EFFECT OF PHYSICAL, CHEMICAL, AND BIOLOGICAL TREATMENT PROCESSES ON BIOSOLID NITROGEN (N) FORMS AND THEIR INFLUENCE ON SOIL N MINERALIZATION DYNAMICS AND N-ACQUIRING ENZYME ACTIVITIES IN AN ACIDIC SOIL

by

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Abstract

Biosolids application to soils has been seen as a sustainable agricultural practice to recycle organic matter and nutrients and mitigate climate change. However, the direct effect of biosolids treatment processes on the resulting biosolids' biochemical properties is poorly understood. Moreover, the potential activities of soil N-acquiring enzymes in response to various biosolids are rarely examined, especially in acidic agricultural soils. Four different types of biosolids were produced by treating an identical batch of raw sewage solids: N-Viro, CaO-treated, heat-dried (HDB), and composted biosolids (CB). Leached and non-leached incubation studies were conducted simultaneously for 154 days to assess N mineralization and N-acquiring enzyme activities (β-1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), and urease) in an acidic soil after biosolids addition. Our results showed that different biosolids treatment processes (alkaline treatment (N-Viro® Process and CaO addition), composting, and heat drying) significantly affected biosolids characteristics in terms of N forms and contents. Similar patterns of N mineralization were observed in leached and non-leached incubation studies, while the non-leached incubation study showed more evident N mineralization or immobilization. N availability decreased in the order CaO-treated > N-Viro > HDB > CB. A first-order kinetic model fitted well to the mineralization data, but the model needs to be further improved to capture the dynamics of N release from biosolids in the early incubation phase. N-acquiring enzyme activities all increased after biosolids addition, suggesting that soil microbial activity was stimulated. During incubation, LAP activities shared a similar trend with NAG activities (i.e., an initial increase followed by a decline); however, urease activities showed a higher persistence.

List of Abbreviations Used

AeD	Aerobically digested
AMC	7-amino-4-methylcoumarin
AnD	Anaerobically digested
APHA	American Public Health Association
ATB	Alkaline-treated biosolids
BFP	Biosolids processing facility
BNR	Biological nitrogen removal technologies
C	Carbon
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide (quicklime)
$Ca(OH)_2$	Calcium hydroxide (slaked or hydrated lime)
CB	Composted biosolids
CH_4	Methane
C_2H_4	Ethylene
CKD	Cement kiln dust
CCME	Canadian Council of Ministers of the Environment
CO	Carbon monoxide
CO_2	Carbon dioxide
C/N	Carbon-to-nitrogen
dw	Dry weight basis
EC	Enzyme commission number
GHG	Greenhouse gas emissions
GMEA	Geometric mean enzyme activity
HDB	Heat-dried biosolids
H_2O	Water
K	Potassium
L	Leached incubation
LAP	Leucine amino peptidase
MAnD	Mesophilic anaerobically digested
MBC	Microbial biomass carbon
MC	Moisture content
MN	Mineral nitrogen
MOE	Ontario Ministry of the Environment
MUB	Methylumbelliferone
N	Nitrogen
N_2	Dinitrogen

NAG	β-1,4-N-acetyl-glucosaminidase
NH_3	Ammonia
NH_4^+	Ammonium
NL	Non-leached Incubation
NO	Nitric oxide
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NSE	Nova Scotia Department of the Environment
OM	Organic matter
ON	Organic nitrogen
P	Phosphorus
PEO	Peroxidase
PHO	Phenol oxidase
*p*NA	*p*-nitroanilide
*p*NP	*p*-nitrophenyl
PVC	Polyvinyl chloride
RS	Dewatered raw sewage sludge
SO_4^{2-}	Sulfate
SOC	Soil organic carbon
SOM	Soil organic matter
SOP	Standard operating procedures
SQI	Soil quality index
THAM	Tris hydroxymethyl aminomethane
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
T-SQI	Treated-soil quality index
USCC	US Composting Council
US EPA	United States Environmental Protection Agency
ww	Wet weight basis

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Chapter 1: Introduction

1.1 Background

Sewage sludge that has undergone treatment processes in order to lower pathogen contents, reduce vector attraction, and stabilize organic matter are called "biosolids". Typical treatment processes include aerobic or anaerobic digestion, composting, alkaline treatment, and heat drying (CCME, 2012). Biosolids are rich in organic matter (OM) and contain essential plant macro- (nitrogen (N), phosphorus (P), potassium (K)) and micro-nutrients (Sharma et al., 2017), which can be used in many beneficial ways in agriculture, forestry, or land reclamation. Due to the rapid growth of global populations over the past decades, there has been a dramatic increase in biosolids production. Currently, it is estimated that nearly 100-125 million wet tonnes of biosolids is produced worldwide. By 2025, the total production of biosolids is expected to increase to roughly 150-200 wet million tonnes (Mohajerani et al., 2017). This enormous amount of biosolids, if not managed properly, can deposit excess nutrients to the environment and cause environmental threats to ecosystems.

The majority of N in biosolids exists in various organic forms such as proteins, amides, amines, and they must be converted to inorganic N forms (ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-)) via mineralization before it can be used by plants or microorganisms (Wang et al., 2003; Liu et al., 2015). During the mineralization process, a group of N-acquiring extracellular enzymes will be released by microbes to help break down polymers into monomers. The presence or potential activity of specific enzymes have been used as valuable indicators of soil N availability (Sinsabaugh and Moorhead, 1994; Schimel and Bennett, 2004) or microbial activity (Sinsabaugh et al., 2008; Forstner et al., 2019).

Up to now, N mineralization in biosolid amended soils has been widely investigated, however, very few studies have taken into account the **direct** effect of treatment processes of

sewage solids on the resulting biosolids' biochemical properties. The potential activities of soil N-acquiring enzymes in response to different N fertilization have also been examined by a considerable quantity of studies, while biosolids as an organic N input is relatively less explored, especially in acidic agricultural soils.

The overall outcomes of our study will contribute towards building knowledge of how treatment processes impact the nature of different types of biosolids applied to soil and to develop effective agronomic strategies to enhance N use efficiency, leading to more profitable crop production, and less environmental impacts of excessive N. In addition, changes in the presence of soil N-acquiring enzymes and their potential effect on soil N cycling after the application of biosolids will be explored.

1.2 Literature Review

1.2.1 Biosolids

1.2.1.1 Beneficial Use of Biosolids

The term "biosolids" refers to the nutrient-rich organic materials derived from the treatment of domestic sewage sludge (also called sewage solids) in wastewater treatment facilities (Singh and Agrawal, 2008). There are a number of disposal methods of biosolids, such as land application, landfilling, and incineration. In Canada, landfilling and incineration have been increasingly restricted as a result of their negative environmental impacts, thus land application has become a dominant use of biosolids and considered to be an economical way of biosolids disposal (Haynes et al., 2009).

There are a variety of benefits of using biosolids as a soil amendment. The use of biosolids leads to recycling of OM and plant-essential nutrients, reducing the need for synthetic fertilizers, agricultural lime, or reclamation materials, and helping mitigate global warming by improving soil carbon sequestration (Chambers et al., 2003; Orndorff et al., 2008; Lu et al., 2012;

Thangarajan et al., 2013). A recent study has shown that the carbon credits obtained from replacing chemical fertilizers with biosolids exceeded the greenhouse gas emissions (GHG) associated with biosolids processing and transportation (Archer et al., 2020), which indicates recycling biosolids is an environmentally sustainable practice from a GHG perspective. In addition, from a circular economy viewpoint, the material loops can be closed by transforming locally available organic wastes into a value-added agronomic product and reintroduced into the same or other value chains. At the same time, the operating costs associated with disposing of biosolids, i.e., landfill tipping fees, can be reduced for wastewater treatment plants (Mosquera-Losada et al., 2017; Bora et al., 2020; Chojnacka et al., 2020; Amorim Junior et al., 2021).

Land application of biosolids is mainly regulated at the provincial and territorial level in Canada (Cogger et al., 2006), unlike in the United States, where it is federally regulated under the 40 Code of Federal Regulations (CFR) Part 503 Rule (US EPA, 1994a). Depending on the heavy metal and pathogen content in the biosolids, many Canadian provinces (e.g., British Columbia, Nova Scotia, New Brunswick, Prince Edward Island, and Nunavut) categorize biosolids into two or three groups (CCME, 2010). In Nova Scotia, biosolids is classified into Class A and Class B biosolids. The quality criteria of these two classes are summarized in Table 1.1. Class A biosolids refer to the biosolids with the highest quality (i.e., low concentrations of heavy metals and contaminants, and undetectable levels of pathogen), which can be used as commercial fertilizer without land application restrictions. Class B biosolids may contain reduced but detectable levels of pathogens; therefore, they are considered as a generated waste and are subject to specific use restrictions (NSE, 2010).

Table 1. 1 Maximum acceptable concentrations of heavy metals, organic contaminants, and pathogens in biosolids in Nova Scotia (NSE, 2010).

	Parameter	Class A	Class B
Heavy Metal (mg kg^{-1} dw)	Arsenic (As)	13	75
	Cadmium (Cd)	3	20
	Chromium (Cr)	210	1060
	Cobalt (Co)	34	150
	Copper (Cu)	400	760
	Lead (Pb)	150	500
	Mercury (Hg)	0.8	5
	Molybdenum (Mo)	5	20
	Nickel (Ni)	62	180
	Selenium (Se)	2	14
	Zinc (Zn)	700	1850
Organic Contaminant (mg kg^{-1} dw)	Dioxins and Furans	0.000017	0.00005
	Polychlorinated biphenyls (PCBs)	0.8	Not specified
Pathogen	Fecal Coliform	< 1000 MPN* g^{-1} dw	< 2,000,000 MPN g^{-1} dw
	Salmonella	< 3 MPN 4 g^{-1} dw	Not specified

*MPN: most probable number; dw: dry weight basis.

1.2.1.2 Biosolids Treatment Processes

The presence of pathogens, heavy metals, and organic contaminants in the raw sewage solids requires additional treatment prior to its safe utilization as a soil amendment (US EPA, 1994b; Smith, 2009). Different treatment processes modify physical and chemical properties of the raw sewage solids and result in different types of biosolids. There are biological, chemical, and physical treatment processes for sewage solids, and each wastewater treatment facility employs a combination of these techniques depending on budget and mandate (Rigby et al., 2016). Biological methods include aerobic or anaerobic digestion, and composting. Chemical methods require the addition of chemicals such as oxidizing or reducing agents, and acidic or alkaline agents (Reimers et al., 2009; Brisolara et al., 2022). Alkaline treatment is one of the most commonly employed chemical methods (de Luca et al., 1996). Physical methods consist of heat drying, air drying, and microwave drying (Mawioo et al., 2017; Chaudhary and Gough, 2021).

Currently, aerobic and anaerobic digestion have been extensively investigated (Jolis et al., 2002; Zhang et al., 2008; Anjum et al., 2016), whereas studies are lacking for alkaline treatment, composting, and heat drying.

1.2.1.2.1 Alkaline Treatment

Alkaline materials such as fly ash, cement kiln dust (CKD), quicklime (CaO) and hydrated lime ($Ca(OH)_2$) can be used as an additive in biosolids processing. To achieve the minimum US EPA (United States Environmental Protection Agency) criteria for class A biosolids, the pH of the mixture should be raised to 12 or higher for at least 72 hours, and in the meantime the temperature should be maintained at 52 °C for at least 12 hours. After the 72-hour period of elevated pH, the mixture can be air dried or heat dried to over 50 % solids (US EPA, 2000).

The application of quicklime to produce Class A biosolids was recommended by the US National Lime Association (National Lime Association, 1999). Slaking is an exothermic process with the release of heat where quicklime is reacted with water to produce hydrated lime ($CaO + H_2O = Ca(OH)_2$). The slaking temperature is a key factor determining the efficiency of slaking. With a higher slaking temperature, the resultant $Ca(OH)_2$ tends to have a finer particle size and greater specific surface area and becomes more reactive (Hassibi, 2009). Typically, adding at least 30 % of alkaline materials to raw sewage solids (on a dry weight basis (dw)) can lower the pathogen content below the detection threshold (Rigby et al., 2016). Keller et al. (2004) reported that helminth eggs and fecal coliforms were not detected in sludge treated with 30-60 % quicklime. Higher doses of lime (> 50 %) are required to obtain a complete disinfection if the mixture needs to be stored for a long duration.

N-Viro® Process involves alkaline treatment combined with accelerated drying (Logan and Harrison, 1995) (Fig.1.1). The product of this process, N-Viro biosolids (N-Rich®), is an odor-free, granular soil-like material and has been registered as a fertilizer under the Canadian Federal

Fertilizers Act and Regulations (Farooque et al., 2011).

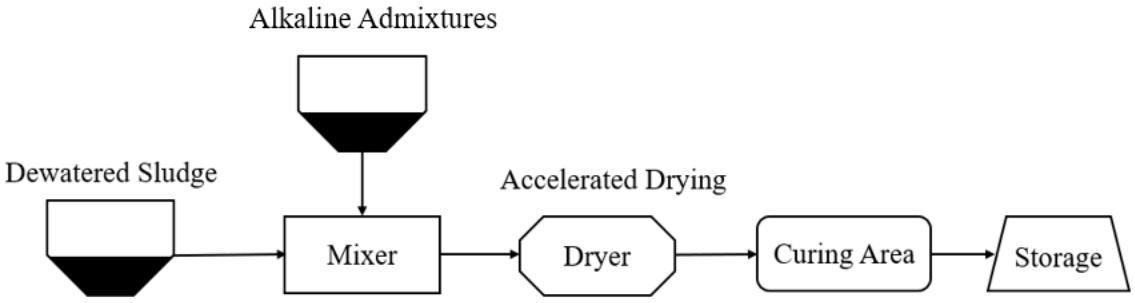


Figure 1. 1 Schematic diagram of the N-Viro® Process.

One advantage of alkaline treatment over heat drying, composting, and anaerobic or aerobic digestion is its low incremental capital cost for a facility to produce Class A biosolids (US EPA, 2000). The major drawback of this method is that this process only inactivates the pathogenic microorganisms, thus there is a potential for pathogen regrowth if the pH decreases below 9.5 with longer storage times (US EPA, 2000; Capizzi-Banas et al., 2004). Meanwhile, the addition of alkaline material could cause N losses through volatilization and make less phosphorus available to plants (US EPA, 2000). However, it would potentially be counterbalanced by the decreased water contents, improved structure, and increased liming value (Keller et al., 2004).

1.2.1.2.2 Composting

Composting is a biological process that is carried out by various microorganisms. Under controlled moisture, self-heating, and aerobic conditions, organic materials are decomposed into a biological stable end product called compost that has some “humus”-like properties (Lobo and Dorta, 2019). There are different types of composting systems, including aerated static pile composting, in-vessel composting, windrow composting, and vermicomposting (Liu and Price, 2011; Lim et al., 2017). In-vessel composting requires less manual labour, smaller land space, and shorter composting period to operate when compared to other systems. Furthermore, as an enclosed system, in-vessel composting has a better control of many environmental conditions.

Nonetheless, the drawbacks of this system are the high expense of energy and the demand for technical expertise to properly handle the equipment (Sangamithirai et al., 2015; Lim et al., 2017; Palaniveloo et al., 2020). The quality of the compost is dependent on many factors, such as moisture content, oxygen supply, C/N ratio, pH, nutritional composition of the feedstock, turning frequency, and temperature. These factors determine the optimal conditions for microbial development and organic matter degradation (Ekinci et al., 2006; Liang et al., 2006; Bernal et al., 2009). During the composting process, the ideal moisture content should be maintained in the range of 50 to 60 % so that the microbial activity and the temperature can be kept at a favorable level. Carbon-rich materials such as sawdust, wood shavings, and straws are commonly-used bulking agents to absorb moisture, increase the C/N ratio, provide structure support, and improve aeration (Banegas et al., 2007; Wu et al., 2010).

Composting has been receiving increased attention as it allows the recovery of nutrients and organic matter from organic waste streams, provides another opportunity for reusing nutrients in agriculture (Senesi and Brunetti, 1996), enhances removal of organic pollutants (Chen et al., 2022), and also helps eliminate pathogenic microorganisms (Fatunla et al., 2017). Many studies have been recently conducted to investigate the feasibility of co-composting raw sewage solids with other organic wastes under different conditions (Tubail et al., 2008; Miaomiao et al., 2009; Ammari et al., 2012). Class A biosolids can be obtained by maintaining the temperature of the mixture at least 55 ℃ for a minimum of three days, so that pathogen can be effectively inactivated (US EPA, 1994a). However, in terms of the nutrient value of the final compost product, the main disadvantages of composting include potentially substantial losses of N via volatilization. It was previously reported in the literature that the initial mass of N can be lost in the range of 20 to 80 % (Martins and Dewes, 1992; Kithome et al., 1999; Lee et al., 2009; Awasthi et al., 2016). Moreover, similar to alkaline-treated biosolids, the addition of extra materials has a diluting effect

on the nutrient contents such as TN, which can reduce the agronomic value of composted biosolids.

1.2.1.2.3 Heat Drying

Heat drying is a physical process that uses direct or indirect heat to kill pathogens and eliminate water content in the biosolids. The main advantage of this method is its high efficiency in producing Class A biosolids (US EPA, 2006). To obtain Class A biosolids, the moisture content of biosolids should be less than 10 %, and the drying temperature must be above 80 ℃ (US EPA, 2006). Unlike alkaline treatment and composting, heat drying does not involve the addition of external materials, which can reduce the volume of waste, and finally contribute to lower transport costs and easier handling and spreading (Stasta et al., 2006).

The operating temperature is a crucial factor that contributes to the N contents in the biosolids. Intensive volatilization of organic compounds can take place if the temperature is set up too high (Deviatkin et al., 2018). The organic compounds can consist of small molecular gasses such as NH_3, CO_2 , C_2H_4, CH_4 and CO, and macro-molecular gases such as aliphatic hydrocarbons, aromatic hydrocarbons, steroids and nitrogen-containing compounds (Liu et al., 2015). O'Shaughnessy et al. (2008) and Deng et al. (2009) reported that substantial N was lost as ammonia via volatilization, but there are several studies showing that N can be recovered from the ammonia by condensation (Deviatkin et al., 2018), absorption with water (Van der Heyden et al., 2015), sulfuric or nitric acids (O'Shaughnessy et al., 2008), or adsorption by palm-shell activated carbon (Guo et al., 2005). Although heat drying is viewed as an energy-expensive technology, there are some developments to make the wastewater treatment process more environmentally sustainable, for example, biogas (a mixture of CH_4 and CO_2) produced from the wastewater treatment has been utilized to assist the drying process (Santos et al., 2021). Solar drying is a more economical and practical option in areas with intense solar radiation and high ambient temperature (Ozdemir et al., 2020). The greenhouse solar drying technology has been

increasingly adopted in some European countries, mainly in Germany, France, Austria, Turkey, and Poland (Bennamoun, 2012), because it requires less land and energy than conventional outdoor drying beds and thermal drying, respectively (Mathioudakis et al., 2013; Boguniewicz-Zablocka et al., 2021).

1.2.1.3 Nitrogen Fractions in Biosolids

Nitrogen in biosolids exist in two forms: organic nitrogen (ON) and inorganic or mineral nitrogen (MN). The dominant fraction of nitrogen in the biosolids is ON, which is found in proteins and their degradation products such as peptides, nucleic acids, amino acids, and amino sugars (Pierzynski et al., 2005). Tian et al. (2013) and Wei et al. (2019) both proved that 80 % of total nitrogen (TN) in the raw dewatered sludge is protein. This considerable amount of protein contents is mainly from human excreta and live and dead bacterial cells. Tian et al. (2002) reported that large numbers of bacterial cells are produced during primary and secondary sludge treatments and 50-60 % of bacterial mass is made up of protein.

From a review of the literature, the mean values of TN for raw sewage sludge (Table 1.2), alkaline-treated (Table 1.3), composted (Table 1.4), and heat-dried biosolids (Table 1.5) were 4.85 % (range: 2.26-7.79 %), 2.19 % (range: 0.65-4.95 %), 2.31 % (range: 1.07-5.96 %), 4.72 % (range: 2.20-6.56 %), respectively. The TN pool was dominated by ON among all the raw sewage sludge and biosolids examined and ranged from 67.74 to 100 %, with a mean value of 92.31 %. These findings agree with results from Rigby et al. (2016), who found the raw sewage sludge and heat-dried biosolids both had, on average, > 4 % TN in the dry solids, while alkaline-treated (3.3 %) and composted biosolids (2.2 %) had relatively lower N concentrations. They also reported the mean value of TN in all types of biosolids was 4.1 % (range: 0.7-15 %), in addition, MN represented only a small percentage of TN (9.7 %). Most of the MN was present as NH_4-N whereas NO_3-N was almost negligible. It should be noted that some calculations of TN and MN vary based

on differences in methodologies, such as use of TKN (total Kjeldahl nitrogen) instead of TN from Dumas methods.

Many factors can contribute to the change in N form and content in biosolids, such as feedstock type and composition, geography and demography, wastewater treatment technologies, storage and management practices, and treatment processes to generate biosolids (Sharma et al., 2017). Wastewaters from domestic sources contain urine and feces with high levels of ON (80-98 % TN) due to the metabolism in human body (Forkes, 2007), whereas industrial wastewater is associated with high levels of heavy metals (Al-Gheethi et al., 2018). Wastewater treatment plants may also receive a mixture of domestic wastewater, industrial wastewater, stormwater runoff, and landfill leachates if combined discharge systems are used (Ngo et al., 2019), which can result in large variations in the characteristics of wastewater influent. Wastewater treatment plants located in rural areas likely receive wastewater with lower concentrations of pollutants. They are generally operated on a smaller scale and equipped with less advanced technologies compared with urban wastewater treatment facilities (Wang and Gong, 2018; Ma et al., 2019). Nitrogen can be lost in many ways throughout the treatment processes, which may not only increase greenhouse gas emissions but also decrease the fertilizer value of biosolids from an agronomic perspective (Liu et al., 2015). A major pathway of N loss is N volatilization, where N is lost as NH_3 to the atmosphere. Volatilization can be promoted not only by rising temperature and pH but also by increasing mechanical mixing and aeration. In addition to volatilization, the decrease of TN content in the biosolids is also largely attributed to the dilution effect from the addition of external materials, such as adding bulking agents during composting and alkaline materials during alkaline treatment. This was a common pattern observed from the literature survey. The highest percentage of MN in the TN was seen in the raw sewage sludge (12.21 %) (Table 1.2), followed by heat-dried biosolids (11.72 %) (Table 1.5), and thereafter composted (7.19 %) (Table 1.4) and alkaline-

treated biosolids (3.13 %) (Table 1.3). Treatments like biological nitrogen removal technologies (BNR) have been recognized as a conventional approach to reduce the ammonium content in the wastewater. There are some biosolids in the literature survey that undergo BNR that had either really low or high TN content (Table 1.2 and 1.5). The possible reason for this outcome is that during this treatment process, nitrogen can be removed either as dinitrogen gas or concentrated to the solids fraction and recycled as N-rich organic amendments (Winkler and Straka, 2019). The products obtained from the activated sludge process tend to have relatively higher levels of TN contents (Table 1.2), because a concentrated population of microorganisms are involved in this treatment and they generate a large quantity of microbial cells (Ni and Yu, 2012). Extended periods of storage can cause a significant decrease in volatile solids and TN contents. Rouch et al. (2011) found that the TN content in dewatered mesophilic anaerobic digested biosolids dropped from 3 % to less than 1 % after being stockpiled for 3 years. Moreover, sample cores collected from the surface of the stockpiled biosolids tend to have lower TN content than samples collected from the middle depth (Little et al., 2020). Heat-dried biosolids can have various physical forms such as powder, granules, and pellets, and come in various sizes with different particle diameters, but how these factors affect the distribution of nitrogen was unclear from the literature, so more comprehensive future research is needed.

Table 1. 2 Total nitrogen (TN), organic nitrogen (ON), and mineral nitrogen (MN: NH_4^+-N+NO_3^--N+NO_2^--N) contents reported in the literature for raw sewage sludge.

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Raw MC: 97.4%	6.80	**5.81**	**0.99**	9800	56 (Including NO_2^--N)	Smith (1998)
Raw MC: 64.4 %	2.26	-	-	-	-	Wang et al. (2008)
Raw (primary)	**4.43**	4.21	**0.22**	2160	24 (Including NO_2^--N)	Parker and Sommers (1983)
Raw (primary), BNR MC: 94.2%	2.69	**2.48**	**0.21**	1800	300	Yoshida et al. (2015)
Raw (primary), activated	**4.52**	4.43	**0.09**	850	34 (Including NO_2^--N)	Parker and Sommers (1983)
Raw (primary), activated Lagoon (4 days) MC: 97.01%	3.62	-	-	5700	-	Smith and Tibbett (2004)
Raw, dewatered MC: 49.03%	3.37	**3.29**	**0.08**	801	0.45	Little et al. (2020)
Raw, BNR, dewatered MC: 81.53%	3.12*	-	-	-	-	Awasthi et al. (2016)
Raw (primary and secondary), dewatered MC: 79.8%	3.49*	**2.65**	-	8400	-	Toledo et al. (2019)
Raw (primary and secondary), BNR, dewatered MC: 78.7%	6.00	**5.24**	**0.76**	7500	100	Yoshida et al. (2015)
Raw (tertiary) MC: 87.8%	6.51	**6.45**	0.06	-	-	Corrêa (2004)
Dewatered	7.07	6.42	**0.65**	6450	**50**	Parnaudeau et al. (2004)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Dewatered MC: 78.7%	6.05	**5.86**	**0.19**	1916	2.82	Rigby et al. (2009)
Dewatered MC: 76.8%	4.60	**4.42**	**0.18**	1800	39.80 (Including NO_2^--N)	Smith (1998)
Dewatered MC: 82.05%	3.02*	-	-	-	-	Wang et al. (2017)
Activated MC: 99.71%	5.90 (Range: 4.9-6.5)	-	-	-	11	Badza et al. (2020)
Activated	**5.61**	5.34	**0.27**	2710	41 (Including NO_2^--N)	Parker and Sommers (1983)
Activated, dewatered MC: 84.6%	6.20*	4.20	**2.07**	20179.0	463.40 (NO_2^--N: 28.5)	Alvarenga et al. (2015)
Activated, dewatered MC: 85.4%	7.79*	-	-	-	-	Ruggieri et al. (2008)
Activated, chemical phosphorus removal, dewatered MC: 83.9%	6.20*	4.40	**1.80**	17891.3	73.90 (NO_2^--N: 7.8)	Alvarenga et al. (2015)
Dewatered Stockpiling (1 year) Sampled from the top 0.7 m MC: 34.07%	3.82	**3.11**	**0.71**	4655	2444	Little et al. (2020)
Dewatered Stockpiling (1 year) Sampled from the top 1.5 m MC: 32.62%	4.41	**3.57**	**0.84**	8178	249	Little et al. (2020)
Dewatered Stockpiling (4 year) Sampled from the top 1.5 m MC: 38%	4.02	**3.29**	**0.73**	7280	15	Little et al. (2020)

*Total Kjeldahl Nitrogen (TKN): ON + NH_4^+-N; A hyphen (-) means that data were not reported;
Nitrogen contents were expressed on a dry weight basis (dw), and values in **bold** were calculated based on the data that are available in the

specific literature;
MC: wet basis moisture content in percent (ww);
Raw: fresh raw sewage sludge; Dewatered: sewage sludge undergo dewatering process; Activated: sewage sludge undergo a biological process using aeration and formation of biological floc composed of bacteria and protozoa; BNR: sewage sludge undergo biological nutrient removal; Lagoon: sewage sludge is settled in lagoon for storage; Stockpiling: sewage sludge is stockpiled for storage.

Table 1. 3 Total nitrogen (TN), organic nitrogen (ON), and mineral nitrogen (MN: NH_4^+-N+NO_3^--N+NO_2^--N) contents reported in the literature for alkaline-treated biosolids.

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
$Ca(OH)_2$ pH: 8.95	3.65	3.60	**0.05**	-	-	Hattori and Mukai (1986)
AeD + CaO Application rate: 15% pH: >12	**3.66**	3.60	0.06	-	-	Mendoza et al. (2006)
AnD + $CaCO_3$	**0.65**	0.62	**0.03**	308	59 (Including NO_2^--N)	Parker and Sommers (1983)
AnD + Lime	2.48	2.47	**0.01**	100	-	Parnaudeau et al. (2004)
Activated, dewatered + Lime pH: 12.2	2.0*	1.90	**0.10**	790.80	103.20 (NO_2^--N:0.4)	Alvarenga et al. (2015)
Raw + CaO Application rate: 30% (dw)	4.01	**4.00**	0.01	-	-	Corrêa (2004)
Raw + CaO	**2.32**	2.32	**0**	62	42 (Including NO_2^--N)	Parker and Sommers (1983)
Raw + Lime	2.49	2.46	**0.03**	200	**100**	Parnaudeau et al. (2004)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Raw + Lime pH: 11.57	Range: 2.12-4.95	-	-	-	-	Penn and Sims (2002)
Raw + Lime pH: 12.5	2.50	2.40	0.10	970	26	Pritchard and Rigby (2010)
Raw + Lime Stockpiled for a year pH: 7.7	2.90	2.87	0.03	350	<5	Pritchard and Rigby (2010)
Raw + Lime pH: 11.6	2.44	**2.22**	**0.22**	2171	43	Rigby et al. (2009)
Dewatered + CaO Application rate: 9 % 13 days of storage pH: 12.1	1.80*	1.78	**0.02**	133.08	34.50	Silva-Leal et al. (2013)
Dewatered + Lime	Range: 1.61-2.65	Range: 1.57-2.64	-	Range: 100-2500	-	Parnaudeau et al. (2004)
Dewatered + Lime	3.97*	**3.84**	-	1310	-	Wang et al. (2018)
Dewatered + Lime pH: 11.2	0.69	0.66	0.03	260	34	White et al. (2018)
N-Viro Biosolids pH: 9.5	0.68	-	-	-	-	Gillis and Price (2011)
N-Viro Biosolids pH: 9.4	1.10	-	-	-	-	Price et al. (2015)
N-Viro Biosolids pH: 9.95	0.97	-	-	<100	-	Shu et al. (2021)
N-Viro Biosolids pH: 8.7-10	Range: 0.70-1.10	-	-	<100-1000	-	Lin et al. (2022)
N-Viro Biosolids pH: 8.7-10.8	0.93*	**0.85**	-	850	-	Obi-Njoku et al. (2022)

*Total Kjeldahl Nitrogen (TKN): ON + NH_4^+-N; A hyphen (-) means that data were not reported;
Nitrogen contents were expressed on a dry weight basis (dw), and values in **bold** were calculated based on the data that are available in the specific literature;
Raw: fresh raw sewage sludge; dewatered: sewage sludge undergo dewatering process; AnD: anaerobically digested; AeD: aerobically digested;
N-Viro Biosolids: a Class A biosolid under Nova Scotia Department of Environment regulations produced by the Walker Environmental, Goffs, Nova Scotia. The patented technology (N-Viro® Process) involves the addition of CaO, cement kiln dust, or other alkaline materials.

Table 1. 4 Total nitrogen (TN), organic nitrogen (ON), and mineral nitrogen (MN: NH_4^+-N+NO_3^--N+NO_2^--N) contents reported in the literature for composted biosolids.

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
AeD + sawdust Composting piles with turning 3 months	1.88	**1.86**	-	180	246	Banegas et al. (2007)
AeD + pinewood splinters C/N ratio: 7.4 Forced aerated tunnels 15 days and 3-month maturation time	3.95*	**3.68**	-	2700	-	Mattana et al. (2010)
AeD + cotton waste C/N ratio: 9.4 Static composting system 49 days and 2-month maturation time	3.79	-	-	-	-	Sánchez-Monedero et al. (2004)
AeD + bulking agent Composting piles 15-28 days and 2 or 3 month maturation time	3.00	-	-	-	-	Marando et al. (2011)
AeD + softwood shavings + sawdust C/N ratio: 38.25 Windrow turning piles	Range: 1.10-1.40	-	-	0-1000	-	Lin et al. (2022)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
AeD + corn straw + tire chips C/N ratio: 5.35 Cone-shaped aerated static piles 2 months	5.96*	-	-	-	-	Esteller et al. (2009)
AeD + AnD + green waste + wood pieces C/N ratio: 11.5	3.40	2.85	**0.55**	5500	-	Parnaudeau et al. (2004)
AnD C/N ratio: 19.50	**1.07**	1.02	**0.05**	505	1115 (Including NO_2^--N)	Parker and Sommers (1983)
AnD + woody mulch material C/N ratio: 12	2.72*	2.46	**0.43**	3100	1170	Alvarez-Campos and Evanylo (2019)
AnD + sawdust Windrow turning piles 3 months	2.15	**2.12**	**0.03**	334	123	Banegas et al. (2007)
AnD + pinewood splinters 15 days and left to mature	3.36*	**2.70**	**0.85**	6560	1880	Tarrasón et al. (2008)
AnD + green wastes C/N ratio: 5.0 Turned-pile system 60 days and over 2-month maturation time	2.57*	**2.56**	**0.03**	104.3	201.1	Gil et al. (2011)
AnD + bulking agent Composting piles 15-28 days and 2 or 3-month maturation time	2.75	-	-	-	-	Marando et al. (2011)
AnD + pinewood splinters C/N ratio: 10.2 Forced aerated tunnels 15 days and 3-month maturation time	2.37*	**2.03**	-	3400	-	Mattana et al. (2010)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Raw + woodchips C/N ratio: 15-22.4	Range: 1.13-2.45*	Range: **1.00-2.04**	-	1300- 4100	-	Obi-Njoku et al. (2022)
Raw + municipal solid waste C/N ratio: 9.2	1.33					Pascual et al. (2002)
Raw + wheat straw Static forced aeration device 28 days + 2-month maturation time	3.75	**3.72**	0.03**	-	-	Liu et al. (2020)
Raw + CaO C/N ratio: 13.93	**1.64**	1.54	**0.10**	970	20 (Including NO_2^--N)	Parker and Sommers (1983)
Raw + yard waste C/N ratio: 8.9 Windrow composting system 21 days and 4-month mature	2.49*	**2.49**	**0.04**	29	411	Oladeji et al. (2020)
Raw + corn straw Forced ventilation system 45 days	1.94*	-	-	-	-	Xue and Huang (2013)
Raw + woodchips + sawdust C/N ratio: 25 Sheltered piles 34 days + 2-month maturation time	1.59	**1.56**	0.03	-	-	Corrêa (2004)
Raw + agricultural wastes + woody materials C/N ratio: 14.2 3 months	3.20*	3.20	**0.04**	349.20	36.70 (NO_2^--N: 1.5)	Alvarenga et al. (2015)
Raw + peanut shells + corn stalks + microbial inoculant C/N ratio: 7.43 21 days	1.76	-	-	-	-	Liu et al. (2017)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Dewatered + wheat straw 130 L Polyvinyl chloride (PVC) reactor C/N ratio: 25.70 56 days	1.63	-	-	2383	-	Awasthi et al. (2016)
Dewatered + wheat straw + lime 130 L Polyvinyl chloride (PVC) reactor C/N ratio: 23.48 56 days	1.62	-	-	2110	-	Awasthi et al. (2016)
Dewatered + wheat straw + lime + biochar 130 L Polyvinyl chloride (PVC) reactor C/N ratio: 11.78 56 days	2.41	-	-	380	-	Awasthi et al. (2016)
Dewatered + wheat straw + natural additives (zeolite, Ca-bentonite and medical stone) C/N ratio: 25.17 Polyvinyl chloride (PVC) reactor 56 days	1.86*	-	-	-	-	Wang et al. (2017)
Dewatered + woodchips C/N ratio: 24.84 Composting piles with turning 86 days	2.50	-	-	-	-	Toledo et al. (2019)
Dewatered + woodchips + eggplant waste C/N ratio: 29.28-36.11 Composting piles with turning 86 days	Range: 1.58-1.90	-	-	-	-	Toledo et al. (2019)

*Total Kjeldahl Nitrogen (TKN): ON + NH_4^+-N; **Alkali-hydrolyzable N, which is regarded as MN;
A hyphen (-) means that data were not reported;
Nitrogen contents were expressed on a dry weight basis (dw), and values in **bold** were calculated based on the data that are available in the specific literature;

C/N ratio: the ratio of total carbon to total nitrogen;
Raw: fresh raw sewage sludge; Dewatered: sewage sludge undergo dewatering process; AnD: anaerobically digested; AeD: aerobically digested.

Table 1. 5 Total nitrogen (TN), organic nitrogen (ON), and mineral nitrogen (MN: NH_4^+-N+NO_3^--N+NO_2^--N) contents reported in the literature for heat-dried biosolids.

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
AeD Heated rotary cylinder 110-130 °C Dried until MC equals to 14 % Granulated from	6.06*	**5.26**	-	8000	-	Mattana et al. (2010)
AnD 105 °C for 30 min Dusty powder form (<0.1 mm in diameter)	4.60	-	-	4000	-	Cogger et al. (1999)
AnD Heated rotary cylinder 80-90 °C Dust form	3.50*	-	-	-	-	Marando et al. (2011)
AnD Heated rotary cylinder 80-90 °C Pelletized form (1 cm in diameter)	3.90*	-	-	-	-	Marando et al. (2011)
AnD Heated rotary cylinder 110-130 °C Dried until MC equals to 14 % Granulated from	5.33*	**4.17**	-	11600	-	Mattana et al. (2010)

Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
AnD Hot air stream 130 °C Dried until MC equals to 14 %	4.45*	**4.11**	**0.34**	3360	40	Tarrasón et al. (2008)
Granulated from MAnD	3.43	-	**0.05**	517	6.85	Rigby et al. (2009)
Raw Furnace 250 °C Dried until no weight loss	6.48	**6.44**	0.04	-	-	Corrêa (2004)
Raw Hot air stream 380-450 °C	4.19	-	-	-	-	Franco-Otero et al. (2012)
Raw Heated rotary cylinder 80-90 °C Fibrous form	2.20*	-	-	-	-	Marando et al. (2011)
Dewatered Rotating drum system 100-105 °C	5.50 4.21*	**3.88**	-	3310	<5	Eldridge et al. (2008)
Granulated from Dewatered 60 °C for 13 hours	2.57*	2.46	**0.11**	1130.7	17.80	Silva-Leal et al. (2013)
OceanGro® Pelletized granulated form	4.91*	4.34	**0.57**	5700	7.21	Alvarez-Campos and Evanylo (2019)
Pelletized form (1-4 mm in diameter)	4.20	-	-	1000	-	Cogger et al. (1999)

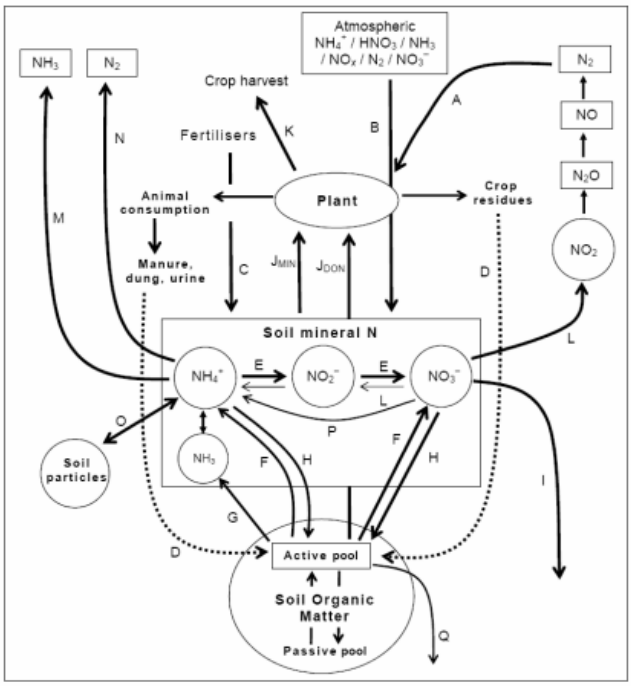
Further Description	TN (%)	ON (%)	MN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Reference
Rotating drum system Inlet: 455-480 °C Outlet: 100 °C (2-4.76 mm in diameter)	5.70	-	-	3500	-	Cogger et al. (2011)
Rotary kiln dryer 450-650 °C for 40 min (1-2 mm in diameter)	6.30	-	-	2500	-	Cogger et al. (2011)
Thermal oil indirect drying 150 °C for 4 hours (1-4.76 mm in diameter)	5.10	-	-	2500	-	Cogger et al. (2011)
BNR Heat treated to pasteurize Pelletized form (4.5 mm in diameter)	6.56	6.22	0.33	3290	6	White et al. (2018)

*Total Kjeldahl Nitrogen (TKN): ON + NH_4^+-N; A hyphen (-) means that data were not reported;
Nitrogen contents were expressed on a dry weight basis (dw), and values in **bold** were calculated based on the data that are available in the specific literature;
Raw: fresh raw sewage sludge; Dewatered: sewage sludge undergo dewatering process; BNR: sewage sludge is processed via biological nutrient removal; AnD: anaerobically digested; AeD: aerobically digested; MAnD: mesophilic anaerobically digested.
OceanGro®: an "Exceptional quality" Class A granular biosolid fertilizer produced by the Ocean County Utilities Authority in Bayville, New Jersey.

1.2.2 Soil N Mineralization

1.2.2.1 Soil N Transformations

The N cycle is one of the most important biogeochemical cycles on the planet that plays a significant role in food production and climate change. However, N loss from agricultural systems not only increases production costs to farm businesses, but also causes environmental problems such as eutrophication and atmospheric pollution (Anas et al., 2020). Therefore, in order to minimize N loss, understanding the impact of different agricultural practices on soil N cycling is important for an effective N use strategy.



A	Symbiotic and asymbiotic N fixation		dissolved organic N (J_{DON})
B	Wet and dry depositions	K	Removal of N from soil by harvest
C	Mineral fertiliser inputs	L	Denitrification (biotic and abiotic)
D	Organic matter inputs	M	Volatilisation
E	Nitrification	N	Anammox (anaerobic ammonium oxidation): $NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O$
F	Mineralisation	O	Ammonium fixation and desorption
G	Ammonification	P	Dissimilatory nitrate reduction to ammonium (DNRA)
H	Immobilisation	Q	Leaching of dissolved organic N
I	Leaching of mineral N		
J	Crop uptake of mineral (J_{MIN}) and		

Figure 1. 2 Schematic diagram of the soil nitrogen cycle (Stark and Richards, 2008).

As shown in Fig 1.2, the major microbially-mediated transformation processes include N fixation (Fig. 1.2 A), mineralization (Fig. 1.2 F), immobilization (Fig. 1.2 H), nitrification (Fig.

1.2 E), denitrification (Fig. 1.2 L), and volatilization (Fig. 1.2 M). N mineralization is a process where complex organic forms of N is converted to soluble inorganic forms of N that can be utilized by plants and microbes.

The end product of the N ammonification process (Fig. 1.2 G) is ammonia (NH_3), which is not stable and will react rapidly with hydrogen from slightly acidic soil to form NH_4. The N nitrification process consists of two successive oxidation reactions in which NH_4 is transformed to NO_2^- firstly and then to NO_3^-. NH_4 and NO_3^- are known to be two primary inorganic forms of N that is available for plant uptake, so mostly N mineralization processes have been assessed by combining N nitrification and ammonification processes (Tanaka et al., 1998; Černohlávková et al., 2009; Wolf et al., 2013). N immobilization is the reverse process of mineralization. Mineralization leads to an increase, while immobilization leads to a decrease in plant available nitrogen in the soil (Villalobos and Fereres, 2016). Hence, evaluating N mineralization in biosolids amended soils and determining N availability from different types of biosolids is essential to improving nitrogen use efficiency.

1.2.2.2 Evaluation of N Mineralization

Potentially mineralizable N is often used as an index of N availability, which describes the soil N supply capacity through the mineralization of soil ON (Schomberg et al., 2009). The most widely accepted method for determining potentially mineralizable N is a laboratory incubation under controlled environment conditions, over a defined period of time, by following either leaching or non-leaching procedures (Benbi and Richter, 2002). Parker and Sommers (1983) evaluated both procedures and concluded that the leached procedure yielded a better model fit, caused by higher variability in the non-leached incubation systems, including subsampling errors and accumulation of toxic substances.

Estimating potentially mineralizable N by mathematical models (Table 1.6) has formed

the basis of prediction of soil N availability (Pereira et al., 2005). It is difficult to model N dynamics in soils treated with organic amendments due to the complex characteristics of the substrate and their interactions with soil. Contrasting results have been obtained from researchers estimating the potentially mineralizable N and the rate of mineralization for biosolids. Models with a good fit have been reported in some studies (Parker and Sommers, 1983; Rasouli-Sadaghiani and Moradi, 2014), but other work has yielded data that are not well explained by these models (Chae and Tabatabai, 1986; Gil et al., 2011).

Table 1. 6 Models for estimating the potentially mineralizable N as a function of t and k.

Model Description	Equation	Parameters[a]	Reference
Linear	$N_m = N_o + k_0 \times t$ (Equation(1))	N_o , k_0	Addiscott (1983)
Simple exponential	$N_m = N_o (1 - e^{-kt})$ (Equation(2))	N_o , k	Stanford and Smith (1972)
Double exponential	$N_m = N_1 (1 - e^{-k_1 t}) + N_2 (1 - e^{-k_2 t})$ (Equation(3))	N_1 , N_2 , k_1, k_2	Molina et al. (1980)
Simple exponential plus linear	$N_m = N_1 (1 - e^{-k_1 t}) + k_0 t$ (Equation(4))	N_1 , k_0, k_1	Bonde and Rosswall (1987)
Simple exponential plus logistic	$N_m = N_1 (1 - e^{-k_1 t}) + \frac{N_3}{1 + e^{-\frac{t-k_3}{k_4}}}$ (Equation(5))	N_3 , k_1, k_3, k_4	Gillis and Price (2016)

N_m: cumulative amount of mineralized N (mg kg^{-1}) at time t

[a]N_o , N_1 , and N_2 : potentially mineralized N pool, labile or readily decomposable organic N pool, and recalcitrant or slowly decomposable N pool (mg kg^{-1}), respectively; N_3 : delayed logistic N pool (mg kg^{-1}).

k_0, zero-order rate constant (mg kg^{-1} day^{-1}) of mineralization of N_2 ,; k, k_1, and k_2 : first-order rate constant (day^{-1}) of mineralization of N_o , N_1 , and N_2 , respectively; k_3: location of inflection point (days); k_4: distance from inflection point to ¾ maximum (days).

Some studies have shown that N mineralization from biosolids can fit a linear zero-order kinetic model (Table 1.6 equation (1)) when the biosolids tend to be characterized by low C/N ratios and more labile OM (Vourlitis and Zorba, 2007; Masunga et al., 2016). The most commonly used model is a non-linear, first-order kinetic model (Table 1.6 equation (2)) proposed by Stanford

and Smith (1972). They defined the N mineralization potential as a single pool of mineralizable N in the soil which can easily decompose with a constant mineralization rate, and the mineralization rate is proportional to the amount of potentially mineralizable N in the soil. Thereafter, models with more than one pool have been used to achieve a better analysis of experimental cumulative mineralized N as a function of time and rate. The double first-order kinetic model (Table 1.6 equation (3)) with two pools was proposed to represent two ON fractions (labile and recalcitrant ON), which decompose independently with different mineralization rate constants (Molina et al., 1980; Lindemann and Cardenas, 1984). Bonde and Rosswall (1987) modified the double model by combining first-order and zero-order kinetics model together (Table 1.6 equation (4)). Gillis and Price (2016) adapted their previous model, a model originally designed for interpreting C mineralization (Gillis and Price, 2011), to study short-term N mineralization (Table 1.6 equation (5)). Their model accounted for large variations in organic amendments with respect to substrate quantity and quality, showing an accurate description of the soil N mineralization data.

Efforts have been made to improve the predictive accuracy of these kinetic N models. Serna and Pomares (1992) and Bernal et al. (1998) suggested that the parameter $N_o \times k$, the amount of N mineralized daily, can be a better predictor of N availability than N_o alone because in the later stages of incubation k tended to decrease while N_o continued increased. Stanford and Smith (1972) and Sharifi et al. (2007) suggested the N released in the first two weeks should be excluded in the model fitting procedures, because it represented the initial N flush caused by rewetting of dried soil. Benbi and Richter (2002) demonstrated that soil incubation needs to be continued until the rate of mineralization k decreases to a low and relatively constant value. Gillis and Price (2016) also proposed that their compartmental model could be refined by including microbial parameters such as microbial biomass or enzyme activity.

1.2.2.3 Nitrogen Mineralization in Biosolids-amended Soils

1.2.2.3.1 Soils Amended with Alkaline-treated Biosolids (ATB)

Application of alkaline-treated biosolids (ATB) is of particular interest in acidic soil ecosystems since alkaline materials have the ability to change soil acidity by neutralization and consequently improve crop production. Furthermore, some alkaline treated products can also supply other essential elements that are important for plant growth, such as P and K. Applying alkaline-treated biosolids or liming the soil prior to biosolids addition (John and Van Laerhoven, 1976; Su and Wong, 2004) were both shown to reduce the bioavailability of heavy metals in the raw sewage solids through immobilization and eventually mitigate metal toxicity (Zornoza et al., 2012). Additionally, it has been widely accepted that the presence of Ca^{2+} ion can contribute to improving soil aggregation and stimulating the activities of soil microorganisms (Ives et al., 2015; Aye et al., 2016; Rowley et al., 2018).

The effects of different types of alkaline materials on soil characteristics can vary. For example, the adsorption rates of Ca^{2+} from different alkaline materials onto the soil colloidal complex influences soil base saturation and ultimately soil pH. CaO is more reactive and dissolves more quickly (Simard et al., 1999; Getahun et al., 2021), thus soil pH was found to increase more sharply under CaO treatment compared with $CaCO_3$ treatment (Mühlbachová and Tlustoš, 2006). Simard et al. (1999) reported that the K level was shown to be significantly higher in soils receiving CKD-treated biosolids than in soils receiving CaO-treated biosolids, which can be explained by the high K content in the CKD (Konsta-Gdoutos and Shah, 2003).

As alkaline conditions are favorable for the solubilization of proteins, and their subsequent biodegradation, the release of nutrients in soils can also be influenced (Andersson et al., 2000; Andersson and Nilsson, 2001; Neina, 2019). An elevated pH will enhance the charge density of compounds, increase the dissociation of acid functional groups, and reduce the bonds between the

organic constituents and clays (Andersson et al., 2000), leading to increased C and N mineralization (Curtin et al., 1998). Rigby et al. (2016) estimated that alkaline-treated biosolids had a mean value of 34 % of mineralizable N, with a range from 3 to 65 %. Lin et al. (2022) demonstrated that 30-45 % of the TN applied was mineralized from N-Viro biosolids over a 3-year consecutive field study. Franco-Hernández et al. (2003) found N mineralization was significantly increased when $Ca(OH)_2$-treated biosolids was added to an acidic soil. In a laboratory incubation study by Ives et al. (2015), 45 % of the TN was mineralized from a neutral soil amended with CaO-treated biosolids after 56 days (at 12.5 ℃). In a field experiment by Rigby et al. (2010), 65 % of the TN was mineralized over the first growing season from an acidic soil amended with CaO-treated biosolids. Similarly, Mendoza et al. (2006) observed 62 % of mineralizable N from an alkaline-treated biosolids in a moderately alkaline sandy soil during a 3-month column leaching study. In contrast, some studies reported no effects or a decreased N mineralization for alkaline-treated biosolids (Rigby et al., 2009; Silva-Leal et al., 2013), which might be attributed to alterations of the microbial community. The elevated temperature and pH caused by the alkalinization process likely suppressed or killed some sensitive microorganisms accountable for N mineralization, hence slowing down the microbial decomposition process (Carneiro et al., 2005; Li et al., 2021). Moreover, the N mineralization process can be inhibited due to high salinity when alkaline-treated biosolids are applied to an alkaline soil (Franco-Hernández et al., 2003; Dendooven et al., 2010). Improved soil aggregate stability following alkaline-treated biosolids can result in organic matter preservation, which can also limit N mineralization (Mahoney et al., 1987; Cambardella and Elliott, 1992; Yucel et al., 2015).

1.2.2.3.2 Soils Amended with Composted Biosolids (CB)

A number of studies have shown that compost applications can improve soil physical properties, including reduced bulk density, increased soil porosity, and elevated water retention

ability and aggregate stability (Kranz et al., 2020). It is also suggested that the presence of acidic functional groups in compost can consume protons and enhance soil buffering capacity, and thus can effectively raise soil pH (Hargreaves et al., 2009; Bougnom et al., 2011), but only slight pH differences haven been observed in composted biosolids (CB) amended soils (Sciubba et al., 2014; Rossini-Oliva et al., 2017).

The quality of the finished compost is closely related to compost maturity and biological stability (Stehouwer et al., 2022). The term "maturity" and "stability" are often used interchangeably in the literature to describe the status of compost, but they are not equivalent. The former term refers to the degree of decomposition of toxic organic substances and is often related to the growth of plants or phytotoxicity (Wu et al., 2000), and the latter refers to a specific state of OM decomposition during composting and is related to the availability of labile organic compounds in the composting mixture (Bernal et al., 2009). A high-quality finished compost can be obtained when both maturity and stability criteria are fulfilled. However, it is acknowledged that there is no single universal standard to evaluate both compost maturity and stability, and thus a combination of tests need to be performed (Komilis et al., 2011). Maturity has been assessed through plant or seed bioassays (Zucconi, 1985; Emino and Warman, 2004). While stability has been generally evaluated by respirometric methods as a result of microbial activity, such as oxygen uptake, carbon dioxide evolution, and self-heating, and/or by studying the easily biodegradable organic matter in the material (Iannotti et al., 1994; Said-Pullicino et al., 2007; Wichuk and McCartney, 2010). Other parameters have also been applied to character compost quality are pH, electrical conductivity, colour, odour, moisture, temperature, C/N, volatile solids, and dissolved organic carbon (Grube et al., 2006; Oviedo-Ocaña et al., 2015). A stable compost is mature if biological activity is low under adequate testing conditions (i.e., sufficient moisture and temperature). However, a compost might test as stable due to a lack of moisture or temperature,

and therefore may be associated with the reduced biological activity but not with the depletion of labile organic matter at the time of testing (MOE (Ontario Ministry of the Environment), 2012).

The maturity and stability of the compost can have a considerable impact on the properties of agricultural soils. Immature compost can release phytotoxic substances that are harmful to plants, such as salts, ammonia, heavy metals, phenolic compounds, and organic acids (Brewer and Sullivan, 2003; Ramírez et al., 2008). The availability of oxygen to the plant roots can also be deprived when immature compost is incorporated into the soil, as soil microbes will utilize the oxygen to break down the unstable OM (Butler et al., 2001; Readyhough et al., 2021). Another concern associated with applying immature compost to soils is a potential for N deficiency in crops. Immature compost can cause soil N immobilization on the native available nitrogen for plants and result in low N mineralization rates. Furthermore, the high fraction of stable or recalcitrant OM in the finished compost can require a longer period of time for microbes to decompose, which can increase soil N retention capacity and slow down the rate of mineral nitrogen release (Khalil et al., 2005; Gale et al., 2006; Huang and Chen, 2009; Sciubba et al., 2013; Franklin et al., 2015).

Rigby et al. (2016) proposed that composted biosolids had the smallest mineralizable N contents among the investigated biosolids, which was 7 % on average, with a range from -10 % to 25 %. Mineralizable N of 10 % has been reported by the US EPA for composted biosolids (US EPA, 1995), similar to findings by Parker and Sommers (1983) (8 %), Oladeji et al. (2020) (11 %), and Lin et al. (2022) (7-13%). Amlinger et al. (2003) and Escudero et al. (2012) reported similar mean values even though they examined other compost amendments, with a range from 5-15 % and 3-13 % respectively. In a field study with tall fescue, Alvarez-Campos and Evanylo (2019) found that the amount of mineralizable N after application of composted biosolids to an alkaline soil was 4.6 % during the growing season. Using the same grass species, Bowden et al.

(2007) conducted a laboratory study, and they observed higher net N immobilization in acidic soils amended with composted biosolids. In their study, soils treated with sewage solids-sawdust-alkaline materials compost had a greater nitrogen immobilization (-15 %) than soils treated with sewage solids-sawdust compost (-5 %). This 10 % difference might be attributed to the N loss via N volatilization during the incubation or the increased soil microbial activity (Chen et al., 2021). Using alkaline materials as additives in the composting process can bring some benefits in terms of pH improvement, reduced bioavailable heavy metals, and lower risks of nutrient leaching (Belyaeva and Haynes, 2009; Awasthi et al., 2016). However, Fang et al. (1999) noticed a substantial ammonium loss during the composting of sewage sludge-coal fly mixtures. Therefore, the rate of additives should be decided carefully.

C/N ratio is often considered a useful indicator of N mineralization (Bonanomi et al., 2019). Compost with high C/N ratios (>25) is generally less mature and can lead to soil N immobilization. However, Pansu and Thuriès (2003) observed notable net N immobilization shortly after incorporating five various composts even though the C/N ratio of each compost was low (11-15). Kaboré et al. (2010) also noticed that even in the most stabilized compost with a C/N ratio of 8.7, N availability remained low after its application. Therefore, we could conclude that C/N ratio did not provide a reliable and accurate information on the mineral nitrogen supply of all types of compost.

The low N availability in composted biosolids can be insufficient to meet the immediate need of crops in the initial crop growth stage, therefore, the combined utilization of compost and mineral fertilizer has been adopted as an integrated nutrient management approach to maintain soil fertility and enhance crop productivity (Oyetunji et al., 2022). Early application of compost (3 months before sowing of the crop) has also been recommended to synchronize N supply with crop demand and avoid potential N loss from the soil in the late season (Sánchez et al., 1997;

Ambus et al., 2002).

1.2.2.3.3 Soils Amended with Heat-Dried Biosolids (HDB)

Substantial amounts of plant macro- and micro-nutrients were found in soils after the application of heat-dried biosolids, however, it is suggested to reduce the application rate to minimize the potential risk of excessive phosphorus loads (Shober and Sims, 2003). Heat-dried biosolids (HDB) can also improve soil structure by enhancing water holding capacity and boost microbial activity (San Miguel et al., 2012). Soil OM increase was not shown in the study of Guiresse et al. (2004), implying that the carbon in the heat-dried biosolids was mostly in readily available forms.

Heat drying treatment can create small-sized particles and consequently increase surface-to-volume ratio in the material itself, which makes heat-dried biosolids contain more easily degradable OM and become more accessible to microbial degradation (Mattana et al., 2010). A much faster N mineralization rate and a greater mineralizable N content were observed in the soil amended with heat-dried biosolids than raw sewage solids (Tarrasón et al., 2008), composted biosolids (Fernández et al., 2007), and alkaline-treated biosolids (Silva-Leal et al., 2013). Eldridge et al. (2008) found that heat-dried biosolids treatment had more than 50 % of its ON mineralized. Smith and Durham (2002) reported 30-60 %, and a mean value of 40 % was published by Rigby et al. (2016), ranging from 26-71 %.

Nonetheless, there were some studies also observed N immobilization after the application of heat-dried biosolids (Smith and Durham, 2002; Marando et al., 2011). Moritsuka and Matsuoka (2017) suggested that biosolids dried at higher temperature (150-200 ℃) tended to have lower MN contents and higher stable ON contents. Higher temperature can also have negative impact on soil microorganisms such as nitrifying organisms. Corrêa et al. (2005) found that heat-dried biosolids have low nitrification rates when incorporated into soils, which can lead to less risk of

NO_3^- leaching. Volatile fatty acids have been detected in the heat-dried biosolids (Rosenfeld et al., 2001) and potentially can cause soil immobilization, because this organic compound was shown to be responsible for the immobilization after the application of manure (Kirchmann and Lundvall, 1993) and digestates (Fagbohungbe et al., 2019).

1.2.3 Soil Enzyme Activities

1.2.3.1 Enzymes Involved in N Mineralization

Enzymes can be categorized into two types depending on their locations: intracellular enzymes which are located both in microbial living cells and extracellular enzymes which are on the surface of clay-humus complexes or in the soil solution (Klose and Tabatabai, 1999). Extracellular enzymes are relatively easier to measure and more sensitive to environmental stress, therefore they have been frequently evaluated by researchers. Whereas the determination of intracellular enzymes requires an additional treatment, for instance, the chloroform fumigation method, in order to release intracellular substances via microbial cells lysis (Brookes et al., 1985; Xu et al., 2020).

Enzymes are often grouped based on the nutrient cycling process (nitrogen, carbon, and phosphorus) they are involved in, and there are two broad types associated with organic matter decomposition: oxidative and hydrolytic enzymes. Phenol oxidase (PHO) and peroxidase (PEO) are the two major oxidative enzymes in relation to lignin degradation (Sinsabaugh, 2010). The mostly widely measured hydrolytic enzymes associated with N mineralization include β-1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), and urea amidohydrolase (urease) (Tabatabai and Bremner, 1972). They are mainly secreted by chitinolytic, proteolytic, and ureolytic microorganisms, respectively (Mobley and Hausinger, 1989; Hui et al., 2020; Balume et al., 2022). NAG (EC 3.1.6.1) releases N-acetyl glucosamine residues by the degradation of fungal chitin and bacterial peptidoglycan (Kögel-Knabner, 2006). LAP (EC 3.4.11.1) is responsible for

breaking down the N-terminus of proteins and polypeptides into leucine and other amino acids. Urea is extensively used in agriculture as a low-cost nitrogen fertilizer but is also produced as a degradation product of nucleic acids. Urease (EC 3.5.1.5) plays a vital role in catalyzing the hydrolysis of urea into ammonia and carbon dioxide (Tabatabai and Bremner, 1972). In the literature, these enzymes are often used as indicators of microbial N demand, and as a result, they are all called N-acquisition or N-acquiring enzymes (Kandeler et al., 2011).

Potential soil enzyme activity is normally measured in the laboratory without substrate limitations, as opposed to *in situ* activity (Wallenstein and Weintraub, 2008). Potential soil enzyme activity reflects the overall enzyme quantities and it is often expressed in two ways: 1) the absolute activity of enzyme as a function of dry soil mass, and 2) the specific activity of enzyme as a function of SOM, SOC, or MBC. In the latter expression, normalization is used to help eliminate the variability in the soil properties under different soil management practices, allowing a reliable comparison of soil microbial functions (German et al., 2011). When similar changes occur in the absolute and specific soil enzyme activities, it means treatments can affect soil enzyme activities without altering a certain soil property (Stark et al., 2014). Ghosh et al. (2020) reported that soils receiving manure had lower specific enzyme activities (per unit of soil SOC) than soils receiving mineral fertilizer. This result signified that there was still a bigger portion of recalcitrant OC undecomposed remaining in the organically managed soils than conventionally managed soils, implying the application of manure can facilitate SOC sequestration. Liu et al. (2017) also found that soils treated with composted biosolids had lower specific enzyme activities (per unit of soil MBC) than untreated controls, indicating that the indigenous microorganisms in the soil might not have adjusted well to the new environment and they were metabolically less active. To our knowledge, there is only one study that has normalized N-acquiring enzyme activities to soil N pools. This study demonstrated that increased NAG and LAP per unit of soil TN in afforested soils

reflected a high percentage of N was cycled (enhanced mineralization) and little N was accumulated (Feng et al., 2018). Much more investigation is needed to link N-acquiring enzyme activities to soil N contents. Additionally, whether specific activity can reveal more clearly soil responses to biosolids application than the absolute enzyme activities still remains unclear.

1.2.3.2 The Potential Activity of N-acquiring Enzymes in Soils Receiving Organic N Inputs

With the application of organic N inputs, responses of different soil N-acquiring enzyme activities differed in both direction and magnitude across a number of studies (Khorsandi and Nourbakhsh, 2007; Saha et al., 2008; Bastida et al., 2009). There are two important meta-analysis studies that investigated the responses of soil N-acquiring enzyme activities to N fertilization, but some limitations existed in the context of N fertilization. For instance, Jian et al. (2016) mainly focused on C-acquiring enzymes and did not specify what kind of organic N inputs they studied, and Chen et al. (2018) were only concerned with inorganic fertilizer and excluded organic amendments as N inputs in the data extraction process. Besides, responses of soil N-acquiring enzymes have been less studied in acidic soils compared to neutral and alkaline soils (Table 1.7-1.9). Thereby, more research on the effect of organic N inputs, such as biosolids, on the N-acquiring enzyme activities in the acidic soil ecosystems is needed to support for these kinds of meta-analysis studies.

1.2.3.2.1 The Potential Activity of NAG

The fluorescence method has received increasing interest over the past decade to determine the potential activity of NAG using a fluorometer (Marx et al., 2001; Saiya-Cork et al., 2002). Methylumbelliferone (MUB) is the most commonly used fluorogenic model substrate (Tabatabai and Dick, 2002), and thus the methylumbelliferone (MUB)-linked substrate (4-MUB-N-acetyl-β-D-glucosaminide) is applied for the NAG assay. Alternatively, the potential activity of NAG can also be measured colorimetrically with a spectrophotometer using *p*-nitrophenyl (*p*NP)-linked

substrate (*p*NP-N-acetyl-β-D-glucosaminide) (Parham and Deng, 2000). The fluorescence method has advantages over spectrophotometric method in terms of sensitivity and the time required for completing an assay, while the downsides of the former method are its high cost and the limited availability of substrates (Marx et al., 2001; Drouillon and Merckx, 2005). As shown in Table 1.7, the potential activity of NAG in control soils ranged from 2.5-220 nmol MUB g^{-1} dry soil h^{-1}, while soils amended with organic amendments ranged from 4.52-260 nmol MUB g^{-1} dry soil h^{-1}; except the study by Bowles et al. (2014), where NAG was not determined by the fluorescence method.

Liu et al. (2017) reported a significant increase of NAG activities in alkaline soils applied with a combination of composted biosolids and chemical fertilizer, in comparison to soils applied with chemical fertilizer alone. The enhanced NAG activity might be associated with the high chitin content derived from the dead fungi during the composting process, since fungi are more likely to break down the complex polymers (hemicellulose, cellulose, and lignin) in the composted materials (Sciubba et al., 2014).

Table 1. 7 Potential activity of NAG reported in the literature for soils treated with organic amendments.

Description	Method	Unit	Activity	Reference
Field experiment Loam soil pH: 6.3-7.2 Treatments: poultry manure, and composted green waste Bulk soil	Parham and Deng (2000) Sodium acetate buffer (pH: 5.5) Substrate: *p*-nitrophenyl-N-acetyl-β-D-glucosaminide Incubation at 37 °C for 1 h Spectrophotometric method: 405nm	mg ρ-nitrophenol kg^{-1} dry soil h^{-1}	Poultry manure: 22-41 Composted green waste: 10-36	Bowles et al. (2014)
Field experiment Sandy soil pH: 8.42 Treatment: composted biosolids with chemical fertilizer Application rates: 30 and 45 t ha^{-1} Bulk soil	DeForest (2009) Buffer: deionized water Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	nmol MUB g^{-1} dry soil h^{-1}	Composted biosolids with chemical fertilizer: 6.5 (30 t ha^{-1}) 7.5 (45 t ha^{-1}) Control with chemical fertilizer alone: 4.3	Liu et al. (2017)
Incubation experiment Forest soil: silty loam, pH: 5.7 Grassland soil: clay loam, pH: 6.3 Treatment: biochar Bulk soil	German et al. (2011) Sodium acetate buffer (pH: 5) Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 20 °C for 3 h Fluorescence method: 365 nm excitation and 450 nm emission	μmol MUB g^{-1} dry soil h^{-1}	Forest soil: Biochar: 0.09-0.15 Control: 0.11-0.16 Grassland soil: Biochar: 0.19-0.26 Control: 0.22-0.27	Pokharel et al. (2018)

MUB: methylumbelliferone

Description	Method	Unit	Activity	Reference
Field experiment 26 years Light loam soil pH: 8.56 Treatment: cattle manure Application rates: 370-450 kg N ha^{-1} Bulk soil	DeForest (2009) Buffer: deionized water Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	nmol MUB g^{-1} dry soil h^{-1}	Cattle manure: 7.36 Mineral fertilizer: 4.96 Half cattle manure + half mineral fertilizer: 4.52 Control: 4.13	Qi et al. (2016)
Incubation experiment 21 days Light loam soil pH: 8.56 Treatment: cattle manure Application rates: 370-450 kg N ha^{-1} Bulk soil	DeForest (2009) Buffer: deionized water Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	nmol MUB g^{-1} dry soil h^{-1}	Cattle manure: 5.3 Mineral fertilizer: 3 Half cattle manure + half mineral fertilizer: 6 Control: 2.5	Qi et al. (2016)
Field experiment Silt loam paddy soil pH: 6.0 Treatment: swine manure Application rate: 41 t ha^{-1} Bulk soil	DeForest (2009) Acetate buffer (pH: 8.5) Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 20 °C for 4 h Fluorescencemethod: 365 nm excitation and 450 nm emission	nmol MUB g^{-1} dry soil h^{-1}	Swine manure: 37 Control: 6	Zhang et al. (2015)

Description	Method	Unit	Activity	Reference
Field experiment 30 years Sandy loam soil pH: 8.7 Treatment: straw and mineral fertilizer Application rates: 0, 2.25, 4.5, and 9 t ha^{-1} Bulk soil	DeForest (2009) Acetate buffer (pH: 8.5) Substrate: 4-MUB-N-acetyl-β-D-glucosaminide Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	nmol MUB g^{-1} dry soil h^{-1}	Straw + mineral fertilizer:10 (0 t ha^{-1}), 10 (2.25 t ha^{-1}), 12 (4.5 t ha^{-1}), 13 (9 t ha^{-1}) Control: 7 (The two highest rates significant increased NAG activity)	Zhao et al. (2016)

1.2.3.2.2 The Potential Activity of LAP

The potential activity of LAP can also be assessed either by a fluorescence method or by a spectrophotometric method. The substrates used in these two types of assays are either linked to a fluorescent artificial compound (fluorophore) or to a colored compound (chromophore). They are 7-amino-4-methylcoumarin (AMC)-linked substrate (L-Leucine-AMC) and *p*-nitroanilide (*p*NA)-linked substrate (leucine *p*-nitroanilide), respectively (Sinsabaugh et al., 1999). The spectrophotometric method has been adopted in analyzing contaminated and reclaimed land soils (Narendrula-Kotha and Nkongolo, 2017), riparian soils (Geng et al., 2017), and forest soils (Zheng et al., 2020), but we found out that using spectrophotometric methods on the organic amendments-treated soils are not available or rarely investigated (Table 1.8). Bailey et al. (2011) compared two kinds of NAG assays and suggested that the fluorescence method would be more accurate and robust to study soils with biochar amendment, which might have encouraged other researchers to perform LAP assay by the fluorescence method. The potential activity of NAG in control soils ranged from 3-5600 nmol AMC g-1 dry soil h^{-1}, while soils treated with organic amendments ranged from 11-14050 nmol AMC g-1 dry soil h^{-1} (Table 1.8).

Gebhardt et al. (2017) found significant increases in LAP activity, by 71 %, in woodchip amended soils, when compared to unamended soils. In contrast, two meta-analysis studies have shown that the change in LAP activity was negligible under the application of either chemical fertilizer or organic amendments (Jian et al., 2016; Chen et al., 2018).

Table 1. 8 Potential activity of LAP reported in the literature for soils treated with organic amendments (unit: nmol AMC g^{-1} dry soil h^{-1}).

Description	Method	Activity	Reference
Greenhouse pots experiment 22 months Sandy loam soil pH: 6.2 Treatment: compost + biochar Bulk soil	Keiblinger et al. (2012) Acetate buffer Substrate: L-Leucine-AMC Incubation at 20 °C for 2.3 h Fluorescence method: 365 nm excitation and 450 nm emission	Compost + biochar: 700 (1 month) 220 (7 months) 1400 (22 months) Control: 625 (1 month) 220 (7 months) 875 (22 months)	Ameur et al. (2018)
Incubation experiment 30 days Silt loam soil pH: 6.9 Treatments: Biochar and oyster shell Application rate: 5 t ha^{-1} Bulk soil	Pritsch et al. (2004) Buffer: distilled water Substrate: L-Leucine-AMC Incubation at 20 °C for 1 h Fluorescence method: 355 nm excitation and 460 nm emission	Biochar: 8500 Control: 5600 Biochar + oyster shell: 14050 Control + oyster shell: 14000	Awad et al. (2018)
Incubation experiment 60 days Clay soil pH: 5.1 Treatment: above-ground residues (leaves, twigs) Bulk soil	Agumas et al. (2021) Buffer: deionized water L-Leucine-AMC hydrochloride Incubation at 30 °C for 3 h Fluorescence method: 360 nm excitation and 460 nm emission	(Enzyme activity increased rapidly first and then reached a maximum value at day 30, then significantly decreased until the end of the incubation period)	Balume et al. (2022)

AMC: 7-amino-4-methylcoumarin

Description	Method	Activity	Reference
Field experiment 26 years Light loam soil pH: 8.56 Treatment: cattle manure Application rates: 370-450 kg N ha^{-1} Bulk soil	DeForest (2009) Buffer: deionized water Substrate: L-Leucine-AMC Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	Cattle manure: 383.42 Mineral fertilizer: 286.11 Half cattle manure + half mineral fertilizer: 332.56 Control: 321	Qi et al. (2016)
Incubation experiment 21 days Light loam soil pH: 8.56 Treatment: cattle manure Application rates: 370-450 kg N ha^{-1} Bulk soil	DeForest (2009) Buffer: deionized water Substrate: L-Leucine-AMC Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	Cattle manure: 350 Mineral fertilizer: 200 Half cattle manure + half mineral fertilizer: 500 Control: 300	Qi et al. (2016)
Field experiment Silt loam paddy soil pH: 6.0 Treatment: swine manure Application rate: 41 t ha^{-1} Bulk soil	DeForest (2009) Acetate buffer (pH close to soil pH) Substrate: L-Leucine-AMC Incubation at 20 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	Swine manure: 11 Control: 3	Zhang et al. (2015)

Description	Method	Activity	Reference
Field experiment 30 years Sandy loam soil pH: 8.7 Treatment: straw Application rates: 0, 2.25, 4.5, and 9 t ha^{-1} Bulk soil	DeForest (2009) Acetate buffer (pH 8.5) Substrate: L-Leucine-AMC Incubation at 25 °C for 4 h Fluorescence method: 365 nm excitation and 450 nm emission	Straw (0 t ha^{-1}) + mineral fertilizer:19.1 Straw (2.25 t ha^{-1}) + mineral fertilizer:19.5 Straw (4.5 t ha^{-1}) + mineral fertilizer: 19.5 Straw (9 t kg ha^{-1}) + mineral fertilizer: 19.5 Control:19.2 (Different application rates did not affect the LAP, and there was no significant difference between control and treatments)	Zhao et al. (2016)

1.2.3.2.3 The Potential Activity of Urease

The potential activity of urease can be measured in many ways using urea as substrate, including an ion electrode method (APHA, 1995), which is based on the concentrations of NH_4^+ ions released into the soil solutions, a spectrophotometric method, as well as a colorimetric method (Table 1.9). There are wide variations in the unit of enzyme activity across studies but they are all convertible. The potential activity of urease in control (unamended) soils ranged from 0-417 mg NH_4^+-N kg^{-1} dry soil h^{-1}, while soils treated with organic amendments ranged from 0-2125 mg NH_4^+-N kg^{-1} dry soil h^{-1} (Table 1.9).

Urease activity was significantly improved, by 15 %, in soils with municipal solid waste compost (Crecchio et al., 2004). A 58-132 % increase in urease activity was also reported by Liu et al. (2017) in soils applied with a combination of composted biosolids (30 and 45 t ha^{-1}) and chemical fertilizer, in comparison to soils applied with chemical fertilizer alone. Bastida et al. (2009) demonstrated a 1.5-fold increase in urease activity from the soils amended with anaerobically digested biosolids (1.28 μmol NH_4^+-N g^{-1} dry soil h^{-1}) and a 2-fold increase from soils amended with composted biosolids (1.80 μmol NH_4^+-N g^{-1} dry soil h^{-1}), relative to unamended soil (0.9 μmol NH_4^+-N g^{-1} dry soil h^{-1}). Enhanced urease activities might be associated with the low NH_4^+ content in the composted biosolid amended soils. However, these observations were in contrast to the findings obtained by Roig et al. (2012) and H. Liu et al. (2020), who, respectively, found the application of anaerobically digested biosolids and composted biosolids did not significantly affect the potential activity of urease. Moreover, Saha et al. (2008) reported that urease activity was greatly inhibited by manure treatment and the highest activity was found in control soils. The likely cause in these studies was the presence of heavy metals in the organic wastes (Tejada et al., 2011).

Table 1. 9 Potential activity of Urease reported in the literature for soils treated with organic amendments.

Description	Method	Unit	Activity	Reference
Field experiment Silt loam soil pH: 6.2 Treatments: vermicompost, chicken manure, horse manure, and sewage sludge Rhizosphere soil	Tabatabai and Bremner (1972) Phosphate buffer (pH: 6.7) Incubation at 37 °C for 24 h Selective electrode method	μg NH_4^+-N g^{-1} dry soil 24 h^{-1}	Vermicompost: 51000 Chicken manure: 40000 Horse manure: 42000 Sewage sludge: 28000 Control: 10000 (4 months after the addition of amendments)	Antonious et al. (2020)
Field experiment Sandy clay loam degraded semiarid soil pH: 7.55 Treatments: anaerobically digested biosolids and composted anaerobically digested biosolid Bulk soil	Kandeler and Gerber (1988) Borate buffer (pH: 10.0) Incubation at 37 °C for 2 h Colorimetric method: 690 nm	μg NH_4^+-N g^{-1} dry soil h^{-1}	Anaerobically digested biosolids: 1.2-2.5 (the highest activity was at day 0) Composted anaerobically digested biosolids: 1.2-1.6 (no significant change during the entire period) Control:0.6-1.5 (the highest activity was at day 0)	Bastida et al. (2008)
Field experiment Clay soil (cultivated vs. uncultivated) pH: 8.30 Treatment: municipal solid waste compost Application rates: 12 t ha^{-1}: 120 kg N ha^{-1} 24 t ha^{-1}: 240 kg N ha^{-1} Bulk soil	Hofmann (1963)	mg NH_4^+-N 100 g^{-1} dry soil 3 h^{-1}	Municipal solid waste compost: 12 t ha^{-1}: (cultivated): 69 24 t ha^{-1}: 65 (cultivated), 60 (uncultivated) Mineral fertilizer (120 kg N ha^{-1}): 61(cultivated); 61 (uncultivated) Control: 59 (cultivated), 58 (uncultivated)	Crecchio et al. (2004)

Rhizosphere soil: soil samples are collected from the area around a plant root; Bulk soil: soil samples are collected outside the rhizosphere.

Description	Method	Unit	Activity	Reference
Field experiment Sandy soil pH: 6.4 Treatments: municipal solid waste compost and cow manure Bulk soil	Nannipieri et al. (1980) Phosphate buffer (pH: 7) Incubation at 30 °C for 1.5 h	μmol NH_4^+-N g^{-1} dry soil h^{-1}	Municipal solid waste compost: 0.31 (inhibition effect) Cow manure: 0.3 (20 t ha^{-1}), 0.25 (80 t ha^{-1}) Mineral fertilizer: 0.32 Control: 0.31	García-Gil et al. (2000)
Incubation experiment 20 weeks Silty clay loam soil pH: 8.3 Treatments: cow manure and corn residues (corn shoot vs corn root) Application rates: 0, 50, and 100 t ha^{-1} Bulk soil	Tabatabai (1994)	mg NH_4^+-N kg^{-1} dry soil h^{-1}	Cow manure significantly increased the activity of urease at both week 0 and 20: 50 t ha^{-1} (week 0): 129.7 50 t ha^{-1} (week 20): 151.3 100 t ha^{-1} (week 0): 172.0 100 t ha^{-1} (week 20): 225.4 Control (week 0): 91.5 Control (week 20): 276.9 Corn residue significantly increased the activity of urease at week 20: Corn shoot: 230 Corn root: 214.3 Control: 209.3	Khorsandi and Nourbakhsh (2007)

Description	Method	Unit	Activity	Reference
Incubation experiment 90 days Clay loam soil pH: 7.1 Treatment: anaerobically digested sewage sludge Application rates: 100, 200, and 300 t ha^{-1} Nitrogen was added in the form of $(NH_4)_2 SO_4$ solution to adjust sewage sludge C/N ratio: 9:1 to 6:1 and 3:1 Bulk soil	Hoffmann and Teicher (1961) Citrate buffer (pH: 6.7) Incubation at 37 °C for 3 h Spectrophotometric method: 578 nm	μg NH_4^+-N g^{-1} dry soil 3 h^{-1}	C/N:3 100 t ha^{-1} (10-35) 200 t ha^{-1} (5-42) 300 t ha^{-1} (2-72) Contol:10 C/N:6 100 t ha^{-1} (9-25) 200 t ha^{-1} (8-35) 300 t ha^{-1} (5-55) Contol:10 C/N:9 100 t ha^{-1} (10-20) 200 t ha^{-1} (8-32) 300 t ha^{-1} (5-50) Contol:10 (Enzyme activity increased rapidly first and then reached a maximum value at day 15, then significantly decreased until the end of the incubation period)	Kızılkaya and Bayraklı (2005)

Description	Method	Unit	Activity	Reference
Field experiment 15 years pH: 7.69 Treatment: farmyard manure Application rate: 15 t ha^{-1} Bulk soil	Tabatabai (1982) Tris hydroxymethyl aminomethane (THAM) buffer (pH: 9.0) Incubation at 37 °C for 2 h	µg NH_4^+-N g^{-1} dry soil 2 h^{-1}	Farmyard manure: 155 Mineral fertilizer: 105 Control: 68	Liang et al. (2014)
Field experiment 2 years Saline soil pH: 8.2 Treatment: biochar poultry manure compost + pyroligneous solution Bulk and rhizosphere soils	Tabatabai (1994) Tris hydroxymethyl aminomethane (THAM) buffer (pH: 9.0) Incubation at 37 °C for 24 h Spectrophotometric method: 578 nm	mg NH_4^+-N g^{-1} dry soil 24 h^{-1}	Biochar poultry manure compost + pyroligneous solution: 2.35 (bulk soil), 2.32 (rhizosphere soil) Control: 1.81(bulk soil), 2.08 (rhizosphere soil)	Lu et al. (2015)

Description	Method	Unit	Activity	Reference
Incubation experiment 28 days Agricultural soil: loam soil, pH: 8.20 Grassland soil: loamy sand soil, pH: 6.5 Treatments: fresh sewage sludge, composted, and heat-dried biosolids Bulk soil	Tabatabai (1994) Incubation at 37 °C for 2 h Colorimetric method	mg NH_4^+-N g^{-1} dry soil h^{-1}	Agricultural soil: Fresh sewage sludge: 39-55 Composted biosolids: 30-50 Heat-dried biosolids: 10-81 Control: 0-35 (The activity of urease was highest at day 4 but then decreased to initial values after 28 days except heat-dried biosolids. Heat-dried biosolids treatment had the highest activity at day 28) Grassland soil: Fresh sewage sludge: 0-50 Composted biosolids: 0-30 Heat-dried biosolids: 6-34 Control: 0-5 (The activity of urease peaked at day 7, and decreased to 0 at Day 28)	Mattana et al. (2014)

Description	Method	Unit	Activity	Reference
Field experiment Sandy soil pH: 5.3 Treatment: cattle slurry Application rate: 150 kg N ha^{-1} Bulk soil	Nannipieri et al. (1980) Phosphate buffer (pH: 8.0) Incubation at 37 °C for 2 h Colorimetric method	μmol NH_4^+-N g^{-1} dry soil h^{-1}	Cattle slurry: 19 Control: 35	Paz-Ferreiro et al. (2009)
Incubation experiment 37 weeks Clay soil pH: 7.76 Treatment: biochar Bulk soil	Tabatabai and Bremner (1972) Incubation at 37 °C for 1 h Spectrophotometric method	μg NH_4^+-N g^{-1} dry soil h^{-1}	Biochar:12 Control: 8	Sakin et al. (2021)
Incubation experiment 50 days Sandy loam soil pH: 7 Treatments: composted biosolids, poultry manure compost, and vermicompost Bulk soil	Spectrophotometric method	mmol NH_4^+-N kg^{-1} dry soil h^{-1}	Composted biosolids: 2.765 (at day 25) 2.857 (at day 50) Poultry manure compost: 6.127 (at day 25) 5.533(at day 50) Vermicompost: 3.564 (at day 25) 3.759 (at day 50)	Zaborowska et al. (2018)

1.2.3.3 Factors Affecting the Activity of N-acquiring Enzymes

1.2.3.3.1 Soil Properties

Soil physico-chemical properties may differ between soil types, and changes in soil properties can affect enzyme activities. Soil particles are one of the important factors as they provide living habitats for microorganisms. It has been reported that urease, invertase, and alkaline phosphatase showed the highest activity in the clay fraction followed by the sand fraction (Stemmer et al., 1998; Kandeler et al., 1999; Marx et al., 2005), whereas the activities of β-glucosidase, α-glucosidase, cellobiohydrolase and β-xylosidase, xylanase were reported to be highest in the sand fraction (Lagomarsino et al., 2009). Many studies found that SOC and TN contents were significantly and positively related to urease activity (Pan et al., 2013; Liang et al., 2014; Guangming et al., 2017; Farooq et al., 2021; Zhu et al., 2021). However, it was not in line with the results from Avellaneda-Torres et al. (2013) and Wu et al. (2020), who reported negative relationships between urease activity and SOC as well as TN. Some studies have demonstrated that significant positive linear relationships exist between the soil NAG and LAP activities and the SOM, SOC, SON, and TN contents (German et al., 2011; Raiesi and Beheshti, 2014; Sinsabaugh et al., 2014; Zhang et al., 2015), but these results were not in agreement with Bowles et al. (2014), who showed soils with lower available MN and TN contents and higher available C tended to result in higher potential NAG and LAP activities. This might be attributed to the shift in microbial demand for substrates. According to the resource allocation theory, enzymes are regulated by microbes to acquire limiting nutrients (Sinsabaugh et al., 2002; Zhang et al., 2016). When soils are under low N availability conditions, microbes will secrete more N-acquiring enzymes to meet microbial demand for N sources. Other studies have also reported that there was no relationship between the NAG and LAP activity and the soil N contents (Cenini et al., 2016; Akinyemi et al., 2020). That could be influenced by C-acquiring enzymes, which allows microbes

to still obtain N by the decomposition of recalcitrant organic compounds using labile C (Craine et al., 2007).

Soil pH can also play a powerful role in enzyme activity. S. Liu et al. (2020) found initial soil pH ranging from 6 to 8 had the strongest relationship with most enzyme activities, which can be explained by the fact that soil pH closer to neutral is generally the best condition for microbial and plant growth. A wide range of studies have reported that elevated soil pH after liming led to increased activity of NAG (Ekenler and Tabatabai, 2003), LAP (DeForest and Moorhead, 2020), and urease (Siddaramappa et al., 1994; Błońska et al., 2016). There are conflicting conclusions in the literature about the optimum soil pH for urease activity. Pan et al. (2013) stated that there was no significant relationship between soil pH and urease activity, but generally the optimum soil pH for urease was near-neutral (Singh and Nye, 1984; Zhang et al., 2014; Fisher et al., 2016).

The presence of plant species and local climate conditions can also impact soil enzyme activities through effects on soil properties (Ushio et al., 2010; Steinweg et al., 2012; Hu et al., 2013). Rhizosphere soil samples have been shown to have higher enzyme activities than bulk soil samples because of the root exudations or enzymes released by the plant roots (Crecchio et al., 2004; Ai et al., 2012; Gianfreda, 2015). Ren et al. (2017) revealed that soil extracellular enzymes are more sensitive to temperature and precipitation changes than substrate availability.

1.2.3.3.2 Substrate Characteristics

Substrate availability is another key factor influencing enzyme activity, and that could be related to the stability of the organic matter, the application rate of the amendment, substrate

forms, and C/N ratio of the amendment. Higher activities of enzymes can often reflect higher substrate availability (Bastida et al., 2008). Amendments with high OM but low degree of stability tended to have greater stimulatory effects for enzyme activities (Mattana et al., 2014). With more abundant simple organic N-containing compounds, the highest application rate of dehydrated poultry manure provided more consistent effects on enhancing enzyme activities (Ninh et al., 2015). Xue and Huang (2013) found that enzyme activities in tree peony garden soils first increased significantly and then decreased with the increasing application rate of composted biosolids. Strong positive relationships between soil labile ON and N-acquiring enzyme activities were observed in afforested soils, as labile organic matter was preferentially used by microorganisms (Zhang et al., 2021). In contrast, the mineral or inorganic forms of substrates can have different influences on soil enzyme activity. Bandick and Dick (1999) and Biswas et al. (2019) mentioned that amendments with more MN reduced urease activity as higher concentrations of end product of the enzymatic reaction can render enzyme synthesis unnecessary.

Grosso et al. (2016) suggested that C/N ratio was closely correlated to microbial C/N ratio. The narrow C/N ratio can result in a decreased soil microbial C/N ratio, which accelerates N mineralization rates, improves enzyme functioning, and benefits bacterial growth (Zornoza et al., 2016). According to a recent global-scale study on the impact of manure on soil biochemical properties, manure application increased urease activity by 104 % (S. Liu et al., 2020). Among all investigated manure types (green manure, farmyard manure, cattle manure, swine manure and poultry manure), swine manure caused the greatest increase of urease (258 %) and NAG (138 %). This may be because swine manure had relatively lower C/N ratio (≈12:1), which highlights the importance of manure characteristics on soil enzyme activity. Similarly, Kızılkaya and Bayraklı (2005) observed the highest values of urease in anaerobically digested biosolids amended soils with lowest C/N ratio.

1.2.2.3.3 Time

Time is also crucial in the process of substrate degradation. A number of studies have demonstrated that biosolid application leads to an initial increase of microbial populations but shortly after application, microbial populations in biosolid amended soils start to decline and eventually can reach a level similar to unamended controls (Lang and Smith, 2007; Lang et al., 2007; Zerzghi et al., 2010). Geisseler et al., (2010) concluded that enzyme activities in amended soils tended to gradually decrease with incubation time as the supply of labile ON was exhausted, and/or the enzyme-clay or enzyme-humus complexes was formed. Kızılkaya and Bayraklı (2005) noticed a rapid increase in urease activity soon after applying anaerobically digested sewage sludge but a progressive decrease after 30 days. A similar tendency was indicated by Mattana et al. (2014), who observed that most enzyme activities peaked after 4 or 7 days and then returned to initial values after 28 days regardless of the biosolids type.

Further research should be done to help figure out the potential reasons underlying the inconsistent responses from a variety of studies, which may improve our understanding of the behaviour of N-acquiring enzymes in the various ecosystems.

1.2.3.4 Soil Quality Index Based on Enzyme Activities

Soil enzymes are involved in nutrient cycling and organic matter turnover, hence they reflect the metabolic requirements of the microbial community (Caldwell, 2005). The activity of soil enzymes has been considered as an early and sensitive diagnostic indicator to assess the effects of various farming practices on soil quality, as they have more rapid response to

environmental changes compared with soil physical or chemical properties. Instead of using single enzyme activity, researchers have recommended the use of numerical indexes to integrate information and to better interpret enzyme activities with lower variability (Mijangos et al., 2010; K. Liu et al., 2020; Netherway et al., 2020). Two commonly used indexes are geometric mean enzyme activity (GMEA) (Table 1.10 equation (6)) and soil quality index (SQI) (Table 1.10 equation (8)).

Table 1. 10 A summary of soil enzymatic indexes.

Equation	Description	Reference
$\mathrm{GMEA} = \sqrt[n]{X_1 * X_2 * X_n}$ (Equation(6))	X represents the activity of each enzyme; n represents the total number of enzymes.	Hinojosa et al. (2004)
$\frac{(\mathrm{GMEA}_{\text{treated soil}} - \mathrm{GMEA}_{\text{untreated soil}})}{(\mathrm{GMEA}_{\text{untreated soil}})} \times 100$ (Equation(7))	The relative increase of GMEA	García-Ruiz et al. (2008)
$\mathrm{SQI} = 10^{\log m + \frac{\sum_{i=1}^{n}(log\, n_i - log\, m)}{n}}$ (Equation(8))	m is the reference (mean value of enzyme activity in the untreated control soil, set to 100 %); n are the measured values for enzyme activity in the treated soils as percentages of the reference. $\bar{n}$ are the mean activities of soil enzymes.	Bloem et al. (2005); Mijangos et al. (2010)
$\mathrm{T-SQI} = 10^{\log m + \frac{\sum_{i=1}^{n}(log\, n_i - log\, m) - \sum_{i=1}^{n}\lvert log\, n_i - log\, \bar{n}\rvert}{n}}$ (Equation(9))		

High GMEA values mean a high microbial functional diversity of soil. In addition, the relative increase of GMEA (%) (Table 1.10 equation (7)) was also used to quantitively measure the improvement of the soil quality (García-Ruiz et al., 2008). Paz-Ferreiro et al. (2012) reported

that soils under the application of high doses of air-dried sewage solids had lower values of GMEA than the control soil. On the contrary, high doses of sewage solids-derived biochar resulted in higher values of GMEA. These findings suggested that enzyme activities were depressed due to the toxic effect of heavy metals in the untreated sewage solids (Gascó et al., 2011), but the conversion of sewage solids into biochar via pyrolysis (at 600 °C, 2h) can create positive impacts on soil quality by making heavy metals less soluble in soils and improving soil physico-chemical properties (OM and available water content).

To overcome the limitations of the traditional SQI, the treated-soil quality index (T-SQI) (Table 1.10 equation (9)) was proposed based on the traditional SQI. T-SQI can show both the magnitude and direction (enhancement or inhibition) of changes caused by an environmental stressor (e.g., organic amendments, contaminants, application of pesticides) on soil enzyme activities compared with those from a reference soil (Sanchez-Hernandez et al., 2018). Urra et al., (2019) used several microbial parameters (e.g., enzyme activities, potentially mineralizable nitrogen, and soil respiration) to calculate SQI, and they concluded that the application of thermally dried and anaerobically digested biosolids resulted in significant higher values of SQI than unamended control soil, thus improving soil quality. T-SQI was more suitable to assess soil quality in studies where the soil has been intentionally treated to increase its biological activity (e.g., to overcome a limiting factor such as soil acidity and nutrient deficiency) (Mijangos et al., 2010). Greater T-SQI was observed in soils with manure application than conventional chemical fertilization, and there was a tight and positive correlation between T-SQI and GMEA (Ghosh et al., 2020).

Currently, no attention has been paid to the application of these indexes in soils treated with other types of biosolids, such as alkaline-treated biosolids and composted biosolids. Future farm management decisions can be supported by these indicators if we are able to verify the

suitability of using these enzymatic indexes under different soil conditions (realistic amendment scenarios).

1.3 Research Objectives and Hypotheses

1.3.1 Knowledge Gaps

Most research in the literature has focused on comparisons of biosolids sourced from different wastewater treatment facilities. However, the heterogeneity of these materials may result in difficulties in modeling of nitrogen mineralization and consequently estimating the N availability from the biosolids to plants. Previous studies in biosolid amended soils have focused on general ecotoxicological parameters with little emphasis on selected N-acquiring enzyme responses. Biosolids, as an organic N input, have not been fully explored in the studies on the effects of N fertilization on N-acquiring enzyme activities. Furthermore, the response of N-acquiring enzymes has been mostly studied for alkaline soils and research with acidic soils has been lacking.

The overall objective of this research is to gain more insights into mechanisms behind the response of N mineralization in biosolids amended soils. The specific objectives include:

1. To investigate how three different biosolids treatment processes (alkaline treatment (N-Viro® Process and CaO addition), composting, and heat drying) affect biosolids characteristics in terms of N forms and contents.
2. To evaluate the effects of different biosolids on N mineralization in an acidic soil.
3. To explore changes in N-acquiring enzyme activities in response to different biosolids.

This study hypothesized that 1) TN contents would be significantly reduced due to biosolids treatments, and ON would represent the highest percentage of total nitrogen in all types of biosolids, 2) alkaline-treated biosolids would have the highest N mineralized, followed by heat-dried and composted biosolids, 3) the application of different biosolids would stimulate microbial

activity, and the selected enzymes activities will increase first and then decrease over time.

1.3.2 Thesis Organization

Chapter 1 is a literature review, presenting all the topics relevant to our research, including the definition of biosolids and soil N mineralization, and the three key enzymes involved in soil N mineralization. Chapter 2 presents how four types of biosolids (alkaline-treated (ATB: CaO-treated and N-Viro), composted (CB), and heat-dried (HDB)) were produced and discusses how distinct treatments modify nitrogen fractions in the sewage solids. Chapter 3 explores N availability of different biosolids in an acidic soil and the responses of soil N-acquiring enzyme activities to biosolids application. Two incubation studies were set up and run concurrently. One incubation study involved periodic leaching procedures and aimed to predict the potentially mineralizable N of biosolids, and the other one allowed destructive measurements for some important soil properties, such as pH, OM, MN, and as well as potential N-acquiring enzyme activities. Chapter 4 provides the overall conclusions of this study and gives future research recommendations.

Chapter 2: Effect of Physical, Chemical, and Biological Treatment Processes on Biosolid Nitrogen (N) Forms

2.1 Introduction

Biosolids are the solid product generated from treating sewage sludge collected in a wastewater treatment facility. They have been shown to provide economic benefits and recycle nutrients to soil, regardless of which kind of biosolids treatment processes are adopted (Chambers et al., 2003; Thangarajan et al., 2013; Archer et al., 2020). Manufacturing chemical fertilizers is an energy-intensive process, moreover, the price of chemical fertilizers has increased dramatically due to the supply chain disruptions during the Covid-19 pandemic and the war between Russia and Ukraine (Mew et al., 2018; Lal et al., 2020; Ben Hassen and El Bilali, 2022). Therefore, it is necessary to encourage farmers to reduce their reliance on artificial chemical fertilizers by implementing sustainable crop management practices, including land application of biosolids as a soil amendment. As a nutrient-rich organic material, biosolids is a good alternative to traditional chemical fertilizers, thereby the production of biosolids has been identified as a promising strategy to mitigate climate change and lower the expense of crop production.

The agronomic values of biosolids are often evaluated from the aspect of nitrogen (N) because N is frequently the most limiting nutrient for crop production (Muchovej and Rechcigl, 1998; Fageria and Baligar, 2005). The agronomic rates for biosolids are designed based on total N and assumed N mineralization rates over a growing season, although this may vary across Canada. If biosolids is applied at a rate that is above the recommended agronomic rate, there are potential environmental risks associated with N losses (US EPA, 1994a; CCME, 2012).

Biosolids treatment processes can make modifications to the characteristics of the raw sewage sludge and result in different types of biosolids. Their properties are also dependent on feedstock type or composition, geography and demography, the specific wastewater treatment

technology used at different facilities, handling practices, and storage conditions (Sharma et al., 2017). The biosolids treatment technologies are generally listed under three broad categories: chemical, biological, and physical. In our study, these three types of treatment processes specifically corresponded to alkaline treatment, composting, and heat drying.

The purpose of this study was to investigate how treatment processes affect biosolid N forms and other chemical properties.

2.2 Materials and Methods

2.2.1 Dewatered Sewage Sludge (RS)

Dewatered raw sewage sludge (RS) (Fig 2.1 (a)), originating from the Halifax Water's wastewater treatment facilities and sent to the Biosolids Processing Facility (BPF) at the Aerotech Business Park, Goffs, Nova Scotia, Canada, was obtained for use in this study. The partially dewatered sewage sludge from several wastewater treatment facilities in the Halifax Regional Municipality are trucked to the BPF on a regular basis. The BPF is operated and maintained by the Walker Environmental Group under a contract to generate an agricultural soil amendment, N-Viro® biosolids (Halifax Water, 2020). Bulk composite samples of the dewatered sewage sludge were collected from several loads using a plastic scoop and placed in polyethylene pails (20 L), and brought to the Innovative Waste Management Research Laboratory at Dalhousie University in February 2021. Samples were stored in a freezer prior to analysis to reduce on-going biological activity. In order to meet the quality standards required for land application and to compare effects of treatment process, the same batch of RS was processed by alkaline treatment, composting, and heat drying. Before making each biosolids, the sewage sludge was taken out of the freezer and left to thaw at room temperature, and then thoroughly homogenized by manual agitation in a container. Each batch of biosolids was frozen until ready for use in the soil incubation studies.

Figure 2. 1 Dewatered raw sewage sludge (a), N-Viro (b), CaO-treated (c), and composted (d), and heat-dried (e) biosolids.

2.2.2 Different Types of Biosolids

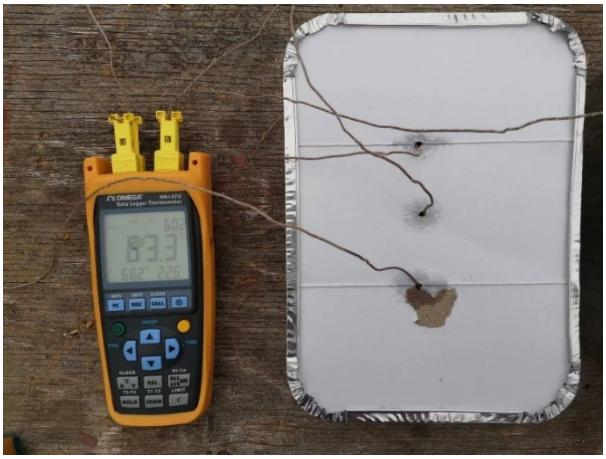
2.2.2.1 Alkaline-treated Biosolids (ATB)

Two types of biosolids were produced using different alkaline materials.

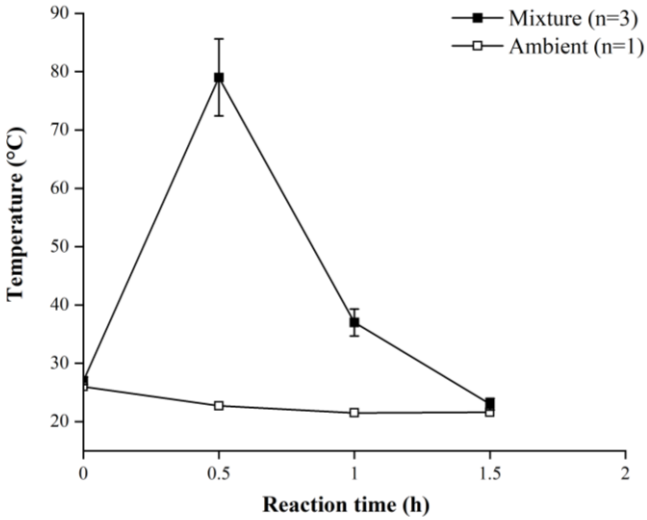
2.2.2.1.1 N-Viro Biosolids (N-Viro)

Advanced alkaline stabilized N-Viro biosolids were obtained directly from the BPF operated by the Walker Environmental Group, Goffs, Nova Scotia, Canada, using the N-Viro® Process (Fig. 2.1 (b)). In this patented process, alkaline admixture is typically blended with dewatered sewage sludge at a rate of 30-45 %, on a wet weight basis (ww) of sewage sludge (N-Viro Systems Canada Inc., 2007). The alkaline admixture consists of a wide range of industrial byproducts such as cement-kiln dust, lime-kiln dust, fly ash, and/or steel-making fines in conjunction with CaO. Following the mixing step, the material is discharged to a rotary drum dryer and dried until the desired moisture content (> 60-65 % solids) is achieved. Before being transferred to a storage area, the material is held in a curing area under a controlled temperature (52-62 ℃) for 12 hours and under an elevated pH level (pH > 12) for 72 hours (Logan and Harrison, 1995).

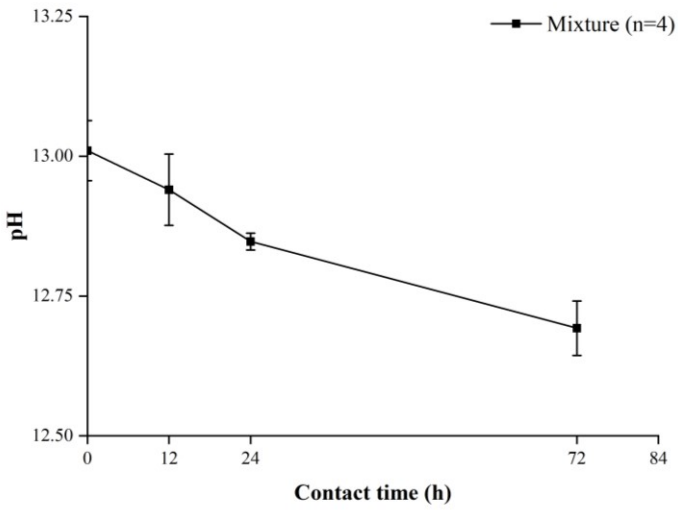
2.2.2.1.2 CaO-treated Biosolids (CaO-treated)



(a)



(b)



(c)

Figure 2. 2 Temperature monitoring by a digital thermometer (a) and changes in mixture and ambient temperatures during chemical reactions (b), and changes in mixture pH over time (hours) (c) (the values of mixture temperature (n=3) and pH (n=4) are means; error bars represent standard deviations of the means).

CaO-treated biosolids (Fig. 2.1 (c)) were made by mixing quicklime (CaO) (pH: 12.5) with dewatered sewage sludge. Reagent-grade CaO powder was purchased from Fisher Scientific. 102 g of CaO was quickly mixed with 238 g of RS at a rate of 30 % on a wet weight basis (1:1 dw) in four individual aluminum foil containers using a glass stir rod, and the containers were then covered with lids. A four-channel, portable data logger thermometer (HH147U, Omega Engineering Inc., CT, USA) with type T thermocouple probes was used for monitoring the

temperature. As shown in Fig. 2.2 (a), thermocouple probes were inserted into the lid and measured at multiple points in the mixture, as well as ambient temperature. The mixture temperature progressively increased and then gradually dropped afterward, and eventually returned to ambient temperature level (Fig. 2.2 (b)). After 1.5 hours of contact, the mixtures of RS and CaO in the four containers were combined together and put into a single container, and then placed into a laboratory oven for the purpose of keeping its temperature above 52 ℃ for 12 hours. Lastly, the mixture was oven dried (55 ℃, > 50 % solids) after being exposed to raised pH for three days. The mixture pH was checked during this period with four replicate samples. The pH decreased by 0.3 units after three days but it was within the preferred range (pH >12) (Fig. 2.2 (c)).

2.2.2.2 Composted Biosolids (CB)

(a) (b) (c)

Figure 2. 3 In-vessel composting system (a), compost pile at the beginning (day 0) (b) and end of the composting (day 35) (c)

An in-vessel composting system (Fig. 2.3 (a)) located at the Bio-Environmental Engineering Complex, Bible Hill, Nova Scotia, Canada, was utilized to generate composted biosolids (CB) (Fig. 2.1 (d)). The in-vessel composting system can hold a capacity of 365 L. It is equipped with four internal equidistantly placed mixing paddles, a vertical stainless steel rod with five temperature thermometers at various depths (7.5, 22.5, 37.5, 52.5, and 67.5 cm from the base), a removable cover, and a small electrically controlled exhaust fan. Sawdust was purchased from Nova Tree, Debert, Nova Scotia, Canada. In March 2021, 40 kg of sawdust was combined with

71 kg of RS at a wet weight ratio of 1:2 to obtain an initial C/N ratio of 35:1 (1:1 dw). The mixture was continuously turned at a speed of 2 rpm to homogenize the material, introduce air into the mixture, and promote decomposition. The moisture content of the compost was maintained at 50-60 % (ww) by adding water as required. The exhaust fan was used to draw air through the in-vessel system and remove odor from the composter through a port in the cover. Homogenized samples were collected on day 0 (Fig. 2.3 (b)), 3, 7, 14, 21, 28, 35 (Fig. 2.3 (c)), consisting of a mixture of nine subsamples taken from different locations within the compost mixture in the vessel. The wet bulk density (BD_W) was measured immediately after collection by the bucket drop method (TMECC, 2002), and then the dry bulk density (BD_d) was calculated based on the MC of the sample. Dry bulk density had an increasing trend throughout the composting period (Fig.2.4 (a)), indicating reductions in particle size and volume through a combination of constant mixing and microbial decomposition (Huerta-Pujol et al., 2010).

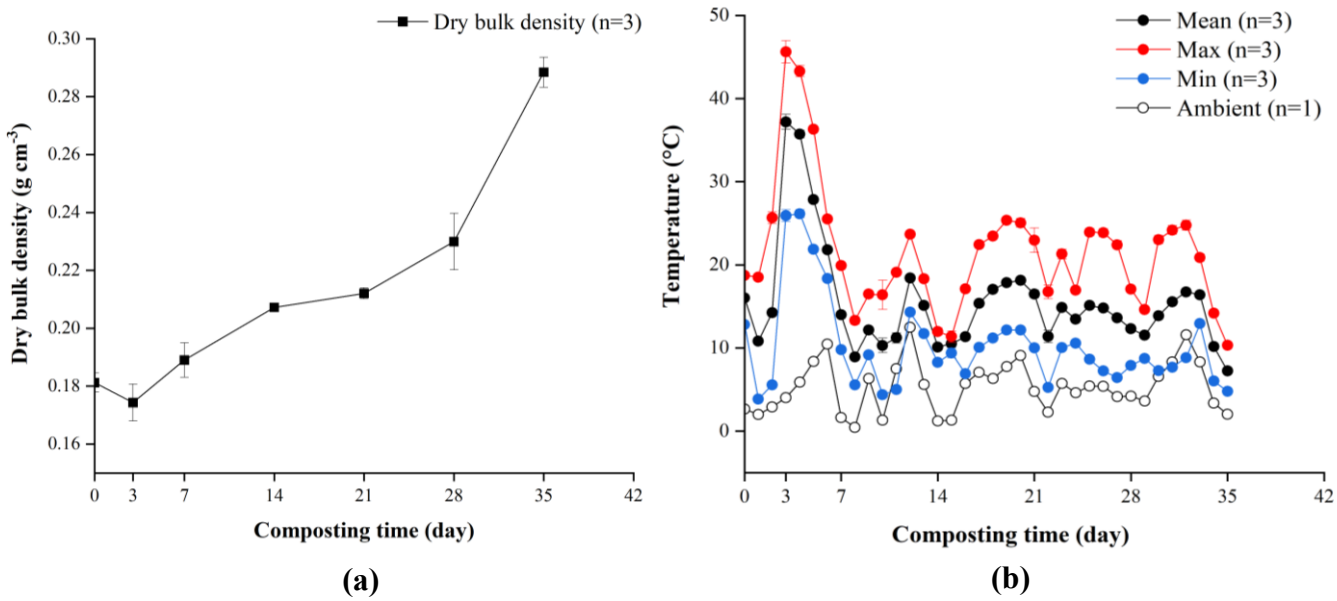


Figure 2. 4 Changes in dry bulk density (a) and temperature (b) during composting (the values are means (n=3) except ambient temperature; error bars represent standard deviation of the means).

The compost pile and ambient temperatures were recorded hourly during the composting process using the thermocouples and a Campbell Scientific CR 100X datalogger (Campbell Scientific, Edmonton, Canada). As the top two thermometers were not always fully covered by or in direct contact with the compost pile, temperature data obtained from these two thermometers were excluded, in order to get a more representative temperature profile (Fig.2.4 (b)). The peak temperature was achieved after three days of composting as a result of the decomposition of easily degradable organic substrates, and then remained at this level for a short period of time. Afterwards, it fell gradually close to the ambient level by day 35. During the whole period of composting, the temperature did not reach the preferred range (> 55 ℃) to meet typical regulatory requirement of Class A biosolids to reduce pathogen content in the sewage sludge. The same situation was also found in the composting of green crabs by using the same in-vessel composting system (Victor, 2021)

The causes of inadequate heating could be related to the small quantity of composting material (127 kg, ww) and high frequency of turning and air exchange. Addition of water during composting can also result in a decrease in temperature. A supplemental heater or an insulation layer might be needed for this kind of in-vessel composting system, especially when the composting is conducted in the cold winter.

2.2.2.3 Heat-dried Biosolids (HDB)

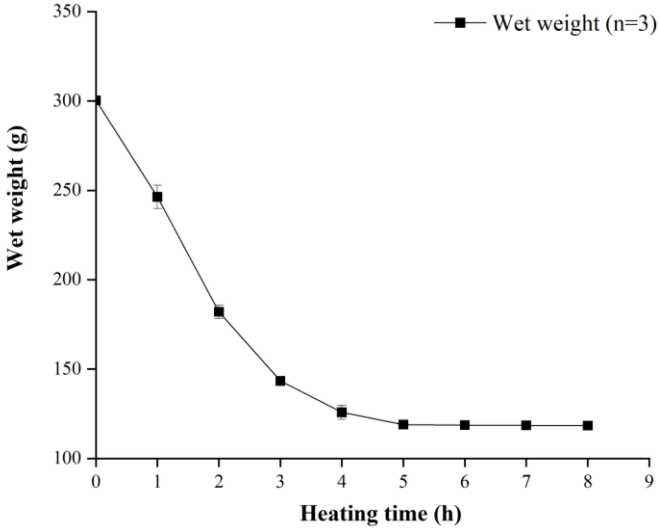


Figure 2. 5 Changes in sample wet weight during heating at 130 ℃ (the values are means (n=3); error bars represent standard deviations of the means).

Three aluminum foil containers with 300 g (ww) of RS each were placed in a laboratory oven. RS was dried at 130 ℃ for 5 hours until the sample reached stable weight (Fig.2.5). HDB (Fig. 2.1 (e)) from three containers were thoroughly mixed to form one composite sample. A temperature of 130 ℃ was selected because it represents the drying temperatures currently employed in the industry (Offer et al., 2010; Horttanainen et al., 2017).

2.2.3 Laboratory Analysis

Prior to chemical analysis, each biosolids type was dried in an oven at 65 ℃ to a constant weight. Dried biosolids were ground to pass through a 2-mm sieve for determination of some key chemical parameters, such as organic matter (OM), pH, total C (TC), total N (TN), and mineral N (MN). OM was measured by the Loss on Ignition (LOI) method (TMECC, 2002), in which dried samples were ignited in an Isotemp programmable muffle furnace (Thermo Fisher Scientific, Waltham, MA, USA) at 550 ℃ until mass constancy was obtained. The initial temperature was 65 ℃ and then it was ramped up to 370 °C, and held at 370 °C for an hour, and finally increased to 550 °C. The OM content was calculated as the mass difference before and after the ignition

process. The pH of the biosolids was determined using a pH meter with a combined glass electrode (Fisher Scientific Accument Excel XL50, Fisher Scientific, Hampton, NH, USA) in a supernatant suspension of a 1:10 (w/v) biosolids to deionized water ratio. TC and TN of the biosolids were measured by combustion using a LECO CN-2000 analyzer (LECO Corporation, St. Joseph, MI). NH_4^+-N and NO_3^--N were measured colorimetrically in 2M KCl extracts (1:200, m/v) by an AutoAnalyzer 3 (Bran-Luebbe Inc., Germany). Before the measurements, the suspension was shaken for 30 min (180 rpm) on a reciprocal shaker (Eberbach Corp., Ann Arbor, MI), and then centrifuged for 10 min (3000×g) (International Equipment Company, USA), and lastly filtered through a filter paper (Whatman #42). All of these parameters were analyzed using four replicate samples.

2.2.4 Statistical Analysis

Data are presented as means of four replicates in the figures and tables. To investigate the differences between different treatments, one-way analysis of variance (ANOVA) was performed in R Studio (version 1.3.1073). The assumption of normality and homogeneity of variances were checked before running an ANOVA by Shapiro-Wilk and Levene's tests, respectively (Levene, 1961; Shapiro and Wilk, 1965). Data were transformed to conform with the assumption of normality if necessary and back-transformed after analysis for presentation. Multiple means comparisons were conducted using the Tukey's test where significant differences were found. Statistical significance was at an alpha (α) value of 0.05.

2.3 Results

2.3.1 Changes in Biosolid N Forms

N contents in the biosolids were significantly influenced by biosolids treatment processes. As shown in Table 2.1, the source material, dewatered sewage sludge (RS), had the highest contents of TN, NH_4^+-N, and ON. The concentrations of TN, NH_4^+-N, and ON all significantly

decreased after alkaline treatment, composting, or heat drying. Both TN and ON contents in the biosolids decreased in the order of RS > HDB > CB > N-Viro > CaO-treated. The NH_4^+-N content also declined in the following order: RS > HDB > CB > N-Viro and CaO-treated. There was no significant difference in NH_4^+-N content between the two types of alkaline-treated biosolids.

Table 2. 1 TN, NH_4^+-N, and ON contents (%) in dewatered raw sewage sludge (RS), heat-dried biosolids (HDB), composted biosolids (CB), N-Viro biosolids (N-Viro), and CaO-treated biosolids (CaO-treated).

Biosolids Type	TN (%)	NH_4^+-N (%)	ON (%)
RS	2.21 (0.04) a	0.16 a	2.05 a
HDB	2.04 (0.03) b	0.15 (0.01) b	1.90 b
CB	1.23 (0.06) c	0.06 c	1.17 c
N-Viro	0.74 (0.05) d	0.04 d	0.71 d
CaO-treated	0.57 (0.01) e	0.04 d	0.53 e
*Significance	<0.001	<0.001	<0.001

ON was estimated from the difference between TN and NH_4^+-N. Mean values (n = 4) are expressed on a dry weight basis (dw) by biosolids type with standard deviations in parentheses. Different letters within each column of the variables represent significant differences ($p < 0.05$).
*Significance at 0.001 ($p < 0.001$)

Fig. 2.6 presents biosolids treatment processes significantly altered different fractions of N in the biosolids. The largest contributor to TN across all the biosolids studied was ON, which ranged from 92.82 % to 95.03 %. The contributions of ON to TN were similar in CB and N-Viro. Heat drying and the addition of CaO addition did enhance the proportion of ON, but the enhancements were not significant. On the other hand, MN or NH_4^+-N only represented a small percentage of TN, ranging from 4.97 % to 7.18 %. The contribution of NH_4^+-N to TN was significantly reduced in CB and N-Viro biosolids, whereas the proportion of ON in both HDB and CaO-treated did not significantly decrease relative to RS.

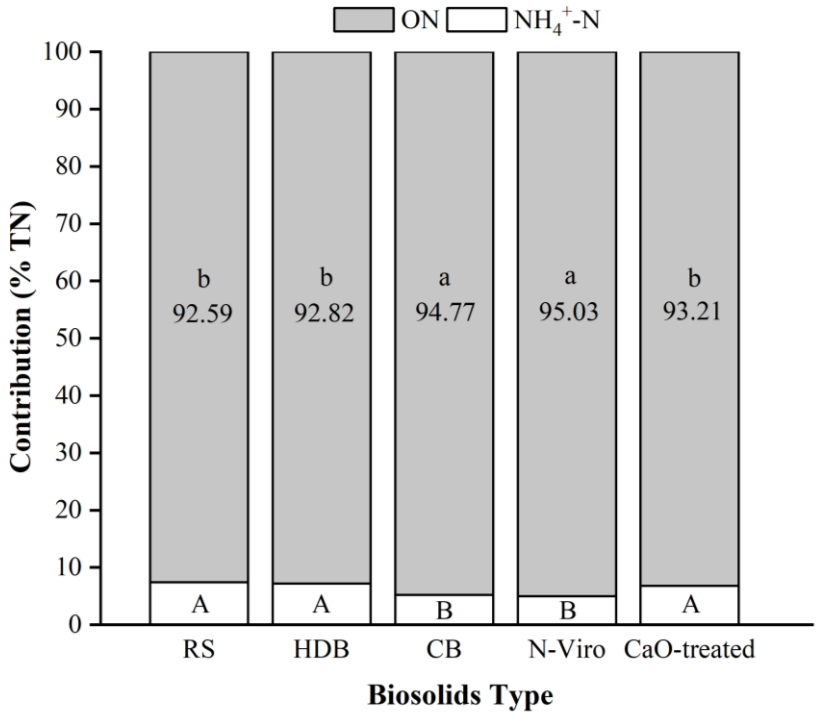


Figure 2. 6 The contributions of ON and NH_4^+-N to TN in dewatered raw sewage sludge (RS), heat-dried biosolids (HDB), composted biosolids (CB), N-Viro biosolids (N-Viro), and CaO-treated biosolids (CaO-treated).

Mean values (n = 4) are expressed on a dry weight basis (dw) by biosolids type. Different small case letters within ON (% TN) fraction represent significant differences across treatments ($p < 0.05$). Different upper case letters within NH_4^+-N (% TN) fraction represent significant differences across treatments ($p < 0.05$). The values presented above are slightly different from those calculated using the values in Table 2.1 because the mean values in Table 2.1 were rounded to 2 decimal places.

2.3.2 Changes in Biosolids Other Chemical Properties

As presented in Table 2.2, biosolids treatment processes contributed to significant alterations to biosolids pH, C/N ratio, TC, and OM. The greatest pH increase was driven by alkaline treatment (6.82 units for CaO-treated and 6.29 units for N-Viro). Composting caused a modest pH increase (0.49 units) as sawdust had a nearly neutral pH level. However, a significant pH decline (0.18 units) was observed in heat drying. Even though RS and HDB had significantly different contents in TN (Table 2.1) and TC, the C/N ratios of both biosolids were not statistically different. A similar finding was obtained between HDB and CaO-treated. RS contained the highest

TC and OM contents, followed by CB, HDB, N-Viro, and CaO-treated. There was no significant difference in TC between CB and HDB. We also found CB had the smallest disparity (1.77 %) with RS concerning OM, when compared to the other three types of biosolids (HDB, N-Viro, and CaO-treated). Alkaline treatment led to the largest differences between RS and ATB with respect to TC and OM, particularly with the addition of pure inorganic chemical CaO.

Table 2. 2 pH, C/N ratio, OM (%), and TC (%) in dewatered raw sewage sludge (RS), heat-dried biosolids (HDB), composted biosolids (CB), N-Viro biosolids (N-Viro), and CaO-treated biosolids (CaO-treated)

Type	pH	C/N Ratio	TC (%)	OM (%)
RS	5.87 (0.06) d	15.22 (0.25) c	33.65 (0.23) a	79.09 (0.16) a
HDB	5.69 (0.03) e	14.60 (0.28) cd	29.82 (0.47) b	61.56 (0.27) c
CB	6.36 (0.05) c	25.31 (1.21) a	31.20 (0.87) b	77.32 (0.62) b
N-Viro	12.16 (0.07) b	21.81 (0.45) b	16.18 (0.99) c	31.08 (2.30) d
CaO-treated	12.69 (0.05) a	13.73 (0.44) d	7.82 (0.24) d	18.78 (0.75) e
Significance	<0.001	<0.001	<0.001	<0.001

Mean values (n = 4) are expressed on a dry weight basis (dw) by biosolids type with standard deviations in parentheses. Different letters for each variable represent significant differences ($p < 0.05$).
*Significance at 0.001 ($p < 0.001$)

2.4 Discussion

The highest concentration of TN was observed in the RS (Table 2.1), with a mean value of 2.21 %. The value fell within a reasonable range, although it was relatively low compared to the average values reported in our literature survey (4.85 %) (Chapter 1 Table 1.2) and Rigby et al. (2016) (> 4 %). The smaller concentrations of TN in the CB and ATB were associated with the dilution effects from the addition of materials with low N contents, such as sawdust and alkaline materials, respectively. CaO and sawdust were both added to RS at a dry weight ratio of 1:1, resulting in a dilution factor of 2, therefore the TN contents in the CaO-treated and CB should be half of that in the RS, if N loss was not taken into account. The TN content in the CB (1.23 %) was close to the theoretically expected value (1.11 %), as the slight increase of TN has considered

a common phenomenon during composting and has been recorded a number of composting studies (Paredes et al., 2002; Alburquerque et al., 2009; Subramanian et al., 2010). Whilst with more intense chemical reactions the TN contents in the N-Viro and CaO-treated biosolids were reduced greatly by 67 and 74 %, respectively. Compared to N-Viro biosolids, CaO-treated biosolids had a significant higher pH level (Table 2.2). The relatively lower value of TN determined for CaO-treated biosolids was probably due to the fact that CaO is more chemically reactive than kiln dust and fly ash (Noller et al., 1980; Catalfamo et al., 1997). HDB had minimal loss in the TN content because heat drying is the simplest, and most straightforward among all the treatment processes in this study. Most living microorganisms can be killed and become inactive after heat drying (20-120 °C) (Neary et al., 1999). Nevertheless, those dead microbial biomasses can still constitute a considerable pool of TN (Jager and Bruins, 1975; Azam et al., 1986). The final NH_4^+-N values in the compost are typically between 0.02 % and 1.0 % (Franke-Whittle et al., 2014), indicating a normal NH_4^+-N level (0.06 %) was achieved in this study. ATB had the lowest level of NH_4^+-N, which is attributed to the substantial loss of N through NH_3 volatilization during treatment and curing. Alkaline treatment provided favorable conditions (high temperature and pH) for NH_3 loss. For example, the maximum temperature reached during the production of CaO-treated biosolids was nearly 80 °C (Fig. 2.2 (b)), in the meantime, the pH of biosolids was always above 12 (Fig. 2.2 (c)).

For CB, the significant decrease in the contribution of NH_4^+-N to TN (Fig. 2.6) could be caused by a number of N cycling pathways, including volatilization (transforming NH_4^+-N into NH_3), nitrification (transforming NH_4^+-N into NO_3^--N), and microbial immobilization (transforming NH_4^+-N and NO_3^--N into ON) (Meng et al., 2017). Moreover, the compost pile went through a moderate thermophilic phase with a rapid temperature surge early during the process, and it was continuously mixed, as well as ventilated throughout the whole period of composting.

However, the significant augmentation in the contribution of ON to TN was likely because of the concentration effect, in which the dry mass of the compost was greatly reduced as a result of the degradation of organic carbon (Tiquia and Tam, 2000; Dias et al., 2010; Kebibeche et al., 2019). Although aerobic composting was conducted, denitrification may also have occurred, especially during the composting of raw materials with high N and moisture content like sewage sludge (Shi et al., 2020). NO_3^--N would be transformed into N gases (nitric oxide (NO), nitrous oxide (N_2O), or dinitrogen (N_2)) via the denitrification process. We noticed during composting that some of the feedstock materials tended to be pushed towards the four corners of the in-vessel composter, where anaerobic pockets could have developed and created an anoxic condition. Studies have reported that the denitrification process is very sensitive to temperature. Braker et al. (2010) reported that the optimal temperature for denitrification is 25-35 °C, which is generally achievable in the composting process. In the future, to avoid the formation of local anaerobic pockets, the optimal angle rotation should be explored for paddles, and paddles need to be adjusted accordingly so that air permeability and mixing thoroughness can be promoted within the in-vessel composting system. N losses through leaching and runoff did not happen in this study.

As shown in Table 2.2, in terms of other chemical properties, treatment processes significantly enhanced biosolids pH with the exception of heat drying. The pH change in the heat drying process may be induced by oxidation of sulfur where sulfate (SO_4^{2-}) is the final product, water evaporation where the concentrations of organic acids increase, nitrification where NH_4^+-N is sequentially converted into NO_2^--N and NO_3^--N, and NH_3 volatilization where NH_4^+-N can be dissociated to NH_3 and H^+ (Franco-Otero et al., 2012; Liu et al., 2019). The relatively higher TC and OM contents in the CB can be partly explained because sawdust as a C-rich bulking agent contributed additional C content and OM to the final composting product CB. Besides, low OM loss during the composting process may be due to the low peak temperature, short period of

composting time, and high contents of complex recalcitrant organic compounds such as cellulose, hemicellulose, and lignin, which affected the microbial degradation. In contrast, great OM loss during the alkaline treatment could be related to the dissolution of organics at high temperatures and elevated pH for a long duration time. N-Viro had a significantly higher OM content than CaO-treated because kiln dust used in producing N-Viro contains around 20-30 % of OM (N-Viro Systems Canada Inc., 2007). In contrast, inorganic chemical compounds such as CaO don't have any organic matter.

2.5 Conclusion

In this study, four types of biosolids (alkaline-treated (CaO-treated and N-Viro), composted, and heat-dried) were generated using the same source of sewage sludge. It is found that biosolids treatment processes significantly changed the contents and forms of N, along with other chemical properties of biosolids. The results from this study were consistent with the literature review in Chapter 1 (Table 1.2-1.5). The highest TN, ON, NH_4^+-N, NH_4^+-N (% TN), TC, and OM contents were found in RS, which did not undergo any treatment processes to reduce hazardous and toxic substances or microbial pathogens. RS is never recommended for agricultural use without additional treatment for pathogens or management for other possible contaminants. The reductions of TN, ON, and NH_4^+-N were in the increasing order of HDB, CB, N-Viro, and CaO-treated. The majority of N in all types of biosolids were in organic forms, but there were significant variations in the contributions of ON or NH_4^+-N to TN between different types of biosolids. As opposed to the other two types of treatments (composting and alkaline treatment), heat drying of RS led to a significant pH decrease, which could be associated with the formation of organic acids during the degradation, oxidation of N and S compounds, and NH_3 volatilization. ATB had relatively higher pH levels as a result of the alkaline materials, with the highest pH in CaO-treated biosolids. ATB and CB varied in each N component and other key chemical

characteristics, albeit they were diluted at the similar rate. The distinctions between these two biosolids were dependent on the nature of the new materials added. Alkaline materials contain very little OM, particularly with CaO. On the other hand, sawdust is a high carbon-based material and consequently can contribute to the abundance of OM and TC, as well as assert a strong influence on the C/N ratio in the end product. As compared to ATB, composting enabled more N to be retained, which maximizes the agronomic value of composted biosolids. Loss of N via volatilization during the process of making ATB can not only weaken the agronomic value of ATB but also bring severe odor issues, which needs to be controlled and managed if the production is operated on a large scale. The form of the added Ca^{2+} in the ATB also had significant effects on the chemical properties of resultant biosolids. With a more intensive exothermic reaction, CaO-treated biosolids tended to have more N lost. However, ATB can serve as a suitable liming material for acidic soils.

Chapter 3: Effect of Different Biosolids on Soil N Mineralization and N-acquiring Enzyme Activities in an Acidic Soil

3.1 Introduction

Biosolids are the solid byproduct generated during the treatment of sewage sludge in a wastewater treatment facility. They are rich in organic matter (OM) and contain essential plant macro- (nitrogen (N), phosphorus (P), potassium (K)), and micro-nutrients. A wide range of benefits from using biosolids as a soil amendment are well documented (Chambers et al., 2003; Thangarajan et al., 2013; Archer et al., 2020), including supplying plant-essential nutrients and organic matter, increasing soil carbon sequestration, and improving soil water holding capacity.

N in biosolids is mainly found in the organic forms (e.g., proteins and their degradation products such as peptides, nucleic acids, amino acids, and amino sugars). These organic N first need to be converted into inorganic or mineral forms (i.e., ammonium (NH_4^+-N) and nitrate (NO_3^--N)) through mineralization before they are available for plant uptake (Wang et al., 2003; Liu et al., 2015). Studying patterns of N release from biosolids-amended soils is a useful approach to developing effective strategies to better manage and apply biosolids as an organic input. These strategies would become very crucial components in the nutrient management plan. Since N mineralization is regulated by a group of N-acquiring enzymes, the potential activities of these enzymes are often considered to be indicators of microbial N demand (Kandeler et al., 2011). β-1,4-N-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), and urease (URE) are the most commonly measured N-acquiring enzymes (Tabatabai and Bremner, 1972), and they are responsible for breaking down proteins and polypeptides, fungal chitin and bacterial peptidoglycan, and urea, respectively (Tabatabai and Bremner, 1972; Kögel-Knabner, 2006).

A number of studies have examined N mineralization in biosolids-amended soils (Smith and Durham, 2002; Pritchard and Rigby, 2010; Alvarez-Campos and Evanylo, 2019; Lin et al., 2022), although these biosolids are typically obtained from different sources. This adds to the

difficulty in understanding the influence biosolids treatment, to generate biosolids, has on N availability from the biosolids due to the heterogeneity of these materials and lack of information on the raw sewage characteristics from each biosolid source. In particular, there is limited information on the response of N-acquiring enzyme activities to biosolids application to soil, especially in acidic soil ecosystems.

In this context, this Chapter includes two parallel leached and non-leached soil incubation experiments, which was designed for assessing N dynamics and N-acquiring enzyme activities in an acidic soil amended with different biosolids generated from sewage solids from the same source.

3.2 Materials and Methods

3.2.1 Soil and Biosolids

Soil samples used in this study were collected (0-15 cm depth) randomly from the buffer areas of an agricultural research site in late August 2020. The research field is located at Bible Hill, Nova Scotia, Canada (45°23′01.7″ N 63°14′35.4″ W). Crop residues on the soil surface were removed prior to soil sampling. The soils are identified as belonging to the Truro Soil Association, Tormentine series, Ortho-Humic Podzols with a sandy loam texture in the Canadian Soil Classification System (Webb et al., 1991). The soil was air-dried and passed through a 2-mm sieve, and stored in sealed buckets before the beginning of incubation studies. Extra intact soil core samples from the 0-7.6 and 7.6-15 cm depths were also taken to determine the bulk density and moisture content.

Biosolids were generated using the same source of raw sewage sludge, which was collected from the Biosolids Processing Facility (BPF) at the Aerotech Business Park, Goffs, Nova Scotia, Canada. Four types of biosolids were used in this experiment:

(1) N-Viro biosolids, which was obtained directly from the BPF operated by the Walker

Environmental Group, using the N-Viro® Process;

(2) CaO-treated biosolids, which was produced by mixing quicklime (CaO) with sewage sludge (30 % ww) followed by oven drying (55 ℃,12 hours);

(3) composted biosolids, which was produced by co-composting sewage sludge with sawdust (2:1 ww) in an in-vessel composting system;

(4) heat-dried biosolids was produced by drying sewage sludge in a laboratory oven (130 ℃, 5 hours) until the sample reached a constant mass.

More detailed information about how each type of biosolids was made can be found in Chapter 2. Biosolids were ground to pass through a 2-mm sieve and stored in the freezer before the incubation started. The characteristics of various biosolids are given in Table 3.1.

Table 3. 1 Physico-chemical characteristics of biosolids.

Parameter	Biosolids			
	HDB	CB	N-Viro	CaO-treated
Dry Matter (%) *	96.79	67.07	72.73	99.44
pH (pH units) *	5.69	6.36	12.16	12.69
OM (%) *	61.56	77.32	31.08	18.78
TN (%) *	2.04	1.23	0.74	0.57
TC (%) *	29.82	31.20	16.18	7.82
C/N Ratio (units) *	14.60	25.31	21.81	13.73
NH_4^+-N (%) *	0.15	0.06	0.04	0.04
Calcium (%)	0.69	0.42	17.93	19.30
Potassium (%)	0.13	0.10	0.74	0.05
K_2O (%)	0.16	0.12	0.90	0.06
Phosphorus (%)	0.82	0.39	0.21	0.25
P_2O_5 (%)	1.89	0.89	0.48	0.58
Magnesium (%)	0.19	0.13	0.29	0.12
Sodium (%)	0.13	0.07	0.08	0.05
Boron (ppm)	<10.00	<10.00	22.72	<10.00
Copper (ppm)	114.97	51.40	29.90	44.76
Iron (ppm)	9853.70	4728.49	4156.56	2961.815
Manganese (ppm)	101.31	127.59	219.07	31.89
Zinc (ppm)	283.83	75.74	117.32	108.95

HDB, heat-dried biosolids; CB, composted biosolids; N-Viro, N-Viro biosolids; CaO-treated, CaO-treated biosolids. Parameters followed by an asterisk (*) were analyzed in four replicates in the Innovative Waste Management Research Laboratory, Dalhousie University. Additional physical and chemical parameters were analyzed in duplicates at the Nova Scotia Department of Agriculture Analytical Services Laboratory, Bible Hill, Nova Scotia. Values are expressed on a dry weight basis (dw) and represent means.

3.2.2 Soil Incubation Experiments

3.2.2.1 Pre-incubation

Figure 3. 1 Pre-incubated soils (un-limed and limed)

10 and 7 kg of air-dried soil were weighed into two 11 L polyethylene containers (Roughneck, Rubbermaid Commercial Products Inc., Oakville, ON, CA), respectively (Fig. 3.1). Limed soil was obtained by mixing 14 g of lime ($CaCO_3$) with 7 kg of air-dried soil. The application rate of lime (4.08 t ha^{-1}) for the soils used in this study was determined by a preliminary experiment (Appendix 1). The air-dried soils were wetted to 50 % WFPS with deionized water before being placed in an incubation chamber equipped with a temperature controller (SOLO 9696, AutomationDirect, USA). The soils were pre-incubated in the dark (at 25 °C and 50 % WFPS) for 14 days to stabilize the microbial activity. Soil moisture content was maintained by adding deionized water to the containers as needed after weighing the containers. Six evenly spaced holes were drilled on the lid of each container for aeration. A 38 L polyethylene tote (Roughneck, Rubbermaid Commercial Products Inc., Oakville, ON, CA) containing 20 L of deionized water was placed in the bottom of the chamber to maintain the air humidity. The temperature and relative humidity in the incubation chamber were recorded during the whole incubation period by the HOBO data logger sensors (HOBO; Onset Computer Corporation, MA, USA) at a one-hour interval, which were 25.06 ± 0.43 °C and 66.05 ± 2.04 % on average,

respectively (Appendix 2).

Table 3. 2 Physico-chemical characteristics of soils after pre-incubation.

Parameter	Soil	
	Un-limed soil	Limed soil
pH (pH units)	5.50	6.87
Buffer pH (pH units)	7.65	7.81
OM (%)	4.74	4.83
Calcium (kg ha^{-1})	2106	3575
K_2O (kg ha^{-1})	395	393.5
P_2O_5 (kg ha^{-1})	919	908
Magnesium (kg ha^{-1})	304.5	296
Sodium (kg ha^{-1})	23	22
Sulfur (kg ha^{-1})	30	32.5
Aluminum (ppm)	1549	1503
Boron (ppm)	< 0.50	< 0.50
Copper (ppm)	4.21	4.59
Iron (ppm)	185.5	163
Manganese (ppm)	95	86
Zinc (ppm)	1.57	1.43

Parameters were analyzed in duplicates at the Nova Scotia Department of Agriculture Analytical Services Laboratory, Bible Hill, Nova Scotia. Values are expressed on a dry weight basis (dw) and represent means.

The characteristics of un-limed and limed soils after pre-incubation are summarized in Table 3.2. After the pre-incubation, un-limed soils received four treatments: N-Viro, CaO-treated, CB, and HDB. Limed soils only received two treatments: CB and HDB (LCB and LHDB), because of the nature of N-Viro and CaO-treated (i.e., high liming capacity). A total of eight treatments were established in the incubation studies, with un-limed and limed soils as two controls (CK and LCK), as well as six amended treatments (N-Viro, CaO-treated, CB, HDB, LCB, and LHDB). CaO-treated, N-Viro, heat-dried, and composted biosolids were applied at rates of 35.36, 27.20, 9.52, and 16.32 t ha^{-1}, respectively, assuming an incorporation depth of 15 cm and bulk density of 1.36 g cm^{-3}. The application rates were all equivalent to 200 kg N ha^{-1}, close to the economically optimal N rate for corn in Eastern Canada (Kablan et al., 2017). Two incubation studies were run

simultaneously under the same controlled conditions for 154 days.

3.2.2.2 Non-leached Incubation Study

Two hundred jars were prepared (8 treatments × 5 sampling times × 5 replicates) for the incubation study without periodic leaching procedures (i.e., non-leached incubation study). Pre-incubated soils (60 g dw) were placed into mason jars and amended with different biosolids. Each treatment was replicated five times in a randomized complete block design (RCBD) to account for potential spatial differences in temperature and humidity (Fig. 3.2). Each mason jar was covered with parafilm perforated with five small holes to allow gas exchange and minimize moisture loss. During the incubation, soil moisture content (60 % WFPS) was maintained by weighing the jars and adding deionized water, if necessary. Eight jars were destructively sampled from each block after 0, 7, 28, 70, and 154 days of incubation. Each soil sample was split into three subsamples: one was air dried and sieved for the measurement of pH and mineral N (NH_4^+-N and NO_3^--N); another was frozen for enzyme assays; the rest was for the determination of MC and OM. Soil physio-chemical properties and enzyme activities were measured in quintuplicate.

(a) **(b)**

Figure 3. 2 Experimental setup illustration: simplified experimental layout (a), side view of the incubation chamber (b).

3.2.2.3 Leached Incubation Study

A second study was conducted by following the periodic leaching procedures, according

to Stanford and Smith (1972), with some modifications. It consisted of 45 cups (8 treatments × 5 sampling times + 5 blanks). Pre-incubated moist soil (30 g dw), 30 g of 10 % HCl washed Ottawa sand (20–30 mesh, Fisher Scientific, Ottawa, ON, Canada), and the corresponding amount of biosolids were first thoroughly mixed with a spatula in a beaker, and then the mixture was transferred to individual cups. Each cup (Nalgene, Fisher Scientific, Rochester, NY, USA) can be easily attached to a Büchner funnel and has a glass fiber filter paper (Whatman GF/A) placed on its perforated base to prevent soil losses during each leaching event. Cups with only sand were served as blanks.

Figure 3. 3 Leaching setup

At days 0, 3, 7, 14, 28, 42, 56, 70, 98, 126, and 154, soils were leached using 200 mL of 0.01 M $CaCl_2$ with small increments followed by an addition of 50 mL of an N-free nutrient solution containing 0.002 M $CaSO_4$, 0.002 M $MgSO_4$, 0.005 M $Ca(H_2PO_4)_2$, and 0.0025 M K_2SO_4 (Fig. 3.3). The first leaching event was used to remove the existing mineral N and bring the mixture to 60 % WFPS. A vacuum of 40 cm Hg was applied to facilitate leaching and remove excess water. After each leaching, the soils were readjusted for moisture content, rewrapped with parafilm, and returned to the incubation chamber. Throughout the incubation, the moisture content

of the mixture (60 % WFPS) was maintained by weighing the cup and adding the required amount of deionized water. Leachate samples were collected and kept frozen until analysis for mineral N.

3.2.3 Laboratory Analysis

3.2.3.1 Analysis of Soil Physico-Chemical Properties

Soil moisture content (MC) was measured gravimetrically by drying soil samples in an oven at 65 ℃ until the constant weight was achieved (Gardner, 1986). Soil bulk density was determined using the core ring method (Blake and Hartge, 1986). Two stainless steel cylinders were hammered into the soil surface (7.6 cm in diameter and 7.6 cm in height), and bulk density was calculated by dividing the dry soil mass by the total core volume. Water filled pore space (WFPS) can be calculated from simple measurements of bulk density and moisture content using the following equation:

$$WFPS = \frac{Gravimetric\ MC\ \times Buld\ denisty}{1 - \frac{Bulk\ density}{Particle\ density}} \times 100\ \%$$

where a particle density of 2.65 g cm $^{-3}$ was assumed. Soil pH was determined using a pH meter with a combined glass electrode (Fisher Scientific Accument Excel XL50, Fisher Scientific, Hampton, NH, USA) in a supernatant suspension of a 1:2 (w/v) soil to 0.01 M $CaCl_2$ ratio. In addition, the leachate samples collected from day 0, 7, 28, 70, and 154 were measured for pH directly in the clear leachate. The relative lime effectiveness (RLE) to neutralize soil acidity was calculated for each treatment using the following equation:

$$\frac{Soil\ pH_{biosolids-amended} - Soil\ pH_{CK}}{Soil\ pH_{LCK} - Soil\ pH_{CK}} \times 100\ \%$$

where LCK and CK were used as reference standards, with acid-neutralizing values of 100 and 0, respectively (Anetor and Akinrinde, 2007). Soil OM was measured by the Loss on Ignition (LOI) method (TMECC, 2002), in which dried samples were ignited in an Isotemp programmable

muffle furnace (Thermo Fisher Scientific, Waltham, MA, USA) at 550 ℃ until mass constancy was obtained. The initial temperature was 65 ℃, and then it was ramped up to 370 °C and held at 370 °C for an hour, and finally increased to 550 °C. The OM content was calculated as the mass difference before and after the ignition process.

Mineral N (NH_4^+-N and NO_3^--N) were extracted with 2 M KCl using a soil : solution ratio at 1:3 (w/v), filtered through a Whatman #42 filter paper and quantified colorimetrically by an AutoAnalyzer 3 (Bran-Luebbe Inc., Germany). The concentrations of mineral N in the leachate samples were directly analyzed using the same instrument described above. After correcting for N mineralization in the control (un-limed soil) and initial soil mineral N, net N mineralization for each biosolids at each sampling time ($N_{\min(t)}$) was calculated using the equation as follows (Griffin et al., 2005; Azeez and Van Averbeke, 2010):

$$N_{\min(t)} = (MN_{amended\,(t)} - MN_{amended\,(t=0)}) - (MN_{control\,(t)} - MN_{control\,(t=0)})$$

where: $MN_{amended\,(t)}$ is the cumulative mineral N in the amended soil at time t, $MN_{amended\,(t=0)}$ is the cumulative mineral N in the amended soil at Day 0, $MN_{control\,(t)}$ is the cumulative mineral N in the control at time t, and $MN_{control\,(t=0)}$ is the cumulative mineral N in the control at Day 0.

The percentage of ON mineralized from each biosolids was calculated using the equation:

$$\%\, N_{\min(t)} = \frac{N_{\min(t)}}{ON} \times 100\ \%$$

where ON is the total organic N in biosolids.

N Mineralization data were fitted to the first-order exponential model described by Stanford and Smith (1972):

$$N_m = N_o\,(1 - e^{-kt})$$

Where: N_m is the cumulative amount of mineralized N (mg kg^{-1}) at time t, N_o is the

potentially mineralized N pool (mg kg^{-1}), *k* is the zero-order rate constant (mg kg^{-1} day^{-1}) of mineralization.

3.2.3.2 Analysis Soil Enzyme Activities

The frozen soil was thawed at room temperature before enzyme assay preparation. Three soil extracellular enzymes involved in N mineralization were investigated in this study: β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and urease (URE). The first two enzymes were assayed according to the method of Allison (2008). A urease assay was performed using the method of Cordero et al. (2019), with some modifications. These methods all involve adding substrates, incubating, and measuring the end products of the enzymatic reaction (Table 3.3). Standard curves were constructed using a range of *p*NA (No. 185310, Sigma-Aldrich, Oakville, ON, Canada), *p*NP (No. AC157051000, Fisher Scientific, Ottawa, ON, Canada), and ammonium concentrations (Ammonium sulfate, No. AAJ6441922, Ottawa, ON, Canada). The end product released during the reaction was quantified by using the respective standard curve.

Table 3. 3 Extracellular enzymes assayed and the corresponding substrates and reaction products (Catalog numbers in parentheses).

Enzyme	Substrate	Incubation Time	End Product
Leucine aminopeptidase	5 mM Leucine *p*-nitroanilide (No. AAA1199406, Fisher Scientific, Ottawa, ON, Canada)	5 h	*p*-nitroanilide (*p*NA)
β-1,4-N-acetyl-glucosaminidase	2 mM *p*NP-N-acetyl-β-D-glucosaminide (No. AC229410010, Fisher Scientific, Ottawa, ON, Canada)	3 h	*p*-nitrophenol (*p*NP)
Urease	80 mM Urea (No. U15-500, Fisher Scientific, Ottawa, ON, Canada)	2 h	Ammonium

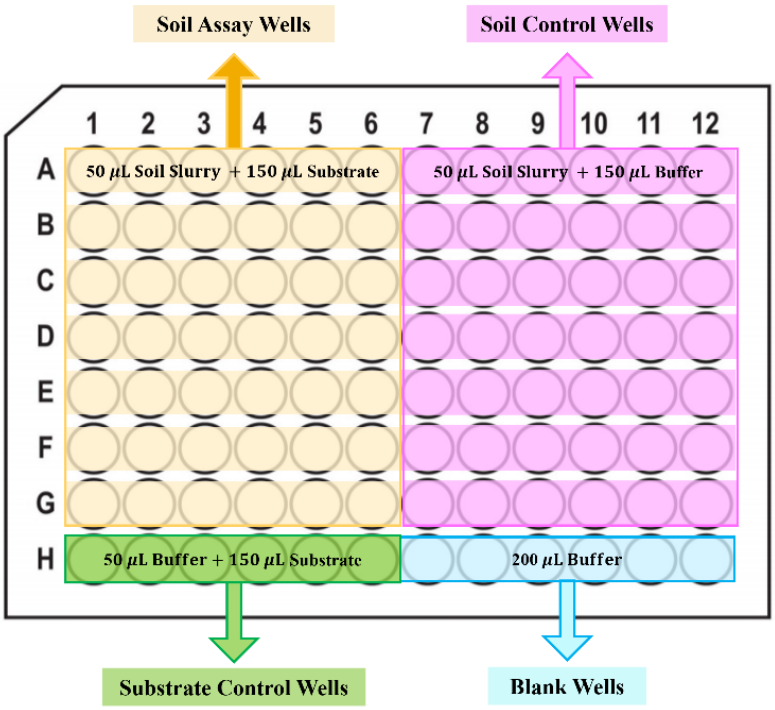


Figure 3. 4 The layout of 96-well microplates for the enzyme assays in this study.

To measure LAP and NAG activities, 0.2 g of wet soil was suspended in 40 mL of 50 mM sodium acetate buffer (pH = 5.0) in a 50 mL sterile centrifuge tube. Before transferring to a 96-well microplate, the soil suspensions were shaken orbitally at 180 rpm on the Innova^{TM} 2100 platform shaker (New Brunswick Scientific, Edison, HI, USA) for an hour. A vortex mixer (2000 rpm, 15 s, Fisher Scientific, USA) was used to facilitate proper mixing. Each microplate contains soil assay wells, soil control wells, substrate control wells, and blank wells (Fig. 3.4). 50 µL of homogenized soil slurry and 150 µL of substrate solution specific to each enzyme were added to soil assay wells. The purpose of the soil control, substrate control, and blank control wells were to account for naturally occurring absorbance of the soil, substrate, and buffer within the well plate. There were six replicate wells per soil sample per assay. Microplates were wrapped with tinfoil and incubated in the dark at room temperature with gentle shaking for 5 h (LAP) or 3 h (NAG). Following incubation, 100 µL of supernatant was removed from each well and pipetted

into a new clear microplate. In the NAG assay plates, 5 µL of 1.0 M NaOH was added to each well to terminate the reaction and develop the color prior to reading. Lastly, the absorbance was determined with a microplate spectrophotometer at 405 nm (Epoch 2, BioTek, Winooski, VT, USA). LAP and NAG activities were calculated after subtracting the values of two controls (i.e., soil and substrate controls) and were expressed as µmol *p*NA g^{-1} dry soil h^{-1} and µmol *p*NP g^{-1} dry soil h^{-1}, respectively.

To measure URE activity, 2 g of wet soil was mixed with 5 mL of 50 mM sodium acetate buffer (pH = 5.0) in a conical flask using a magnetic stirrer. 250 µL soil slurry was incubated with 100 µL urea solution in 2 mL sterile microcentrifuge tubes, representing soil assays. Additionally, soil controls (250 µL soil slurry + 100 µL buffer), substrate controls (100 urea µL + 250 buffer µL), and blank controls (350 µL buffer) were also included. After 2 h of incubation, 1 mL of 2M KCl was added to each tube. The mixture was shaken for 30 minutes and then centrifuged (Sorvall Legend Micro 17, Thermal Scientific, USA) at 2900 g for 5 minutes. Afterward, 150 µL of supernatant from each tube was placed into the corresponding well on a 96-well microplate. Following the addition of 75 µL of colour reagent (a mixture of sodium hydroxide, sodium salicylate, and sodium nitroprusside dihydrate) and 30 µL of oxidation solution (dichloroisocyanuric acid sodium salt dihydrate), samples were incubated again at 25 °C for 30 min. The absorbance of the supernatant was measured at 650 nm, and the urease activities were expressed as mg NH_4^+-N kg^{-1} dry soil h^{-1}.

3.2.4 Statistical Analysis

All statistical analyses were performed using repeated measures analysis of variance (ANOVA) by PROC MIXED in SAS version 9.4 (SAS Institute, Cary, NC, USA). Treatment and incubation time were considered as fixed effects and block as a random effect in the ANOVA. The assumption of normality and homogeneity of variances were tested prior to analysis using the

Proc Univariate procedure of SAS. Data were transformed as necessary to meet assumptions, but non-transformed data are presented for ease of interpretation. The covariance structure was chosen based on the lowest Akaike Information Criterion value. Multiple means comparisons were conducted using the Tukey's test where significant differences were found. Statistical significance was at an alpha (α) value of 0.05.

Model fitting was performed by OriginPro 2023 (OriginLab Corporation, Northhampton, MA, USA). Parameters in the model were estimated using the non-linear least-square technique (Marquardt-Levenberge algorithm) through minimizing the sum of squares error. The models were evaluated based on the adjusted coefficient of determination (*Adj* R^2) and mean square error (MSE). Higher *Adj* R^2 and lower MSE values indicate a better fit of the data to the model. Pearson's correlation analysis was also conducted to evaluate the relationships between variables.

3.3 Results

3.3.1 Soil pH and Leachate pH Changes During Incubation

3.3.1.1 Changes in Soil pH

The ANOVA analysis (Table 3.4) shows that the main effects of incubation time and treatment, and the interaction effect between incubation time and treatment on soil pH were all significant ($P < 0.0001$). Table 3.5 indicates that soil pH responded differently at each incubation time depending on the treatment applied. At the end of the incubation, soil pH dropped significantly in all treatments compared to the start of incubation, excluding N-Viro and CK. A slight increase in soil pH was found in N-Viro, whereas a slight decrease in CK. CaO-treated led to an apparent decline in soil pH at day 7, while the significant decreases in soil pH were delayed under HDB and LHDB treatments and under CB, LCB, and LCK treatments for about two months and five months, respectively.

Table 3. 4 ANOVA p-values for the main and interaction effect of incubation time and treatment on soil properties in two separate incubation settings (i.e., leached (L) and non-leached (NL)).

	pH (pH units)		OM (%)	Cumulative MN (mg N kg^{-1} soil)	MN (mg N kg^{-1} soil)	Net N mineralized (% organic N)	
	L	**NL**	**NL**	**L**	**NL**	**L**	**NL**
Incubation time	<0.0001	<0.0001	**<.0001**	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	<0.0001	<0.0001	**<.0001**	<0.0001	<0.0001	<0.0001	<0.0001
Incubation time × Treatment	**<0.0001**	**<0.0001**	0.2899	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

*Significant effects that require multiple means comparison are highlighted in bold.

Table 3. 5 Effect of Incubation Time x Treatment interaction on soil pH (pH units).

Treatment	Day				
	0	**7**	**28**	**70**	**154**
CaO-treated	8.42 (0.03) A	7.89 (0.19) B	7.84 (0.10) BC	7.68 (0.07) C	7.66 (0.14) C
N-Viro	6.59 (0.03) DEFG	6.57 (0.07) DEFGH	6.68 (0.03) DE	6.60 (0.10) DEF	6.76 (0.04) D
Heat-dried (HDB)	5.32 (0.05) M	5.29 (0.04) MN	5.20 (0.05) MNOPQ	5.10 (0.03) OPQ	5.04 (0.06) Q
Composted (CB)	5.35 (0.08) M	5.28 (0.06) MNO	5.25 (0.04) MNOP	5.16 (0.06) MNOPQ	5.05 (0.02) Q
Lime + Heat-dried (LHDB)	6.53 (0.10) EFGHI	6.47 (0.10) FGHIJ	6.39 (0.10) IJKL	6.26 (0.08) KL	6.25 (0.03) KL
Lime + Composted (LCB)	6.51 (0.09) EFGHI	6.58 (0.09) DEFGH	6.39 (0.03) IJKL	6.40 (0.04) HIJKL	6.23 (0.05) L
Lime (LCK)	6.53 (0.04) EFGHI	6.55 (0.08) EFGHI	6.47 (0.06) FGHIJ	6.42 (0.06) GHIJK	6.30 (0.07) JKL
Control (CK)	5.27 (0.07) MNOP	5.24 (0.05) MNOP	5.18 (0.04) MNOPQ	5.13 (0.09) NOPQ	5.09 (0.03) PQ

Mean values (n = 5) are presented, with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

Table 3. 6 The relative lime effectiveness (RLE) (%) for the neutralization of soil acidity.

Treatment	Day				
	0	7	28	70	154
CaO-treated	250.00	202.29	206.20	197.67	212.40
N-Viro	104.76	101.53	116.28	113.95	138.02
Heat-dried (HDB)	3.97	3.82	1.55	-2.33	-4.13
Composted (CB)	6.35	3.05	5.43	2.33	-3.31
Lime + Heat-dried (LHDB)	100.00	93.89	93.80	87.60	95.87
Lime + Composted (LCB)	98.41	102.29	93.80	98.45	94.21

Mean values (n = 5) are presented.

Table 3. 7 Effect of Incubation Time x Treatment interaction on leachate pH (pH units).

Treatment	Day				
	0	7	28	70	154
CaO-treated	7.35 (0.21) A	6.87 (0.15) B	6.28 (0.11) C	6.00 (0.07) CDE	5.62 (0.15) FGH
N-Viro	6.18 (0.12) C	5.28 (0.28) HIJKLMN	5.65 (0.10) DEFG	5.57 (0.15) FGHI	5.22 (0.13) IJKLMN
Heat-dried (HDB)	5.29 (0.13) GHIJKLMN	4.38 (0.05) P	5.19 (0.20) JKLMN	5.09 (0.13) KLMN	4.47 (0.11) OP
Composted (CB)	5.29 (0.18) GHIJKLM	4.45 (0.19) P	5.00 (0.11) LMNO	5.12 (0.10) KLMN	4.64 (0.19) OP
Lime + Heat-dried (LHDB)	6.11 (0.06) C	5.36 (0.07) FGHIJKL	5.64 (0.08) EFGH	5.42 (0.10) FGHIJK	4.94 (0.22) MNO
Lime + Composted (LCB)	6.01 (0.07) CD	5.32 (0.25) FGHIJKL	5.55 (0.16) FGHIJ	5.42 (0.08) FGHIJK	4.93 (0.12) NO
Lime (LCK)	6.07 (0.04) C	5.25 (0.12) IJKLMN	5.66 (0.07) DEF	5.43 (0.08) FGHIJK	5.19 (0.16) JKLMN
Control (CK)	5.30 (0.11) FGHIJKLM	4.42 (0.09) P	5.08 (0.24) KLMN	5.25 (0.15) IJKLMN	4.68 (0.16) OP

Mean values (n = 5) are presented, with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

Table 3. 8 The relative lime effectiveness (RLE) (%) for the neutralization of soil acidity.

Treatment	Day				
	0	7	28	70	154
CaO-treated	267.62	297.33	207.22	419.10	185.04
N-Viro	114.88	104.61	98.97	179.78	105.12
Heat-dried (HDB)	-1.31	-5.58	19.59	-88.76	-42.91
Composted (CB)	-1.04	3.16	-12.71	-75.28	-7.87
Lime + Heat-dried (LHDB)	105.48	114.32	96.56	96.63	50.00
Lime + Composted (LCB)	92.95	109.22	81.44	95.51	47.64

Mean values (n = 5) are presented.

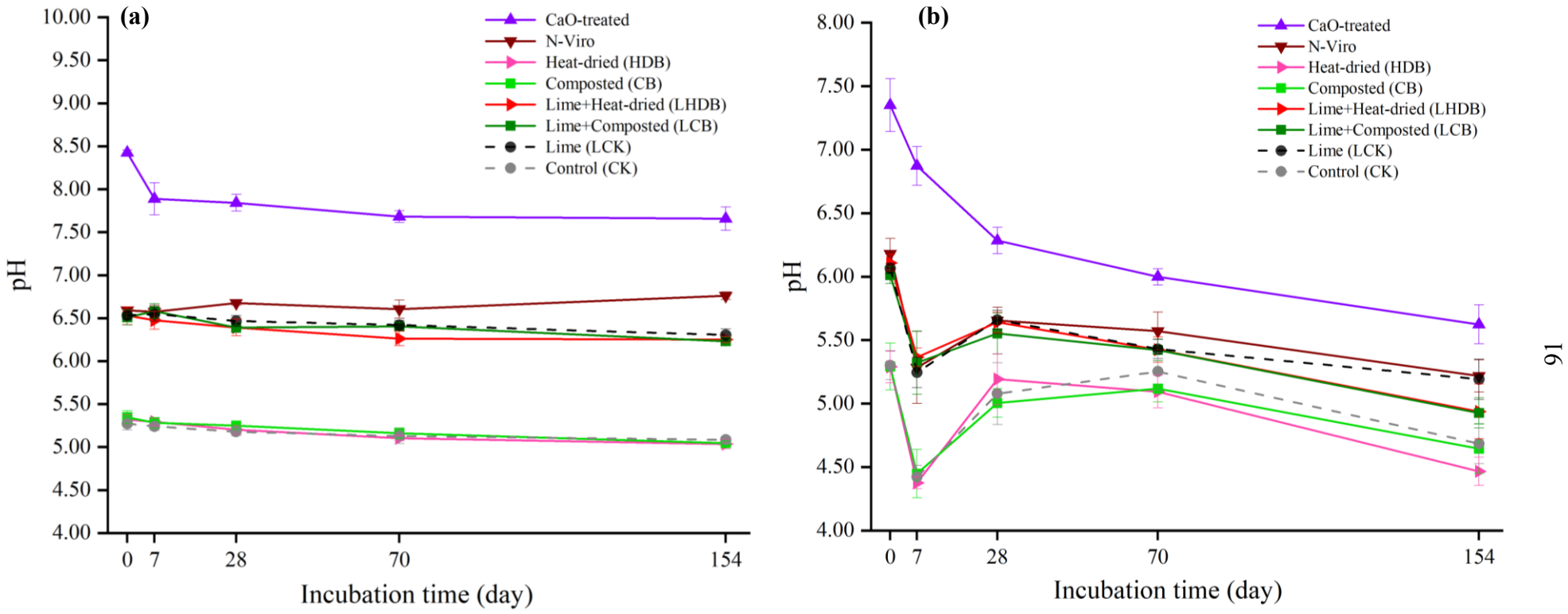


Figure 3. 5 Change in soil pH (a) and leachate pH (b) during incubation (note the different y-axis scales). Points represent means (n=5). Error bars represent standard deviations of the means.

The soil pH under the CaO treatment was always significantly higher than that under all the other treatments throughout the course of the incubation (Fig. 3.5 (a)). A pH value ranging from 6 to 6.5 was maintained in treatments that had received lime in the pre-incubation period (LHDB, LCB, and LCK). N-Viro treatment kept soil pH higher than 6.5. Treatments without pH adjustment (HDB, CB, and CK) had relatively low pH levels (pH: 5-5.5). All treatments shared a similar change over time exhibiting a weak tendency for the soil pH to decrease with increasing incubation time, except for the N-Viro treatment that had a longer-lasting relative effectiveness as an alternative liming material. The RLE values varied with treatments and incubation times (Table 3.6). Although small fluctuations occurred during the incubation, the same order was followed by the RLE values of treatments at the beginning and the end of the experiment: CaO-treated > N-Viro > LHDB > LCB > CB > HDB. The sole application of CB and HDB resulted in negative RLE values towards the later stages of the incubation, whereas the inherent lime in LCB and LHDB generated RLE values close to 100 %. Two alkaline-treated biosolids held high values of RLE (> 100 %) during the whole incubation period, and by the end of the incubation, their RLE values were 1.5-fold and 2-fold higher than that of LCB and LHDB, respectively.

3.3.1.2 Changes in Leachate pH

The ANOVA analysis (Table 3.4) shows that the main effects of incubation time and treatment, and the interaction effect between incubation time and treatment on leachate pH were all significant ($P < 0.0001$).

Overall, more dramatic pH changes, as well as greater variations, occurred in the leachate samples when compared to that in the soils. In addition, leachate samples collected from the leaching events were more acidic than non-leached soils (Fig. 3.5 (b)). pH measured in the leachates decreased significantly across all treatments after 7 days of incubation (Table 3.7). Except for CaO-treated, all treatments underwent increases in pH between day 7 and day 28.

Thereafter, they all showed a decreasing trend and eventually reached pH values significantly lower than their initial pH values. The leachate pH measured in the CaO-treated treatment was always significantly greater than in other treatments at all incubation times. Starting from day 70, the differences in leachate pH between HDB and LHDB, CB and LCB, and CK and LCK were not significant anymore. On the last day of the incubation, there were no significant differences among N-Viro, LHDB, LCB, and LCK treatments. Negative RLE values were also found under CB and HDB treatments but appeared earlier in the leached incubation study than in the non-leached incubation study (Table 3.8). On day 154, the magnitude of lime application in LCB and LHDB was greatly decreased by about half. CaO-treated and N-Viro treatments had more than three times and two times, respectively, greater RLE values than LCB and LHDB.

3.3.2 Soil OM Changes During Incubation

Soil OM was significantly affected by incubation time and treatment ($P < 0.0001$), with no significant interaction ($P > 0.05$) (Table 3.4). Almost all the treatments showed a decreasing trend at first for soil OM and then a reverse trend (Fig. 3.6). With the addition of lime, the OM contents were smaller in LHDB, LCB, and LCK the majority of the incubation time, when compared to HDB, CB, and CK, respectively.

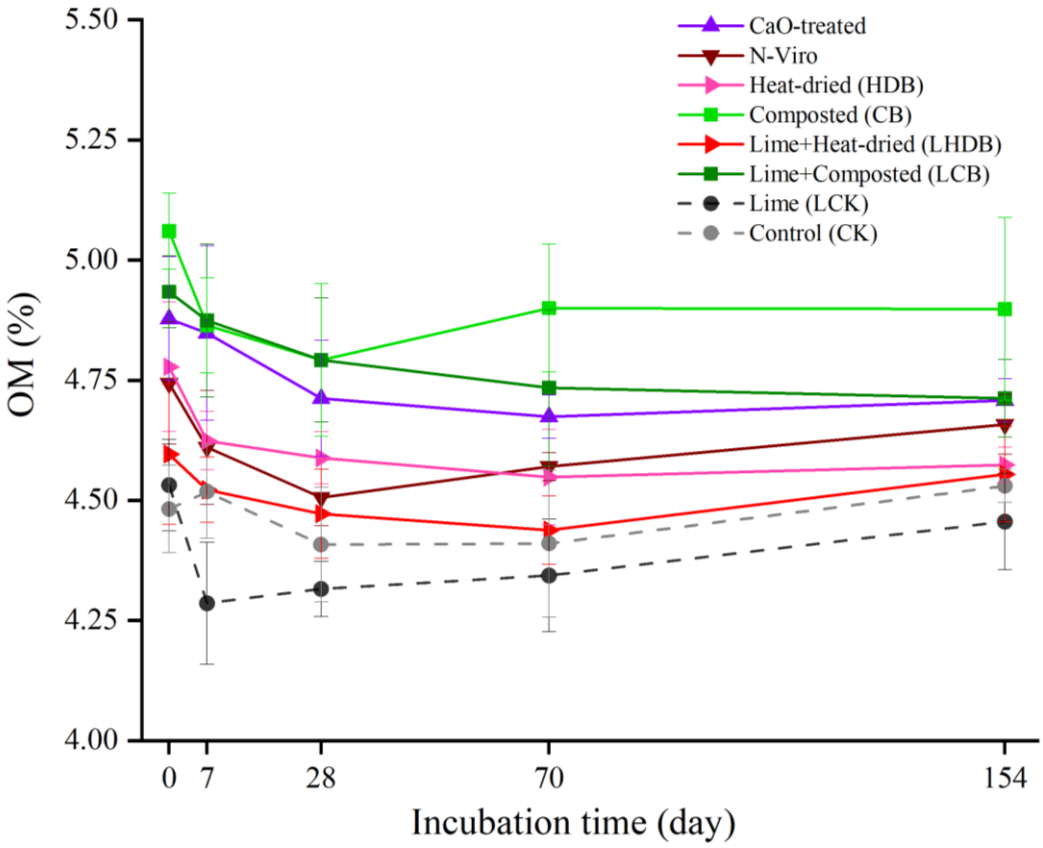


Figure 3. 6 Soil OM changes during non-leached incubation. Points are expressed on a dry weight basis (dw) and represent means (n=5). Error bars represent standard deviations of the means.

As shown in Table 3.9, the highest soil OM content was observed at the beginning of the incubation in almost all the treatments. After 7 and 28 days of incubation, there were significant reductions in soil OM. Nonetheless, the soil OM content remained relatively stable from day 28 onward. After a continuous decrease, a slight and insignificant soil OM increase was detected at day 154 (final day). Without any organic treatments applied, two controls (i.e., LCK (4.39 %) and CK (4.47 %)) had significantly lower soil OM contents, and CK was not significantly different from LCK and LHDB. In the amended treatments, soil OM increased in the order of LHDB (4.52 %) < N-Viro or HDB (4.62 %) < CaO-treated (4.76 %) < LCB (4.81 %) < CB (4.90 %). No significant difference was found between CB and LCB, whereas a significant difference was found

between HDB and LHDB.

Table 3. 9 Effect of Incubation Time and Treatment on soil OM (%).

Main Factors		OM
Incubation Time	0	4.75 (0.22) A
	7	4.64 (0.22) B
	28	4.57 (0.19) C
	70	4.58 (0.20) BC
	154	4.64 (0.16) BC
Treatment	CaO-treated	4.76 (0.13) B
	N-Viro	4.62 (0.11) C
	Heat-dried (HDB)	4.62 (0.11) C
	Composted (CB)	4.90 (0.15) A
	Lime + Heat-dried (LHDB)	4.52 (0.11) D
	Lime + Composted (LCB)	4.81 (0.14) AB
	Lime (LCK)	4.39 (0.13) E
	Control (CK)	4.47 (0.11) DE

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

3.3.3 N Mineralization in Soils

Incubation time, treatment, and their interaction effect had significant influences on soil MN release and net N mineralized (% organic N) from biosolids in both incubation settings, according to the ANOVA results ($P < 0.0001$) (Table 3.4).

3.3.3.1 N mineralization in Soils from Non-leached Incubation Study

The MN concentrations and net N mineralized, respectively (Fig. 3.7 (a) and 3.8 (a)), in HDB and LHDB, CB and LCB, and CK and LCK tended to change in parallel over time. The evolution of soil MN over time can be generally divided into three phases: 1) initial rapid increase or decrease; 2) modest increase; 3) slow and steady increase (Fig. 3.7 (a)). In the first phase (day 0-7), HDB, LHDB, and LCK showed decreases in soil MN (Fig. 3.7 (a)). Soil MN release in all treatments consistently increased after day 7, although the rate of increase varied across

treatments. At the end of incubation, the highest amount of soil MN was released from CaO-treated and decreased in the order CaO-treated > N-Viro > LHDB > HDB > LCB > CB > LCK > CK (Table 3.10).

Table 3. 10 Effect of Incubation Time and Treatment on soil MN from the non-leached incubation study (mg N kg^{-1} soil).

Treatment	Day				
	0	**7**	**28**	**70**	**154**
CaO-treated	71.33 (2.00) STU	114.93 (5.17) HI	133.75 (2.88) F	173.79 (4.61) BC	223.98 (2.61) A
N-Viro	78.14 (3.68) PQRST	79.19 (4.76) QRS	100.71 (3.28) KL	137.65 (5.15) EF	181.26 (3.42) B
Heat-dried (HDB)	79.25 (3.26) PQRST	64.48 (1.46) U	82.62 (2.76) PQR	117.24 (2.86) GHI	153.14 (3.25) D
Composted (CB)	74.00 (3.58) RSTU	85.82 (3.59) OPQ	87.78 (4.13) OP	108.96 (5.64) IJK	142.71 (2.51) DEF
Lime + Heat-dried (LHDB)	89.31 (5.48) NOP	80.37 (3.22) PQRS	101.47 (3.94) KL	130.15 (5.00) FG	168.24 (5.98) C
Lime + Composted (LCB)	92.90 (4.77) LMNO	99.52 (2.16) KLM	99.77 (2.52) KLM	115.02 (5.42) HI	145.75 (9.58) DE
Lime (LCK)	88.48 (7.36) OPQ	85.96 (2.54) OPQ	92.58 (1.40) MNO	113.80 (5.18) HIJ	141.43 (3.73) DEF
Control (CK)	68.07 (3.01) TU	75.63 (1.60) RST	81.85 (2.33) PQR	101.33 (6.52) JKLMN	122.69 (4.16) GH

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

Figure 3.8 (a) and Table 3.11 show that net N immobilization dominated in LCB treatment, as presented by the negative values of net N mineralized at all sampling times. N-Viro, LHDB, and HDB also experienced net N immobilization at the early stages of incubation, with net N mineralized being close to zero or negative at day 7 or/and day 28.

In contrast, net N mineralization dominated in soils amended with CaO-treated biosolids over the entire incubation period. After 22 weeks of incubation, the highest percentage of N mineralized was from CaO-treated biosolids (107.73 %), followed by N-Viro (52.34 %), LHDB (26.72 %), HDB (21.18 %), CB (15.12 %), and LCB (-1.91 %) (Table 3.11). Although a slightly greater amount of N was mineralized from LHDB than HDB, the application of lime did not result

in a significant difference between these two treatments during the whole incubation. On the other side, lime behaved differently with CB, contributing to a significantly lower proportion of N mineralized from LCB than CB since day 70. Net N mineralized in CB and LCB firstly decreased (close to or below 0) and subsequently gradually increased, whereas other treatments all showed consistent increases (Fig. 3.8 (a)). Besides, the insignificant increases of net N mineralized from day 7 to 154 were only found in CB and LCB.

Table 3. 11 Effect of Incubation Time and Treatment on net N mineralized (% organic N) from biosolids from the non-leached incubation study.

Treatment	Day			
	7	**28**	**70**	**154**
CaO-treated	39.61 (5.68) D	53.46 (3.17) C	76.06 (5.07) B	107.73 (2.87) A
N-Viro	-7.03 (5.13) JK	9.48 (3.54) GH	28.33 (5.55) E	52.34 (3.69) C
Heat-dried (HDB)	-24.53 (1.60) M	-11.44 (3.03) KL	5.20 (3.14) GHI	21.18 (3.57) EF
Composted (CB)	4.57 (3.86) GHI	0.00 (4.43) HIJ	1.83 (6.06) HIJ	15.12 (2.69) FG
Lime + Heat-dried (LHDB)	-18.13 (3.54) LM	-1.78 (4.33) IJK	8.34 (5.49) GHI	26.72 (6.07) E
Lime + Composted (LCB)	-1.01 (2.31) HIJK	-7.42 (2.71) JKL	-11.95 (5.81) KL	-1.91 (10.29) IJK

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

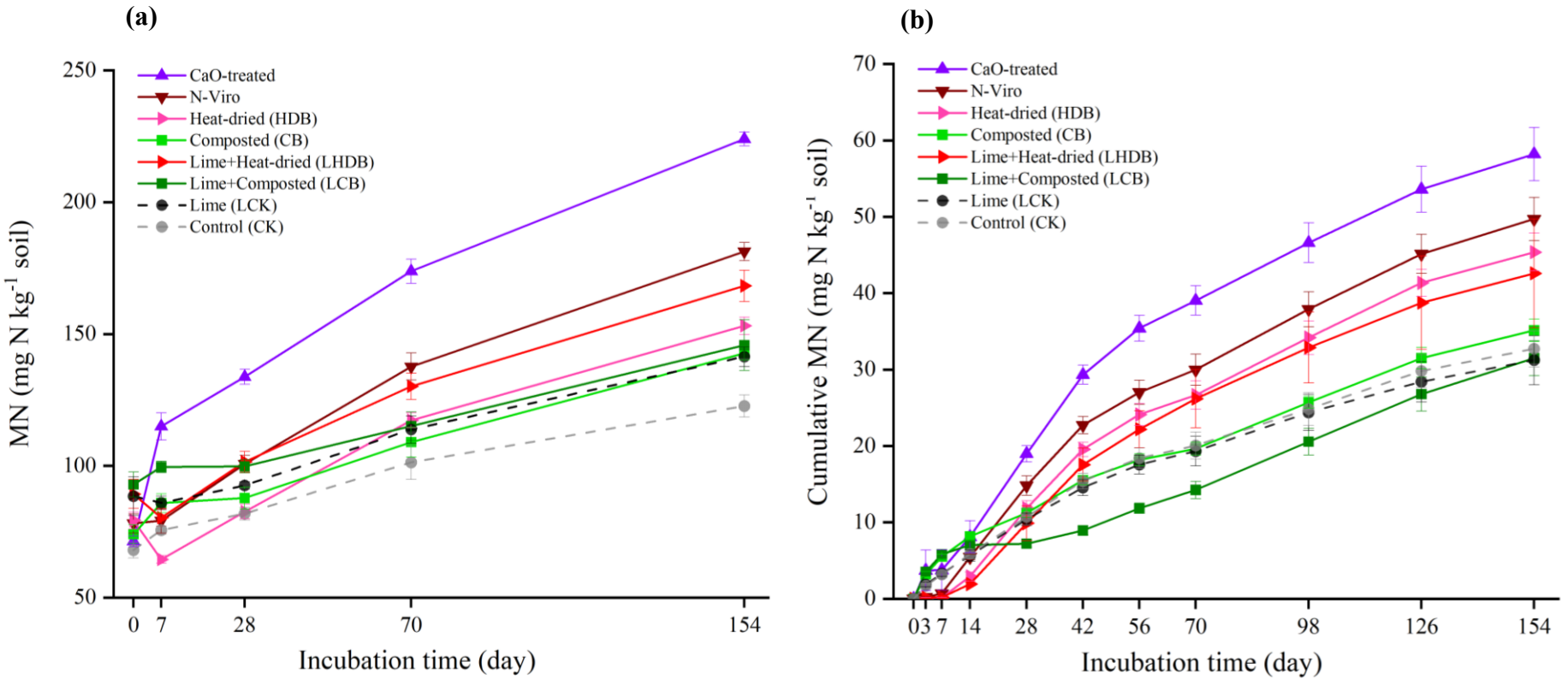


Figure 3. 7 Mineral N (MN) in non-leached incubated soils (a) and cumulative MN from leached incubated soils (b) (note the different y-axis scales). Points are expressed on a dry weight basis (dw) and represent means. Error bars represent standard deviations of the means.

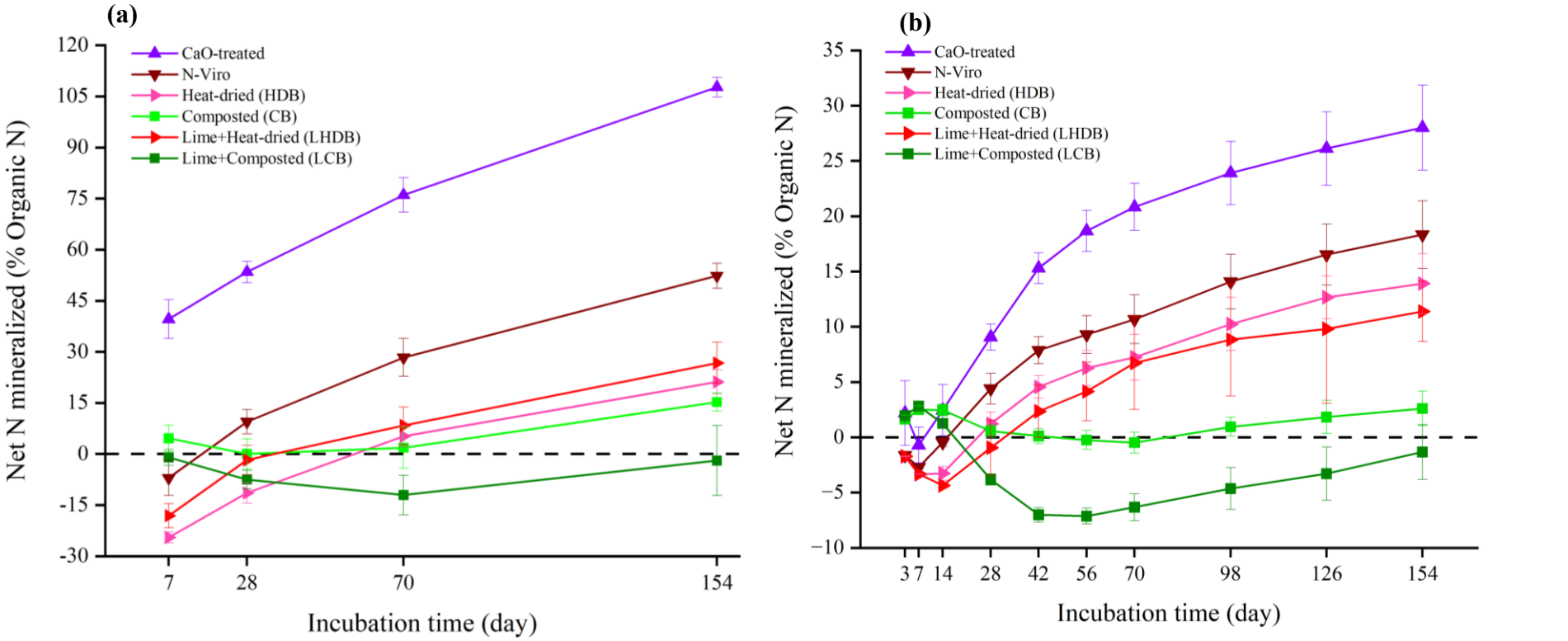


Figure 3. 8 Percentage of organic N (ON) mineralized by amendments during non-leached (a) and leached (b) incubation (note the different y-axis scales). Points are expressed on a dry weight basis (dw) and represent means. Error bars represent standard deviations of the means. (Horizontal dashed line at 0 indicates the amount N mineralized is equivalent to the amount of N immobilized).

3.3.3.2 N mineralization in Soils from Leached Incubation Study

MN collected in the initial leaching is not considered to have been mineralized, so it was not included in the cumulative release of MN from leached incubated soils. Similar patterns of cumulative MN were observed between HDB and LHDB, CB and LCB, and CK and LCK (Fig. 3.7 (b)). Similar patterns of net N mineralized were observed between HDB and LHDB, and CB and LCB (Fig. 3.8 (b)). The cumulative release of MN in all the treatments over time can be generally divided into three phases: 1) initial rapid or slight increase; 2) modest increase; 3) slow and steady increase.

Table 3. 12 Effect of Incubation Time and Treatment on soil cumulative MN from the leached incubation study (mg N kg^{-1} soil).

Treatment	Initial MN Leached (Day 0)	Day			
		7	28	70	154
CaO-treated	52.84 (2.57)	2.56 (1.48) N	19.12 (1.07) H	39.22 (1.93) CD	58.45 (3.46) A
N-Viro	46.62 (6.47)	0.69 (0.14) O	14.78 (1.27) I	29.94 (2.05) EFG	49.70 (2.86) AB
Heat-dried (HDB)	42.19 (4.15)	0.14 (0.03) P	11.83 (0.99) IJK	26.63 (1.90) FG	45.35 (2.50) BC
Composted (CB)	41.13 (4.56)	5.51 (0.32) M	11.25 (0.57) JK	19.62 (0.84) H	35.14 (1.45) DE
Lime + Heat-dried (LHDB)	48.97 (3.05)	0.18 (0.11) OP	9.76 (2.28) KL	26.07 (3.84) G	43.06 (2.49) BC
Lime + Composted (LCB)	51.60 (3.88)	5.81 (0.31) M	7.18 (0.47) LM	14.23 (1.13) IJ	31.45 (2.30) EFG
Lime (LCK)	45.07 (2.99)	3.29 (0.36) N	10.38 (0.82) K	19.27 (1.94) H	31.16 (3.23) EFG
Control (CK)	35.78 (4.71)	3.18 (0.45) N	10.72 (1.03) JK	20.05 (1.79) H	32.70 (2.34) DEF

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

At the end of incubation, the total MN detected from leached incubation (initial MN leached + cumulative MN) was almost 1.75-2.01-fold smaller than that from the non-leached incubation (Table 3.12). Cumulative MN decreased in the order CaO-treated > N-Viro > HDB >

LHDB > CB > LCB > CK > LCK. There were no significant differences in the release of MN between CaO-treated and N-Viro, HDB and LHDB, CB and LCB, and CK and LCK, respectively. A substantial amount of MN released from N-Viro was similar to that released from HDB and LHDB.

Table 3. 13 Effect of Incubation Time and Treatment on net N mineralized (% organic N) from biosolids from the leached incubation study.

Treatment	Day			
	7	**28**	**70**	**154**
CaO-treated	-0.70 (1.62) IJKL	9.06 (1.18) DE	20.84 (2.12) B	28.01 (3.84) A
N-Viro	-2.71 (0.16) JKLM	4.40 (1.39) FGH	10.68 (2.20) DE	18.33 (3.07) BC
Heat-dried (HDB)	-3.37 (0.03) KLM	1.21 (1.08) HIJ	7.24 (2.06) EF	13.90 (2.71) CD
Composted (CB)	2.48 (0.34) GHI	0.56 (0.59) HIJK	-0.48 (0.93) IJKL	2.58 (1.57) GHI
Lime + Heat-dried (LHDB)	-3.30 (0.12) KLM	-0.93 (2.51) IJKL	6.72 (4.20) EFG	11.38 (2.72) DE
Lime + Composted (LCB)	2.80 (0.33) GHI	-3.79 (0.51) LM	-6.26 (1.20) M	-1.31 (2.48) IJKL

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

Net N immobilization dominated in LCB treatment at most sampling times and occurred in other treatments at the early stages of incubation (day 7 or/and day 28) except in the CB treatment where net immobilization occurred at a relatively later stage of incubation (day 70) (Figure 3.8 (b) and Table 3.13). The net N mineralized in amended soils was in decreasing order: CaO-treated biosolids (28.01 %) > N-Viro (18.33 %) > HDB (13.90 %) > LHDB (11.38 %) > CB (2.58 %) > LCB (-1.31 %) (Table 3.13). The application of lime did not result in a significant difference between HDB and LHDB during the whole incubation, which is in agreement with the results from our non-leached incubation study. With the addition of lime, no significant difference was observed between CB and LCB at the beginning and end of the 154-day incubation, but significantly lower net N was mineralized from LCB than CB at day 28 and 70. The increases of

net N mineralized during the whole incubation period (from day 7-154) were significant in all treatments except CB and LCB, which agrees with previous observations in our non-leached incubation study.

3.3.3.3 Modeling N Mineralization from Biosolid Amended Soils

The parameter estimates calculated using the first-order exponential model are listed in Table 3.14. The highest estimated N_o value was obtained for LCB (105.43 mg N kg^{-1}), followed by LHDB (72.78 mg N kg^{-1}), HDB (69.69 mg N kg^{-1}), CaO-treated (68.59 mg N kg^{-1}), N-Viro (67.55 mg N kg^{-1}), and CB (43.59 mg N kg^{-1}). CB and HDB's potentially mineralizable N in limed soil was 2.42 and 1.04-fold greater than in un-limed soil.

Table 3. 14 Parameter estimates for N mineralization from soils receiving different biosolid treatments using a first-order kinetic model.

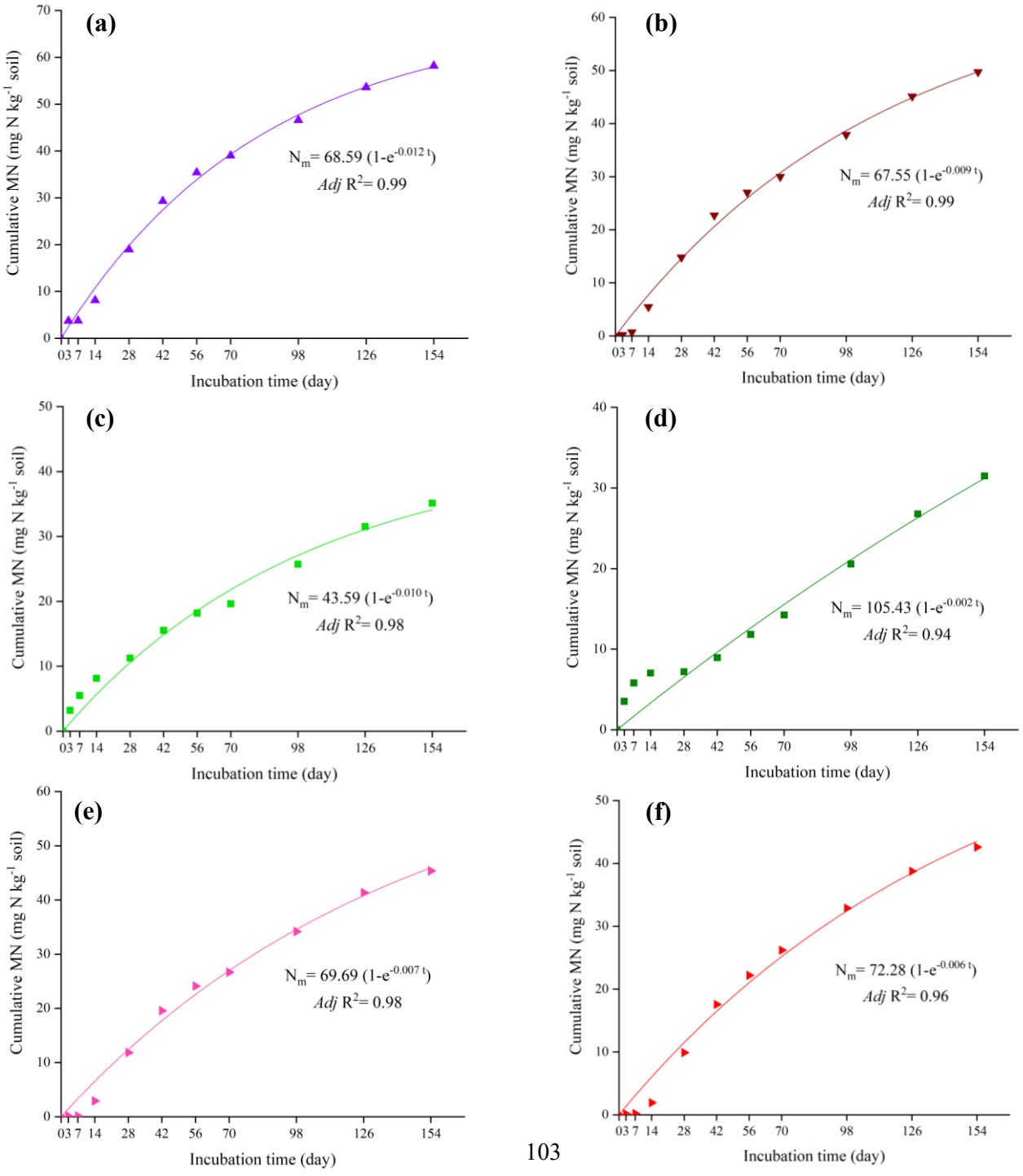
Treatment	N_o (mg N kg^{-1})	k (day $^{-1}$)	*Adj.* R^2	MSE
CaO-treated	68.59 (2.57)	0.012 (0.001)	0.99	5.98
N-Viro	67.55 (3.54)	0.009 (0.001)	0.99	4.60
Heat-dried (HDB)	69.69 (5.20)	0.007 (0.001)	0.98	4.63
Composted (CB)	43.59 (2.26)	0.010 (0.001)	0.98	2.97
Lime + Heat-dried (LHDB)	72.28 (11.07)	0.006 (0.001)	0.96	10.42
Lime + Composted (LCB)	105.43 (46.53)	0.002 (0.001)	0.94	5.24
Lime (LCK)	37.60 (1.73)	0.011 (0.001)	1.00	2.56
Control (CK)	39.58 (1.61)	0.011 (0.001)	0.98	2.08

Mean values (n = 5) are presented, with standard errors in parentheses.

As shown in Figure 3.9, the rate of N mineralization showed a decreasing trend with incubation time. Large fluctuations in N mineralization rate mainly appeared in biosolids-amended soils (i.e., biosolids-amended or biosolids + lime-amended) from day 0 to day 70. However, from day 70 onwards, all treatments remained relatively stable. The estimated mineralization rate constant k was the lowest in LCB (0.002 day^{-1}) and increased in the following order: LHDB (0.006 day^{-1}), HDB (0.007 day^{-1}), N-Viro (0.009 day^{-1}), CB (0.010 day^{-1}), CaO-treated (0.012 day^{-1}).

The first-order kinetic model fitted well with our data, which was represented by the high

adjusted coefficient of determination (*Adj* R^2) values for each treatment of 0.99, 0.99, 0.98, 0.98, 0.96, and 0.94 for CaO-treated, N-Viro, HDB, CB, LHDB, and LCB treatments, respectively. The curve fits in limed soils without organic amendments had an *Adj* R^2 value of 1.0. However, the model predicted a wide range of N_o values for LCB (58.90-151.96 mg N kg^{-1}) and LHDB (61.21-83.35 mg N kg^{-1}).



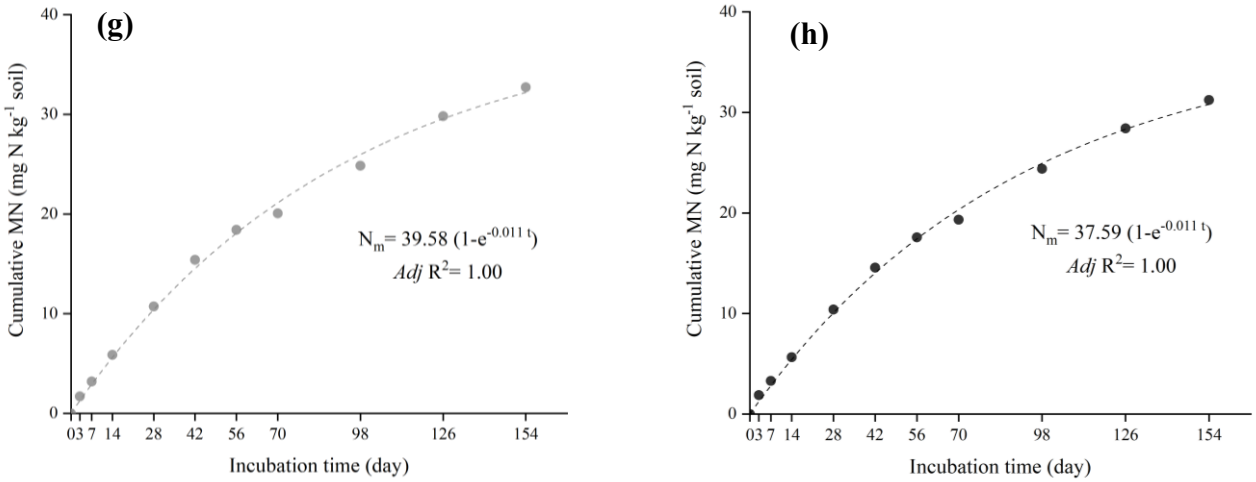


Figure 3. 9 Cumulative mineral nitrogen (MN) from leached incubated soils: (a) CaO-treated; (b) N-Viro; (c) CB; (d) LCB; (e) HDB; (f) LHDB; (g) CK; and (h) LCK. Points represent observed data (means), and lines represent a first-order kinetic model fit to the observed data. Error bars were not shown for visual clarity.

3.3.4 N-acquiring Enzyme Activities in Soils

3.3.4.1 LAP Activity

As shown in Table 3.15, soil LAP activities varied significantly among treatment, incubation time, and their interactions. The changes in soil LAP activity were significant only under N-Viro, HDB, and LHDB treatments throughout the entire incubation (Table 3.16). The dramatic decreases in LAP activity were found under N-Viro and HDB treatments at the end of incubation. In addition to the above-mentioned decrease, LHDB treatment also experienced a significant increase in LAP activity at the early stage of incubation (day 7). At each incubation time, the LAP activities in CK and LCK were similar to those in all the other treatments. The soil LAP was more active at day 7, 28, and 70, and was present at low or even undetectable levels at the start and end of incubation (Fig. 3.10 (a)). Similar responses (i.e., an initial increase followed by a decrease, with the peak values occurring at day 28) were observed for the LAP activity in all treatments except CK.

Table 3. 15 ANOVA p-values for the main and interaction effect of incubation time and treatment on soil LAP (µmol *p*NA g^{-1} soil h^{-1}), NAG (µmol *p*NP g^{-1} soil h^{-1}), and urease (mg NH_4^+-N kg^{-1} soil h^{-1}) activity.

	LAP Activity	NAG Activity	Urease Activity
Incubation time	<.0001	<.0001	<.0001
Treatment	0.0002	<.0001	<.0001
Incubation time × Treatment	**0.0208**	**<.0001**	**<.0001**

Significant effects that require multiple means comparison are highlighted in bold.

Table 3. 16 Effect of Incubation Time x Treatment interaction on soil LAP activity (µmol *p*NA g^{-1} soil h^{-1}).

Treatment	Day				
	0	**7**	**28**	**70**	**154**
CaO-treated	0.02 (0.13) BCDEFG	0.00 (0.05) DEFG	0.16 (0.22) ABCDEFG	0.14 (0.19) ABCDEFG	0.01 (0.03) CDEFG
N-Viro	0.01 (0.06) BCDEFG	0.16 (0.16) ABCDEFG	0.26 (0.13) ABCD	0.23 (0.31) ABCDEF	0.00 G
Heat-dried (HDB)	0.07 (0.09) ABCDEFG	0.25 (0.19) ABCDE	0.30 (0.16) ABC	0.27 (0.17) ABCD	0.00 (0.01) FG
Composted (CB)	0.02 (0.04) BCDEFG	0.03 (0.14) ABCDEFG	0.21 (0.06) ABCDEFG	0.11 (0.12) ABCDEFG	0.01 (0.07) BCDEFG
Lime + Heat-dried (LHDB)	0.00 G	0.32 (0.32) AB	0.37 (0.22) A	0.10 (0.12) ABCDEFG	0.01 (0.07) DEFG
Lime + Composted (LCB)	0.12 (0.31) ABCDEFG	0.02 (0.08) BCDEFG	0.17 (0.10) ABCDEFG	0.10 (0.12) ABCDEFG	0.00 G
Lime (LCK)	0.00 (0.02) DEFG	0.05 (0.03) ABCDEFG	0.16 (0.07) ABCDEFG	0.05 (0.10) ABCDEFG	0.00 (0.02) EFG
Control (CK)	0.00 (0.01) FG	0.10 (0.13) ABCDEFG	0.06 (0.06) ABCDEFG	0.13 (0.09) ABCDEFG	0.03 (0.24) ABCDEFG

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

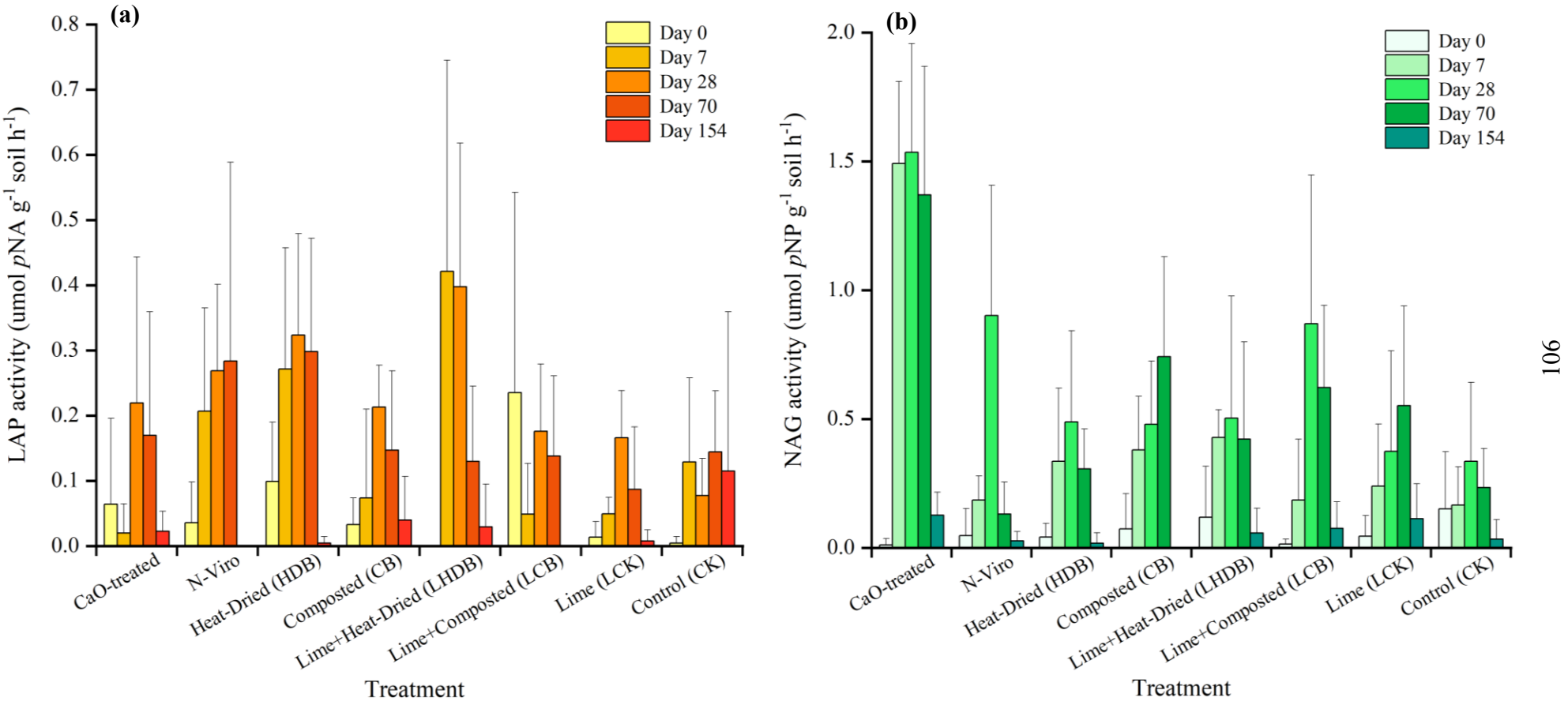


Figure 3. 10 Change of LAP (a) and NAG (b) activity in soils during incubation. Values are expressed on a dry weight basis (dw) and represent means (n=5). Error bars represent standard deviations of the means (n=5).

3.3.4.2 NAG Activity

Soil NAG activities varied significantly among treatment, incubation time, and their interactions (Table 3.15). During the incubation, significant changes in soil NAG activity were detected in all treatments except for LHDB and CK, where NAG activities remained relatively stable (Table 3.17). Similar to LAP, NAG activity in most treatments showed a constant increase until day 28 and subsequently dropped to a low or undetectable level across all treatments (Fig. 3.10 (b)). At each incubation time, CK showed similar NAG activity values to the other treatments, with the exception that NAG activities were markedly higher in CaO-treated than in CK at day 7, 28, and 70, respectively.

Table 3. 17 Effect of Incubation Time x Treatment interaction on soil NAG activity (µmol *p*NP g^{-1} soil h^{-1}).

Treatment	Day				
	0	7	28	70	154
CaO-treated	0.00 (0.03) LM	1.48 (0.32) AB	1.51 (0.42) A	1.33 (0.50) ABC	0.10 (0.09) EFGHIJKLM
N-Viro	0.01 (0.11) IJKLM	0.18 (0.10) DEFGHIJKLM	0.84 (0.51) ABCD	0.09 (0.12) FGHIJKLM	0.01(0.04) IJKLM
Heat-dried (HDB)	0.02 (0.05) HIJKLM	0.29 (0.28) DEFGHIJKLM	0.45 (0.35) ABCDEFGHI	0.29 (0.15) DEFGHIJKLM	0.00 (0.04) LM
Composted (CB)	0.02 (0.14) HIJKLM	0.35 (0.21) CDEFGHIJKL	0.45 (0.25) ABCDEFGHI	0.66 (0.39) ABCDEF	0.00 M
Lime + Heat-dried (LHDB)	0.04 (0.20) GHIJKLM	0.42 (0.11) BCDEFGHIJK	0.43 (0.47) ABCDEFGHIJ	0.35 (0.38) CDEFGHIJKL	0.03 (0.10) HIJKLM
Lime + Composted (LCB)	0.01 (0.02) KLM	0.12 (0.24) DEFGHIJKLM	0.79 (0.58) ABCDE	0.59 (0.32) ABCDEFG	0.03 (0.10) HIJKLM
Lime (LCK)	0.02 (0.08) IJKLM	0.16 (0.24) DEFGHIJKLM	0.27 (0.39) DEFGHIJKLM	0.50 (0.39) ABCDEFGH	0.06 (0.14) FGHIJKLM
Control (CK)	0.08 (0.22) FGHIJKLM	0.13 (0.15) DEFGHIJKLM	0.24 (0.31) DEFGHIJKLM	0.21 (0.15) DEFGHIJKLM	0.01 (0.08) JKLM

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

3.3.4.3 Urease Activity

Incubation time, treatment, and the interaction of incubation time and treatment all significantly affected soil urease activity (Table 3.15). As presented in Table 3.18, no significant changes were seen in LCB, LCK, and CK during the entire incubation period. Soil urease activities in CaO-treated, N-Viro, HDB, and LHDB treatments substantially increased after 7 days of incubation. After the initial increases, such high levels of urease activities were sustained up to the end of incubation, except for HDB treatment (Fig. 3.11). Under HDB treatment, urease activity peaked at day 7, followed by a dramatic decrease and a continuous gradual decrease afterward. The urease activities in CB treatment between days 0, 7, and 28 were not significantly different, while after 70 days, the urease activity was significantly lower than that at the start of incubation.

Table 3. 18 Effect of Incubation Time x Treatment interaction on soil urease activity (mg NH_4^+-N kg^{-1} soil h^{-1}).

Treatment	Day				
	0	7	28	70	154
CaO-treated	0.04 (0.15) P	2.44 (0.71) GHIJKLM	2.74 (0.38) FGHIJKL	4.07 (0.56) CDEFGH	3.97 (0.60) CDEFGHI
N-Viro	0.97 (0.72) NO	8.05 (2.47) AB	8.50 (0.93) A	9.70 (1.50) A	10.84 (1.98) A
Heat-dried (HDB)	0.65 (0.39) O	11.11 (1.35) A	5.53 (1.06) BC	3.60 (0.80) CDEFGHIJ	2.55 (0.39) GHIJKLM
Composted (CB)	4.01 (0.41) CDEFGH	3.64 (0.47) CDEFGHIJ	2.77 (0.72) FGHIJKL	2.21 (0.56) IJKLMN	2.02 (0.46) JKLMN
Lime + Heat-dried (LHDB)	1.19 (0.69) MNO	4.24 (0.84) CDEFG	4.99 (0.59) CDE	4.25 (0.91) CDEFG	5.11 (0.53) CD
Lime + Composted (LCB)	5.62 (1.35) BC	5.13 (1.13) CD	4.53 (0.81) CDEF	5.39 (0.61) BCD	5.47 (0.79) BC
Lime (LCK)	2.31 (0.31) HIJKLM	2.46 (0.57) GHIJKLM	3.27 (0.38) DEFGHIJ	3.54 (0.21) CDEFGHIJ	3.03 (0.63) EFGHIJK
Control (CK)	1.43 (0.20) LMNO	1.65 (0.42) KLMNO	1.42 (0.54) LMNO	1.49 (0.28) LMNO	1.35 (0.49) LMNO

Mean values (n = 5) are presented on a dry weight basis (dw), with standard deviations in parentheses. Means followed by different letters represent significant differences ($p < 0.05$).

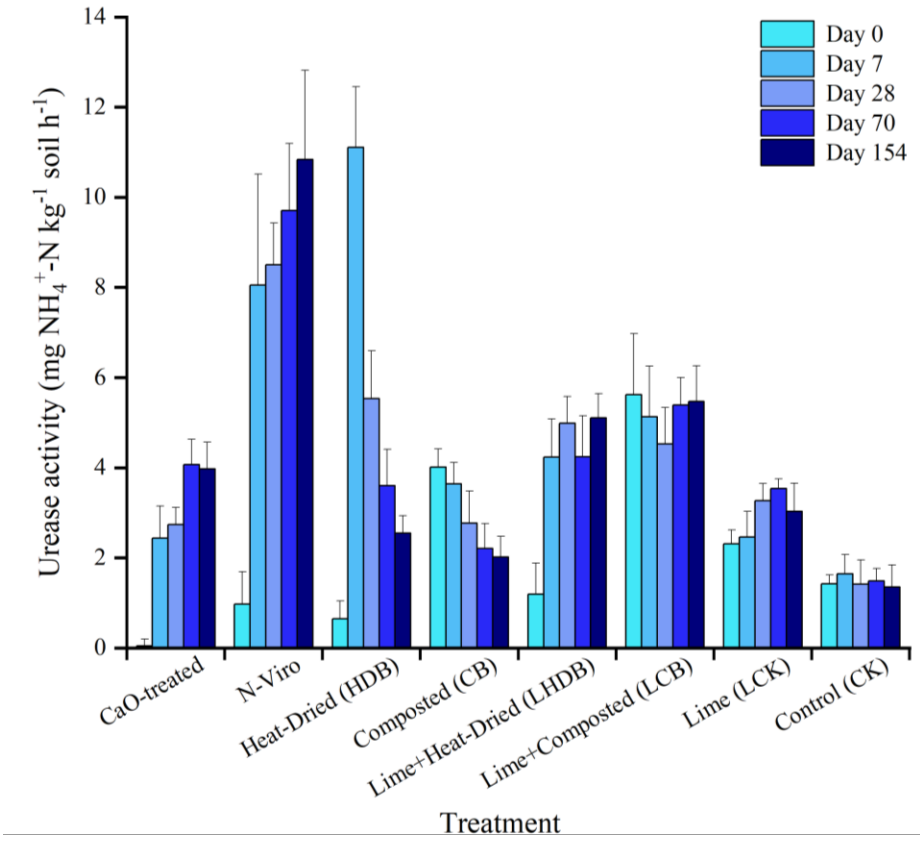


Figure 3. 11 Changes in urease activity over time in soils with different treatments. Values are expressed on a dry weight basis (dw) and represent means (n=5). Error bars represent standard deviations of the means.

Overall, an upward trend in soil urease activity was observed in treatments including pH adjustment, such as CaO-treated, N-Viro, LHDB, LCB, and LCK (Fig. 3.11). LHDB, LCB, and LCK had greater urease activity than HDB, CB, and CK, respectively, at most sampling times (Table 3.18).

3.4.5 Relationships Between Enzyme Activities with Other Soil Properties

Table 3.19 presents the Pearson's correlation coefficients. It shows that LAP activity was inversely related to all other soil chemical properties (weak but significant relationships with OM, NO_3^-N, MN, and net N mineralized). On the contrary, NAG activity was positively correlated with all the other soil chemical properties, and there was a highly significant ($p < 0.001$) correlation between NAG and soil pH. Urease activity had positive associations with pH, NO_3^-N, MN, LAP

activity, and NAG activity, but it had negative associations with OM, NH_4^+-N, and net N mineralized. Net N mineralized was strongly and positively correlated with pH, NO_3^-N, and MN ($p < 0.001$).

Table 3. 19 Pearson's correlation coefficients (r) between soil chemical and biological properties in soil samples (n=200) from the non-leached incubation study.

	Urease	NAG	LAP	Net N mineralized	MN	NH_4^+-N	NO_3^--N	OM
pH	0.1	0.34^{***}	-0.03	0.62^{***}	0.30^{***}	0.29^{***}	0.24^{***}	0.08
OM	-0.01	0.05	-0.14^{*}	0.05	-0.01	0.25^{***}	-0.05	
NO_3^--N	0.22^{**}	0.04	-0.15^{*}	0.83^{***}	0.99^{***}	-0.39^{***}		
NH_4^+-N	-0.23^{**}	0.17^{*}	-0.01	0.01	-0.24^{***}			
MN	0.19^{**}	0.01	-0.17^{*}	0.86^{***}				
Net N mineralized$^+$	-0.13	0.18^{*}	-0.29^{**}					
LAP	0.26^{***}	0.2^{**}						
NAG	0.04							

+Net N mineralized (n=120, CK and LCK data were excluded); * = Significance at 0.05 ($p < 0.05$), ** = significance at 0.01 ($p < 0.01$), *** = significance at 0.001 ($p < 0.001$). The units of each variable are those given in Tables 3.4 and 3.15. NO_3^--N, NH_4^+-N, and MN have the same units.

3.4 Discussion

3.4.1 Soil pH and Leachate pH Changes During Incubation

It was clear from the results that treatments could be divided into three broad categories according to the changes in pH and RLE values during incubation: (1) High pH (i.e., CaO-treated); (2) Modest pH (N-Viro, LHDB, LCB, and LCK); (3) Low pH (HDB, CB, and CK) (Fig. 3.5). Group 1 caused a much more profound effect on raising pH values than group 2 and 3, and this may be due to the fact that CaO-treated biosolids increase Ca^{2+} concentrations. CaO-treated biosolids had the highest pH values (pH: 12.69) among all the studied biosolids. Furthermore, it could be relevant that CaO-treated biosolids had a finer particle size, larger surface area, and greater solubility (Li et al., 2019; Mahmud and Chong, 2022). In group 2, LHDB, LCB, and LCK showed insignificant differences both in soil pH and leachate pH. However, N-Viro behaved differently from the other three treatments in soil pH after 28 days (i.e., significantly higher pH

and more than 100 % RLE), indicating its strong capacity to improve soil pH over the long term. This can be closely related to the composition of the alkaline admixtures (i.e., CKD) in the N-Viro biosolids, which contains a combination of mild alkali compounds ($CaCO_3$) and strong alkali compounds (CaO and $Ca(OH)_2$). pH values among Group 3 were statistically similar. Thus, we can conclude that HDB and CB had negligible liming effects and were insufficient to achieve optimal soil pH (i.e., 6.5–7.5) for plant growth. Applying ATB (CaO-treated or N-Viro) or lime alone, or alternatively, lime in combination with HDB and CB, would effectively correct soil acidity.

In the leached incubation study, leaching soil cups with diluted $CaCl_2$ solution can be considered as precipitation events. The relatively smaller pH values in the leachate samples can be explained by the removal of soil exchangeable base cations during heavy precipitation events, such as Ca^{2+}, Mg^{2+}, K^+, and Na^+ (Meng et al., 2019). Besides the volume, the frequency of precipitation can also have a huge impact on the pH level. A total of eleven leaching events were administered during the incubation study, significantly accelerating soil acidification. According to a mapping study across Canada, Atlantic Canada was predicted to experience increased extreme precipitation events because of climate change (Simonovic et al., 2017). Soils in humid regions, such as the Atlantic region, can potentially have more severe acidification problems if not limed as needed.

Overall, pH values for soil and leachate samples tended to decrease in both incubation settings as nitrification proceeded. Leachate pH in all treatments fell dramatically at day 7, but afterwards a significant surge was observed in most of the treatments, with the exception of CaO-treated, LHDB, and LCB. The initial fall might be attributed to the first two short-interval leaching events (day 0 and 3), and the subsequent increase can be related to the relatively long-interval leaching events (day 7 and 14). Soils undergoing leaching events with a longer interval were more

likely to have biochemical processes and respond to treatments. The first two leaching events likely removed a large amount of soil base cations, and then ammonification contributed to the increase of pH between day 7 and 28. After day 28, nitrification started to dominate the soil N cycle over the remainder of the incubation period.

3.4.2 Soil OM Changes During Incubation

Treatments including compost (i.e., CB and LCB) contained the highest soil OM due to the high OM contents (77.32 %) in the compost material. Although CaO-treated had the lowest OM content (18.78 %), soil OM in CaO-treated treatment did not differ statistically from CB and LCB treatments. This is because CaO is highly reactive and may cause the disruption of organic substances in moist soil (Mühlbachová and Tlustoš, 2006). HDB biosolids (61.52 %) had a significantly higher OM content than N-Viro biosolids (31.08 %); however, no significant difference was found in soil OM between HDB and N-Viro treatments. That might also link to the reaction between alkaline materials and soil organic compounds. Lower soil OM contents were observed in treatments with the lime application (LHDB, LCB, LCK) compared to those without lime application (HDB, CB, and CK), suggesting that the preincubation of limed soil for 14 days promoted the decomposition of soil OM despite the fact that some insignificant differences existed. Soils amended with biosolids all had significantly higher OM than control soils, demonstrating that applying organic amendments increases soil OM. The same results were also reported by other studies (Molina-Herrera and Romanyà, 2015; Oldfield et al., 2018; Yang et al., 2021).

Soil OM decreased greatly at day 7 and 28 because of enhanced microbial respiration and decomposition of labile soil OM. During the later incubation stages, soil OM stayed considerably stable, indicating that the soil OM present might be predominantly recalcitrant.

3.4.3 N Mineralization in Soils

Two alkaline-treated biosolids (CaO-treated and N-Viro) had relatively higher N availability, indicating elevated pH increased the solubility of organic N in the soil, and enhanced net N mineralization (Neina, 2019). The initial soil MN flush from the CaO-treated treatment implies that CaO-treated biosolids might pose a risk of NO_3^- leaching or runoff if its application is not timed to match plant uptake. Farmers would need to reduce the application rate or split the application to avoid these negative consequences. Under actual field conditions, CaO-treated biosolids would not be able to achieve such a high value of net N mineralization (107.73 %) due to losses of N through plant uptake, leaching, runoff, gaseous emissions (Cheng et al., 2017; Sun et al., 2020). High concentrations of NH_4^+-N in CaO-treated amended soils were observed within the first 28 days of incubation (Appendix 3 Fig. A3.1), which could lead to NH_3 volatilization, especially in soils with high pH values (pH ≈ 8). CaO might result in an additional mineral N released from the native soil organic N, and consequently contribute to an overestimation of the amount of N mineralized from the CaO-treated biosolids. The potentially mineralizable N N_o of N-Viro (67.55 mg N kg^{-1}) was similar to that of CaO (68.59 mg N kg^{-1}), but N-Viro had a lower mineralization rate constant k, which means N was mineralized slower, and N leaching might be less of a risk.

Net N immobilization was more evident in CB and LCB than in HDB and LHDB. Three reasons might lead to these distinct effects: (1) C/N ratio is positively correlated to fungal growth (Zhang et al., 2019). CB had a higher C/N ratio (25.31 %) than HDB (14.60 %); thus, it was more likely to increase soil fungal biomass and cause N to be immobilized. Some studies have reported that more N was immobilized in fungi-dominated soils than bacteria-dominated soils (Schimel and Bennett, 2004; de Vries et al., 2011; Tahovská et al., 2013); (2) CB had already undergone N mineralization in the composting process and therefore contained more recalcitrant organic N

fractions than HDB. The recalcitrant organic N compounds are difficult to be decomposed by microbes, and the accumulation of these compounds would slow down N mineralization (Khalil et al., 2005); (3) The physico-chemical properties of the soil were affected after the addition of organic amendments, which in turn would influence the N cycling. CB might have a greater potential for improving soil aggregation than HDB by providing more binding agents such as humic-like substances, microbial polysaccharides, and fungal hyphae (Mikha and Rice, 2004). This finding was consistent with previous studies on municipal solid waste compost (Annabi et al., 2007) and manure compost (Sarker et al., 2018). N immobilization in HDB and LHDB during the early phase of incubation can be linked to the high NH_4^+-N concentration (0.15 %) in the HDB because NH_4^+-N is the preferred N source for most soil microorganisms (Geisseler and Scow, 2014). Without the addition of either alkaline materials or bulking agents, the heat drying process led to fewer modifications to the raw sewage solids compared to alkaline treatment and composting. Therefore, there was more easily degradable OM (e.g., simple organic N monomers such as amino acids and amino sugars) in the HDB, which facilitated microbial attack and induced the initial N immobilization in soil (Andrés et al., 2011; Marando et al., 2011). Many studies have found that microbes can take up amino acids immediately and use them as N and C sources (Geisseler et al., 2010, 2012; Tahovská et al., 2013).

The combined effect of CB and lime on the net N mineralized (% organic N) differed from the single effect of CB, and this difference was more prominent over time. In both incubation settings, we observed significantly larger amounts of N were immobilized from LCB than CB (non-leached study: day 70-154; leached study: day 28-70), which was also reflected by the great difference of mineralization rate constant k between these two treatments (LCB: 0.002 day $^{-1}$; CB: 0.010 day $^{-1}$). This result suggested that liming promoted the native soil OM mineralization during the early incubation phase and resulted in a relatively larger pool of recalcitrant soil OM

remaining. Another possible explanation for this could be that liming stimulated microbial activity, and consequently, microorganisms had a higher demand for soil available N. Reduced N mineralization or enhanced immobilization in limed-amended soils with a large C/N ratio was also reported by Cheng et al. (2013). The two leaching events in the leached incubation study (at day 98 and 126) might have diminished the effects of liming, making the net N mineralized (% organic N) from LCB similar to that from CB at the end of incubation. LCB's potentially mineralizable N N_o (105.40 mg N kg^{-1}) was much higher than its cumulative mineralized N N_m (31.45 mg N kg^{-1}) following 22 weeks of incubation, indicating a great amount of the mineralizable organic N fraction might still remain available for further mineralization after 22 weeks. It should be noted that CB and LCB started to slowly re-mineralize the previously immobilized N after two months (Fig. 3.8). Moreover, net N mineralization in LCB did not take place until five months later. Knowing the intensity and time of the remineralization have a crucial impact on enhancing the synchronization between N supply and crop N demand and improving N use efficiency (Chaves et al., 2006; Romero et al., 2015). For example, farmers should adopt the early application of CB (five months before sowing of the crop) and HDB (one month before sowing of the crop) for limed soils so that the immediate N need of crops can be met at the early growth stage.

Smaller release of MN and net mineralized N were obtained from the leached study as compared to the non-leached study because native MN, and possibly large pools of soluble organic N (SON), was removed in the leached study by the initial leaching that occurred at the beginning of the incubation. Due to leaching events, soil microbes were not able to utilize a considerable pool of available MN and some of the soluble OM, leading to reduced N immobilization in the leached incubation study (Dessureault-Rompré et al., 2018). This result is in accordance with the results of Sharifi et al. (2019), who found that the net immobilization in anaerobic digestate-amended soils was more evident in the greenhouse study than in the leached incubation study.

Moreover, N-free solution added after the leaching solution may be not sufficient to compensate the leached ions and maintain soil microbial growth.

The N_o values highly varied over a wide range in LCB and LHDB, which indicates a need for more precision in the parameter estimates and accuracy of the model we used. The zero-order linear model fit N mineralization from LCB treatment better since the data represented the relatively early phase of N mineralization rather than the plateau phase where released MN reaches a stable level and has minor changes (Fig. 3.9 (d)). HDB and LHDB showed logistic behaviors at the beginning of incubation (with an inflection point at around day 28) (Fig. 3.9 (e) and (f)), which was likely due to the slow adaptation of the indigenous soil microorganisms to the new substrates introduced by HDB (Grigatti et al., 2011). In order to decrease the standard error of the estimated N_o and make the model more robust, we might need to use a more complex model (e.g., simple exponential plus logistic) and include more data points. This result agrees with previous observations made by Gil et al. (2011), who demonstrated that the model with more parameters (simple exponential plus linear) explained the N mineralization kinetics in compost (i.e., composted bovine manure and composted biosolids)-amended soils better than the simple exponential model over a longer incubation duration.

3.4.4 N-acquiring Enzyme Activities in Soils

3.4.4.1 LAP and NAG Activity

Soil pH plays an important role in soil enzyme activity because different enzymes have their optimal pH range at which their activity is relatively high (Kivlin and Treseder, 2014). We observed persistent highest levels of NAG activity under CaO treatment from day 7 to 154 (soil pH =7.66 - 7.89). This positive relationship between NAG and soil pH has been reported in other studies (Deng and Tabatabai, 1997; Liu et al., 2017; Xu et al., 2017; Sun et al., 2022). In general, NAG showed higher activity in CB and LCB as compared to HDB and LHDB, which can be

explained by the greater availability of chitin originating from the fungal biomass (McDaniel et al., 2013; Liu et al., 2017) and the greater OM content (Sainju et al., 2022) in the composted biosolids. In contrast, we found higher LAP activity in HDB and LHDB compared to that in CB and LCB, and this is likely attributed to the fact that there were more labile N (protein)-rich substrates in the heat-dried biosolids. Therefore, soil enzymes have different mechanisms in response to how biosolids are generated. The behaviours of the soil LAP and NAG activity in response to biosolids in this study coincide with other studies. A low level of LAP activity was found in litter-amended soils under field as well as under controlled laboratory incubation conditions (Rinkes et al., 2013). Sun et al. (2022) observed significantly higher NAG activities in soils amended with N-Viro and composted biosolids than urea; however, LAP activity was not affected by three annual applications of biosolids. Mattana et al. (2014) reported similar changes (i.e., an initial increase followed by a decrease) in almost all soil enzyme activities they studied, irrespective of the type of biosolids. Most enzymes showed an initial increase in activity followed by a gradual decline, and eventually enzyme activities returned almost to their initial values. Likewise, Kızılkaya and Bayraklı (2005) assessed the effect of anaerobically digested biosolids on soil enzyme activities. They found that all enzyme activities reached their maximum values within the first month and showed continuous decreases for the rest of the incubation period. Overall, the LAP and NAG activities in CK were lower than those in biosolids-amended soils, indicating that the addition of biosolids stimulated microbial activity and increased the microbial demand for N.

Compared to urease activity, LAP and NAG activities showed higher variability among replicates and were unable to be detected sometimes, revealing that the same standard operating procedures (SOP) for LAP and NAG assays needs further improvement. LAP and NAG assays were each performed following the same SOP but the urease assay used a different SOP. The

improvements can include the following aspects: 1) make a more homogenized soil slurry in a homogenizer; 2) optimize LAP and NAG assays by testing out the optimum substrate concentration; 3) optimize LAP and NAG assays by testing out the optimum soil slurry volume: substrate volume ratio in each assay well; 4) compare the results from the spectrophotometric method with that from the fluorescence method if possible. As mentioned in Chapter 1's section 1.2.3.2, the fluorescence method has been used as the main technique in the recent soil extracellular enzyme research. Since using the spectrophotometric method to measure LAP and NAG activities on the organic amendments-treated soils is rarely reported in the literature, the LAP and NAG assays might not work well in the spectrophotometric method. Soil enzymes are secreted by soil microorganisms, and they can also be from root secretion and organic (plant and animal) residues (Tabatabai, 1994; Burns et al., 2013). The formation of enzyme-clay or enzyme-humus complexes can constrain LAP and NAG activities in the later stages of incubation (Geisseler et al., 2010). The potential enzyme activities might also vary with the functional lifespan of the enzymes. Short-lived enzymes tend to lose activity rapidly (Schimel and Weintraub, 2003; Schimel et al., 2017). Furthermore, heavy metals in the biosolids, such as Cu, Zn, and Pb, can potentially damage microbial cells, inactivate enzyme reactions, and consequently lower enzyme activity (Guo et al., 2012; Feng et al., 2016).

3.4.4.2 Urease Activity

The upward trend of observed urease activity in treatments with pH adjustment implied that soil pH also played an essential role in urease activity. The combined application of alkaline materials with organic amendments not only enhanced urease activity successfully but also maintained an elevated level of urease activity for an extended period. The responses of urease in our study were consistent with the findings of previous work by Laxminarayana (2021), which reported greater urease activity in acidic soils receiving lime in combination with farm yard

manure than in soils receiving only farm yard manure or lime. We also observed a lower urease activity under CaO-treated treatment with respect to that under N-Viro, LHDB, and LCB treatments, regardless of whether the differences in urease activity were significant or not. This result shows that the relationship between soil pH and urease activity is not linear. The optimum soil pH for urease is probably near-neutral (Singh and Nye, 1984; Zhang et al., 2014; Fisher et al., 2016). Higher soil pH levels (> 7.5) could potentially suppress urease activity.

Soils amended with biosolids all initially experienced significant increases in urease activity, likely due to the introduction of organic substrates (Schlatter et al., 2017) and microorganisms (Kao et al., 2006). Despite the decrease in urease activity during the later incubation stage, Mattana et al. (2014) and Kızılkaya and Bayraklı (2005) both reported a significant increase in urease activity in neutral soils receiving composted biosolids and heat-dried biosolids, and anaerobically digested biosolids, respectively, during the early incubation stage. However, biosolids-derived pollutants can have detrimental impacts on soil enzyme activity (Sharma et al., 2017). A study by Su and Wong (2004) found that applying alkaline-treated biosolids or liming the soil before biosolids addition reduced the bioavailability of heavy metals. In our study, the downward trend of urease activity in the HDB and CB treatments indicated that urease activity was inhibited during more prolonged incubation in the absence of pH adjustment. This inhibiting effect may be related to the increased solubility and mobility of pollutants in soils amended with biosolids only (Benítez et al., 2001; Basta et al., 2005; Samara et al., 2022).

3.4.4.3 Relationships Between Enzyme Activities with Other Soil Properties

A recent study conducted by Sun et al. (2022) evaluated the response of enzyme activity to three consecutive years of biosolids application (mesophilic anaerobic digested biosolids, N-Viro, and composted biosolids) in a corn-cultivated field. They demonstrated that the NAG activity had positive and significant relationships with soil NH_4^+ concentrations ($r = 0.38$) and soil

pH (r = 0.28), which was in good agreement with our results, where the correlation coefficients for these two variables were 0.17 and 0.34, respectively. Ekenler and Tabatabai (2002) also demonstrated that increased NAG activity was associated with enhanced soil N availability.

The negative relationship between the MN in soil and LAP activity (r = -0.17) was in line with the study of Bowles et al. (2014), indicating that there were probably low amounts of labile organic N fractions (e.g., amino acids) left in the soil for LAP to hydrolyze. Another possible explanation for this is that proteolytic microorganisms tend to invest fewer resources towards obtaining N from degrading peptides when the soil has a large pool of available mineral N, because N is not a limiting nutrient for microbial growth anymore (Wallenstein et al., 2012; Zhang et al., 2016).

The negative relationship between urease and NO_3^--N has been generally reported (Piotrowska et al., 2006; Mondini et al., 2008; Yang et al., 2008; Biswas et al., 2019), but it was not the case in our study, where urease and NO_3^--N had a significant positive relationship (r = 0.22). Although the aforementioned result was inconsistent with most previous studies, we found greater urease activity with the decline of net N mineralized (% organic N) (r = -0.13), suggesting that urease activity can be promoted when the N availability is below the microbial N demand and can be repressed when the N availability exceeds the microbial N demand (Geisseler et al., 2010). The hydrolysis of urea mediated by urease theoretically will contribute to the accumulation of NH_4^+-N and subsequent increase of NO_3^--N as a result of nitrification, thus the positive relationship between urease and NO_3^--N was reasonable and has also been observed in Bai et al. (2009) and Kátai et al. (2022). We did observe a significant negative relationship (r = -0.58, $P < 0.001$) after seven days of incubation (Appendix 4 Table. A4.1). Pooling the data set from all incubation times likely impacted the overall correlation analysis so that the differential results were obtained. These results also implied that time is an important contributing factor to the

changes in enzyme activity. The behaviour pattern of enzyme activities could reveal the substrate availability, soil microbial community composition, and soil fertilization and cropping practices (Vepsäläinen et al., 2001). More long-term field- or laboratory-based research is required to monitor the changes in soil enzyme activities at multiple time points (e.g., different stages of the growing season) so that we could have a clearer picture of the relationships between soil enzyme activities and other soil characteristics. We detected a weak and positive but insignificant correlation between urease and pH ($r = 0.1$), which was similar to the findings of Błońska et al. (2016) and Kátai et al. (2022). While contrasting results were obtained in the study conducted by Piotrowska et al. (2006) and Bai et al. (2009), who both found a moderate, negative, and significant correlation ($r = -0.52$ and -0.57, respectively). This could be ascribed to the fact that the urease activity was restricted in the studied alkaline soils ($pH > 8$), whereas most soil treatments in our study had acid-to-neutral pH values ($pH < 5$-7).

3.5 Conclusion

The results of this study showed that N mineralization in an acidic soil differed among the biosolids type, with the highest net N mineralization from alkaline-treated biosolids at the end of the incubation period. Net N mineralization from CaO-treated biosolids was significantly higher than that from N-Viro biosolids, which might be associated with the properties of the added alkaline materials (e.g., composition, particle size, reactive surface area, and solubility). Both heat-dried biosolids and composted biosolids showed relatively low net N mineralization, with composted biosolids in limed soil (i.e., LCB) exhibiting net N immobilization during most of the incubation time. These findings suggest that CaO-treated biosolids have the highest N leaching or runoff potential if its application is not timed to match plant uptake. Farmers would need to reduce the application rate or split the application. Heat-dried and composted biosolids have less risks of N loss, but in order to meet the microbial and plant needs, farmers should increase the application

rates or adopt the early applications; or alternatively, implement the integrated nutrient management approach by applying them in combination with inorganic fertilizer. When considering the application of composted biosolids to limed soils, farmers would need to adjust their original fertilization strategies since more N was retained in LCB than in CB.

N mineralization in biosolids-amended soils followed first-order kinetics (*Adj* R^2= 0.94-0.99). However, the large variability in the predicted potentially mineralizable N for LCB and LHDB may reflect the need to improve the model accuracy by using a more complex model or/and including more data points. Although two alkaline-treated biosolids had similar potentially mineralizable N, mineral N was released more slowly from N-Viro-amended soils than from CaO-treated biosolids-amended soils. Overall, MN release from biosolids-amended soils and net N mineralized from various biosolids showed similar trends in leached and non-leached incubation studies. Due to the potential removal of soluble organic matter and base cations during the leaching events, N mineralization and immobilization were less evident in the leached incubation study. This observation implies that more frequent and/or intense precipitation events can enhance the risk of N losses and reduce the pool of substrates for plant and microbial uptake. In addition, the measured pH for the leachates was much lower than the soils, indicating extreme precipitation events can accelerate soil acidification. HDB and CB had negligible liming effects and could not adequately raise soil pH to a desirable level for plant growth. N-Viro biosolids can effectively correct soil acidity and gradually supply N, which enables it to be environmentally and economically attractive to farmers.

The activities of N-acquiring enzymes (LAP, NAG, and urease) in biosolids-treated soils all increased to a certain extent during the incubation when compared to the control soil. We can conclude that the addition of biosolids can stimulate microbial activity. The responses of soil N-acquiring enzyme activities to biosolids application were related, not only to the biochemical

properties of biosolids, but also to the specific preferences of enzymes. LAP showed relatively higher activity in HDB and LHDB as there are more labile and easily degradable N-rich substrates (proteins and their degradation products) in the heat-dried biosolids. A significant positive correlation was found between NAG activity and soil pH; therefore, soils amended with CaO-treated biosolids had the highest NAG activity most of the incubation time. We also observed high NAG activity under CB and LCB treatments, which could be relevant to the greater fungal abundance and OM content in the composted biosolids. Urease activity was greater in soils with modest pH (i.e., N-Viro, LHDB, LCB, and LCK) or in soils with high proportions of labile N (i.e., HDB and LHDB). LAP and NAG activities showed an initial increase followed by a gradual decline, and eventually both enzyme activities returned close to their initial values. In contrast, the changes in urease activity over time was less drastic.

Chapter 4: Conclusion

The main purposes of this study were to examine the effects of different biosolids treatment processes on the N forms in the resultant biosolids and their influence on soil N mineralization and N-acquiring enzyme activities in an acidic soil.

We have shown that biosolids treatment processes had significant effects on the contents and forms of N as well as other chemical properties of biosolids (Chapter 2). The majority of N in all types of biosolids remained in organic forms. However, each N form and the contributions of ON or NH_4^+-N to TN vary between different biosolids types. Minimal TN loss was observed in the HDB relative to the RS, as heat drying is the simplest among all the treatments studied. In contrast, more pronounced TN decreases were found in the ATB and CB, which was likely caused by the volatilization of NH_3 and the addition of external materials. Although RS were diluted by sawdust and alkaline materials at a similar rate in the composting and heat drying process, respectively, there were large variations in each N form and other key chemical properties between ATB and CB, due to the nature of the materials added. The type of alkaline material (CaO or kiln dust and fly ash) also had an impact on the chemical properties of the resultant ATB.

Two parallel leached and non-leached laboratory soil incubation experiments were carried out to evaluate N dynamics and N-acquiring enzyme activities in an acidic soil amended with different biosolids generated from the same source (Chapter 3). N mineralization followed very similar patterns during leached and non-leached incubation studies. The potential removal of soluble OM and base cations during the leaching events caused lower mineralization and immobilization in the leached incubation study. N availability differed among various biosolids, and it increased in the order CB < HDB < N-Viro < CaO-treated. The results implied that N management practices should be developed for different biosolids to synchronize soil N supply with crop N demand. The N mineralization data fitted well to a simple first-order kinetic model.

The discrepancy between the observed and predicted MN was mainly observed in the early incubation phase (Day 0-28), but this discrepancy diminished over time. The observed mineralization for CB was more rapid than that predicted by the model, whereas the observed mineralization for HDB and ATB were slower than that predicted by the model. These findings suggested that adaptation or selection of specific microbial groups may occur in soils shortly after biosolids application.

Furthermore, the application of biosolids stimulated microbial activity and promoted the selected enzyme activities, due to the introduction of organic substrates and microorganisms. LAP, NAG, and urease varied significantly among treatment, incubation time, and their interactions. LAP showed relatively higher activity in HDB-amended soils, and it was likely that the heat drying process was able to retain more readily biodegradable N-rich substrates. NAG showed relatively higher activity in soils amended with CaO-treated and CB, which could be related to the high soil pH (pH > 7.5) and greater fungal populations caused by alkaline treatment (i.e., CaO addition) and composting process, respectively. Urease showed relatively higher activity in soils with modest pH (pH < 7) or soils with larger labile N pools, which can be induced by applying N-Viro and HDB. LAP and NAG appeared to be more active at day 7, 28, and 70 and showed low or even undetectable levels at day 0 and 154, while urease activity responded to biosolids less rigorously than LAP and NAG.

Recommendations for the future work are as follows:

1. The net N mineralization (% ON) in CaO-treated biosolids was overestimated in our non-leached incubation study. Since soils were incubated at an optimal temperature (25°C) and were amended with CaO-treated (soil pH ≈ 8), there was a high potential for N volatilization. Therefore, future work should look at the volatilization of NH_3 from the CaO-treated amended soils.

2. This study was carried out under controlled laboratory conditions in the absence of plants, so further work is needed to investigate N dynamics and N-acquiring enzymes in un-limed or limed soils amended with different biosolids generated from the same source under realistic field conditions. In addition, more long-term field- or laboratory-based research is required to monitor the changes in soil enzyme activities so that we can have a better understanding of the relationships between soil enzyme activities and other soil characteristics.

3. In this study, we found the weaknesses of the simple exponential first-order kinetics model to describe the N release from biosolids in the early stage of incubation. The model performance should be further improved with more complex models (e.g., simple exponential plus logistic) or/and by adding more sampling times in the early incubation phase.

4. We found that LAP and NAG exhibited higher variability in activity among replicates. Future work is thus required to improve the methodology for measuring LAP and NAG potential activities spectrophotometrically.

5. We only measured mineral N contents in the incubated soils at each sampling time. Future studies can also examine other organic N pools and express potential enzyme activities relative to those specific N pools.

6. In this study, only soil samples were measured for potential enzyme activities. The inherent microorganisms in the biosolids could also have influences on the soil enzyme activities. Therefore, we suggest that the biosolids-borne microorganisms should be examined in future research, in order to better understand the contribution of biosolids-borne microorganisms to the impact of various biosolids treatment processes on soil enzyme activities.

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Appendix 1: Preliminary Study for Determining the Application Rate of Lime ($CaCO_3$) to Raise Soil pH.

1. Introduction

Soil acidification can lead to decreased nutrient availability, increased risks of aluminum (Al) and manganese (Mn) toxicity, and consequently reduced crop yield (Meng et al., 2019). Liming acidic soils is the most common management practice to raise the soil pH to the ideal range (i.e., 6.5–7.5) for crop production (Liu et al., 2020). This preliminary study aimed to determine how much lime ($CaCO_3$) should be applied to our acidic soil.

2. Materials and Methods

Acidic soil was collected at the depth of 0-15 cm from a field located in Bible Hill, Nova Scotia, Canada (45˚23' N, 63˚14' W)). The soil was air-dried and passed through a 2-mm sieve for an incubation experiment. The soil is described as a Gleyed Humic Ferric Podzol in the Tormentine (Truro Series) with a sandy loam textural classification. Finely-ground reagent grade lime ($CaCO_3$) was purchased from Fisher Scientific. 60 g of soil was measured into cups. $CaCO_3$ was applied to 60 g soil at rates of 0, 0. 5, 1, 2, 4, 8, 16 mg g^{-1} air-dried soil, which were equivalent to the field study rates of 0, 1.02, 2.04, 4.08, 8.16, 16.32, 32.64 t ha^{-1} (Fig. A1.1). The treatments were arranged in RCBD with three replicates. Parafilm with holes to ensure aerobic conditions. Soils were incubated at 25 ℃ and 60 % WFPS (18 % gravimetric water content). The incubation was carried out in a temperature controlled, dark incubation chamber held constant at 25 ℃, and soils were moistened to 60 % WFPS. Soil samples were taken from each cup after homogenizing the soil at 0, 14, 21, and 28 days after the start of incubation and then analyzed for pH. Soils were moistened every five days during the incubation. Three replicates per treatment were destructively sampled to determine soil pH.

(a) **(b)** **(c)**

Figure A1. 1 Soils received different rates of $CaCO_3$: low (0 t ha^{-1}) (a), modest (4.08 t ha^{-1}) (b), and high (32.64 t ha^{-1}) (c).

The pH of the soil was measured using a pH meter with a combined glass electrode in a supernatant suspension of a 1:2 (m/v) soil to $CaCl_2$ ratio. The amount of lime sufficient to raise the pH of the soil to the desired target pH level (near 6.5) was selected for liming the experimental soils.

3. Results

Lime application rate at 2 mg g^{-1} (4.08 t ha^{-1}) gave soil pH values favorable for crop production from day 14 to 28 (dashed line in Fig. A1.2). This indicates that liming at 2 mg g^{-1} (4.08 t ha^{-1}) is required to correct the acidity of the experimental soil and maintain the soil pH level within the ideal range after 14 days.

3. Conclusion:

In order to adjust soil pH to the optimal level for crop production, lime ($CaCO_3$) should be applied to our acidic soil at a rate of 2 mg g^{-1} (4.08 t ha^{-1}).

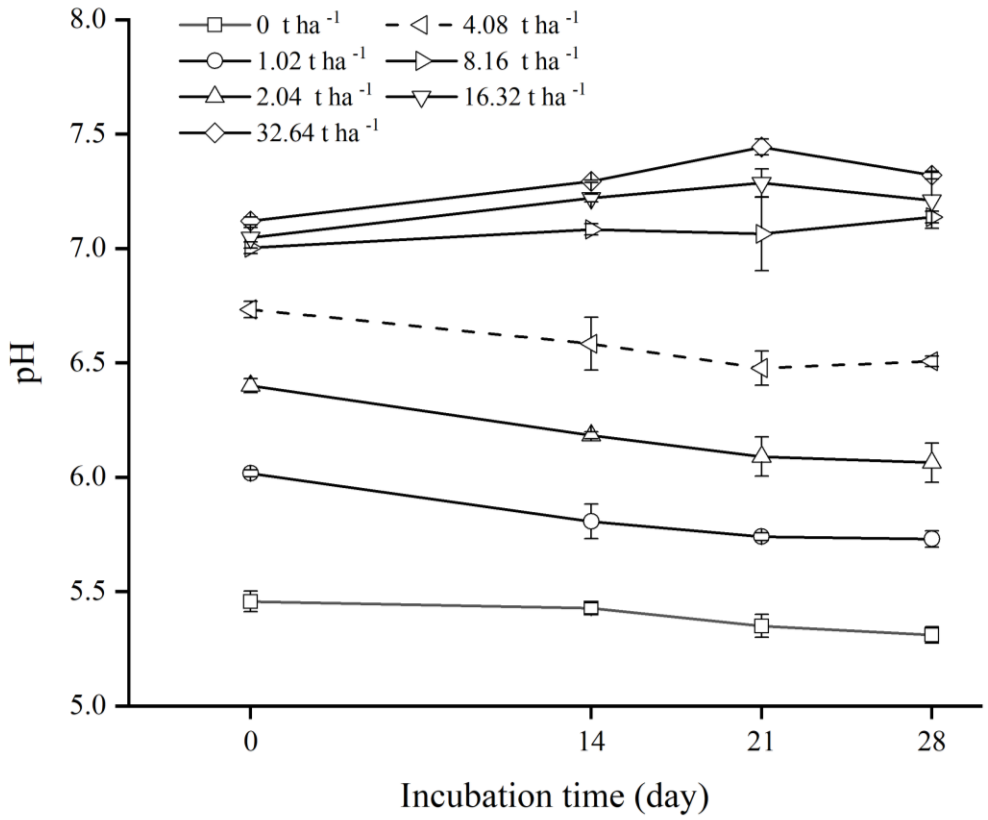

Figure A1. 2 Kinetics of soil pH in $CaCO_3$-amended soils. Points represent means (n=3). Error bars represent standard deviations of the means.

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Meng, C., Tian, D., Zeng, H., Li, Z., Yi, C., Niu, S., 2019. Global soil acidification impacts on belowground processes. Environmental Research Letters 14, 074003. doi:10.1088/1748-9326/ab239c

Appendix 2: Incubation Chamber Conditions

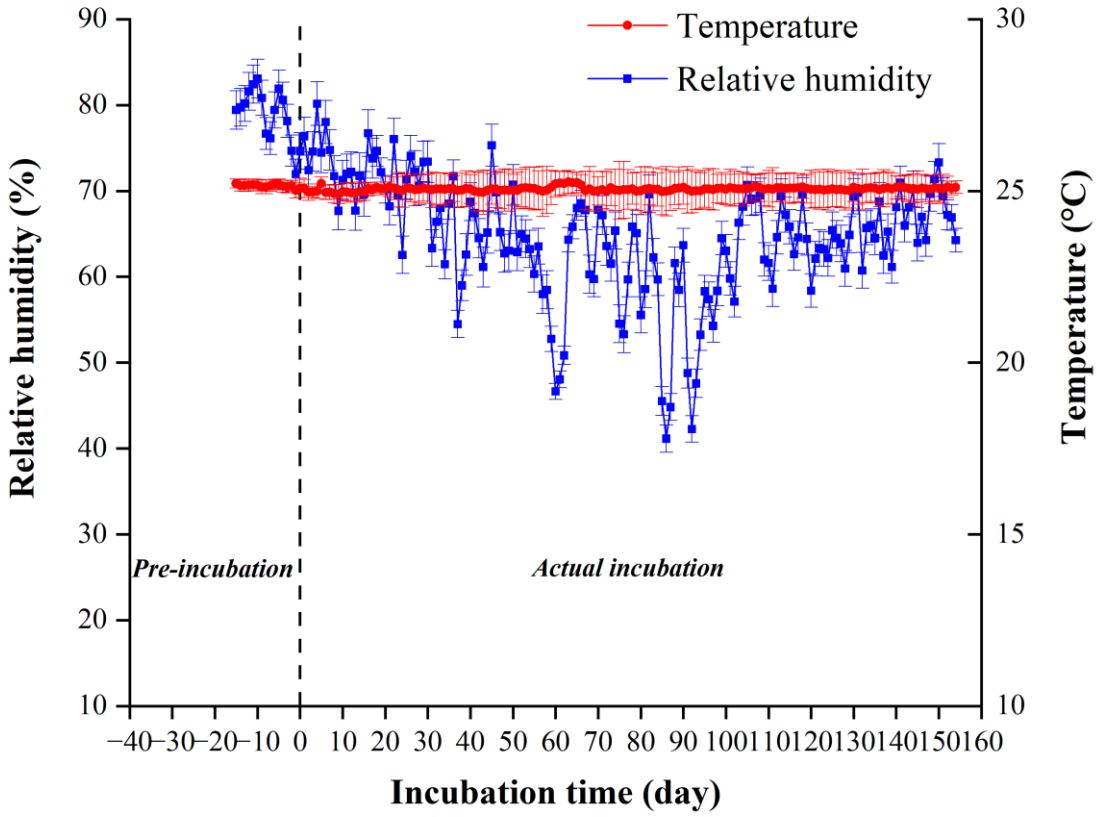

Figure A2. 1 The mean (n=5) temperature and relative humidity in the incubation chamber during the whole incubation period. Error bars represent standard deviations of the means.

Appendix 3: Soil NH_4^+-N Changes During the Non-Leached Incubation Study

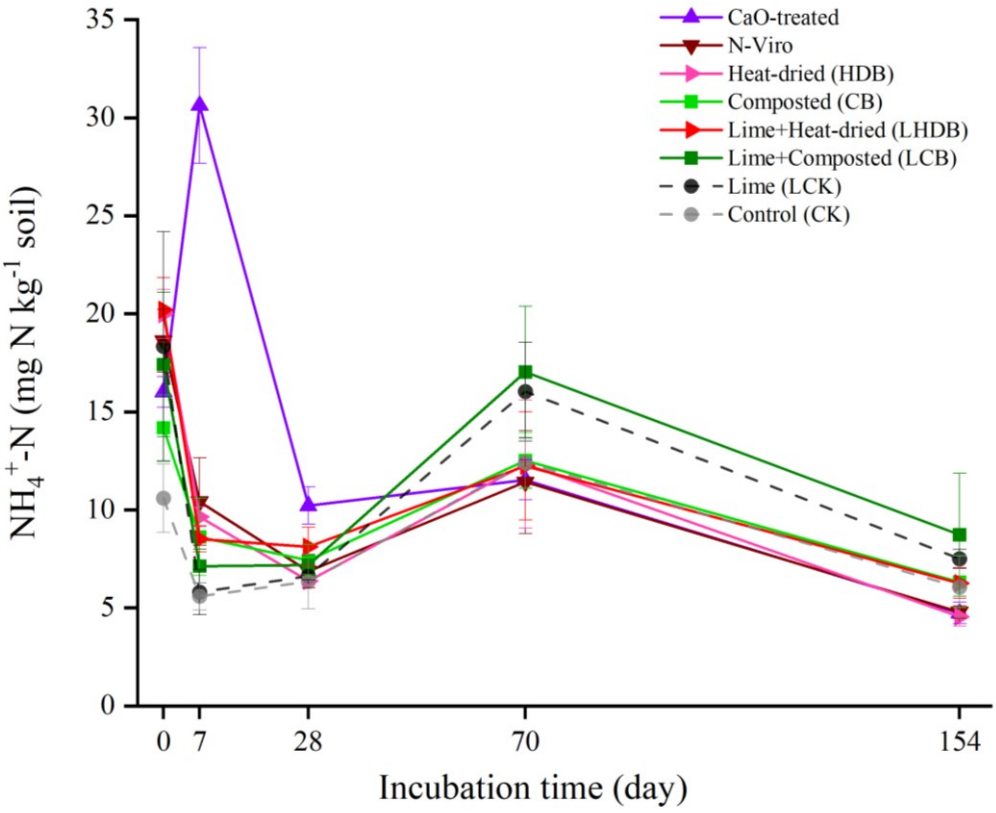

Figure A3. 1 Soil NH_4^+-N changes during non-leached incubation study. Points are expressed on a dry weight basis (dw) and represent means (n=5). Error bars represent standard deviations of the means.

Appendix 4: Pearson's Correlation Results

Table A4. 1 Pearson's correlation coefficients (r) between soil chemical and biological properties in Day 7's soil samples from the non-leached incubation study (n=40).

	Urease	NAG	LAP	Net N mineralized	MN	NH_4^+-N	NO_3^--N	OM
pH	-0.24	0.62***	-0.15	0.69***	0.78***	0.69***	0.56***	0.11
OM	0.1	0.25	-0.25	0.54**	0.43**	0.40**	0.29	
NO_3^--N	-0.58***	0.26	-0.46**	0.66***	0.86***	0.21		
NH_4^+-N	-0.13	0.87***	-0.16	0.83***	0.69***			
MN	-0.5**	0.66***	-0.43**	0.91***				
Net N mineralized$^+$	-0.66***	0.77***	-0.52**					
LAP	0.26	-0.16						
NAG	-0.23							

+Net N mineralized (n=30, CK and LCK data were excluded); * = Significance at 0.05 ($p < 0.05$), ** = significance at 0.01 ($p < 0.01$), *** = significance at 0.001 ($p < 0.001$). The units of each variable are those given in Tables 3.4 and 3.15. NO_3^--N, NH_4^+-N, and MN have the same units.