EFFECT OF LONG-TERM APPLICATION OF NITROGEN AND LIMING ON SOIL CARBON DYNAMICS IN A SEMI-ARID GRASSLAND

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DECLARATION

I, Kwenama Buthelezi, hereby declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original work.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.

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As the candidate's supervisor, I have/~~have not~~ approved this dissertation for submission.

Signed [redacted]

Dr NN Dube ……………………………………..Date: 22/01/2022

DISSERTATION OUTPUT

Results from Chapter 3 and 4 have been published in a peer-reviewed journal. The article citation is:

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ABSTRACT

Grassland ecosystems cover up a considerable portion of the earth surface (40 %) and provide a range of ecological services. These ecosystems are mostly limited by nitrogen and phosphorus and thus often supplemented with fertilizers and lime to maximize grassland productivity. Upon addition of mineral fertilizer, biomass production is improved and subsequently more return of carbon in a form of plant litter. This addition may result in a change in soil fertility subsequently affecting biomass production, stoichiometry and soil carbon (C) dynamics. The long-term addition of nitrogen and lime influences soil biogeochemical properties, and potentially hindering the sustainability of the ecosystem. The study was done at Ukulinga long-term (70 years) fertilized veld to examine the effect of long-term application of lime and nitrogen fertilizer on plant-soil interactions in relation to nutrient cycling and soil carbon storage dynamics. This study focused on nitrogen applied annually as ammonium nitrate and ammonium sulphate at 70 and 211 kg N/ha and lime applied as dolomite at 2250 kg/ha every five years. Ten treatments were selected: (1) control (0 lime or N fertiliser kg/ha), (2) L = lime (2250 kg/ha), (3) AS70 = ammonium sulphate at 70 kg/ha ; (4) AS211 = ammonium sulphate at 211 kg/ha ; (5) AN70 = ammonium nitrate at 70 kg/ha ; (6) AN211 = ammonium nitrate at 211 kg/ha ; (7) AS70L = ammonium sulphate at 70 kg/ha + lime; (8) AS211L = ammonium sulphate at 211 kg/ha + lime; (9) AN70L = ammonium nitrate at 70 kg/ha + lime and (10) AN211L = ammonium nitrate at 211 kg/ha + lime. Soil samples were taken in the autumn of 2020 using soil auger at 0-10 cm depth, sieved using (2mm sieve) and stored in plastic jars. Soil analysis was conducted at Cedara Soil Fertility Analytical Service for the analysis of extractable phosphorus (P), exchangeable bases, total cations, exchangeable acidity, acid saturation, trace elements (zinc (Zn), copper (Cu) and Manganese (Mn)) and particle size analysis. Aggregate stability was done using the wet sieving method and aggregate associated C was analysed using the Walkley-Black method. Plant samples were taken by cutting 0.25 x 0.25 m^2 area in each of the chosen plots dried on an oven and measured. Roots samples were taken using soil core, washed the soil and dried the roots to a constant mass. Plant tissue was analysed for carbon (C), nitrogen (N) and phosphorus (P).

Long-term nitrogen addition affected the soil properties and enhanced plant biomass production. Nitrogen fertilization increased soil acidity, reduced exchangeable bases and cation exchange capacity, while lime increased soil pH and exchangeable bases concentration. Total soil organic carbon was not changed by nitrogen and liming. Overall, studied soils had very high total soil organic carbon ranging from 49.7 – 57.6 g/kg across treatments. Nitrogen fertilization significantly decreased large macro-aggregates (>2 mm) and increased micro-aggregates (53–250 μm) while liming did not significantly affect water-stable aggregates (WSA) compared to the control. While aggregate stability decreased under long-term nitrogen addition, C stored in micro-aggregates increased. Liming increased aggregate stability and with high C stored in large macro-aggregates compared to N fertilised only treatments. Lack of response of total carbon concentration and stocks suggests possible carbon saturation. However, changes in C associated with differently sized aggregates suggests that long-term N fertilization and liming resulted in the redistribution of carbon. Above ground and below ground production was enhanced by nitrogen application with alteration of plant elemental stochiometric ratio. Lime and N decreased below-ground C:N ratio while shoot C: N was increased by AS70. Root N:P was increased by N and N combined with lime treatments. The correlation between plant production and soil parameters was very weak. Aggregates stability in this study seemed to be a function of exchangeable bases rather than carbon. In conclusion, long-term nitrogen application caused soil acidification and had a detrimental effect on soil structure due to loss of basic cations and NH_4^+ induced dispersion thus hindering the long-term sustainability of the ecosystem. While N fertilisers improve plant productivity, the addition of lime to N fertilised grasslands can help maintain good soil condition

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Grassland ecosystems cover up a large portion of the earth surface (40 %) and provide a range of ecological services (ES) (Hewins et al., 2018). Various management practices are implemented to maximize resource utilization and optimise delivery of grassland ES. These management practices include grazing, mowing, burning and fertilization which have been shown to influence the delivery of (i) regulating (mitigation of greenhouse gas emission and soil organic carbon sequestration) and (ii) supporting (nutrient cycling) ES in grasslands (Conant et al., 2017; Maia et al., 2009; Dubeux et al., 2007). The provision of ecological services by grasslands depends on the climate, temperature, soil type and management (Zhang et al., 2013b).

In Southern Africa, 23 % of the grassland is under cultivation, 60 % has been irreversibly transformed, and 2 % is protected, the remaining area is used as livestock rangeland (O'connor and Kuyler, 2009; Fairbanks et al., 2000). Several grassland practises are reported to increase soil C stocks however, it is not always the case because of the function of climate, soil, and vegetation characteristics shaping soil C stocks responses (Conant et al., 2017). Across the managements, the main driving indicator of positive response of C stocks is enhanced net primary production (NPP), however, disturbances such as overgrazing, fire, invasive or any management involving removal of plant biomass and reduced net primary production could cause a decline in soil carbon stocks (Conant, 2010). Soil disturbance for agricultural purposes imposes a detrimental impact on greenhouse gas emission which has been reported that in South Africa agricultural sector alone emits 29.62 million tonnes of greenhouse gases (Ritchie and Roser, 2020).

Any management techniques aiming to increase forage production have the potential to increase soil C stocks, by sequestering atmospheric C in the soil. Irrigation, fertilization, and intensive grazing are improved methods to enhance productivity and reduce the amount of C input removal, promoting more C inputs or below-ground allocation, thus increasing C stocks (Conant et al., 2001). Fertilization increases below-ground as well as above-ground production which both leads to high soil C. Poeplau et al. (2018) showed that 1.15 kg N is needed to sequester 1 kg of SOC in temperate grassland, which was in line with the relationship reported by Kätterer et al. (2012) in croplands. There are several sources of fertilizer applied in nutrient-deficient grassland ecosystems. Nitrogen fertilizer application is well documented on grasslands (Chen et al., 2020; Li et al., 2019; Li et al., 2018), with a positive response on biomass production and below-ground allocation. Numerous studies have reported increased soil C storage following continuous application of N due to enhanced biomass production and litter returns (Lin et al., 2019, Fornara et al., 2013, Vargova et al., 2020).

Some sources of N such as urea, ammonium-based fertilizers are reported to cause soil acidification, resulting in Al and Fe toxicity and a subsequent decline in exchangeable bases and cation exchange capacity (CEC) (Kidd et al., 2017; Wang et al., 2017 b). This has negative implications on plant production and could cause nutrient deficiency. Furthermore, it could affect microbial activity responsible for OM mineralization which plays a crucial role in nutrient cycling. In this manner, grassland soils are treated with lime to reduce the effect of acidity on nutrient supply.

Liming has long been the common agricultural practice which counteracts soil acidity and helps in sustaining plant production, soil stability and carbon sequestration (Fornara et al., 2011; Holland et al., 2018). There are contrasting evidence of liming on C sequestration, and aggregate stability. This argument comes from the fact that liming increases decomposition and soil respiration due to pH-induced effects on soil microbes (Biasi et al., 2008; Badalucco et al., 1992). The impact of liming on soil C storage varies with soil type, land use, climate, and management factors (Holland et al., 2018). There are well-known benefits of liming such as increased nutrient availability for crops which increases biomass yield, thus, increasing litter returns to the soil. Long-term effect of liming on soil C stocks may have variable outcomes depending on management. For example, co-application of lime and mineral fertilizers reduced C stocks in Rengen Grassland Experiment (Sochorová et al., 2016) and potentially alter plant elemental composition.

Changes in plant composition affect the quality of litter, which in turn could significantly influence soil biogeochemical properties including nutrient cycling, C and N storage (Heyburn et al., 2017b). Nitrogen addition has been shown to increase foliar N content which could decrease C:N, increase N:P ratio. This affects the decomposability of litter when returned to the soil, for example, fertilized and nutrient-rich soil associated with high productivity is linked with high decomposition rates, low plant nutrient use efficiency, and low level of C accumulation (Heyburn et al., 2017b; Wardle et al., 2004). Liming results in enhanced conditions for microbial activity and availability of substrates.

1.2 Justification of the study

Ukulinga grassland trials are one of the longest running trials in Africa which have been running for 70 years (Morris and Tainton, 2002), initially aimed to examine plant species response and productivity. There have since been detailed studies of plant productivity (Le Roux and Mentis, 1986), species diversity (Grunow et al., 1970; Scott and Rabie, 1956), soil biology and respiration (Ward et al., 2017a; Zeglin et al., 2007), soil N and P cycling (Schleuss et al., 2020) conducted on the fertilized veld, with their long-term implications. Studies on soil response have only highlighted the basic characteristic (carbon, nitrogen, pH, cations etc.) without a thorough investigation of carbon and nutrient dynamics in these trials. Long-term implication of fertilization and lime is crucial in understanding the sustainability of fertilized grassland ecosystem. The long-term effect of nitrogen and liming addition on carbon storage and nutrient content needs to be addressed in link with plant productivity.

1.3 Research questions

- What is the long-term effect of nitrogen fertilization and liming on the soil fertility status of Ukulinga grassland?
- How does soil aggregation and soil organic carbon storage respond to long-term N and lime addition?
- How does long-term N and lime addition influence plant-soil interactions in relation to nutrient cycling?

1.4 Objectives

The general objective of this study was to determine the effect of long-term N and lime application on soil C dynamics. The specific objectives were:

- To investigate the dynamics of selected soil fertility parameters under long-term N and lime addition at Ukulinga grassland trial
- To determine the mass of water-stable aggregates and their carbon contents after long-term N and lime addition at Ukulinga grassland trial.
- To examine the long-term effect of N and lime addition to plant nutrient stoichiometry and implications for C cycling.

CHAPTER 2

EFFECT OF GRASSLAND MANAGEMENT PRACTICES ON PLANT PRODUCTION AND SOIL PROPERTIES IN RELATION TO NUTRIENT CYCLING: A REVIEW

2.1 Introduction

Grasslands are among the largest ecosystem in the world estimated to cover up to 52.5 million square kilometres (Suttie et al., 2005) which is 40.5 percent of the terrestrial area and consist of rangelands, shrublands, pastureland and croplands sown with pasture and fodder crops (Rumpel et al., 2015). Most of the natural grasslands have been demolished by cultivation and other land-uses, the remaining grasslands are used for intensive grazing for livestock which affects grassland diversity and can lead to land degradation. Farmers have been facing the dilemma to increase livestock production on a limited grazing land, which shifted the innovation of native management and subjected grasslands to the use of fertilizers and reseeding (Watkinson and Ormerod, 2001). In addition to fertilization, mowing, grazing, burning, and liming are used to improve soil properties to achieve optimum conditions for plant growth (Eze et al., 2018).

Different grassland practices facilitate diverse responses with regard to plant productivity and plant species richness. For example, heavy grazing pressure resulted in a low number of species and specific species types of domination on grazed grassland in Southern Sweden when compared to abandoned grassland (Dupré and Diekmann, 2001). Several previous studies have examined the effect of burning (Zhang et al., 2008), mowing (Yang et al., 2019) and fertilization (Socher et al., 2012) management on plant response and species richness. The common direct effect of these practises includes frequent destruction of plant organs resulting in a decline in sensitive species and enrichment of more disturbance resistant taxa (Grime, 2006). The response of biomass production could reflect the nutrient status and cycling, moreover the overall interaction between soil-plant ecosystem, directly from nutrient removal with biomass harvesting (Oelmann et al., 2009).

Changes in above ground biomass response affect the below-ground processes and thus nutrient cycling. For example, clipping of plant biomass (Mawdsley and Bardgett, 1997) and chronosequence grazing (Holland and Detling, 1990) nearly always reduces root biomass because of reduced C allocation. Seagle et al. (1992) showed that long-term heavy grazing reduced below-ground C allocation and stimulated net N mineralization and plant N uptake. A review by Bardgett et al. (1998) concluded that linkage between above-ground and below-ground interactions may either be an effect of short-term through shift in input of root exudate and long-term effect through alteration of the below-ground biomass, NPP, the quality of plant litter input and the composition of the plant community.

Grassland management systems are believed to influence soil properties, consequently affecting soil quality (Askari and Holden, 2014). Maintaining soil quality and long-term productivity of a sustainable grassland agriculture system has been the main goal (Mayel et al., 2021). Studies have investigated the effect of grazing (Banerjee et al., 2000; Dormaar and Willms, 2000) and fertilization (Hoeffner et al., 2021; Cui and Holden, 2015) on soil properties. Greater knowledge of soil response to grassland management is essential to evaluate different techniques for achieving a long-term sustainable grassland agroecosystem. This review assesses the long-term effect of grassland management on soil physical, biological and chemical parameters to understand how the implementation of various management practices influences soil nutrients, particularly C dynamics.

2.2 Grassland management practices

2.2.1 Grazing

Grazing land consists of vegetated land dominated by either native or introduced plants which have the potential to be grazed by animals (Vallentine, 2000; Allen et al., 2011). Grazing management objective is to manipulate vegetation to maximize animal production and profitability. Animal grazing has mediated effects on plants through poaching, squashing and spatial defoliation which impacts the growth and survival of pasture plant affecting photosynthesis and C supply. There are two recognized contrasting grazing management: extensive and intensive. Extensive grazing management operates on a large area per animal which is mostly self-sustaining and utilizes low resource and labor inputs while intensive grazing management is usually associated with improved pastures which exploit high labor and resource inputs attempting to increase the quality/quantity of forage and thus animal production (Vallentine 2000). It is important to start looking closer at the long-term impact of these management practices given that the duration may be short-term or medium-term depending on the objective of the owner. The question will be how the management practices seem to affect soil directly or indirectly. Stocking rate is important in maintaining sustainable production, of which at continuous grazing, maintaining low stocking rate is crucial to minimize degradation (Greenwood et al., 1997). Higher stocking rates have been shown to negatively affect soil aggregate stability and soil compaction (Yingzhi et al., 2004), however, may increase OM content through dung and urine addition. Therefore, a great stocking rate in intensive grazing is important for supplying readily available nutrients in dung relative to litter, thus increasing nutrient flow and cycling.

2.2.2 Mowing

Mowing is the removal of the above-ground biomass using machines. Mowing is usually done annually to produce hay during winter for livestock. This practice removes nutrients from the soil with the harvested biomass which affects nutrients level and biomass production (Socher et al., 2012). Effect of mowing on plant diversity differs with altitude, grassland type, fertilization rates and mowing dates (Tälle et al., 2016). Species richness under mowing management has been found comparable to that under grazing (Socher et al., 2013). However, mowing is believed to enhance seed germination by removing litter (Socher et al., 2012).

Mowing management is usually characterized by time interval between cuts, frequency and plant cut heights. Cutting of grass at lower plant height (less than 6 mm) reduces root production and depth, while shoot density, shoot growth rate increase at lower mowing heights (Salaiz et al., 1995). This is attributed to higher photosynthate priority for leaves and shoot than to roots. Mowing intervals and frequency varies with management techniques. Grasslands receiving no mineral and organic fertilizers are usually extensively mowed (once or twice per year) unless they are supplemented with fertilizers (Blüthgen et al., 2012). In this manner, mowing is done at the peak of growing season on extensive mowing, of which on intensive mowing management, its meadows are cut up to six times a year while receiving high number of fertilizers (Tallowin and Jefferson, 1999).

2.2.3 Burning

Burning of grasslands is done to manage species composition and production (Anderson and Bailey 1980) and as an alternative for pesticides (Warren et al., 1987) Notably, burning creates competition within the species, however had been reported to increase diversity (Fynn et al., 2004). Timing of burning with seasonal change is very crucial to achieve the objective. Burning is conducted during dormant period to maintain grass species richness (Towne and Craine, 2014). Burning in winter results in decline in grass species richness and lower soil nitrogen than in spring (Fynn et al. 2004).

Maximum fire temperatures and duration is a very crucial variable for plant tissue response. Some studies reports that temperatures >60 ^{o}C are lethal for plant tissue and longer exposure can cause severe effects (Bilbao et al., 2006). Fire temperature may have an influence on seed germination and plant mortality; however, fire intensity has been mentioned as the most important variable in analyzing fire effect on plant community and population (Fidelis et al., 2010). Fire intensity could also relate with rate of spread, temperature, and flame height (Trollope et al., 2002)

2.2.4 Fertilization

Grasslands are commonly located on marginal lands with low soil fertility. To provide the required nutrients, both mineral and organic fertilizers has been widely used in grassland management often in combination with grazing and mowing. Nitrogen fertilization has significantly increased in grassland management since N is the most limiting nutrient for plant growth. Nitrogen addition is linked with high biomass production responses and reduction in species number (Clark and Tilman, 2008). However, it is known that significant amount of N may be lost in intensively managed system through ammonia volatilization, denitrification, and by nitrate leaching (Vermoesen, 1999; Bussink, 1992; François et al., 1992). N losses in grasslands increase with increasing N fertilization (Nevens and Rehuel, 2003), particularly in grazed grasslands. Phosphorus is a non-renewable nutrient; therefore, P fertilizer is also applied. In agricultural ecosystems, fertilizers are commonly applied in combination with others (N:P: K). The application depends on various factors including soil tests, soil type and specific plant requirements. There are several forms of fertilizers, nitrogen can be applied in 3 forms of mineral N: nitrates supply NO_3^- ions, ammonium salts supply NH_4^+ ions, and amides contain N in $-NH_2$ form (Van Cleemput et al., 2008). Phosphorus is applied in combination with N as ammonium phosphate or as single superphosphate.

Measuring fertilizer impact as a single factor is uncomplicated, rather than in combination with other land uses because they tend to interact with each other. For example, mineral nutrients removed in plant by mowing and grazing is supplemented by fertilizer addition, which challenges the observation of soil nutrient responses. In this manner, each of these management differs in level of disturbance posing difficulty in defining land-use intensity independency (Blüthgen et al., 2012).

2.3 Effect of grass management on plant production

2.3.1 Effect of grazing and mowing

Grazing alters species composition, richness, plant traits, and also modifies nutrient cycling directly through defoliation, trampling and dung and indirectly through modification of species composition and interaction (Altesor et al., 2005). This can be attributed to selective grazing where grazers tend to pick palatable and competitively dominant vegetation based on plant quality resulting in reduced diversity (Hobbs and Huenneke, 1992). Han et al. (2008) reported a decline in palatable species under intensive grazing. This is attributed to lessened competition from palatable species and creating of favorable conditions for seed germination benefiting less palatable species (Peco et al., 2005). The intensity of grazing on plant species richness and composition is well known (Arévalo et al., 2007). Milchunas et al., (1994) observed that after 50 years of grazing, heavily grazed treatments had lowest forage production (57 g/m^2 $/yr^1$) compared to light and moderate grazed (71 g/m^2 $/yr^1$ and 60 g/m^2 $/yr^1$ respectively). Therefore, species richness and diversity are a function of grazing intensity and grazer's preference. Altesor et al. (2005) found that grazing increased diversity both in number of species richness and evenness while above ground net primary production (ANPP) was the function of the season. During warm season ANPP declined in grazed sites which was attributed to a possible dominance of cool season forbs posing competition for water and nutrients for the warm season grasses (Altesor et al., 2005). Grassland dominated by more palatable and productive species preferred by grazers, their decline may result in net loss of forage production (Han et al., 2008)

Mowing significantly alters plant and below ground production by creating more favourable light and energy for plant growth (Kitchen et al. 2009). Removal of above ground production could raise questions about nutrient cycling disturbance, and how it affects plant production as plant litter input is reduced. Previous studies show that intensity and mowing heights greatly influence plant production by compensatory growth (Turner et al., 1993; Zhao et al., 2008). Such differences maybe the result of different factors such as nutrient availability, plant species, and environmental conditions. These effects may change over long-term period, by consistently reducing plant litter input causing imbalance in soil nutrients in turn reducing plant production. Yang et al., (2020) showed that optimal mowing height for biomass production and forage yield ranges from 6-12 cm. Cutting grass on lower height removes plant photosynthetic organs completely, damages plant growth points, and increases plant mortality to lower biomass (Hunt, 2001) (Figure 2.1A). Mowing changes species composition (Figure 2.1B) by enhancing growth of non-dominant species by removing competition and improving light interception for lower species. Inconsistency of suitable stubble heights obtained from different studies (Zhong and Bao, 1999) calls for further investigation of mowing stubble height for sustainable grassland ecosystems.

Elemental composition and stoichiometric ratio of plant tissue is affected by mowing and grazing (Turner et al., 1993). This is a result of standing-dead plant shoots which stimulates growth of young shoot with high N and P concentration (Ziter and MacDougall, 2013) and an increase in nutrient uptake, transfer, and translocation from below ground organs (Turner et al., 1993; Jaramillo and Detling, 1988). Consequently, changes in plant elements concentration affects plant C: N:P stochiometric ratios which plays an important role in ecological processes through their effect on plant growth and substrate quality for organisms (Mooshammer et al., 2012).

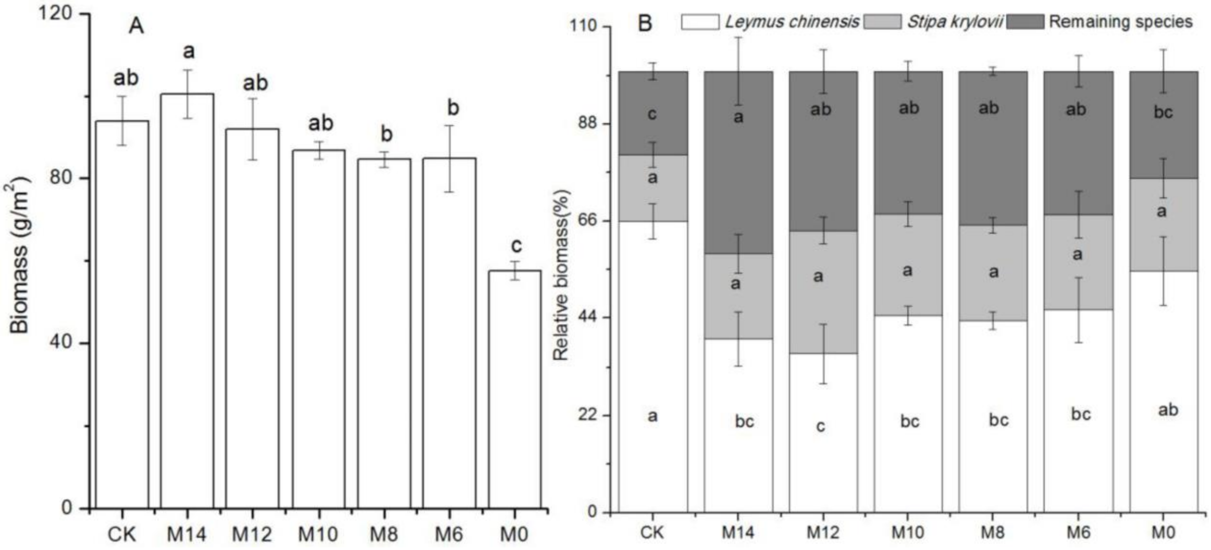

Figure 2. 1 Effects of mowing at different stubble heights during 2013–2016 on plant aboveground biomass production (A) and the relative biomass (%) of two dominant species and remaining species (B) in 2017. (Yang et al., 2020). *Different lowercase letters denote significant difference. Six stubble heights are 14 cm (M14), 12 cm (M12), 10 cm (M10), 8 cm (M8), 6 cm (M6) and less than 0.3 cm (M0) height to ground surface, CK – Control*

2.3.2 Effect of burning

Burning is done seasonally or annually, and plants recovery from burning is ambivalent depending on the species and climate (Pathak et al., 2017; Parmenter, 2008). To assess the production, samples are taken before the next cycle of burning. Annual burning prior to onset of rain in subtropical climate of Northeastern India showed that the burnt site was responsible for mean biomass production rate of 25·4 kg/ha /day which surpasses that of unburned site 8·6 kg/ha/day (Pathak et al., 2017). This trend is also reported by other studies on semi-arid (Alhamad et al., 2012) and temperate grasslands (Brys et al., 2005). They further argue that the peak above and below-ground accumulation rate was during the 30th to 150th days after burning, respectively, supported by the nutrient decline in bulk soil. This explains that during this time plant absorb much of the nutrients released during burning through the ability of chemical conversion of nutrients bound in dead plant tissue, mainly in a form of water-soluble components of ash that become readily available for plants (Hedo et al., 2015; Wang et al., 2016) or result from the effects of soil warming on microbial activity and nutrient mineralization (Henry et al., 2006)

Species richness have been reported to be enhanced by burning in semi-arid grassland (Alhamad et al., 2012) and mantained by correct timing usually during dormant season (Fynn et al., 2004). Fire play an important role in reducing species competition and creating new space for emerging species because of reduced litter cover (Figure 2.2e). Burning positively affected biomass, species richness, and vegetative cover (Figure 2.2 a, b, d). Prescribed fires results in a short-term increase in soil N and P availability (Wan et al., 2001; Ojima et al., 1994; Rau et al., 2007; Picone et al., 2003) subsequently increasing foliar nutrient concentration. All these affect plant stoichiometric ratios.

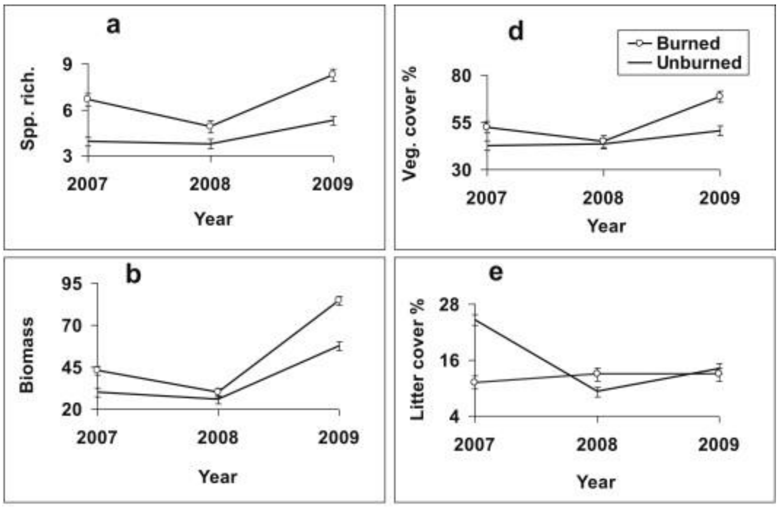

Figure 2. 2 Response of (a) species richness, (b) aboveground plant biomass (g 0.25 m-2, (d) vegetative cover %, (e) litter cover %, to burning treatment grasslands community in the 2006/7, 2007/8 and 2008/9 growing seasons.

The plant stoichiometric N:P ratio have been used to describe N-or P- plant limited communities (Güsewell, 2004), when N:P ratio <10 and >20 corresponds to N- and P- limited biomass production, respectively. According to Koerselman and Meuleman (1996) based on analysis of 40 fertilized studies, the N:P ratio is <14 and >16 in explaining limitation, where in between plant growth is limited by both N and P. These observations were based on short-term effect, long-term frequent burning may reduce tissue N concentration and increase C:N ratio (Kitchen et al., 2009) of the roots and organic matter (Ojima et al., 1994, Fynn et al., 2004), as a result of change in soil N availability (Lü et al., 2012b). Reports from previous studies show a decline (Henry et al., 2006) and no change (Britton et al., 2008, Cui et al., 2010) in N:P ratio in California annual grassland and alpine heath respectively.

2.3.3 Effect of fertilization

It is no doubt that fertilizer positively affects biomass production by providing depleted nutrients required for plant growth since most of the grassland's ecosystems are N and P limited (Tessier and Raynal, 2003; Mamolos et al., 2005). Biomass production is also constrained by water availability in this ecosystem, which is the function of climate (Churkina and Running, 1998; Bernhardt-Römermann et al., 2011). In a long-term experiment in Netherlands, plant species richness started to recover after 25 years of N, P, K and lime addition which is somethings that's rarely reported in long-term experiments (Figure 2.3). Several experiments have shown a rapid loss of species richness and diversity with mineral or organic fertilizer within the first years after fertilization (Benizri and Amiaud 2005; Reynolds et al., 2007; Hejcman et al., 2010a; Boch et al., 2021; Kotas et al., 2017) (Figure 2.4). Decrease in plant species richness and diversity is mostly reported as function of nitrogen, which is responsible for photosynthesis, thus promoting high production subsequently enhancing growth of strong competitor species and suppress others (Scott and Rabie 1956). The dominant species utilizes more nutrients and overcome inferior species through light interception and height. Therefore, a decrease in species richness and diversity following fertilization is a function of shoot and root competition in utilizing nutrients, light, moisture and space (Rajaniemi, 2002).

Plant composition is used to evaluate fertilizer effect on crop response and have been reported to be controlled by soil pH, soil moisture and nutrient in European grasslands (Klimek et al., 2007; Olofsson and Shams, 2007; Prach, 2008). Change in plant species composition may not result in increase in total biomass production because of the differences in species-specific nutrient uptake and nutrient use efficiency within the plant community (Hejcman et al., 2010b). Through direct supply of N, P, and K, species composition response should be predictable with increasing foliar N with nitrogen fertilizer (Nguyen et al., 2002) and P with P fertilizer (Hrevušová et al., 2009). This causes significant reduction of below and above ground C:N and C:P ratio (Gill et al., 2006; Kirkby et al., 2013). Foliar stochiometric ratio tend to be predictable with the abudance of elements when fertilizer is added. For example, grassland supplemented with P will reduce plant N:P and C:P ratio, while N addition reduces plant C:N and N:P ratio (Heyburn et al., 2017b). Soils with high nutrient status are expected to be associated with lower plant C:N and C:P ratios which are easily decomposable with positive effect on microbial activity (Zechmeister-Boltenstern et al., 2015).

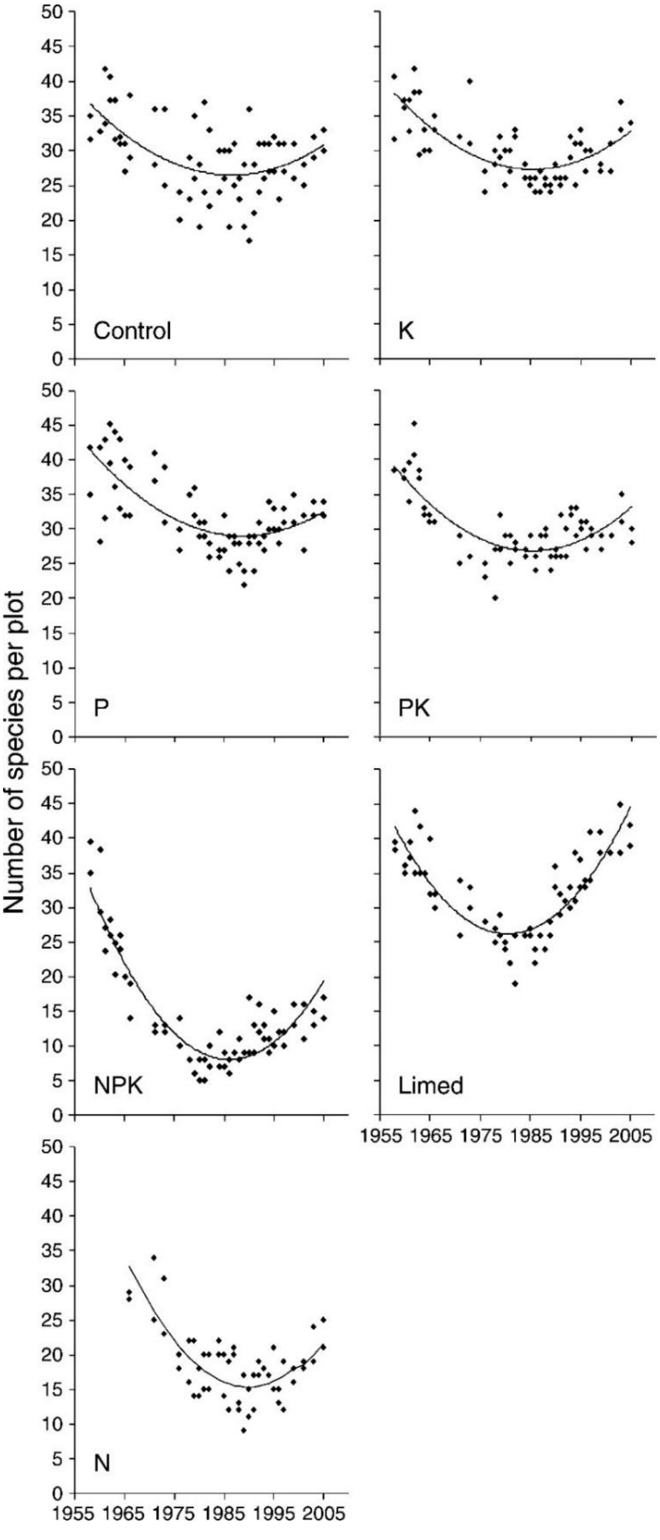

Figure 2. 3 Long-term changes of plant species richness in unfertilized control, K, P, PK, NPK, unfertilized limed, and N grassland plots in Netherlands (Pierik et al., 2011).

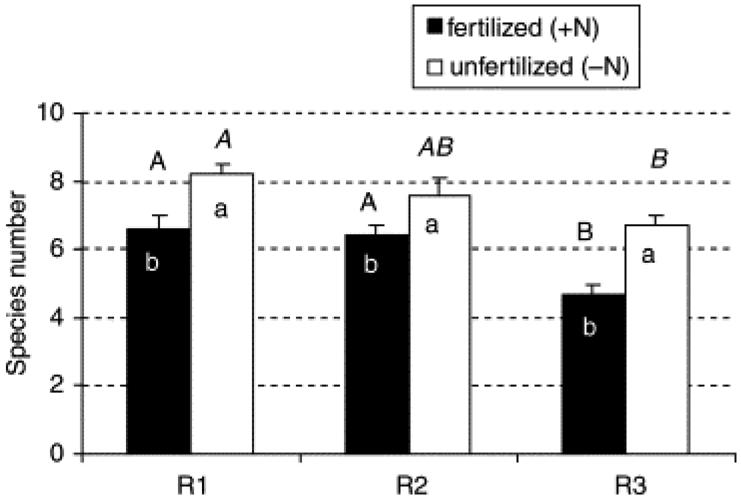

Figure 2. 4 Species richness in the unfertilized and fertilized plots for the three sampling dates (R1–R3). *Capital and lower-case letters correspond to the grouping of means by the Newman–Keuls' test at p=0.05 for sampling date and fertilization, respectively (Benizri and Amiaud, 2005)*

2.4 Effect of grassland management practises on soil properties

Grassland management effect on biomass production, species richness and elemental composition highlighted in the previous section has implications for soil properties and processes. Management is the manipulation of soil, which is a medium that is reflected by production. Without adequate quality of the soil, production is regarded as poor. We will look at the effect of the management practices of grassland by reviewing selected soil properties as a reflection.

2.4.1 Grazing

Indirect effects of grazing on soil are through forage production, cow dung and urine which tend to affect plant input in a form of litter (Dubeux Jr, 2005). Surely changes in vegetation caused by grazing animals should somehow affect and modify soils they grow on. Effect of grazing intensity, type of animal on soil physical, chemical and biological properties have been documented (Johnston et al., 1971; Smoliak et al., 1972; Yuan and Hou 2015; Savadogo et al., 2007). Cui et al. (2005) observed that heavy grazing increases pH, however, declines the SOC in a long-term (20 years) degraded grazing site (8.34 mg/g) when compared to non-grazed site (11.64 mg/g) in Inner Mongolia semiarid grassland. Grazing intensity effect on soil properties greatly differ among soil types, vegetation, grazing animal, stocking rate, length of grazing experiment (Lavado et al., 1996; Cui et al., 2005).

Long-term grazing increases bulk density within 0-10 cm depth under high grazing intensity (Binkley et al., 2003; Evans et al., 2012; Mapfumo et al., 1999) especially large herbivores (McNaughton et al., 1997) through action of trampling (Greenwood and McKenzie, 2001). This may result in soil compaction, poor porosity, aggregation, and infiltration with adverse effects on plant growth (Yingzhi et al., 2004). Evans et al. (2012) showed the effect of long-term grazing (20 and 30 years) in a study done on a rangeland in Kamloops, British Columbia. They found that grazing in spring increased aggregate stability with a mean weight diameter (MWD) of 1.5 mm and 32 % and 10 % of aggregates in the 2-6 mm and 1-2 mm size fractions, respectively, compared to MWD of 1.0 mm and 20 % and 6 % under fall grazing (Table 2.1).

Table 2. 1 Effect of grazing on selected soil physical parameters after 20 and 30 years of the field experiment on rangeland north of Kamloops, British Colombia (Evans et al., 2012).

		Grazing season		Grazing	
year	Depth (cm)	Spring	Fall	Without	With
			Bulk density (mg m^{-1})		
20	0-7.5	1.09(0.02) *	1.03(0.02)*+	1.03(0.02)	1.08(0.02)*
	7.5-15	1.07(0.02)	1.02(0.03)+	1.03(0.03)	1.06(0.02)
	15-30	1.09(0.02)	0.98(0.03)**	1.02(0.02)	1.05(0.03)
30	0-7.5	0.94(0.04)	0.90(0.02)	0.87(0.03)	0.98(0.03)**
	7.5-15	0.91(0.02)	0.90(0.02)	0.89(0.02)	0.92(0.02)
	15-30	0.92(0.02)	0.87(0.03)+	0.89(0.02)	0.90(0.02)
			MWD (mm)		
20	0-7.5	2.3(0.09)	2.3(0.11)	2.4(0.09)	2.2(0.11)
30	0-7.5	1.5(0.10)	1.0(0.07)**	1.3(0.11)	1.2(0.11)
			2-6 mm size fraction (kg kg^{-1} soil)		
20	0-7.5	0.52(0.025)	0.53(0.030)	0.54(0.025)	0.50(0.030)
30	0-7.5	0.32(0.025)	0.20(0.018)**	0.27(0.026)	0.25(0.027)
			1-2 mm size fraction ((kg kg^{-1} soil)		
20	0-7.5	0.10(0.004)	0.09(0.005)*	0.09(0.005)	0.09(0.004)
30	0-7.5	0.10(0.007)	0.06(0.004)**	0.08(0.004)	0.08(0.009)
			0.25-1 mm size fraction (kg kg^{-1} soil)		
20	0-7.5	0.07(0.005)	0.07(0.004)	0.06(0.004)	0.07(0.005)
30	0-7.5	0.08(0.005)	0.08(0.003)	0.08(0.004)	0.08(0.005)

		<0.25 mm size fraction (kg kg^{-1} soil)			
20	0-7.5	0.32(0.020)	0.32(0.024)	0.30(0.019)	0.34(0.024)
30	0-7.5	0.50(0.024)	0.66(0.019)**	0.57(0.030)	0.59(0.030)

Values in parentheses are the standard error of the mean (n=16). +, *, ** significant at $p< 0.10$, $p<0.05$, $p<0.01$, respectively,

This is caused by severe impact of moisture in spring season where soil is less resilient to grazing and trampling (Naeth et al., 1991). The increase in aggregates stability was attributed to positive correlation of MWD with polysaccharide and root biomass which enhance formation and stabilization of larger aggregates. However, this was contrary to Dormaar et al. (1998) who reported decrease in MWD under grazing pressure, the differences are the result of stocking rate (0.6 and 1.2-4.8 AUM ha^{-1}) of which high stocking rate (heavy grazing) decreases MWD.

Grazer-induced impact on nutrient cycling within grassland communities is documented across the world and different climates (Frank and Groffman, 1998; Augustine and McNaughton, 2006, Frank, 2008). Nitrogen and carbon cycling is accelerated in urine and fecal excretion through; (i) supply of readily available forms to plant and microbes than N and C in plant leaves, (ii) lower C:N ratio of plant litter roots and organic matter (Shariff et al., 1994). Livestock faeces are a potential source of N, P, S, Mg and Ca (Nicol, 1987) therefore affect soil chemical properties. Dubeux Jr (2005) reported significant contribution of urine (59 kg N/ha) and dung (84 kg N/ha) to N availability in bahiagrass pastures.

There are contrasting reports across literature on soil C stocks and N with increasing grazing intensity (Steffens et al., 2008). Some studies have reported a decrease (Abril and Bucher, 1999; Neff et al., 2005), no change (Barger et al., 2004; Binkley et al., 2003) and increase (Reeder and Schuman, 2002; Conant et al., 2005) in C stocks. Grazing modifies the magnitude and relative allocation of C to above and belowground biomass, changes microclimate and functionally diversity of plants consequently altering C storage (Hobbie, 1992; Vendramini et al., 2014). According to a DAYCENT model, that simulates plant and soil C, N, P, and S dynamics and trace gas N_2O, NO, and CH_4 fluxes, at 2 sites (Mandanan and Cheyenne) Del Grosso et al. (2001) found a slight decrease (-2.7 %) in soil C and increase in N (2.0 %) for moderate grazing compared to grazing exclusion while heavy grazing increased (3.8 %) C and N (6.0 %) at Mandanan (Figure 2.5). Larger increase in C (20.2 and 25.5 %) and N (24.5 and 32.3 %) was observed in continuous light and continuous heavy grazing at the Cheyenne site

(Figure 2.5). The N_2O showed correlation to changes in soil N content, small increases in N_2O fluxes can substantially reduce C sequestration benefits and in relation to decrease in mineral N availability (Conant et al., 2005)

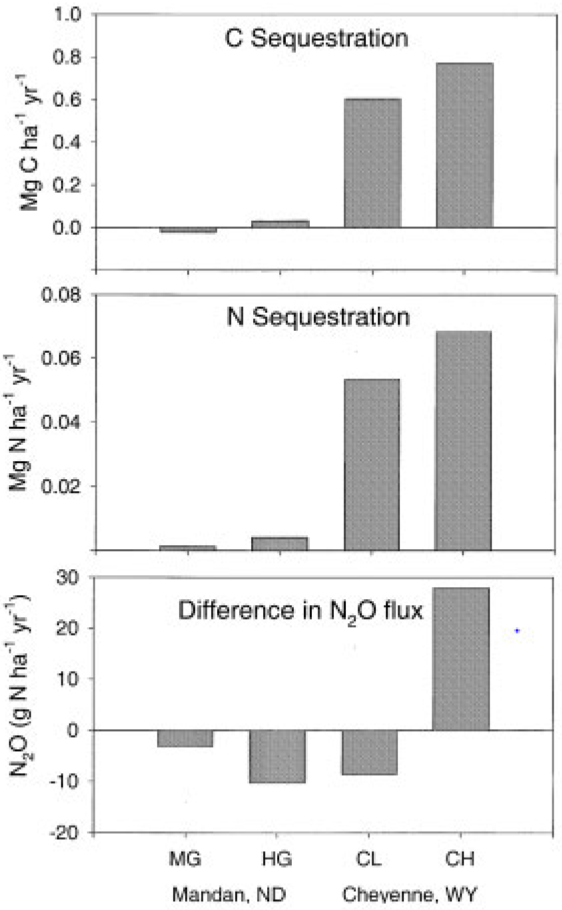

Figure 2. 5. DAYCENT model on changes in soil C stocks, N stocks, and N2O following grazing at 2 rangeland sites. *MG – moderate grazing; HG – heavy grazing; CL – continuous light grazing; CH – continuous heavy grazing (Conant et al., 2005).*

2.4.2 Mowing

Mowing mimics grazing in some way using machinery, therefore, it has significant consequences on ecosystem. There are several reports on effect of mowing on soil physical aspects such as moisture, temperature, compaction and bulk density (Chai et al., 2019; Schrama et al., 2013; Kramberger et al., 2015). Through removal of above ground biomass, mowed grasslands are exposed to litter reduction and increased exposure to sunlight. It is therefore expected that soil will be sensitive to temperature fluctuations and soil evaporation because of more light absorbance (Simons et al., 2017).

Grassland ecosystems are believed to have desirable impact on soil structure through roots-induced effect on granulation, vegetation protective cover, organic exudates (Pavlů et al., 2016). Soil compaction is usually expected in wet fine textured soils (van Klink et al., 2015). The use of modern machinery in cutting grass can cause severe stress to soil structure. Therefore, increasing number of wheeling events (intensive mowing) may result in soil compaction, higher bulk density values, reduced porosity (Horn et al., 2003; Krebstein et al., 2013). Reducing mowing intensity and allowing recovery after disturbance can minimize effects on soil physical properties.

The amount of plant residues returned to the soil is reduced due to removal of above ground biomass, therefore having an impact on nutrient biochemical cycle. Long-term mowing of plants without fertilizer application could lead to nutrient depletion and reduced SOM because of limited returns of plant residues which could affect biochemical cycles (Oelmann et al., 2009). Li et al., 2017 examined the nitrogen mineralization, fungal and bacterial communities on a long-term semi-arid mowed grassland. In their study, SOC (37.4 mg/kg, TN (3.3 mg/kg) and microbial biomass was high under moderate mowing compared to the control (Table 2.2). Fungal community abundance had played a superior role in N mineralization compared to bacterial abundance. Such differences between fungal and bacterial communities are attributed by less diverse carbon inputs and plant richness on mowing because of plant cover removal (Gilmullina et al., 2020; Chen et al., 2021). Similar results of increasing SOC and total N with long-term mowing are reported (He et al., 2012; Ziter and MacDougall, 2013) suggesting the importance of mowing intensity on soil C response.

Berliner and Kioko (1999) reported a decline in plant available P and inorganic N on a 30-year-old mowing trials in central Kenya. This was attributed to the export of nutrient in hay and no stimulation effects of herbivores (Berliner and Kioko 1999). Exchangeable bases and micronutrients (Fe and Cu) and effective cation exchange capacity (ECEC) have been found to increase with mowing (Berliner and Kioko 1999, Wang et al., 2018a). Such finding can be confusing regarding no return of plant residues to the soil, however, it suggested that this might be a result of greater plant uptake of Ca from subsoil and enrichment to the surface via root uptake (Han et al. 2014) or root turnover and mortality (Liu and Huang 2002).

Table 2. 2 Effects of mowing on soil chemical properties (Li et al., 2017).

Treatment	pH	Moisture (%)	AN (mg/kg)	AP (mg/kg)	RK (mg/kg)	SOM (g/kg)	TN (g/kg)
CK	7.32a	7.2a	75a	1.0a	262ab	30.9c	3.0ab
M1/2	7.33a	7.0a	86a	1.3a	254b	34.9ab	3.0ab
M2/3	7.39a	7.9a	76a	1.3a	275a	33.6bc	3.0ab
M1	7.32a	8.5a	86a	1.2a	269ab	37.6a	3.3a
M2	7.24a	7.6a	57b	1.2a	122c	30.8c	2.9b

The values are the mean ± SE, n = 3. Different lowercase letters in the same row indicate significant differences ($P < 0.05$). AN alkali-hydrolysable nitrogen, AP available phosphorus, RK rapidly available potassium, SOM soil organic matter, TN total nitrogen. N: B *M1/2 Herbage was harvested once every second year on August 16. M2/3 Herbage was harvested twice every 3 years, once every year for two successive on August 16 and zero mowing for the third year M1 Herbage was harvested once every year on August 16. M2 Herbage was harvested twice every year on June 23 and September 12.*

2.4.3 Burning

Common practice with burning and mowing is the removal of plant cover, which exposes soil to bare sunlight resulting in increased soil temperatures subsequently altering soil moisture. Short term (2 years) impact of burning reduced soil water content, OC, total N and exchangeable P in semi-arid grassland in Bloemfontein, South Africa (Snyman, 2002). This is mainly attributed to decrease in plant cover and increasing in soil temperature, consequently the rate of organic matter and mineralization of organic P (Suárez and Medina 2001). The combustion of organic matter during fire could result in breakdown of aggregates resulting in increase in bulk density and compaction subsequently decreasing aggregate stability (Kennard and Gholz 2001; Granged et al., 2011a). The impact of prescribed fire on bulk density is not well understood as authors have reported no change (Phillips et al., 2000; Grady and Hart, 2006), reduced (Brye, 2006; Chief et al., 2012) and higher values (Kennard and Gholz, 2001; Hubbert et al., 2006) after fire. There are many factors involved in determining bulk density in these ecosystem (e.g fire intensity and severity, cementing agents, fuel accumulation, texture, moisture)(Alcañiz et al., 2018).

The physical aspects of the soil on the mechanisms involved after burning could be visible. Infiltration rate, saturated hydraulic conductivity, and mean weight diameter have been reported to decrease (0-10 cm) immediately after burning (Hubbert et al., 2006, Are et al., 2009). This is the result of short-term change in bulk density and fine textured particles with reduced permeability (Are et al., 2009; Vogelmann et al., 2012). Soil moisture also play an important role in controlling soil temperature at deeper depths. Moist soil decreases fire temperatures with depth due to thermal conductivity while saturated soil has stronger effect due to increasing heat capacity (Badía et al., 2017). Biological communities have different threshold to fire tolerance, which affects their activity and population.

Many studies focused their attention on soil chemical properties on burnt grassland which undergoes several changes in vegetation and ash formation as well as OM incorporation to the soil. The nutrient status (Ca, Mg, K) has been reported to be increased after fire (Kennard and Gholz 2001; Arocena and Opio 2003) due to the release of base cations from combustion of organic matter (Guinto et al., 2001) consequently resulting in increasing pH (Arocena and Opio 2003; Úbeda et al., 2005). The pH is likely to rise due to OH- losses following the complete oxidation of OM which releases cations (Alcañiz et al., 2018). The reviews on the impact of fire on soil properties report variable results on SOC stocks, microbial activity and nutrient status (Neary et al., 1999; DeBano, 2000; Alcañiz et al., 2018). Indirect impact occurs on a long-term influenced by organic matter transformation, compatibility and reduced porosity (Alcañiz et al., 2018).

Generally burning reduces SOC post fire (Granged et al., 2011b; Hatten et al., 2005) due to an impact of fire temperature. It has been shown that fire temperature of 200-300 ^{o}C reduces soil organic carbon content and at 500 ^{o}C soil OC could be completely lost (Fernández et al., 1997; Terefe et al., 2008). As such, low fire intensity (<200 ^{o}C) showed a slight increase in TC, N, K and P in Mediterranean climate of north-east Spain (Úbeda et al., 2005). Water-repellent characteristic due to impact of fire could also accelerate nutrient loss through erosion. In conclusion, using prescribed low fire intensity could maintain major ecosystem processes and reverse negative impacts possessed.

2.5 Soil response to grassland fertilization

2.5.1 Physical properties

The urge to increase biomass production through fertilization also changes soil properties and their behavior. The physical aspect of the soil could indicate the productivity, however there are limited studies that examine long-term impact of fertilizer (mineral and organic) addition. Soil bulk density has been reported to decrease with mineral fertilizer application because of increasing SOC in the upper soil layer, which is positively correlated with water content, water holding capacity and aggregate stability (Hopkins et al., 2009; Blanco-Canqui et al., 2009). This could be the effect the kind of fertilizer applied (mineral or organic). Mineral fertilizers on their own have no effects on bulk density (Głąb, 2014; Wang et al., 2021; Lynch et al., 2005). Decrease in BD is reported with organic fertilizers through an impact direct supply of organic manure which enhances OM content (van Eekeren et al., 2009; Lee et al., 2009). Lower bulk density values can relate to greater porosity, infiltration rate, gaseous exchange and soil stability.

The soil structure is one of the essential soil parameters because it facilitates many biological, chemical, and physical processed. Good and stable soil structure is a function of moisture, vegetative cover, root exudates, texture, and SOC. Nitrogen fertilizer alone has been shown to increase proportion of macroaggregates (>2 mm) which was associated with increases in soil arbuscular mycorrhizal fungal hyphae (Wilson et al., 2009; Aoyama et al., 1999). Differences in aggregation exists between mineral and organic fertilizer due to organic matter composition (Pachepsky et al., 1996). As suggested by Czachor et al. (2015), mineral fertilizer has less aggregate resistance compare organic fertilizer while Yu et al. (2012) showed that organic fertilizer increased mass proportion of macroaggregates from 8.8 % to 17.7 to 30.8 %. This corresponds to organic constituents' excretion from manure favoring formation of larger aggregates (Ciarkowska, 2010). The specific alteration of physical aggregation on N fertilized is limited, but the mechanism is related to pH induced effects and organic matter. Literature suggests that soil pH can be positively related to water-stable aggregates (WSA) through the effect of microbial activity where inhibition of bacterial proliferation slows down the degradation of the organic stabilizing agent (Bethlenfalvay et al., 1999).

2.5.2 Chemical properties

Adequate chemical fertility is required to support plant production are crucial in ensuring high productivity of grasslands. Continuous addition of ammonium-based N fertilizers increases soil acidity due to nitrification of NH_4^+ which releases H^+ ions (Schwab et al., 1990; Ying et al., 2017). Soil acidity constrains biomass production by affecting exchangeable capacity of the soil and limiting the availability of exchangeable bases (Ca, Mg and K). Changes in soil CEC and exchangeable bases with soil acidification associated with N fertilization is well documented (Stroia et al., 2011; Wang et al., 2017b). As the pH drops, retention of hydroxyl-

Al lowers the negative charge of the soil colloid resulting lower CEC, meanwhile the Al and Fe concentration in soil solution increases leading to exchangeable bases cation depletion. At lower pH, Al associated with clay minerals and P precipitate as Al phosphate which forming an interstitial cement that binds aggregates together (Haynes and Naidu, 1998). Soil available P is positively correlated with applied P, which is challenging on soil receiving no P supplement. For example, Messiga et al. (2013) observed the reduction in water extractable P with increasing N rate. This phenomenon is attributed to an increase in exchangeable Al and Fe triggered by increasing acidity upon N addition resulting in P fixation (Breeuwsma and Silva 1992).

Some of the reported physical, chemical, and biological properties affected by mineral fertilization (NPK) addition on long-term grassland trials are highlighted in Table 2.3. Soil nitrogen is positively affected by N addition in some studies (Li et al., 2018) or no change (Semelová et al., 2008; Chen et al., 2020). Nitrification process is sensitive to acidic soils and limited by high Al and Mn or H^+ concentration. High total N concentration may be accounted for by N addition and reduced nitrification. However, Fornara et al., (2013) reported high loss of soil N on plots receiving N fertilizer. Increase in organic N on soils is caused by the reduction in microbial mineralization of organic pool, effectively slowing down decomposition with N fertilization (Janssens et al., 2010).

Nitrogen induced effects on soil C sequestration have been well documented in many experiments (Fornara et al., 2013; Cenini et al., 2015) and meta-analysis (Lu et al., 2011) reporting neutral, positive and negative of soil C with N addition. In Germany and Netherlands, Poeplau et al. (2018) observed that fertilization (NPK) increased SOC stocks of a temperate grassland with annual sequestration rate was 0.13- 0.37 Mg/ha/yr. However, they noted increment of SOC was in 10-30 cm depth, while there was no detected increment in 0 -10 cm and on solely application of N. They argued that the differences might be the differences and change of bulk density overtime; and largest SOC gradient in the upper 10 cm of the soil profile. This study is contradicting the results reported by Fornara et al., 2013, where separate N application was effective in soil C sequestration compared to combination of fertilizers (NPK). Kätterer et al. (2012) also reported an increase in SOC of 1 kg/ha for every kg N/ha applied. This could be caused by the result of long-term accumulation of partially undecomposed plant material. Contrary, several studies reported no change in soil C (Chen et al., 2020; Lu et al ., 2011) after N addition. This may be caused by alteration of plant tissue chemistry, where N decreases litter lignin:N ratio which decomposes faster causing more C release and labile C substrate input to the soil (Liu and Greaver, 2010). The common explaination of no change of C after N addition could also be explained by the concept of C saturation. C saturation could occur when the soil could not store more added C inputs enhanced with N addition (Stewart et al., 2007). A meta-analysis in China showed a net increase in macro-aggregate (15 %) associated C and in bulk soil (5 %) C due to N enrichment (Lu et al., 2021). Inhibition of microbially mediated breakdown of various binding organic agents such as microbial- and plant-derived polysaccharides, which are all C-rich materials, may explain high mineral-associated organic C (Chen et al., 2019; Lu et al., 2021).

The C sequestered in the soil profile is located in differently sized aggregates. It has been shown that C associated with large macroaggregates is labile and more prone to microbial decomposition than microaggregate associated C (Aoyama et al., 1999). This argument arise from the observed increased respiration and N mineralization after discruption of soil aggregates (Beare et al., 1994). Changes in C stocks can be highly variable due to complexities of C pools and mean resident time (MRT) depending on recalcitrate of OM, protection in aggregates, and interaction between soil mineral and OM (Lützow et al., 2006; Dungait et al., 2012). Riggs et al. (2015) examined the decomposition of C and its pools on aggregates fractions on 5 different grassland sites at U.S. Central Great Plains region. They found that most of the C (>50 %) was associated with middle aggregate classes (small macroaggregates and microaggregates). Decomposition rate of unoccluded labile C was increased by N then non labile (recalcitrant) C in studed grasslands. The MRT of labile C decreased from 9.92 to 8.53 days while that of non-labile increased from 9.30 to 10.42 years (Riggs et al., 2015). A number of factors would be the cause of this. Plant litter substrate with low C:N ratio decomposes faster than substrate with high C:N ratio (Cornwell et al., 2008), or could be effect of N on microbial community (Carreiro et al., 2000). Changes in plant community composition and species richness due to addition of mineral fertilisers has been reported to affect SOC dynamics (Heyburn et al., 2017b). This is due to associated changes in litter quality parameters such as C:N ratio (Berendse, 1998) that influence decomposability. Moreover, strong contrasts in plant stoichiometry, even with the same species, can result to differences in nutrient availability (Poeplau and Kätterer, 2017). Change in plant tissue chemistry may affect soil C and N dynamics. A decrease in litter lignin:N ratio accelerates decompotisionof plant litter causing more C and N release and labile C substrate input to the soil (Liu and Greaver, 2010).

Therefore, theres no guarantee on how soil C would respond to fertilizer addition because of differences of microclimate such as moisture, temperature, pH and microbial activity.

Table 2. 3 Response of soil properties to fertilizer addition in grassland experiments.

Fertiliser Treatment	Years	climate	Application rate	Physical properties		Chemical properties		Biological properties		reference
				Parameter	Response	Parameter	Response	Parameter	Response	
N	<5	temperate	10 g N/m2/year	-	-	pH, Mg, Ca Exch. Al K TC	Decrease Increase No effect No change	Litter and C decomposition rate Enzyme activity	Decrease Increase	(Keeler et al., 2009)
NPK	8	Mediterranean	10 g/m2 annually	-	-	Soil C	Increase	-		(Lin et al., 2019)
N	9	-	10 g/m2 annually	-	-	pH, TOC, TN	No change	Microbial biomass C Microbial biomass N Bacteria diversity	Decrease No change Increase	(Chen et al., 2020)
N	9	Semi-arid	5, 10, 15 g/m2/year	-	-	ECEC; Ca & Mg Fe; Mn; Cu	Decrease Increase			(Wang et al., 2017b)
N	14	Semi-arid	2, 4, 8, 16, and 32 g/ m2 /yr	-	-	pH	Decrease	Microbial Biomass C Copiotropic bacteria; Saprotrophic Fungi	Decrease Increase	(Li et al., 2019)
N	15	temperate	4, 16 g/m2/yr	-	-	pH organic N	Decrease Increase	Soil respiration utilization of gram-positive bacteria and actinomycetes	Decrease Increase	(Li et al., 2018)

								13C utilization of gram-negative bacteria and fungi	Decrease	
N	19	-	100kg/ha	-	-	Soil C	Increase	-		(Fornara et al., 2013)
NPK	44	-	200kgN/ha/yr	-	-	-		Ammonia oxidizing bacteria	Increase	(Zhou et al., 2015)
N	54	-	160 kgN/ha/yr	-	-	Exch. Al	Increase	Actinobacteria Aquificae; Chlorobi;Cyanobacteria;Firmicutes; Nitrospirae; Plactomycetes; Verrucomicrobia	Increase Decrease	(Pan et al., 2014)
NPK	60	-	50, 100, 200 kg/ha/yr	-	-	pH SOC; Available P	Decrease Increase	-		(Vargova et al., 2020)
NPK	62	-				Exch. Ca Mg, P, K, N	Increase No change	-		(Semelová et al., 2008)
N	120	temperate	90–140 kg N	-	-	pH; Exch. K, Al; Total N; SOC stocks	Decrease	-		(Kidd et al., 2017)

N- Nitrogen, P-Phosphorus, K- Potassium

2.5.3 Biological properties

Microbial activity is the key component in nutrient cycling in soil ecosystem. There are various microbial communities existing in the soil and are strongly related to plant species (Kourtev et al., 2003). Field studies showed that N enrichment favors fungal communities rather than bacterial communities (Wang et al., 2017a; van der Bom et al., 2018) (Table 2.3). Studies have shown positive (Yandong et al., 2005), negative (Sarathchandra et al., 2001) and neutral (Johnson et al., 2005) soil microbial biomass (SMB) response to grassland fertilization. The variations depend on several soil properties including pH, total N, moisture, organic matter, and rate of N addition amongst others (Drenovsky et al., 2004; He et al., 2013). Soil C:N ratio play a crucial role in balancing C and N substrate. Soils with C/N ratio below 30:1 have restricted C supply required by microorganism, which is believed to be one of the mechanism responsible for negative response of SMB to the increase soil N (He et al., 2013). Decrease in root biomass under high N addition lowers the amount of root exudate released which have important function in promoting growth of microorganisms (Dijkstra et al., 2005), this phenomenal explain negative SMB response.

Microbial species response to N addition differs because of environmental tolerance, for instance, an increase in ammonia oxidizing bacteria (Zhou et al., 2015), Copiotropic bacteria and Saprotrophic Fungi (Li et al., 2019) due to greater capability of adapting to soil acidity. Increased enzyme activity (Keeler et al., 2009) is linked with high soil moisture because of high OM. A 2-year study in an upland grassland found increase AM fungal colonisation and no change in PMEase activity when grasslands were treated with nitrogen and lime attributed to the ameriolating effect of the latter on soil pH (Johnson., 2005)

2.6 Soil response to grassland liming

2.6.1 Physical properties

Impact of liming on soil physical parameters are through pH changes affected the composition of cations responsible for structural formation. Improved soil stability after lime addition is well documented (Bennett et al., 2014), where increased calcium and magnesium concentration triggers flocculation of clay particles which may stabilize to macro-aggregates through microbial activity (Rengasamy and Marchuk 2011; Holland et al., 2018), consequently influencing SOC storage on aggregates (Figure 2.6). Improved soil structure and mean weight diameter (MWD) improves roots penetration (Forster et al., 2021). However, some reports involving liming show that structural stability may be reduced (Munoz et al., 2012; Aye et al., 2016). The decrease may be explained by SOC exposure to microbial degradation at high pH leading to depletion of labile C which is the main source of binding agent for macroaggregates or subjection to frequent soil disturbance (Aye et al., 2016). Liming increase aggregates stability is soils with high clay content and aggregate stability (Keiblinger et al., 2016). Soil physical properties tend to affect chemical properties and mechanisms, for example, soil structure disturbance may disrupt OM stored in more stable microaggregates, exposing it to microbial decomposition which could result in decline in OM.

2.6.2 Chemical properties

Liming requirements depend on soil acidity, texture, buffering capacity and organic matter. Supply of Ca^{2+} cations with liming neutralizes the effect of H^+ ions in soil solution and leads to increase in soil pH (Holland et al., 2018). This increase in soil pH leads to an increase in ECEC due to deprotonation of pH-dependent charge sites from organic and mineral components (Edmeades 1982). The effect of liming on some chemical parameters reported on several articles are highlighted on Table 2.4. Soil OM input may increase through induced effect of pH, exchangeable bases, and plant response to liming. There is an uncertainty around net effect of liming on SOC in relation to induced effect of increasing exchangeable Ca and pH Increase in microbial activity leads to more OM mineralization which could decrease SOC (Grieve et al., 2005, Aye et al., 2016); while enhanced soil structure creates physical protection of SOC to microbial attacks (Egan et al., 2018a); positive plant response increases C inputs thus increasing SOC (Figure 2.6).

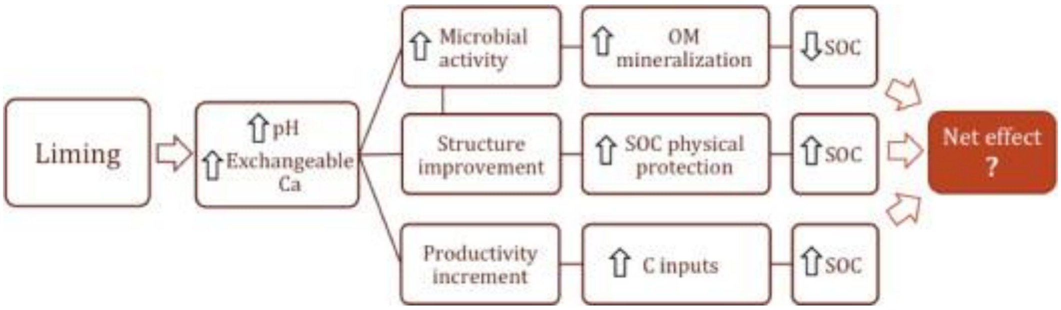

Figure 2. 6 Potential effects of liming on soil organic carbon (SOC). *The schema summarizes results from several papers reviewed by (Paradelo et al., 2015).*

The net effect on SOC due to interdependent processes depend on the number of factors including initial soil pH, liming rate, clay content and mineralogy, soil use, climate, and others (Paradelo et al., 2015). Increase in SOC because of liming have been shown to enhance C sequestration and C stocks in a 129-year grassland trial due to increased root mass and root exudates (Fornara et al., 2011). A meta-analysis by (Eze et al., 2018) revealed that climate had an influence on overall effect on liming on soil C stocks. Positive response of soil C stocks on temperate climate was attributed to an increased in length of growing seasons thereby enhancing plant growth and C addition. However, this is only effective when C additions surpasses OM decomposition induced by enhanced microbial activity at this climate. Carbon storage on grasslands is accomplished through physical protection on micro-aggregate fractions which are associated with stable OM (Egan et al., 2018a).

Table 2. 4 Response of soil properties to lime addition on grassland experiments

Treatment	Years	climate	Application rate	physical		chemical		biological		reference
				parameter	response	parameter	response	parameter	response	
Lime	2	-	0.6 kg m^{-2}	-	-	-	-	MBC; basal respiration rate; phosphatase activity	Decrease	(Johnson et al., 2005)
Lime	5 34	-	3, 6, 12.5, 25 and 50 t ha^{-1} 12.5, 25, 50, 75 and 100 t ha^{-1}	MWD MWD	Decrease Increase	pH, SOC, total N pH, SOC, total N	No effect Decrease	MBC Soil respiration	Increase	(Aye et al. ,2016)
Lime	22	-	5000 kg ha^{-1}	-	-	pH soil N N & C stocks	Increase Increase No effect	Arbuscular and mycorrhizal fungal	Increase	(Heyburn et al,. 2017a)

Lime	23	-	5 t. ha^{-1}	-	-	-	-	archaeal and bacterial abundance fungal abundance	Increase Decrease	(Egan et al., 2018b)
lime	23	-	5 t ha^{-1}	Large macroaggregate (>2 mm) silt+clay fractions (SCA; < 53 µm) small macro-aggregates (SMA; > 250 µm–2 mm)	Decreased Increase Increase	pH C & N in microaggregates C & N in LMA Total C & N bulk soil	Increase Increase Decrease No change	Ammonia oxidizing bacteria amoA gene copies	Increase	(Egan et al., 2018a)
Lime	27	maritime climate	-	Bulk density	Decrease	-	-	-	-	(Forster et al., 2021)
Lime	129		4 t ha^{-1}	-	-	Soil C C sequestration	increase	-	-	(Fornara et al., 2011)
Lime	-		1.25, 2.5, and 5 t ha^{-1}	-	-	ECEC Exchangeable Mg and K	Increase decrease	-	-	(Edmeades 1982)

2.6.3 Biological properties

Effect of liming on soil biological properties is through increased soil pH and microbial activity. This enhances nutrient cycling through root decomposition and N mineralization (Heyburn et al., 2017a). Liming has been shown to increase ammonia oxidizing bacteria, archaeal and bacterial abundance, arbuscular and mycorrhizal fungal organisms (Heyburn et al., 2017a; Egan et al., 2018a; Egan et al., 2018b) due to positive soil pH effect on root exudates favoring decomposition by bacteria. Fungal abundance and AM fungi is mostly dominant in acidic soils (Bothe 2015) which explains the decline in limed grasslands (Millard and Singh 2010; Egan et al., 2018b). However, Holland et al. (2018) highlighted that the abundance increases of AM fungal root colonization at pH 5-6 while to decline was observed at soil pH >7. Given the variation of soil biota response to liming, there is a significance impact on soil biological processes (Table 2.4).

An extensive literature on liming induced increase in soil pH have cascading impact on soil N and C transformation which in turn influence N and C supply and cycling. Soil respiration is linked to microbial activity and mineralization of OM. A decrease in microbial biomass C on a 2-year experiment was linked to a low basal respiration (Johnson et al., 2005) while 5- and 34-year-old experiment reported an increase in both MBC and soil respiration (Aye et al., 2016). It likely that continuous liming application will increase mineralization, but the overall impact depends on C:N ratio of plant debris returned to soil (Bailey 1995). Increase in mineralization will have negative impact in C and N regulations.

2.7 Conclusion

It is important to evaluate the grassland response to anthropogenic processes. From this review, soil physical, chemical and biological processes are interlinked to one another. Soil properties therefore affect soil water, temperature, air, and nutrients which subsequently have an impact in above and below-ground production. As a result of different management practice, either the same or different response can be obtained and can be a function of environmental impact such as climate, topography, and plant community. The long-term impact of these grasslands management practices depends on the intensity of each practice (grazing, fire, fertilizer, and mowing) and its sustainability is related to frequency. Long-term sustainability of grazing relies on implementation of rest grazing periods or integrating with other managements practices such as rotational management with mowing with minimizing invasive machinery. The use of fertilizer to compensate lost nutrient through above ground removal is also substantial. Mowing, fertilization, liming and grazing can be used in rotational management for long-term sustainability. Limiting the effect of mowing involves minimizing use of machinery, cutting grass at prescribed height, and reduced frequency. All these practices can be self-sustained if used with precaution. Nutrient cycling is dependent on biomass production and microbial activity, while the storage of C and N depends on the physical stability of aggregates.

CHAPTER 3

SOIL FERTILITY STATUS IS INFLUENCED BY THE LONG-TERM APPLICATION OF NITROGEN AND LIME AT UKULINGA SEMI-ARID GRASSLAND

3.1 Introduction

Fertility status of grassland soils greatly impacts their productivity. Most important is the nitrogen level which has been identified to limit biomass and net primary production in these ecosystems (Fargione and Tilman, 2006; LeBauer and Treseder, 2008). Nitrogen addition improves nutrient storage and N availability thus resulting in high biomass production due to increased photosynthesis (Pons and Anten, 2004). As such N fertilizers have been intensively used in grasslands to improve soil fertility, increase forage and hay production for livestock. Worldwide use of nitrogen (N) fertilizers in grassland has increased significantly since the early 1950s and currently projected to about 115 million tons (Bumb and Baanante, 1996). Nitrogen is commonly applied as urea (most preferred source due to high N concentration (46 %), ammonium sulphate (21 %) and ammonium nitrate (34 %). Nitrogen fertilizers, however, not only differ in total N content but also in the predominant ionic form (NO_3^- and NH_4^+) in which N is supplied to plants (Santos et al., 2013) and inevitably extent of soil acidification. Ammonium based fertilizer are subject to nitrification of NH_4^+ which releases H^+ ions which occupies the exchange sites previously occupied by bases subsequently causing loss through leaching and acidity (Are et al., 2018). Although N fertilizers are not acidic, their input to the soil are acid forming. Numerous reports have examined the acidifying effect of different N fertilizers (Stone et al., 1991; Malhi et al., 2000). For example, a 3-year study showed that applying different sources of N at 336 kg N/ha annually had acidifying effect in order $(NH_4)_2SO_4$ > NH_4Cl > NH_4NO_3 ~ anhydrous NH3 ~ urea > urea form (Wolcott et al., 1965). Algae bloom, leaching to groundwater, ocean and soil acidification are some of negative impacts caused by excessive use of N particularly by agricultural sectors (Ngatia et al., 2019).

Numerous studies have reported increased soil C storage following continuous application of N (Lin et al., 2019; Fornara et al., 2013; Vargova et al., 2020). Nitrogen enhances biomass production, change root trait which have important consequences for soil nutrient cycling and the sequestration of C and N in grassland soils (Egan et al., 2018a; Heyburn et al., 2017a). However, N fertilization can also stimulate organic matter mineralization which could lead to decline in N and SOC (Congreves et al., 2017). Other studies found no effect of 10-year addition of N on SOC even though it increased plant productivity at an alpine site in Colorado, USA (Neff et al., 2002). Long term fertilization (135 years) at Rothamsted experiments resulted in increase of TN and available N overtime, but did not affect SOC overtime (Glendining et al., 1996). Therefore, the effect of long-term addition of N has produced inconsistent outcome in literature, suggesting that further research is needed for understanding detailed mechanisms and processes involved. Despite some positive aspects of N addition, it does come with downfalls to both soil and the environment.

Soil acidification interferes with soil physicochemical properties, soil biogeochemical processes and thus soil fertility. For example, an increase in soil exchangeable acidity accompanied by reduction in exchangeable bases, cation exchange capacity (CEC) and base saturation after long-term N addition, particularly ammonium N fertilizer, has been widely documented (Barak et al., 1997; Schroder et al., 2011). A meta-analysis by Lucas et al. (2011) showed that on average N addition decreased soil exchangeable Ca, Mg, and K in boreal forest, temperate forest, and grassland biomes by 24 %. While addition of N fertilizers results in losses of exchangeable bases due to soil acidification, it increases the availability of micronutrients causing toxicity to plants and microorganisms (Fageria et al., 2010). High Fe and Al concentrations due N induced soil acidity influences soil P solubility by sorption reactions (Ahmed et al., 2019). As such studies have reported decreased soil available P with N addition (Lu et al., 2012; Yang et al., 2015). However, others have reported an increase (Fan et al., 2019; Liu et al., 2021) in soil P. The differences the soil available P may be attributed to high P uptake by plants which reduces soil P or P solubilization with Al and Fe at lower pH values (Yang et al., 2015).

Biogeochemical cycles of micronutrients have an influence on soil fertility in terrestrial ecosystems (Richardson et al., 2017). The opposite effect of nitrogen addition on soil exchangeable bases and trace elements may result in nutrient imbalances with serious implications for ecosystem function (Feng et al., 2019). Soil acidity affects the solubility and mobility of certain elements such as Cd, Zn, Mn, Fe and Al. The background concentration of these elements depends on geological characteristics of soils (Atafar et al., 2010) and could be a result of industrial waste, mining activity and/or application of fertilizers and pesticides for agriculture. Limited studies had examined the effect of N addition rate and type on trace element concentration in the soil. Nitrogen addition has been reported to promote Fe, Mn, Zn and Cu accumulation in rice or maize grains (Hao et al., 2007) which indicates that the transportation ability of trace elements is enhanced by the presence of N. Possible mechanism involved is increased solubility at lower pH levels triggered by acidifying effect of ammonium-based fertilisers. Lime is applied to maintain optimum pH level to avoid toxicity and prevent immobilization which potentially causes deficiency. Laser (2007) examined the effect of N and lime on the trace element concentrations of pastures. He observed that Mn decreased and no change in Cu on limed soils, while N did not affect Cu, Zn, and Mn. Hejcman et al., (2009) reported decreased mobility of Cd, Fe, Mn, and uptake of Zn on limed soils because of increase in pH.

Unfortunately, studies on effects of fertilization on grassland ecosystems are limited and often based on short-term impacts (<10 years) which may not be representative of the long-term response on studied sites (Silvertown et al., 2006; Kidd et al., 2017; Poeplau et al., 2018). According to Silvertown et al. (2006) ecosystems take time to stabilize in response to fertilization implying the importance of long-term responses. This study investigated the changes in selected chemical and biochemical parameters following long-term nitrogen fertilization and liming at Ukulinga experimental veld established in 1951. Although the experiment is still under observation, no study examined these parameters focusing on N fertilizer rate, type, and combination with lime. We aim to explain how exchangeable bases, trace elements and selected biochemical parameters are affected by these treatments and what are the implications for soil fertility and C cycling.

3.2 Materials and Methods

3.2.1 Study site

The study was done in a long-term grassland trial located at Ukulinga, a research farm of the University of KwaZulu-Natal in Pietermaritzburg, South Africa (29° 24′E, 30° 24′S) (Figure 3.1). The area is semi-arid with mean annual precipitation of 790 mm and located on a plateau at 838 m.a.s.l. It is characterised by warm summers with a mean monthly maximum of 26.4 °C in February and winters are mild (3.2 °C in July) with occasional frost (Kirkman et al., 2014). The soil is derived from localized dolerite intrusions into a shale parent material (Rutherford et al., 2006). The vegetation of the area is southern tall grass veld or, at a larger spatial scale, KwaZulu-Natal hinterland thornveld, which is an open savanna of *Acacia (Vachellia) sieberiana* with patches of *Hyparrhenia hirta* L. and other herbaceous species (Ward et al., 2017b).

Figure 3. 1 Location of Ukulinga grassland experiment site (Abdalla et al., 2016).

3.2.2 Experimental design

The long-term veld fertiliser experiment trial started in 1951 with the application of nitrogen, phosphorus and lime. The experiment was laid out in a randomized block design with 9.0 x 2.7 m size plots replicated three times. This study focused on nitrogen applied annually as ammonium nitrate and ammonium sulphate at 70 and 211 kg N/ha and lime applied as dolomite at 2250 kg/ha every five years. Ten treatments were selected including (1) control (0 lime or N fertiliser kg/ha), (2) L = lime (2250 kg/ha), (3)AS70 = ammonium sulphate at 70 kg/ha ; (4) AS211 = ammonium sulphate at 211 kg/ha ; (5) AN70 = ammonium nitrate at 70 kg/ha ; (6) AN211 = ammonium nitrate at 211 kg/ha ; (7) AS70L = ammonium sulphate at 70 kg/ha + lime; (8) AS211L = ammonium sulphate at 211 kg/ha + lime; (9) AN70L = ammonium nitrate at 70 kg/ha + lime and (10) AN211L = ammonium nitrate at 211 kg/ha + lime.

3.2.3 Soil sampling and analysis

Five subsamples (0-10 cm) were collected from each plot and mixed to make a composite sample. The samples were air-dried and sieved to <2 mm then stored in plastic jars for further analysis. Soil samples were sent to Cedara Soil Fertility Analytical Service for the analysis of extractable P, exchangeable bases, total cations, exchangeable acidity, acid saturation, trace elements (Zn, Cu and Mn) and particle size analysis (Manson and Roberts 2000). Extractable P, K, zinc, copper and manganese was analysed using Ambic-2 consisting of 0.25 M NH_4CO_3 + 0.01 M Na_2EDTA + 0.01 M NH_4F + 0.05 g L-1 Superfloc (N100), adjusted to pH 8 with a concentrated ammonia solution. A 25 ml of the AMBIC solution was added to 2.5 g soil and stirred at 400 r.p.m for 10 min. The extract was filtered using Whatman No.1 filter paper. Phosphorus was analysed on a 2 ml aliquot of filtrate at a wavelength of 670 ɳm using molybdenum blue procedure (Murphy and Riley, 1962). Zn, Cu and K were analysed by atomic absorption. Total N was analysed by the Automated Dumas dry combustion method using a LECO CNS 2000 (LECO Corp., St. Joseph, MI). Soil organic carbon was analysed using Walkley-Black procedure (Walkley and Black 1934). Extractable Ca, Mg was determined by scooping 2.5 ml of soil and adding 25 ml of 1 m KCl solution. The suspension was stirred at 400 r.p.m for 10 min. The extracts were filtered using Whatman No.1 paper and diluted 5 ml of the filtrate with 20 ml of 0.0356 M $SrCl_2$ followed by analysis of Ca and Mg by atomic absorption. Soil pH was analysed by scooping 10 ml of soil into sample cups and added 25 ml of 1 M KCl solution. The suspension was stirred at 400 r.p.m for 5 min and allowed to stand for 30 minutes. The pH was measured using a gel-filled combination glass electrode. Soil bulk density was determined using the core method (Grossman and Reinsch, 2002) with the samples collected from the middle of the plots using a steel core sampler with 7.5 cm diameter 5 cm height. The soil was removed from the steel cores, dried in an oven overnight followed by measurement of dry weight. Bulk density was calculated by dividing soil dry weight by the volume of the soil core.

Bulk density (g/cm^3) = mass of oven dry soil (g)/ volume of soil core (cm^3).

Particle size distribution was determined using a pipette method (Gee and Or, 2002).

3.2.4 Statistical analysis

Statistical analysis was done using GENSTAT 18th edition. One-way analysis of variance (ANOVA) was done to determine effect of N and lime addition on soil properties. Treatment means were compared using Turkey's multiple comparisons at significance level $p<0.05$.

3.3 Results

3.3.1 Effect of N fertilization and liming on particle size distribution and bulk density

The trial soils were classified as clay soil texture. Clay contributed 50 % while silt and sand consisted of 25 % respectively (Table 3.1) Lime had the highest concentration of silt (30.33 %) followed by AS70L and AN70L (29.33 and 28.33 % respectively). There was no significant difference on sand % between the control and the other treatments. The bulk density ranged from 0.807 to 0.955 across the treatments, however there was no significant difference between the treatments (Table 3.1).

Table 3. 1 Particle size distribution and bulk density as affected by long-term N and lime addition.

Treatment	Clay	Silt	Sand	Bulk density (g cm^{-3})
		%		
C	50.33	25.67^{ab}	24.33^{ab}	0.876
L	46.67	30.33^{d}	23.00^{ab}	0.934
AS70	49.67	23.33^{a}	27.00^{b}	0.883
AS211	51.67	23.00^{a}	25.67^{ab}	0.849
AN70	50	24.67^{a}	25.67^{ab}	0.955
AN211	50.67	22.67^{a}	26.33^{ab}	0.807
AS70L	49.33	29.33^{cd}	21.33^{a}	0.861
AS211L	48.33	23.67^{a}	27.67^{b}	0.854
AN70L	48	28.33^{bcd}	23.67^{ab}	0.917
AN211L	48.33	26.00^{abc}	24.00^{ab}	0.921
p-value	**0.267**	**<.001**	**0.013**	**0.888**

Values within a treatment followed by a different lowercase letter in the columns are significantly different ($p < 0.05$) according to Tukey's LSD procedure. *C = control (0 kg/ha), L = lime (2250 kg ha^{-1}), AS70 = ammonium sulphate at 70 kg ha^{-1}; AS211 = ammonium sulphate at 211 kg ha^{-1}; AN70 = ammonium nitrate at 70 kg ha^{-1}; AN211 = ammonium nitrate at 211 kg ha^{-1}; AS70L = ammonium sulphate at 70 kg ha^{-1} + lime; AS211L = ammonium sulphate at 211 kg ha^{-1} + lime; AN70L = ammonium nitrate at 70 kg ha^{-1} + lime and AN211L = ammonium nitrate at 211 kg ha^{-1} + lime.*

3.3.2 Effect of N fertilization and liming on soil chemical properties

Soil pH and acid saturation

Long-term nitrogen addition and liming significantly ($p<0.05$) altered soil pH (Figure 3.2). After 70 years, soil pH varied significantly from 3.27 to 6.56 amongst treatments.

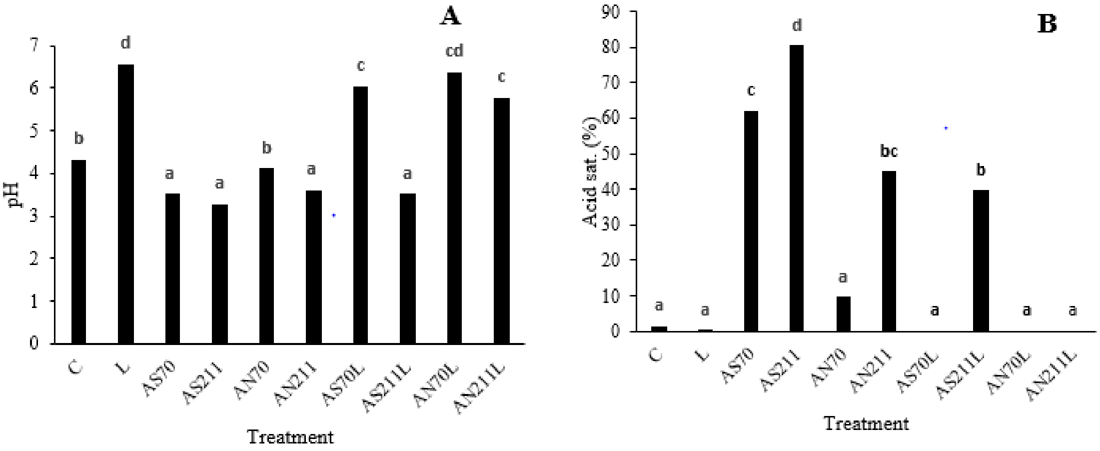

Figure 3. 2 Soil pH (A) and acid saturation (%) (B) after 70 years of lime and nitrogen application. *Means represented by same letter are not significantly different ($p <0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

Addition of N fertilization as ammonium sulphate significantly decreased soil pH irrespective of rate (Figure 3.2A). Only the high application rate of ammonium nitrate (AN211) significantly decreased soil pH compared to the control. Lime (L) and all lime and nitrogen fertilizer combinations, except AS211L, significantly increased soil pH ($p<0.001$) compared to the control (Figure 3.2). Acid saturation percentage was highest on AS211 (80.3), AS70 (62.0), AN211 (45.0) and AS211L (39.7) treatments while the control and limed treatments had the lowest acid saturation (Figure 3.2 B). High N rates, irrespective of fertility type, resulted in high acid saturation compared to lower application rates.

Soil organic C, total N and soil C:N ratio

Soil organic C was not significantly altered by liming and N addition when compared with the control (Figure 3.3A). However, there were significant differences between N fertiliser types and application rates. Treatment AN70 (46.6 g C/kg) reduced SOC compared to AN211 (57.6 g C/kg) and both ammonium sulphate treatments (57.2 and 56.1 g C/kg for AS70 and AS211, respectively) (Figure 3.3A).

Ammonium nitrate at 211 kg/ha and the AS at both rates had higher TN than the control (Figure 3.3B). Total soil N in AN211(4.15 g N/kg) was comparable to AS70 and AS211 and all were significantly higher than AN70. Only combinations of lime with higher rate (211 kg/ ha) of both AS and AN led to a significant increase in TN (Figure 3.3B).

Soil C:N ratio was significantly affected by treatments (Figure 3.3C). Soil C:N ratio ranged from 13.3 to 16.0 across all the treatments. Control had the highest soil C:N ratio, lime and N decreased soil C:N ratio ($p<0.05$) when compared to the control. Comparison between the treatments, lime had high C:N ratio while AS211L had the lowest (Figure 3.3C).

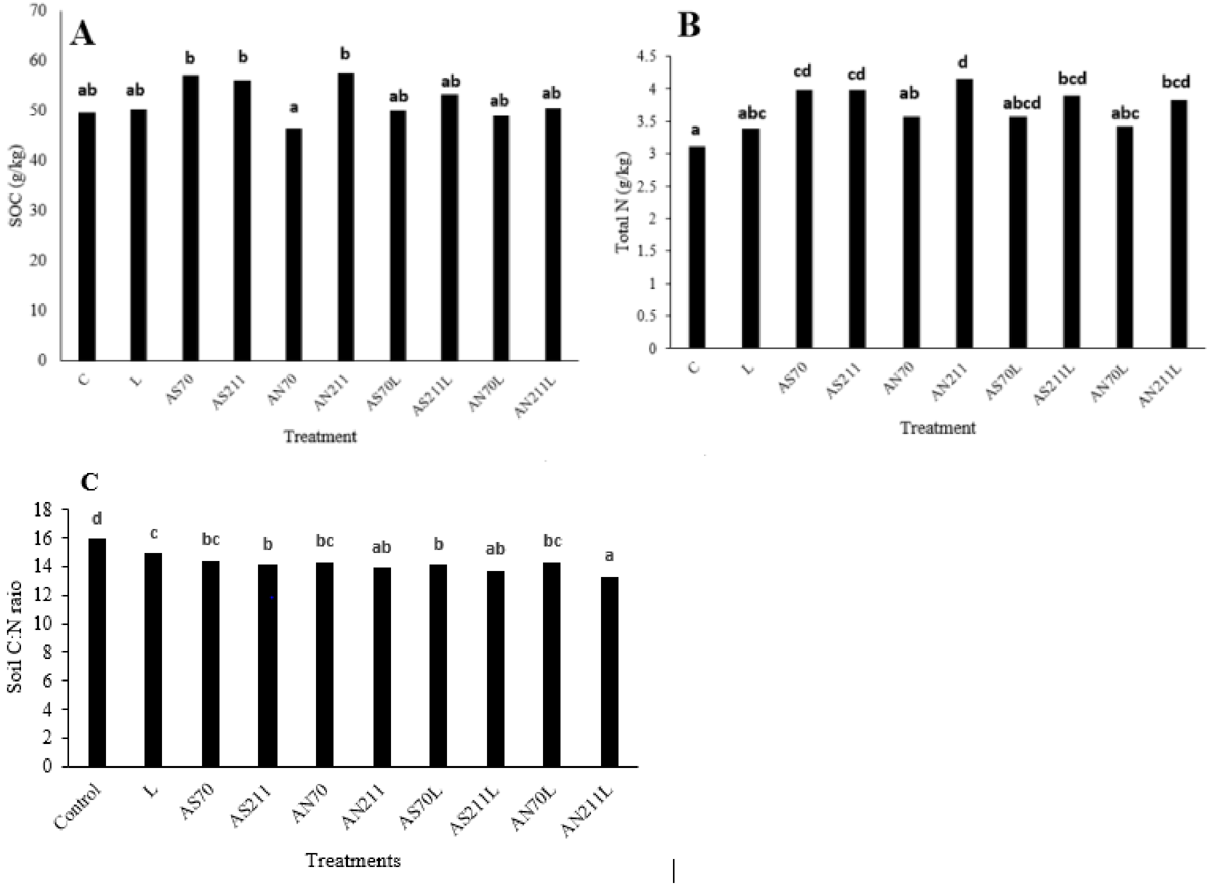

Figure 3. 3 Mean concentration of SOC (A), soil TN (B) and soil C:N ratio (C) as affected by long-term liming and N fertilization. *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

Soil exchangeable bases and effective cation exchange capacity

Long-term N addition and liming affected the concentration of exchangeable Ca, Mg and ECEC (Figure 3.4A, B and C).

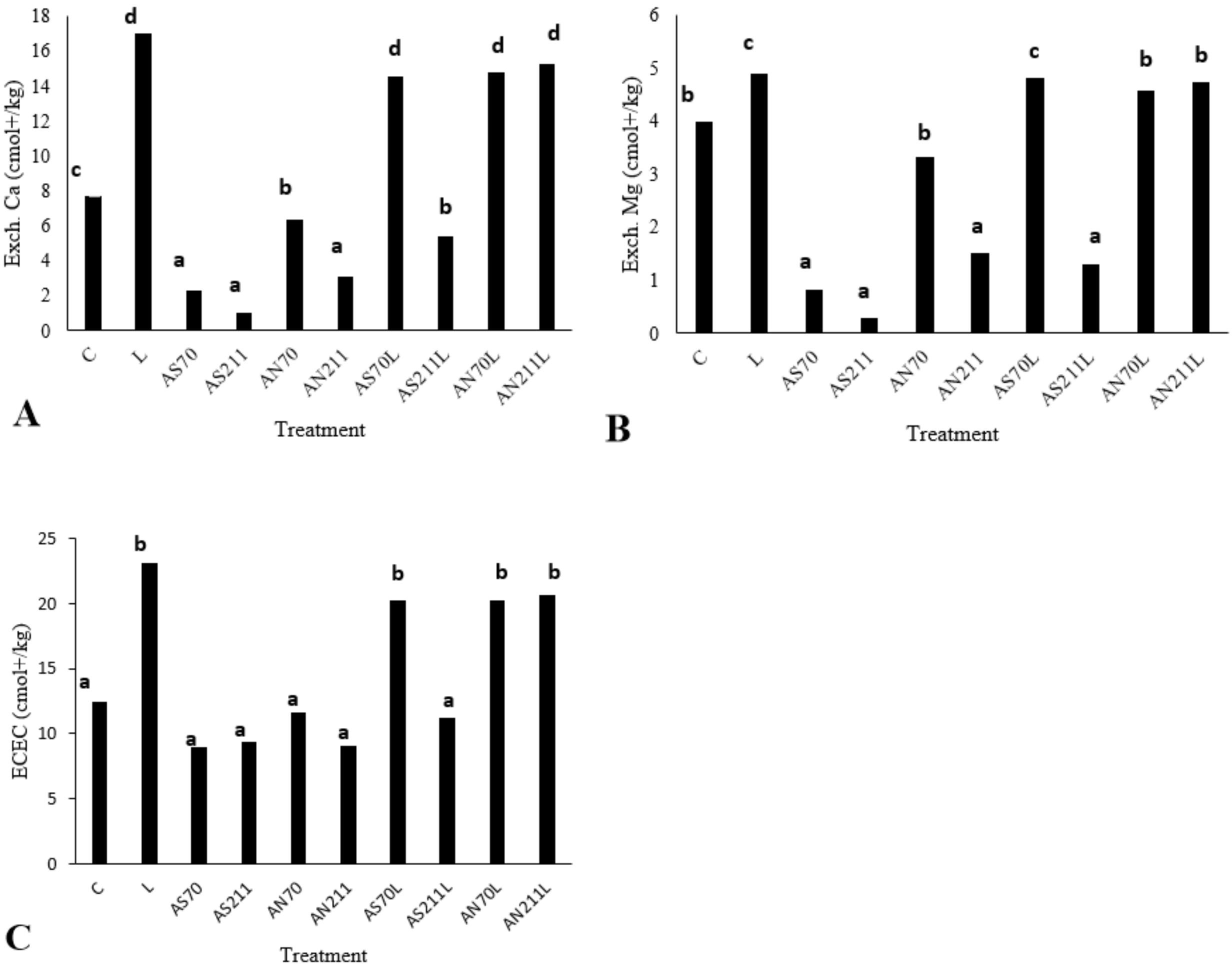

Figure 3. 4 Exchangeable Ca (A), Mg (B) and effective cation exchange capacity (C) as affected by liming and N fertilization. *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

Concentration of Ca and Mg ranged from 7.71 to 17.04 cmol+/kg and 0.28 to 4.90 cmol+/kg, respectively across all treatments. Limed treatments significantly increased the concentration of exchangeable Ca except AS211L compared to the control, while AS70, AS211 and AN211 significantly decreased exchangeable Ca when compared to the control (Figure 3.4A). Exchangeable Mg was lower in AS70, AS211, AN211 and AS211L treatments (Figure 3.4B). Only the L and AS70L treatments had significantly higher exchangeable Mg than the control. We found that neither long-term N addition nor liming significantly affected exchangeable K. Exchangeable bases followed the order Ca > Mg > K in all treatments. Effective cation exchange capacity was increased by L, AS70L, AN70L and AN211L compared to the control.

Soil available P

Soil available P ranged from 6.94 to 37.94 across the treatments. It was significantly increased by both N fertilizer treatments and rates except for AN70 (Figure 3.5). Comparably, limed treatments, including lime and N fertiliser combinations (except AS211L), had lower P concentration compared to AS70, AS211 and AN211 but were not significantly different from the control. Treatment AS211L had higher extractable P compared to the control and other limed treatments.

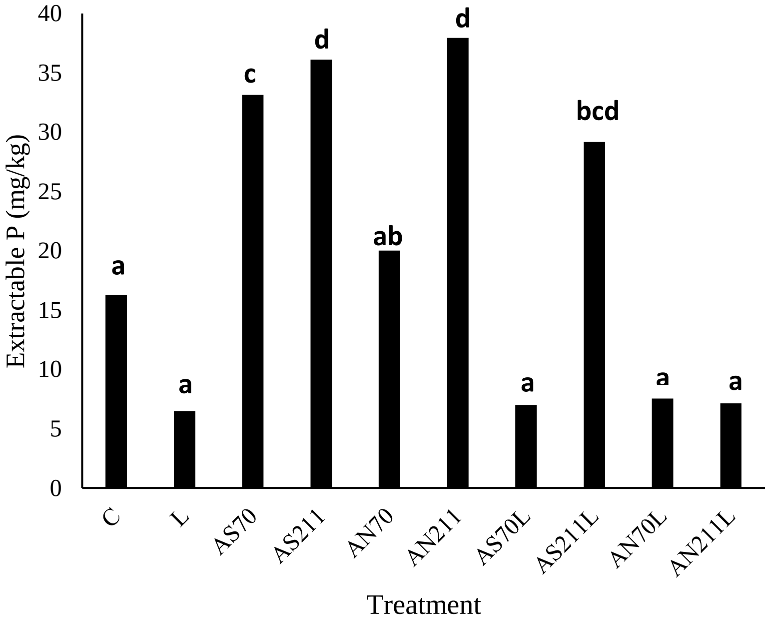

Figure 3. 5 Soil extractable P depending on liming and N fertilization. *Means represented by same letter are not significantly different (p <0.05) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

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Soil micronutrients

Micronutrients were affected by nitrogen and lime application (Table 3.2). Zinc was only significant for AN211L ($p<0.01$) while all other treatments were not different from the control. Zinc concentration in AN211L (0.42 mg Zn kg^{-1}) was significantly lower than AS70 (1.33 mg kg^{-1}). Treatment AS70 (99.3 mg kg^{-1}) lead to a significant two-fold increase ($p<0.001$) in Mn concentration compared to the control (33.4 mgkg^{-1}).

Table 3. 2 Trace metals concentration as affected by N fertilization and liming

Treatments	Zinc	Manganese	Copper
		mg/kg	
C	1.51 ^{b}	33.35 ^{ab}	7.79 ^{b}
L	0.72 ^{ab}	2.08 ^{a}	2.09 ^{a}
AS70	1.33 ^{b}	99.32 ^{c}	9.05 ^{b}
AS211	1.02 ^{ab}	40.62 ^{ab}	9.40 ^{b}
AN70	1.22 ^{ab}	54.22 ^{bc}	8.83 ^{b}
AN211	0.90 ^{ab}	69.37 ^{bc}	10.43 ^{b}
AS70L	0.81 ^{ab}	3.49 ^{a}	2.79 ^{a}
AS211L	0.98 ^{ab}	64.64 ^{bc}	7.93 ^{b}
AN70L	0.84 ^{ab}	1.98 ^{a}	2.27 ^{a}
AN211L	0.42 ^{a}	7.10 ^{a}	1.87 ^{a}
p-value	**0.013**	**<.001**	**<.001**

Values within a treatment followed by a different lowercase letter in the columns are significantly different ($p<0.05$) according to Tukey's LSD procedure. *Means represented by same letter are not significantly different ($p<0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

Except for AS211L (64.6 mg/kg), L (2.08 mg/kg) and lime + N fertiliser combinations, AS70L (3.49 mg/kg), AN70L (1.98 mg/kg) and AN211L (7.10 mg/kg) significantly decreased Mn compared N fertilised plots which had 99.3, 40.6, 54.2 and 69.4 mg/kg for AS70, AS211, AN70 and AN211, respectively. The concentration of copper was not affected by N addition; however, it was reduced by all limed treatments, except by AS211L, compared to the control (Table 3.2).

3.4 Discussion

Soil acidification was the major effect of long-term N fertilization at Ukulinga veld experiment. Naturally, studied soils are acidic soils, however, nitrogen fertilizer decreased pH further below the critical levels for Al toxicity (from 4.31 in control to 3.27 in AS211), especially at high application rate and when ammonium sulphate is applied. Similar results have been reported in several studies on the effect of long-term application of ammonium-based fertilizer (Malhi et al., 1998; Rasmussen and Rohde, 1989; Xu et al., 2020). Long-term application of nitrogen decreased soil pH by >16 % in 0-10 cm depth. Long-term application of N increased soil acidity especially high application rates, with ammonium sulphate having more acidifying effect than ammonium nitrate due to the higher ammonium added in former than the latter at a given application rate (Figure 3.2). Ammonium based fertilizers are known to acidify the soil by nitrification and plant uptake of NH_4^+ which releases H^+ protons (Are et al., 2018; Bouman et al., 1995; Matson et al., 1999). This argument is supported by high acid saturation on N fertilized treatments compare to limed treatments. This reduction in the soil pH poses a serious implication on grassland species composition and diversity which have been shown to decrease under nitrogen addition (Fang et al., 2012; Ward et al., 2017b; Midolo et al., 2019). Liming managed to increase soil pH towards neutral even when combined with N counteracting the effect of N fertilizers. However, liming was ineffective to ameliorate soil pH when combined with higher rate of ammonium sulphate.

High acid saturation was expectedly accompanied by a decrease in exchangeable bases and ECEC with N fertilization (Figure 3.4). Effective cation exchange capacity of soil that have variable negative charge depends on soil pH, and ionic strength (Edmeades 1982). Organic matter may have played a role as a pH-dependent charge surface which is represented by an increase in ECEC when lime is applied. At low pH, it is mainly the permanent charge of the clay particles responsible for adsorbing exchangeable cations (Solly et al., 2020) which could have accounted for acidic treatments. This mechanism is caused by the deprotonation of pH-dependent charge sites arising from organic or mineral components of the soil (Edmeades 1982). The contribution of different exchangeable cations to ECEC was affected by pH; greater than 70 % and 21 % of ECEC originates from the exchangeable Ca^{2+} and Mg^{2+} respectively at pH higher than 5.5 of which ECEC had a contribution from both clay and OM. This is not surprising as Ca^{2+} is the major cation present in lime, which dominates most of the exchange sites at high pH and has high selectivity preference upon liming (Edmeades 1982). These results are coherent with several studies reporting a decline in exchangeable bases with N addition because of increasing solubility of Al and Fe (Lungu et al., 2008) and an increase with lime addition (Caires et al., 2015).

While some authors reported decline (Luo et al., 2019), increase (Eze et al., 2018; Fornara and Tilman, 2012) in SOC concentration and C stocks in grasslands with N application and liming (Fornara et al., 2011), SOC content was not significantly affected by these treatments in this study. Current findings are consistent with earlier results from Ukulinga Research Farm (Zeglin et al., 2007). Lack of SOC response may be attributed to possible carbon saturation. Studied soils have very high C, ranging from 49.7 g/kg to 57.6 g/kg across treatments, which may have decreased bulk soil C stabilization (Six et al., 2002). Carbon saturation prevents addition of new SOC even in fertilized plots with high C inputs. Soil carbon stocks are high in the surface layer of most grassland soils, which causes the carbon saturation deficit to be relatively low, therefore increasing soil carbon stocks by carbon inputs from primary production may be limited (Whitehead et al., 2018). Angers et al. (2011) showed that soil pH and clay and silt particles are strongly related to carbon saturation deficit. Our soils were acid which could explain that they might have reached carbon saturation, where decomposition of SOM is limited on low pH (Angers et al., 2011). Bulk density is dependent on factors such as organic matter, texture and nutrient content (Chaudhari et al., 2013). Soil C:N ratio, however, decreased due to N and lime addition suggesting that both liming, and N addition did not significantly contribute additional C sequestration because of lower N use ratio (Yang et al., 2011).

High total N concentration may be explained by significant amounts of N added (70 kg N/ha and 211 kg N/ha) annually via fertilization and reduced nitrification due to N-induced acidity. The recorded increase in soil N with increasing N rates for both N fertilizers is coherent with earlier findings (Ward et al., 2017a; Wang et al., 2018b). Nitrification process is sensitive to acidic soils and limited by high Al and Mn or H^+ concentrations (Robson and Abbott, 1989). Moreover, an increase in soil total N may be due to great returns of organic N to the soil roots and litter (Glendining et al., 1996). Lime did not significantly change total N concentration.

Phosphorus concentration was doubled by N fertilizers compare to the control, while lime reduced it (Figure 3.5). The availability and cycling of P in the soil are determined by complex and interrelated soil chemical and biological processes. It is convincing that the processes involved in this study were precipitation and dissolution of P transformation where phosphate sorption decreased for Al oxides at $pH < 4$, attributed to the absence of proton-reactive but phosphate non-reactive triple-coordinated surface hydroxyls at the oxide surface (Fan et al., 2019). This view is supported by soil pH values less than 4 under N addition (Figure 3.2) suggesting Fe rather than Al had a strong influence on soil P fractions. Studies have reported a shift in the soil buffering system from Al towards Fe with N addition, particularly at $pH <4$ (Bowman et al., 2008). Fan et al. (2019) found similar results and attributed increased available P under N addition to decreased organic Fe and Al contributing to desorption and decomposition of organic P. Another possible explanation for increasing P concentration under N addition may be related to an increase of fungi and phosphatase activity which accelerate organic P decomposition thus releasing available P. The classic view of maximum P solubility is obtained at pH 4.5 – 6.5 which is contradicted in this study as plots with pH 6 – 6.5 had the lowest available P concentration, while acidic plots with $pH< 4$ showed high P availability. The possible explanation low available P following liming would be the formation of Ca phosphate mineral on plots with high pH values triggered by high Ca concentration which can decrease the pH required for precipitation of Ca phosphate mineral to be less than 7 (Penn and Camberato, 2019). A similar observation in decline in P concentration with lime addition is reported by Amarasiri and Olsen (1973) and Fan et al. (2019). These plots are P limited which is common in semi-arid climates (Kong et al., 2020). Soil microbial activity, litter decomposition, nutrient mineralization, and soil nutrient availability is limited in this climate, affecting P cycling (You et al., 2018). It has been highlighted that P concentration and microbial P is related to P inputs and moisture (Frossard et al., 2000) which may have been affected in these plots receiving no P input.

Trace metals were affected by long-term N and lime addition (Table 3.2). These concentrations are below the average content of trace elements of uncontaminated soils (Wyszkowski and Brodowska, 2020). The effect of liming and N fertilization on copper and manganese could be a function of pH (Laser 2007) as manganese and copper concentrations were high on acidic plots, than in limed plots. The copper content in soil can be affected by texture and tends to be adsorbed by SOM, carbonates, clay minerals, and oxyhydroxide of Mn and Fe (Hejcman et al., 2009; Borah et al., 2020) and caused immobility which could explain lower Cu concentration. Hejcman et al. (2009) reported an increase in Mn, Zn and Cu with long-term addition of ammonium nitrate and a decline on limed soils. They also argue that the pH had a strong effect on the mobility of these elements.

3.5 Conclusion

In this chapter, we conclude that the long-term application of nitrogen and liming influenced the fertility status of the fertilized veld. Long-term nitrogen fertilisation increased soil acidity, total nitrogen and phosphorus. Ammonium sulphate fertilizer tend to be more acidifying than AN fertilizer. This is also supported by failure of acid amelioration on at higher AS rates. Soil acidification due to N fertilization reduced exchangeable bases and ECEC. Liming increased pH, exchangeable bases, and decreased P. Nitrogen application has a detrimental effect on soil fertility which could limit plant growth, however, liming can remediate the effect through induced pH effect. Long-term application of N and lime did not affect SOC content. Lack of change in total organic carbon upon application of both nitrogen fertiliser and lime suggests that addition of new SOC from residues inputs was limited possibly due to C saturation. Through pH induced effect, lime remediate the toxicity of Mn and Cu which is elevated at lower pH values triggered by N application. Due to decline in phosphorus concentration in limed soils, P fertilizer should be employed to avoid P deficiency as readily available P to reach optimum requirements in the soil and ensuring substantial production.

CHAPTER 4

LONG-TERM NITROGEN FERTILIZATION AND LIMING EFFECT ON CARBON STORAGE IN WATER STABLE AGGREGATES IN A SEMI-ARID GRASSLAND SOIL

4.1 Introduction

Grasslands store about 30 % of terrestrial soil carbon (C) (Cenini et al., 2015) and contribute significantly to C and nutrient cycling. Nitrogen fertilization increases plant growth thus increasing above-ground C storage. Inconsistent results have however been reported with regards to SOC stock response to N addition with some studies reporting no response (Hassink, 1994; Zeglin et al., 2007; Lu et al., 2011), decrease (Luo et al., 2019) and increase (Fornara et al., 2013; Du et al., 2014; Cenini et al., 2015; Eze et al., 2018; Crème et al., 2020). Consistent with earlier findings by Zeglin et al. (2007), Chapter 3 reported no response of total SOC to N addition and liming at Ukulinga grassland trial. No response of soil organic carbon (SOC) stocks have been attributed to decoupling of above-ground and below-ground C dynamics due to complex C related processes associated with N addition causing lack of soil C storage (Lu et al., 2021). Some soils have been found to be carbon saturated with respect to C inputs (Stewart et al., 2007). High C inputs and/or high soil C levels have been associated with decreased bulk soil C stabilization in long-term agroecosystem experiments (Nyborg et al., 1995; Reicosky et al., 2002; Gulde et al., 2008) suggesting a saturation level for soil C (Six et al., 2002).

Complex interactions between organic matter and its environment largely impacts the persistence of organic carbon in ecosystems (Schmidt et al., 2011). Size and turnover of labile and recalcitrant C pools are largely influenced by differences in soil management, ecosystems and soil properties making it difficult to predict the net effect of N enrichment on soil C storage (Neff et al., 2002). Stabilization of organic matter in soil aggregates is the principal mechanism for long-term soil C sequestration (Verchot et al., 2011). Soil aggregation is one of the main factors that encourage the persistence of SOC by forming a protecting barrier that impedes microbial access and increases water filtration, thus reducing water runoff and C losses through erosion (Bai et al., 2021). Generally, increases in SOM are associated with C-rich macro-aggregates but long-term C sequestration depends on stabilization of carbon in micro-aggregates (Tisdall and Oades, 1982; Six et al., 2000a). According to Wu et al. (2004) (SOC) in different size aggregates characterizes the relationship between organic matter balance and mineralization rate with dual significance in soil fertility and carbon sink. Organic C stored in differently sized stable aggregates has shown a strong response to management compared to bulk SOC (Denef et al., 2007; Wang et al., 2016). For example, Chen et al. (2021) reported a significant increase in mineral associated C while no response was observed for bulk SOC following N addition to B. *platyphylla* forest. Accoring to Lu et al. (2021) the combined effects of individual aggregate C drives the general response of soil C pools to N enrichment. This suggests that separating bulk soil into different functional C pools, related to differently sized aggregates, may be used as a diagnostic indicator for potential changes) and reveal observed complex responses of SOC to N addition. Investigating carbon pools in soil aggregates thus provides an understanding of the dynamics of carbon sequestration and mineralization in aggregates (Whalen and Chang, 2002). Despite many studies on the effect of N enrichment on bulk soil C dynamics, there is lack of evidence on the mechanisms explaining the response of soil aggregates (Lu et al., 2021).

Grasslands and pastures are subjected to acidic conditions due to fertilizer application or extensive cation leaching (Paradelo et al., 2015). Application of ammonium-based fertilisers are particularly responsible for significant soil acidification in human-managed pastured. The N-induced acidity results in loss of basic cations and high aluminum toxicity. Loss of Ca due to N-induced acidity interferes with the bridging of clays and organic materials thus decreasing stability of water stable aggregates (Oades, 1984). However, contradicting results have been reported where low soil pH was associated with high aggregate stability (Bethlenfalvay et al., 1999). This positive effect on water-stable aggregates was attributed to possible inhibition of bacterial proliferation slowing down of degradation of the organic stabilizing agent under with increasing soil acidity. Fertilization-induced release of NH_4^+ is also associated with reduced aggregate stability (Haynes and Naidu, 1998). Exchangeable Ca contributes significantly to aggregation through flocculation of clay particles (Rengasamy and Marchuk, 2011). Adsorption of NH_4^+ (low hydration energy) in place of high hydration energy cations (e.g., Mg^{2+} and Ca^{2+}) results in the collapse of the interlayer (Rigol et al., 1999) due to NH_4^+ -induced dispersion. Liming is employed to remediate the effect of acidity on soil's physical and chemical properties. A review by Paradelo et al. (2015) showed that several factors determine the net effect of liming on SOC. For example,(a) liming can increase microbial activity by creating more favourable pH conditions, which will enhance OM mineralization, thus decreases SOC stocks (b) favourable pH condition increases plant productivity and net primary production (NPP) resulting in more significant OM inputs in the form of dead roots and decaying crop residues consequently increasing SOC and (c) liming is known to ameliorate soil structure thus increasing clay assemblages and clay-organic matter bonds creating physical and physico-chemical protection of SOC.

Despite many studies on the effect of N enrichment on bulk soil C dynamics, there is lack of evidence on the mechanisms explaining the response of soil aggregates (Lu et al., 2021). Moreover, most long-term studies on grassland management focus on separate effects of liming and nitrogen on SOC, C in aggregates and aggregates stability while little attention has been given to their interaction effects on these parameters. Johnson et al. (2005) showed that a combination of lime and N affect soil microbial biomass, soil pH, and total N in a short-term upland grassland experiment in Scotland. Studies from Ukulinga veld fertilization experiment have focused on plant productivity (Le Roux and Mentis, 1986), plant diversity (Grunow et al., 1970; Scott and Rabie, 1956), soil biological properties (Zeglin et al., 2007; Ward et al., 2017a), and soil as affected by fertilizer and liming addition. Soil properties such as SOC, MWD, and aggregate stability are reported as essential soil characteristics and not profoundly examined on the possible processes involved. This chapter aims to examine the long-term effects of nitrogen fertilizer type and rate and liming on water-stable aggregates (WSA) fractions and carbon stored in them. We hypothesize that; (1) Nitrogen application will increase carbon concentration in bulk soil and (2) Liming will increase the WSA, MWD, and increase C concentration in macro-aggregates.

4.2 Material and methods

4.2.1 Site description

The description of study site and experimental design is given in section 3.2.1 and 3.2.2 of Chapter 3.

4.2.2 Soil sampling and analysis

Soil sampling procedure is explained in section 3.2.3 of Chapter 3. Soil samples for microbial biomass were sampled and immediately stored in a cooler box and transported to the lab for analysis.

Aggregate stability was determined using the wet sieving method as described by (Six et al., 2000a). Air-dried samples were sieved through an 8mm sieve. An 80 g of 8 mm sieved soil sample was placed on a 2 mm sieve and submerged in a bowl with water for 5 minutes, followed by sieving for 2 minutes by moving the sieve up and down 50 times. The material remaining on the 2 mm sieve was transferred by backwashing into a pre-weighed pan and dried for 48 hours at 60 ^{o}C. By repeating wet sieving using 250 µm and 53 µm sieves, four aggregate fractions were obtained. The water-stable soil aggregates (WSA) classes were separated into large macro-aggregates (LMA; > 2 mm), small macro-aggregates (SMA; > 250 µm–2 mm), micro-aggregates (MiA; 53–250 µm) and silt + clay fractions (SCA; < 53 µm). Mean weight diameter (MWD) for WSA was calculated using the equation:

$MWD = \sum_{i=1}^{n} XiWi$ (1) (Abdalla et al., 2016)

where Xi is the mean diameter for each fraction size, Wi is the proportional weight of the fraction from the total dry weight of soil used, and n is the number of aggregate classes separated. Aggregate stability was expressed as MWD. Organic C in water-stable soil aggregate fractions and bulk soil was analyzed using the Walkley- Black dichromate oxidation method (Walkley and Black, 1934). Carbon measured in each pool was then expressed as a fraction of bulk SOC which allowed for the calculation of the percent recovery of soil C. The recovery of total SOC after fractionation was 87.7 – 99.5 % across all treatments. Soil carbon stocks in bulk soil were calculated using the formula

$SOC_{stocks} = \sum_{i=1}^{n} BDi \,.\, SOCconc \,.\, Depth_{volume}$ (2)

Where SOC_{stocks}= soil organic carbon stocks (t/ha), BD = Bulk density (g/cm^3), SOC_{conc} = soil organic carbon concentration (g/kg) and $depth_{volume}$ is the sampling depth (cm)

Statistical analysis

Statistical analysis was done using GENSTAT 18th edition. One-way analysis of variance (ANOVA) was used to compare effects of different treatments on soil properties. Treatment means were compared using Turkey's multiple comparisons at significance level $p<0.05$.

4.3 Results

4.3.1 Mean weight diameter and weight of water-stable aggregate fractions

Long-term nitrogen addition and liming significantly ($p<0.05$) affected MWD and weight of water stable aggregates. Mean weight diameter was only significantly decreased by the AS70 and AN211 treatments compared to the control (Figure 4.1). While liming did not significantly affect MWD when compared to the control, the MWD in the lime treatment (4.03) was significantly higher than in the AS70 (2.01), AN70 (2.67), AN211 (2.27), and AS211L (2.84).

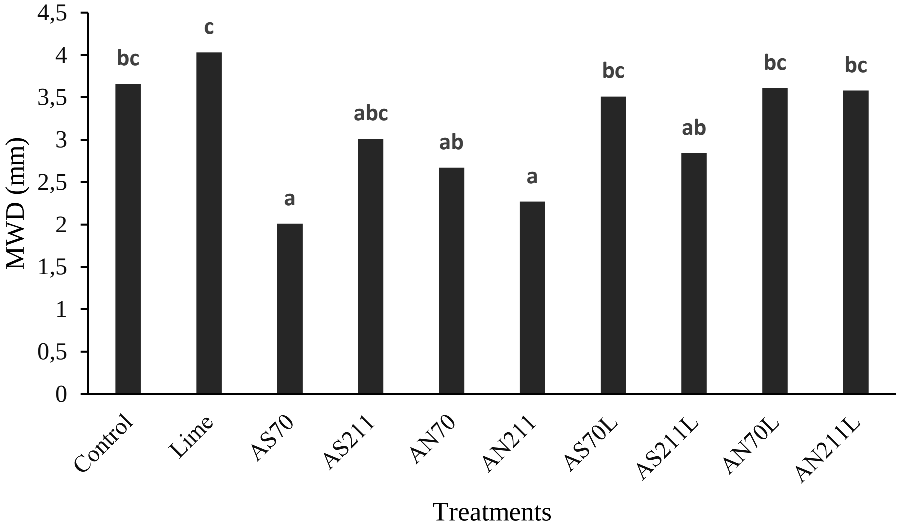

Figure 4. 1 Mean weight diameter of soil aggregates affected by nitrogen and liming. *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg ha^{-1}), AS70 = ammonium sulphate at 70 kg ha^{-1}; AS211 = ammonium sulphate at 211 kg ha^{-1}; AN70 = ammonium nitrate at 70 kg ha^{-1}; AN211 = ammonium nitrate at 211 kg ha^{-1}; AS70L = ammonium sulphate at 70 kg ha^{-1} + lime; AS211L = ammonium sulphate at 211 kg ha^{-1} + lime; AN70L = ammonium nitrate at 70 kg ha^{-1} + lime and AN211L = ammonium nitrate at 211 kg ha^{-1} + lime*

Long-term nitrogen fertilizer and liming application influenced water-stable aggregates (Figure 4.2). Except for AS70, all nitrogen fertilizer treatments significantly decreased large macro-aggregates (LMA; >2 mm) fractions (Figure 4.2A). Lime had relatively high LMA fraction compared to the control, but the difference was not significant. Except for the (AS211L), the combination of lime with nitrogen fertilizer treatments had higher large macro-aggregates when compared with the treatments without lime. The trend of small micro-aggregate (SMA) results (Figure 4.2B) was inverse to that of the LMA (Figure 4.2A).

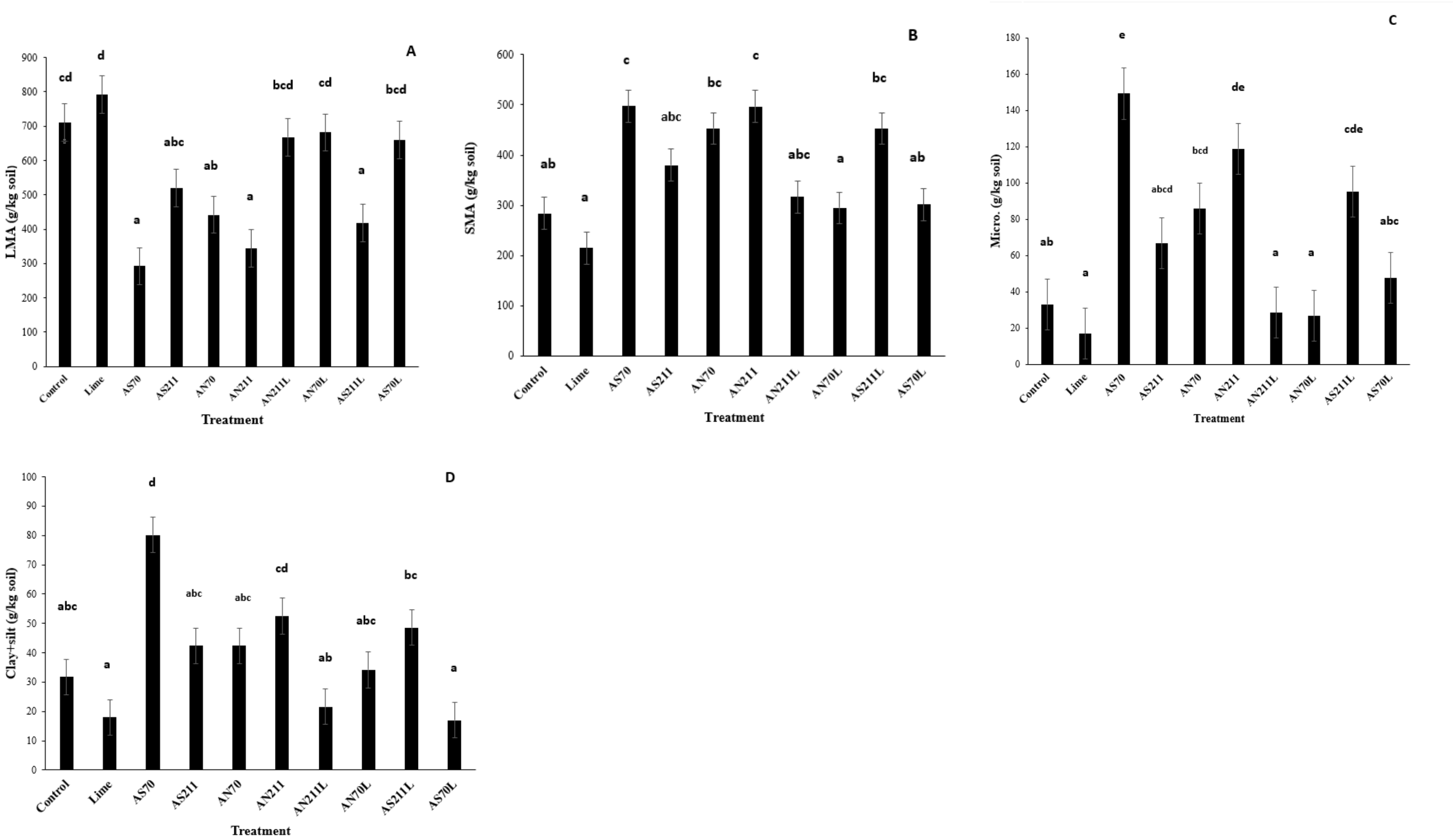

Figure 4. 2 Masses of large macro-aggregates (A) (LMA; >2 mm), (B) Small macro-aggregates, (C) micro-aggregates, and (D) silt+clay fractions for different treatments. Error bars represent standard error (n=3). *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

The fertilizer treatments without lime, except the AS211L, increased SMA (> 250 μm–2 mm) when compared with the control (Figure 4.2B). The results of micro-aggregates (MiA; 53–250 μm) (Figure 4.2C) and clay + silt fractions (Figure 4.2D) followed the same trend as that of SMA (Figure 4.2B), where the AS70, AN211, and AS211L for both, and AN70 for MiA, had higher fractions than the control. The addition of lime to the fertilizer treatments reduced the SMA and MiA fractions except in the AS211L wherein both fractions increased. Similarly, lime addition reduced the silt+clay fractions in the AS70 and AN211 treatments, while lime + AS11 treatment increased this fraction compared to the same treatments without lime. The WSA were in the order; (LMA; >2 mm) > (SMA; >250 μm–2 mm) > (SCA; < 53 μm) > (MiA; 53–250 μm), with the macroaggregate (> 250 μm) fractions making up >50% of the total soil weight in all treatments.

4.3.3 Organic carbon in water-stable aggregates

The SOC concentration in the AS70 and AN211 treatments was significantly lower in the LMA fraction and higher in the SMA when compared to the control (Table 4.1). Lime (L) significantly increased C storage (3.65 %) in LMA compared to AS70 (1.52 %), AN70 (1.91 %), and AN211 (1.67 %). Overall the control, L, AS70L, and AN70L, and AN211L had >3 % C stored in the LMA fraction (Table 4.1). In all treatments, more than 60 % of SOC was stored in large macro-aggregates except for AS70, AN70, AN211, and AS211L where more ca

was stored in small macro-aggregates (>45 % of SOC). The results of SOC in the other fractions followed an inverse trend to that in the LMA, where the AS211L, AN211, and AS70 treatments significantly increased C in micro-aggregate (53–250 μm) fraction in the order; AS211L< AN211 <AS70. Limed plot had lowest C concentration in MiA; 53–250 μm (0.09 %) compared to AS70 (2.67 %), AN211 (2.49 %) and AS211L (2.31 %). The SCA (< 53 μm) had the least carbon concentration of all the aggregate fractions of which only AS70 had a significantly higher concentration compared to the control (Table 4.1). Both micro-aggregates and clay and silt fraction (<250 μm) stored less than 20 % of the total C. There was a significant difference between L and nitrogen fertilized plots, where nitrogen fertilized plots increased C with an increase in N level in clay + silt compared to limed plot (Table 4.1).

Table 4. 1 Organic carbon in water-stable aggregates as affected by long-term nitrogen fertilization and liming.

Treatment	% Organic C in WSA			
	LMA; >2 mm	SMA; >250 µm–2 mm	MiA; 53–250 µm	SCA;< 53 µm
Control	3.40 cd	1.26 ab	0.17 abc	0.13 abc
Lime	3.65 d	0.96 a	0.09 a	0.079 ab
AS70	1.52 a	2.67 c	0.86 e	0.37 d
AS211	2.58 abcd	2.02 abc	0.47 cd	0.24 cd
AN70	1.91 abc	2.02 abc	0.39 bcd	0.15 abc
AN211	1.67 ab	2.49 c	0.65 de	0.25 cd
AS70L	3.41 cd	1.35 ab	0.22 abc	0.064 a
AS211L	2.08 abcd	2.31 bc	0.53 d	0.22 bc
AN70L	3.34 bcd	1.58 abc	0.15 ab	0.15 abc
AN211L	3.07 abcd	1.48 abc	0.12 ab	0.067 ab
p -value	**<.001**	**<.001**	**<.001**	**<.001**

Values followed by a different lowercase letter in the same column are significantly different ($p < 0.05$) according to Tukey's LSD procedure. *C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

4.4 Discussion

Nitrogen application decreased the proportion of large macroaggregates and increased microaggregates fraction which reduced mean weight diameter. This was due to the acidifying effect of ammonium N which led to rapid depletion of Ca^{2+} and Mg^{2+} (Figure 3.3) which performs an important role in aggregation through flocculation of clay particles (Rengasamy and Marchuk, 2011). Ammonium-based fertilisers release NH_4^+ (with low hydration energy) to the soil which is adsorbed in place of high hydration energy cations (e.g., Ca^{2+} and Mg^{2+}) causing dispersion of soil aggregates as the interlayer collapses (Rigol et al., 1999). N-induced acidity may have reduced nitrification making NH_4^+ a predominate form of N in studied plots. As such, NH_4^+-induced dispersion may also explain reduced fraction of large macroaggregates and MWD under separate N fertilization (Figure 4.1 and 4.2A). Similarly, Blanco-Canqui and Schlegel (2013) reported a decrease in aggregate stability upon addition of N at >100 kg N/ha/yr. Moreover, the acidity caused by increased N fertilizer application rate reduces fungal biomass, root growth, and length which is responsible for excreting binding agents, which could further explain the reduction in MWD and macroaggregates in N fertilized plots. Bai et al. (2020) found a strong correlation between MWD and exchangeable Mg^{2+} and fungal biomass.

Conversely, liming increased basic cations (Figure 3.3), thus encouraging the formation of cation bridging as positively charged cations function as the binding agent between negatively charged clay surfaces and organic compounds (Bai et al., 2020). An increase in soil pH when lime was applied may have led to improved conditions for nitrification thus decreasing NH_4^+ concentration thus counteracting N-induced effects on soil aggregation, especially where lime was combined with AS70 and AN211. These results imply that liming helps maintain a stable soil structure while N addition can make the soil susceptible to erosion and degradation. Interestingly, AS211L did not increase MWD as expected with lime application, instead, it reduced LMA, possibly due to a high acidifying effect than the other N fertilizer treatments.

Several studies recorded higher TOC in macro-aggregates than in micro-aggregates after long-term application of mineral and/or organic fertilizers (Bhattacharyya et al., 2010; Tripathi et al., 2014). Generally, 80 % of the total C is associated with macro-aggregates (>250 μm) in the upper soil layers of grasslands (Chen et al., 2019; Schwendenmann and Pendall, 2006). While Are et al. (2018) showed a positive correlation between soil acidity, organic C, clay, and silt on aggregate stability, the relationship of total SOC with aggregate stability was not significant in this study. However, aggregate size distribution and the SOC stored in the different aggregate sized fractions were affected by N fertilizer application and liming. SOC in bulk soil was not affected by treatments (Figure 3.3A), it can be said that SOC was redistributed into smaller fractions upon the breakdown of aggregates rather than being lost by decomposition suggesting that C dynamics were driven more by fertilizer and liming induced effect on aggregates. Applying N fertiliser without lime led to more carbon in SMA, MiA and SCA (Table 4.1) suggesting long-term storage of C as it is generally protected from microbial attack and thus have longer resident time compared to that stored in macro-aggregates (Six et al 2000b). As such high C in SMA and clay and silt were observed under N fertilization. Soil pH-mediated effects resulting from N fertilization can increase mineral surface activity resulting in an increase in mineral associated SOM (Riggs et al., 2015). Carbon in micro-aggregates is protected from microbial attack and thus has longer resident time compared to that stored in macroaggregates (Six et al., 2000b). Lime and the control had marginally higher mean values of OC in the LMA fraction than N fertilized plots though not significant. These results are consistent with the concept of aggregate hierarchy, which states that increasing aggregate class size results in an increase in C concentration in which microaggregates are bound together into macroaggregates by binding agents (Six et al., 2000b; Tisdall and OADES, 1982).

4.5 Conclusion

The findings of this study showed that long-term N and lime application resulted in the redistribution of organic C in water-stable aggregates. Separate application of N fertilizers decreased MWD, large macro-aggregates and increased micro-aggregates while separate liming had no effect on WSA. Nitrogen application had a strong influence on labile organic carbon which was shifted to stable soil fractions confirmed by high C in micro-aggregates (<250 µm). These findings suggests that long-term N addition may result in poor soil physical condition and possible stabilization of C in stable fractions. Contrary, liming and combination of lime with N fertilizer, except at high rates of ammonium sulphate led to the formation of large macro-aggregates and storage of high organic C in them. Changes in C associated with WSA fractions suggests their importance as diagnostic indicators of N fertilizer and liming induced changes in SOC. Findings also show that ammonium-based N fertilization is associated with soil acidification, dispersion of LMA resulting in an increase of micro-aggregates and C stored in them. Liming improved soil aggregation, increased MWD compared to N fertilized plots, despite having comparable SOC concentration. This emphasizes induced soil pH changes as the main driver of observed response to N-fertilization and liming at Ukulinga research farm. Liming can counteracts acidifying and the dispersive effect on NH_4^+ associated with ammonium-based fertilizers thus restoring macro-aggregation in N fertilized grasslands. Liming may thus be used to ameliorate N-induced effects on soil pH and structure when N fertilizers are applied on grasslands. This will ensure that while the goal is mainly to improve grassland productivity using N fertilizers, caution is also taken to maintain good soil condition.

CHAPTER 5

EFFECT OF LONG-TERM LIMING AND NITROGEN APPLICATION ON PLANT BIOMASS AND COMPOSITION C:N:P STOICHIOMETRY: IMPLICATION ON SOIL CARBON CYCLING IN A SEMI-ARID GRASSLAND

5.1 Introduction

Nitrogen fertilizers are related to an increase in biomass production of grasslands (Chen et al., 2018) through increasing N availability which enhances foliar N and chlorophyll content promoting rapid plant growth (Pons and Anten 2004). Based on a global comparison of grasslands biomass response to N, shoot: root ratio of more than 5.28 reflects overall decrease in biomass response to N enrichment (Lee et al., 2010). Despite the reported reduction in plant species and diversity on long-term N additions (Stevens et al,. 2004; Niu et al., 2018), greater increase in biomass shows positive effect of N on overall C sequestration through above-ground net primary production (NPP). Increase in N availability may alter stoichiometric balance of nutrients in plants and soil, potentially limiting P relative to N (Vitousek et al., 2010). For example, plant growth and biomass production are considered N limited when shoot N:P ratio is <14 and P limited when the ratio is >16 (Koerselman and Meuleman, 1996; Güsewell 2004) . Root N:P is rarely reported on any nutrient limitation (Zhan et al., 2017). N application increases tissue N concentration through enhanced uptake (Lobos Ortega et al., 2016) which results in alteration of tissue composition. Lü et al., (2012a) showed that N addition reduced C:N ratio and increased N:P ratio in semi-arid grassland, while under increased water supply, C:P and N:P ratio decreased. Other long-term N application showed that inorganic N reduces plant below-ground C:N ratio (Gill et al., 2006; Fornara and Tilman 2012). However, stochiometric ratios widely varies with species. N enrichments have potential of increasing soil acidity, which leads to suppression of plant growth in grasslands (Chen et al., 2013; Chen et al., 2015). Soil acidification could decrease concentration of mineral cation, resulting in change in plant species and tissue composition especially at high intensities of soil acidification.

Liming is introduced to grasslands to counteract impact of N induced acidification creating optimal condition for plant growth and enhance nutrient bioavailability. Liming have been reported to have no effect on biomass production, root biomass with decreases in plant C:N and biomass: N ratio (Lochon et al., 2019). On the contrary, liming increased shoot C:N ratio, and had negative effect on C:P ratio, below and above-ground N:P ratio, and N, C and P (%) content of above ground biomass in a grassland studied by Heyburn et al. (2017b). The differences are maybe a function of liming-induced increase in soil cation exchange capacity and nutrient availability. Liming on its own, does not contribute much on biomass production than when it's combined with other nutrients which supply limiting nutrients such as N, P and K. This is achieved through liming-induced effect on soil pH, microbial activity, soil N mineralization, which influence N uptake by plants. Forage production was increased in Arraba temperate mountainous grasslands (Mijangos et al., 2010), due to species adaptation.

Enhanced biomass production with N and lime application has a potential to sequester C, through returns of plant debris to the soil. Over long-term, C storage and sequestration depends on the balance between net primary production and decomposition (Hu et al., 2001). Soil N mineralization rate is negatively related to plant C:N ratio (Heyburn et al., 2017b), which is expected that soil N availability and cycling increases because of positive N-induced effects on soil N mineralization rates. Moreover, nutrient supply of microbial activity is related to plant stoichiometry, for example low C:N ratio of plant detritus inputs in fertilized grassland increase the activity of the C-acquiring enzyme β-1,4-glucosidase because of more C being processed and accumulated (including microbial biomass C) into organo-mineral soil pools. Therefore, when C is limited, microbial metabolism switches from N to C resource supply (Cenini et al., 2015). Therefore, there must be the link reflecting decomposability rate of plant litter, microbial activity, and nutrient cycling of which the overall processes regulate C storage in the soil.

Labile carbon is one of the most soil indicators of soil quality important in controlling nutrient availability to crops and microbes (Malobane et al., 2020). Change in labile soil C could result in nutrient in below-ground affecting soil stoichiometry ratio. Main contributor of labile C is plant litter; however, its decomposition rate depends on soil C:N ratio. It has been shown that higher C:N ratio slows down the mineralization process (Tong et al., 2009). This chapter aims to link grassland biomass, tissue composition to soil stoichiometry and labile C (water extractable organic C) response to long-term liming and N application in link with C sequestration and storage.

5.2 Material and methods

5.2.1 Site description

Site description is provided in Section 3.2 of Chapter 3.

5.2.2 Soil sampling and analysis

Soil sampling description (Section 3.2.1) and soil pH (Figure 3.2), SOC, N and soil C:N ratio (Figure 3.3) and extractable P (Figure 3.5) methodology is given in Chapter 3.

5.2.3 Cold-water extractable carbon (CWEOC)

Soil samples of 3 g air-dried sample was weighed and place in a 30 mL polypropylene centrifuge tubes and extracted with 30 mL of distilled water at 30 rpm for 30 min. Then the suspension was centrifuged at 3500 rpm for 30 min. The organic C in the supernatant was determined using the Walkley Black method.

5.2.4 Above-ground biomass and below-ground biomass sampling

Plant above and below-ground biomass samples were collected in March 2021. In the middle of the plots, 0.25 x 0.25 m^2 quadrants were created per chosen experimental plots, then the all above-ground plant samples were clipped to ground level using scissor and stored in plastic pockets. The harvested grass samples were oven dried at 60 ^oC for 48 hours and the dry weight was measured. The yield was calculated as dry weight of grass per area and presented as kg/ha . Corresponding below-ground biomass samples were obtained from the experimental plots using 3 soil cores to a depth of 10 cm from each plot. Cores from each plot were combined in one plastic bag. The roots were separated from the soil by washing and sieving through 0.5 mm sieve. Live roots were oven dried at 60 ^oC to a constant weight and expressed as dry weight kg/ha. The plant tissue samples were analyzed for C, N and P content (Manson and Roberts 2000).

Briefly, 0.125 g of milled (1 mm sieved) samples was weight and placed into a small thin foil cups and pace in a furnace for analysis. Mass of an empty beaker is pre-weighed, and 0.5 g of milled sample was added to beaker and placed in an oven 110 ^oC for 2 hours. The beakers were cooled in a desiccator for 30 min and weighed. The beakers were taken into the furnace at 450 ^oC for 4 hours. The beaker and ashed contents were removed, cool and then wetted with a few drops of distilled water, and 2 ml of conc. HCI added to each sample. Evaporated slowly to dryness on a water bath in the fume cupboard with the extractor fan on. Using a Fortuna Optifix dispenser, 25 ml of a freshly prepared 1:9 HCl solution was added and stirred each sample using a rubber policeman, rinsing the rod in a beaker of distilled water in between each sample.

Filtered through Advantech 5B: 90 mm diameter filter papers into a clean rack of sample cups. The filtrate was diluted with de-ionized water at a ratio of 5:20, then the diluted solution was analyzed for P on ICP-OES. Plant C and N analysis were done using Leco CNS 2000.

5.2.5 Statistical analysis

One-way ANOVA was used to test the effect of N and lime application on below-ground biomass, above-ground biomass, litter as well as plant elemental composition. A two-way ANOVA was used to test the interacting between the plant tissue (shoot and root) and treatment (N and lime) on stochiometric ratio. Significant differences among treatment means were analysed using Tukey's multiple comparison post hoc test. All the statistical analysis were used using GENSTAT 18th edition. Pearson correlation was used to determine the relationship of plant C:N:P stoichiometry and measured soil properties.

5.3 Results

5.3.1 Cold water extractable organic C

Concentration of CWEOC ranged from 30 to 88 mg/kg (Figure 5.1) Lime had the highest amount of cold-water extractable C but was not significant to the control. AN70L and AN211L had the lowest CWEC concentration and was significantly different from lime treatment.

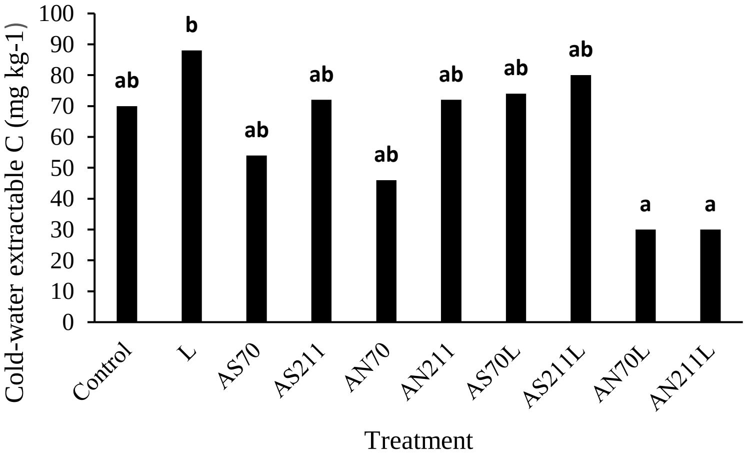

Figure 5. 1 Cold-water extractable C affected by long-term application of N and lime. *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

5.3.2 Plant biomass

Plant biomass was significantly affected by treatments (Table 5.1). Above-ground biomass ranged from 12737 t/ha to 35791 t/ha. Nitrogen fertilization as AS70 and AN211 significantly increased above-ground by 202 % and 218 %, respectively. Although relatively higher, lime and lime + N fertiliser treatments were not significantly different from the control. Both N fertilization and liming affected below-ground biomass ($p<0.05$). Separate liming and N fertilization, except for AN211, increased below ground biomass while their combination had no effect when compared to the control (Table 5.1). Litter quantity did not significantly differ across treatments.

Table 5. 1 Above ground (shoot), below ground (root) and litter biomass as affected by long-term liming and N application.

Treatments	Above-ground biomass	Below-ground biomass	Litter
		Kg/ha	
Control	16324^{abc}	75.8 ^{ab}	3497
Lime	12737^{a}	259 ^{cd}	1572
AS70	33075de	238.7 ^{cd}	2789
AS211	23801^{bcd}	353.2 ^{d}	2397
AN70	16251^{abc}	247.8 ^{cd}	1721
AN211	35719^{e}	90.0 ^{ab}	1962
AS70L	15612^{abc}	148.2 ^{abc}	2635
AS211L	25795^{cde}	174.6 ^{bc}	3350
AN70L	13363^{ab}	38.1 ^{a}	2726
AN211L	15253^{abc}	104.6 ^{ab}	3728
P value	**<.001**	**<.001**	**0.034**

Values followed by a different lowercase letter in the same column are significantly different ($p <0.05$) according to Tukey's LSD procedure. *C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium* *sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

5.3.3 Plant elemental stoichiometry

Plant tissue analysis shows that C, N and K accumulation was affected by N fertilizers (Table 5.2). Both N fertilizers and their combination with lime, expect for low N rates (AS70L and AN70L), increased C in the shoots. Ammonium nitrate fertilizer led to highest C accumulation in shoot biomass compared to ammonium sulphate. Only AN211 (1.16 % N) was significantly different from the control for shoot N concentration (Table 5.2). The AS70 had the lowest (0.656 %) shoot N concentration and was significantly different from AN211 (1.16 %), AS211L (1.01 %), and AN211L (1.05 %). There was no significant difference in shoot P concentration ($p>0.05$). Shoot K concentration was only significantly different between AS70, AS70L and AN211.

Nitrogen and liming altered the elemental concentration of the roots. Nitrogen concentration was high in AN211L (1.4 %) and was significantly different to control, lime; AN70, and AN211. Carbon in the roots was not affected by N nor liming application (Table 5.2) but noticeably, control had the roots with highest C concentration while lime had the least C in their roots. Treatments with nitrogen fertilizer significantly decreased P concentration in roots except for AS211 and AS70L.

Table 5. 2 Elemental plant tissue concentration for above-ground (shoot) and below-ground (roots)

Treatment	Above-ground biomass				Below-ground biomass		
	C	N	P	K	C	N	P
				%			
Control	42.40 ^{a}	0.8401 ^{abc}	0.08	1.008 ^{ab}	43.43	0.68 ^{ab}	0.071 ^{d}
Lime	44.31 ^{ab}	0.7113 ^{ab}	0.082	1.290 ^{ab}	24.99	0.63 ^{a}	0.061 ^{cd}
AS70	46.07 ^{bc}	0.6563 ^{a}	0.061	0.863 ^{a}	30.76	0.63 ^{a}	0.045 ^{abc}
AS211	46.97 ^{bc}	0.8527 ^{abcd}	0.065	1.096 ^{ab}	30.33	1.17 ^{bc}	0.60 ^{cd}
AN70	47.38 ^{c}	0.9243 ^{abcd}	0.063	1.065 ^{ab}	35.79	0.87 ^{ab}	0.035 ^{a}
AN211	47.55 ^{c}	1.1691 ^{d}	0.074	0.933 ^{a}	38.08	0.68 ^{ab}	0.033 ^{a}
AS70L	45.01 ^{abc}	0.7095 ^{ab}	0.058	1.425 ^{b}	37.20	0.98 ^{abc}	0.055 ^{bcd}
AS211L	46.34 ^{bc}	1.0132 ^{bcd}	0.067	0.980 ^{ab}	31.83	1.07 ^{abc}	0.042 ^{ab}
AN70L	45.34 ^{abc}	0.7996 ^{abc}	0.078	1.230 ^{ab}	41.63	1.09 ^{abc}	0.048 ^{abc}
AN211L	45.62 ^{bc}	1.0537 ^{cd}	0.066	1.155 ^{ab}	37.60	1.40 ^{c}	0.040 ^{ab}
P value	**<.001**	**<.001**	**0.073**	**0.014**	**0.173**	**<.001**	**<.001**

Values followed by a different lowercase letter in the same column are significantly different ($p < 0.05$) according to Tukey's LSD procedure. *C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

There was a significant effect of the treatments and interaction of treatments and plant tissue for all the stoichiometric ratios (Figure 5.2). Only AS70 significantly increased the C:N ratio of the shoots. Lime, AS211, AS70L and AN70L had relatively high C:N ratio than the control, though not significant (Figure 5.2). Root C:N ration was significantly decreased by lime, AS211, AS211L and AN211L compare to the control. Overall C:N ratio of the roots was highest on the control treatment.

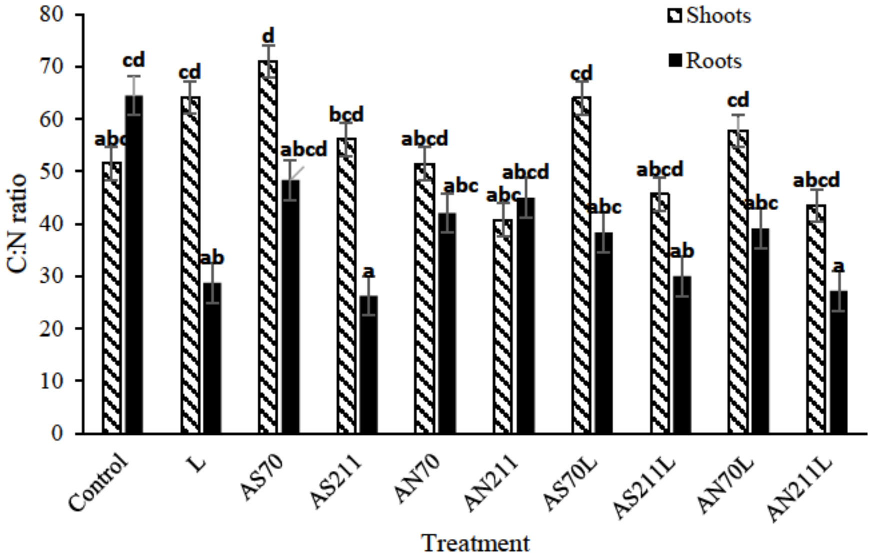

Figure 5. 2 Response of shoot and root C:N in different treatments. . *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure.C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

There was a significant effect of treatment, plant tissue and treatment x plant tissue on N:P ratio ($p<.001$). There was no significant effect of liming, N fertiliser and their combination on shoot N:P ratio (Figure 5.3). Except for AS70, all the N fertiliser and N fertiliser +lime treatments significantly increased N:P ratio in roots. The N:P ratio was lowest in the control and lime treatments for both roots and shoot, while on AN70L it was in shoot only. Amongst the fertilized treatments AN211L had the highest root N:P ratio which when compared to other AN treatment it was significantly different to AN70 and AN70L (Figure 5.3).

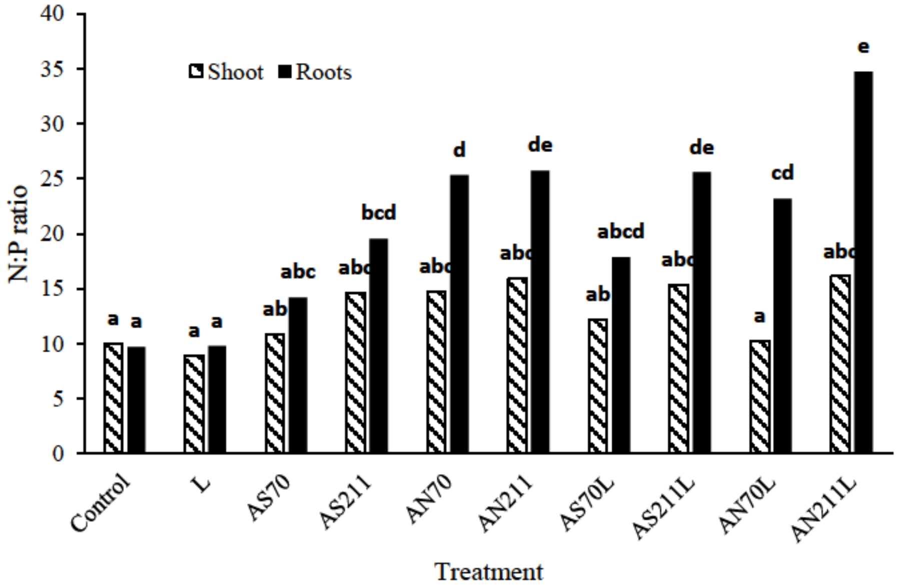

Figure 5. 3 Response of shoot and root N:P in different treatments. *Means represented by same letter are not significantly different ($p < 0.05$) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

Shoot C:P ratio showed no difference between the treatments, however that was a strong interaction between treatment and plant tissue ($p<.001$). Only AN211 significantly increased C:P in roots when compared to the control (Figure 5.4).

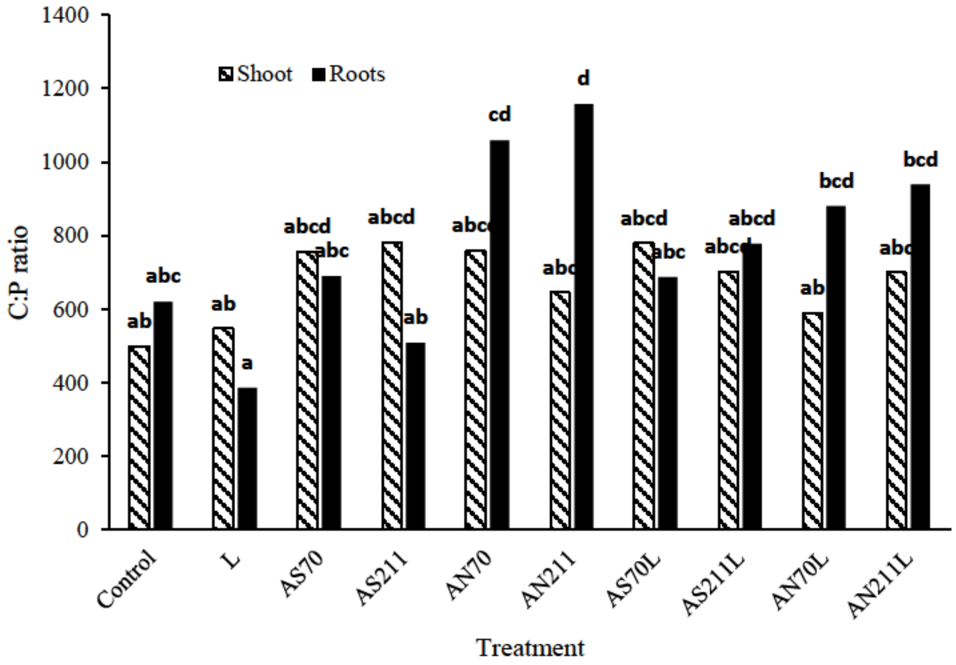

Figure 5. 4 Response of shoot and root C:P in different treatments. *Means represented by same letter are not significantly different (p <0.05) according to Tukey's LSD procedure. C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

5.3.4 Plant nutrient uptake

Only AN211 and AS211L significantly increased N uptake when compared to the control (Table 5.3). Phosphorus and K uptake were only significantly increased by AN211 treatment. Nutrient uptake followed the order N > K > P in these experiments.

Table 5. 3 Uptake of nitrogen; phosphorus and potassium as affected by by nitrogen and liming application.

Treatment	N uptake	P uptake	K uptake
		Kg/ha	
Control	140.6 ^{abc}	14.35 ^{ab}	162.7 ^{a}
L	90 ^{a}	10.62 ^{ab}	166 ^{a}
AS70	215.4 ^{cd}	20.45 ^{bc}	284.9 ^{ab}
AS211	203.3 ^{bcd}	15.34 ^{ab}	261.5 ^{ab}
AN70	150.4 ^{abc}	10.14 ^{a}	171.5 ^{a}
AN211	415.7 ^{e}	26.55 ^{c}	335 ^{b}
AS70L	110.7 ^{ab}	9.09 ^{a}	222.6 ^{ab}
AS211L	262.4 ^{d}	17.46 ^{abc}	254.6 ^{ab}
AN70L	108.5 ^{ab}	10.56 ^{ab}	162.1 ^{a}
AN211L	160.2 ^{abc}	9.97 ^{a}	176.2 ^{a}

Values followed by a different lowercase letter in the same column are significantly different ($p < 0.05$) according to Tukey's LSD procedure. *C = control (0 kg/ha), L = lime (2250 kg/ha), AS70 = ammonium sulphate at 70 kg/ha; AS211 = ammonium sulphate at 211 kg/ha; AN70 = ammonium nitrate at 70 kg/ha; AN211 = ammonium nitrate at 211 kg/ha; AS70L = ammonium sulphate at 70 kg/ha + lime; AS211L = ammonium sulphate at 211 kg/ha + lime; AN70L = ammonium nitrate at 70 kg/ha + lime and AN211L = ammonium nitrate at 211 kg/ha + lime*

5.3.4 Correlation analysis

5.3.4.1 Soil properties

Total N was positively correlated with C associated with clay+silt (SCA) ($r = 0.530$, $p < 0.05$), microaggregates (MiA) ($r = 0.600$, $p < 0.001$) and SMA ($r = 0.598$, $p < 0.001$) fractions; and positively correlated with SOC ($r = 0.889$, $p < 0.001$) and P ($r = 0.599$, $p < 0.001$) (Table 5.4). A negative correlation of TN was observed with pH ($r = -0.451$, $p < 0.05$), soil C:N ratio ($r = 0.603$, $p < 0.001$), and LMA associated C ($r = -0.371$, $p < 0.05$). LMA stored in C was also negatively correlated with MBC (r = -0.448, p <0.05), microaggregate C (r =-0.824, p <0.001), P (r = -0.65, p <0.001) and SMA (r = -0.811, p <0.001), but positively related with pH (r = 0.676, p <0.001). There was a strong positive relationship of SOC with SCA (r =0.632, p <.001), MiA (r = 0.591, p <.001), P (r =0.626, p <.001) and SMA (r =0.536, p <.05) WSA fractions.

5.3.4.2 Plant stoichiometry

Above-ground biomass (AG) C:P showed a negative correlation with soil C:N ratio (r -0.488, p <0.05) while N:P was negatively correlated to LMA C (r = -0.511, p < 0.05) and soil C:N ratio (r = -0.587, p <0.001) (Table 5.4). There was no relationship between AG C:N and measured soil parameters, however, there was a negative correlation with below ground C:P, N:P and AG N:P. Below ground (BG) stoichiometry had a relation with soil parameters and above ground stoichiometry (Table 5.4). BG C:P had a negative correlation with LMA C (r = -0.390, p< 0.05), AG C:N (r = -0.475, p <0.05), and a positive relationship with MBC, BG N:P, C:N and AG N:P. Below ground N:P was positively related with AG N:P, while being negatively related to AG C:N, CWEOC (r =-0397, p < 0.05) and soil C:N (r =-0.685, p <.001). There was no relationship of AG or BG stoichiometry with SOC.

Table 5. 4 Pearson correlation analysis of above ground stoichiometry with soil parameters and carbon.

	Total N %	BG C/P	BG N/P	BG C/N	AG C/P	AG N/P	AG C/N	CWEOC	SCA C	LMA C	MiA C	P	SMA C	SOC	Soil C/N
Total N %	-														
BG C/P	0.096	-													
BG N/P	0.293	**0.693****	-												
BG C/N	**-0.375***	**0.392***	-0.248	-											
AG C/P	0.304	0.175	0.272	-0.130	-										
AG N/P	0.345	**0.467***	**0.647****	-0.140	**0.688****	-									
AG C/N	-0.056	**-0.475***	**-0.603****	-0.027	0.122	**-0.618****	-								
CWEOC	0.078	-0.325	**-0.397***	-0.019	-0036	-0.051	0.030	-							
SCA C	**0.530***	0.530	-0.060	0.101	0.079	-0.013	0.194	-0.019	-						
LMA C	**-0.371***	**-0.390***	-0.284	-0.106	-0.337	**-0.511***	0.298	0.079	**-0.605****	-					
MiA C	**0.600****	0.190	0.080	0.081	0.242	0.233	0.011	0.046	**0.885****	**-0.824****	-				
P	**0.599****	0.113	0.060	0.049	0.128	0.275	-0.172	0.174	**0.798****	**-0.651****	**0.812****	-			
SMA C	**0.598****	0.426*	0.363	0.074	0.196	0.353	-0.237	-0.063	**0.739****	**-0.811****	**0.876****	**0.710****	-		
SOC	**0.889****	-0.057	-0.036	-0.156	0.090	0.062	0.110	0.126	**0.632****	-0.240	**0.591****	**0.626****	**0.536***	-	
Soil C/N	**-0.603****	-0.295	**-0.685****	**0.568***	**-0.488***	**-0.587****	0.252	0.055	-0.053	0.367	-0.264	-0.178	-0.354	-0.174	-
pH	**-0.451***	-0.195	-0.087	-0.194	-0.236	-0.400	0.252	-0.166	**-0.701****	**0.676****	**-0.758****	**-0.884****	**-0.695****	**-0.495***	0.077

P 0.05- 0.001*; P<0.001**; *BG- below ground; AG- above ground; CWEOC – cold-water extractable carbon; HWEOC- hot-water extractable carbon; LMA C – large macroaggregate carbon; SCA C- clay+silt carbon; SMA C- small macroaggregate carbon, MiA C– microaggregate C.*

5.4 Discussion

Nitrogen application facilitates above-ground growth which decreases shoot:root ratio (Li et al., 2015). The increase in above-ground production by AS70 and AN211 treatments is attributed to an increase in nutrient availability following N addition. High response of above ground biomass production suggests Ukulinga grassland is N limited and that N addition favoured plant growth. Other studies have also reported a significant increase in aboveground biomass upon addition of N fertilizers (Ai et al., 2017; Nivelle et al., 2018)). This could also be explained by better adaption and competitive advantage of the species in utilizing N. The plots that showed greater biomass response were reported to be dominated by nitrophilous species (*Eragrostis curvula*, and the 'invader' grasses *Panicum maximum* and *Paspalum dilatatum)* (Le Roux and Mentis 1986; Morris and Fynn 2001). The results are in line with previous study on this experiment reporting that ammonium sulphate gives higher yields of grass than ammonium nitrate at low N rates (Grunow et al., 1970). However, at higher N rates, there is better biomass production response to ammonium nitrate than ammonium sulphate mainly because species respond well when there is a predominate supply of NO_3^- while NH_4^+ impairs nutrient uptake and caused toxicity (Santos et al., 2013). Our results also show that plant biomass response to N depends on type and rate of N fertilization. Lack of response to separate lime application potentially reflects P deficiency indicated by low extractable P in this treatment compared to N fertilized plots (Figure 3.5). These results are suggesting both N and P as limiting factors to plant productivity in at Ukulinga grassland. However, high soil available P under N addition (Figure 3.5) may have alleviated N induced P pressures resulting in increased above ground biomass production. Root biomass of the ammonium sulphate and lime treatments showed a positive response (Table 5.1) compared to ammonium nitrate. This is contradictory to the results reported by (Santos et al., 2013) where ammonium nitrate increased root length, surface area, dry matter, and N use efficiency of *Panicum maximum.* More resource allocation to below ground biomass under ammonium sulphate and lime treatments could be a result of nutrient deficiency (Ai et al., 2017). Moreover, under low-N conditions, which is the case for lime treatments, plants tend to increase their allocation of photosynthetic products to below-ground organs to obtain N (Grechi et al., 2017). Overall, observed differences in plant biomass between above-ground and below-ground organs is a reflection of the balance between above-ground resources (i.e., light and CO_2) and below-ground resources (water and nutrients) (Yang et al., 2010). This argument explains high C allocation in shoot than root in this long-term N enrichment (Table 5.2).

The plant biomass allocation is the key information linking above-ground productivity and C sequestration (Hovenden et al., 2014). However, low rates of N + lime treatment application did not significantly affect tissue C concentration. This may be because photo-assimilates were transported to below-ground plant parts (Domanski et al., 2001; Kuzyakov 2001). Only AN211 and AN211L significantly increased shoot N concentration which is consistent with preferred uptake of nitrogen in the form of nitrate. This was supported by significantly high N uptake when ammonium nitrate is applied (Table 5.3). Increased N availability upon N addition to grasslands results in increased absorption of N via roots resulting in increased foliar N concentration (Zhang et al., 2013a). Plant tissue N concentration can also be affected by plant specific N use strategies and efficiency (Zhang et al., 2004). It is commonly observed that N application changes plant species composition (Zeng et al., 2010), as a reflection of different nutrient uptake. Plant species have different nutrient uptake regardless of nutrient application (Fujita et al., 2010), and element uptake is predetermined by biomass yield rather than concentration of the element in the biomass (Hejcman et al., 2010b). The lack of response to shoot P concentration combined with greater shoot biomass may indicate more efficient utilization of P. P allocation pattern is driven by soil P availability and affects leaf photosynthetic rates of plant species (Guilherme Pereira et al., 2018). Similar to other studies (Güsewell, 2004), P concentration in roots differed more across treatments compared to N suggesting that N concentration is more stable than that of P. Long-term application of N at Ukulinga grassland has resulted in a decrease in root P concentration which is consistent with results shown by a meta-analysis by Sardans et al. (2017).

Change in plant composition resulted in plant below and above-ground stoichiometric variation across treatments (Table 5.2). Although numerous studies have evidently reported reduced plant above-ground C:N ratio under long-term N addition to grasslands (Gill et al., 2006; Fornara and Tilman 2012; Heyburn et al., 2017b.), this study found no significant response to N addition, except for lower rate of ammoniun sulphate which increased C:N ratio in the shoots. A similar insignificant trend was also observed for shoot N:P and C:P ratio which was also consistent with effects of lime. This implies that grass species of Ukulinga grassland could maintain a stable shoot C:N:P ratio despite changes in nutrient availability.

A significant trend was observed for root C:N:P ratio which varied with nutrient availability. N fertilization increased root N:P and C:P ratio due to low P in roots under N addition. Separate application of lime and its combination of lime with high rates (211kg N/ha) of both ammonium sulphate and ammonium nitrate decreased root C:N ratio and low N availability under lime.

The N:P ratio variation could reflect ecological strategies and growth-rate capacities of the species (Zechmeister-Boltenstern et al., 2015). Considering suggested N:P ratio values in estimating P and N limitation in the soil (Koerselman and Meuleman 1996; Güsewell 2004; Craine et al., 2008), control and lime treatments are considered N limited (N:P<10) and N treated plots with co-limitation of N and P (N:P>14). This however remains debatable as Zhang et al (2004) reported a threshold leaf N:P ration of <21 as N-limiting and >23 as P-limiting in grasslands ecosystems. It is thus unclear whether these ratios are sufficient to estimate limiting nutrients because grass species can have either low N requirement or high P requirement compared to other species (Craine et al. 2008). Current results suggest that Ukulinga grassland may have a different critical N:P index for biomass production which may be important in planning N fertilization.

Root N:P, shoot C:P and N:P had a negative relationship with soil C:N, this is attributed to high N returning to the soil therefore decreasing soil C:N. It is has been mentioned that low C:N and N:P ratio in plant debris could increase microbial C-use efficiency, which then determines more microbial C being accumulated and stabilized into soil organo-mineral fractions, thus contributing to greater soil C stocks (Heyburn et al., 2017b). High C:N ratio of plant tissue in this study could explain that the decomposability was limited even though soil C:N ratio were low enough to support litter decomposition. The pH induced limitation on microbial decomposers be the explanation of low decomposition aided with accumulation of litter and high labile (CWEOC) in the surface. Positive correlation of SOC with total N, SCA C, MiA, P and SMA C implies that majority of the C in the soil is predominated by organic N, whereas most of the C is stored in SCA and MiA. Negative correlation of SOC with pH implies through pH induced effect of N, N could potential increase SOC however the relationship is weak. This correlates with the other studies (Aula et al., 2016; Liao., 2016)

5.5 Conclusion

This study shown how long-term N application and liming affects biomass-production, nutrient composition, and plant tissue stoichiometry of the semi-arid grassland. It was found that below-ground plant element ratios are much more responsive than above-ground which could reflect microbial decomposition and predicting soil nutrient availability. Changes in plant elemental composition did not affect C in the soil. In conclusion, higher litter accumulation in plots shows that the decomposition in the plots is limited due to high plant stoichiometric ratio and pH-induced effect on microbial activity. Furthermore, there was no relationship between plant stoichiometry and SOC, indicating that further addition of plant litter does not contributes to more C sequestration.

CHAPTER 6

SUMMARY OF FINDINGS AND CONCLUSIONS

6.1 Summary of findings

Long-term application of nitrogen fertilizer significantly affected soil parameters. The most obvious effect was on the change of pH wich had an influence on other parameters. Lime increased soil pH and mostly had the opposite effect than that of N.addition. In this study, 70 years of N addition increased soil acidity and caused significant reduction of exchangeable bases, while lime increase them. The effect was more severe in plots that recieved high N application rates. Soil N was high in N treated plots because of added mineral N. Increase in soil acidity results in Ca, Mg and K leaching because of replacement by Al and Fe at lower soil pH. Interestingly, N addition resulted in high soil available P compared to the control. Convincing explanation is the involvement of dissolution of Al-P, where phosphate sorption decreased for Al oxides at $pH < 4$ Regardless of the negative impact on soil properties and imbalance of macro and micro elements caused by N addition; plant biomass responded positively to nitrogen. This implies that though this grassland is N limited there is a better adaptation of species dominating the plots.

Sole application of lime increased Ca^{2+} and HCO_3^- ions concentration when lime dissolves, consequently replacing Al^{3+} ions in the complex sites which increases soil pH, exchangeable bases and ECEC. Despite the increase in soil pH, biomass production was not changed by liming. This was due to limitation of N and P, resulting in low growth than plots receiving N. Co-application of lime and N had positive effect on exchangeable bases and ECEC when pH is < 4. The overall outcome on biomass production is inconsistent. Evidence shows that it is a function of acidifying effect and power of liming in reversing acidity. For instance, core application of lime and high application of ammonium sulphate had lower pH than other treatments with combination of lime and N, therefore, it had low ECEC and exchangeable bases than other co-application treatments.

Negative effect of N application on soil pH resulted in reduction of water-stable aggregate stability. Mass of large macro-aggregate (>2 mm) fraction was reduced by N application while the proportion of small macro-aggregates (>250 μm–2 mm), micro aggregates (53–250 μm) and Clay+Silt (< 53 μm) was increased. Correlation analysis evidently showed a negative relationship between LMA, SMA, microaggregate, and clay+silt carbon. This is caused by the reduction of exchangeable bases (especially Ca^{2+}) replaced by NH_4^+ (with low hydration energy) causing dispersion of soil aggregates as the interlayer collapses, thus limiting the formation of cation bridging of positively charged cations and negatively charged clay surfaces and organic compounds. This mechanism is supported by an increase in aggregates stability when lime is applied, indicating that aggregate stability in this experiment is a function of cation bridges gluing smaller particles into larger aggregates rather than organic matter. Also, positive correlation of soil pH and LMA C supports this. This implies that ammonium fertilizer increases the susceptibility of the soil to erosion and compaction while liming maintained structural stability.

Carbon distribution in aggregates was attributed by the change in proportion of each aggregate fraction with N addition rather than change in soil C content (%). Disruption of macroaggregates leads to redistribution of C into smaller aggregates where N was added. This arise from the evidence that soil C content was not different among the treatments suggesting that the soil has reached C saturation. Any of the carbon added in a form of litter accumulates on the soil surface and slowly decomposes over time, however the soil can no longer store any of the added C. Even the C stock stored in respect to each treatment does not significantly differ to the control.

Biomass response with respect to N application was positive, while co-application of N and lime showed inconsistent response. Furthermore, below ground production was improved by separate lime and N application. The main source of C input is dead roots and leaves. This is also observed in the relationship between root N:P ratio with labile C in the form of CWEOC. However, there was no relationship between plant stoichiometry and SOC implying that any added C in a form of plant litter does not change soil carbon content. While N increased foliar C content, N was not changed, which resulted in high shoot C:N ratio in AS70 and no change was detected on N:P and C:P. On the contrary root C:N ratio decreased while N:P increased, C:P was only high in AN treatment. N uptake was not significantly different from the control expect for AN211 and AS211L treatments, which mean that C accumulation in the shoots surpasses N accumulation resulting in high C:N ratio. Root C:N ratio decreased, while N:P increased indicating that P and N is limited, and low tissue C. Limitation of N and P creates competition between plant and microbes which restrict microbial activity associated with lower OM decomposition.

6.2 Conclusions

Application of ammonium-based fertilizer as a source of nitrogen resulted in soil acidification, reduced exchangeable bases and ECEC and increased soil available P. On the other hand, liming increased pH, exchangeable bases, and decreased P. Ammoniumbased N application has a detrimental effect on soil fertility which could limit plant growth, however, liming can remediate the effect through induced pH effect. Due to decline in phosphorus concentration in limed soils, P fertilizer should be employed to avoid P deficiency as readily available P to reach optimum requirements in the soil and ensuring substantial production. Co-application of lime and nitrogen balances the impact of N induced acidity as lime maintains pH to optimum. This also give balance in exchangeable bases and nutrient availability. Lack of change in total organic carbon upon application of both nitrogen fertiliser and lime suggests that addition of new SOC from residues inputs was limited possibly due to C saturation. Liming improved soil aggregation, increased MWD compared to N fertilised plots, despite having comparable SOC concentration. This emphasizes induced soil pH changes as the main driver of observed response to N-fertilization and liming at Ukulinga research farm.   This study gives a basic understanding of what might affect MWD, however, the mechanisms are not addressed. Nitrogen application improved biomass production and changed the stoichiometric ratio of shoots and roots. Lime had relatively high shoot C:N ratio while the combination of lime+N had was inconsistent. Nitrogen application greatly influenced root N:P ratio possibly due to greater N uptake which increases foliar N content relative to P. Plant stochiometric ratio had no relationship with SOC, implying that the soil had reached it C saturation.

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