Slow release brown coal-urea fertiliser for improving crop yield, nitrogen use efficiency and soil health

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Abstract

Achieving optimum crop yield by minimum application of nitrogen (N) fertiliser is increasing in important due to the detrimental effects of N losses on the environment. Addition of humic-rich brown coal (BC) as an organic amendment with urea, and application to soil, can alter N cycling and availability to crop plants. The release of N from synthetic fertiliser, such as urea, is usually rapid and susceptible to dissipation from soil without benefit to the crop. More gradual release of fertiliser-N may reduce the off-site movement of mineral N, thereby enhancing N supply to crops and minimising negative off-site impacts. In this thesis, novel slow release fertilisers were formed by blending brown coal with urea which delayed fertiliser N release through strong retention facilitated by the extensive surface area, porous structure and chemical functional groups in the BC. Subsequent investigations characterised the role of BC as a carrier for synthetic urea and the effect of their interaction on the N dynamics, soil health, yield and N use efficiency of several crop plants in both glasshouse and field conditions. Initially, four different brown coal-urea (BCU) granules, with C:N ratios of 1-10, were prepared by pan granulation. Advanced spectroscopic and X-ray powder diffraction (XRD) techniques confirmed loading of urea-N into the BC structure. Nitrogen-release from BCU granules was slower than from urea, resulting in higher N retention over a longer period. This trend increased with higher loading of BC, emphasising the significant role of BC in N retention. These findings supported the hypothesis that BC is suitable for developing slow release N fertilisers.

Incubation experiments demonstrated that blending and subsequent granulation of urea with BC substantially increased fertiliser N retention in soil by decreasing gaseous and leaching losses of N compared to urea alone, irrespective of soil types. The BCU granule containing the highest proportion of BC had lower N leaching and gaseous emissions and maintained considerably higher mineral and mineralisable N in topsoil. Possible modes of action of the BCU blends were proposed, emphasising the role of BC in enhancing N retention over a longer period of time. The results support the notion that BCU blends can be used as a slow release and enhanced efficiency fertiliser for increasing availability and use efficiency of N by crop plants.

Plant-based experiments were conducted in both glasshouse and field (Department of Primary Industries (DPI), Wollongbar Research Institute, NSW, Australia) conditions to confirm the results obtained in laboratory experiments. Blending of urea with BC showed a beneficial effect on the growth, biomass and economic yield, and N uptake by crop plants studied. Like incubation experiments, a similar trend was found in reducing gaseous losses of N from soil due to addition of BCU. As a result, significantly higher amounts of mineral N was available to crop plants in BCU amended soil. The increased N availability and its subsequent uptake by crop plants facilitated better crop growth and increased biomass and economic yield of studied crop plants by approximately 30 and 25%, respectively. In addition to yield benefit, incorporation of BCU granules also improve soil health by increasing organic carbon content, moisture holding capacity and microbial activity of soil. A ^{15}N isotope tracer study demonstrated that granulation of BC with urea significantly reduced the fertiliser derived N losses and enhanced microbial immobilisation of the applied fertiliser-N. The decreased losses and increased fertiliser-N retention were mainly responsible for increasing fertiliser-N use efficiency of crop plants in BCU over urea. The overall results presented in this thesis revealed that application of N fertiliser in the form of granular BCU could reduce the chemical N fertiliser application rate by approximately 25% without any loss of crop yields as well as enhancing fertiliser-N recovery while diminishing its losses to the environment.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature:

Print Name: Biplob Kumar Saha

Date: 16-12-2017

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original papers published in peer reviewed journals and one submitted publications. The core theme of the thesis is to minimise gaseous and leaching losses of nitrogen for improving its use efficiency, crop yield and soil health through the granulation of brown coal with urea. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Chemistry under the supervision of Associate Professor Antonio Frank Patti.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapter 2 to 7 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution**	**Co-author(s), Monash student Y/N***
2	Hybrid brown coal-urea fertiliser reduces nitrogen loss compared to urea alone	Published	70%. Concept and collecting data and writing first draft		No
3	Nitrogen dynamics in soil fertilized with slow release brown coal-urea granules	Submitted	70%. Concept and collecting data and writing first draft		No
4	Slow release brown coal-urea granule for increasing biomass yield and nitrogen uptake by silver beet	Not Submitted	70%. Concept and collecting data and writing first draft		No
5	A cost effective slow release nitrogen fertiliser for improving yield and nitrogen use efficiency of sweet corn	Not Submitted	60%. Concept and collecting data and writing first draft		No

6	Fate and recovery of nitrogen applied as brown coal-^{15}N urea in a sub-tropical agroecosystem	Not Submitted	60%. Concept and collecting data and writing first draft		No
7	Mechanistic understanding on the granulation effect of brown coal with urea on the dynamics and mineralisation of N in soil-plant systems	Not Submitted	70%. Concept and collecting data and writing first draft		No

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 16-12-2017

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: Antonio F. Patti **Date:** 16-12-2017

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Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to heart. With utmost respect, I dedicate this thesis to my parents whose love and unselfish support over many years laid the foundations for the discipline and application necessary to complete this work.

List of abbreviations

ANOVA= Analysis of variance

BC= Brown coal

BCU= Brown coal-urea

C= Carbon

CEC= cation exchange capacity

CH_4= Methane

cm= Centimeter

CO_2= Carbon di-oxide

DAF= Days after fertiliser addition

DAS= Days after sowing

DPI= Department of Primary Industries

FDA= Fluorescein diacetate hydrolysis

FNUE= Fertiliser nitrogen use efficiency

FTIR= Fourier Transform Infrared Spectroscopy

g= Gram

GC= Gas chromatography

GHG= Greenhouse gas

ha= Hectare

HAs= Humic acids

IPCC= Intergovernmental panel on climate change

kg= Kilogram

L=Litre

Leu-Ap= Leucine aminopeptidase

m= Meter

MBC= Microbial biomass carbon

MBN= Microbial biomass nitrogen

µg= Microgram

mL= Mililitre

mm= Milimeter

N= Nitrogen

N_2O= Nitrous oxide

NH_3= Ammonia

NH_4^+= Ammonium ion

NMR= Nuclear magnetic resonance spectroscopy

NO= Nitric oxide

NO_3^- = Nitrate ion

NUE= Nitrogen use efficiency

OA= Organic amendments

P= Phosphorous

PMN= Potentially mineralisable nitrogen

ppbv= Parts per billion by volume

PVC= Poly vinyl chloride

PXRD= Powder X ray diffraction

RDW= Root dry weight

SDW= Shoot dry weight

SOC= Soil organic carbon

t= Ton

TC= Total carbon

TN= Total nitrogen

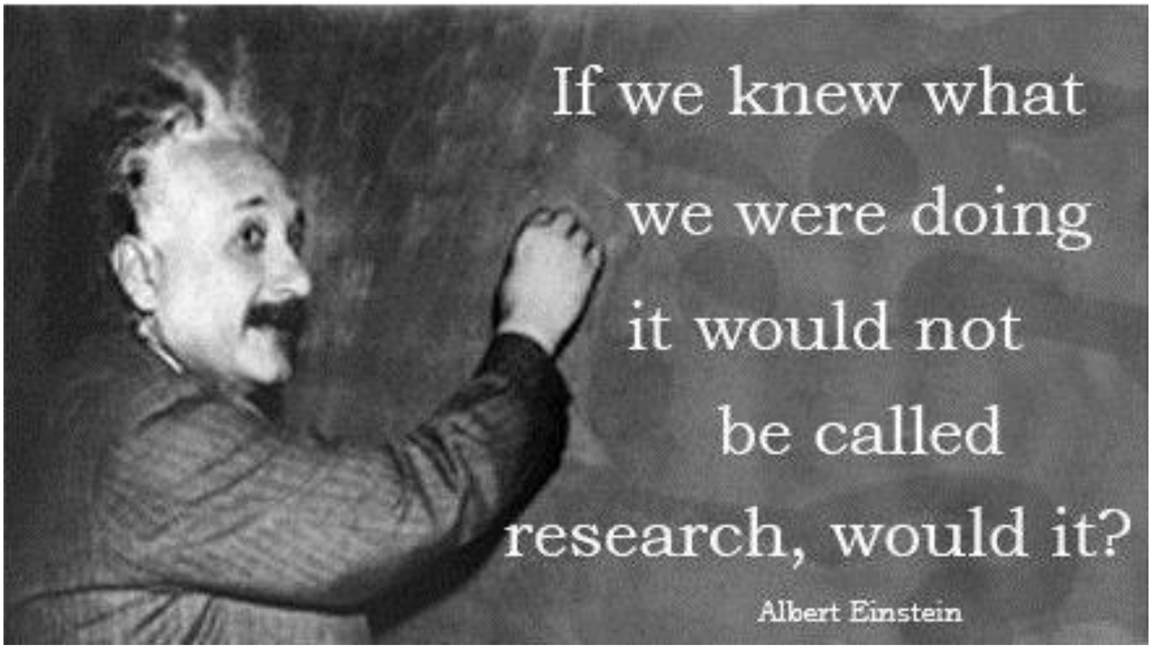
V= Volume

WA= Water absorbency

WFPS= Water filled pore space

WHC= Water holding capacity

WRC= Water retention capacity



If we knew what
we were doing
it would not
be called
research, would it?
Albert Einstein

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1. Chapter: Introduction and Literature Review

1.1. Introduction

Nitrogen (N) is one of the most important and limiting nutrients for ecosystem provisioning services (Jackson et al., 2008). Urea is the dominant and main source of N input in many crop production systems worldwide because of its high N content. Unfortunately, the use efficiency of urea fertiliser is very low and the losses of N could be very high under certain conditions (Raun et al., 2002). The low N use efficiency of urea is associated with N losses by leaching, denitrification and volatilisation (Fageria and Baligar, 2005). This is a major challenge for farmers worldwide since the lost N represents both an economic cost and an environmental burden. This is largely as a result of off-site N transport in water polluting both surface- and groundwater, whilst nitrous oxide contributes to greenhouse gas accumulation in the atmosphere (Vitousek et al., 1997). The widespread loss of organic matter from arable soils has been implicated as one factor contributing to low fertiliser N-use efficiency because soils depleted in organic carbon are often impaired in N retention (Accoe et al., 2004; Gärdenäs et al., 2011). Furthermore, studies show that the carbon (C) sequestration potential of soil is intricately linked with sequestration of other nutrients, especially N (Dong et al., 2009).

There is an increasing interest in the application of organic materials (Das and Adhya, 2014; Hargreaves et al., 2008; Li et al., 2015), as they can contribute to climate change mitigation through C sequestration and supply organic matter to soil at the same time (Diacono and Montemurro, 2011). Organic matter improves soil water holding capacity, soil buffering capacity, cation exchange capacity, soil structure, and increases the availability of plant nutrients by increasing microbial biomass and their activities (Ge et al., 2009). Traditionally, organic materials in the form of, for example, cow-dung, compost, farmyard manure, poultry litter, municipal wastes and humic substances were applied in large amounts to improve crop productivity (Hargreaves et al., 2008; Imbufe et al., 2005; Loecke et al., 2004; Mondini et al., 2008). Efficient use of manure as fertiliser is complicated by the imbalance of nutrients, variability in sources, difficulties in estimating nutrient availability, relatively low nutrient concentration and very high application rates which limit the distances manure can profitably be transported for use as a fertiliser (Albiach et al., 2001; Edmeades, 2002; Hargreaves et al., 2009; Quilty and Cattle, 2011). However, fortification of traditional synthetic fertilisers with humified organic matter could enhance fertiliser use efficiency and return organic matter back into soil, restoring soil health and improving crop yield (Ahmad et al., 2008).

1.2. Impact of N loss on the environment

Nitrogen is one of the most important nutrient elements for ecosystem productivity. High application rates of N fertiliser are very common for improving crop production. The use of N fertiliser is expected to increase, particularly in developing countries. Globally, intensive agricultural production has dramatically altered N cycling. This intensive land use with high N fertiliser addition can potentially degrade soil organic C and increase N losses in the environment (Khan et al., 2007). Nitrogen is primarily lost via gaseous emissions or leaching in many agricultural production systems. The major N loss pathways of an agricultural system are shown in Figure 1.1.

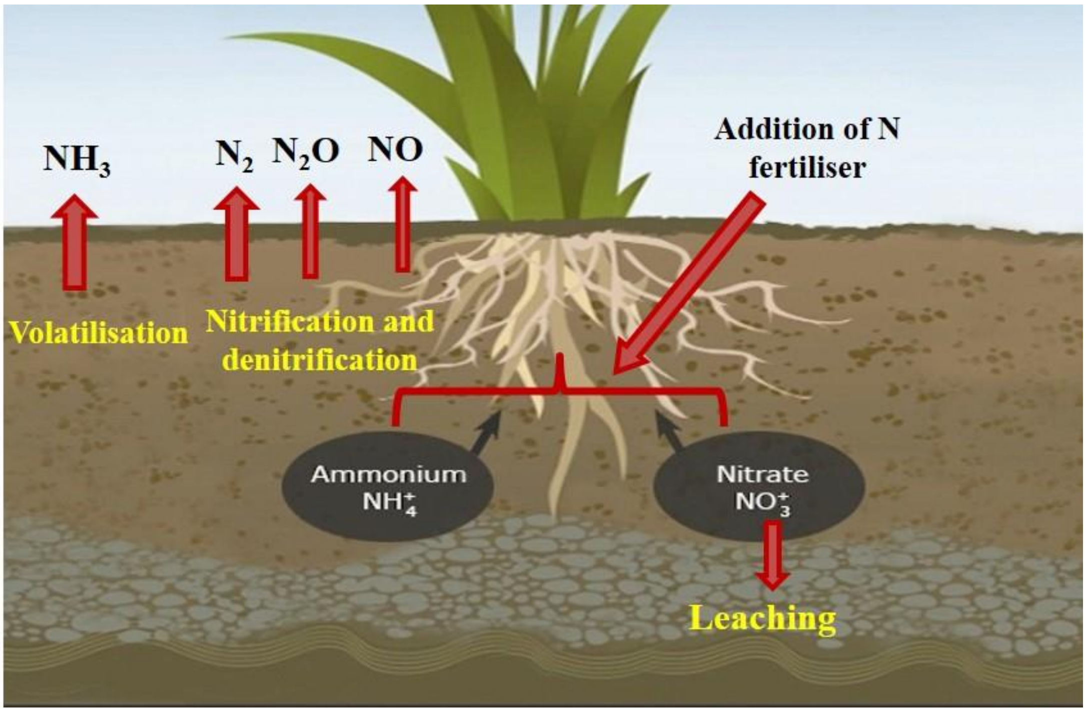

Figure 1.1. Major N loss pathways in an agricultural system

Volatilisation of ammonia (NH_3) is one of the major N loss pathways in the global N cycle. It is a form of reactive N which can negatively affect ecosystems, biodiversity, and human health through its accumulation and indirect nitrous oxide (N_2O) emissions (Dean et al., 2014). Globally, agriculture sectors account for more than 40% of total NH_3 emissions (Bouwman et al., 1997). Volatilised NH_3 is considered to be one of the factors indirectly influencing the formation of acid rain, acidification of soils, acidification and eutrophication of lakes and rivers, which can result in decreasing biodiversity and an indirect source of N_2O (Barthelmie and Pryor, 1998).

Agriculture sectors contribute 35% of all N_2O emissions in the world. Millar et al. (2010) reported that N_2O emissions from soil increases exponentially with increasing rate of N fertilisation. Nitrous oxide is an important greenhouse gas with a very long atmospheric lifetime and high global warming potential, 298 times greater than CO_2 in the atmosphere (Forster, 2007). It is also associated with depletion of the ozone layer, ocean current movements through melting of ice, and weather and climate change. The atmospheric concentration of N_2O is increasing at a rate of 0.7 ppb (v/v) year^{-1} (IPCC, 2001). Therefore, the increased concentration of N_2O could potentially contribute to higher temperatures and decrease stratospheric ozone concentrations (Ravishankara et al., 2009).

Nitrate (NO_3^-) leaching from agricultural land is one of the most important environmental concerns due to its negative impact on surface waters and groundwater. Excessive use of N fertilisers in agricultural soils can contaminate groundwater due to leaching of nutrient ions, especially NO_3^- through macropores with percolating water as well as soil acidification (Di and Cameron, 2005). Overuse of N fertilisers at rates higher than plant requirements can enhance the rate of NO_3^- leaching by many times (Di and Cameron, 2002). Generally, as a cation ammonium (NH_4^+) is strongly held by cation exchange sites in soil, whereas the mobility of NO_3^- in the soil profile is very high due to lack of anion exchange sites (Liu et al., 2005). The movement and transport of N in soil-water-plant systems are very complex processes and controlled by many environmental factors. Therefore, it is very important to understand the fate of fertiliser N in the soil-water-plant system, not only for improving N use efficiency in biological systems, but also because of its adverse effect on surface and ground water.

1.3. Processes and soil factors influencing transformation and losses of N from agroecosystem

The processes involved in the transformation and loss of N from agroecosystem include mineralisation, nitrification, NH_3 volatilisation and denitrification. The conversion of organic N to mineral forms of N is called mineralisation and the process occurs as a result of biological activity (Kox and Jetten, 2015). The first step of N mineralisation is the ammonification which converts organic N to NH_4^+ by heterotrophic microorganisms. The release of ammonium N during ammonification process mainly depends on the N supply and its availability to microbial populations, composition of organic materials, C:N ratio, soil and environmental factors (Kox and Jetten, 2015). Ammonia volatilisation is another physicochemical process where NH_3-N remains in the equilibrium condition between the gaseous and hydroxyl forms of N. This conversion process is mainly pH dependent. The nitrification and denitrification are the principal processes associate with the production and consumption of gaseous forms of N in soil (Conrad, 2002). The biological oxidation of NH_4^+ or NH_3 to NO_3^- via NO_2^- as an intermediary product is known as nitrification (Reddy and Patrick, 1984). Only a very few groups of aerobic chemoautotrophic bacteria governing this process in soil. The gaseous loss of N during nitrification process can occur by chemical transformation of NH_2OH and NO_2^- (Russow et al., 2009) or by the use of NO_2^- in a nitrifier denitrification pathway (Wrage et al., 2001). Denitrification is defined as the biological reduction of NO_3^--N to N_2 under anaerobic conditions with N_2O and NO emitted as intermediary gaseous products (Robertson and Tiedje, 1987). The denitrification process is carried out by a wide variety of heterotrophic bacteria, whereby they use NO_3^- rather instead of oxygen (O_2) as a terminal electron acceptor during respiration. As a result most of the denitrifiers commence denitrification only when O_2 is unavailable due to increased soil moisture. The process is catalysed by a series of enzymes and influenced by many soil and environmental factors predominantly O_2 and moisture content of soil Wrage et al. (2001).

The factors influencing N losses from soil-plant system are mainly a consequence of climatic, soil and plant factors. However, N losses are also largely influenced by N sources, methods and rate of application. The most important soil factors affecting N losses are pH, texture and moisture content.

1.3.1. Soil pH

Soil pH is one of the most important factors controlling N losses from soil. Loss of N via volatilisation is largely influenced by soil pH. Ammonia (NH_3) volatilisation generally occurs at soil pH greater than 7.0 (Cevallos et al., 2015). It was revealed from experimental results that a significant amount of N is lost as NH_3 from heavily limed or calcareous soils (Larsen and Gunary, 1962; Terman and Hunt, 1964). A review study conducted by Pesek et al. (1971) reported that the worldwide volatilisation loss of N is directly correlated with $CaCO_3$ and pH of soil. In another study, Dannenmann et al. (2008) found a positive correlation between soil pH and denitrification rate. The optimum soil pH for most of the denitrifying microorganisms lies between 6.0 to 8.0 (Kyveryga et al., 2004). The mineralisation rate was significantly higher in alkaline soil having higher soil pH compared to acidic soil with lower soil pH (Hanan et al., 2016). Similarly, Cevallos et al. (2015) reported that NO_3^- leaching rate was lower at soil pH 6.0 compared to soil pH 8.0. Likewise, Rahman et al. (2014) found that the transport of NH_4^+ in soil increased remarkably with an increase in soil pH. Meng et al. (2015) also indicated that the addition of lime to soil accelerates the NO_3^- leaching from soil.

1.3.2. Soil texture

Soil texture is another important physical property, influencing N losses from agricultural soil. Soil texture regulates the retention and infiltration rate of water in the soil profile and thereby controls the NO_3^- leaching. Nitrate leaching is considerably higher in light textured soils compared to heavy textured soils (Kücke and Kleeberg, 1997). Course textured soils had higher NO_3^- leaching was reported by Sogbedji et al. (2000). Similarly, significantly higher amounts of NO_3^- leaching were observed in coarse textured sandy soil due to the high permeability and infiltration of water (De Paz and Ramos, 2004). Soil texture can also affect the nitrification and denitrification of N by altering the soil structure, water holding capacity, pore size distribution, water infiltration rate and aeration (Aulakh et al., 1991). In another study, Aulakh et al. (1992) reported that the denitrification rate is positively correlated with the clay content of soil might be due to high moisture content and low oxygen content of wet soil. Jamali et al. (2016) also found a considerably higher N_2O emissions in a clay soil compared to a sandy soil due to increased moisture holding capacity.

1.3.3. Soil moisture

Soil moisture content is also a very important factor influencing soil microbial activity and crop growth. Soil water is responsible for the movement and transport of nutrients to plants and in the soil profile (Xu et al., 2012). Soil moisture is one of key factors governing the nitrification and denitrification of N (Bouwman, 1996). The soil moisture content at field capacity is suitable for greater activity of nitrifying and denitrifying microbial communities in soil (Pesek et al., 1971).

1.4. Strategies to mitigate N losses from agricultural systems

Last few decades there has been a sharp increase in N fertiliser inputs to agricultural soils and this increase is expected to continue in the foreseeable future. On a global scale, the application of N fertiliser is expected to increase at a rate of 1.4% per year (http://www.fao.org/3/a-i4324e.pdf). The increasing N input to agricultural soils has rekindled the debate about its impact on atmospheric, terrestrial and aquatic environments. Considerable research efforts in recent years have been aimed at developing mitigation strategies to reduce gaseous and leaching losses of N from intensively managed agricultural systems (Chen, et al., 2008; Lassaletta et al., 2014; Trenkel, 2010; Behin and Sadeghi, 2016; Naz and Sulaiman, 2016; Paramashivam et al., 2016; Rose et al., 2016).

1.4.1. Use of urease and nitrification inhibitors

One such approach to reduce N losses is the use of urease and nitrification inhibitors. The urease inhibitors *viz*., phosphoryl amides (e.g. N-(*n*-butyl) phosphoric triamide, cyclohexylphosphoric triamide, phenyl phosphorodiamidate (PPDA) and N-butyl thiophosphoric triamide (nBTPT) occupy the urease active site inactivating the urease enzyme and thus reducing the loss through NH_3 volatilisation (Chen et al., 2008; Zaman and Blennerhassett, 2010). Nitrification inhibitors such as dicyandiamide (DCD), nitropyrin and 3, 4 dimethyl pyrazole phosphate (DMPP) offer potential for decreasing gaseous N emissions through controlling the rate of nitrification and so reducing the potential for denitrification (Abbasi and Adams, 2000; Di et al., 2007; Lam et al., 2017; Hube et al., 2017). For example, nitrification inhibitors reduced NO_3^- leaching and N_2O emissions by 55-65% and 30-80%, from agricultural soils, respectively (Singh et al., 2009; Lam et al., 2017). The factors influencing the efficacy of these inhibitors are soil textures, soil organic matter content (Singh et al., 2009), sorption capacity of organic matter

(Sahrawat et al., 1987), application method and rate (Rajbanshi et al., 1992), soil moisture, pH and soil temperature (Kelliher et al., 2008). Application of fertilisers containing inhibitors or inhibitor-coated fertilisers are being widely used in intensive agricultural systems where it is estimated that they can reduce gaseous and leaching losses of N compared to application of N fertiliser on its own (Pfab, 2009). However, several studies reported that the effect of these inhibitors on gaseous N losses is highly variable and, in some cases, increased NH_3 volatilisation by increasing soil NH_4^+ concentrations (Merino et al., 2001; Nastri et al., 2000).

1.4.2. Use of controlled release fertilisers

In recent years, many new technologies have been reported to enhance the use efficiency of N fertilisers by reducing its losses via different pathways. To minimise gaseous and leaching loss of N from agricultural soils, one of the proposals currently being considered is use of controlled release fertilisers. Controlled release fertilisers are designed and manufactured to release nutrients from fertiliser in a controlled and delayed manner by synchronising with plant demand to achieve higher nutrient use efficiency. Many researchers have proposed that fertiliser efficiency can be greatly improved by developing formulations where N is released in a slow and/or controlled manner (Khan et al., 2015; Naz and Sulaiman, 2016; Rose et al., 2016; Yamada et al., 2014).

One such approach for controlling the nutrient release involves coating technology. Coating technology is considered to be one of the ways of consistently supplying N to plants and reducing N losses in the environment (Bröckel and Hahn, 2004). Coatings for commercial N fertilisers can be made from various materials that are capable of reducing its dissolution and release rate of water soluble fertilisers, including organic polymers or inorganic minerals (Wang et al., 2013). Several experimental results revealed that the N delivering efficiency of these fertilisers is higher than conventional fertilisers (Dave et al., 1999; Trenkel, 2010). Reduction of NH_3 and N_2O emission by the addition of slow release fertilisers has been reported in many studies (Byrnes and Freney, 1995; Chu et al., 2004; Shi et al., 2009; Trenkel, 2010). For example, González et al. (2015) reported that addition of polymer coated urea reduced NO_3^- leaching from the soil up to 26%. Similarly, several agronomic studies have demonstrated that use of slow release fertiliser increased biomass yield (Zhao et al., 2013), grain yield (Sanderson and Fillmore, 2012), N uptake (Farmaha and Sims, 2013; Haase et al., 2006) and N-use efficiency (Zhao et al., 2013) of crop plants compared to commercial conventional fertilisers.

Until recent times, only a small number of different polymeric and non-polymeric materials have been tested as slow release fertilisers (Behin and Sadeghi, 2016; Khan et al., 2015; Saleh et al., 2003; Shivay et al., 2001). Unfortunately, the nutrient release pattern can vary significantly depending on the coating materials that are used. Moreover, some fertilisers sometimes failed to show slow release behaviour due to influence of environmental factors such as temperature and moisture content of soil (Jin et al., 2013). However, most of the coating materials are non-degradable, highly expensive and may have adverse environmental effects (Naz and Sulaiman, 2016).

1.4.3. Use of improved management practices

Recent research has identified various management practices to abate losses and improve the use of N in intensively managed agricultural systems. Cultivation of genetically improved varieties can also increase N use efficiency and minimise N loss (Zebarth et al., 2008). Nitrogen cycling in soil is intimately linked to soil water management. The quality and distribution of water during the growing season are important factors influencing the growth and nutrient uptake by crop plants. Therefore, improved water management could also improve N utilisation by plants through decreasing N losses via NO_3^- leaching and denitrification (Machado et al., 2008). In addition split application of N is another approach commonly used to improve fertiliser use efficiency of crops (Li et al., 2017). However, none of these approaches have been adopted widely by the farmers, because of cost and/or difficulties in on-farm implementation of those practices (Chen et al., 2008).

1.5. Integrated use of organic materials and synthetic fertilisers in N management

The concept of integrated nutrient management simultaneously uses synthetic and organic sources of nutrients (Wu and Ma, 2015). Integrated application of organic materials with synthetic N fertilisers has received considerable attention in the last decade with the aim to maintain sustainable agricultural production with minimum application of synthetic fertilisers (Kumar and Yadav, 2009). Combining the application of organic and synthetic fertilisers can improve the physical, chemical and biological properties of soils by influencing the bulk density, buffering capacity and microbial activity of soil (Quilty and Cattle, 2011). Integrated nutrient management can also reduce soil erosion, improve water infiltration, soil aeration and plant root growth (Johnson et al., 2004). Furthermore, it can also reduce the loss of N through leaching, runoff, volatilisation and emissions due to increased use efficiency of N fertilisers (Zhang et al., 2012).

It has been demonstrated by previous studies that integrated application of organic and synthetic N increased crop productivity (Chander et al., 2013; Garai et al., 2014; Nath et al., 2011; Patil et al., 2013; Rahman et al., 2013), reduced fertiliser-N requirements, minimised N losses (Zhang et al., 2012), increased N uptake (Bairwa et al., 2013) and improved soil fertility (Esilaba et al., 2005) by increasing soil organic carbon (SOC) content (Das and Adhya, 2014). While integrated nutrient management can lead to increased yield, economic and environmental benefits, it is difficult to optimise nutrient inputs due to variation in nutrient content to match crop demand.

1.6. Role of organic amendments in agriculture

Currently, a large number of commercially available products commonly known as organic amendments (OA) are marketed to farmers for agricultural use (Quilty and Cattle, 2011). These products are mainly derived from naturally occurring organic matter. The organic materials used to manufacture OA can include composted and fresh organic wastes from agricultural, industrial and municipal operations, seaweed, meat blood and bone meal, and humic substances (Conn and Franco, 2004; Curnoe et al., 2006; Eghball and Power, 1999; Hargreaves et al., 2008; Imbufe et al., 2005; Mondini et al., 2008; Nastri et al., 2006; Sivasankari et al., 2006). The addition of OA to soil has been reported to provide a number of benefits including improvements in growth and yield of crops (Wu and Ma, 2015) through the supply of plant nutrients, the control of pests and diseases, enhancement of the efficiency of synthetic fertilisers, and improvements to soil health (Quilty and Cattle, 2011). Despite the claims of manufacturers and farmers regarding the beneficial effect of OA application, there has been very little scientific research reporting the possible effects of OA application in broadacre agricultural farming systems (El-Tarabily et al., 2003; Horii et al., 2007; Imbufe et al., 2005; Mondini et al., 2008; Tenuta and Lazarovits, 2004). The increasing interest in the application of OA in agricultural crop production and the proliferation of OA in the marketplace is possibly due to the modern emphasis on maintaining and improving soil health by building SOC, as well as the increased demand of consumers for organically produced food (Loecke et al., 2004).

Table 1.1 List of organic amendments with their application rate in agricultural crop production

Organic amendments	Application rate
Compost	0.5 to 30 t ha^{-1}
Vermicasts (Liquid)	10 to 100 L ha^{-1}
Vermicasts (Solid)	2 to 50 t ha^{-1}
Humic substances (Liquid)	1 to 30 t ha^{-1}
Humic substances (Liquid)	0.025 to 1 t ha^{-1}
Meat, blood and bone meal (Liquid)	1 to 30 t ha^{-1}
Meat, blood and bone meal (Solid)	0.1 to 1.2 t ha^{-1}
Fish hydrolysate	2 to 60 t ha^{-1}
Seaweed extracts	0.5 to 20 t ha^{-1}
Bio-inoculants	1 to 20 t ha^{-1}
Municipal waste	25 to 100 t ha^{-1}
Beef cattle feedlot manure	10 to 145 t ha^{-1}
Sewage sludge	30 to 80 t ha^{-1}
Paper mill waste	22 to 78 t ha^{-1}
Chicken manure	10 to 20 t ha^{-1}
Farm yard manure	5 to 30 t ha^{-1}
Biochar	10 to 50 t ha^{-1}

Source: Quilty and Cattle, 2011

1.6.1. Role of OA as a source and retention ability plant nutrients

One of the common characteristics of OA is that they can act as a significant source of plant nutrients, depending on their chemical composition. Most of the OA can effectively provide plant nutrients, as most of them are sourced from agricultural wastes (Hargreaves et al., 2009). Addition of OA increases cation exchange capacity of the soil which is vital for retaining essential nutrient ions for plants growth (Odlare et al., 2008). A significant proportion of the nutrients are present in the organic molecular structures (Jeng et al., 2006) and gradually become available to plants following microbial mineralisation (Cayuela et al., 2008; Dilly, 2001; Mondini et al., 2008). Addition of OA at agronomically appropriate rates can effectively supply nutrients to crops to maintain sustainable yields (Jeng et al., 2006; Mondini et al., 2008). Application of OA to soil showed a significant increase in mineral and total N (Nastri et al., 2006; Padmavathiamma et al., 2008; Sierra et al., 2007). Therefore, the environmental factors and chemical processes governing the mineralisation of OA in soil need to be considered for the application of the products as a source of plant nutrients.

1.6.2. Role of OA on SOC and soil structure

There is a general consensus that SOC is one of the most important factors influencing soil processes and is a useful indicator of soil health (Bronick and Lal, 2005; Lal, 2006). Reversing the continuous loss of SOC due to intensive agricultural production is one of the most important environmental concerns worldwide (Favoino and Hogg, 2008; Lal, 2007; Mikha et al., 2006; Park et al., 2007). Soil OC is the second largest C pool in the earth after the oceans (Swift, 2001), and the possibility of increasing SOC content by adding OA may play an important role in combating climate change (Lal, 2001). Incorporation of OA, such as sewage sludge (Pedra et al., 2007), vermicompost (Ferreras et al., 2006), composts (Hati et al., 2006), food waste (Rashid and Voroney, 2004) and biochar (Lehmann et al., 2006), has been shown to increase SOC content. Moreover, addition of OA also increases microbial activity and microbial biomass carbon in soil (Albiach et al., 2001; Karaca et al., 2006).

Addition of OA to soil is not only important for maintaining SOC but also contributes to improved soil physical, chemical, and biological properties (Ashagrie et al., 2007). Traditionally, plant biomass has been used as mulch to minimise soil erosion and improve soil surface condition. Johnson et al. (2004) reported that addition of lignin enriched OA increased the aggregate stability and reduced erodibility of soil. OA can also increase soil structural stability, maintain and improve the physical condition of soil, which was identified by Clark et al. (2009). OA application also plays an important role in decreasing soil crusting and bulk density which are essential for healthy soil and seedling germination (Karami et al., 2012; Zhao et al., 2009).

1.6.3. Role of OA on soil microbial activity

The soil microbial biomass plays a vital role in nutrient cycling by influencing the mineralisation and immobilisation of nutrients (Abbott and Murphy, 2007). The diversity in microbial communities and their activity governs the rate of OC mineralisation and nutrient release in soil which is a key factor for soil health (Burauel and Baßmann, 2005). Incorporation of OA influences the growth, activity and diversity of microbial communities in soil (Chakraborty et al., 2011). Continuous application of OA over a longer period of time has a significant impact on increasing soil microbial communities and their enzyme activities (Barbarick et al., 2004). For example, Allison and Martiny (2008) reported that microbial community structures are very sensitive to nature of OA added due to their variability in chemical composition, whereas increased microbial activity with OA application compared to chemical fertilisation was reported by Barbarick et al. (2004). Similarly, OA addition enhanced soil microbial biomass C and enzyme activities in soil (Bastida et al., 2008; Reeve et al., 2012).

1.6.4. Role of OA on gaseous N loss

Certain organic amendment addition to soil may play an important role in controlling the gaseous loss of N directly or indirectly through nitrification and denitrification (Köster et al., 2011). There is a very close relationship between OA addition, microbial activity and gaseous N loss. Higher rates of N loss through denitrification occurred in soils treated with OA compared to synthetic fertiliser treated soil only due to increased microbial activity (Dambreville et al., 2006; Walker and Shannon, 2006). OA are usually applied in large amounts along with synthetic fertilisers to obtain better crop yields which eventually increases SOC, mineral N and denitrification rates resulting in N loss as N_2 or N_2O or leaching of NO_3^- (Crutzen et al., 2008). It is widely accepted that addition of OA containing higher available N to soil, especially animal manures and slurries, increases N_2O emissions compared to synthetic fertilisers (Rochette et al., 2004). This might be due to the fact that manures and slurries contains high amounts of available C and N which triggers microbial growth and enhances N_2O production through denitrification. However, there are also reports of decreased N_2O emissions from soils amended with manure (Flessa and Beese, 2000; Velthof et al., 1996) and recalcitrant organic carbon like biochar (Bruun et al., 2011; Castaldi et al., 2011; Nelissen et al., 2014; Wang et al., 2011) compared with synthetic fertilisers. For example, Velthof et al. (1996) reported reduced N_2O emissions from soil amended with cattle slurry compared to mineral fertilizers. Similarly, Nelissen et al. (2014)

found decreased N_2O and NO emissions from soil incorporated with different types of biochar compared to unamended chemical fertiliser treated soil might be due to improved soil aeration, electrostatic N immobilisation and microbial inhibition of denitrification.

1.7. Limitations of using OA in crop production

Although OA addition to soil has been shown a number of economic and environmental benefits, the use of OA in broadacre agriculture is still limited. The slow adoption of OA in broadacre agricultural farming system might be due to lack of consistency in results, very high application rate to achieve benefits, variation in composition and imbalanced nutrient release, high transport cost and public perception. Large variability among research findings on the effect of OA in agriculture due to inconsistent product composition has been identified by Hargreaves et al. (2009). The inconsistent effect of OA represents a substantial challenge to researchers and farmers in confidently predicting the effects of OA application to various agricultural systems. The heterogeneous nature of soil could also contribute to the conflicting results of OA in crop production. Therefore, a very good understanding of the capability and suitability of different types of OA along with improvements in the consistency of their composition and nutrient release is required to enhance the rate of adoption of OA application in broadacre agriculture.

1.8. Granulation of mineral fertilisers with organic materials

Blending and subsequent granulation of conventional synthetic fertilisers with organic materials is usually practised to obtain a desirable concentrations of nutrients in the final blended product. The materials are blended in such a manner so that the blend can deliver immediate and prolonged nutrient supply to plants and help to minimise the loss of nutrients. Blending of organic material with synthetic fertiliser to meet crop nutrient requirements has several benefits over the application of organic material or synthetic fertiliser as a sole nutrient source. Organo-mineral blends supply organic matter to soil whilst simultaneously enhancing nutrient availability to crop plants by slowing down the nutrient release due to increased adsorption by organic matter (Richards et al., 1993). As a result, nutrients are available to plants throughout the plant growing cycle with the implication that less fertiliser needs to be applied to the soil, thereby reducing input costs and the potential for environmental pollution. Moreover, organic-synthetic blended fertiliser often contain a large amount of humic acid, which can improve nutrient cycling and uptake by crop plants (Rao et al., 2007). Granulation of organo-mineral blends offers an opportunity to improve nutrient concentration and characteristics of OA to facilitate precise land application with farm machinery and affordable transportation, thus creating scope for large scale commercial production and revenue source to producers.

Recently, there has been increasing trend towards using organo-mineral granules in agricultural crop production because of their suggested potential benefits on nutrient cycling, crop yield and soil health. González et al. (2015) found that blending of urea with biochar slowed down the release rate of urea-N in soil. Richards et al. (1993) reported that granulation of NH_4NO_3 with peat reduced NO_3^- leaching from soil. Similarly, Sikora and Enkiri (2000) also blended ammonium nitrate with biosolids compost and found that the blended product significantly increased yield and N uptake by fescue. In another experiment, Antille et al. (2014) found that application of biosolids derived organo-mineral blends increased biomass yield of ryegrass and organic matter content of soil compared to commercial synthetic fertilisers. More recently, Mazeika et al. (2016) reported that addition of an organo-mineral blend formulated from poultry litter and ammonium chloride significantly increased yields of potato and rapeseed compared to sole application of chemical fertiliser. A recent study conducted by Rose et al. (2016) also found that fertilisation of soil with brown coal-urea (BCU) granules decreased N_2O emissions compared to commercial urea treated soil.

The fortification of synthetic fertilisers with organic matter in a granular form could enhance consistency in nutrient release and increase fertiliser use efficiency as well as return organic matter back into soil, restoring soil health and improving crop yield in a more sustainable manner. In Australia, Victorian brown coal (BC) is a readily available, inexpensive source of natural organic matter with high organic C and humic acids (HAs), suitable for land application (Rose et al., 2106).

1.8.1. Brown coal as an organic amendment

A number of studies indicates that combining natural organic materials high in humic substance content with N fertilisers, leads to more efficient nutrient uptake by plants and decreased N losses in the environment from excess N fertiliser application (Debska et al., 2002; Kwiatkowska et al., 2008). In recent years, organic amendments other than traditional manures have been investigated for their potential to restore soil organic matter levels and provide associated benefits (Kim Thi Tran et al., 2015). Brown coal, also known as lignite, is a low rank coal with low ash and very high moisture content (Kwiatkowska et al., 2008). Moreover, the Latrobe Valley, in south-eastern Australia has very large reserves of BC which is very cheap and easily available.

1.8.2. Properties of Victorian BC

Brown coal (BC) is formed from plant remains through the coalification process under heat and pressure (Woskoboenko et al., 1991). There is growing interest in the utilisation of BC and products derived from it in agricultural production due to their particular physical and chemical properties. Compared to other low rank coals found throughout the world, Victorian BC is high quality because it is low in ash, sulphur and heavy metals (Gloe, 1991). Usually, BC is acidic in nature, high in humic acid content and contains ion exchange groups capable of complexing or adsorbing nutrients (Kwiatkowska et al., 2008). The high intra-particle porous structure of BC contributing to high surface area further enhances its capacity for adsorption and absorption of nutrient ions (Karczewska et al., 1996b). The chemical structure of BC is highly complex, and includes aromatic rings, bridges such as alkyl, ether or ester linkages and side chains such as alkyl, carboxyl and hydroxyl groups and exhibits properties similar to those of soil humic acids (Kwiatkowska et al., 2008). The porous structure and functional groups of BC are can contribute to nutrient retention and support microbial colonisation (Kim Thi Tran et al., 2015). For example, NH_3 may be abiotically fixed on to the porous structure of humic acid (Bosatta and Agren, 1995). Similarly, due to the presence of functional groups in BC, specially carboxylic and phenolic groups that act as ion exchange site, BC has the ability to complex with metal and nutrient ions, including NH_4^+ (Wei De et al., 1988).

1.9. Impact of BC in agriculture

A large number of studies have demonstrated the suitability of BC as a soil amendment to improve the physical and chemical properties of soil for better crop production (Debska et al., 2002; Karczewska et al., 1996b; Kim Thi Tran et al., 2015; Little et al., 2014; Paramashivam et al., 2016; Piccolo et al., 1996; Rose et al., 2016). Brown coal is a very rich source of HAs which are a major component of soil organic matter. The HAs isolated from BC possess similar properties to the humic acids extracted from soil (Ayuso et al., 1996).

1.9.1. Effect of BC on soil properties

Addition of BC to soil can influence soil properties in many ways due to its complex structure. For example, it can change soil physical and chemical properties by changing soil pH (Yazawa et al., 2000), increasing SOC (Schmidt et al., 1996) and electrical conductivity in soil (Imbufe et al., 2005) by decreasing heavy metal bioavailability in soil (Janoš et al., 2010; Pusz, 2007). Humic acids improve soil physical properties through formation of stable clay-humic aggregates. For instance, Piccolo and Mbagwu (1989, 1994) observed a significant increase in water-stable aggregates in soil treated with HAs isolated from BC. Moreover, application of BC as a soil amendment also increase aeration, water infiltration and percolation, reduce runoff and erosion loss of soil (Piccolo et al., 1996). In another study, Piccolo et al. (1997) found that addition of HAs extracted from oxidized BC to soil reduced runoff erosion by 36% due to increased water retention capacity.

1.9.2. Effect of BC on soil microbial activity

The soil microbial community plays an important role in the availability of nutrients for plant growth by influencing the mineralisation, immobilisation and transformation of nutrients from organic matter. The microbial biomass and their activity can be influenced by the addition of soil organic amendments (Anderson et al., 2011; Paranychianakis et al., 2013). The effect of BC on the soil microbial community has been less studied than other areas. Emmerling et al. (2000) observed that soil application of BC increased microbial and enzyme activity in soil, whereas Vallini et al. (1997) found that addition of coal-derived HAs stimulated the aerobic bacterial population. Similarly, Visser (1985) reported increased abundance of amylolytic, proteolytic and denitrifiers in soil amended with soil derived HAs. Puglisi et al. (2009) also identified the microbial community by denaturing gradient gel electrophoresis and found that the diversity of the microbial community increased due to the addition of HAs. Hartz and Bottoms (2010) also found increased bacterial and fungal abundance in soil as response to application of various coal derived commercial humate products. In contrast, no significant variation in microbial biomass C, N and community structure was observed following addition of lignite and lignite derived commercial products (Kim Thi Tran et al., 2015; Little et al., 2014).

1.9.3. Effect of BC on growth, nutrition and yield of crop plants

Brown coal and HAs derived from it can be considered as organic amendments for increasing crop production. Addition of BC and HAs to soil has been shown to promote root and shoot growth as well as increased yield of a variety of grain and vegetable crops (Arancon et al., 2006; Eyheraguibel et al., 2008; Lee and Bartlett, 1976; Nardi et al., 2002; Piccolo et al., 1993; Puglisi et al., 2009). It has been claimed that HAs, isolated from BC and other sources, usually increase plant yields and enhance nutrients uptake by plants (Ayuso et al., 1996; Nardi et al., 2002; Nikbakht et al., 2008). For instance, Michael and Lua (1997) found that the addition of HAs showed increased uptake of N, P and S by plants. Recently, Paramashivam et al. (2016) found that addition of lignite in combination with urea and biosolids increased pasture growth compared to soil treated with urea alone. Conversely, in a pot trial experiment, Little et al. (2014) found no significant effect on pasture growth following application of raw BC and different commercial BC derived products compared to commercial urea. Similarly, addition of BCU granule produced by superheated steam drying process did not show any significant increase in pasture biomass in relation to commercial urea (Rose et al., 2016).

1.9.4. Effect of BC on N cycling

It is suggested that BC or HAs derived from coal can play an important role in the global N cycling by influencing the distribution, bioavailability and ultimate fate of organic N (Paramashivam et al., 2016). Humic substances can incorporate N into their structure either directly through chemical reactions with the acid functional groups or indirectly through microbial activities and subsequent decomposition of microbial biomass. It is evident from several reports that NH_3 may be abiotically fixed onto the porous structure of BC (Bosatta and Agren, 1995; Lapierre et al., 1994; Nommik and Vahtras, 1982).

Bollmann and Laanbroek (2001) hypothesised that BC may reduce volatilisation losses of N from urea fertilised soil by inhibiting the urease enzyme activity, thereby increasing urea-N availability to plants. As a result, the increased NH_3 could, in turn, enhance the NH_3 oxidising archea and bacterial communities in soil. Husted et al. (1991) reported that addition of OA containing acid functional groups significantly reduced volatilisation losses of NH_3. For instance, soil incorporation of lignite decreased NH_3 losses by 60-68% from cattle manure amended feedlots (Chen et al., 2015; Sun et al., 2016). A recent study conducted by Rose et al. (2016) found that fertilisation of soil with BCU granules decreased N_2O emissions compared to commercial urea treated soil. On the other hand, other studies reported increased N_2O emissions from soil treated with raw lignite (Paramashivam et al., 2016; Sun et al., 2016). In the same study, Rose et al. (2016) reported that application of BCU granules to soil decreased NO_3^- leaching and maintained higher amounts of mineral N in the topsoil compared to urea treated soil only. Similarly, a slight decrease in NO_3^- leaching from soil treated with lignite was reported by Paramashivam et al. (2016).

It is evident from a large number of experimental results that addition of BC and HAs increased mineral N availability and its uptake by a variety of vegetable, pasture and grain crops (Adani et al., 1998; Nardi et al., 2000; Piccolo et al., 1992; Pinton et al., 1999; Rauthan and Schnitzer, 1981; Rose et al., 2016; Sánchez Sánchez et al., 2009). However, the effect of adding BC and BC derived HAs on plants and soils largely depends on the origin, application rate, soil and plant types (Rose et al., 2014). Therefore, it is very difficult to generalise about the effect and mechanisms by which BC and HAs influence plant growth and properties of soil. Nevertheless, several mechanisms have been proposed. Some studies reported the formation of hydrogen bonds between acid functional groups, particularly carboxylic or phenolic groups and urea-N, under acidic pH (Gao et al., 1999). Other studies described the physical adsorption of NH_4^+ on to the porous structure of BC (Rose et al., 2016) and formation ionic salts of NH_4^+ and oxygen containing functional groups of BC (Jochová et al., 2004). Likewise, BC has a higher number of active functional groups with a higher resultant cation exchange capacity, which may increase retention of NH_4^+ ions and decrease its mobility in the soil profile (Wong et al., 1996).

1.10. Conclusions

The dynamics of N movement in agroecosystems are very complex and controlled by interdependent physical, chemical and biological processes. The role of BC and coal derived products in determining plant available N, growth and yield is well known. Some studies reported the effect of BC on yield and gaseous loss of N. However, the mechanisms responsible for decreasing or increasing gaseous losses of N by BC are largely unknown and dependent on the individual properties of both BC and soil. Moreover, to date, there are a few reports on the granulation effect of BC with urea on dynamics and mineralisation of N in soil and on crop growth and yield in glasshouse conditions. However, no experimental results were found regarding BCU granule addition on gaseous N loss and crop yield in field conditions. There is a lack of information with regard to the blending effect of BC with urea on gaseous losses (N_2O and NH_3) and leaching losses (NH_4^+ and NO_3^-) from different soil types. A clear understanding is a prerequisite for the application of BCU granules as a nitrogenous fertiliser as a tool to combat environmental pollution by reducing N losses via different pathways.

1.11. Research hypothesis aims and objectives

There is evidence that addition of BC and coal derived HAs to soil can increase growth and availability of N to crop plants under certain conditions. However, the interactive effect of BC with urea due to blending on N dynamics is still unclear. In most of the work using BC and HAs as soil amendments, the total N balance is hardly considered, especially the N derived from the soil organic pool. In addition, the processes controlling the transport and movement of N between organic and synthetic pools are yet to be fully elucidated.

This thesis presents the results of research conducted to increase our understanding of the physical and chemical interaction of BC with urea, formulated as granules. The dynamics, mineralisation and availability of N supplied as BCU granules to plants was studied. The hypothesis underpinning this work is that blending and subsequent granulation of BC with urea will increase the availability and retention of N by BC and will decrease the gaseous and leaching losses of N via different pathways by slowing the release rate and mobility of N in soil. In turn, this will lead to more efficient use by the plant of the N provided and retained.

This may occur by cation exchange mechanisms, whereby the NH_4^+-N can be exchanged with the acid functional groups of BC or any form of N can be bind on the porous surface area of BC by adsorption, decreasing its mobility and leaching loss in soil. Moreover, BC has the potential to slowdown urea hydrolysis by inhibiting the urease enzyme activity and thereby decrease the volatilisation loss of N.

1.12. Thesis outline

This thesis describes the physical and chemical interaction of BC with urea due to blending on the dynamics and movement of N and their influence on crop growth and soil health. To understand the background of the present research, a comprehensive literature review and the research gaps are discussed in Chapter 1.

In order to understand the interaction between and BC and urea-N and evaluate their efficacy as commercial fertiliser it is necessary to optimise the formulations having commercial fertiliser standards that can be handled by currently used farm machinery. Four different BCU granules were prepared using propriety granulation technology. The physical and chemical characterisation, and N release pattern from the BCU granules are described in Chapter 2.

Following the characterisation of BCU granules, the next step was to observe the effect of BCU granules on the dynamics and mineralisation of N in soil systems. This provided baseline information about the blending effect of BC with urea on the movement and gaseous losses of N in soil compared to commercial urea alone. Chapter 3 describes three sequential soil column incubation experiments which investigate the effect of different BCU blends on the dynamics and mineralisation of N in three contrasting soil types of Australia.

Measurement of gaseous and leaching losses of N in soil column experiments provides information about the effect of BCU blending on N availability in soil and identify suitable BCU formulations. However, these findings cannot be directly implemented to a soil-plant based agroecosystem. Therefore, Chapter 4 describes a glasshouse pot trial experiment which tested the effect of BCU blends on the growth, biomass yield and N uptake by silver-beet in two contrasting soils.

The glasshouse study was important for identifying the soil and plant responses and assessing the application rate of BCU blends in a controlled environment compared to commercial urea. However, without validation of this experiments in field conditions, any role of blending BC with urea as nitrogenous fertiliser in agroecosystems remains merely speculative. Chapter 5 describes the effect of BCU blends on the growth, yield and N uptake by sweet corn in field conditions.

Although the soil column study, glasshouse and field studies gave a clear idea on the potential of BCU granules as slow release N fertiliser compared to commercial urea, it is not possible to obtain a complete understanding about the movement of N among various

N pools. Moreover, the effect of BCU addition on the total N balance is necessary to identify the specific effect of BCU granules on N use efficiency. Therefore, Chapter 6 describes the fate and recovery of applied N as BCU granules in the soil-plant system through a ^{15}N labelled microcosm experiment.

All the above mentioned experiments provide valuable information regarding the blending effect of BC with urea on N dynamics, soil health, growth, yield and N uptake by crop plants both in laboratory, glasshouse and field conditions. Chapter 7 explains some of the mechanisms responsible for the observed results obtained in the previous experiments.

Chapter 8 brings together all the key findings, new knowledge and understanding that has been developed through this thesis from the experiments conducted. The results from each experiment led to many more research questions, some of which are outside the area and scope of this thesis. Some of these questions are explained, which may provide the basis for further future research.

2. Chapter: Hybrid brown coal-urea fertiliser reduces nitrogen loss compared to urea alone

This thesis chapter is based predominantly on the final manuscript published in the peer-reviewed Science of the Total Environment. The full reference for the published paper is:

Saha, B.K., Rose, M.T., Wong, V., Cavagnaro, T.R. and Patti, A.F. (2017) Hybrid brown coal-urea fertiliser reduces nitrogen loss compared to urea alone. Science of the Total Environment, 601–602: 1496-1504.

Declaration for Thesis Chapter 2

Declaration by candidate

In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
I designed the study in consultation with my supervisors, performed 100% of the lab work, and was the primary author of this article.	70

The following co-authors contributed to the work:

Name	Nature of contribution
Michael T. Rose	Co-designed the study, co-authored the manuscript
VanessaWong	Co-designed the study, co-authored the manuscript
Timothy R. Cavagnaro	Co-designed the study, co-authored the manuscript
Antonio F. Patti	Co-designed the study, co-authored the manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate’s and co-authors’ contributions to this work.

Candidate’s signature : BKSaha Date: 16-12-2017

Main Supervisor’s signature : Antonio F. Patti Date: 16-12-2017

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Hybrid brown coal-urea fertiliser reduces nitrogen loss compared to urea alone

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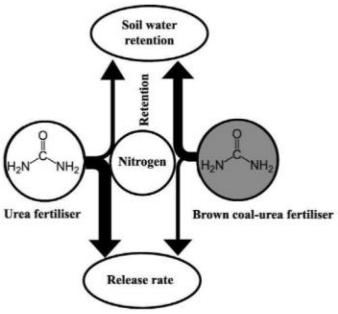
HIGHLIGHTS

- Granulation of brown coal with urea decreased the rate of fertiliser N release under laboratory conditions.
- Brown coal retained N for longer period by adsorption and ion exchange.
- Addition of brown coal-urea blends increased water holding and retention capacity of soil.
- Soil incorporation of BCU granules improved biomass yield and N uptake by plants.
- Brown coal can be used as a substrate to develop slow release nitrogenous fertiliser.

GRAPHICAL ABSTRACT



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ABSTRACT

Synthetic nitrogen (N) fertilisers, such as urea, are susceptible to rapid dissipation from soil. More gradual release of mineral N from fertiliser may reduce the off-site movement of mineral N, thereby enhancing N supply to crops and minimising negative off-site impacts. We hypothesised that granulation of urea with humified brown coal (BC) delays mineral N release and maintains higher concentrations of N in soil than conventional urea granules. Four different brown coal-urea granules, with C:N ratios of 1-10, were prepared by pan granulation. Advanced spectroscopic and X-ray powder diffraction (XRD) techniques confirmed loading of urea-N into the BC structure. Nitrogen-release from BCU granules was slower than from urea, resulting in higher N retention over a longer period for increasing growth and N uptake by crop plants. This trend increased with higher loading of BC, emphasising the significant role of BC in N retention. These findings support the hypothesis that BC is suitable for developing slow release N fertilisers.

1. Introduction

Nitrogen is one of the most important, and limiting, nutrient elements for ecosystem provisioning services (Jackson et al., 2008). Urea is the dominant and main source of N input in many crop production

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systems world-wide because of its high N content. Unfortunately, the use-efficiency of urea fertiliser is very poor and recovery of N in soil-plant systems seldom exceeds 50% of applied N (Raun et al., 2002). The low N-use efficiency of urea is associated with N losses by leaching, denitrification and volatilisation (Fageria and Baligar, 2005). This is a major challenge for farmers worldwide, since the lost N represents both an economic cost and an environmental burden, as off-site N transport in water pollutes both surface and ground water, whilst nitrous oxide contributes to greenhouse gas accumulation in the atmosphere (Vitousek et al., 1997). The widespread loss of organic matter from arable soils has been implicated as one factor contributing to poor fertiliser N-use efficiency, because soils depleted in organic carbon are often impaired in their ability to retain N (Accoe et al., 2004). Furthermore, studies show that the carbon sequestration potential of soil is intricately linked with sequestration of other nutrients, especially N (Dong et al., 2009).

There is an increasing interest in the application of organic fertilisers (Das and Adhya, 2014; Hargreaves et al., 2008; Li et al., 2015), as they can contribute to climate change mitigation through C sequestration, and supply organic matter to soil at the same time (Diacono and Montemurro, 2011). Organic matter improves soil water holding capacity, soil buffering capacity, cation exchange capacity, soil structure, and increases the availability of plant nutrients by increasing microbial biomass and their activities (Ge et al., 2009). Traditionally, organic fertilisers in the form of cow-dung, compost, farm yard manure, poultry litter, municipal wastes and humic substances were applied in various forms and in large amounts to improve crop productivity (Hargreaves et al., 2008; Imbufe et al., 2005; Loecke et al., 2004; Mondini et al., 2008). Efficient use of manure as a fertiliser is complicated by the imbalance of nutrients, variability in sources, difficulties in estimating nutrient availability, relatively low nutrient concentration and very large application rate limiting the distances manure can profitably be transported for use as a fertiliser (Albiach et al., 2001; Edmeades, 2002; Hargreaves et al., 2009; Quilty and Cattle, 2011). However, fortification of inorganic fertilisers with humified organic matter could enhance fertiliser use efficiency and return organic matter back into soil, restoring soil health and improving crop yield in a more sustainable manner (Ahmad et al., 2008).

Considerable scientific evidence indicates that combining natural organic materials high in humic substances with N fertiliser, leads to more efficient nutrient uptake by plants and decreased N losses in the environment from excess N fertiliser application (Debska et al., 2002; Kwiatkowska et al., 2008). In recent years, organic amendments other than traditional manures have been investigated for their potential to restore soil organic matter levels and provide associated benefits (Kim Thi Tran et al., 2015). Brown coal (BC), also known as lignite, is one such substrate, as it is high in humic acid content and contains ion exchange groups capable of complexing or adsorbing nutrients (Kwiatkowska et al., 2008). The high porosity of BC further enhances its capacity for adsorption and absorption of nutrient ions on its porous surfaces (Karczewska et al., 1996). The chemical structure of BC include aromatic rings, bridges such as alkyl, ether or ester linkages and side chains such as alkyl, carboxyl and hydroxyl groups and exhibits properties similar to those of soil humic acids (Kwiatkowska et al., 2008). The porous structure and functional groups of BC are very important for better nutrient retention and microbial activity. For example, ammonia-N may be abiotically fixed to humic acid (Bosatta and Agren, 1995) when the C:N ratio is higher than 10 (Knicker et al., 1997).

Brown coal may reduce volatilisation loss of N from urea-fertilised soil by inhibiting urease enzyme activity, thus increasing urea-N availability for plants (Bollmann and Laanbroek, 2001; Sun et al., 2016). More recently, Rose et al. (2016) demonstrated that granulation of urea with BC alters the dynamics of fertiliser N in soil compared with unmodified urea, including reductions in mineral N leaching and nitrous oxide emissions. There is therefore, strong evidence to support ongoing research into optimisation of brown coal-urea fertilisers to provide a product with both slow release and immediate nutrient availability properties. Moreover, refinements in the preparation of such organo-mineral fertiliser granules are required to maintain granule integrity for soil application through currently used machinery. This study aimed to manufacture and characterise a number of different BC-urea fertiliser blends, and to quantify the subsequent N release patterns as a basis for the formulation of improved fertiliser granules that minimise N losses in agricultural use. Two field experiments were conducted to assess the performance of BCU granules for improving crop N acquisition.

2. Materials and methods

2.1. Preparation of brown coal-urea granules

Brown coal-urea (BCU) granules were prepared in Feeco International, Pakenham, Victoria using propriety granulation technology. Air-dried Loy Yang BC collected from the Latrobe Valley, Victoria, Australia, was used for the preparation of the fertiliser granules. The physical and chemical characteristics of the BC used in this study are given in Table 1.

Four different brown coal-urea (BCU) granules were prepared by mixing BC and urea on the basis of C:N ratios presented in Table 3. The urea blended in this study was supplied by Elders Agribusiness Company, Australia (elders.com.au). Various amount of urea and BC were blended to match with the theoretical C:N ratio based on the C and N content of BC and urea, respectively. The BC and urea were powdered prior to mixing in a pin mixer to form seed granules. Starch and molasses were used as binders to assist the granulation process and increase granule strength. Seed granules were subsequently developed in a pan granulator and dried in a rotary drum oven between 180 and 200 °C to reduce the moisture content to 4–5%. A schematic of the granulation process of BCU preparation is shown in Fig. 1.

Table 1
Physical and chemical properties of the Victorian brown coal used in this study.

Property	
Moisture (%)	62.2
pH (H_2O)	4.45
Ash (%)	1.46
Volatile matter (%)	51.4
Total carbon (%)	66.8
Hydrogen (%)	4.93
Nitrogen (%)	0.58
Sulfur (%)	0.42
Oxygen (%)	24.3
Silica (%)	0.34
Iron (%)	0.13
Calcium (%)	0.04
Magnesium (%)	0.08
Sodium (%)	0.11
Chlorine (%)	0.14
Phosphorous (%)	0.01
Potassium (%)	0.02
Aluminium (%)	0.02
Manganese (mg kg^{-1})	53
Copper (mg kg^{-1})	<1
Molybdenum (mg kg^{-1})	2.69
Cobalt (mg kg^{-1})	2.13
Nickel (mg kg^{-1})	6.72
Zinc (mg kg^{-1})	3.19
Selenium (mg kg^{-1})	18.4
Cadmium (mg kg^{-1})	0.82
Lead (mg kg^{-1})	7.23
Chromium (mg kg^{-1})	<1
CEC ($cmol_c$ kg^{-1})	76.3

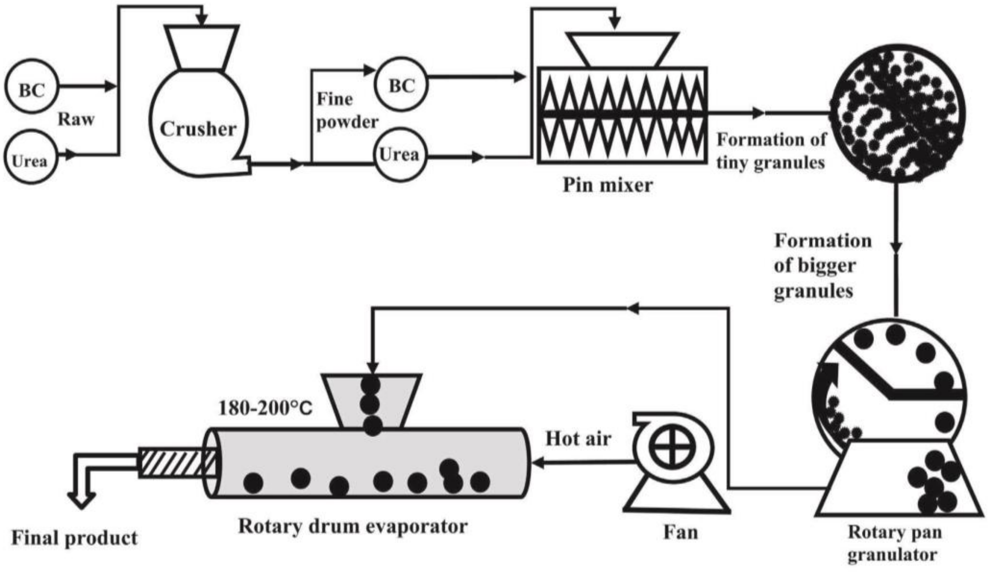

Fig. 1. Schematic of granulation process of BCU granules preparation.

2.2. Characterisation of BCU granules

2.2.1. Physical and chemical properties of BCU granules

Granule particle size, moisture content, crush strength, and CHN composition were measured as follows. The size of fifty randomly selected granules from each blend was measured using slide calipers. The moisture content of the product was calculated based on the product mass before and after oven drying at 105 °C for 4 h according to Eq. (1) (product moisture content).

$$\text{Moisture content } (\%) = 100 \times \frac{\text{wet product mass} - \text{dry product mass}}{\text{wet product mass}} \quad (1)$$

Single particle compressive crush strength tests were carried out on granules using a bench top scale Instron testing machine (Chattilon, 50LBF, AMETEK). The maximum force applied to break the granule was measured. A minimum of 20 granules from each of the BCU blends were tested and the mean and standard deviation of the crush strength were determined. The weight of 20 individual granules were measured and averaged. The C, H and N content of the samples were determined by combustion using a high-frequency induction furnace CHN analyser (Vario Micro Cube).

2.2.2. Structure analysis of BCU of the granules

The chemical structures of the BCU granule (C:N = 1.5), unmodified urea and BC were characterised using Fourier Transform Infrared Spectroscopy (FTIR), ^{13}C Solid State nuclear magnetic resonance (NMR) spectroscopy and powder X-ray diffraction (PXRD). The FTIR spectra of very finely powdered samples were obtained in the wave length range of 400 to 4000 cm^{-1} with a FTIR spectrophotometer using the Attenuated Total Reflectance (ATR) technique. ATR spectra were acquired using a golden gate single bounce diamond ATR mounted in a nitrogen purged Bruker Equinox 55 FTIR spectrometer, equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. The samples were kept in good optical contact on the diamond surface with a consistent application of low pressure. A background spectrum of a blank diamond was used to generate transmission spectra. Solid State ^{13}C NMR spectra were determined using a Bruker 100 (^{13}C) MHz spectrometer with cross polarisation-magic angle spinning (CP/MAS). Mineralogical and structural characteristics of urea, BC and BCU granules were determined by X-ray diffraction analysis of powdered samples using a Bruker D8 Advanced Powder X-ray Diffractometer at a scanning speed of 2°θ min^{-1}.

2.2.3. Water absorbency of BCU granules

Accurately weighed BCU granules (5 ± 0.1 g) were immersed in 20 mL of deionised water and allowed to soak at room temperature for 10 min. The swollen granules were isolated from non-absorbed water and weighed (Wu et al., 2008). The water absorbency (WA) was calculated using Eq. (2) (water absorbency measurement):

$$WA = \frac{M - M_0}{M_0} \quad (2)$$

where, M and M_0 denote the weight of wet granules and dry granules, respectively.

2.2.4. Water-holding capacity of soil amended with BCU granules

Loamy soil collected from Horsham, Victoria was used in this study. The physical and chemical properties of soil are presented in Table 2. Individual quantities (5 ± 0.1 g) of each different BCU granule were well-

Table 2
Physical and chemical properties of soil.

Property		
Australian soil order	Vertosol	Sodosol
USDA soil order	Xerertic Vertisol	NA
Texture	Clay loam	Sandy loam
Bulk density (g cm^{-3})	1.10	1.40
Moisture (%)	7.26	14.6
pH (H_2O)	8.32	6.27
Total carbon (%)	1.31	2.1
Nitrogen (%)	0.09	0.16
Ammonium nitrogen (mg kg^{-1})	20.6	7.1
Nitrate nitrogen (mg kg^{-1})	7.7	11.2
Phosphorus (Colwell) (mg kg^{-1})	39	9.3
Calcium (cmol$_c$ kg^{-1})	30.8	1.3
Magnesium (cmol$_c$ kg^{-1})	6.4	1.7
Potassium (cmol$_c$ kg^{-1})	2.18	5.3
Sulfur (mg kg^{-1})	1.8	2.7
Iron (mg kg^{-1})	24	11

mixed with 60 g of dry soil and kept in individual poly vinyl chloride (PVC) tubes (1.8 cm diameter and 25 cm long); the bottom of the tube was sealed using a permeable nylon fabric and weighed (W1). Deionised water was added to the soil columns until saturation and drainage occurred. The tube was weighed (W2) again when free drainage has ceased. Two control treatments, viz., with no BCU granules and with straight BC granules were also included to compare the results with BCU granules. The experiment was laid out in a completely randomised design with five replicates. The water-holding capacity (WHC%) of the soil was calculated using the equation of Wu et al. (2008) (water holding ratio measurement).

$$\text{WHC}\ (\%) = 100 \times \frac{\text{W2} - \text{W1}}{(\text{W2} - \text{W1}) + 65} \tag{3}$$

2.2.5. Urease activity and water retention capacity of soil amended with BCU granules

To 100 g of soil, 5 ± 0.1 g of the different BCU granules were mixed in a 250 mL plastic container. The soil was then saturated by adding 70 mL of deionised water slowly and weighed (W1). Two control treatments, viz., with no BCU granules or with straight BC granules were also included to compare the results with BCU granules. The experiment was laid out in a completely randomised design with five replicates. The containers were maintained at room temperature and weighed (Wi) at every 5 days interval over a period of 30 days. The water retention capacity (WRC%) of soil was calculated using the equation of Wu et al. (2008). The urease enzyme activity in soil was measured according to the method of Kandeler and Gerber (1988) 7 days after different N fertiliser additions (water retention capacity measurement).

$$\text{WRC}\ (\%) = 100 \times \frac{\text{W1} - \text{Wi}}{\text{Weight of soil (g)}} \tag{4}$$

2.2.6. Dissolution of N from BCU granules in water

A N dissolution incubation experiment was undertaken following the method of Dai et al. (2008). One gram of equivalent-N from each fertiliser blend preparation was accurately weighed and transferred into nylon mesh bags. The bags were subsequently placed into 250 mL plastic containers containing 200 mL of deionised water. The containers were incubated at 25 ± 1 °C for 14 days. The bags were taken out of the containers at days 1, 3, 5, 7 and 14 of the incubation, and transferred into 250-mL new plastic containers containing 200 mL of deionised water. The experiment was laid out in a completely randomised design with five replicates. The solutions were filtered through 0.45 μm membrane filter and collected for urea-N, ammonium-N and nitrate-N analyses. The solutions were analysed colorimetrically for urea-N using *P*-dimethylaminobenzaldehyde following the method of Singh and Saksena (1979). Ammonium-N was quantified by reacting with salicylate and hyphochlorite in a buffered alkaline solution contain sodium nitroprusside as a reductant (Forster, 1995). Nitrate-N (Nitrite) was determined by reduction of nitrate using vanadium (III) combined with colourimetric detection by acidic Griess reaction (Miranda et al., 2001).

2.2.7. Field experiments

Field experiments were conducted to determine the effect of BCU blends on the biomass yield and N uptake by canola and wheat compared to commercial urea fertiliser. The field trials were carried out in the experimental field of Southern Farming System (SFS), Inverleigh (38°10′29″S and 144°03′99″E), Victoria, Australia. A temperate monsoon climate prevailed in this area with mean annual temperature and precipitation of 18.5 °C and 551 mm, respectively. The key physicochemical properties of this soil are presented in Table 2. The individual plot size was 15 m^2 (10 m × 1.5 m). The field was divided into four blocks and each block was treated as a replicate. A 1 m buffer zone was maintained in between two blocks. Before seed sowing the soil was treated with Boxer Gold to minimise the weed growth and Lorsban to control pests. Seeds were uniformly sown with a seed driller, maintaining a uniform seed to seed and row to row distance. For the canola trial, N was added at the rate 70 kg ha^{-1} from urea and BCU 2. For the wheat trial, the soil was treated with various amounts N to get a yield response. The soil was fertilised with urea-N at 100% of the recommended dose (75 kg N ha^{-1}) and 75, 50 and 25% of the recommended N dose. For BCU blends, the soil was treated with 75, 50 and 25% of the recommended N dose from BCU 1, BCU 2 and BCU 3, respectively. In both the canola and wheat trial a control treatment with no N was also included. In addition, monoammonium phosphate (MAP) was incorporated at a rate of 100 kg ha^{-1} in all the plots except control plots. The experiments were laid out following a randomised complete block design. The canola biomass was harvested at flowering stage. The biomass N content of canola was determined by combustion using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The biomass N uptake by canola was calculated following the equation described by Finzi et al. (2007). Wheat was grown to maturity and grain yield was measured at harvest. The grain protein content of wheat was determined using Near Infrared Reflectance (NIR).

2.2.8. Statistical analysis

Statistical analyses were performed using the statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). All tests of significance were carried out at $P < 0.05$. Normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Levene's test. One way analysis of variance (ANOVA) was performed and the multiple comparisons among the different treatments were undertaken using a Scheffé test.

3. Results and discussion

3.1. Physical and chemical properties of BCU granules

The physical and chemical properties of the BCU granules and urea are presented in Tables 3–4. The moisture content of BCU granules varied from 2.9 to 3.6%. The moisture content of BCU granules was similar to that of urea (2.1%). The size of >90% of granules of different BCU blends fall in the range between 2 and 4 mm, with the average size of BCU granules equivalent to that of urea except for BCU 4 which was smaller and had the highest BC content. The individual granule weight of BCU 2 was significantly higher compared to other granules including urea. As expected, all BCU granules contained a higher %C and lower %N

Table 3
Physical properties and characterisation of BCU granules (values are mean ± standard error, N = 5).

Granules	C:N^{T}	C:N^{A}	Moisture (%)	Size (mm)	Crush strength (kg)	Water absorbency (g g^{-1})	Weight granule^{-1} (mg)
BCU 1	1.0	1.8	2.89 ± 0.11a	3.33 ± 0.21a	4.75 ± 0.37a	0.62 ± 0.02b	49 ± 1.2ab
BCU 2	1.5	2.7	3.56 ± 0.09a	3.43 ± 0.19a	6.69 ± 0.44a	0.85 ± 0.02b	58 ± 1.3a
BCU 3	3.0	5.4	3.40 ± 0.05a	3.19 ± 0.14a	2.38 ± 0.06b	1.03 ± 0.06b	34 ± 1.4c
BCU 4	10.0	10.8	3.21 ± 0.13a	2.89 ± 0.13c	0.42 ± 0.03c	1.70 ± 0.05a	18 ± 1.1d
Urea	ND	ND	2.19 ± 0.06a	3.36 ± 0.23a	5.28 ± 0.20a	ND	45 ± 1.3b

ND: not determined, T = theoretical and A = actual.
The values with different letters differ significantly according to Scheffé-test at $P < 0.05$.

Table 4
Chemical characterisation of BCU granules (values are mean ± standard error, N = 5).

Granules	C (%)	H (%)	N (%)	C granule^{-1} (mg)	N granule^{-1} (mg)
BCU 1	39.81 ± 0.05b	5.83 ± 0.09a	21.45 ± 0.08b	19.51 ± 0.07b	10.54 ± 0.02b
BCU 2	45.43 ± 0.07b	5.82 ± 0.2a	17.31 ± 0.08b	26.24 ± 0.05a	10.11 ± 0.02b
BCU 3	48.40 ± 0.16ab	4.81 ± 0.08a	8.33 ± 0.11c	16.22 ± 0.04c	2.83 ± 0.02c
BCU 4	53.83 ± 0.09a	5.22 ± 0.1a	5.74 ± 0.11c	9.69 ± 0.02d	1.12 ± 0.01c
Urea	19.22 ± 0.08c	6.41 ± 0.04a	45.72 ± 0.06a	8.61 ± 0.02d	20.50 ± 0.06a

The values with different letters differ significantly according to Scheffé-test at $P < 0.05$.

compared to commercial urea. BCU 1 granule contained the highest %N (21.45%) and lowest %C (39.81%) and BCU 4 granule containing the lowest %N (5.74%) and highest %C (53.83%). A significantly ($P < 0.05$) higher amount of C was measured in the individual granule of BCU 2 compared to other BCU granules and urea, while each urea granule contained significantly ($P < 0.05$) higher and about double the amount N compared to BCU 1 granules. No significant ($P > 0.05$) differences were observed in the N content of individual granules of BCU 1 and BCU 2 but the N content of these granules were about ten times higher than that of BCU 4.

Brown coal has previously been explored as a potential substrate for a slow release nitrogenous fertiliser by exploiting its extensive reactive surface area and humic acid content (Rose et al., 2016). Addition of humified BC to soil altered N cycling and dynamics in soil (Kim Thi Tran et al., 2015; Paramashivam et al., 2016; Rose et al., 2016; Sun et al., 2016). In this study, four formulations of urea-enriched BC granules were successfully made using the pan granulation technology (Fig. 1) to increase the understanding of mechanisms of N retention by BC. The N content of the dried products at all C:N ratios was close to the theoretical N content determined from the initial amount of urea addition to the wet coal (Table 3). The slightly lower N content in the BCU granules compared to the expected values may be due to the gaseous loss of N in drying process as has previously been observed for BCU granules produced using a simultaneous granulation and super-heated steam drying process (Rose et al., 2016). However, in contrast to Rose et al. (2016), no urea crystals were observed on the surface of BCU granules, indicating homogenous mixing of BC with the urea. This might be due to the effect of the pin mixing process of granulation, which improves agglomeration and densification to facilitate better N distribution and retention by BC after granulation. More than 90% of the BCU granules produced were of a suitable size for commercial use (2–4 mm), with low moisture content for practical long term storage and transport.

The mean crush strength of different BCU granules ranged from 0.42 to 6.69 kg. Most of the BCU granules maintained the standard fertiliser crush strength except BCU 4 which was lower. The crush strengths of BCU 1 and 2 were not significantly different ($P > 0.05$) from that of commercial urea. Formulation BCU 2 produced the hardest granules with a maximum crush strength and numerically higher than that of urea and the other BCU granules. The crush strength of fertiliser granules is another important characteristic determining its suitability for handling by farm machinery, and also its stability and degradation in the environment. The crush strengths of the dried BCU 1 and BCU 2 granules were statistically similar to commercial urea and other available fertilisers currently used in agriculture (Table 3). For handling purposes the granules crush strength should be at least 1.4 kg, and a crush strength ≥ 2.27 kg is highly desirable (Hignett, 1985).

3.2. Water absorbency, holding and retention capacity of BCU granules in soil

Blending of BC with urea increased the water absorbency, water holding capacity and retention of water in the soil used in this study (Table 3 and Fig. 2). The water absorbency increased gradually with an increase in BC content of the granules. The water absorbency of urea and BC granules were not measured due to their instability in water. Among the granules, BCU 4 showed significantly higher water absorbency compared to other granules. Significantly higher water holding capacity was observed in soil amended with BC only and BCU granules compared to urea and control soil. Among the different granules, BCU 4 had the greatest increase in water holding capacity and cumulative water retention capacity of soil.

As such, three of the four BCU granules were found to be physically suitable as alternatives to currently available commercial N fertilisers. On average, the BCU granule can absorb 1 g of water by each g of granule. Thus, addition of BCU granules in soil could potentially increase the soil moisture content and water retention capacity of soil. This is supported by the water holding capacity and water retention capacity data in which BCU granules amended soil showed significantly higher water retention in soil compared to urea and the control treated soil. This is similar to the findings of Xie et al. (2011), who reported that the addition of organo (wheat straw)-mineral fertiliser increased the water holding capacity of soil. Therefore, blending of BC with urea could be an option for increasing soil moisture and water retention for longer period of time in dry land and rain-fed agriculture, however

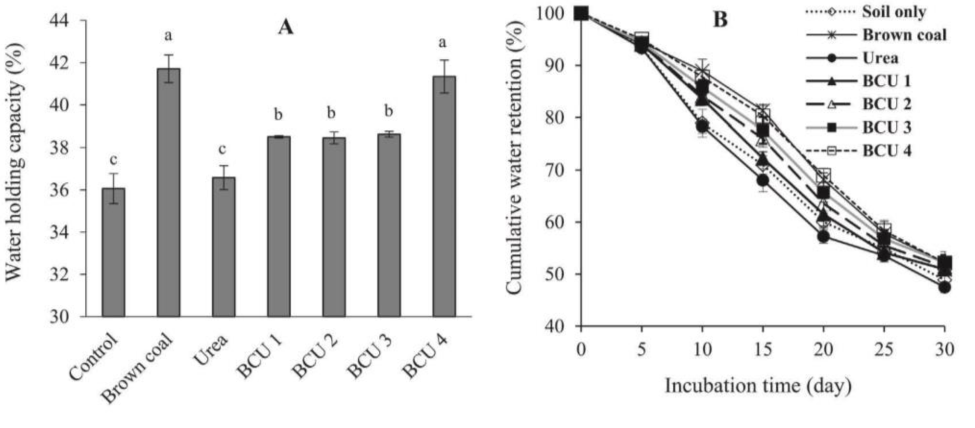

Fig. 2. Effect of BCU granules on the water holding capacity (A) and cumulative water retention capacity (B) of soil (values are mean ± standard error, N = 5).

the extent of the potential benefits at realistic application rates under field conditions requires further investigation.

3.3. Structural analysis of BCU granules

The FTIR spectra of BC, BCU ($C:N = 1.5$) and urea are shown in Fig. 3. Blending of BC with urea decreased the peak intensity at 1250 and 1710 cm^{-1} which are assigned to carboxylic groups. Blending of BC with urea also decreased the phenolic group peak intensity at 3396 cm^{-1}. The increased peak intensities at 1053 and 3251 cm^{-1} indicate loading of urea-N into BC due to C—N and N—H stretching, respectively. The introduced urea mainly interacts with the carboxylic and phenolic groups of the BC.

The NMR spectra of BC, BCU ($C:N = 1.5$) and urea are shown in Fig. 4. Each NMR spectrum is grouped into three major regions for the integration purpose: 220–170, 170–77 and 77–0 ppm, corresponding to carboxyl/carbonyl, aromatic and aliphatic carbons respectively. Both the BC and BCU blend contain higher amount of carboxyl and aromatic C relative to their aliphatic C. No differences were observed in aliphatic carbon of BC and BCU granules. Blending of BC with urea showed some interesting differences in the aromatic and carboxyl C compared to BC. The marked increase in the peak at position of 73 ppm clearly indicates the loading of N in BC by reacting with R-OH. Moreover, the decreased peak intensity at 125 ppm represents a decrease in carboxyl-C of BC. On the other hand, the increased peak intensity at 163 ppm could be the result of loading of the urea molecule in BC, based on a slight shift of the peak at 161 in the urea spectrum. The NMR spectra of BCU clearly indicate the loading of urea-N by chemical reaction with COOH or R-OH group of BC.

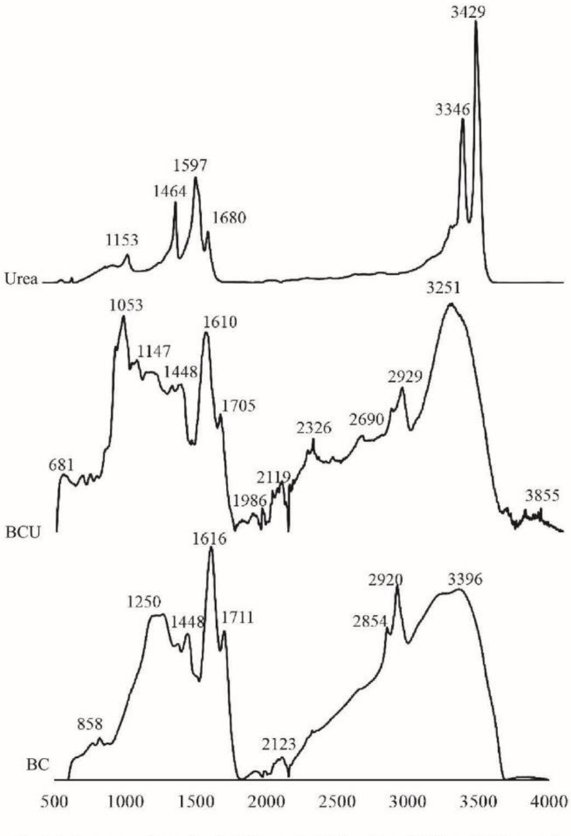

Fig. 3. FTIR spectra of urea (top), BCU granule ($C:N = 1.5$, middle) and BC (bottom).

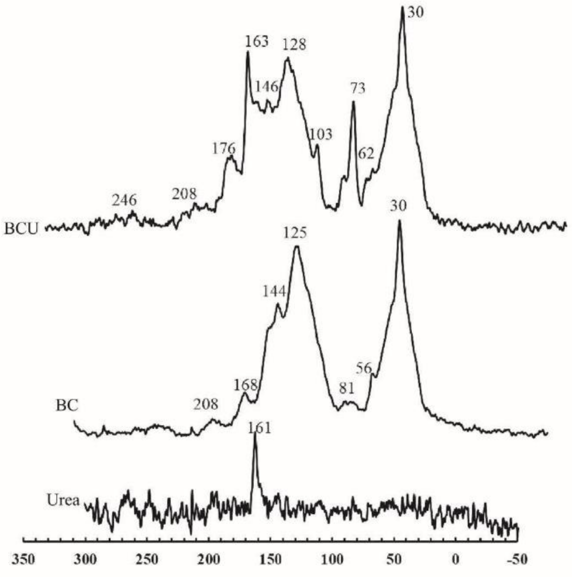

Fig. 4. ^{13}C CP/MAS NMR spectra of urea (bottom), BC (middle) and BCU granule ($C:N = 1.5$, top).

To monitor the structural changes and loading of urea into BC due to blending, the materials were analysed by XRD, as shown in Fig. 5. The BCU blend adopted a higher degree of crystalline order due to loading of urea or urea-N on to BC.

Results from FTIR and NMR of BC and the BCU blends support the hypothesis that the urea interacts with BC by chemical reaction with the carboxyl and phenolic groups of BC (Figs. 3–4). The decreased and shifted peak intensities in carboxyl and phenolic groups and appearance of new peaks in the BC clearly indicates the loading of urea-N or possibly other forms of N (e.g. NH_4^+-N or NH_3-N) into the structure of BC. This is supported by XRD analysis which showed an increased crystallinity in

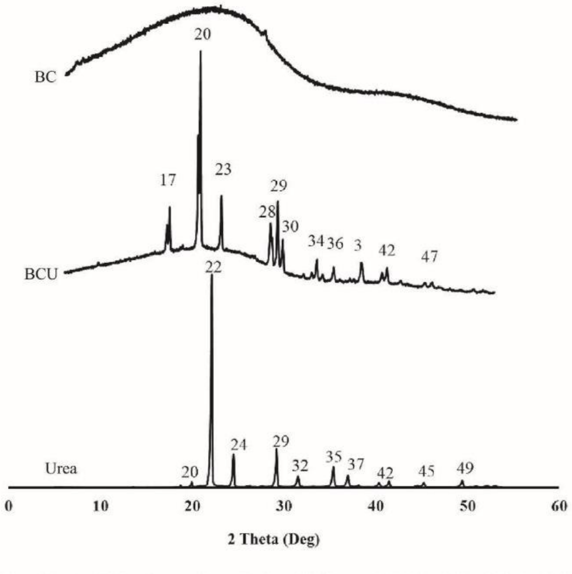

Fig. 5. Powder XRD patterns of urea (bottom), BCU granule ($C:N = 1.5$, middle) and BC (top).

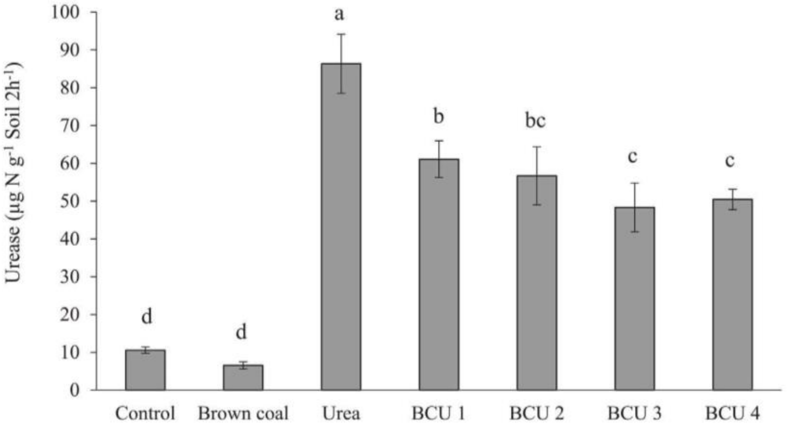

Fig. 6. Urease enzyme activity in soil 7 days after amendment with various N fertilisers (values are mean ± standard error, N = 5).

the structure of BCU granules compared with raw BC due to loading of urea-N into BC (Fig. 5). As previously highlighted, the enhanced agglomeration and densification of granules through pin mixing, may also have contributed to the altered crystalline structure of BCU formulations by loading of urea-N or NH_4^+-N onto BC. These findings corroborate those of Kwiatkowska et al. (2008), who also identified similar chemical changes in mineral enriched BC using FTIR, and those of Manikandan and Subramanian (2013), who reported changes to FTIR and XRD spectra under loading of urea-N into biochar.

3.4. Urease activity in soil

The urease enzyme activity in soil was significantly influenced by the addition of N fertilisers (Fig. 6). Urease activity in the soil amended with BCU granules was substantially lower than in soil amended with commercial urea. Among the different BCU granules, the granules with a higher proportion BC generated a lower urease activity in soil with respect to the granules with a lower proportion of BC and higher N loading. A similar finding was reported by Bollmann and Laanbroek (2001) who found that incorporation of brown coal to soil inhibited the urease enzyme activity in soil.

3.5. Dissolution of N from BCU granules in water

Blending of BC with urea decreased the rate of N release into water compared to urea (Fig. 7A). In the case of urea, the release of N was very rapid and it reached almost 100% by day 3 of the incubation period. For the BCU granules, N release was delayed and after 14 days of incubation, only 73% of added N was released in water as urea-N. The BCU blends containing a higher proportion of BC content displayed lower and slower N release. A similar trend was also observed for the release of NH_4^+-N from the granules. The release rate of NH_4^+-N increased with increasing incubation time (Fig. 7B). No release of NH_4^+-N was recorded for urea. The release of NH_4^+-N for the granules indicates the partial hydrolysis of urea-N to NH_4^+-N. No nitrate-N was released from either urea or from BCU granules during the incubation period.

The delayed N release of N into water from the BCU granules supports the findings of Rose et al. (2016) who reported that granulation of BC with urea reduced the release rate of N in soil, resulting in N retention over a longer period of time. Importantly, our results show that after 14 days of incubation an average of only 73% of added N was released into water as urea-N from the BCU blends.

Evidence from FTIR, NMR and XRD structural analysis suggests that the remainder of the N was retained by BC either by strong physical adsorption or by ion exchange with the acidic functional groups of BC. This could include (i) adsorption of urea-N or NH_4^+-N on the reactive surface area and polar functional groups of BC by hydrogen bonding, decreasing the mobility of N (Sun et al., 2016), and/or (ii) partial hydrolysis of urea, releasing ammonia subsequently captured and measured as ammonium. The lower retention of N by the high N granules appears to be a consequence of oversaturation of sorption sites within the BC matrix. This effectively increases the surface area of the crystalline urea and would promote faster dissolution and N release in water. The release of NH_4^+-N from the granules indicates the partial hydrolysis of urea-N to NH_4^+-N, likely to be caused by natural acidity of the BC and/or the presence of urease from microbial activity inherent in the BC (Kim Thi Tran et

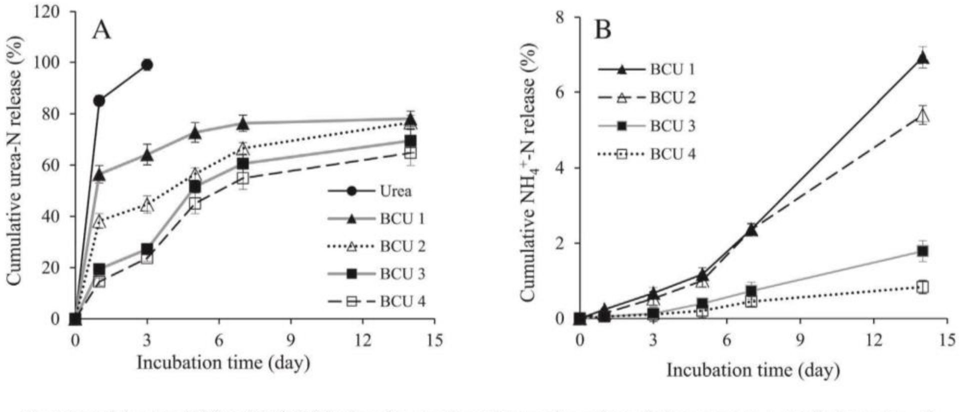

Fig. 7. Cumulative urea-N (A) and NH_4^+-N (B) release in water from BCU granules and urea (values are mean ± standard error, N = 5).

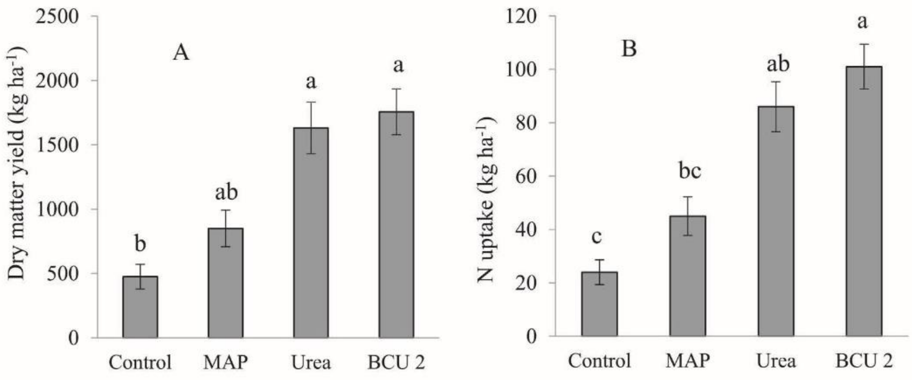

Fig. 8. Effect of BCU granules and urea on the dry matter yield (A) and N uptake (B) by canola (values are mean ± standard error, N = 4).

al., 2015). The NH_4^+-N can exchange onto carboxyl site of the BC, assisting in its retention in the soil. Overall, these findings provide strong mechanistic support for the development of BCU blends as a slow release fertiliser for better N retention in the plant-soil system. Therefore, BCU blends can be used as a slow release N fertiliser for better N retention in soil-plant system for a longer period of time. As a results, it is anticipated that addition of BCU blends may also increase N availability and its use efficiency to crop plants by decreasing its losses in the environment.

3.6. Field experiments

Addition of BCU granule and urea to soil caused a significant increase in the dry matter yield of canola compared to control treatment which received no N fertiliser (Fig. 8A). No significant difference in the dry matter yield of canola was observed between the plots treated with urea and BCU 2. Nevertheless, N uptake by canola was substantially higher in plots receiving N fertiliser as BCU compared to other treatments including those receiving an equivalent amount of fertiliser N as urea (Fig. 8B). No significant differences in the grain yield of wheat were observed between any treatments, but slightly increased grain yield was noticed with the increase in N application rate (Table 5). In contrast, significant variation in grain protein content was determined among the various treatments (Table 5). The increased application of N fertiliser increased the grain protein content of wheat. Although no significant difference in grain protein content was measured between urea and BCU treatments at N application rate higher than 55 kg N ha^{-1}, grain protein was significantly higher from plots receiving BCU compared to commercial urea when the N was applied at the rate of 37.5 and 20 kg N ha^{-1}. The increased grain protein and N uptake by plants fertilised with BCU granules might be due to increased N availability in soil for a longer period of time, as has been previously observed under glasshouse conditions in a sandy soil (Rose et al., 2016). The field results here demonstrated the potential agronomic value of BCU fertiliser and justify further research into the dynamics of N release, transport and plant availability from BCU fertiliser in different soils under various environmental conditions.

Table 5
Effect of BCU granules and urea on the grain yield and protein content of wheat.

Treatments	Yield (t ha^{-1})	Grain protein (%)
Control	5.43	9.4c
Urea (20 kg N ha^{-1})	5.53	9.4c
BCU 3 (20 kg N ha^{-1})	5.88	9.8b
Urea (37.5 kg N ha^{-1})	5.90	10.0b
BCU 2 (37.5 kg N ha^{-1})	6.03	10.5a
Urea (55 kg N ha^{-1})	6.08	10.5a
BCU 1 (55 kg N ha^{-1})	6.10	10.5a
Urea (75 kg N ha^{-1})	6.30	10.6a

4. Conclusions

Urea and BC were successfully blended and granulated using pan granulation technology. The granules formed were of an appropriate size and sufficiently strong for agricultural use. The loading of urea-N into BC was confirmed by FTIR, NMR and XRD characterisation. Granulation of urea with BC delayed the fertiliser N release, enhanced N retention in the granule and increased the moisture holding and retention capacity of soil compared to urea. Nitrogen retention increased concurrently with BC loading, emphasising the significant role of BC in N retention. Field experiments demonstrated increased N uptake by canola and grain protein content of wheat in BCU amended soil compared with the equivalent amount of N supplied in the form of urea. Based on the overall results and international fertiliser standards, BCU granules containing 8–17% N with a C:N ratio of 5.4 to 2.7 (i.e. BCU 3 and BCU 2 in this study) were shown to be the most suitable slow-release, enhanced efficiency N fertiliser for future research and application. This research has increased our understanding of how BC can be used as a substrate to develop improved slow release N fertilisers with a lower environmental hazard. Additional work is now necessary to study the effect of BCU granules on plant growth and N uptake in various soil types and under field conditions.

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2.1. Concluding remarks

This research has increased our understanding of how BC can be used as a substrate to develop improved slow release N fertilisers with a lower environmental hazard. The characterisation and N release data provides a clear understanding of physical and chemical interaction of BC and urea due to blending. As the following chapters will investigate the blending and granulation effect of BC with urea on the dynamics, mineralisation and uptake of N by crop plants in various soil types, this chemical information obtained in this chapter will provide a basis for the results which will be obtained in the following chapters, from which potential mechanisms can be identified. In this chapter, some preliminary field trial results were included as a basis of the effect of BCU on the plant growth and N uptake for future large scale and detailed study.

3. Chapter: Nitrogen dynamics in soil fertilized with slow release brown coal-urea granules

This thesis chapter is based predominantly on the final manuscript submitted in the peer-reviewed ACS journal *Environmental Science and Technology*. The full reference for the published paper is:

Saha, B.K., Rose, M.T., Wong, V., Cavagnaro, T.R. and Patti, A.F. Nitrogen dynamics in soil fertilized with slow release brown coal-urea granules, submitted.

Declaration for Thesis Chapter 3

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
I designed the study in consultation with my supervisors, performed 100% of the lab work, and was the primary author of this article.	70

The following co-authors contributed to the work:

Name	Nature of contribution
Michael T. Rose	Co-designed the study, co-authored the manuscript
VanessaWong	Co-designed the study, co-authored the manuscript
Timothy R. Cavagnaro	Co-designed the study, co-authored the manuscript
Antonio F. Patti	Co-designed the study, co-authored the manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

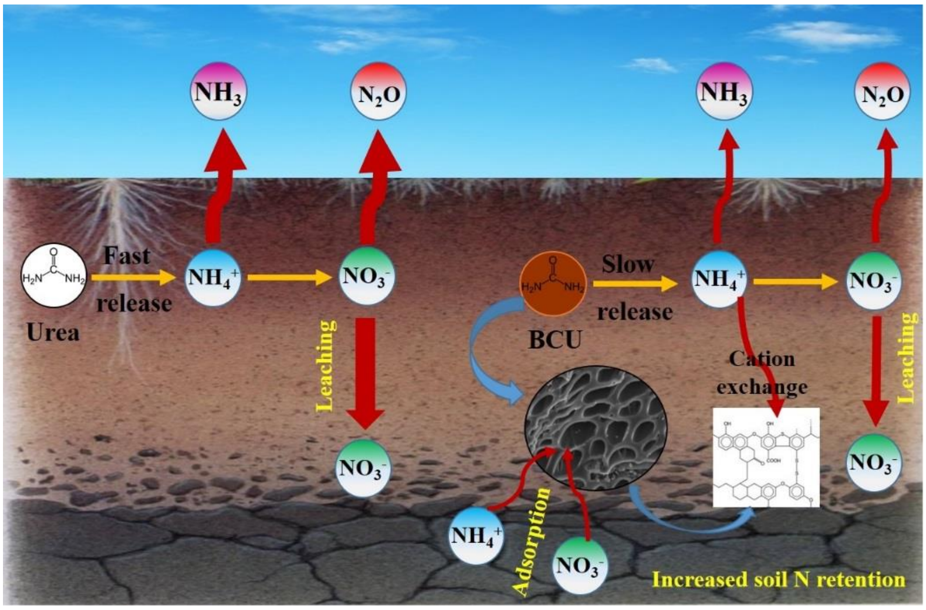
Candidate's signature : BKSaha Date: 16-12-2017

Main Supervisor's signature : Antonio F. Patti Date: 16-12-2017

ABSTRACT: Reducing the release rate of urea can increase its use efficiency and minimize negative effects on the environment. A novel fertilizer material that was formed by blending brown coal (BC) with urea, delayed fertilizer N release in glasshouse condition through strong retention facilitated by the extensive surface area, porous structure and chemical functional groups in the BC. However, the role of BC as a carrier of synthetic urea and the effect of their interaction with various soil types on the dynamics and mineralization of N remains largely unclear. Therefore, a soil column incubation study was conducted to assess the release, transformation and transportation of N from several different brown coal-urea (BCU) granules, compared to commercial urea. Blending and subsequent granulation of urea with BC substantially increased fertilizer N retention in soil by decreasing gaseous emissions and leaching of N compared to urea alone, irrespective of soil type. The BCU granule containing the highest proportion of BC had lower leaching and gaseous emissions and maintained considerably higher mineral and mineralizable N in topsoil. Possible modes of action of the BCU granules have been proposed, emphasizing the role of BC in enhancing N retention over a longer period of time. The results support the notion that BCU granules can be used as a slow release and enhanced efficiency fertilizer for increasing availability and use efficiency of N by crop plants.

Keywords: Brown coal, urea, slow release fertilizer, dynamics and mineralisation of N, fertilizer N use efficiency.

Graphical abstract



3.1. Introduction

Nitrogen (N) is one of the most important and limiting nutrients for agricultural crop production systems (Jackson et al., 2008). As a result, high rates of synthetic N fertilizers are commonly applied to improve agricultural crop production world-wide. Unfortunately, more than 50% of the added N fertilizers are commonly lost through leaching, denitrification and volatilization, resulting in poor fertilizer N use efficiency (Fageria and Baligar, 2005). The movement and transport of this lost N can cause serious environmental pollution by contaminating groundwater through nitrate leaching (124-160 kg N ha^{-1} yr^{-1}) and by contributing to greenhouse gas emissions (120-143 kg N ha^{-1} yr^{-1}) to the atmosphere (Cameron and Wild, 1984; Silva et al., 1999; Herzog et al., 2008; van der Salm et al., 2007). This lost N is also responsible for economic inefficiency by increasing the cost of agricultural production without contributing to higher yields. From both an economic and an environmental perspective, it is therefore necessary to increase the use efficiency of fertilizer N by reducing its losses via different pathways.

Considerable research efforts been aimed at developing suitable mitigation strategies to reduce gaseous and leaching losses of N from intensively managed agricultural systems (Behin and Sadeghi, 2016; Naz and Sulaiman, 2016; Paramashivam et al., 2016; Rose et al., 2016). Mitigation strategies, such as using controlled release or enhanced efficiency fertilizers or combined application of organic and synthetic fertilizers to minimise the loss of N for improving its use efficiency by crops plants have been extensively studied in the last few decades (McCrory and Hobbs, 2001; Petersen et al., 2012). However, the broadacre application of these technologies has not been adopted in intensive farming systems due to lack of consistency and cost in research findings confirming the efficacy of these strategies (Chen et al., 2008).

Granulation of synthetic fertilizers with organic materials is increasingly becoming more popular because of its potential benefits on crop yields and soil health compared to the application of organic material or synthetic fertilizer as a sole nutrient source (Antille et al., 2014; Mazeika et al., 2016). Indeed, the depletion of soil organic carbon (SOC) due to intensification of agricultural crop production can be another reason for declining fertilizer N use efficiency (Duan et al., 2014). This is because, SOC plays an important role in the retention of fertilizer N in soil and limits off-site N losses (Accoe et al., 2004). Organo-mineral granules supply organic matter simultaneously with added nutrients, which helps to reduce immediate nutrient release due to increased adsorption by organic matter and/or microbial nutrient immobilization (Richards et al., 1993). As a result, nutrients are available to plants for longer periods during the plant growing cycle with the implication that less fertilizer needs to be applied to the soil, thereby reducing the potential for environmental pollution and the cost of production (Wild et al., 2011).

There is a growing interest in the utilization of brown coal (BC) and BC-derived products in agricultural crop production due to their particular physical and chemical properties (Little et al., 2014). The surface area, porous structure and functional groups of BC (Wei De et al., 1988) have been demonstrated to increase the prevalence of carboxyl and phenolic functional groups in BC-amended soil (Kwiatkowska et al., 2008), enhanced nutrient retention and uptake (Mora et al., 2010), and facilitate cation binding (Simmler et al., 2013) with minimal effects on microbial activity (Kim Thi Tran et al., 2015). It is also evident that the addition of BC alone or in combination with urea can significantly influence the dynamics and mineralization of N in soil (Paramashivam et al., 2016; Rose et al., 2016; Saha et al., 2017; Sun et al., 2016). In a recent study it is found that granulation of urea with BC substantially reduced the release rate of fertilizer N compared to urea alone (Saha et al., 2017). Multiomics experimental results showed decreased NH_3 emissions (Chen et al., 2015; Sun et al., 2016), N_2O emissions and leaching loss of N (Rose et al., 2016), whereas increased N_2O emissions (Paramashivam et al., 2016; Sun et al., 2016) were also reported by the addition of BC alone or in combination with urea. A better understanding of the effect of blending and subsequent granulation of BC with urea on the dynamics and mineralization of N in various soil types is critical to enable successful use of BCU granules as slow release N fertilizer. In this study, we tested the hypothesis that blending and subsequent granulation of urea with BC reduces N losses by leaching and gaseous emissions, compared with conventional urea fertilizer, in three different soil types.

3.2. Materials and methods

3.2.1. Site and soil description

Three sequential soil column incubation experiments were carried out in the glasshouse of the Plant Science Complex, Monash University, Clayton, Victoria, 3800, Australia. Three contrasting soils were evaluated in this study. The first, a Ferrosol, was collected from an experimental field of Department of Primary Industries (DPI), Wollongbar Research

Institute, New South Wales (28°48′50″S and 153°23′51″E), the second, a Tenosol, from a farmer's field in the Wimmera (37°16′37″S and 143°16′49″E), Victoria and the third, a Vertosol, from an experimental field of Department of Primary Industries (DPI), Horsham Research Institute, Victoria (36°43′8″S and 142°11′46″E), Australia (Isbell, 2002). The soil samples were collected from a depth of 0-15 cm. The collected soil samples were mixed, air dried, homogenised by sieving to less than 0.2 cm, and stored for the incubation study. The physical and chemical properties of soils are presented in Table 3.1.

Table 3.1. Physical and chemical properties of soils

Property	Ferrosol	Tenosol	Vertosol
Texture	Clay loam	Sandy loam	Clay loam
Bulk density (g cm^{-3})	1.31	1.41	1.34
pH (Water)	4.69	7.23	8.32
Total carbon (%)	4.6	0.77	1.31
Total nitrogen (%)	0.54	0.13	0.24
Ammonium nitrogen (mg kg^{-1})	6.1	6.1	20.6
Nitrate nitrogen (mg kg^{-1})	140	2.2	7.7
Phosphorus (Colwell) (mg kg^{-1})	39	10	39
Exchangeable calcium ($cmol_c$ kg^{-1})	7.5	4.57	30.8
Exchangeable magnesium ($cmol_c$ kg^{-1})	1.3	0.73	6.4
Exchangeable potassium ($cmol_c$ kg^{-1})	0.30	0.24	2.18
Extractable sulfur (mg kg^{-1})	79.5	2.7	1.8
DTPA-iron (mg kg^{-1})	394	44	24
Extractable aluminium (mg kg^{-1})	61	2	2

3.2.2. *Brown coal-urea granules*

Four brown coal-urea (BCU) granules previously described and characterized (Saha et al., 2017), were tested in the soil column incubations. Total C and N contents of the BCU granules are listed in Table 3.2.

Table 3.2. C and N concentration of BCU granules

Granules	C concentration (%)	N concentration (%)
Brown coal-urea 1 (BCU 1)	40	22
Brown coal-urea 2 (BCU 2)	46	17
Brown coal-urea 3 (BCU 3)	49	9
Brown coal-urea 4 (BCU 4)	54	5

3.2.3. *Incubation experiment*

A two month incubation of soil samples was undertaken in polyvinyl chloride (PVC) tubes of 15 cm height and 4.25 cm radius with a volume of 851 cm^3. The equivalent of 1,115; 1,200 and 1,141 g dry soil was added to each PVC tube to match with the field soil bulk density of 1.31, 1.41 and 1.34 g cm^{-3} for the Ferrosol, Tenosol and Vertosol, respectively. Thirty five PVC columns for each soil type were covered at one end with nylon mesh and then packed with acid washed sand (1 cm depth) and overlying soil. Deionized water was added to maintain the soil moisture content at 60 % water-filled pore space (WFPS) throughout the incubation period. The soil was pre-incubated at 22±1 °C for one week to restore microbial activity. After this, the top (0-5 cm) of each soil column was uniformly amended with urea, BCU 1, BCU 2, BCU 3 and BCU 4. The amount of N applied was the same for all treatments, at the rate of 250 mg N kg^{-1} soil, either from granulated urea alone or one of the BCU granules. Two additional treatments were also included: one contained granulated raw BC at a rate equivalent to the BCU 4 and an un-amended control. Each experiment was laid out following a completely randomized design with five replicates. During the incubation period, three leaching events occurred: at days 15, 30, and 45 after treatment application. At each leaching event, soils were adjusted to 60% WFPS and then leached out with approximately 150 mL of deionized water. Leachate was collected in 50 mL plastic containers and then frozen for future analysis of mineral N (SI Table S1). Emissions of NH_3 and greenhouse gas (N_2O) were sampled separately at days 1, 3, 6, 10, 14, 16, 29, 31, 44 and 46 after fertilizer addition (DAF) using a static closed chamber (Nelissen et al., 2014). The NH_3 volatilization was measured using polyurethane foam absorbers (Alves et al., 2011). The headspace concentration of N_2O was measured at three time events (0, 30, and 60 min after closing the PVC tubes) during each measuring day and N_2O concentrations were analyzed using an Agilent 7890A gas chromatograph (GC). At the end of the incubation, the soil columns were dissected into three 5 cm sections. The depth of the different soil profile layers were: top (0-5 cm), middle (6-10 cm) and bottom (11-15 cm). Soil was removed from each section and taken to the laboratory for chemical analysis. The details of NH_3 volatilization and greenhouse gas determination, and chemical analysis of soil and leachate can be found in the Supporting Information (SI).

3.2.4. Statistical analysis

The statistical analyses were performed using statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). All tests of significance were carried out at $P<0.05$. The normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Levene’s test. Some data were not normally distributed and showed unequal variance. Those data were log or Ln transformed before performing analysis of variance (ANOVA). One way ANOVA was performed and the multiple comparisons among the different treatments were done using a Tukey test (SI Table S6). In all the analysis, soil was not considered as factor because the experiments were conducted sequentially.

3.3. Results and discussion

3.3.1. Leaching loss of mineral N (NH_4^+-N and NO_3^--N) from soil

Addition of urea and BCU granules significantly increased the concentration of mineral N in leachates obtained from the columns of all three soil types, compared to columns not receiving any N fertilizer (Figure 3.1). Compared to urea alone, granulation of urea with BC significantly reduced the amount of mineral N leached out from the different soils in all leaching events, except the leaching event at 30 DAF in the Tenosol, where no significant variations in mineral N concentrations were observed among urea, BCU 1 and BCU 2 (SI Figures S1 and S2). In general, increasing the ratio of BC to N reduced the amount of mineral N lost through leaching. The leaching of mineral N was much higher in the Ferrosol compared to the Tenosol and Vertosol. On average, addition of BCU granules reduced leaching losses of mineral N by 56, 65 and 42% from the Ferrosol, Tenosol and Vertosol, respectively compared to urea fertilized soil.

The lower NH_4^+-N concentration in leachates obtained from the BCU granules treated soil might be due to its adsorption to BC, which has an extensive porous surface area and high cation exchange capacity (76.3 $cmol_c\ kg^{-1}$) due to presence of large number of acidic functional groups (Saha et al, 2017). In addition, soil incorporation of BC has been shown to decrease the release rate of urea-N by reducing urea hydrolysis through the inhibition of urease enzyme activity in soil (Dong et al., 2009; Saha et al., 2017). The lower availability of NH_4^+-N may also decrease the nitrification of NH_4^+ to NO_3^- resulting in lower NO_3^--N concentration in leachates obtained from BCU treated soil compared to urea alone. Likewise, a slight decrease in NO_3^--N leaching was observed from soil amended with lignite as a result of slow hydrolysis of urea and partial retention of N by lignite (Paramashivam et al., 2016; Rose et al., 2016). The lower leaching loss of mineral N from BCU granules containing the higher amount of BC emphasizes the role of BC in N retention. The leaching loss of mineral N was considerably higher in the Ferrosol compared to Tenosol and Vertosol (Figure 3.1). The Ferrosol is dominated by iron and aluminium (oxy) hydroxides which are variable charge minerals, unlike the 2:1 aluminosilicates likely to be present in the Vertosol. At low pH, the variable charge minerals are predominantly positive which may reduce the potential for NH_4^+ adsorption. NH_4^+ usually competes with other cations (Fe^{2+}, Fe^{3+}, Al^{3+}, Ca^{2+}, K^+ and Na^+) for exchange sites in soil. Therefore, an increase in Fe^{2+}, Fe^{3+} and Al^{3+} concentration may displace the colloidal-bound NH_4^+ from the soil matrix resulting an increased leaching in the Ferrosol compared to other soils. A similar finding was reported by Kithome et al. (1999) who found a very low NH_4^+ adsorption by zeolite, silicate clay with similar properties to common soil silicate clay minerals, in an acidic solution (pH 4.0).

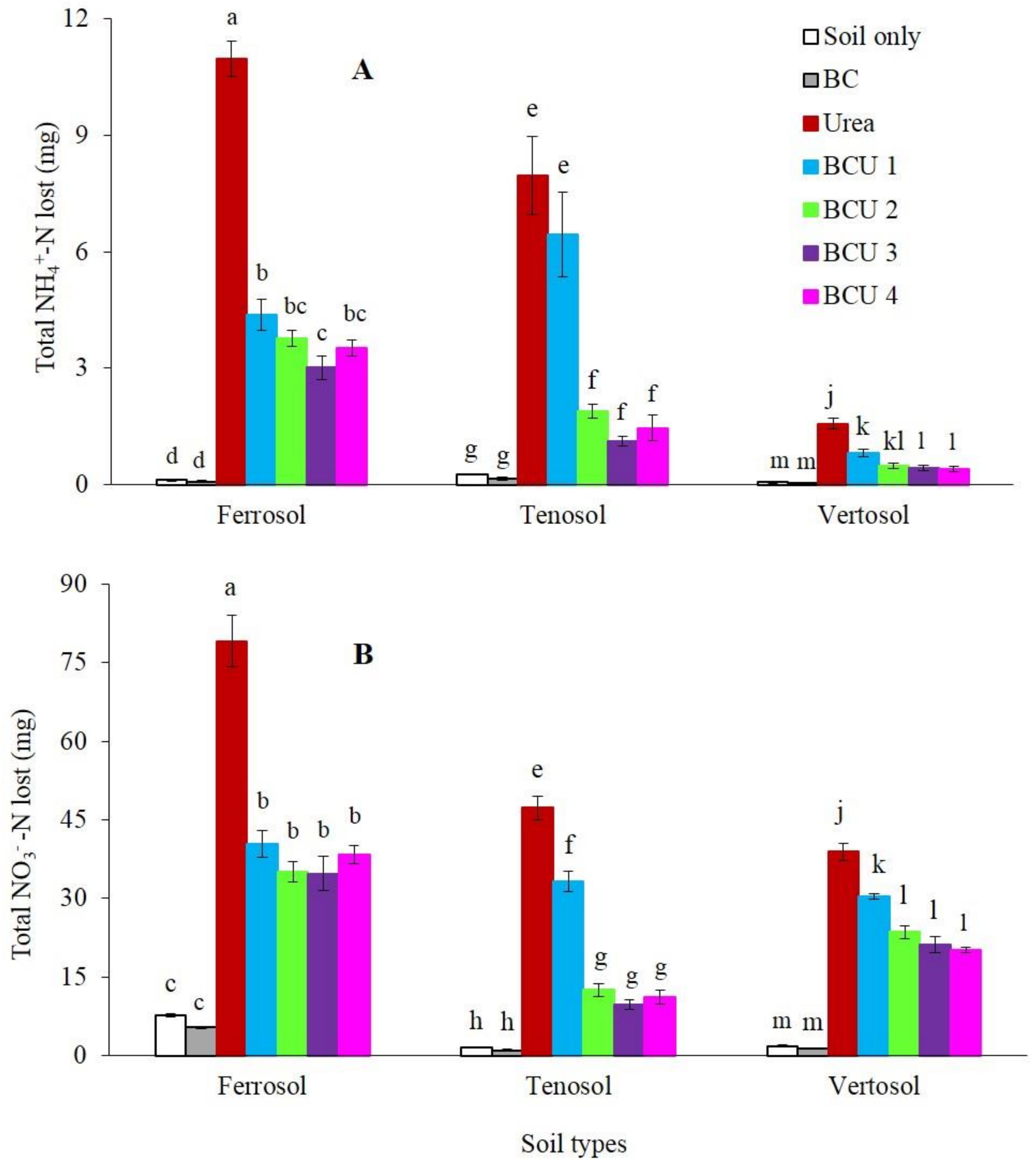


Figure 3.1. Leaching loss of NH_4^+-N (A) and NO_3^--N (B) from soil (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different ($P<0.05$). Valid statistical comparisons cannot be made between soil types.

3.3.2. N_2O emissions from soil columns

Application of N fertilizer significantly increased the emissions of N_2O from the different soils compared to soil which received no N fertilizer (Figure 3.2). The daily N_2O emissions from urea and BCU treated soil reached a peak at 14 DAF in the Tenosol and Vertosol whereas in the Ferrosol, N_2O emissions for the urea treated soil peaked at 3 DAF (SI Figure

S3). A dramatic increase in N_2O emissions was monitored after each leaching event in all soil types. The increased soil moisture and subsequent reduction in air-filled pore space after each leaching event triggered the activity of denitrifying microbes and enhanced N_2O emissions in soil (Nelissen et al., 2014; Ruser et al., 2006). The addition of BCU granules resulted in a significant decrease in daily and cumulative N_2O emissions compared to straight urea, irrespective of soil type. In general, the granules with higher BC had significantly lower N_2O emissions from soils compared to the granules with lower BC. Compared to urea, on average, BCU granules suppressed the cumulative N_2O emissions by 35, 42 and 31% from the Ferrosol, Tenosol and Vertosol, respectively.

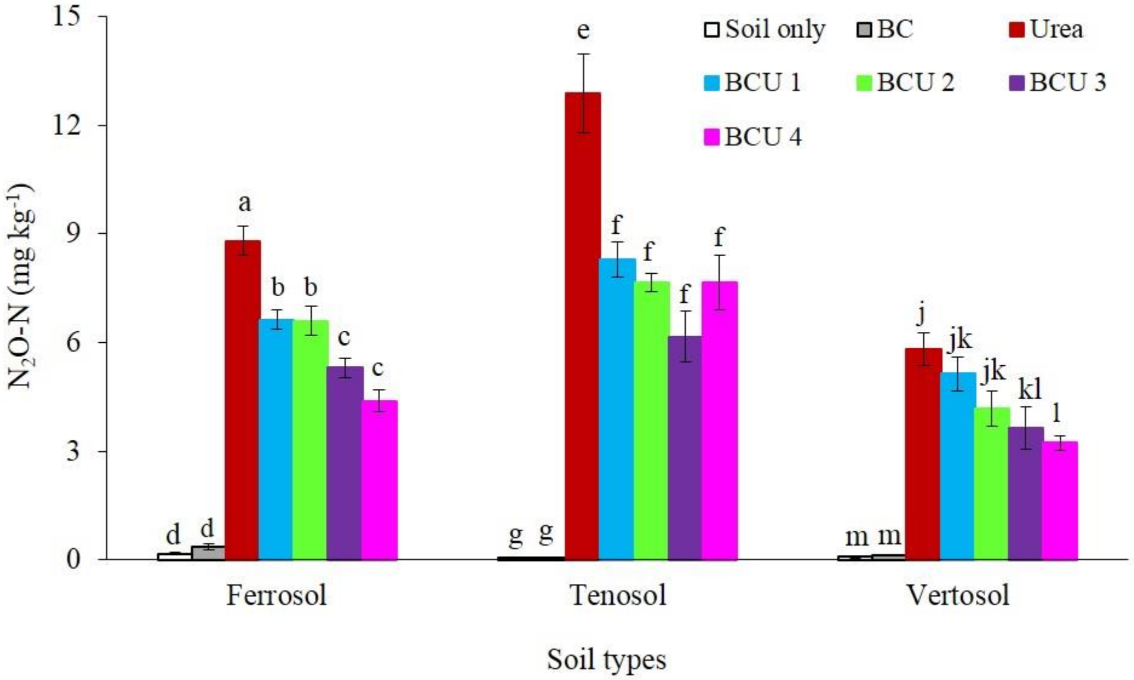


Figure 3.2. Cumulative N_2O-N emissions from different soil types (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

The soil N_2O emissions are largely influenced by both the properties of BC and soil. However, the mechanisms by which BC decreased N_2O emissions are still not clear. The reduction in N_2O emissions from the BCU amended soil could be the result of slower release of urea-N and reduced availability of NO_3^- during the incubation period. This is in good agreement with Rose et al. (2016) who found that granulation of BC with urea significantly decreased N_2O emissions from soil compared to urea. Incorporation of BCU granules to soil can potentially alter the dynamics and mineralization of N by changing soil moisture, pH and C/N ratio of the soil (Saha et al., 2017) (SI Table S4). Acidic pH (4.5) of BC may be responsible for the decreased pH in soil fertilized with BCU granules (SI Table S5) might have constrained the activity of denitrifiers and thereby decreased the N_2O emissions (Kyveryga et al., 2004). Soil incorporation of lignite may also enhance microbial activity by increasing soil moisture content and providing additional organic C as substrate for microbial colonization and food. The increased microbial population and enhanced microbial immobilization of the available urea-N could be an alternative mechanism of less N_2O production in BCU amended soil.

3.3.3. NH_3 volatilization from soil columns

The volatilization loss of NH_3 from the different soils was significantly influenced by the application of N fertilizers. A sharp increase in NH_3 volatilization was measured initially one day after fertilizer addition and reached its peak rapidly within one week of fertilization in all soils (SI Figure S4). NH_3 volatilization then declined rapidly and remained almost constant until the end of incubation, irrespective of soil type. The addition of BCU granules resulted in a significant decrease in daily and cumulative volatilization loss of NH_3 from the Tenosol and Vertosol compared to urea alone treated soil (Figure 3.3). However, no significant variation was found in the NH_3 volatilization among the urea and BCU treated soils in the Ferrosol. The volatilization loss of NH_3 was considerably higher in the Vertosol compared to the Tenosol and Ferrosol. The granules with higher BC had significantly lower NH_3 volatilization from soil compared to the granules with lower BC. Compared to urea, on average, BCU granules suppressed the cumulative NH_3 emissions by 19, 64 and 42% from the Ferrosol, Tenosol and Vertosol, respectively. The results of our study supports the findings of Sun et al. (2016) and Chen et al. (2015) who also reported that soil incorporation of lignite decreased NH_3 emissions by 60-68% from cattle manure amended pens compared to control pens where no lignite was added. The inhibition of urease enzyme activity in BCU amended soil may be the main mechanism of decreasing volatilization loss of NH_3 (Saha et al., 2017). Alternatively the decline in pH due to addition of extra BC may be the cause of lower volatilization in BCU amended soil. In addition, adsorption of NH_3-N on the porous structure of BC can be an alternate mechanism of reducing volatilization loss of NH_3 in soil (Bosatta and Agren, 1995; Lapierre et al., 1994).

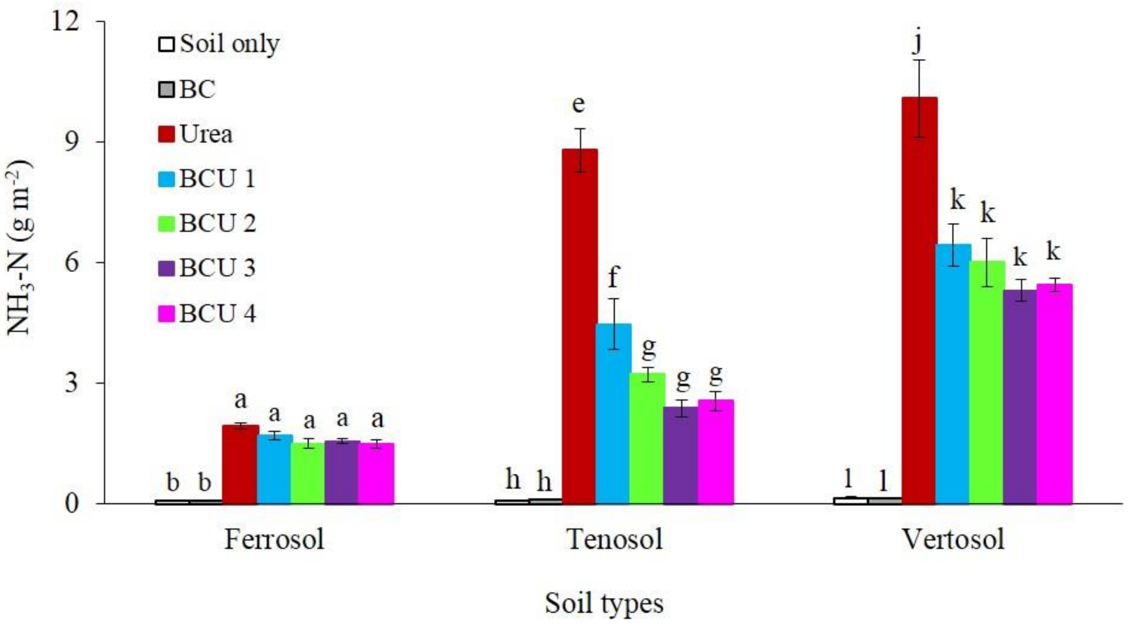


Figure 3.3. Cumulative NH_3-N emissions from soil (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

Among the various soil types, the volatilization loss of N was substantially lower in the Ferrosol than the Tenosol and Vertosol (Figure 3.3). The lower NH_3 volatilization in the Ferrosol is likely to be the result of the low soil pH (pH = 4.69) because soil pH is one of the most important factors controlling volatilization loss of N in soil (SI Table S5). Cevallos et al. (2015) observed that most NH_3 volatilization occurs at soil pH > 7.0, supporting the results of our study where NH_3 volatilization was substantially higher in the Tenosol (pH = 7.24) and Vertosol (pH = 8.32). The higher $CaCO_3$ content could be another reason for increased NH_3 volatilization in the Vertosol. NH_4^+ reacts directly with $CaCO_3$ to produce ammonium bicarbonate or ammonium carbonate, both of which are unstable and rapidly break down to produce NH_3 gas, CO_2 gas, water and calcium. Both the alkaline soil pH and high $CaCO_3$ content induced the conversion of NH_4^+ to NH_3 gas as well as increased volatilization loss of NH_3 in the Vertosol compared to other soils. Pesek et al. (1971) also reported that the volatilization loss of N is positively correlated with $CaCO_3$ content and pH of soil.

3.3.4. Mineral and mineralizable N content of soil profile

Significantly higher amounts of mineral N and potentially mineralizable N (PMN) were measured from the soil fertilized with urea and BCU granules compared to BC and control soil (Figures 3.4-3.5). Addition of BCU granules maintained significantly higher amounts of mineral and mineralizable N in the top layer (0-5 cm) of the soil compared to urea alone treated soil (SI Figures S5-7). However, no significant variation in mineral and mineralizable N was found among urea and BCU granules in soil depth from 6 to 15 cm in all the soil types (SI Table S6). In the Ferrosol and Vertosol, the mineral and potentially mineralizable N concentrations decreased with the increase in soil profile depth whereas an opposite trend was noticed in the Tenosol for urea and BCU 1 treatments. Generally, the BCU granules with higher amounts of BC retained higher mineral and mineralizable N in soil than the granules containing lower amounts of BC. Compared to urea, on average, incorporation of BCU granules increased the NH_4^+-N content by 54, 37, and 44%, and NO_3^--N content by 14, 49 and 31%, in the Ferrosol, Tenosol and Vertosol, respectively (Figure 3.4).

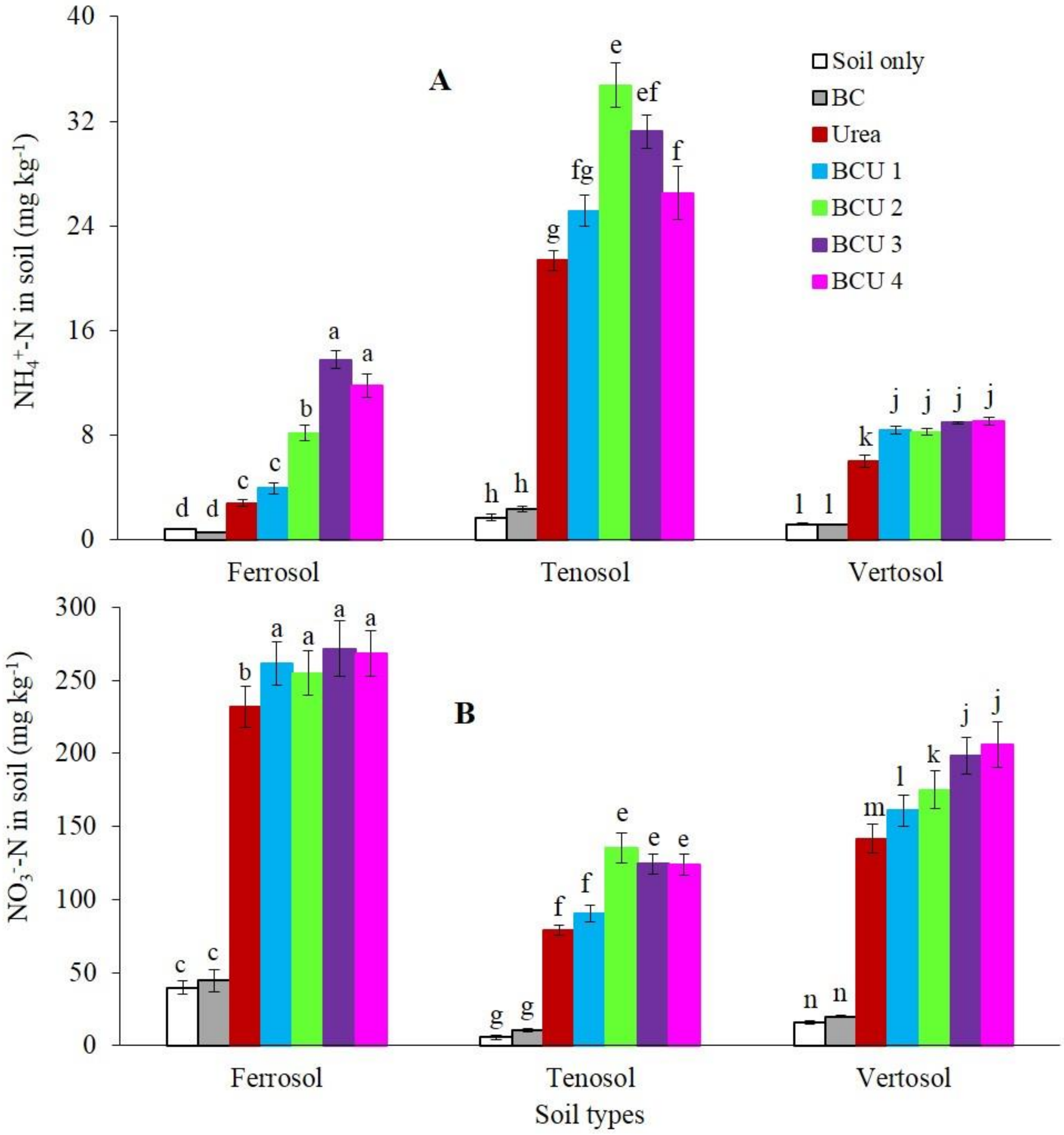


Figure 3.4. Average NH_4^+-N (A) and NO_3^--N (B) content of soil profile at the end of incubation (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

Substantially higher amounts of mineral and mineralizable N in BCU amended soil might be the result of strong and prolonged retention of urea-N by BC and reduced N losses through leaching and gas emissions. Similarly, the role of increased numbers of cation exchange sites and surface area of BC may result in improved N retention, explaining the higher mineral-N content in soil fertilized with BCU granules containing higher amounts of BC. Moreover, the higher mineralizable N content in BCU granules amended soil indicated that still there was some N retained by BC which will be available for mineralization at a later stage. A similar mechanism was also proposed by Dong et al. (2009) who reported that humic acids can incorporate N into their structures either directly through chemical reactions or indirectly through microbial activities and subsequent decomposition of the microbial biomass.

The effect of BCU granules on the dynamics and mineralization of N in soil was also largely influenced by the properties of the different soils used in this study. The soil properties, especially organic matter content, clay content, pH and texture, play an important role in the mineralization, transport and movement of N in soil. The organic matter and clay content of the Ferrosol and Vertosol was much higher than the Tenosol. The higher amount of mineral and mineralizable N in the Ferrosol and Vertosol may be due to higher organic matter and clay content, which favoured higher and prolonged N retention via increased cation exchange sites compared to the Tenosol.

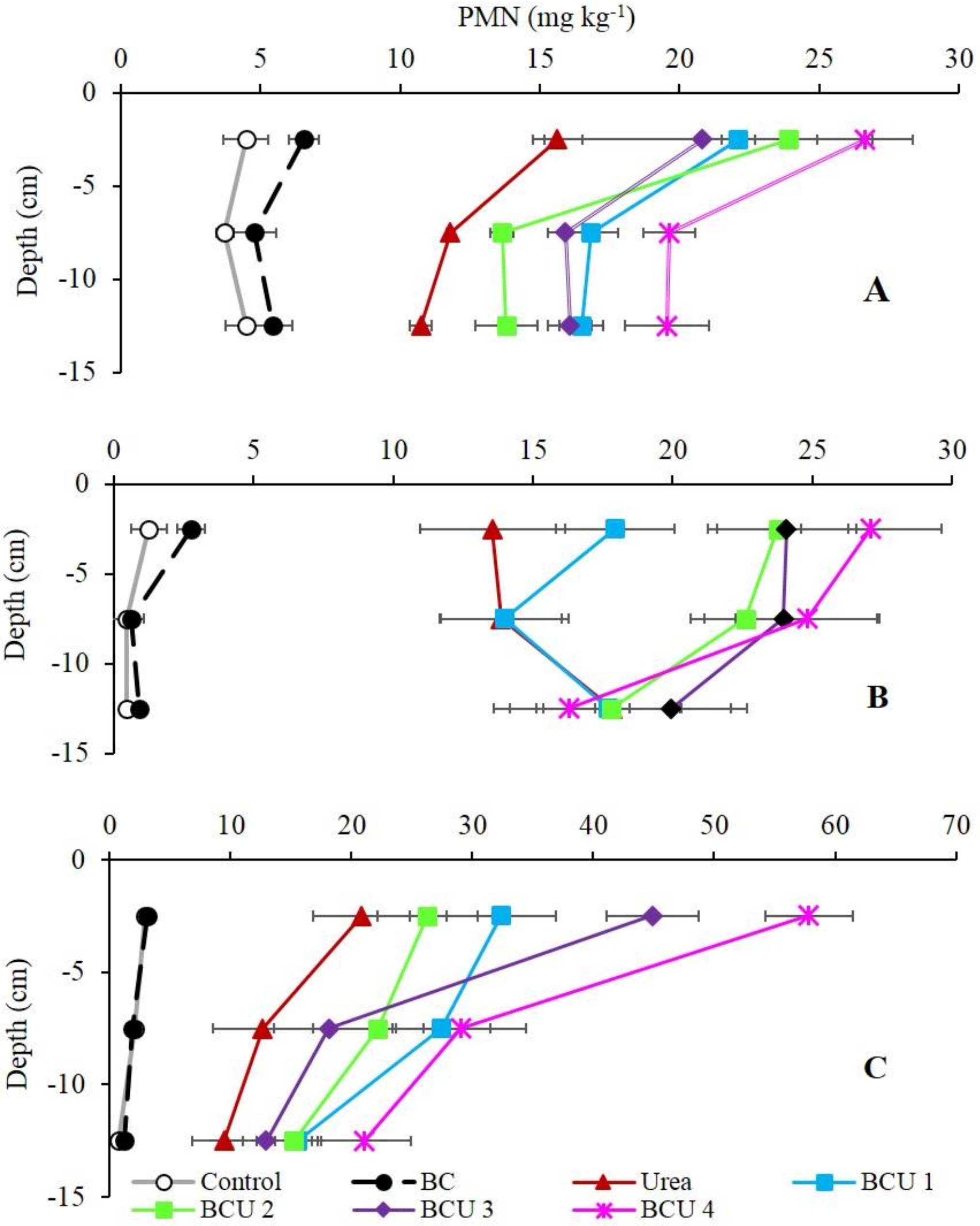


Figure 3.5. PMN concentration of soil profile at the end of incubation in a Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

3.3.5. Total N concentration of soil

Incorporation of urea and BCU granules showed a considerably higher amount of total N in the top (0-5 cm) of the soil column compared to the middle (6-10 cm) and bottom (11-15 cm) layer in all the soil types (SI Figure S8). Significant differences in top soil (0-5 cm) total N were found in the soil fertilized with BCU over urea irrespective of soil type.

Application of BCU granules 2, 3 and 4 maintained significantly higher amount of average total N than the urea treatment only in the Vertosol (Figure 3.6). Among the three different soils, the average total N concentration was remarkably higher in the Ferrosol than the other soils.

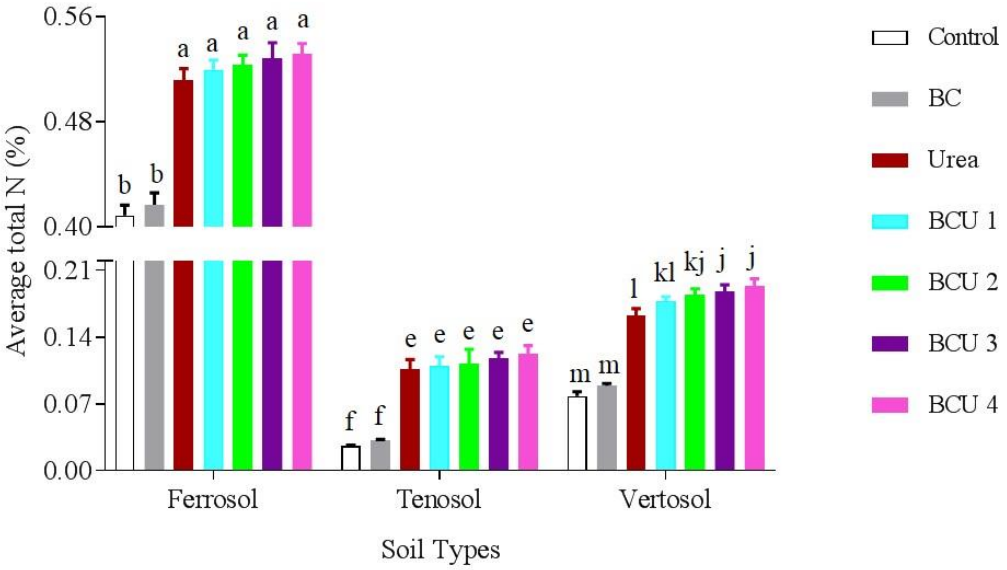


Figure 3.6. Average total N concentration of soil profile at the end of incubation (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

3.3.6. Total C concentration of soil

Significant changes in the 0-5 cm layer total C concentration was observed due to the addition of BC and BCU granules in the various soils. A decreasing trend in soil C levels was noticed with the increase in soil depth in all the soil types (SI Tables S2-4). Significantly higher amount of total C was obtained from the BC and BCU granules 3 and 4 treated soils compared to urea and control treatments (Figure 3.7). The granules with higher amounts of BC maintained significantly higher amounts of total C in soil compared to the granules with lower amounts of BC. A higher amount of total C was found in the Ferrosol compared to the Tenosol and Vertosol.

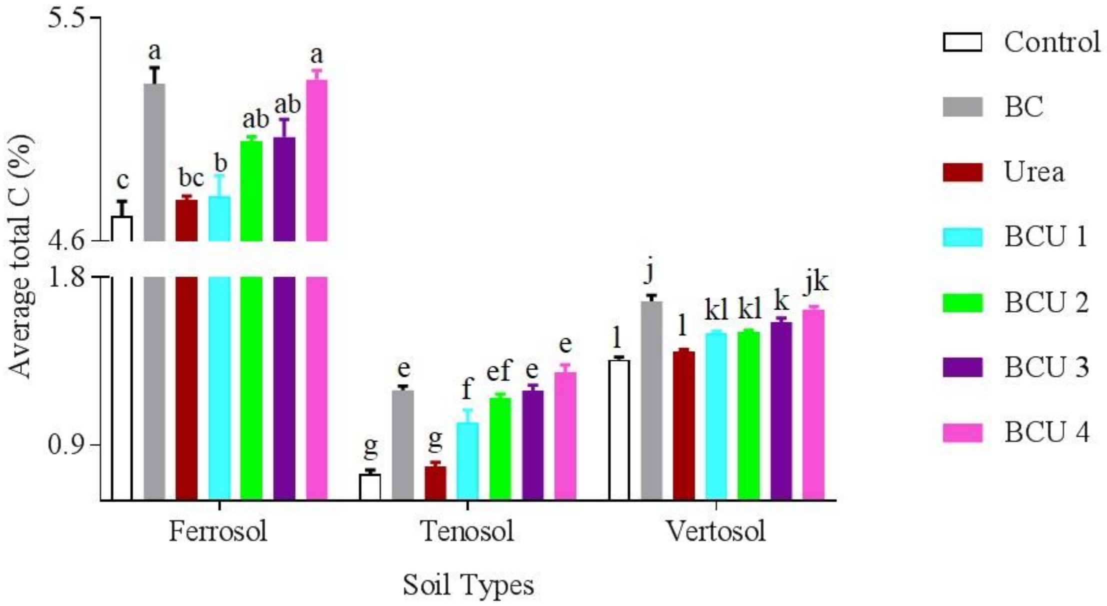


Figure 3.7. Average total C concentration of soil profile at the end of incubation (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different ($P<0.05$). Valid statistical comparisons cannot be made between soil types.

3.3.7. *Mass balance of N*

The mass balance of N presented in Figure 3.8 provides an overview regarding the effect of BCU granule addition on the dynamics and mineralization of N in various soils. The effect of BCU granules varied substantially depending on soil types. Granulation of BC with urea reduced the leaching loss of mineral N about twice as much as in the Ferrosol and Vertosol. However, in the Tenosol, the reduction in N lost by leaching was around three times compared to urea. The N_2O emissions was almost twice in the urea treated soil compared to BCU granules in all the soil types. Compared to urea, incorporation of BCU granules decreased NH_3 volatilization loss of N by around 50% in the Tenosol and by about 40% in the Vertosol. In contrast, no significant effect of BCU granules was observed in reducing NH_3 loss in the Ferrosol. Considerably higher amounts of soil mineral and mineralizable N were determined from the BCU granules amended soil compared to straight urea irrespective of soil types. A ^{15}N tracer experiment will be done in future to calculate the complete mass balance and total recovery of fertilizer-N from soil.

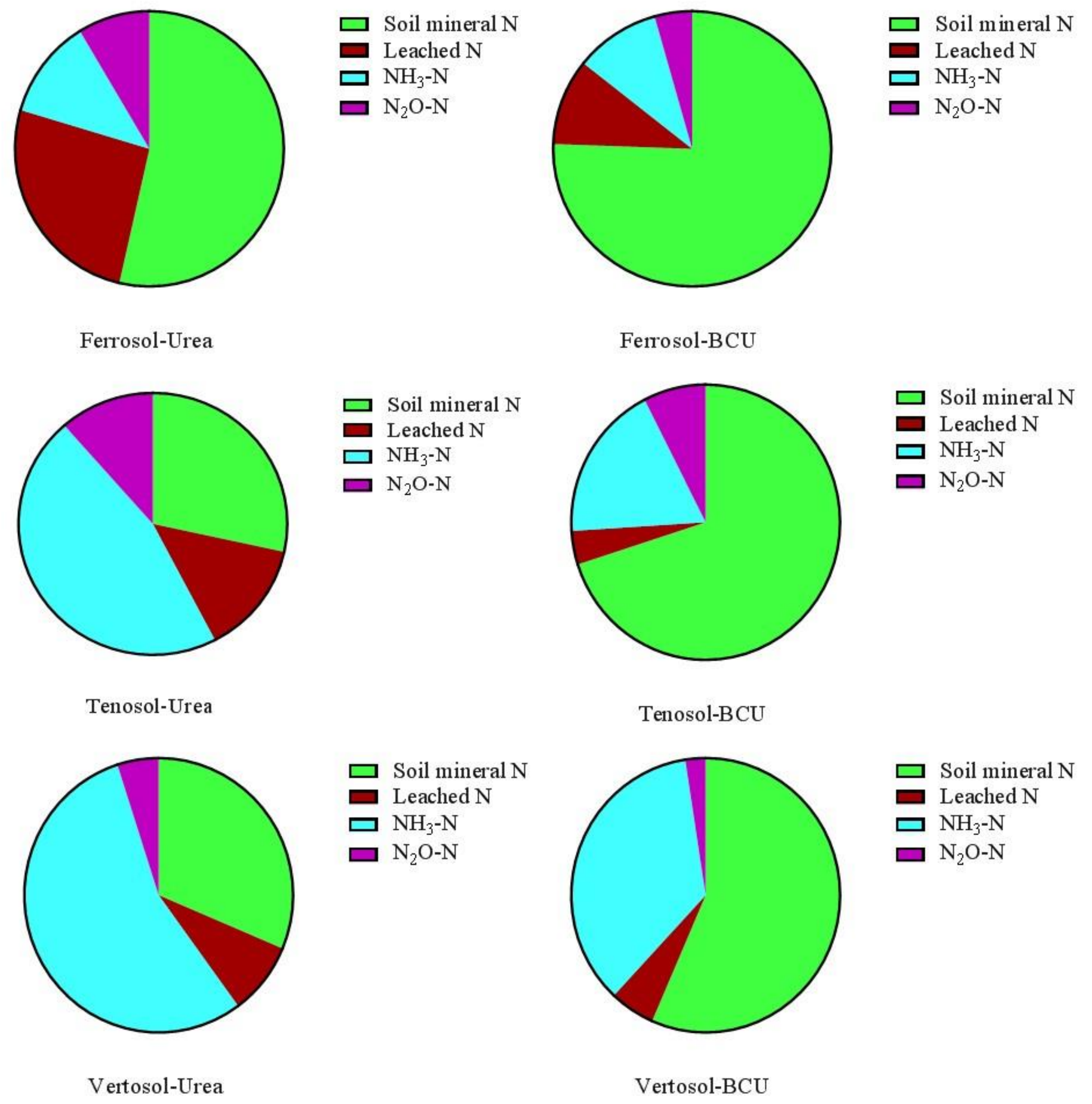


Figure 3.8. Comparison of measured N balance in various soils treated with urea and BCU (Average of BCU1 to BCU4).

In conclusion, granulation of BC with urea influenced the distribution, bioavailability and ultimate fate of N in soil when compared to urea alone. Granulation of urea with BC decreased the release of fertilizer-N and substantially increased mineral and mineralizable N in soil by decreasing its gaseous and leaching losses. As a result, fertilizer N will theoretically be available to crop plants over a longer time period. We hypothesize that this will lead to increased fertilizer N use efficiency as well as potentially reducing the required application rates of N fertilizers. The soils responded differently to the addition of different BCU granules. The BCU granules were more efficient in Ferrosol and Tenosol for maintain better N by reducing its gaseous losses. The granules containing higher proportions of BC had lower leaching and gaseous emissions and maintained higher mineral N in soil, emphasizing the role BC in better N retention. Based on the overall results, BCU granules 2 and 3 could be considered as most suitable for slow release and enhanced efficiency nitrogenous fertilizer for future work. Overall, these findings provide mechanistic support for the development of BCU granules as an enhanced efficiency and slow release nitrogenous fertilizer for better N retention in the plant-soil system over a longer period of time.

3.4. Supporting information (SI)

A detailed description of experimentation, analyses, greenhouse gas sampling, NH_3 emissions measurement and calculations were presented. The information includes tables on leachate collection, soil C concentration, post-harvest soil pH and summary of ANOVA; figures on mineral and mineralizable N measurement from leachate and soil, soil total N at various depth of different soils, daily N_2O and NH_3 emissions from soil.

3.4.1. Leachate analysis

Leachates were filtered through Advantech filter paper 42 prior to analysis for mineral N species by spectrophotometry in microplate formats using plate reader. Ammonium ion concentrations were quantified by reacting with salicylate and hyphochlorite in a buffered alkaline solution containing sodium nitroprusside as a reductant (Forster, 1995). Nitrate was determined by reduction using vanadium (III) combined with detection by acidic Griess reaction (Miranda et al., 2001).

Table S.1. Volume of leachate collected during different leaching events from soil columns (values are mean ± standard error, N = 5).

	Ferrosol			Tenosol			Vertosol		
	L1	L2	L3	L1	L2	L3	L1	L2	L3
Control	35.0±2	33.0±3	25.8±2	34.8±2	36.4±1	36.2±2	27.4±2	27.8±2	36.4±1
BC	30.2±3	29.2±2	21.6±2	30.1±3	34.2±2	33.6±3	23.8±2	20.2±1	32.4±1
Urea	41.0±1	31.0±2	30.8±2	33.2±2	37.4±2	35.8±3	24.4±1	24.0±3	37.0±3
BCU 1	37.4±1	31.0±3	28.0±1	29.2±3	34.1±2	33.6±2	22.2±2	23.2±2	32.4±2
BCU 2	29.0±3	30.4±2	27.2±2	25.2±3	30.2±4	30.4±2	20.8±1	20.2±2	28.2±2
BCU 3	29.4±1	26.2±1	29.4±4	23.6±1	31.5±3	32.2±3	16.8±2	22.2±2	36.4±2
BCU 4	31.8±4	29.0±2	29.4±2	24.2±3	29.2±3	30.5±3	18.8±3	22.0±2	31.4±2

L1= Leaching at 15 DAF, L2= Leaching at 30 DAF and L3= Leaching at 45 DAF

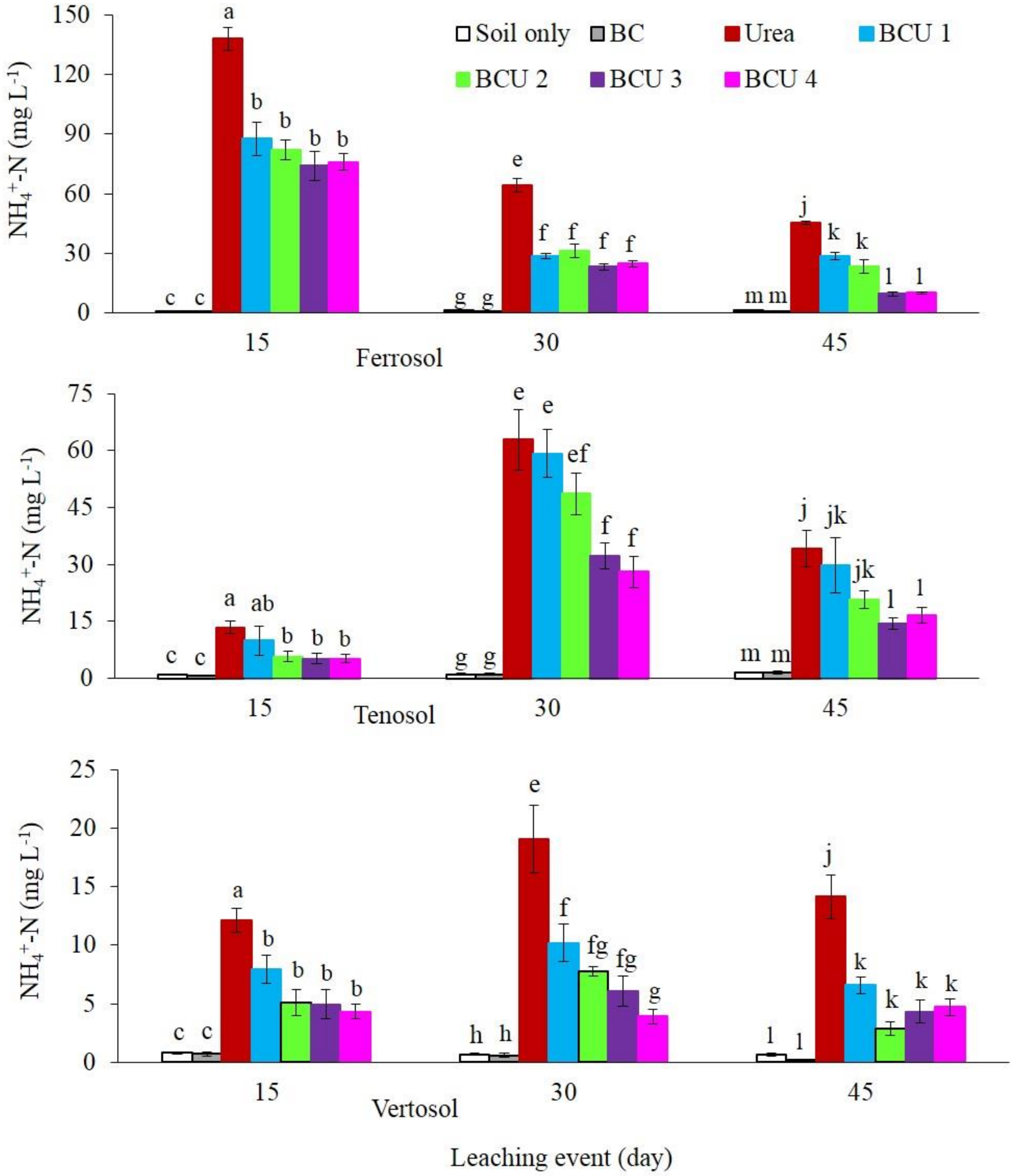


Figure S.1. Ammonium-N concentration in leachates at different leaching events in different soils (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different ($P<0.05$). Valid statistical comparisons cannot be made between soil types.

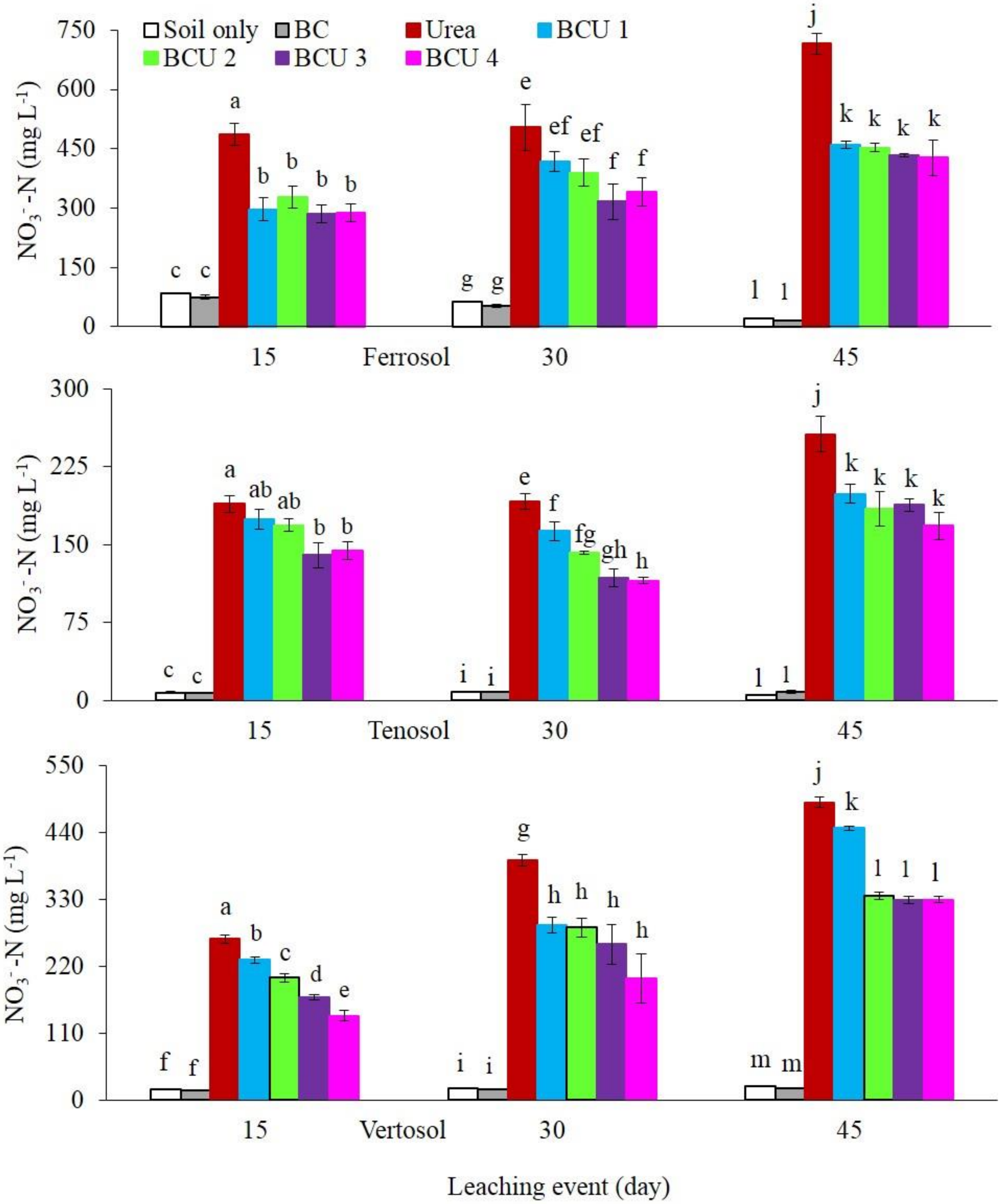


Figure S.2. Nitrate-N concentration in leachates at different leaching events in different soils (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

3.4.2. Measurement of N_2O Emissions

The PVC tubes were closed with an air tight lid having a rubber septum with a headspace volume of 637 mL for the measurements of N_2O flux. The headspace concentration of N_2O was measured at three time events (0, 30, and 60 min after closing the PVC tubes) during each measuring day. At each measurement, a 12 mL gas sample was withdrawn from the headspace of the PVC tubes by an air tight syringe (SGE, 25MDR-LL-GT). The gas sample was then transferred into a 12 mL air tight glass vial which was pre-evacuated and flushed with argon and then re-evacuated. The gas sample was collected in the glass vial and the N_2O concentration was measured within one week. Gas samples were analyzed for N_2O using an Agilent 7890A gas chromatograph (GC) fitted with a Gerstel MPS autosampler. The N_2O concentration was determined with the help of a calibration curve prepared from the reference gas with a known concentration of 1 ppm. Linear interpolation of the gas concentrations were used to calculate the flux and cumulative N_2O according to the method and equations detailed by van Zwieten et al. (2010).

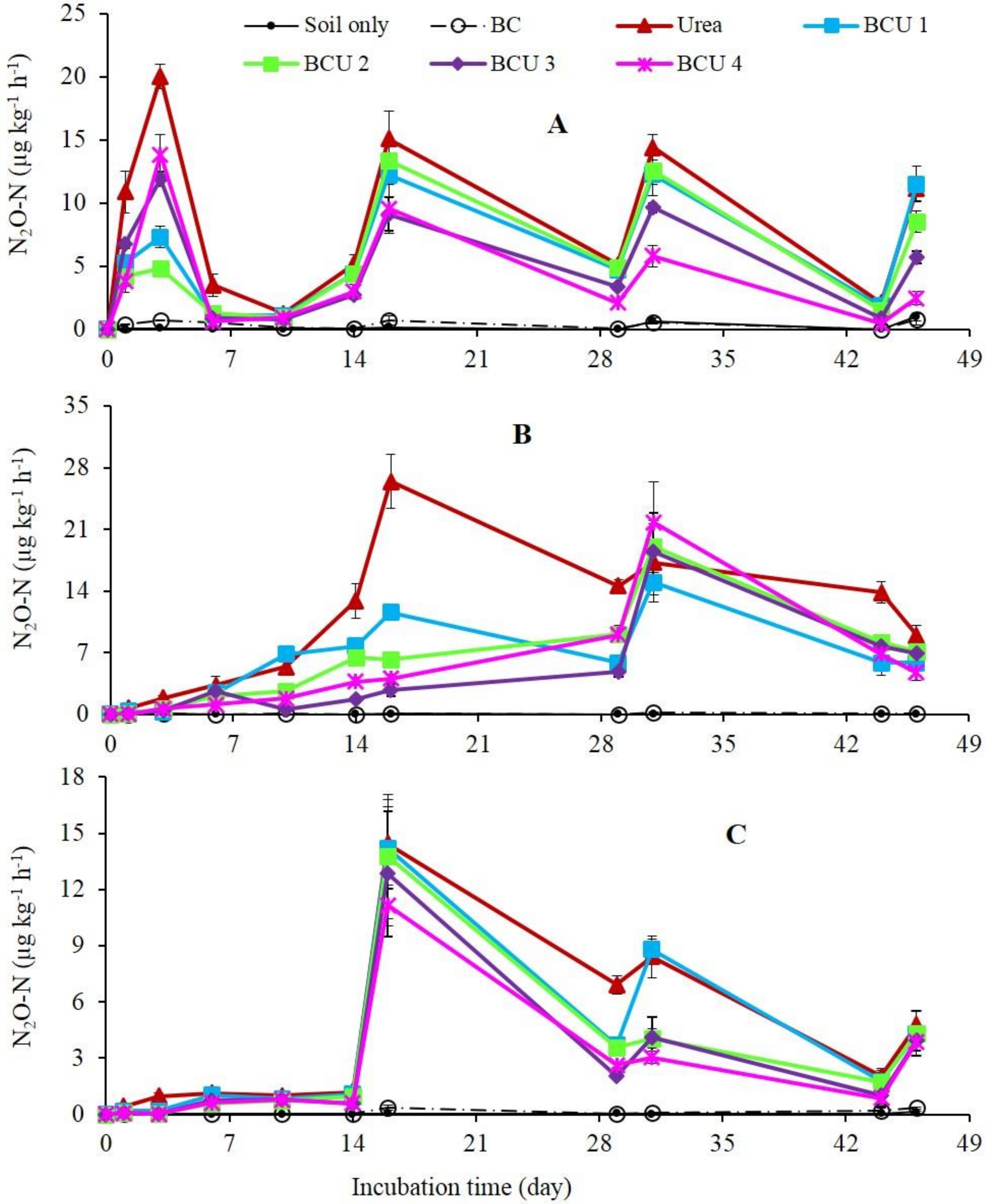


Figure S.3. Daily N_2O-N emissions from Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

3.4.3. Measurement of NH_3 Emissions

The NH_3 volatilization was measured using polyurethane foam absorbers. The absorbers were placed into plastic petri dish and soaked with 20 mL of 0.05 M sulphuric acid solution. The NH_3 emissions were measured for 12 h during each sampling event. To prevent contamination with environmental NH_3, the absorbers were maintained in plastic bags until installation on the PVC tubes. Above each PVC tube one absorber was installed at the top. The petri dishes on the upper side of the absorbers prevented capture of NH_3 from atmosphere. After this, the absorbers were removed and deep-frozen in plastic bags immediately. At the end of the experimental period, the absorbers were disassembled and components were washed with deionized water. For each absorber, the petri dish was washed with approximately 30 mL of water, using a wash bottle, above the foam placed in a Buckner funnel attached to a Kitassato and a vacuum pump. For a complete removal of the acidic solution, the absorber was washed a second time with deionized water. The solution collected in the Kitassato was then transferred to a volumetric flask and the final volume was made up to 100 mL by adding deionized water. The NH_4^+-N concentration was determined colorimetrically by reacting with salicylate and hyphochlorite in a buffered alkaline solution contain sodium nitroprusside as a reductant (Forster, 1995). The NH_3 flux (mg N m^{-2} h^{-1}) was then calculated using the equation of Singh et al. (2009).

$$\text{Ammonia flux} = \frac{\mathrm{C} \times \mathrm{V}}{a \times \mathrm{D}} \qquad (1)$$

Where, C is NH_3 concentration in the acid trap (mg dm^{-3}); V is the volume of acid (dm^3); a is total cross-section area (m^2) of soil column and D is duration (h) of each sampling.

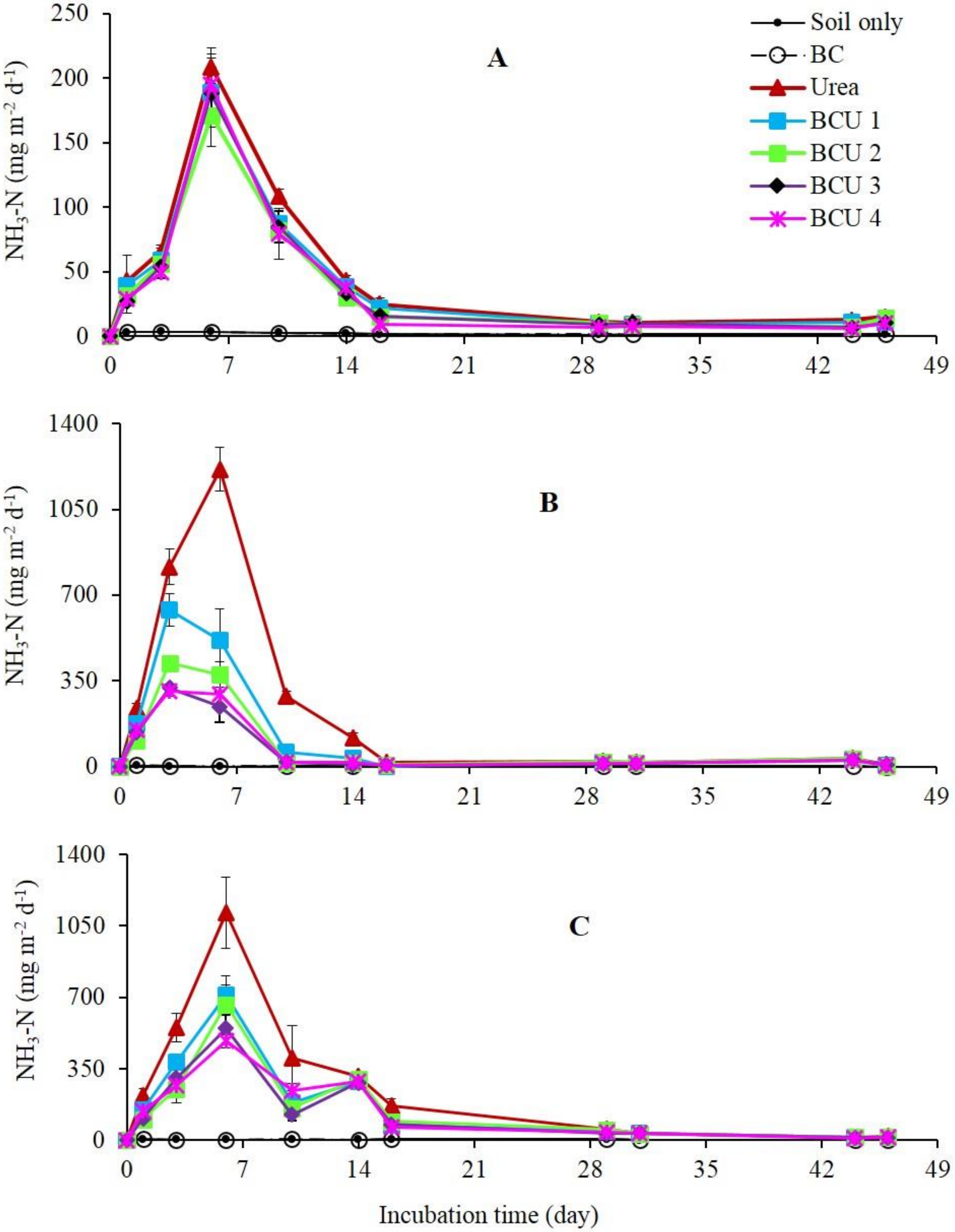


Figure S.4. Daily NH$_3$-N emissions from Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

3.4.4. Soil analysis

Soil pH was determined at a soil-to-water ratio of 1:5 (WP 80 Reference pH Meter, Anpros Phy LTD., Victoria, Australia). Mineral N was extracted from soils with 2 M KCl using a 1:2.5 soil: extractant ratio. The soil extracts were filtered through Advantech filter paper 42 prior to analysis for mineral N species. The mineral N concentrations of soil were also measured following colorimetric method used for leachate analysis. Potentially mineralizable nitrogen (PMN) was assessed as described by Waring and Bremner (1964), modified by Stenberg et al. (1998). Total C and N in soil was measured using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The soils were finely ground using a mortar and pestle prior to C and N analysis.

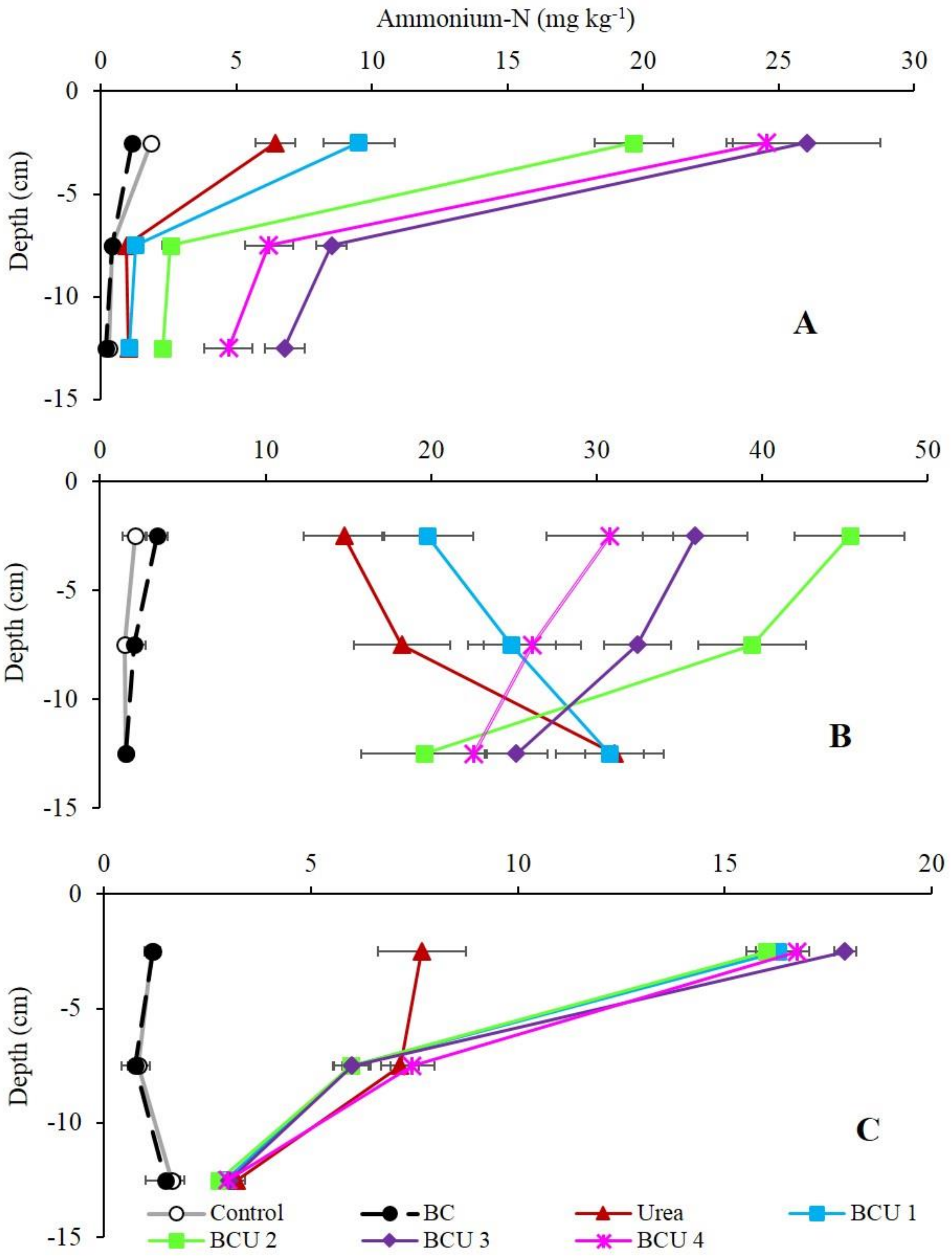


Figure S.5. Ammonium-N content of soil profile at the end of incubation in a Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

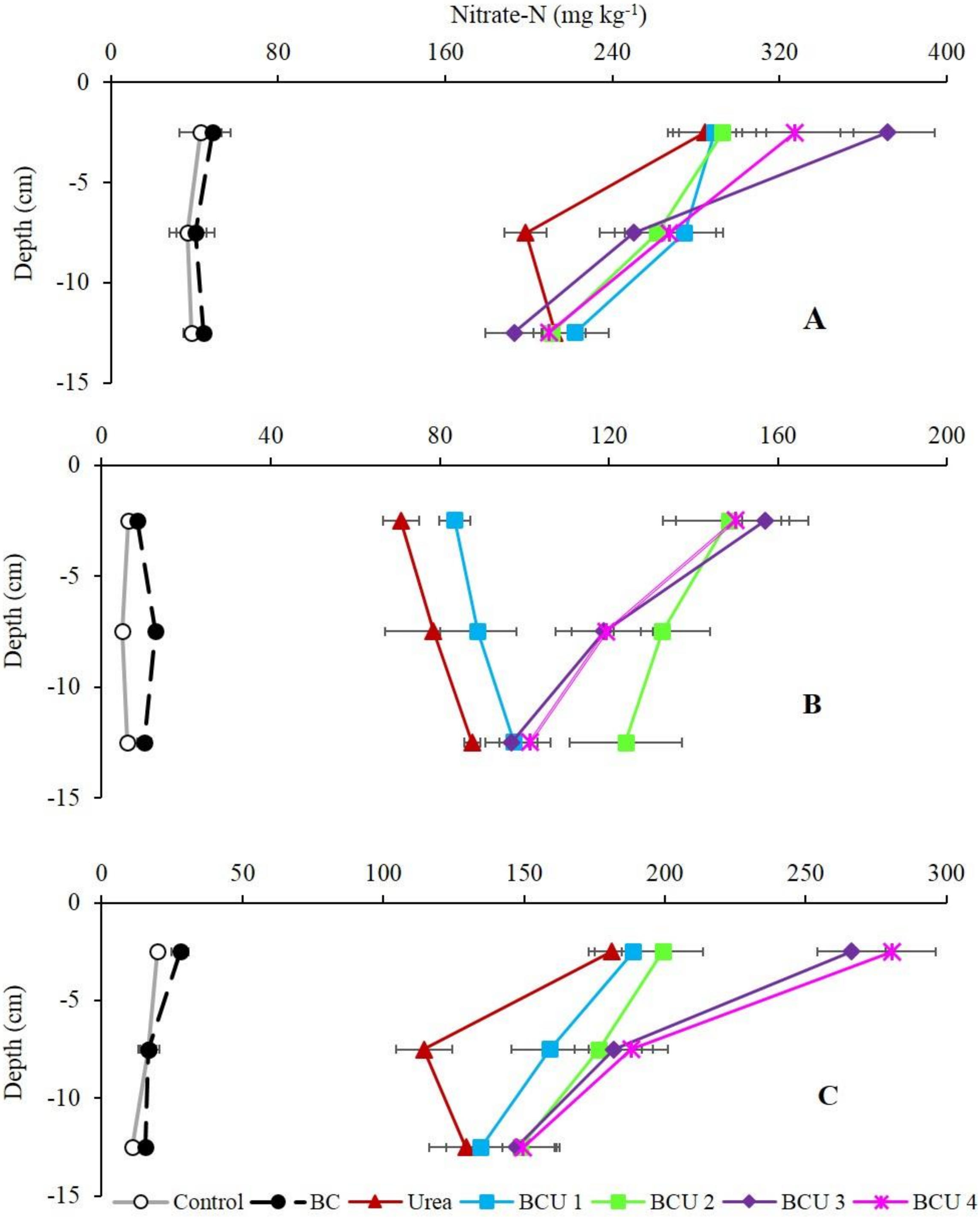


Figure S.6. Nitrate-N content of soil profile at the end of incubation in a Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

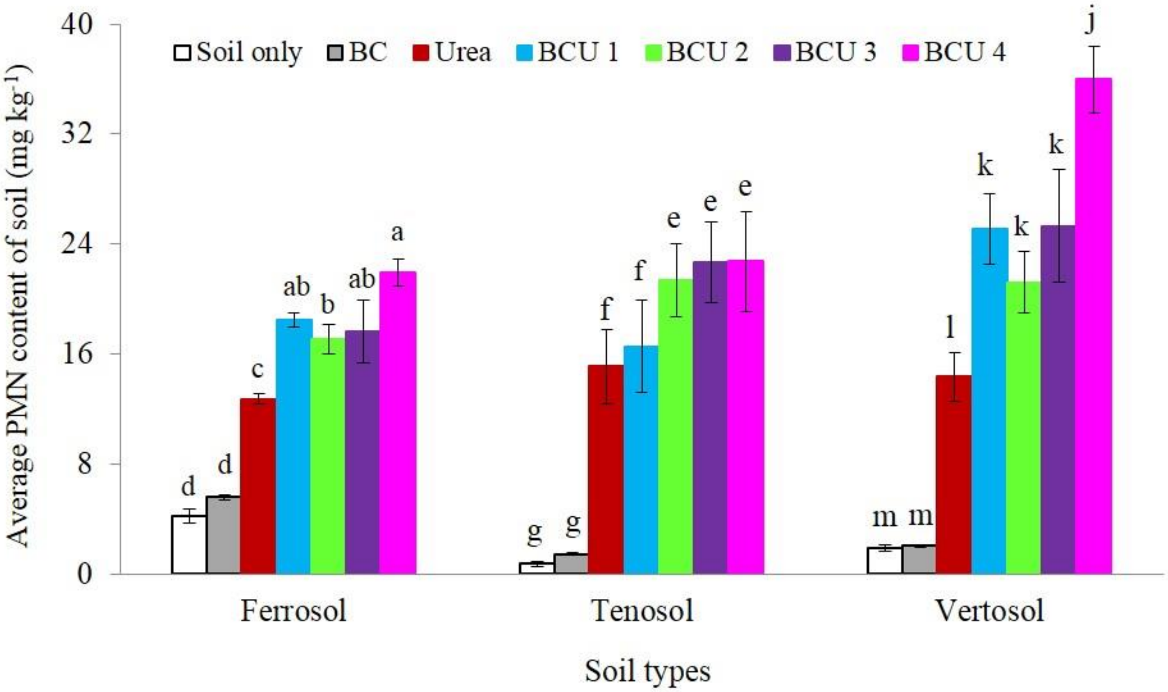


Figure S.7. Average PMN concentration of soil profile at the end of incubation in different soils (values are mean ± standard error, N = 5). Letters above columns are different if the values are significantly different (*P*<0.05). Valid statistical comparisons cannot be made between soil types.

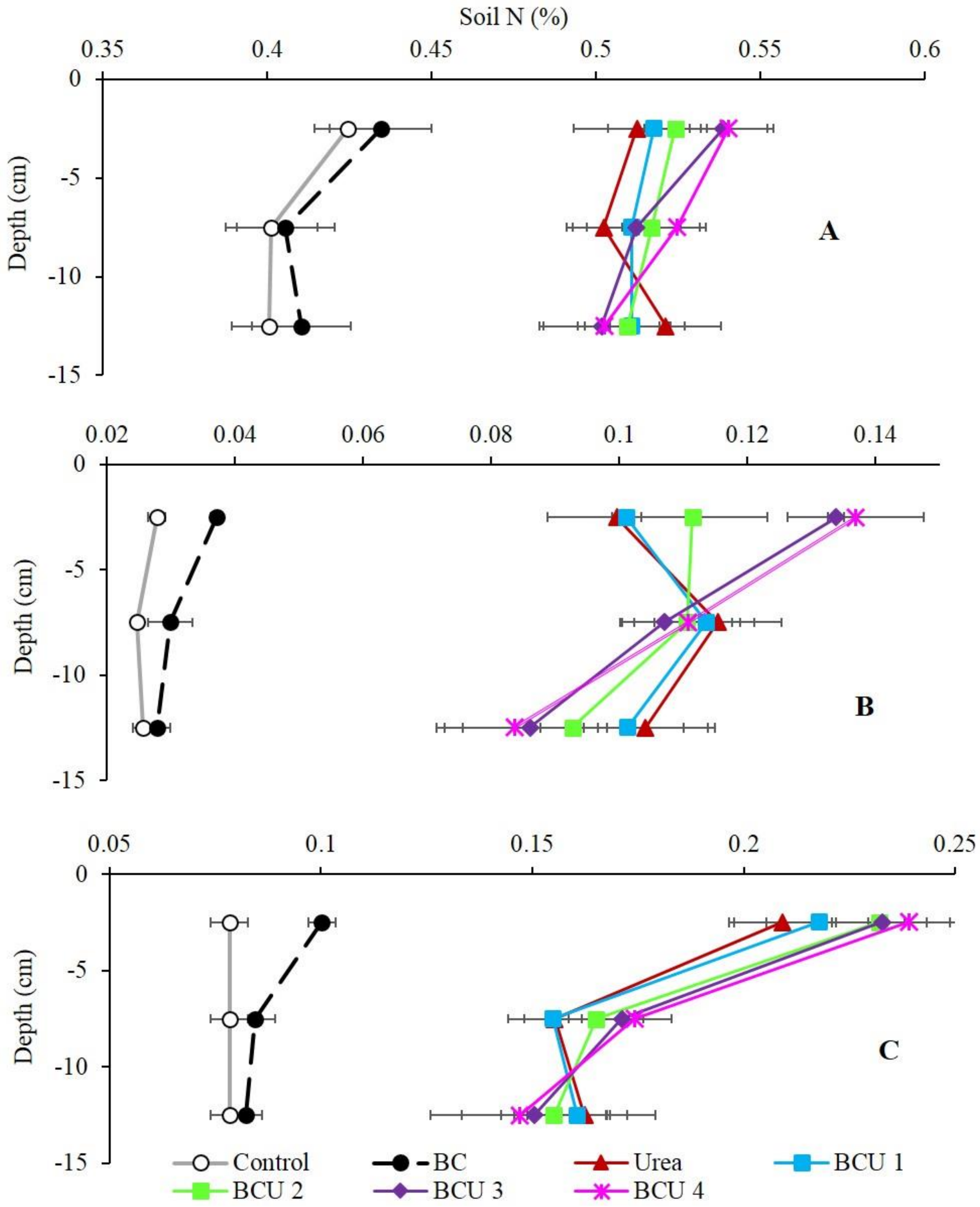


Figure S.8. Total N concentration of soil profile at the end of incubation in Ferrosol (A), Tenosol (B) and Vertosol (C) (values are mean ± standard error, N = 5).

Table S.2. Total C concentration of Ferrosol at various depth (values are mean ± standard error, N = 5).

Treatments	Total C concentration of Ferrosol (%)		
	Top (0-5 cm)	Middle (6-10 cm)	Bottom (11-15 cm)
Control	4.69±0.16c	4.71±0.18a	4.71±0.05a
BC	6.01±0.14a	4.97±0.07a	4.73±0.08a
Urea	4.79±0.05c	4.79±0.06a	4.72±0.04a
BCU 1	4.88±0.11bc	4.73±0.18a	4.73±0.08a
BCU 2	5.28±0.08bc	4.96±0.06a	4.78±0.04a
BCU 3	5.36±0.12b	4.87±0.07a	4.83±0.05a
BCU 4	5.99±0.16a	4.93±0.08a	4.83±0.06a

Letters adjacent to values are different if the values within a column are significantly different (P<0.05).

Table S.3. Total C concentration of Tenosol at various depth (values are mean ± standard error, N = 5).

Treatments	Total C concentration of Tenosol (%)		
	Top (0-5 cm)	Middle (6-10 cm)	Bottom (11-15 cm)
Control	0.79±0.03d	0.67±0.03b	0.74±0.04b
BC	1.55±0.10a	1.27±0.13a	0.76±0.03b
Urea	0.81±0.02d	0.75±0.04b	0.78±0.04b
BCU 1	1.06±0.07c	0.93±0.13ab	1.06±0.08a
BCU 2	1.18±0.03bc	1.20±0.02a	1.06±0.03a
BCU 3	1.17±0.03bc	1.28±0.08a	1.13±0.03a
BCU 4	1.42±0.15ab	1.29±0.07a	1.17±0.09a

Letters adjacent to values are different if the values within a column are significantly different (P<0.05).

Table S.4. Total C concentration of Vertosol at various depth (values are mean ± standard error, N = 5).

Treatments	Total C concentration of Vertosol (%)		
	Top (0-5 cm)	Middle (6-10 cm)	Bottom (11-15 cm)
Control	1.38±0.03e	1.36±0.03c	1.34±0.03b
BC	2.07±0.06a	1.48±0.02b	1.45±0.04a
Urea	1.45±0.02e	1.39±0.03bc	1.36±0.03b
BCU 1	1.58±0.05d	1.43±0.03b	1.49±0.03a
BCU 2	1.60±0.02cd	1.46±0.02b	1.46±0.03a
BCU 3	1.64±0.03c	1.51±0.04a	1.54±0.03a
BCU 4	1.88±0.04b	1.47±0.02b	1.53±0.02a

Letters adjacent to values are different if the values within a column are significantly different ($P<0.05$).

3.4.5. Post-harvest soil pH

Addition of BCU blends significantly affected the top soil pH at the end of incubation in all three soils (Table 3.16). Incorporation of BCU blends significantly lowered the post-harvest top soil pH compared to urea and control treatments in all the soil types. Overall, the higher the amount of BC in the blends, the lower the soil pH irrespective of soil types.

Table S.5. Effect of BCU blends addition on the top (0-5 cm) soil pH at the end of incubation (values are mean ± standard error, N = 5).

Treatments	Ferrosol		Tenosol		Vertosol	
	Initial	Post-harvest	Initial	Post-harvest	Initial	Post-harvest
Control	4.69	4.71±0.01a	7.24	7.19±0.02a	8.32	8.30±0.01a
BC	4.69	4.41±0.01b	7.24	6.12±0.01d	8.32	7.65±0.02f
Urea	4.69	4.39±0.01b	7.24	6.65±0.02b	8.32	8.04±0.02b
BCU 1	4.69	4.32±0.02c	7.24	6.46±0.02c	8.32	7.98±0.01c
BCU 2	4.69	4.33±0.01c	7.24	6.42±0.02c	8.32	7.93±0.02d
BCU 3	4.69	4.35±0.02c	7.24	6.41±0.03c	8.32	7.75±0.02e
BCU 4	4.69	4.31±0.03c	7.24	6.38±0.03c	8.32	7.71±0.02e

Letters adjacent to values are different if the values within a column are significantly different (*P*<0.05).

Table S.6. *P*-value results from one-way ANOVAs for response variables

Studied parameters	Ferrosol	Tenosol	Vertosol
Soil NH_4^+-N (0-5 cm)	0.000	0.000	0.000
Soil NH_4^+-N (6-10 cm)	0.000	0.000	0.000
Soil NH_4^+-N (11-15 cm)	0.000	0.000	0.000
Soil NO_3^--N (0-5 cm)	0.000	0.000	0.000
Soil NO_3^--N (6-10 cm)	0.000	0.000	0.000
Soil NO_3^--N (11-15 cm)	0.000	0.000	0.000
Soil PMN (0-5 cm	0.000	0.000	0.000
Soil PMN (6-10 cm)	0.000	0.003	0.000
Soil PMN (11-15 cm)	0.000	0.220	0.000
Soil total N (0-5 cm)	0.000	0.000	0.000
Soil total N (6-10 cm)	0.000	0.000	0.000
Soil total N (11-15 cm)	0.000	0.000	0.000

4. Chapter: Slow release brown coal-urea granule for increasing biomass yield and nitrogen uptake by silver beet

4.1. Introduction

Nitrogen is one of the key nutrient elements for the production of leafy vegetables. An optimum supply of N can accelerate plant growth and enhance crop production, while excessive and inappropriate application of N fertilisers can accumulate NO_3^- in the edible parts which could be detrimental for human health (Collins and McCoy, 1997). Addition of N fertiliser is essential to obtain profitable yields and quality of leafy vegetables. However, N is one of the most challenging elements to manage in the soil-plant system to ensure adequate N supply through fertilisation. Urea is the dominant and main source of N input in crop production systems world-wide because of its high N concentration (http://www.fao.org/3/a-i4324e.pdf). Unfortunately, the efficiency of urea fertiliser use is very poor and recovery of N in soil-plant systems seldom exceeds 50% of applied N (Raun et al., 2002). The low use efficiency of urea is associated with its losses by leaching, denitrification and volatilisation (Fageria and Baligar, 2005).

One of the major challenges facing farmers worldwide is improving N fertiliser use efficiency, and reversing the widespread loss of soil organic matter. The lost N represents both an economic inefficiency and an environmental problem, as off-site N transport in water pollutes both surface and groundwater, whilst N_2O contributes to greenhouse gas accumulation in the atmosphere. The loss of SOC has also impacted on crop productivity. Negative effects include reductions to soil water-holding capacity; decreased nutrient availability and cycling; and a reduced capacity to buffer changes in pH, salinity and other chemical stressors (Accoe et al., 2004). Because soils depleted in organic carbon often have a decreased N retention capacity, retaining fertiliser applied N in the plant-soil system has become increasingly difficult. Furthermore, recent studies have shown that C sequestration potential of a soil is intricately linked with the sequestration of other nutrients, especially N (Dong et al., 2009; Kirkby et al., 2016). Therefore, improving fertiliser N use efficiency and increasing SOC are essential goals on a global scale to provide food security, maintain soil health and minimise adverse effects on the environment.

Granulation of organic material with synthetic fertiliser to meet crop nutrient requirements has several benefits over the application of organic material or synthetic fertiliser as a sole nutrient source (Mazeika et al., 2016). Organo-mineral blending reduced the application rate of both synthetic fertiliser and organic matter, and decreased the rate of nutrient release due to increased adsorption by organic matter which prolonged the availability of nutrients in soil (Richards et al., 1993). As a result, the nutrients were available to plants for longer in the plant growing cycle with the implication that fertiliser application to the soil could be reduced thereby reducing the potential for environmental pollution and cost of production. Moreover, organic-synthetic fertiliser blend generally contain a large amount of humic acid, amino acid, and enzymes, which can improve nutrient cycling and its uptake by crop plants (Ayuso et al., 1999; Atiyeh et al., 2002; Arancon et al., 2006; Rao et al., 2007).

Recently, there has been an increasing trend of using organo-mineral granules in agricultural crop production because of their potential benefits on nutrient cycling, crop yield and soil health. Sikora and Enkiri (2001) blended ammonium nitrate with biosolids compost and found that the blended product significantly increased yield and N uptake by fescue. In another study, Antille et al. (2014) found that the application of biosolids-derived organo-mineral blends increased biomass yield of ryegrass and the organic matter content of the soil increased, compared to commercial synthetic fertilisers. It is evident from the results presented in Chapter 2 and 3 that granulation of urea with BC increased N availability in soil by reducing its losses via leaching and gaseous emissions. Therefore, there is a strong reason to hypothesise that granulation of urea with BC could be a potential alternative to conventional N fertilisers for increasing N-use efficiency by plants. In this chapter, the objective was to test the effect of selected BCU granules previously described on the growth and N uptake by silver beet in glasshouse conditions.

Silver beet (*Beta vulgaris* L.) is a commonly grown leafy vegetable in Australia. Its leaf is eaten like spinach and the stem is cooked like celery. The seedlings are also served as salads. The leaves are highly nutritious and a very good source of different vitamins. Silver beet was chosen as a test plant for a glasshouse study as it is very responsive to N fertiliser and relatively tolerant of cold, heat, drought and disease. It also grows very quickly. *Fordhook giant* is one of the popular varieties cultivated in Australia. The stem is white and the leaves are blistered dark green. Silver beet can be grown in any climatic and soil conditions with adequate irrigation water. A slightly acidic to neutral soil pH (6.0 to 7.5) is suitable for better growth of silver beet (Ali and Ali, 2011).

4.2. Materials and methods

4.2.1. Soil collection and characterisation

Two soils were used in this study. The first, a Dermosol, was collected from a grazed pasture near Stony Creek, Gippsland, in south-eastern Victoria, and the second, a Tenosol, from a farmer's grazed field in the Wimmera region, Victoria, Australia. Both soils were collected from the 0-15 cm soil layer. Immediately following collection, the soils were air-dried and sieved to <2 mm. Subsamples (200 g) of each soil were then analysed for a range of key physicochemical properties (Table 4.1).

Table 4.1. Physical and chemical properties of soils used in this study

Property	Tenosol	Dermosol
Texture	Sandy loam	Loam
Moisture (%)	2.66	6.24
pH (Water)	7.23	5.46
Total carbon (%)	0.77	1.19
Total nitrogen (%)	0.13	0.17
*Ammonium nitrogen (mg kg^{-1})	6.1	3.7
*Nitrate nitrogen (mg kg^{-1})	2.2	2.6
Phosphorus (Colwell) (mg kg^{-1})	10	31
*Calcium (mg kg^{-1})	915	260
*Magnesium (mg kg^{-1})	89	71
*Potassium (mg kg^{-1})	92	52
*Sulfur (mg kg^{-1})	2.7	3.7
*Iron (mg kg^{-1})	44	122

*Indicates the soluble form of the nutrients

4.2.2. *Plant growth experiment*

Plastic, free-draining pots (25 cm depth and 16 cm diameter) were filled with 7.9 kg of Dermosol and 8.2 kg Tenosol to match the field bulk densities for the two soils, which were 1.3 g cm^{-3} and 1.4 g cm^{-3}, respectively. The experiment was laid out following completely randomised design with five replicates. To each soil, the following treatments were added:

Treatments:

T1: Control (No N added)

T2: Brown coal (No N added)

T3: Urea (N at a rate of 100 kg ha^{-1})

T4: Brown coal-urea 1 (BCU 1) (N at a rate of 100 kg ha^{-1})

T5: Brown coal-urea 2 (BCU 2) (N at a rate of 100 kg ha^{-1})

T6: Brown coal-urea 1 (BCU 1) (N at a rate of 50 kg ha^{-1})

T7: Brown coal-urea 2 (BCU 2) (N at a rate of 50 kg ha^{-1})

T8: Urea (N at a rate of 50 kg ha^{-1})

Equal amounts of N were added to the soil hence resulting in varying amounts of granules depending on their N concentration (Table 4.2). In addition, P (single superphosphate) and K (potassium sulphate) were added at a rate of 40 and 60 kg ha^{-1}, respectively. All the fertilisers were mixed with the topsoil in the granular form and uniformly covered the whole surface area of soil. The pots were then left to equilibrate at field capacity for 3 days before the sowing of seeds. To each pot, 10 seeds of silver beet were sown to ~2 mm below the soil surface. Soil moisture was maintained at field capacity by regular addition of tap water as required, usually every 2 days. After germination, only one plant was allowed to grow in each pot (Figure 4.1).

Table 4.2. C and N concentration of BCU granules and urea used in this study

Fertilisers	C concentration (%)	N concentration (%)
Brown coal-urea 1 (BCU 1)	40	22
Brown coal-urea 2 (BCU 2)	46	17
Urea	20	46

4.2.3. Gas sampling, plant harvesting and soil analysis

Five gas sampling periods were undertaken at the early growth stage of the plants to measure the loss of N as N_2O and NH_3 emissions. The greenhouse gas flux was calculated according to the method and equations detailed by van Zwieten et al. (2010). Ammonia emissions were measured using polyurethane foam absorbers and flux was calculated using the equation of Singh et al. (2009). For measuring the chlorophyll content leaf samples were collected from the third leaf from the top. Leaf chlorophyll was extracted using 80% aqueous acetone. Leaf chlorophyll content was measured spectrophotometrically at 45 days after fertiliser addition following the method of Arnon (1949).

Plants were destructively harvested at 10 weeks after sowing. The plants and soil were carefully removed from the pots. The soil was gently shaken from the roots, after which the shoots and roots were separated. The roots were then thoroughly washed with water to remove any adhering soil. The shoots and roots of each plant were oven-dried for 7 days at 55°C, following which shoot dry weight (SDW) and root dry weight (RDW) were measured. The dried plant materials were then ground to a fine powder and N concentrations were determined using a CHN analyser (Vario Micro Cube). The biomass N uptake by silver beet was calculated following the equation described by Finzi et al. (2007).

$$Nitrogen\ uptake\ (g/pot) = \frac{\text{N concentration (\%)}}{\text{Dry biomass yield (g)}} * 100$$

Nitrogen use efficiency (NUE) and fertiliser N use efficiency (FNUE) were calculated according to the formula described by Moll et al. (1982). Soil samples were collected and taken to the laboratory for chemical analyses. The soil was analysed for pH (WP 80 Reference pH Meter, Anpros Phy LTD., Victoria, Australia), NH_4^+-N and NO_3^--N (Forster, 1995), potentially mineralisable nitrogen (PMN) (Waring and Bremner (1964), soil organic C and total N (CHN analyser Vario Micro Cube).

$$Nitrogen\ use\ efficiency\ (\%) = \frac{\text{N yield in plant}}{\text{N applied}} * 100$$

Figure 4.1. Experimental set up and GHG sampling (left) and comparison of plant growth of silver beet among different treatments at 100 kg N ha^{-1} (right) at 10 weeks

4.2.4. Statistical analysis

The statistical analyses were performed using the statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). The normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Levene's test. Some data (Plant height and root biomass) were not normally distributed and showed unequal variance. Those data were log or Ln transformed before performing the analysis of variance (ANOVA). A two-way ANOVA including factors fertiliser type (including the BC and control) and soil type were run. As the interaction term was significant ($p<0.001$), a one-way ANOVA was run by combining the two factors (fertiliser type and soil type). The multiple comparisons among the different treatments were done using a Scheffé test.

4.3. Results

4.3.1. Plant growth parameters and biomass yield

Incorporation of BCU granules to soil significantly influenced the growth parameters, root biomass and biomass yield of silver beet in both soils. There was a significant interaction between soil type and N fertilisers for most of the growth parameters and biomass yield (Appendix 1). The growth parameters and biomass yield varied significantly between BCU and urea treated soils. The tallest plant and maximum number of leaves were recorded from the pots treated with BCU granules at a rate 100 kg N ha^{-1} (Table 4.3). Significantly higher amounts of fresh and dry biomass yields were measured from the plants fertilised with BCU granules compared to urea, BC and control treatments (Figure 4.2). In the Dermosol, the biomass yield was significantly higher in BCU 2 treatments over urea at the high N application rate whereas the difference was not significant for low N application rate. Importantly, statistically similar biomass yields were recorded from both soils receiving 50 kg N ha^{-1} as BCU and 100 kg N ha^{-1} as urea. Compared to urea, BCU granules increased biomass yield by 27% and 23% in the Tenosol (pH 7.24) and Dermosol (pH 5.4), respectively. Like shoot biomass yield, a similar trend was observed in root biomass in both the soils (Table 4.3). Regarding soil types, the growth and biomass yield was significantly higher in the Tenosol than the Dermosol.

Table 4.3. Effect of BCU granules and urea on the plant height, leaves plant^{-1}, root weight of silver beet

Treatments	Plant height (cm)		Leaves plant^{-1} (No.)		Fresh root wt. (g)		Dry root wt. (g)	
	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol
Control	33b	23b	7.0c	6.8c	21d	2.2c	3.1d	0.58c
Brown coal	34b	23b	7.2c	6.0c	22d	2.6c	3.2d	0.51c
Urea-100	44a	38a	8.8bc	8.6ab	56b	21.9ab	10.3b	4.54a
BCU 1-100	46a	42a	11.0ab	10.6ab	96a	25.7a	19.4a	4.18a
BCU 2-100	44a	41a	12.0a	11.6a	85a	25.3a	17.8a	5.45a
Urea-50	39ab	37a	7.8c	9.2ab	34c	11.2b	5.7bc	2.36b
BCU 1-50	40ab	33ab	9.6abc	8.8ab	42bc	13.4b	8.1bc	3.23b
BCU 2-50	41ab	38a	9.4abc	10.6ab	47bc	17.1b	8.4bc	3.81b

The values with in a response variables are significantly different according to Scheffé-test at *P*<0.001.

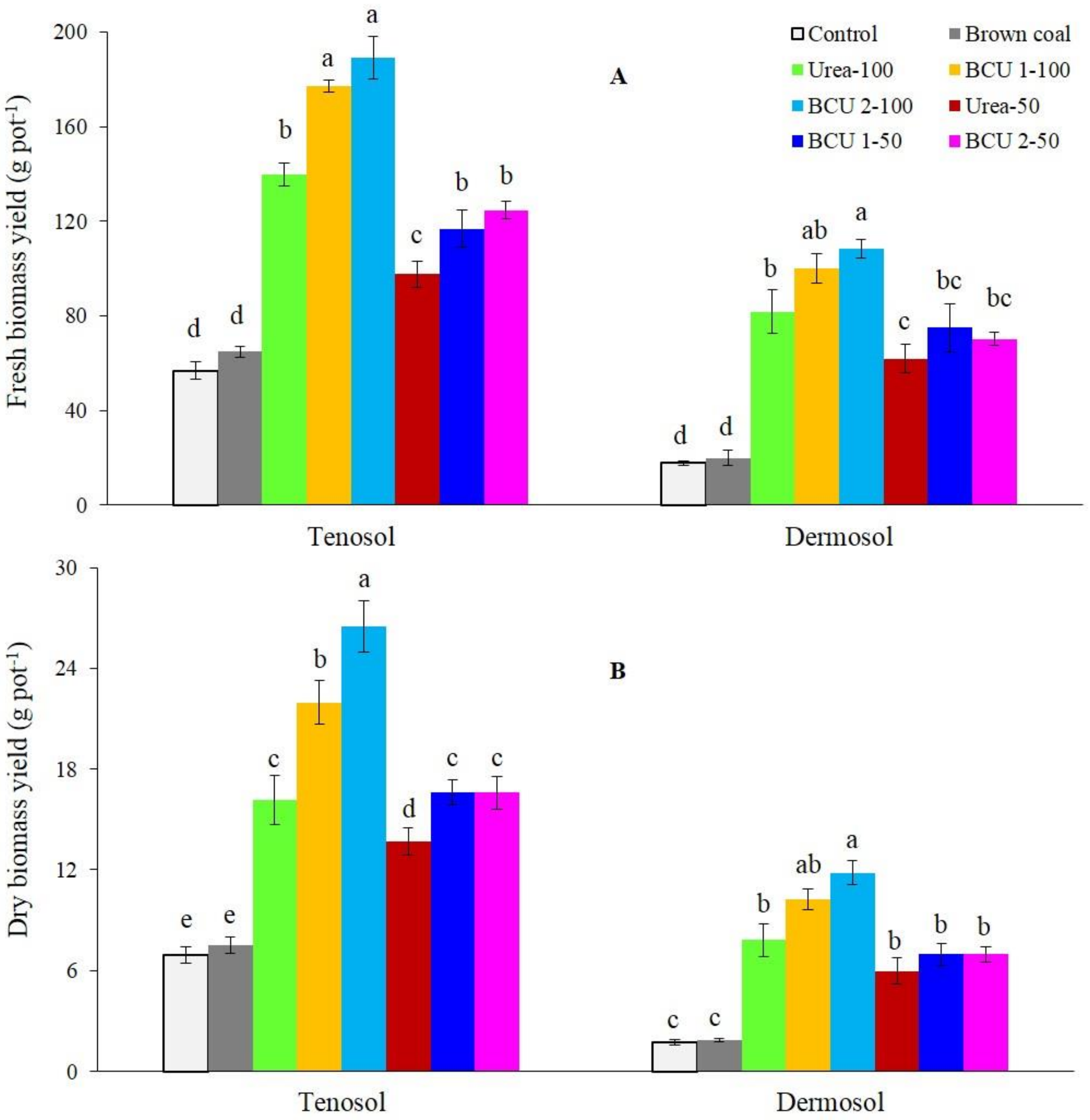


Figure 4.2. Effect of BCU granules and urea on the fresh (A) and dry (B) shoot biomass yield of silver beet. Bars with different letters differ significantly according to Scheffé-test at *P*<0.001 and the error bars indicate the standard error among the replicates (n=5).

4.3.2. *Total chlorophyll content*

Addition of BCU granules caused a significant increase in the leaf total chlorophyll content of silver beet (Appendix 1 and Figure 4.3). Significantly higher amounts of total chlorophyll were measured from the plants fertilised with BCU granules for both high and low N application rate compared to urea at the same rate irrespective of soil type. Interestingly, statistically similar amounts of chlorophyll were determined from the plants receiving 100 kg N ha^{-1} as urea and 50 kg N ha^{-1} as BCU granules in both soils.

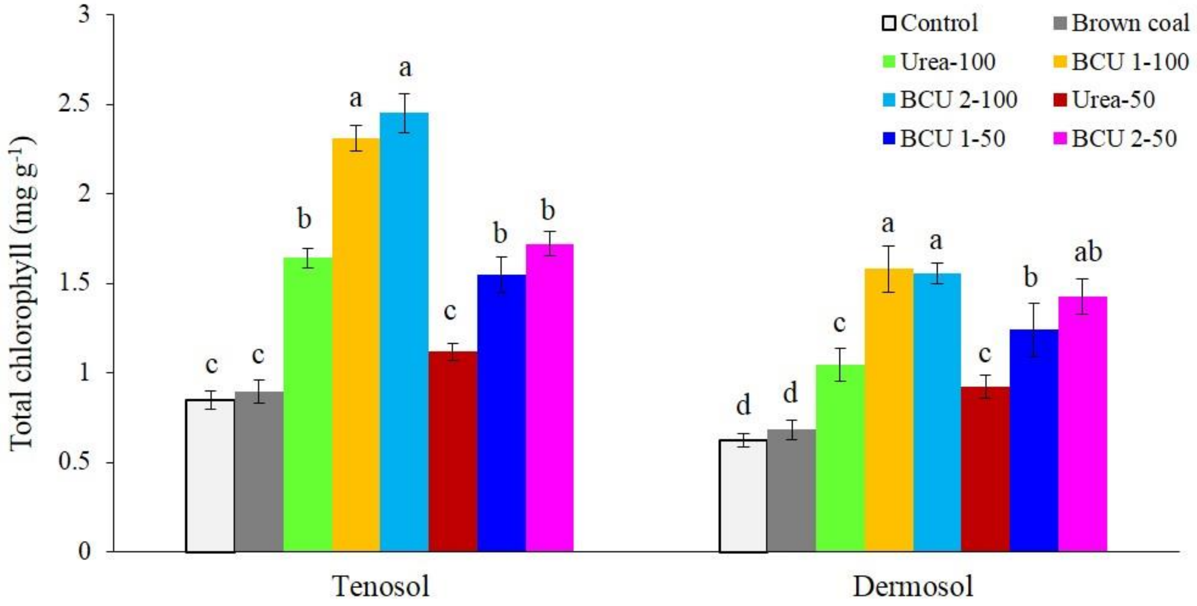


Figure 4.3. Effect of BCU granules and urea on the total chlorophyll content of silver beet. Bars with different letters differ significantly according to Scheffé-test at $P<0.001$ and the error bars indicate the standard error among the replicates (n=5).

4.3.3. *C and N concentration in shoot and root*

The shoot and root N concentration among the treatments of silver beet grown in the Tenosol and the Dermosol were significantly different (Appendix 1). There was more shoot N in the plants receiving N as BCU compared to plants receiving equivalent amount N as urea in both soil type (Figure 4.4). In case of root N, the plants grown in the Dermosol soil amended with 100 kg N ha^{-1} as BCU 2 showed significantly higher amounts of N compared to the plants fertilised with same amount of N as straight urea (Table 4.4). However, no significant difference was found in the root N concentration of BCU and urea amended plants at low N application rate in the Tenosol. No significant change was noticed in the shoot and root C concentration among the various treatments in both soils (Table 4.4).

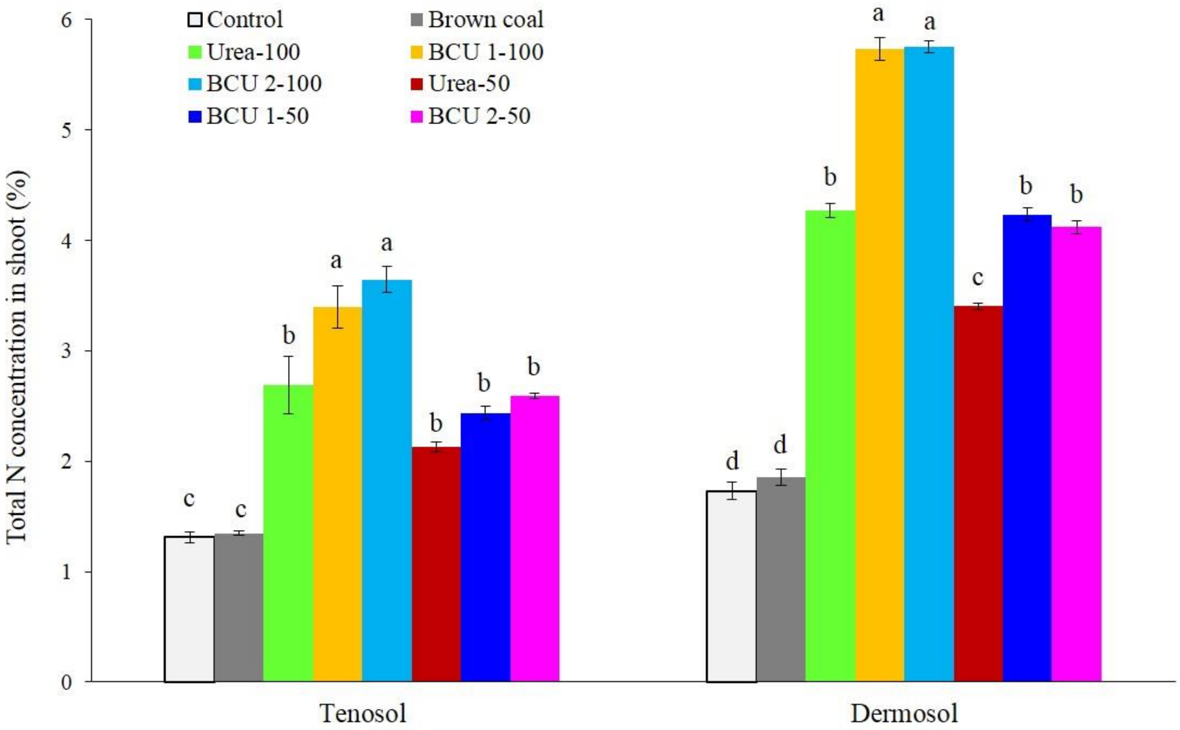


Figure 4.4. Effect of BCU granules and urea on the total N concentration in shoot of silver beet. Bars with different letters differ significantly according to Scheffé-test at *P*<0.001 and the error bars indicate the standard error among the replicates (n=5).

Table 4.4. Effect of BCU granules and urea on the root N, shoot and root C concentration of silver beet

Treatments	Shoot C (%)		Root N (%)		Root C (%)	
	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol
Control	33.2	35.5	1.42b	1.31d	29.8	25.6
Brown coal	34.1	35.4	1.42b	1.36d	29.0	26.8
Urea-100	35.4	35.7	1.57ab	1.71b	28.4	27.2
BCU 1-100	36.5	36.8	1.66a	1.86ab	30.5	27.7
BCU 2-100	36.5	37.9	1.72a	2.03a	29.6	25.3
Urea-50	36.3	36.1	1.55ab	1.41c	30.6	27.1
BCU 1-50	36.6	37.4	1.59ab	1.46c	31.5	27.5
BCU 2-50	35.9	36.2	1.72a	1.97ab	29.1	26.7

The values with in a response variables are significantly different according to Scheffé-test at $P<0.001$.

4.3.4. Nitrogen uptake by silver beet

The N uptake by silver beet was significantly influenced by the addition of various N fertilisers in both soils (Appendix 1 and Figure 4.5). The N uptake was significantly greater in plants receiving 100 kg N ha^{-1} as BCU compared to plants receiving the equivalent of amount of N as urea. Compared to urea, the application of BCU granules failed to show any significant variation in N uptake, when N was applied at the rate 50 kg N ha^{-1}. Like biomass yield and chlorophyll content, similar N uptake was calculated from the plants receiving 100 kg N ha^{-1} as urea and plants receiving 50% less N as BCU. Generally, the N uptake was greater in plants fertilised with BCU granules containing higher amounts of BC compared with the BCU granules having lower amounts of BC. The N uptake was considerably higher in the Tenosol than the Dermosol.

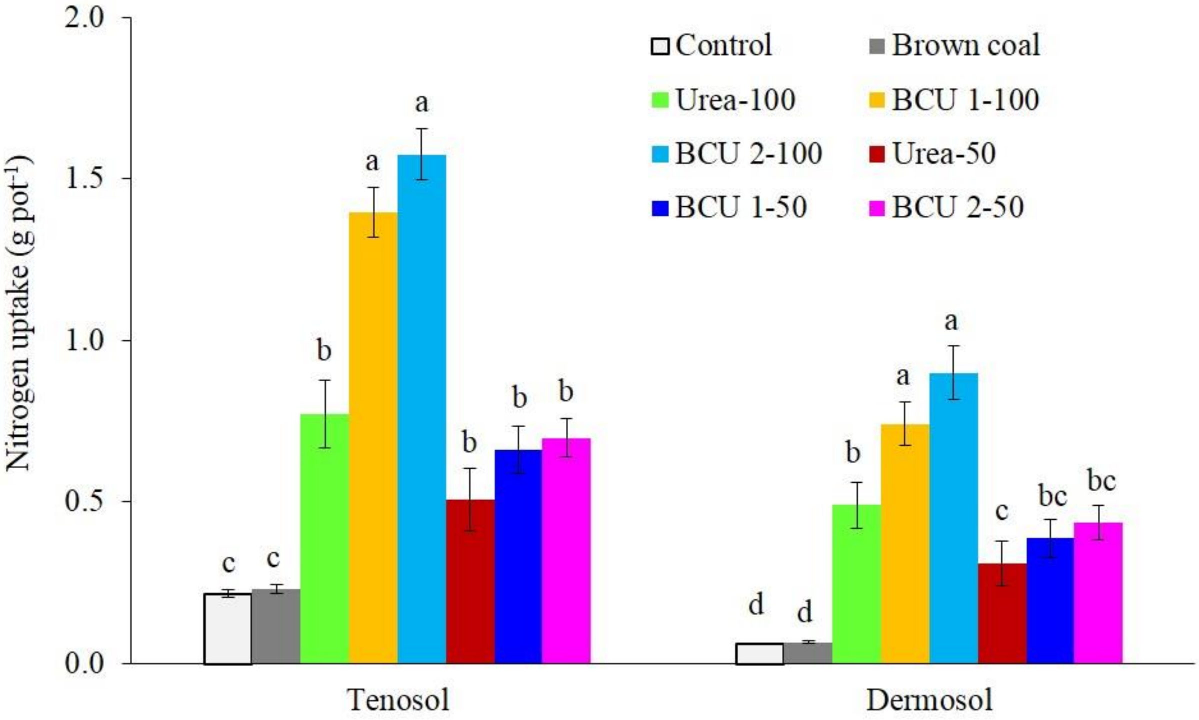


Figure 4.5. Effect of BCU granules and urea on the N uptake by silver beet. Bars with different letters differ significantly according to Scheffé-test at *P*<0.001 and the error bars indicate the standard error among the replicates (n=5).

4.3.5. N_2O emissions from soil

Application of N fertilisers (as either straight urea or BCU granules) significantly increased the emissions of N_2O from soil compared to soil which received no N fertilisers (Appendix 1). A sharp increase in daily N_2O flux was monitored just after N fertilisation and peaked at day 14 and 21 after fertiliser addition to the Tenosol and Dermosol, respectively. After this, the daily N_2O emissions declined rapidly and remained almost constant until the last gas sampling (Figure 4.6). The addition of BCU granules resulted in a decrease in cumulative N_2O emissions from both soils. Compared to urea, BCU granules significantly suppressed the N_2O emissions from both the Tenosol and Dermosol when N was applied at the rate of 100 kg ha^{-1} (Figure 4.7). In the case of the low N application (50 kg ha^{-1}), only the BCU 2 showed significantly lower N_2O emissions than urea in both the soils. Compared to urea, on average BCU granules suppressed the cumulative N_2O emissions by 31 and 34% for the Tenosol and Dermosol soils, respectively. In general, the granules containing a higher amount of BC had considerably lower N_2O emissions from soil with respect to the granules having lower proportions of BC.

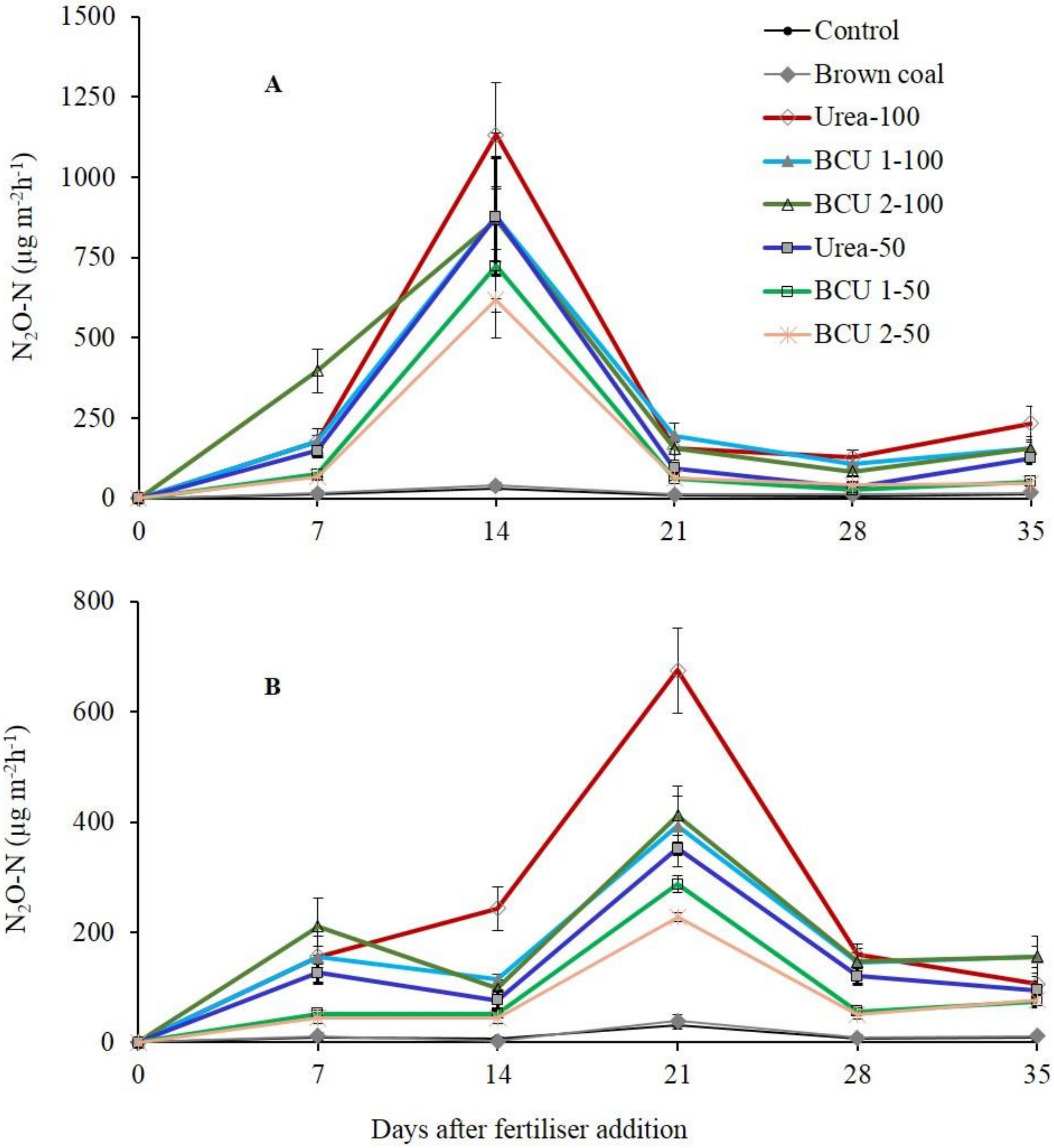


Figure 4.6. Effect of BCU granules and urea on the daily N_2O-N emissions from Tenosol (A) and Dermosol (B) during early growth stage of silver beet. The error bars indicate the standard error among the replicates (n=5).

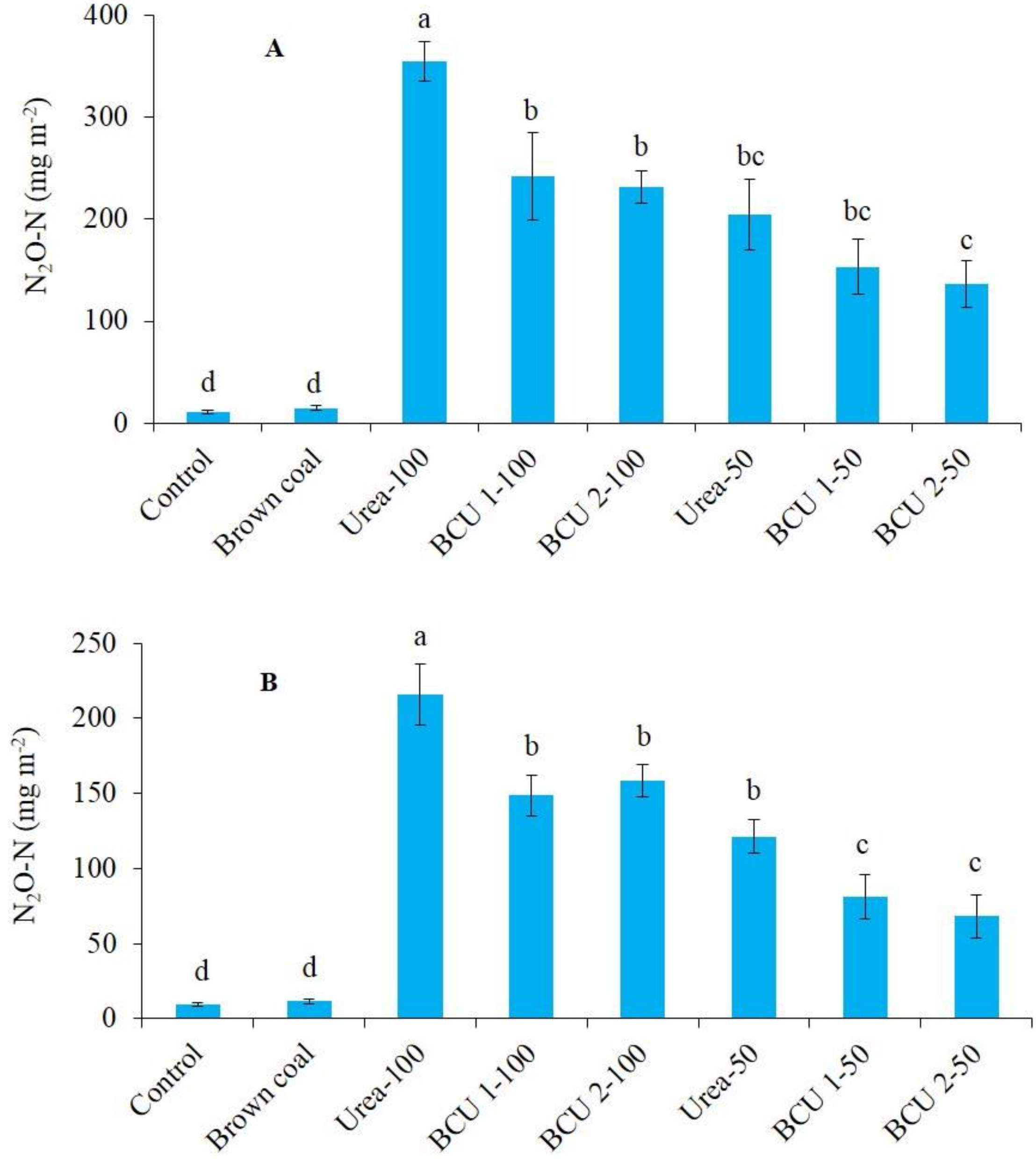


Figure 4.7. Effect of BCU granules and urea on the cumulative N_2O-N emissions from Tenosol (A) and Dermosol (B) during early growth stage of silver beet. Bars with different letters differ significantly according to Scheffé-test at $P<0.001$ and the error bars indicate the standard error among the replicates (n=5).

4.3.6. NH_3 volatilisation from soil

The loss of NH_3 via volatilisation from the two soils was influenced significantly by the application of N using different fertilisers (Appendix 1). Addition of BCU granules resulted in a decrease in daily and cumulative volatilisation of NH_3 for both the Tenosol and Dermosol soils compared to urea alone. A sharp increase in NH_3 volatilisation was measured initially one day after fertiliser addition and reached its peak rapidly within one week of fertilisation in both the soils. The daily NH_3 emissions reached a peak at 3 days after fertiliser (DAF) addition in the Tenosol whereas for Dermosol the emissions peaked at 6 DAF (Figure 4.8). The NH_3 volatilisation then declined rapidly and remained almost constant until the end of gas sampling period irrespective of soil type. In the Tenosol, the cumulative NH_3 volatilisation was significantly lower in soil receiving N as BCU compared to soil receiving an equal amount of N as urea in both high and low N application rate (Figure 4.9). In contrast, the NH_3 volatilisation was not significantly different between the BCU and urea treatments in the Dermosol. Compared to urea, on average, BCU granules decreased the cumulative NH_3 volatilisation by 36 and 12% from the Tenosol and Dermosol, respectively. The volatilisation loss of N as NH_3 was significantly higher in the Tenosol compared to Dermosol.

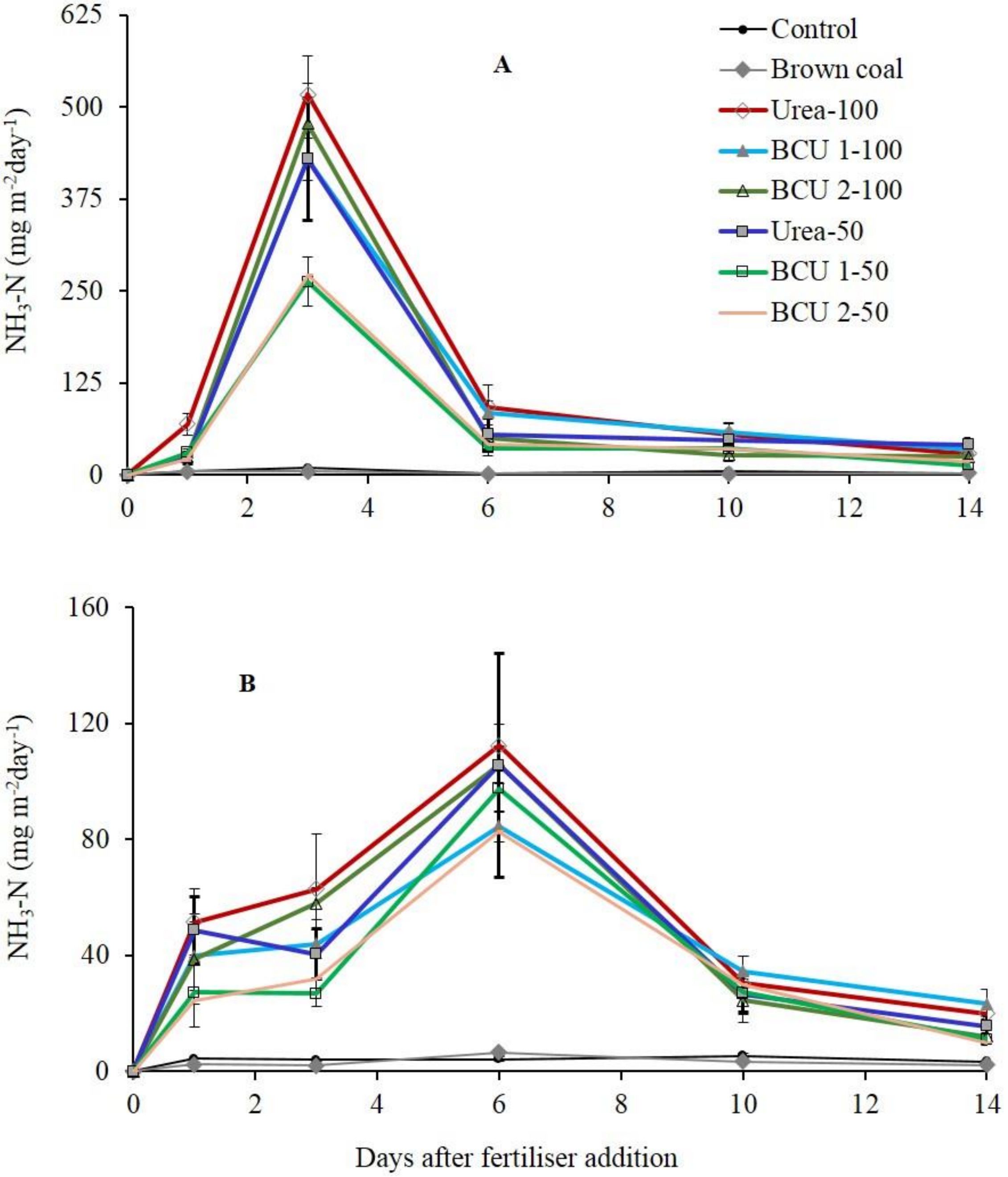


Figure 4.8. Effect of BCU granules and urea on the daily NH_3 emissions from Tenosol (A) and Dermosol (B) during early growth stage of silver beet. The error bars indicate the standard error among the replicates (n=5).

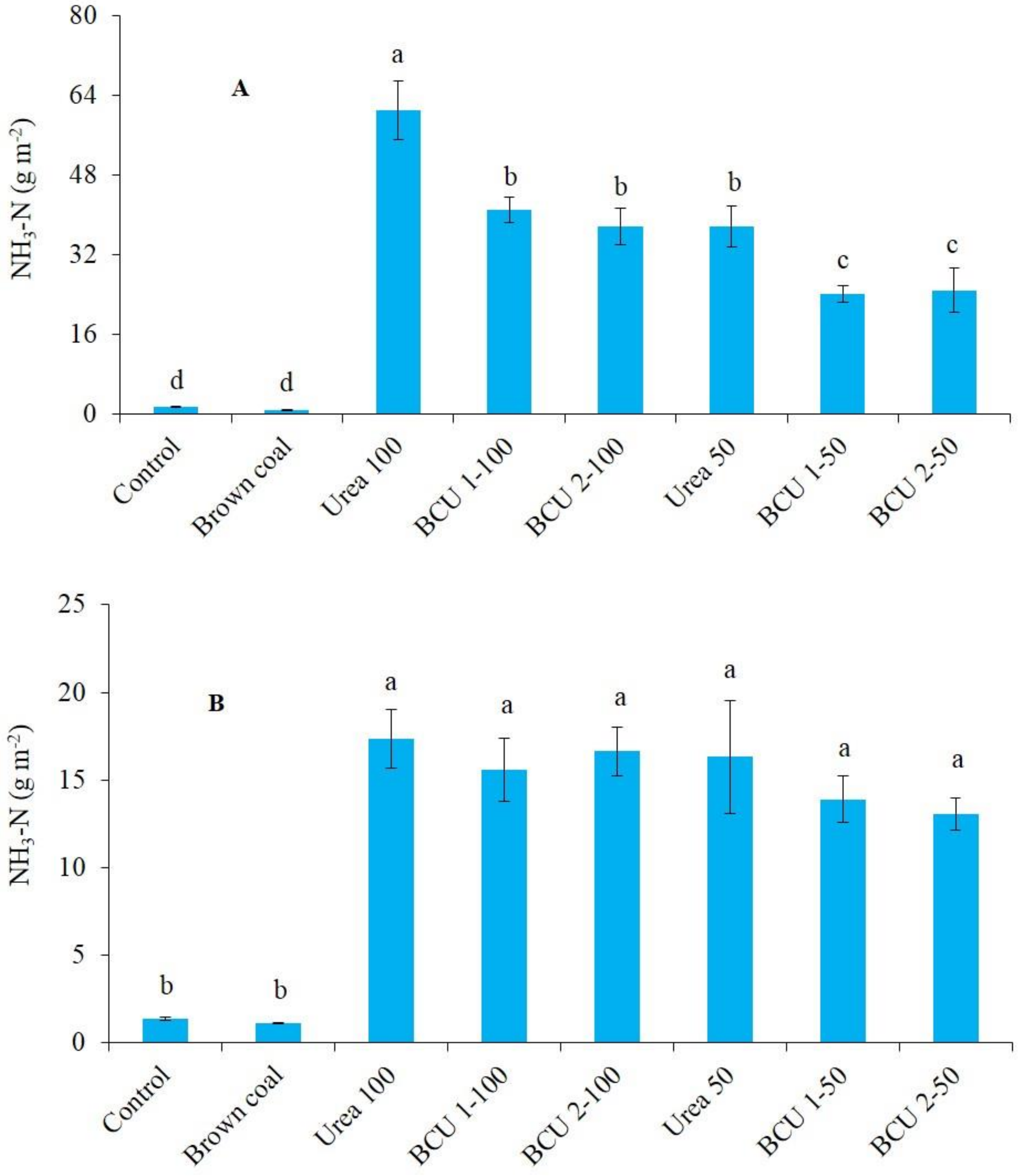


Figure 4.9. Effect of BCU granules and urea on the cumulative NH_3 emissions from Tenosol (A) and Dermosol (B) during early growth stage of silver beet. Bars with different letters differ significantly according to Scheffé-test at $P<0.001$ and the error bars indicate the standard error among the replicates (n=5).

4.3.7. pH, NH_4^+-N, NO_3^--N, PMN, total N and C content in soil at harvest

Significantly higher amounts of NH_4^+-N, NO_3^--N and PMN were measured from the soils fertilised with urea and BCU granules compared to BC and control treatments (Appendix 1). Addition of BCU granules at a rate of 100 kg N ha^{-1} maintained significantly higher amounts of NH_4^+-N, NO_3^--N and PMN in soil compared to other treatments, including urea, at the same N rate in both soils (Table 4.5 and Figure 4.10). No significant difference in total N concentration was measured among the different treatments in the Tenosol and Dermosol. Significantly greater amounts of soil C were also found in the soils amended with BCU granules and BC compared to urea and control treatments. The BCU granules containing higher proportion of BC maintained higher amounts of NH_4^+-N, NO_3^--N, PMN and C content in soil compared with BCU granules having lower amount of BC. In the Tenosol, the soil pH differed significantly among the various treatments. The soil amended with BC and BCU 2 caused a significant decrease in soil pH compared with control treatments. In contrast, no significant variation was measured in post- harvest soil pH among the different treatments in Dermosol.

Table 4.5. Effect of BCU granules and urea on the pH, NH_4^+-N, NO_3^--N, total nitrogen, carbon concentration of soil at harvest

Treatments	NH_4^+-N		NO_3^--N		Soil total N (%)		Soil total C (%)		Soil pH	
	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol	Tenosol	Dermosol
Control	1.22c	1.65b	0.92b	0.94b	0.09	0.10	0.90b	1.22c	7.33a	5.44
Brown coal	1.49c	1.81b	1.16b	1.54b	0.10	0.12	1.85a	2.47a	6.91b	5.18
Urea-100	3.59b	2.95b	4.06ab	7.99ab	0.11	0.13	0.93b	1.27c	7.06ab	5.26
BCU 1-100	3.97ab	6.55a	5.46ab	16.32a	0.14	0.15	2.12a	2.17a	6.96ab	5.19
BCU 2-100	5.54a	7.37a	8.61a	13.25a	0.14	0.15	2.27a	2.60a	6.90b	5.29
Urea-50	2.42bc	2.49b	2.75b	2.44b	0.12	0.11	0.99b	1.15c	7.06ab	5.33
BCU 1-50	2.79bc	3.21b	3.74ab	6.98ab	0.15	0.14	1.21b	1.75b	7.03ab	5.28
BCU 2-50	3.03bc	4.15ab	3.44ab	3.32b	0.15	0.13	1.29b	1.69b	7.18ab	5.28

The values with in a response variables are significantly different according to Scheffé-test at $P<0.001$.

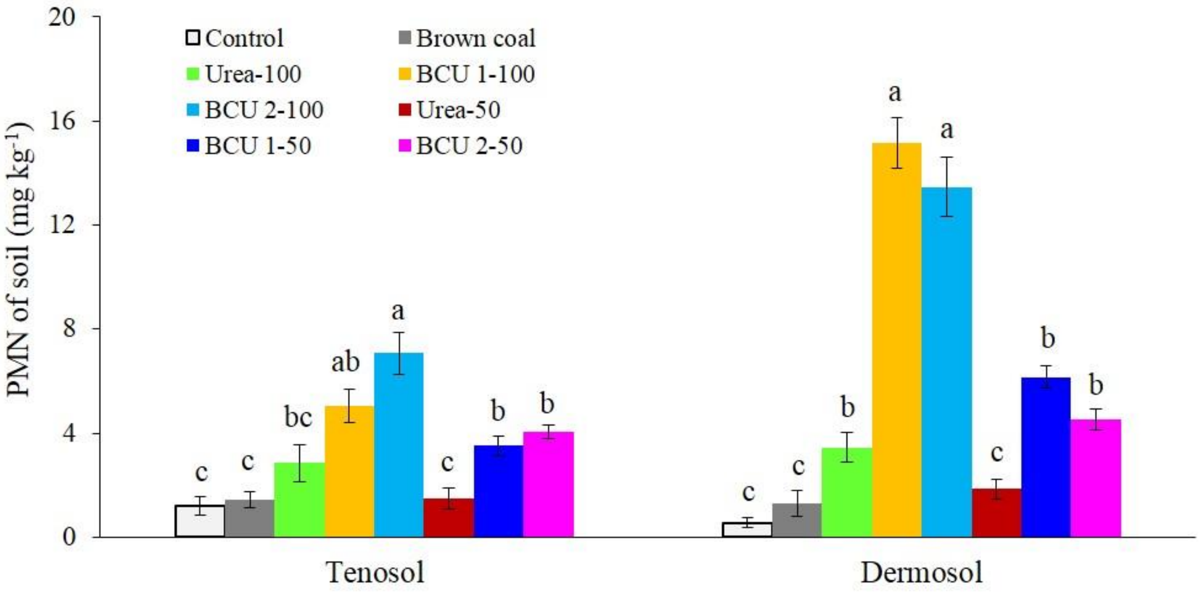


Figure 4.10. Effect of brown coal-urea granules and urea on the potentially mineralisable nitrogen (PMN) concentration in soil at harvest. Bars with different letters differ significantly according to Scheffé-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=5).

4.3.8. Nitrogen use efficiency (NUE) and fertiliser N use efficiency (FNUE)

The NUE and FNUE of silver beet was significantly influenced by the application of N fertilisers and their application rate (Appendix 1). In both soils, the NUE and FNUE were decreased substantially with the increase in N fertiliser application rate (Table 4.6). No statistical significant differences were observed in the NUE and FNUE between BCU and urea for both low and high application rate, irrespective of soil types. On average, addition of BCU granules increased NUE by 29% and 20%, and FNUE by 43% and 25% from the Tenosol and Dermosol, respectively, compared to straight urea treated soil.

Table 4.6. Effect of BCU granules and urea on the NUE and FNUE of silver beet

Treatments	NUE		FNUE	
	Tenosol	Dermosol	Tenosol	Dermosol
Urea-100	88b	41c	55b	33b
BCU 1-100	138a	48bc	104a	40ab
BCU 2-100	148a	58abc	114a	49ab
Urea-50	129a	56abc	62b	40ab
BCU 1-50	164a	68ab	97a	53ab
BCU 2-50	166a	72a	99a	56a

The values with in a response variables are significantly different according to Scheffé-test at $P<0.001$.

4.4. Discussion

Brown coal and coal derived products are gaining popularity as organic amendments for improving crop yield and soil health by influencing the dynamics and mineralisation of nutrients, especially N in soil. Previous studies presented in Chapter 2 and 3 indicated that BC has the potential for decreasing fertiliser-N release due to increased N retention by exploiting its extensive surface area and high cation exchange capacity. In this study, N was applied at the rate of 50 and 100 kg N ha^{-1} in two contrasting soils to investigate the efficacy of BCU compared to commercial urea on silver beet.

4.4.1. Gaseous loss of N

Application of N fertiliser as BCU significantly decreased the daily and cumulative N_2O emissions from both soils compared to commercial urea and control soil where no N were added (Figure 4.7-4.8). The BCU granules containing the higher amount of BC were superior to the BCU granules with lower amount of BC in terms of reducing N_2O emissions from both the soils. The N_2O emissions were considerably higher in soil that received 100 kg N ha^{-1} than the soil that received 50 kg N ha^{-1}. High fertiliser N applications and increased soil N availability favours the activity of denitrifying microbial community in the soil and lead to a higher amount of N_2O emissions, compared to low N application (Nelissen et al., 2014; Ruser et al., 2006). In contrast, the reduction in N_2O emissions from the BCU treated soil is likely to be the result of slower N release and prolonged retention of N as NH_4^+ by the cation exchange sites of BC. It is evident from the results presented in Chapter 2 and 3 that granulation of urea with BC substantially reduced the release rate of urea-N due to its strong retention by BC. Moreover, BC can potentially decrease urea hydrolysis by inhibiting the urease enzyme activity in soil (Saha et al., 2017). The decreased urea hydrolysis in BCU amended soil will reduce the availability of NH_4^+ for nitrification, and, as a result, less NO_3^- will be available for denitrification and N_2O production. The results of our study are similar to those of Rose et al. (2016) who reported that granulation of urea with BC had a significant effect in decreasing N_2O emissions compared to soil amended with urea alone.

Application of BCU granules caused a significant reduction in NH_3 volatilisation in the Tenosol compared to urea treatment, whereas no significant difference in NH_3 emissions were observed between BCU and urea treatments in the Dermosol (Figure 4.9-4.10). In both soils, the NH_3 volatilisation reached to its peak within one week of fertiliser addition. The addition of N fertilisers coupled with adequate soil moisture was responsible for the rapid hydrolysis of urea-N to NH_3. Bollmann and Laanbroek (2001) reported that addition of BC reduced volatilisation losses of N from urea fertilised soil, and this may partly be a consequence of BC inhibition of the urease enzyme activity (Saha et al., 2017). Moreover, it is also evident from several reports that NH_3 may be abiotically fixed onto the porous structure of BC (Bosatta and Agren, 1995; Lapierre et al., 1994). Similarly, Husted et al. (1991) reported that addition of organic amendments containing acid functional groups significantly reduced volatilisation losses of NH_3. The results of our study are supported by the findings of Sun et al. (2016) and Chen et al. (2015) who also reported that soil incorporation of lignite decreased NH_3 emissions by 60-68% from cattle manure amended pens compared to control pens where no lignite was added. Among the soil types, the volatilisation loss of N was remarkably higher in the Tenosol over the Dermosol (Figure 4.16-4.17). The lower NH_3 volatilisation in the Dermosol is likely the result of low pH (5.46) and calcium content of soil because, the soil pH is one of the most important factors controlling volatilisation loss of N. Cevallos et al. (2015) observed that most of the NH_3 volatilisation occurs at soil pH >7.0. The findings of Cevallos et al (2015) are supported by our study where the NH_3 volatilisation loss was higher in Tenosol, with a pH of 7.23. The higher calcium content of Tenosol could be another reason for increased NH_3 volatilisation. It is also evident from the results of Pesek et al. (1971) that volatilisation loss of N is positively correlated with $CaCO_3$ content of soil. Both the N_2O and NH_3 emissions peaked bit earlier in Tenosol than the Dermosol. The higher soil pH (7.23) of the Tenosol could be the reason for triggering the activity of denitrifiers a bit earlier than the acidic Dermosol (pH 4.56). Similarly, Dannenmann et al. (2008) found a positive co-relation between soil pH and denitrification rate. The optimum soil pH for most of the denitrifying microorganisms lies between 6.0 to 8.0 (Kyveryga et al., 2004). Addition of BC had a tendency to decrease soil pH (Table 4.5) which would also contribute to reduced NH_3 volatilisation.

4.4.2. Growth characteristics and biomass yield

Addition of BCU granules to soil had a significant effect on the growth characteristics and biomass yield of silver beet in both soils. A significant increase in leaf number, chlorophyll content, root biomass and biomass yield was measured from the BCU treatments compared to other treatments receiving an equal amount of N as urea and the treatments where no N fertiliser was added (Figure 4.2-4.4 and Table 4.4). A similar finding was reported by Mazeika et al. (2016) who found that addition of an organo-mineral pelleted fertiliser formulated from poultry litter, peat, di-ammonium phosphate and ammonium chloride significantly increased yields of potato and rapeseed compared to sole application of synthetic fertiliser. Victorian brown coal contains very large amounts of humic acids (50-60%) and lower amounts of some essential nutrients such as N and S, and minor amount of micronutrients which may stimulate plant growth (Perry et al., 1984). It can be speculated that the addition of humified organic matter like BC to soil showed a positive effect on the structure, moisture and cation exchange capacity (CEC) of soil which ultimately helped to improve plant growth in BCU amended soil (Piccolo et al., 1993). Furthermore, application of humic acids has been shown to improve plant growth by regulating many soil processes including nutrient adsorption and release (Arancon et al., 2006). The higher chlorophyll content in BCU granule amended plants was an indication of higher N uptake by plants compared to urea. This is in agreement with the findings of Fernández-Luqueño et al. (2010) who reported that combined application of humified vermicompost and urea increased chlorophyll content compared with urea fertilised soil. Incorporation of BCU granules enhanced root growth and root biomass over urea alone. Application of N as BCU favoured plant growth and substantially increased biomass yield of silver beet over urea. Full dose (100 kg N ha^{-1}) of either BCU or urea application were superior in growth parameters and biomass yield of silver beet in both the soils compared with the application of 50% less N as urea and BCU. At full dose (100 kg N ha^{-1}), both the urea and BCU enhanced N supply and increased absorption of N by silver beet in both the soils. The growth and biomass yield of silver beet was considerably lower in the Dermosol than the Tenosol. This might be the effect of acidic soil pH (5.46) of the Dermosol. Slightly acidic to neutral soil pH (6.0 to 7.5) is suitable for improved growth and biomass yield of silver beet (Ali and Ali, 2011). Moreover, soil pH is a key soil parameter responsible for controlling nutrient availability and microbial activity, both of which generally improved when pH is increased from acidic to neutral (DeLuca et al., 2006).

On the other hand, the rate of N release varied considerably between urea and BCU. In the case of urea, the N release rate was faster and thereby showed higher N losses immediately after fertiliser addition. As a result, less of the fertiliser-N was available for plant uptake at the later growth stage. This is supported by the gaseous N loss data which showed that the gaseous loss N as N_2O and NH_3 was significantly higher in urea treated soil compared to BCU amended soil (Figure 4.7-4.8). On the other hand, the mineralisation and release of N from BCU was very slow and steady due to its strong retention by the surface area and cation exchange site of BC which ultimately minimised gaseous N losses and increased its availability to plants over a longer period of time. The results of this study are also strongly supported by the findings of our previous study results presented in Chapter 2 where granulation of urea with BC substantially slowed down the release of urea-N and NH_4^+-N compared to commercial urea alone. Thus, the growth and yield benefit on application of BCU granule might be due its capability to retain fertiliser-N for longer periods of time as well as growth promoting humic acids for improvement of growth and yield of silver beet. A similar finding was reported by Nikbakht et al. (2008) who found that the application of humic acids significantly improved plant growth and yield of gerbera.

4.4.3. Biomass N uptake and NUE

Nitrogen uptake and its use efficiency was significantly influenced by the application of different N fertilisers. The N concentration and its uptake was substantially higher in BCU treated plants compared to urea fertilised plants (Figure 4.5-4.6). It is evident from a large number of experimental results that addition of BC and HAs increased mineral N availability and its uptake by a variety of vegetable, pasture and grain crops (Nardi et al., 2000; Piccolo et al., 1993; Pinton et al., 1999; Sánchez Sánchez et al., 2009). The increased N uptake in the BCU fertilised plants is associated with its increased soil availability of N and thereby its uptake through increased biomass yield. The NUE decreased gradually with the increase in N application rate which might be due to increased mineralisation and losses of N. The BCU treatments had higher NUE compared to commercial urea treatments in both soils (Table 4.7). A similar result was reported by Duan et al. (2014) who also found that combined application of manure and synthetic fertiliser increased NUE of wheat up to 63% over sole application of synthetic fertiliser. The N use efficiency was about two times higher in plants receiving 50 kg N ha^{-1} as BCU compared to the plants receiving 100 kg N ha^{-1} as urea. This is because of slow and steady release of N in BCU treatments and thereby it's increased soil availability and enhanced crop uptake. Furthermore, the higher biomass yield in BCU amended soil also contributed to increasing NUE. In addition, the decreased gaseous N losses also improved NUE in BCU treated plants compared with urea fertilised plants. These results suggest that addition of 50% less N as BCU can produce similar yield benefits like 100% urea. This is supported by our results, where no statistical difference was observed in the chlorophyll content, biomass yield and N uptake by plants fertilised with 50 kg N ha^{-1} as BCU and 100 kg N ha^{-1} as commercial urea.

4.4.4. Post-harvest soil analysis

Total C concentration of post-harvest soil was greater in BCU and BC amended pots compared to urea (Table 4.6). The C concentration of BCU granules was around two times higher than the urea granules. Therefore, addition of C rich BCU granules to soil was responsible for increased total C status in soil. BCU 1, with lower amount of C, had a lower concentration of total C in soil than the BCU 2 with the higher amount of total C. Moreover, increased root growth and microbial biomass in BCU amended soil during the study period resulted in a marginal increase of total C in soil. Many researchers have reported an increase in SOC content with the application of organic matter high in total C (Carey et al., 2009; Mitchell et al., 2007). The data presented in Figure 4.11 showed that there was significant gain in PMN in soil due to the use of BCU granules. However, such an increase in PMN level in soil indicated that the mineralisation and release of fertiliser-N was much slower in BCU granule amended soils. Granulation of BC with urea has been demonstrated to act as an enhanced efficiency fertiliser that can offer a balanced N release pattern to plants with immediate and slow release properties, providing soil organic matter for improving soil health and microbial activity.

4.5. Conclusions

Application of different BCU granules to silver beet in two contrasting soils showed differences in growth, biomass yield, N uptake, N use efficiency and a selection of measures of soil health. Blending and subsequent granulation of urea with BC substantially influenced the release and availability of fertiliser N in both the Tenosol and Dermosol soils. Compared to urea, BCU granules significantly decreased N losses via N_2O and NH_3 emissions and maintained significantly higher amounts of plant available N and mineralisable N in soil. As a result, greater amounts of fertiliser N were available to silver beet over a longer time period, increasing the N uptake and its use efficiency. Addition of N as BCU resulted in 27% and 23% more biomass yield in the Tenosol and Dermosol soils compared to commercial urea alone, respectively. In addition, incorporation of extra C as BCU granules will help to improve soil organic matter content in soil. The enhanced efficiency BCU granule may reduce the N application rate by 30-40% compared with urea and thereby minimise N losses and negative impact on the environment. The overall results indicate that brown coal-urea blends in appropriate proportions are effective in enhancing productivity, biomass yield, and N uptake by silver beet and fertility of soil in terms of nutrient perspectives. It appears that the efficacy of commercial urea can be improved substantially by granulation with humified organic matter like brown coal.

5. Chapter: A cost effective slow release nitrogen fertiliser for improving yield and nitrogen use efficiency of sweet corn

5.1. Introduction

Intensification of agricultural production all over the world has been increasing at a rapid pace to meet food demand for an increasing population. As a result, worldwide application of synthetic fertiliser, specifically N, has increased in the last few decades and is expected to continue in the foreseeable future (Zhang and Zhang, 2007). Although the application of chemical N fertilisers increased significantly, the recovery of this added N by crop plants is very poor, signifying that fertiliser-N is not utilised by plants and is lost to environment via different pathways (Raun et al., 2002). Urea is one of the most widely used N fertilisers to enhance growth and yield of crop plants due to its very high N content. The very poor use efficiency and low recovery of urea-N in soil-plant systems is associated with its losses by leaching, denitrification and volatilisation (Fageria and Baligar, 2005). The N lost through this process represents a serious economic loss and environmental burden, as leached N movement to water contaminates water bodies, whilst N_2O accumulation in the atmosphere causes destruction of the ozone layer and contributes to acid rain and acidification of the environment (Akiyama et al., 2010).

Considerable research efforts in recent years have been aimed at developing mitigation strategies to reduce gaseous and leaching losses of N from intensively managed agricultural systems (Galloway et al., 2004; Naz and Sulaiman, 2016). Several such approaches include the use of controlled-release N fertilisers, and urease and nitrification inhibitors (Chen et al., 2008; Davidson and Gu, 2012). Application of slow release fertilisers controls the release of fertiliser-N by matching with the crop demand for the whole growing season to enhance use efficiency and N uptake by crop plants (Malhi et al., 2010). The slow release behaviour of fertiliser also prevent fertiliser-N from being lost via leaching and gaseous emissions from the soil as well as minimise the cost of crop production by reducing the fertiliser application rate, labour and fuel costs associated with repeated applications of fertilisers (Akiyama et al., 2010; Simonne and Hutchinson, 2005).

There is an increasing interest in the application of organic materials (Das and Adhya, 2014; Hargreaves et al., 2008; Li et al., 2015), as they can contribute to climate change mitigation through C sequestration and supply organic matter to soil at the same time (Diacono and Montemurro, 2011). Efficient use of manure as a fertiliser is complicated by the imbalance of nutrients, variability in sources, difficulties in estimating nutrient availability, relatively low nutrient concentration and very high application rates which limit the distances manure can profitably be transported for use as a fertiliser (Albiach et al., 2001; Edmeades, 2002; Hargreaves et al., 2009; Quilty and Cattle, 2011). However, granulation of synthetic fertilisers with organic matter could enhance fertiliser use efficiency and concurrently return organic matter back into soil, restoring soil health and improving crop yield in a more sustainable manner (Ahmad et al., 2008).

Granulation of suitable organic materials with synthetic fertiliser to meet crop nutrient requirements has several benefits over the application of organic material or synthetic fertiliser as a sole nutrient source. Organo-mineral blending can reduce the application rate of organic matter and decrease nutrient release rate due to increased adsorption by organic matter which prolonged the availability of nutrients in the soil (Richards et al., 1993; Saha et al., 2017). Moreover, organic-synthetic fertiliser blend can contain a large amount of humic acid, amino acid, and enzymes, which can improve nutrient cycling and uptake by crop plants (Rao et al., 2007). Furthermore, granulation offers an opportunity to improve nutrient content and characteristics of organic matter, which facilitate precise land application with farm machinery and affordable transportation, thus creating scope for large scale commercial production and revenue source to producers.

Recently, there has been increasing trend towards using organo-mineral granules in agricultural crop production because of their potential benefits on nutrient cycling, crop yield and soil health. González et al. (2015) found that blending of urea with biochar decreased the release rate of urea-N in soil compared with commercial urea. Similarly, Mazeika et al. (2016) reported that addition of an organo-mineral blend formulated from poultry litter and ammonium chloride, significantly increased yields of potato and rapeseed compared to sole application of synthetic fertilisers. Furthermore, Rose et al. (2016) observed that fertilisation of soil with brown coal-urea (BCU) granules decreased N_2O emissions compared to straight urea treated soil. A recent study conducted by Saha et al. (2017) (Chapter 2) found that granulation of urea with BC significantly slowed down the fertiliser-N release by reducing urease activity and urea hydrolysis compared to urea alone. This study will evaluate the efficacy of BCU granules prepared using a pan granulation technology on the growth, yield, use efficiency and uptake of

N by sweet corn in field conditions compared to conventional urea. Sweet corn was tested in this trial because it is a very high value crop and responsive to N fertiliser.

5.2. Materials and methods

5.2.1. Soil and experimental site

The field trial was carried out at the research station of Department of Primary Industries (DPI), Wollongbar Research Institute (28°90′95″S and 153°41′54″E), NSW, Australia from November 2015 to April 2016. The soil is acidic with pH 4.7, with high concentrations of iron and is classified as a Ferrosol (Isbell, 2002). Initial soil samples were collected and analysed for a range of key physicochemical properties (Table 5.1). The daily rainfall, soil moisture and temperature data is presented in Appendix 2.

Table 5.1. Physical and chemical properties of soils

Property	
Australian soil order	Ferrosol
Texture	Clay loam
Bulk density (g cm^{-3})	1.31
pH (Water)	4.69
Carbon (%)	4.6
Nitrogen (%)	0.54
Ammonium nitrogen (mg kg^{-1})	13.1
Nitrate nitrogen (mg kg^{-1})	81.2
Phosphorus (Colwell) (mg kg^{-1})	39
Exchangeable calcium (cmol$_c$ kg^{-1})	7.5
Exchangeable magnesium (cmol$_c$ kg^{-1})	1.3
Exchangeable potassium (cmol$_c$ kg^{-1})	0.30
Extractable sulfur (mg kg^{-1})	79.5
DTPA-iron (mg kg^{-1})	394
Extractable aluminium (mg kg^{-1})	61

5.2.2. Plant growth experiment

The individual plot size was 34 m^2 (10 m×3.4 m). The field was divided into four blocks and each block was considered as a replicate. A 1 m buffer zone was maintained in between two blocks. Before seed sowing the soil was treated with Atradex® WG (Atrazine 900 g kg^{-1}) to minimise weed growth and Lorsban 500 EC (Chlorpyrifos 500 g L^{-1}) to control soil borne insect pests. Seeds were uniformly sown with a seed drill, maintaining a seed to seed distance of 0.2 m. To each plot, four rows of sweet corn (Hybrix 5 Elite) were planted, maintaining a row spacing of 0.8 m in between rows. Two BCU granules (BCU 1 having 20% N and BCU 2 having 10% N) and two rates of N (180 and 90 kg ha^{-1}) were evaluated in this field trial. The experiment was laid out following a completely randomised block design with four replicates. To each block, the following treatments were added:

Treatments:

T1: Control (No N added)
T2: Brown coal (No N added)
T3: Urea (N at a rate of 90 kg ha^{-1})
T4: Brown coal-urea 1 (BCU 1) (N at a rate of 90 kg ha^{-1})
T5: Brown coal-urea 2 (BCU 2) (N at a rate of 90 kg ha^{-1})
T6: Urea 1 (N at a rate of 180 kg ha^{-1})
T7: Brown coal-urea 1 (BCU 1) (N at a rate of 180 kg ha^{-1})
T8: Brown coal-urea 2 (BCU 2) (N at a rate of 180 kg ha^{-1})

Equal amounts of N were added to the soil, which involved applying varying amounts of BCU granules depending on their N concentration. In addition, P (single superphosphate) and K (potassium sulphate) were added at a rate of 40 and 60 kg ha^{-1}, respectively. The fertilisers were mixed with the top (0-10 cm) soil in granular form and uniformly covered the whole surface area of soil one day prior to seed sowing.

Figure 5.1. Experimental set up (left) and greenhouse gas (GHG) sampling (right) of sweet corn in the field

5.2.3. Gas sampling, soil sampling and plant harvesting

To each plot, two static gas chambers were installed to collect the greenhouse gas (GHG) and volatilised NH_3 samples (Figure 5.1). Twelve GHG samples were collected at one week intervals and similarly five extra GHG samplings were done in the following day after each heavy rainfall event to observe the effect of rainfall on GHG emissions. Headspace concentrations of GHG were measured at 0, 30 and 60 min after closing the static gas chambers during each measuring day. The GHG flux was calculated according to the method and equations detailed by van Zwieten et al. (2010). The cumulative GHG emissions during the sweet corn growing period were calculated by a linear integration of hourly fluxes. Ten NH_3 emission sampling periods were undertaken at the early growth stage of sweet corn. Ammonia emissions were measured using polyurethane foam absorbers and the flux was calculated using the equation of Singh et al. (2009).

$$\text{Ammonia flux} = \frac{\mathrm{C} \times \mathrm{V}}{a \times \mathrm{D}} \qquad (1)$$

Where, C is NH_3 concentration in the acid trap (mg dm^{-3}); V is the volume of acid (dm^3); *a* is total cross-section area (m^2) of soil column and D is duration (h) of each sampling.

From each plot, 10 soil samples were collected at 7, 14, 21, 28, 42, 56, 70 and 98 days after fertilisation to measure the N release pattern, enzyme and microbial activity in the soil. A destructive harvest from 1 m^2 area of each plot was undertaken at 42 days after sowing (DAS) of seed to observe the effect of BCU blends on the early growth of sweet corn (Figure 5.2). A destructive final harvest was undertaken at 98 days after seed sowing. The cobs were separated from the plants and counted. Shoot biomass was also quantified excluding cob biomass. Subsamples of 10 cobs and biomass of 10 plants from each plot were taken randomly to measure the dry cob and biomass yield and nutrient status of corn. The biomass and cob subsamples were oven-dried for 7 days at 80°C, following which biomass dry weight and cob dry weight was measured. The dried plant materials were then ground to a fine powder and nutrient concentrations were determined following standard methods, described in Section 5.2.4.

Figure 5.2. Growth of sweet corn after 42 DAS (left) and during flowering (right)

5.2.4. Plant analysis

For measuring the chlorophyll content leaf samples were collected from the third leaf from the top. Leaf chlorophyll content was measured at 42 days after fertiliser addition following the method of Arnon (1949). The biomass and cob N concentration was determined by combustion using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The biomass and cob N uptake was calculated following the equation described by Finzi et al. (2007). Nitrogen use efficiency (NUE) was calculated according to the formula of Moll et al. (1982).

5.2.5. Soil analysis

Soil pH was determined at a soil-to-water ratio of 1:5 (WP 80 Reference pH Meter, Anpros Phy LTD., Victoria, Australia). Mineral N was extracted from soils with 2 M KCl using a 1:2.5 soil: extractant ratio. Soil extracts were filtered through Advantech filter paper 42 prior to analysis for mineral N species by spectrophotometry using plate reader in microplate formats. Ammonium was quantified by reacting with salicylate and hyphochlorite in a buffered alkaline solution containing sodium nitroprusside as a reductant (Forster, 1995). Nitrate plus nitrite was determined by reduction using vanadium (III) combined with detection by acidic reaction (Miranda et al., 2001). Potentially mineralisable nitrogen (PMN) was assessed as described by Waring and Bremner (1964), modified by Stenberg et al. (1998). Total C and N in soil were measured using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The soils were finely ground using a mortar and pestle prior to C and N analysis. The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by a fumigation extraction method of Vance, with extraction of organic C and N by 0.5 M K_2SO_4 (1:4, soil : solution) and determination of the C and N content in the K_2SO_4 soil extracts with a liquid organic C and N analyser (Formacs Series, Combustion TOC/TN analyser).

5.2.6. Enzyme assays

The potential activities of enzymes involved in the cycling of C, N and P compounds in soil were assessed from the plots of each treatments in two analytical replicates: β-glucosidase, leucine aminopeptidase (Leu-AP) and phosphatase. The above mentioned enzyme activities were determined colorimetrically using a microplate fluorimeter following the methods of Marx et al. (2001). The total microbial activity in soil was monitored by measuring the fluorescein diacetate hydrolysis (FDA) according to the method of Schnurer and Rosswall (1982). The urease enzyme activity in soil was measured according to the method of Kandeler and Gerber (1988).

5.2.7. Statistical analysis

The statistical analyses were performed using statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). The normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Levene’s test. Some data (β-glucosidase, FDA) were not normally distributed and showed unequal variance. Those data were log or Ln transformed before performing the analysis of variance (ANOVA). A one-way ANOVA including factor fertiliser type (including the BC and control) were run. The individual block effect was considered during performing ANOVA. The multiple comparisons among the different treatments were done using a Tukey test. The Pearson’s linear correlation analyses were carried out between different parameters.

5.3. Results

5.3.1. Biomass yield, chlorophyll content and N uptake by sweet corn at 42 DAS

Addition of N fertilisers as BCU and urea significantly influenced the fresh and dry biomass yield, chlorophyll content, biomass N content and its uptake by sweet corn at 42 DAS (Figures 5.3-5.5). Greater amounts of fresh and dry biomass yield, chlorophyll content and biomass N content and its uptake was measured from the plots amended with 180 kg N ha^{-1} either as BCU or urea compared to plots fertilised with 90 kg N ha^{-1} and the plots where no N fertiliser was added. In contrast, significantly higher biomass N uptake was calculated from the plots fertilised with BCU 1 (180 kg N ha^{-1}) compared to the plots that received the equivalent amount of N as urea.

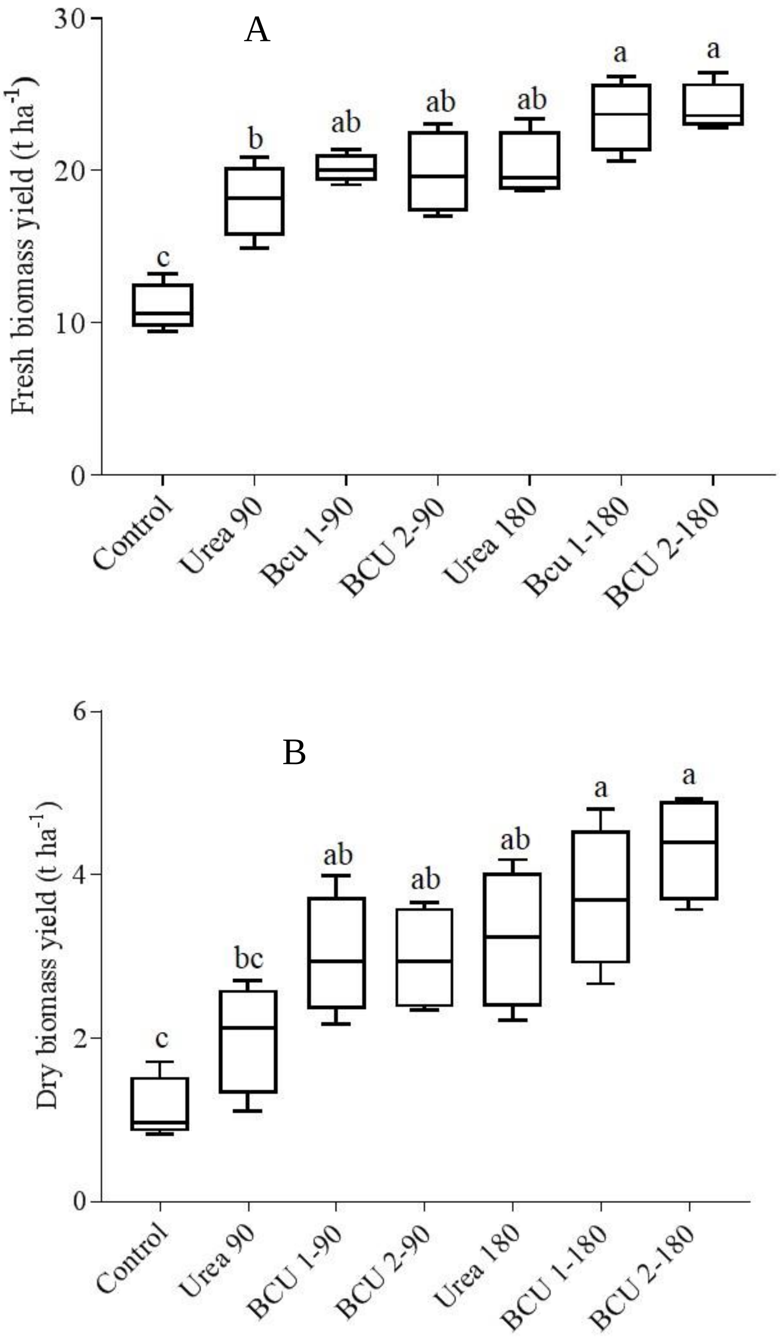


Figure 5.3. Effect of BCU granules and urea on the fresh biomass yield (A) and dry biomass yield (B) of sweet corn at 42 days after fertiliser addition. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

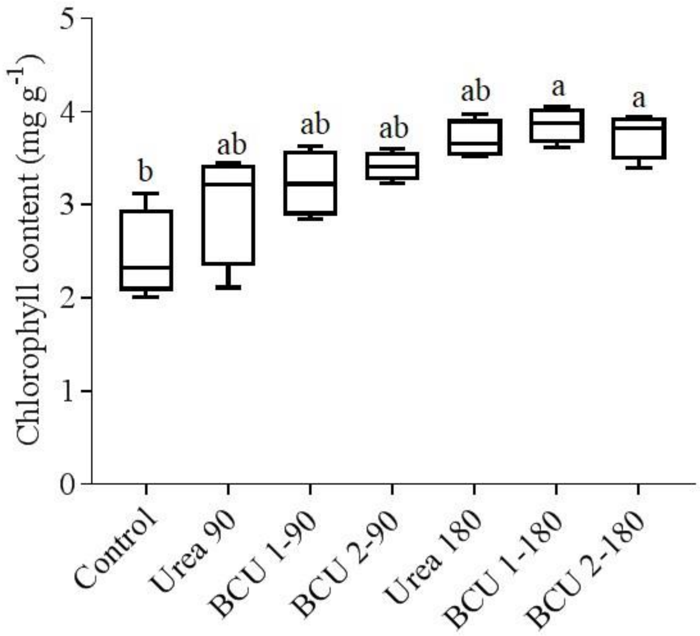


Figure 5.4. Effect of BCU granules and urea on the chlorophyll content of sweet corn at 42 days after fertiliser addition. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

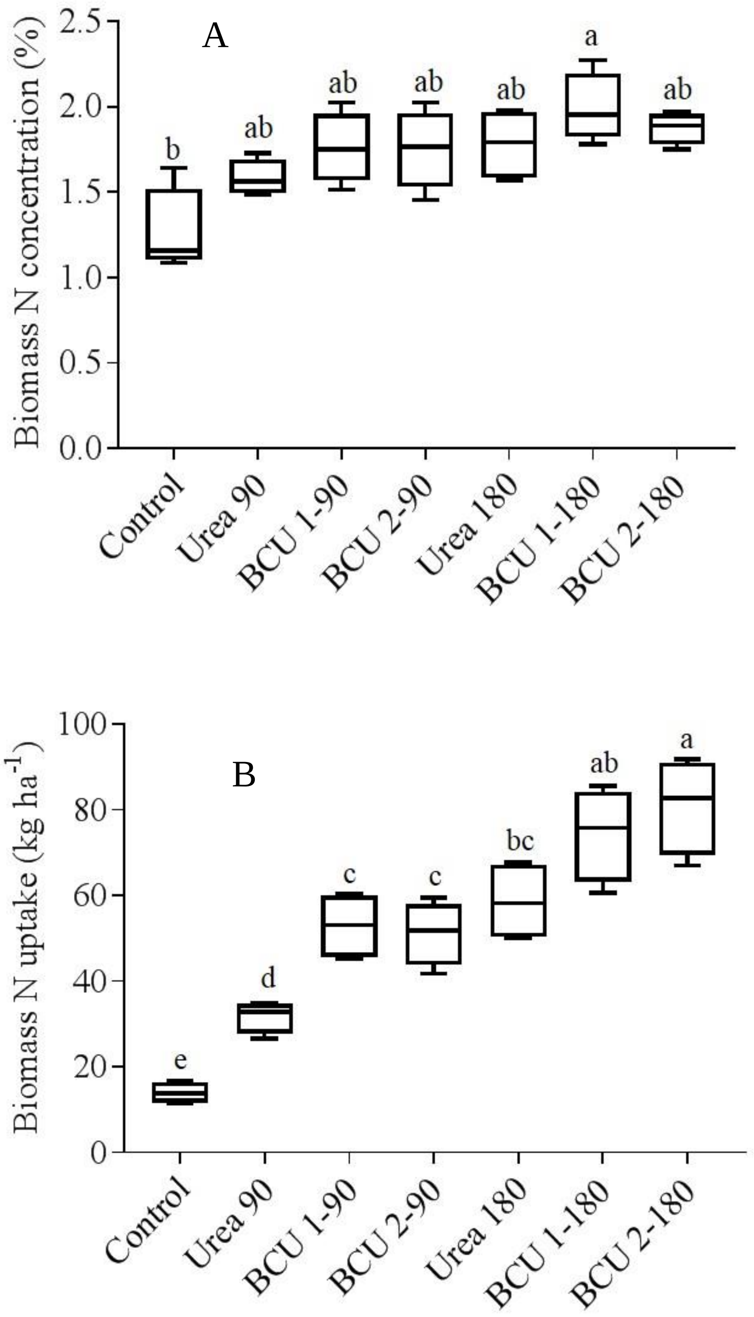


Figure 5.5. Effect of BCU granules and urea on the biomass N concentration (A) and biomass N uptake (B) by sweet corn at 42 days after fertiliser addition. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.2. Enzyme activity in soil

Urease activity: A sharp increase in urease enzyme activity was observed immediately after N fertiliser addition in all the treatments (Figure 5.6A). The urease enzyme activity peaked at 7 DAS, after which it decreased rapidly and remained almost same until the harvesting of sweet corn. The urease activity was substantially higher in urea amended soil up to 21 DAS compared to BCU and other treatments including the control. A slight increase in urease activity was found in BCU amended soils from 21 DAS to 56 DAS over urea fertilised soils. Enhanced urease activity was measured from the high N application rate compared to the low N application rate.

Leu-AP activity: The Leu-AP activity was very high at the early growth stage initially after N fertilisation and reached to its maximum value at 7 DAS in all the treatments (Figure 5.6B). The Leu-AP activity then fluctuated around the same level until 42 DAS followed by a sharp decrease. The Leu-AP activity was considerably higher in urea amended soil up to 14 DAS compared to BCU and other treatments including control. Subsequently, the Leu-AP activity increased slightly in the BCU amended soil over urea-amended soil until the harvesting of sweet corn. The Leu-AP was higher in high N fertilised soil than the low N treated soil.

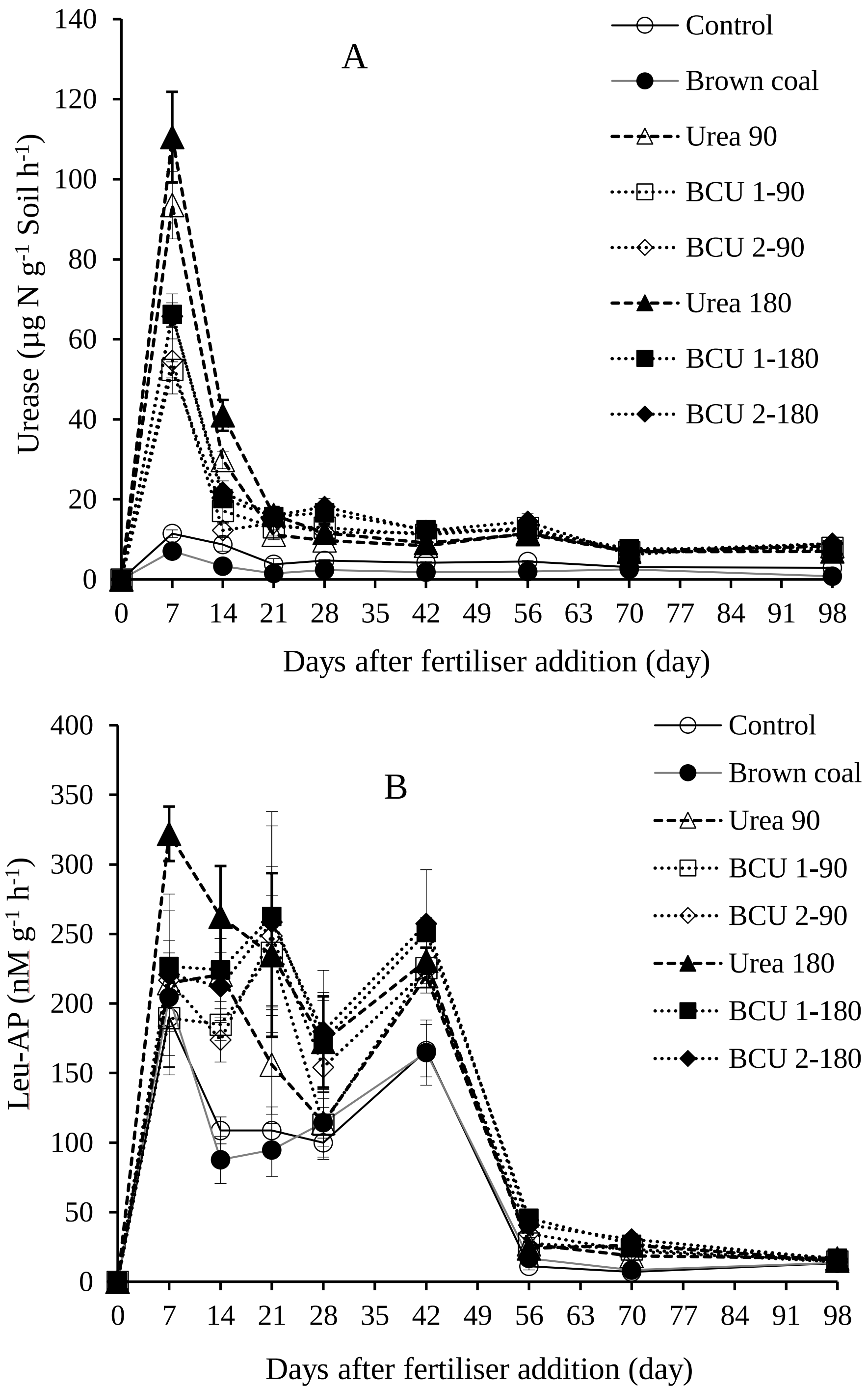


Figure 5.6. Effect of BCU granules and urea on the urease (A) and Leu-AP (B) enzyme activity in soil. Bars indicate the standard error among the replicates (n=4) in a box plot design.

Phosphatase activity: The phosphatase enzyme activity was very slow up to 7 DAS. After which, the phosphatase activity increased rapidly and peaked at 14 DAS in all the treatments (Figure 5.7A). No significant difference was observed between urea and BCU treated soil. However, the phosphatase enzyme activity was slightly higher in BCU amended soil than the urea amended soil. The phosphatase activity fluctuated, following a similar pattern until the end of the experimental period for all the treatments. No observable difference was found between high and low N amended soils.

β glucosidase: The β glucosidase activity also showed a similar pattern to the phosphatase activity. The β glucosidase activity was substantially higher in BCU amended soil compared to urea fertilised soil during the whole growing season of sweet corn (Figure 5.7.B). Considering different BCU applications, the β glucosidase activity was considerably higher in soil fertilised with higher C containing BCU compared to lower C containing BCU.

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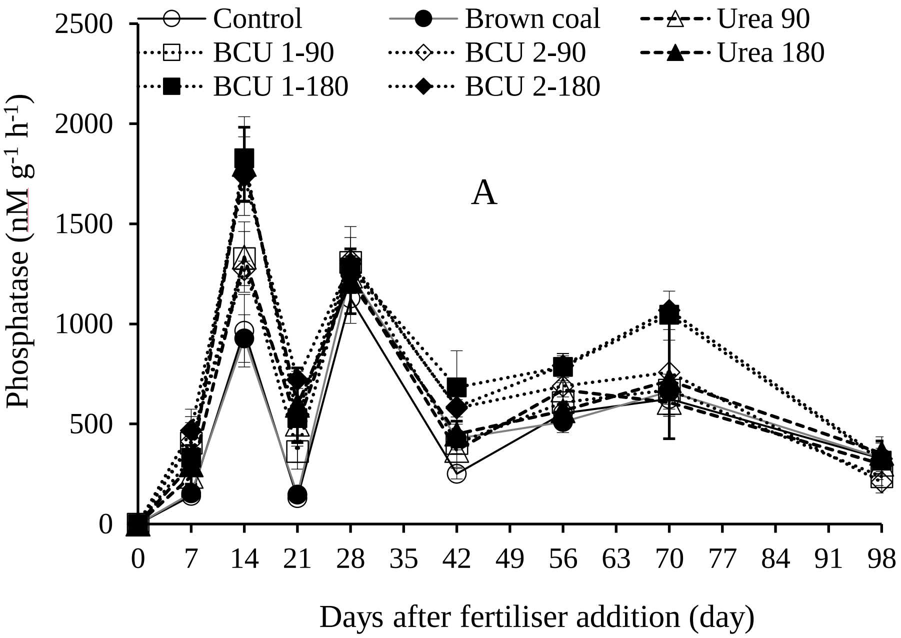


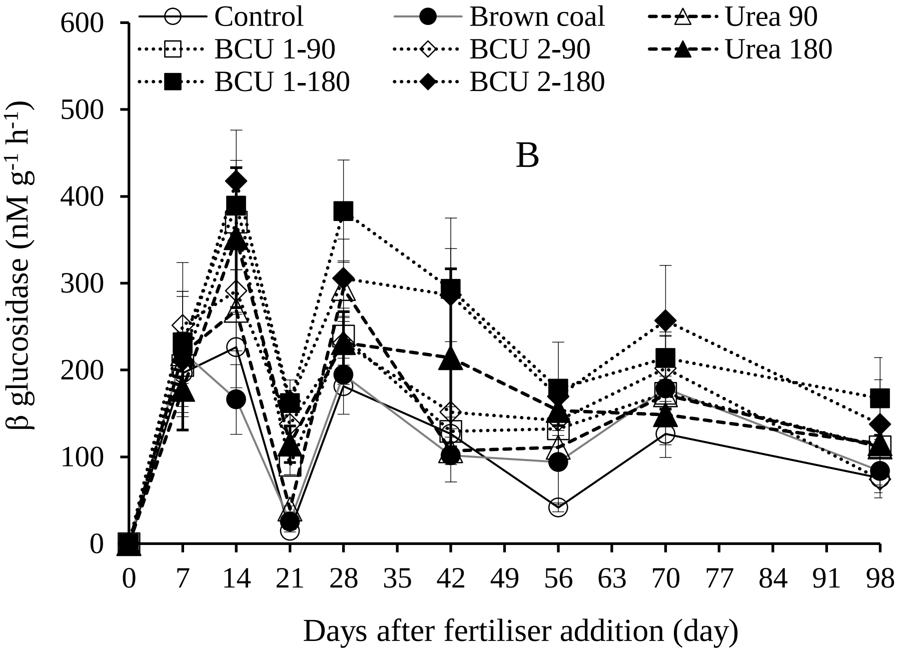


Figure 5.7. Effect of BCU granules and urea on the phosphatase (A) and β glucosidase (B) enzyme activity in soil. Bars indicate the standard error among the replicates (n=4) in a box plot design.

Fluorescein diacetate hydrolysis activity (FDA): A sharp increase in FDA activity was observed immediately after fertiliser addition (Figure 5.8). The FDA activity peaked at 7 DAS, after which it decreased rapidly at 14 DAS. Again a dramatic increase in FDA activity was monitored at 21 DAS and remained almost same until 42 DAS followed by sharp decrease in FDA activity at 56 DAS. After which the FDA activity decreased gradually until the harvesting of sweet corn. Overall, the FDA activity was slightly higher in BCU amended soil compared to urea and other treatments including control. A slight increase in FDA activity was monitored in urea amended soils from 28 DAS to 42 DAS over BCU fertilised soils.

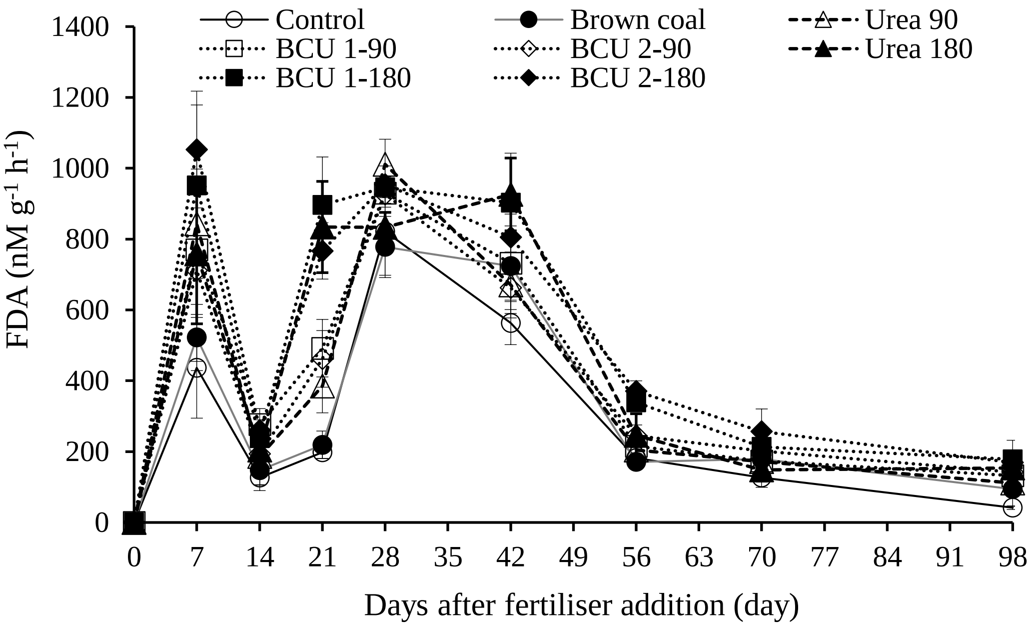


Figure 5.8. Effect of BCU granules and urea on the fluorescein diacetate hydrolysis (FDA) enzyme activity in soil. Bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.3. *Release of NH_4^+-N, NO_3^--N and PMN in soil*

Granulation of BC with urea showed a significant effect on the mineralisation and release of mineral (NH_4^+-N and NO_3^--N) and potentially mineralisable N in soil (Figures 5.9-5.10). The release of NH_4^+ and NO_3^- N was significantly higher in urea fertilised soil up to 21 DAS compared to BCU, after which it reduced dramatically. In contrast, the release of NH_4^+ and NO_3^- N was slightly higher in BCU amended soil over urea from 28 to 70 DAS. The N release pattern clearly indicates that granulation of BC with urea exhibited both immediate and slow release properties. On the other hand, no significant variation in PMN concentration was observed in urea and BCU amended soil at 7 DAS. Subsequently, a substantially higher amount of PMN was determined from the soil amended with BCU fertilised soil over urea for both high and low N application rates until the harvesting of the sweet corn. A significantly higher amount of PMN was measured from the soil treated with high N application rate. Among the BCU, the BCU granules having the higher amount of BC reduced the release rate of fertiliser-N and showed more PMN concentration compared to BCU granules having the lower proportion of BC.

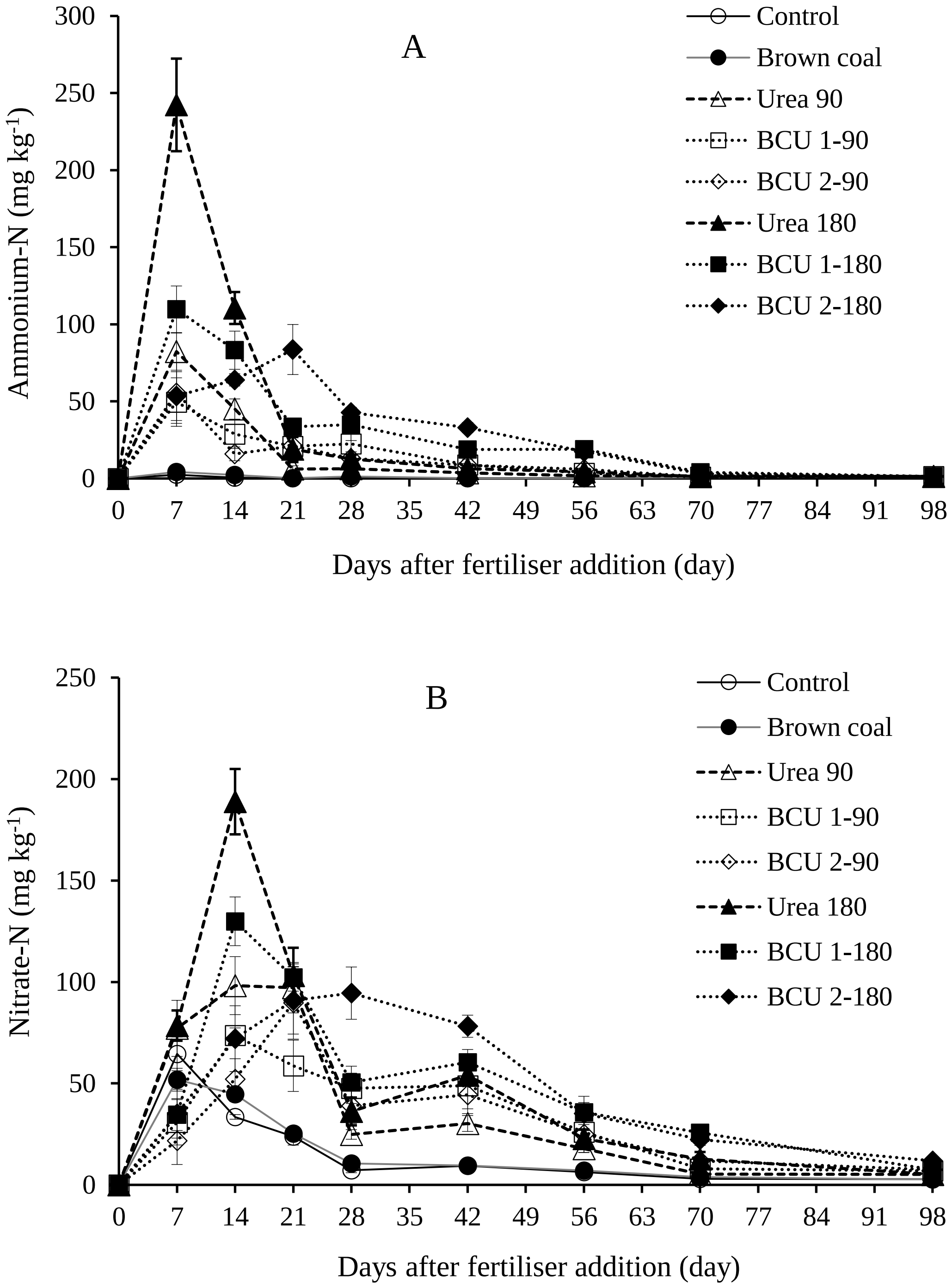


Figure 5.9. Effect of BCU granules and urea on the NH_4^+-N (A) and NO_3^--N (B) concentration of soil during the growing season of sweet corn. The error bars indicate the standard error among the replicates (n=4) in a box plot design.

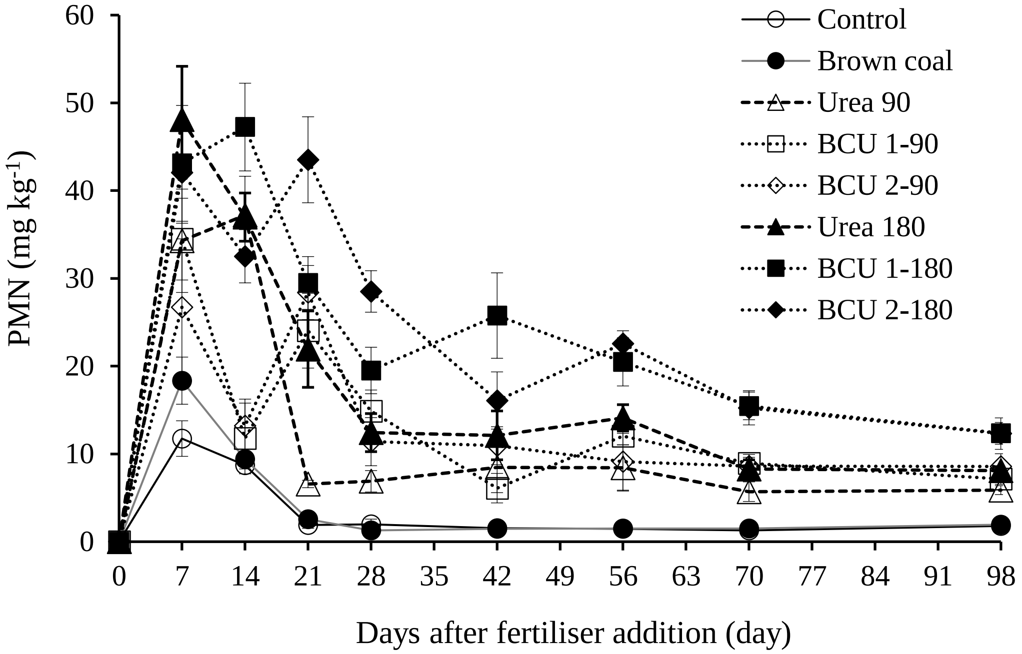


Figure 5.10. Effect of BCU granules and urea on the PMN concentration of soil during the growing season of sweet corn. The error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.4. Greenhouse gas emissions from soil

5.3.4.1 N_2O emissions from soil

Application of N fertilisers (either as straight urea or BCU granules) significantly increased the emissions of N_2O from soil compared to soil which received no N fertilisers (Figure 5.11A). A rapid increase in N_2O emissions were measured initially after N fertilisation followed by a sharp decline at 14 DAS. After which, a large increase in N_2O flux was monitored at 21 DAS and gradually the N_2O emissions peaked at 29 DAS followed by a heavy rainfall event at 28 DAS. The N_2O emissions then decreased dramatically and remained almost the same until the harvesting of the sweet corn. At the early growth stage, a large increase in N_2O emissions were measured after each heavy rainfall and daily N_2O emissions were substantially higher in soil receiving N as urea compared to soil receiving same amount of N as BCU.

The addition of BCU granules resulted in a significant decrease in cumulative N_2O emissions from both high and low N amended soil over urea (Figure 5.11B). Compared to urea, on average, addition of BCU granules suppressed the cumulative N_2O emissions by 59% from soil.

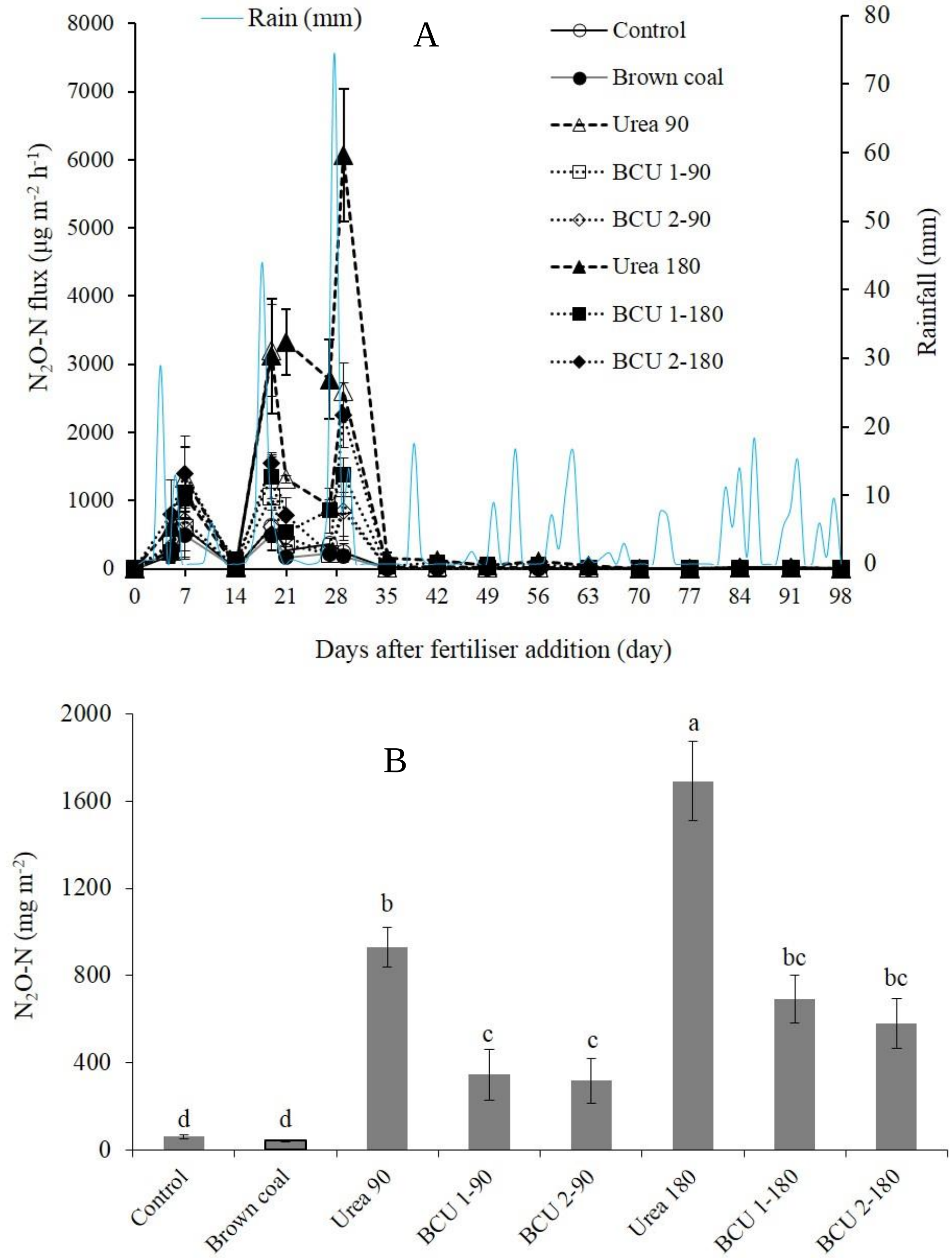


Figure 5.111. Effect of BCU granules and urea on the daily (A) and cumulative (B) N_2O-N emissions from soil during the growing season of sweet corn. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.5. NH_3 volatilisation from soil

Addition of N fertilisers either as urea or BCU triggered NH_3 volatilisation from soil compared to treatments where no N fertiliser was added. A sharp increase in NH_3 volatilisation was observed initially after N fertilisation and reached its maximum value at 3 DAS followed by a rapid decline at 14 DAS (Figure 5.12A). The NH_3 volatilisation then decreased gradually and remained almost constant until the last sampling. The daily NH_3 volatilisation was considerably higher in urea amended soil compared BCU fertilised soil.

The volatilisation loss of NH_3 from soil was significantly influenced by the application of different N fertilisers (Figure 5.12B). Addition of BCU granules significantly reduced the cumulative volatilisation loss of NH_3 from soil amended with 180 kg N ha^{-1} over commercial urea fertilised soil at the same rate. In contrast, no significant difference in NH_3 volatilisation was monitored among urea and BCU treated plots when N was added at the rate 90 kg ha^{-1}. The NH_3 emissions were significantly higher in the high N application rate than the low N application rate. The NH_3 volatilisation was slightly lower in BCU granules containing higher proportion of BC than the BCU granules containing lower proportions of BC. Compared to urea, on average, incorporation of BCU granules decreased the cumulative NH_3 volatilisation loss by 29%.

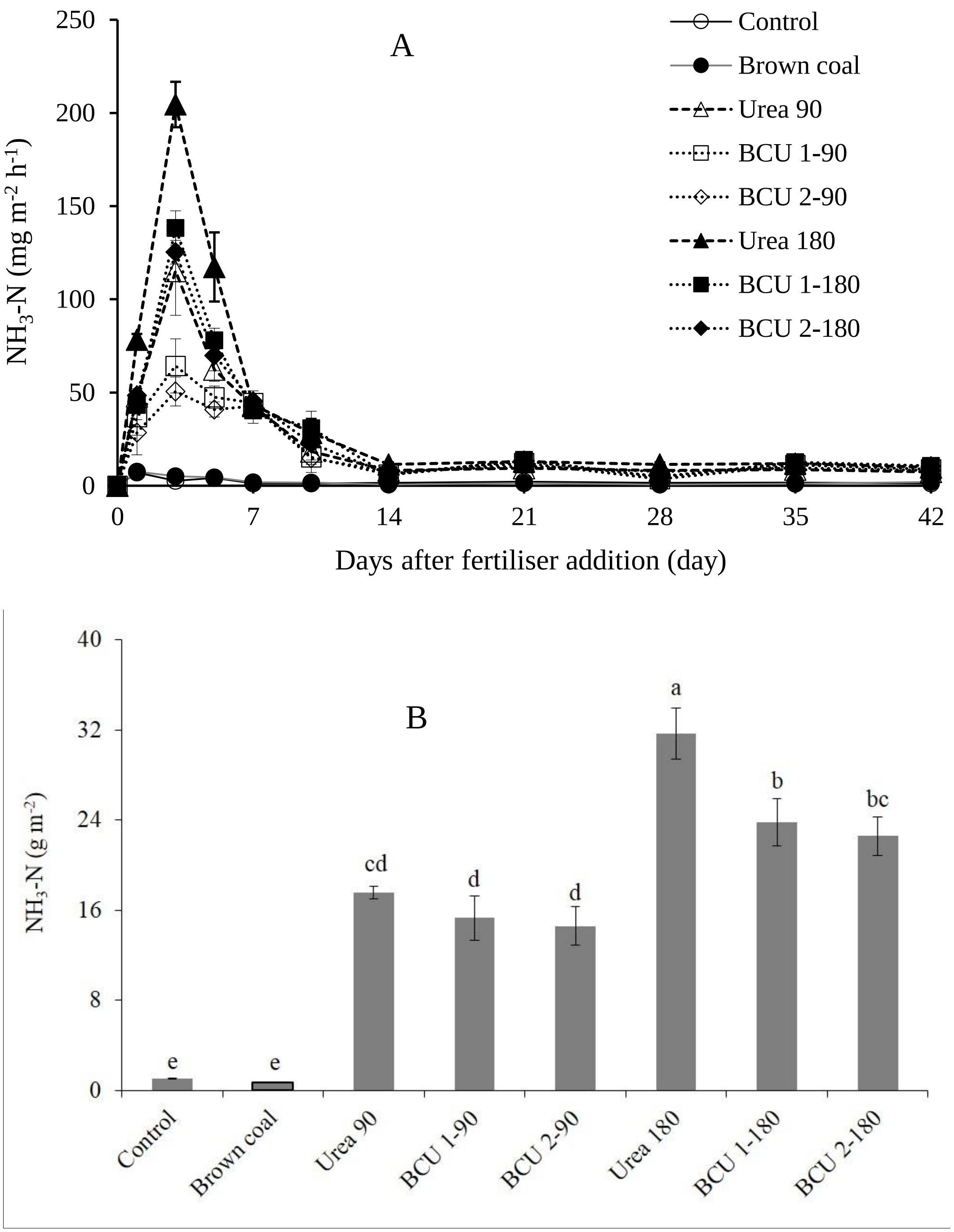


Figure 5.122. Effect of BCU granules and urea on the daily (A) and cumulative (B) NH_3-N volatilisation from soil during the growing season of sweet corn. Bars with different letters differ significantly according to Tukey-test at *p*<0.05 and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.6. Biomass and cob yield of sweet corn at harvest

Incorporation of different N fertilisers to soil significantly influenced the fresh and dry biomass yield of sweet corn (Figure 5.13). The biomass yield increased considerably with the increase in N application rate. Addition of N fertilisers either as urea or BCU showed a significant effect on the fresh and dry cob yield of sweet corn (Figure 5.14). A significantly higher amount of cob yield was obtained from the plots receiving 180 kg N ha^{-1} as BCU 1 compared to the plots receiving equivalent amount of N as urea. A similar trend was also observed in case of the low N application rate. No significant difference in cob yield was measured between urea and BCU 2 for both low and high N application rate. Interestingly, statistically similar amounts of cob yield was obtained from the plots fertilised with 90 kg N ha^{-1} as BCU 1 and the plots receiving 180 kg N ha^{-1} as urea. Compared to urea, on average addition of BCU granules to soil increased cob yield of sweet corn by 17%.

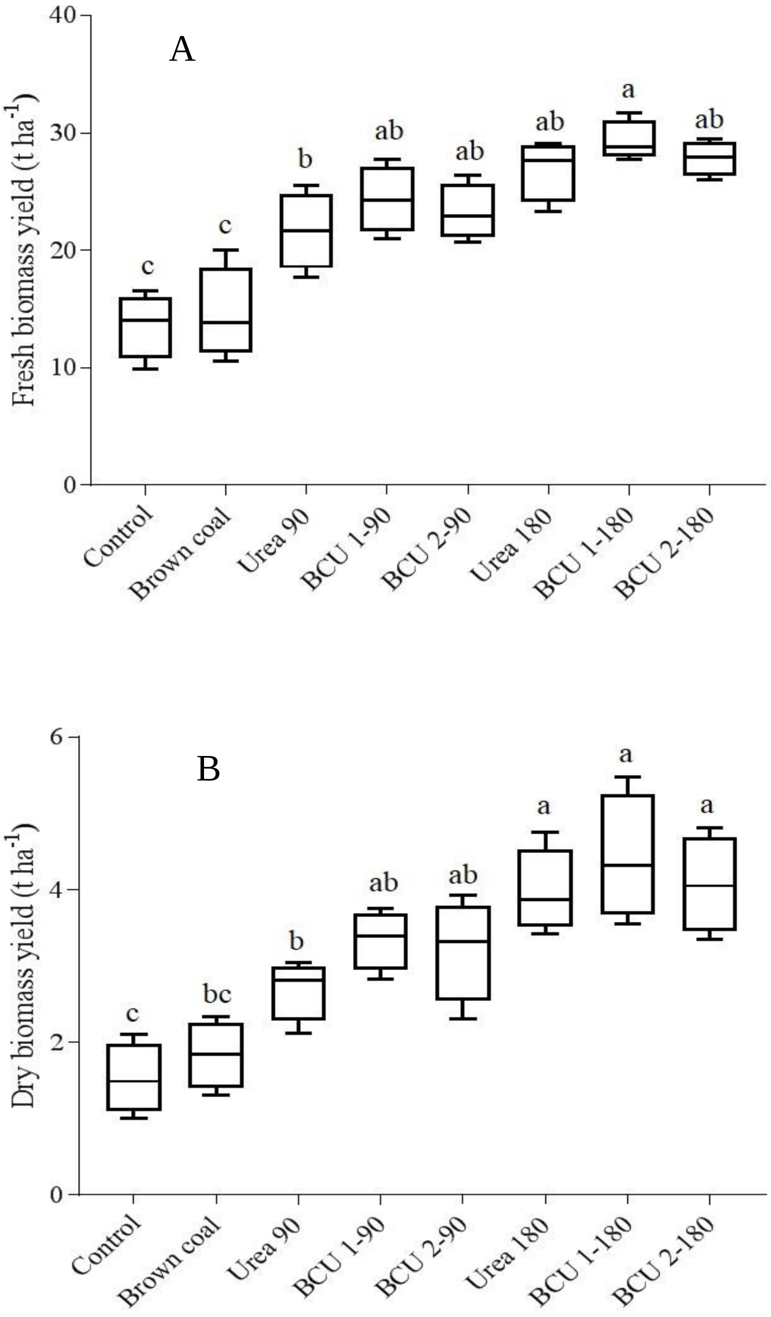


Figure 5.133. Effect of BCU granules and urea on the fresh (A) and dry (B) biomass yield of sweet corn at harvest. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

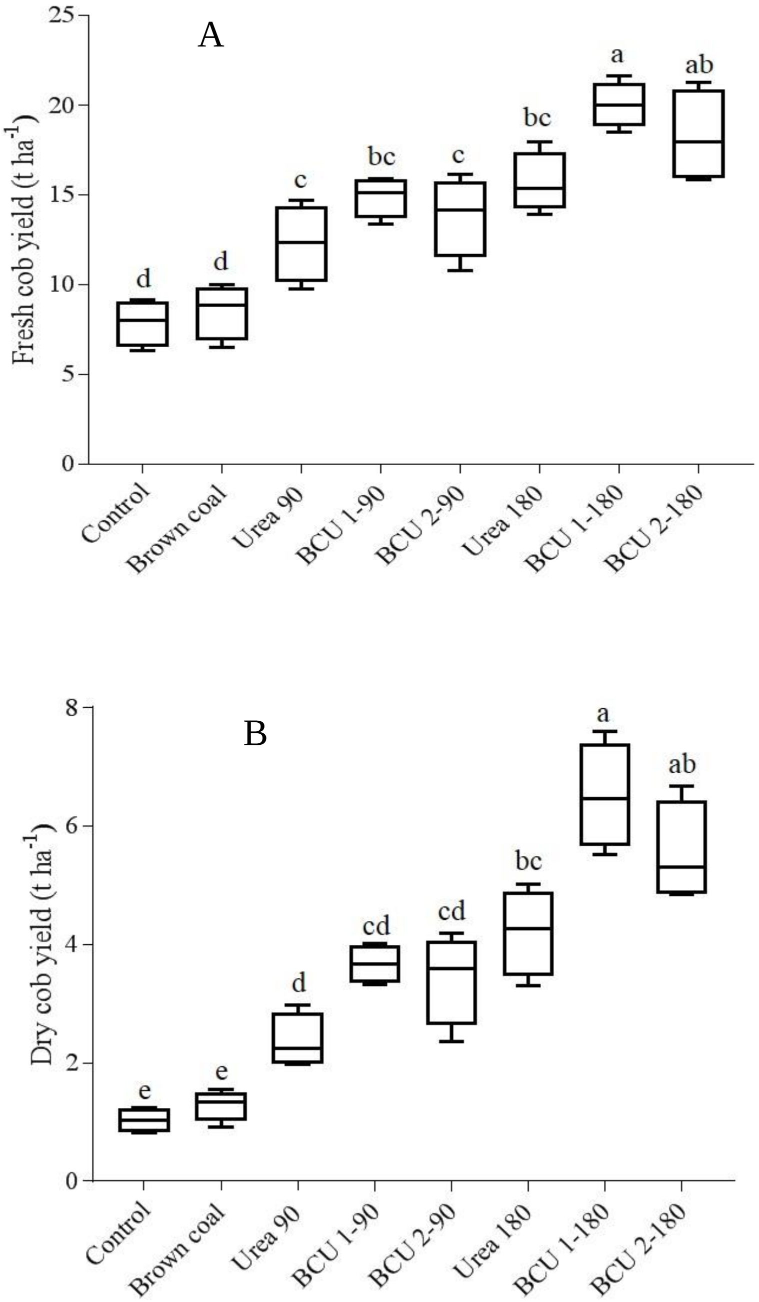


Figure 5.144. Effect of BCU granules and urea on the fresh (A) and dry (B) cob yield of sweet corn at harvest. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.7. Biomass and cob N concentration of sweet corn at harvest

Significant difference was observed in the biomass and cob N concentration of sweet corn among the various treatments (Figure 5.15). The biomass and cob N concentration was significantly higher in plots amended with N fertilisers either as urea or as BCU compared to the plots where no N fertiliser was added. No significant difference was measured in the biomass N concentration among the urea and BCU amended plots for both the low and high N application rate. Nevertheless, the cob N concentration was significantly higher in plants receiving 180 kg N ha^{-1} as BCU 1 over the plants receiving equivalent amount of N as urea. The increased application of N fertiliser enhanced the cob N concentration of sweet corn. No statistical difference was observed in the cob N concentration among the plants receiving 90 kg N ha^{-1} as BCU and the plants receiving 180 kg N ha^{-1} as urea.

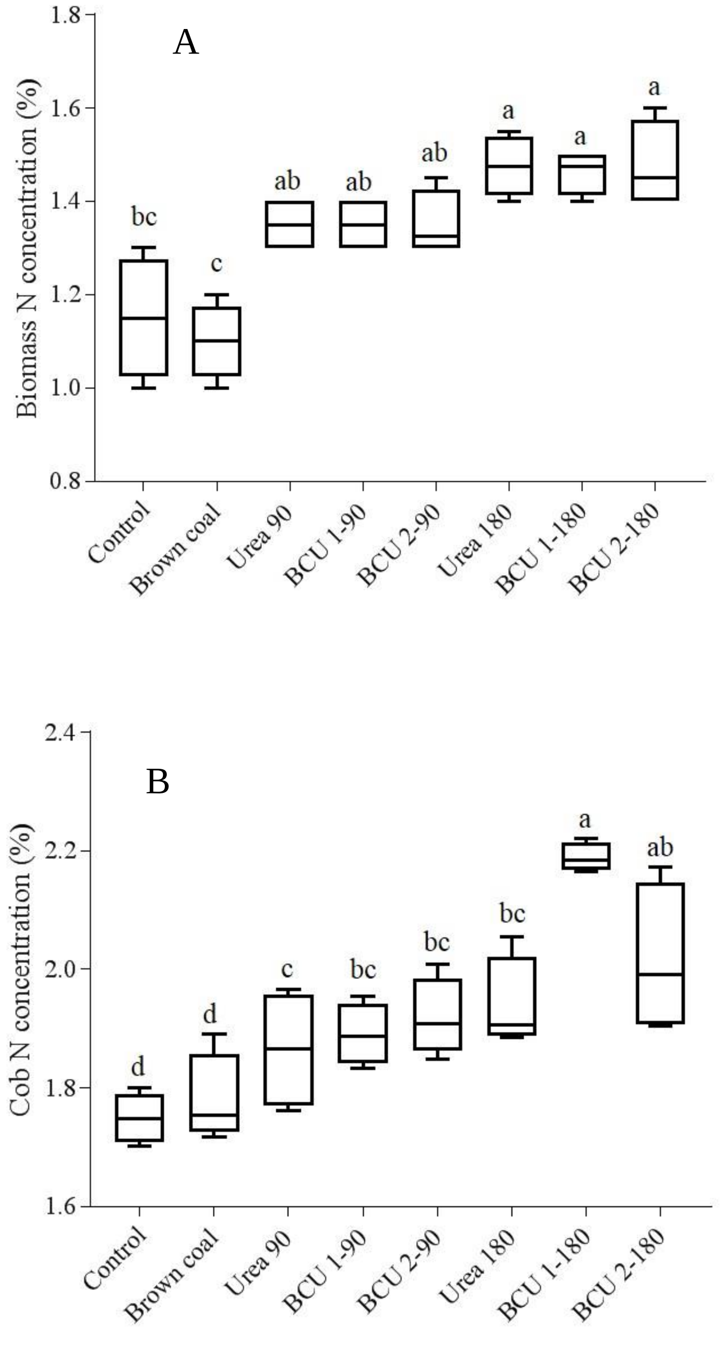


Figure 5.155. Effect of BCU granules and urea on the biomass (A) and cob (B) N concentration of sweet corn at harvest. Bars with different letters differ significantly according to Tukey-test at *p*<0.05 and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.8. Biomass and cob N uptake by sweet corn at harvest

Addition of urea and BCU significantly influenced the biomass and cob N uptake by sweet corn (Figure 5.16). Significantly higher amounts of biomass and cob N uptake were calculated from the plants fertilised with N fertilisers compared to plants receiving no N fertilisers. No significant difference was observed in biomass N uptake between urea and BCU amended plants. In contrast, significantly higher amount cob N uptake was measured from the plants fertilised with 180 kg N ha^{-1} as BCU 1 compared to plants receiving equivalent amount of N as urea. The biomass and cob N uptake was substantially higher at high N application rate than the low N application rate.

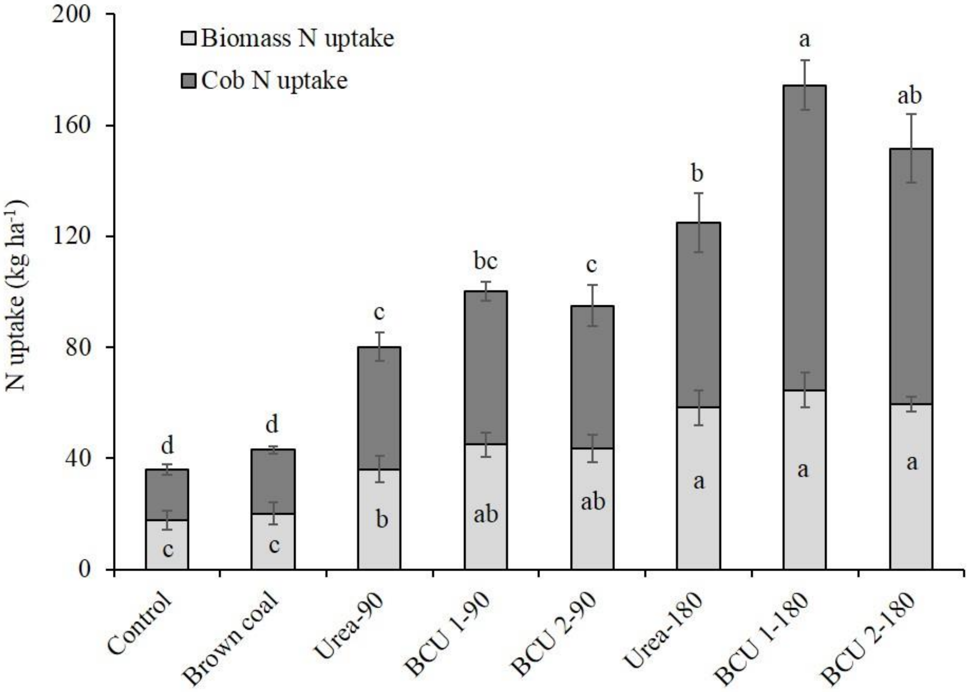


Figure 5.166. Effect of BCU granules and urea on the N uptake by sweet corn at harvest. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.9. Microbial biomass C and N content of soil at harvest

The microbial biomass C and N content of soil was significantly altered due to addition of N fertilisers either as urea or BCU (Figure 5.17). Significantly higher amounts of microbial biomass C were determined from the soil amended with BCU compared to urea for both low and high N application rates. On the other hand, the microbial biomass N was significantly higher in BCU fertilised soil compared to urea only in the high N application rate whereas in the low N application rate, the biomass N content did not differed significantly between urea and BCU. Considering the different BCU granules, the granules having a higher proportion of BC showed greater amounts of microbial biomass C and N with respect to the granules containing the lower amount of BC.

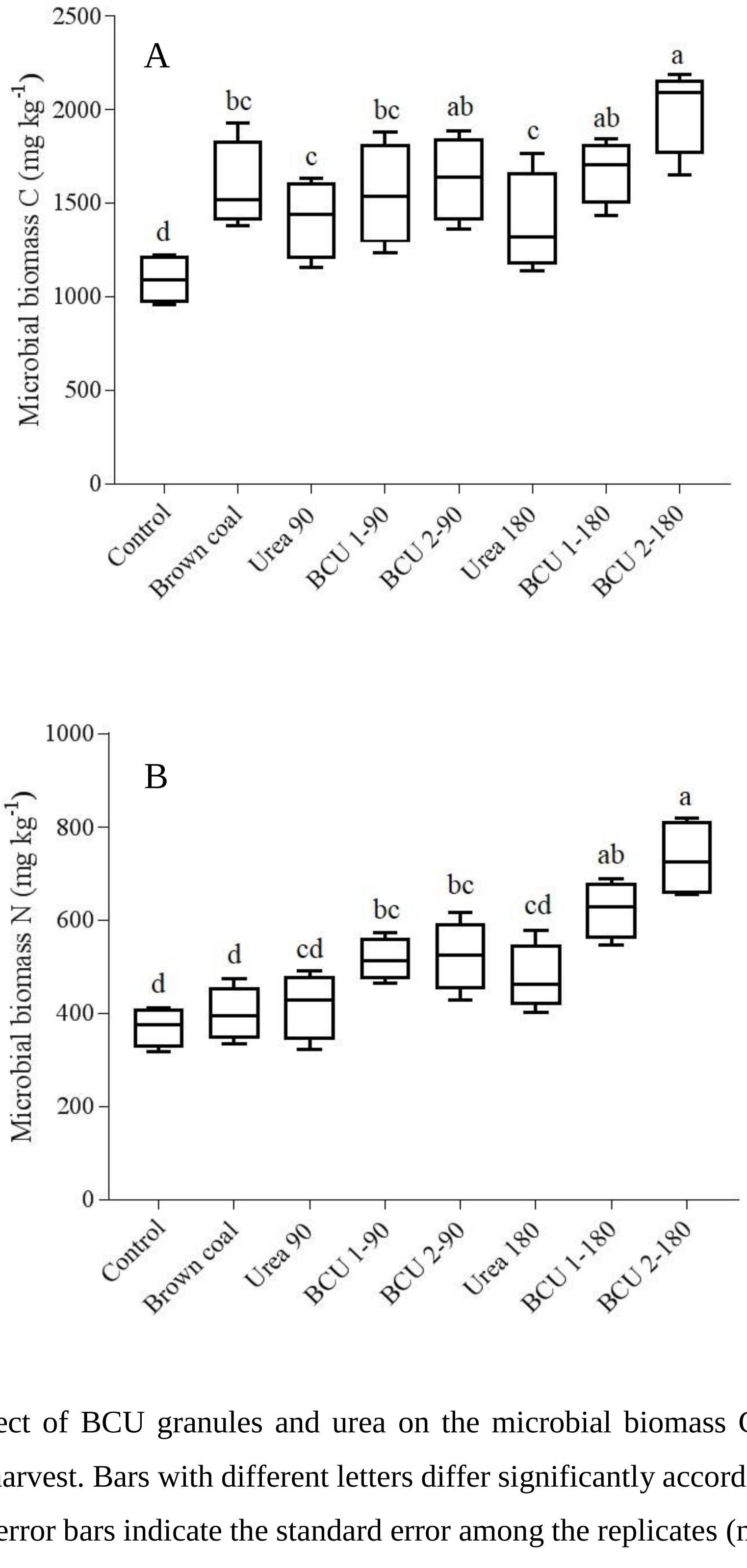
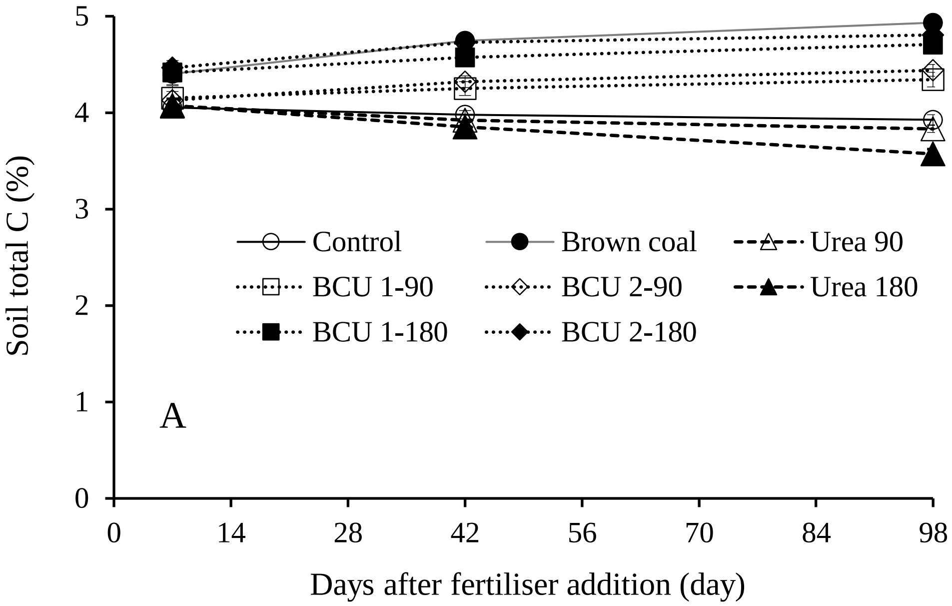


Figure 5.177. Effect of BCU granules and urea on the microbial biomass C (A) and N (B) content of soil at harvest. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.10. Total C and N concentration of soil

Incorporation of BCU and urea significantly influenced the total C and N concentration of soil (Figure 5.18). Significantly higher amounts of total C were determined from the BC and BCU amended soils over urea and control treatments at 42 and 98 DAS. In the case of BC and BCU treatments, the total C concentration increased gradually during the growing period of sweet corn while an opposite trend was observed in urea and control treatments. On the other hand, total N concentration decreased gradually during the growing period of sweet corn in all the treatments. No significant variation was monitored in total N concentration among the urea and BCU treatments in all sampling events. The BCU granules containing the higher proportion of BC showed a greater amount of total C and N concentration in soil with respect to the granules containing a lower proportion of BC.



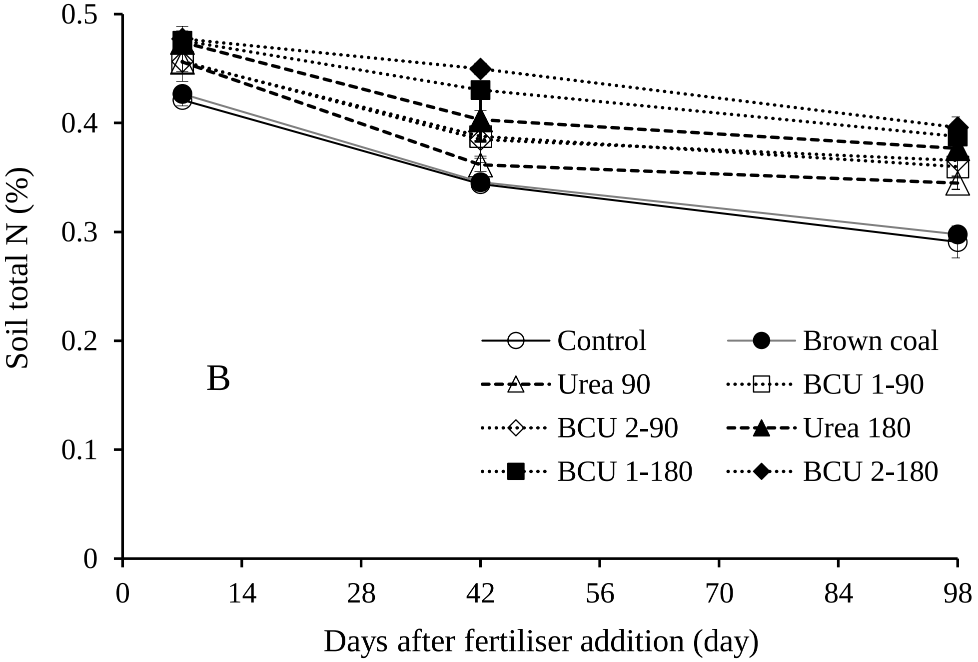


Figure 5.1818. Effect of BCU granules and urea on the total C (A) and total N (B) concentration of soil at harvest. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.11. Changes in soil pH

Addition of N fertilisers either as urea or BCU significantly influenced the soil pH at different sampling events (Figure 5.19). A significant and sharp increase in soil pH was measured from the urea fertilised plots compared to all other treatments including control at 7 and 14 DAS. A slight increase in soil pH also monitored in BCU amended soils at 14 DAS. After which no significant difference was found among the various treatments from 21 to 98 DAS. However, the soil pH was slightly higher in BCU amended soil than the urea fertilised soil from 28 to 70 DAS.

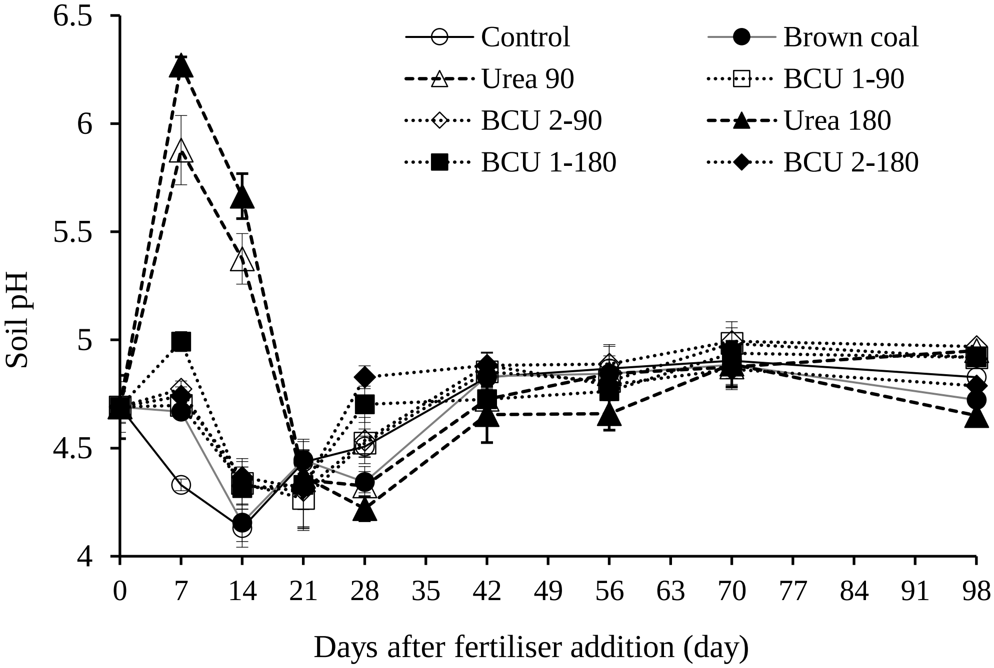


Figure 5.199. Effect of BCU granules and urea on the soil pH during the growing season of sweet corn. Bars with different letters differ significantly according to Tukey-test at *p*<0.05 and the error bars indicate the standard error among the replicates (n=4) in a box plot design.

5.3.12. Nitrogen use efficiency of sweet corn

The N use efficiency of sweet corn varied significantly due to application of different N fertilisers (Table 5.2). The N use efficiency decreased significantly with the increase in N application rate from 90 to 180 kg N ha^{-1} in both the urea and BCU amended soil. The N use efficiency was significantly higher in BCU 1 fertilised soil compared to urea fertilised soil for both low and high N application rate. Compared to urea, on an average addition of BCU increased the N use efficiency by 34 and 26% over urea for low and high N application rate, respectively.

Table 5.2. Effect of BCU granules and urea on the NUE of sweet corn

Treatments	**Nitrogen use efficiency (NUE)**
Urea-90	56.2 ± 0.89 cd
BCU 1-90	77.9 ± 1.19 a
BCU 2-90	73.9 ± 3.11 ab
Urea-180	45.4 ± 2.96 d
BCU 1-180	60.6 ± 2.72 bc
BCU 2-180	53.5 ± 2.51 cd

The values with in a response variables are significantly different according to Tukey-test at *P*<0.001.

5.3.13. Correlation studies among key soil properties

Significant and positive linear correlations were observed among the different parameters studied at various sampling events. Soil pH was positively correlated with NH_4^+-N content, NO_3^--N content, NH_3 volatilisation, leucine amino peptidase (Leu-AP) and urease enzyme activity. Soil pH was significantly correlated with NH_4^+-N content of soil up to 42 DAS (Table 5.3). On the other hand, soil pH was significantly correlated with NO_3^--N content only in 7, 14 and 42 DAS (Table 5.4). Similarly, soil pH was significantly correlated with NH_3 volatilisation and Leu-AP at the early growth stage up to 21 DAS (Tables 5.5-5.6). Highly significant correlation was observed between soil pH and urease activity in all most all the sampling events except 42, 56 and 70 DAS (Table 5.7).

Table 5.3. Pearson's r values of correlations (n = 8) between pH and NH_4^+-N content of soil

Correlation matrix		NH_4^+-N content of soil							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Soil pH	D 7	0.788**							
	D 14		0.601**						
	D 21			0.468**					
	D 28				0.527**				
	D 42					0.474**			
	D 56						0.112		
	D 70							0.150	
	D 98								0.221

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.4. Pearson’s r values of correlations (n = 8) between pH and NO_3^--N content of soil

Correlation matrix		NO_3^--N content of soil							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Soil pH	D 7	0.445^{*}							
	D 14		0.681^{**}						
	D 21			0.152					
	D 28				0.357^{*}				
	D 42					0.049			
	D 56						0.168		
	D 70							0.122	
	D 98								0.003

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.5. Pearson’s r values of correlations (n = 8) between pH and NH_3 volatilisation of soil

Correlation matrix		NH_3 volatilisation							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Soil pH	D 7	0.664^{**}							
	D 14		0.676^{**}						
	D 21			0.263^{*}					
	D 28				0.118				
	D 42					0.078			
	D 56						ND		
	D 70							ND	
	D 98								ND

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01; ND = Not determined.

Table 5.6. Pearson's r values of correlations (n = 8) between soil pH and leucine aminopeptidase enzyme activity

Correlation matrix		Leucine aminopeptidase enzyme activity							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Soil pH	D 7	0.413*							
	D 14		0.515**						
	D 21			0.323*					
	D 28				0.191				
	D 42					0.011			
	D 56						0.096		
	D 70							0.201	
	D 98								0.145

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.7. Pearson's r values of correlations (n = 8) between soil pH and urease enzyme activity

Correlation matrix		Urease enzyme activity							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Soil pH	D 7	0.771**							
	D 14		0.705**						
	D 21			0.435*					
	D 28				0.460**				
	D 42					0.085			
	D 56						0.174		
	D 70							0.111	
	D 98								0.345*

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Like soil pH, urease enzyme activity was also positively correlated with NH_4^+-N, NO_3^--N, NH_3 volatilisation and Leu-AP activity of soil. Significantly positive correlations were calculated between urease activity with NH_4^+-N content and NH_3 volatilisation in the all the sampling events (Tables 5.8-5.9) whereas with NO_3^--N the correlations were significant in almost all the sampling events except 7 and 21 DAS (Table 5.10).

Table 5.8. Pearson's r values of correlations (n = 8) between urease enzyme activity and NH_4^+-N content of soil

Correlation matrix		NH_4^+-N content							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Urease enzyme activity	D 7	0.766**							
	D 14		0.684**						
	D 21			0.606**					
	D 28				0.698**				
	D 42					0.688**			
	D 56						0.504**		
	D 70							0.363*	
	D 98								0.315*

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.9. Pearson’s r values of correlations (n = 8) between urease enzyme activity and NH_3 volatilisation from soil

Correlation matrix		NH_3 volatilisation							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Urease enzyme activity	D 7	0.732**							
	D 14		0.646**						
	D 21			0.830**					
	D 28				0.755**				
	D 42					0.697**			
	D 56						ND		
	D 70							ND	
	D 98								ND

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01; ND = Not determined.

Table 5.10. Pearson’s r values of correlations (n = 8) between urease enzyme activity and NO_3^--N content of soil

Correlation matrix		NO_3^--N content							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Urease enzyme activity	D 7	0.188							
	D 14		0.654**						
	D 21			0.187					
	D 28				0.645**				
	D 42					0.657**			
	D 56						0.718**		
	D 70							0.395*	
	D 98								0.549**

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Similarly, Leu-AP enzyme activity was also significantly and positively correlated with NH_4^+-N, NO_3^--N and NH_3 volatilisation of soil in most of sampling events (Tables 5.11-5.13).

Like other parameters cob yield was also significantly correlated (r =0.966^{**}) with N uptake by sweet corn.

Table 5.11. Pearson's r values of correlations (n = 8) between leucine aminopeptidase activity and NH_4^+-N content of soil

Correlation matrix		NH_4^+-N content							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Leucine aminopeptidase enzyme activity	D 7	0.374^{*}							
	D 14		0.627^{**}						
	D 21			0.444^{*}					
	D 28				0.592^{**}				
	D 42					0.743^{**}			
	D 56						0.416^{*}		
	D 70							0.149	
	D 98								0.102

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.12. Pearson’s r values of correlations (n = 8) between leucine aminopeptidase activity and NO_3^- content of soil

Correlation matrix		NO_3^--N content							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Leucine aminopeptidase enzyme activity	D 7	0.196							
	D 14		0.591^{**}						
	D 21			0.130					
	D 28				0.169				
	D 42					0.754^{**}			
	D 56						0.414^{*}		
	D 70							0.325^{*}	
	D 98								0.745^{**}

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01.

Table 5.13. Pearson’s r values of correlations (n = 8) between leucine aminopeptidase activity and NH_3 volatilisation from soil

Correlation matrix		NH_3 volatilisation							
		D 7	D 14	D 21	D 28	D 42	D 56	D 70	D 98
Leucine aminopeptidase enzyme activity	D 7	0.776^{**}							
	D 14		0.702^{**}						
	D 21			0.644^{**}					
	D 28				0.619^{**}				
	D 42					0.697^{**}			
	D 56						ND		
	D 70							ND	
	D 98								ND

*Correlation is significant at *P* = 0.05; **Correlation is significant at *P* = 0.01; ND = Not determined.

5.4. Discussion

5.4.1. Growth, biomass yield and N uptake by sweet corn at 42 DAS

Addition of N fertilisers did not show any significant difference in the growth, biomass yield, chlorophyll content and biomass N uptake among the BCU and urea treatments for both high and low N application rates at the early growth stage of sweet corn (Figures 5.3-5.5). The higher availability of mineral N at the early growth stage could be the reason for the observed results in our study. Similar findings were also reported by Rose et al. (2016) who found that the application of BCU granules produced by a steam drying process did not show any significant difference with urea on the growth and biomass yield of wheat in a six week glasshouse study. A recent study conducted by Saha et al. (2017) reported that the addition of BCU failed to produce any significant difference in the dry matter yield of canola at the flowering stage with respect to commercial urea.

5.4.2. Enzyme activity in soil

Application of N fertilisers either as urea or BCU had a considerable influence on the important C, N and P cycling enzyme activities in soil (Figures 5.6-5.8). A substantial decrease in urease and Leu-AP activity was determined from the soil amended with BCU compared to urea alone fertilised soil in the early growth stage of sweet corn. In contrast, an opposite trend was observed in phosphatase, β glucosidase and FDA activity. Urease and Leu-AP are two important key enzymes controlling the hydrolysis and mineralisation of urea in soil, respectively. It is evident from the findings of Bremner and Douglas (1971) that addition of compounds high in phenolic groups have a strong capacity to inhibit N mineralisation and urease activity in soil. The presence of a large number of phenolic groups in the BC matrix might be the reason for reduced urease and Leu-AP activity in BCU amended soil. A similar finding was also reported by Saha et al. (2017) who reported that the addition of BCU significantly reduced the urease enzyme activity in soil compared to urea alone. FDA is one of the widely accepted methods for measuring total microbial activity in soil (Nayak et al., 2007). The increased FDA, phosphatase and β glucosidase enzyme activities in BCU amended soil might be due to the addition of highly humified organic matter as BC. The high organic C and humic acid content of BC may trigger the C and P cycling enzyme activity by adding substrate for microbial colonisation. The results of our study in accordance with the findings of Das and Adhya (2014) who found that combined application of urea and compost significantly increased the microbial activity and FDA activity in soil compared to sole application of urea.

5.4.3. Release of mineral and mineralisable N in soil

Blending and subsequent granulation of urea with BC showed a significant influence on the release of mineral and mineralisable N in soil during the growing period of sweet corn. The release of mineral N was substantially higher in urea fertilised soil at the early growth stage of sweet corn up to 21 DAS followed by a very sharp decrease (Figures 5.8-5.9). In contrast, the release of mineral N was slower in BCU amended soil than urea amended soil at the early growth stage, increased gradually over urea and maintained almost a constant release rate until the harvesting of sweet corn. The mineralisable N concentration was considerably higher in BCU amended soil over urea during the whole growing season. The mineralisation and release pattern of N indicated that granulation of urea with BC reduced the release rate of urea-N and acted as slow release N fertiliser. The slow release of N from BCU ultimately increased availability mineral N throughout the growing season of the sweet corn. The release of mineral N was significantly and positively correlated with urease and Leu-AP activity in soil (Tables 5.8-5.12). Urease is the major enzyme controlling the hydrolysis of urea to NH_4^+ and Leu-AP is another important enzyme responsible for mineralisation of N in soil. The reduced activity of both urease and Leu-AP in soil amended with BCU might be the reason for slow release of mineral N compared to urea. Aside from the potential for direct inhibition of these enzymes by the BC, the strong and prolonged retention of urea-N by BC matrix through physical adsorption or chemical complexing on to the porous and extensive surface area of BC could also limit the substrate availability and hence activities of the enzymes. The results of our study are in good agreement with previous findings reported in Saha et al. (2017) who also found that granulation of BC with urea reduces the release rate of urea-N compared to commercial urea in a water dissolution study. Similarly, Rose et al. (2016) reported that granulation of BC with urea reduced the release rate of N in soil, resulting in higher N retention over a longer period of time. A similar mechanism was also proposed by Dong et al. (2009) who also postulated that the addition of humic substances can incorporate N directly into their structure through cation exchange reactions.

5.4.4. Gaseous loss of N as N_2O and NH_3

Incorporation of N fertiliser as BCU significantly decreased the daily and cumulative N_2O and NH_3 emissions from soil compared to commercial urea fertilised soil (Figures 5.13-5.14). A sharp increase in daily N_2O and NH_3 emissions were monitored immediately after N fertilisation in all the treatments. High availability of mineral N to soil microorganisms is one of the most important controllers of the microbial processes that produces N_2O and NH_3 (Paranychianakis et al., 2013). A significant increase in N_2O emission after N fertiliser addition also reported by Nelissen et al. (2014). The BCU granules containing the higher amount BC were superior to the BCU granules with lower amounts of BC in terms of reducing N_2O and NH_3 emissions from soil. The reduction in N_2O and NH_3 emissions from the BCU amended soil could be the result of slower N release and prolonged retention of N as NH_4^+ by the cation exchange sites of BC. It is evident from the results presented in Chapter 2 and 3 that granulation of urea with BC substantially reduced the release rate of urea-N due to its strong retention by BC. Moreover, BC can potentially decrease hydrolysis and mineralisation of fertiliser-N by inhibiting the urease enzyme activity and Leu-AP activity in soil.

It is evident from the correlation study that volatilisation losses of N as NH_3 was significantly and positively correlated with Leu-AP (Table 5.13) and urease enzyme activity (Table 5.9) in soil. The slower urea hydrolysis by inhibiting urease activity in BCU amended soil could be the result of less NH_3 volatilisation in soil. Moreover, the NH_3 volatilisation was also significantly and positively correlated with soil pH (Table 5.5). The decreased soil pH (Figure 5.21) in BCU fertilised soil could be another reason for lower NH_3 volatilisation compared to the urea fertilised soil. A similar finding was reported by Bollmann and Laanbroek (2001) who found that addition of BC reduced volatilisation losses of N from urea fertilised soil by inhibiting the urease enzyme activity. However, several researchers also found that NH_3 can be abiotically fixed onto the porous structure of BC (Bosatta and Agren, 1995; Lapierre et al., 1994; Nommik and Vahtras, 1982). The results of our study are in good agreement with the findings of Sun et al. (2016) and Chen et al. (2015) who also reported that incorporation of lignite to soil decreased NH_3 emissions by 60-68% from cattle manure amended pens compared to control pens where no lignite was added.

A significant and sharp increase in N_2O emissions was observed after each heavy rainfall event at the early growth stage of sweet corn in all the treatments (Figure 5.13A). A substantial increase in soil moisture was recorded after each heavy rainfall (Appendix 2). Increased soil moisture is an important factors influencing the nitrification and denitrification processes to produce N_2O (Xu et al., 2012) and would have influenced the emission profile in this experiment The decreased urea hydrolysis and strong retention of NH_4^+ in the BC matrix in BCU amended soil will reduce the availability of NH_4^+ for nitrification, and as a result, less NO_3^- will be available for denitrification and N_2O production. The results of our study are in accordance with the findings of Rose et al. (2016) who reported that granulation of urea with BC had a significant effect in decreasing N_2O emissions compared to straight urea amended soil.

5.4.5. Biomass and cob N concentration and uptake by sweet corn at harvest

Application of N fertiliser in different forms significantly influenced the biomass and cob N concentration and uptake by sweet corn (Figures 5.17-5.18). No significant difference was observed in biomass N concentration and uptake between urea and BCU amended plants. This might be due to the translocation of N from the biomass to cob development and grain formation. In contrast, the cob N concentration and uptake was substantially higher in BCU 1 amended soil compared to urea amended soil for both high and low N application rates. It is evident from the results that granulation of urea with BC significantly reduced gaseous loss of N and considerably increased availability of mineral N to sweet corn during the whole growing season. The slow mineralisation and release of N could be another reason for increased N availability throughout the growing season of sweet corn. The higher N uptake in the BCU fertilised soil is associated with enhanced soil availability of mineral N and therefore, its uptake through increased biomass and cob yield. Similar results were reported by several groups, that addition of BC and HAs have the potential to increase availability and uptake of N by a variety of crop plants (Nardi et al., 2000; Piccolo et al., 1993; Pinton et al., 1999; Sánchez Sánchez et al., 2009).

5.4.6. Total C and N concentration of soil

Incorporation of BCU and urea significantly influenced the total C and N concentration of the soil (Figure 5.20). A substantial and gradual increase in soil total C was determined from the

BC and BCU amended soils during the growing season of sweet corn while an opposite trend was observed in urea and control treatments. In contrast, a gradual decline in soil total N was measured during the growing period of sweet corn in all the treatments. However, the total N concentration was substantially higher in BCU amended soil compared to urea fertilised soil. The BCU granules containing a higher proportion of BC showed the greater amount of total C and N concentration in soil with respect to the granules containing the lower proportion of BC. Therefore, addition of the C rich BCU granule to soil may have contributed to the total C build up in soil. BCU 1 having a lower amount of C also showed a lower status of total C in the soil compared with the BCU 2 treatment, having the higher amount of total C. Considerably higher amounts of potentially mineralisable N, microbial biomass C and N were measured from the BCU amended soil over urea. The increased root growth and microbial biomass in the BCU amended soil during the study period resulted in a marginal increase in total C and N in the soil. It is well known that the application C rich organic matter to soil considerably increased SOC content (Carey et al., 2009; Mitchell et al., 2007). The data presented in Figure 5.10 depicted that the PMN concentration was considerably higher in BCU amended soil throughout the growing season of the sweet corn. However, such a pattern of PMN concentration in the soil clearly indicated that the mineralisation and release of fertiliser-N was much slower in BCU amended soil.

5.4.7. Biomass and cob yield of sweet corn at harvest

Application of N fertilisers either as urea or BCU significantly influenced the cob yield of sweet corn (Figures 5.15-5.16). The high N application (180 kg N ha^{-1}) either as urea and BCU facilitated higher biomass growth and cob yield than the low N application rate (90 kg N ha^{-1}). The high N application rate increased nutrient supply and enhanced uptake of N by sweet corn which contributed to the improved growth and yield. Substantially higher cob yield was obtained from the plants receiving N as BCU compared to the plants receiving equivalent amount of N as urea for both high and low N application rate. Interestingly, statistically identical amounts of cob yield were obtained from the plots fertilised with 90 kg N ha^{-1} as BCU 1 and the plots receiving 180 kg N ha^{-1} as urea. Considering the BCU granules, the granules having higher amount of BC produced lower amount of biomass and cob yield compared the granules having lower amount of BC.

The results of this study are supported by the findings of Mazeika et al. (2016) who found that addition of an organo-mineral pelleted fertiliser formulated from poultry litter, peat, di-ammonium phosphate and ammonium chloride significantly increased yields of potato and rapeseed compared to sole application of synthetic fertilisers. Addition of highly humified organic matter like BC has been shown to influence structure, moisture, cation exchange capacity (CEC) and many other soil processes including nutrients adsorption and release which ultimately help to improve plant growth in BCU amended soil (Arancon et al., 2006; Piccolo et al., 1993).

The mineralisation and release of N differed considerably between urea and BCU. The hydrolysis, mineralisation and release of N was faster and resulting in higher N losses immediately after fertilisation compared to straight urea. This is supported by the enzyme activity data which showed that urease and Leu-AP activity was higher in urea fertilised soil. As a result a lower amount of fertiliser-N will be available for plant uptake at the later growth stage. This is also supported by the gaseous N loss data which showed that the gaseous loss N as N_2O and NH_3 was significantly higher in urea treated soil at the early growth stage of sweet corn. On the other hand, the mineralisation and release of N from BCU was very slow and steady due to its strong and prolonged retention by the surface area and cation exchange site of BC which ultimately minimised gaseous N losses and increased its availability to plants over a longer period of time. This can be explained by the reduced enzyme (Urease and Leu-AP) activity and mineral N release data shown in Figures 5.6 and 5.9. Similar findings reported by Saha et al. (2017), showed that that granulation of urea with BC substantially slowed down the release of urea-N and NH_4^+-N compared to commercial urea alone in a water dissolution incubation study. The increased availability of mineral N throughout the growing season enhanced N uptake by sweet corn in BCU amended soil. A highly significant relationship was found between cob yield and N uptake by sweet corn ($r = 0.966^{**}$). Therefore, the enhanced N uptake could be another good reason for increased biomass and cob yield of sweet corn in BCU amended soil compared to straight urea fertilised soil. Thus, the growth and yield benefit on application of BCU granule might be due its capability to retain and supply fertiliser-N throughout the growing period as well as the potential of humic acids in improving soil fertility and crop productivity.

5.4.8. *NUE of sweet corn*

Nitrogen use efficiency of sweet corn was significantly influenced by the application of different N fertilisers. The NUE gradually decreased with the increase in N application rate which may be due to increased mineralisation and higher losses of N at high N application. The BCU treatments had higher NUE compared to commercial urea treatments (Table 5.2). On average, addition of N fertiliser as BCU increased the NUE of sweet corn by around 30 to 40% over the straight urea. The results of our study are in good agreement with the findings of Duan et al. (2014) who found that combined application of manure and synthetic fertiliser increased NUE of wheat up to 63% over sole application of synthetic fertiliser. The N use efficiency was considerable higher in plants receiving 90 kg N ha^{-1} as BCU compared to the plants receiving 180 kg N ha^{-1} as urea. This is because of the slow and steady release of N from BCU increased soil availability of N and thereby enhanced its uptake by sweet corn. In addition, significantly lower gaseous N losses could be another reason for higher NUE in BCU treated plants compared urea fertilised plants. This results suggest that addition of 30-40% less N as BCU can produce similar yield benefits to 100% urea at the same level of N application. This can be supported by our results, where no statistical difference was observed in the cob yield and N uptake by sweet corn fertilised with 90 kg N ha^{-1} as BCU and 180 kg N ha^{-1} as commercial urea.

5.4.9. *Economic analysis of sweet corn*

The total number of cobs used in this economic analysis was counted during harvest. The price of individual cob was fixed based on supermarket value. The other values used in this analysis were tentative and based on the discussion of growers. Addition of N fertilisers either as urea or BCU had a considerable effect on the economic analysis of sweet corn (Table 5.14). It is evident from the economic analysis that a high N application rate (180 kg N ha^{-1}) was more profitable than the low N application rate (90 kg N ha^{-1}). This might be due to fact that by adding 50% less N, it was not possible to gain optimum yield and higher economic return like 100% N application. Addition of N fertiliser as BCU was more economic and profitable over sole application of urea. The increased N use efficiency and its uptake facilitated higher cob yield in

BCU amended plants which ultimately increased net return and benefit over straight urea. The highest profit was obtained from the plots amended with 180 kg N ha^{-1} as BCU 1. Considering different BCU granules, the BCU granules containing a lower proportion of BC were more profitable compared to the BCU granules having the higher proportion of BC. Compared to urea, on average, the addition of BCU increased profit by around \$680 and \$1,933 AUD for the low and high N application rate, respectively. More importantly, almost similar net returns were obtained from the plants fertilised with 90 kg N ha^{-1} as BCU 1 and plants receiving 180 kg N ha^{-1} as straight urea. In addition to yield benefits, incorporation of BCU in to soil will also gradually increase soil C content and in the long run can be turned into a significant C credit. Furthermore, the decreased gaseous N losses as N_2O and NH_3 can also be converted to C credit. As a result, extra economic returns could be earned by converting this C credit to financial returns. Overall, the results of this study showed that the granulation of urea with BC is a good option for increasing profits from the direct and indirect economic returns compared to the sole application of urea.

Table 5.14. Economic analysis of sweet corn production per hectare in Australian Dollar (AUD)

3.a

Treatments	Cost of urea ha^{-1}	BCU cost ha^{-1}	Other fertilisers	Seed	*Tractor cost	Weed control	Pest control	*Labour cost	*Marketing and transport	Total input cost
Urea-90	243.7	0	400	315	350	110	400	3500	6000	11318.7
BCU 1-90	0	268.7	400	315	350	110	400	3500	6300	11643.7
BCU 2-90	0	280.5	400	315	350	110	400	3500	6200	11555.5
Urea-180	487.5	0	400	315	350	110	400	3500	6500	12062.5
BCU 1-180	0	537.5	400	315	350	110	400	3500	6800	12412.5
BCU 2-180	0	562.5	400	315	350	110	400	3500	6650	12287.5

Labour cost includes labour involved for the production to harvest of sweet corn per hectare; marketing and transport cost involved packing and transport from farm to supermarket

3.b

Treatments	Number of cobs ha^{-1}	*Price cob^{-1}	Gross returns	Net returns	Benefit over urea
Urea-90	65820	0.3	19746	8427.3	
BCU 1-90	70147	0.3	21044.1	9400.4	973.1
BCU 2-90	67926	0.3	20377.8	8822.3	385.0
Urea-180	72186	0.3	21655.8	9593.3	
BCU 1-180	81238	0.3	24371.4	11958.9	2365.6
BCU 2-180	77934	0.3	23380.2	11092.7	1499.4

* Indicate estimated value, Gross returns = (Number of cobs ha^{-1}× Price cob^{-1}); Net returns = (Gross returns - Total input cost)

5.5. Conclusions

Soil incorporation of BCU exhibited considerable effects on the growth, biomass and cob yield, N uptake, NUE and economic returns for sweet corn, grown in field conditions. Granulation of urea with BC substantially influenced the release and availability of fertiliser N in soil by influencing the hydrolysis and mineralisation of fertiliser-N. Addition of BCU significantly reduced the hydrolysis and mineralisation of urea-N at the early growth stage of sweet corn by inhibiting the urease and Leu-AP enzyme activity. Due to reduced hydrolysis and mineralisation of N as well as its retention by the BC matrix resulted in a significant decrease in gaseous loss of N as N_2O and NH_3. Compared to urea, BCU granules considerably decreased N losses via N_2O and NH_3 emissions and significantly increased plant available N in soil. Moreover, the slow and steady release of fertiliser-N increased the availability of mineral N throughout the whole growing season, enhancing N content and its uptake by sweet corn. The increased yield and reduced gaseous losses of N makes the BCU fertiliser granule more economic, profitable and environment friendly over the use of conventional urea. Based on the overall results, the BCU 1 (20% N) can be considered as a more efficient and economically profitable alternative to conventional urea fertiliser (46% N). The use efficiency of commercial urea fertiliser can be improved substantially by granulating it with humified organic matter like BC. It is evident from the results obtained so far that BCU fertiliser granules have been demonstrated to act as an enhanced efficiency fertiliser that offer a balanced N release pattern to plants with immediate and slow release properties, as well as providing soil organic matter for improving soil health and microbial activity.

In the following Chapter, the results of a ^{15}N study will be reported to observe the fate of applied N as BCU in the soil-plant system, undertaken as part of the field trial on corn growth that has just been described.

6. Chapter: Fate and recovery of nitrogen applied as brown coal-^{15}N urea in a sub-tropical agroecosystem

6.1. Introduction

Urea is the dominant and most widely used nitrogen (N) fertiliser for agricultural crop production world-wide. However, one major drawback is that less than 50% of the added fertiliser-N is utilised by crop plants due to its very poor use efficiency (Galloway et al., 2004; Raun et al., 2002). The gaseous and leaching losses of N are largely responsible for low N use efficiency (NUE) which leading to variety of adverse environmental effects such as ground water contamination and greenhouse gas accumulation in the atmosphere (Fageria and Baligar, 2005). In the recent years, a large number of studies have focused on improving fertiliser N use efficiency and reversing the widespread loss of soil organic matter as well as minimising the negative impacts on the environment (Cassman et al., 2002; Loecke et al., 2004; Raun et al., 2002; Sikora and Enkiri, 2001; Tilman et al., 2002) because soil organic C plays an important role in the retention and movement of N in soil (Dong et al., 2009). From economic and environmental perspectives, it is necessary to improve the fertiliser N use efficiency and reduce its losses to the environment. This is a major challenge and complicated task because of the highly dynamic nature of N turnover in soil and the complex network of versatile factors influencing the availability and uptake of N by crop plants.

A variety of approaches has been shown to improve NUE, such as use of slow or controlled-release N fertilisers, urease and nitrification inhibitors, combined application of organic-synthetic fertiliser and organo-mineral granulation (Chen et al., 2008; Davidson and Gu, 2012). However, granulation of synthetic fertilisers with organic matter could enhance fertiliser use efficiency and return organic matter back into soil, restoring soil health and improving crop yield in a more sustainable manner (Ahmad et al., 2008). Granulation of organic material with synthetic fertiliser has been shown to reduce gaseous and leaching losses of fertiliser-N by decreasing the nutrient release due to increased adsorption by organic matter which prolonged the availability of nutrients in the soil (Richards et al., 1993; Saha et al., 2017).

In the previous Chapter (s) it was shown that granulation of BC with urea significantly reduced the release and losses of fertiliser-N through its prolonged retention. As a results, substantially higher amount of mineral and mineralisable N was available for plant uptake throughout the growing cycle. The increased N availability enhanced N uptake by crop plants and favoured plant growth. There is, therefore, strong reason to hypothesise that granulation of BC with urea influences the dynamics, turnover, fate and recovery of fertiliser-N in the soil-plant system.

To consistently improve NUE and crop uptake of N through BCU granulation, it is necessary to understand how BCU affects the fate, distribution and recovery of fertiliser-N in soil including: sorption to the soil clay particles, microbial immobilisation, retention by soil organic matter, complexation with metal ions, gaseous emissions, movement in soil and plant uptake. Numerous studies have used ^{15}N labelled fertiliser to trace ^{15}N in various pools in soil in order to determine the fate and recovery of applied N in soil-plant system (Allen et al., 2004; Halitligil et al., 2002; Lehmann et al., 2004; Müller et al., 2007; Rütting et al., 2011). However, to the best of our knowledge very few studies have used this technique to assess the whole N balance in soil fertilised with organo-mineral granules under field conditions. Therefore, this study used ^{15}N labelled BCU fertiliser to evaluate the fertiliser N use efficiency by measuring ^{15}N uptake by sweet corn to quantify soil N dynamics by determining ^{15}N immobilisation in microbial biomass and soil organic matter, synthetic N accumulation in soil and N losses through leaching and gaseous emissions.

6.2. Materials and methods

6.2.1. Preparation of ^{15}N labelled brown coal-urea granules

The ^{15}N labelled brown coal-urea (BCU) granules were prepared by Feeco International, Pakenham, Victoria using propriety granulation technology. The detailed description of BCU preparation and characterisation is described elsewhere (Saha et al., 2017).

6.2.2. Soil and experimental site

The microcosm experiment was carried out in the field of Department of Primary Industries (DPI), Wollongbar Research Institute (28°90′95″S and 153°41′54″E), NSW, Australia. The soil is acidic with pH 4.7, contains high concentrations of iron and is classified as a Ferrosol. Basic soil properties (0-15 cm) are listed in Table 6.1. The daily rainfall, soil moisture and temperature data is presented in Appendix 2.

Table 6.1. Physical and chemical properties of soils

Property	
Australian soil order	Ferrosol
Texture	Clay loam
Bulk density (g cm^{-3})	1.31
pH (Water)	4.69
Carbon (%)	4.6
Nitrogen (%)	0.54
Ammonium nitrogen (mg kg^{-1})	13.1
Nitrate nitrogen (mg kg^{-1})	81.2
Phosphorus (Colwell) (mg kg^{-1})	39
Exchangeable calcium ($cmol_c$ kg^{-1})	7.5
Exchangeable magnesium ($cmol_c$ kg^{-1})	1.3
Exchangeable potassium ($cmol_c$ kg^{-1})	0.30
Extractable sulfur (mg kg^{-1})	79.5
DTPA-iron (mg kg^{-1})	394
Extractable aluminium (mg kg^{-1})	61

6.2.3. Microcosm experiment

Polyvinyl chloride (PVC) rings were used to prepare microcosms where ^{15}N labelled BCU granules were applied to sweet corn. The PVC ring was 50 cm height with 30 cm diameter which were installed in the field blocks to a depth of 50 cm. The microcosm experiment was conducted simultaneously with the corn trial having similar treatments. Before seed sowing, the soil was treated with Atradex® WG (Atrazine 900 g kg^{-1}) to minimise weed growth and Lorsban 500 EC (Chlorpyrifos 500 g L^{-1}) to control pests. One BCU granule (BCU having 20% N enriched with 10 atom percent ^{15}N) and two rates of N (180 and 90 kg ha^{-1}) were applied in this trial. Ten atom percent ^{15}N enriched urea with the same N application rate was applied to compare with BCU. Two control treatments, viz., with no N and straight BC were also included to compare the results with urea and BCU granules. In addition, P and K were added at a rate of 40 and 60 kg ha^{-1}, respectively. All the fertilisers were mixed with the top (0-15 cm) soil as granular form and uniformly covered the whole surface area of soil. The experiment was laid out following a completely randomised block design with four replicates. Five seeds of sweet corn (Hybrix 5 Elite) were uniformly sown at 2 cm depth by drilling with a stick. After germination only one seedling was allowed to grow up in each microcosm (Figure 6.1).

Figure 6.1. Microcosm set up in the field (left) and growth of sweet corn (right)

6.2.4. *Gas sampling, soil sampling and plant harvesting*

To each microcosm, a static gas chamber was installed to collect the GHG and volatilised NH_3 samples. Twelve GHG samples were collected at one week intervals and similarly five extra GHG samples were undertaken on the following day after each heavy rainfall event to observe the effect of rainfall on GHG emissions. Headspace concentrations of GHGs were measured at 0, 30 and 60 min after closing the static gas chambers during each measuring day. At each measurement, a 12 mL gas sample was withdrawn from the headspace of the PVC tubes by an air tight syringe (SGE, 25MDR-LL-GT). The gas sample was then transferred into a 12 mL air tight glass vial which was pre-evacuated and flushed with argon and then re-evacuated. The gas sample was collected in that glass vial and N_2O concentration was measured within one week. Gas samples were analysed for N_2O using an Agilent 7890A gas chromatograph (GC) fitted with a Gerstel MPS autosampler. The GHG flux was calculated according to the method and equations detailed by van Zwieten et al. (2010). The cumulative GHG emissions during the sweet corn growing period were calculated by a linear integration of hourly fluxes. Six NH_3 emission sampling periods were undertaken at the early growth stage of sweet corn. Ammonia emissions were measured using polyurethane foam absorbers and flux was calculated using the equation of Singh et al. (2009). A destructive final harvest was undertaken at 98 days after seed sowing. The cobs were separated from the plants and shoot biomass was also quantified. The biomass and cob subsamples were oven-dried for 7 days at 80°C, following which biomass dry weight and cob dry weight was measured. The dried plant materials were then ground to a fine powder and nutrient concentrations were determined following standard methods. At the end of the experiment, soil samples were collected from each microcosm to a depth of 45 cm with a hand auger (Figure 6.2). The collected soil was separated into three 15 cm sections. The depth of the different soil profile layers were top (0-15 cm), middle (16-30 cm) and bottom (31-45 cm). Soil was removed from each section and taken to the laboratory for chemical analyses. The soil was analysed for NH_4^+-N, NO_3^--N, potentially mineralisable N (PMN), total C and N.

Figure 6.2. Collection of soil with hand auger

6.2.5. *Plant analysis*

The biomass and cob N concentration was determined by combustion using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The biomass and cob N uptake was calculated following the equation described by Finzi et al. (2007). Fertiliser N use efficiency (NUE) was calculated according to the formula of Moll et al. (1982).

6.2.6. *Soil analysis*

Mineral N was extracted from soils with 2 M KCl using a 1:2.5 soil: extractant ratio. Soil extracts were filtered through Advantech filter paper 42 prior to analysis for mineral N species by spectrophotometry in microplate formats. Ammonium was quantified by reacting with salicylate and hyphochlorite in a buffered alkaline solution containing sodium nitroprusside as a reductant (Forster, 1995). Nitrate plus nitrite was determined by reduction using vanadium (III) combined with detection by acidic reaction (Miranda et al., 2001). Potentially mineralisable nitrogen (PMN) was assessed as described by Waring and Bremner (1964), modified by Stenberg et al. (1998). Total C and N in soil was measured using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The soils were finely ground using a mortar and pestle prior to C and N analysis. The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by a fumigation extraction method of Vance et al. (1987), with extraction of organic C and N by 0.5 M K_2SO_4 (1: 4, soil: solution) and determination of the C and N content in the K_2SO_4 soil extracts with a liquid organic C and N analyser (Formacs Series, Combustion TOC/TN analyser).

6.2.7. Isotope analysis

6.2.7.1 Measurement of ^{15}N of N_2O

The concentration of ^{15}N of N_2O in gas mixtures was determined at the Stable Isotope Facility at the University of California, Davis. The $\delta^{15}N$ was measured using a ThermoScientific GasBench with Precon gas concentration system interfaced to a ThermoScientific Delta V plus Isotope Ratio Mass Spectrometry (IRMS) (Bremen, Germany). Gas samples were purged from vials through a double-needle sampler into a helium carrier stream (20 mL min^{-1}). Then, N_2O was isolated and concentrated in preparation for isotopic analysis. The N_2O was trapped and concentrated in liquid nitrogen cryo-trap and passed to another cryo-trap. Finally, the trapped N_2O was separated by IRMS and isotope ratio of N_2O sample was calculated using a reference N_2O peak.

6.2.7.2 Measurement of ^{15}N-NH_4^+ in NH_3 volatilised sample

The volatilised NH_3 was trapped into 0.25 M H_2SO_4 and converted to NH_4^+. The ^{15}N-NH_4^+ in the volatilised sample was converted to N_2 using hypobromide. The N_2 was then analysed using the GC-IRMS interfaced with a cryo-prep module described by Zhang et al. (2007).

6.2.7.3 Measurement of ^{15}N in plant, soil and microbial biomass N samples

The plant and soil samples were ground in an agate mortar to obtain a fine and homogeneous powder. The N concentration and ^{15}N isotopic composition were measured from plant and soil samples using an elemental analyser (Carlo Erba/Fisons/Interscience) coupled via a ConFlo II interface to an isotope ratio mass spectrometer (EA-IRMS, Finnigan Delta S). The ^{15}N derived from fertiliser was calculated using the following equation (Nason and Myrold, 1991).

$$N_{\text{Derived from fertiliser}}\ (\%) = (^{15}\text{N at. \% in sample}/^{15}\text{N at. \% applied as fertiliser}) \times 100$$

$$N_{\text{Derived from soil}}\ (\%) = \text{Total N in the sample (\%)} - \text{N derived from fertiliser (\%)}$$

6.2.8. Statistical analysis

The statistical analyses were performed using statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). The normality testing of the data was conducted using the Kolmogorov-Smirnov goodness of fit test and equality of variances was tested using the modified Levene's test. Some data were not normally distributed and showed unequal variance. Those data were log or Ln transformed before performing the analysis of variance (ANOVA). A one-way ANOVA including factor fertiliser type (including the BC and control) were run. The multiple comparisons among the different treatments were done using a Tukey test.

6.3. Results

6.3.1. N_2O emissions

Addition of different N fertilisers substantially increased the daily and cumulative N_2O emissions from soil compared to soil where no N fertilisers were added (Figure 6.3). A rapid increase in daily N_2O emissions was measured initially after N fertilisation followed by a sharp decline at 14 DAS. Then, the N_2O emissions increased very sharply and reached to its maximum value at 21 DAS just one day after a heavy rainfall event. The N_2O emissions then decreased dramatically and fluctuated around the same level until the harvesting of the sweet corn (Figure 6.3.A). A large increase in N_2O emissions was observed after each heavy rainfall at the early growth stage of sweet corn. Addition of different forms of N fertilisers significantly increased the gaseous losses of N_2O derived from soil compared to the soil where no N fertilisers were added. Application of N fertiliser as BCU granules resulted in a significant decrease in cumulative $^{15}N_2O$ emissions derived from added fertiliser-N for both high and low N amended soil over commercial urea (Figure 6.3.B). Addition of BCU granules significantly reduced the gaseous loss of N as N_2O from both fertiliser and soil source compared to commercial urea fertilised soil. The loss of fertiliser-N as N_2O was decreased by around 50% due to addition of BCU than the sole application of urea.

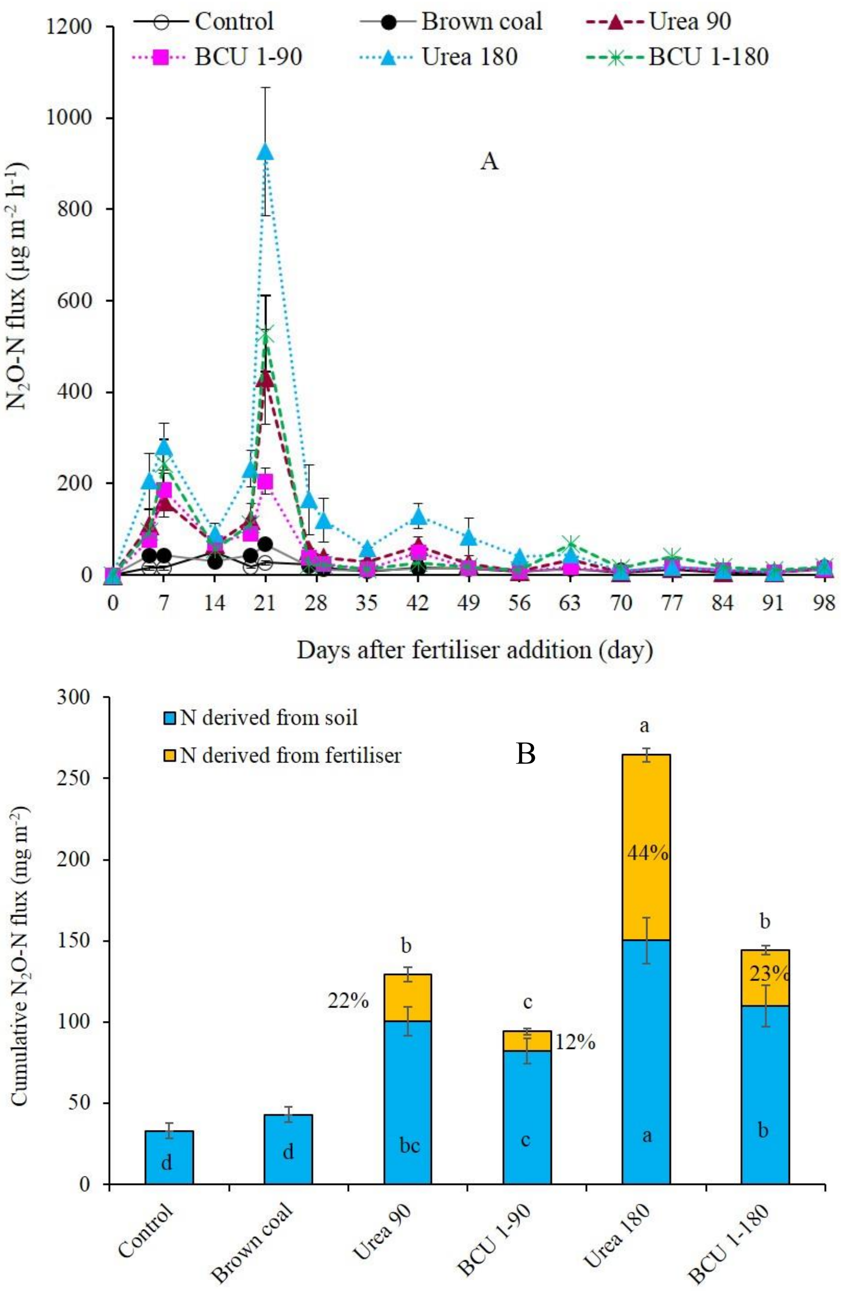


Figure 6.3. Effect of BCU granules and urea on the daily (A) and cumulative (B) N_2O-N emissions from soil during the growing season of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.2. NH_3 volatilisation

Soil incorporation of BCU granules showed a considerable influence on the daily and cumulative NH_3 emissions from the soil (Figure 6.4). A sharp increase in NH_3 volatilisation was observed initially after the N fertilisation and peaked at 7 DAS followed by a quick decline at 14 DAS (Figure 6.4.A). Subsequently, the NH_3 volatilisation decreased gradually and remained almost the same until the last sampling period. Application of N fertilisers either as urea or BCU significantly increased the volatilisation loss of soil-N compared to soils receiving no N fertilisers. The volatilisation loss of $^{15}NH_3$ derived from applied fertiliser-N was significantly higher in straight urea fertilised soil compared to BCU amended soil (Figure 6.4.B). In contrast, no significant variation was observed in the volatilisation loss of NH_3 derived from soil source among the urea and BCU treatments. The addition of BCU granules reduced the cumulative volatilisation loss of $^{15}NH_3$ derived from applied fertiliser-N by around 60% than the commercial urea. The volatilisation loss of $^{15}NH_3$ from derived from fertiliser-N was substantially higher in high N application rate over the low N application rate.

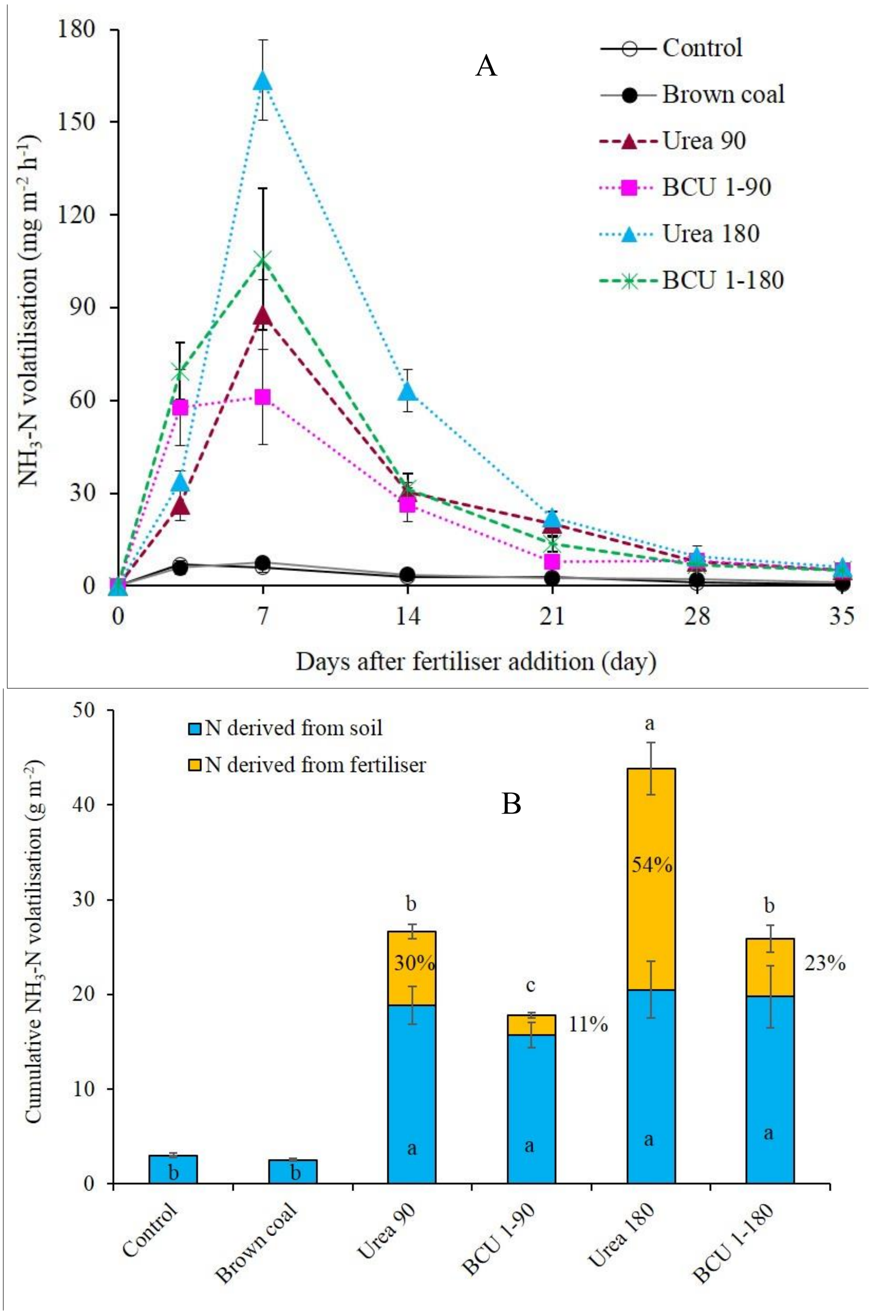


Figure 6.4. Effect of BCU granules and urea on the daily (A) and cumulative (B) NH_3-N volatilisation from soil during the growing season of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.3. *Biomass and cob yield of sweet corn*

Application of N fertilisers as either urea or BCU significantly influenced the fresh and dry biomass and cob yield of sweet corn (Figures 6.5-6.6). Both the fresh and dry biomass yields were considerably higher in BCU amended soil compared to urea fertilised soil in both high and low N application rate, however, these effects were not significant. The biomass yield was considerably higher in the high N application rate than the low N application rate (Figure 6.5). In contrast, a significantly higher cob yield was obtained from the plants receiving 180 kg N ha^{-1} as BCU compared to the plants receiving the equivalent amount of N as urea. On the other hand, no significant difference in cob yield was measured between urea and BCU for the low N application rate. Importantly, statistically similar amount of cob yield were obtained from the plants receiving 90 kg N ha^{-1} as BCU and the plants receiving 180 kg N ha^{-1} as urea (Figure 6.6). Compared to urea, on average addition of BCU granules to soil increased cob yield of sweet corn by 23%.

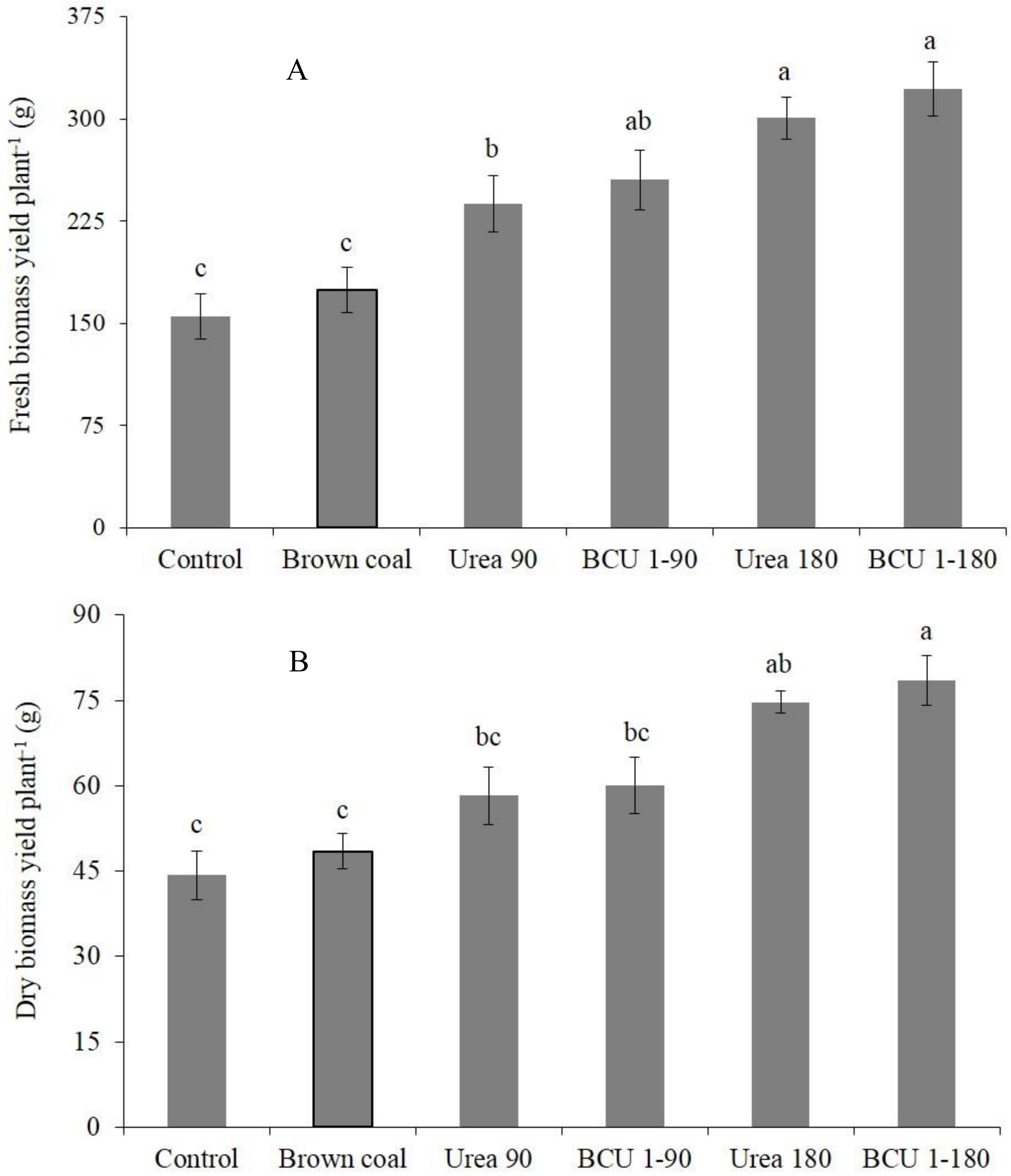


Figure 6.5. Effect of BCU granules and urea on the fresh (A) and dry (B) biomass yield of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

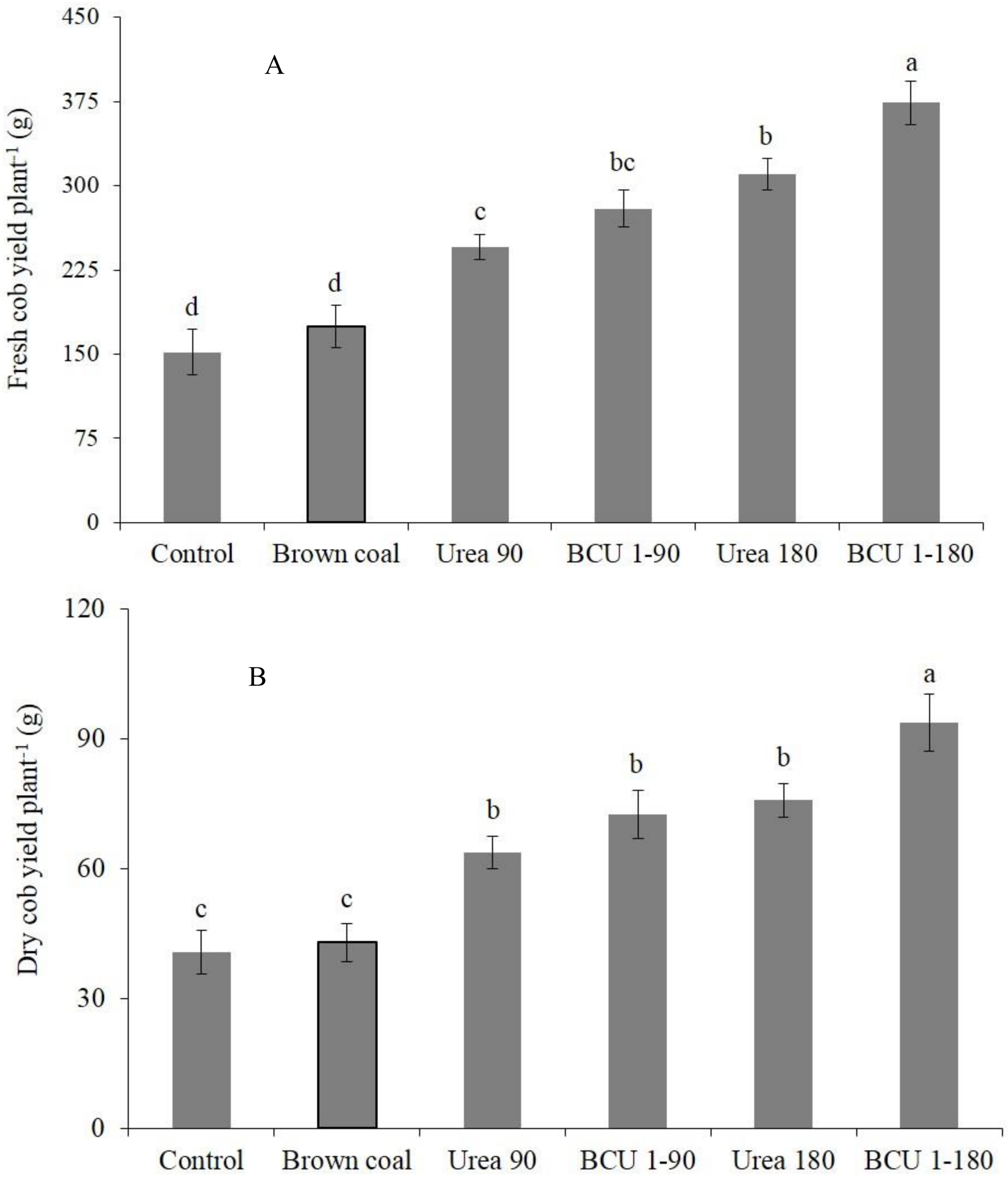


Figure 6.6. Effect of BCU granules and urea on the fresh (A) and dry (B) cob yield of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.4. Biomass and cob N concentration of sweet corn

The biomass and cob N concentration of sweet corn varied substantially among the various treatments (Figure 6.7). The biomass and cob ^{15}N derived from applied fertiliser was significantly higher in plants receiving 180 kg N ha^{-1} as BCU compared to plants receiving equivalent amount of N as urea only and other treatments including control. Conversely, no significant differences were measured in the biomass and cob N sourced from soil among the urea and BCU amended plants for both low and high N application rate. Nevertheless, the cob ^{15}N concentration derived from the applied fertiliser was considerably higher in plants receiving the high rate of fertiliser-N than the low rate of BCU fertiliser-N application.

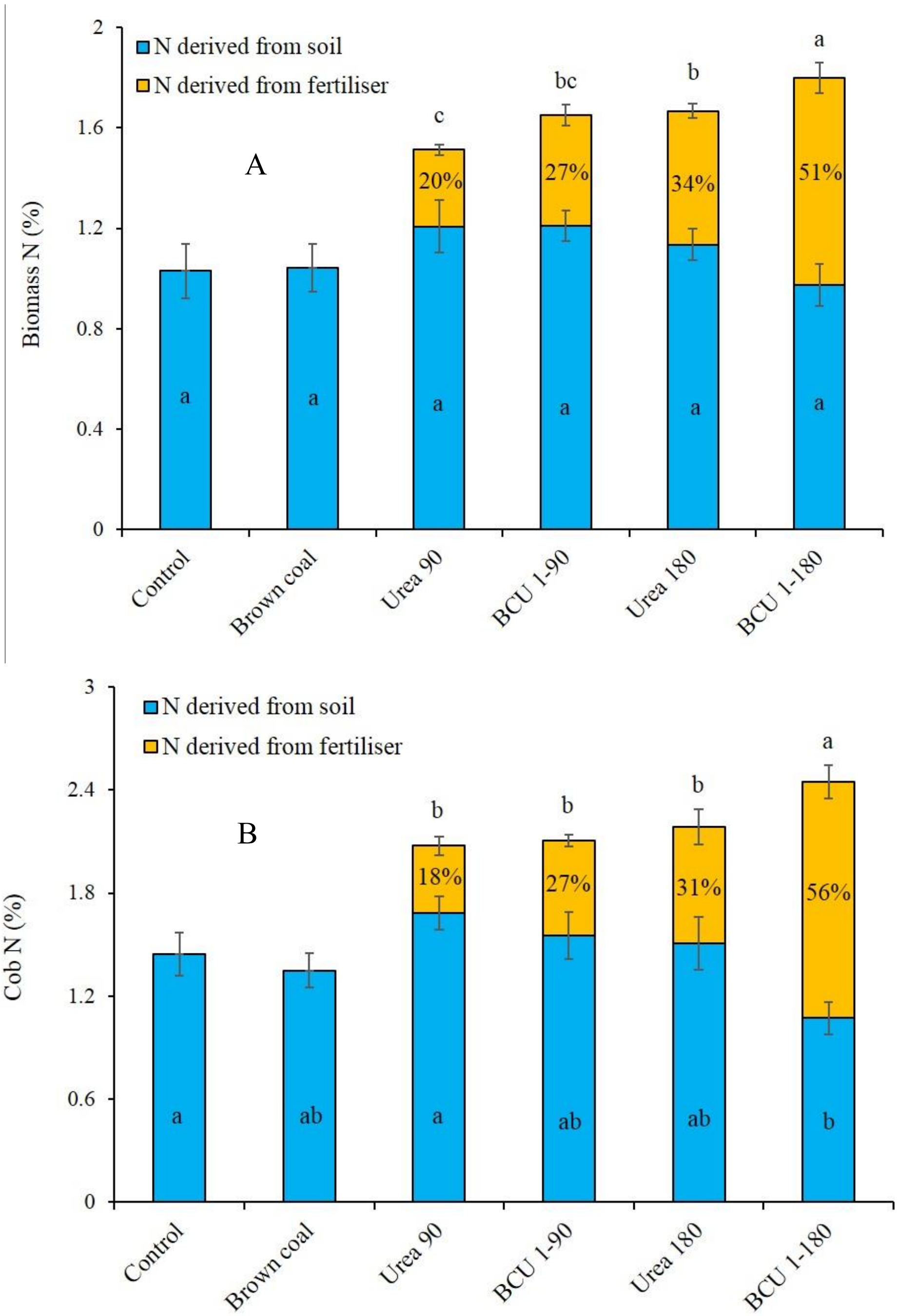


Figure 6.7. Effect of BCU granules and urea on the biomass (A) and cob (B) N concentration of sweet corn at harvest. Bars with different letters differ significantly according to Tukey-test at *P*<0.05 and the error bars indicate the standard error among the replicates (n=4).

6.3.5. *Biomass and cob N uptake by sweet corn*

Addition of N fertilisers either as urea or BCU significantly influenced the biomass and cob N uptake by sweet corn (Figure 6.8). No significant variation was observed in biomass and cob N uptake sourced from soil-N among the urea and BCU amended plants. In contrast, the uptake of applied fertiliser-^{15}N by biomass and cob of sweet corn were significantly higher in plants fertilised with 180 kg N ha^{-1} as BCU compared to plants receiving an equivalent amount of N as urea and all other treatments included in this experiment. The high fertiliser-N application rate facilitated higher N uptake by sweet corn over the low N application rate. Compared to urea, on average, addition of BCU granules enhanced fertiliser-^{15}N uptake around 25 and 8% by cob and biomass of sweet corn from the high and low N application rate, respectively.

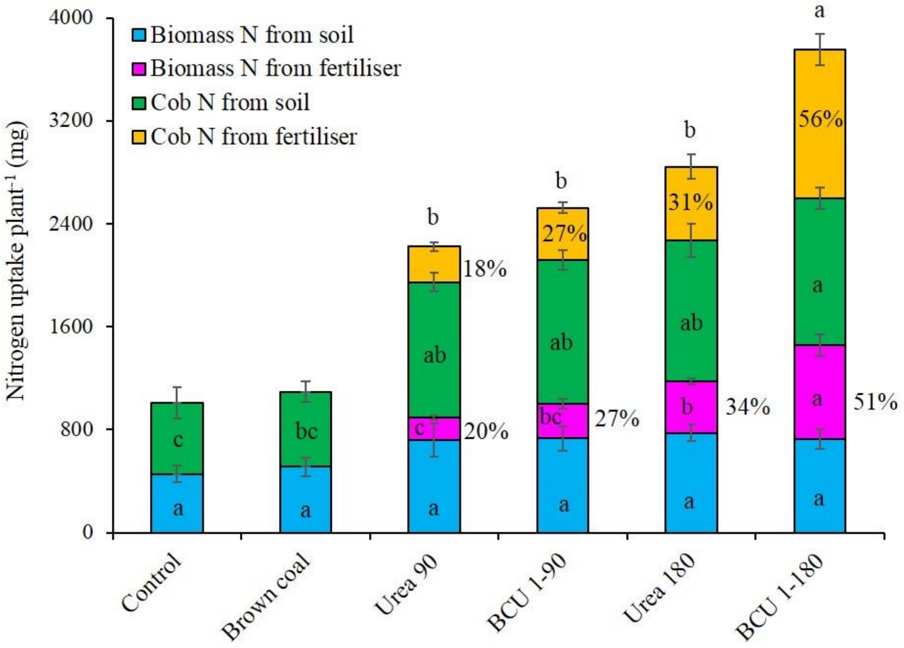


Figure 6.8. Effect of BCU granules and urea on the N uptake by sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.6. *Ammonium-N, NO_3^--N and PMN content of soil*

Soil incorporation of BCU granules exhibited a considerable effect on the mineral (NH_4^+-N and NO_3^--N) and potentially mineralisable N (PMN) concentration of post-harvest soil of sweet corn (Figures 6.9-6.10). The mineral and mineralisable N content of the top (0-15 cm) soil was substantially higher compared to deeper layer of the soil profile. In general, the mineral and mineralisable N concentrations decreased gradually with increasing depth in the soil profile except in the urea treated soil where the mineral N concentrations were higher in the bottom (31-45 cm) layer compared with the middle (16-30 cm) layer of soil profile (Figure 6.9). The mineral and mineralisable N content of top soil was significantly higher in the BCU treatment compared to urea alone. In contrast, no significant variation was observed in the mineral and mineralisable N content of deeper soil layers among the BCU and urea treatments.

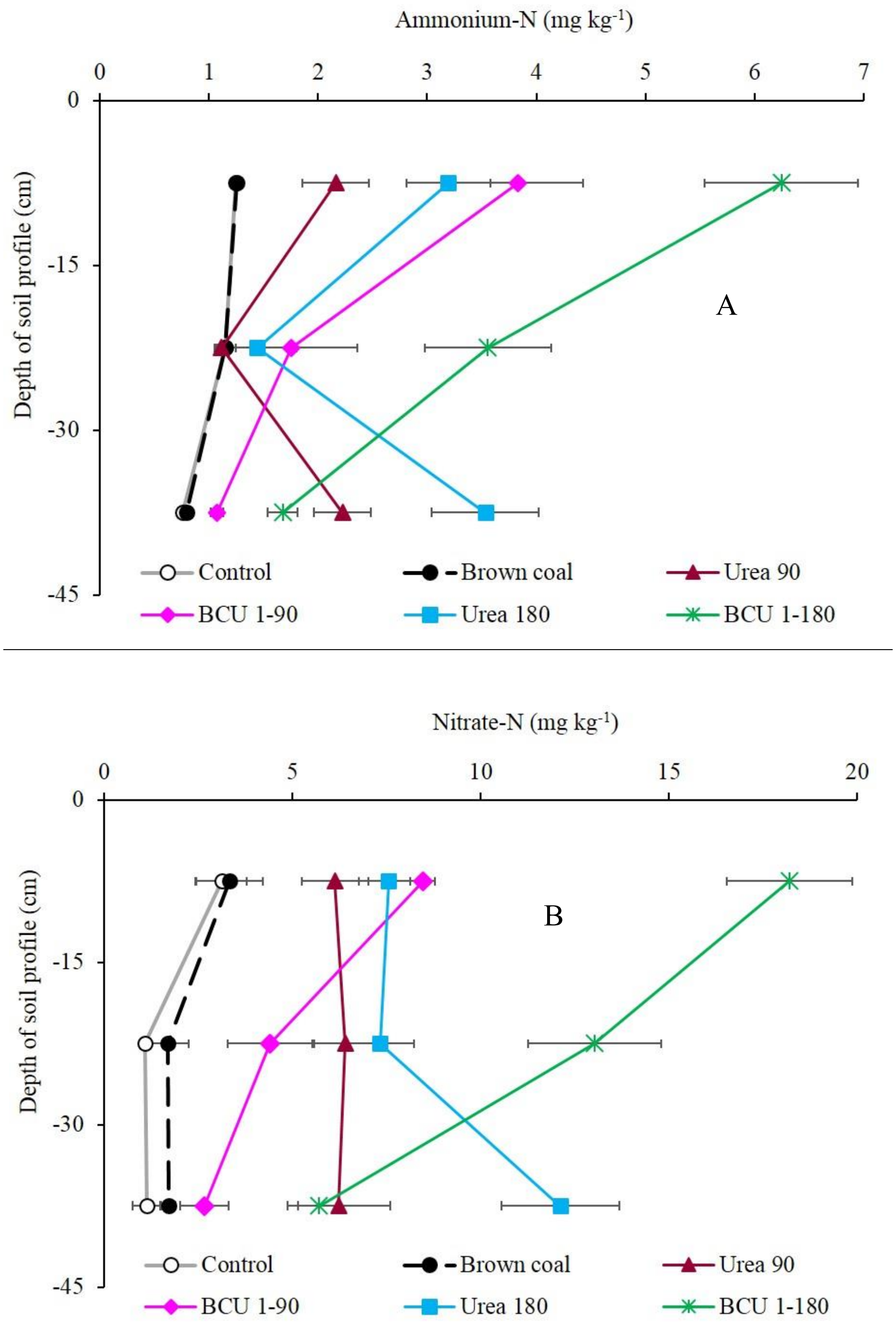


Figure 6.9. Effect of BCU granules and urea on the NH_4^+-N (A) and NO_3^--N (B) content at various depth of soil profile at harvest.

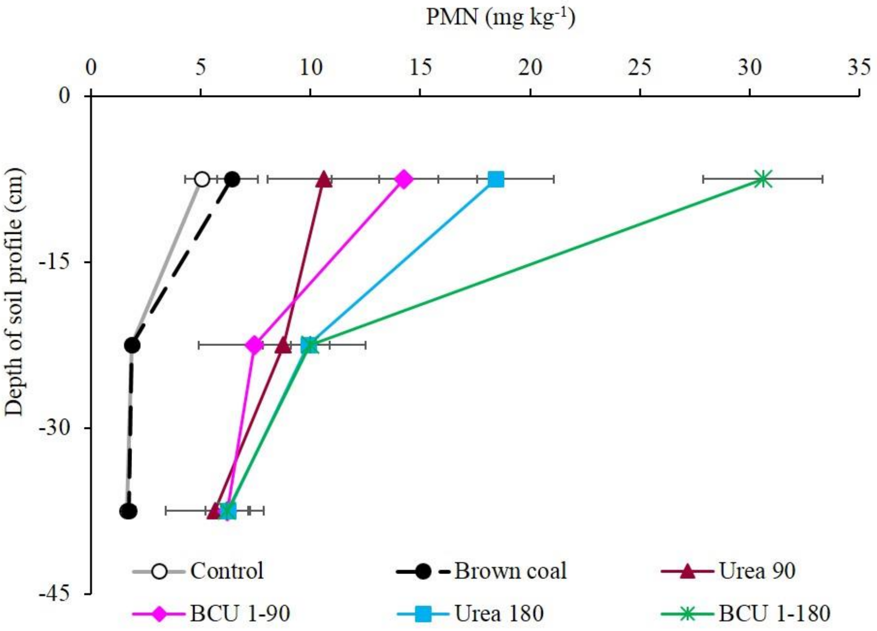


Figure 6.10. Effect of BCU granules and urea on the PMN concentration at various depth of soil profile at harvest.

6.3.7. Microbial biomass C and N content of soil at harvest

The microbial biomass C and N content of soil varied according to the addition of the N fertilisers as either urea or BCU (Figure 6.11). A significantly higher amount of microbial biomass C was determined from the soil amended with BCU over urea for both low and high N application rates. Conversely, no significant differences were observed in the microbial biomass N derived from soil in the BCU and urea amended with soil receiving equivalent amounts of N. However, the soil derived microbial biomass N was considerably higher at low N application rate compared with the high N application rate. The microbial immobilisation of applied fertiliser-^{15}N was significantly higher in BCU fertilised soil compared to urea only in the high N application rate whereas in the low N application rate, the microbial biomass ^{15}N derived from fertiliser did not differ significantly between urea and BCU. The high N application as BCU favored microbial activity and enhanced microbial consumption of fertiliser-^{15}N by 31% compared to urea at the same rate.

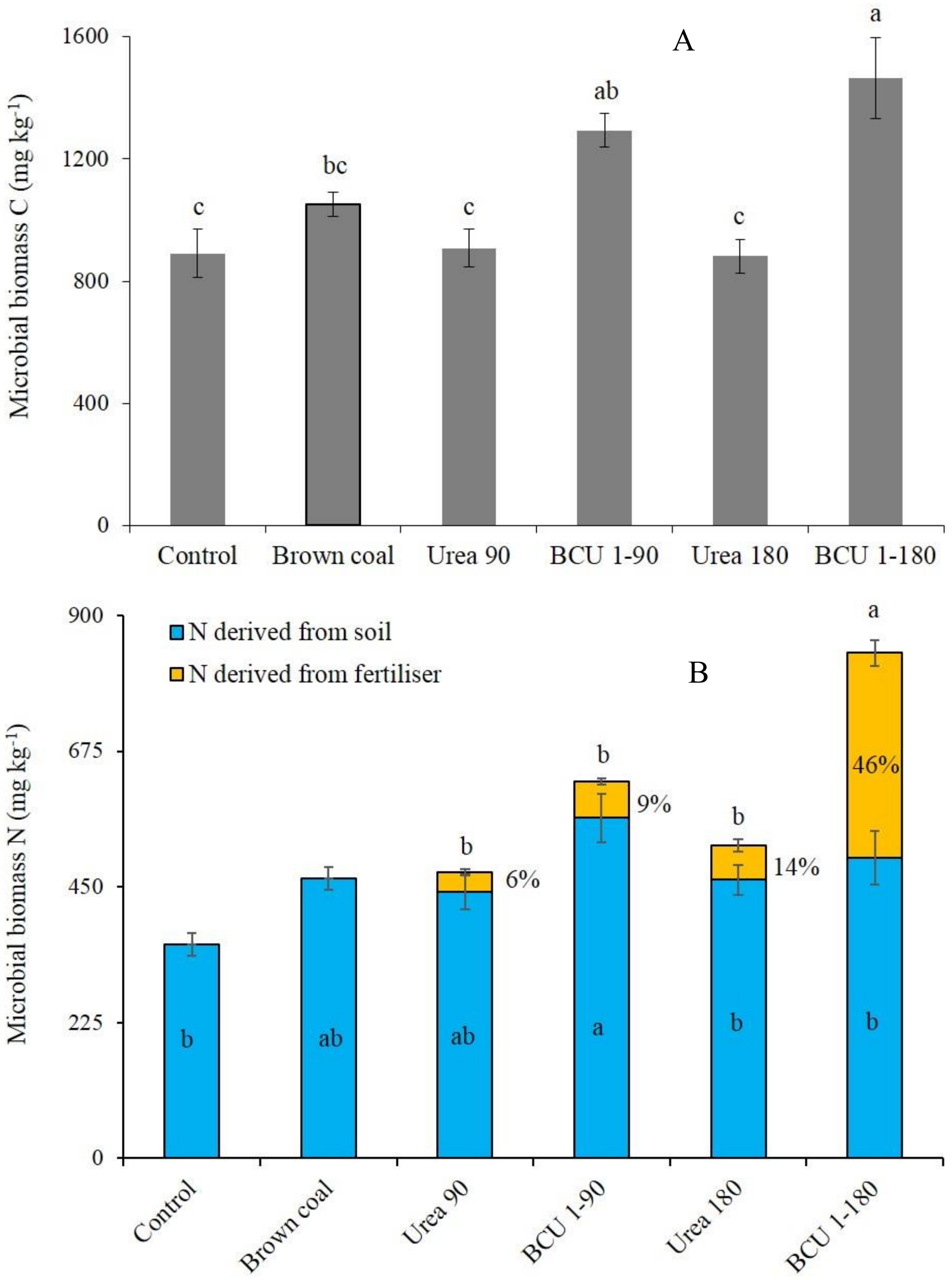


Figure 6.11. Effect of BCU granules and urea on the MBC (A) and MBN (B) content of soil at harvest. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.8. Total C concentration of soil at harvest

Generally the total C concentration of soil decreased gradually with the increase in depth in the soil profile. No significant variation in soil total C concentration was observed among the different treatments used in this study at various depths in the soil profile (Figure 6.12).

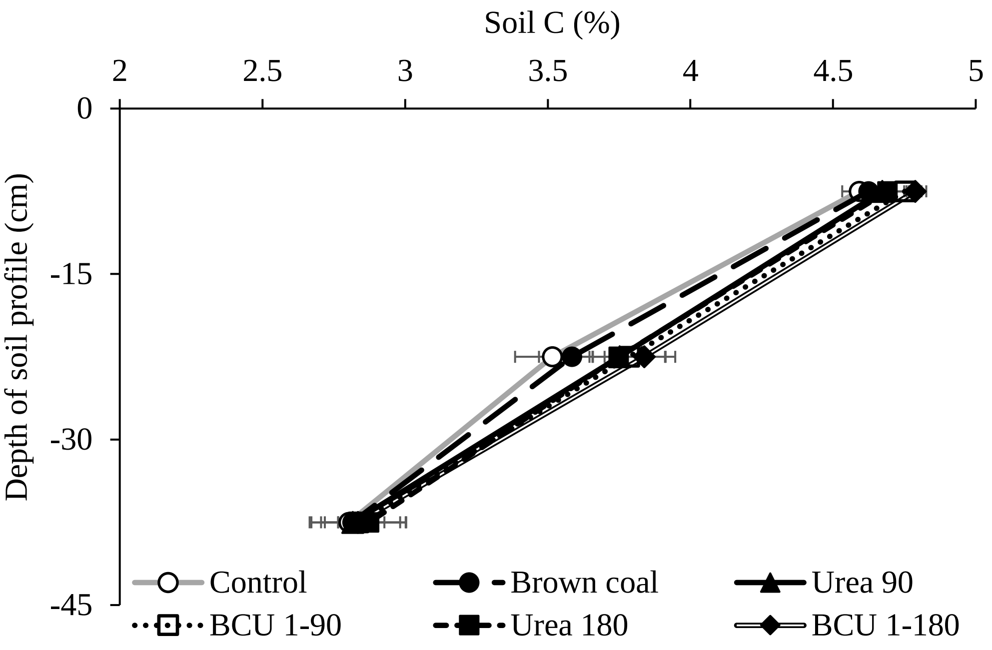


Figure 6.12. Effect of BCU granules and urea on the total C concentration at various depth of soil profile at harvest.

6.3.9. Total N concentration of soil at harvest

Application of different forms of N fertilisers significantly influenced the total N concentration in the different treatments at various depths of soil profile (Figure 6.13). In general, the total N concentration derived from both soil and fertiliser source declined gradually with the increasing depth of soil profile. A significantly higher amount of total ^{15}N derived from applied fertiliser was determined from the soil amended with 180 kg N ha^{-1} as BCU compared to soil receiving equivalent amount of N as urea in the top layer of soil profile. In contrast, an opposite trend was observed for the middle and bottom layers of the soil profile where total ^{15}N derived from fertiliser was significantly higher in urea treated soil than BCU amended soil. No significant variation in total N derived from soil was found throughout the soil profile between the urea and BCU amended soil except the middle layer of soil profile where the total N derived from soil was significantly higher in urea amended soil over BCU fertilised soil for both high and low N application rate.

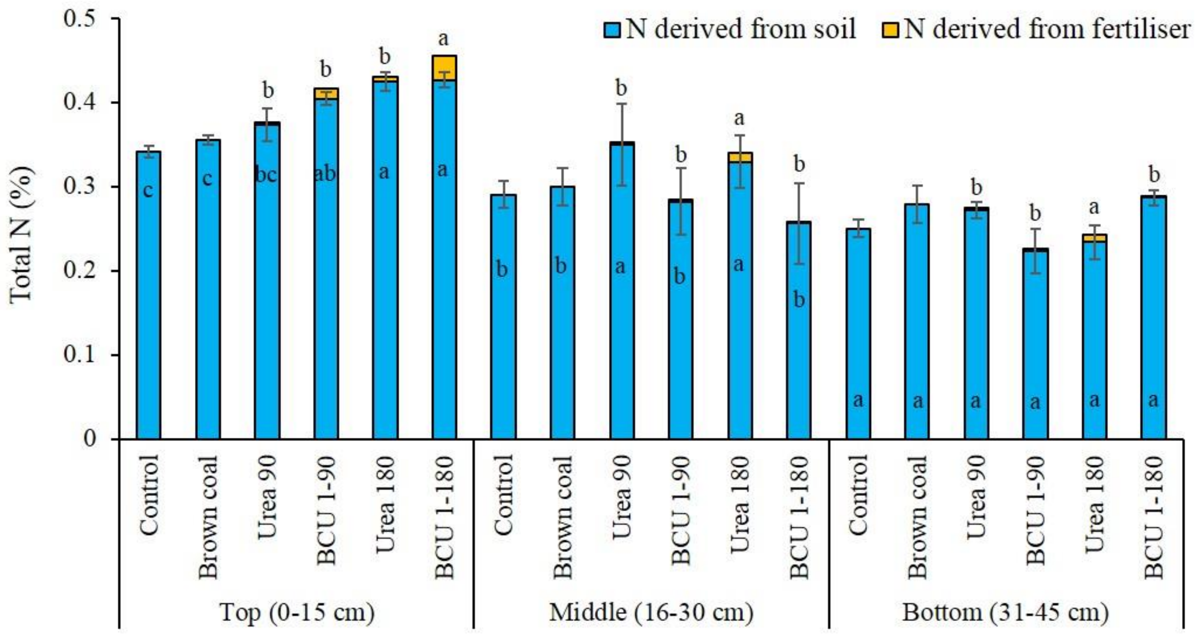


Figure 6.13. Effect of BCU granules and urea on the total N concentration at various depth of soil profile. Bars with different letters differ significantly according to Tukey-test at *P*<0.05 and the error bars indicate the standard error among the replicates (n=4).

6.3.10. Fertiliser N use efficiency of sweet corn

The fertiliser ^{15}N use efficiency of sweet corn differed significantly due to application of N fertilisers either as urea or BCU (Figure 6.14). The N use efficiency decreased slightly with the increase in N application rate in both the urea and BCU amended soil. The fertiliser ^{15}N use efficiency was significantly higher in BCU fertilised soil compared to urea fertilised soil for both low and high N application rate. On an average addition of BCU increased the fertiliser ^{15}N use efficiency of sweet corn by approximately 25% compared to straight urea.

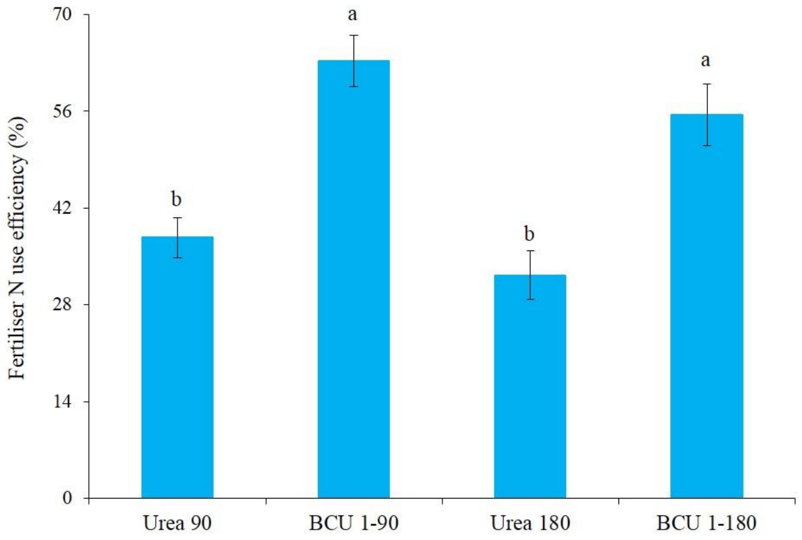


Figure 6.14. Effect of BCU granules and urea on the fertiliser N use efficiency of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

6.3.11. Recovery of fertiliser N

The distribution and recovery of applied ^{15}N in the different N fractions (as a percentage of added N) varied substantially due to the addition of N fertilisers either as urea or BCU (Figure 6.15). The total recovery of applied fertiliser-^{15}N was considerably higher in BCU amended soil over urea treated soil. The recovery of applied fertiliser-^{15}N by sweet corn was around 25% higher in soil amended with BCU compared to sole application of urea. The soil treated with BCU retained approximately five times more fertiliser-^{15}N than the soil amended with urea alone. However, a relatively large proportion of applied urea-^{15}N was not recovered after the harvesting of the sweet corn, and the unaccounted proportions were about 35% for the urea treatments and nearly 13% for the BCU treatments. The gaseous losses (N_2O and NH_3) of applied fertiliser-^{15}N were three times higher in straight urea fertilised soil than the BCU amended soil. In contrast, addition of N fertiliser as BCU increased the microbial immobilisation of fertiliser-^{15}N by twice as much as urea.

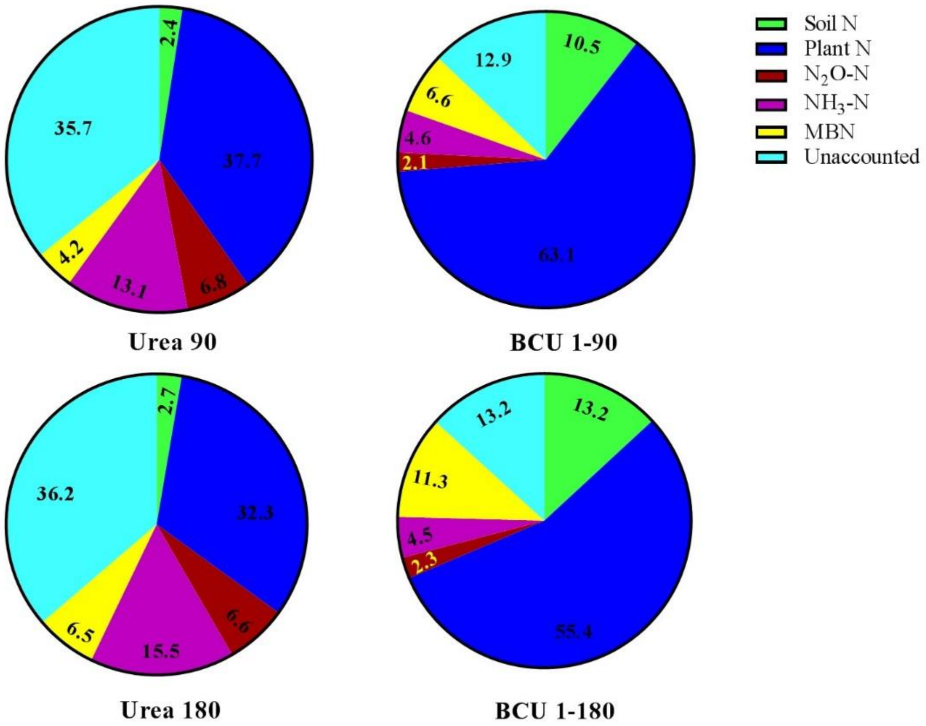


Figure 6.15. Recovery of applied fertiliser-N from the urea and BCU amended soil

6.4. Discussion

6.4.1. *Gaseous losses of N as N_2O and NH_3*

The application of N fertiliser either as straight urea or BCU significantly increased the gaseous losses of N (N_2O and NH_3) from both soil and fertiliser sources compared to soil receiving no N fertilisers. The addition of fertiliser N triggered the activity of nitrifying and denitrifying bacteria in soil which might enhance the mineralisation of both soil and fertiliser N and resulted in higher N_2O and NH_3 emissions. This can be supported by the daily flux of gaseous N loss where a sharp increase in N_2O and NH_3 emissions were observed immediately after N fertilisation.

Considerably higher N_2O emissions after each heavy rainfall event might be due to increased soil moisture which enhanced the activity of denitrifying bacteria in soil. Adequate soil moisture, fertiliser N and a source of labile C are essential for improved activity of denitrifiers for producing higher N_2O (Nelissen et al., 2014; Ruser et al., 2006). Application of BCU significantly decreased the gaseous loss of N_2O derived from the fertiliser-^{15}N compared to commercial urea alone (Figure 6.3). The field results of our study are supported by the findings of Rose et al. (2016) who also found that granulation of BC with urea significantly decreased N_2O emissions from soil compared to urea in a laboratory soil column experiment. Compared to urea, on average, addition of BCU granules to soil suppressed the fertiliser-^{15}N loss as NH_3 by 60%. The results of our study is in close agreement with the results of Sun et al. (2016) and Chen et al. (2015) who also reported that soil incorporation of lignite reduced NH_3 volatilisation by up to 68% from cattle manure amended pens compared to control pens where no lignite was added.

The reduction in applied fertiliser-^{15}N losses as N_2O and NH_3 emissions from the BCU amended soil could be the result of strong retention of N by BC, reduced urea hydrolysis and slower release of urea-N. The strong and prolonged retention of fertiliser-^{15}N onto the porous structure and cation exchange of NH_4^+ with the acid functional groups of BC could be the main reason for decreasing the gaseous losses of fertiliser-N in BCU amended soil (Bosatta and Agren, 1995; Lapierre et al., 1994; Nommik and Vahtras, 1982). In addition, slow release of fertiliser-^{15}N due to reduction of urea hydrolysis by inhibiting urease enzyme activity in BCU amended soil may also suppress gaseous losses of ^{15}N derived from fertiliser. A similar mechanism was also described by Saha et al. (2017) who reported that granulation of BC with urea substantially reduced fertiliser N losses compared to urea by slowing down urea hydrolysis through inhibition of urease activity and strong retention of N with BC. The enhanced microbial immobilisation of fertiliser-^{15}N could be an alternative mechanism of less N_2O and NH_3 production in BCU amended soil. This can be supported by microbial biomass N data, where a considerably more added fertiliser-^{15}N was recovered by the microbial biomass in the BCU fertilised soil compared with straight urea treated soil. Moreover, the acidic pH of BC could reduce the activity of denitrifiers in the microsites around the BCU granules and thereby decrease N_2O emissions via denitrification (Kyveryga et al., 2004).

6.4.2. Yield, N uptake and its use efficiency by sweet corn

Granulation of BC with urea significantly increased the availability and uptake of applied fertiliser-^{15}N by sweet corn compared to the application of urea only. We propose that the higher fertiliser-^{15}N uptake by both biomass and cob in the BCU fertilised soil was associated with the enhanced and prolonged availability of mineral N throughout the growing season of the sweet corn. Several researchers have also reported that addition of BC and products derived from it has the potential of increasing availability and uptake of

N by a variety of crop plants (Nardi et al., 2000; Piccolo et al., 1993; Pinton et al., 1999; Sánchez Sánchez et al., 2009). However, these earlier studies did not use a combined urea-BC granule and involved adding the lignite alone or humic acids derived from it. Our study has demonstrated that blending and subsequent granulation of BC with urea was more effective and beneficial than raw application of BC. In addition, the mineralisation and release of fertiliser-N differed considerably between urea and BCU. The mineralisation and release of fertiliser-N from BCU was very slow and steady due to its strong and prolonged retention by the high surface area and cation exchange sites of the BC which ultimately increased its availability to plants over a longer period of time. This can be supported by the gaseous ^{15}N loss data where fertiliser-N losses were much lower in BCU amended soil which might contributed for increasing its availability to sweet corn. Moreover, the higher microbial biomass ^{15}N and potentially mineralisable N derived from fertiliser in BCU amended post-harvest soil was a clear indication of prolonged retention and slower release of plant available fertiliser-N until the harvest of sweet corn which could be available for the next cropping season.

The increased and prolonged availability of fertiliser-^{15}N from BCU amended soil and its subsequent uptake by sweet corn facilitated better growth and contributed significantly higher cob yield over the sole application of urea. The higher growth and yields of sweet corn in BCU fertilised soil were mainly the result of increased availability and uptake of added fertiliser-^{15}N because no significant variation was found in the soil derived N in the urea and BCU treatments. The results of our study are consistent with the findings of Mazeika et al. (2016) who found that addition of an organo-mineral pelleted fertiliser formulated using humified poultry litter and peat with di-ammonium phosphate and ammonium chloride significantly increased yields of potato and rapeseed compared to sole application of synthetic fertilisers. Interestingly, in this current study, statistically identical amounts of cob yields were obtained from the plants fertilised with 90 kg N ha^{-1} as BCU and the plants receiving 180 kg N ha^{-1} as straight urea. This can be supported by the fertiliser N use efficiency data which shows the fertiliser N use efficiency of sweet corn was 25% higher in BCU fertilised soil compared to application of urea alone. The results of our study are also consistent with the findings of Duan et al. (2014) who found that combined application of manure and synthetic fertiliser increased N use efficiency of wheat up to 63% over the sole application of synthetic fertiliser. The reduced gaseous losses and slow release of fertiliser-N from BCU increased soil availability of N and thereby enhanced its uptake by sweet corn. The increased N uptake and higher cob yield might be responsible for increasing fertiliser-N use efficiency of sweet corn grown in BCU amended soil. This results indicate that application of 90 kg N ha^{-1} as BCU was a good as 180 kg N ha^{-1} as urea.

6.4.3. *Recovery of ^{15}N-labeled urea*

Figures 6.15 shows that after harvesting the sweet corn, the ^{15}N recovery by plant and soil for all treatments varied within a range of 40.1-73.6% of the applied fertiliser-N. Addition of N fertiliser as BCU had the highest recovery of applied fertiliser-^{15}N by plant and soil. Granulation of BC with urea considerably enhanced recovery and microbial immobilisation of applied fertiliser-^{15}N over sole application of urea. This microbial immobilised N could slowly become available to crop plants and potentially increase the recovery of applied fertiliser-^{15}N by the plant. A similar finding was also reported by Choi et al. (2001) where the combined application of compost and ^{15}N urea substantially increased the recovery of applied fertiliser-^{15}N by the plant. The soil also showed a prolonged retention and enhanced microbial immobilisation of fertiliser-^{15}N.

6.5. Conclusions

Application of N fertiliser as BCU considerably influenced the gaseous losses, microbial immobilisation and plant recovery of ^{15}N in a corn-soil system. Addition of BCU showed the lowest urea-derived ^{15}N losses during corn growth period, while applying urea alone had less effect on it. In addition, granulation of BC with urea significantly reduced the fertiliser derived gaseous losses of ^{15}N as N_2O and NH_3 compared to the sole application of urea. Moreover, soil incorporation of BCU increased immobilisation of the applied fertiliser-^{15}N due to stimulated microbial activity. Microbial immobilisation of the applied fertiliser-^{15}N not only decreased gaseous losses, but also led to the higher retention of the applied fertiliser-^{15}N in the soil for longer periods of time. The decreased gaseous ^{15}N losses and increased fertiliser-^{15}N retention by BC and soil microbial biomass were mainly responsible for increased yield and N use efficiency of the sweet corn. Soil treated with BCU enhanced fertiliser-N use efficiency by around 25% over urea. Moreover, the unaccounted proportions of applied fertiliser-^{15}N was remarkably lower in soil amended with high-efficiency BCU (13.1%) than the low-efficiency urea (35.9%). The results obtained in the present study revealed that application of N fertiliser in the form of granular

BCU substantially improved crop yield as well as enhanced fertiliser-^{15}N recovery while diminishing its losses to the environment.

This study provides some very interesting results on potential improvements in fertiliser-N use efficiency of sweet corn, but further studies in large scale with various crops under various soil and climatic conditions are necessary before using BCU granule as a potential alternative of commercial urea fertiliser. In the next chapter, the potential mechanisms responsible for the results obtained in previous chapters of the thesis on the granulation effect of BC with urea on the dynamics, mineralisation, fate and recovery of applied fertiliser-N in soil-plant system will be discussed in detail for a better understanding.

7. Chapter: Mechanistic understanding on the granulation effect of brown coal with urea on the dynamics and mineralisation of N in soil-plant systems

7.1. Introduction

Application of brown coal (BC) and humic substance rich products in agricultural crop production is increasing due to the potential benefit for improved soil fertility and crop productivity. Moreover, such organic materials, containing high levels of humic acids, provide ion exchange groups that complex or adsorb nutrients, as well as improving soil properties (Dick et al., 2002). One of the important properties of BC is its high surface area for adsorption of nutrient ions on its porous surface (Karczewska et al., 1996a). Also, the porosity of BC influences water and air conductivity which is important for controlling the nutrient cycling processes in soil. In addition, BC can provide a high surface area for microbial colonisation and as a growth substrate for microbes (Cohen and Gabriele, 1982).

Granulation of BC with urea can play an important role in the global N cycle by influencing the distribution, bioavailability and ultimate fate of fertiliser-N. It is evident from the results presented in previous chapters that granulation of BC with urea significantly reduced fertiliser N losses and retained the fertiliser-N over a longer period of time by decreasing the release rate of fertiliser-N. Moreover, granulation of BC with urea substantially decreased leaching and gaseous losses of N and resulted in increased availability and uptake of N by crop plants. The increased N uptake facilitated improved crop growth and produced higher yields. Several studies have proposed mechanisms for the observed effects of BC in N retention and transport in soil-plant system. For example, Bollmann and Laanbroek (2001) assumed that BC may reduce volatilisation loss of N from urea fertilised soil by inhibiting the urease enzyme activity, thus increasing N availability to crop plants. Similarly, Dong et al. (2009) hypothesised that BC can incorporate N into its structure either directly through chemical reactions or indirectly through microbial activities and subsequent decomposition of microbial biomass. Together these studies indicate that granulation of BC with urea could be a potential option for increasing fertiliser-N use efficiency by minimising its losses.

The dynamics of N movement in the agroecosystem are very complex and controlled by interdependent chemical and biological processes. The role of BC and coal derived products in determining plant available N, growth and yield is well established. However, the mechanisms responsible for decreasing gaseous and leaching losses of N by BC are largely unknown and dependent on the individual properties of both BC and soil. To the best of our knowledge, no studies have been published to understand the potential mechanisms responsible for reducing N losses via different pathways and increasing N availability to crop plants due to granulation of urea with BC. However, a clear understanding is a prerequisite for the large scale application of BCU granules as a slow release and enhanced efficiency N fertiliser for agricultural crop production. This thesis chapter will discuss some of these potential mechanisms to understand the effect of BC in N retention, mineralisation and movement in soil-plant systems.

7.2. Mechanism 1: Adsorption of different forms of N onto BC

The use of BC as a carrier of fertiliser by blending with any forms of N fertilisers depends on the adsorption behaviour and chemical reactivity of different forms of N with BC. For physical adsorption, the volume, size of pores and surface area of BC are important and play a significant role. Accordingly, it is necessary to examine the adsorption and desorption behaviour of BC with different forms of N.

7.2.1. Methodology

7.2.1.1 Collection and preparation of BC

Air-dried Morwell coal was supplied by Feeco International, Pakenham, Victoria. The physical and chemical properties of BC used in this adsorption and desorption study are shown in Chapter 2. The BC was finely ground and sieved to <2 mm before use.

7.2.1.2 Preparation of N solution

Different stock solutions containing 30 g N L^{-1} were prepared separately using analytical grade urea, di-ammonium phosphate (DAP), potassium nitrate and ammonium sulphate by adding deionised water. The stock solution was diluted with deionised water to make solutions with N concentrations of 1, 2, 5, 10, 15 and 20 g N L^{-1}for the adsorption study.

7.2.1.3 Batch equilibrium, adsorption and desorption of N onto BC

A stock solution containing 30 g N L^{-1} was used in the batch equilibrium study. Therefore, 0.9 g N (30/1000 x 30) was added to each 50 mL falcon tube containing 5 g of BC and shaken on a platform mixer at 180 rpm for 1, 2, 4, 8, 12, 16, 20 and 24 hours at room temperature to establish the equilibrium point. The experiment was conducted using five replicates. The equilibrium point was calculated based on the maximum amount of N adsorbed. After this point no more adsorption onto BC was monitored with the increase in shaking time period. The tubes were centrifuged and the supernatant was collected and stored at 4°C until analysis. Based on the equilibrium point, the adsorption and desorption study was carried out at 20 hours of shaking. The adsorption study was undertaken following the same shaking procedure with the ratio of BC to stock solution, as described above. After collecting the supernatant, the pellets were kept for the desorption experiment. In the desorption experiment, 30 mL of deionised water was added into each falcon tube and was shaken again for 20 hours in the same way described above. The sample was centrifuged and supernatant collected to measure the amount of N desorbed from BC matrix. To observe the effect of pH on the adsorption of different forms of N onto BC, the pH of the stock solution was adjusted to 4.5, 7.0 and 9.5 by adding 1 M HCl or 5M NaOH to the stock solution.

7.2.1.4 Pellet and supernatant analysis

The supernatant was analysed for urea, and NH_4^+ and NO_3^- N. NH_4^+ was quantified by reacting with salicylate and hyphochlorite in a buffered alkaline solution contain sodium nitroprusside as a reductant (Forster, 1995). Nitrate plus nitrite was determined by reduction using vanadium (III) combined with detection by acidic reaction (Miranda et al. 2001). Urea was measured colorimetrically using P-dimethylaminobenzaldehyde following the method of Singh and Saksena (1979).

7.2.2. Results

7.2.2.1 Batch equilibrium

The data presented in Figure 7.1 showed that for all forms of N the equilibrium point was reached 16 hours after shaking. Based on this equilibrium point the shaking time for all the adsorption studies was carried out for 20 hours.

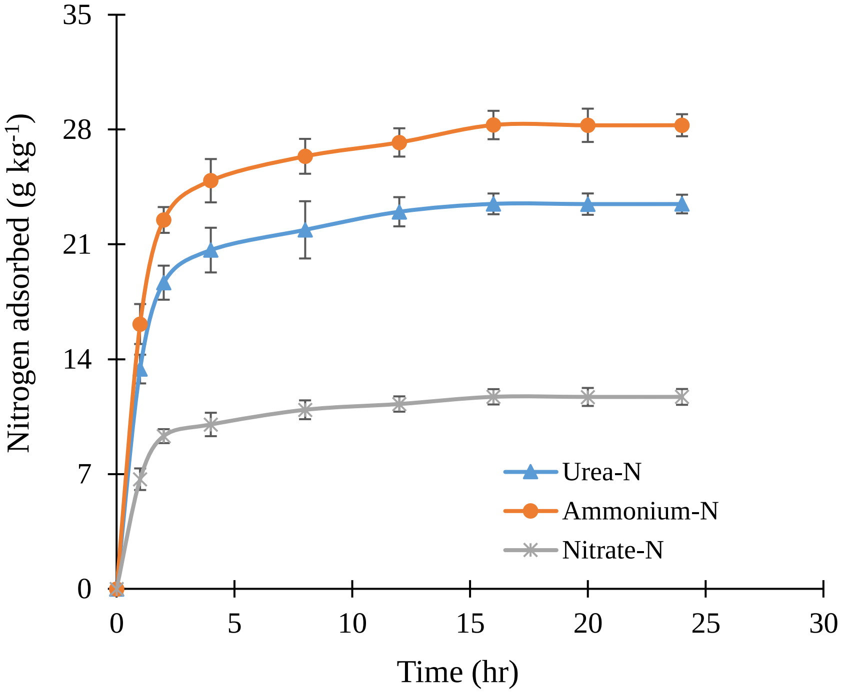


Figure 7.1. Batch equilibrium of different forms of N adsorption onto BC

7.2.2.2 Adsorption of different forms of N onto BC

The data revealed that the adsorption of different forms of N onto BC increased rapidly with the increase in initial N concentration and then the adsorption rate declined gradually to reach its equilibrium over time (Figure 7.2). The N sorption capacity of BC was much greater for NH_4^+-N compared to all other forms of N. The adsorption of urea-N was also much higher than the DAP and NO_3^--N. The difference in NH_4^+-N adsorption between ammonium sulphate and DAP might be due to effect of pH of the respective solution. The presence of NH_4^+ in the supernatant of the urea adsorption treatment indicates the partial hydrolysis of urea releasing NH_4^+. The higher N sorption as urea might be due to fact that both urea and NH_4^+ were adsorbed onto BC whereas for DAP, only NH_4^+ was adsorbed.

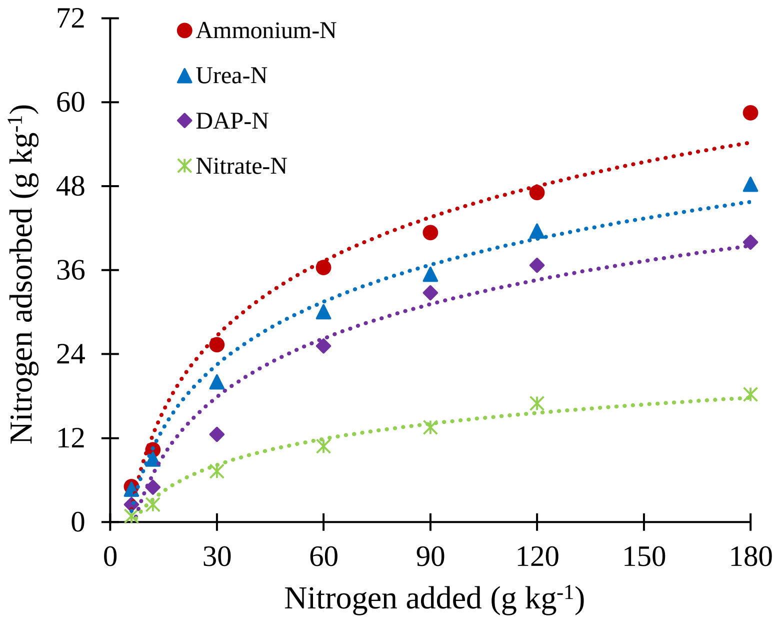


Figure 7.2. Adsorption of different forms of N onto the BC at respective solution pH

7.2.2.3 Desorption of different forms of N from BC

A desorption study was carried out to measure the amount of N adsorbed onto BC after it was thoroughly washed with deionised water. On average, about 56% NH_4^+-N, 63% urea-N, 64% DAP and 94% NO_3^--N was desorbed from BC to deionised water from the initially adsorbed N (Figure 7.3). The results suggest that mechanisms, such as chemical sorption by ion exchange and physical adsorption on the porous surface area of BC, are involved in the adsorption process. In general, the adsorption potential of BC was much higher for NH_4^+ than other forms of N. The presence of large number of acid functional groups of BC and cation exchange of NH_4^+ with these functional groups is likely to be the main reason for its increased adsorption. The hydrolysis of urea to NH_4^+ and its subsequent adsorption through cation exchange could be the cause of higher adsorption of urea on to BC. Ammonium, urea and DAP all released NH_4^+ in the solution directly or indirectly which could be held strongly by BC as a result of cation exchange and less readily desorbed from BC. The overall results of the adsorption and desorption study suggest that urea is a better option and also has over twice the N concentration compared to DAP for blending with BC. Therefore, formulating fertiliser blends of BC and urea could serve as a fertiliser with combined properties that will enable both rapid and slow release behaviour.

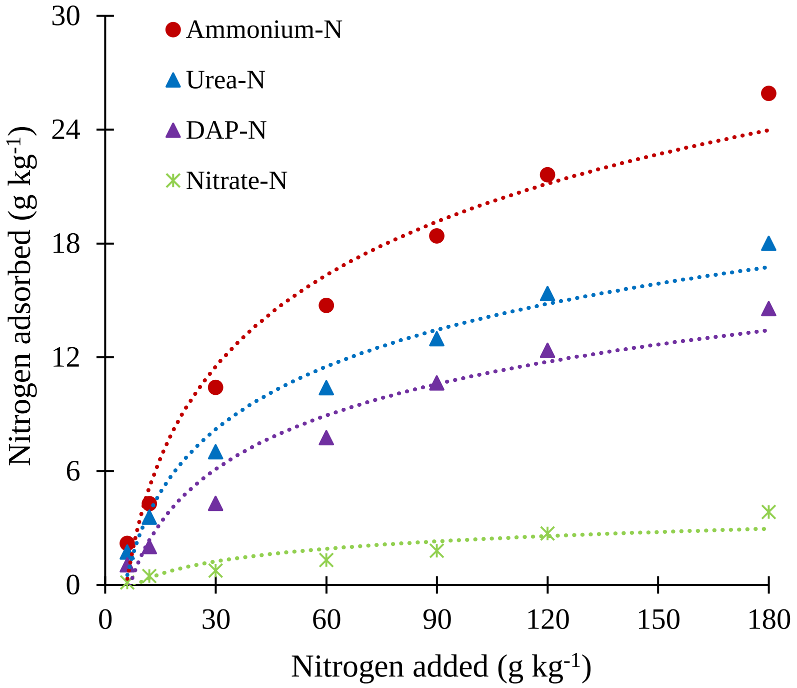


Figure 7.3. Adsorption of different forms of N onto BC after desorption with deionised water

7.2.2.4 Effect of solution pH on the adsorption of different forms of N onto BC

The results presented in Figure 7.4 revealed that the adsorption of different forms of N was substantially influenced by solution pH. Among the different forms of N, the adsorption of urea, DAP and NH_4^+-N was considerably affected by the solution pH. However, the adsorption of NO_3^--N did not vary significantly with the change in solution pH. The adsorption of N as urea, DAP and NH_4^+-N was substantially lower at solution pH 4.5 and increased considerably at solution pH 7.0 followed by a sharp decrease at solution pH 9.5. The higher adsorption of N as urea, DAP and NH_4^+-N at pH 7.0 might be due to increased cation exchange and availability of binding sites due to enhanced protonation of functional groups of BC. In contrast, the decreased N adsorption at pH 9.5 could be results of higher N losses as NH_3 volatilisation which increase considerably above pH 7.0. Similar findings were also reported by Kithome et al. (1999) who reported that the adsorption of NH_4^+ increased linearly with the increase in solution pH up to 7.0. The increased ionic competition for the binding sites at higher pH due to release of Na^+ could be another reason for less NH_4^+ adsorption at pH 9.5. This suggests that the adsorption of N as urea, DAP and NH_4^+ occurred both physically and chemically, because chemical adsorption was influenced by solution pH. However, for NO_3^--N, physical adsorption was the dominant process and as a result, was not influenced by the solution pH.

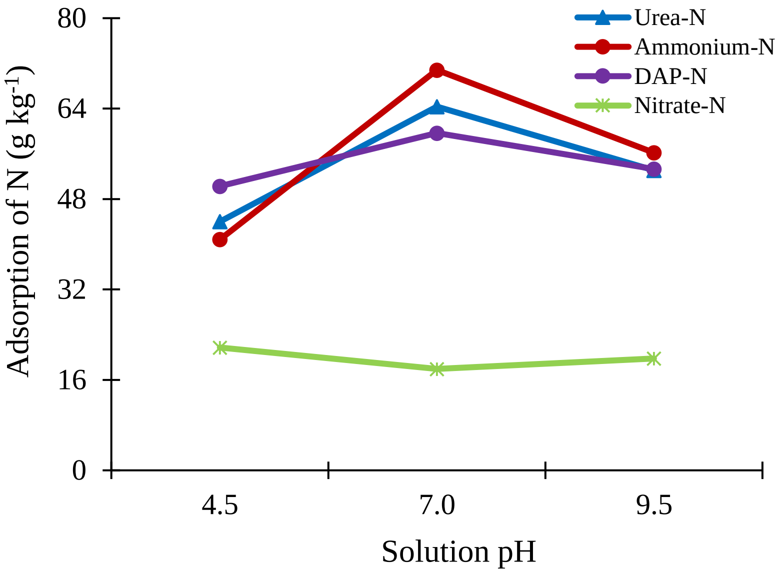


Figure 7.4. Effect of solution pH on the adsorption of different forms of N onto BC

7.3. Mechanism 2: Retention of N with the acid functional groups of BC by cation exchange reaction

The cation exchange capacity and the presence of numerous acid functional groups in BC can play a significant role in the retention of N as NH_4^+-N. A cation exchange study was carried out to measure amount of NH_4^+-N exchanged with the acid functional groups and binding sites of BC.

7.3.1. Methodology of cation exchange study

A NH_4^+-N stock solution containing 10 g N L^{-1} was prepared by dissolving ammonium sulphate in deionised water. Thirty mL of NH_4^+-N solution was added to each falcon tube containing 5 g of BC and shaken on a platform mixer at 180 rpm for 20 hours at room temperature to saturate the cation exchange sites of BC with NH_4^+-N. Then, the tubes were centrifuged and the supernatant was discarded. The NH_4^+ saturated BC was then washed with deionised water several times to remove the physically bound NH_4^+ from the porous surface of BC. Finally, 30 mL of 2 M potassium chloride (KCl) solution was added to each falcon tube containing BC saturated with NH_4^+. The falcon tube was then shaken for 1 hour to displace the exchanged NH_4^+ with K^+, centrifuged and the supernatant was collected to measure the amount of NH_4^+ displaced from BC matrix by K^+. The experiment was replicated five times. The NH_4^+-N concentration was measured colorimetrically following the same method discussed in Section 7.1.1.4.

7.3.2. Results

The results revealed that 1 kg of BC has the capacity to retain about 8,500±300 mg (49.12 $cmol_c\ kg^{-1}$) of NH_4^+-N on the binding sites, determined by the cation exchange reaction. The results clearly suggest a mechanism of strong retention of N as NH_4^+ by BC which could both reduce the mobility, and hence leaching of N in soil and increase the availability of N in soil for longer periods of time. The strong retention of N by BC will also help reduce the release rate fertiliser-N from the BCU granule in soil.

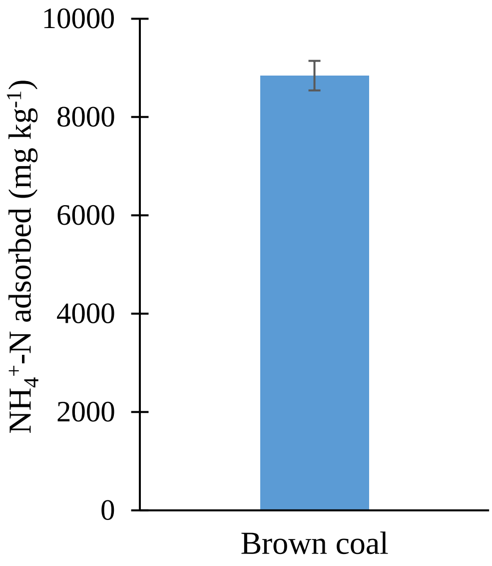


Figure 7.5. Amount of NH_4^+ exchanged with the cation exchange sites of BC

7.4. Mechanism 3: Reduction of urea hydrolysis by inhibiting urease enzyme activity

Urea is the most commonly used synthetic N fertiliser and its release is greatly influenced by urease enzyme activity in soil. Usually after soil application, urea is rapidly hydrolysed to NH_3 and subsequently forms NH_4^+ through soil urease activity. During this transformation process, a large part of added urea is lost via NH_3 volatilisation depending on environmental conditions, pH, soil moisture and urease activity of the system (Suter et al., 2011). Many attempts have been made to find compounds that inhibit urease activity in soil to minimise the volatilisation loss of N (Kobashi et al., 1975; Patil and Kshirsagar, 2006; Pugh and Waid, 1969; Tarun et al., 2005). Numerous compounds have been identified that reduce the urease activity in soil. Many of the compounds which contain phenolic groups and quinones were found to be particularly effective (Bremner and Douglas, 1971) urease inhibitors. BC itself contains high amounts of phenolic groups (Kwiatkowska et al., 2008) and hence, possesses urease enzyme inhibiting properties, thus reducing urea hydrolysis in soil (Dong et al., 2009).

7.4.1. Methodology

A 7 day incubation study was undertaken in polyvinyl chloride (PVC) tubes of 15 cm height and 4.25 cm radius with a volume of 851.18 cm^3. The equivalent of 1200 g dry soil was added to the tubes to reach a bulk density of 1.4 g cm^{-3}. The physical and chemical properties of the soil are presented in Table 2.2 in Chapter 2. Thirty five PVC columns were covered at one end with nylon mesh and then packed with 1 cm of washed sand and soil. Water was added to the soil to obtain a moisture content of 60% water filled pore space (WFPS). The soil was pre-incubated at 22±1°C for one week to restore the microbial activity (Figure 7.6). After this, the topsoil was amended with 250 mg N kg^{-1} using straight urea, BCU 1, BCU 2, BCU 3 and BCU 4 at a depth of 5 cm, to give a final nominal delivery of 300 mg of N per column. Two controls were also included: one contained raw BC at a rate equivalent to the BCU 4 and an unfertilised control. The experiment was laid out following a completely randomised design with five replicates. After 7 days the soil was removed from each PVC tube and taken to the laboratory to determine urease enzyme activity in each column. The urease enzyme activity in soil was measured according to the method of Kandeler and Gerber (1988). The details of urease enzyme measurement was described in section 5.2.6 of Chapter 5.

Figure 7.6. Incubation of soil in PVC tubes at 22±1°C in the glasshouse

7.4.2. Results

The urease enzyme activity in soil was significantly influenced by the addition of different N fertilisers (Figure 7.7). Urease activity in soil amended with BCU granules was significantly lower than the soil fertilised with commercial urea. Among the different BCU granules, the granules containing higher amounts of BC showed reduced urease activity in soil compared to the granules having the lower proportion of BC. This indicates, the potential role of BC in inhibiting the urease activity in soil. A similar finding was reported by Dong et al. (2009) who found that incorporation of humic acid to soil significantly inhibited urease enzyme activity in soil.

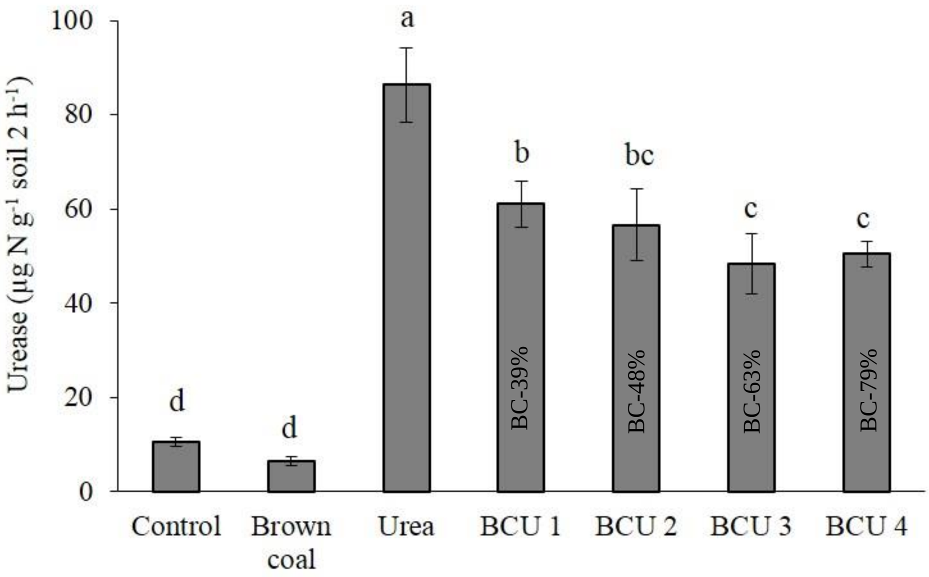


Figure 7.7. Urease enzyme activity in soil 7 days after amendment with various N fertilisers (values are mean ± standard error, N = 5)

7.5. Conclusions

Brown coal-urea granules can be used as an enhanced efficiency N fertiliser with both instant and slow release properties. The adsorption and desorption study results indicated that different forms of N adsorbed onto BC both physically, on the extensive porous surface area, and chemically, by ionic exchange as NH_4^+ with the acid functional groups in the BC. The physically adsorbed N could be available for plant uptake initially after BCU addition to soil whereas the chemically exchanged N as NH_4^+ could be available to crop plants over a longer period of time due to strong retention by BC. On the other hand, reducing urea hydrolysis by inhibiting the urease enzyme activity could also decrease the rate of fertiliser-N release in soil. The mechanistic study supports the potential role of BC in fertiliser-N retention over a longer period of time and reduction of fertiliser-N losses via different pathways compared to commercial urea. This research has increased our understanding on how BC can be used as a substrate to develop enhanced efficiency slow release N fertilisers and decrease adverse environmental impacts.

8. Chapter: Conclusions and future research

8.1. General discussion

Increasing the use efficiency of N fertilisers is a major concern world-wide because of the detrimental effect of nitrogen losses on the environment. A gradual release of mineral N from the fertiliser may reduce the off-site movement of mineral N, thereby enhancing N supply to crops and minimising negative off-site impacts. This study has demonstrated a novel approach involving the granulation of brown coal (BC) with urea, thus giving a product with both slow release and immediate nutrient availability properties. BC was shown to delay fertiliser N release due to its strong retention attributed to its extensive surface area, porous structure and acidic functional groups, particularly carboxyl and phenolic groups. It is also known that the addition of BC alone or in combination with urea can substantially influence the release, gaseous and leaching losses of fertiliser-N in soil (Paramashivam et al., 2016; Rose et al., 2016; Saha et al., 2017; Sun et al., 2016).

Determination of the interactions between N and organic matter, and the effect on the losses and use efficiency of fertiliser-N is a topic which has received significant attention over the past 50 years (Atkinson et al., 1958; Saha et al., 2017). This thesis represents a continuity of research works utilising current technologies, to establish a link among the fundamental processes involved in the N dynamics in soil and how they relate to plant growth and N uptake.

This thesis illustrates the findings of an interdisciplinary study on the preparation, characterisation and N release from different BCU granules, and their effect on the N dynamics in various soil types, key soil health indicators, yield and N use efficiency of several crop plants under glasshouse and field conditions. The majority of the research was conducted in soil-plant systems, where there were challenges such as variability in properties and composition of soils used, sampling from highly heterogeneous environments.

The preparation and characterisation of different BCU granules was illustrated in Chapter 2. Initially, urea and BC were successfully blended and granulated using pan granulation technology following four different C:N ratios. The granules formed were of an appropriate size and sufficiently strong (2.38 to 6.69 kg) for agricultural use meeting the fertiliser standards. For agricultural use the granules crush strength should be at least 1.4 kg, and a crush strength ≥ 2.27 kg is highly desirable (Hignett, 1985). The loading of urea-N into BC was confirmed by FTIR, NMR and XRD characterisation. Granulation of urea with BC delayed fertiliser N release, enhanced N retention in the granule and increased the moisture holding and retention capacity of soil compared to commercial urea. Nitrogen retention increased concurrently with BC loading, emphasising the significant role of BC in reducing N losses. This research has increased our understanding of how BC can be used as a substrate to develop improved slow release N fertilisers with a lower environmental hazard.

In Chapter 3, the effect of BCU granules on the dynamics and mineralisation of N in various soils were monitored in series of soil column leaching incubation experiment. The results revealed that granulation of BC with urea showed a considerable effect on the dynamics and mineralisation of N by influencing the distribution, bioavailability and ultimate fate of N in soil. Blending of urea with BC slowed down the release of fertiliser N and substantially increased mineral and mineralisable N in soil by decreasing its gaseous and leaching losses. The granules with a higher proportion of BC had lower leaching and gaseous emissions and maintained higher mineral N in soil, emphasising the role BC in improved N retention. Overall, these findings provide strong mechanistic support for the development of BCU granules as an enhanced efficiency and slow release nitrogenous fertiliser for improved N retention in the plant-soil system over a longer period of time.

Chapters 4 and 5 mainly focused on the effect of BCU addition on the growth, yield and N use efficiency of silver beet in glasshouse (Chapter 4) and sweet corn in field conditions (Chapter 5). Soil incorporation of BCU exhibited considerable effects on the growth, yield, use efficiency and N uptake by the crop plants studied. Addition of BCU significantly reduced the hydrolysis of urea-N by inhibiting the urease and Leu-AP enzyme activity. The slow and steady release of fertiliser-N in the BCU amended soil increased the availability of mineral N throughout the whole growing season, enhancing N uptake by crop plants, and N content in the plant components.

In Chapter 6, a ^{15}N study was conducted to observe the fate, distribution and recovery of applied fertiliser-N in the soil-plant system. Application of N fertiliser as BCU considerably influenced the recovery of ^{15}N in the soil-plant system. Addition of BCU showed the lowest urea-derived ^{15}N losses from soil compared to sole application of urea. Moreover, soil incorporation of BCU increased immobilisation of the applied fertiliser-^{15}N due to stimulated microbial activity. Microbial immobilisation of the applied fertiliser-N not only lowered its losses, but also led to the higher retention of the applied fertiliser-N in the soil for longer periods of time. Soil treated with BCU substantially enhanced fertiliser-N use efficiency over urea. The overall results suggest that addition of slow release and enhanced efficiency BCU could reduce N fertiliser application rates by 25% without any loss of crop yields as well as enhanced fertiliser-^{15}N recovery while diminishing its losses to the environment.

Brown coal has previously been explored as a potential substrate to prepare a slow release N fertiliser by exploiting its extensive reactive surface area and humic acid content (Rose et al., 2016). Addition of BC to soil altered N cycling and dynamics in soil (Kim Thi Tran et al., 2015; Rose et al., 2016). It was evident from the adsorption study and structural analysis (FTIR, XRD and NMR) that BC can adsorb N physically onto the porous structure and chemically through cation exchange with the carboxyl and phenolic groups. Moreover, the slow release of urea-N from the BCU granules might also be the result of decreased urea hydrolysis and mineralisation due to the inhibition of urease and Leu-AP enzyme activity by BC. The delayed N release from BCU granules is in good agreement with the findings of Rose et al. (2016) who reported that granulation of BC with urea reduced the release rate of N in soil, resulting in N retention over a longer period of time.

The strong retention and slow release of fertiliser-N by BC resulted in significant decreases in gaseous (N_2O and NH_3) and leaching losses of N in soil. The results of our study are in close agreement with the results of Sun et al. (2016) and Chen et al. (2015) who also reported that soil incorporation of lignite reduced NH_3 volatilisation by up to 68% from cattle manure amended pens compared to control pens where no lignite was added. In addition, the enhanced microbial immobilisation of applied fertiliser-N could also decrease leaching potential by reducing the mobility of N in soil profile.

Increased fertiliser-N uptake by plants in the BCU fertilised soil was associated with the enhanced and prolonged availability of mineral N throughout the growing season of the crop. The growth and yield benefit obtained from BCU fertilised soil was mainly the result of increased availability and uptake of added fertiliser-N, because no significant variation was monitored in the soil derived N among the urea and BCU treatments. The increased fertiliser-N uptake and higher yield might be responsible for increasing fertiliser-N use efficiency of crop plants grown in BCU amended soil. This was supported by the ^{15}N recovery data which showed that the addition of BCU had the highest recovery of applied fertiliser-N from plant and soil. A similar finding was also reported by Choi et al. (2001) where the combined application of compost and ^{15}N urea substantially increased the recovery of applied fertiliser-N by plant and retention in the soil. The result of this study indicated that the granulation of BC with urea promoted the transport of absorbed ^{15}N from soil to plant.

8.2. Conclusions

The overarching aim of this study was to prepare and characterise different BCU granules, observe their effect on soil health, and on the growth, yield and N use efficiency of several crop plants both in glasshouse and field conditions to determine a suitable and sustainable alternative to commercial N fertilisers with slow release and enhanced efficiency properties with a view to minimise the N losses in the environment. A number of techniques and series of study designs were conducted to address this research question.

BCU granules were produced successfully using pan granulation technology with similar properties to commercial N fertilisers. The results revealed that granulation of urea with BC substantially delayed the release of fertiliser-N due to its strong and prolonged retention on the porous structure and cation exchange with the acid functional groups of BC. The FTIR, NMR and XRD characterisation data also supported the loading of urea-N onto BC. Granulation of BC with urea showed a considerable effect on the dynamics and mineralisation of N by influencing the distribution, bioavailability and ultimate fate of N in soil. Compared to urea, the application of BCU substantially increased mineral and mineralisable N in soil by decreasing its gaseous and leaching losses. As a result, greater amounts of fertiliser N were available to crop plants over a longer time period. In addition to N retention, the reduction in hydrolysis and mineralisation, and slow and gradual release of urea-N due to the inhibition of urease and Leu-AP enzyme activity could be another reason for increasing fertiliser-N availability in BCU amended soil. The reduction of N losses and its retention increased concurrently with BC loading, emphasising the significant role of BC in N retention in the soil environment.

Addition of BCU significantly increased the availability and uptake of fertiliser-N by crop plants which facilitated better growth and contributed significantly higher yield over the sole application of urea. The increased fertiliser-N uptake and higher yield might be responsible for increasing fertiliser-N use efficiency and ^{15}N recovery by crop plants grown in BCU amended soil. The increased yield and reduced gaseous losses of N makes the BCU blends more economic, profitable and with improved environmental outcomes compared to conventional urea. It appears that the efficacy of commercial urea can be improved substantially by granulating it with humified organic matter like BC. Brown coal-urea has been demonstrated to act as an enhanced efficiency fertiliser that offers a balanced N release pattern to plants with immediate and slow release properties, providing soil organic matter for improving soil health and microbial activity. Therefore, granulation of BC with urea could be a promising alternative to commercial urea alone to enhance urea-N efficiency and crop yield in a sustainable manner while decreasing N losses and its potentially negative impacts on the environment.

8.3. Future research

In this study, only one kind of BC was used to prepare the BCU granules. Other low rank coal could be used, as well as other sources of organic matter with a high content of humic substances (e.g. peat, compost). It is well known that the efficacy of any N fertiliser depends on many factors including crop types, soil properties, climatic conditions and application methods. Other crop and soil types need to be tested in field studies in different climatic conditions. The potential to include other plant nutrients in the formulation is an option, particularly nutrients (e.g P and K) that might affect N uptake (Wu et al., 2005). Additional field and laboratory studies can also evaluate the effect of these fertiliser blends on soil health, particularly the effects on microbial populations and associated enzyme activity in the soil. Such research work is likely to be scientifically rewarding and industrially relevant.

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Appendices

Appendix 1. *P*-value results from two-way ANOVAs for all response variables

Studied parameters	Factor		
	Fertiliser	Soil	Fertiliser × Soil
Plant height	0.000	0.000	0.086
Leaves plant^{-1}	0.000	0.815	0.422
Fresh biomass yield	0.000	0.000	0.000
Dry biomass yield	0.000	0.000	0.000
Fresh root weight	0.000	0.000	0.000
Dry root weight	0.000	0.000	0.000
Total chlorophyll	0.000	0.000	0.000
Shoot N	0.000	0.000	0.000
Root N	0.000	0.018	0.030
Shoot C	0.221	0.129	0.253
Root C	0.227	0.133	0.132
N uptake	0.000	0.000	0.000
Cumulative N_2O	0.000	0.000	0.000
Cumulative NH_3	0.000	0.000	0.000
Soil ammonium	0.000	0.053	0.051
Soil nitrate	0.000	0.020	0.266
Soil PMN	0.000	0.000	0.000
Soil total N	0.063	0.722	0.326
Soil total C	0.000	0.000	0.006
Post-harvest soil pH	0.016	0.091	0.030
NUE	0.000	0.000	0.000
FNUE	0.000	0.000	0.000

Appendix 2. Weather data of experimental site during the growing period of sweet corn