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Response of Soil Biochemical and Physical Properties to Long Term Prairie Cordgrass and Kura Clover Intercropping System

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RESPONSE OF SOIL BIOCHEMICAL AND PHYSICAL PROPERTIES TO LONG TERM PRAIRIE CORDGRASS AND KURA CLOVER INTERCROPPING SYSTEM

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

RESPONSE OF SOIL BIOCHEMICAL AND PHYSICAL PROPERTIES TO LONG TERM PRAIRIE CORDGRASS AND KURA CLOVER INTERCROPPING SYSTEM

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Prairie cordgrass (*Spartina pectinata*) (PCG) is a warm-season perennial grass that can be used as a biofuel feedstock and can be grown on marginal lands. Previous studies on intercropping of a perennial legume i.e., kura clover (*Trifolium ambiguum*) (KC) with PCG can improve soil biochemical properties, increase biomass production, mitigate greenhouse gas (GHG) emissions while reducing the chemical fertilizer requirement. However, there is a lack of evidence about the effects of PCG production on soil biochemical and physical properties during the cropping season and at deeper soil depths in reference to support plant growth, environmental implications and enhance the soil health of marginal lands. The overall objective of this study was to investigate the effects of PCG-KC intercropping on (1) soil biochemical properties at different sampling times during the season, and at different soil depths up to 60 cm, and (2) near-surface (0-10 cm depth) hydro-physical properties. This field experiment was initiated in 2010 as a randomized complete block design with four replications of five treatments i.e., PCG intercropped with KC (PCG-KC), and PCG fertilized with different N fertilizer rates: 0 (PCG-0N), 75 (PCG-75N), 150 (PCG-150N), and 225 N kg ha^{-1} (PCG-225N). For seasonal biochemical properties assessment, surface soil samples were collected at 0-10 cm depth at three different times during the crop season: pre-emergence (PE), active

growth (AG), and after harvest (PH) stages. To investigate the long-term effects on soil total carbon (TC) and total nitrogen (TN), deep soil cores were collected from 0-5, 5-15, 15-30, 30-45 and 45-60 cm depth after the crop harvest. The intact soil cores from 0-10 cm depth were collected at the AG stage to measure the soil physical properties.

Overall, microbial biomass carbon (MBC) and nitrogen (MBN), and urease activity, were observed to be higher at the PE stage as compared to AG and PH stages. No treatment effect was observed for both MBC and MBN. Cold water-extractable C (CWC) showed a gradual increase from PE to AG to PH. Whereas, hot water extractable C (HWC), β-glucosidase activity, FDA, and glomalin, were highest at the AG stage and lowest at PE stage. The treatment effect was not significant for CWC, HWC and glomalin whereas, treatments had significant effect for all the enzymes, PCG-KC treatment had 45% higher mean β-glucosidase activity when compared to control (PCG-0), 56 and 42% higher mean urease compared to PCG-0 and PCG-75 N, and 13-19% higher FDA activity compared all other treatments. Total PLFA, bacterial, and fungal PLFA, CWN increased from PE to AG stage and showed similar or decreasing trend from AG to PH stages whereas HWN content decreased from AG to PH stage. Treatment effect was not significant for total PLFA and bacterial PLFA. There was a significant interaction between treatment and stage for CWN and HWN; PCG-KC showed higher mean values of CWN compared to PCG-0, PCG-75N and PCG-150N during the PE stage and was similar to all other N treatments except PCG-225N. PCG-KC had higher mean HWN when compared to control i.e., PCG-0 during AG stage and PCG-225N at PH stage. Nitrate concentration was higher at PE stage when compared to AG and PH stage which is contrary to the ammonium concentration that showed an increasing trend from PE to

PH stage. Both the parameters had significant interaction between treatment and stage. The PCG-KC treatment had showed 34.3 and 24.4% lower nitrate concentration than PCG-150 and 225N during the AG stage and 24.3% lower than PCG-150N at PH stage. For ammonium PCG-KC treatment had a higher mean value than the control i.e., PCG-0 during PE stage and PCG-75N at PH stage.

There was a gradual decrease in both TC and TN concentrations with soil depth. In the 0-5 cm layer, soil TC concentration was approximately 43% higher when compared to 45-60 cm soil depth. Similarly, TN concentration was approximately 83% higher in 0-5 cm soil layer when compared to deeper soil depth i.e., 45-60 cm. There was no treatment effect and its interaction with depth.

The intercropping of KC with PCG had 0.024 cm^3 cm^{-3} of macroporosity (MP), 50% higher K_{sat}, and 1% lower λ as compared to the other treatments. Moreover, PCG-KC treatment had 1.42 g kg^{-1} of TN and 21.4 g kg^{-1} of TC in the surface layer which was comparable with PCG-75 treatment, however, were significantly higher as compared to the PCG-0.

Overall, this study showed that the long-term maintenance of KC with PCG can provide soil health benefits through enhancing or maintaining the soil biochemical and hydro-physical properties. Therefore, long-term adoption of the perennial grass-legume system can enhance soil functional processes including, nutrient cycling, C sequestration, water storage, and availability, while maintaining biomass yields for biofuel production on marginal lands.

CHAPTER 1

INTRODUCTION

Growing perennial lignocellulosic crops on marginal lands provides opportunities for bioenergy production and better utilization of the land that is not suitable for annual crop production (Stoof et al., 2015). Moreover, they provide several agroecological benefits such as soil conservation, improving water quality, enhancing carbon (C) sequestration, reducing soil erosion, mitigating greenhouse gas emissions, nutrient capturing and recycling, reducing nutrient losses, increase soil organic matter (Awasthi et al., 2017). The use of cellulosic feedstocks for bioenergy production can also have a favorable impact on the environment and might account for a significant share of future energy portfolios (Mehmood et al., 2017). Therefore, perennial bioenergy crops have a great potential for production on marginal lands without competing with food crops, while reclaiming the lands and protecting the environment without threatening food security.

1.1 Prairie cordgrass and kura clover perennial intercropping

Prairie cordgrass (*Spartina pectinata*) (PCG) is a rhizomatous C4-perennial warm-season grass that can grow well on marginal lands. It can grow throughout most of the United States and most importantly in the Northeast, Great Lakes, and Midwest states (USDA, NRCS, 2022). It has broad climatic adaptations (Kim 2015) and can even perform well under flooded and alkaline conditions (Sedivec et al., 2001). However, the efficient use of nitrogen (N) is essential for sustainable PCG biomass production. Where under application of N fertilizer can reduce the biomass yields (Guretzky et al., 2011), over-application can lead to increased greenhouse gas emissions contributing to global

warming, leakage of reactive N into ground or surface water bodies causing water pollution, and also increase the cost of biomass production (Duran et al., 2016). To combat this problem, mixed or intercropping leguminous crops have been encouraged as a source of N in sustainable energy production systems.

Kura clover (*Trifolium ambiguum* Bieb.) (KC) is a perennial leguminous crop that can be intercropped with PCG to supply N and enhance biomass yields (Abagandura et al., 2020). It has the ability to form a symbiotic relationship with the N-fixing bacteria in the soil, such as *Rhizobium*, and therefore, also plays a great role in maintaining soil fertility. According to Zemenchik et al., (2001), intercropping KC with cool-season grasses can replace N fertilizer requirement by 74 - 336 kg N ha^{-1} yr^{-1}. In addition, KC can withstand poorly drained soils and is very well adapted to lower fertility and low soil pH conditions (Speer and Allinson 1985). Adding legume crop as an intercrop with grasses provides additional below and above-ground residue inputs enhancing the soil organic matter, soil pore properties and hydrological properties (Franzluebbers et al., 2000). Overall, intercropping KC with PCG may provide several agroecological benefits and can enhance the sustainable productivity of marginal lands.

1.2 Soil health significance and assessment

Soil health is defined as the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans (Natural Resources Conservation Service-USDA-NRCS, 2012). It is considered an important indicator of sustainable cropland management. The concept of soil health includes integrating and optimizing chemical, physical, and biological processes of soil with an overall goal to enhance or maintain soil productivity with minimum impacts on environmental quality

(Moebius-Clune et al., 2016). The chemical indicators of soil health provide information about the capacity of the soil to provide nutrients for plants as well as chemicals or compounds harmful for the plant growth and environment (Cardoso et al., 2013). The most commonly used chemical indicators for soil health assessment include soil pH, cation exchange capacity, nutrient levels, salinity, sodicity, heavy metals, etc. (Moebius-Clune et al., 2016). The physical indicators of soil health assessment provide information about soil texture, porosity, bulk density, surface hardness, aggregate stability, soil hydrological processes, such as water infiltration and water holding capacity, etc. The biological indicators include microbiological and biochemical properties that provide information about the microbial activity and diversity that play an important role in C and nutrient cycling. The biological indicators that are commonly measured include soil organic matter (SOM), active C, microbial biomass, microbial community structure and activity, etc.

1.3 Intercropping and biochemical indicators of soil health

Soil microbes play an important role in maintaining soil health by performing a variety of biochemical processes in the soil including, organic matter decomposition, plant nutrient availability, formation, and stability of soil aggregates (Sahu et al., 2017). The microbial biomass and diversity in the soil depends on soil type, climate, and management practices (Ren et al., 2019). Most importantly, land use practices, vegetation, and plant productivity influence the microbial biomass, community structure, and soil microbial processes (Steenwerth et al., 2002; Calderón et al., 2000; Jin et al., 2010). For example, Jin et al., 2010 suggested that both quality and quantity of belowground residue input affect the soil microbial biomass and community structure.

Intercropping perennial grass and legume can influence soil health by affecting the bio-functionalities of soil microorganisms (Sun et al., 2019) through high-quality residue inputs (Yang et al., 2020), soil nutrient use efficacy (Hinsinger et al., 2011), reduced synthetic fertilizer inputs (Lemaire et al., 2014), and effects on plant root functions with different root morphologies (Bukovsky-Reyes et al., 2019).

For assessment of soil health, soil organic C and total N as well as labile SOM fractions are important biochemical indicators. Soil organic C is the C component in the organic compounds and total N includes both organic and inorganic form of N in the soil (McGill et al., 1981). The most labile fractions of SOM are water-extractable organic substances for which solubility depends on the temperature of the solvent. Important water-extractable organic compounds include cold water-extractable organic carbon (CWC) and nitrogen (CWN), and hot water-extractable organic carbon (HWC) and nitrogen (HWN). The CWC and N are quantitatively very close to dissolved organic carbon which is measured directly in soil using different lysimeters and suction cups (Rees 2005; Ostrowska et al., 2010). The HWC and N consists of more stable components that form the close reserve of nutrients and energy for plants and microorganisms (Bu et al., 2011). Intercropping with legumes can increase C and N pools through root exudates and N fixation. Moreover, legumes have low C/N ratios and easily degradable chemicals, both of which might increase microbial activity thereby breakdown of plant residues is rapid (Bini et al., 2013). With intercropping, C and N sources will be different as compared to monocultures fertilized with synthetic N fertilizers i.e., biologically fixed N is mainly released in diverse forms of organically bound N.

Microbial biomass carbon (MBC) and nitrogen (MBN) measures the C and N contained within the living component of SOM (i.e. bacteria and fungi). Intercropping systems have higher diversity of roots and residues of different composition could impact energy supply for the microbial biomass and activities through the release of root exudates such as amino acids and organic compounds, etc. For example, the intercropping of durum wheat with legumes like chickpea, lentil (Dalai et al., 1977), *S. hermaphrodita* plants with the perennial legume species *Trifolium pratense, T. repens, Melilotus albus*, and *Medicago sativa* (Nabel et al., 2018) increased the soil microbial biomass.

Soil enzymes play an important role in maintaining carbon and nutrient cycling, and functional diversity. Therefore, measurement of soil enzyme activities including β-glucosidase, urease and fluorescein diacetate hydrolysis (FDA) activity is considered as important biochemical indicator for soil health assessment. Previous studies have reported higher dehydrogenase (DHA), β-glucosidase, and urease enzyme activities after biomass harvest under PCG-KC intercropping as compared to fertilized PCG (Sekaran et al., 2020). Solanki et al., 2019 in their study on sugarcane and soybean intercropping concluded higher enzymatic activity when compared to monocultures.

Phospholipid fatty acid analysis (PLFA) provides the snapshot of microbial abundance and community structure i.e., proportions of microbial types such as gram-negative bacteria, gram-positive bacteria, total fungi, actinomycetes, and arbuscular mycorrhizal fungi (AMF) at the time of sampling (Dong et al., 2008). Microbial communities are influenced by environmental conditions, soil type, and management practices (Ritcher et al., 2018). Intercropping of legumes and grasses could affect the

microbial communities due to the addition of low C/N residues and higher availability of readily available C, which stimulate the activity of soil microbes (Franco-Andreu et al., 2017).

1.4. Sampling time and soil depth effects on biochemical indicators of soil health

The soil biochemical properties in agricultural systems are well known to be substantially influenced by climatic conditions, soil type, crop type, and management practices (Wardle, 1992; Murphy et al., 2007; Bastida et al., 2008). Moisture and temperature, as well as crop residue inputs, particularly rhizodeposition, have been linked to seasonal responses of biological and chemical properties of soil (Dalal and Mayer 1987; Sarathchandra et al., 1988; Franzluebbers et al., 1995). Furthermore, soil microbial biomass and enzyme activity fluctuate seasonally depending on soil type, crop species, land use, and management approaches (Srivastava and Singh 1989; Bardgett et al., 1999). For example, Krämer and Green (2000) suggested that the seasonal fluctuations in biochemical properties could be due to a combination of soil moisture, temperature, root activity, and organic matter return to soils via litterfall and rhizodeposition. Van Geste et al., 1992 reported that the hot and dry seasons can have declining impacts on biochemical properties due to low soil moisture and high temperature conditions. A study performed on winter wheat by Liang et al., (2015) reported that low soil temperature led to decreased soil respiration between seedling and tillering stage, due to optimum soil temperature at filling stage soil respiration increased because of the increase in microbial and root activities, whereas at the senescence stage there was a significant decline in soil respiration due to low microbial activities.

Perennial cropping systems can affect the soil biochemical properties. Some of these effects are apparent in the short-term, and others only become apparent after several years. In the long-term, a return of relatively high root and shoot biomass of perennial crops creates conditions that are favorable for the build-up of the microbial biomass and its activity (Wardle, 1992). The amount of C and N increases over a long run of perennial grass and legume mixtures due to higher belowground inputs through root turnover and rhizodeposition which favour C and N storage, and slower soil organic carbon mineralization due to the absence of soil tillage (Soussana et al., 2004). There is very limited research reporting long term perennial cropping system affects of prairiecord grass on soil health of marginal lands.

Soil biochemical activity is expected to be higher on the surface layer of the soil, and it gradually decreases with the depth due to the difference in the litter composition, soil bulk density, water content, and root turnover rates (Hooper and Vitousek 1998). Example, cultivation of temperate-zone perennial grasses – Miscanthus, switchgrass, or native mixes increased SOC by an average of ∼0.1–1 Mg ha^{-1} yr^{-1} in the top 30 cm (e.g., Kahle et al., 2001; Lemus & Lal 2005; Liebig et al., 2008).

1.5. Intercropping and hydrological-physical indicators of soil health

Cultivation of perennial crops can improve soil hydro-physical properties and processes (e.g., bulk density, soil water properties) (Stewart et al., 2015). Kirkegaard et al., 2004 reported ten-year-old stands of perennial reed canary grass (*Phalaris arundinacea* L.) increased steady-state infiltration by three times and increased the time to water ponding by about three times than that under row crops due to improved porosity and the reduced effects of cultivation. Duration of perennial crop establishment is an

important factor influencing the changes in soil hydro-physical properties, Anderson et al., 2007 reposted that it might take ten years or more for grasslands that are restored under the conservation reserve program (CRP) to achieve saturated hydraulic conductivities (K_{sat}) that are comparable to those of naturally established native grasslands. Saturated hydraulic conductivity is the ease with which pores of a saturated soil transmit water. Under the long-term perennial system K_{sat} value increase, which is probably because as the stands grow, continuous root growth and residue inputs alter the hydro-physical characteristics of the soil. Saturated hydraulic conductivity (K_{sat}) levels in switchgrass soil can be up to seven times greater than in corn or soybean fields (Rachman et al., 2004). However, the amount of time it takes for significant changes to emerge is uncertain. Long-term maintenance of perennial plants under marginal land conditions is expected to enhance soil mechanical and moisture qualities, although results may vary depending on the location (Blanco-Canqui et al., 2015).

Moreover, soils managed with perennial bioenergy plants usually exhibit lower bulk density (ρb) and penetration resistance (PR) in the soil surface layer (Tolbert et al., 2002). Higher amount of root and shoot biomass in perennial systems may improve SOM content by the addition of more residue inputs, which directly or indirectly affects soil physical parameters and processes such as aggregation, water-holding capacity, saturated hydraulic conductivity, and resistance to water and wind erosion (Zebarth et al., 1999; Franzluebbers 2002; Celik et al., 2004).

Hudson (1994) reported that higher water holding capacity is found in soils with high organic matter than soils of analogous texture with low organic matter. Long-term management of the perennial grasses on the marginal lands tends to improve soil physical

properties coupled with complete ground cover, which helps in mitigating the problem of soil erosion. Surface vegetation, according to Grewal and Abrol (1986), shielded soil directly from the erosive pressures of raindrops and surface run-off by increasing soil physical and hydrological properties. According to the findings from Liu (2006), ryegrass which is a gramineous forage with many root systems, had better water retention effects on soil than those of legumes and composite forages.

Previously published studies on native grasslands and restored prairies have reported perennial vegetation increased porosity, macroporosity, and coarse mesoporosity in the soil profile (Rachman et al., 2005; Udawatta et al., 2006; Udawatta et al., 2008b). A study conducted on two perennial plants purple alfalfa (*Medicago sativa*) and korshinsk peashrub (*Caragana korshinskii*) concluded that restoration of these perennial plants improved soil macroporosity, macropore counts, largest pore area, surface area density, macropore branch density, junction density, and connectivity (Li et al., 2016). Several scientific studies have reported these improvements in soil physical properties are due to deep root systems of the permanent vegetation, bulk organic matter addition, duration of the growing season, and minimum soil disturbance (Acosta-Martinez et al., 2004; Culman et al., 2010; Wienhold et al., 2001).

1.6. Objective

Previously in South Dakota, intercropping KC with PCG on marginal lands have reported that PCG-KC mixtures have potential for biofuel feedstock production, improving soil biological conditions and have potential for producing higher soil enzymatic activities and glomalin protein for better plant growth.

However, there is a lack of understanding on long term impacts of intercropping KC with PCG vs PCG fertilized with different N rates on C and N pools at different soil depths. Also, previous studies have explored the influence of PCG-KC intercropping on soil biochemical properties, however, there is a lack of understanding on seasonal changes under KC- PCG intercropping vs fertilized PCG. Moreover, despite the wide application of the X-ray CT technique in studying soil pores under diverse management scenarios, there is a lack of understanding on the long-term impacts of KC-PCG intercropping on soil physical and hydrological properties. Especially, understanding the soil pore systems using high-resolution X-ray CT and the correlation with soil pore structure.

Therefore, the aim of this study was to investigate the effects of long-term PCG-KC intercrop vs fertilized PCG on soil health (biochemical and hydro-physical properties) during the crop growth period. Specific objectives for each study as listed below:

Study 1. Influence of prairie cordgrass and kura clover intercropping on soil biochemical properties at different sampling times and soil depths

Study objectives: (i) to assess the influence of fertilized and intercropped PCG on soil biochemical properties (0-10 cm depth) at different growth stages, the specific parameters analyzed for this objective were soil C, N and microbial community and activity, (ii) to assess the influence of fertilized and intercropped PCG on soil biochemical properties specifically soil TC and TN after harvest at 0-60 soil depth.

Hypotheses: (i) Intercropping PCG-KC may have higher soil C, N, microbial community and activities, (ii) Soil C, N, microbial community and activities may be higher during the active growth stage (summer) as compared to pre-emergence (spring) and post-harvest (Autumn) stages, (iii) Intercropping PCG-KC may have higher soil TC and TN at all depths and, (iv) Soil TC and TN may decrease with soil depth from 0-60 cm.

Study 2. Soil hydro-physical soil properties and CT-measured soil pore characteristics under prairie cordgrass production

Study objective: To assess the impacts of intercrop (PCG-KC) and PCG monocultures managed with different N rates on soil hydro-physical properties.

Hypothesis: Intercropping treatment (PCG-KC) may have better soil hydro-physical properties and soil pores characteristics as compared to fertilized PCG.

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CHAPTER 2

INFLUENCE OF PRAIRIE CORDGRASS AND KURA CLOVER INTERCROPPING ON SOIL BIOCHEMICAL PROPERTIES AT DIFFERENT SAMPLING TIMES AND SOIL DEPTHS

ABSTRACT

Soil biochemical properties are affected by the climatic conditions, soil properties, type of crop and management practices. Prairie cordgrass (PCG) is a perennial grass that has shown a significant potential as a biofuel feedstock crop for marginal lands. This study aims to evaluate the effect of intercropping of kura clover (KC) with PCG (PCG-KC) and PCG N fertilization rates on soil biochemical properties: (i) at different sampling times during the crop season, and (ii) at different soil depths in the year 2021. The study was conducted on an 11-year long-term PCG trial in Brookings, SD with five treatments: PCG-KC and four N fertilizer rates i.e., 0, 75, 150, and 225 N kg ha^{-1} in a randomized complete block design with four replications. For objective (i) soil samples were collected at three different times during the crop season: pre-emergence (PE), active growth (AG), and after harvest (PH) stages, at a soil depth of 0 -10 cm from each treatment; and for objective (ii), deep soil cores were collected at 0 - 60 cm depth for biochemical assessments. Greater microbial biomass C (MBC) and N (MBN) amounts were observed at the PE stage when compared to the other two stages. Water extractable C and N: CWC showed a gradual increase from PE to PH and treatments did not have any effect on the CWC content. HWC was highest at AG stage and lowest at the PH stage. At PE and AG stages, PCG-KC and N fertilized treatments had similar amounts of CWN; while HWN amounts were statistically similar for PCG-KC and N fertilized treatments at AG and PH stages. Intercropped treatment and PCG-225N had higher

urease activity than other PCG treatments. And the highest urease activity was observed at the PE stage as compared to other stages. Intercropping had 44.8% higher β-glucosidase activity than PCG-0N and activity was observed to be higher during AG and PH stages. Fluorescein diacetate (FDA) had shown (~ 20%) higher concentration in PCG-KC treatment than other treatments. Total PLFA and glomalin were observed to be higher at AG and PH stages, with no treatment effect. Nitrate showed 41% higher concentration at PE stage when compared to AG stage whereas NH_4^+ -N concentration increased from PE to PH stage. For deeper depth assessment, the TC and TN had shown a ~41% and 85% decrease in concentration with soil depth i.e., from topsoil (0-5 cm) to bottom layer (45-60 cm) respectively. All the treatments had similar TC and TN concentrations at all soil depths. In conclusion, PCG-KC enhanced the soil biochemical activity at the surface depth and most of the soil biochemical activities were higher during the AG and PH stages compared to the PE stage. Moreover, PCG-KC had similar amounts of TC and TN at all depths as in N fertilized treatments. Therefore, this study suggests that PCG-KC intercropping system can improve the soil health of marginal lands used for biofuel feedstock production.

2.1 Introduction:

Soil health has gained much attention in the recent years due to evidence of positive influences on agronomic performance and ecosystem functions (Culman et al., 2013; Hurisso et al., 2018; Wade et al., 2020). Among different soil health indicators, soil microbial diversity and activity, and carbon (C) and nitrogen (N) pools are the most important components. Microbes in the soil regulate a variety of biochemical processes, such as soil organic matter (SOM) breakdown, nutrient cycling, and aggregates formation

(Sahu et al., 2017). Seasonal changes have a big impact on soil microbial characteristics, either directly through changes in soil moisture and temperature or indirectly through changes in landscape, and vegetation. Increase in temperature accelerates microbial respiration (Lloyd and Taylor 1994) and breakdown of organic materials (Kirschbaum 1995; Gougoulias et al., 2014). Several studies have found that seasonal temperature and moisture changes alter soil microbial community function, such as N mineralization and enzyme activity (Ebersberger et al., 2003), respiration (Luo et al., 1996), SOM decomposition (Wolf et al., 2007), and therefore, C and N cycling (Jin and Evans 2007). For example, in a hot and dry period, the rising temperature may decline microbial biomass and activity due to removal of moisture from the soil (Van Gestel et al., 1992).

Besides the responses to environmental conditions, the soil microbial biomass and community structure also change with soil type and management practices. Management approaches such as intercropping of perennial grass and legume have shown a great potential to enhance soil health and productivity while reducing chemical pollution of soil and waterbodies (Franzluebbers et al., 2014), increasing plant root functions (Isaac et al., 2019), enhancing soil nutrient and spatial use efficiency (Betencourt et al., 2011) and promoting bio-functionalities of soil microorganisms.

Prairie cordgrass (PCG) is a warm-season perennial grass that has a significant potential as a biofuel feedstock. It spreads both vegetatively through rhizomes and non-vegetatively through seed. The PCG can grow well under marginal land conditions and can be grown in most of the states and most importantly in the Northeast, Great Lakes, and Midwest states (USDA, NRCS, 2022). It performs well under low temperatures, poorly drained soils (with frequent flooding), and alkaline conditions. Proper N fertilizer

management plays an important role in obtaining desirable PCG biomass yield and improving soil health through the activation of many enzymes in the soil (Mohapatra et al., 2017). Inefficient use of N fertilizer can lead to environmental pollution (Duran et al., 2016) and increase the input cost. To minimize such problems, leguminous crops have been encouraged as a source of N for sustainable bioenergy crop production. Roots form a symbiotic relationship with N-fixing bacteria such as *Rhizobium*, which can convert atmospheric N into plant available ammonium form . According to Zemenchik et al., 2001, intercropping KC with cool-season forage grasses can replace N fertilizer between 74 and 336 kg N $ha^{-1}year^{-1}$. It can often withstand poorly drained soils and is better adapted to lower soil fertility and pH conditions (Speer and Allinson 1985). It can improve soil biochemical properties by increasing the aboveground and root biomass and root exudate inputs with different biochemical compositions to the soil (Mishra et al., 2012).

Vegetation can influence the soil microbial biomass and its community structure through variety of processes, including the production of litter, alternations of the microenvironment and microclimate changes caused by shading, rhizodeposition, interactions with root-symbiotic organisms, and secretion of root exudates (Prescott and Grayston 2013; Urbanova et al., 2015). Temperature and moisture play a major role in influencing the number of biochemical properties and it is expected that soil microbial biomass and activity is greater in summer (June) when compared to autumn (November) and spring (April) due to large amount of C supply to soil, via root exudates, and fine root turnover (Yao et al., 2011). Previous studies investigating the PCG-KC intercropping

vs N fertilized PCG systems in South Dakota lack information on changes in biochemical activities during the crop season as well as at deeper soil depths.

Therefore, the objective of this study was to assess the effects of PCG production on marginal lands with N fertilizer application vs legume intercrop (KC) on soil biochemical properties (soil C, N, microbial community and activities) at different sampling times during the crop season (i.e., PE stage (spring), AG stage (summer) & PH stage (autumn)) in the top-soil (0-10 cm) and at deeper (0-60 cm) soil depths. We hypothesized that; (i) the intercropping PCG-KC may have higher soil C, N, microbial community and activities due to additional biomass inputs with different C:N compositions and differences in N sources; (ii) soil C, N, microbial community and activities may be higher during the AG stage (summer) due to root exudates, moderate temperature and moisture conditions; (iii) Intercropping PCG-KC may have higher soil TC and TN at all depths due to additional KC residual inputs and differences in C:N ratio; (iv) and the TC, TN is may decrease gradually with depth due to difference in litter composition and the nutrients absorbed by the deep root system at lower soil depths.

2.2. Materials and Methods

2.2.1. Site description and treatment details

The field experiment was established in 2010 at South Dakota State University Felt Research Farm (44° 22′ N; 96° 47′ W) located near Brookings (SD, USA). The soils at this location consist of moderately well-drained silty clay loam McIntosh soil (Fine-silty, mixed, superactive, frigid Aquic Calciudolls). The experiment was established in a randomized complete block design with four replications of five treatments: PCG-KC

(intercrop) and four N fertilizer application rates: 0 (PCG-0), 75 (PCG-75), 150 (PCG-150) and 225 (PCG-225) kg N ha^{-1} yr^{-1}. The individual plots measured 3.0 m wide x 5.7 m long. Plants of PCG were generated from germplasm collected in SD (natural population) and the KC was an experimental line developed by AgResearch New Zealand. Kura clover was inoculated with *R. leguminosarum* biovar trifolo strains 162C11, 162C13, and 162C14 mixture in the greenhouse. In the PCG-KC mixture treatment, the KC was transplanted on 30 cm centers between and within rows for a total density of 111,111 KC plants ha^{-1}. PCG seedlings were transplanted within the KC on 60 cm centers (populations of 26,896 plants ha^{-1}). Monoculture PCG stands were also established at the same population densities as of intercropping stands. From 2012 to 2013, 2, 4-D (2, 4-Dichlorophenoxyacetic acid) was applied at a rate of 0.6 kg a.i. per hectare to KC treatments to suppress the clover growth. From 2011 to 2021, N fertilizer rate treatments were applied in the PCG monoculture treatments once a year in April or May as granular urea (46% N) and, no other mineral fertilizers were applied. After senescence, the biomass was harvested using a sickle-bar mower to measure the biomass yield.

2.2.2. Soil sampling

Biochemical properties at different sampling times:

In 2021, soil samples were collected at three different sampling times (stages of crop growth) i.e., PE in April (spring), AG in June (summer) and PH in November (autumn). Six soil cores were collected at 0–10 cm depth randomly within individual plot using soil core sampler of diameter 2.5 cm and pooled to make one composite sample. After removing visible root and plant debris, they were placed in a ziplock plastic storage

bag and placed on ice while transportation to lab. These samples were kept fresh and stored in a refrigerator at 4 °C until processed for biochemical assays and a part of composite samples was stored at −80 °C for phospholipid fatty acid (PLFA) analysis. All the microbial parameters were analyzed within 2–3 weeks of sampling.

Biochemical properties at different depths:

In 2021, November (PH stage), intact soil cores were collected at a depth of 0-60 cm using hydraulic push probe. One core per plot was collected and was split into five depths: 0-5 cm, 5-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm. The soil samples were analyzed for bulk density, moisture content, soil total carbon, and total nitrogen

2.2.3. Soil analyses

Soil microbial biomass carbon (MBC) and nitrogen (MBN):

Soil MBC and MBN were determined by chloroform-fumigation method as described by Brookes et al., (1985) and Vance et al., (1988). For each sample, three subsamples were taken, (i) subsample for determining gravimetric soil moisture (48 hours @ 100°C), (ii) non-fumigated sample (8 g oven-dry equivalent) for immediate extraction with 40 ml 0.5 M K_2SO_4, and (iii) fumigated sample (8 g oven-dry equivalent). Soil samples were fumigated with ethanol-free chloroform for 24 h at 25ºC in an evacuated extractor and extracted with 40 ml 0.5 M K_2SO_4. Both the fumigated and non-fumigated samples were shaken for 1 h on a reciprocal shaker at 160 rpm and the extractants were filtered using Whatman no. 42 filter paper. Determine total dissolved C and N on a TOC analyzer. The MBC and MBN were calculated by the difference between C and N in the fumigated and non-fumigated samples and with a correction factor of 0.45 and 0.54, respectively (Beck et al., 1997). Cmic was calculated as

$$Cmic = EC/k_{EC},$$

where, EC = (organic C extracted from fumigated soils) − (organic C extracted from nonfumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Nmic was calculated as

$$Nmic = EN/k_{EN},$$

where, EN = (total N extracted from fumigated soils) − (total N extracted from nonfumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985).

The results were expressed as mg C kg^{-1} soil and mg N kg^{-1} soil.

Soil enzyme activity analysis:

Urease enzyme (EC 3.5.1.5) activity was measured using the method provided by Kandeler and Gerber (1988). Five grams of soil was placed into each of the three 50 mL incubation flasks. Two of them were treated with substrate [2.5 mL of urea solution (720 mM) and 20 mL of borate buffer (0.1 M, pH 10)], and the third one was considered as a control (only 20 mL borate buffer were added to it), and flasks were incubated for 2 h at 37 °C. After incubation, 2.5 mL of urea solution was added to control and all the samples with 30 mL of KCl solution. Samples were shaken for 30 min at 160 rpm on a rotatory shaker and the soil suspensions were filtered using N-free folded filter papers. After filtering the soil suspension, to determine the released ammonium (NH_4^+), 1 mL of filtrate was added with 9 mL of distilled water into a test tube. Five mL of sodium salicylate–sodium hydroxide solution and 2 mL of sodium dichloroisocyanurate solution (3.91 mM) were added and allowed for 30 min for color development at room temperature. The absorbance of the color was measured at 660 nm using a spectrophotometer and the results were expressed as mg NH_4^+ kg^{-1} soil hr^{-1}.

The β-glucosidase enzyme (EC 3.2.1.21) activity was determined by using the method of Eivazi and Tabatabai (1988). In this method, 1 g of fresh soil was placed in three 50 mL Erlenmeyer flasks and 0.2 mL toluene was added to each flask, mixed, and left to set for 15 min. Then, 4 mL of 0.05 M modified universal buffer (MUB, pH 6) was added to each flask, and 1 mL of 50 mM p-nitrophenyl-d-glucoside (PNG) solution was added to just two of the flasks, with the third serving as a control, and incubated for 1 hour at 37 °C. After incubation, the reaction was stopped by adding 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM buffer (pH 12) to the control flask, along with 1 mL PNG solution. Soil suspensions were filtered using Whatman No. 2 filter paper. The formation of p-nitrophenol (pNP) (yellow color) was determined spectrophotometrically at 405 nm. The β-glucosidase enzyme activity is expressed as mg pNP kg^{-1} soil h^{-1}.

Fluorescein diacetate (FDA) hydrolytic activity was determined by the procedure of Green et al., 2006. One gram of soil was incubated with 50 mL of 0.1 M Tris (hydroxyl methyl) aminomethane (THAM) buffer (at pH 7.6) and 0.50 mL of FDA substrate solution for 3 hr at 37ºC. At the end of 3 h incubation, 2 mL of acetone was added to terminate the reaction and the absorbance of the supernatant solution was measured at 490 nm and expressed as mg p-Naphthylamine kg^{-1} soil hr^{-1}.

Glomalin-related soil protein (GRSP) analysis:

Glomalin related soil protein content was determined using the method by Wright and Upadhyaya (1998). Each tube containing 3.0 g of air-dried soil sample was added with 24 ml of 20 mM sodium citrate (pH 7.0) and was thoroughly mixed. The tubes were autoclaved for 30 minutes at 121°C (15 psi) and were cooled, and centrifuged (10,000 x g). The Pierce BCA protein assay kit was used to measure the amount of protein in the

solutions (Thermo Scientific, IL, USA). The absorbance was measured at 562 nm in a spectrophotometer. The results of GRSP were reported as g kg^{-1} of dry soil.

Phospholipid fatty acid (PLFA) analysis:

The microbial community structure in soil samples was determined using a PLFA analysis (Clapperton et al., 2005) by taking 0.5 – 1 g of soil. The extraction was done through various steps such as lipid extraction, lipid separation, trans-esterification, and GC analysis.

Lipid extraction: total soil lipids were extracted by shaking the soil sample in 4 ml of Blight & Dyer reagent (200 ml 50 mM K2HPO4 buffer in deionized H2O, 500 ml methanol, 250 ml chloroform) and 19:1 phosphatidylcholine (Avanti Polar Lipids, US) internal PLFA standard followed by sonication at room temperature. Samples were centrifuged in the 5804 R centrifuge (Eppendorf, US) at 4000 rpm for 15 min to separate solid and liquid phases. Supernatant was added with 1 ml of each deionized water and chloroform and centrifuged again at 4000 rpm for 15 min. The separated liquid phase was placed in a SpeedVac™ vacuum concentrator (Thermo Scientifc, US) for drying at low/ambient temperature for 1 h.

Lipid separation: The samples were dissolved with 1 ml chloroform and transferred to conditioned HyperSep™ solid-phase extraction (SPE) columns (Termo Scientifc, US), containing 50 mg silica per 1 ml column and allowed to gravity drain. A 1.5 ml clean glass catch vial was placed below each column and phospholipids were eluted using 0.5 ml of the 5:5:1 chromatography eluent solution (methanol: chloroform: deionized

water) to the SPE columns. The collected solution was dried in a SpeedVac™ vacuum concentrator for approx. 1 h at ambient temperature.

Trans-esterifcation and GC analysis: A 0.2 ml of trans-esterifcation reagent was added to the dried samples followed by incubation at 37°C for 15 min. A 0.4 ml of 0.075 M acetic acid and 0.5 ml of chloroform was added to each tube and bottom phase after vortex was transferred to a GC vial followed by drying in SpeedVac™ vacuum concentrator for 20–30 min at ambient temperature. The samples were further resuspended using 100 µl of hexane and analyzed using an Agilent 2030-GC equipped with a CP-7693 auto-sampler and a fame ionization detector (FID). Fatty acid peaks were identified by comparing the retention times to MIDI PLFAD2 calibration mix using SHERLOCK software v.6.2 (MIDI Inc, US). Fatty acids were used as functional group signatures for various microorganisms and each PLFA was expressed as nmol g−1 soil. The identified bacteria biomarkers included ten gram (+) (15:1 iso ω6c, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:1 iso ω9c, 17:0 iso, 17:0 anteiso, 18:0 iso, 18:1 ω9c, 20:00), ten gram (−) (16:1 ω9c, 16:1 ω7c, 17:1 ω8c, 17:0 cyclo ω7c, 16:0 2OH, 18:1 ω7c, 18:1 ω5c, 19:0 cyclo ω7c, 20:1 ω9c, 21:1 ω9c), four actinomycetes (16:0 10-methyl, 17:1 ω7c 10-methyl, 18:1 ω7c 10-methyl, 18:0 10-methyl), AM fungi (16:1 ω5c), and fungi (18:2 ω6c) biomarkers.

Soil labile carbon (C) and nitrogen (N):

Water extractable C and N fractions were estimated by the procedure described by Ghani, Dexter, and Perrott (2003). The cold-water extractable C (CWC) and N (CWN) were extracted with deionized water at room temperature (hereafter referred to as cold water extraction). This involves shaking of 3-g soil samples with 30 ml of deionized water in 50 ml centrifuge tubes for 30 min. The soil-water suspension was then

centrifuged, and the supernatant was filtered through a Whatman no. 42 filter paper. A further 30 ml of water was added to the remaining residue and put on a vortex shaker for 10 s. The suspension was left in hot water bath at 80°C for 12–15 hr. The suspension was then centrifuged at 3,000 rpm for 25 min at 25°C. The filtrate obtained was used for measurement of hot water extractable organic carbon (HWC) and nitrogen (HWN). The CWC, CWN, HWC, and HWN fractions were determined using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS) and results were expressed as mg C kg^{-1} dry soil and mg N kg^{-1} dry soil.

Soil inorganic nitrogen

Nitrate and ammonium analyses were performed at a commercial soil analysis lab i.e., at Ward Laboratories, Inc. (Lincoln, NE). The method used for the measurement of nitrate (NO_3-N) and ammonium (NH_4-N) content involved extraction with 2.0 M KCl solution. Nitrate was determined by reduction to nitrite via a copperized cadmium column. The nitrite was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the product was measured at 520 nm. Ammonia was determined by heating with salicylate and hypochlorite in an alkaline phosphate buffer. The presence of EDTA was used to prevent the precipitation of calcium and magnesium. Sodium nitroprusside was added to enhance sensitivity. The absorbance of the reaction product was measured at 660 nm wavelength. The results were reported as mg N kg^{-1} dry soil.

Soil total carbon (TC) and total nitrogen (TN)

A subsample of the intact soil cores was oven-dried at 105ºC for 48 h to determine the gravimetric moisture content using oven drying method and bulk density (ρ_b) using the core method (Grossman and Reinsch 2002). The rest of the soil was air-dried, ground, and passed through 2 mm sieve. Soil total C and total N contents were determined by a dry combustion method using a C/N Analyzer (TruSpec carbon/hydrogen/nitrogen analyzer; LECO Corporation, St. Joseph, MI, USA). The results of TC and TN are expressed as g C kg^{-1} dry soil and g N kg^{-1} dry soil, respectively.

2.2.4. Dry biomass yield and quality

In October 2021, biomass was harvested from individual plot from 1 m^2 area using a sickle-bar mower. To measure the biomass yield, all fresh harvested biomass was weighed, and then biomass subsamples from each plot were dried at 60 °C for 72 h to measure the dry biomass weight. For forage quality assessment, the analyses such as acid detergent fiber (ADF), neutral detergent fiber (NDF) and total digestible nutrients (TDN) were conducted on dry biomass samples. Samples were analyzed for forage quality at Ward Laboratories, Inc. (Lincoln, NE). The results of the ADF, NDF, and TDN assessment were reported as percentage (%).

2.2.5. Statistical analyses

The treatment and sampling time effects were assessed using repeated-measures analysis of variance (ANOVA). The Tukey's test was used to detect statistically significant differences between means. All variables were tested for normality and homogeneity. For deep core analysis, repeated-measures ANOVA was conducted with

(Treatment and Depth as fixed factors. The Tukey's test was used to detect statistically significant differences between means. All statistical analyses were performed with *RStudio version 1.4.1717* (R Core Team (2021)). The level of significance that was considered for all the analyses in this study was at $\alpha = 0.05$.

2.3. **Results**

2.3.1. Microbial biomass carbon (MBC) and nitrogen (MBN)

The MBC was ~40-43% higher at PE stage when compared with the other two sampling times (Fig. 2.1). Treatments had no significant effect on MBC. Similarly, for MBN a significant difference was found between the sampling times (Fig. 2.2). The PE and PH stages had 51.8% and 55.6% higher MBN, respectively when compared with the AG stage. Treatments had no significant effect on MBN.

Fig. 2.1. Microbial biomass carbon (MBC, mg C kg^{-1} soil) at three different sampling times (stages) i.e., pre-emergence, active-growth and post-harvest as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference ($P \leq 0.05$) between three sampling times.

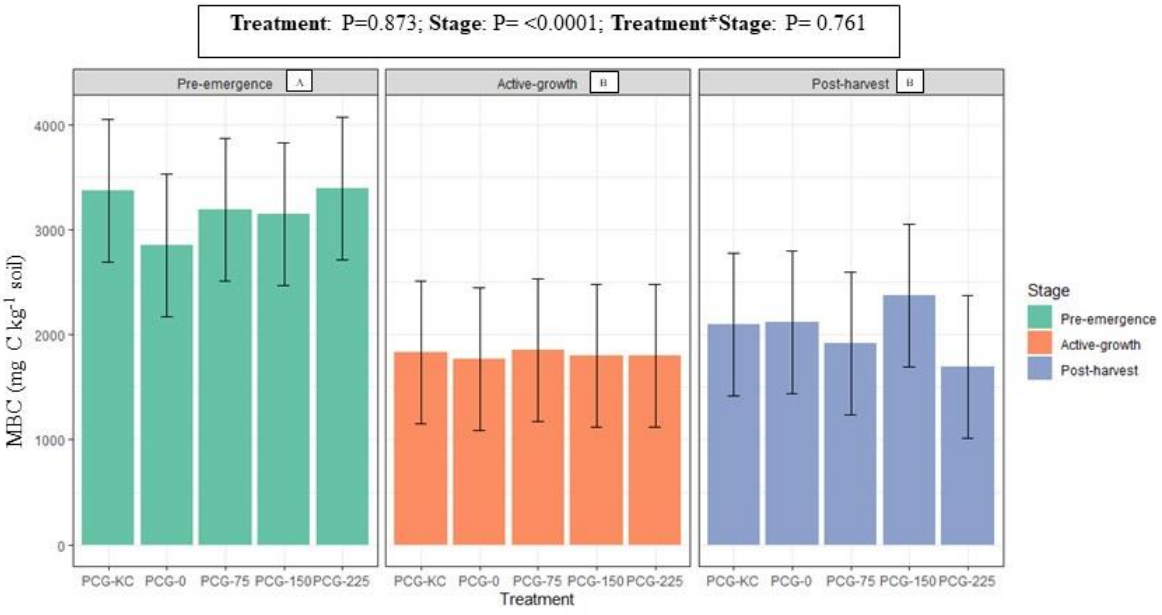
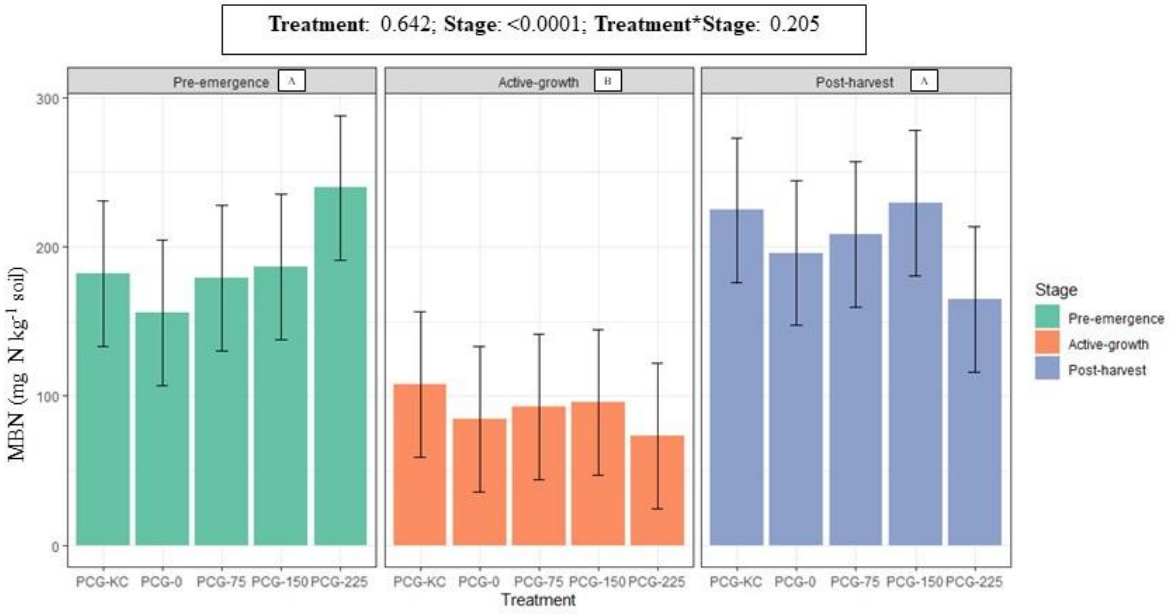


Fig. 2.2. Microbial biomass nitrogen (MBN, mg N kg^{-1} dry soil) at three different sampling times (stages) i.e., pre-emergence, active-growth and post-harvest stage as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference (P ≤ 0.05) between different sampling times.



2.3.2. Soil enzymatic activity

Urease activity was 36 and 29 % higher during the PE stage when compared to the AG and PH sampling times, respectively (Table 2.1). Intercropping treatment (PCG-KC) had 56 and 42% higher mean urease activity when compared to PCG-0 and PCG-75N, respectively, but similar to PCG-150N and PCG-225N treatments. Contrary to urease activity, AG and PH had 51.8% and 59.3% higher β-glucosidase activity as compared to the PE sampling time, respectively. Treatments also had a significant effect on the β-glucosidase activity. Intercropping treatment (PCG-KC) had ~45% higher mean β-glucosidase activity than control i.e., PCG-0 treatment, but was similar to the treatments with N fertilizer application. The PH sampling time had 19 and 12.5% higher

FDA activity when compared with the AG and PE sampling times, respectively. Treatments had a significant effect on the FDA i.e., with PCG-KC had 13-19% higher FDA activity as compared to all the PCG N fertilization treatments.

Table 2.1. Urease (mg NH_4^+ kg^{-1} soil hr^{-1}), β-Glucosidase (mg pNP kg^{-1} soil hr^{-1}), and fluorescein diacetate (FDA) (mg p-Naphthylamine kg^{-1} soil hr^{-1}) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH), as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}).

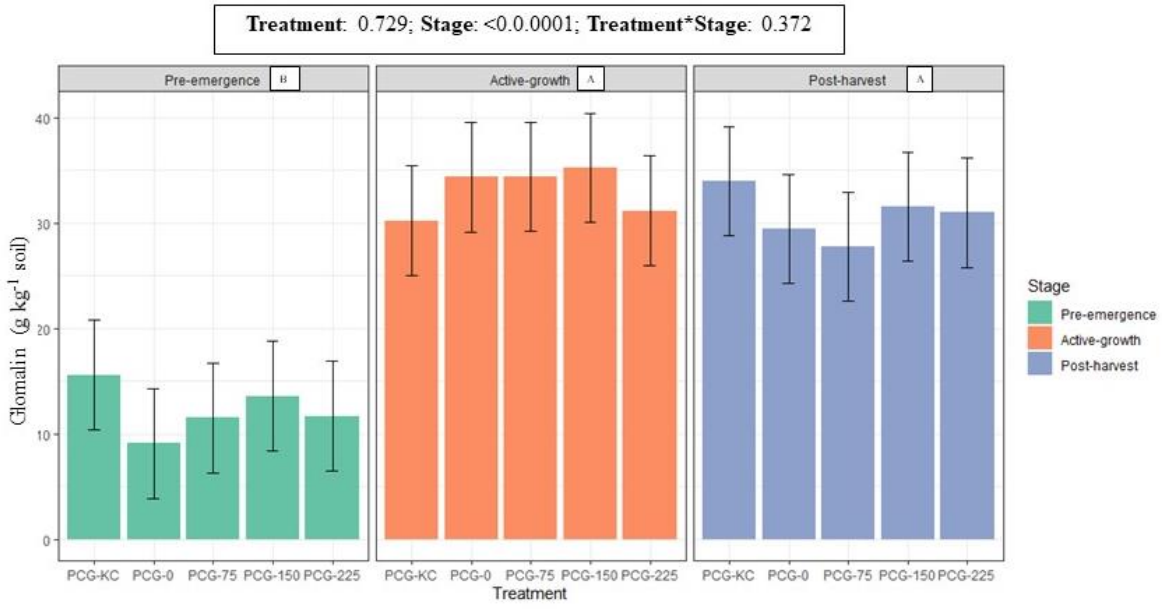
Treatments	Stage			
Urease	*PE*	*AG*	*PH*	*Treatment means*
mg NH_4^+ kg^{-1} soil hr^{-1}				
€PCG-KC	4.65	2.81	3.88	3.78^{ab†}
PCG-0 N	2.94	1.48	1.09	1.67^{d}
PCG-75 N	3.03	2.17	1.36	2.19^{cd}
PCG-150 N	3.49	2.54	3.31	3.11^{bc}
PCG-225 N	5.46	3.16	3.86	4.16^{a}
Stage means	3.82^{A‡}	2.43^{B}	2.70^{B}	
	Analysis of Variance (P>F)			
Treatments	<0.0001			
Stage	<0.0001			
Treatment*Stage	0.286			
β-Glucosidase				
mg pNP kg^{-1} soil hr^{-1}				
€PCG-KC	2.93	7.93	8.23	6.36^{a†}
PCG-0 N	2.31	3.86	4.38	3.51^{b}
PCG-75 N	2.71	5.67	7.73	5.37^{a}
PCG-150 N	3.06	5.40	6.94	5.13^{a}
PCG-225 N	2.72	5.32	6.44	4.83^{ab}
Stage means‡	2.74^{B‡}	5.63^{A}	6.74^{A}	
	Analysis of Variance (P>F)			
Treatments	0.0091			
Stage	<0.0001			
Treatment*Stage	0.481			
FDA				
mg p-Naphthylamine kg^{-1} soil hr^{-1}				
€PCG-KC	0.167	0.155	0.186	0.16^{a†}
PCG-0 N	0.121	0.119	0.151	0.13^{b}
PCG-75 N	0.149	0.123	0.169	0.15^{b}
PCG-150 N	0.144	0.137	0.140	0.14^{b}
PCG-225 N	0.143	0.135	0.165	0.15^{b}
Stage means	0.14^{B‡}	0.13^{B}	0.16^{A}	
	Analysis of Variance (P>F)			
Treatments	<0.0001			
Stage	<0.0001			
Treatment*Stage	0.626			

€ PCG-KC: prairie cordgrass and kura clover intercropping; PCG-0N to 225 N: prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg N ha^{-1}. †Means followed by different lowercase letters within a column indicate significant difference between treatments at $P \leq 0.05$. ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

2.3.3. Glomalin-related soil protein (GRSP)

The AG stage and PH stages had 2-3 times higher GRSP as compared to that at the PE stage (Figure 2.3). Intercropping and N fertilizer rate treatments had no significant influence on the GRSP.

Fig. 2.3. Glomalin (g kg^{-1} soil) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG), and post-harvest (PH), as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference (P ≤ 0.05) between sampling times (stages).



2.3.4. Phospholipid fatty acids (PLFA)

Total PLFA at AG and PH stages were 27 and 17% higher than the PE sampling time, respectively (Fig. 2.4). The highest total bacterial (Table 2.2) and total fungi biomass were detected at the AG stage (Table 2.3). On average across treatments, the total bacterial PLFA at AG stage was 87.9 nmol g^{-1} soil, with 48.8 nmol gram –ve bacteria g^{-1} soil and 39.1 nmol gram +ve bacteria g^{-1} soil. Treatments had a significant

effect on the total fungal PLFA; PCG-75N treatment had the highest levels and PCG-KC treatment had similar total fungi as PCG-0, 150 and 225 N treatment. Arbuscular mycorrhizal (AM) fungi were highest at AG stage and lowest at PE stage. Treatments also had significant effect on AM fungi, with highest levels observed under PCG-75 N treatment, while PCG-KC had similar to PCG-0N, PCG-150N, PCG-225N. Similarly, actinomycetes was observed to be 42 and 38.2% higher at AG and PH sampling times as compared to PE.

Fig. 2.4 Total PLFA (nmol g^{-1} soil) at three different sampling times (stages) i.e., pre-emergence, active-growth, and post-harvest, as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference ($P \leq 0.05$) between sampling times (stages).

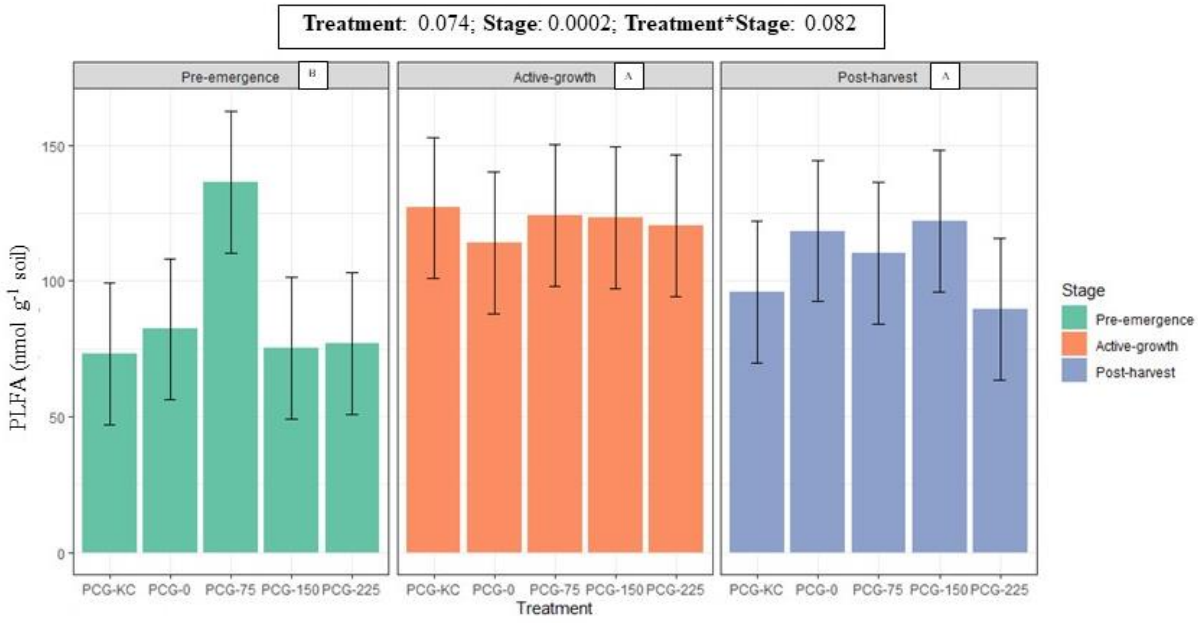


Table 2.2. Total bacterial PLFA, actinomycetes, gram (-ve) and gram (+ve) bacteria, total fungi, and AM fungi (nmol g-1 soil) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH), as influenced by prairie cordgrass (PCG), managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	Stage			
nmol g^{-1} soil	*PE*	*AG*	*PH*	Treatment means
Total Bacterial PLFA				
€PCG-KC	56.9	95.1	73.7	75.2
PCG-0 N	64.5	80.1	85.9	76.8
PCG-75 N	105.6	87.7	83.6	92.3
PCG-150 N	60.6	88.8	90.2	79.9
PCG-225 N	62.5	88.1	67.8	72.8
Stage means	70.0^{B}	87.9^{A‡}	80.2^{AB}	
	Analysis of Variance (P>F)			
Treatments	0.109			
Stage	0.013			
Treatment*Stage	0.066			
Gram (-ve) bacteria				
€PCG-KC	31.9	52.4	38.7	41
PCG-0 N	35.0	44.2	46.7	42
PCG-75 N	60.0	49.1	46.8	52
PCG-150 N	32.1	49.2	47.8	43
PCG-225 N	33.9	49.4	37.0	40
Stage means	38.5^{B}	48.8^{A‡}	43.4^{AB}	
	Analysis of Variance (P>F)			
Treatments	0.059			
Stage	0.012			
Treatment*Stage	0.066			
Gram (+ve) bacteria				
€PCG-KC	25.1	42.7	31.1	33
PCG-0 N	29.6	35.8	39.1	35
PCG-75 N	45.7	38.7	36.7	40
PCG-150 N	28.3	39.5	42.4	37
PCG-225 N	28.6	38.6	30.8	33
Stage means	31.5^{B}	39.1^{A‡}	36^{AB}	
	Analysis of Variance (P>F)			
Treatments	0.145			
Stage	0.015			
Treatment*Stage	0.054			

€PCG-KC denotes prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}). ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

Table 2.3. Total fungi, AM fungi and actinomycetes, as influenced by prairie cordgrass (PCG), managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH).

Treatments	Stage			
nmol g^{-1} soil	*PE*	*AG*	*PH*	Treatment means
Total fungi				
€PCG-KC	10.8	9.75	6.55	7.21^{b†}
PCG-0 N	5.10	12.67	8.78	8.85^{ab}
PCG-75 N	5.32	15.00	6.91	10.89^{a}
PCG-150 N	4.67	11.64	7.88	8.06^{b}
PCG-225 N	4.70	9.60	4.70	6.33^{b}
Stage means	6.11^{B‡}	11.7^{A}	6.97^{B}	
	Analysis of Variance (P>F)			
Treatments	0.0022			
Stage	<0.0001			
Treatment*Stage	0.314			
AM fungi				
€PCG-KC	3.74	8.35	6.69	6.26^{bc†}
PCG-0 N	4.53	7.67	7.43	6.54^{ab}
PCG-75 N	7.91	8.19	6.91	7.67^{a}
PCG-150 N	4.14	7.55	5.29	5.66^{bc}
PCG-225 N	3.87	6.88	4.70	5.15^{c}
Stage means	4.83^{C‡}	7.73^{A}	6.21^{B}	
	Analysis of Variance (P>F)			
Treatments	<0.0001			
Stage	0.001			
Treatment*Stage	0.104			
Actinomycetes				
€PCG-KC	11.0	22.0	21.4	18.15
PCG-0 N	12.6	21.2	20.1	17.99
PCG-75 N	20.1	21.5	19.7	20.46
PCG-150 N	10.0	22.8	23.7	18.88
PCG-225 N	9.65	22.6	17.2	16.49
Stage means	12.6^{B‡}	22.0^{A}	20.4^{A}	
	Analysis of Variance (P>F)			
Treatments	0.355			
Stage	<0.0001			
Treatment*Stage	0.111			

€PCG-KC denotes prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}). †Means followed by different lowercase letters within a column indicate significant difference between treatments at $P \leq 0.05$. ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

2.3.5. Soil labile C and N

The PH sampling time had 28 and 48% higher CWC than AG and PE sampling, respectively (Table 2.4). Treatments had no significant effect on the CWC content. The AG stage had 44.4 and 33% higher HWC content than PH and PE sampling times, respectively. Similar to the CWC, treatments had no significant effect on the HWC content.

A significant interaction between sampling times and treatments was observed for both CWN and HWN (Table 2.5). At PE stage, the CWN content was the highest in PCG-KC (2.56 mg N kg^{-1}soil), which was similar to PCG-150N and PCG-225N treatment. While at the AG stage, CWN was the highest with PCG-225N treatment (8.28 mg N kg^{-1} soil), however, PCG-KC was statistically similar to PCG-0N and other N rate treatments. On average over treatments, AG and PH sampling times had significantly higher CWN content when compared to the PE time.

On average over the treatments, the HWN was higher at the PE and AG sampling times as compared to the PH stage. At the AG stage, PCG-0 had the lowest HWN while there was no significant difference between N fertilized and KC intercropped PCG treatments. On the other hand, at the PH stage, PCG-KC had 24 % higher HWN as compared to PCG-225N. Overall, on average over three sampling times, PCG-KC had significantly 21% higher HWN than PCG-0N treatment but was statistically similar to N fertilized treatments.

Table 2.4. Cold-water extractable carbon (CWC, mg C kg^{-1} soil) and hot water extractable carbon (HWC, mg C kg^{-1} soil) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH), as influenced by prairie cordgrass (PCG), managed with intercropping of kura clover (KC) and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	**Stage**			
mg C kg^{-1} soil	*PE*	*AG*	*PH*	Treatment means
CWC				
€PCG-KC	42.9	58.3	86.0	62.4
PCG-0 N	32.7	51.0	68.6	50.7
PCG-75 N	37.0	52.1	72.7	54.0
PCG-150 N	36.9	52.1	57.4	49.0
PCG-225 N	35.7	42.7	70.7	50.0
Stage means	37.0^{C} ‡	51.1^{B}	71.1^{A}	
	Analysis of Variance (P>F)			
Treatments	0.146			
Stage	<0.0001			
Treatment*Stage	0.236			
HWC				
€PCG-KC	172	270	172	199
PCG-0 N	159	221	159	175
PCG-75 N	172	248	172	186
PCG-150 N	167	264	167	191
PCG-225 N	182	268	182	192
Stage means	170^{B} ‡	254^{A}	141^{C}	
	Analysis of Variance (P>F)			
Treatments	0.130			
Stage	<0.0001			
Treatment*Stage	0.2055			

€PCG-KC denotes prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}). †Means followed by different lowercase letters within a column indicate significant difference between treatments at $P \leq 0.05$. ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

Table 2.5. Cold-water extractable nitrogen (CWN, mg N kg^{-1} soil) and hot water extractable nitrogen (HWN, mg N kg^{-1} soil) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH), as influenced by prairie cordgrass (PCG), managed with intercropping of kura clover (KC) and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	Stage			
mg N kg^{-1} soil	*PE*	*AG*	*PH*	Treatment means
CWN				
€PCG-KC	2.56^{a†}	3.02^{b†}	3.60	3.06^{b†}
PCG-0 N	1.66^{b}	2.34^{b}	3.33	2.44^{b}
PCG-75 N	1.74^{b}	2.50^{b}	3.52	2.58^{b}
PCG-150 N	1.98^{ab}	4.01^{b}	2.90	2.96^{b}
PCG-225 N	2.11^{ab}	8.28^{a}	3.47	4.62^{a}
Stage means	2.00^{B‡}	4.03^{A}	3.37^{A}	
	Analysis of Variance (P>F)			
Treatments	<0.0001			
Stage	<0.0001			
Treatment*Stage	<0.0001			
HWN				
€PCG-KC	13.3	15.0^{a†}	11.9^{a†}	13.3^{a†}
PCG-0 N	11.9	9.78^{b}	10.0^{ab}	10.5^{b}
PCG-75 N	12.4	11.9^{ab}	10.5^{ab}	11.5^{ab}
PCG-150 N	11.8	13.8^{a}	11.1^{ab}	12.2^{ab}
PCG-225 N	14.0	14.5^{a}	9.01^{b}	12.4^{a}
Stage means	12.6^{A‡}	13.0^{A}	10.5^{B}	
	Analysis of Variance (P>F)			
Treatments	0.013			
Stage	<0.0001			
Treatment*Stage	0.028			

€PCG-KC denotes prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}). †Means followed by different lowercase letters within a column indicate significant difference between treatments at $P \leq 0.05$. ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

2.3.6. Soil inorganic nitrogen

There was a significant interaction observed between sampling time and treatments for the effect on soil NO_3^--N concentration (Table 2.6); at AG sampling time PCG-150 N had higher NO_3^--N concentration when compared to PCG-0 N and PCG-75N treatments, at PH stage NO_3^--N concentration was 78% higher than PCG-0N, while at PE stage treatments did not show any significant affect. Overall, PCG-KC treatment had lower NO_3^--N concentration than PCG-225N but was similar to other N fertilized treatments.

Soil NH_4^+-N concentration increased from PE to PH stage with a significant interaction between sampling time and treatment. At PE stage, PCG-KC treatment had 66.4% higher soil NH_4^+-N concentration when compared to control (PCG-0), quantitatively similar to PCG-75N and PCG-150N, but 44.1% lower than PCG-225N treatment. At the AG stage, NH_4^+-N was the 23 and 24% higher in PCG-0 and PCG-150N treatments as compared to PCG-75N, while PCG-KC was statistically similar to all the N fertilization treatments. On average over the sampling times, PCG-225N had the highest NH_4^+-N concentration while PCG-KC was statistically similar to N fertilization treatments.

Table 2.6. Nitrate (NO_3^--N, mg N kg^{-1} soil) and ammonia (NH_4^+-N, mg N kg^{-1} soil) concentration, as influenced by prairie cordgrass (PCG), were managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}) at three different sampling times (stages) i.e., pre-emergence (PE), active-growth (AG) and post-harvest (PH).

Treatments	Stage			
mg kg^{-1}	*PE*	*AG*	*PH*	Treatment means
NO_3^--N				
€PCG-KC	6.67	3.03^{ab†}	3.14^{ab†}	4.28^{bc†}
PCG-0 N	6.85	0.81^{b}	2.37^{b}	3.34^{c}
PCG-75 N	6.90	0.77^{b}	5.35^{ab}	4.34^{bc}
PCG-150 N	7.18	13.4^{a}	10.8^{a}	8.03^{ab}
PCG-225 N	8.43	10.4^{ab}	7.16^{ab}	8.67^{a}
Stage means	7.20^{A‡}	4.23^{B}	5.76^{AB}	
	Analysis of Variance (P>F)			
Treatments	0.013			
Stage	0.010			
Treatment*Stage	0.015			
NH_4^+-N				
€PCG-KC	3.19^{b†}	3.35^{ab†}	6.93	4.5^{ab†}
PCG-0 N	1.07^{c}	3.94^{a}	6.83	4.0^{b}
PCG-75 N	1.78^{bc}	3.04^{b}	6.49	3.7^{b}
PCG-150 N	2.42^{bc}	3.98^{a}	7.14	4.5^{ab}
PCG-225 N	5.71^{a}	3.27^{ab}	6.67	5.2^{a}
Stage means	2.83^{B‡}	3.52^{B}	6.81^{A}	
	Analysis of Variance (P>F)			
Treatments	0.034			
Stage	<0.0001			
Treatment*Stage	<0.0001			

€PCG-KC denotes prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of (0, 75, 150, and 225 kg ha^{-1}). †Means followed by different lower-case letters within a column indicate significant difference between treatments at $P \leq 0.05$. ‡Means followed by different uppercase letters within a row indicate significant difference between sampling times at P<0.05.

2.3.7. Soil TC and TN concentrations at different soil depths in the year 2021

There was a significant effect of the soil depth on soil TC and TN concentrations (Fig. 2.5 and 2.6). The soil TC content drastically decreased from the top soil (0-5 cm) to the deep soil layer (45-60 cm). The highest TC concentration was found in the top 0-5 cm soil layer i.e., 36.99 g C kg^{-1} dry soil, and the 45-60 cm depth had the lowest TC concentration i.e., 14.5 g C kg^{-1} dry soil. Fertilization treatments had no significant effect on TC and TN concentrations.

Similarly, there was a gradual decrease in soil TN from top to lower soil depths. The highest TN concentration was found at 0-5 cm and 5-15 cm soil depths and lowest concentration found was at 45-60 cm i.e., 0.47 g N kg^{-1}dry soil.

Fig. 2.5. Soil total carbon (g C kg^{-1} dry soil) concentration at different soil depths i.e., 0-5 cm, 5-15 cm, 15-30 cm, 30-45 cm and 45-60 cm under prairie cordgrass (PCG) with intercropping of kura clover (KC) and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}) in the year 2021. Different uppercase letters next to soil depths (in the second y-axis) indicate significant difference between soil depths.

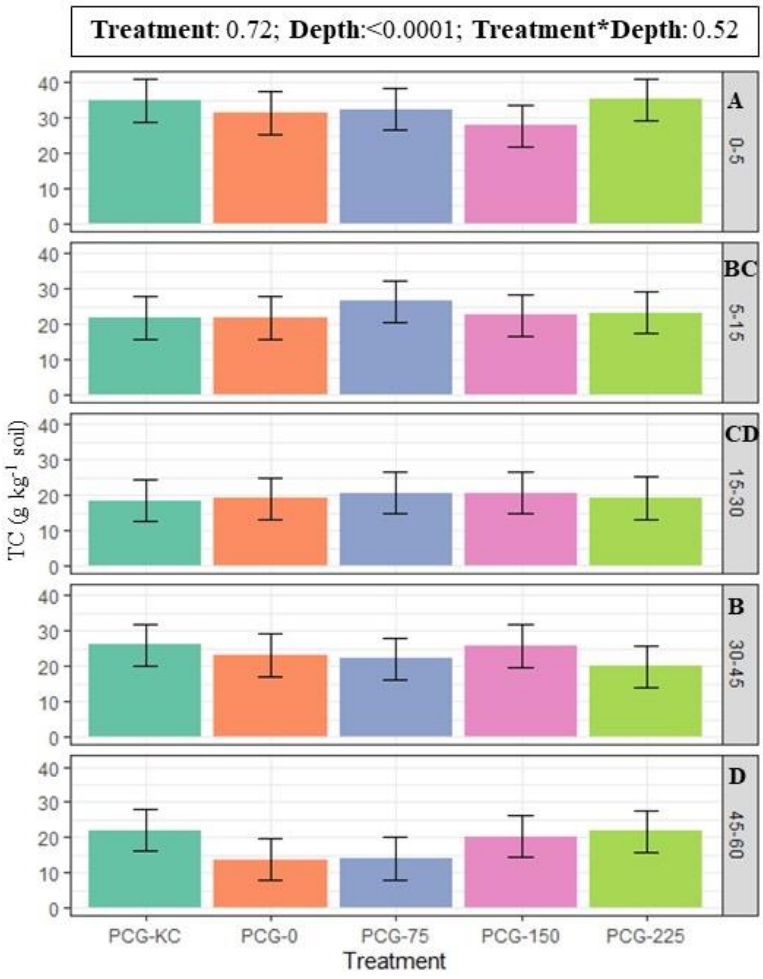
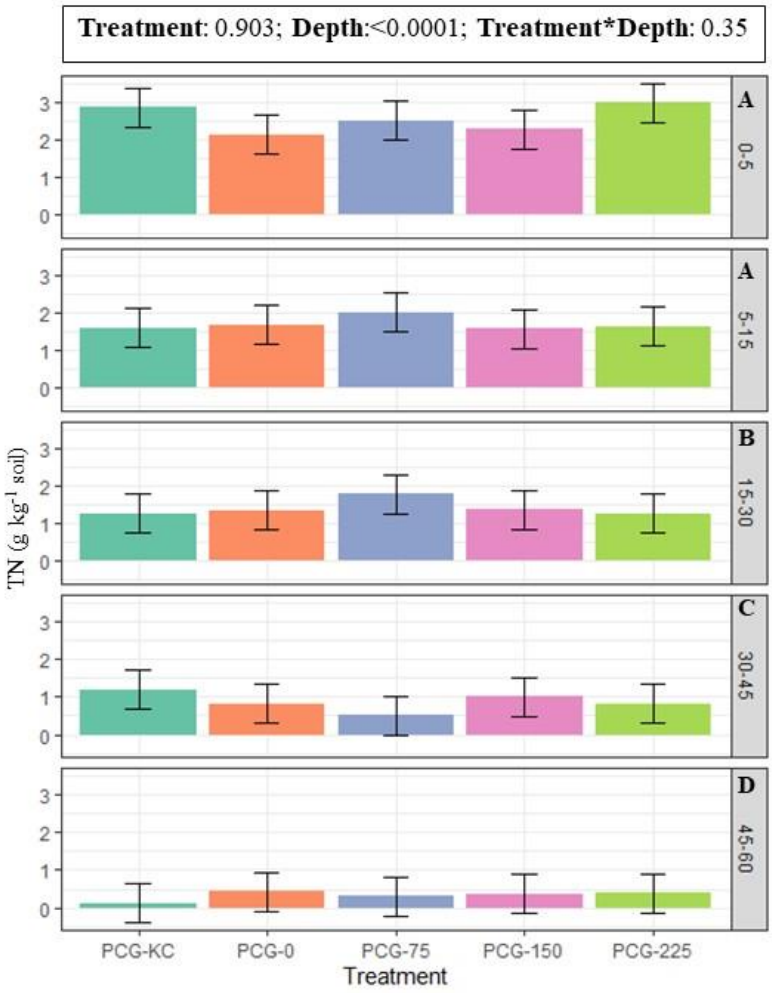


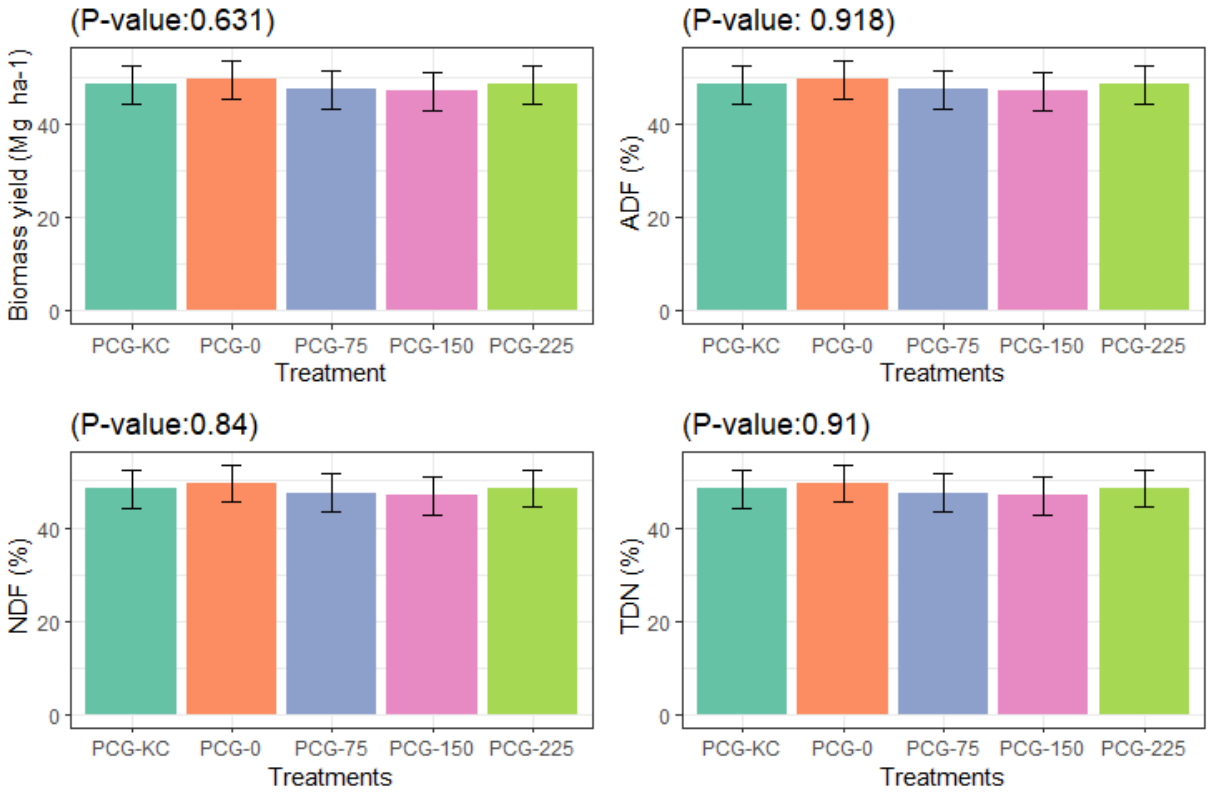
Fig. 2.6. Soil total nitrogen (g N kg^{-1} dry soil) concentration at different soil depths i.e., 0-5 cm, 5-15 cm, 15-30 cm, 30-45 cm and 45-60 cm under prairie cordgrass (PCG) with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters next to soil depths (in the second y-axis) indicate significant difference between the soil depths.



2.3.8. Dry biomass yield and tissue analyses

In 2021, overall, dry biomass production ranged from 3 to 4.3 Mg ha^{-1} (Fig. 2.6). No significant differences in yield were observed among treatments. Similarly, there was no significant effect of treatment on forage quality including, acid detergent fiber (ADF), neutral detergent fiber (NDF) and total digestible nutrients (TDN).

Fig. 2.8. (a) Dry biomass yield (Mg ha^{-1}) and tissue (b) acid detergent fiber (ADF, %), (c) neutral detergent fiber (NDF, %) and (d) total digestible nutrients (TDN, %) in the year 2021, as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}).



2.4. Discussion

2.4.1. Soil microbial biomass C and N

Soil microbial biomass plays an important role in linking plants and soil. Environmental factors such as moisture and temperature changing with seasons and as well as quality and quantity of litter input influence the amount of SOC and microbial biomass and result in changes in key biogeochemical cycles (Morrissey et al. 2014). Seasonal variations in temperature and precipitation have a significant impact on the composition and functions of soil microbial communities, such as N cycling (Parker and Schimel 2011), and SOM

mineralization (Jia et al. 2014). In this study highest MBC and MBN activity was found in the PE stage (spring season) when compared to the other two stages i.e., AG stage (summer) and PH stage (autumn), this may be due to the late harvesting of the previous crop which could have possibly accumulated lots of organic matter on the soil surface and conserved the moisture with moderate soil temperature (Supplementary figures Fig. S.1. and S.2.). According to our results, PE stage had significantly higher moisture content compared to the AG stage but similar to PH stage. Seasonal changes in microbial biomass may also be related to changes in the amount of C that can be obtained from the number of fine roots, root secretions, and dead cover (Babur and Dindaroglu 2020). Evangelou et al. (2021), also found similar declining trend in MBC and MBN during summer followed by an autumn increase with the onset of rains irrespective of differences in vegetation, management practices, and SOC levels in Mediterranean agroecosystems.

2.4.2. Soil enzymatic activity

Urease enzyme catalyzes the hydrolysis of urea. The observed higher urease enzymatic activity during PE stage when compared to the other stages could be due to the accumulation of bulk of organic matter from the previous year and higher microbial biomass during the PE stage which thought to have a strong positive correlation with the urease activity (Myers and McGarity 1968; Dalal 1975; Burns 1978). The accumulation of SOM on the surface layers usually stimulates biological activity and consequently increases enzymatic activity (Klein and Koths 1980). β-glucosidases activity plays a critical role in the global carbon (C) cycle (Knight and Dick 2004). Previous research suggests that C allocation via rhizodeposition has a significant impact on soil bacteria

during the active growth season (Žifčáková et al. 2016). In this study, β-glucosidase activity was higher during the AG and PH stage than the PE stage as the belowground carbon allocation via plant roots is limited to the vegetation period (Högberg et al. 2010) and may be due to the root exudates which would have influenced the soil enzymatic activity. Fluorescein diacetate (FDA) analysis determines the amounts of active fungi and bacteria and to locate acetyl esterases in living protist cells (Brunius 1980, Lundgren 1981). The FDA was higher during the PH stage when compared to PE and AG stages this might be due to the release of nutrients into the soil from decaying organic matter with a high contribution of crop biomass which would increase the microbial activity even further and could account for the continuing increase in enzymatic activity (Rodríguez-Kábana et al., 1982). In comparison to N fertilization, intercropping KC with PCG had a comparable or even greater enzyme activity compared to PCG monocultures treated with different N rates as leguminous crops have a low C:N ratio, as well as readily accessible substances including sugars and amino acids, which can enhance microbial activity and population (Dinesh et al. 2004, Piotrowska-Długosz and Wilczewski 2014).

2.4.3. Glomalin related soil protein

Glomalin is a mycorrhizal glycoprotein (Wright and Upadhyaya 1996) that favorably contributes to soil nutrient delivery, with a focus on soil organic carbon (SOC) pools. As a result, mycorrhizal symbioses provide plants with soil micro-and macronutrients (Wright et al. 2006; Emran et al. 2021; Gispert et al. 2018). In this study higher glomalin activity was found during AG and PH stage i.e., during summer and autumn which can be due to more C contents in the soil and greater photosynthetic activity during those stages. Glomalin was highly produced in soils rich in their C content

and much more when photosynthetic processes increase as in the summer season (Palmqvist 2002). The SOC pool may be a source for glomalin formation when low photosynthate production occurs. A study conducted by Emran et al. (2021) found that glomalin production increased from winter to autumn with a corresponding decrease in SOC contents. This pattern might indicate that there is a balance between the production of new glomalin molecules to increase organic C pools and their subsequent consumption and transformation into mineralized C forms such as CO_2. As a result, depending on the environmental and soil circumstances at any given time of year, the C resources utilized to form glomalin units might have been soil carbon pools, photo-assimilated carbon, or both.

2.4.4. Phospholipid fatty acids (PLFA)

PLFA provide information about the active soil microbial community (Tunlid and White) which includes total bacterial biomass, total fungi, AM fungi, and actinomycetes. In this study, fatty acid profiles and concentrations showed a seasonal influence, with all fatty acids having higher concentrations in the summer than in the spring and autumn. Temperature, humidity, and nutrient inputs from plants all have a substantial influence on soil biological parameters, therefore seasonal changes in lipid profiles are common (Liebig et al. 2006). It may be due to the interference of plant residues or rhizodeposition and to changes in humidity affecting the lipid extraction efficiency (Joergensen and Wichern 2008). Other than the seasonal effect, treatments also showed significant differences. PCG-KC intercropping treatment showed higher PLFA than other PCG monocultures treated with different N rates probably due to the supply of readily

available C substrates, through root exudates and SOM, and availability of mineral N through N-fixation (Yang et al. 2012).

2.4.5. Soil labile C and N

Labile SOM pools, such as extractable organic C and N, are particularly significant because they influence ecosystem production in the near term and are sensitive to changes in management methods (Ramesh et al. 2019). We found labile C and N pools were significantly higher during summer followed by autumn and spring season due to the deposition of more liters and plant density. Water soluble carbon (WSC) concentrations in the mineral horizon peaked in early summer (AG), and autumn (PH), and declined in spring (PE). Higher temperature and precipitation in early summer may result in increased microbial activity and production of labile C due to enhanced microbial breakdown of larger insoluble compounds to soluble ones (Marschner and Kalbitz 2003) in the organic horizon and their leaching to the lower horizons. The release of leached C from the organic horizon coincided with root development and the production of root exudates, which frequently surpasses the demand of soil microorganisms at the start of the growing season (Marschner and Kalbitz 2003, Uchida et al. 2012), resulting in higher peak during summer. Hot water extractable C concentrations also increased substantially in spring (PE) and peaked in summer (AG) in the mineral horizon and declined in autumn (PH). This was most likely owing to an increase in microbial biomass, which may have occurred because of the early summer's abundance of accessible carbon. This trend demonstrates that leaching of soluble plant residues, microbial metabolites, and the release of root exudates have the greatest impact on the water-extractable pool during the start of the growing season. CWN and HWN

also followed the same trend in this study as that of HWC. It may be due to the presence of low C:N ratios and increase temperature and precipitation which resulted in fast decomposition of the organic matter during the summer and resulted in peak extractable N during the active growth stage. In this study, a comparatively higher mean values in labile C and N were observed under PCG-KC as the mechanism mediated in the rhizosphere, the fibrous rooting system under PCG, and the dense rhizomatous root system under KC may have contributed a considerable amount of the carbon, because of their longer photosynthetic activity and higher root biomass, perennial plants provide much more C inputs than annual crops (Marshall et al., 2016).

2.4.6. Soil inorganic N

Nitrate form of inorganic N is very mobile and diffuses more rapidly in the soil and has a great potential to be lost into ground or surface water through leaching or runoff water causing environmental degradation. The inorganic N concentration can change significantly during the growing season with plant growth and the changes in soil moisture, temperature, and microbial activities. In this study, we found a significant interaction between sampling times and treatments for both soil NO_3^--N and NH_4^+-N concentrations. Soil NH_4^+-N showed an increasing trend from spring to autumn this can be due to mineralization of organic nitrogen to ammonia by microorganisms during the active growth stages with the availability of root exudates. Intercrop treatment has shown relatively higher values of NH_4^+-N compared to control in PE stage whereas similar to other treatments during the AG stage this may be due to the N fixation capacity of the leguminous crop.

2.4.7. TC and TN at different soil depths

This study showed the long terms benefits of intercropping on TC, and TN contents at 0-60 cm soil depth i.e., similar TC and TN contents as observed in N fertilized treatments. In the current study, perennial grass system exhibited the highest TC and TN content in the 0–5 cm layer and the lowest concentrations were found in the deeper soil depths. Guo and Gifford (2007) reported that roots play an important role in the contribution to increase in TC and TN. The profile distribution of fine roots coordinates with the distribution of C and N. Thus, the extensive fine root system that grows continuously under perennial systems may be responsible for the higher TC and TN content in the PCG systems. The higher residual inputs in the surface soil maybe contributed to the increased TC and TN content (WU et al. 2004; Liu 2005). However, with the increasing depths, few residues were introduced, and the nitrogen was absorbed by the deep root system from the lower soil depths, as a result gradual decrease in the concentrations of TC and TN. In this study the treatments did not show any significant effect on the TC and TN contents which could be due to no treatment effect on the biomass production.

2.4.8. Dry biomass yield and tissue analysis

Lack of treatment effect on the biomass yield and quality suggests that intercropping KC with PCG has the equal ability to generate the same amount of biomass yield and quality which can be obtained with application of higher N rates to the PCG monocultures, which also indicate the N availability to PCG through biological N fixation. This could possibly help in cutting down the use of synthetic fertilizer and therefore helps in reducing the input cost.

2.5. Conclusion

The current study examined the response of soil biochemical properties and microbial community structure at different stages of the crop growth and long-term effects at different soil depth under PCG-KC mixture and different N fertilization rates to PCG. Microbial biomass C and N, urease activity was higher during the PE stage (Spring) whereas all other parameters were higher during AG or PH stages. Intercropping KC with PCG showed a good potential for producing higher soil enzymatic activities, microbial community structure and HWN and CWN than that of PCG monocultures fertilized with different N fertilizer rates. Thus, it indicates that the PCG-KC mixture created more favorable environment for the growth of plant roots and soil microorganisms that secrete enzymes into the soil. Long-term period i.e.,10 years of PCG-KC intercropping had similar TC and TN as with N fertilized PCG at 0-60 cm soil depths. Thus, long-term maintenance of PCG-KC mixture can reduce fertilizer requirement through N fixation, improve soil biochemical activities and microbial community structure which are sensitive indicators of soil health, and it also specifies that under long term management of these PCG-KC mixtures can increase C and N storage in soil. Overall, N application also had a greater impact on soil labile C, biochemical activities, and soil microbial community structure, especially at 75N. However, when the negative environmental effects are considered with the use of synthetic N fertilizer, PCG-KC treatment is desirable, as it is producing the same amount of biomass yield and showing similar soil biochemical contents when compared to the other N fertilized PCG.

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Supplementary information

Fig. S.1. Soil moisture (%) content at three different sampling times (stages) i.e., pre-emergence, active-growth, and post-harvest, as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference ($P \leq 0.05$) between sampling times (stages).

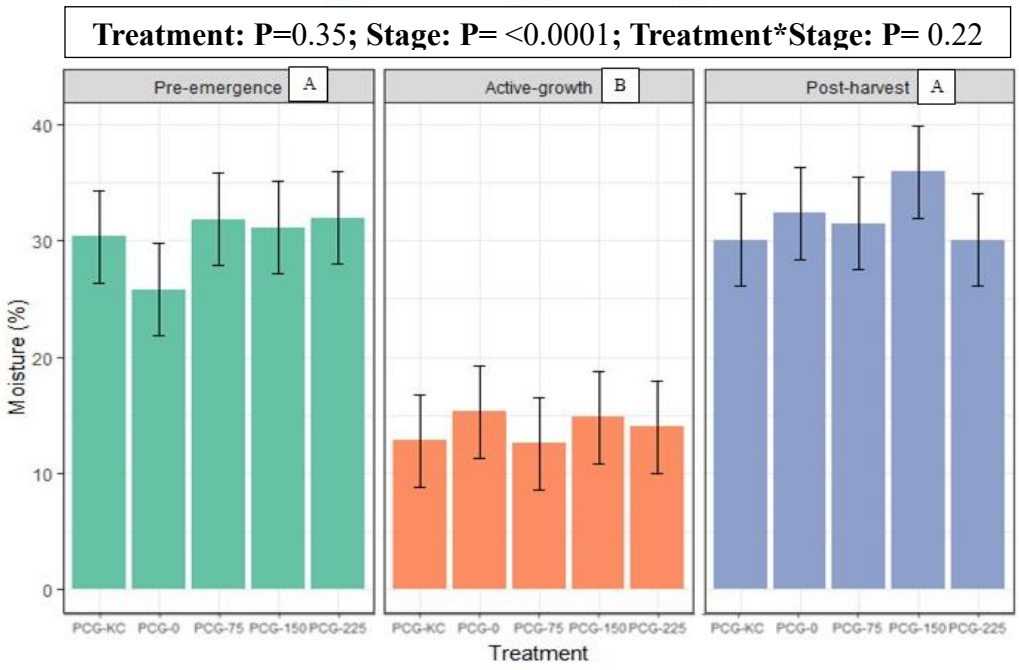
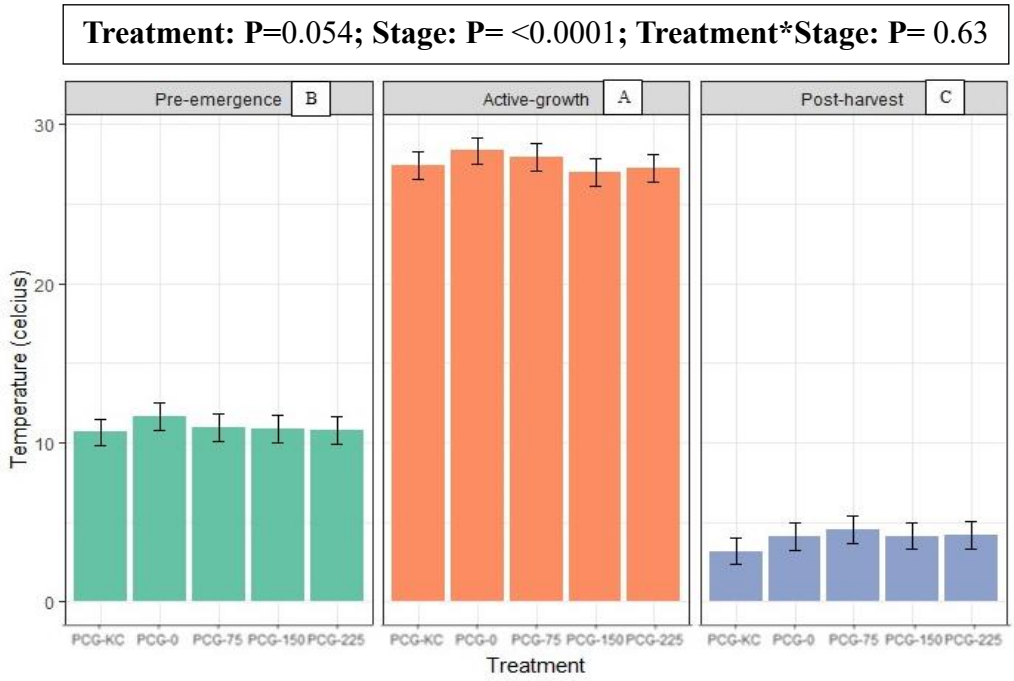


Fig. S.2. Soil temperature (degree Celsius) at three different sampling times (stages) i.e., pre-emergence, active-growth, and post-harvest, as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different N fertilizer rates (0, 75, 150, and 225 N kg ha^{-1}). Different uppercase letters indicate significant difference ($P \leq 0.05$) between sampling times (stages).



CHAPTER 3

SOIL HYDRO-PHYSICAL PROPERTIES AND CT-MEASURED SOIL PORE CHARACTERISTICS UNDER PRAIRIE CORDGRASS

ABSTRACT

Prairie cordgrass (PCG), a C_4-perennial crop, has the potential for biofuel crop under marginal lands. Intercropping of a perennial legume with PCG can mitigate greenhouse gas (GHG) emissions by reducing the use of chemical fertilizer while maintaining the soil's physical and hydrological conditions. The objective of this study was to compare the soil GHG fluxes and physical and hydrological conditions under PCG intercropped with kura clover (PCG-KC), and PCG fertilized with different rates of N [0 (PCG-0), 75 (PCG-75), 150 (PCG-150), and 225 (PCG-225) N kg ha^{-1}] in a randomized complete block design with four replications. Soil samples (0-10 cm depth) and gas samples were collected from all the treatments. Soil water retention, saturated hydraulic conductivity (K_{sat}), saturated thermal conductivity (λ), soil total carbon (TC), and total N (TN) contents were measured. Soil pore parameters were measured using X-ray computed tomography (XCT). The PCG-KC resulted in 1.42 g kg^{-1} of TN and 21.4 g kg^{-1} of TC in the surface layer which was comparable with using 75 N kg ha^{-1} of N fertilizer, however, a significant increase as compared to the PCG-0. In addition, the intercropping PCG-KC resulted in 0.024 cm^3 cm^{-3} of macroporosity (MP), increased K_{sat} by 50%, and decreased λ by 1% as compared to the other treatments. This study, therefore, showed that PCG-KC can reduce the negative environmental impact of using N fertilizers while maintaining the soil physical and hydrological conditions.

***Keywords*:** Soil physical properties, prairie cordgrass, kura clover, nitrogen fertilizer, saturated hydraulic conductivity, computed tomography scanning

3.1. Introduction

The harmful environmental effects and depletion of fossil fuels have created an interest in sustainable energy systems and sparked the search for an alternative plant-based biofuel (*source*: EPA.gov). Plant-based biofuels have been suggested as potentially renewable and carbon (C) neutral forms of bioenergy. Perennial grasses are a prime tool in agricultural systems, especially for improving soil health and mitigating greenhouse gas (GHG) emissions (Wick et al., 2017). A bioenergy system would be sustainable with minimal inputs but can supply energy without negative impacts on C emissions (Sanchez et al., 2015). According to the U.S. Department of Energy (DOE), a biofuel like ethanol produces up to 48% less carbon dioxide (CO_2) than conventional gasoline while the use of biodiesel releases only one fourth the amount of CO_2 than that the conventional diesel releases, making it a much more environmentally friendly option (EESI). Prairie cordgrass (*Spartina pectinata*) (PCG) is a warm-season perennial grass that has significant potential as biofuel and feedstock production which spreads both vegetatively through rhizomes and non-vegetatively through seed. The PCG can grow well under the marginal land conditions and found in most parts of the USA except California, Nevada, and Arizona in the southwest, and Louisiana to South Carolina in the southeast and Canada (Weaver 1954). The PCG has a broad climatic adaptation. It can be able to handle low temperature, poorly drained soils, tolerate alkaline conditions, and land with frequent flooding (*source*: nrcs.usda.gov). Prairie cordgrass is useful for stabilizing soil,

bio feedstock production, and revegetating wetlands (*source*: nrcs.usda.gov). Nitrogen (N) fertilizer management plays an important role in obtaining desirable PCG biomass yield. However, inappropriate rate and application method of N fertilizer can lead to environmental pollution (Duran et al., 2016) and increase the input cost. However, to minimize such problems, the use of leguminous crops has been encouraged as a source of N in sustainable bioenergy production systems. Legumes can be able to form a symbiotic relationship with nitrogen-fixing soil bacteria called rhizobia. This results in the formation of nodules on the plant root, within which the bacteria can convert atmospheric nitrogen into ammonia that can be used by the plant. The amount of N fixed by the leguminous crops is expected to meet the N requirement of the grass species that may help in biomass production and offer potential environmental benefits such as improved soil structure, erosion control, and nutrient cycling (Sekaran et al., 2020).

Kura clover (*Trifolium ambiguum* Bieb.*)* (KC) is a perennial legume that can fix atmospheric N. It is a relatively low-growing, persistent, winter-hardy and has excellent potential for livestock grazing. It mixes well with the commonly grown perennial grasses and can often withstand poorly drained soils and better adapted to lower fertility and pH than the alfalfa (*Medicago sativa*) (*source*: nrcs.usda.gov). It spreads vegetatively by rhizomes or stolons which can colonize unoccupied areas if the management is proper (Beuselinck et al., 1994). A given range of calculated N fertilizer replacement values for intercropping KC with cool-season forage grasses is between 74 and 336 kg N ha^{-1} according to (Zemenchik et al., 2001). Intercropping kura clover with Prairie cordgrass (PCG-KC) can reduce the use of synthetic fertilizer through biological N fixation. The addition of atmospheric N through biological fixation affects soil physical

environment by increasing the above ground and root biomass due to the immediate supply of plant nutrients in sufficient quantities (Ranjan Mishra et al., 2012). This in turn increases the soil organic matter content that has an impact on soil total carbon (TC) and total nitrogen (TN) which may affect the soil pore spaces and improve or maintain the soil hydrological properties. Soil total carbon has been identified as a key indicator of soil quality, which influences a wide range of soil physical, chemical, and biological properties (Carter et al., 2003). A decrease in TC leads to a decrease in soil's structural stability (Castro Filho et al., 2002). Generally, TC and soil hydrological properties are strongly influenced by land-use patterns (Saha et al., 2020) and soil management practices such as tillage (Parras-Alcántara et al., 2016), crop rotation, and organic and inorganic fertilizer applications (Srinivasarao et al., 2014). Therefore, in our study, we focused on the effects of long-term N fertilizer applications and biological N fixation on TC and soil hydraulic properties, and GHG emissions.

Intercropping of PCG-KC is a sustainable management practice to improve the production of plant-based biofuel crops without negatively impacting the environment. However, limited or no studies are available that assessed the impacts of PCG managed with intercropping of KC and different N rates on soil properties. To our best knowledge, reported work in the literature that specifically investigated soil pore-structure and soil hydro-physical properties for a legume-perennial grass intercropping is almost negligible. We hypothesized that the intercropping of PCG-KC may improve or maintain the soil hydro-physical conditions as compared to the other treatments. The specific objective of this study is to assess the impacts of intercropping of KC and different N rates in PCG on

soil hydro-physical properties and compare these properties with those measured under PCG without intercropping, and different N fertilizer rates.

3.2. Materials and methods

3.2.1. Site description and treatment details

This study was established in 2010 at South Dakota State University Felt Research Farm (44° 22′ N; 96° 47′ W) located near Brookings, South Dakota (SD), USA. The soils of the study site are McIntosh silty clay loam soil, which is relatively well-drained (Fine-silty, mixed, superactive, frigid Aquic Calciudolls). The study site had a randomized complete block design with four replications and five treatments: PCG-KC (intercrop) and four N application rates: 0 (PCG-0), 75 (PCG-75), 150 (PCG-150), and 225 (PCG-225) N kg ha^{-1}. Plants of PCG were generated from germplasm collected in SD (natural population) and the KC was an experimental line developed by AgResearch New Zealand. The PCG and KC were transplanted on 60 cm and 30 cm centers in the field in the late spring of 2010. The KC was inoculated with R. leguminosarum biovar trifolo strains 162C11, 162C13, and 162C14 mixture in the greenhouse. In the PCG-KC mixture treatment, the KC was transplanted on 30 cm centers between and within rows for a total density of 111,111 KC plants ha^{-1}. The PCG seedlings were transplanted within the KC on 60 cm centers (populations of 26,896 plants ha^{-1}). Monoculture PCG stands were also established at the same population densities of mixed stands. From 2012 to 2013, the 2, 4-D (2, 4-Dichlorophenoxyacetic acid) was applied at a rate of 0.6 kg a.i. per hectare to KC treatments to suppress the clover growth. From 2011 to 2021, different doses of N fertilizer were given to the PCG monoculture treatments once a year in April

or May as granular urea (46% N). In all years, no other mineral fertilizers were used. The individual plots measured 3.0 m wide and 5.7 m long. After considerable senescence, the complete plot in each treatment was harvested using a sickle-bar mower to measure the PCG yield. The PCG was harvested at a stubble height of around 10-cm once a year to maintain appropriate winter ground cover and rate of regrowth following the harvest.

3.2.2. Soil sampling and analyses

Intact soil cores from each treatment plot were collected at the 0-10 cm depth using cylindrical plexiglass cores during summer of 2021. One intact core per plot was collected.

The intact soil cores were first prepared for X-ray computed tomography analysis. The scanned cores were then used for soil water retention measurements up to the soil matric potential of -30 kPa. Saturated hydraulic conductivity and saturated thermal conductivity measurement followed the soil water retention measurement with the re-saturated cores. A subsample of the intact soil core was then oven-dried at 105^{o}C for 48 h to determine the bulk density (ρ_b) using the core technique (Grossman and Reinsch 2002), and the rest of the soil was air-dried, ground, and passed through 2 mm sieve. Soil total C and total N contents in the bulk soil were determined by a dry combustion method using a C/N Analyzer (TruSpec carbon/hydrogen/nitrogen analyzer; LECO Corporation, St. Joseph, MI, USA).

Saturated hydraulic conductivity (K_{sat}) was estimated using constant head method in the laboratory. A constant water level was maintained on top of an undisturbed soil core. The volume of water that flows through the sample is measured over time and

measured data of the water flow rate (Q; $L^3 T^{-1}$) through a sample, the sample cross-section area (A, L^2) the difference in the hydraulic head (dh, L) and the distance over which *dh* is applied (dl, L).

$$q = Q/A = -Ks \times dh/dl \quad [1]$$

where, q = Q/A is the specific discharge [L/T], dh/dl is the hydraulic gradient, and K_s is the saturated hydraulic conductivity.

Thermal conductivity (λ) is the ability of a material to transfer heat. Meter tempos thermal analyzers have been used to measure thermal conductivity of soil cores. Typically, a probe for this measurement consists of a needle with a heater and temperature sensor inside. A current pass through the heater and the system monitors the temperature of the sensor over time. Analysis of the time dependence of sensor temperature, when the probe is in the material under test, determines thermal conductivity. TR-3 (single-needle type) probe was used to measure thermal conductivity. Range of conductivity measured using TR-3 (10 cm [large]) single needle is 0.1-4.0 W m^{-1} K^{-1}.

$$K = \frac{q}{4\pi} \; x \; \frac{\ln(t2) - \ln(t1)}{(T2 - T1)} \quad [2]$$

where, *k* (W m^{-1} K^{-1}) is the thermal conductivity of the material, *q* (W m^{-1}) is the generated heat per unit length of the material, t_1 and t_2 (s) are the measured times, and T_1 and T_2 (K) are the temperatures at t_1 and t_2.

For X-ray computed tomography (XCT) scanning, the intact cores were saturated in the laboratory using capillarity and then drained at −4.0 kPa using a low-tension table to remove water from macropores to improve image contrast for XCT scanning. Samples were then packed with polythene with appropriate labels and stored under cold conditions

prior to scanning. The cores were carried to the University of Missouri Veterinary Health Center at Columbia, MO for XCT scanning. Cores were placed horizontally on the scanner table to perform a spiral scanning with a peak voltage current of 135 kV and an X-ray tube current of 200 mA. Each 3D image had a voxel size of (0.35×0.35× 0.28) mm^3 in all directions and a pixel resolution of 31.6 μm. The X-ray beam width or "slice" thickness was 0.3 mm. The entire sample was imaged with a field of view 512 by 512 mm pixels. The 16-bit monochrome images were saved in the TIFF format for further processing.

Image processing was carried out using the software ImageJ and the bundle of plugins distributed in FIJI (Schindelin et al., 2012). To prevent any artefacts on the boundaries of soil core samples that may have formed during sampling or transport, the Region of Interest (ROI) tool was used to crop the 3-D images to diameter and height of 6.5 and 6.5 cm, respectively. For noise reduction in the picture stack, a 3D median filter with a radius of 2 voxels was used (Luo et al., 2010). Images were normalized using the "enhance contrast" technique to improve the contrast between the soil solid material and pores. The segmentation approach was based on (Phansalkar et al., 2011) adaptive local thresholding method. The threshold value of each pixel was computed using the mean and standard deviation of the grey values of nearby pixels in this method. Pores were defined as pixels with grey values less than the threshold. The dispersed features with one-voxel width were removed using a closure technique. The photos were also visually reviewed to ensure that the segmented images were of good quality. This technique produced a binary picture with white and black pixels representing pores and soil matrix, respectively.

Using the particle analyzer plugin within the BoneJ plugin (Doube et al., 2010) in ImageJ software, the porosity and number of pores were estimated while taking the picture resolution into account. The 3D fractal dimension is a measure of self-similarity and complexity. A box-counting technique was used to determine surface detail (Perret et al., 2003) and the degree of anisotropy, which determines how pores tend to be oriented, was calculated by the BoneJ plugin (Doube et al., 2010). The tortuosity (τ) was calculated from macropore skeletons created with the ImageJ software's Skeletonize 3D plugin. The skeletons (the centerline of macropores with a thickness of one voxel) were examined in ImageJ using the Analyze Skeleton plugin (Doube et al., 2010) and was computed as the ratio of total real lengths of all macropores to the sum of the shortest distance between two ends for all macropores (Katuwal et al., 2015).

Soil water retention (SWR) was measured after the scanning of cores completed. Cheesecloth was wrapped at the bottom of each soil core and was kept for saturation for 24 h and SWR was measured at four soil matric potentials (Ψ_m) that included: 0, -0.5, -5.0, and -30 kPa using a combination of tension table and pressure plate extractors (Soil moisture equipment corp) (Klute and Dirksen 1986). Soil water content (g g^{-1}) was obtained gravimetrically at each Ψ_m by oven drying soil samples at 105°C for 48 hr, and this moisture content was converted to volumetric water content (cm^3 cm^{-3}) by multiplying by (ρ_b) and dividing by density of water (ρ_w). The permanent wilting point water content in this study was defined as water retained at −1500 kPa and was determined using WP4C Water Potential Meter (Meter Group Inc.). Approximately, 30 g of 2 mm sieved air-dried soil sample was mixed with 6 ml of water and equilibrated (Basche et al., 2016). Three measurements of the same sample were taken with WP4C to

obtain the -1500 kPa water content. Van Genuchten function was fitted to the measured retention data to obtain soil water retention curves (SWRC) parameters for each treatment.

3.2.4. Statistical analyses

Data for soil hydro-physical properties and GHG fluxes were analyzed by ANOVA, Post Hoc tests, Pearson correlation coefficients (δ) in *RStudio version 1.4.1717*. The treatments were considered as fixed effects and replications as random effects. Pearson's correlation analysis was conducted to analyze the relationships among various soil hydro-physical, and XCT scanning properties. The level of significance that was considered for all the analyses in this study was at $\alpha = 0.10$.

3.3. Results

3.3.1. Soil TC, TN, ρ_b, K_{sat}, and λ.

Data on TC, TN, ρ_b, K_{sat}, and λ under different treatments are shown in Table 3.1. A significant treatment effect was observed for the SOC and TN values at a 10% significance level. Intercropping treatment (PCG-KC) showed a higher mean value for TN (1.42 g kg^{-1}) when compared to the other treatments. Similarly, for TC, PCG-KC has shown a higher mean value (21.4 g kg^{-1}) when compared to the control i.e., PCG-0N (19.3 g kg^{-1}) and PCG-150N (20.2 g kg^{-1}) treatment.

Treatment did not impact soil ρ_b and K_{sat}. The ρ_b values ranged from 1.13 to 1.19 g cm^{-3}. Although non-significant, the intercropping PCG-KC treatment showed a higher mean value of 16.6 cm min^{-1} for K_{sat} when compared to the other treatments. However,

the saturated thermal conductivity for PCG-KC (1.057 W m^{-1} K^{-1}) was lower than PCG-0N (1.15 W m^{-1} K^{-1}) and PCG-150N (1.14 W m^{-1} K^{-1}) treatments.

Table 3.1. Soil bulk density (ρ_b), total nitrogen (TN), total carbon (TC), saturated conductivity (K_{sat}), and saturated thermal conductivity (λ) as influenced by prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	TC	TN	ρ_b	K_{sat}	λ
	g kg^{-1}	g kg^{-1}	g cm^{-3}	cm min^{-1}	W m^{-1} K^{-1}
PCG-KC†	21.4^{b††}	1.42^{a}	1.13^{a}	16.6^{a}	1.06^{bc}
PCG-0N	19.3^{d}	1.17^{b}	1.19^{a}	7.91^{a}	1.15^{a}
PCG-75N	22.0^{a}	1.38^{ab}	1.14^{a}	9.70^{a}	1.02^{c}
PCG-150N	20.2^{c}	1.35^{ab}	1.14^{a}	8.23^{a}	1.14^{ab}
PCG-225N	21.3^{b}	1.38^{ab}	1.14^{a}	7.70^{a}	1.06^{bc}
	Analysis of variance (P>F)				
Treatments	0.098	0.098	0.916	0.428	0.075

†PCG-KC denotes prairie cordgrass and kura clover intercropping and PCG-0N to 225 N denotes prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}).
††Means with different letters within a column are significantly different at P<0.10.

3.3.2. X-ray Computed tomography (XCT)-measured pore characteristics

X-ray Computed tomography derived porosity (Macroporosity (MP), Mesoporosity (MesoP) and Total porosity (TP), number of macropores (NMP), number of mesopores (NMesoP), number of total pores (NTP), tortuosity (τ), Junction (J), Triple point (TrP), Quadruple point (QP), and Max branch length (MBL)) under different treatments are shown in Table 3.2 and Table 3.3. Figure 3.1 shows the variation in MP, MesoP, TP, and numbers of macropores, mesopores and total pores. From Table 4.3 and Fig. 4.1, we can observe that intercropping treatment i.e., PCG-KC had shown 42.8% higher MP and 37.9% higher TP than the PCG-150N treatment; no significant difference was observed with other N rates. Total macropores, total mesopores, and total pores did not show any significant differences among the treatments. Moreover, a significant

treatment effect was not observed for the tortuosity, junction, triple points, quadruple point, and max branch length values at a 10% significance level.

Table 3.2. X-ray Computed tomography (XCT)-measured average macroporosity, mesoporosity, total porosity, total number of macropores, total number of mesopores and total number of pores as influenced by Prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	Porosity (m^3 m^{-3})			Total macro-pores	Total meso-pores	Total pores
	Macroporosity	Mesoporosity	Total porosity			
PCG-KC†	0.0240^{a††}	0.0073^{a}	0.0313^{a}	523^{a}	1664^{a}	2143^{a}
PCG-0N	0.0206^{ab}	0.0068^{a}	0.0274^{ab}	435^{a}	1499^{a}	1934^{a}
PCG-75N	0.0227^{a}	0.0067^{a}	0.0295^{ab}	417^{a}	1315^{a}	1686^{a}
PCG-150N	0.0168^{b}	0.0059^{a}	0.0227^{b}	436^{a}	1442^{a}	2041^{a}
PCG-225N	0.0219^{ab}	0.0072^{a}	0.0286^{ab}	436^{a}	1540^{a}	1801^{a}
	Analysis of variance (P>F)					
Treatments	<0.05	0.745	<0.05	0.345	0.4	0.455

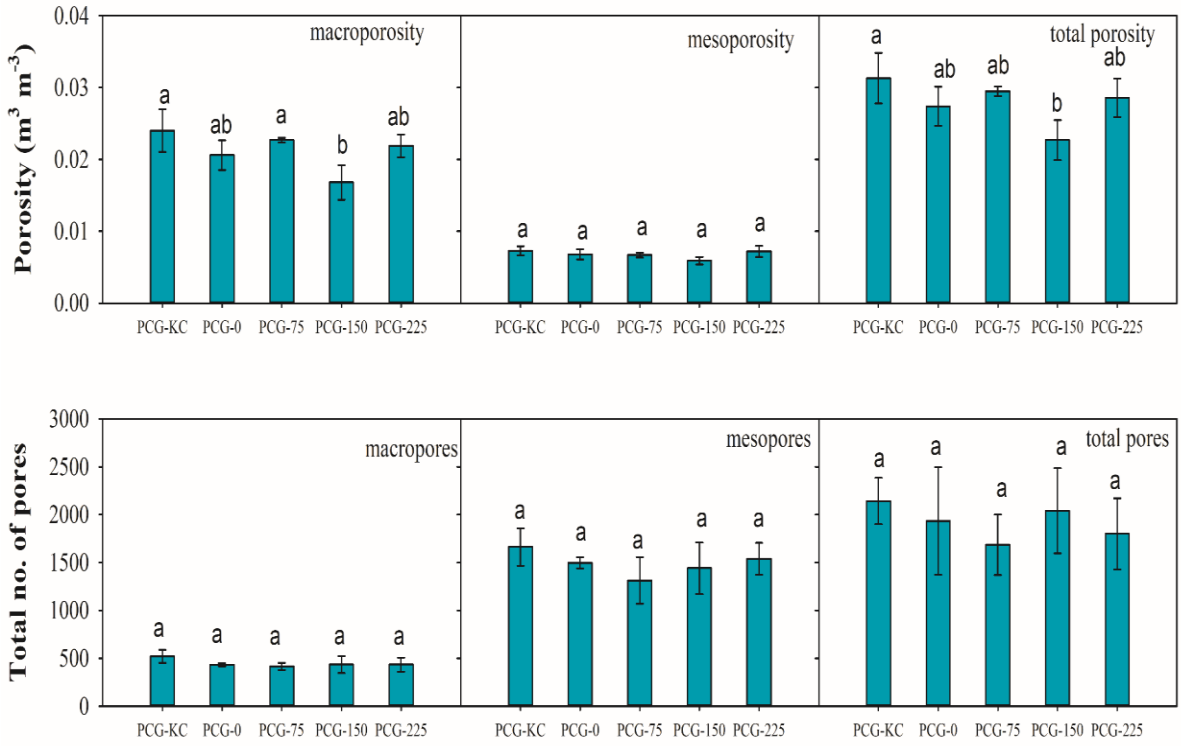
†PCG-KC denotes Prairie cordgrass and kura clover intercropping and PCG-0N to 225 N denotes Prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}).
††Means with different letters within a column are significantly different at P<0.10.

Table 3.3. X-ray Computed tomography-derived average Tortuosity (τ), Junction (J), Triple point (TrP), Quadruple point (QP), and Max branch length (MBL) of pores as influenced by Prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}).

Treatments	τ	J	TrP	QP	MBL (mm)
KC-PCG†	1.285^{a††}	7447^{a}	5175^{a}	1463^{a}	0.796^{a}
PCG-0N	1.281^{a}	7000^{a}	4935^{a}	1351^{a}	0.808^{a}
PCG-75N	1.283^{a}	8043^{a}	5551^{a}	1628^{a}	0.769^{a}
PCG-150N	1.276^{a}	7125^{a}	4989^{a}	1400^{a}	0.805^{a}
PCG-225N	1.281^{a}	7924^{a}	5459^{a}	1612^{a}	0.776^{a}
	Analysis of variance (P>F)				
Treatments	0.184	0.494	0.525	0.353	0.817

†PCG-KC denotes Prairie cordgrass and kura clover intercropping and PCG-0N to 225 N denotes Prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}).
††Means with different letters within a column are significantly different at P<0.10.

Fig. 3.1. X-ray Computed tomography (XCT)-measured average macroporosity, mesoporosity, total porosity, total number of macropores, total number of mesopores and total number of pores as influenced by Prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}).



3.3.3. Soil water retention

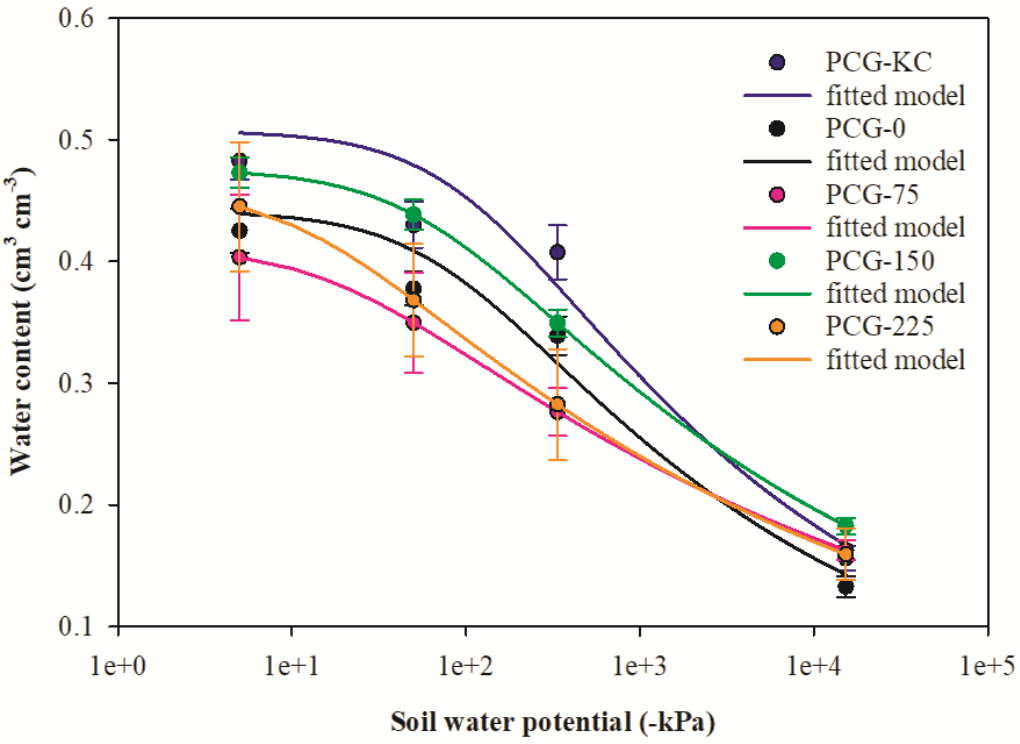
Soil water retention under different treatments is shown in Table 3.4. The SWR was not influenced by any of the treatments. The water retention at 0kPa varied from 0.45 to 0.52 cm^3 cm^{-3}. Similarly, for the pressures of 0.5, 5, 30 and 1500 (-kPa), the water contents in the soil varied from 0.42 to 0.49 cm^3 cm^{-3}, 0.35 to 0.43 cm^3 cm^{-3}, 0.32 to 0.40 cm^3 cm^{-3}, and 0.14 to 0.15 cm^3 cm^{-3}, respectively. Treatments were insignificant to the soil water retention curves (Fig. 3.2).

Table 3.4. Soil water retention at low tension as influenced by Prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha [1]).

Treatments	Soil Water Potential (-kPa)				
	0	0.5	5	30	1500
	----------water content (m^3 m^{-3})----------				
PCG-KC[†]	0.46[a††]	0.44[a]	0.37[a]	0.34[a]	0.15[a]
PCG-0N	0.46[a]	0.44[a]	0.38[a]	0.35[a]	0.14[a]
PCG-75N	0.49[a]	0.46[a]	0.39[a]	0.36[a]	0.15[a]
PCG-150N	0.52[a]	0.49[a]	0.43[a]	0.40[a]	0.15[a]
PCG-225N	0.45[a]	0.42[a]	0.35[a]	0.32[a]	0.15[a]
	Analysis of variance (P>F)				
Treatments	0.741	0.703	0.459	0.433	0.968

[†]PCG-KC denotes Prairie cordgrass and kura clover intercropping, and PCG-0N to 225 N denotes Prairie cordgrass grown with different N rates of 0, 75, 150, and 225 kg ha^{-1}).
[††]Means with different letters within a column are significantly different at P<0.10.

Fig. 3.2. Soil water retention curves as influenced by Prairie cordgrass (PCG) managed with intercropping of kura clover (KC), and different nitrogen rates (0, 75, 150, and 225 N kg ha^{-1}).



3.3.5. Correlation among XCT derived pore characteristics and measured soil hydro-physical properties

The correlation matrix among the XCT-derived pore characteristics and measured soil hydro-physical properties is shown in Table 3.5. From the table, we can observe that K_{sat} had shown a high negative correlation (-0.40) with a number of mesopores (Nmeso). Saturated thermal conductivity had shown a high negative correlation (-0.42, -0.70, and -0.64) with MP, TC, and TN respectively. TN on the other hand showed a high negative correlation (-0.47 and -0.64) with NTP and λ respectively. Whereas soil TC showed a high negative correlation (-0.49 and -0.70) with NTP and λ, respectively, and high positive correlation (0.85) with the TN.

Table 3.5. Pearson correlation coefficients among the soil hydro-physical properties such as saturated hydraulic conductivity (K_{sat}), thermal conductivity (λ), total nitrogen (TN), and soil total carbon (TC), and XCT derived pore characteristics of macroporosity (MP), mesoporosity (MesoP), total porosity (TP), number of macro pores (NMP), number of mesopores (NmesoP), number of total pores (NTP) and tortuosity (τ).

	TC	*TN*	MesoP	MP	TP	NMP	NMesoP	NTP	K_{sat}	λ	τ
TC	1.00										
TN	0.85***	1.00									
MesoP	0.14	0.12	1.00								
MP	0.25	0.22	0.80***	1.00							
TP	0.17	0.13	0.85***	0.99***	1.00						
NMP	-0.24	-0.07	0.47*	0.23	0.29	1.00					
NMesoP	-0.21	-0.06	0.62**	0.34	0.40	0.70***	1.00				
NTP	-0.49*	-0.47*	0.45*	0.18	0.27	0.65***	0.80***	1.00			
K_{sat}	0.14	0.23	-0.13	0.01	-0.03	0.12	-0.40	-0.35	1.00		
λ	-0.70***	-0.64**	-0.30	-0.42*	-0.37	-0.05	-0.10	0.21	-0.16	1.00	
τ	-0.01	0.15	0.53**	0.67***	0.66***	0.08	0.10	-0.06	0.00	-0.08	1.00

*Significant at the 0.05 level
**Significant at the 0.01 level
***Significant at the 0.001 level

3.4. Discussion

Intercropping PCG-KC treatment has contributed to almost the similar amount of TC and TN contents in the surface soil layer as the N rates of 225N and 75 N, respectively. In a bioenergy system, high N fertilization rates and intercropping with legumes improved biomass output, resulting in a rise in TC. The intercropping resulted in increased TC than the control and PCG-150N, and similar TC as that of PCG-225N. (Alvarez 2005) have reported that N application could increase the TC only when crop residues are maintained on the soil surface. However, as the aboveground biomass was collected in all years, the effects of N fertilization rates and KC intercropping on TC may have been impacted. Higher TN under increased N rates and PCG-KC intercrop in this study were attributed to N availability through N fertilizer and through N-fixation by the latter. This study highlights the contributions of TC and TN contents under KC intercropping in comparison with variable N rates on PCG monoculture. Intercropping KC with PCG might be one of the most effective approaches to rehabilitate marginal land soils.

Soil physical properties such as ρ_b and K_{sat} showed no treatment differences, and this may be probably due to the role of long-term grass-legume management which may have led to improved soil structure and the proportion of macropores and coarse mesopores (Table 3.1) which help water move easily through the soil for all the treatments. It is noted that we observed very high values of K_{sat} in this study irrespective of the treatments. Long-term management of perennial plants is expected to enhance soil mechanical and moisture qualities, although outcomes may vary depending on the location (Bonin et al., 2012). In perennial systems, a higher amount of biomass is located

below ground, which decreases soil erosion, bulk density and may also increase water penetration qualities by forming root bio pores (Bonin et al., 2012). The formation of bio-pores might result in very high K_{sat} values. Perennial crop systems can improve or maintain soil hydraulic properties in the long run. Even though there is no treatment effect for the K_{sat}, intercropping treatment had shown a higher mean K_{sat} value. This may be due to higher pore connectivity, MP, and mesoporosity (MesoP). Water and air circulation in soils are primarily controlled by continuous pores that run from the surface to the bottom of the soil column (Allaire-Leung et al., 2000). In addition, the non-significant difference in K_{sat} may be due to the higher coefficient of variation of K_{sat} among the replications (Dirk Mallants et al., 1997). The soil λ is the ratio of the magnitude of the conductive heat flow through the soil to the magnitude of the temperature gradient (W m^{-1} K^{-1}) under saturated conditions. It measures the soil's capacity to transfer heat in the same way that hydraulic conductivity measures the soil's ability to conduct water. The thermal conductivity of soil is influenced by a variety of factors, including air-filled porosity, water content, bulk density, texture, mineralogy, organic matter content, soil structure and soil temperature. An increase in a soil's λ is caused by an increase in either its saturation or dry density. Mineral composition, temperature, texture, and time are all secondary variables that affect soil λ (Salomone and Marlowe 1989; Mitchell 1991; Becker et al., 1992). In this study, a significant difference was observed between the treatments for λ. The PCG-KC had shown lower λ when compared to PCG-0N which is ideal for crop growth and development due to minimum fluctuations in soil temperature.

A significant difference was observed between the treatments for MP and total porosity (TP), whereas MesoP did not show any treatment effect (Table 3.2). PCG-KC has shown higher MP and TP when compared to the PCG-150N treatment but similar to the other N treatments. Greater root growth and subsequent root breakdown, the addition of SOM, and improvement in soil physical qualities owing to permanent vegetation as compared to seasonal crops can also be ascribed to the significantly higher number of pores observed in different treatments. This may be due to the addition of high organic matter content and root development which improved the MP in the soil. According to the literature, permanent vegetation improves soil porosity as compared to continually cropped agricultural fields because of the presence of more roots and organic matter (Udawatta et al., 2006). The extensive exudation of organic compounds by roots is promoted by root growth via shoot renewal (Hamilton III et al., 2008), which improves aggregate stability and hence soil porosity. High TC in the soil might also have contributed to the improved soil structure.

Other pore characteristics such as tortuosity, which is the degree of intricacy of the sinuous porous route of soil (Pagenkemper et al., 2014) influence the movement of water and air through the soil. Junction where different pores join at one point, triple and quadruple points represent three and four pores joining together, respectively, and the max branch length (MBL) indicates the most elongated pore in the soil core. Plant development is aided by the existence of these elongated pores, which improve root penetration and water and gas transport through soils (Pagliai et al., 2004). No significant difference was found among the treatments for the pore properties due to the deep root system and long-term maintenance of this grass-legume intercropping which might have

maintained the soil structure. Previously published research showed that the perennial system improves XCT-measured soil porosity as compared to agricultural row crops (Udawatta et al., 2006). Changes in soil physical qualities are caused by permanent plant roots, organic matter addition, the duration of the growing season, and management approaches, according to research. However, for the present study, a significant difference in pore characteristics is not observed among the treatments.

Soil water retention is a major soil hydraulic property that ensures soil functioning in the ecosystem, and it is greatly influenced by the soil management (Calanca et al., 2006). SWR mainly depends on the pore size distribution of the soil. The finer the soil particles, high is the cohesiveness and to ensure optimal crop development and production. However, in this study, SWR did not show any significant difference among the treatments, this may be due to long term management of the perennial grasses on the field which could have led to the maintenance of soil hydrological properties without many differences among the treatments. Moreover, the findings from a long-term perennial grass system (71-year) study concluded that N fertilizer application simply maintained the SWR capacity (Blanco-Canqui et al., 2015). Grass roots help improve or maintain the soil structure in the long run by increasing porosity and adding organic material that helps bind soil particles together.

It is reported that soil organic matter is a major influence in soil quality and production (Cannell and Hawes 1994). Soil nitrogen (N) and N retention in ecosystems are affected by the quantity and quality of TC (Hart et al., 1994). From Table 3.5 we could observe that TC is highly correlated with TN (a correlation coefficient of 0.85 is observed). The λ decreases as soil porosity increases (Lu et al., 2014). For this study, we

observed a significant negative correlation between λ and MP. The K_{sat} is reported to be correlated with XCT derived pore characteristics (Li et al., 2018, Zhang and Schaap 2019). For example, (Singh et al., 2020) derived a linear regression model to predict K_{sat} as a function of the number of macropores. For the long-term perennial grass ecosystem in this present study, we observed that K_{sat} is not significantly correlated with any of the XCT derived parameters.

3.5. Conclusions

This study was conducted to compare the soil physical and hydrological conditions under PCG-KC, and PCG fertilized with different rates of N. Soil TC, TN, ρ_b, K_{sat}, λ, SWR and pore characteristics were estimated in this study. The PCG-KC treatment had contributed almost the same amount of TC and TN on the surface layer as that of the PCG-225N treatment, which can conclude that the long-term maintenance of this intercropping system may potentially help in storing more carbon and reduce the input fertilizer cost. Study treatments did not impact soil hydro-physical properties; however, it can be concluded that long-term maintenance of PCG with KC intercropping can help in maintaining these soil properties without needing any additional N fertilizer due to the deep root systems of these perennial plants. Our data contributed to a thorough understanding of soil hydro-physical properties under long-term grass-legume intercropping which was cultivated on marginal soil and provided new knowledge on the measured soil hydro-physical properties and its correlation with the XCT derived parameters. It is recommended that further study on long-term perennial systems for bioenergy production on marginal land is required. This can help to bridge our current

knowledge gap on soil C and N interactions in these land-use systems. This study concludes that PCG-KC can minimize the use of synthetic fertilizer and helps in maintaining the soil pore characteristics and hydrological properties.

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CHAPTER 4

CONCLUSIONS

This study was conducted at South Dakota Felt Research Farm, Brookings to investigate the impact of prairie cordgrass and kura clover intercropping on soil biochemical, soil physical and hydrological properties. The following conclusions were drawn from this study:

4.1. Study 1 – Soil biochemical properties

This study was conducted to evaluate the impact of PCG-KC intercropping and N fertilization treatments on soil biochemical properties and microbial community structure at three different sampling times corresponding to different crop growth stages and long-term (10 years) effects at different soil depths.

1. Intercropping KC with PCG showed good potential for producing higher or similar soil enzymatic activities, microbial community structure, and hot and cold-water extractable N production than that of PCG monocultures with different N fertilization. Thus, it suggests that the PCG-KC mixture created a more favorable environment for the growth of plant roots and soil microorganisms that enhance soil health.
2. Microbial biomass C and N, urease activity was higher during the pre-emergence stage (Spring) whereas all other parameters were higher during active (Summer) or post-harvest (Autumn) growth, which clearly indicates that root growth and exudates increases the microbial community and increases biochemical properties.
3. The PCG-KC treatment and fertilized PCG monocultures had similar soil total C and N but it decreased with the increasing soil depth due to less litter accumulation

and lower microbial population. Higher concentration of TC and TN were found in the surface layers (0-5 cm) whereas the lower concentrations were observed at deeper depth (45-60 cm).

4. Overall, N application also had a greater impact on soil labile C, biochemical activities, and soil microbial community structure, especially at 75N. However, when the negative environmental effects are considered with the use of synthetic N fertilizer, PCG-KC treatment is desirable, as it is producing the same amount of biomass yield and shows similar soil biochemical contents when compared to the other N fertilized PCG.

4.2. Study 2 - Soil hydro-physical properties and measured soil porosity

A long-term (>10 years) experimental site was selected to evaluate the impact of PCG-KC intercropping on soil hydro-physical properties and soil pore structure using X-ray computed microtomography (XCT).

1. Intercropping PCG-KC treatment had contributed almost the same amount of SOC and TN on the surface layer as that of the PCG-225N treatment, which can conclude that the long-term maintenance of this intercropping system may potentially help in storing more carbon and reduce the input fertilizer cost.
2. Study treatments did not impact soil hydro-physical properties; however, it can be concluded that long-term maintenance of PCG with KC intercropping can help in maintaining these soil properties without needing any additional N fertilizer due to the deep root systems of these perennial plants.
3. Our data contributed to a thorough understanding of soil hydro-physical properties under long-term grass-legume intercropping which was cultivated on

marginal soil and provided new knowledge on the measured soil hydro-physical properties and its correlation with the XCT derived parameters.

4. This study concludes that PCG-KC can minimize the use of synthetic fertilizer and helps in maintaining the soil pore characteristics and hydrological properties.

4.3. Overall conclusion

Overall, this study highlights the importance of long-term maintenance of PCG-KC mixtures on marginal lands. Among the treatments evaluated in this study, PCG-KC mixture can be considered as desirable treatment over N fertilizer application since it can reduce the fertilizer input and negative environmental impacts, improves soil biochemical properties, and maintains soil hydro-physical properties.