March 11, 2019

Project Report of NREC Project

Impacts of Manure and Inorganic Fertilizer on Soil Fertility, Water Quality, and Crop Yield in South Dakota

Sandeep Kumar, Jose Guzman, Peter Kovacs and Asmita Gautam (MS Student)

Summary. The experimental site for SDSU soil fertility project is located at the Southeast Research Farm of the South Dakota State University located at Beresford, and Brookings Felt farm, South Dakota. The experimental sites were established in 2003 at Beresford and 2008 at Brookings to assess the influences of manure and inorganic fertilizer on the long-term corn (*Zea mays* L.) – soybean (*Glycine max*. L.) rotation. Crop rotation now is proposed to be corn-soybean-springwheat. The experimental site has 24 plots with 4.6 to 20 m dimensions into complete randomized block design. Study treatments included: three manure (dairy and beef manure) [P-based recommended manure application rate (P), N-based recommended manure application rate (N), nitrogen-based double of recommended manure application rate (2N)], two fertilizers [recommended fertilizer (F) and high fertilizer (HF)], and a control (CK) with no manure application]. The manure was applied in the spring in a manual application and incorporated by disk at 6-cm deep for 1 to 3 days before planting at either site. Manure of the study was analyzed by South Dakota Agricultural Laboratories. Fertilizer treatments for 190 kg ha^{-1} yield goal for corn in 2018 and 44.8 kg ha^{-1} for soybean were used for both the sites; however, no nutrient recommendation of fertilizer for soybean was used.

Findings form the Beresford site in 2018 show that in the top 20 cm soil depth, microbial total biomass was 43% greater in the 2N treatment compared to other treatments (average of 2882 ng g^{-1}). Higher microbial diversity index, 14% on average, was also observed in the 2N treatment compared to the other treatments (on average 1.35 ratio). Chemical properties, soil organic matter, inorganic N, K, and phosphorus was in general, greatest in the 2N treatment, followed by the (F) and (P) manure treatments, and lowest in the inorganic fertilizer and (CK) treatments. However, soil phosphorus thresholds (risk for water pollution) were exceeded in the (F) and (2N) treatments. Differences in soil physical properties across the treatments were only observed in the top 10 cm soil depth. Bulk density under the (2N) (1.10 g cm^{-3}) was 14% lower on average when compared to the inorganic fertilizer and control (CK) treatments. Higher soil enzymatic activities were observed with 2N treatment compared to control (CK). Results suggest that soil health overall was higher under manure applications when compared to inorganic fertilization. However, risks for nutrient transport away from the field were also high when manure applications exceeded recommend rates based on soil phosphorus.

Study Objectives.

1. Assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on soil fertility parameters such as N dynamic (NO_3^--N and NH_4^+-N), P, K, soil organic carbon (SOC), micronutrients (Fe, Mn, Zn and Cu), bulk density, microbial activity, aggregate stability, pH and EC at two sites (Beresford and Brookings) and water quality parameters (*similar as the previous year*).
2. Assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat rotation on crop growth parameters, nutrients in plants, and N use efficiency, agronomic N use efficiency and physiological N use efficiency.
3. Develop site-specific best fertilizer management practices using the Soil Management Assessment Framework (SMAF) tool by utilizing the data from FY-2017 and Objectives 1 and 2 of this proposal.

Methods

Soil samples were collected before planting in 2018 at both the sites to determine the nutrient status to apply manure and fertilizer to the treatments. Corn crop was planted at both the sites and calculated manure and fertilizers were applied based on the treatment requirement.

Soil samples were collected from 0-10, 10-20, 20-30 and 30-40 cm depths in 4 replicates and mixed together to make a composite sample for each plot in 2018 to analyze selected soil health indicators. Detailed talks are mentioned below in the results section.

Results and Impacts

Task 1. *Measurement of Soil health indicators.*

Soil samples were extracted from 0-10, 10-20, 20-30 and 30-40 cm depths in 4 replicates and mixed together to make a composite sample for each plot in 2018 to analyze selected soil health indicators. Composited soil samples were labeled, sealed in plastic zip-lock bags, and transported to the laboratory. Wet aggregate stability of the soil for the 0-20 cm depths was measured using the procedure of Kemper and Rosenau (1986). The pH of the soil is a measure of the concentration of the hydrogen ion (H^+) concentrations. Soil pH and electrical conductivity (EC) was determined using a suspension sample with soil (air-dried) to the water (soil: water) ratio of 1:1 procedure with an Orion star pH and EC meter. The method outlined by Stetson, Osborne, et al. (2012) was used to determine carbon (C) and nitrogen (N) concentrations after removing visible crop residues and sieved through a 0.5 mm. Total C (TC) and nitrogen (TN) was analyzed by combustion using a Tru-Spec-CHN analyzer (LECO Corporation, St. Joseph, MI). Soil inorganic carbon (SIC) was measured using 1M 10 ml of HCI addition to the one gram of the 0.5 mm sieved soil samples. The loss of the weight from the initial weight of the total was given as SIC. Soil organic carbon (SOC) was calculated by subtracting SIC from TC and expressed in g kg^{-1}. Soil enzymes like urease, β –glucosidase, alkaline phosphatase, arylsulfatase were analyzed using a spectrophotometer. Phospholipid fatty acid (PLFA) analysis was also conducted for 2 soil depths for both sites as a measurement of soil health indicator. High throughput 16S RNA-soil sequencing was also carried out to identify the soil microbial composition. The data processing for soil sequencing is under progress.

In addition, intact soil core samples were collected for analyzing the soil pore-size distributing using the computed tomography study (CT-Scanning). These cores were scanned at the University of Missouri-Columbia, and the analysis of pore-sizes are under progress. This outcome will provide an useful indicator of soil health.

Task 2. *Measurements of Soil Nutrients and crop yield.*

The impact of manure and inorganic fertilizer applications on soil nutrients and crop yield was also studied. Soil samples at depths of 0 to 15 cm and 15 to 60 cm, were taken at the beginning of the experiment before manure application and mineral fertilization year by using augers and soil data were used to calculate the amount of manure rates. For soil nutrient analysis 1 month after planting, disturbed soil samples were extracted from four replications of each plot from 0-10, 10-20,20-30 and 30-40 cm depths and mixed together to make a composite sample for the respective depth. Soil samples were carried in plastic zip-lock bags, labeled, transported to the lab, and stored for analysis. Soil was air dried, mechanically grinded, and sieved through 2-mm sieve to analyze and also stored at 4°C. Soil analysis was performed to determine N, P, K, Soil organic matter (SOM), Sulfur, Zinc, pH, Salts, and Texture. Soil pH was determined through a suspension sample with a soil (air-dried) to water (soil: water) ratio of 1:1 procedure and measured with a pH meter. Available phosphorus in soil was determined by extracting samples with 0.5 M $NaHCO_3$ and determining using Olsen method (Olsen et al., 1954). Total N in soil was determined by Kjeldahl digestion–distillation method (Bremner and Mulvaney, 1982). Available potassium is displaced from exchange sites by 1N (pH 7.0) NH_4OAc and the concentration of K measured by flame emission on the A.A. spectrophotometer. This procedure is designed to determine soluble sulfate-sulfur plus a fraction of the absorbed sulfate. Crop yield and biomass yield were recorded after the harvest. Post-harvest soil samples were collected and analyzed for their nutrient status. Grain and biomass samples were also collected and analyzed for their nutrient concentration. These data will be statistically analyzed and prepared for the peer-reviewed publication later this fall 2019.

Task 3. *Measurements of Soil C Pools.*

The impact of manure and inorganic fertilizer applications on soil carbon (C) pools were also studied. Soil samples at depths 0-10, 10-20, 20-30 and 30-40 cm were taken from five spots from each plot at harvest in November 2018. Soil samples were carried in plastic zip-lock bags, labeled and transported to the lab. Soil was sieved through 2-mm sieve to remove any visible plant materials and then stored at 4 °C. Soil analysis was performed to determine soil C pools (mineralize C, non-labile organic C, particulate organic C, light organic C, active organic C, dissolved organic C, and microbial biomass C). Mineralize C was determined using titration method. Non-labile organic C was measured as the difference between total C content and active C. Soil light C and particle C pools were separated by density fractionation, which is one of the physical fractionation methods used widely. Active C pool was measured using the permanganate oxidizable C. Dissolved organic C and soil microbial biomass C was determined using the chloroform fumigation-extraction method. Soil organic C stock and distribution and C management index will be calculated for all the C pools. These data will be statistically analyzed and prepared for the peer-reviewed publication later this fall 2019.

Task 4. *Application of Soil Management Assessment Framework (SMAF) tool.*

Data have been analyzed in the lab, however, data analysis using statistical software still under progress. The soil health assessment is calculated using the Soil Management Assessment Framework (SMAF) scoring function analyses Scores are given for each soil property measured, then combined into one simple value (0 to 1) for an easy interpretation of overall soil health. Soil properties being measure are physical (bulk density, penetration resistance, water retention), chemical (pH, P, N, K, salinity), and biological (PLFA, soil enzymes, carbon).

Changes in project or personnel. One MS Student (Asmita Gautam), partially budgeted Research Assistant 1 (Abagandura Gandura), postdoctoral researcher (Udayakumar Sekaran) and temporary researcher (Tess Owens) worked under this project in 2018. Due to the excessive soil moisture in the spring, lysimeters for measuring nutrient leaching could not be installed on time before leaching events occurred.

Products/Publications/Presentations.

- Ozlu, E., and Kumar, S. 2018. Response of Soil Organic Carbon, pH, Electrical Conductivity, and Water Stable Aggregates to Long-Term Annual Manure and Inorganic Fertilizer. Soil Science Society of Am. J. doi:10.2136/sssaj2018.02.0082.

- Ozlu, E., and Kumar, S. 2018. Response of surface GHG fluxes to long-term manure and inorganic fertilizer application in corn and soybean rotation. Science of The Total Environment 626, 817-825.
- Gautam, A., Jose Guzman, Sandeep Kumar, Peter Kovacs and Peter Sexton. 2019. Long-Term Impacts of Manure Application and Inorganic Fertilization on Soil Health in South Dakota. Poster presentation at Soil Science Society of America Annual Meeting in San Diego.

Initial Budget and Expenditure.

#	Initial Budget ($)	Expenditure ($)
1(July 01, 2017-December 2018)	74,998.00	37,226.71 (as of May 2018)
2 (Jan 2018-December 2018	40,500.00	21,941.82
3 (Jan 2019-December 2019)	40,238.00	40,238.00

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Sandeep Kumar, Jose Guzman, Peter Kovacs, Asmita Gautam (MS Student), Gandura Abagandura (Postdoctoral Researcher), Udayakumar Sekaran (Postdoctoral Researcher)

Summary. The experimental site for SDSU soil fertility project is located at the Southeast Research Farm of the South Dakota State University located at Beresford, and Brookings Felt farm, South Dakota. The experimental sites were established in 2003 at Beresford and 2008 at Brookings to assess the influences of manure and inorganic fertilizer on the long-term corn (*Zea mays* L.)-soybean (*Glycine max*. L.) rotation. Crop rotation now is proposed to be corn-soybean-spring wheat. The experimental site has 24 plots with 4.6 to 20 m dimensions into complete randomized block design. The study treatments include: three manure rates [low manure (LM), manure application based on the phosphorous requirement; medium manure (MM), manure application based on nitrogen requirement; high manure (HM), two times prescribed nitrogen rate], two chemical fertilizer rates [medium fertilizer (MF), recommended inorganic fertilizer rate; high rate of the fertilizer (HF)], and control (CK, without any manure or fertilizer application). The manure was applied in the spring in a manual application and incorporated by disk at 6-cm deep for 1 to 3 days before planting at either site. South Dakota Agricultural Laboratories analyzed manure of the study. Fertilizer treatments for 190 kg ha^{-1} yield goal for corn in 2018 and no fertilizer application was done for soybean in 2019 for both sites.

Data from this study showed that bulk density under HM was 19 and 9% lower compared to the CK in 2018 and 2019, respectively. Data from 2019 showed that manure application significantly increased soil wet aggregate stability (0-10 cm) compared with the CK. Soil organic carbon (SOC) was significantly higher in HM treatment by 51.7, 9.27, 11.7, and 33.3% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively, as compared to the CK. In 2018 and 2019, water stable aggregates (WSA) was increased by 18, 22, 46, 21, 28, and 44% in LM, MM and HM treatments, respectively, as compared to the CK. Data showed that higher rates of organic manure application (HM) significantly increased urease, β-glucosidase, and alkaline phosphatase enzyme activities compared to the CK for 0-10 cm soil depth in 2018. Similar trend was observed for 2019. For 10-20 cm depth, soil enzyme activity values were lower than the 0-10 cm soil depth. In 2018, β-glucosidase enzyme activity was significantly higher with MM, HM, and HF treatments (4.79, 4.86 and 4.75 µmol $PNPg^{-1}$ soil h^{-1}, respectively) than the CK (3.74 µmol $PNPg^{-1}$ soil h^{-1}) treatment. Furthermore, in 2019, HM treatment significantly increased the β-glucosidase enzyme activity by 38 and 25% than the CK and HF treatments, respectively, at 10-20 cm depth. The PLFA biomass at 0-10 and 10-20 cm depths was higher with the HM compared with the CK. In 2018, the HM increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass by 70, 84, 65, 108, 72, 118, 92, and 1.36% than the CK treatment. Similarly, in 2019, application of HM and MM treatments significantly increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass than the CK. The SQI values in 0-10 cm soil depth were higher in LM, MM, and HM treatments by 7.95, 6.75 and 9.80 %, respectively, compared to the HF. In 10-20 cm soil depth, the SQI values were higher in LM, MM, and HM treatments by 10.1, 8.73 and 16.3%, respectively, compared to the CK.

Results suggest that overall soil health was higher under manure applications when compared to inorganic fertilization. However, risks for nutrient transport away from the field were also high when manure applications exceeded recommend rates based on soil phosphorus. For C pools in 2018 and 2019, results also showed that all manure and inorganic fertilizer significantly increased active C at 10-20 cm depth compared to CK. Treatments that included manure had significantly higher C mineralization than inorganic fertilized treatments and CK at 0-10 cm. The manure treatments also had higher dissolved organic C and N than inorganic fertilized treatments at both sites. Particulate organic C and N also increased by manure at both depths compared to inorganic fertilized and CK at both sites. Carbon management index increased by manure at both depths compared to inorganic fertilizer at Brookings. The results demonstrate that manure can increase C and N compared to inorganic fertilized and unfertilized treatments.

Study Objectives.

1. Assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on soil fertility parameters such as N dynamic (NO_3^--N and NH_4^+-N), P, K, soil organic carbon (SOC), micronutrients (Fe, Mn, Zn and Cu), bulk density, microbial activity, aggregate stability, pH and EC at two sites (Beresford and Brookings) and water quality parameters.
2. Assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat rotation on crop growth parameters, nutrients in plants, and N use efficiency, agronomic N use efficiency and physiological N use efficiency.
3. Develop site-specific best fertilizer management practices using the Soil Management Assessment Framework (SMAF) tool by utilizing the data from FY-2017 and Objectives 1 and 2 of this proposal.

Methods

Soil samples were collected before planting in 2018 and 2019 at both sites to determine the nutrient status to apply manure and fertilizer to the treatments. Corn crop was planted at both sites and calculated manure and fertilizers were applied based on the treatment requirement.

Soil samples were collected from 0-10, 10-20, 20-30 and 30-40 cm depths in 4 replicates and mixed together to make a composite sample for each plot in 2018 and 2019 to analyze selected soil health indicators. Detailed tasks are mentioned below in the results section.

Results and Impacts

Task 1. *Measurement of Soil health indicators.*

Soil samples were extracted from 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm depths in 4 replicates and mixed together to make a composite sample for each plot in both years to analyze selected soil health indicators. Composited soil samples were labeled, sealed in plastic zip-lock bags, and transported to the laboratory. Higher manure application significantly decreased the bulk density as compared to the CK for the 0-10 cm soil depth. Bulk density was lowered in HM treatment by 14.7 and 9.20 % in 2018 and 2019, respectively, as compared to the CK. However, fertilizer application rate did not impact BD in any soil depths. Soil organic carbon (SOC) was significantly higher in HM treatment by 51.7, 9.27, 11.7, and 33.3% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively, as compared to the CK. Similarly, MM treatment has higher SOC by 3.47 and 44.9% in 10-20 cm, 30-40 cm soil depths, respectively, as compared to the CK. The SOC was higher in LM and HF at 10-20 cm soil depth by 4.24 and 3.47%, respectively, as compared to the CK. TN was significantly higher in HM treatment by 48.3, 8.84, 8.92, and 15.7% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively as compared to the CK. Similarly, MM treatment has higher TOC by 4.08 % in 10-20 cm soil depth, respectively, as compared to the CK. At 10-20 cm soil depth, TOC was higher in LM and HF by 4.42 and 3.40%, respectively, as compared to the CK.

Aggregate stability generally increases with the increase in amount of manure applied. In 2018, water stable aggregates (WSA) was increased by 18, 22, and 46% in LM, MM and HM treatments, respectively, as compared to the CK. In 2019, WSA was increased by 21, 28, and 44% in LM, MM and HM treatments, respectively, as compared to the CK. In 2018, mean weight diameter (MWD) was increased in LM, MM, and HM treatments by 48, 69 and 114%, respectively, compared to the CK. In 2019, MWD was increased in LM, MM, and HM treatments by 40, 50, and 71%, respectively, compared to the CK.

Soil enzymes like urease, β –glucosidase, alkaline phosphatase, arylsulfatase were analyzed using a spectrophotometer. For 0-10 cm soil depth, manure application under HM treatment significantly increased the soil β-glucosidase activity by 44 and 64% compared to the CK in 2018 and 2019, respectively. For 0-10 cm soil depth in 2018 and 2019, the HM treatment significantly increased the urease enzyme activity by 54 and 100% times than the CK, respectively. Alkaline phosphatase activity was also increased by 1.2 and 2.29 times with HM treatment than the CK in 2018 and 2019, respectively. However, there was no significant increase observed from inorganic fertilizer application (MF and HF) and CK for soil β-glucosidase and urease enzyme activity in either year. However, alkaline phosphatase enzyme activity was increased by 78% under HF as compared to the CK in 2019. For 10-20 cm depth, soil enzyme activity values were lower than the 0-10 cm soil depth. In 2018, β-glucosidase enzyme activity was significantly higher with MM, HM, and HF treatments (4.79, 4.86 and 4.75 µmol $PNPg^{-1}$ soil h^{-1}, respectively) than the CK (3.74 µmol $PNPg^{-1}$ soil h^{-1}) treatment. Furthermore, in 2019, HM treatment significantly increased the β-glucosidase enzyme activity by 38 and 25% than the CK and HF treatments, respectively, at 10-20 cm depth. However, no significant difference was observed for urease activity at 10-20 cm soil depth in 2018, whereas, in 2019, the HM treatment increased the urease activity by 1.08 times higher than the CK treatment. Alkaline phosphatase activity in 2018 for 10-20 cm soil depth was increased in all fertilizer treatments irrespective of the source of fertilizer when compared to the CK, however, the MM and HM treatments increased the alkaline phosphatase activity by 29 and 43%, respectively, as compared to the inorganic fertilizer (MF) treatment. In 2019, no differences were observed among the treatments for alkaline phosphatase activity.

The total phospholipid fatty acid (PLFA) biomass at 0-10 and 10-20 cm depths was higher with the HM compared with the CK. In 2018, the HM increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass by 70, 84, 65, 108, 72, 118, 92, and 1.36% than the CK treatment. However, no significant differences were observed between inorganic fertilizer and CK treatments. Similarly, in 2019, application of HM and MM treatments significantly increased the total PLFA, total bacterial, actinomycetes, Gram-negative bacteria, Gram-positive bacteria, total fungi, arbuscular mycorrhizal fungi (AMF), and saprophyte PLFA biomass than the CK. Similar to 2018, there were no significant differences between inorganic fertilizer and CK treatments in 2019. High throughput 16S RNA-soil sequencing was also carried out to identify the soil microbial composition. In comparison to HF and CK, the HM application stimulated some microbial groups (*Chitinophagaceae*, *Burkholderiaceae*, *Beijerinckiaceae*, and

Cellulomonadaceae) those are often involved in phosphorus solubilisation, nitrogen mineralization, methane degradation, and degradation of complex organic compounds.

In addition, intact soil core samples were collected for analyzing the soil pore-size distributing using the computed tomography study (CT-Scanning). These cores were scanned at the University of Missouri-Columbia, and the analysis of pore-sizes are under progress. This outcome will provide an useful indicator of soil health. In addition, aggregate associated C and N in each aggregate size fraction were determined from Beresford site in 2019. Manure application has significantly increased aggregate associated SOC in most of the size fractions as compared to the CK. Aggregate associated SOC was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in HM treatment by 62.7, 58.4, 58.7, 71.9, 47.7, and 54.1%, respectively as compared to the CK. Similarly, aggregate associated SOC was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in MM treatment by 18.2, 15.6, 13.1, 27.7, 17.3, and 20%, respectively, as compared to the CK. LM had higher aggregate associated SOC in 4-2 mm, 2-1 mm, 1-0.5 mm, and 0.25-0.053 mm size aggregate fractions by 18.5, 13.1, 22.1, and 15.5%, respectively as compared to the CK. However, fertilizer application rates did not impact aggregate associated SOC.

Manure application significantly increased aggregate associated N in most of the size fractions as compared to the CK. Manure application significantly increased aggregate associated N in most of the size fractions as compared to the CK. Aggregate associated N was increased in 8-4 mm, 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in HM treatment by 66.5, 63.5, 61.8, 67.1, 48.5, and 53.2%, respectively, as compared to the CK. Similarly, aggregate associated N was increased in 4-2 mm, 2-1 mm, 1-0.5 mm, 0.5-0.25 mm and 0.25-0.053 mm size fractions in MM treatment by 11.7, 21.5, 15.3, and 14.9%, respectively, as compared to the CK. LM treatment had higher aggregate associated N in 4-2 mm, and 1-0.5 mm size aggregate fractions by 17.4, 14.3, and 18.4% as compared to the CK.

Task 2. *Measurements of soil nutrients and crop yield.*

The impact of manure and inorganic fertilizer applications on soil nutrients and crop yield was also studied. Soil samples at depths of 0 to 15 cm and 15 to 60 cm, were taken at the beginning of the experiment before manure application and mineral fertilization by using augers and soil data were used to calculate the amount of manure rates. For soil nutrient analysis 1 month after planting, disturbed soil samples were extracted from four replications of each plot from 0-10, 10-20,20-30 and 30-40 cm depths and mixed together to make a composite sample for the respective depth. Soil samples were carried in plastic zip-lock bags, labeled, transported to the lab, and stored for analysis. Soil was air dried, mechanically grinded, and sieved through 2-mm sieve to analyze and also stored at 4°C. Soil analysis was performed to determine N, P, K, Soil organic matter (SOM), Sulfur, Zinc, pH, Salts, and Texture. In 2018, SOM was significantly higher in HM treatment by 36.8, 33.7, 7.69, 7.69% in 0-10 and 10-20 cm soil depths, respectively, as compared to CK and MF. In 2019, SOM was significantly higher in HM treatment by 29.4, 6.5, 6.8% in 0-10, 10-20, and 20-30 cm soil depths, respectively, as compared to CK. In 2018, MF, HM, and HF treatments increased NO_3-N content by 318, 279, and 236% than the CK treatment in 0-10 cm soil depth, respectively. In 2019, NO_3-N content was significantly higher in HM treatment by 156, 92, 74, and 124% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively, as compared to the CK. Compared to MF treatment, the NO_3-N content was significantly higher in HM treatment by 130, 79, 126, and 136% in 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm soil depths, respectively. At 0-10 cm soil depth in 2018, available P was significantly higher in HM, MM, and LM treatments as compared to HF, MF, and CK treatments. HM treatment significantly increased the available K in 2018 and both available P and K content in 2019 at all depths compared to all the other treatments. In 2018, the corn yield was significantly higher in HM, MM, HF, LM, and MF treatments by 104, 104, 97, 85, and 81% as compared to the CK, respectively.

Task 3. <u>*Measurements of Soil C Pools.*</u>

With an objective to understand the long-term effects of different rates of manure and synthetic fertilizer on soil carbon (C) and nitrogen (N) pools in corn-soybean cropping system, a study was conducted at Beresford, South Dakota. Soil samples were collected from 0-10 and 10-20 cm soil depths in 2018 to measure permanganate oxidizable C, microbial biomass C and N, dissolved organic C and N, particulate organic C and N, and C and N mineralization. Further, carbon management index (CMI) was calculated from this data. On average, manure treatment had higher soil C and N pools as compared to synthetic fertilizer and control. At 10-20 cm depth, HM had 113% and 98% higher DOC than MM and LM, respectively, and 215% higher DON compared to LM. The principal component analysis showed that manure rate has a significant influence on the majority of C and N pools. No significant differences in C and N pools were observed between the synthetic fertilizer and CK treatments. Therefore, this study suggests that manure application can be beneficial to enhance C and N pools compared to synthetic fertilizer and zero fertilizer application.

Task 4. <u>*Application of Soil Management Assessment Framework (SMAF) tool.*</u>

The soil health assessment was calculated using the Soil Management Assessment Framework (SMAF) scoring function analyses. Scores are given for each soil property measured, then combined into one simple value (0 to 1) for an easy interpretation of overall soil health. The soil quality index (SQI) values in 0-10 cm soil depth were higher in LM, MM, and HM treatments by 7.95, 6.75 and 9.80 %, respectively, compared to the HF. In 10-20 cm soil depth, the SQI values were higher in LM, MM, and HM treatments by 10.1, 8.73 and 16.3%, respectively, compared to the CK. However, fertilizer application rate did not impact SQI at either depth.

<u>Changes in project or personnel.</u> One MS Student (Asmita Gautam) and two partially budgeted postdoctoral researchers (Abagandura Gandura and Udayakumar Sekaran) worked under this project in 2019. Due to the excessive soil moisture in the spring, lysimeters for measuring nutrient leaching could not be installed on time before leaching events occurred.

Products/Publications/Presentations.

- Gautam, A. MS student. (Graduated Fall 2019). Long-term impacts of manure and inorganic fertilization on soil physical, chemical and biological properties. MS Thesis.
- Ozlu, E. MS student. (Graduated Fall 2016). Long-term impacts of annual cattle manure and fertilizer on soil quality under corn-soybean rotation in eastern South Dakota. MS Thesis.
- Ozlu, E., and Kumar, S. 2018. Response of Soil Organic Carbon, pH, Electrical Conductivity, and Water Stable Aggregates to Long-Term Annual Manure and Inorganic Fertilizer. Soil Science Society of America Journal, 82(5): 1243-1251.

- Ozlu, E., and Kumar, S. 2018. Response of surface GHG fluxes to long-term manure and inorganic fertilizer application in corn and soybean rotation. Science of the Total Environment 626, 817-825.
- Gautam, A., Guzman, J., Kumar, S., Kovacs, P. and Sexton, P. 2019. Long-term impacts of manure application and inorganic fertilization on soil health in South Dakota. Poster presentation at Soil Science Society of America Annual Meeting in San Diego, CA.
- Abagandura, O.G., Butail, P.B., Mahal, N.K., Gautam, A. and Kumar, S. 2019. Carbon and nitrogen pools as affected by long-term manure and synthetic fertilizer application in corn and soybean rotation. Oral presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.
- Gautam, A., Guzman, J.G., Kovacs, P., Sexton, P. and Kumar, S. 2019. Long-term impacts of manure application and inorganic fertilization on soil aggregate stability, soil organic carbon and nitrogen in different aggregate factions in South Dakota. Oral presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.
- Gautam, A., Sekaran, U., Guzman, J.G., Kovacs, P., Kumar, S. and Sexton, P. 2019. Long-term impacts of manure application and inorganic fertilization on microbial properties in South Dakota. Poster with 5-minute Rapid presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.

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Impacts of manure and inorganic fertilizer on Soil P availability, N losses, Fertility, and Crop Yield in South Dakota

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Summary. Long term impact of manure and inorganic fertilization on soil health was studied at the Southeast Research Farm of the South Dakota State University located at Beresford, and Brookings Felt farm, for SDSU soil fertility project. The experimental sites were established in 2003 at Beresford and 2008 at Brookings to assess the influences of manure and inorganic fertilizer on the long-term corn (*Zea mays* L.)-soybean (*Glycine max*. L.) rotation. The experimental site has 24 plots with 4.6 to 20 m dimensions into complete randomized block design. The study treatments include: three manure rates [low manure (LM), manure application based on the phosphorous requirement; medium manure (MM), manure application based on nitrogen requirement; high manure (HM), two times prescribed nitrogen rate], two chemical fertilizer rates [medium fertilizer (MF), recommended inorganic fertilizer rate; high rate of the fertilizer (HF)], and control (CK, without any manure or fertilizer application). The manure was manually applied and incorporated in the spring by disk at 6-cm deep for 1 to 3 days before planting at both sites. South Dakota Agricultural Laboratories analyzed manure of the study. Fertilizer treatments for 190 kg ha^{-1} yield goal for corn in 2018 and no fertilizer application was done for soybean in 2019 for both sites. Corn-soybean crop rotation is now proposed to be corn-soybean-spring wheat-cover crop. The spring wheat was introduced into the corn-soybean rotation in 2020 spring and cover crops were added in the rotation in 2020 fall at the Brookings and Beresford sites. The introduction of the spring wheat and cover crops into the rotation is to diversify crop rotation and increase year around soil coverage, and target nutrient scavenging. The present study will also help in assessing the impacts of incorporating the cover crops in enhancing the soil health and water quality under corn-soybean-spring wheat rotation managed with different manure and fertilizer treatments.

Data for the year 2020 under long term experimental sites showed that soil pH determined for post-harvest spring wheat soils at a depth of 0-60 cm ranged from 7.42 to 8.25 at Brookings site and 6.95 to 8.30 at Beresford site. In general, manure application decreased the bulk density at both sites. At Beresford, lower bulk density (1.32 Mg m^{-3}) was observed under high manure compared to control treatment (1.61 Mg m^{-3}) at 0-10 cm soil depth. At Brookings, the high manure had a lower average bulk density (1.40 Mg m^{-3}) when compared to high fertilizer (1.46 Mg m^{-3}) and the control treatments (1.43 Mg m^{-3}) at 0-10 cm soil depth. At Brookings, nitrate-N (NO_3-N) content increased with the high manure application (13.4 ppm) at 0-10 cm soil depth compared to control (8.1 ppm). However, overall NO_3-N content increased with high fertilizer (21.4 ppm) compared to all other treatments. Manure application also increased the enzyme activity as compared to the control for 0-5 cm soil depth, indicating the availability of higher substrates in soils due to manure application at both the sites. β glucosidase activity was higher under 2N treatments by 80 and 278 % as compared to control at Brookings and Beresford, respectively. Higher arylsulphatse (124.4 and 81.4 µg p-nitrophenol g^{-1} soil h^{-1}), β-glucosidase (8.34 and 6.96 µg p-nitrophenol g^{-1} soil h^{-1}) and acid phosphatse activity (117.0 and 108.5 µg p-nitrophenol g^{-1} soil h^{-1}) were observed under high manure application when compared to control during spring wheat at Beresford and Brookings, respectively. Urease activity and fluorescein diacetate hydrolysis had significant increase under manure treatments compared to control at both the sites. The results demonstrate that manure can increase enzyme activity compared to inorganic fertilization.

However, risks for nutrient transport away from the field were also high when manure applications exceeded recommend rates based on soil phosphorus. Therefore labile, moderately labile and stable pools of soil P fractions have been studied to identify the impact of manures and fertilizers on nutrient pools and for which data analysis is under progress. Data of previous years have showed that the treatments which included manure had significantly higher nutrient mineralization than inorganic fertilized treatments and control at surface soil depth. The manure treatments also had higher hot water and cold water soluble organic C and N fractions than inorganic fertilized treatments at both sites. The results demonstrate that manure can increase soil labile organic C and N compared to inorganic fertilized and unfertilized treatments. Greenhouse gas emissions were measured using a static chamber method during spring wheat and data analysis is under progress. Biomass samples were collected at harvest at both the sites for calculating nutrient uptake and use efficiencies of spring wheat with their respective yields. Post-harvest soil samples are also collected and analyzed for basic soil properties.

Study Objectives.

The primary goal of this project is to provide information to producers on the optimum rates of inorganic fertilizer and manure for enhancing soil fertility and crop yields without losing extra N and P losses. The specific objectives of the project are to:

1. Assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on soil P fractions (organic and inorganic) at two sites (Beresford and Brookings).
2. Assess the impacts of manure and inorganic fertilizer applications on soil health indicators (microbial carbon, enzymes, phospholipid fatty acids, water retention and storage, porosity, and water infiltration rate).
3. Assess the impacts of manure and inorganic fertilizer applications on N loss via leaching, NH_3 volatilization, N_2O emissions, and N retention in plant and soil pools.
4. Assess the impacts of manure and inorganic fertilizer applications on crop growth parameters, nutrients in plants, and N use efficiency.

Methods

Soil samples were collected before planting in 2020 at both sites to determine the nutrient status and to apply manure and fertilizer to the treatments. Spring wheat was planted at both sites and calculated manure and fertilizers were applied based on the treatment requirement. Soil samples were collected from 0-5 and 5-15 cm depths in 4 replicates and mixed together to make a composite sample for each plot in June 2020 to analyze selected soil health indicators. Post-harvest spring wheat soil samples (0-60 cm) and plant samples were collected in August, 2020 at both the sites and cover crops were planted. Soil samples were also collected after establishment of cover crops in November, 2020. Greenhouse gas samples were collected during spring wheat and cover crop at regular intervals. Detailed tasks are mentioned below for 2020.

Task 1. To determine soil phosphorus fractions.

Impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on soil P fractions (organic and inorganic) at two sites (Beresford and Brookings). Soil samples were collected from 0-5 cm (surface), and 5-15 cm (sub surface) depth from each plot in June 2020 (during spring wheat). Samplings after the harvest of spring wheat (Sep, 2020) and during cover crop (Nov-Dec, 2020) were collected at both the sites to determine available phosphorus content. At both the sites, phosphorus fractions (labile, moderately labile and non-labile) were determined using the fractionation procedure for 0-30 cm soil depth. Total

P concentration was determined ($HClO_4$–HNO_3 digestion). Both organic and inorganic P levels were determined by measuring with $NaHCO_3$, NaOH and HCl extracts. The residual P content was determined by subtracting the amount of extracted P from the total P content. Microbial biomass P was determined by the fumigation-extraction method. The total aboveground biomass were collected at crop harvest to calculate dry matter production at both the sites. Soil P stock will be calculated for each soil depth. In addition, P concentrations in straw and grain are analyzed, as well as P accumulation will be calculated. Straw and grain P content ranged from 0.07 to 0.14 % and 0.39 to 0.48% at Brookings site and 0.06 to 0.16% and 0.25 to 0.53%. Data analysis is yet under progress.

Task 2. To Assess Soil Health

Impacts of manure and inorganic fertilizer applications on soil health indicators such as C and N fractions, enzyme activities, microbial community structure (MCS), and physical and hydrological properties. Soil samples at 0-5 and 5-15 cm depths were taken from each plot for the two sites (Beresford and Brookings). Collected soil samples were stored in a cooler, transported to the lab and stored at 4°C for soil enzyme activities and microbial biomass analysis and at -20 °C for MCS analysis. Part of a sample was air-dried and ground to pass through a 2-mm sieve for further analysis. Soil total C and total N concentrations were determined by combustion using a C and N analyzer after grinding the soil to 0.1 mm. Total C and N ranged from 2.20 to 2.97 and 0.16 to 0.22 mg kg^{-1}at Brookings site and 1.49 to 2.17 and 0.04 to 0.17 mg kg^{-1}at Beresford site during spring wheat growth.

Microbial biomass C (MBC) and (MBN) N, active C (AC), water-soluble C (WSOC) and N (WSON) were also determined. While the content of MBC and MBN was analyzed by using the fumigation-extraction method, AC in soils were measured using the permanganate oxidizable C. Water-soluble C and WSON were determined using a distilled water in a soil/solution ratio of 1:10. Manure application has significantly increased MBC and MBN at both sites. MBC was MBN was increased in manure treatments by 17 and 20%, respectively as compared to the fertilizer treatments at Beresford site, during spring wheat crop growth. Similarly at Brookings site, MBC and MBN was significantly increased in high manure (2N) treatments by 173 and 150%, respectively as compared to the CK. Post-harvest soils had higher MBC content under high manure treatment (2N), whereas MBN content was higher in LM (P) treatments at both the sites. In addition glomalin related soil protein was analyzed and it had positive correlations with microbial biomass with significantly higher soil protein content of 2.42 and 2.70 mg kg^{-1} during spring wheat and 4.73 and 3.24 mg kg^{-1} under 2N treatments, at Brookings and Beresford, respectively. Post-harvest total soil carbon content analyzed for 0-60 cm soil depths ranged from 0.2 to 2.8 mg kg^{-1} soil at both the sites. Other carbon fractions are analyzed and data analysis is in progress.

Enzyme assays following standard protocols with sample incubation at 37°C for a fixed time period in a specific buffer pH range, with the needed substrate was used to calculate the respective enzymes. Moisture content was determined from loss in weight following drying at 105°C for 48 h. β glucosidase activity was higher under 2N treatments by 80 and 278 % as compared to control at Brookings and Beresford, respectively. Aryl sulphatase activity was also significantly higher under 2N treatments by 173 and 186% as compared to control Brookings and Beresford, respectively. Application of fertilizers and manures had significant effect on acid phosphatase activity, urease activity and fluorescein diacetate hydrolysis compared to control at both the sites.

Phospholipid fatty acid (PLFA) analysis was used to assess the composition of MCS. Briefly, phospholipids were separated from neutral lipids and glycolipids in silica gel columns. Fatty acid methyl esters was created through mild acid methanolysis. The PLFA methyl esters analyzed on an Agilent gas chromatography and the sum of all PLFAs were used to estimate total microbial biomass and the data analysis is under progress for both the sites.

Intact soil core samples were also collected after harvest of spring wheat for analyzing the soil pore-size distributions to serve as a supporting indicator for soil health. These cores were scanned at the University of Missouri-Columbia using the computed tomography study (CT-Scanning), and the analysis of pore-size distributions is under progress.

Task 3. Soil nitrogen losses assessment

To assess the impacts of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on N loss via leaching, NH_3 volatilization, N_2O emissions, and N retention in plant and soil. Soil N_2O emissions were measured using a static chamber method. At regular intervals during crop growth green-house gas fluxes are extracted and in the laboratory, the samples were analyzed for N_2O along with CH_4, and CO_2 using a gas chromatograph (GC). Soil samples were collected for measuring the NO_3-N and NH_4-N contents using the KCl extraction method, and data yet to be calculated. Plant dried samples were ground to pass through a 0.5 mm sieve and have been digested with H_2SO_4-H_2O_2 to measure N level in the plant. Straw N content of spring wheat ranged from 0.6 to 1.4%.

Task 4. To Assess Crop growth and yield

To assess the impact of manure and inorganic fertilizer applications under corn-soybean-spring wheat-cover crop rotation on crop growth parameters (crop height, different crop growth stages, crop yield, 1000 grain weight), nutrients in plants, and N use efficiency, agronomic N use efficiency and physiological N use efficiency. Collected plant samples at harvest were dried at 70ºC and ground to pass a 20-mesh sieve for measuring nutrients levels. Nutrient uptake was calculated based on the per cent nutrient in gran/straw and dry matter content of grain/straw. The N use efficiency was calculated as a ratio of (N uptake by the crop on N treated plots - N uptake by the crop on the control) to the total N applied. The agronomic N use efficiency will be determined by dividing (the yield in N treated plot - the yield in control plot) by the rate of applied N. The physiological N use efficiency for grain will be calculated by dividing the sun-dried grain weight from all plant harvest by the total N accumulation at maturity. The physiological N-use efficiency for biomass will be calculated by dividing the plant matter accumulation at maturity by the total N accumulation at maturity. Test weight (1000 grain weight) of spring weight ranged from 25.1 to 31.8 grams at both the sites. Grain nitrogen, phosphorus and potassium content of grain ranged from 2.04 to 2.97%, 0.25 to 0.53% and 0.31 to 0.51% at Beresford site. At Brookings site, nitrogen, phosphorus and potassium ranged from 2.2 to 3.1%, 0.39 to 0.48 % and 0.36 to 0.45% in grain.

Changes in project or personnel. One PhD Student (Anuoluwa Sangotaya) and a Research Assistant (Jemila Chellappa) worked under this project in 2020.

Products/Publications/Presentations.

- Gautam, A., Jose Guzman, Péter Kovács and Sandeep Kumar. 2020. Manure and inorganic fertilization impacts on soil nutrients, aggregate stability, organic carbon and nitrogen in different aggregate fractions. Archives of Agronomy and Soil Science. (Under review)

- Gautam, A., Udayakumar Sekaran, Jose Guzman, Péter Kovács, Jose Gonzalez and Sandeep Kumar. 2020. Responses of soil microbial community structure and enzymatic activities to long-term application of mineral fertilizer and beef manure. Environmental and Sustainability Indicators. (*In Press*)
- Gautam, A. MS student. (Graduated Fall 2019). Long-term impacts of manure and inorganic fertilization on soil physical, chemical, and biological properties. MS Thesis.
- Gautam, A., Jose Guzman, Sandeep Kumar, Peter Kovacs and Peter Sexton. 2019. Long-Term Impacts of Manure Application and Inorganic Fertilization on Soil Health in South Dakota. Poster presentation at Soil Science Society of America Annual Meeting in San Diego, CA.
- Abagandura, O.G., Butail, P.B., Mahal, N.K., Gautam, A., Kumar, S. 2019. Carbon and Nitrogen Pools As Affected By Long-Term Manure and Synthetic Fertilizer Application in Corn and Soybean Rotation. Oral presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.
- Gautam, A., Guzman, J.G., Peter Kovacs, P., Sexton, P, and Kumar, S. 2019. Long-Term Impacts of Manure Application and Inorganic Fertilization on Soil Aggregate Stability, Soil Organic Carbon and Nitrogen in Different Aggregate Factions in South Dakota. Oral presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.
- Gautam, A., Sekaran, U., Guzman, J.G., Peter Kovacs, P., Kumar, S., and Sexton, P. 2019. Long-Term Impacts of Manure Application and Inorganic Fertilization on Microbial Properties in South Dakota. Poster with 5-minute Rapid presentation at 2019 ASA-CSSA-SSSA International Annual Meeting, Nov. 10-13, San Antonio, Texas.