SOIL NITROGEN DYNAMICS AND MINERALIZATION

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Abstract: A group of scientist in the United Kingdom recently proposed that mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass. This was termed the Regulatory Gate hypothesis, which challenges one of the long held theories in soil microbiology that mineralization processes in soil are predominantly driven by microbial activities. In this study, the main objective was to test the Regulatory Gate hypothesis through evaluation of soil nitrogen (N) dynamics and mineralization by monitoring KCl-extractable nitrogen content in three soils following a series of treatments with or without laboratory incubations. The soils were originated from a century-old continuous winter wheat experiment. For over a century, one of the soils had been added cattle manure once every four years (Manure) and another had been fertilized with inorganic phosphorus every year (P). An untreated control (Check) was included for comparison. These soils had significantly different fertility levels, evidenced by nutrient levels as well as grain production of wheat. Content of KCl-extractable N were low in the air-dried soil, but increased about 4-fold following rewet and incubation at room temperature for 10 days. Additional 10 days incubation did not lead to significant changes in the KCl-extractable N in the Check soil, but led to significant reduction in the Manure soil. Autoclaving led to release of KCl-extractable N in all soils tested, with Manure soil significantly higher than P or Check soil. Rewet followed by 10-day incubation resulted in the release of inorganic N that was significantly higher than the air-dried soils. Data obtained this study seems to be supporting the Regulatory Gate hypothesis, but simultaneous N mineralization and immobilization processes complicate the interpretation of data. Although changes of total N due to these treatments were undetectable, the significant changes of KCI-extractable N demonstrated the complexity of N dynamic of soil and warrant further studies to reveal the underline basis for the observed changes and relative contributions for mineralization and microbial immobilization of N in soil.

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CHAPTER I

INTRODUCTION

Nitrogen is one of the most frequently deficient nutrients in soils and also one of the most expensive inputs applied in agricultural systems. Moreover, 65% of the applied nitrogen in agricultural production is lost to runoff, gaseous emissions, erosion, and leaching (Bhattacharjee et al., 2008). There is a negative and positive effects in N deficiency when an over abundant amount that is present in soil. Because there is an increasingly need for the use nitrogen product to promote plants for food production, the over use of fertilizers and pesticides contribute to a significant amount of the contamination of water, soil and natural areas, which in turn threatened the quality of human and animal health (Pedraze and Brooks, 2008).

Applications of animal manure promote the growth of crops. However, nitrogen in manure is organically bound. This means that the nitrogen must be mineralized before it is available for plant use (Balkcom et al., 2009). Even though the addition of nitrogen would help mineralize (process of converting inorganic N to organic N in soils thus allowing N plant uptake, Bregliani et al. (2008) soil and make it more fertile, the addition.

of organic material also promotes immobilization which converts inorganic N to organic and makes N unavailable for plant uptake (Balkcom et al., 2009) therefore altering plant fertility.

In soil there is only a small amount of N that is found in inorganic form, which is readily available for up takes for plants. Many biological and biochemical processes affect content of available N. These processes can be calculated and used to estimate to find the potential of available N rather than a how much of the N can be mineralized in a certain pool (Bregliani et al., 2008). In the study by Bregliani et al. (2008), they observed that the change in extractable organic and inorganic N is dependent on temperature and soil type. If the extractable N is solely dependent on temperature and soil type, we should be able to track how much influence it has on the mineralization of N on soils that is under optimum conditions in a controlled environment.

A group of scientist in the United Kingdom developed a new prospective, proposing that mineralization of native soil organic matter is not regulated by the size, activity, or composition of the soil microbial biomass (Kemmit et al., 2008). This termed the Regulatory Gate Hypothesis. This hypothesis considers that soil organic matter is fully controlled by abiotic or nonliving chemical and physical factors, which challenges one of the long held theories in soil microbiology that mineralization processes in soil are predominantly driven by microbial activities and biotic factors such as bacteria and fungi (Kemmit et al., 2008). According to Pietri and Brookes (2008), nitrogen (N) mineralization is the biological processes of converting organic N to inorganic N by soil biomass and it is one of the key indicators in the activity of microbes and soil fertility. The Regulatory Gate hypothesis questioned the study of Jenkinson and Powlson (1976)

that the determinant factor of mineralization is microbial activity in soil. Kemmitt et al. (2008) shows that chloroform fumigation does not significantly affect mineralization of organic matter. Kemmit et al. (2008) raised a question that sparked controversy: Is mineralization really driven by microbial activity? In another word, could nitrogen mineralization be solely driven on abiotic factors and processes?

The purpose of this study is to test the Regulatory Gate hypothesis through evaluation of soil N dynamics and mineralization potential by monitoring KCl-extractable N content in soils following a series of treatments with or without laboratory incubations. The three soils used in this study were from a century-old continuous winter wheat experiment. Understanding nutrient cycling and conditions affect mineralization of N is prevalent in understanding treatments that will enhance N availability in soils. This study will assistance in introducing and supporting new possibilities and directions in the evaluation of the causes that influences mineralization in N by backing up the Regulatory Gate Hypothesis.

CHAPTER II

REVIEW OF LITERATURE

Importance of Nitrogen

Nitrogen (N) is an essential element for all living systems, which is often limiting in agricultural production. Although air is rich in N (78%), most organisms cannot break the triple bond of atmospheric N_2 molecules. Therefore, N fertilizer is often applied to maximize the production of crop yields (Davis et al., 2013). In soil, 90% of N is organically bound, while less than 10% in inorganic forms that is available for direct plant uptake (Balkcom et al., 2009; Bregliani et al., 2010). It is generally accepted that the organic part of the nutrients in soils is on reserve for when needed and under field conditions inorganic N is continuously being released from organic N through mineralization processes. It is important to focus on the inorganic form of N, such as ammonium and nitrate, because not only these are plant available forms but also nitrate is known to be transported throughout soil that could potentially lead to N loss as well as eutrophication of nearby water bodies. Therefore, understanding fluctuation of inorganic N levels in soil is important in soil management for sustainable agricultural production

Table 1. Important forms of Nitrogen Compounds in the Soil Ecosystem

Compound	**Chemical Formula**
Nitrate	NO_3^-
Nitrogen Dioxide (g)[†]	NO_2
Nitrite	NO_2^-
Nitric oxide (g)	NO
Nitrous oxide (g)	N_2O
Dinitrogen (g)	N_2
Ammonia (g)	NH_3
Ammonium	NH_4^+
Organic N	RNH_3

[†]g, Gases which occur both free in the soil atmosphere and dissolved in soil water.
(Adapted and modified from Robertson and Groffman, 2007)

while preserving environmental quality. Some important N compounds in soil ecosystems are listed in Table 1.

Nitrogen Cycle

Nitrogen cycle is composed of multiples process by which nitrogen is converted between its various chemical forms. These could be abiotic and biotic processes. Important processes in the nitrogen cycle include dinitrogen fixation, ammonification, nitrification, and denitrification. Conversion of nitrogen between different forms could result in mineralization (converting organic N to inorganic N) or immobilization (converting inorganic N to organic N) of N in an environment (Fig. 1).

Earth's atmosphere contains 78% of nitrogen, making it the largest pool of nitrogen. However, atmospheric nitrogen has limited availability for biological systems, and required to be fixed to bioavailable forms for direct plant uptake. Nitrogen fixation of atmospheric N can be is done by chemical (i.e. industrial or lightening strikes) or biological processes, including free-living, associative or symbiotic bacteria. Ammonification refers to processes that convert organic N to ammonium (NH_4^+), which result in mineralization of N. Many of these conversions are primarily carried out by the activities of microorganisms in the soil. Nitrification is the conversion of ammonia to nitrate (NO_3^-), which is performed primarily by nitrifying bacteria that proliferate in warm, moist, and well-aerated soil. Nitrification is commonly restricted to topsoil. On the other hand, denitrification is the process of converting NO_3^- to N_2 and other gases that

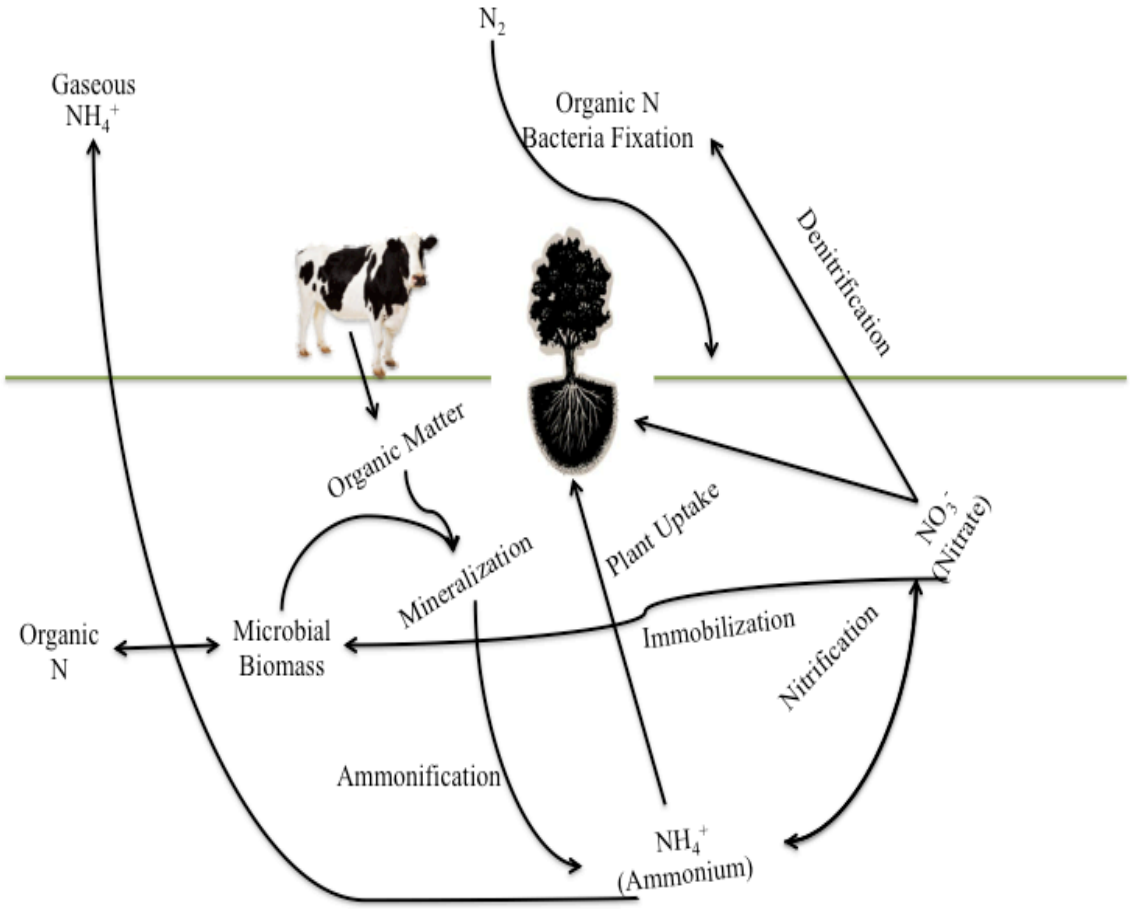

Figure 1. Nitrogen cycle and key transformation processes.
(Adapted and modified from Brady and Weil (2000) and Sylvia et al. (2005)

are commonly lost to the atmosphere. Denitrification usually thrives in warm wet conditions where oxygen is lacking in soil. Nitrogen mineralization occurs when N transformation processes lead to conversion of organic N to inorganic forms. On the contrary, immobilization occurs when N is converted to reduced bioavailability, such as assimilation of available N by living organisms, including plants and microorganisms. Because mineralization and immobilization can occur concurrently simultaneously, these competing processes can ultimately cause an increase in inorganic N when mineralization is taking place at high rates or a decrease in inorganic N when immobilization exceed rates of mineralization. Collectively, N transformation processes ultimately determine the bioavailability of N in soil, and thus the level of inorganic N content.

Extensive studies have been conducted to understand N mineralization. This process is generally described as soil organic matter being decomposed by bacteria and fungi using enzymatic processes that releases ammonium and nitrate into the soil solution (Deenik 2006). Through microbial fixation and mineralization, N_2 and organic N are gradually made available for plant uptake (Gonzalez-Prieto et al., 1996). During growing seasons it is challenging to predict N necessary for optimum crop production from organic N sources (Agehara and Wancke, 2005). There is a concern for more information regarding N mineralization and the amounts of N fertilizer that is being applied for crop production because mineralization of N has been difficult to assess for agricultural application (Balkcom et at., 2009). As shown in Table 2, physical, chemical, biochemical and microbial properties of soils all affect the rate of mineralization of N (Deng and Tabatabai, 2000). Understanding factors affect N transformation processes

Table 2. Factors that Affect Potential Nitrogen Mineralization.

Factor	Effect
Temperature and moisture	Microbial activity thrives at lower temperatures and optimum moisture environments
Soil type	Mineralization tends to be higher in course-texture soil and decreases as the clay content increase
Soil quality	Soils with high N mineralization tend to be more fertile that soils with low N mineralization
Management practices	Extensive cultivation with minimal organic matter input depletes soil organic matter this decreases potential for N mineralization

(Adapted from Deenik, 2006)

would facilitate our understanding of managing N resources to maximize crop yield with minimum inputs and environmental impact.

Soil Moisture and Temperature Effects on Nitrogen Mineralization

Soil moisture and temperature are vital environmental factors affecting mineralization of N (Deenik, 2006). In most climate regimes under normal temperature range, microbial activity is limited at low temperatures and increases with increasing temperature. As a result, N mineralization rates are higher in soils with higher temperatures where soil microbes are more active. Mineralization of N in dry soil would be limiting due to limited water availability and microbial activity. On the other hand, N mineralization is limited when soils are saturated because limited availability of oxygen in these soils would limit microbial activity and thus N mineralization rates (Deenik, 2006). The determinant of nutrient supplies and productivity of soil in reference to N are dependent on the soils ability to mineralize organic matter (Gonzalez-Prieto et al., 1996). Soil fertility and microbial activity are important factors affect the mineralization of N (Pietri and Brookes, 2008). They play an important role in determining N mineralization potential and assessing the needs of applying N to enhance agricultural production (Davis et al., 2013).

Human and Environmental Impact on Nitrogen Cycle

According to Galloway (1998), the human population increases about 1 billion every 12 years. By 2020 the population is projected to be about 7.7 billion. According to United States census 2013, we have already superseded to a little over 7.78 billion people

(U.S. census, 2013). This increase in population causes a demand to maintain a standard of living and to have the required resources for survival (Galloway, 1998). The production of food and energy has become a major concern. About 90% of food production requires arable-farmed land. In the last 30 years, the population out grew the amount of land that is used for production causing world in turn to start running out of new arable land. This concern discussed by Galloway (1998) is already happening at an even faster pace than predicted.

Vitousek et al. (1997) published a technical report on increase of human impact on the N cycle on a global scale. Due to human alterations to the global N cycle, composition, productivity, and other properties modifies natural ecosystems considerably after the addition of N. Species composition, diversity, dynamics, terrestrial, freshwater, and marine ecosystems are all apart and effected by N cycling. These findings made it necessary to evaluate N cycle due to the extensive human alterations and impact on the environment. Several forms of N are of environmental concern (Table 3). A global increase concentration of atmospheric N_2O is of concern because this is a greenhouse gas. Nitrate is known to be transported to groundwater and cause potential pollution of drinking water and coastal eutrophication. Transportation of nitrate in the environment has contributed to the loss of nutrients, which are crucial for long-term preservation of soil fertility. These increases of N transfer throughout rivers to estuaries and coastal oceans caused an increase of organic carbon storage within the terrestrial ecosystem. Additionally, the loss of biological diversity has accelerated in plants animals and microorganisms that depend on the use of N, and contributes to changes and functions of estuarine ecosystems and long-term declines in coastal marine fisheries. More recently,

Table 3. Forms of nitrogen of concerns in the environment

Form	**Source**	**Dominant transport Vectors**	**Potential environmental effects**
Nitrate (NO_3^-)	Nitrification Fertilizer Disturbance that stimulates nitrification Combustion (acid rain)	Groundwater	Pollution of drinking water and Coastal eutrophication
Ammonia (NH_3, NH_4^+)	Fertilizer Animal waste	Surface runoff Atmosphere	Pollution of drinking water and Eutrophication
Nitrous oxide (N_2O)	By-product of nitrification, denitrification, anammox	Atmosphere Groundwater	Greenhouse gas and Ozone destruction in stratosphere
Nitric oxide	By-product of nitrification, denitrification, anammox	Atmosphere	Ozone precursor in troposphere
Dissolved organic N	By-product of mineralization	Surface runoff Groundwater	Eutrophication (?)

(Adapted from Robertson and Groffman, 2007).

Vitousek et al. (2002) published another study that targeted the importance of N to the ecosystem and raised concerns that N limitation is widespread in the ecosystem due to the loss of N to stream water, groundwater and the atmosphere. Although the majority of human alterations of the N cycle are in place to cater to human needs, the long-term effects are of major concern because human alterations have steadily increased rates of N input into the terrestrial N cycle (Vitousek et al., 1997 & 2002). Human alteration to the N cycle has the capacity to change the Earth's ecosystem because of the effects of N supply limits processes of the ecosystem and primary production of the natural world.

Nitrogen in Agriculture

Modern agricultural production is inseparable from supplement of N. Nitrogen can be supplied to aid the growth of plants from the atmosphere through biological fixation or precipitation, from commercial chemical fertilizers, soil organic matter, or supplement using crop residue or animal manure. Ammonium (NH_4^+) and nitrate (NO_3^-) are the forms of N that are available to plants and are the most commonly applied in chemical fertilizer N forms. When excessively applied, negative effects may occur to the plant and the environment. Excess N fertilizer allows for N to leaching into groundwater, lost in runoff, or lost in erosion of soil. Nitrogen enrichment in water bodies could lead to eutrophication and water quality deterioration in the environment.

Nitrogen cycling in agriculture is an important component of global N cycle. Processes involved in N cycling are highly integrated, inter-connected, and do not work independent of each other (Johnston, 1995). The processes in the global N cycle coexist and are greatly influenced by properties of soils, climate, and environmental factors as a

whole. Atmospheric N is one of the largest pools of N on earth. Although there are no plants that are able to directly use atmospheric N_2 gas, some plants can form symbiotic relationships with bacteria to fix N_2 gas for use as an N nutrient for development. In agricultural production, 65% of N comes from atmospheric N_2 through symbiotic N fixation of legume plants and bacteria. Other sources of N, such as decomposed plant material and precipitation, are also important in support sustainable agricultural production. Of particular importance is the understanding of different N transformation processes that govern supply of N as an available form for direct plant uptake. It has long been recognized that N mineralization is governed by microbial activities. Recent research data indicates the need to initiate discussions on this long-held belief.

Regulatory Gate Hypothesis

Kemmitt et al. (2008) proposed a new perspective that mineralization of soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass, which was termed the Regulatory Gate Hypothesis. This suggests that mineralization and the trickle down processes of mineralization are independent of microbial population and their ability to mineralize soil organic matter. The potential mechanisms of mineralization possible involve chemical oxidation or hydrolysis, diffusion form inaccessible soils pores or aggregates, desorption form the solid phase, or action of extracellular established enzymes (Kemmitt et al., 2008). This suggests that the rate-limiting steps of mineralization are abiotic and soil organic matter is mineralized at a relatively constant rate over a period of time without fresh substrate being added. This proposal sparked conversation on the topic. Some questioned the integrity of the study

because it ignores the biological factor that was believed to control mineralization of soil organic matter (Toosi et al., 2012). Kuyakov et al. (2009) challenged the hypothesis based on two principle of soil microbial ecology. One of the principles is the excessive pool principle, which simply states that a large pool of microbial biomass stay dormant until an available input of substrate is added. The other principle called the redundant principle, which states that many microbial taxonomic groups have specific similar functions.

The proposed hypothesis is not defined precisely what specific abiotic process that mineralization is dependent on. Kuyakov et al. (2009) argued that the experiment designed did not allow investigation of limiting agents of mineralization because it only focused on microbial biomass properties. In fact, their study really focused on finding the reasons behind the post fumigation CO_2 flush and the non-fumigated soils showed similar CO_2 evolution rates regardless of microbial activities. This is not consistent with expectations that soil with larger population of microbial biomass should be able to mineralize soil organic matter at a quicker speed than soil with lower microbial biomass (Brooks et al., 2009).

Toosi et al. (2012) conducted a study using ^{13}C isotope and evaluated dissolved organic matter (DOM) in native soils by adding two different forms of fresh organic matter under sterile and non-sterile conditions. They hypothesized that if the Regulatory Gate Hypothesis is functional, that regardless of the organic matter input that the solubilized organic matter would be independent of biological activity. Although their results were able to differ with the long held theories, they concluded that DOM is controlled by many abiotic and biotic factors but that microbial activity did not have a

considerable effect on the DOM that was produced from the input OM and only varied the mineralogy. Their results suggest that physico-chemical processes can primarily control DOM. On the other hand, Kemmitt et al. (2008) research is specially based on mineralization of C and not as much emphasis on the mineralization of N. Therefore, majority of the work done on the Regulatory Gate Hypothesis emphasizes the mineralization of C. This study, however, is focused on mineralization of N. We hope to evaluate The Regulatory Gate Hypothesis and mineralization of soil organic matter from a different aspect.

CHAPTER III

METHODOLOGY

Soil and site description

The soils were taken from a century-long continuous winter wheat (Triticum aestivum L.) experiment located in central Oklahoma, USA. The experiment was initiated in 1892 on a Kirkland (fine, mixed, thermic Udertic Paleustolls) silt loam with a mean particle size distribution of 37.5% sand and 22.5% clay. The manure treatment plot was initiated in 1899. The chemical fertilizer treatment plots were initiated in 1929. There are six plots currently under investigation, including manure (M), phosphorus (P), nitrogen and P (NP), NPK, NPK plus lime (NPKL) and Check (CK), an untreated control plot. Cattle manure from a feedlot was applied in October prior to planting every 4 years at 269 kg N ha^{-1} and was incorporated into soil immediately following application in order to reduce potential surface runoff. The average ratio of N:P of the applied manure is 3.3:1. Chemical fertilizer plots received an annual application of 67 kg N, 14.6 kg P, and 28 kg K ha^{-1} before planting in October.

Preincubation

A 10-g of air-dried (Ck, M, and P) was placed in a 50-ml flask and covered with aluminum foil. 45 flasks for each soil were prepared. Soils were added water to reach 40% water holding capacity. The total weight were recorded. Soils were preincubated at 23^{o}C for 10 days. Moisture loss during incubation was adjusted by adding sterilized water. 45(15 CK, 15 M, and 15 P soil) of these flasks were sterilized three times in three consecutive days and 30 minutes each time**.** After each autoclave, the soil was weighted again and sterile water was added to adjust the loss of water. All soils (both sterile and non-sterile) prepared as described above were adjusted to 60% water holding capacity by addition of sterile water. Soils were incubated at 23^{o}C for 0, 2, 4, 6, and 10 days. Water in an open container was placed inside the incubator to keep the humidity at a near saturation level. After incubation, soil was added 100 mL 1N KCl (1:5 ratio) and shaken at 150 rpm for 30 min. Soil suspension was subsequently filter through Whatman no 4 filter paper. The filtrates were used for quantification of inorganic N by steam distillation followed by acid titration as described below. Triplicate treatments were performed for each experiment and the whole experiments were repeated on different days.

Effect of autoclave on KCl-extractable N in air-dried soils

A 10-g of air-dried (CK, M, and P) was placed in a 50-ml flask and covered with aluminum foil. Soil was autoclaved for eight times in eight consecutive for 30 minutes each time or one time for 60, 120, or 180 min. Experiments were conducted in triplicates and on two different days. KCl extractable N was measured as described above.

Quantification of KCl Extracted Nitrogen

Filtrates obtained above were analyzed for inorganic N (NO_3^--N and NH_4^+-N) by the steam distillation method (Keeney and Nelson, 1987). A 40 mL aliquot was steam distilled following an addition of 0.2 g of Devard's Alloy and 200 mg MgO. KCl extractable N was determined using boric acid as an indicator and HCl titration.

CHAPTER IV

RESULTS

Soil properties

Selected basic characteristics of the soils are presented in Table 1, including pH, organic carbon (OC), total nitrogen (TN) and ratios of OC to TN in soils used in this study. Soils pH values ranged from 4.8 to 5.6, with Manure (M) treated being the highest and Phosphorus (P) treatment being the lowest. The OC ranged from 6.67 to 8.56 g C kg^{-1} soil. Total N ranged from 0.66 to 0.82 g N kg^{-1} soil, the highest of these values was in M treated soils following by P treated, and then the Check soil. Ratios of C/N ranged from 9.7 to 10.4. Of the soils tested, the M treated soil had a higher pH, OC, TN, as well as C:N ratio.

Effect of autoclaving on KCl-extractable nitrogen

As shown in Figure 1, KCI-extractable N increased with increasing times of autoclaving and this increase was linear for up to five times of autoclaving. The releasing

rates of N were similar in the M and P soils, but considerably lower in the CK.soil. For each initial autoclaving event, 3.72 and 3.63 mg N kg^{-1} were released from M and P soils, respectively. In the CK soil, each initial autoclaving event released 2.1 mg N kg^{-1}. Nitrogen releasing rates decreased noticeably after undergone about five daily autoclaving. It is interesting that CK had more extractable N than P soil initially, but autoclaving released considerably more N from P soil than CK. The cumulative N extracted following eight daily autoclaving was the highest in M (31.7 mg N kg^{-1}), followed by P (23.5 mg N kg^{-1}), and the least in CK soil (19.0 mg N kg^{-1}).

To further examine the nature and impact of autoclaving on extractable N, soils were autoclave one time for 60 min or 120 min prior to quantifying KCl extractable N. The obtained results were compared with data resulting from 2 or 4 times of autoclave that was 30 min each time, and thus, resulting a cumulative 60 min or 120 min of autoclaving (Table 2). Given the same cumulative autoclaving time, greater release of KCl-extractable N was shown in multiple autoclaving when compared with one-time autoclaving event. The degree of impact was greatest in P-treat soil, followed by CK, and the least in M treated soil.

Effect of incubation on KCl extractable N

Rewet of air-dried soils followed by 10-day incubation led to significant increase in the extractable N content. However, incubation for additional 10 day did not lead to the release of significant amount of extractable N. In fact, significant reduction of extractable N was observed in M treated control soil (Tables 3 and 4). The largest release of KCl-extractable N was 31.85 mg N kg^{-1} soil in the control P soil following rewet and

Table 4. Soil pH, content of organic carbon (OC) and total nitrogen (TN), and ratios of OC to TN in soils used in this study.[†]

Treatment	pH[‡]	OC	TN	C/N ratio
		g C kg^{-1} soil	g N kg^{-1} soil	
Check	4.9	6.67	0.66	10.1
Phosphorus	4.65	7.39	0.76	9.7
Manure	5.6	8.56	0.82	10.4

[†] Different letters indicate significantly different means at $P < 0.05$ according to least significant difference test.

[‡] Measured in 0.05 M $CaCl_2$, soil:solution=1:2.5.

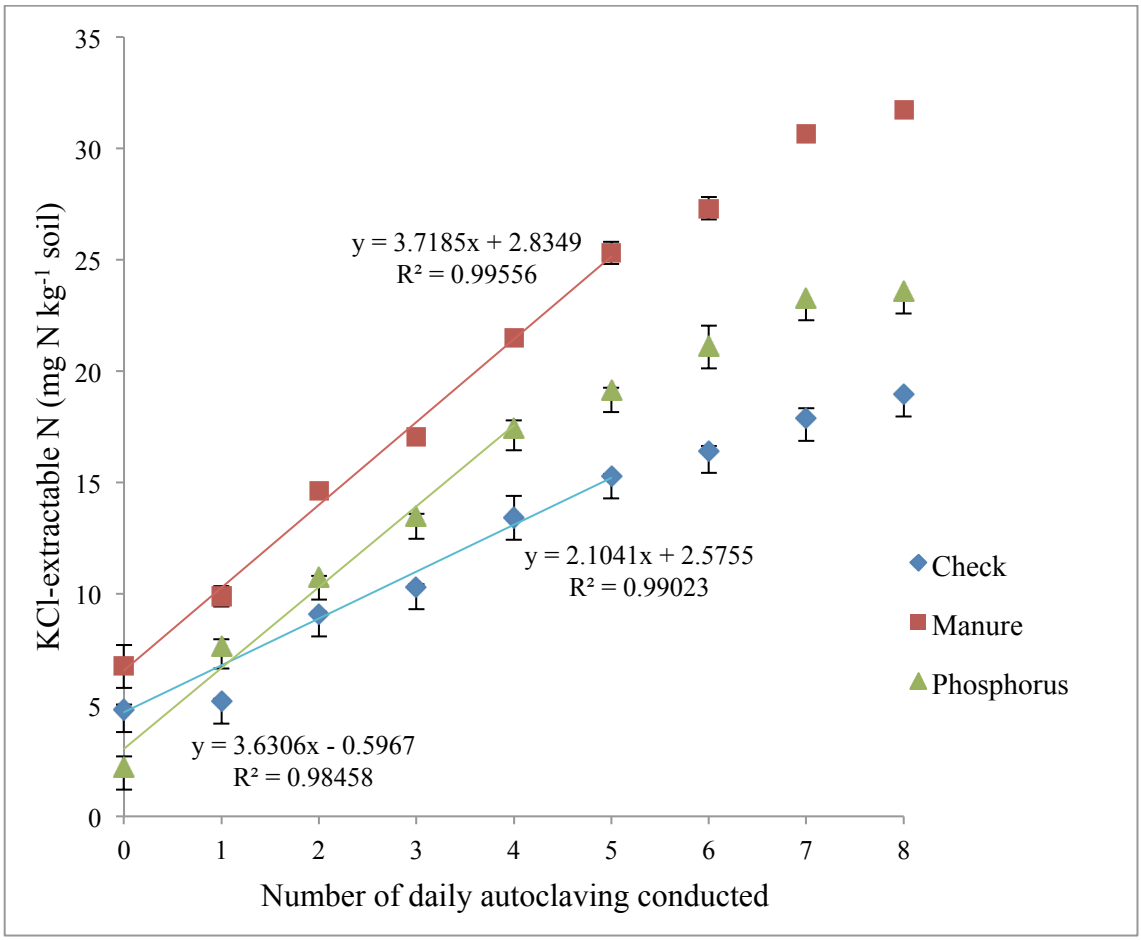

Figure 2. KCl extractable nitrogen (mg N kg^{-1} soil) in 10 g of air-dried soil that was autoclaved daily for 30 min each time and for eight consecutive days. Extraction of nitrogen was conducted by adding 100 mL of 1M KCl to 10 g soil, then shaken for 60 min at 150 rpm min^{-1}, and filtered with Whatman no. 4 filter paper. Each treatment was done in triplicates and the experiment was repeated on two different days. Data reported are mean±standard error (n=6).

Table 5. The release of inorganic nitrogen by autoclaving air-dried soils in consecutive days up to 4 times or one time for 60 or 120 minutes. KCl-extractable nitrogen content (mg N kg^{-1} soil). †

	Number of autoclave x time (min) of each autoclave event = cumulative autoclave time (min)			
Soil	2 x 30 = 60	4 x 30 = 120	1 x 60 = 60	1 x 120 = 120
Check	7.77 ± 0.13	13.42 ± 0.13	5.84 ± 0.18	11.19 ± 0.28
Phosphorus	10.73 ± 0.31	17.44 ± 0.12	9.33 ± 0.50	13.73 ± 0.29
Manure	13.99 ± 0.45	21.50 ± 0.13	14.07 ± 0.53	20.17 ± 0.21

†10-g air-dried soil was autoclaved for four consecutive days for 30 minutes per day and another soil set was autoclaved for one time at 60 minutes and 120min. Inorganic nitrogen was extracted by adding 100 mL 1M KCl to 10 g soil, then shaken at 150 rpm min^{-1} for 60 minutes, and then filtered with Whatman no. 4 filter paper. The filtrates were determined by steam distillation followed by acid titration. Each treatment was conducted in triplicates and the experiment was repeated in two different days.

Table 6. Mean KCl-extractable nitrogen in soil that were air-dried, rewet and incubated for 10 days, and rewet and incubated for 10+10 days. †

Treatment‡		Extractable N in soils specified (mg N kg^{-1} soil) §		
		Check	Phosphorus	Manure
Air-dried (AD)	Control	4.8 ± 0.2	2.2 ±1.0	6.7 ±0.2
	Autoclaved	8.9 ± 0.4	12.06 ±.54	17.1 ±0.5
		(4.13)	(7.70)	(10.41)
Rewet/incubate 10 days and +/- autoclave (RP)	Control	18.9 ± 0.6	34.05 ±.97	24.9 ±1.8
	Autoclaved	23.4 ± 0.5	31.70 ±.51	43.5 ±1.8
		(4.52)	(2.35)	(18.54)
Rewet/incubate 10 days, +/- autoclave, and additional 10 days incubation (RPA)	Control	21.2 ± 0.7	35.07 ±.98	7.5 ±2.5
	Autoclaved	22.7 ± 0.5	35.98 ±.58	43.5 ±1.0
		(1.55)	(0.91)	(35.98)

† Inorganic nitrogen was extracted by adding 100 mL 1M KCl to 10 g soil, then shaken at 150 rpm min^{-1} for 60 minutes, and then filtered with Whatman no. 4 filter paper. The filtrates were determined by steam distillation followed by acid titration. Each treatment was conducted in triplicates and the experiment was repeated in two different days.

‡ Autoclave was conducted in three consecutive days for 30 minutes each time with 10 g soil in 50-mL Erlenmeyer flasks. Rewet was done by adding water to 40-60% of water holding capacity. Soil moisture was maintained at desired content based on weight loss throughout the incubation.

§ Figures in parenthesis are calculated differences (autoclaved - control) in KCl-extractable nitrogen in soils of various treatments.

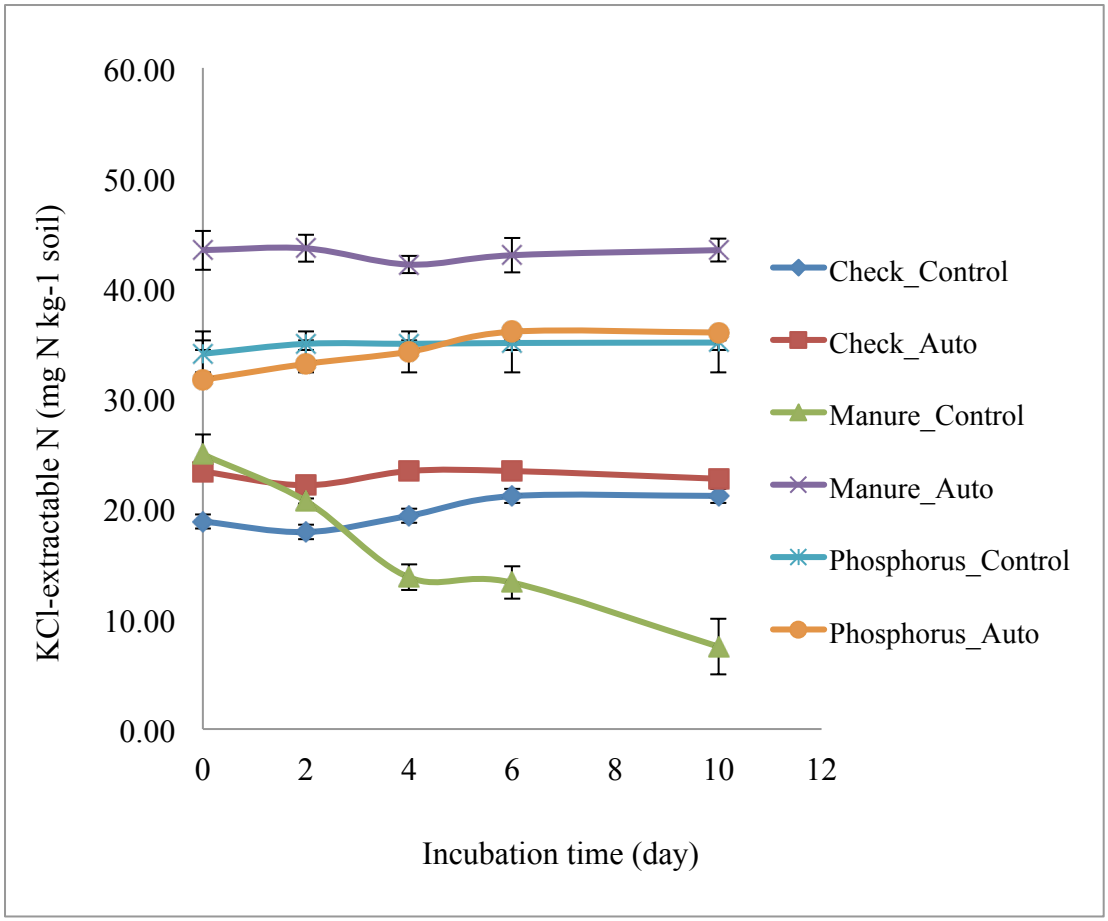

Figure 3. Mean KCl-extractable nitrogen (mg N kg^{-1} soil) in autoclaved or non-autoclaved soils during incubation at 60% water holding capacity and 23°C for up to 10 days. Autoclave was conducted for 10 g field-moist soils for 30 min per day in three consecutive days. Inorganic N was extracted by adding 100 mL of 1M KCl to 10 g soil, then shaken for 60 min at 150 rpm min^{-1}, and filtered with Whatman no. 4 filter paper. The filtrates were determined for inorganic N concentration by steam distillation with addition of Devard's Alloy, MgO and NaOH, and then followed by HCl titration. Each treatment was conducted in triplicates and the whole experiment was repeated in two different days. Bars indicate standard deviations (n=6).

10 d incubation (Table 4). Limited increased in extractable N was detected following additional 10 day incubation.

To confirm the observed phenomenon, KCI-extractable N was measured every two days during incubation for up to 10 days in the control or autoclaved soils. KCI-extractable N decreased with increasing incubation time in the control M soil, but remained fairly constant in all other soils tested (Figure 2). Of the soils and treatments tested, the highest extractable N detected was 23.4 mg N kg^{-1} soil (or 3.55% of TN) for Check soil, 35.98 mg N kg^{-1} soil (or 4.72% of TN) for P treated, and 43.5 mg N kg^{-1} soil (or 5.3% of TN) for M treated soil. For two of the treatments (AD and RP), the difference in extractable N between autoclaves and control soils also indicate autoclave released N in soils under the same treatment. Data suggest that autoclave release N was limited in the CK and P soils, ranging from 2.35 to 7.70 mg N kg^{-1} soil. Considerable amount of extractable N was released by autoclaving in the M soil, showing 10.41 and 18.54 mg N kg^{-1} soil in the two treatments tested.

Table 7. Incubation released KCl-extractable nitrogen (mg N kg^{-1} soil).†

Treatment		Differences in extractable N before and after 10 days incubation (mg N kg^{-1} soil)		
		Check	Phosphorus	Manure
Rewet/preincubate 10 days (RP)	Control	14.07	31.85	18.18
	Autoclaved	14.46	19.60	26.31
Rewet/preincubate 10 days +/- autoclave + additional 10 days incubation (RPA) ‡	Control	2.31	1.02	-17.43
	Autoclaved	0.06	4.28	0.01

†See footnote of Table 3 for detail description of the experiment.

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CHAPTER V

DISCUSSION

With the increasing concern on global sustainable agricultural production to support growing world population, it is important to conserve natural resources and soil quality. Nitrogen in soil is one of the most limiting nutrients that support plant growth. Understanding N cycling, in particular N mineralization, is important in understanding soil nutritional status and how management practices affect soil fertility.

It is a long-held belief that mineralization processes in soil are driven by microbial activity. Recently proposed Regulatory Gate hypothesis states that mineralization of native soil organic matter, including N mineralization, is not regulated by the size, activity or composition of the soil microbial biomass (Kemmitt et al., 2008). This challenges one of the long held theories in soil microbiology.

In this study, evaluation of soil nitrogen (N) dynamics and mineralization were conducted by monitoring KCl-extractable N content in soils following a series of treatments, including air-drying, reweting and autoclaving, with or without subsequent laboratory incubations. The soils were from a century-old continuous winter wheat experiment with three treatments: unfertilized (CK), cattle manure addition once every four years (Manure), or treated with phosphorus (P) every year. Results showed that

KCI- extractable N decreased with increasing incubation time in the unsterilized Manure soil, but remained fairly constant in all other soils tested. Measurements of extractable inorganic N are synthesized results of immobilization and mineralization. The observed decrease in measured extractable N during incubation suggested that immobilization exceeded mineralization in M treated soils. In the other two soils tested, immobilization and mineralization balanced out, resulting in the measured extractable N being fairly constant over incubation time. The obtained results also suggested that M treated soil supported considerable amount of fast growing microbial community, which promoted N immobilization. In theory, enhanced microbial activity would also promote N mineralization as well. The markedly higher immobilization over mineralization also suggested high growth efficiency of microbial community in the M treated soil. The significantly different microbial diversity and community in the M soil from the CK and P treated soils were reported by Sun et al. (2003), which is consistent with observations in this study.

Rewet of air-dried soil followed by a 10 d incubation led to significant increase in the content of extractable N. This is, to certain degree expected because similar trend had been observed before in other studies. The observed phenomenon is commonly interpreted as air-drying process led to killing a portion of microbial community and rewet with incubation stimulated microbial growth and mineralization of microbial debris. As a result, increased extractable N in soil was observed. On the other hand, increased microbial growth should also lead to increased immobilization. The observed

discrepancy could be supporting the Regulatory Gate Hypothesis, suggesting critical roles of chemical and physical processes in the release of extractable N from soil during incubation.

However, incubation for additional 10 d did not lead to the release of more extractable N (Check or sterilized Manure), or resulted in significant reduction of extractable N. This suggests that processes involved in N transformation in these soils have reached some sort of balance between immobilization and mineralization, and thus led to the observed steady levels of extractable N. The highest extractable N detected was 23.4 mg N kg-1 soil (or 3.55% of TN) for Check and 43.5 mg N kg-1 soil (or 5.3% of TN) for Manure. The fact that extractable N content was highest following incubation of sterilized soils suggested that microbiological activities were not the key players in the mineralization of native soil organic matter. Therefore, it can be concluded that data obtained in this study supports Regulatory Gate hypothesis.

Different soil treatments affected extractable N following autoclaving. Overall the M-treated soils contained the most KCl-extractable N, following the P-treated and the check soil contained the least. Comparing soils tested, the higher amount of inorganic N released by autoclave or incubation by manure treatment is consistent with observation reported by other studies (Deng and Tabatabai, 2000; Parham et al., 2003). It is interesting that KCl extractable-N in Check, Phosphorus and Manure treated soil was released by each autoclave event, resulting KCI-extractable N increased with increasing times of autoclaving and this increase was linear for up to five times of autoclaving. The

releasing rates of N were similar in the M and P soils, but considerably lower in the CK soil. For each initial autoclaving event, 3.72 and 3.63 mg N kg^{-1} were released from M and P soils, respectively. In the CK soil, each initial autoclaving event released 2.1 mg N kg^{-1}. Nitrogen releasing rates decreased noticeably after undergone about five daily autoclaving. It is interesting that CK had more extractable N than P soil initially, but autoclaving released considerably more N from P soil than CK. KCI-extractable N increased with increasing times of autoclaving, with r^2 values ranging from 0.95 to 0.99. The effects of autoclaving to extractable N were similar in Manure- and P-treated soils, evidenced by similar slopes of the linear relationships shown in Figure 1. This increase in extractable N continued until it reached a steady equilibrium and plateaued around day 7 and 8. This followed similar patterns in studies conducted in fumigated soils (Kemmitt et al., 2008). In our case it was of a rapid increase in the release of KCl-extractable N, after the first autoclave. The obtained results suggested that the amount and form of N in soil affected the releasing of N by autoclaving. This is evidenced by cumulative N extracted following eight daily autoclaving was the highest in M (31.7 mg N kg^{-1}), followed by P (23.5 mg N kg^{-1}), and the least in CK soil (19.0 mg N kg^{-1}).

To further understand mechanisms of N release during autoclaving and to reveal whether the length of autoclaving or number of autoclaving events play more critical roles in the release of extractable N. Comparisons were made with soils being autoclaved for 2 and 4 times with 30 min each time or autoclave one 60 and 120 minutes. The obtained results were not significantly different, suggesting that N release from soil were

governed heat exposure time as well as the form and quantity of N in soil. Therefore, it is possible that an N releasing curve resulting from autoclaving may be used to illustrate N dynamics in soil. In this study, CK released more N in the initial autoclaving event that P treated soil, but ultimately released less cumulative extractable N. This suggests that CK soil may contain more easily released N than P treated soil.

Cooper et al. (2011) observed significant declines in microbial biomass with increasing temperatures and increased rates of respiration. What are the possible driving forces that causes N to be mineralized by minimized microbial activities? According to the results presented in this study, the mineralization of N is largely driven by abiotic factors that are independent of microbial biomass. Our study is consistent with those reported by Kemmitt et al. (2008), except N was evaluated in the place of C. We also suggest that mineralization of N is not proportional to the size, activity or composition of microbial biomass. Though our focus was not on the microbial biomass structure itself, previous studies (Kemmitt et al., 2008; Jinkinson and Powlson, 1976; Winogradasky, 1924) are sufficient to conclude that microbial activity in fumigated soils is limited. Winogradasky (1924) explained that after fumigation, soils would require some unidentifiable remarkable properties to be able to mineralize soil organic matter. Since microbial biomass is limited, there has to be a series of abiotic factors that allow N to transform to extractable form. Kemmitt et al. (2008), describes this in two steps, the first is non-biological step and the second is a biological step that is caused by a trickle down of substrate from step one. The approach taken on the modeling of this study was not the

same used in Kemmitt et at. (2008), but the same concept of fumigation was used through autoclave and gave similar effects.

The Regulatory gate hypothesis is the first to put the observations into perspective. So what could be the possible abiotic factors that aid in N mineralization. One thought could be enzymatic activity in soils, which are recognized to be influenced and interconnected with physical chemical and biological composition of soil (Gianfreda & Ruggiero, 2006). Enzyme activities in soil are known to be stable and many remain active following autoclaving (Parham and Deng, 2000). Extensive studies have showed that enzyme activity is expressed and affected my natural environmental factors and agricultural and management practices, and are proposed to be sensitive indicators of soil quality and assess to determining soil fertility, quality, microbial growth, and activities in soils (Frankenberger & Dick, 1983; Gianfreda & Ruggiero, 2006). Due to these enzymatic processes causing decomposition of organic matter, it releases ammonium in to the soil solution (Deenik, 2006). Organic inputs are also said to increase the mineralization on plant available N.

In summary, abiotic factors could play crucial role in releasing KCl extractable N from soil. This has significant implication in understanding soil N dynamics and in managing soil as natural resources for sustainable agricultural production.

CHAPTER VI

CONCLUSSION

Understanding N mineralization dynamics is useful in agriculture because it will aid decision making in the application of fertilizers to ensure competitive agricultural production while maintaining and preserving environmental quality. It is well recognized that many biotic and abiotic factors facilitate cause decomposition of organic materials in soil. The fact that extractable N content was highest following incubation of sterilized soils suggested that biochemical parameters such as cell-free enzyme activities (NOT microbiological activities) played a predominant role in the mineralization of native soil organic matter. The procedure differences of the commonly used fumigation method versus autoclave method did not have a significant impact on the results generated. By autoclaving the soils, we eliminated the risk of having chemically altered results. Therefore, the data from this study supports Regulatory Gate Hypothesis. For future studies, more focused attention may be directed to thoroughly understand the impact of the non-microbiological activities on the release of inorganic N in soil.

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