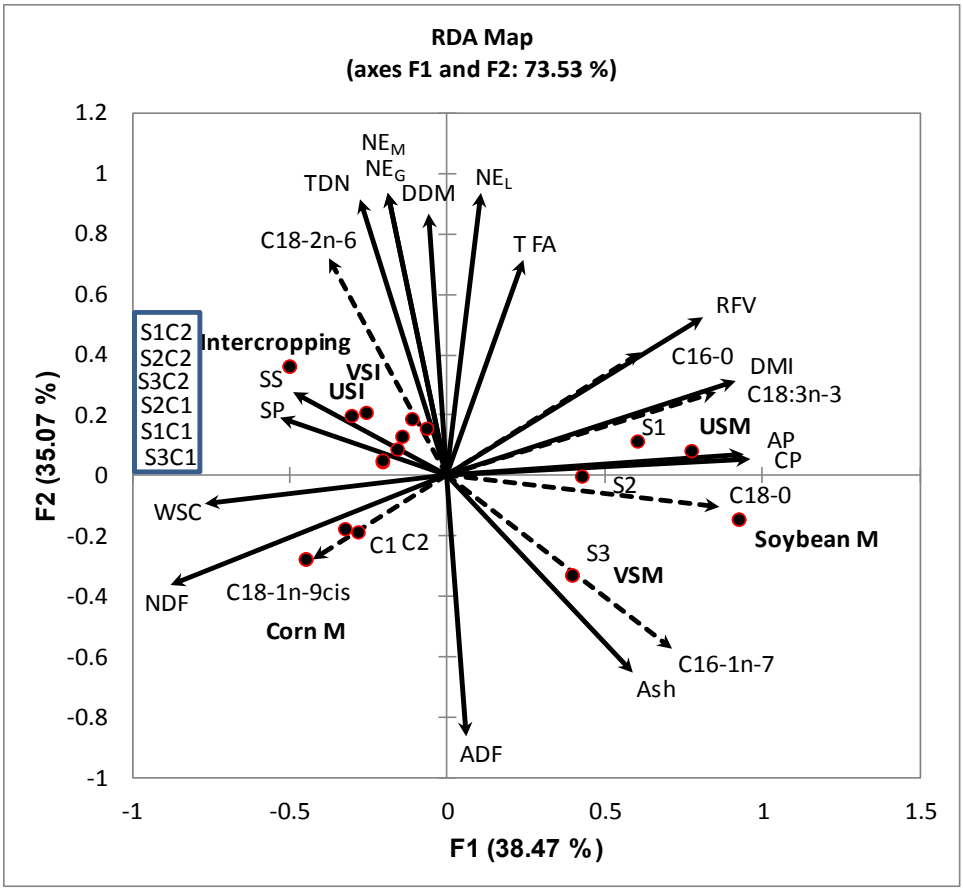

Figure 3.5: Redundancy analysis (RDA), showing the relationship between the fodder quality parameters and FA contents of forage obtained from corn and soybean cultivated as either monocrop or intercrop under cool climatic conditions.

The FA contents are represented by bold dashed arrows and fodder quality as bold solid arrows. CP (crude protein); AP (available protein); SP (soluble protein); SS (soluble sugars); TDN (total digestible nutrients); NE_L (net energy lactation); NE_M (net energy maintenance); NE_G (net energy gain); WSC (water soluble carbohydrates); NDF (neutral detergent fiber); ADF (acid detergent fiber); DMI (dry matter intake); DDM (digestible dry matter); RFV (relative feed value) T FA (total fatty acids). Corn M = corn monocropping; Soybean M = soybean monocropping; VSM = vine soybean monocropping; USM = upright soybean monocropping; I (C+US) = corn intercropped with upright soybean; I (C+VS) = corn intercropped with vine soybean.

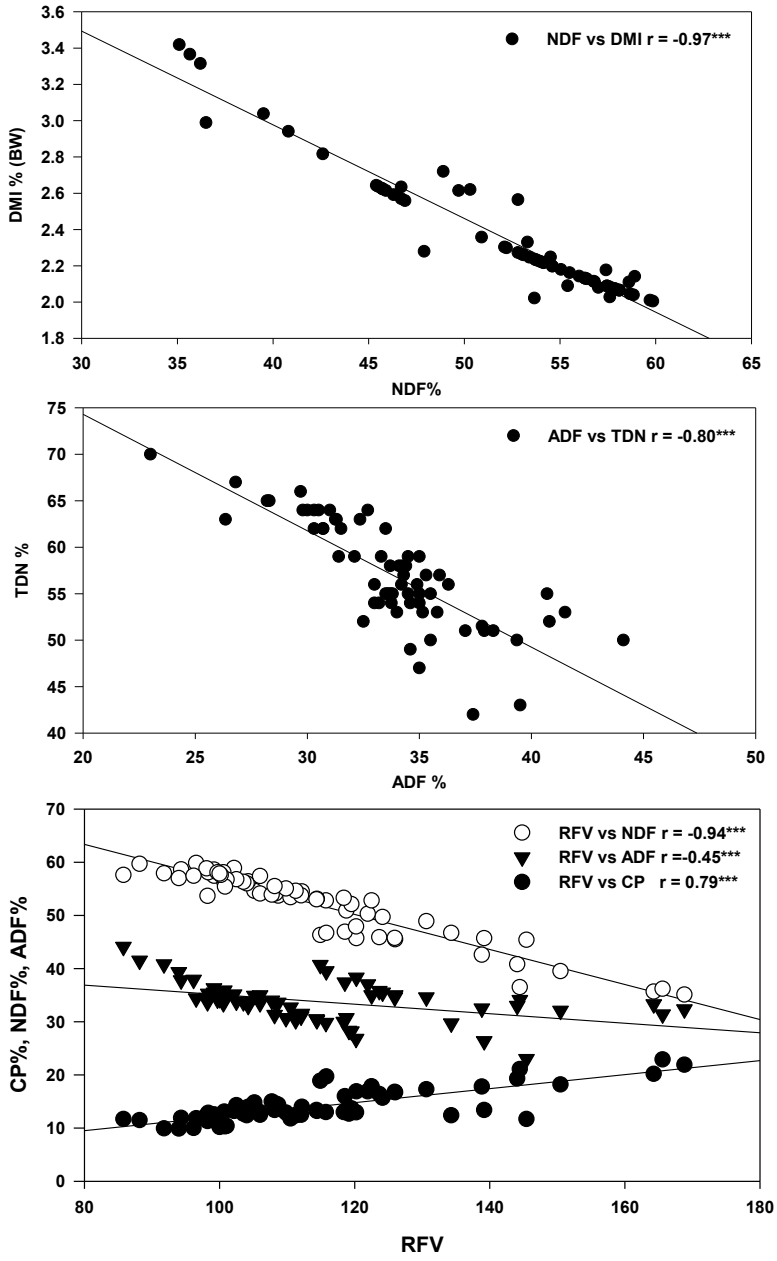

Figure 3.6: A Pearson correlation between NDF and DMI; ADF and TDN; RFV and CP, ADF and NDF for different corn-soybean monocropping and IC treatments.

CP = crude protein, NDF = neutral detergent fiber; ADF = acid detergent fiber; RFV = relative feed value; DMI = dry matter intake and TDN = total digestible nutrients. ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) n=33 for all parameters

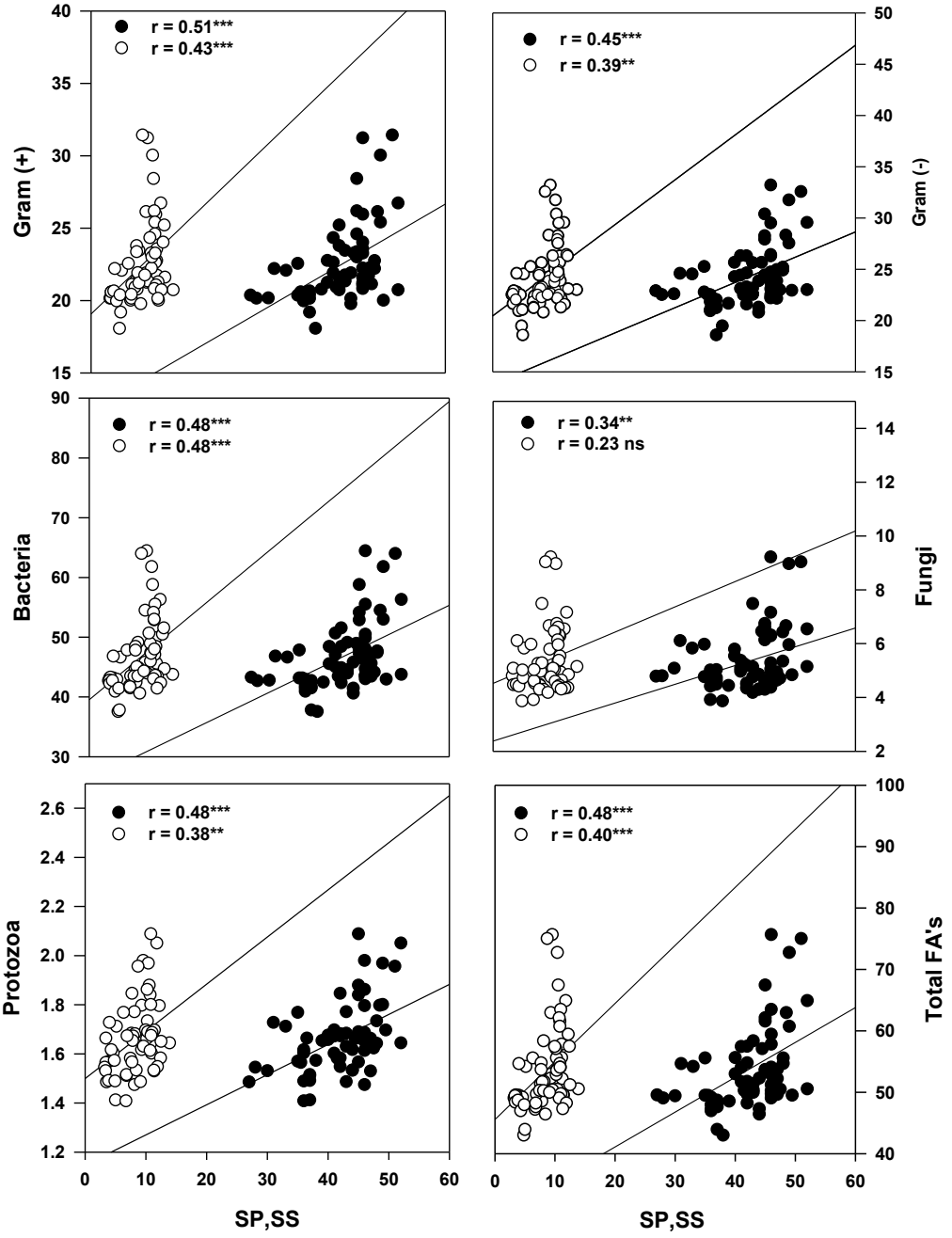

Figure 3.7: A Pearson correlation between SP and SS with microbial PLFA's for different corn-soybean monocropping and IC treatments.

SP = soluble protein, SS = soluble sugars. ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) n=33 for all parameters

Table 3.11: Pearson correlation coefficients between forage FAs and quality.

	C16:0	C16:1n7	C18:0	C18:1n9	C18:2n-6	C18:3n-3	T FA
CP	0.62***	0.64***	0.86***	-0.36**	-0.35**	0.86***	0.31*
AP	0.65***	0.64***	0.83***	-0.31*	-0.37**	0.82***	0.31*
SP	-0.15^{ns}	-0.46***	-0.19^{ns}	0.50***	0.05^{ns}	-0.20^{ns}	0.13^{ns}
ADF	-0.28*	0.49***	0.19^{ns}	0.09^{ns}	-0.55***	-0.14^{ns}	-0.55***
NDF	-0.62***	-0.38**	-0.71***	0.53***	0.07^{ns}	-0.86***	-0.41***
WSC	-0.40***	-0.54***	-0.47***	0.44***	0.14^{ns}	-0.58***	-0.17^{ns}
SS	-0.09^{ns}	-0.45***	-0.20^{ns}	0.49***	0.08^{ns}	-0.18^{ns}	0.18^{ns}
Ash	0.10^{ns}	0.89***	0.52***	0.07^{ns}	-0.81***	0.26*	-0.37**
TDN	0.20^{ns}	-0.68***	-0.34**	-0.12^{ns}	0.70***	-0.03^{ns}	0.52***
NEL	0.40***	-0.43***	-0.02^{ns}	-0.37**	0.61***	0.31*	0.60***
NEM	0.25*	-0.62***	-0.26*	-0.19^{ns}	0.69***	0.06^{ns}	0.54***
NEG	0.24*	-0.62***	-0.26*	-0.19^{ns}	0.69***	0.07^{ns}	0.54***
DDM	0.28*	-0.49***	-0.19^{ns}	-0.10^{ns}	0.55***	0.14^{ns}	0.55***
DMI	0.64***	0.44***	0.77***	-0.54***	-0.11^{ns}	0.90***	0.41***
RFV	0.65***	0.26*	0.65***	-0.51***	0.05^{ns}	0.86***	0.53***

ns = correlation is non-significant; *Correlation is significant ($p \leq 0.05$); **($p \leq 0.01$); ***($p \leq 0.001$) n=66 for all parameters)

3.5. Discussion

3.5.1. Forage nutritional quality:

Overall IC significantly increased the CP contents than corn MC in this study. That increase accounted for almost 22% in IC treatments compared with corn MC treatments. The high CP content observed in soybean MC treatments mean the increase in CP % is due to mixing of soybean with corn as an IC system for forage produced under cool climatic conditions. The present results were consistent with other studies done on IC under warmer growth temperature conditions. For example, Baghdadi et al. (2016) reported 30 % increase in CP % due to IC compared to corn MC. Liu et al. (2006) also reported increases in CP ranging from (31 to 59%) due to IC of corn with alfalfa compared to corn MC. This increase in CP content of forage produced following cultivation by IC have been reported by several researchers (Abdulraheem et al., 2012; Anil et al., 2000; Dahmardeh et al., 2009; Javanmard et al., 2009; Lithourgidis et al., 2007, 2006; Strydhorst et al., 2008). Htet et al.,(2017, 2016a) observed that corn intercropped with legumes produced forage with significantly increased CP content compared to forage obtained from corn MC. The higher protein contents obtained from forage produced under IC systems can reduce the requirement for protein supplements during animal feed formulation (Anil et al., 2000). Other important features of a high-quality forage are the concentrations of ADF and NDF (Bingol et al., 2007; Contreras-Govea et al., 2006; Lithourgidis et al., 2007, 2006). During ration formulation, the NDF content is very crucial because it's a major determinant influencing the animal forage consumption (Bingol et al., 2007; Lithourgidis et al., 2006). My results demonstrate a decrease in ADF and NDF contents (-14 to -15 %) and (-9 to -11

%) in IC as compared to corn MC, when cultivated under cool climatic conditions (Table 3.2 & 3.3). This is consistent with earlier findings showing that IC corn with soybean decrease the NDF contents significantly compared to forage obtained from corn cultivated as a monocrop (Htet et al., 2016b). The presence of leguminous crops in the IC system reduced the ADF and NDF concentration in the present study (Table 3.2 & 3.3). The leguminous crops are lower in NDF and ADF concentration compare to grasses (Costa et al., 2012). NDF concentration in forage can also be affected by the crop maturity, because maturity changes the levels of hemicellulose, lignin and cellulose which are essential parts of the plant cell wall (Mugweni et al., 2000). Forage quality is negatively related to NDF and ADF, and lower values are required for both in higher quality forage. This is consistent with the findings in this study, when forage was produced as intercrop with corn and soybean. These finding are also supported by the work published by other authors in the literature (Aasen et al., 2004; Lauriault and Kirksey, 2004; Sleugh et al., 2000; Strydhorst et al., 2008). However, in these systems the crops were not produced under cool climatic conditions. Conversely, some studies reported IC had no effect (Costa et al., 2012) or increased (Gill and Omokanye, 2018) the NDF and ADF levels in the forage produced. These findings are in contrast to the results observed in the present study conducted under cool climatic conditions. Similar to the NDF values, the WSC were lower in IC treatments compared to corn MC treatments. Legumes especially soybeans are known to contain low WSC but have high protein concentration (Contreras-Govea et al., 2006). In this study IC treatments produced WSC lower than corn MC, but higher than soybean MC. These findings are in agreement with the results reported for soybean intercropped with millet

(Jahanzad et al., 2014) and corn intercrop with beans (Dahmardeh et al., 2009; Htet et al., 2016a). These finding suggest that increasing the ratio of legumes in cereal-legume IC systems will decrease the WSC (Contreras-Govea et al., 2006). Legumes contain higher ash contents than grasses (Paulson et al., 2008). This was observed in the present study where both vine and upright soybean cultivated as monocrops had superior ash contents compared to corn cultivated as monocrops. Consequently, the ash content was enhanced in the mixed forage obtained from IC silage corn with forage soybeans. The enhanced ash contents we observed in the forage obtained from corn intercropped with soybean in agreement with the reports in the literature for corn-kale, corn-sunflower and corn-runner bean IC systems (Anil et al., 2000). Present results were also supported by (Dahmardeh et al., 2009) who got the same trend and increase (10 %) due to IC treatments.

In cool climates, forage energy is a significant nutritive consideration in beef cattle production because it is beneficial in improving animal growth and productivity. TDN refers to those nutrients that livestock can utilize and are negatively correlated with the ADF contents in the forage (Sadeghpour et al., 2013). As ADF contents increases in the forage, TDN decreases indicating animals are less able to utilize those nutrients present in the forage. The negative correlation is similar to the present result (Figure 3.6), where IC treatments generally increased TDN contents 8 to 10 % compared to corn MC; and 12 % compared to soybean MC. These finding are consistent with those of Salama and Zeid (2016) and Gill and Omokanye (2018). The forage having a range of 55 to 65% TDN is considered a good quality forage. All the IC treatments in the present study were in the range of 55-65 % during both growth years. There is a rule of thumb for TDN (55-60-

65%), briefly a mature beef cow needs 55 %, 60 % and 65 % energy for mid, late pregnancy and after calving period respectively to maintain her body condition score (Yurchak and Orkine, 2004). TDN as well as CP percentage are critical in forage quality and have positive correlation with the forage price (Rostamza et al., 2011). IC also enhanced the NE_L (9%), NE_M (13-16%) and NE_G (27-29 %) levels relative to corn MC treatments during 2016 and 2017 (Table 3.4 & 3.5). Lauriault and Kirksey, (2004) found an increase in NE_L of forage because of pea-wheat or pea-oat IC, while no effect was observed in pea-barley and pea-rye IC systems. Sadeghpour et al. (2014, 2013) also found an increase in the NE_L of forage produced from barley and annual medic IC compared to barley cultivated as a monocrop. Crude protein and sugars are highly digestible nutients (Stoltz et al., 2013), and were observed to enhance the quality of the forage when produced under corn-soybean IC systems in cool climatic conditions. Concomittant with the CP and SS contents, the digestible dry matter increased 5-6% in the IC treatments evaluated in this study. This indicate the superior DDM content of the forage may be associated with the enhanced level of CP and SS present in the mixed forage obtained from soybean and corn IC (Stoltz et al., 2013). Present results are in harmony with Dahmardeh et al., (2009) who concluded that IC of corn and cowpea can increase the DDM than corn MC. Sadeghpour et al. (2013, 2014) reported similar results for DDM when annual medic intercropped with barley. Cereals and grasses intercropped with legume crop (forage cow pea) can produce a forage with higher DDM (Salama and Zeid, 2016). The higher DDM in IC forage compared to corn MC forage may be due to higher CP and sugar contents (Stoltz et al., 2013), because both are highly digestible nutrients. Animal productivity is directly related to voluntary

intake of forage (Ullah, 2010). Higher voluntary intake means higher DMI that ultimately results in higher nutrient intake. DMI was higher in soybean MC treatments and lower in corn MC treatments. However, the IC treatments were intermediate between both soybean and corn MC. These results agree with forage cow pea intercropped with pearl millet and grasses (Salama and Zeid, 2016). DMI is higher for leguminous forage than non-leguminous forage (Ullah, 2010). In the present study, there was a negative correlation observed between DMI and NDF indicating as NDF increases the quality and DMI of forage decreases (Figure 3.6), supported by (Caballero et al., 1995).

RFV is an important index that used to forecast the energy value and forage consumption (Lithourgidis et al., 2006) and can be calculated from DMI and DDM. Similar to the trends for net energies value, the RFV was also higher (12-18 %) for IC treatments compared to corn MC treatments. Higher RFV's have been reported for barley legume IC compared to when barley was cultivated as monocrops (Sadeghpour et al., 2014, 2013; Salama and Zeid, 2016; Strydhorst et al., 2008). The RFV is known to increase as NDF and ADF values decreases in the forage (Strydhorst et al., 2008). RFV's for beef cows should be in the range 90-115 RFV as suggested by Schroeder (1996) and are consistent with the values observed for the IC treatments in the present study (Tables 3.4-3.5). In the present study RFV showed significant positive correlation with CP while a significant negative correlation with NDF and ADF.

3.5.2. Forage mineral contents:

Legumes contain higher total macro and micro minerals than grasses (Paulson et al., 2008). Ca is two to three times higher in legumes than all other major forages (Paulson et al.,

2008). IC increased the Ca contents in the forage compared to MC (Htet et al., 2016b). Higher Ca contents in IC compared to corresponding MC has been observed previously (Carr et al., 2004; Gill and Omokanye, 2018). Conversely, no difference between IC and MC treatments for forage nutrients such as Na, K, P and Mg have also been reported (Htet et al., 2016b). IC (1M3S) improved the forage nutrient (Mg, P and Ca) composition compared to corn MC (Htet et al., 2017). The maximum Ca contents were recorded for soybean MC treatments that showed the contributions are coming from soybean (Table 3.6). All the IC treatments studied showed produced forage with adequate quantity of Ca (1.8 g/kg) required for dry gestating beef cows (NRC. 2000). All the IC treatments evaluated in the present study met the required Mg (1.2 g/kg) and (2.0 g/kg) quantity for a dry gestating beef cow and for a lactating beef cow respectively (NRC. 2000). P and Mg was also in the range of required quantity for dry gestating beef cow and lactating beef cow (NRC. 2000). This indicate the forage produced by the IC treatments were of superior mineral contents when cultivated under cool climatic conditions (Table 3.6-3.8). Consistent with these findings, an increase in the K, Ca and Mg contents of forage produced as intercropped have been reported previously in the literature compared to corn MC (Serbester et al., 2015). The important micronutrient Zn (30 mg/kg), Fe (50 mg/kg), Cu (10 mg/kg) and Mn (40 mg/kg) content observed in the IC treatments in this study (Table 3.7-3.80) are also in the range required for a mature beef cow (NRC. 2000).

3.5.3. Forage fatty acid composition:

In the present results unsaturated FAs (UFA) especially PUFAs were higher in all the treatments compared to saturated FAs (SFA), that may be because of low temperature in

region (Table 3.9 & 3.10). Plants respond to lower temperature by increasing UFA especially PUFA contents (Falcone et al., 2004; Iba, 2002; Routaboul et al., 2012) because they play a role to maintain the chloroplast fluidity (Elgersma, 2015). It is known that forages are often the main source of PUFA and that forages with higher PUFAs can modify the FA profile of the dairy animal milk (Hatfield et al., 2007; Khan et al., 2012). Milk FA profile is of prime importance as it is a significant part of human diet (Elgersma, 2015) and that FA profile depends on the animal's diet (Kalac and Samkova, 2010). PUFA contained two major FAs (18:2n-6 and 18:3n-3) in the forage. The contribution of 18:2n-6 is almost 91 % and 18:3n-3 is 9 % in the maize silage (Khan et al., 2015b). When the conditions are normal in the rumen, the hydrogenation of C18:2n-6 results into an increase in the concentration of cis-9, trans-11 C18:2 and trans-11 C18:1 which are very valuable for human health (Khan et al., 2015b). In the present study, an increase (40% to 100 %) in C18:3n-3, while a decrease (14% to 15%) in C18:2n-6 was observed in the forage due to corn-soybean IC (Table 3.9 & 3.10). The higher (18:3) and lower (18:2) may be the contribution of intercropped soybean varieties, because soybean showed higher C18:3n-3 but lower C18:2n-6 FA contents in the forage compared to corn. The corn forage was high in C18:2n-6 but low in C18:3n-3 FA contents and in agreement with earlier findings (Khan et al., 2015b). An increase of omega 3 in the intercropped forage may assist in modulating the PUFA profile of the milk produced from animals fed a ration containing this forage in the formulation by increasing the n-3/n-6 and decreasing n-6/n-3 ratio (Kliem et al., 2008). Dairy cows fed with diet high in 18:3 was observed to produced milk enhanced with PUFA especially alpha linoleic acid (ALA) and *c*9, *t*11 conjugated FAs (Mach et al., 2013). High

n-6/n-3 ratio can cause coronary heart disease, particularly blood clotting (Enser et al., 1998). The conjugated linoleic acid (CLA) which formed by the isomerization of linoleic acid (C18:2n-6) with the help of ruminal bacteria (Harfoot and Hazlewood, 1997) and the inclusion of optimum level of CLA and other PUFA's in the feed of dairy cows can increase pregnancy rate (de Veth et al., 2009; Lopes et al., 2009). The milk rich in CLA can be advantageous for human health (Belury, 2002). Thus, by increasing of (18:3) and (18:2) in the ruminant feed, we can enhance the availability of CLA in the ruminant products (Rochfort et al., 2008).

3.5.4. Relationship between forage quality and forage FA contents:

All the quality parameters showed a significant ($p \leq 0.05$) positive correlation with the major forage FA contents, while a negative correlation was observed between NDF, ADF, WSC, SS, SP and total and major FA contents (Table 3.11, Figure 3.5). This showed that FA contents are negatively correlated with plant maturity. As the plant maturity increases, the ADF and NDF contents increases, while FA contents decreases in forage produced under cool climatic conditions. The FA contents decrease with plant maturity, due to low leaf/stem ratio, flower initiation and leaf senescence (Khan et al., 2015a, 2012). ADF and NDF are significantly ($p \leq 0.001$) but negatively correlated with RFV. However, a significant positive correlation between RFV and CP showed that the forage contain higher CP contents is of higher quality than a forage with high NDF and ADF contents. They can decrease the animal feed intake and TDN in the forage as the DMI is negatively correlated with NDF (Caballero et al., 1995) and TDN is negatively correlated with ADF (Sadeghpour et al., 2013) Figure 3.6. Soil microbial community composition showed a significant

positive correlation with potassium contents of the forage (Figure 3.2). These findings indicate that the increased bioaccumulations of K in forage crops produced under cool climatic conditions may be because of higher mineralization of nutrients in the soil by the active soil microbial community (bacteria, protozoans and fungi).

3.6. Conclusion

Overall, the present study demonstrated that IC was superior than corn MC treatments in terms of forage quality, mineral composition and FA composition. IC also increased the total dry matter yield compared to MC treatments. The CP, DMI, DDM, TDN, NE_L, NE_M, NE_G values were higher in IC compared to corn MC, while ADF and NDF values were reduced, when silage corn was intercropped with forage soybeans under cool climatic conditions. The trend showed that soybean varieties IC with C2 perform better compared to C1 in terms of forage quality. In comparison between soybean varieties, upright soybeans performed superior compared to vine soybean variety. IC resulted in higher omega 3 and lower omega 6/omega 3 compared to MC of corn. Mineral uptake was also superior because of IC and positive contribution from soybean plant. Upright (S1, S2) soybean varieties produced forage with superior macronutrients, while vine soybeans (S3) produced forage with higher micronutrients. The active microbial community structure was significantly associated with the potassium, SP and SS content in forage soybean and silage corn cultivated as mono or intercrops under cool climatic conditions. Overall, the results of this study demonstrate that silage corn intercropped with forage soybeans can be used to produce forage with improve nutritional composition under cool climatic conditions.

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Chapter 4

4. Development of a novel imaging technique using LA-ICP-MS to show the spatial distribution of elements in soil cores

4.1. Abstract

Laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS) is a relatively new analytical technique that has been demonstrated as a robust mapping method to create qualitative images delineating the spatial distribution of elements including micro and macro nutrients in different samples. Primarily, LA-ICP-MS imaging has been applied to biological samples. To our knowledge, though this approach has been popular in the assessment of the elemental composition in environmental and geological samples; However, there is no demonstration of this technique in imaging the spatial distribution of elements in soil cores. To address this issue, we developed a novel imaging method to visualize the spaital distribution of select macro and micro nutrients in soil cores as a proof concept. Soil cores were collected in plastic liners at depths of 15cm, frozen at -20 degrees; then cut uniformly in halves using a band saw to create flat, even soil core surfaces with homogenous distribution of the intact soil particles. The cores were ablated using a 213nm laser optimized with the following parameters: spot size of 100 μm, laser energy of 7 mJ, and scan speed of 220 μms^{-1}. ICP–MS analysis was also performed simultaneously following acid digestion of the elements using one half of the soil core to quantify the elements present in the soil. The developed method was then used to assess the spatial distribution of the selected elements at a depth of 15cm following different land use

management systems. Two–dimensional images of the soil cores were created for Ca, Mg, P, K, Na, Zn, Fe, Co, and Mn using the iolite software; demonstrating proof of concept of the method in imaging macro and micronutrients in the soil. Application of different crop management systems and fertilizer applications was demonstrated to significantly alter the levels of Ca, Mg, P, K, Na Zn, Fe, Co, and Mn across the different land use management systems. The new imaging method was very effective in showing the spatial distribution and hot spots of the different mineral elements within the top 15cm depth of the soil in response to changes in the management systems. The results show that the developed method has great potential in elemental imaging of nutrients in in the soil using soil cores. This could have huge implications in environmental impact assessments, soil resource evaluation, agriculture crop production and the effectiveness of land use or land management systems in modulating the spatial distribution of elements within the soil.

Keywords: Environment and elemental imaging, soil core, nutrient, management, mapping

4.2. Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS) is a relatively new elemental imaging technique used to discern the spatial distribution of metals and non-metals in biological and environmental samples (Becker et al., 2008; Bulska and Wagner, 2016; Dean, 2005; Hill et al., 2005; Russo et al., 2002; Wu et al., 2009). This techniques confer several superior advantages in terms high sample throughput, sensitivity, spatial resolution and minimal sample preparation (Mokgalaka and Gardea-Torresdey, 2006; Ohata et al., 2002) compared to other elemental imaging techniques (micro synchrotron X-ray fluorescence (μ-SR-XRF) spectroscopy (Hachmöller et al., 2016), synchrotron-radiation XRF (Carlier et al., 2016), micro proton-induced X-ray emission (μ-PIXE) spectroscopy (Buso et al., 2005; Novak et al., 2012), energy-dispersive X-ray spectrometry (EDXS) (Topolovec et al., 2013), and secondary-ion mass spectrometry (SIMS) (Brunelle et al., 2005). Elemental imaging by LA–ICP–MS has been widely used to study biological tissues, where animal and human brain were frequent subjects. For example, Becker et al. (2008), and Sussulini and Becker (2015) performed imaging of essential and toxic elements in the rat brain. Hanć et al. (2017) used the technique to investigate the Ca, Mg, Zn, Cu, Mn, Pb, Al, Ba and Sr distribution patterns in bird feathers. While Theiner et al. (2016) used the technique to demonstrate the effects of drug accumulation in multicellular tumor spheroids.

Though LA-ICP-MS technique has been used routinely to study environmental and soil samples. There is no report to our knowledge of a method developed for imaging the spatial

distribution of elements in soil cores using this technique. This is of major significance considering soil cores are one the most common tool used to gather soil samples to evaluate the soil nutrient composition for crop production, resource prospecting, environmental impact assessment, and land use management system responses among others. Minerals are of prime importance for plant growth owing to their essentials roles for optimum plant growth and development (Uchida, 2000). For instance, phosphorus (P) and copper (Cu) are required by growing plants for photosynthesis, respiration, and energy storage, and therefore any deficiency can delay the crop maturity (Uchida, 2000). Potassium (K), on the other hand, plays a crucial role as an enzyme activator and a water regulator. Ferrous iron (Fe) is involved in chlorophyll production as do magnesium (Mg), and their deficiencies can cause leaf chlorosis (Uchida, 2000). However, excesses of these nutrients can also result in toxicity and impaired plant growth (Uchida, 2000). Higher Cu concentrations in the plant cell can produce reactive oxygen species that can impede plant metabolism (Salt et al., 2002). Therefore, it is important to know the spatial location of theses essential and toxic elements in the soil profile. Knowledge of the spatial location can confer many advantages: 1) we could assess the spatial location and concentration of essential nutrients, as well as toxic elements in the soil to guide land use. Management decisions could be made to select crops with the most suitable root morphology for better nutrient harvesting. 2) Furthermore, we could investigate contaminants (heavy metals) levels and their locations in agricultural soils caused by agro-chemicals spill or soil affected by industrial operations such as smelting. Soil contamination can cause accumulation of potentially toxic metals in crops that can affect crop productivity, quality, human and animal health.

The effectiveness of phytoremediation or other techniques to removed toxic metals from soil following anthropogenic contamination could also be assessed. Therefore, LA–ICP–MS is a powerful tool for mapping the element distribution both in environmental, as well as in biological solid samples (Becker et al., 2014; da Silva and Arruda, 2013; Ek et al., 2004; Hanć et al., 2013; Koch and Günther, 2011). The major advantages of LA–ICP–MS is the ability of multi–elemental analysis with high sensitivity, fast sample throughput, and high spatial resolution (Ahmed et al., 2017; Falciani et al., 2000). Even though, the sample preparation can be relatively straight forward and easy, the quantification of elements is still a major challenge because the precision and accuracy of LA–ICP-MS is still inferior than ICP–MS (Ohata et al., 2002).

The availability of appropriate matrix match reference standards, optimizing the instrument operational condition, homogeneity of sample, elemental fractionation and matrix effects can affect the ability to reproduce elemental images with high accuracy and spatial resolution, and are major areas of current investigations in the scientific community (Austin et al., 2011; Hare et al., 2012; Hoesl et al., 2014; Reifschneider et al., 2013). However, the accuracy and precision of the methods germane to the type of sample to investigate and elements of interests for analysis can be improved through the optimization of instrument conditions, and the adoption of suitable correction strategies and reference materials (Liu et al., 2013). For the quantification of mineral distribution, internal standards are needed. Therefore, due to unavailability of suitable matrix matched reference standards, validation using suitable wet chemistry, as well as a complimentary imaging technique with high spatial resolution should be done. In wet chemistry analysis, samples are

digested with an acid (HNO_3) and run separately on ICP–MS for quantification using suitable external standards for calibration. ICP–MS is a robust technique for the analysis of minerals in the soil to trace and ultra–trace levels due to its ability to analyze multi–elements with high detection power and sensitivity requiring very small sample volume (Ahmed et al., 2017; Falciani et al., 2000).

Microwave digestion in tightly sealed vessels followed by ICP–MS analysis is one of the most often used methods for soil sample analysis. The reason for using high pressure and sealed vessels is the lower digestion process time and to avoid any sample contamination (Falciani et al., 2000). This wet chemistry method is a suitable analytical technique to use complimentary to LA-ICP-MS in developing and validating any new elemental imaging technique. To our knowledge, this is the first study attempting to use LA-ICP-MS as technique to image the spatial distribution of elements in undisturbed soil cores. The purpose of the present work was to develop a method for elements (Ca, K, P, Mg, Zn, Fe, Na, Mn and Co) mapping (imaging) to understand their spatial distribution within the first 15 cm of the soil depth by LA–ICP–MS, following quantitation of the elements in acid digested samples and analysis by ICP–MS. The second objective was to evaluate the effectiveness of the new imaging method in assessing the effects of different nutrient management practices on spatial nutrient distributions in the plant root zone.

4.3. Methodology

4.3.1. Experimental treatments and soil sample collection

The experiment was conducted at Pynn's Brook Agricultural Research Station, Pasadena, Newfoundland (NL) (49.0130° N, 57.5894° W), managed by the Department of Fisheries, and Land Resources, Government of NL, Canada. Undisturbed soil core samples were collected (n=4) from the experimental area established to evaluate the effects of different agronomic practices on forage production using organic (dairy manure: DM) and inorganic fertilizer (IF) as nutrient sources, as well as inter and monocropping (MC) systems. For the current study, a total of four different treatments were selected including: 1) corn–MC fertilized with IF; 2) corn–soybean intercropping (IC) fertilized with IF and 3) corn fertilized with DM; 4) corn fertilized with DM and biochar added as a soil amendment (BC) (Figure 4.2). Undisturbed soil cores were collected using 3.8 cm x 15.0 cm plastic liners (Osprey Scientific Inc. Edmonton, AB. Canada) inserted in the auger of the soil core sampler (AMS, Inc. USA). Samples were collected close to the crop maturation period. Core samples were properly labeled, and then transferred to the lab in the sealed bags and stored at –20 °C to prevent core disturbance until further analysis.

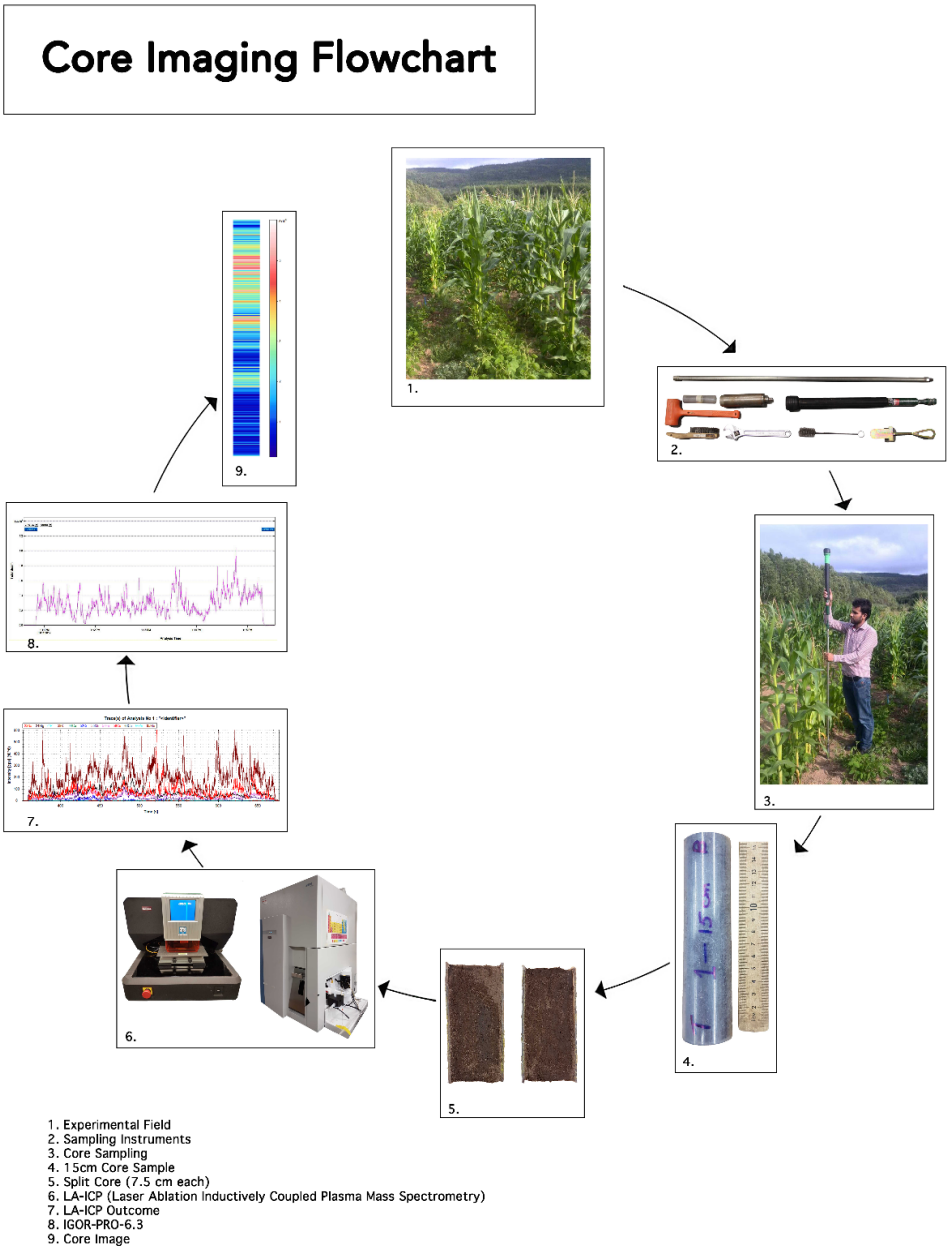

Figure 4.1: Flow chart showing all the steps involved from undisturbed core sampling until the final image

Figure 4.2: Experimental treatments A) corn–MC fertilized with IF; B) corn fertilized with DM; C) corn–soybean intercropping (IC) fertilized with IF and D) corn fertilized with DM and biochar

4.3.2. Sample preparation for LA–ICP–MS imaging

A total of four soil core samples were collected from each experimental treatment. Each core (15 cm) sample was then cut into two halves longitudinally using a band saw, and each halve consisted of 7.5 cm in length, so it could fit in the LA drawer for image analysis. The individual core width and length were 3.81 cm × 7.5 cm. However, the top 7.5cm (0-7.5cm) and bottom 7.5cm (7.6-15cm) were stitched during the image analysis to obtain a total depth of 15cm for each sample treatment. Each core sample was then ablated from

top to the bottom across the entire sample surface by using the single line scan method as described in Table 4.1.

4.3.3. Imaging of macro and micro elements in soil core by LA–ICP–MS

Laser ablation of soil core was performed at 213 nm wavelength using a solid-state laser (Nd: YAG) to get the spatially distributed elements in the soil core in an oxygen purged laser ablation chamber. The LA-ICP-MS method was optimized using a test core sample and the final instruments parameters suitable for sample analysis determined as follow: The laser was warm up for 20 s before starting the ablation by firing the close shutter. The optimization of spot size, scanning speed, laser energy and repetition rate was performed following ablation of the test sample to get a high-resolution image with good signal intensity and short time of scanning. The laser repetition rate was 20 Hz with 7 mJ energy applied to ablate a spot size of 100 μm, and the ablation done using helium as a carrier gas. The conditions used both for LA and ICP–MS methods are given in Table 1. Core ablation was done by parallel line scans. Ablated soil core samples were then transferred to the ICP-MS by using helium as a carrier gas. The data from LA was recorded on ICP–MS (iCAP Q. Thermo Scientific, Canada). All the selected macro and micro elements were recorded with a dwell time of 0.01s. The ICP–MS was tuned daily to check its performance. The created data files were imported to the Iolite software (Iolite Version 3.4) as an add–on to Igor Pro (WaveMetrics, Inc. Igor Pro 6.37) as a universal file type (CSV) format. The elements maps (images) were created by using data reduction scheme including baseline subtraction. The procedure from core sampling to final image creation is depicted in Figure 4.1.

Table 4.1: LA-ICP-MS system operating conditions for optimized imaging of soil core samples

Laser ablation	
Instrument	ESI NWR 213, Nd-YAG
Scan method	Single line scan
Spot size (μm)	100
Scan speed (μms^{-1})	220
Repetition rate (Hz)	20
Laser energy (mJ)	7
Mass flow ($mL\ min^{-1}$)	800
Wavelength	213
Carrier gas	Helium
Laser mode	Continuous
ICP-MS	
Instrument	iCAP Q. Thermo Scientific, ICP-MS
Auxiliary gas flow ($L\ min^{-1}$)	0.79
Nebulizer gas flow ($L\ min^{-1}$)	1.01
Plasma gas flow ($L\ min^{-1}$)	14
RF Power (W)	1548
Lens setting	Autolens calibrated
Detector mode	STD
Dwell time (s)	0.01

4.3.4. Quantification of micro and macro elements by ICP–MS

Quantification of micro and macro elements was performed by the method describe by Hassan et al., (2007) with some modifications. A multi element (43) highly pure ICP–MS standard solution IV–ICPMS–71A obtained from Inorganic™ Ventures, Inc. (Christiansburg, VA 24073, USA) was used for external calibration during quantitative analysis. Seven-point calibration (10, 20, 50, 100, 200, 300 and 500 ppb) was done for 9 different elements with R^2 values ranging from 0.988-0.998 (Figure 4.3-4.4). The output generated from standard curves was used to quantify the mineral elements present in the soil cores and values expressed as $g\ kg^{-1}$ for Ca, Mg, P, K, Na (Figure 4.6-4.10) and $mg\ kg^{-1}$ for Fe, Cu, Zn, Co (Figure 4.11-4.14). All the required solution preparation and storage

was done in polypropylene bottles, which were

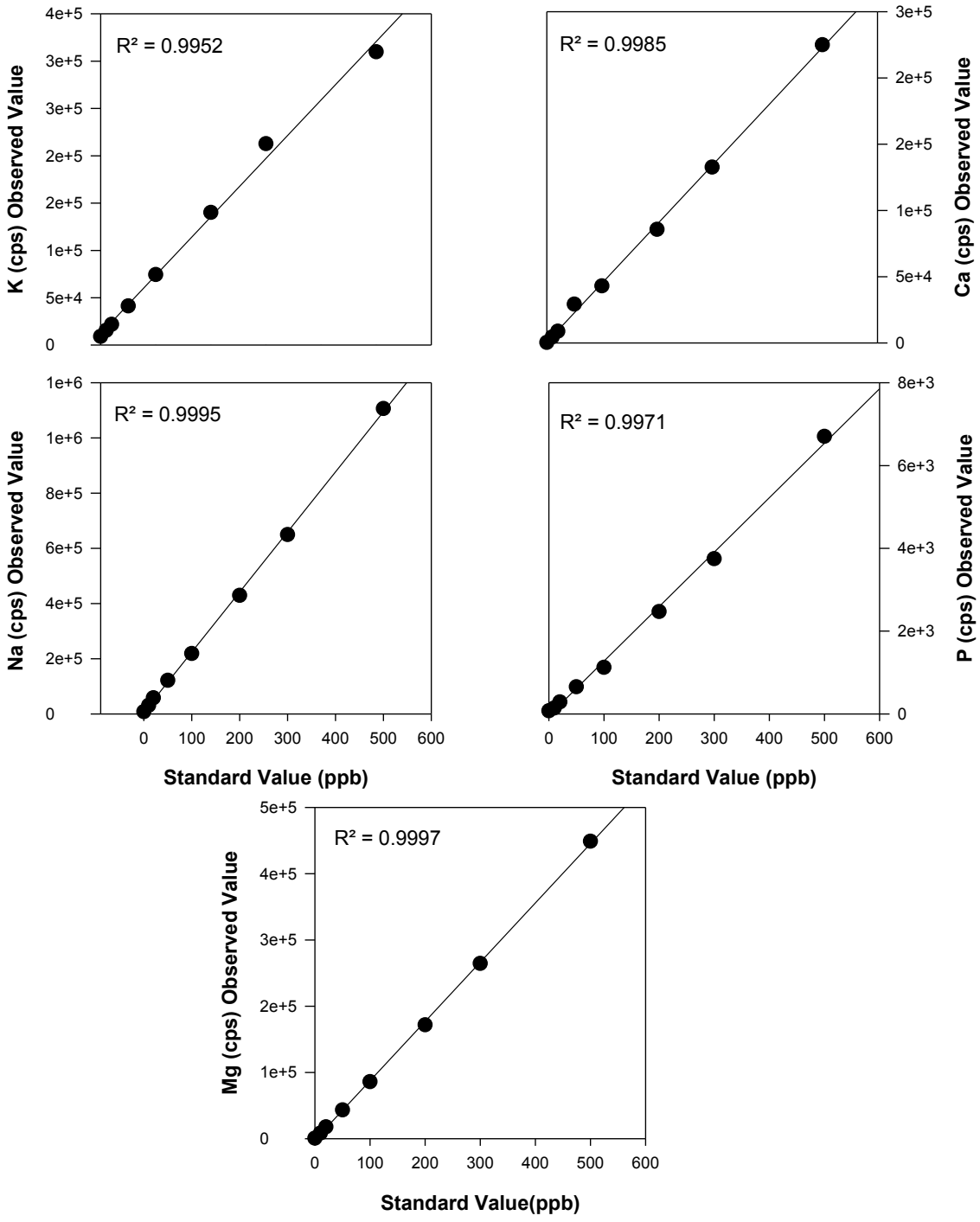

Figure 4.3: Calibration curves for selected macro elements run on ICP-MS

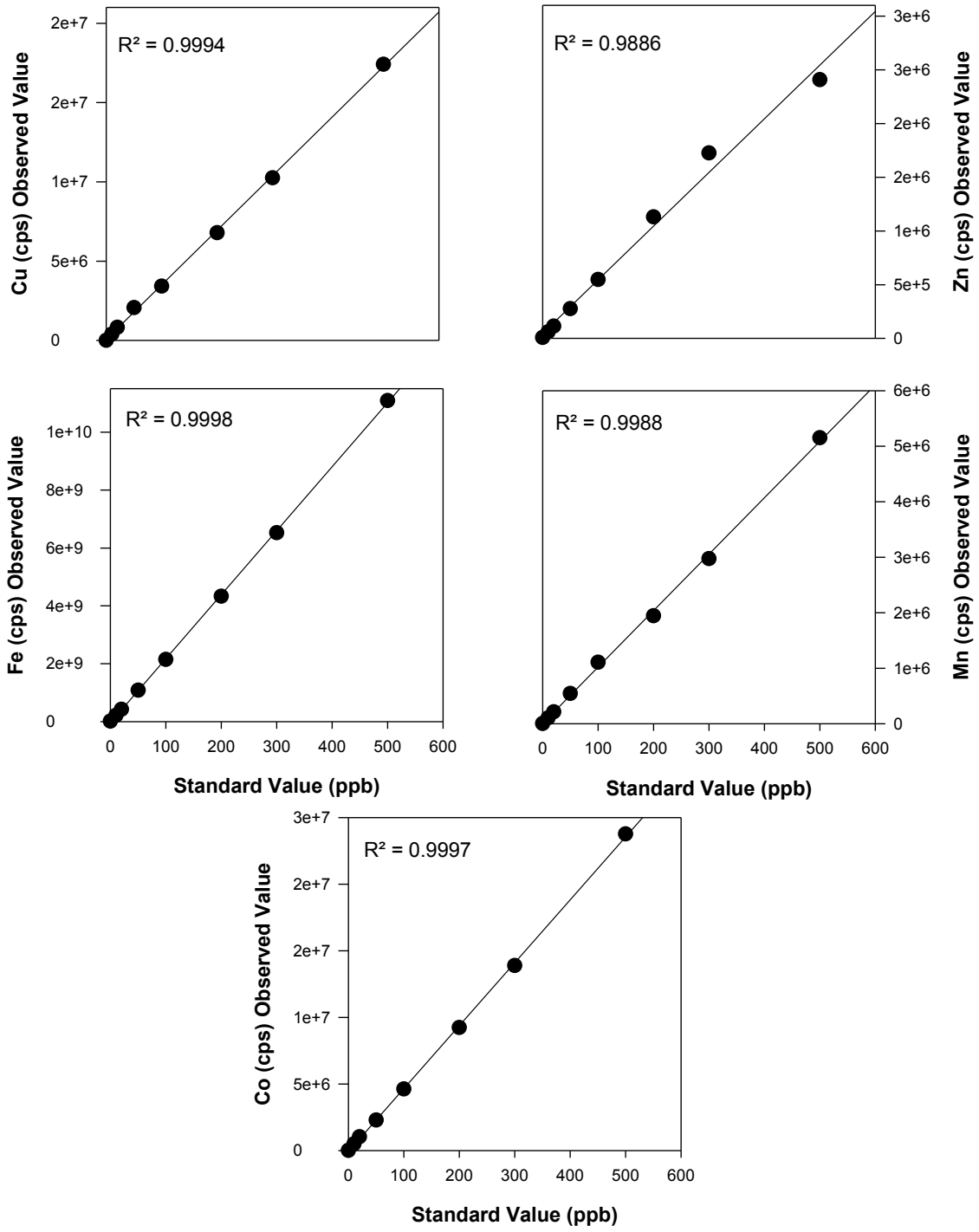

Figure 4.4: Calibration curves for selected micro elements run on ICP-MS

precleaned with 10% HNO_3, and then rinsed several times with ultra–pure water (Barnstead™ MegaPure™ Glass Stills, Thermo Scientific™). The argon purity was 99.99 % (Air Liquide Canada Inc.). The internal standard (0.1 mg L^{-1} Rh) was prepared in 1.5% HNO_3. An aliquot of dried and sieved soil core samples (100 mg) was weighed and placed into pre-cleaned Teflon digestion vessels and digested with 10 mL concentrated (67% – 70%) trace metal grade HNO_3 (Catalog No. A509P212, Fisher Scientific, Ontario, Canada). The soil samples used for quantitative analysis were obtained from the second half of the soil cores obtained after cutting the frozen samples in the liners with a band (the other half was used for the laser ablation- image analysis). All the digestion vessels were tightly packed and placed in a Multiwave Go Microwave Digestion System (Anton Paar United States) which was operated using the following conditions: temperature ramped to 180 °C for 10 min and held at this temperature for 20 min to allow complete sample digestion (Table 4.2).

Table 4.2: Steps involved in soil core sample digestion

Steps	Ramp (mm: ss)	Temperature (^oC)	Power (%)	Hold (mm: ss)
1	10:00	180	100	20:00

After the complete sample digestion, the vessels were allowed to cool down to room temperature for about 10 min. Vessels were opened carefully in the fume hood and transferred to 50 mL Nalgene centrifuge tubes and samples centrifuged for 5 min at 4000 rpm to remove any undissolved soil particles that could block the nebulizer. The supernatant was collected after centrifugation and stored at ± 4 ^oC. The determination of mineral nutrients in the supernatant was done by ICP–MS after sample dilution using

external calibration standards. The instrumental parameters used were: i) auxiliary gas flow 0.79 L min^{-1}; ii) nebulizer gas flow 1.01 L min^{-1}; iii) plasma gas flow 14 L min^{-1}; iv) RF power 1548 W; v) detector mode KED; and vi) dwell time 0.01 s.

4.3.5. Statistical analysis

To evaluate the effects of different nutrient management practices on nutrient distribution within the soil core, one–way analysis of variance (ANOVA) was performed using Statistix–10 software package (Analytical Software, FL, USA). The means were compared using Fisher's LSD test (alpha = 0.05). Graphs were created using Sigma Plot 13.0 software program (Systat Software Inc., San Jose, CA).

4.4. Results and Discussion

4.4.1. Imaging and quantification of essential soil elements

Our first objective was to develop a suitable method to image and quantify mineral nutrients in undisturbed soil cores collected at a depth of 0–15 cm representative of the plant root zone. The spatial distribution of the analyzed elements' as well as quantification of the elements in the core samples (0–15 cm) using LA–ICP–MS and ICP–MS is shown in one–dimensional images and bar charts, respectively (Figure 4.5–4.14). Each image represent line scans (ablation) along the whole soil core from 0–15 cm. There are several parameters that can influence the LA process, such as scan speed, spot size (Hu et al., 2011), laser energy (Shaheen and Fryer., 2012) and repetition rate (Gonzalez et al., 2008). The spot size can affect the instrument sensitivity and elemental fractionation, as well as, the matrix effects, and maybe reliant on the ICP mass load (Zhu et al., 2012). Large spot size can increase the mass-load-induced matrix effect and finally the elemental fractionation because the aerosol transfer to the ICP will increase (Kroslakova and Günther, 2007) that can hamper the precise detection of some elements (Zhu et al., 2012). Conversely, very small spot size can also hamper the LA-ICP-MS process due to severe fractionation (Hu et al., 2011). At higher repetition rate, the effects of spot size are less important than at lower repetition rate (Diwakar et al., 2014). At higher repetition rate >10Hz and spot size >70μm, better results were achieved for elemental ratios (Diwakar et al., 2014). Higer repetition rate can result in higher ablation mass due to more power and laser pulses overlapping on a specific spot (Diwakar et al., 2014). So, optimization is required for all these parameters before imaging so that a high-resolution image can be

obtain in a short scan time. Qualitative results (core images) obtained from LA-ICP-MS showed different essential (micro and macro) elements distribution in the first 15 cm of depth of the plant root zone. Complimentary quantification of the same elements imaged in the soil cores were obtained through ICP-MS following acid digestion (Figure 4.5–4.14). Arroyo et al. (2010) showed a very high correlation between these two complementary techniques LA–ICP–MS and ICP–MS quantification with regression coefficient (R^2 = 0.9983 & 0.9827) using soils as environmental samples. Arroyo et al. (2010) indicated that both methods (digestion–ICP–MS and LA–ICP–MS) generate similar accuracy with a <10% relative standard deviation. Most of the work using LA-ICP-MS in elemental imaging have been applied to biological tissues to image and quantify essential as well as toxic elements in animal and plant samples (Becker et al., 2008; Hanć et al., 2017; Sussulini and Becker, 2015). However, to our knowledge, there is no report of the use of this technique to study the elemental distribution in the plant root zone using undisturbed intact soil cores. Using wet chemistry technique (ICP-MS) complimentary with the elemental imaging technique (LA-ICP-MS), it is possible to image and quantify the spatial distribution of elements in soil cores considering the challenges associated with the availability of suitable matrix match reference standards (Hassan et al., 2007; Liu et al., 2013). We can use this technique to check the contamination of different toxic elements such as (Pb, Sn, Sr) in agricultural as well as commercial soils; and strongly believe that site specific remediation techniques can be developed for those contaminants. Farm management strategies can be altered using outputs from elemental imaging by modifying the cropping systems, fertilizer, herbicide as well as weedicides use to overcome the

elemental toxicity that may be harmful for plants, and human beings as well as for the environment. This technique can also help to decide land uses for different crops since knowing the essential element location and quantity can lead to finalize decisions as to whether fertilizers should be applied or not and which crops would be suitable in the present situation of nutrient richness or deficiency (e.g. deep rooted or shallow rooted crops that would be best for cultivation).

4.4.2. Comparison between different nutrient management practices

The second objective focused on the effectiveness of the newly developed elemental imaging approach to assess the effects of different nutrient and crop management practices on micro and macro nutrients availability and spatial distribution in the root zone. DM application significantly increased the Ca concentration in the soil compared to all other IF, and it was followed by DM+B and IC. However, no significant differences were observed between IC and corn MC. Spatially, Ca was more evenly distributed within 5–13 cm soil depth in DM fertilized corn plots, with high intensity between 8-9 cm and 10-11 cm. However, Ca was not well distributed in IC system and was present as a hot spot from 6–7.5 cm in the root zone. As for the DM+B treatment, Ca was distributed as mosaic at 2.5–4.0, 6.5–8, 9, 12 and 14–14.5 cm depths. Although, the concentrations were lower in MC; Ca was more evenly distributed in the first segment of the soil profile (0.5–11 cm depth) (Figure 4.5A). Mg distribution was uniform over the entire core from 0–15 cm in podzolic soil fertilized with DM, while its distribution was mainly within the first half of the core in DM+B and MC (Figure 4.6A). In contrast to the other three treatments, IC showed the distribution of Mg from 6 cm to 14 cm depth. The concentration was

significantly higher in IC followed by DM+B, then mono and DM treatments (Figure 4.6B). The P concentration was significantly affected by different management systems. High P concentrations were noted in DM and DM+B than in the IC and MC systems as expected. In addition, the P content was not significantly different between the IC and MC treatments. Infact, the P spatial distribution was opposite to each other for both mono and IC systems. P deposition was in the upper 9 cm in IC, while it was located at 4–15 cm in the MC treatments. However, in the case of DM and DM+B systems, the distribution was not so clear; P dispersal was in patches (5–7cm & 9–13cm) and (2.5–5.2cm & 6–8cm) respectively as depicted in Figure 4.8A. P is an immobile nutrient (Holford, 1997) and can easily be utilize by plant only if present in plant root zone. Potassium (K) concentration was significantly higher in IC and DM+B. In contrast, MC showed significantly lower K concentration than the other nutrient management practices (Figure 4.9B). Most of the K was located at 4-5cm in the IC treatment compared to be more evenly distributed when DM is applied either with or without BC (Figure 4.9A). Na distribution was not well defined (Figure 4.7A) and was present all over the soil core, with the exception of hot spot at 8-9 cm in the DM treatment. Surprisingly, the nutrients were more evenly distributed within the first 10 cm of the soil following monocropping compared to the intercropping treatment (Figure 4.6-4.14). The micro nutrient concentrations were higher in IC treatments than all other treatments, except Zn which was significantly higher in DM+B and DM respectively (Figure 4.11B–4.14B). The spatial distribution of micronutrients showed a specific trend in the IC and MC systems. In IC treatments, the distribution was mostly between 6–15cm, while it was 1–11 cm in case of MC system (Figure 4.11A–4.14A).

Spatially, Fe was distributed evenly throughout the studied soil core (Figure 4.11A). Zn was present from 2–13cm in DM, and at 2–8cm in DM+B. Mn and Co was distributed between 8–13cm respectively for DM, and in patches for DM+B. The investigation of spatial distribution of nutrients is very important because of mobility and immobility of these nutrients in the soil profile. Most of the essential macro and micro nutrients are present in plant root zone referred as "A horizon" ranging from 5 to 25 cm in most of the soil. This region is also referred to as the mineral horizon (Balasubramanian., 2017). Most of the plant roots are present in the first 15 cm, especially for shallow rooted crops. So, if the plant essential nutrient would be present in the root zone area it would be easier for plant to take up those essential nutrients. Nutrients can be classified based on mobility and immobility in the soil profile. K, Ca and Mg are less mobile nutrients in the soil (Pandey., 2010), while, Fe, Mn, Zn and P are immobile nutrients in the soil profile (Holford, 1997; Jones and Jacobsen, 2006; Pandey., 2010). In the present study, the image technique demonstrate that the nutrients evaluated varied distinctly within the first 15cm of the root zone in response to the crop management systems. This imaging method demonstrate unequivocally how the different crop management systems spatially modulate the distribution of the evaluated micro and macronutrients within the root rhizospheres.

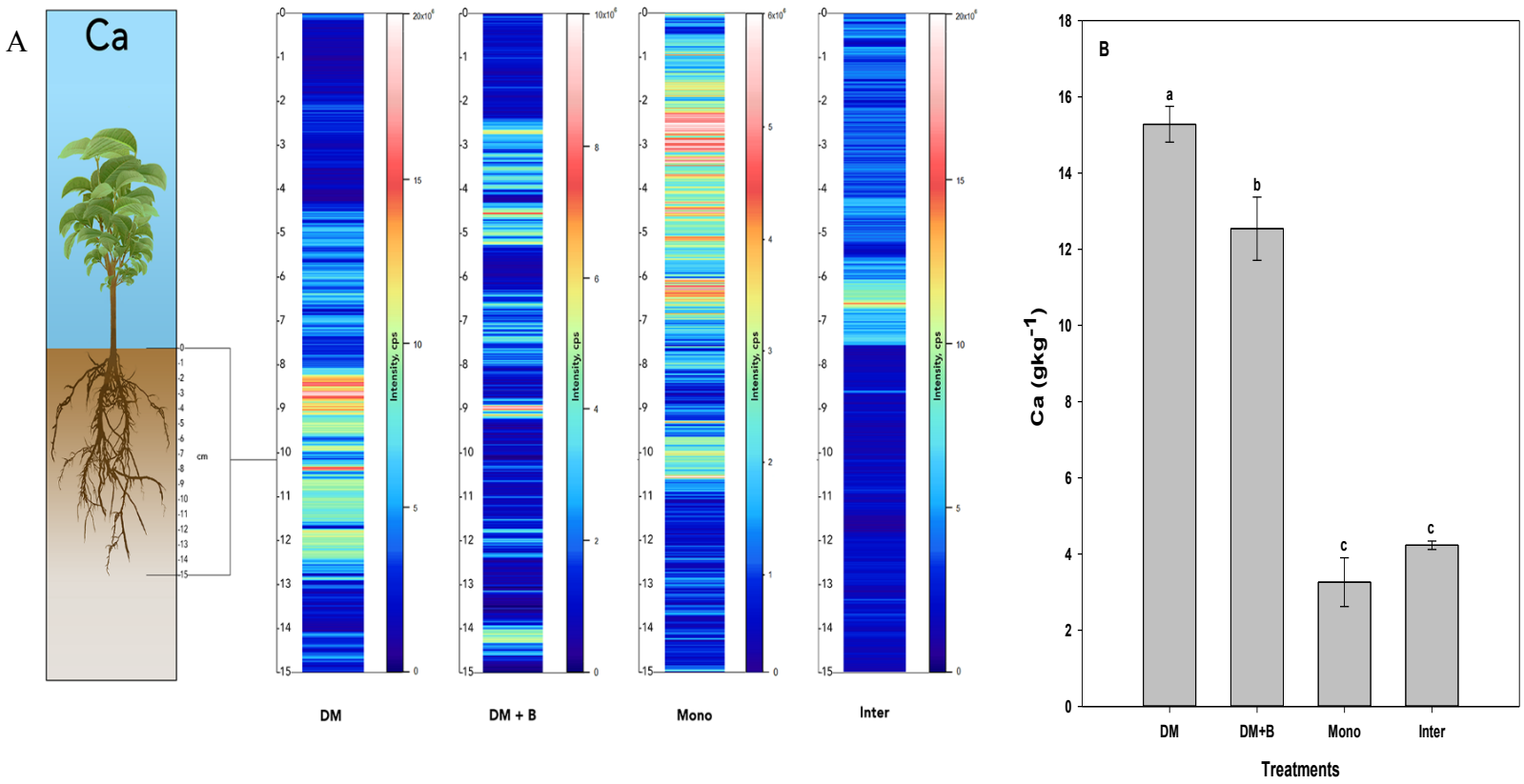

Figure 4.6: Qualitative images (A) of selected spatially distributed Ca, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

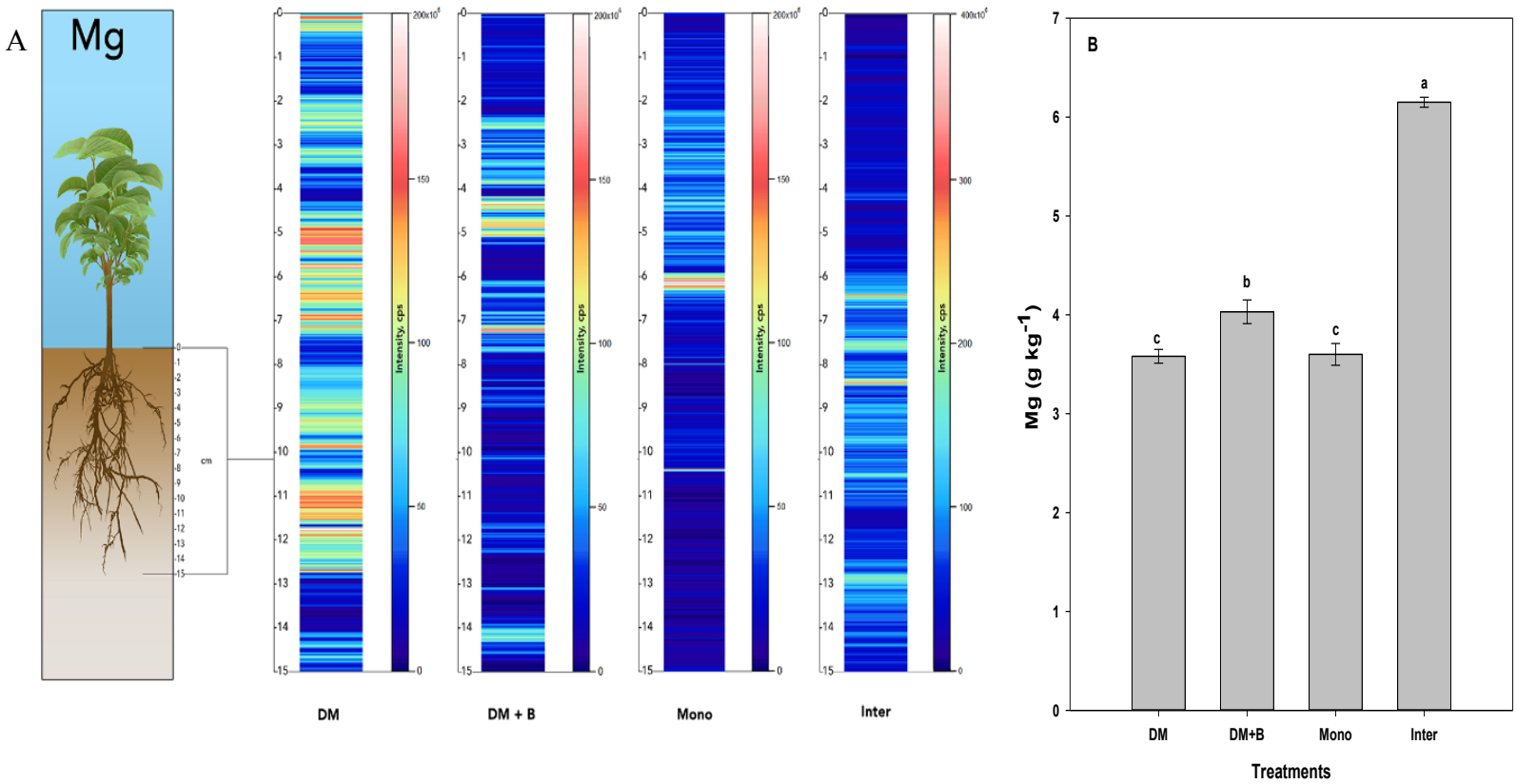

Figure 4.7: Qualitative images (A) of selected spatially distributed Mg, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

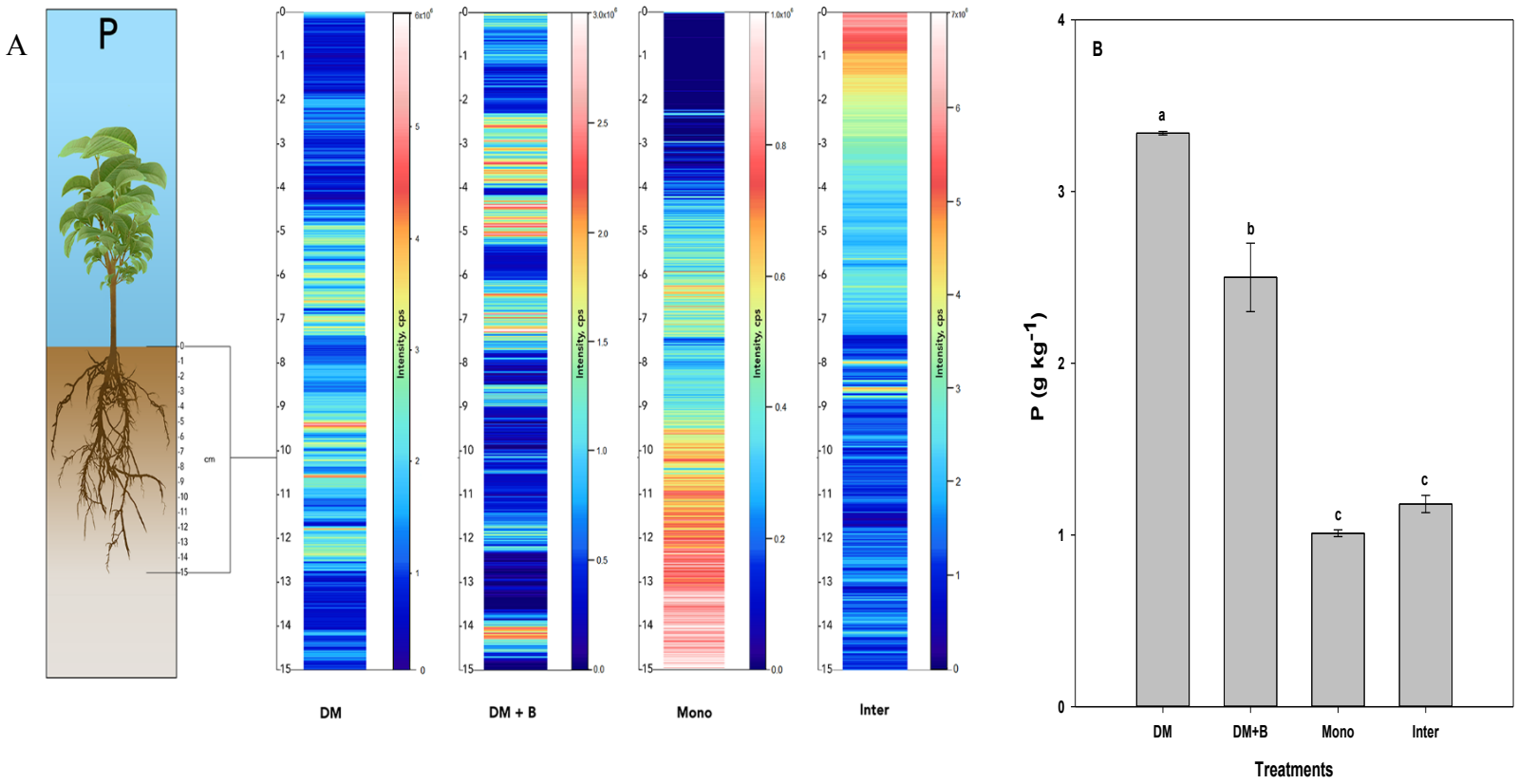

Figure 4.8: Qualitative images (A) of selected spatially distributed P, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

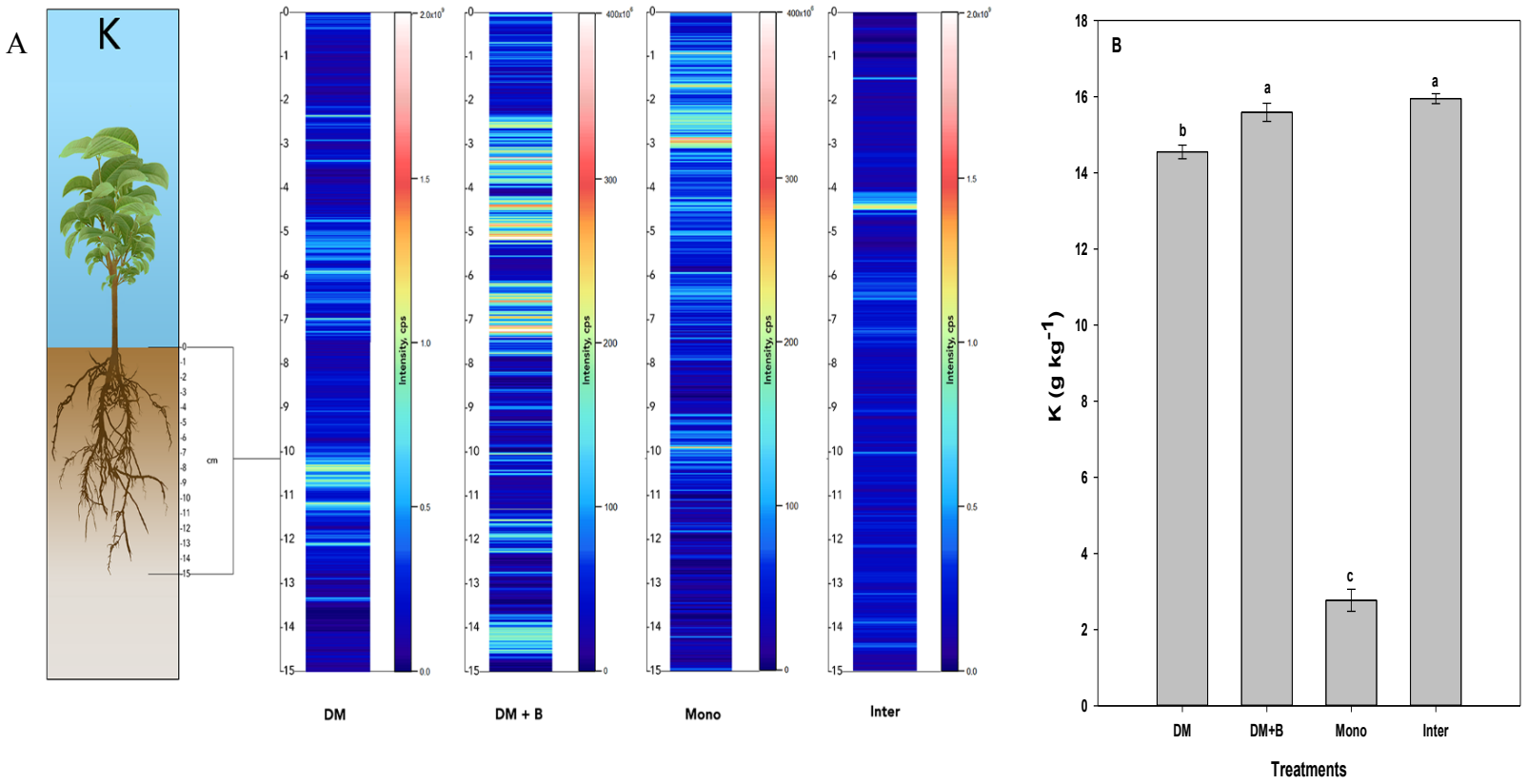

Figure 4.9: Qualitative images (A) of selected spatially distributed K, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

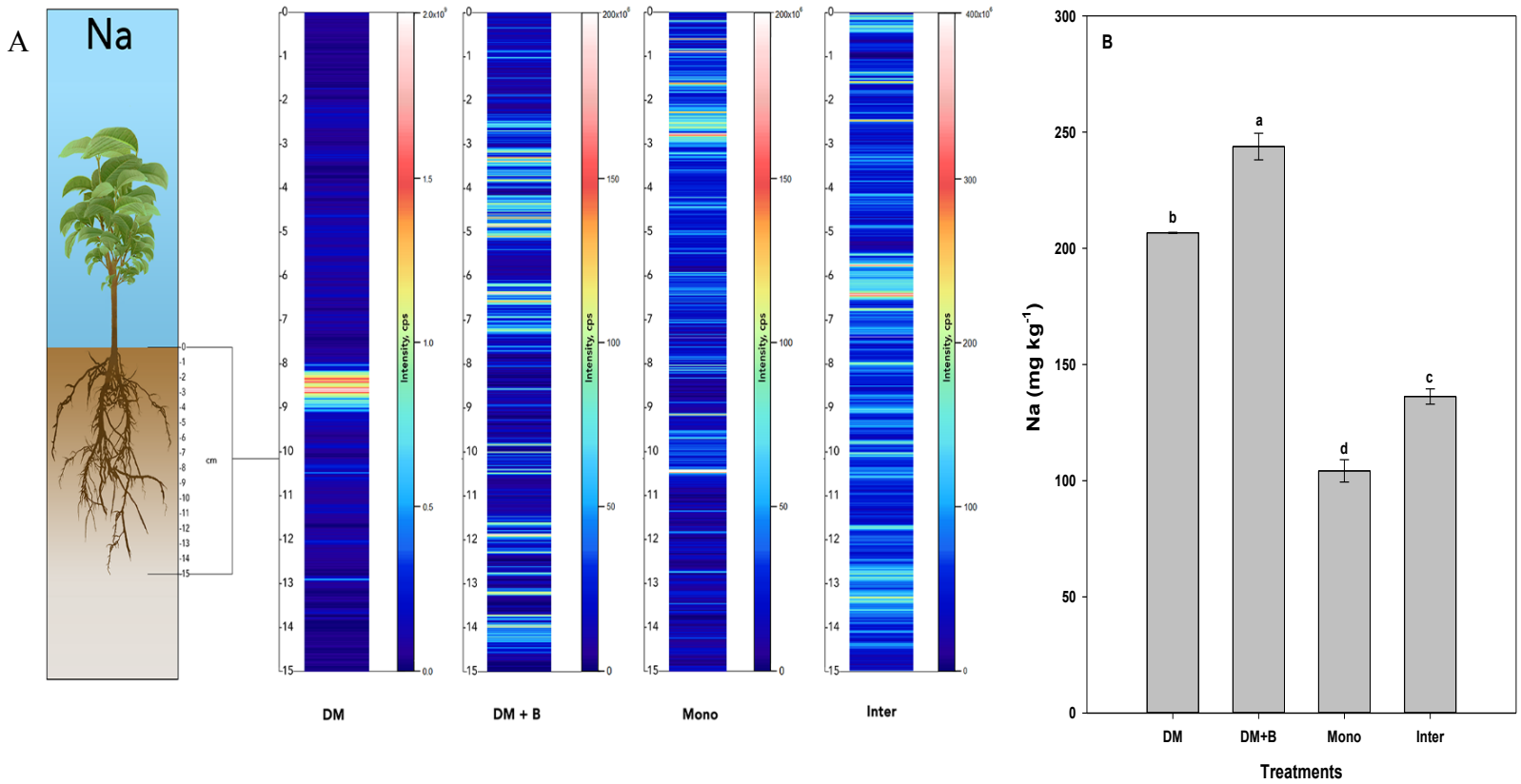

Figure 4.10: Qualitative images (A) of selected spatially distributed Na, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

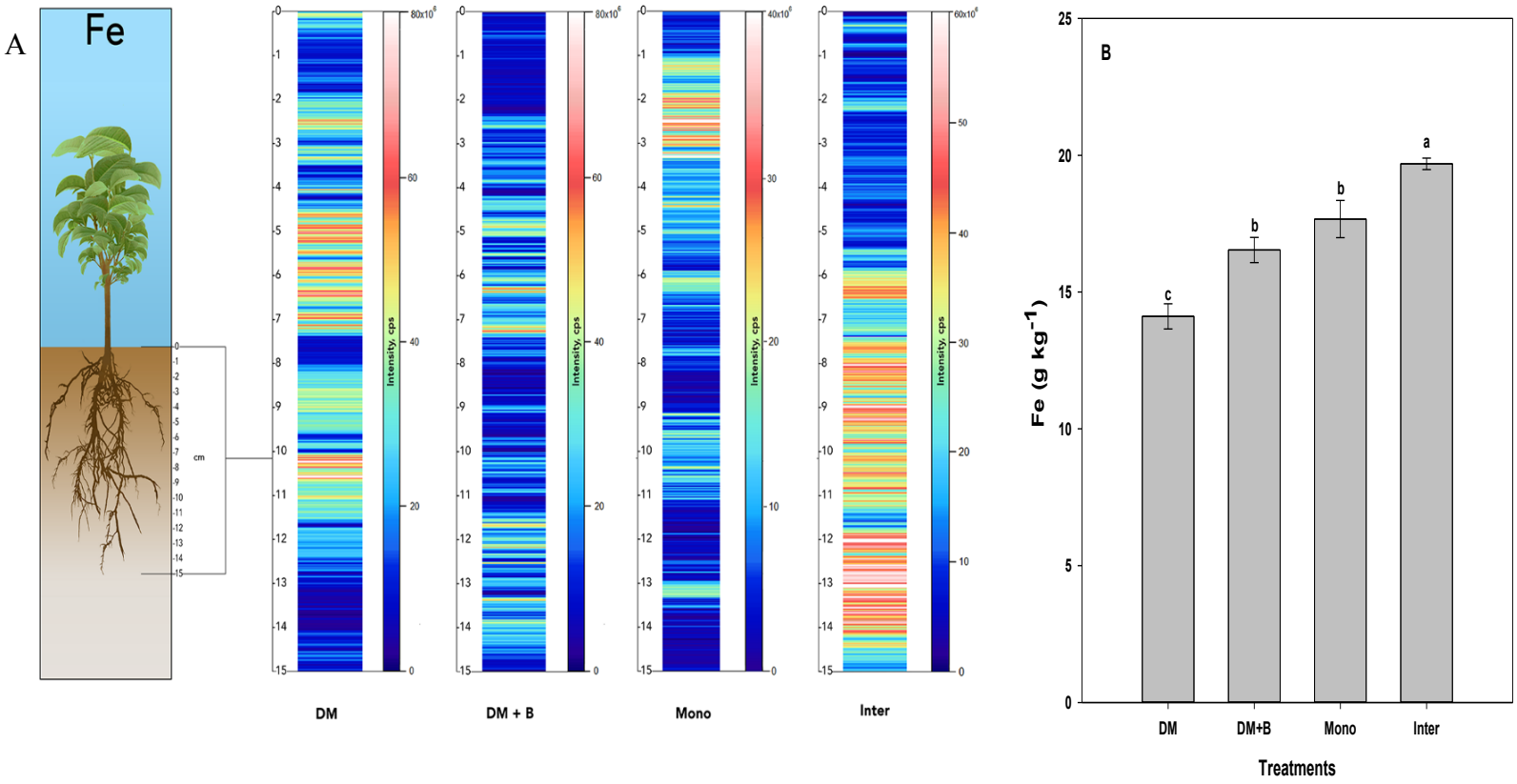

Figure 4.11: Qualitative images (A) of selected spatially distributed Fe, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

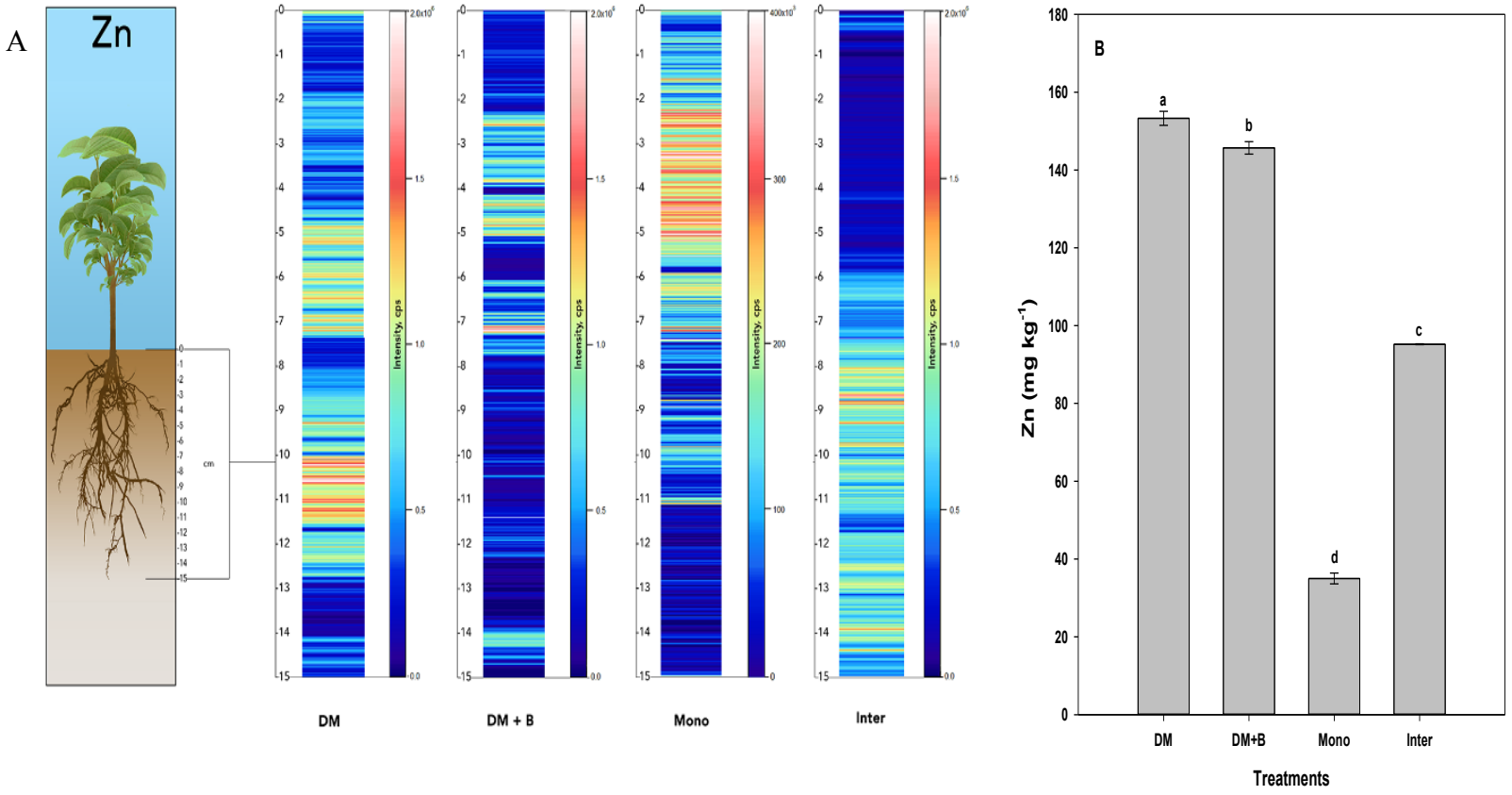

Figure 4.12: Qualitative images (A) of selected spatially distributed Zn, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

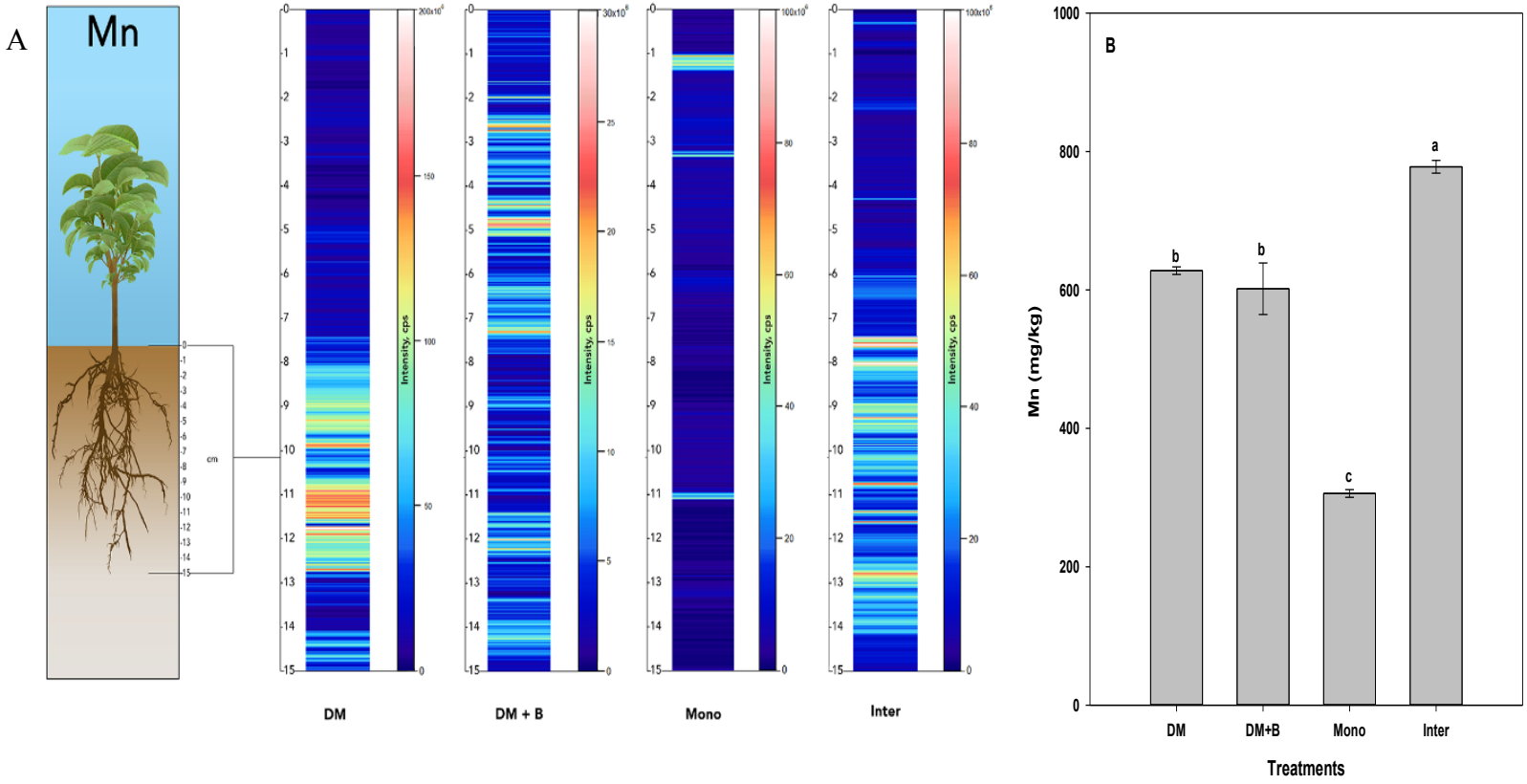

Figure 4.13: Qualitative images (A) of selected spatially distributed Mn, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

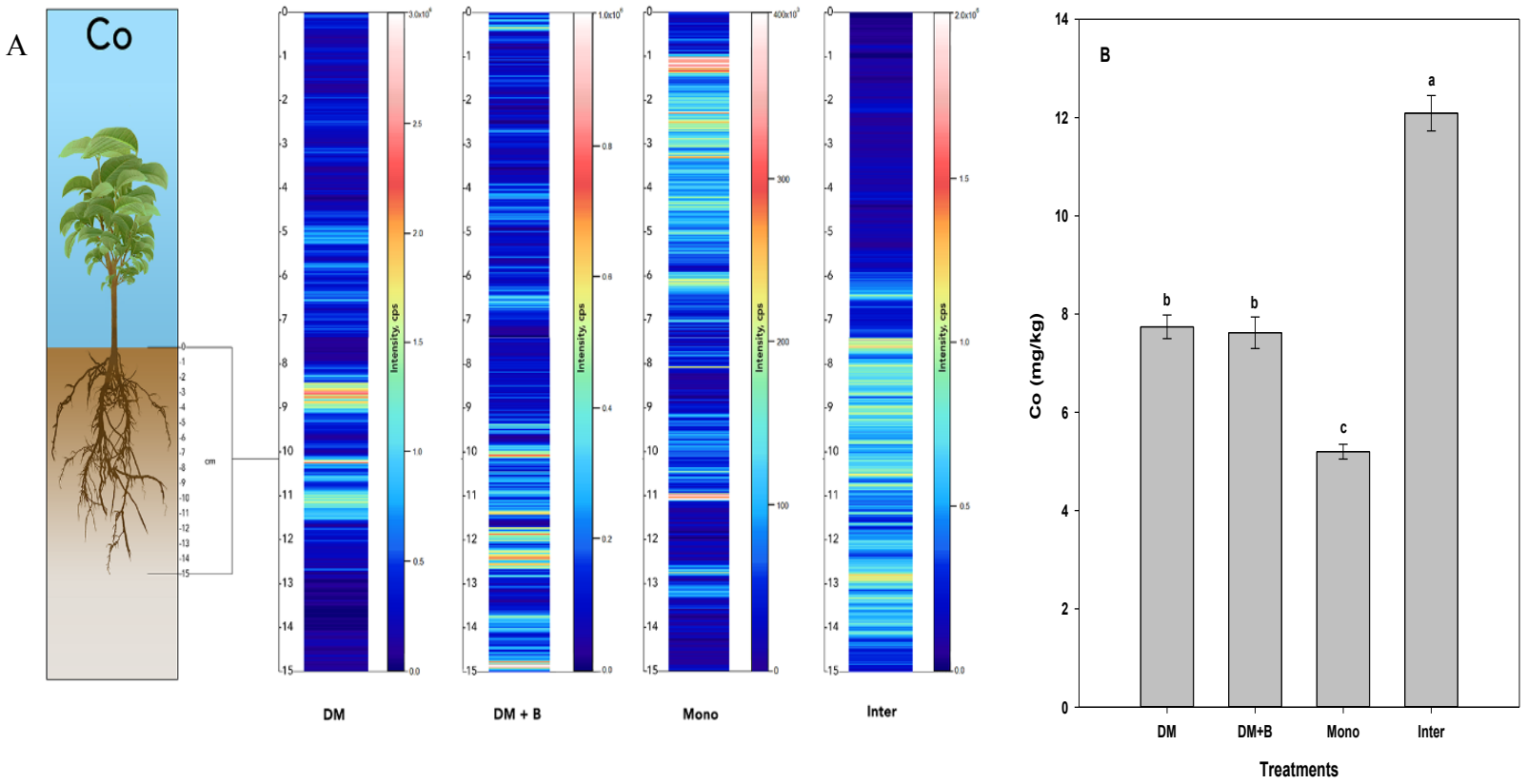

Figure 4.14: Qualitative images (A) of selected spatially distributed Co, and its quantification (B) bar charts measured in different nutrient management practices measured by LA–ICP–MS and ICP–MS, respectively.

4.5. Conclusion

In the present study, we developed a technique to image non-destructively the spatial distribution of different macro and micro–elements in soil cores at a depth representing the plant root zone (0-15 cm). LA–ICP–MS and ICP–MS were used to image and quantify the elements present in the soil cores respectively. LA–ICP–MS is a simple and appropriate technique to map the spatial distribution of nutrients non-destructively in the sample and ICP–MS was used to accurately quantify the amount of nutrients in the plant root zone following microwave acid digestion. Application of this technique to different crop and fertilizer management systems showed the spatial distribution or localization of macro and micro nutrients in the first 15cm of the plant root zone varied dramatically with fertilizer treatments and the cropping system used. For example, P was present in the first 8 cm in IC and in the 2nd half of the core (8-15 cm) in MC treatments. DM treatment showed P localized between 5-13cm, but the addition of BC increased the P distribution in bands all over the core. However, more research is needed to calibrate and validate the qualitative imaging technique with a suitable matrix matched reference material. This will be the main target of our future research. Once the methodology is validated, it could help to examine contaminated soils and choose the best remedies to get rid of those harmful elements from the contaminated soil and the environment. Additionally, this technique could be very useful in assessing the effects of crop management systems and fertilizer application on the spatial distribution of elements in the plant root zone, as well as inform or guide the best decisions for land use and management strategies.

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Chapter 5

5. General discussion and conclusion

5.1. Discussion

The objectives of present thesis were:

i. To determine the effect of silage corn and forage soybean IC on FP under the unique climate of NL. Canada.

ii. To understand the role of IC to improve soil health (RS-pH, RS-APase, RS-$P_{available}$ and rhizosphere soil microbial community composition) status under the short and cool climate of NL. Canada.

iii. To assess the quality (protein, fiber, mineral, energy and fatty acids contents) of corn-soybean forage grown either as MC or IC in Western Newfoundland.

iv. To investigate the associations between soil health indicators (rhizosphere active soil microbial community composition and soil chemical properties (RS-APase, RS-$P_{available}$) and agronomic performances (FP).

v. To study the correlations between forage nutritional or functional quality {FA composition (omega 3 and omega 6)} and the soil active microbial community structure.

vi. To develop an appropriate imaging method to visualize the spatial distribution of essential soil nutrients in the plant root zone; and assess the effectiveness of the method in discerning changes in spatial distribution of nutrients in the root zones in response to changes in land use or crop management systems

This thesis covered the above objectives via three main experiments described in (Chapter 2, 3 & 4). Determination of plant chlorophyll contents, plant height, FP, RS-pH, RS-APase, RS-$P_{available}$ and rhizosphere soil microbial community composition and their associations are presented in chapter 2. While, IC effect on forage nutritional quality, fatty acid composition, forage mineral composition and their association are presented in chapter 3. Chapter 4 comprised the development of a novel method for imaging the spatial distributions of nutrients in soil core samples, as well as, assessing the effectiveness of the method to track changes in spatial distribution of elements in the root zone in response to changes in land use or crop management strategies.

5.1.1. Effect of IC on agronomic performance (chlorophyll contents, plant height and forage production)

Chlorophyll contents, plant height and FP are the indicators of agronomic performance of a crop. Intercropping can result in superior crop growth and productivity because of efficient utilization of resources both below and above ground (Li et al., 2001; Zhou et al., 2011). Plant chlorophyll contents play a crucial role in plant photosynthesis (Anten, 2005). Plant height and chlorophyll contents are considered as important drivers of FP. Three possible outcomes of intercropping in terms of FP could be LER>1, LER<1 and LER=1 (Eskandari, 2012; He et al., 2013). The increase in FP observed was due to cereal legume IC (Eskandari, 2012; Eslamizadeh et al., 2015), but that increase was observed in the dominant (corn) crop at the expense of the dominated crop (soybean) (Li et al., 2013). IC can use environmental resources more efficiently than MC (Dhima et al., 2007) and that

might be one possible reason for the higher productivity in case of cereal legume IC compared to MC.

5.1.2. Effect of IC on soil health status (RS-APase, RS-pH, RS-$P_{available}$ and rhizosphere soil active microbial community composition)

The major form of P in agricultural soils is organic P which account for 30-80% of the total P of the soil and can only be utilized by plants after hydrolyzation by phosphate enzymes (Gilbert et al., 1999; Tarafdar and Claassen, 1988). As such a significant correlation was observed in arid agricultural soil between RS-$P_{available}$ and RS-APase (Sardans et al., 2008) confirming the role of acid phosphatase enzyme in the mineralization of organic P (Conn and Dighton, 2000; Dick et al., 2000). Cereal legume IC results in an increase in the acid phosphatase activity (Inal et al., 2007; Wang et al., 2014) and available P (Li et al., 2004; Xiao et al., 2013). Legumes are considered as the main contributor towards higher acid phosphatase in the root rhizosphere because of significant release from their roots (Gunes et al., 2007; Li et al., 2004). The reduction in RS-pH in cereal legume-based IC (Wang et al., 2014) is attributed to the contributions from the legumes in the IC due to release of a large quantities of H^+ ions and organic acids from their roots (Li et al., 2007; Tang et al., 1997). Both higher RS-APase and low RS-pH in IC system are the possible drivers of P availability in the plant root rhizosphere (Li et al., 2007). Rhizosphere soil microbial community could also be an important contributor to increase P availability, because a close association was observed between dominant active soil microbes and $P_{avaialable}$ (He et al., 2013) . IC results in an increase in the active soil microbial community (Li et al., 2016; Zhou et al., 2011); and the diversity of the active microbial community is recognized as an

indicator of the good soil health status and quality (Kong et al., 2011). Bacterial population is usually the dominant community in the soil microbes, and the dominance of G+ bacteria over G- is an indicator of organic carbon deficiency in the soil (Bossio et al., 2005; Herman et al., 2012); while the dominance of G- over G+ bacteria and high fungal population indicate the presence of complex organic matter in the soil (Herman et al., 2012; Mathew et al., 2012).

5.1.3. Effect of IC on forage quality (protein, fiber, mineral, energy and fatty acids contents)

Supply of quality forage is the foremost priority of the dairy farmers to reduce the animal cost of production and farm expenses as well as enhancing the farm productivity because low quality (low in crude protein) forage require additional concentrates to overcome this issue and can accounts for most of the farm feed input expenses. Cereal legume IC improved the forage quality by enhancing the CP contents (Abdulraheem et al., 2012; Baghdadi et al., 2016; Dahmardeh et al., 2009; Htet et al., 2016a, 2017), while simultaneously reducing the ADF and NDF contents of the forage (Htet et al., 2016b; Strydhorst et al., 2008). IC can improve the WSC of the forage (Htet et al., 2016a), because of a positive contribution from the corn used as a companion crop in IC (Jahanzad et al., 2014). In cool climates, the provision of energy rich forage is an important factor to improve the growth and development of beef and dairy cattle. TDN refer to the nutrients that are easily utilized and supply energy during animal pregnancy or gestation.

As such, TDN is negatively associated with ADF; and fodder with 55-65% TDN is considered a good forage for animal feed formulation or direct feeding (Sadeghpour et al., 2013; Yurchak and Orkine, 2004). Cereal legume IC can also result in increased DDM (Sadeghpour et al., 2014, 2013). This may be due to the high CP and sugar contents in the intercropped forage (Stoltz et al., 2013). Higher animal productivity is related to voluntary DMI of forage (Ullah, 2010). IC can produce forage with improved DMI (Salama and Zeid, 2016) because legumes forage DMI is superior to that of non-legume-based forage (Ullah, 2010). RFV is an imperative index that tells the forage energy value and consumption (Lithourgidis et al., 2006); and is negatively associated with the NDF and ADF contents of the forage (Strydhorst et al., 2008).

Legumes are rich in minerals compared to grasses, resulting in enhanced mineral levels in IC forage (Paulson et al., 2008). Forage used in animal feed formulations are major sources of PUFA in the animal's diet and play important roles in modifying the FA profile of milk (Hatfield et al., 2007; Khan et al., 2012) because milk FA profile depends on animal diet (Kalac and Samkova, 2010). The present study was conducted at relatively low plant growth temperatures, and plants are known to respond to low temperature by increasing the biosynthesis of PUFA as a survival or acclimation strategy (Routaboul et al., 2012). Two FAs are considered the major contributors towards PUFA (C18:3n-3 an omega 3 FA and C18:2n-6 an omega 6 FA). IC can increase the omega 3 FAs that ultimately can modify the animal milk profile by increasing the omega 3/ omega 6 ratio in the milk produced (Kliem et al., 2008). Indirectly, C18:2n-6 can results in higher pregnancy rate because of CLA production (Harfoot and Hazlewood, 1997; Lopes et al., 2009).

5.1.4. Soil core imaging and quantification using LA-ICP-MS and ICP-MS

LA-ICP-MS was used to develop a novel method to image the spatial distribution of elements in the plant root zones to discern alterations in the spatial distribution of nutrients in response to changes in the crop management systems and/or addition of production inputs (fertilizers or soil amendments). In this new method we specifically develop the new method to image the elements in the soil core samples. To our knowledge this is the first-time report of element imaging in soil cores using LA-ICP-MS. In fact, LA-ICP-MS is a new technique that can be used to image the spatial distribution of metals and non-metals in biological as well as environmental samples (Becker et al., 2008; Bulska and Wagner, 2016; Dean, 2005; Hill et al., 2005; Russo et al., 2002; Wu et al., 2009) and it is superior than other imaging techniques due to high sensitivity, high resolution and nominal sample preparation (Mokgalaka and Gardea-Torresdey, 2006; Ohata et al., 2002). Four management systems were compared via imaging the core sample from their root zones. The results showed that some elements were dispersed evenly all over in the 15 cm core and some elements were present like hot spots. The knowledge of nutrient availability can be useful for future decision making regarding choosing a shallow or deep-rooted crop. Some macro and micro nutrients are mobile, and some are immobile in the soil profile (Holford, 1997; Jones and Jacobsen, 2006; Pandey., 2010). Most probably the immobile nutrients can only be utilized by plant if they are present in the root zone of that specific shallow or deep-rooted plant.

5.2. Conclusion and recommendations

Over all IC enhanced forage production, forage quality and improved the soil health status as compared to MC following cultivation under the short and cool climate of NL. Canada. IC forage was superior in FP compared to corn or soybean MC during the two growing seasons evaluated in this study. Forage was of higher quality with increased levels of CP, SS, SP, WSC, TDN, NE_L, NE_M, NE_G, DMI, DDM and improved RFV in intercropping; but was lower in ADF and NDF contents compared to MC forage. Mineral contents and FA profile especially (omega 3 and omega 6) were superior for IC forage than MC forage and could ultimately increase the dairy animal milk production with improved milk FA profile. IC not only enhanced the yield and quality of forage, but also improved the soil health status. Higher RS-APase activity was recorded in the root rhizosphere, presumably resulting in an increase in the RS-$P_{available}$ by hydrolyzing the organic P source in soil for the plants in IC treatments. The active microbial biomass or composition was improved under IC treatments with enhanced levels of (bacterial, fungal and protozoan's population). Overall, upright (S1, S2) soybean varieties performed better under cool climate of Newfoundland Canada compared to the vine (S3) soybean variety. Yukon-R (C1) variety performed better in FP compared to DKC26-28RIB (C2). Collectively, we can conclude that silage corn intercropped with forage soybeans could be used as a suitable practice to increase forage biomass production with superior nutrient quality by improving the soil health status under cool climate of Western Newfoundland, Canada. Secondly, we developed an LA-ICP-MS imaging technique with ICP-MS quantification as a wet method

that can be a powerful tool to study the nutrient distribution in the plant root zone. Further studies are required:

i. To get optimum seeding rate to enhance productivity and nutrient quality of the forage produced in this IC system. Experiments should be conducted in controlled environment to know the heating unit requirement of soybean varieties used, maximum yield potential and comparison between field and controlled conditions for determining the mechanisms associated with enhanced forage quality or production and the soil health status under cool climate cropping systems.

ii. To calibrate and validate the qualitative imaging technique LA-ICP-MS with a suitable matrix matched reference material, which will be the main target of our future research.

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