Soil microbial activity and nutrient bioavailability influenced by soil application of organic, bio and chemical fertilizers

by

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ABSTRACT

SOIL MICROBIAL ACTIVITY AND NUTRIENT BIOAVAILABILITY AS INFLUENCED BY SOIL APPLICATION OF ORGANIC, BIO AND CHEMICAL FERTILIZERS

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An incubation study was conducted with the bulk soil samples from a field experiment on switchgrass. LysteGro, JumpStart and MYKE Pro were used in this study as commercial formulations of MSWC, *Penicillium bilaiae* (phosphorus solubilizing fungi-PMF) and *Glomus intraradices* (Arbuscular Mycorrhizal Fungi -AMF*)*, respectively. The basal soil respiration (BSR) rate (µg CO_2 $g^{-1}day^{-1}$) improved in all treatments in the initial period of incubation study but started to decrease afterwards. Lyste Gro had the highest BSR during the entire period. The highest cumulative respiration was observed in MSWC treatment (508.9 CO_2 µg g^{-1}) followed by urea alone (466.1 CO_2 µg g^{-1}). Soil NH_4-N and NO_3-N increased significantly with MSWC, PSF and urea application. The NH_4-N content significantly decreased, whereas NO_3-N content in soil improved progressively and significantly with the length of incubation period. The mineral N content increase was 59.3% and 54.1% over control in MSWC and urea treatments, respectively. The mean P content in soil increased from 16.84 µg g^{-1} in control to 22.84 and 21.85 µg g^{-1} soil, in MSWC and PSF treated soils, respectively.

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Abbreviation	**Definition**
AMF	Arbuscular mycorrhizal fungi
$BaCl_2$	Barium chloride
$BaCO_3$	Barium carbonate
BSR	Basal soil respiration
C	Carbon
CO_2	Carbon dioxide
g	Grams
H_2O	Water
HCl	Hydrochloric acid
INM	Integrated nutrient management
MW	Municipal Waste
K	Potassium
Kg	Kilogram
$Kg\ ha^{-1}$	Kilogram per hectare
M	Molarity
Mg	Magnesium
Mn	Manganese
mm	Millimeter
mL	Milliliters
$mg\ kg^{-1}$	Milligram per Kilogram
$mg\ CO_2\ kg^{-1}day^{-1}$	Milligram of carbon dioxide per kilogram per day
MBWC	Municipal Bio-waste Compost
N	Nitrogen

NO_3	Nitrate
NH_4^+	Ammonium
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
OM	Organic matter
P	Phosphorus
PSB	Phosphate solubilizing bacteria
PMF	Phosphate mobilizing fungi
S	Sulphur
Zn	Zinc
$\mu g\ g^{-1}$	microgram per gram

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Appendix

1. Introduction

Maintaining good soil health and efficient crop nutrient are important tasks associated with sustainable agricultural production. The dependence on chemical fertilizers has increased with the advent of green revolution since mid-20th century. Crop response to applied fertilizer is very quick as they are good source of readily available plant nutrients. They are composed of higher nutrient percentage, thus required in small quantity which makes them more acceptable (Han et al 2016). The use of chemical fertilizers became an integral part of present-day agriculture as they restore the soil nutrients to achieve high crop productivity (Sharma, 2017; Bishnoi, 2018).

But, the use of excessive fertilizer over a long period can be harmful for soil health and for the entire ecological system. Prolonged application of inorganic fertilizers adversely affects the soil physical-chemical, and biological properties (Ghosh, 2004). Continuous imbalanced fertilizer use reduced crop yields, their quality, soil fertility and ultimately diminishing net returns (Ansari, 2008). In addition to detrimental effects of non-judicious use of chemical fertilizers on soil health, it also accounts for ecological implications including soil, water and air pollution. NPK fertilizers have found to be the source of air and ground water pollution (Youssef and Eissa, 2014; Chen, 2006). Also, acidification or basification caused by excessive use of fertilizers may lead to soil degradation along with a decline in soil microbial population. Soil acidification due to the decline in soil pH was recorded with urea application, caused by release of H^+ after the absorption of NH_4^+ by the plants (Magdof et al, 1997).

Efforts are being made in the wake of sustainability in different aspects of agriculture whether it is sustainable crop production or sustainable soil health. Integrated nutrient management (INM) offers a viable solution for maintaining soil health, minimizing environmental pollution and get sustainable crop production. INM includes use of chemical fertilizers, but in an integrated way along with organic manures (including animal waste, compost etc.), biofertilizers and crop residues.

Basically, biofertilizers are carrier based live formulations of microorganisms like bacteria and fungi, which are integrated through seeds or applied directly to soil. These microbes help in mobilization of nutrients into forms which are readily available for the plant use. Biofertilizers are eco-friendly, pollution free and low-cost inputs, which help in integrated plant nutrient management by fulfilling crop nutrient needs and maintaining soil fertility status (Bargaz et al., 2018; Vessey, 2003).

In case of phosphorus (P) application through synthetic phosphorus fertilizers, plant only takes up a small amount of P while the rest is fixed in the soil. Hence, increasing the amount of insoluble phosphorus forms in the soil which the plants cannot use. Certain bacterial and fungal species have the ability to transform these to soluble forms. Biofertilizers, therefore, contain these bacterial and fungal species in order to enhance P solubilization in soils (Gupta 2004). Phosphate solubilizing bacteria (PSB) including *Bacillus circulans*, *Bacillus megaterium* var *Phosphaticum* and *Bacillus subtilis*, and certain fungi such as *Aspergillus awamori*, *Penicillum* spp. and *Trichoderma* spp. do this conversion by releasing some organic acids which decreases the soil pH and solubilize phosphorus. A group of fungi known as Arbuscular Mycorrhiza (*Sclerocystis* spp., *Glomus* spp., *Scutellospora* spp., *Acaulospora* spp. and *Gigaspora* spp.) mobilize phosphorus by absorbing phosphates from the layers of soil and making them available to the plants (Itelima et al, 2018).

In recent decades, large amount of municipal waste (MW), especially in populated areas, inevitably has caused environmental pollution, thus requiring proper management strategy. A good management practice is to convert MW into compost (Municipal Waste Compost [MWC]), which not only keeps the environment clean but also works as an efficient organic fertilizer in agricultural soils providing good quality nutrients and high content of stabilized organic matter. Researchers (Price et al., 2009; Hargreaves et al., 2008) have observed high soil organic matter and increased nutrient availability to the plants after MWC application. Furthermore, incorporation of MWC to agricultural land improves the soil nutrients concentrations, decreases chemical fertilizer dependence and enhances the environmental sustainability of agriculture (Khoshgoftarmanesh and Kalbasi, 2002; Mathur et al., 1993; Papafilippaki et al., 2015; Meena et al., 2016). Adrien (2006) also revealed an increase in formation water-stable aggregates in soils under MWC application along with increased C and N contents.

Since, chemical fertilizers, organic fertilizers and biofertilizers have their own merits and demerits, a proper management strategy needs to be developed for crop production. Keeping this in view a field experiment was planned in 2019 using switchgrass (*Panicum virgatum*) as a biomass crop with control, Lyste Gro, Jump Start, MYKE Pro and chemical nitrogenous fertilizer (urea) as treatments at the Guelph Turfgrass (GTI) research plots, University of Guelph. An incubation study was conducted at the end of 2019 with the objective to study the effect of different chemical, bio and organic fertilizers on microbial activities and nutrient release from associated soil samples collected from different treatments, as mentioned above.

Chapter 2: Review of Literature

The supply of essential plant nutrients in a balanced way is a must to achieve higher and quality crop production to feed the ever- increasing human population. Now a-days these nutrients are provided to agricultural crops mostly with the help of inorganic fertilizers. But, non-judicious use of chemical fertilizers poses a serious threat to soil health, human health as well as to the environmental health. Hence, it is required to develop some alternative strategies for nutrient management keeping in view both higher crop yield and good soil health. The use of biofertilizers, organic manures and municipal bio-waste composts (MBWC) in crop production offer an alternative for the chemical fertilizers. The present review of literature is focused on effect of MBWC and phosphorus solubilizing and mobilizing biofertilizers on soil heath and nutrient availability.

2.1. Phosphorus solubilizing and mobilizing biofertilizers

Nitrogen fixers (N-fixers), phosphorus solubilizers, mycorrhizal fungi and growth promoting rhizobacteria are most commonly used micro-organisms as biofertilizers. Application of biofertilizers has shown to enhance nutrient and water absorption by plants, which further improves plant growth and increases plants immunity against abiotic and biotic stresses (Itelima et al, 2018). This improvement in nutrient availability is either through N fixation, or through P and K solubilization/ mineralization (Sinha et al., 2014).

Phosphorus is the second most important plant nutrient after Nitrogen which is required by plants in various biochemical reactions. Narsian and Patel (2000) reported that only a small amount of P applied through fertilizers is taken up by plants and rest is fixed as insoluble P complexes in the soil. Various phosphate solubilizing bacteria (PSB) are capable of solubilizing insoluble phosphorus compounds (both organic and inorganic) to plant available soluble P form (Kalayu, 2019). A number of fungi species are also capable of converting these insoluble phosphates into soluble forms and are therefore used as biofertilizers (Gupta 2004).

Phosphorus biofertilizers are divided in to two main groups:

2.1.1 **Phosphate solubilizing microbes** includes bacterial species like *Bacillus spp.* and fungi like *Aspergillus spp* and *Penicillum spp*. These microorganisms release some organic acids in soil and decrease soil pH, which solubilizes the insoluble phosphate complexes to forms available for plant use.

Penicillum spp.

The positive correlation of total soil phosphorus with phosphorus solubilizing fungi was put forth in 1981 with a soil survey which was conducted at seventeen sites in southern Alberta. In these soils, phosphorus solubilizing bacteria and fungi constituted about 0.5% and 0.1% of the total bacterial and fungal population respectively. Among these microbes, they found out fungi to be more active in carrying out phosphate-solubilization and further microscopic examination indicated that a large number of these phosphate-solubilizers were from the *Penicillium* spp. (Kucey, 1983).

Different reports indicated that various species of bacteria and fungi are capable of solubilizing the insoluble or precipitated forms of P in the soil (Kucey and laggett,1989; Whiteaw, 2000). Out of these, fungi are found to be more efficient as they produced relatively more amount of acid than bacteria in both liquid and solid media (Venkateswarlu et al., 1984). The commercial formulations of *Penicillium bilaiae* has been available in Canada since 1991 under the trade names Provide® and thereafter as JumpStart® (Gleddie, 1993).

In a liquid culture study, it was observed that *Penicillum bilaiae* and *Penicillum cf. fuscum* solubilized rock phosphate, which was correlated with the decline in pH by acids produced by these fungi (Asea et al, 1988). In another study it was demonstrated that *Penicillium bilaiae* reduced the pH of solution from 7.0 to 4.9 in buffered and 5.0 to 4.1 in non-buffered media in just 12 days. The production of relatively more citric and oxalic acid was noticed in buffered solution which was responsible accelerated pH decline and improved solubility of rock phosphate (Takeda and Knight, 2006).

Various studies (Cunningham and Kuiack, 1992; Kucey, 1988; Richardson, 2001) on *Penicillium bilaiae* treated soils suggested that soil acidification is induced by production of oxalic and citric acid, which is directly responsible for the dissolution of P minerals in the soil. Whereas, Tarafdar et al (1995) attributes the exudation of phosphatases enzyme by the fungus for the enhanced P solubility.

Yet, in another experiment (Gómez-Muñoz et al., 2018) with *P. bilaiae* conducted in potted conditions under maize crop found no evidence of soil P mobilization by the fungi. They rather concluded that the increase in nutrient uptake was due to increased root growth in the presence of the fungi *P. bilaii*. Similarly, an earlier report (Legget et al., 2015), also indicated that the increase in biomass is not always related with a higher P absorption by the plant but could be due to the modifications in root morphology influenced by the growth promoting effect of the rhizospheric fungi.

Beneficial effect of *P. bilaiae* inoculation on biomass production, P uptake and yield has been reported in wheat (Kucey, 1987), canola (Kucey and Leggett, 1989) and alfalfa (Beckie at al., 1998). *P. bilaiae* inoculation in pea plant showed increase in root length, increase in proportion of roots containing root hairs and lastly an increase in P content of the plant (Gulden and Vessey, 2000; Vessy and Hesinger, 2001). Wanget al (2016) put forth that the combined effect of both increase in plant root length and P solubilization under *P. bilaiae* inoculated soil might be responsible for higher yields and improved nutrient availability.

Additionally, *P. bilaiae* has shown to work better in combination with *S. meliloti*. When inoculated together they have shown to increase nodule number and yield of alfalfa, and nitrogen and phosphorus content in hay (Rice et. al., 2000). Similarly, the combined inoculation of *P. bilaiae* and *Bacilus simplex* has shown to increase P, Mg, S and Mn content in low P soil cultivated under wheat. However, no significant effect on wheat growth was recorded with this inoculation. This combined inoculation was tested for other microbes also and was proven to be beneficial with respect to plant P content, hence making consortium of microbial inoculants a good choice to use in agriculture (Hansen et al, 2019).

The above review indicated that *P. bilaiae* releases organic acids like oxalic and citric acid which causes acidification and improves P solubility in soil and culture media. However, some reports also suggest that it improves the root growth and abundance of root hair which ultimately enhances the nutrient assimilation and crop growth.

2.1.2 **Phosphate mobilizing fungi** (PMF) includes arbuscular mycorrhiza microbes. These include *Sclerocystis Spp., Glomus Spp., Gigaspora Spp., Scutellospora Spp. and Acaulospora Spp*. These microbes absorb P from soil layers and mobilize it into the soil (Itelima et al, 2018).

***Glomus* spp.**

Arbuscular mycorrhizal fungi (AMF) form fine, tree-shaped hyphal structures within the cortical cells of the root of the host plant known as ‘arbuscules’. These arbuscules in the roots create an association of the host plant and the fungi for the exchange of carbon and nutrients (Hodge, 2000). These associations between AMF and plant is beneficial for the host plant as it improves plant’s nutrition, increases its resistance to drought & salinity and makes it tolerant to high heavy metal content (Gosling et al, 2006; Selvakumar and Thamizhiniyan, 2011). In nature, AMF interact with majority (70-80%) of plant species and this symbiosis can improve the nutritional status and growth of plants under both optimal and limited water conditions (Smith and Read, 2008; Jansa et al., 2011). Of all nutrients, this association significantly enhances availability of phosphorus to the plant (Miyasaka and Habete, 2001; Vassilev et al., 2001). Improvement in P and N uptake by AMF inoculated berseem plants was also reported by 63.

Raiesi, F. et al. (2006), which also led to production of higher plant biomass. Similarly, in an experiment conducted on wheat on sandy loam soil, inoculation with *G. caledonium* significantly increased the plant biomass, P uptake and crop yield (Hu et al., 2010).

A field study in maize (Zea mays L.) conducted by Cozzolino, V. et al (2013) with commercial mycorrhizal inoculant (*Glomus intraradices*) found out that the mycorrhizal inoculation along with nitrogen and potassium treatment (without addition of phosphorus) gave comparable yields as that obtained under NPK treatment, thus suggesting that AMF can be a potential component of integrated nutrient management in crop production. *Glomus intraradices* inoculated maize crop has shown higher Zn uptake (Jansa et al., 2003). It also improves the plants capability to absorb higher amounts of P from scarcely soluble P complexes such as iron phosphate, aluminum phosphates and rock phosphate (Bolan, 1991).

The different possible mechanisms responsible for enhanced P uptake in mycorrhizal inoculated plants include i.) increase in the root surface area for absorption and exploration of larger soil volume. ii.) rapid transmission of P into hyphae due to increased P ion attraction and decrease in the concentration threshold needed for P absorption. iii.) production of organic acids and phosphatase enzymes which solubilize soil P (Bolan, 1991).

2.2. Municipal Bio Waste Compost (MBWC)

Management of soil health in a sustainable way is a major issue in the present agriculture scenario. Soil organic matter plays a very significant role in maintaining the soil quality by improving the biological and chemical properties of the soil (Murphy, 2015; Pedra et al. 2007). OM is also important for maintaining good physical properties of soil, and its decline has often led to degradation of the soil structure. (Diacono and Montemurro, 2010). Organic matter needs of soil can be fulfilled from the large quantities of organic waste which is produced in urban areas like municipal solid waste and sewage sludge. Along with organic matter, urban waste also contains essential plant nutrients which can be helpful in crop production (Debiase et al., 2016; Walter et al., 2006).

Municipal solid waste compost (MSWC) is found to be helpful in maintaining soil fertility by enhancing the soil organic matter (OM) content but only after repeated and prolonged use (Garcia-Gil et al., 2014; Crecchio et al., 2014). In an experiment which was done to compare the effect of MSWC and olive pomace compost on alfalfa and cocksfoot, a buildup in total organic carbon was seen but only after 3 year of repeated compost application (Montemurro et al., 2006). Not only prolonged application but large amounts of MSWC is also required for improvement in organic matter content (Hargreaves et al., 2008). Continuous application of MSWC for 8 years, led to a significant increase in the soil basal respiration rate (which is an indicator of soil microbial activity) over control (Pascual et al. 1999). Better soil respiration after compost application may be due to the enhanced resource (organic carbon and other nutrients) availability, which leads to better growth of microbes and stimulation of microbial activities (Iovieno, 2009; Vinhal-Freitas et al., 2010). An incubation study done on municipal compost, concluded that CO_2 evolution depends on readily decomposable organic matter rather than total amount of organic matter in the compost (Horrocks et al., 2016).

Another incubation study done on soils treated with MSWC and traditional cow dung manure observed both the treatments to have positive impact on microbial biomass carbon, microbial soil respiration, urease and acid phosphatase enzyme activity. However, cow dung manure was found to be superior than MSWC, but no detrimental effect of higher dose of MSWC application was recorded on soil quality (Bhattacharyya et al., 2003).

And if we take into consideration the nutrients, it is reported that MSWC contains about 16-21% of nitrogen as NH_4-N and NO_3-N, which can be readily up taken by plants (Iglesias-Jimenez and Alvarez, 1993). Also, significant increase of NH_4-N-N and NO_3-N in soil after MSWC application was recorded (Cuevas et al., 2000; Ramadass and Palaniyandi, 2007). Additionally, other macronutrients like P, K, Na and Ca have shown an increase with MSWC application (Achiba et al., 2009; Lee et al., 2004; Shanmugam, 2005). With MSWC, Mg content also increased in poorly drained soils (Zheljazkov and Warman, 2004). A study was conducted by Meena et al. (2016) to examine the impact of integrated use of MSWC along with rice straw compost, gypsum enriched compost and chemical fertilizers on saline soil, found out that use of composts along with 25 per cent recommended chemical fertilizer can improve the N, P, K availability and microbial biomass in soil, and can also reduce the soil salinity (which was reflected by declined electrical conductivity of soil). City refuse compost has also shown to be better source of P than inorganic fertilizers in sesquioxide-rich and volcanic-ash soils (these soils require higher amounts of P due to high P fixation capacity by active aluminum and iron compounds present in them) (Iglesias-Jimenez et al., 2013). This increase in available inorganic P could be due to enhanced phosphatase enzyme activity in MSWC soils (Perucci, 1990., Iglesias-Jimenez et al., 1993). In experiment by P. Zhang et al. (2006), once in 4-year MSWC application at rate of 200 t/ ha increased P content from 7.2 mg kg^{-1} in control to 86 mg kg^{-1}. Similarly, Horrock et al. (2016) reported that after one year of MSWC application, the soil Olsen P improved significantly (increase of 0.15 ppm P per tonne of soil).

The above review revealed that the MSWC may be used in crop production as a source of organic matter and essential plant nutrients but for it to have significant effect on soil organic matter content, prolonged application is required. It also needs to be checked for toxicity as Papafilippaki et al. (2015) indicated that MSWC can be sources of environment pollution due to presence of some toxic heavy metals in it.

Chapter 3: Method and Material

An incubation study was conducted by collecting soil samples from an ongoing experiment on *Panicum virgatum* (switchgrass) (cultivar ‘Cave-in-Rock’) with different chemical, bio and organic fertilizers. The experiment is being conducted at the Guelph Turfgrass Institute (GTI) (Guelph, ON).

The details of the treatments are given in Table 1. below:

3.1 Treatment: The following treatment was applied on July 15-16, 2019.

Table 3.1 Different fertilizer treatments and their rate of application

S. No.	**Treatments**	**Details**
T1	Control	No input
T2	Lyste Gro	Lyste Gro (municipal biosolids organic fertilizer): surface-applied at a rate of 60 kg N/ha corrected for a 50% N volatilization rate
T3	Jump Start	*Penicillium bilaiae* produced by Monsanto, applied at a rate of 2.05 × 10^5 cfu/L; plots along with 30 kg N/ha as Urea
T4	MYKE Pro	*Glomus intraradices* produced by Premier Tech, applied at a rate of 3000 spores/m^2; plots also received urea at a rate of 30 kg N/ha
T5	Chemical Fertilizer	Urea fertilizer applied at a rate of 60 kg N/ha

Fig. 3.1. Layout of the Field Experiment

3.2 Description of field experiment:

Figure. 3.1 shows the layout of the switchgrass plots at GTI in Guelph, Ontario (43°32'59.99" N, 80°12'29.89" W) The plots have sandy loam (52% sand, 43% silt, 5% clay) texture with pH 7.5 and organic matter content of 2.7%. The alphabets L, B, F, J and C in the figure represent LysteGro, MYKE Pro, Fertilizer, Jumpstart treatment and Control plots respectively.

3.3 Collection and preparation of soil samples:

Bulk soil samples were collected on November 7, 2019 from the above-mentioned field experiment to conduct the incubation study. Surface soil samples (0-15 cm) were collected at random from four different locations within a treatment plot and then a composite sample was taken in order to have a good representative sample. Each treatment was replicated 3 times in the field. These composite soil samples were air-dried, grounded and passed through sieve of 2 mm mesh for further incubation study.

3.4 Execution of Incubation Study:

60g air-dried sieved soil collected from three replicated plots of each treatment was taken in small containers. The moisture content in the containers were adjusted to 22 per cent by adding the required amount of distilled water. These small containers were placed in large jars along with a vial containing 25 ml 0.125 M NaOH and were sealed and placed into the incubator at temperature of 20^0C. Similarly, 700g of soil was taken in large jars and moisture content was adjusted to 22 per cent by adding the required amount of distilled water. These jars were sealed and kept in the incubator.

3.5 Nutrient availability assessment:

Incubation study was started on January 14, 2020, and soil samples were taken before incubation (week 0), and then on week 1, week 3, week 5 and week 7 of the incubation. Around 100g of soil sample was taken from the bulk 700g soil for each treatment and kept in freezer for further nutrient assessment.

Later, soil samples were analyzed at the SGS laboratory (Guelph) for nitrate-nitrogen, ammonium-nitrogen, available phosphorus and potassium.

SGS lab uses Ontario accredited methods for the estimation of these nutrients which are as follows:

1. Nitrate-nitrogen & ammonium-nitrate: Soil is mixed with potassium chloride (at ratio of 1:5) which is shaken for half an hour and then filtered. Extract is then analyzed using auto analyzer which measures the color intensity produced after treating extract with chemicals.
2. Available phosphorus: Olsen method which uses sodium bicarbonate was used for analyzing P. One part of soil is mixed with 20 parts of 0.5 M sodium bicarbonate solution (pH 8.5), which is shaken for 30 minutes. After adding chemicals (molybdate and stannous chloride solution) to the extract, a blue color is formed which is read on photoelectric colorimeter.
3. Potassium: Ammonium acetate was used to extract potassium in soil which is further measured using flame photometer.

3.6 Estimation of soil respiration:

Soil respiration was estimated as the amount of CO_2 produced at different intervals of the incubation. This was done after one, three, five and seven weeks after the start of incubation.

Following method was used for estimating CO_2:

- NaOH solution from the jars containing 60g of soil was removed.
- NaOH was replenished again in the vial and the jars were sealed and kept in incubator
- In the removed NaOH solution, 2 ml of 1.5M $BaCl_2$ added along with two-three drops of phenolphthalein
- This was titrated with 0.06M HCl to colorless endpoint.
- Titration was also done for the blank NaOH samples
- The above steps were done for week 1, week 3, week 5 and week 7
- CO_2 was calculated by standard calculations through the following reactions:
  - Reaction of CO_2 released by the soil in the incubator with NaOH
    $NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$
  - Reaction of Na_2CO_3 with $BaCl_2$ leads to precipitation of Na_2CO_3
    $BaCl_2 + Na_2CO_3 \rightarrow BaCO_3 + NaCl$
  - Titration reaction with HCl
    $NaOH + HCl \rightarrow NaCl = H_2O$

3.7 Statistical analysis:

The statistical analysis of the data was done using GLM procedure in the SAS software.

Chapter 4: Results and Discussion

The effect of LysteGro (MSWC), biofertilizers as (*Penicillium bilaiae*) and MYKE Pro (*Glomus intraradices*) with 30 kg N ha^{-1} as urea and 60 kg N ha^{-1} as urea application on soil respiration, nitrogen mineralization and availability of phosphorus (P) and potassium (K) was determined in an incubation study conducted for 7 weeks.

Statistical analysis was carried out using GLM procedure in the SAS software. The p values were determined for incubation period, fertilizer treatment and time-treatment interaction for basal soil respiration (BSR, mg CO_2 kg^{-1}day^{-1}), cumulative respiration (mg CO_2 kg^{-1}) evolution and nutrients (NO_3-N, NH_4-N, Mineral-N, P and K content (mg kg^{-1}).

Table 4.1. Analysis of variance for the effect of different treatments on different soil parameters

Source of variation	df	Parameters						
		BSR rate	Cumulative respiration	NH_4-N	NO_3-N	Mineral -N	P	K
Week (W)	4	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	0.112
Treatment (T)	4	< .0001	0.0007*	< .0001	< .0001	< .0001	< .0001	< .0001
Week×Treatment (W×T)	16	< .0001	0.6576	0.0002*	0.2645	0.4155	0.004*	1

(* p value < 0.05)

4.1. Soil Respiration:

Basal soil respiration (BSR) measured as CO_2 evolution is an important parameter to understand the soil biological activities. In this study the BSR and cumulative respiration were recorded and presented as under:

4.1.1. Basal soil respiration (BSR) rate: The data pertaining to BSR rate (mg CO_2 kg^{-1}day^{-1}) affected by different organic, bio and inorganic amendments is presented in Fig. 4.1. The data shows significance for incubation period, treatment and for week-treatment interaction. Different treatments significantly improved the BSR (p=<.001). The LysteGro treated soil had the highest release of CO_2 throughout the incubation period. The highest BSR rate of 23.7 mg CO_2 kg^{-1}day^{-1}

was recorded in LysteGro treatment followed by 22.4 mg CO_2 $kg^{-1}day^{-1}$ in urea application during the initial stage of incubation study (week1).

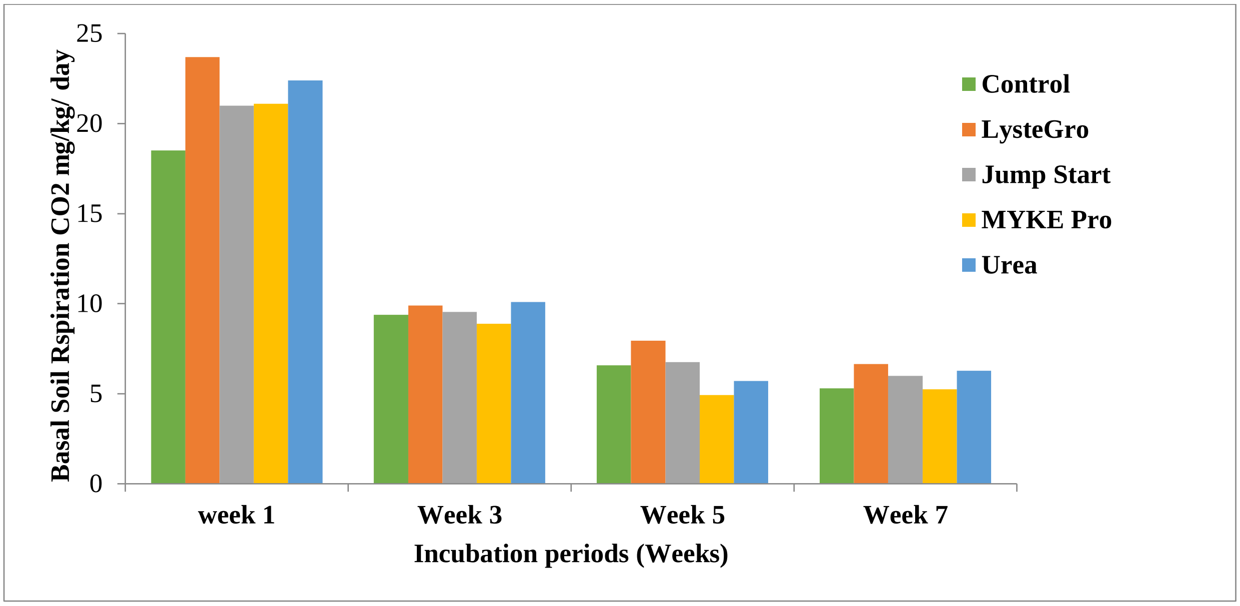

Fig. 4.1. Effect of different treatments on Basal Soil Respiration expressed as mg CO2 kg-1day-1

A slight improvement in BSR rate was also noticed in Jump Start and MYKE Pro treatments over control, which was respectively 1.5 and 1.6 mg CO_2 $kg^{-1}day^{-1}$ higher than control at week 1 of incubation. The BSR rate decreased with the progress of incubation period (p=<.0001). The peak of BSR rate was obtained in week1 and least during week 7, for all treatments except urea, which showed a slight increase at week 7 when compared to week 5. The mean BSR rates were 21.3, 9.6, 6.4 and 5.9 mg CO_2 $kg^{-1}day^{-1}$ at week 1, 3, 5 and 7, respectively, indicating a sharp decline with the progress of incubation period.

4.1.2. Cumulative respiration: The data presented in Fig.4.2. highlights that the cumulative soil respiration recorded as CO_2 efflux (mg CO_2 kg^{-1}) and it significantly differs with different treatments (p=0.0007). The highest CO2 efflux (mg CO_2 kg^{-1}) was recorded in LysteGro applied soil followed by urea and JumpStart treatment throughout the incubation period. Initially MYKE Pro produced significantly higher amount of CO_2 but became less than control at week 7. The peak was observed in LysteGro treatment (508.9 mg/kg CO_2) followed by urea alone (466 mg CO_2 kg^{-1}) at week7 of incubation study. A significant (p=<0.0001) improvement in cumulative respiration was also recorded with incubation period. All the treatments show a linear increase (R2> 0.97) in CO_2 production through the incubation.

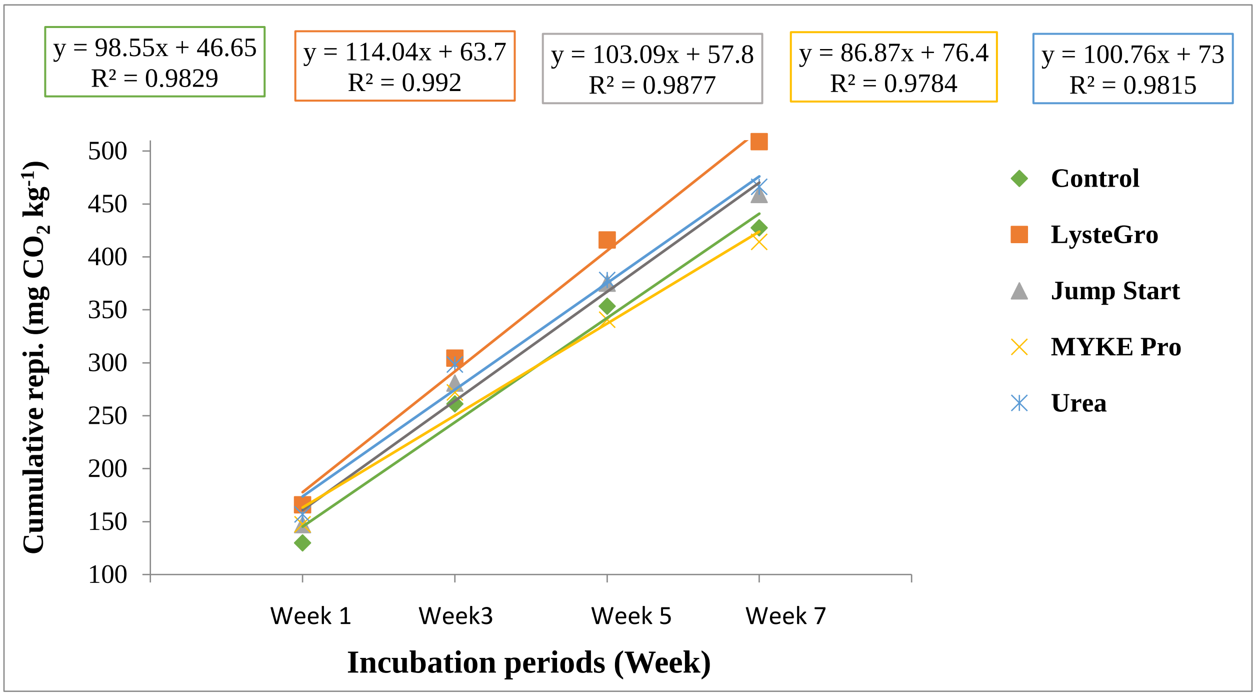

Fig. 4.2 Effect of soil application of different fertilizers on cumulative respiration (mg CO_2 kg^{-1})

The availability of suitable environment, substrates and nutrients may be responsible for the enhanced respiration rate in LysteGro treatment. Various reports indicated that the enhanced soil respiration caused by compost application may be due to the enhanced resource availability, responsible for the growth and stimulation of microbial activities (Iovieno, 2009). An enhanced CO_2 evolution with the rate of soil added municipal compost was also recorded by Horrocks et al. (2016) in an incubation study. It was concluded that CO_2 production depends upon the amount of readily decomposable organic matter rather than total amount of organic matter in the compost. Another earlier study conducted by Pascual et al. (1997) indicated that municipal bio-waste and organic compost amendments significantly increased the soil respiration over control. The synergistic effect of household residues compost application on cumulative soil respiration was also obtained by Vinhal-Freitas et al. (2010) and it was suggested that the positive effects were mainly due to the increased microbial activity in response of the improved organic C and nutrients availability from compost addition.

The results indicated that BSR rate and cumulative respiration also increased by urea application. Different types of reports are available in the literature about the effect of nitrogen application through chemical fertilizers on soil respiration. In one of the studies, soil microbial respiration reduced with the application of urea as a N source in two out of three soils used in the incubation study, however in a grassland soil with narrow C:N ratio, the application of urea even at a rate of 500 mg kg^{-1} found to be enhanced the soil respiration rate by 20% over control (Ramirez et al., 2010). Staley et al. (2018) observed that urea application at higher rate (500 mg kg^{-1}) proved toxic for soil microbes and decreased the microbial activities, however the low level (100 mg kg-1) of urea may have stimulated the N cycling by improving the microbial activity and diversity. For this incubation study, soil C:N ratio was not quantified however, as the soil was taken from a 6-year-old perennial biomass field, low C:N ration can be expected based on a similar study conducted at the same site (Marsal F. et al., 2016).

The BSR was higher at initial level of incubation study and it declined gradually with the length of incubation period, irrespective of different treatments including MSWC, urea, and bio-fertilizers with half the dose of urea. It might be due to the progressively short supply of organic substances required for the microbial growth. It was also recorded by Horrocks et al. (2016) that CO_2 evolution decreased with progress of incubation period, however it was increasing very rapid during initial period of the study. Bhattacharyya et al., (2001) observed that the soil respiration in MSWC treated soil approached to its height level at 30 days of incubation and thereafter gradually decreased up to 120 days of incubation. The initially increased soil respiration was attributed to the readily available substrates for microbial growth, and with declined availability of substrates over time the microbial activity came down (Garcia-Gil, 2000). Sharma et al. (2015) gave two reasons for decrease in soil respiration with time in incubation studies; (i) the depletion of soluble carbohydrates and (ii) built up of some impeding substances like polyphenols, which may adversely affect the microbial growth and activity.

4.2. Nitrogen Mineralization:

Nitrogen mineralization is a microbial process in which organic nitrogen is transformed to plant available inorganic nitrogen. In this process, NH_4^+ and NO_3^- ions are produced through ammonification and nitrification reactions, respectively. The results pertaining to NH_4–N and, NO_3–N and total mineral nitrogen for the incubation study is presented below.

4.2.1. NH_4–N Content: The data pertaining to the effect of different fertilizers treatments on NH_4–N content at different periods of incubation study is presented in Fig. 4.3. Treatment differences were significant (p=<.0001). The highest mean NH_4-N content throughout the incubation was recorded in urea (2.54 mg kg^{-1}) followed by LysteGro (2.43 mg kg^{-1}) as compared to 1.59 mg kg^{-1} in control. The JumpStart and MYKE Pro treatments also produced higher NH_4-N content over control.

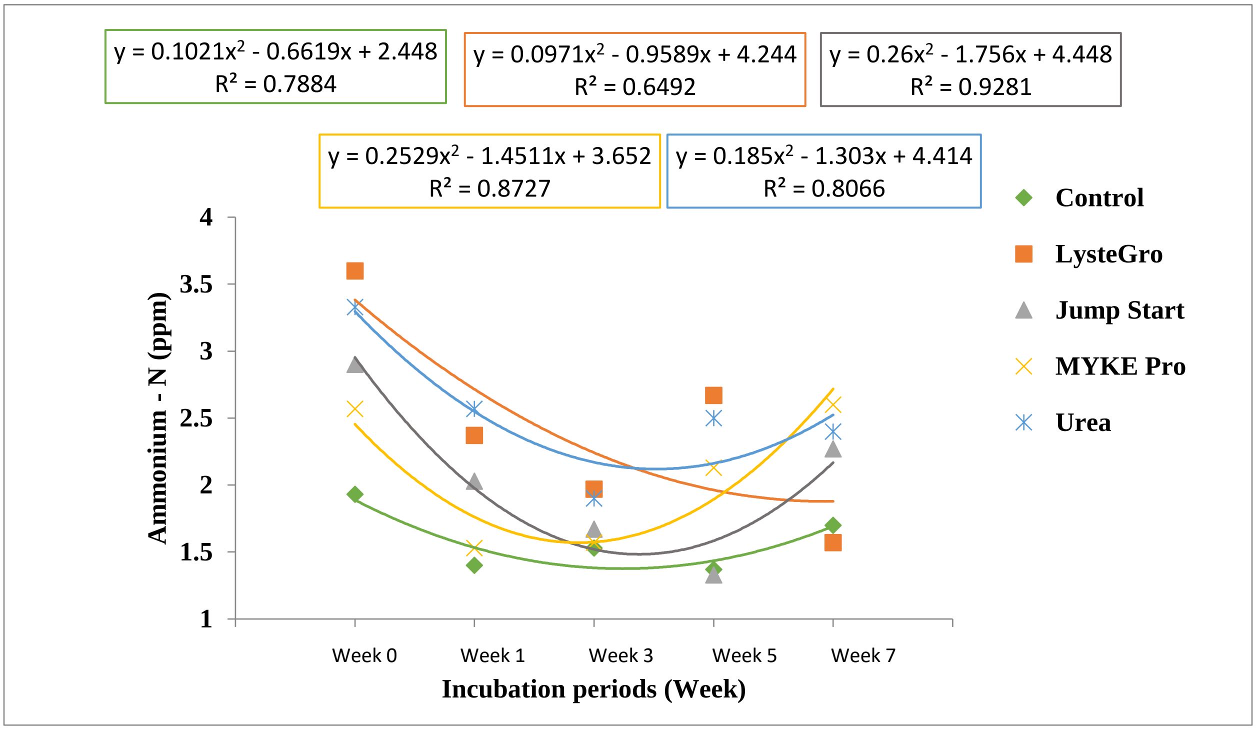

Fig. 4.3 Effect of different treatments on NH_4-N content (mg kg-1) in soil at various incubation period

Time effect was also significant (p=<.0001). In LysteGro treatment, a decreasing trend in NH_4-N was recorded with the incubation period. While in other treatments (Urea, MYKE Pro, Jump Start) and control, initially a decrease was seen but it started to increase after week 3-week 5.

4.2.2. NO_3-N Content: The data on soil NO_3-N content as affected by different treatments is presented in Fig. 4.4. It is pertinent from the data that different treatments had significant (p=<.0001) and positive effect on NO_3-N content as compared to control. The mean soil NO_3-N content increased significantly from 10.32 mg kg^{-1} in control to 16.53 mg kg^{-1} and 15.81 mg kg^{-1} in LysteGro and urea application, respectively. The combined effect of half dose of urea application along with biofertilizers also found to improve the NO_3-N recovery over control, however they were less than Lyste Gro and urea treatments.

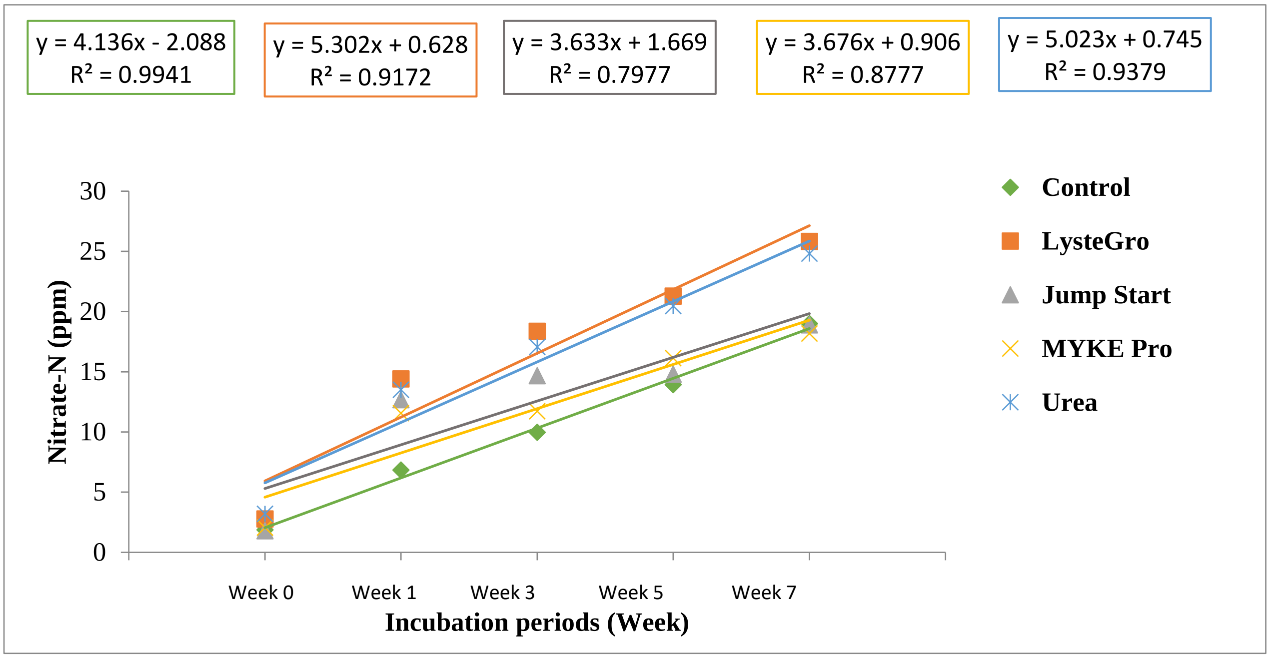

Fig. 4.4 Effect of different treatments on NO_3-N content (mg kg-1) in soil at various incubation periods

The mean effect of different treatments indicated that NO_3-N content was 60.17%, 53.19%, 21.80% and 15.6% higher in LysteGro, urea, half dose of urea with Jump Start and MYKE Pro treatments, respectively over control.

A significant (p=<.0001) impact of incubation period was also noticed on soil NO_3-N content. The NO_3-N content increased in all treatments with incubation period and a liner relationship (R2 > 0.877) was recorded between incubation period and NO_3-N content. The highest soil NO_3-N content of 25.83 mg kg^{-1} followed by 24.83mg kg^{-1} was obtained in LysteGro and urea application, respectively at 7th week of incubation. This is pertinent that LysteGro and urea treatments had similar increase in slope with time. Also, they had the highest slope among all the treatments indicating significant treatment effects.

4.2.3. Total Mineral Nitrogen: The data indicates that NO_3-N makes up major proportion of the mineral nitrogen pool as NH_4-N contributed only 2-3 mg kg^{-1} to it. The total mineral nitrogen content (NH_4-N+NO_3-N) differs significantly (p=<.0001) with various treatments (Fig. 4.5.).

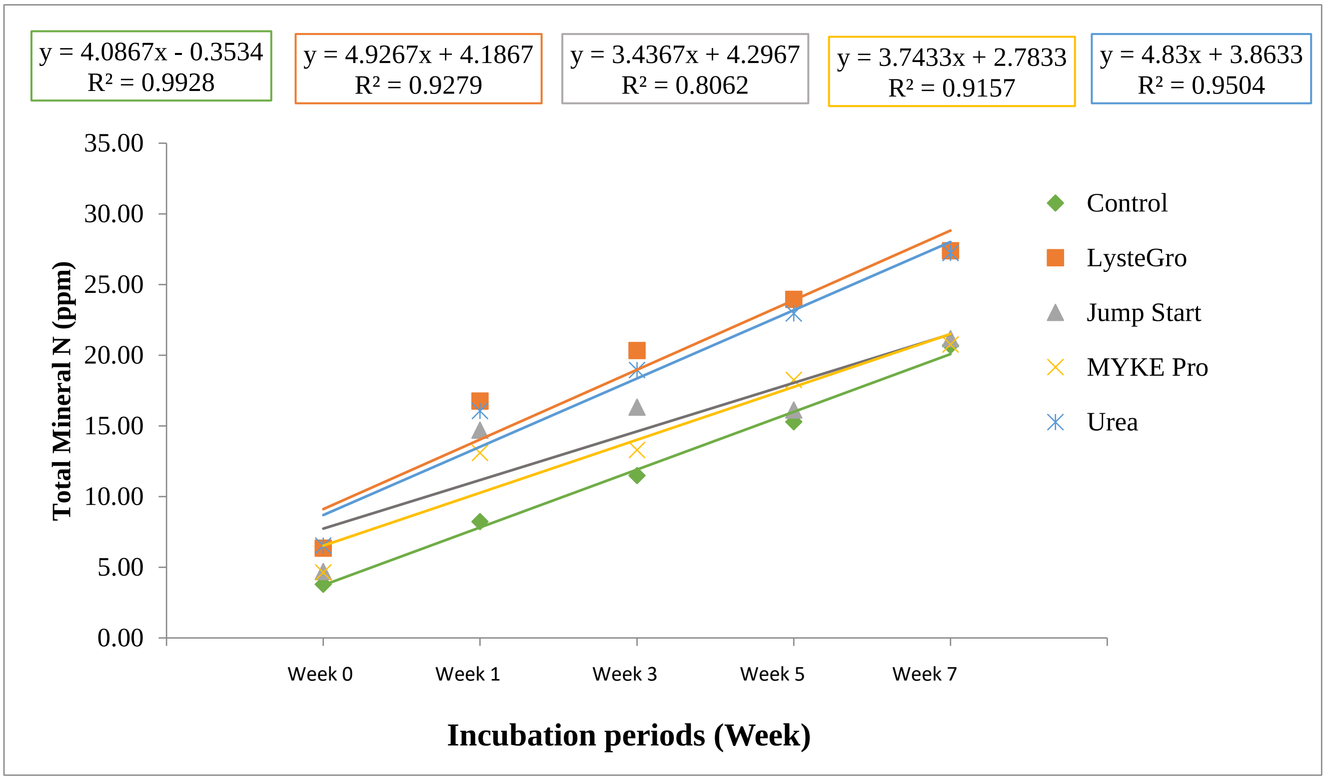

Fig. 4.5 Effect of different treatments on total mineral-N content (mg kg-1) in soil at various incubation periods

The mean mineral-N content increased from 11.91 mg kg^{-1} in control to 18.97, 18.35, 14.61 and 14.01 mg kg^{-1} in LysteGro, urea, Jumpstart and MYKE Pro treatments, respectively. The corresponding increase values were 59.3%, 54.1%, 22.7% and 17.6% higher than that of control.

The mineral N content in soil increased (p=<.0001) with increase in the incubation period. A liner relationship (R2 > 0.806) was obtained between incubation period and NO_3-N content. The data indicated that the mineralization was quite fast till week 1 and thereafter it increased with a relatively slower rate till the 7th week.

The interaction effect of different fertilizer treatments and incubation period was non-significant. The peak of total mineral-N was obtained in LysteGro (27.40 mg kg^{-1}) followed by urea treatment (27.23 mg kg^{-1}) at week 7 of incubation. At this stage of incubation, the total mineral-N content was almost equal in control (20.70 mg kg^{-1}), JumpStart (21.17 mg kg^{-1}) and MYKE Pro (20.77 mg kg^{-1}) treatments.

The increase of NO_3-N, NH_4-N and mineral N content in case of LysteGro was probably due to supply of easily mineralizable N by this MSWC. The elevated soil microbial activities as reflected by soil respiration (both BSR and cumulative) might be related to the higher NO_3-N and NH_4-N content in soil amended with this compost over control as a positive correlation ($r=0.813$, $p<0.01$) was obtained between cumulative soil respiration and mineral-N content. It was highlighted by Iglesias- Jimenez and Alvarez (1993) that MSWC contains 16-21% of total N as NH_4-N and NO_3-N, hence can be used as source of inorganic N in agriculture. Increased availability of NO_3 and NH_4 in soil with MSWC application has been reported in various studies (Singh, Y. et al, 1988; Cuevas et al., 2000; Ramadass and Palaniyandi, 2007; Alvarez 1993; Horrocks et al. 2016).

Many reports have indicated the increased recovery of NH_4-N, NO_3-N and total mineral/inorganic N in soil with urea application, which is probably due to increased mineralization in these soils (Noguera et al., 2010; Malhi et al 2006). Prosser (1990) suggested that urea applied to soil undergoes hydrolysis to form ammonia which is further transformed to NO_3^- through the nitrification process. The low proportion of NH_4-N in total mineral N content of is mainly due to rapid oxidation process, which converts NH_4-N to NO_3-N (Fageria, 2014; Gupta 2015; Nascente et al., 2017).

4.3. Available Phosphorus (P) content:

The data in Fig. 4.6. indicates that the amount mineralized P (available P) in soil differs among various fertilizer treatments. Application of different fertilizers significantly ($p=<.0001$) improved P mineralization over control irrespective of incubation periods. The highest available P content was recorded in LysteGro followed by Jump Start treatment. The mean P content throughout the incubation in soil increased from 16.84 mg kg^{-1} in control to 22.84 mg kg^{-1}, 21.85 mg kg^{-1}, 20.50 mg kg^{-1} and 20.57 mg kg^{-1} respectively in LysteGro, Jump Start, MYKE Pro and Urea treatments, respectively.

The available P content also differs significantly (p=<.0001) with different incubation periods. The mean available P content increased throughout the incubation period for Urea treatment. But for Lyste Gro, MYKE Pro and JumpStart treatments it was highest at week 3 and declined slightly afterwards. Singh Y. et al (1988) also saw a similar trend (initial increase than decrease) in available P in an incubation study of soil with various organic matter, they attributed this decrease due to the absorption of mineralized P on clay minerals in soil.

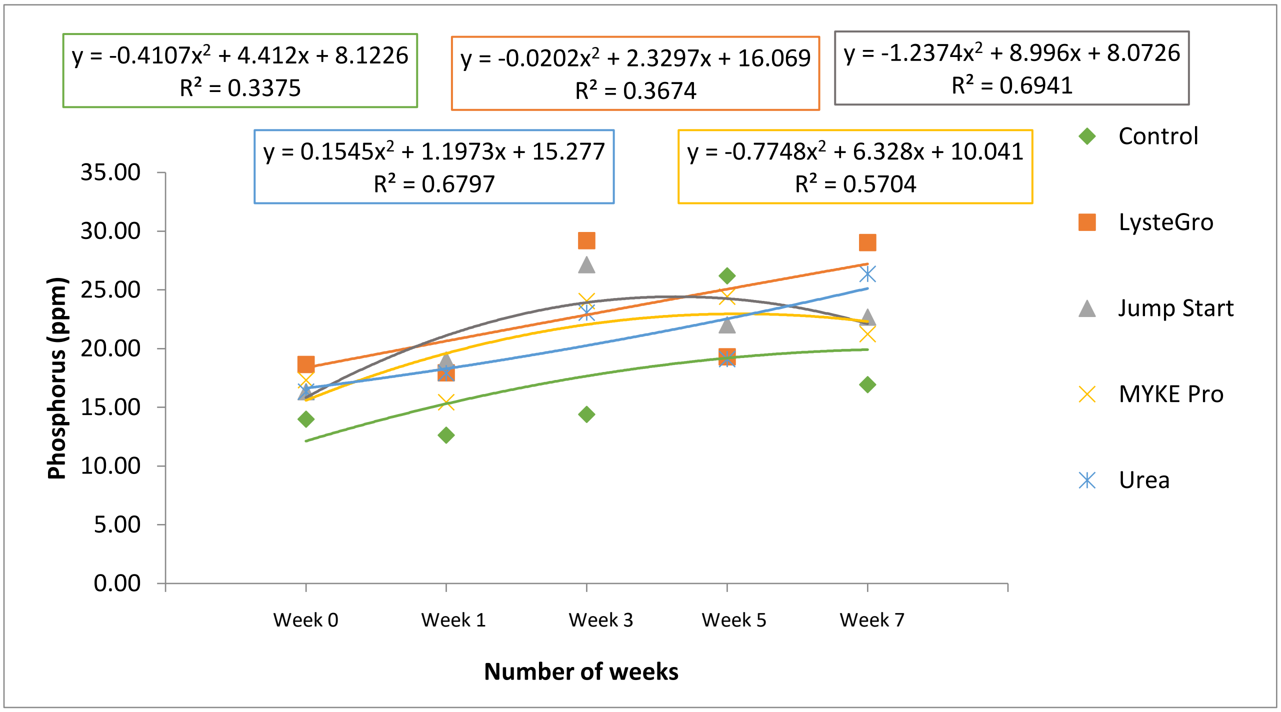

Fig. 4.6 Effect of different treatments on P content (mg kg^{-1}) in soil at various incubation periods

The initial increased P availability in LysteGro treatment was mainly due to the increased supply of P and improved microbial activities by the application of compost. Various reports are available in the literature demonstrating the improved P availability with the incorporation of MSWC to soils. (Zang et al. 2006; Ramadass and Palaniyandi (2007). Similarly, Horrock et al. (2016) also reported that after one year of MSWC application, the soil Olsen P improved significantly. The increase in Olsen P with each tonne of soil MSWC application was 0.15 mg kg^{-1}. It was further highlighted by Iglesias-Jimenez et al., (1993) in an earlier report that MSWC is as efficient as inorganic P fertilizers with respect P supply. It was suggested that MSWC may stimulate the transformation of organic P into its inorganic forms due to enhanced phosphatase enzyme activity (Stevenson, 1986; Peucci, 1990).

Further, studies are also available for increase in P availability with application of fungi of *Penicilium* spp and *Glomus* spp. Cunningham and Kuiack (1992) reported that *P. bilai* produced oxalic and citric acid which caused acidification and solubilized the insoluble P complexes and enhanced the P availability. Similarly, mycorrhizal association also improved the P uptake from the poorly soluble iron and aluminum phosphates and rock phosphate (Bolan, 1991; Miyasaka and Habte, 2001; Vassiev et al., 2001).

The increase in P availability with incubation period might be due to increased microbial activities. A significant positive correlation was obtained between cumulative respiration and P availability ($r=0.54$, $p< 0.5$).

4.4. Available K:

The available K content in the response of different fertilizer treatments is depicted in Fig. 4.7. A significant ($p=<.0001$) effect on K availability was obtained with the application of different fertilizers treatments. The highest available K content was recorded in LysteGro (MSWC) treated soil followed by Jump Start and urea treatments. The mean K availability recorded was 58.47 mg kg^{-1}, 73.25 mg kg^{-1}, 57.44 mg kg^{-1}, 63.53 mg kg^{-1} and 64.38 mg kg^{-1} in control, LysteGro, Jump Start, MYKE pro and urea applied soils, respectively. The data indicated that the LysteGro application enhanced the K availability by 25.2 % over control.

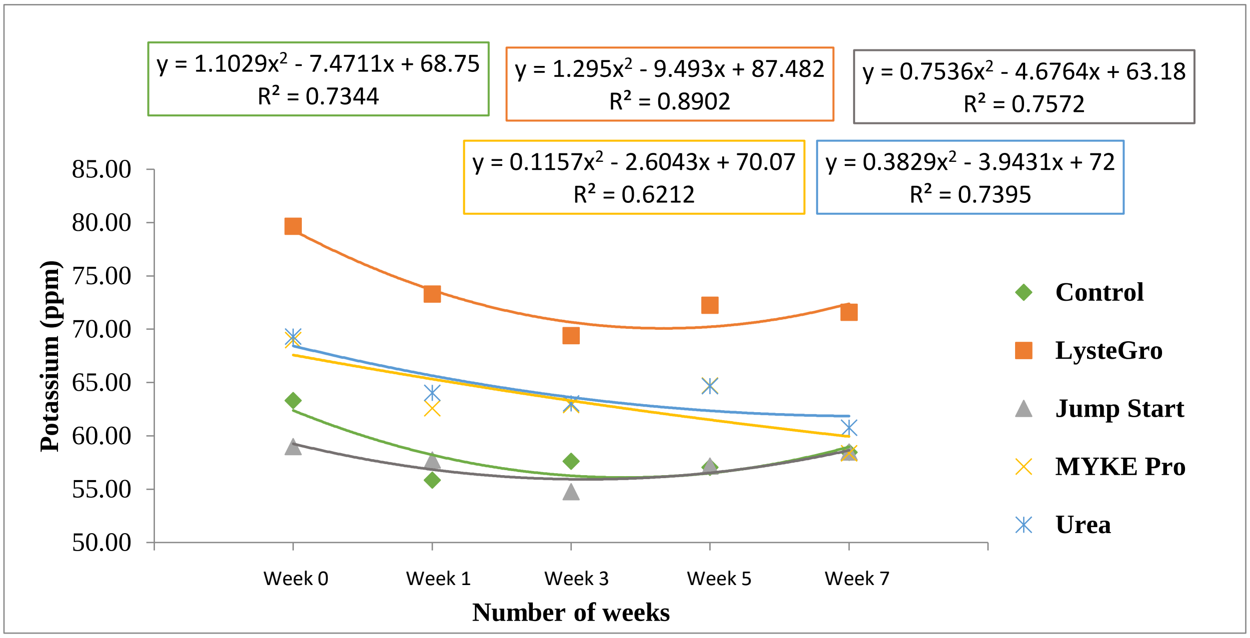

Fig.4.7. Effect of different treatments on K content (mg kg^{-1}) in soil at various incubation periods

In a long-term experiment deHaan (1981) demonstrated that the available K content in MSWC was comparable to K mineral fertilizers. Application of MSWC for conjunctive five years enhanced the soil K availability by 26 % over the control treatment (Hartl et al. 2003). A significant increase in soil available K with MSWC application was also reported by different researchers (Warman et al., 2004; Ramadass and Palaniyandi, 2007; Castro et al. 2009; Blanchet et al., 2016; Ranjbar et al., 2016)

Chapter 5: Summary

Chemical fertilizers play a significant role in improving the crop productivity however their long time non-judicious use may cause a threat to soil health and ecosystem sustainability. The integrated nutrient management (INM) approaches includes the use of organic manures and biofertilizers along with chemical fertilizers, which prove helpful in reducing the chemical fertilizer dependence. An experiment on switchgrass with municipal solid waste compost (MSWC), bio-fertilizers (*Penicillium bilaiae* and *Glomus intraradices* with 30 kg N ha^{-1}as urea) and, 60 kg N ha^{-1} as urea is in progress at the Guelph Turfgrass Institute (GTI), University of Guleph, Ontario, Canada. LysteGro, JumpStart and MYKE Pro were used in this study as commercial formulations of MSWC, *Penicillium bilaiae* (phosphorus solubilizing fungi-PMF) and *Glomus intraradices* (Arbuscular Mycorrhizal Fungi -AMF*)*, respectively.

An incubation study for different intervals up to 7 weeks was conducted by taking soil samples from the ongoing experiment to evaluate the contribution of different treatments to soil respiration and nutrient availability. Basal soil respiration (BSR), NH_4-N, NO_3-N, mineral N, available P, K, were determined by following the standard methods.

The BSR rate (µg CO_2 g^{-1}day^{-1}) improved with the application of MSWC, PSF, AMF and urea application at initial period of incubation study. With progress in incubation period the BSR rate decreased drastically and differences within the treatments also narrowed down. The reduction of BSR rate could be due to the reduced supply of substrates required for the growth and activities of the microbial population. The highest BSR rate of 23.7 µg CO_2 g^{-1}day^{-1} was obtained in MSWC in 1st week of incubation

The cumulative respiration also increased significantly with the soil application of MSWC and urea. The peak was observed in MSWC treatment (508.9 µg g^{-1}) followed by urea treatment (466.1 µg g^{-1}). The availability of suitable environment, substrates and nutrients for microbes may be responsible for the improved of respiration in MSWC treated soil. Further, the increased N supply with urea may also be beneficial for nitrogen limiting microbe thus enhancing the soil respiration.

The data indicates that different fertilizer treatments had a significant effect on the NH_4-N content of the soil. The mean NH_4-N content increased from 1.59 µg g^{-1} in control to 2.43 µg g^{-1}, 2.04 µg g^{-1}, 2.08 µg g^{-1} and 2.54 µg g^{-1}, respectively in MSWC, PSF, AMF and urea application, respectively. The NH_4-N content significantly decreased with the progression in incubation period.

The mean soil NO_3-N content also enhanced significantly from 10.32 µg g^{-1} in control to 16.53, 15.81 and 12.57 µg g^{-1} in MSWC, PSF and urea application, respectively. The NO_3-N content in soil improved progressively and significantly with the length of incubation period.

The amount of mineral N increased significantly over control with the application of different fertilizer treatments. The increase was about 59.3% and 54.1% over control in MSWC and urea treatments, respectively. The mineral N content in soil also increased with increase in the incubation period. It is hypothesized that MSWC supplies easily mineralizable organic carbon, which is responsible for improved amount of mineral N in soil. Similarly, the supply of N by urea leads to improvement in mineral N of soil. The increase in mineral N with progress of incubation period was may be due to continuous nitrification process which increases the NO_3-N content.

Application of MSWC and PSF significantly improved P mineralization over control. The mean P content in soil increased from 16.84 µg g^{-1} in control to 22.84 µg g^{-1} and 21.85 µg g^{-1} soil in MSWC and PSF added soils, respectively. An increasing trend in P mineralization was recorded till week 3, after which it almost stabilized.

Application of MSWC significantly improved the available K content in soil as compared to control. Improvement in available K content over control was also recorded in AMF and urea treatments. The mean K availability recorded was 73.25 µg g^{-1} in MSWC treated soil, against 58.47 µg g^{-1} in untreated control. This increase in K availability was 25.2 per cent over control. No effect of incubation period was noticed on K availability.

The present study indicated that MSWC and bio-fertilizer could be a good component of INM. The evaluation of MSWC along with biofertilizers or its enrichment with biofertilizers in improving the biological, chemical and physical soil fertility, will also be helpful in widening the scope of using this product in agriculture. Furthermore, there is need to evaluate MSWC for toxic heavy metals, which could be harmful human and environmental health.

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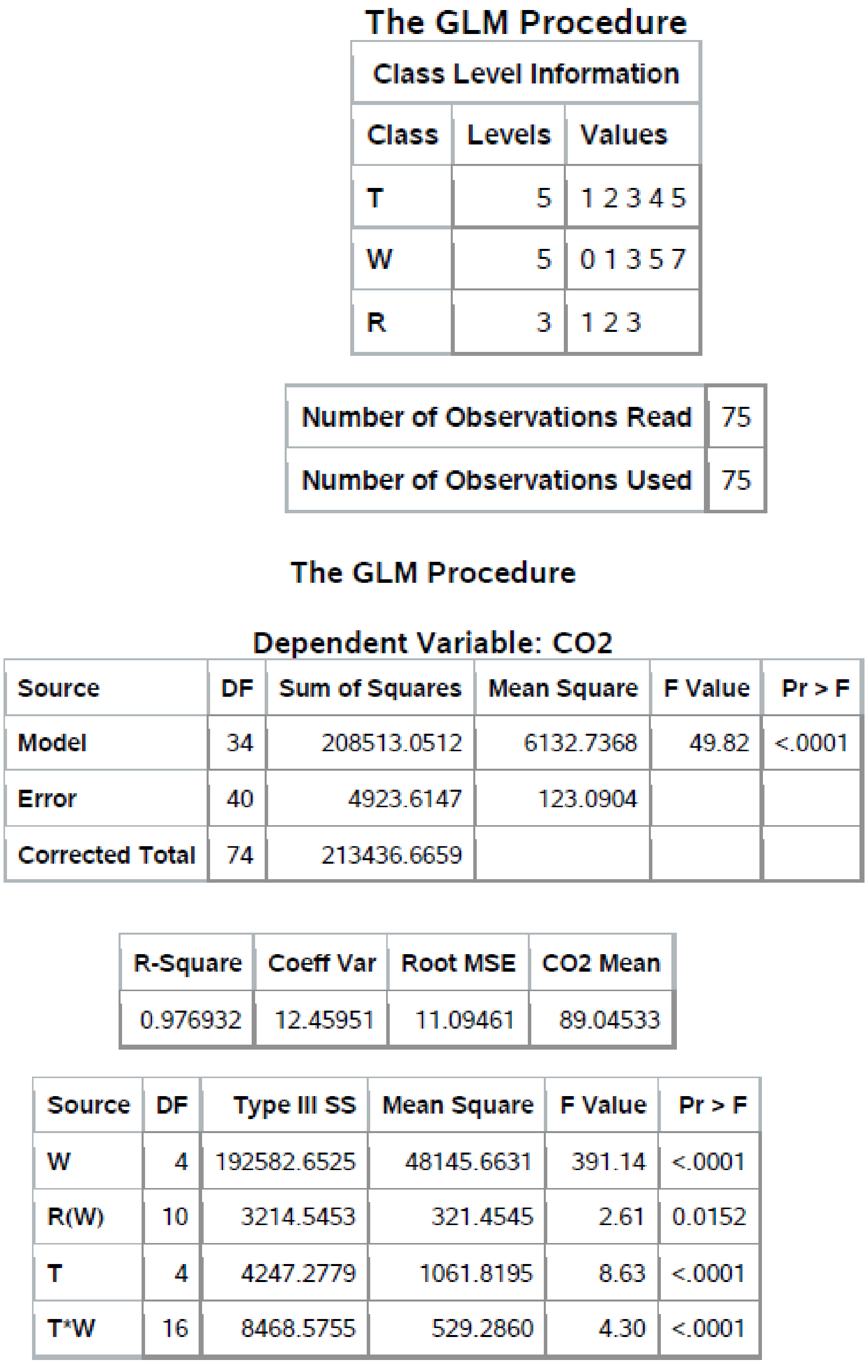
Treatment	Treatment	Week	Replication	CO_2-Evolved mg/Kg/day	Cumulative CO2 mg//Kg	NO_3-N (ppm)	NH_4-N (ppm)	Mineral N (ppm)	P (ppm)	K (ppm)
Control	1	0	1	0.00	0.00	1.5	1.8	3.3	16	74
	1	0	2	0.00	0.00	2	2.1	4.1	15	53
	1	0	3	0.00	0.00	2.1	1.9	4	11	63
	1	1	1	140.30	140.30	6.3	1.3	7.6	16.73	63.87
	1	1	2	126.44	126.44	7.1	1.3	8.4	11.92	45.44
	1	1	3	122.60	122.60	7.1	1.6	8.7	9.26	58.31
	1	3	1	153.66	293.96	11	1.4	12.4	14.98	65.6
	1	3	2	116.44	242.88	10.2	1.4	11.6	12.92	49.85
	1	3	3	124.45	247.05	8.7	1.8	10.5	15.32	57.42
	1	5	1	99.16	393.12	13.6	1.5	15.1	31.31	66.21
	1	5	2	83.69	326.56	14.2	1.1	15.3	26.98	47.68
	1	5	3	93.50	340.56	14	1.5	15.5	20.33	57.27
	1	7	1	79.13	472.25	18.9	2	20.9	19.69	66.82
	1	7	2	67.64	394.20	18.6	1.4	20	21.52	48.87
	1	7	3	75.89	416.45	19.5	1.7	21.2	9.65	59.73
LysteGro	2	0	1	0.00	0.00	4	4	8	19	83
	2	0	2	0.00	0.00	1.5	3.2	4.7	16	68
	2	0	3	0.00	0.00	2.8	3.6	6.4	17	88
	2	1	1	159.46	159.46	18.2	2.1	20.3	20.86	88.26
	2	1	2	175.81	175.81	9.5	2.2	11.7	13.07	60.93
	2	1	3	162.38	162.38	15.5	2.8	18.3	12.42	70.78
	2	3	1	136.26	295.72	20.2	2.1	22.3	27.25	74.91
	2	3	2	112.70	288.51	18.9	1.9	20.8	27.12	60.87
	2	3	3	167.03	329.41	16	1.9	17.9	17.8	72.46
	2	5	1	108.28	404.00	23.3	3	26.3	27.11	85.89
	2	5	2	102.26	390.76	23.7	2.5	26.2	27.29	58.33
	2	5	3	123.47	452.88	16.9	2.5	19.4	24.89	72.52

	2	7	1	93.14	497.15	29.5	1.3	30.8	29.11	79.52
	2	7	2	86.39	477.15	30.5	1.8	32.3	21.28	63.24
	2	7	3	99.66	552.54	17.5	1.6	19.1	13.33	72.02
Jumpstart	3	0	1	0.00	0.00	1.5	3.3	4.8	17	66
	3	0	2	0.00	0.00	2	2.5	4.5	16	62
	3	0	3	0.00	0.00	1.9	2.9	4.8	16	49
	3	1	1	160.66	160.66	12.8	2	14.8	21.77	66.09
	3	1	2	154.23	154.23	11.5	1.9	13.4	15.15	60.97
	3	1	3	126.05	126.05	13.7	2.2	15.9	20.18	46.17
	3	3	1	94.59	304.77	13.9	1.7	15.6	30.86	62.62
	3	3	2	76.79	295.69	15.1	1.5	16.6	27.9	56.87
	3	3	3	80.13	241.62	15	1.8	16.8	28.71	44.85
	3	5	1	94.59	399.36	13.1	1.8	14.9	25.72	63.99
	3	5	2	94.59	390.28	17.8	1.1	18.9	21.88	60.63
	3	5	3	94.59	336.21	13.5	1.1	14.6	18.49	46.88
	3	7	1	93.28	492.64	16.1	2	18.1	21.4	67.54
	3	7	2	84.32	474.60	18.2	2.9	21.1	19.89	59.05
	3	7	3	73.96	410.17	22.4	1.9	24.3	26.79	48.95
MYKE Pro	4	0	1	0.00	0.00	2.3	2.8	5.1	28	78
	4	0	2	0.00	0.00	1.5	2.8	4.3	18	70
	4	0	3	0.00	0.00	2.4	2.1	4.5	10	59
	4	1	1	164.86	164.86	11.7	1.4	13.1	25.53	74.62
	4	1	2	130.10	130.10	13.2	1.4	14.6	19.15	53.1
	4	1	3	147.61	147.61	9.8	1.8	11.6	9.2	60.15
	4	3	1	144.24	309.10	10.7	0.8	11.5	35.62	75.21
	4	3	2	94.64	224.74	12	2.2	14.2	29.52	53.71
	4	3	3	134.32	281.93	12.5	1.7	14.2	22.5	59.79
	4	5	1	84.14	393.24	17.8	2.1	19.9	19.87	77.8
	4	5	2	50.12	274.87	14.1	2	16.1	19.38	53.76

	4	5	3	72.32	354.25	16.5	2.3	18.8	18.66	62.65
	4	7	1	84.30	477.54	19.5	2.7	22.2	37.68	67.06
	4	7	2	54.61	329.47	19.8	2.4	22.2	28.62	48.34
	4	7	3	81.02	435.27	15.2	2.7	17.9	20.81	59.77
Urea	5	0	1	0.00	0.00	4.3	3.7	8	24	88
	5	0	2	0.00	0.00	3.4	2.8	6.2	15	59
	5	0	3	0.00	0.00	1.9	3.5	5.4	10	61
	5	1	1	163.96	163.96	11.4	2.5	13.9	23.64	82.09
	5	1	2	161.76	161.76	11.4	2.5	13.9	19.25	52.75
	5	1	3	145.07	145.07	17.7	2.7	20.4	10.92	57.3
	5	3	1	148.06	312.02	19.3	2.4	21.7	26.59	86.92
	5	3	2	149.47	311.23	18	2.2	20.2	22.82	49.18
	5	3	3	126.72	271.79	13.9	1.1	15	19.85	53.08
	5	5	1	94.11	406.13	20.1	3.2	23.3	27.93	85.71
	5	5	2	77.20	388.43	21.5	2.3	23.8	19.3	53.26
	5	5	3	68.47	340.26	19.8	2	21.8	10.13	55.11
	5	7	1	86.40	492.53	24.8	2.5	27.3	32.31	81.41
	5	7	2	90.30	478.73	22.7	2.4	25.1	26.72	48.26
	5	7	3	86.77	427.04	27	2.3	29.3	20.06	52.68

APPENDIX B- ANOVA Tables

The SAS System



The GLM Procedure

Class Level Information		
Class	Levels	Values
T	5	1 2 3 4 5
W	5	0 1 3 5 7
R	3	1 2 3

Number of Observations Read	75
Number of Observations Used	75

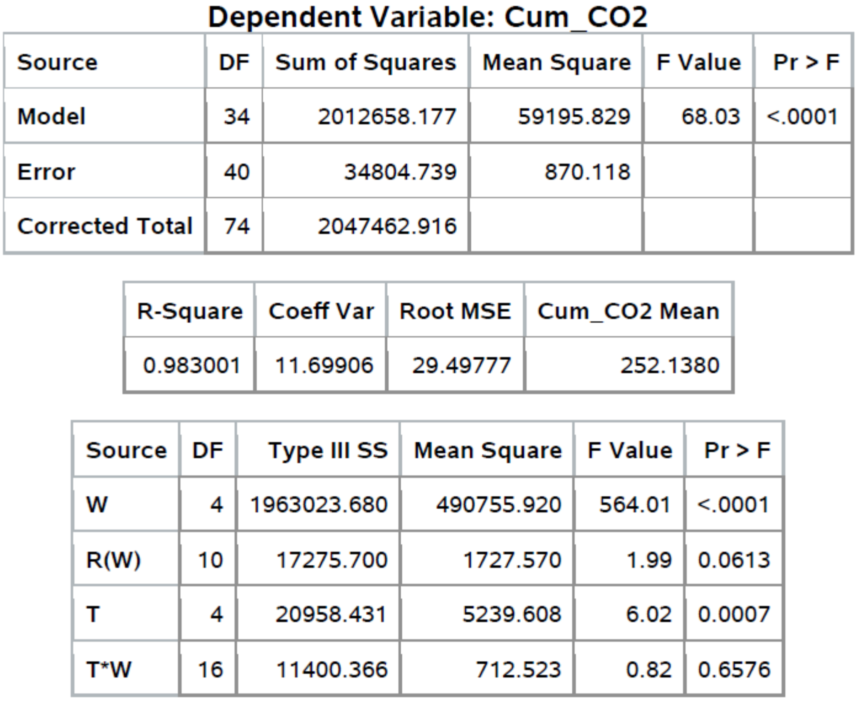
The GLM Procedure

Dependent Variable: CO2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	208513.0512	6132.7368	49.82	<.0001
Error	40	4923.6147	123.0904		
Corrected Total	74	213436.6659			

R-Square	Coeff Var	Root MSE	CO2 Mean
0.976932	12.45951	11.09461	89.04533

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	192582.6525	48145.6631	391.14	<.0001
R(W)	10	3214.5453	321.4545	2.61	0.0152
T	4	4247.2779	1061.8195	8.63	<.0001
T*W	16	8468.5755	529.2860	4.30	<.0001

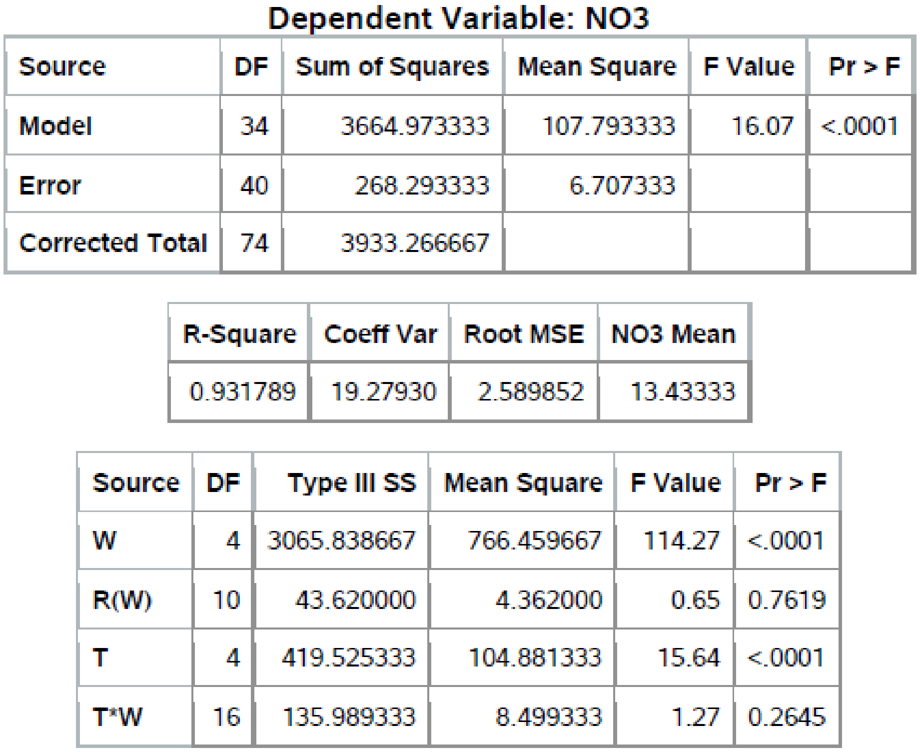


Dependent Variable: Cum_CO2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	2012658.177	59195.829	68.03	<.0001
Error	40	34804.739	870.118		
Corrected Total	74	2047462.916			

R-Square	Coeff Var	Root MSE	Cum_CO2 Mean
0.983001	11.69906	29.49777	252.1380

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	1963023.680	490755.920	564.01	<.0001
R(W)	10	17275.700	1727.570	1.99	0.0613
T	4	20958.431	5239.608	6.02	0.0007
T*W	16	11400.366	712.523	0.82	0.6576



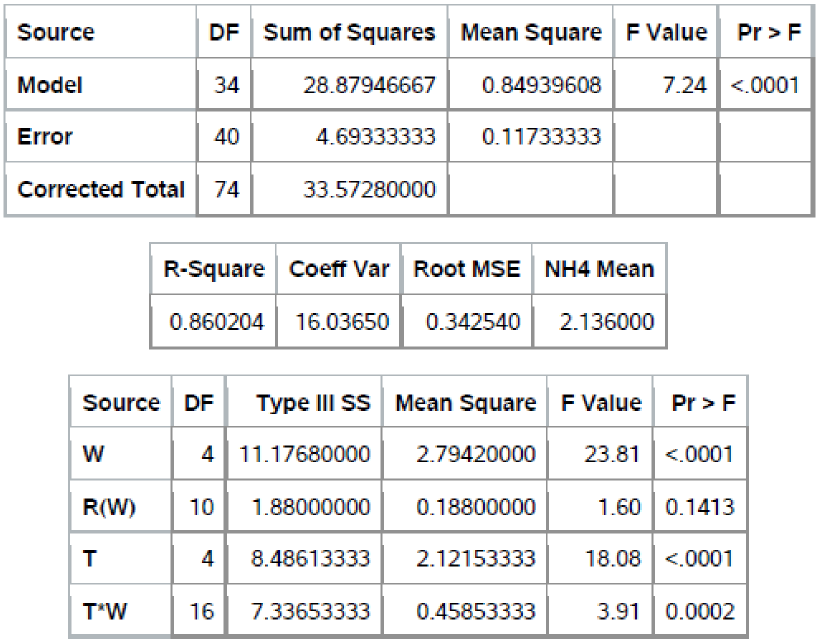
Dependent Variable: NO3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	3664.973333	107.793333	16.07	<.0001
Error	40	268.293333	6.707333		
Corrected Total	74	3933.266667			

R-Square	Coeff Var	Root MSE	NO3 Mean
0.931789	19.27930	2.589852	13.43333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	3065.838667	766.459667	114.27	<.0001
R(W)	10	43.620000	4.362000	0.65	0.7619
T	4	419.525333	104.881333	15.64	<.0001
T*W	16	135.989333	8.499333	1.27	0.2645

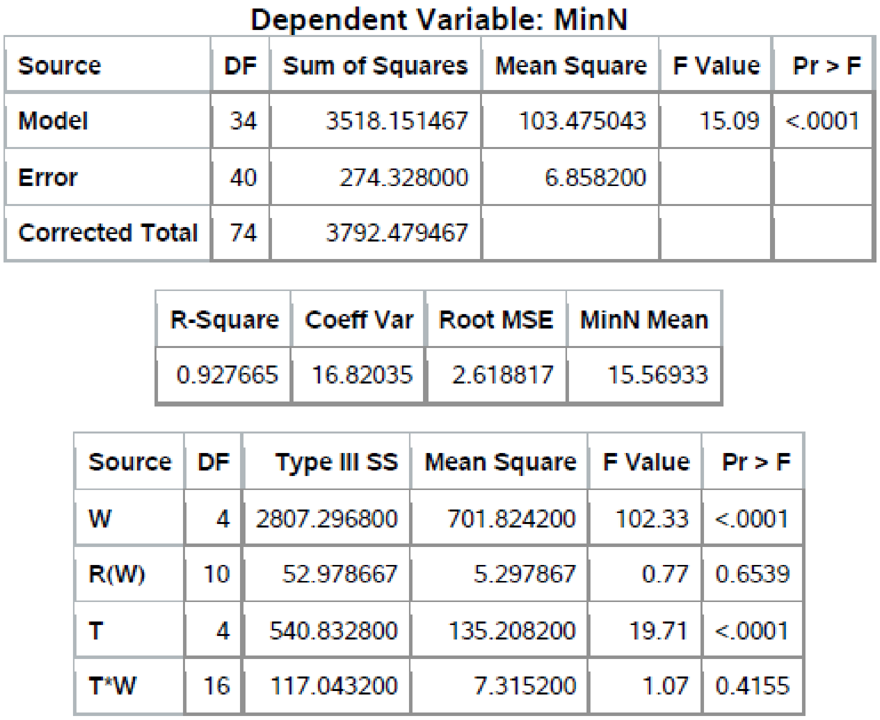
Dependent variable: NH4



Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	28.87946667	0.84939608	7.24	<.0001
Error	40	4.69333333	0.11733333		
Corrected Total	74	33.57280000			

R-Square	Coeff Var	Root MSE	NH4 Mean
0.860204	16.03650	0.342540	2.136000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	11.17680000	2.79420000	23.81	<.0001
R(W)	10	1.88000000	0.18800000	1.60	0.1413
T	4	8.48613333	2.12153333	18.08	<.0001
T*W	16	7.33653333	0.45853333	3.91	0.0002

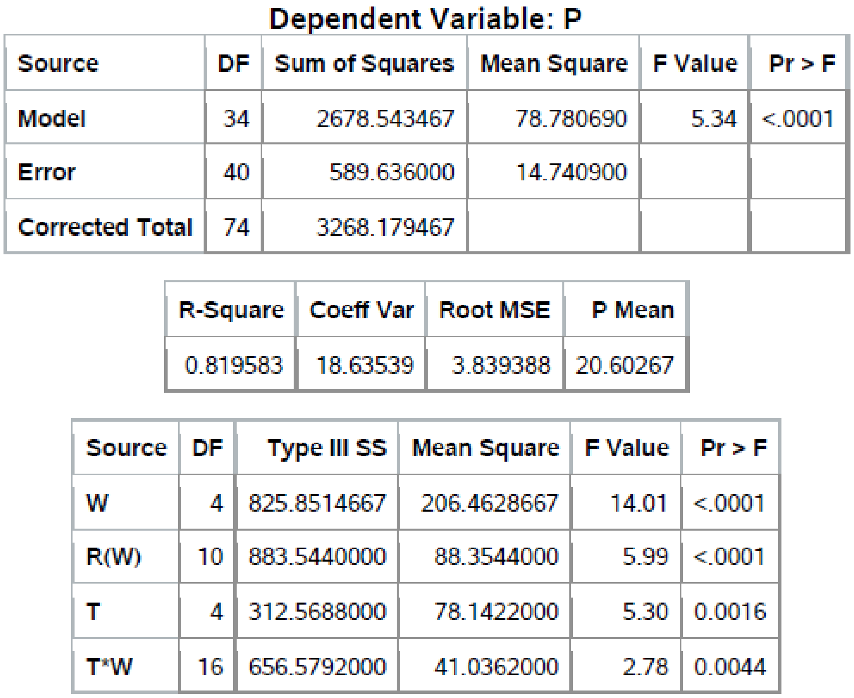


Dependent Variable: MinN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	3518.151467	103.475043	15.09	<.0001
Error	40	274.328000	6.858200		
Corrected Total	74	3792.479467			

R-Square	Coeff Var	Root MSE	MinN Mean
0.927665	16.82035	2.618817	15.56933

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	2807.296800	701.824200	102.33	<.0001
R(W)	10	52.978667	5.297867	0.77	0.6539
T	4	540.832800	135.208200	19.71	<.0001
T*W	16	117.043200	7.315200	1.07	0.4155

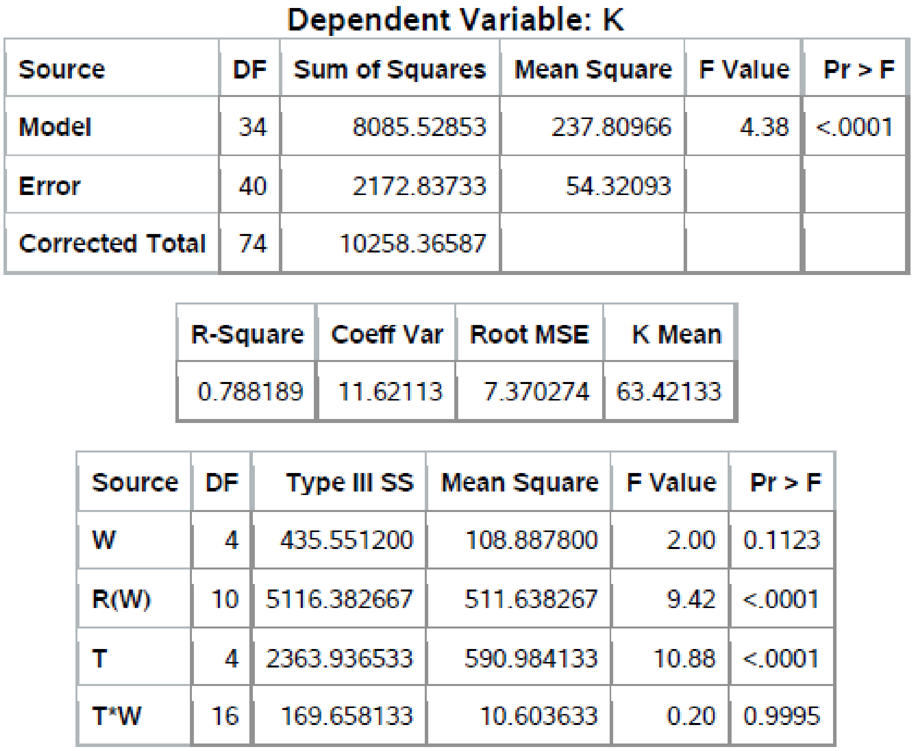


Dependent Variable: P

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	2678.543467	78.780690	5.34	<.0001
Error	40	589.636000	14.740900		
Corrected Total	74	3268.179467			

R-Square	Coeff Var	Root MSE	P Mean
0.819583	18.63539	3.839388	20.60267

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	825.8514667	206.4628667	14.01	<.0001
R(W)	10	883.5440000	88.3544000	5.99	<.0001
T	4	312.5688000	78.1422000	5.30	0.0016
T*W	16	656.5792000	41.0362000	2.78	0.0044



Dependent Variable: K

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	8085.52853	237.80966	4.38	<.0001
Error	40	2172.83733	54.32093		
Corrected Total	74	10258.36587			

R-Square	Coeff Var	Root MSE	K Mean
0.788189	11.62113	7.370274	63.42133

Source	DF	Type III SS	Mean Square	F Value	Pr > F
W	4	435.551200	108.887800	2.00	0.1123
R(W)	10	5116.382667	511.638267	9.42	<.0001
T	4	2363.936533	590.984133	10.88	<.0001
T*W	16	169.658133	10.603633	0.20	0.9995