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Effects of rotational grazing intensity on the quantity and quality of soil organic carbon in Inner Mongolian grasslands

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Abstract

The aim of this thesis is to assess the effects of rotational grazing at varying grazing intensity on soil organic carbon (SOC) quantity and quality in Inner Mongolia. People of the autonomous region of Inner Mongolia, China, have a long tradition of pastoralism. The semi-arid region contains one of the largest grassland ecosystems in the world and is severely affected by land degradation and the loss of soil organic matter due to overgrazing. The present study quantifies different SOC fractions down to one metre depth as an indicator of ecosystem health, at a rotational grazing site in Xilinhot, Inner Mongolia. Soil samples were collected from pastures under rotational grazing at three grazing intensities in addition to a reference plot, where grazing is excluded. Four grassland treatments were thus defined by the livestock densities 0 sheep/ha/year, 0.64 sheep/ha/year, 1.28 sheep/ha/year and 2.56 sheep/ha/year for non-grazed (NG), light grazing (LG), moderate grazing (MG) and high grazing (HG) intensity, respectively.

Eight years of ongoing rotational grazing showed a tendency of highest SOC content in NG, and SOC depletion with increasing livestock density. HG was consistently an exception to this trend, where SOC content tended to increase in comparison to MG and reach concentrations close to NG. Changes in SOC content across treatments were not significant. However, the pattern was seen for both total SOC, particular organic carbon (POC), mineral associated organic carbon (MOC) and hot water extractable carbon (HWE) in the upper 20 or 30 cm of the soil. HWE was the only fraction that showed significant impact of treatment and was significantly higher in NG and LG compared to MG and HG. Following the general trend of total SOC, HWE also tended to increase from MG to HG in the upper 20 cm. Results suggest that the upper 20 cm of the soil are most sensitive to grazing.

In studies of continuous grazing, both MG and HG, which are representative for local practices of pastoralism in Inner Mongolia, are shown to reduce SOC levels. The present study shows that even rotational grazing with HG is below the threshold of sustainable grazing, i.e. the pressure that maintains SOC storage and overall ecosystem health. All three rotationally grazed treatments could thus be considered alternatives to grazing exclusion and continuous grazing. Overall, changes in SOC content and their stability were minimal and should not be used forcefully as an argument for choosing a particular management practice among the four treatments. However, tendencies in SOC fractions indicate possible future SOC enhancement under HG. As HG also more closely represents the generally preferred livestock densities, HG rotational grazing may be a viable management practice in Xilinhot.

Sammendrag

Hensikten med denne masteroppgaven er å vurdere påvirkningen rotasjonsbeite av varierende beiteintensitet har på kvalitet og kvantitet av jordorganisk karbon (soil organic carbon, SOC) i Indre Mongolia. Befolkningen i den autonome regionen Indre Mongolia, Kina, har en lang tradisjon med beitedrift. Det tørre steppesklimaet inneholder et av verdens største steppøkosystemer og er sterkt påvirket av tap av jordorganisk materiale og landdegradering grunnet overbeite. Dette studiet kvantifiserer forskjellige SOC-fraksjoner ned til en meters dybde som en indikator på økologisk tilstand ved et forsøksområde for rotasjonsbeite i Xilinhote, Indre Mongolia. Jordprøver ble samlet fra eng under tre intensitetsnivåer av rotasjonsbeite, i tillegg til en referanse uten beiting. Fire behandlinger ble dermed definert etter husdyrtetthet på 0 sau/ha/år, 0.64 sau/ha/år, 1.28 sau/ha/år og 2.56 sau/ha/år for henholdsvis ikke-beiting (NG), lett beitetrykk (LG), moderat beitetrykk (MG) og høyt beitetrykk (HG).

Åtte pågående år med rotasjonsbeite viste en tendens med høyest SOC-innhold i NG, og tap av SOC med økende husdyrtetthet. HG var konsistent et unntak til denne trenden, der SOC-innholdet som regel økte ved MG og nådde konsentrasjoner i nærheten av NG. Endringer i SOC mellom behandlinger var ikke signifikante. Derimot var denne trenden lik for både SOC, partikulært organisk karbon (POC), mineralassosiert organisk karbon (MOC) og hot water extractable carbon (HWEBC) i de øvre 20 eller 30 cm av jorda. HWEBC var den eneste fraksjonen der behandling hadde signifikant påvirkning, og var signifikant høyere i NG og LG enn MG og HG. I samsvar med den generelle trenden i SOC, viste også HWEBC en tendens til å øke fra MG til HG i de øvre 20 cm. Resultatene viste tegn til at de øvre 20 cm av jorda er mest sensitive for beitepåvirkning.

I studier av kontinuerlig beite fører dyretetthet tilsvarende både MG og HG, som er representative for lokal beitedrift i Indre Mongolia, til reduserte SOC-nivåer. Denne oppgaven viser at selv rotasjonsbeite med HG er under terskelen for bærekraftig beiting, altså beitetrykket som opprettholder SOC-lagrene og økologisk tilstand. Alle de tre behandlingene med rotasjonsbeite kan dermed vurderes som alternativer til beiteeksklusjon eller kontinuerlig beite. Generelt var endringer i SOC-nivåer og -stabilitet minimale og burde ikke brukes som et tungt argument for å velge en spesifikk beitestrategi mellom de fire behandlingene. Tendenser i SOC-fraksjonene tyder derimot på mulige fremtidige økninger i SOC under HG. Ettersom HG også i større grad representerer den generelt foretrukne husdyrtettheten, kan rotasjonsbeite med HG være en fordelaktig praksis for beitedrift i Xilinhote.

Preface

During the final course of my bachelor's degree, I walked through the grassland near a research site in Inner Mongolia when I was asked whether I had any interest in a master's degree. My answer was that an elaboration of the summer course I attended, concerning sustainable soil and grassland management, would be at the top of my wish list. After two and a half years I am now completing my master's degree in Environment and Natural Resources at the Norwegian University of Life Sciences (NMBU), and I am closing what is hopefully only the first chapter in my work with soil sciences. I thank the Norwegian Directorate for Higher Education and Skills (HK-DIR) who supported this project through the Utforsk project UTF-2016-long-term/10089 (SiNorSoil: Sustainable Soil Management in Response to Pollution and Climate Change). Writing this 60-credit thesis has been educational, motivating and challenging. There are many that I have crossed paths with along my way that I would like to thank.

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Table of abbreviations

NG	Non-grazing (reference)
LG	Light grazing intensity
MG	Moderate grazing intensity
HG	High grazing intensity
SOM	Soil organic matter
SOC	Soil organic carbon
IC	Inorganic carbon
TotC	Total carbon
TotN	Total nitrogen
POM	Particular organic matter
MOM	Mineral associated organic matter
POC	Particular organic carbon
MOC	Mineral associated organic carbon
PON	Particular organic nitrogen
MON	Mineral associated organic nitrogen
HWEC	Hot water extractable carbon
LOI	Loss on ignition
BD	Bulk density
ASD	Aggregate size distribution
SAG	Aggregate stability
GSD	Grain size distribution
Appx	Appendix

1| Introduction and hypotheses

1.1| Grasslands and soil

About one third of the world's terrestrial surface is covered by nearly continuous grass vegetation, comprising areas such as steppes, savannas and prairies (Oertel et al., 2016; Smith, 1999). These vegetation communities, commonly categorised as parts of the grassland biome, provide essential ecosystem services such as erosion prevention and water retention (regulating services), carbon storage in soil (supporting service), maintaining pools of biodiversity (cultural service) in addition to making up 70 percent of the world's agricultural land (provisioning service) (Abdalla et al., 2018; Fan et al., 2019; Gaujour et al., 2012; Smith, 1999). These services however depend on the continued productivity and preservation or restoration of grasslands which are currently under threat by land degradation and climate change (Gaujour et al., 2012; Han et al., 2008).

One of the largest grassland ecosystems in the world, located in the autonomous region of Inner Mongolia, China, is strongly affected by land degradation due to overgrazing (Jiang et al., 2006; Kawamura et al., 2005). Land and soil degradation of grasslands result in a long-term weakening or even loss of several important ecosystem services (FAO, 2021; Olsson et al., 2019). As modern settlement and anthropogenic activity has increased in the region, the resilience of the ecosystem has decreased drastically (Jiang et al., 2006). From 1949 to 2000, livestock numbers increased 18-folds, while the grazing area per animal significantly decreased - a change that not only impacts vegetation or animals, but ultimately also the livelihoods of the local population (Jiang et al., 2006; Xu et al., 2012).

Overgrazing reduces the ability to capture, store and recycle materials and nutrients, which in turn causes ecosystem degradation and hinders accumulation of soil organic matter (SOM) (Wiesmeier et al., 2009), one of the essential factors that counteract land degradation. The soil is estimated to be the largest terrestrial pool of organic carbon, containing up to four times the amount of carbon that makes up CO₂ in our atmosphere, and over three times more carbon than what is stored in global above-ground vegetation (Jobbagy & Jackson, 2000; Lal, 2004). This soil organic carbon (SOC) content is however not equally distributed. In productive rainforest areas primary production and organic matter input to soil is high, but SOC accumulation is limited due to high heterotrophic respiration. Whereas high turnover rates limit SOC accumulation potential in rainforest areas, decomposition rates of temperate grasslands are low

compared to organic matter input rates. This provides relatively high SOC accumulation potential.

Grassland soils are estimated to store up to 30 percent of below-ground SOC stocks globally and have the potential to store more than 10 kg SOC/m² in the upper meter (Jobbagy & Jackson, 2000; Risch et al., 2007). Another reason for the high carbon storage potential in grasslands is the plant physiology in these biomes, which supports relatively quick allocation of carbon from the atmosphere into the ground. Perennial grasses have the majority of their biomass below the soil surface in long and thin short-lived roots, and allocate about two thirds of the carbon content in their roots (Olsson et al., 2019). The grasslands of the Mongolian plateau are dominated by these perennial grasses (Su et al., 2017).

Vegetation enhances SOC sequestration, which in turn represents a positive feedback, as SOC sequestration enhances the vegetation cover by improving soil health. SOC makes up on average 50 percent of the organic matter content in the soil (Pribyl, 2010), and there are several reasons for why it is often used as an indicator for soil health. The carbon and energy that SOC provides for soil biota is essential for sustained microbial activity. Organic matter also improves nutrient cycling and provides important nutrients for plant growth due to its release of phosphorous, nitrogen and sulphur when decomposed (Weil & Brady, 2017). With increased microbial activity and organic matter exudation from plants, organic compounds become available to bind mineral particles together into aggregates, like the protein glomalin (Weil & Brady, 2017). As such, SOC improves soil structure and increases aggregate stability, which in turn increases water infiltration and retention (Powlson et al., 2011; Weil & Brady, 2017). Subsequently, SOC also decreases risk of erosion and surface run-off, in combination with vegetation and below ground biomass which also stabilizes the soil (Powlson et al., 2011; Weil & Brady, 2017). Because of the impact SOC has on soil properties, it is a good indicator of soil degradation status when comparing landscapes of similar ecosystems and climates.

In addition to providing a robust ecosystem, carbon accumulation is also a relevant characteristic in light of the current climate crisis (IPCC, 2019). Carbon sequestration and enhanced SOC levels have been recognised as an important contributor in mitigating climate change (IPCC, 2019). The term carbon sequestration is used synonymously with carbon accumulation in this thesis, i.e. the uptake of carbon by vegetation or soil which leads to increased SOC stock. Human influence on the climate has caused rising ocean levels, extreme weather, changes to ecosystems and increasingly warmer temperatures (IPCC, 2021). Although

carbon accumulation in soil is only one aspect in facing this challenge, it is an important one. Grassland management is deemed amongst the land use options with largest CO₂ reduction potential, as a result of carbon sequestration (IPCC, 2019). Input or output of SOC does not determine the carbon accumulation potential of grasslands if seen in isolation. As with all ecosystems, grasslands emit CO₂ through heterotrophic respiration. Soil respiration includes respiration from roots and microbes that decompose SOM and release nutrients, and is an essential part of the soil ecosystem. Globally, grasslands come third in the line of greenhouse gas emission rates after wetlands and forest lands (Oertel et al., 2016).

Whether the ecosystem contributes as a net sink or source of CO₂, i.e. the net SOC sequestration, is determined by total ecosystem respiration subtracted from carbon input through photosynthesis (Oertel et al., 2016). Soils are not static, and annual fluctuations in productivity are affected by climatic factors such as temperature and precipitation (Sanderman et al., 2015). Land use also impacts the balance between SOC input and output, like grazing by livestock. Grazing is the world's most extensive form of land use and alters SOC stocks in several interconnected ways (Bardgett & Wardle, 2003; Piñeiro et al., 2010). Because the practice of pastoralism is a major common factor among many grasslands, the terms grassland and grazing lands are used interchangeably in this thesis unless stated otherwise.

1.2| [Effects of grazing on soil](#)

Grazing by livestock such as sheep and cattle affects the ecosystem both directly and indirectly (Piñeiro et al., 2010; Raiesi & Riahi, 2014). Most obviously, livestock grazes and forages, tramples the ground and deposits faeces and urine across the accessible area. Consumption of vegetation directly removes above-ground biomass, and thus decreases litter input, in turn affecting future decomposition of litter into SOC (Harrison & Bardgett, 2008). The selectivity of grazing animals may also alter plant community composition, e.g. by stimulating growth of certain grazing resistant species of different decomposability or with different carbon allocation to below-ground biomass (Gaujour et al., 2012; Liang et al., 2021b). The sheer weight and movement of grazers could lead to soil compaction, aggregate disruption and reduced topsoil structure (Raiesi & Riahi, 2014; Wiesmeier et al., 2009). The stability of aggregates determines their water storage and aeration properties (Six et al., 2004).

Trampling could on the other hand also increase incorporation of plant litter into the upper soil layers, which is believed to stabilize SOM (Wei et al., 2021). Nutrients in some of the consumed biomass will be returned as manure or faeces left by the animals, adding nitrogen, phosphorous,

potassium and organic matter with an entirely different quality to the grassland. All these effects could further change pedoclimatic conditions, like exposing the upper soil layer and changing soil moisture, light accessibility and temperature (Gaujour et al., 2012). In turn, this might change nutrient cycling and the availability of nutrients to plants and microorganisms, further impacting both the content and quality of SOC (Harrison & Bardgett, 2008). The net impacts of grazing on grassland are heavily influenced by livestock density and seasonality, which determines the quantity and quality of SOC (IPCC, 2007). Although high livestock rates provide animal products, high grazing pressure in Inner Mongolia is found to impair regulating services (Fan et al., 2019).

Grazing intensity, dependant on livestock density and grazing time per area, is relative to the productivity of the system (Abdalla et al., 2018). Continuous grazing from 1.5 to 3 sheep/ha/year is commonly practiced by local herders in Xilinhot (Hoffmann et al., 2016). Sheep densities of about 2.3 sheep/ha/year (Cao et al., 2013; Wang et al., 2016) to more than 4.5 sheep/ha/year (Hoffmann et al., 2016) have been classified as high or intensive in Inner Mongolia. In arid and fluctuating warm or cool climates such as the Inner Mongolian grasslands, high grazing densities in general seem to decrease SOC content and reduce foraging quality (Abdalla et al., 2018; Zhou et al., 2017). This is largely due to decrease in both above- and below-ground biomass, change in species composition and other negative effects on previously mentioned soil characteristics (Gao, Y. Z. et al., 2008; Liang et al., 2021b; Steffens et al., 2008).

High grazing intensity is in other words associated with deterioration of grasslands, and several studies have investigated the effect of grazing exclusion (Dong et al., 2020; Steffens et al., 2008). Although cessation of grazing generally increase SOC stocks (Wang et al., 2018), it might decrease stimulation of other ecosystem services and ultimately not be the best way to reverse the degradation process (Fan et al., 2019). This is exemplified by Zhang et al. (2020) who found that summer grazing at moderate intensity improved plant production, as the initially reduced above-ground nutrient pool of plants was recovered by fast plant regrowth and enhanced nutrient concentrations. Similarly, SOC sequestration potential might benefit from the same intermediate disturbance by livestock. Grazing reduction has been studied as an alternative to complete exclusion and is shown in some cases to increase plant diversity and SOC sequestration without decreasing forage or soil quality (Dong et al., 2020; Liang et al., 2021a; Wang et al., 2016; Zhang et al., 2018). This seems to support the so-called intermediate

disturbance hypothesis (Austrheim et al., 2016), where moderate grazing supports ecosystem services to significantly higher degrees than exclusion or too high stocking rates.

1.3| Storage of soil organic carbon

Different forms, or qualities, of SOC are often divided into labile and recalcitrant carbon. Labile SOC is often assumed to be represented by particulate organic carbon (POC), which includes hot water extractable carbon (HWEC), and usually consists of younger SOC with a turnover rate from tens of days to several years (Dong et al., 2020; Leifeld et al., 2009). Recalcitrant SOC, with fractions like mineral associated organic carbon (MOC), is usually more resistant to microbial decomposition and thus is a more stable carbon pool with a turnover rate from decades to centuries (Kleja et al., 2007; Zhang & Zhou, 2018).

Any net increase in SOC will be positive for climate change mitigation. However, the more labile SOM fraction, such as particular organic matter (POM), will be largely decomposed and returned to the atmosphere as CO₂ or contribute to stable SOC (Lavallee et al., 2020). POM thus simultaneously contributes to nutrient turnover and continued productivity of the grassland. Grasslands seem to be dominated by the more stable mineral associated organic matter (MOM) (Cotrufo et al., 2019). However, the MOM-fraction has a lower carbon to nitrogen-ratio than POM, indicating a lower carbon accumulation potential in MOM compared to POM (Cotrufo et al., 2019). The implications of this are that both MOM and POM are important contributors to carbon accumulation, due to different stability and storage properties.

Any change in soil carbon input does not directly indicate long-term change in soil or ecosystem carbon stock, as aggregate turnover, mineral binding and recalcitrance strongly affect decomposition rate and thus the time scale of carbon storage (von Lützow et al., 2006). Generally, the topsoil is regarded as more sensitive than deeper soil layers to management change, such as trampling, which can decrease aggregate stability (Raiesi & Riahi, 2014; Steffens et al., 2010). This decreases physical protection against decomposition which could impact the SOC content of the topsoil (Lavallee et al., 2020). On the other hand, trampling may also stimulate conversion of litter into POM and MOM, thus promoting SOM stabilisation (Wei et al., 2021).

Although both POM and MOM can be occluded within aggregates, MOM is even further protected against microbial decomposition as it is stabilized by mineral association (Lavallee et al., 2020). Several studies have observed changes to especially labile SOC, such as POC, within the upper centimetres of the soil (Cao et al., 2013; Steffens et al., 2010). Cessation of

grazing in Inner Mongolia has been found to increase SOC in the easily decomposable POM fraction (Steffens et al., 2010), increase HWE (Dong et al., 2020), increase SOM quantity in certain aggregate fractions (Steffens et al., 2009), and sustain other forms of labile SOC with quick turnover rates (Cao et al., 2013).

Storage of SOC is not only dependent on carbon allocation through plants, but the formation of organic material in which carbon is stored. Nitrogen is a required component for biomass production, and consequently also for the input of dead organic matter to the soil (Piñeiro et al., 2010). As such, nitrogen is often considered a limiting factor for furthered biomass production and carbon sequestration (Hu et al., 2016; Piñeiro et al., 2010). Storage of organic carbon in soil is mediated by microbes that require nitrogen (Cotrufo et al., 2019). Nitrogen mineralisation rate, which is the time required for organic nitrogen to be converted into microbial- and plant available inorganic nitrogen, is correlated with SOC stocks (Dong et al., 2020). Correlation between inorganic nitrogen and HWE is especially high as nitrogen mineralisation is mediated by microbes which use labile carbon as an energy source (Dong et al., 2020). HWE and total nitrogen content is also seen to correlate strongly, suggesting that quality and lability of SOM is connected (Bankó et al., 2021; Weigel et al., 2011).

Microbes prefer SOM of higher nitrogen content, i.e. a lower C/N-ratio, as energy sources (Cotrufo et al., 2019). The rate at which plant residue is decomposed, and SOC potentially lost, thus depends on the C/N-ratio of organic matter. Studies of the Inner Mongolian grasslands show decreased nitrogen content at high grazing intensity due to lower organic matter input and increased erosion (Steffens et al., 2008; Zhou et al., 2017). Because sheep excrete nitrogen through easily decomposable manure and urine, however, grazing affects nitrogen mineralisation and provides an additional pathway for nitrogen turnover (Dong et al., 2020; Shand & Coutts, 2006). Grazing can thus increase soil available nitrogen concentrations and facilitate both litter decomposition and primary production (Hao & He, 2019).

Strengthening the nitrogen pathway through livestock grazing may stimulate vegetation growth and further grazing opportunities (Harrison & Bardgett, 2008). In addition, China is known for its increasingly higher nitrogen pollution levels. Although measured nitrogen deposition in Inner Mongolia has not reached the levels of several other Chinese regions, the nitrogen levels have exceeded the critical value for grassland ecosystems (Zhang, Y. et al., 2017). Zhang, Y. et al. (2017) even suggest high nitrogen levels as another cause for land degradation due to loss of species richness and changed plant community composition. Contrary to this, nitrogen is also

presented as a limiting factor in these grasslands to a higher degree than phosphorous (P) (Yang et al., 2017). Aridity might also limit plant growth in Inner Mongolian steppes, in which case increased SOM content and therefore increased water retention could be of higher importance. Drought may enhance effects of grazing, however it does not seem to negatively affect perennial forbs which are favoured by livestock in Inner Mongolia (Liang et al., 2018).

1.4| Grazing management and experimental site

Grazing has complex, interconnected and sometimes contradictory effects on the ecosystem. Because of this, defining only one sustainable management practice that encompass all grazing areas is problematic. However, sustainable grazing involves ensuring the continued production of livestock and vegetation, and the continued provision of existing ecosystem services. Grazing practices can be managed in optimal ways for carbon accumulation (IPCC, 2007; Lal, 2004), but must be seen in context of grazing season, climatic and environmental factors, vegetation and breeds, which makes up the grazing regime (Austheim et al., 2016; IPCC, 2007). Each grazing regime has its own optimal herbivore density according to the productivity of the system to achieve intermediate disturbance.

Rotational grazing is a regime that involves short grazing periods followed by exclusion of animals (Briske et al., 2008). Rotational grazing has gained attention across the world where it is also referred to as time-controlled grazing, the Savory grazing system, short duration grazing, management-intensive grazing, multi-paddock grazing, cell grazing (Conant et al., 2003; Queensland gov., 2017; Sanjari et al., 2008; Teague et al., 2011) and possibly other variations. It is considered a potentially more sustainable alternative to continuous grazing, as the rotational aspect might allow for an ecological recovery period of compensatory growth, where plants are stimulated and given the time to regrow, thus providing an advantage to complete grazing exclusion or to continuous grazing (Sanderman et al., 2015; Sanjari et al., 2008). The effects of rotational grazing on SOC pools and quality are, however, not commonly agreed upon (Briske et al., 2008). Furthermore, rotational grazing has not been investigated in Inner Mongolia to the same degree as in other regions or countries, and needs to be explored in more detail (Dong et al., 2020).

The Chinese government has implemented measures to more strictly control grazing in Northern China. This is in order to restore grassland ecosystems, and although cessation of grazing has shown to significantly increase SOC stocks and ecosystem health (Steffens et al., 2008; Wang et al., 2018), the policy also has significant challenging impacts on local farmers'

livelihood (Xu et al., 2012). Livelihood diversity and adaptability are important aspects for increasing livelihood sustainability (Xu et al., 2012), but so is facilitating alternative grazing practices. Lower intensity rotational grazing could be an alternative to the currently practiced “Fencing grassland, forbidding grazing and moving user”-policy, which in some cases demands complete cessation of pastoral farming (Dong et al., 2020; Du et al., 2016; Xu et al., 2012).

Exploring alternatives that support continued pastoralism is important, as grazing contributes to food security and is especially valuable in its use of resources otherwise unavailable for human consumption (Herrero et al., 2010; IPCC, 2014). Grazing by herbivores also affect a multitude of other ecosystem services and is of societal importance should it not result in SOC increase or mitigation effect on climate change (Austrheim et al., 2016; Herrero et al., 2009). Another argument in favour of exploring grazing management alternatives is that they are relatively easy to implement. They do not require development of new technologies and it is seen to be one of the most cost-effective climate change mitigation options in the agricultural sector (IPCC, 2014).

In a study by Dong et al. (2020), rotational grazing was suggested as a more sustainable alternative to continued livestock practices or grazing exclusion. Grazing patterns similar to rotational grazing have long been practiced in Inner Mongolia, but as part of nomadic traditions, and without scientific surveillance. In 2012, an experimental site was established by Inner Mongolia University for rotational grazing with sheep near Xilinhot, Inner Mongolia. This site operates with lower stocking rates than what has previously been studied in countries like Australia and USA (Sanderman et al., 2015; Sanjari et al., 2008). After eight years of experiment initiation, there has been reported significantly decreased shoot biomass and changed plant community composition in the treatments with higher livestock rates compared to controls (Liang et al., 2021b). Other researchers confirm slight changes in SOC and nitrogen properties between the different rotational grazing intensities from this experimental site (Dong et al., 2020; Fan et al., 2019; Liang et al., 2021b), however there are important knowledge gaps to be filled.

As an optimal grazing regime in this area, Dong et al. (2020) recommends the treatment with lowest sheep density based on analyses of SOC and nitrogen within the upper 10 centimetres of the soil. How SOC quality and quantity is affected at lower depths in Inner Mongolian steppes is more unclear (Steffens et al., 2010). There is also a request from the local academic community to learn more, especially about the effects on SOC quality (personal

communication, Frank Li Yonghong, 14.11.2019). This thesis will as such assess the effect of rotational grazing, and possibly provide a steppingstone of knowledge towards an area management practice that is more sustainable, both in regard to the ecosystem and for the farmers.

1.5| Goal and hypotheses

The thesis compares rotational grazing treatments in Inner Mongolia, with three different grazing intensities, low (LG), moderate (MG) and high (HG), and one reference plot without grazing (NG). The main research objective is to assess how SOC stock and the distribution of labile (POC, HWEC) and stable (MOC) carbon fractions vary among different grazing intensities. Based on the research findings presented above, the hypotheses for this thesis are:

- i) SOC content is higher in LG plots than in NG, MG and HG plots,
- ii) Changes in SOC are highest in the topsoil and decrease with depth,
- iii) Differences in SOC between treatments are due to changes in POC content,
- iv) Aggregate stability correlates with OC content and is highest in LG plots, and
- v) Nitrogen content correlates with SOC content and is greatest in LG plots, while nitrogen mineralization potential is highest in HG plots.

The research is based on field work from 2020 at the experimental site in Xilinhot, operated by the Inner Mongolia University. The parameters that were analysed are SOC stocks, SOC fractions, soil aggregation, as well as nitrogen content at different soil depths.

2| Materials and methods

2.1| Study area

2.1.1| Location and climate

The Xilinhhot Rotational Grazing Experiment is conducted by Inner Mongolia University. The research site is within the city borders of Xilinhhot, Xilingol League, in Inner Mongolia Autonomous Region (Figure 1). The site is close to the National Climate Observatory, about 470 km north of Beijing city (Google Maps, 2021) (Appx 1). The coordinates are 44°08" N, 116°19" E and the elevation is 1129 meters above sea level.

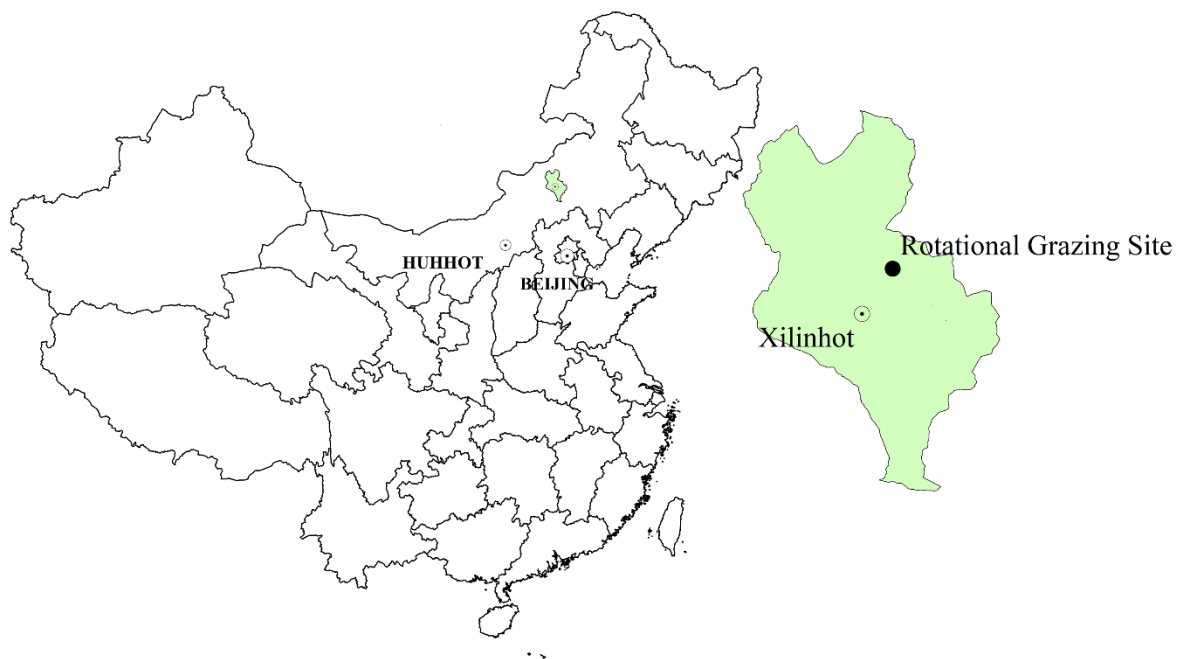


Figure 1: Map of China with the city of Xilinhhot highlighted in green, modification to map by (Dong et al., 2020). The location of the Rotational Grazing Site is marked to the north-east of the city centre.

The area consists of dry and flat grasslands, dominated by the perennial grass species *Stipa grandis* and *Leymus chinensis*. The soil is classified as Kastanozems in the World Reference Base for soil resources by Dong et al. (2020) (WRB 2014), or the equivalent Chestnut soil or Calcic Orthic Aridisol in the Chinese and USA soil taxonomic system, respectively (Fan et al., 2019). The soil is typical for dry and calcic grasslands (FAO, 2015). The climate is continental temperate semiarid (Xu et al., 2012), with a mean annual precipitation of 306.1 mm/year with most precipitation received during summer (TimeanddateAS, 2021). Mean annual temperature is 3 °C, with temperatures during the year fluctuating between 28 °C and -26 °C (TimeanddateAS, 2021). The annual deposition of nitrogen (N) is 14.7 kg / ha / year, based on measurements from 2013 - 2015 in the south of Xilingol League (Zhang, Y. et al., 2017).

2.1.2| Experimental design

The experimental site, which has been in operation since 2013, involves rotational grazing treatments during the summer seasons (June - September) and grazing cessation during winter (Dong et al., 2020) (Figure 2). The setup consists of twelve fenced plots of 120 m x 120 m each with different grazing treatments (Figure 3). In addition to a reference treatment with no grazing (NG), there are three treatments of different grazing intensities where 28 Inner Mongolian Ujimqin sheep (*Ovis aries*) graze 3, 6 and 12 days per month in what will be referred to as low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity respectively (Fan et al., 2019). The grazing pressure equals 0.64 sheep/ha/year, 1.28 sheep/ha/year and 2.56 sheep/ha/year respectively for LG, MG and HG. There are three replicates for each treatment, and the treatments are randomly distributed over the plots.



Figure 2: Air photo of the experimental rotational grazing site in Xilinhot, Inner Mongolia. Can be seen in relation to grazing plots in Figure 3. Photo: (Supplementary material, Liang et al. (2021a)).

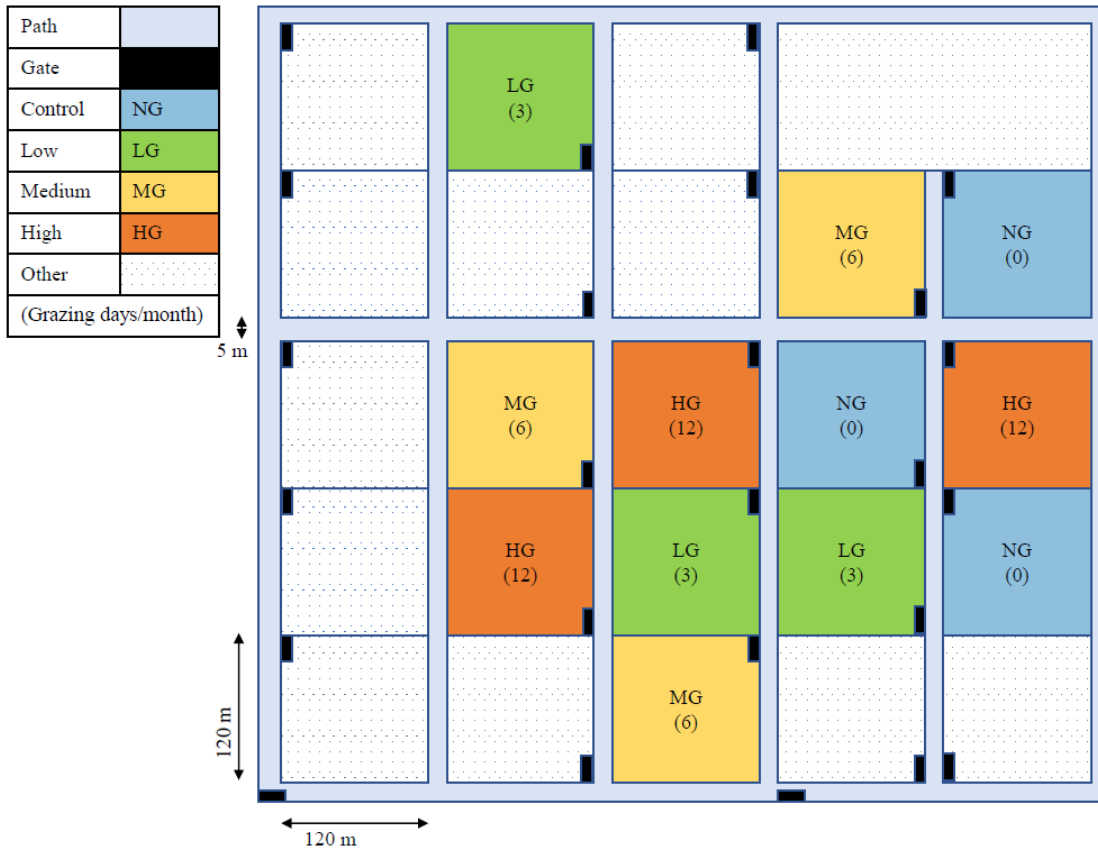


Figure 3: Setup of rotational grazing experiment, showing the plot number and relative location of grazing plots with no grazing (NG, blue, 0 grazing days/month), low grazing (LG, green, 3 grazing days/month), moderate grazing (MG, yellow 6 grazing days/month) and high grazing (HG, red, 12 grazing days/month). The white areas belong to another experiment.

2.2| Soil sampling procedure

The soil samples were collected in July 2020, the eighth year after experiment initiation. Due to covid-19 related travel restrictions, students of Inner Mongolia University considerably provided sampling aid based on agreed instructions. Each of the twelve plots were divided into four quadrants, and five random sampling locations were selected within each quadrant (Figure 4). At all sampling locations, soil samples were collected with an auger at depths 0 - 5 cm, 5 - 10 cm, 10 - 15 cm, 15 - 20 cm and 20 - 30 cm. All five samples from the same depth in each of the quadrants were bulked into one sample, resulting in a total of 240 soil samples (4 treatments x 3 replicates x 4 quadrants x 5 depths). One set of additional samples was collected from the middle of the twelve plots at depths 30 - 50 cm and 50 - 100 cm (mix of soil from 35 cm and 45 cm depth, and mix of soil from 60, 75 and 90 cm depth, respectively), resulting in another 24 samples.

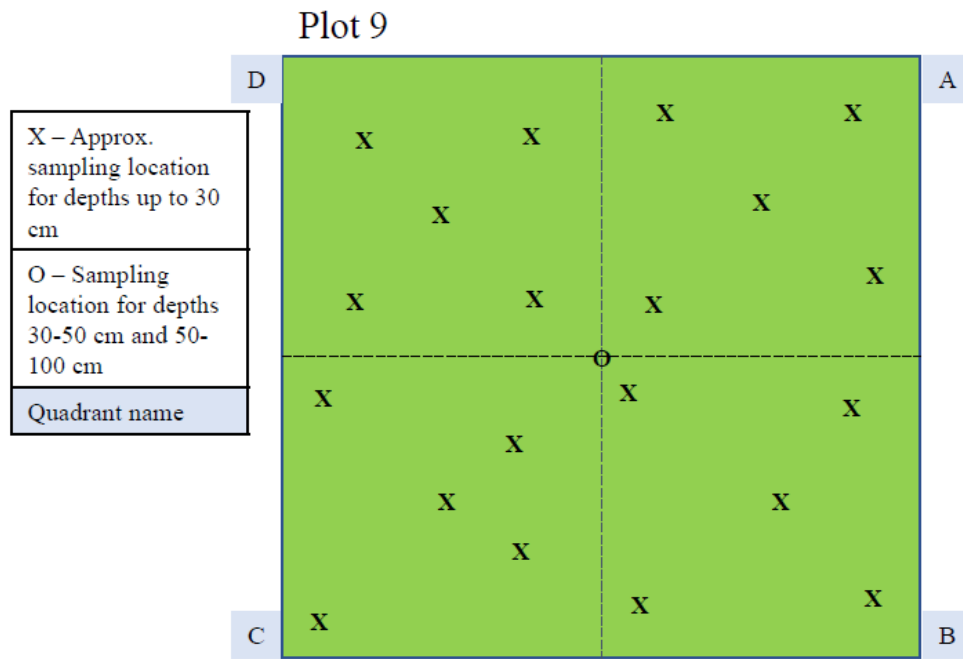


Figure 4: Schematic figure of grazing plot #9 as an example of quadrants and locations for sub-sampling. X'es show location for random sampling at the 5 shallowest depths (up to 30 cm). O in the middle shows location for sampling at the 2 greatest depths (30 - 50 cm and 50 - 100 cm respectively).

The soil samples (about 200 g each) were stored in airtight plastic bags and labelled according to their plot number, quadrant, depth and date (yy.mm.dd) of sampling (e.g., 1A (0 - 5), 20.06.12). This resulted in a total of 264 soil samples. Undisturbed soil samples for bulk density determination were not collected based on own instructions. However, undisturbed samples were collected at depths 0 - 10 cm, 10 - 20 cm, 20 - 30 cm and 30-40 cm with 100 cm³ cylinders during the same period for another project, and relevant analysis results were shared (personal communication, Wu Yan Tao, 12.03.2020).

2.3| Preparation

After air-drying, the soil samples were stored for three months prior to analysis, due to long, covid-19 related shipment duration. The samples were deemed very dry upon arrival at the laboratory and thus the only pre-treatment of the samples was sieving by hand with a 2 mm mesh sieve. All analyses are based on this homogenous, sieved soil. Some analyses were performed on the complete set of 264 soil samples, whilst others were performed on a reduced set. The selection of samples is presented in Appx 3.

2.4| Bulk density

The sampling details of the bulk density dataset ($BD_{(W_u)}$, $n = 144$, Appx 3) does not match the sampling depth otherwise used for this thesis ($n = 264$, Appx 3). To estimate a full dataset for bulk density ($BD_{(SOC)}$), the model of Bellamy et al. (2005) (Equation 1) is used. It is calculated

based on soil organic carbon (SOC) data and further adjusted based on Wu Yan Tao's data ($BD_{(Wu)}$) (Equation 8). The bulk density estimation based on soil organic carbon ($BD_{(SOC)}$) will be used further in the results section.

Equation 1:

$$BD_{(Bellamy)} \left(\frac{g}{cm^3} \right) = 1.3 - 0.275 \times \ln \left(\frac{SOC}{10} \right)$$

2.5| pH

In accordance with Krogstad & Børresen (2015), soil pH (pH_{H_2O}) was measured in a soil-water suspension (10 mL soil in 25 mL deionized water) that was mixed and shaken in plastic beakers with lids and left to achieve equilibrium overnight. The beakers were shaken once the next morning to retrieve homogenous suspensions, and left for 10 minutes for the soil particles to settle. A PHM210 standard pH-meter was calibrated with buffer solutions pH 4 and pH 6.87 before use. The electrode was carefully inserted into the soil solution, avoiding contact with the sedimented soil. The stabilised readings of the pH-meter were recorded. Between each measurement, the electrode was rinsed thoroughly with water and blotted dry.

2.6| Grain size distribution

To analyse grain size distribution, pre-treatment methods were executed following Krogstad and Børresen (2015), while the Beckman Coulter (2011) instrument was used for particle size distribution analysis. In 1 L glass beakers, 10 g of soil was suspended in 20 ml deionized water. Next, 10 ml 35% H_2O_2 was added and stirred together to oxidize organic matter. The beakers were covered with watch glasses and left overnight in a fume cupboard. 10 ml H_2O_2 was added the second and third day as well. The beakers were then heated on a hotplate to increase the reaction rate, while stirring occasionally with a glass rod to avoid spilling.

When oxidation was nearly finished, as indicated by ceased frothing, deionized water was added to the 200 ml mark. The solution was left to evaporate at about 80 °C to remove the remaining H_2O_2 . When 110 mL of the solutions had evaporated, the beakers were removed from the hotplate. 10 mL of 2 M HCl was added to remove carbonates which could cement mineral particles together. The beakers were filled with deionized water and 2 - 3 drops of 1 M $MgCl_2$ was added to increase sedimentation velocity.

After sedimentation, the clear liquid was removed with a suction tool and the washed sediments were transferred to 200 mL beakers. These were filled with deionized water to the 100 mL-

mark, and 50 mL 0.05 M sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$). The samples were stirred with a magnetic stirrer and 2 - 20 mL of the solution was transferred directly from the beaker with a pipette to the LS 13320 Beckman Coulter Laser Diffraction Particle Size Analyser, where the Aqueous Liquid Module was used.

2.7| Aggregate size distribution

The total weight of bulk soil was noted before fractionation according to aggregate size. Aggregate and grain size distribution was determined by sieving soil through stacked 1 mm mesh and 0.25 mm mesh sieves. The resulting soil in the 1 - 2 mm (macro-), 0.25 - 1 mm (meso-) and < 0.25 mm (micro-) size classes were weighed, and the fractions were calculated (ASD). The macro- and mesoaggregate size fractions were saved for conducting the aggregate stability analysis. It is uncertain whether aggregate size distribution represented only aggregates or both aggregate and grain size distribution. Because of this, the mineral particle fraction measured for macro- and meso-size aggregates was subtracted from these two size classes. This subtraction resulted in a corrected aggregate size distribution (ASD_{corr}), while the < 0.25 mm size class represent both aggregate and possibly grain size distribution.

2.8| Aggregate stability

The Wet Sieving Apparatus along with included instructions from Eijkelkamp (2008) was used to separate stable and unstable aggregate fractions (Figure 5). The cans of the apparatus were filled with approximately 60 mL deionized water. The smaller cans with mesh bottom (0.25 mm) were filled with 4 g of soil in the fraction of 1 - 2 mm and lowered into the bigger, waterfilled cans. By initiating the apparatus, the soil was continuously raised and lowered below the water surface of the bigger cans for 10 minutes. The smaller cans with mesh bottom were then raised and remaining water was left to drip into the cans. The water with suspended soil in the bigger cans, representing unstable aggregates, were transferred to pre-weighed containers.



Figure 5: Wet sieving apparatus from Eijkelkamp (photo: (Eijkelkamp))

The big cans were rinsed with deionized water and filled with 2 g/L dispersing solution. Because the soil pH was above 7, sodium hexametaphosphate ($\text{Na}_6[(\text{PO}_3)_6]$) was used instead of NaOH ($\text{pH} < 7$). The smaller cans with mesh bottom and the remaining soil were again submerged in the bigger cans and moved for another 10 minutes. Any aggregates hard to dissolve were prodded with a spoon until they disintegrated. The content of the bigger cans, equalling dissolved stable aggregate fractions, as well as the coarse rest fraction remaining in the smaller cans, equalling particles and not aggregates, were transferred to separate pre-weighed containers. All containers were dried at 105 °C overnight, weighed, and the fractions were calculated (Equation 2, Equation 3).

Equation 2:

$$\begin{aligned} & \text{Stable aggregates after drying (g)} \\ &= \text{Total soil weight (g)} - (\text{Unstable aggregates after drying (g)} \\ &+ \text{Rest fraction after drying (g)}) \end{aligned}$$

and

Equation 3:

$$\begin{aligned} & \text{Stable or unstable aggregate fraction (\%)} \\ &= \frac{\text{Stable or unstable aggregates after drying (g)}}{\text{Unstable aggregates after drying (g)} + \text{Stable aggregates after drying (g)}} \end{aligned}$$

Stable aggregates of 1 - 2 mm and 0.25 - 1 mm size were then analysed for total C content with the Leco CHN628 analytic instrument. Note that it is unclear to which degree the stable aggregates contain inorganic carbon (IC), and if the distribution is similar to IC distribution in the bulk soil. Because of this uncertainty, total carbon values will be presented instead of SOC-values corrected for IC.

2.9| Loss On Ignition (LOI)

Calculations and methods for loss on ignition (LOI) were performed in accordance with Krogstad and Børresen (2015). Labelled crucibles were weighed before adding about 5 g dry soil, noting the total weight. The crucibles were dried at 105 °C overnight and weighed again, resulting in a dry matter percentage. The crucibles were calcinated overnight (approximately 16 hrs including preheating time of oven) at 550 °C. After weighing, the LOI content was calculated and corrected for soil-bound water, which only evaporates at temperatures higher than 105 °C and increases with clay content (Equation 4). Clay content thus determines the subtraction factor, given by Krogstad and Børresen (2015).

Equation 4:

$$LOI_{(corr)} (\%) = \left(\frac{\text{Soil after drying (g)} - \text{Soil after calcination}}{\text{Soil after drying (g)}} \times 100\% \right) - \text{Factor}$$

2.10| Soil Organic Carbon (SOC) calculations and SOC stocks

There are several ways to estimate SOC. It is often assumed that soil organic matter contains 58 % carbon (Pribyl, 2010). This percentage is widely used, and sometimes credited to researcher van Bemmelen (Pribyl, 2010). Organic carbon content could thus be estimated as a mass percentage of bulk soil by multiplying $LOI_{(corr)}$ by 0.58, resulting in $SOC_{(Van Bemmelen)}$.

Another method for determining SOC-values are via element analysers, although this procedure is more costly than LOI and in this case not performed on all soil samples. Total carbon and nitrogen content (totC, totN) of the bulk soil was analysed with the Leco CHN628 analytic instrument for a selection of samples (n = 70, Appx 3). TotC was also measured with the CHN-instrument on a selection of ash-remains from the LOI-process (n = 21, Appx 3). Because these samples were calcinated and without any organic carbon left, the resulting values are assumed to represent inorganic carbon (IC) content of the bulk soil. IC is not expected to vary with treatment (Cao et al., 2013) and the mean value for each depth was thus subtracted from totC. This created another dataset for organic carbon ($SOC_{(TotC - IC)}$). The linear relationship between $SOC_{(TotC - IC)}$ and $LOI_{(corr)}$ was used to determine a complete SOC-dataset specific to the experimental site, $SOC_{(Xilinhot)}$ (Equation 5, Figure 8).

Equation 5:

$$SOC_{(Xilinhot)} (\%) = 0.67 + 0.42 \times LOI_{(corr)} (\%)$$

This $SOC_{(Xilinhot)}$ -value is used when calculating organic carbon stocks (Equation 6).

Equation 6:

$$\begin{aligned} & SOC_{(Xilinhot)} \text{ stocks } \left(\frac{kg}{m^2} \right) \\ &= Bulk \text{ density }_{(SOC)} \left(\frac{g}{cm^3} \right) \times \frac{SOC(\%)}{100} \times Soil \text{ depth (cm)} \times 10 \end{aligned}$$

2.11| Hot Water Extractable Carbon (HWEC)

Hot water extractable carbon (HWEC) was determined according to Dong et al. (2020), with slight adjustments to centrifugation force*. Centrifuge tubes of 50 mL were filled with 4.5 g

soil and 45 mL deionized water, and briefly shaken to suspend the soil. The tubes were covered with lids and left in a hot water bath at 80°C for 16 hours. The samples were centrifuged at 3803 RCF (Relative Centrifugal Force) for 10 minutes and the supernatants filtered through 45 µm cellulose nitrate membrane filters. The dissolved organic carbon (DOC) in the filtrate was analysed by a TOC-analyser (TOC V CPN, Shimadzu, Kyoto, Japan).

* Centrifugation of lower force was tested as an adjustment to the method. Duplicates were analysed for a selection of samples, where one sample was centrifuged at 1690 RCF and the other sample at 3803 RCF, for 10 minutes. This was to investigate the effect on results as well as intensity of filter clogging, and to which degree a stronger centrifugation would lessen the necessary pressure for successful filtration.

As mentioned in the previous section, totC and totN content of the bulk soil was analysed with the Leco CHN628 analytic instrument for a selection of samples (n = 70, Appx 3). By evaluating the correlation and using linear regression between totN and HWEC (Appx 14B), which was analysed for the complete dataset (n = 264, Appx 3), it was possible to estimate a complete dataset for totN_(HWEC). The SOC/N-ratio was achieved by dividing SOC_(Xilinhot) with totN_(HWEC).

2.12| Carbon Density Fractionation - Particular and Mineral associated Organic Carbon (POC and MOC)

Density fractionation to separate particulate organic carbon (POC) and mineral associated carbon (MOC) of the 2 mm to 20 µm size fraction was determined according to Dong et al. (2020). Additional steps were taken for separating the fraction < 20 µm. A suspension was made by mixing 15 g soil with 30 mL sodium polytungstate (SPT) solution ((Na₆W₁₂O₄₀)*H₂O) of 1.8 g/cm³ density in 50 mL tubes. The suspension was left to settle for 10 minutes, and then centrifuged at 1690 RCF for 10 minutes. The supernatant was washed with deionized water in a 20 µm sieve, ensuring removal of SPT by measuring a conductivity of < 100 µS/cm.

The soil fraction left on the sieve equals particular organic matter (POM) and was transferred to a pre-weighed container. The tubes were shaken, and the steps repeated to transfer any remaining POM to the same container. The remaining solution and soil in the tube was similarly washed in the sieve*, leaving mineral associated organic matter (MOM) which was transferred to a pre-weighed container. The containers with POM and MOM were dried at 105 °C overnight and analysed for totC and totN content with the Leco CHN628 analytic instrument. Because

carbonates were not removed before analysis, MOC is calculated based on the assumption that POC does not contain any inorganic carbon, and that all inorganic carbon measured in the bulk soil exists in the mineral associated fraction. MOC is thus estimated as the analysed content of mineral associated carbon (both inorganic and organic) in bulk soil, subtracted by the content of inorganic carbon in bulk soil (Figure 6).

TotC		
IC	SOC	
MC (mineral associated OC and IC)		POC
IC	MOC = MC-IC	POC

Figure 6: Visualisation of mineral associated organic carbon (MOC) fraction in relation to particular organic carbon (POC) and inorganic carbon (IC). TotC = Total carbon content. SOC = Soil organic carbon.

* Exception from normal procedures: For a selection of samples (n = 6, Appx 3), additional steps were taken to investigate the usually reported “lost fraction” of < 20 µm that is washed away. The following procedure is based on retaining any water dissolved carbon, which results in a “filter”-fraction (20 µm > filter fraction > 12 µm), and a rest-fraction (< 12 µm).

An underlying basin for collecting wash water was inserted before transferring SPT-solution and MOM from the tubes to the sieve. The washing liquid was stored in beakers and left to sediment over a few days. The liquid was transferred to another set of pre-weighed beakers, using a suction tool to not disturb the sedimented layer. The sedimented soil was washed with deionized water in a pre-weighed white ribbon filter (12 µm). The fraction remaining in the filter (filter fraction) was dried, weighed and analysed for totC. The volume of wash water removed by suction was noted and the SPT-soil solution was left to dry and analysed for totC.

SPT does not contain carbon. 1 L SPT solution of 1.8 g/mL density consists of 990 g solid SPT and 810 mL water (SometuGermany, n.d.). Approximately all 30 mL SPT-solution is kept during the additional steps and dried in beakers with the soil. This equals 990 g * 0.03 mL = 29.7 g SPT per sample. The weight of soil in the dried rest fraction thus equals total dry weight subtracted by dry SPT (29.7 g). The C content of the soil rest fraction could then be calculated in a similar fashion to the filter fraction (Equation 7).

Equation 7:

$$\begin{aligned} & \text{Filter or rest fraction } C \text{ (\% of bulk soil)} \\ &= \frac{\text{Filter or rest fraction (g)}}{\text{Total soil sample (g)}} \times \text{TotC (filter or rest fraction)} \end{aligned}$$

2.13| Potential N mineralization rate

Potential nitrogen (N) mineralization rate was determined following Dong et al. (2020). About 2.3 g deionized water was combined with 8 g soil in a 50-mL centrifuge tube to reach a soil moisture content of 80% field capacity. Two sets were made from each soil sample. The first set of samples was kept frozen during the whole 8-week period. The other set was incubated at room temperature in the dark. Lids on the incubated samples were kept loose to allow for air exchange, i.e. enabling oxidation which is required to convert organic N to inorganic and plant available N. The lids were accidentally kept tight the first week, potentially hindering access to oxygen and subsequently slowing the N mineralization rate. Every two weeks the incubated samples were weighed, and deionized water was added to restore their initial weight.

After eight weeks, the incubated samples were frozen. Both sets were retrieved and thawed four days after the eight-week period. 20 mL 2M KCl was added to the samples, and they were shaken mechanically for 1 hour at 180 strokes per minute. The samples were left to settle for 10 minutes, and the supernatants were filtered through blue ribbon filter. The filtrate was analysed for nitrate and ammonium through Flow injection analysis (FIA). Potential N mineralization was then calculated by subtracting NO_3 and NH_4 measured in the frozen tubes from NO_3 and NH_4 measured in the incubated tubes, respectively.

2.14| Statistical analysis

All statistical analyses and plotting of figures were performed in the data program R, version 3.6.2 (2019). Linear mixed effects models (R extension package lme4 (Bates et al., 2015)) were used to test all data with plot as random terms. This was to account for temporal correlation of data gathered from the same plots (i.e., to avoid pseudo-replication). All variables were tested for the fixed effects of soil depth and treatment (7 levels and 4 levels respectively, except for data from limited sample selections (Appx 3)), starting with the full model but reducing it to exclude the interaction term or one of the two factors if possible.

Differences were assessed with least square means for multiple comparisons (lsmeans) using the p-value adjustment method “sidak”, followed by compact letter display of pairwise comparisons (cld in the lsmeans package (Piepho, 2004)). Means were determined to be different with an overall p-value of 0.05.

Additionally, linear regression was used to find correlation and the linear equation for some variables. Diagnostic plots (inspection of residual and QQ plots) were used to evaluate the assumptions of normal distribution and homogeneity of variances.

3| Results

Appx 2 shows a full summary of the data averages and sample size. Note from the method section that soil was sampled with depth intervals of 5 cm in the upper 20 cm of the soil, but with greater intervals between 20 and 100 cm soil depth. Because of this, the x-axis of figures in the results section does not reflect equally divided depths.

3.1| Carbon content and different Soil Organic Carbon (SOC) fractions

3.1.1| Total Carbon (TotC)

Total Carbon (totC) includes both soil organic carbon (SOC) and inorganic carbon (IC) content, presence of the latter being highly likely due to average pH between 8 and 9. Although not statistically significant, NG consistently shows the highest totC-values for all depth intervals, whereas LG shows lowest totC content from depths 0 to 20 cm (Figure 7). TotC varies significantly with depth, and ranges from an average of 2.22% (30 - 50 cm) to 2.77% (20 - 30 cm) in the upper 50 cm. The lowest values are 1.50% TotC at 50 - 100 cm depth.

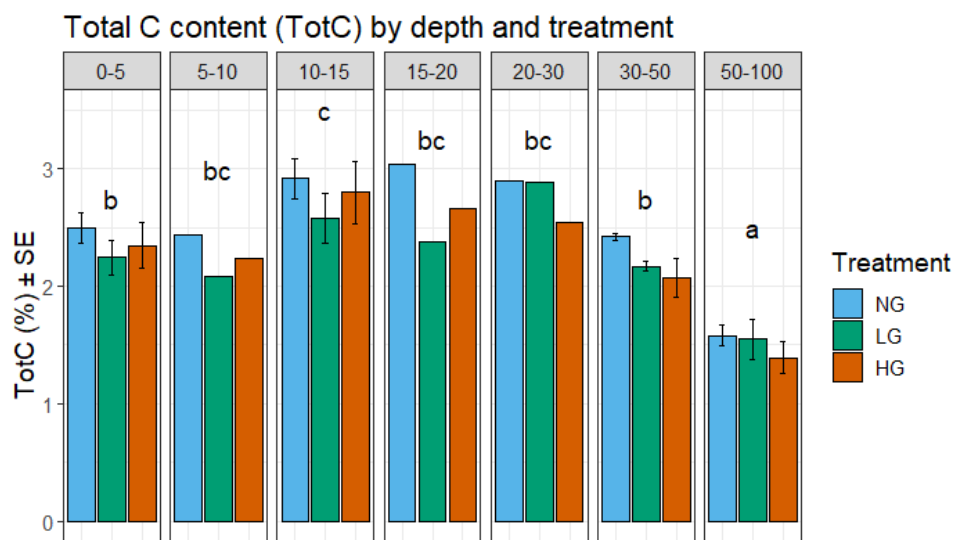


Figure 7: Average TotC ± standard error (SE) by treatment and depth. Treatment is shown in blue, green, and red respectively for non-grazing (NG) as the reference, and two of the three treatments, low grazing (LG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.

3.1.2| Inorganic Carbon (IC) and pH

Inorganic carbon (IC) was less than 0.6 % of totC in the topsoil but increased significantly to 43.3 % of totC at 50 - 100 cm depth. High IC content in lower soil depths likely indicates calcic horizons in the Calcic-Orthic Aridisol (Liang et al., 2021b). Carbonate content is also indicated by high pH values, which range from 7.3 to 9.2. Average pH is 8.0 in the upper 5 cm of the soil

and increased significantly to 9.0 in soil sampled from 50 to 100 cm depth (Appx 2, Appx 4). Neither IC nor pH varied significantly with treatment. Average IC across all treatments was thus used as a correction factor when calculating $\text{SOC}_{(\text{TotC} - \text{IC})}$, and when estimating MOC.

Table 1: Average inorganic carbon (IC) content \pm standard error (SE) by depth as a percentage of totC and bulk soil respectively.

Depth (n)	IC (% of totC) \pm SE (significance)	IC (%) \pm SE
0 - 5 (3)	0.57 ± 0.34 (a)	0.01 ± 0.01
5 - 10 (3)	1.71 ± 0.89 (ab)	0.04 ± 0.02
10 - 15 (3)	7.48 ± 3.44 (ab)	0.19 ± 0.09
15 - 20 (3)	14.11 ± 3.67 (bc)	0.36 ± 0.12
20 - 30 (3)	25.31 ± 2.78 (cd)	0.66 ± 0.09
30 - 50 (3)	35.14 ± 3.07 (de)	0.71 ± 0.02
50 - 100 (3)	43.26 ± 3.03 (e)	0.56 ± 0.03

3.1.3| Soil Organic Carbon (SOC)

The two estimates of soil organic carbon, $\text{SOC}_{(\text{Van Bemmelen})}$ and $\text{SOC}_{(\text{Xilinhhot})}$, both depend on corrected loss on ignition data ($\text{LOI}_{(\text{corr})}$, (Equation 4)) which further depends on clay content. Clay made up 7-14% of the grain size distribution (Appx 2), and did not vary significantly with treatment or depth (Appx 5). This corresponds to a correction factor of 2 (Krogstad & Børresen, 2015). Correlation between $\text{SOC}_{(\text{TotC} - \text{IC})}$ and $\text{LOI}_{(\text{corr})}$ gives $R = 0.86$ (Figure 8). Linear regression between these parameters provided Equation 5 (Figure 8), which was used to calculate $\text{SOC}_{(\text{Xilinhhot})}$. In contrast to $\text{SOC}_{(\text{Van Bemmelen})}$, $\text{SOC}_{(\text{Xilinhhot})}$ is thus based on CHN-analysis in addition to LOI, and will be used for further calculations and result comparisons.

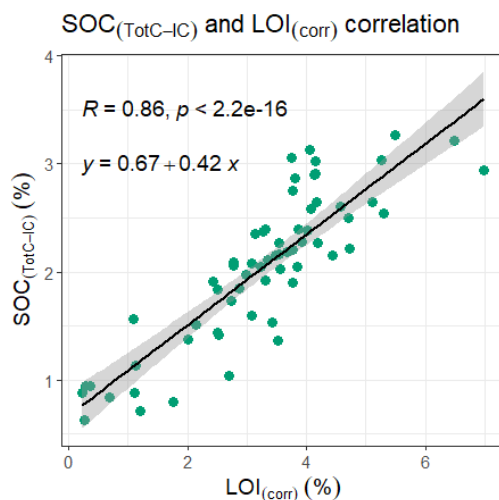


Figure 8: Correlation coefficient and linear relationship between $\text{SOC}_{(\text{TotC} - \text{IC})}$ and $\text{LOI}_{(\text{corr})}$ for a subset based on the total number of samples available for $\text{SOC}_{(\text{TotC} - \text{IC})}$ ($n=63$, see Appx 3 for sample selection).

The different estimations of SOC are presented in Table 2. $\text{SOC}_{(\text{TotC} - \text{IC})}$ shows slightly higher values than $\text{SOC}_{(\text{Xilinhhot})}$, which again shows slightly higher values than $\text{SOC}_{(\text{Van Bemmelen})}$, although not to a significant extent.

Table 2: Average SOC \pm standard error (SE) with n = number of samples, based on three different SOC estimations * ($\text{SOC}_{(\text{Van Bemmelen})}$, $\text{SOC}_{(\text{Xilinhot})}$ and $\text{SOC}_{(\text{TotC-IC})}$) by depth as a percentage of bulk soil.

Depth	$\text{SOC}_{(\text{Van Bemmelen})} \pm \text{SE (n)}$	$\text{SOC}_{(\text{Xilinhot})} \pm \text{SE (n)}$	$\text{SOC}_{(\text{TotC-IC})} \pm \text{SE (n)}$
0 - 5	1.98 ± 0.08 (48)	2.10 ± 0.06 (48)	2.35 ± 0.09 (18)
5 - 10	2.14 ± 0.09 (47)	2.22 ± 0.07 (47)	2.21 ± 0.10 (3)
10 - 15	2.25 ± 0.09 (48)	2.30 ± 0.06 (48)	2.56 ± 0.11 (18)
15 - 20	2.11 ± 0.09 (47)	2.19 ± 0.06 (47)	2.31 ± 0.16 (3)
20 - 30	1.93 ± 0.08 (47)	2.06 ± 0.06 (47)	2.07 ± 0.09 (3)
30 - 50	1.34 ± 0.13 (12)	1.64 ± 0.09 (12)	1.44 ± 0.05 (9)
50 - 100	0.58 ± 0.12 (12)	1.09 ± 0.09 (12)	0.85 ± 0.04 (9)
* SOC estimations	$\text{LOI}_{(\text{corr})} \times 0.58$	$0.67 + 0.42 \times \text{LOI}_{(\text{corr})}$	TotC - IC

$\text{SOC}_{(\text{Xilinhot})}$ increases on average from 2.10% in the upper 5 cm of the soil to a peak of 2.30 % at depth 10 to 15 cm before decreasing significantly to the lowest percentage of 1.09% in the 50 to 100 cm depth interval (Appx 2) $\text{SOC}_{(\text{Xilinhot})}$ did not vary significantly with treatment. However, MG shows continuously lower $\text{SOC}_{(\text{Xilinhot})}$ concentrations than any other treatment in the upper 30 cm of the soil (Figure 9). There is a strong tendency of decreasing $\text{SOC}_{(\text{Xilinhot})}$ concentrations from NG to LG and MG, before $\text{SOC}_{(\text{Xilinhot})}$ content increases in HG. Based only on treatment average across all depths, MG shows the lowest SOC content with 1.95 %, followed by HG, LG and NG at 2.10 %, 2.17 % and 2.19 % respectively.

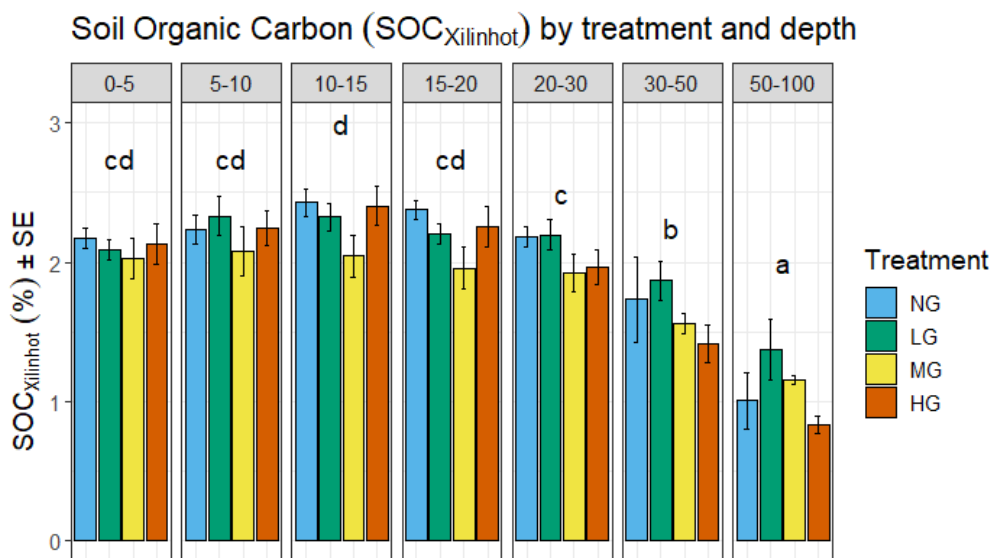


Figure 9: Average soil organic carbon ($\text{SOC}_{(\text{Xilinhot})}$) \pm standard error (SE) by treatment and depth. Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the reference, and the three treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For each sampling depth between 0 to 30 cm, n = 47 or 48. For each sampling depth between 30 and 100, n = 12. See also Appx 3 for sample selection.

3.1.4| Particular Organic Carbon (POC), Mineral Associated Organic Carbon (MOC) and fraction < 20 μm

Particular organic carbon (POC) decreases significantly with depth, from 0.37 % in the topsoil to 0.06 % in the deepest depth interval (Appx 2). The differences between treatments are not significant, potentially due to a low sampling number (only one sample from each treatment at depths 5 - 10, 15 - 20 and 20 - 30 cm, Appx 3). However, POC content decreased with 16.9 %, 45.13 %, 22.3 % and 37.6 % from NG to LG in the four upper depth intervals (Figure 10). In the upper 30 cm of the soil, POC content also consistently increases from LG to HG.

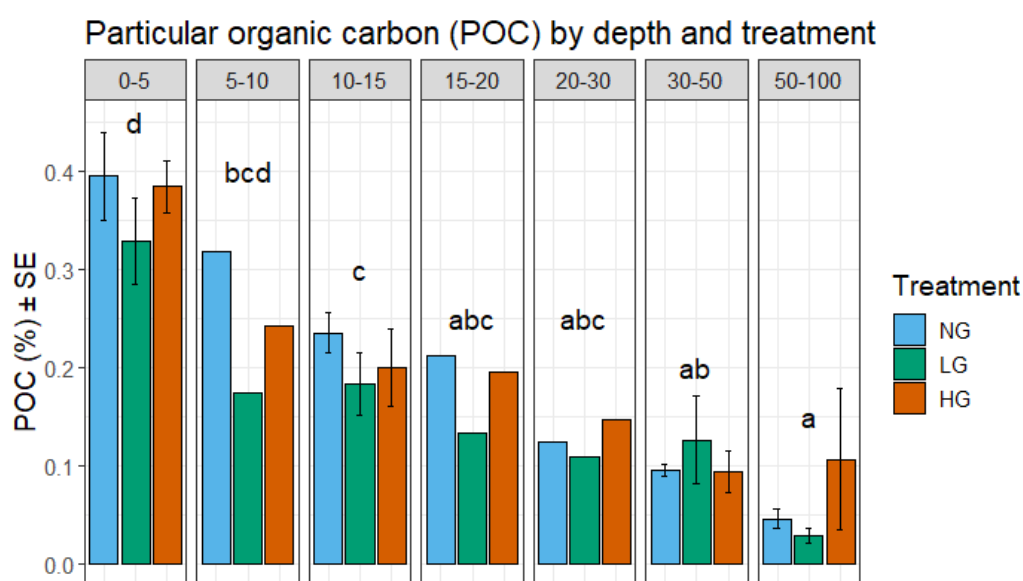


Figure 10: Average particular organic carbon (POC) \pm standard error (SE) as a percentage of bulk soil by treatment and depth. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, and two of the three treatments; low grazing (LG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.

Mineral associated organic carbon (MOC) decreases significantly with depth and range from 0 % at lower depths to a maximum of 1.28 % of the bulk soil in the upper 10 to 15 cm (Figure 11, Appx 2). MOC shows negative values for the deepest depth interval with an average of - 0.11 % because the values were calculated by subtracting inorganic carbon (IC) content in bulk soil from mineral associated carbon (Figure 6). Considering uncertainties and different analysis methods of IC and mineral associated organic matter (MOM), these MOC values are not significantly different from 0. MOC shows no significant effect of treatment, although MOC content in LG tends to decrease compared to NG and HG.

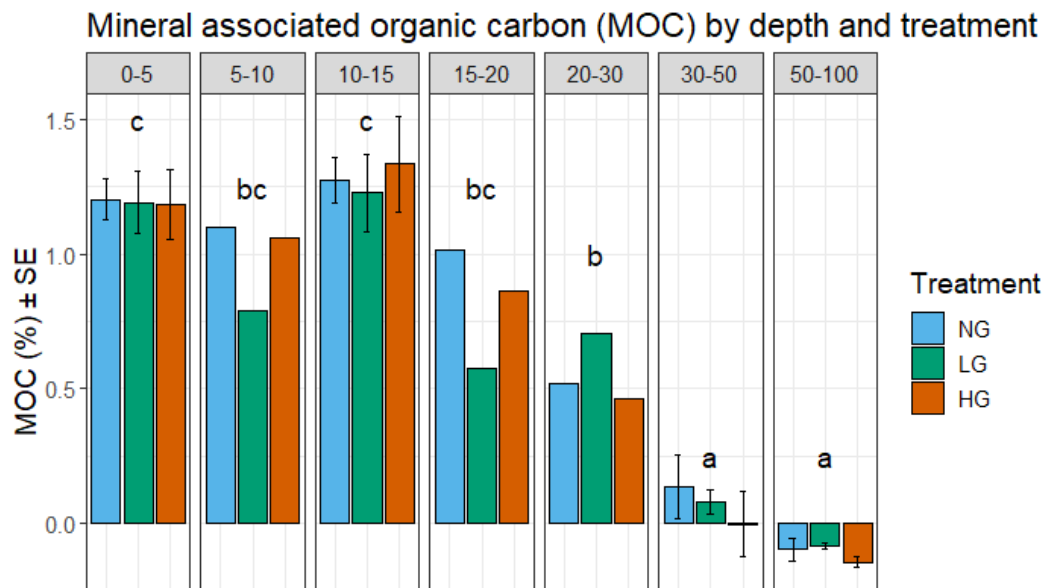


Figure 11: Average mineral associated organic carbon (MOC) \pm standard error (SE) as a percentage of bulk soil by treatment and depth. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, and two of the three treatments; low grazing (LG) and high grazing (HG) intensity. Depth intervals are separated in grids. Note that negative values due to calculations are unrealistic and suggest values close to 0. Different letters indicate significant differences between depths ($p < 0.05$). For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.

Within the upper 30 cm of the soil, there is between 3.2 and 6.2 times higher MOC content than POC. The sum of analysed MOC and POC does not equal $\text{SOC}_{(\text{Xilinhot})}$. Almost 25 % of SOC is lost in the topsoil where both POC and MOC content relative to SOC-percentage is highest (Table 3). POC, MOC and recovery of SOC in the density fractionation process decreases with increasing depths.

Table 3: Average C content \pm standard error (SE) in particular and mineral associated organic matter (POM, MOM) and average soil organic carbon (SOC), particular organic carbon (POC) and mineral associated organic carbon (MOC) \pm standard error (SE) as percentages of bulk soil. n = number of samples. POC and MOC is also presented in relation to SOC content, and to recovery from the fractionation process. Note that negative values are due to calculations and not significantly different to 0.

Depth	C (% of POM) \pm SE (n)	C (% of MOM) \pm SE (n)	
0 - 5	13.41 \pm 0.33 (18)	1.55 \pm 0.07 (18)	
5 - 10	12.83 \pm 1.19 (3)	1.33 \pm 0.11 (3)	
10 - 15	13.02 \pm 0.68 (18)	1.79 \pm 0.10 (18)	
15 - 20	13.07 \pm 1.07 (3)	1.64 \pm 0.18 (3)	
20 - 30	13.70 \pm 2.00 (3)	1.69 \pm 0.09 (3)	
30 - 50	12.90 \pm 0.49 (9)	1.14 \pm 0.07 (9)	
50 - 100	15.28 \pm 1.48 (9)	0.68 \pm 0.02 (9)	
Depth	POC (% bulk soil) \pm SE (n)	MOC (% bulk soil) \pm SE (n)	SOC _(Xilinhot) (% bulk soil) \pm SE (n)
0 - 5	0.37 \pm 0.02 (18)	1.19 \pm 0.06 (18)	2.10 \pm 0.06 (48)
5 - 10	0.24 \pm 0.04 (3)	0.98 \pm 0.10 (3)	2.22 \pm 0.07 (47)
10 - 15	0.21 \pm 0.02 (18)	1.28 \pm 0.08 (18)	2.30 \pm 0.06 (48)
15 - 20	0.18 \pm 0.02 (3)	0.82 \pm 0.13 (3)	2.19 \pm 0.06 (47)
20 - 30	0.13 \pm 0.01 (3)	0.56 \pm 0.07 (3)	2.06 \pm 0.06 (47)
30 - 50	0.10 \pm 0.02 (9)	0.07 \pm 0.05 (9)	1.64 \pm 0.09 (12)
50 - 100	0.06 \pm 0.02 (9)	-0.11 \pm 0.02 (9)	1.09 \pm 0.09 (12)
Depth	POC/SOC-ratio	MOC/SOC-ratio	Recovery (POC + MOC)
0 - 5	0.18	0.57	0.74
5 - 10	0.11	0.44	0.55
10 - 15	0.09	0.56	0.64
15 - 20	0.08	0.37	0.45
20 - 30	0.06	0.27	0.33
30 - 50	0.06	0.04	0.11
50 - 100	0.05	-0.10	-0.04

The soil fraction less than 20 μm was only included in the density fractionation process for a selected 6 samples in the 0 - 5 cm soil layer, divided on two treatments (Appx 3). The filter fraction (20 μm > filter fraction > 12 μm) and rest fraction (< 12 μm) all showed detectable values for C according to the CHN-analyser. C of the filter and rest fraction ($C_{\text{(filter)}}$ and $C_{\text{(rest)}}$) did not vary significantly with treatment, although NG showed 1.35 times higher $C_{\text{(filter)}}$ values than HG (Figure 12).

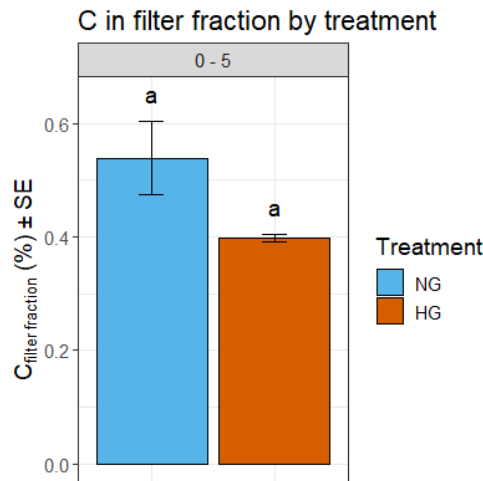


Figure 12: Average C measured in the filter fraction \pm standard error (SE) as a percentage of bulk soil by treatment in the upper 5 cm of the soil. Treatment is shown in blue and red respectively for non-grazing (NG) as the reference and high grazing (HG) intensity. Different letters indicate significant differences between treatments ($p < 0.05$). $n = 6$. See also Appx 3 for sample selection.

Averages for C_{filter} and C_{rest} are given in Table 4. The amount of C measured from the fractionation process increased significantly when C_{filter} and C_{rest} was included. When taking C_{filter} and C_{rest} into consideration, the recovery of SOC from density fractionation process increased significantly from 0.74 (Table 3) to 0.96 (Table 4) in the top 5 cm of the soil.

Table 4: Average C measured in the filter ($20 \mu\text{m} > \text{filter fraction} > 12 \mu\text{m}$) and rest ($< 12 \mu\text{m}$) fraction \pm standard error (SE) as percentages of the fraction and bulk soil, and as a ratio to soil organic carbon (SOC) in the bulk soil. $n =$ number of samples.

Fraction (n)	C (% of fraction) \pm SE	C (% of bulk soil) \pm SE	$C_{\text{frac}}/\text{SOC}$	Recovery
Filter (6)	4.37 ± 0.22	0.47 ± 0.04	0.22	(POC + MOC + filter + rest)
Rest (6)	0.02 ± 0.00	0.00 ± 0.00	0.00	0.96

3.1.5| Hot water extractable carbon (HWEC)

Evaluation of hot water extractable carbon (HWEC) analysis showed advantages of using centrifugation at 3803 RCF compared to 1690 RCF. Increased RCF prevented filter clogging. This decreased necessary pressure for successful filtration and filter changes to obtain sufficient amounts of analysis liquid. The two centrifugation procedures correlated perfectly ($R = 1$), and centrifugation at 3803 RCF is thus used for the complete dataset in this thesis. Note that increased RCF resulted in 78.49 mg/kg lower HWEC on average than 1690 RCF (Appx 6).

There is a significant interaction between grazing treatments and soil depth for concentration of HWEC. In the upper layers of the soil, HWEC is significantly lower in MG and HG than in NG (0 - 20 cm) and LG (0-15 cm) (Figure 13). There are consistent trends of decreased HWEC from NG to LG and from LG to MG. From MG to HG however, HWEC content tends to increase. Depth decreases HWEC significantly, reducing the content by 87% from 724 mg/kg in the top 5 cm to 94 mg/kg in the deepest half metre.

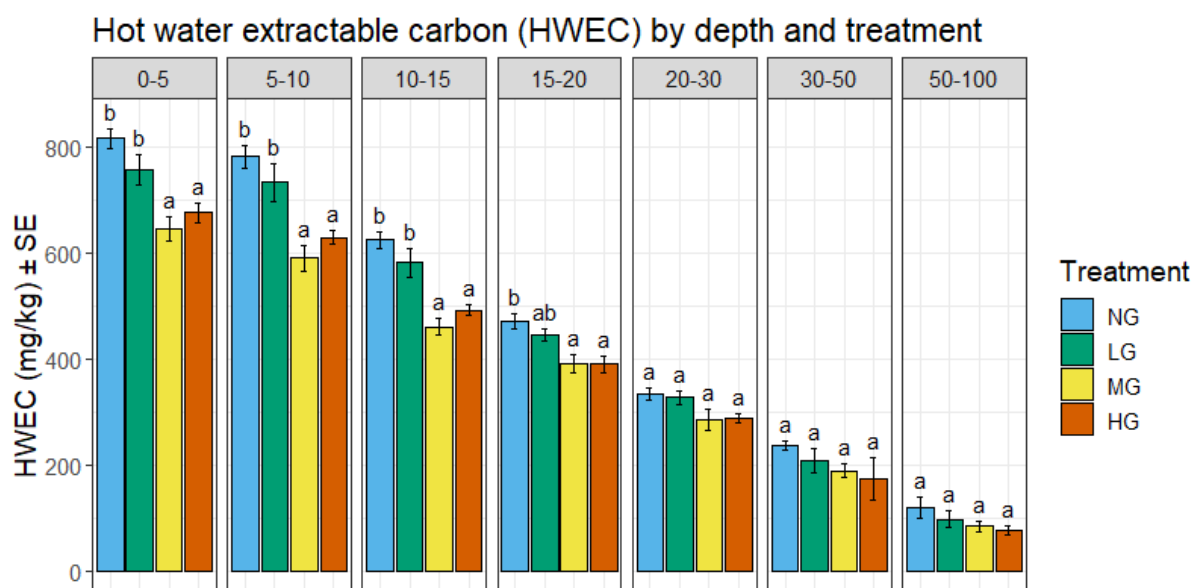


Figure 13: Average hot water extractable carbon (HWE) \pm standard error (SE) in mg/kg bulk soil by treatment and depth. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, and the treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between treatments at each depth ($p < 0.05$). For each sampling depth between 0 to 30 cm, $n = 47$ or 48. For each sampling depth between 30 and 100, $n = 12$. See also Appx 3 for sample selection.

HWE is assumed to be a part of POC and makes up on average 0.07 % of the bulk soil in the top 5 cm and 0.01 % of the bulk soil in the bottom half meter (Table 5).

Table 5: Average Hot Water Extractable Carbon (HWE) \pm standard error (SE) as percentages of bulk soil, and as a ratio to soil organic carbon (SOC). n = number of samples. Intensity of green colour signifies treatment with highest HWE-values for the specific depth.

Treatment	NG	LG	MG	HG
Depth	HWE (%) \pm SE			
0 - 5 (12)	0.08 \pm 0.00	0.08 \pm 0.00	0.06 \pm 0.00	0.07 \pm 0.00
5 - 10 (12)	0.08 \pm 0.00	0.07 \pm 0.00	0.06 \pm 0.00	0.06 \pm 0.00
10 - 15 (12)	0.06 \pm 0.00	0.06 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00
15 - 20 (12)	0.05 \pm 0.00	0.04 \pm 0.00	0.04 \pm 0.00	0.04 \pm 0.00
20 - 30 (12)	0.03 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00
30 - 50 (3)	0.02 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00
50 - 100 (3)	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
Treatment	NG	LG	MG	HG
Depth	HWE/SOC-ratio			
0 - 5 (12)	0.04	0.04	0.03	0.03
5 - 10 (12)	0.04	0.03	0.03	0.03
10 - 15 (12)	0.03	0.03	0.02	0.02
15 - 20 (12)	0.02	0.02	0.02	0.02
20 - 30 (12)	0.02	0.02	0.01	0.01
30 - 50 (3)	0.01	0.01	0.01	0.01
50 - 100 (3)	0.01	0.01	0.01	0.01

3.1.6| Comparison of various carbon fractions

Different analysed carbon fractions are presented in Figure 14 for NG, LG and HG plots where POC- and MOC-values are available. The sum of carbon fractions for all treatments in the 0 - 5 cm depth interval is 1.57 % of bulk soil, and it decreases to 0.51 % in the bottom layer. With increasing depth, the IC/SOC ratio increases significantly. In the upper 20 cm of the soil, organic C fractions seem to be lower in LG compared to NG and HG.

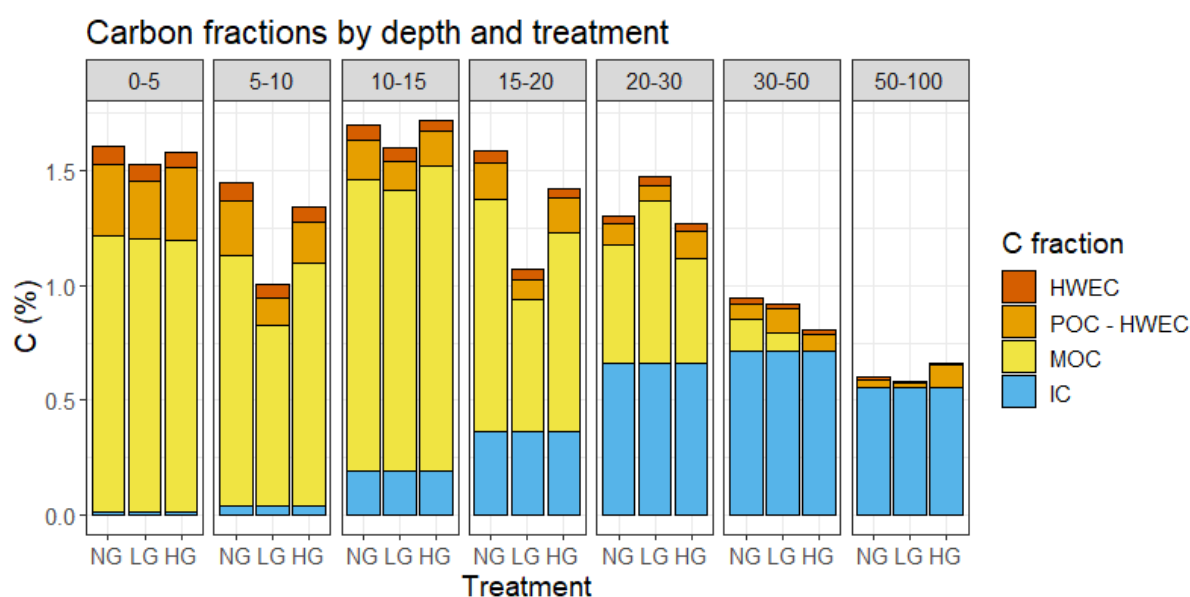


Figure 14: Average carbon fractions \pm standard error (SE) as a percentage of bulk soil by treatment and depth. Depth intervals are separated in grids. Treatment is shown on the x-axis for non-grazing (NG) as the reference, and two of the three treatments; low grazing (LG) and high grazing (HG) intensity. The four C types presented are shown in different colours: Hot water extractable carbon (HWEC, red), particular organic carbon (POC) which includes and thus has the values of HWEC subtracted (orange), mineral associated carbon (MOC, yellow) and inorganic carbon (light blue). Note that the full dataset is not available for all C types presented and a subset has been done. As such, the plot does not include the mean of all earlier presented HWEC values. Also note that calculation of MOC provided unrealistic negative values at lower depths (Figure 11), and these have been changed to zero for this figure. For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.

Compared to average totC results where MG also is excluded (Appx 2), the discrepancy of the presented values in Figure 14 is 0.79 % less C in the top 5 cm of the soil. If C in the filter and rest fraction is included to the sum of analysed C fractions as well, this discrepancy decreases to 0.31 % less C in the top 5 cm compared to totC (Table 6). The C recovery deviation from totC is still considerable.

Table 6: Sum of carbon fractions excluding and including filter and rest fraction with letters of significance, and comparison with totC-values. All values presented as percentages of bulk soil. See Appx 3 for sample selection.

Depth	Sum C fractions* (%)	Deviation from totC (%)	Sum C fractions* including filter and rest fraction (%)	Deviation from totC (%)
0 - 5	a 1.57	0.79	b 2.05	0.31
5 - 10	1.27	0.99		
10 - 15	1.67	1.09		
15 - 20	1.36	1.33		
20 - 30	1.35	1.42		
30 - 50	0.89	1.33		
50 - 100	0.51	0.99		
* C fractions = Particular organic carbon (POC) where hot water extractable carbon (HWEC) is included + mineral associated organic carbon (MOC) + inorganic carbon (IC).				

3.2| Soil Organic Carbon (SOC) stocks

3.2.1| Bulk density

Bulk density values estimated with Equation 1 by Bellamy et al. (2005) corresponded well with available bulk density data from Wu Yantao. However, adjusting the intercept of the original equation from 1.3 to 0.74 lowered average mean deviation between bulk density for the calculated, complete dataset ($BD_{(SOC)}$) and available $BD_{(Wu)}$ -data. Equation 8 was thus used to calculate the complete bulk density dataset. The slope was kept similar to the original equation because $BD_{(Wu)}$ -data at depths lower than 40 cm was not available for comparison. Further optimizing the slope could potentially improve Equation 8 in future bulk density estimations.

Equation 8:

$$Bulk\ density_{(SOC)} \left(\frac{g}{cm^3} \right) = 0.74 - 0.275 \times \ln \left(\frac{SOC_{(Xilinhot)}}{10} \right)$$

Bulk density ($BD_{(SOC)}$) was estimated based on $SOC_{(Xilinhot)}$ -values, and as such there was a significant negative correlation between the two parameters ($R = -0.97$). Within the upper 40 cm, both the analysed and estimated bulk density values ($BD_{(Wu)}$ and $BD_{(SOC)}$) mostly showed similar statistical significance with depth and no significant effect of treatment (Appx 7). $BD_{(SOC)}$ will thus be used further in the results section. The absolute average deviation between the two methods is $0.07\ g/cm^3$ across all depths and treatments, while the highest average deviation for the treatment and depth combinations was $0.12\ g/cm^3$ at depth 20 - 30 cm for MG and HG.

Bulk density ($BD_{(SOC)}$) is shown in Figure 15. Although samples from MG shows the highest density in the upper 30 cm, the differences are not statistically significant. Depth has no significant effect on estimated bulk density within the first 30 cm of the soil, where it is an average of 1.16 g/cm³. Bulk density does however increase significantly to 1.24 g/cm³ in the 30 - 50 cm depth interval and to 1.36 g/cm³ in the deepest depth interval.

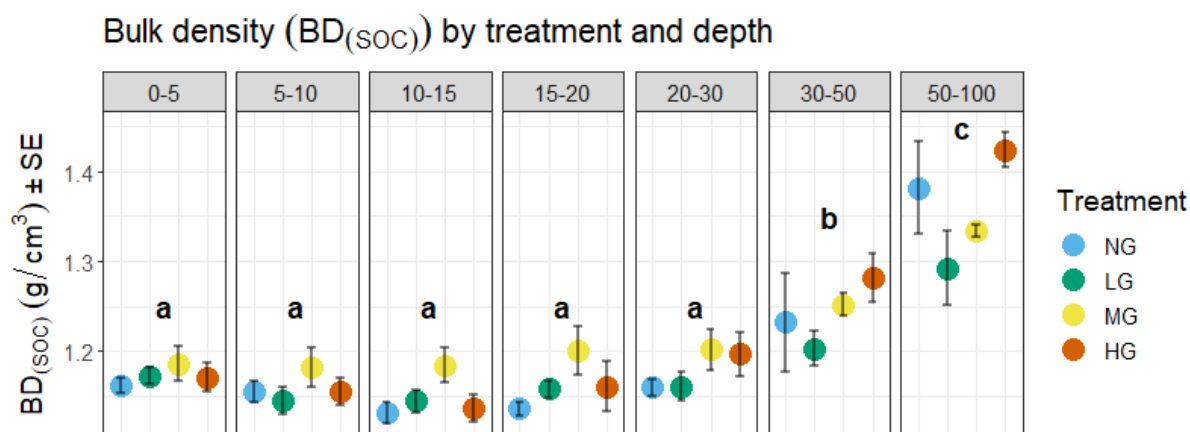


Figure 15: Average bulk density \pm standard error (SE) by treatment and depth. Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the reference, and the three treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For each sampling depth between 0 to 30 cm, $n = 47$ or 48. For each sampling depth between 30 and 100, $n = 12$. See also Appx 3 for sample selection.

3.2.2| Soil Organic Carbon (SOC) stocks

Comparison of SOC between treatments at similar depths could potentially lead to overestimation of stocks if bulk density increases while SOC content remains equal (Wendt & Hauser, 2013). Because bulk density did not vary significantly between treatments, the estimation of SOC stocks were compared based on soil volume, and not corrected for soil equivalent mass. Soil organic carbon ($SOC_{(Xilinhot)}$) stocks range from 1.23 kg/m² in the upper 5 cm of the soil to 7.31 kg/m² at depth 50 - 100 cm. Only the SOC stocks from 30 - 100 cm depth show significant difference between treatments (Figure 16). Note again that the grids do not show the same depth intervals, and that depths 0 - 20 cm are not directly comparable to depths 20 - 100 cm. SOC stocks from 0 - 20 cm soil depth are also shown in closer detail in (Appx 8).

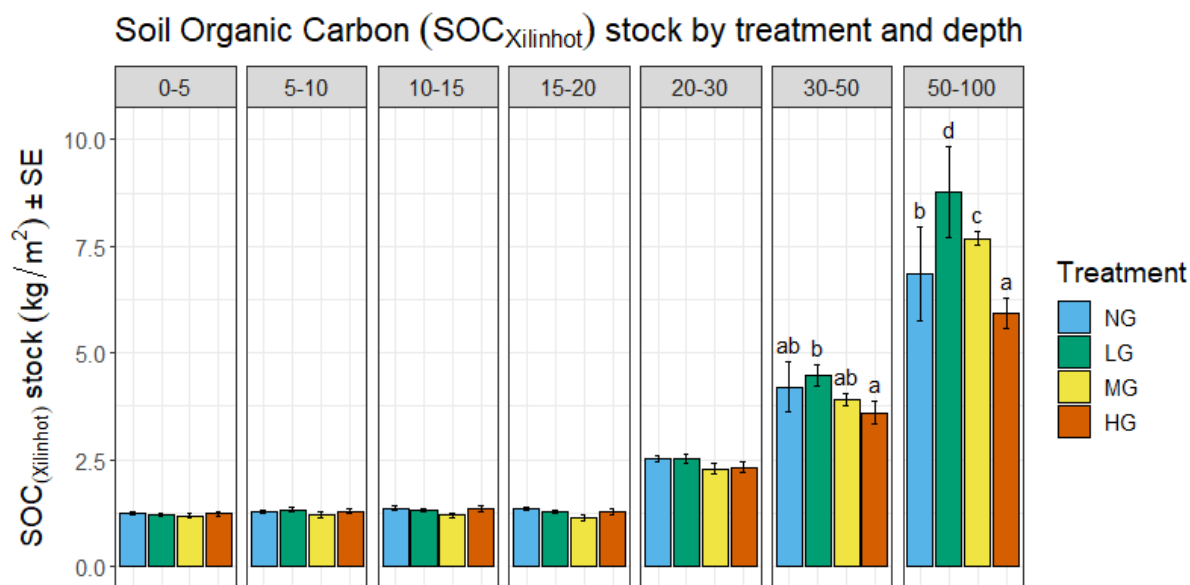


Figure 16: Average soil organic carbon (SOC) stocks \pm standard error (SE) in kg/m^2 by treatment and depth, calculated from $\text{SOC}_{\text{Xilinhot}}$ and BD_{SOC} . Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the reference, and the three treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. For each sampling depth between 0 to 30 cm, $n = 47$ or 48. For each sampling depth between 30 and 100, $n = 12$. Note the differences in depth interval. Letters of significance are not included for depth, as different depth intervals for stock are not comparable. Letters in grids 30 - 50 and 50 - 100 cm show significance of treatment ($p < 0.05$). See also Appx 3 for sample selection.

Although treatment does not significantly impact SOC stocks from 0 to 30 cm, MG consistently shows the lowest values at these depths (Table 7). In the upper 30 cm, NG shows highest SOC stocks, while LG shows highest SOC stocks in the lower 20 cm.

Table 7: Average SOC stocks in $\text{kg/m}^2 \pm$ standard error (SE) with n = number of samples, by treatment and depth. Intensity of green colour signifies treatment with highest stock values for the specific depth.

SOC stock $\text{kg/m}^2 \pm \text{SE}$ (n)				
Depth	NG	LG	MG	HG
0 - 5	1.26 \pm 0.03 (12)	1.22 \pm 0.03 (12)	1.19 \pm 0.06 (12)	1.24 \pm 0.06 (12)
5 - 10	1.29 \pm 0.04 (12)	1.32 \pm 0.06 (11)	1.21 \pm 0.08 (12)	1.29 \pm 0.05 (12)
10 - 15	1.37 \pm 0.04 (12)	1.32 \pm 0.04 (12)	1.19 \pm 0.07 (12)	1.36 \pm 0.06 (12)
15 - 20	1.35 \pm 0.03 (11)	1.27 \pm 0.03 (12)	1.15 \pm 0.07 (12)	1.29 \pm 0.07 (12)
20 - 30	2.53 \pm 0.06 (12)	2.53 \pm 0.10 (11)	2.28 \pm 0.13 (12)	2.32 \pm 0.12 (12)
30 - 50	4.20 \pm 0.60 (3)	4.48 \pm 0.25 (3)	3.90 \pm 0.14 (3)	3.60 \pm 0.27 (3)
50 - 100	6.85 \pm 1.09 (3)	8.78 \pm 1.07 (3)	7.68 \pm 0.15 (3)	5.94 \pm 0.35 (3)

3.3| Aggregate size distribution and stability

3.3.1| Aggregate size distribution

Aggregate size distribution was corrected for grains ($\text{ASD}_{\text{corrected}}$) in order to more correctly compare the size classes. The deviation between ASD and $\text{ASD}_{\text{corrected}}$ was however below 1%

for all samples (Appx 10). Furthermore, $ASD_{(corrected)}$ uncertainty was high due to mineral rest fraction of microaggregates not being analysed. Uncorrected ASD was thus used for further evaluation of results.

Aggregates in the meso-size fraction (1 - 0.25 mm) make up 45.13 %, 54.82 % and 66.54 % on average within the three shallowest 5 cm depth intervals (Appx 2, Figure 17). At depths 0 - 15 cm, mesoaggregate content is significantly higher than the amount of macro-size aggregates (2 -1 mm). Mesoggregates dominate in the upper 20 cm, while microaggregates (< 0.25 mm) dominate from 20 - 100 cm depth. Treatment did not cause significant variations in aggregate size distribution, although the total amount of macro- and mesoaggregates is greater in NG than HG within the upper 10 cm of the soil (Appx 9).

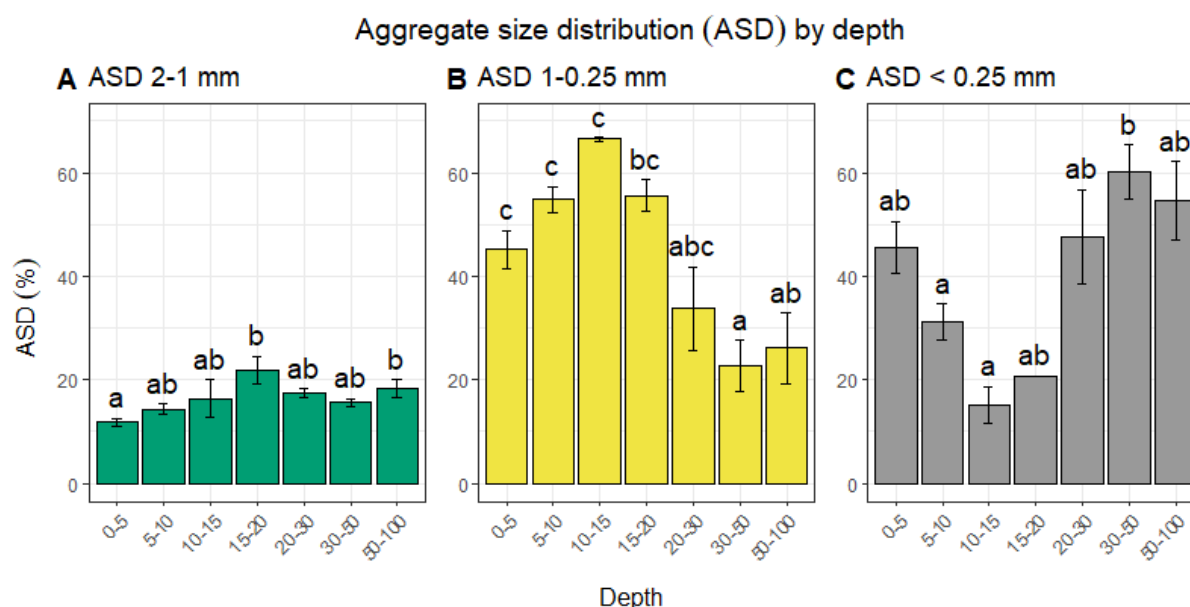


Figure 17: Average aggregate size distribution (ASD) \pm standard error (SE) as a percentage of bulk soil by depth. Aggregates of size 2-1 mm is shown in green, aggregates of size 1-0.25 mm is shown in yellow and anything beneath 0.25 mm is shown in gray. Different letters indicate significant differences between depths ($p < 0.05$). For 0 - 5 cm, $n=24$. For 5 - 10 cm, $n=23$. For the three intervals between 10 to 30 cm, $n = 2$. For the two intervals 30 to 100 cm, $n = 9$. See Appx 3 for sample selection.

There was a significant negative correlation and linear relationship between $SOC_{(Xilinhot)}$ content and macroaggregate content, but positive relationship between $SOC_{(Xilinhot)}$ and mesoaggregate content (Appx 11). $SOC_{(Xilinhot)}$ did not show any significant linear relationship with the microaggregate fraction.

3.3.2| Aggregate stability

Treatment did not affect aggregate stability significantly, although aggregates tended to be slightly more stable in HG (Appx 13). Although aggregate stability varies significantly with depth, there is no clear trend of stability increasing or decreasing as depth increases (Figure 18).

The highest average aggregate stability was found at depth interval 20 - 30 cm for macroaggregates, and at 10 - 15 cm depth for mesoaggregates (Appx 2). There was no correlation between aggregate stability and aggregate size distribution ($R < 0.1$). Content of stable mesoaggregates was significantly higher than the content of stable macroaggregates in bulk soil. However, this simply reflects the distribution of total meso- and macroaggregates in soil, as there was no significant difference in stable aggregates as a percentage of the aggregate size fractions.

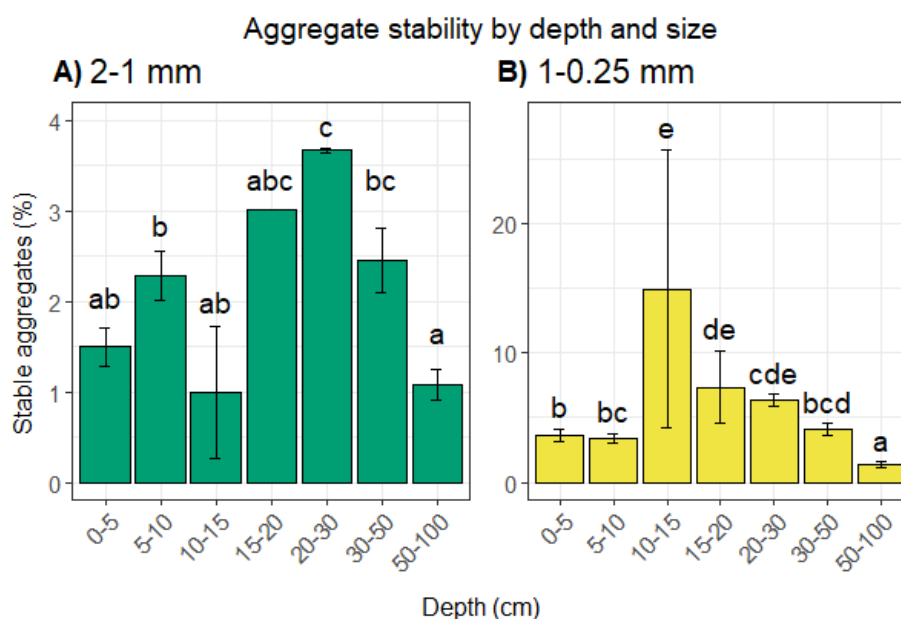


Figure 18: Average aggregate stability for aggregates size 1-0.25 mm (A, yellow) and 2-1 mm (B, green) as a percentage of bulk soil by depth. Note different values on y-axis. Different letters indicate significant differences between depths ($p < 0.05$). For 0 - 5 cm, $n=24$. For 5 - 10 cm, $n=23$. For the three intervals between 10 to 30 cm, $n = 2$. For the two intervals 30 to 100 cm, $n = 9$. See also Appx 3 for sample selection.

3.3.3| Total C in stable aggregates

Total C content (totC) measured in the stable aggregate fractions varies significantly with depth but is not significantly affected by treatment (Table 8). TotC in stable aggregates was higher in the 0 - 30 cm soil depth for both stable aggregate size fractions, and lowest in the deepest soil layer. The highest C content in stable aggregates was seen at 20 - 30 cm depth for both macro- and meso-size aggregates. Aggregates in the meso-size fraction contributed more to the total SOC-content than the aggregates in the macro-size fraction did (see TotC/SOC, Table 8). This mirrors the generally higher content of stable mesoaggregates (Figure 18). Furthermore, there is a weak positive correlation between $\text{SOC}_{(\text{Xilinhot})}$ content and stable mesoaggregates as a percentage of bulk soil (Appx 12), which simply reflects correlation between $\text{SOC}_{(\text{Xilinhot})}$ and total mesoaggregate content in bulk soil (Appx 11).

Note again that totC values are presented for stable aggregates because it is unclear whether the distribution of inorganic carbon in aggregates and bulk soil is equal. If all C in stable aggregates is organic, then stable aggregates contributed to 21 - 56 % of total SOC_(Xilinhot) (Table 8).

Table 8: Averages TotC in stable aggregates as percentages of bulk soil shown by depth and as ratios to SOC. Different letters indicate significant differences between depths ($p < 0.05$). See also Appx 3 for sample selection. (†, one value from dataset removed).

Depth (n)	TotC (% in SAG) \pm SE	TotC _(SAG) (% in bulk soil) \pm SE †	TotC _(SAG) /SOC	
2-1 mm				
0-5 (24)	1.89 \pm 0.10 (bc)	0.75 \pm 0.05	0.09	
5-10 (23)	1.67 \pm 0.07 (c)	0.89 \pm 0.04	0.11	
10-15 (2)	1.40 \pm 0.04 (bc)	0.93 \pm 0.02	0.11	
15-20 (2)	1.56 \pm 0.21 (bc)	0.87 \pm 0.16	0.16	
20-30 (2)	1.91 \pm 0.34 (bc)	0.61 \pm 0.04	0.16	TotC _(SAG) /SOC
30-50 (9)	1.39 \pm 0.10 (b)	0.28 \pm 0.03	0.12	
50-100 (9)	0.61 \pm 0.08 (a)	0.15 \pm 0.05	0.07	
1-0.25 mm				2-0.25 mm
0-5 (24)	1.61 \pm 0.05 (c)	0.18 \pm 0.01	0.36	0.45
5-10 (23)	1.72 \pm 0.09 (bc)	0.24 \pm 0.02	0.40	0.51
10-15 (2)	1.61 \pm 0.21 (abc)	0.26 \pm 0.02	0.40	0.51
15-20 (2)	1.69 \pm 0.28 (bc)	0.36 \pm 0.02	0.40	0.56
20-30 (2)	1.87 \pm 0.29 (bc)	0.32 \pm 0.03	0.30	0.46
30-50 (9)	1.28 \pm 0.07 (b)	0.20 \pm 0.02	0.17	0.29
50-100 (9)	0.45 \pm 0.06 (a)	0.08 \pm 0.01	0.14	0.21

Figure 19 shows significant positive correlation and linear relationship between stability and C content of mesoaggregates, but no significant relationship for macroaggregates.

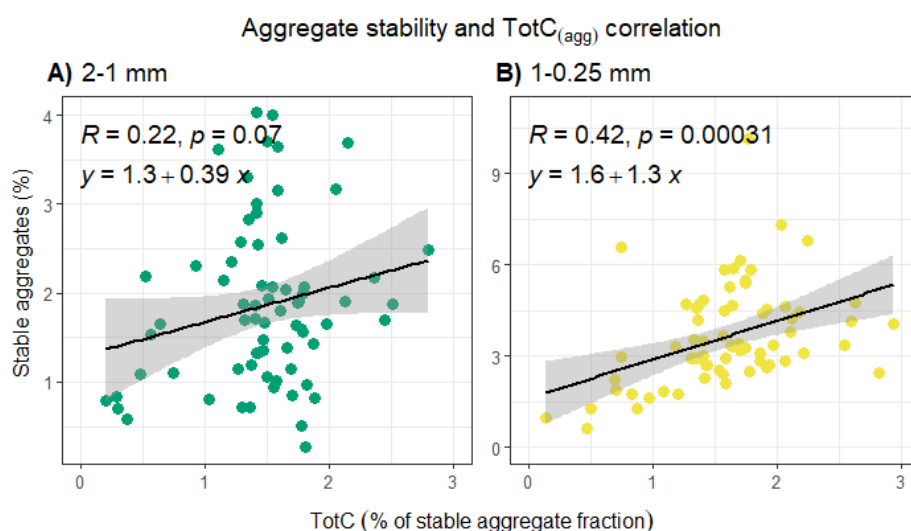


Figure 19: Correlation coefficient and linear relationships between totC (% of stable aggregates) and amount of stable aggregates (% of bulk soil) in the 1-2mm (A, green), 0.25-1 mm (B, yellow) size class. For 0 - 5 cm, n=24. For 5 - 10 cm, n=23. For the three intervals between 10 to 30 cm, n = 2. For the two intervals 30 to 100 cm, n = 9. See Appx 3 for sample selection.

3.4| Total nitrogen (TotN) content and N mineralization

3.4.1| Total nitrogen content (TotN)

TotN ranges as an average by depth from 0.21 % to 0.18 % in the top 20 cm of the soil, before decreasing significantly to 0.06 % within the deepest depth interval (Figure 20). NG plots show the highest nitrogen levels except for in the 20 - 30 cm depth interval, although not to a significant extent. TotN correlated strongly with $SOC_{(Xilinhot)}$ (Appx 14A).

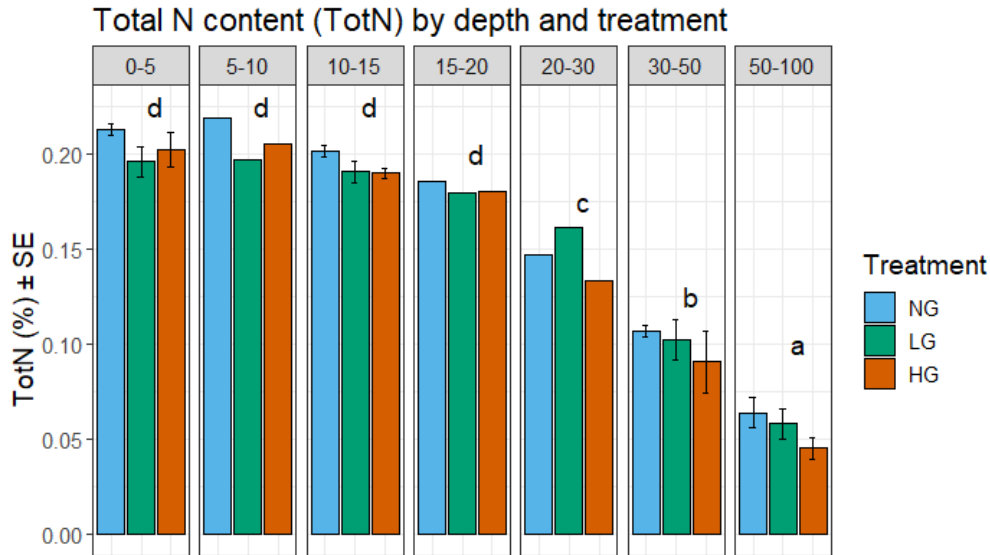


Figure 20: Average total nitrogen content (totN) ± standard error (SE) as a percentage of bulk soil by treatment and depth. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, and two of the three treatments; low grazing (LG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.

3.4.2| SOC/N-ratio

TotN was only analysed for a sample selection ($n=63$, Appx 3). To estimate a complete dataset, the relationship between HWEC and totN was evaluated. These parameters were highly correlated (Appx 14B) and provided Equation 9 through linear regression. The estimated dataset for totN ($totN_{(HWEC)}$) was further used in calculating SOC/N-ratio for a complete dataset.

Equation 9:

$$TotN_{(HWEC)}(\% \text{ of bulk soil}) = 0.401 + 0.075 \times \log(HWEC(\% \text{ of bulk soil}))$$

The average ratio between $SOC_{(Xilinhot)}$ and $totN_{(HWEC)}$ (SOC/N) increases from 10.25 in the topsoil to peak values of 24.30 at 50 to 100 cm depth (Figure 21, Appx 2). Although both $SOC_{(Xilinhot)}$ and totN decreases with depth, it is apparent that $totN_{(HWEC)}$ decreases to a larger extent than $SOC_{(Xilinhot)}$.

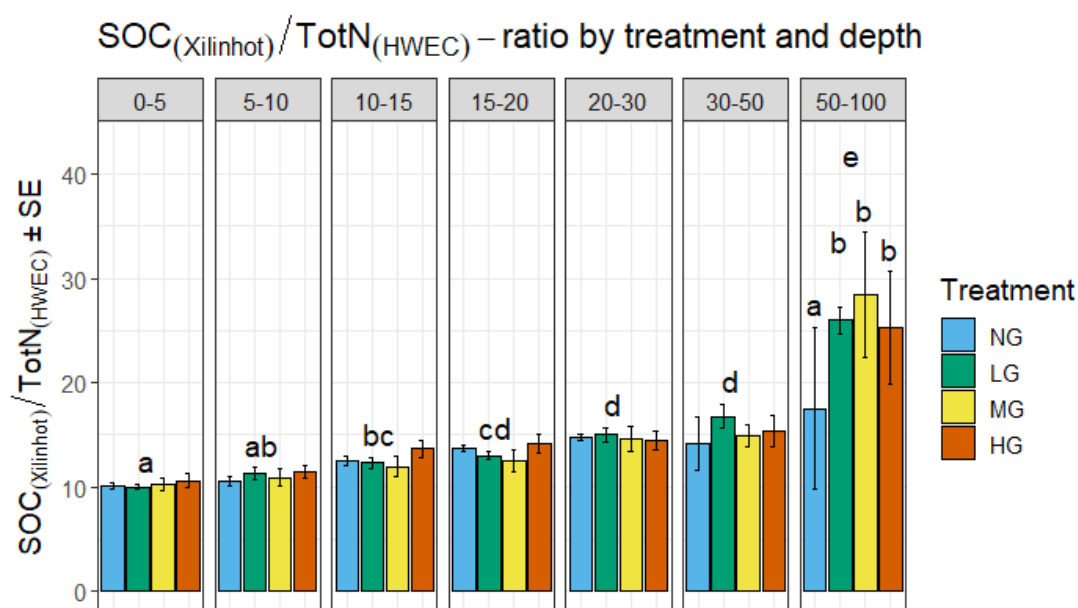


Figure 21: Ratio of soil organic carbon ($\text{SOC}_{(\text{Xilinhot})}$) to total nitrogen content ($\text{TotN}_{(\text{HWEC})}$) by treatment and depth. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, and the treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths, apart from depth 50 - 100 where significant differences between treatments are included as well ($p < 0.05$). For each sampling depth between 0 to 30 cm, $n = 47$ or 48 . For each sampling depth between 30 and 100, $n = 12$. See also Appx 3 for sample selection.

3.4.3| Nitrogen mineralization (NO_3 , NH_4)

Depth has a significant effect on nitrate (NO_3) mineralization, i.e. nitrification, but proves no significant differences in immobilization of ammonium (NH_4) (Figure 22). There was however a significant difference between treatment (only NG and HG) for NH_4 , but no significant effect for NO_3 . N mineralization in LG was only measured for selected samples in the upper 5 cm (Appx 3). At this depth, LG showed the highest NH_4 immobilization and nitrification values, although not to a significant extent (Appx 15). Net N mineralization did not show significant differences between treatments either (Appx 16).

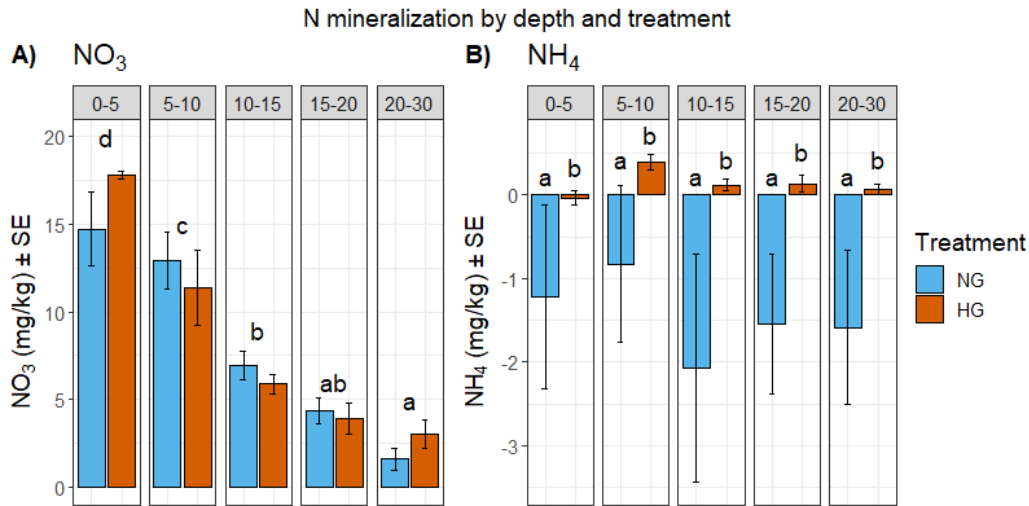


Figure 22: Average NO₃ (A) and NH₄ (B) mineralization in mg/kg ± standard error (SE) by treatment and depth. Treatment is shown in blue and red respectively for non-grazing (NG) as the reference and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths for NO₃ and between treatments in each depth for NH₄ ($p < 0.05$). Negative NH₄-mineralization indicates immobilization or nitrification. For each sampling depth, $n = 6$. See also Appx 3 for sample selection.

HWEC correlated strongly with NO₃ ($R = 0.82$), but showed an even higher correlation with $\log(\text{NO}_3)$ ($R = 0.87$, Figure 23).

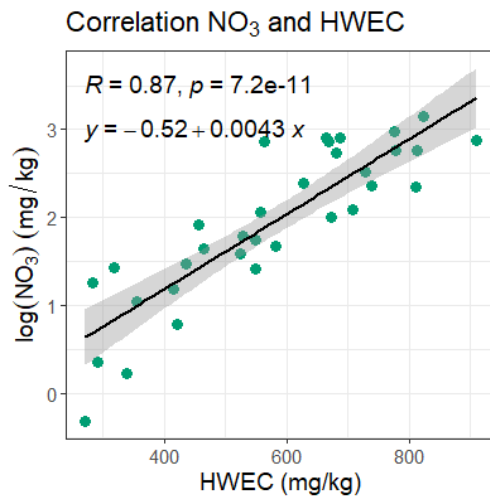


Figure 23: Correlation between hot water extractable carbon (HWEC) and the logarithm of mineralized NO₃, both given in mg/kg. $n = 33$, see Appx 3.

3.4.4| Particular Organic Nitrogen (PON) and Mineral associated Organic Nitrogen (MON)

Similar to MOC and POC, mineral associated organic nitrogen (MON) showed comparably higher values than particular organic nitrogen (PON) for all depths (Appx 17), being on average 5.2 times higher than the PON content in the top 5 cm and increases to the highest difference of 11.4 times higher in the 20 - 30 cm depth interval. For both PON and MON, values decreased significantly with depth, but treatment did not have any significant effect. Note that PON and MON was measured on samples dried at more than 100 °C, which might cause some N loss. The values might not represent the full N content but are still comparable with each other.

4| Discussion

The aim of this thesis is to assess the effects of rotational grazing at varying grazing intensity on soil organic carbon (SOC) quantity and quality in Inner Mongolia. Grazing, or the lack thereof, affects interactions between plants, soil and the microbiome. The presented results can be explained by highlighting some of these complex interactions. Evaluating which of these processes that dominate or cancel each other out is important, as the balance between pathways ultimately decides the rate and manner of carbon input versus carbon output, and thus the net carbon storage of the soil.

4.1| Soil Organic Carbon (SOC) content

Rotational grazing did not cause significant variations in SOC concentrations between treatments of low, moderate and high grazing intensity (LG, MG and HG respectively) or the grazing excluded reference plot (non-grazing, NG). This is in accordance with Dong et al. (2020), who studied changes in SOC content at the same experimental site 3 years prior to the present study (5 versus 8 years after experiment initiation). Despite lacking significant differences in SOC content, a consistent pattern of SOC concentration changes were found between treatments in the upper 30 cm of the soil. SOC content was mostly higher in ungrazed than grazed plots, slightly decreased in LG plots and consistently lowest in MG plots. SOC concentrations seemed to increase from MG to HG in the surface soil, suggesting that tendency of SOC reduction did not follow a linear relationship with grazing intensity (Figure 9). Within the upper 20 cm of the soil, SOC concentrations in HG mostly surpassed both MG and LG.

4.1.1| Response time

Similar SOC concentrations regardless of grazing pressure is in contrast with established effects of carbon depletion under higher livestock density (Li et al., 2008; Steffens et al., 2008; Zhao et al., 2007). As several previous studies of Inner Mongolian grasslands concern grazing at higher pressures or without periodical grazing cessation, it is not surprising that SOC concentrations at the experimental site do not vary to the same extent. Simply assuming too low grazing pressure for ecosystem property alteration is not a viable explanation, as several other studies from the Xilinhot experimental site have found significance of treatment (Fan et al., 2019; Liang et al., 2021b). Historical degree of soil degradation might dictate the recovery rate because ecosystems depleted with SOC presumably have poor nutrient recycling, water retention and gas exchange, properties that are necessary for primary production and SOC accumulation (Cui et al., 2005; Wang et al., 2018).

The overall lack of significant SOC variation can be attributed to long response time of soil chemical and physical properties. Research on conversion from continuously grazed steppe to non-grazed steppes illustrate the delayed response of SOC change. Steffens et al. (2008) conclude that soil parameters needed several decades of livestock exclusion to recover from grazing induced land degradation, while five years was not sufficiently long to observe differences in SOC stocks. However, Cao et al. (2013) show that 4 years of continuous grazing at similar livestock densities to the experimental site significantly decreased POC and other labile SOC fractions in MG and HG compared to NG and LG. The study was conducted under similar climatic conditions in a neighbouring region to the Xilingol League and indicates potentially lower impact of rotational grazing compared to continuous grazing.

4.1.2| Above-ground biomass

The trends in SOC variation reflect the numerous studies reporting SOC depletion under increasing grazing intensity compared to grazing cessation (Li et al., 2008; Steffens et al., 2008; Zhao et al., 2007), apart from slight SOC increases in HG. Potential enrichment of SOC in NG is presumably caused by higher net primary production, which is observed in ungrazed areas of Inner Mongolia (Schönbach et al., 2011; Wiesmeier et al., 2009). Instead of being foraged by sheep, standing above-ground biomass in non-grazed plots will be left as plant residue and increase ground cover. Litter accumulation may affect temperature and water retention in soil, which might further enhance primary production and SOC accumulation. Wiesmeier et al. (2009) suggests that grazing exclusion promotes development of so-called “islands of fertility”, which are vegetation patches of augmented SOC and water content. These are a result of heterogeneous plant distribution, which can be repressed by high livestock densities. Build-up of litter can also inhibit plant diversity and growth (Xiong & Nilsson, 1999), but no clear evidence of this has been found in NG (Liang et al., 2021b).

Presence of livestock is proven to decrease above-ground biomass, both at the Xilinhote rotational grazing site (Liang et al., 2021b) and in Inner Mongolian steppes at large (Dong et al., 2020; Wang et al., 2016; Yang et al., 2017). This effect is strongest under high livestock density, where above-ground biomass decreased by 39 % in HG compared to NG at the rotational grazing site (Liang et al., 2021b) (Figure 2). Less return of biomass to the ground surface signifies decreased soil organic matter (SOM) input and decreased SOC quantity (Schönbach et al., 2011). Yet, SOC concentrations of HG found in the present study seems greater than in LG and MG and close to NG. This tendency indicates other determining factors for SOC accumulation.

4.2| Labile SOC

Density fractionation of SOC uncovered consistent trends in particular organic carbon (POC) and mineral associated organic carbon (MOC) that resembled variations in total SOC content. Although differences were not of statistical significance, both POC (representing labile SOC) and MOC (representing stable SOC) tended to decrease in LG compared to NG and HG in the upper 30 and 20 cm of the soil, respectively (Figure 10, Figure 11). Matching trends in POC content and total SOC content is in accordance with Martinsen et al. (2011). If the observations are indicators to real changes in the soil, LG seems to deplete POC and MOC content in the upper 30 cm of the soil. These findings partly correspond to previous research, where rotational grazing has been observed to both decrease and be without impact on POC compared to grazing exclusion (Dong et al., 2020; Sanderman et al., 2015). Cessation of grazing is documented to increase POC content in coarse and medium aggregates (Steffens et al., 2010) and stabilize POC in stable microaggregates (Six et al., 2004).

During density fractionation, carbon in the filter fraction was measured on NG and HG samples. Although treatment did not impact filter carbon significantly, it showed higher carbon content in NG than HG samples (Figure 12). Filter carbon represents the more labile fraction of POC, which potentially could explain resemblance to trends in hot water extractable carbon (HWEC). HWEC is the most labile SOC fraction, and the only fraction that showed significant effects of both treatment and depth in the topsoil of the present study. HWEC was significantly lower in MG and HG than in NG (0 - 20 cm) and LG (0 - 15 cm). MG consistently showed lowest HWEC content, mirroring the generally lower SOC content in MG plots (Figure 13). Additionally, HWEC content seemed to increase slightly from MG to HG, mirroring the pattern of total SOC content change (Figure 9). HWEC is both shown to increase and decrease with increasing grazing intensity Dong et al. (2020). However, when high livestock densities are documented to increase labile SOC through heightened turnover rates and microbial biomass (Wu et al., 2012), it does not usually reflect trends in total SOC, as was observed in this study.

4.2.1| Plant species composition

The meta-analysis by Abdalla et al. (2018) states that species richness affects sensitivity to grazing and SOC accumulation rate. In arid steppes typical of Inner Mongolia, vegetation mainly consists of C3-plants (Zhang et al., 2014). C3-plants differ from C4-plants in their photosynthetic pathways, adaptability to environment and forage quality (Zhang et al., 2014). Grazers prefer perennial, tall grasses compared to annual, short grasses, which at the Xilinhote rotational grazing site corresponds to a preference of C3 to C4-grasses (Liang et al., 2021b).

Despite this preference, herbivore selectivity resulted in increased relative abundance of C3-grasses (Liang et al., 2021b). This contrasts with the generally reported grazing-induced increases in C4-abundance (Zhang et al., 2014). Although findings were not further elaborated upon by Liang et al. (2021b), it could exemplify compensatory growth, which light pressure pastoralism has been seen to promote elsewhere in Inner Mongolia (Zhang et al., 2018; Zhang et al., 2020). Species and distribution of native grasses affects carbon allocation properties, and they are thus key factors in understanding SOC properties (Speed et al., 2014).

4.2.2| Below-ground biomass

Gao, Y. Z. et al. (2008) reports that the root biomass pool of grasslands is estimated to be between two to thirty times the aboveground biomass pool. Additionally, a review by Piñeiro et al. (2010) states that root carbon content generally increases with grazing intensity in low precipitation regions. This is representative for Inner Mongolia and could explain SOC enhancement in HG. In a meta study on Chinese grasslands however, Hao and He (2019) summarizes that grazing intensity decreases below-ground biomass. Regardless of below-ground biomass decreases, root/shoot-ratio is generally seen to increase, as grazing-induced biomass reduction rate is stronger above than below ground (Gao, Y. Z. et al., 2008; Hoffmann et al., 2016; Wang et al., 2018). This was the case in Xilinhote as well, where Liang et al. (2021b) documented twice as high root/shoot-ratio in HG plots compared to NG plots, simply due to reduction in above-ground biomass. Root biomass tended to be higher in grazed plots than in NG at the experimental site, but differences were not significant (Liang et al., 2021b). Herbivore selectivity is still relevant for interpreting the cause of SOC change, as differences in SOC, labile fractions specifically, are often attributed to differences in rooting systems. In other words, changes to SOC content are due to more properties than just biomass alone.

Plants allocate much of their assimilated carbon to root exudation (Bardgett & Wardle, 2003). This allocation is highly correlated to labile SOC content, especially HWE (Dong et al., 2020). According to Liang et al. (2021b), C4-grasses are more light-use efficient, and consequently have higher carbon input rates through vegetation and roots than C3-grasses. C4 herbage mass was highest in NG plots, albeit C3-grasses still dominated in all pastures at the experimental site (Liang et al., 2021b). Shift in plant composition could thus explain the higher HWE concentrations in NG and LG compared to MG and HG. Changes in rates of carbon loss could also explain trends in total and labile SOC. Lavelle et al. (2020) and Bardgett and Wardle (2003) point out that more root exudation might stimulate the destabilization and subsequent decomposition of SOC through increased microbial activity and priming effects in the

rhizosphere. Additionally, Liang et al. (2021b) reported reduced soil respiration rates with increasing grazing pressure and increased relative abundance of C3. Low respiration rates in HG could thus further suppress loss of SOC and POC compared to LG and MG.

4.3| Depth dependency of SOC

All SOC fractions decreased with depth, which is expected due to lower contact with litter carbon input from the surface and diminishing roots with depth. Total SOC concentrations were consistently high in the upper 30 cm of the soil, where NG mostly contained highest SOC stocks. At 30 to 100 cm depth, however, this relation shifted and SOC stocks became significantly higher in LG (Table 7). HWE C concentrations were significantly higher in NG and LG than MG and HG in the upper 20 cm of the soil and tended to increase from MG to HG. However, changes in HWE C between treatments were less pronounced in deeper soil layers and HWE C content did not seem to increase from MG to HG. Instead, tendencies indicated a linear decrease in HWE C content with increasing grazing intensity. Increasing SOC/N-ratio with depth, to be discussed in light of nitrogen properties, also demonstrate depth dependency.

Depth dependant responses to grazing indicate the different impacts on soil properties between direct grazing effects and indirect grazing effects mediated through vegetational shifts and changes in root properties. Zhang et al. (2018) summarizes that SOC concentrations are highest in the upper 30 or 40 cm of the soil, and that consequently, most researchers of carbon dynamics have not sampled soil below this depth. However, evaluating properties of a deeper profile is valuable to uncover shifts in trends such as these. Although below-ground carbon input through roots seem to impact SOC content more than carbon input through litter at the surface (Liang et al., 2021b), there are certain direct grazing effects that primarily only impact surface soil. This could exacerbate the trends seen in SOC.

4.3.1| Trampling

Trampling is known to increase mineral and SOC interactions, which stabilizes SOC and slows decomposition (Wei et al., 2021). Wei et al. (2021) found that trampling decreased litter mass and proportionally increased the incorporation of litter derived carbon to the SOC pool, contributing both to MOC and POC accumulation. Furthermore, and contrary to destabilizing effects of root exudation, Wei et al. (2021) found that trampling-induced increases in microbial biomass did not initiate significant positive priming effects. Trampling, which is not present in the NG plots, is thus an important accelerator for carbon sequestration and presumably strongest in HG plots. Despite trampling-facilitated incorporation of plant residue to the SOC pool,

trampling has also shown to increase decomposition rates, and ultimately decrease SOC content (Martinsen et al., 2011; Sanjari et al., 2008). Trampling may also decrease stabilization of SOM through physical stress and breakage of aggregates (Wang et al., 2018). The effects of trampling were not specifically quantified at the experimental site, but it is likely that the SOC decreasing pathway of trampling did not dominate due to results from analysis of aggregates.

4.4| Aggregate protection of SOC

Aggregate stability tended to increase slightly in HG compared to other treatments in the upper 10 cm (Appx 13). However, neither aggregate size, aggregate stability nor aggregate carbon content was significantly affected by livestock density. Stability and carbon content of aggregates correlated significantly for mesoaggregates (1 - 0.25 mm), but the relation was relatively weak (Figure 19) and only seemed to reflect the correlation to all aggregates in the meso-size fraction regardless of stability. Carbon content in stable macro- (2 - 1 mm) and mesoaggregates made up about 50 % of SOC content in the upper 30 cm (Table 8). This suggest that carbon content in microaggregates and unstable aggregates, which were not analysed, contribute largely to the total SOC pool. Grazing pressure did not impact bulk density significantly, in line with Dong et al. (2020). Still, NG generally showed lowest density, while MG showed highest density (Figure 15). Bulk density correlated significantly with SOC, as is in accordance with Steffens et al. (2008), and thus reflected the tendencies of SOC change between treatments.

4.4.1| Stabilization of SOC

Hoffmann et al. (2016) summarizes that total SOC content in the aggregates did not decrease when continuously grazed pastures were converted to grazing excluded areas, in line with the present findings. Contrary to findings in this study, soil compaction and lower aggregate stability in Inner Mongolia is commonly reported as an outcome of higher sheep density (Steffens et al., 2009; Wiesmeier et al., 2009; Zhang et al., 2020). Aggregate formation and stability is highly dependent on plant residue and roots, as they release organic compounds and exudates which bind particles together and increase proportion of stable microaggregates (Bronick & Lal, 2005; Six et al., 2004; Wang et al., 2018). Stabilisation of SOC is also highly dependent on aggregate formation, causing a feedback relation. The lacking variations in aggregate properties presumably contribute to explaining SOC content, which did not vary significantly with grazing treatment either. Unchanging aggregate attributes could however be explained by other factors at play, such as carbonate and fine mineral particle content.

Soil of the Xilinhot rotational grazing site contained almost 50 % silt and 10 % clay, with no significant difference between treatments, in accordance with Dong et al. (2020). Although not usually reported, grazing has in instances increased the fraction of coarse minerals (Pei et al., 2008). Generally, MOC and aggregate formation seems to be more dependent on silt and clay content rather than grazing treatment (Conant et al., 2003; Steffens et al., 2010). Decrease in silt and clay fraction could thus lower mineral association and SOM adsorption capacity (Conant et al., 2003; Ding et al., 2013). Similarity in texture between grazing treatments could thus potentially limit MOC and aggregation variability. Steffens et al. (2010) also suggests that changes in livestock management at a certain point do not necessarily increase carbon sequestration because of a limited carbon capacity in aggregates. Presence of carbonates have also been seen to stabilize aggregates and adsorb SOC, potentially decreasing soil sensitivity to management changes (Bronick & Lal, 2005; Rowley et al., 2018).

4.5| Nitrogen and SOC coupling

Differences in total nitrogen content did not vary significantly between treatments at the rotational grazing site. Like all SOC fractions, nitrogen concentrations tended to be highest in NG, lowest in LG (MG was not analysed) and then increase in HG within the upper 20 cm of the soil (Figure 20). This tendency shifted from 30 to 100 cm depth, where total nitrogen seemed to decrease linearly with grazing, like HWEC. Nitrogen content was greatest in the upper 20 cm of the soil, and decreased with depth to a higher degree than SOC. This resulted in increasing SOC/N-ratio with depth (Figure 21). Treatment did not alter SOC/N-ratio significantly in the upper 50 cm of the soil, where it ranged from 11 to 15. From 50 to 100 cm soil depth however, the SOC/N-ratio in NG plots was significantly lower than in the grazed plots. Nitrogen and SOC correlated significantly and with a strong linear relationship, confirming the stoichiometric coupling in SOM.

Net N mineralization was analysed in depths 0 to 30 cm and tended to be lower in NG than HG plots, although not to a significant extent (Appx 16). This is reasonably indicated by a SOC/N-ratio of below 20, which according to Hagemann et al. (2016) usually leads to net mineralization. It also corresponds to previous observations in Inner Mongolia, where grazing generally is seen to increase soil available nitrogen, or net N mineralization (Yang et al., 2017; Zhou et al., 2017). NH_4 mineralization was significantly lower in NG than HG. The negative NH_4 mineralization in NG indicates net immobilization but could also be explained by the further conversion of N to NO_3 by nitrification. NH_4 mineralization did not vary with depth, which was the opposite of NO_3 mineralization. NO_3 mineralization decreased

significantly with depth, but not with treatment, agreeing with findings by Dong et al. (2020) from the same experimental site. Mineralized NO_3 and HWEC content correlated strongly (Figure 23), to an even higher degree than what Dong et al. (2020) reported.

4.5.1| Turnover rates

In China and Inner Mongolia, grazing generally seems to deplete the total nitrogen pool (Hao & He, 2019; Zhou et al., 2017). Sensitivity to nitrogen content alteration could however vary with vegetation. Wiesmeier et al. (2009) reported that continuous grazing depleted nitrogen to a higher degree in *Leymus* dominated sites compared to *Stipa* dominated sites. Both *Leymus chinensis* and *Stipa grandis* were present at the experimental site, but relative amount of *Stipa grandis* increased with grazing intensity (Liang et al., 2021b). As grazing intensity increased *Stipa* content, it could potentially also increase resistance to nitrogen loss compared to ungrazed plots. This shift in plant composition could thus counteract the expected nitrogen loss with grazing intensity and explain the tendency of increased total nitrogen from LG to HG. Yang et al. (2017) suggest that typical steppe grasslands in Inner Mongolia are mostly nitrogen limited. However, surface soil layers had low SOC/N-ratio and are thus presumably enriched in nitrogen compared to bottom layers. Furthermore, Zhang, Y. et al. (2017) suggests that atmospheric nitrogen deposition in Inner Mongolia have exceeded appropriate levels for the ecosystem.

Sheep return more easily decomposable organic matter with a lower C/N-ratio through urine and faeces to the soil (Bardgett & Wardle, 2003; Gao, Y. H. et al., 2008). Powlson et al. (2011) questions to which degree carbon in manure is incorporated to the soil and suggest a high rate of carbon loss as CO_2 . Although nitrogen can be lost in gas form as well, Gao, Y. H. et al. (2008) reports that available nitrogen content in soil increases through urine and faeces. In this way, increased nitrogen input in grazed pastures could cause significantly higher ammonification in HG plots than NG plots. As described by Harrison and Bardgett (2008), increased nitrogen input and mineralization due to livestock could cover a larger part of the plant nitrogen demand and limit the necessity for assimilating nitrogen from deeper soil layers. Manure deposition and nitrogen-related decreases in root biomass could be one explanatory factor for the observed shifts in SOC fraction trends between grazing treatments above and below 30 cm depth. Sheep also excrete a large part of the nutrient elements they ingest, and returns P, Ca, Mg and K to the soil (Shand & Coutts, 2006). This could positively influence primary production in HG. More waste products can create more rapid cycling of nutrients and SOM, which could cause net higher SOC depletion or increased biomass and thus net SOC storage (Harrison & Bardgett, 2008).

The pH at the experimental site was very high, ranging from an average of 8 to 9 (Appx 4). Variations between treatments were not significant. However, NG consistently showed higher pH-values than LG, and MG consistently showed higher values than HG in the upper 30 cm. Grazing intensity has been observed to have both increasing, decreasing and no effect on pH in Inner Mongolia (Pei et al., 2008; Wiesmeier et al., 2009; Zhang, J. et al., 2017). Too acidic or alkaline soil conditions can inhibit microbial activity and subsequently the turnover of organic matter and nitrogen mineralization (Oertel et al., 2016). Lavalley et al. (2020) presents acidification as a consequence of added nitrogen, which at the Xilinhote rotational grazing site could neutralize pH and promote microbial growth. The high correlation between NO_3 and HWEC indicates how labile SOC provides energy to microorganisms and enables nitrogen mineralization (Dong et al., 2020). Microbial activity enables nutrient mobilization and biomass production, but also promotes decomposition of SOC. Whether future changes in pH and other factors have a net positive SOC accumulating effect depends on this producer-decomposer feedback.

4.5.2| Balance of pathways

The balance between vegetation and soil pathways affects the observed trends in SOC, MOC, POC and HWEC change of the upper 20 to 30 cm of the soil (Figure 14). However, the mechanisms were not quantified, and the balance is difficult to predict due to complex interactions. The differences in SOC properties between NG and HG could potentially be erased by slightly lower root biomass but higher carbon allocation abilities of C4-grasses in NG plots, compared to slightly higher biomass but lower carbon allocation abilities of C3-grasses in HG plots. NG potentially has the benefit of higher above-ground biomass, while HG potentially has higher SOC incorporation and stabilization due to trampling and decreased root respiration (Liang et al., 2021b; Wei et al., 2021).

Livestock density increases twice as much from MG to HG (plus 6 grazing days/month) than from LG to MG (plus 3 grazing days/month). The threshold for positive effects of trampling as a SOM accumulating factor could potentially be found between MG and HG. The importance of assessing this balance between pathways is also suggested by Zhang et al. (2018), who found the best management to be moderately grazed plots when concerning carbon sequestration. Although tendencies of higher SOC fractions in NG and HG compared to LG and MG were observed in several analyses, the trends were not significant. Difficulties in finding strong pathways that explain these trends is thus to be expected, and in line with the minimal changes between treatments.

4.6| Revisiting the hypotheses

Findings and correspondence to hypotheses of the present study are summarized below.

i) SOC content is higher in LG plots than in NG, MG and HG plots

This hypothesis is not fully supported, as it is only correct for the bottom 30 - 100 cm of the soil. In the upper 30 cm of the soil, NG contained highest SOC stocks followed by HG. In the 30 - 100 cm depth interval, LG had significantly higher SOC stocks than HG (30 - 100 cm) and MG (50 - 100 cm).

ii) Changes in SOC are highest in the topsoil and decrease with depth

This hypothesis is not fully supported, as it is only true for the most labile carbon fraction. HWEC is higher in NG and LG than in MG and HG. Although the trend was seen at all depths, the difference was only significant in the upper 20 cm of the soil, consistent with the hypothesis. For SOC stocks and SOC/N-ratio, however, the significant differences were only found at depths 30 - 100 and depths 50 - 100 cm, respectively.

iii) Differences in SOC between treatments are due to changes in POC content

This hypothesis is not fully supported, because neither SOC nor POC content between treatments varied significantly. Future differences in POC content could, however, be indicated by the changes in HWEC content.

iv) Aggregate stability correlates with SOC content and is highest in LG plots

This hypothesis is not fully supported, as SOC content only correlates with stable aggregates in the meso-size fraction (1 - 0.25 mm) and there are no significant differences between treatments.

v) Nitrogen content correlates with SOC content and is greatest in LG plots, while nitrogen mineralization potential is highest in HG plots

This hypothesis is not fully supported. Although SOC correlates strongly with nitrogen content, total nitrogen content did not vary significantly between treatments. Net nitrogen mineralization was slightly higher in HG plots compared to NG plots, but the differences were not significant.

4.7| Implications of grazing management

Several studies show that the labile fraction decreases in response to increasing continuous grazing intensity (Dong et al., 2020; Steffens et al., 2010), even when grazing management does not necessarily affect the total SOC storage (Cao et al., 2013). Labile SOC is generally the first fraction to respond to management changes, and the largest contributor to increased C

accumulation in Inner Mongolia (Wiesmeier et al., 2015). Both POC and HWE C are considered early indicators of grazing impact (Dong et al., 2020); Zhang et al. (2018). If HWE C is any indicator of future SOC storage, then NG and LG are expected to be the two preferred management practices. Based on trends in POC content, however, LG might bring upon future depletion in C storages compared to NG and HG. Overall, MG showed least desirable soil properties, like highest compaction and lowest total SOC and HWE C content.

4.7.1| Grazing and ecosystem restoration

Findings in the present thesis must be seen in relation to other research that focus on impact of abiotic factors, vegetation, climate gas emissions and finally the impact that rotational grazing has on livestock and farmers. Sheep's grazing behaviour are highly adaptability to different stocking rates (Hoffmann et al., 2016). However, the forage nutrient, protein and energy content retrieved under the different rotational grazing treatments is not clarified. At the Xilinhot rotational grazing site, Fan et al. (2019) concluded that NG and LG caused stronger regulating services such as erosion prevention, water storage, nutrient cycling, and C sequestration, while MG and HG had stronger provisioning services, i.e. herbage intake by livestock. Liang et al. (2021b) found increased above-ground biomass with decreased grazing intensity, also implying decreasing risk of wind erosion, reduced evaporation, and increased water content in spring from slower thawing (Abdalla et al., 2018; Hoffmann et al., 2016; Sanderman et al., 2015). Both Fan et al. (2019) and Liang et al. (2021b) recommended LG as the optimal management, corresponding with the intermediate hypothesis.

4.7.2| Grazing and climate mitigation

Carbon accumulation in soil tends to be rapid in the early stages after land management change, but decelerate towards a new carbon flux equilibrium (Freibauer et al., 2004; Meyer et al., 2012; Wang et al., 2018). Dong et al. (2020), Meyer et al. (2012) and Steffens et al. (2010) all stress that soil has a limited potential for carbon sequestration, as increase in SOC content with grazing management changes usually is in the labile carbon fraction and not necessarily stabilized in the long-term. Furthermore, storage of SOC is a reversible process. Poor future land management, or land disturbances such as increased temperature, humidity or drought can also cause the reemission of carbon into the atmosphere (IPCC, 2019).

Future climatic changes in Inner Mongolia could both increase carbon accumulation potential and increase carbon emissions (Wu et al., 2018). Sanderman et al. (2015) found that 60 % of the variance in SOC in their rotational grazing study could be explained by mean winter

temperature and mean annual rainfall alone, which changes with climate change. Climate change, with rising temperatures and altered precipitation patterns is likely to influence the vegetative composition and abundance of C3 versus C4-grasses (Zhang et al., 2014). Plant selectivity by herbivores is perhaps one of the main ways that sheep affect soil parameters, and ultimately the functioning of the pastoral ecosystem in Xilinhot. Additionally, carbon enhancing land management could be counteracted by potential increases in other greenhouse gasses (Freibauer et al., 2004).

4.8| Methodology

There were few findings of statistical significance, in several cases due to a limited dataset and thus limited statistical power. Although all discussed soil processes are in effect to some degree, the indications of future effects might be overestimated. Shortcomings of this thesis relates to possible mistakes during soil sampling, analysis, or data processing. Soil samples were collected in July, which is among the months with highest precipitation, and then stored in plastic bags during a 2 and a half month long shipping period. Changes to soil attributes due to moisture content and storage time could influence analysis results. Below are some remarks to methodology used in this study.

4.8.1| Estimation of unavailable data

Within the upper 40 cm, both the analysed and estimated bulk density values ($BD_{(Wu)}$ and $BD_{(SOC)}$) mostly showed similar statistical significance with depth and no significant effect of treatment (Appx 7). $BD_{(SOC)}$ provides a reliable estimate at depths below 50 cm, granted adjustment of the intercept. Analysing undisturbed soil taken from field sampling is the most reliable and generally practiced method for achieving bulk density data. Gathering undisturbed soil samples may require more time and effort than gathering samples for soil organic carbon analyses. For this reason, estimating bulk density based on Equation 8 is a viable alternative, provided a selection of bulk density data is available as a correction for the soil in question.

By comparing different methods for estimating SOC it seems that the often-used Van Bemmelen factor of 0.58 might underestimate the actual SOC content (Table 2). Analysing samples with CHN-analyser can on the other hand be cost-expensive. A compromise is to analyse a sample selection on CHN-analyser and the complete sample set by loss on ignition (LOI), followed by a simple regression to calculate the actual SOC content. Calculated SOC ($SOC_{(Xilinhot)}$) correlated significantly with analysed SOC ($SOC_{(TotC-IC)}$) and the strong linear relationship ($R = 0.86$) legitimizes this method for estimating SOC content.

4.8.2| Correction for inorganic carbon

As indicated by the high pH, there was reason to assume presence of calcium carbonate (CaCO_3). Alkaline soils are expected in arid climates like Xilinhot and suggests presence of carbonates which precipitate and accumulate when low precipitation or high transpiration levels cause lack of leeching (Schlesinger & Bernhardt, 2020). Rising pH with depth is in line with Cui et al. (2005) and explained by less percolation of water at lower depths, and thus higher precipitation of carbonates, or less dissolved organic matter which in the topsoil might inhibit carbonate formation (Schlesinger & Bernhardt, 2020). Removal of carbonates before conducting SOC analysis, or using a correcting factor for carbonates, is crucial to avoid overestimation of SOC.

Analysing total carbon content of ash remains from LOI is a simple way to obtain a correction factor for inorganic carbon, and an established method used in similar research (Wiesmeier et al., 2009). There were, however, still detectable values of nitrogen in the calcinated samples, suggesting an incomplete LOI process and potentially some remaining SOC in the samples. Furthermore, Heiri et al. (2001) suggest that some inorganic carbon from specific carbonate containing minerals might also be lost during LOI below 550 °C. There are in other words uncertainties to this manner of obtaining inorganic carbon data, but the values will be used seeing as LOI is a widely relied upon method in soil research and provides a good foundation for comparison with other studies.

4.8.3| Recovery of labile SOC

There are several analytical methods for separating the particular and mineral associated organic matter (POM and MOM). Lavalley et al. (2020) recommend both density and size fractionation to reflect differences in persistence and formation of POM and MOM. Fractionation was conducted based on density only for one size fraction (from 20 μm to 2 mm), to comply with previous studies from the Xilinhot rotational grazing site (Dong et al., 2020). Lavalley et al. (2020), however, also recognize density-based fractionation as an alternative process, when sand content is less than 45 %, which is barely the case for the present study. During the density fractionation process soil is washed in 20 μm sieves, thus losing the < 20 μm fraction (called filter and rest fraction in this thesis). Loss of this fraction is reflected by low recovery of SOC from density fractionation, which decreased with depth and is close to zero in the deepest soil layer (Table 3).

Total SOC and the sum of POC and MOC was measured by two different methods, which can explain some, but not all, of the high recovery loss. When including analysis of filter and rest fraction in the topsoil, the recovery increased to almost 100 % (Table 4). As only soil from 0 - 5 cm depth was analysed for this additional experiment, it is unclear to which degree the < 20 µm fraction is responsible for SOC content discrepancy in deeper soil layers as well. Carbon content in the filter fraction was higher than carbon content in the POM fraction. Filter carbon also made up a higher percentage of bulk soil than HWEC in the topsoil. This is no surprise, as HWEC is measured on only the very fine < 0.45 µm fraction, while the filter fraction contains all particles between 20 and 12 µm. This suggests the importance of including the normally reported “lost” fraction in the density fractionation process. As demonstrated by comparing different measurements of total carbon content, SOC and SOC fractions (Figure 7, Figure 9, Figure 14), small uncertainties of each analysis procedure adds up to considerable differences. Evolving the density fractionation process to include the water extractable carbon in wash water could avoid future systematic underestimation of SOC.

5| Conclusion

This study quantifies different SOC fractions down to one metre depth in Inner Mongolian grasslands managed for rotational grazing. After 8 years of ongoing grazing experiment, there were few significant changes to SOC quality and quantity between the four treatments non-grazed (NG), light grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Even HG seemed to be below the threshold of livestock density that is observed to decrease SOC and overall ecosystem health in the region. Despite lacking significance to changes in SOC, certain tendencies were observed in all measured carbon fractions in the upper 20 to 30 cm of the soil. Concentrations tended to be highest in NG and decrease with increasing intensity, apart from HG which tended to show second highest concentrations. Hot water extractable carbon (HWE) was the only fraction that showed significant impact of treatment. HWE was significantly higher in NG and LG compared to MG and HG, but also followed the trend of increasing concentrations from MG to HG. These trends were only present in the upper 20 to 30 cm of the soil, demonstrating highest sensitivity to grazing in the surface soil.

Both MG and HG livestock densities, which are representative for local pastoralism in Inner Mongolia (Hoffmann et al., 2016), are shown in other studies to reduce SOC levels under continuous grazing (Cao et al., 2013). The fact that few variations between treatments were of significance reveal that rotational grazing did not cause negative repercussions compared to ungrazed grassland. All the three grazing management practices could be considered as a sustainable alternative to grazing exclusion and continuous grazing, as they all enable continuation of pastoralism and maintain SOC storages. Overall, differences in SOC stocks and their stability were minimal and cannot be used forcefully as an argument in favour of choosing between the four treatments. However, tendencies in SOC fractions suggest potential future increases in SOC under HG compared to LG and MG. Furthermore, livestock density in HG is closer to generally preferred herd sizes in Xilinhote than LG and MG. As such, rotational grazing of high livestock density could be a viable management alternative. Ultimately, evaluating consequences of rotational grazing on total ecosystem services, livestock and the livelihoods of local herders is required for outlining the overall most ideal grazing management in Xilinhote.

To improve the understanding of the SOC tendencies uncovered in this thesis, a suggestion for future research at the Xilinhote experimental site is to assess trampling effects on incorporation, stabilization and decomposition of organic matter in soil. At the experimental site, livestock density of the treatments is determined by changing number of grazing days, not number of

sheep. In effect, this provides a study of grazing on an ungrazed to continually grazed continuum. Practitioners of rotational grazing in other countries have pointed out that having large livestock herds over a short duration of time tends to reduce herbivore selectivity (Sanjari et al., 2008). This results in a plant composition similar to ungrazed areas, without such a strong shift in species dominance. Rotational grazing where increased livestock density is a function of herd size, not grazing days, could be an interesting experiment to further explore potential benefits of this management practice.

6| References

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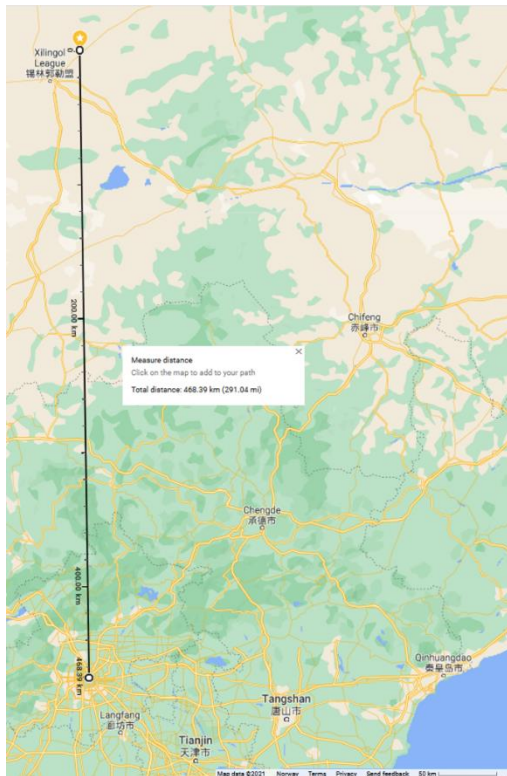
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7| Appendix

7.1| Materials and methods appendix



Appx 1: Distance between the Rotational Grazing Site (Xilinhot) and Beijing City Centre (Google Maps, 2021)

7.2| Results appendix

Appx 2: Averages of soil attributes \pm standard error (SE) at the experimental site divided by depth. Variables marked with asterisk (*) vary significantly with treatment for some depths. All values presented as percentages are of the total bulk soil unless stated otherwise. n represents number of observations for the different soil attributes and depths, see also Appx 3 for sample selection. See bottom of table for abbreviations and further footnotes.

Depth (n)	pH _{H2O} ± SE	BD _(SOC) (g/cm3) ± SE	SOC _(Xilinhot) (%) ± SE	SOC stock (kg/m2) ± SE *	HWEC (mg/kg) ± SE *		
0 - 5 (48)	8.00 ± 0.04	1.17 ± 0.01	2.10 ± 0.06	1.23 ± 0.02	724.16 ± 14.76		
5 - 10 (47)	8.15 ± 0.03	1.16 ± 0.01	2.22 ± 0.07	1.27 ± 0.03	682.96 ± 16.55		
10 - 15 (48)	8.35 ± 0.02	1.15 ± 0.01	2.30 ± 0.06	1.31 ± 0.03	539.74 ± 12.97		
15 - 20 (47)	8.44 ± 0.01	1.16 ± 0.01	2.19 ± 0.06	1.26 ± 0.03	423.17 ± 8.84		
20 - 30 (47)	8.49 ± 0.01	1.18 ± 0.01	2.06 ± 0.06	2.41 ± 0.05	308.57 ± 7.44		
30 - 50 (12)	8.46 ± 0.04	1.24 ± 0.02	1.64 ± 0.09	4.04 ± 0.18	202.15 ± 12.43		
50 - 100 (12)	8.97 ± 0.05	1.36 ± 0.02	1.09 ± 0.09	7.31 ± 0.46	94.13 ± 7.81		
Depth (n)	ASD _{2-1 mm} (%) ± SE	ASD _{1-0.25 mm} (%) ± SE	ASD _{< 0.25 mm} (%) ± SE	SAG _{2-1 mm} (%) ± SE†	SAG _{1-0.25 mm} (%) ± SE†	TotC _{SAG 2-1 mm} (%) ± SE	TotC _{SAG 1-0.25 mm} (%) ± SE
0 - 5 (24)	11.75 ± 0.75	45.13 ± 3.74	45.66 ± 4.99	1.46 ± 0.12	3.39 ± 0.27	0.18 ± 0.01	0.75 ± 0.05
5 - 10 (23)	14.32 ± 0.99	54.82 ± 2.51	31.07 ± 3.50	2.05 ± 0.16	3.73 ± 0.25	0.24 ± 0.02	0.89 ± 0.04
10 - 15 (2)	16.39 ± 3.57	66.54 ± 0.37	15.17 ± 3.53	0.99 ± 0.73	14.94 ± 10.75	0.26 ± 0.02	0.93 ± 0.02
15 - 20 (2)	21.91 ± 2.61	55.53 ± 3.08	20.69 ± 0.01	3.01 ± NA	7.36 ± 2.80	0.36 ± 0.02	0.87 ± 0.16
20 - 30 (2)	17.43 ± 0.83	33.69 ± 8.19	47.55 ± 9.17	3.66 ± 0.02	6.31 ± 0.48	0.32 ± 0.03	0.61 ± 0.04
30 - 50 (9)	15.54 ± 0.76	22.69 ± 4.88	60.06 ± 5.25	2.74 ± 0.31	4.12 ± 0.35	0.20 ± 0.02	0.28 ± 0.03
50 - 100 (9)	18.25 ± 1.80	26.10 ± 6.76	54.56 ± 7.67	1.16 ± 0.18	1.96 ± 0.61	0.08 ± 0.01	0.15 ± 0.05
Depth (n)	TotC (%) ± SE	TotN (%) ± SE	SOC/N ± SE *	POC (%) ± SE	MOC (%) ± SE ‡	PON (%) ± SE	MON (%) ± SE
0 - 5 (18)	2.36 ± 0.09	0.20 ± 0.00	10.25 ± 0.25	0.37 ± 0.02	1.19 ± 0.06	0.02 ± 0.00	0.11 ± 0.00
5 - 10 (3)	2.25 ± 0.10	0.21 ± 0.01	11.06 ± 0.30	0.24 ± 0.04	0.98 ± 0.10	0.01 ± 0.00	0.10 ± 0.01
10 - 15 (18)	2.76 ± 0.12	0.19 ± 0.00	12.61 ± 0.35	0.21 ± 0.02	1.28 ± 0.08	0.01 ± 0.00	0.10 ± 0.00
15 - 20 (3)	2.69 ± 0.19	0.18 ± 0.00	13.35 ± 0.39	0.18 ± 0.02	0.82 ± 0.13	0.01 ± 0.00	0.09 ± 0.01

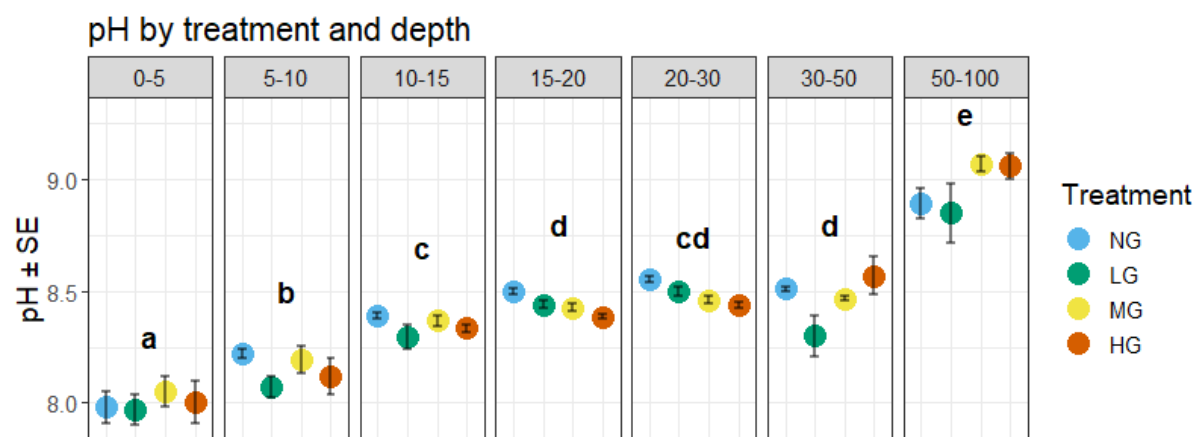
20 - 30 (3)	2.77 ± 0.12	0.15 ± 0.01	14.73 ± 0.41	0.13 ± 0.01	0.56 ± 0.07	0.01 ± 0.00	0.07 ± 0.01
30 - 50 (9)	2.22 ± 0.07	0.10 ± 0.01	15.30 ± 0.77	0.10 ± 0.02	0.07 ± 0.05	0.01 ± 0.00	0.04 ± 0.00
50 - 100 (9)	1.50 ± 0.07	0.06 ± 0.00	24.30 ± 2.70	0.06 ± 0.02	-0.11 ± 0.02	0.00 ± 0.00	0.02 ± 0.00
Depth (n)	Clay (%) ± SE	Silt (%) ± SE	Sand (%) ± SE	Depth (n)	NO₃ (mg N/kg) ± SE	NH₄ (mg N/kg) ± SE *†	
0 - 5 (2)	8.25 ± 0.46	46.01 ± 7.10	45.75 ± 7.56	0 - 5 (9)	17.65 ± 1.12	-1.17 ± 0.46	
5 - 10 (2)	10.91 ± 3.19	50.18 ± 10.79	38.91 ± 13.98	5 - 10 (6)	12.15 ± 1.25	-0.22 ± 0.50	
10 - 15 (2)	9.94 ± 0.33	48.59 ± 2.69	41.48 ± 3.02	10 - 15 (6)	6.42 ± 0.50	-0.98 ± 0.78	
15 - 20 (2)	10.45 ± 0.45	48.23 ± 1.91	41.33 ± 2.35	15 - 20 (6)	4.14 ± 0.53	-0.70 ± 0.53	
20 - 30 (2)	11.04 ± 2.41	49.41 ± 8.55	39.56 ± 10.96	20 - 30 (6)	2.32 ± 0.56	-0.76 ± 0.56	
30 - 50 (2)	11.97 ± 0.50	57.60 ± 4.33	30.44 ± 3.83				
50 - 100 (2)	9.97 ± 1.78	55.30 ± 3.09	34.74 ± 4.87				
<p>* Varies significantly with treatment for some depths. †, one unrealistic negative value was removed from the dataset. ‡, negative values are due to calculation (MOC) or signifies immobilization (NH₄). BD_(SOC) = Bulk density estimated based on SOC_(Xilinhot). SOC_(Xilinhot) = Soil organic carbon calculated based on own correction factor. HWEC = Hot water extractable carbon. SOC/N = the ratio calculated based on SOC_(Xilinhot) and totN_(HWEC). ASD = Aggregate size distribution for the various size fractions. SAG = Stable aggregates in the various size fractions. TotC = Total carbon content measured either in SAG or in the bulk soil. TotN = Total nitrogen content measured in bulk soil. POC = Particulate organic carbon. MOC = Mineral associated organic carbon. PON = Particulate organic nitrogen. MON = Mineral associated organic nitrogen.</p>							

Appx 3: Table of soil sample selection for analysed soil properties.

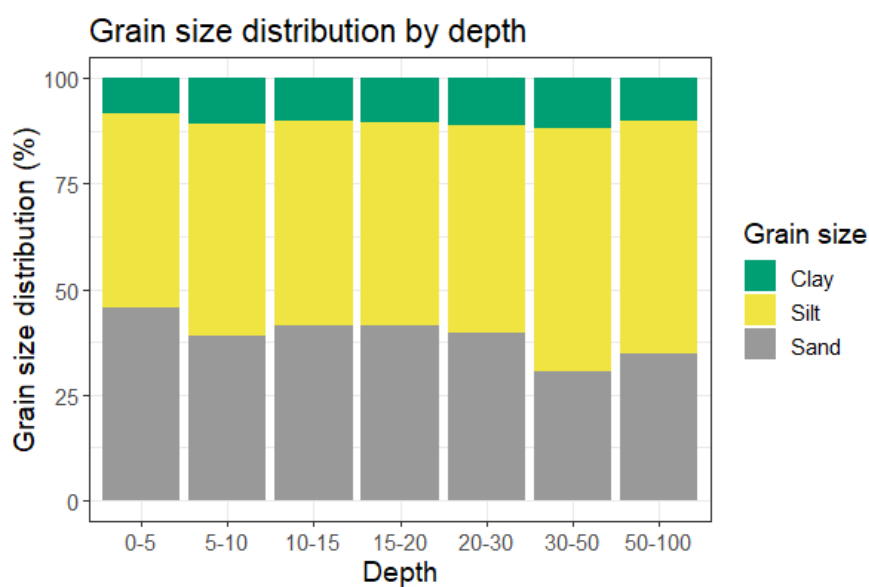
	Analysis selection									
Treatment and depth	BD _(Wu)	BD _(SOC) , pH _{H2O} , LOI, SOC _(Xilinhot) , HWE _{C(3803} RCF), TotN _(HWE_C) SOC/N	GSD	ASD, SAG, TotC	IC	HWE _C ₍₁₆₉₀ RCF)	TotC, TotN	POC, MOC, PON, MON	Filter fraction, Rest fraction	N min.
NG	36	66	7	21	7	3	21	21	3	15
0 - 5	9	12	1	6	1	1	6	6	3	3
5 - 10		12	1	6	1	1	1	1	0	3
10 - 15	9	12	1	1	1	1	6	6	0	3
15 - 20		12	1	1	1	0	1	1	0	3
20 - 30	9	12	1	1	1	0	1	1	0	3
30 - 50*	9	3	1	3	1	0	3	3	0	0
50 - 100		3	1	3	1	0	3	3	0	0
LG	36	66	0	18	7	0	21	21	0	3
0 - 5	9	12	0	6	1	0	6	6	0	3
5 - 10		12	0	6	1	0	1	1	0	0
10 - 15	9	12	0	0	1	0	6	6	0	0
15 - 20		12	0	0	1	0	1	1	0	0
20 - 30	9	12	0	0	1	0	1	1	0	0
30 - 50*	9	3	0	3	1	0	3	3	0	0
50 - 100		3	0	3	1	0	3	3	0	0
MG	36	66	0	12	0	0	7	0	0	0
0 - 5	9	12	0	6	0	0	1	0	0	0
5 - 10		12	0	6	0	0	1	0	0	0
10 - 15	9	12	0	0	0	0	1	0	0	0

15 - 20		12	0	0	0	0	1	0	0	0
20 - 30	9	12	0	0	0	0	1	0	0	0
30 - 50*	9	3	0	0	0	0	1	0	0	0
50 - 100		3	0	0	0	0	1	0	0	0
HG	36	66	7	21	7	3	21	21	3	15
0 - 5	9	12	1	6	1	1	6	6	3	3
5 - 10		12	1	6	1	1	1	1	0	3
10 - 15	9	12	1	1	1	1	6	6	0	3
15 - 20		12	1	1	1	0	1	1	0	3
20 - 30	9	12	1	1	1	0	1	1	0	3
30 - 50*	9	3	1	3	1	0	3	3	0	0
50 - 100		3	1	3	1	0	3	3	0	0
Total n	144	264	14	72	21	6	70	63	6	33
* = Depth interval is 30 - 40 cm for BD _(Wu) . BD _(Wu) = Bulk density data from Wu Yantao. BD _(SOC) = Bulk density estimated based on SOC _(Xilinhhot) . SOC _(Xilinhhot) = Soil organic carbon calculated based on own correction factor. HWEC = Hot water extractable carbon. SOC/N = the ratio calculated based on SOC _(Xilinhhot) and totN _(HWEC) . ASD = Aggregate and grain size distribution for the various size fractions, not corrected for mineral particle content. SAG = Stable aggregates in the various size fractions. TotC = Total carbon content measured either in SAG or in the bulk soil. TotN = Total nitrogen content measured in bulk soil. POC = Particulate organic carbon. MOC = Mineral associated organic carbon. PON = Particulate organic nitrogen. MON = Mineral associated organic nitrogen. N min. = N mineralisation.										

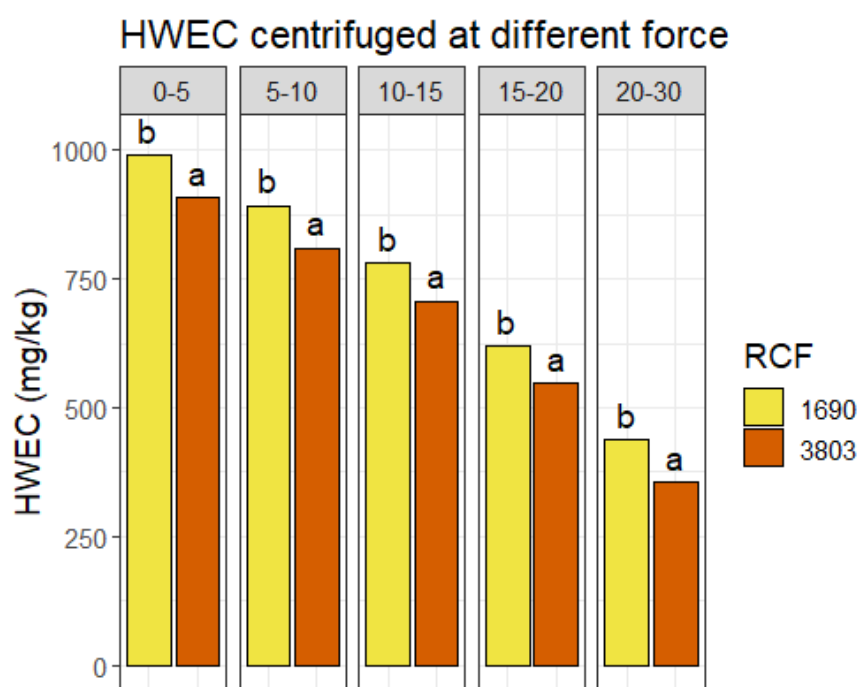
7.2.1| Carbon content and different Soil Organic Carbon (SOC) fractions, appendix



Appx 4: Average pH \pm standard error (SE) by treatment and depth. Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the reference plot, and the three treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For each sampling depth between 0 to 30 cm, $n = 47$ or 48. For each sampling depth between 30 and 100, $n = 12$. See also Appx 3 for sample selection.

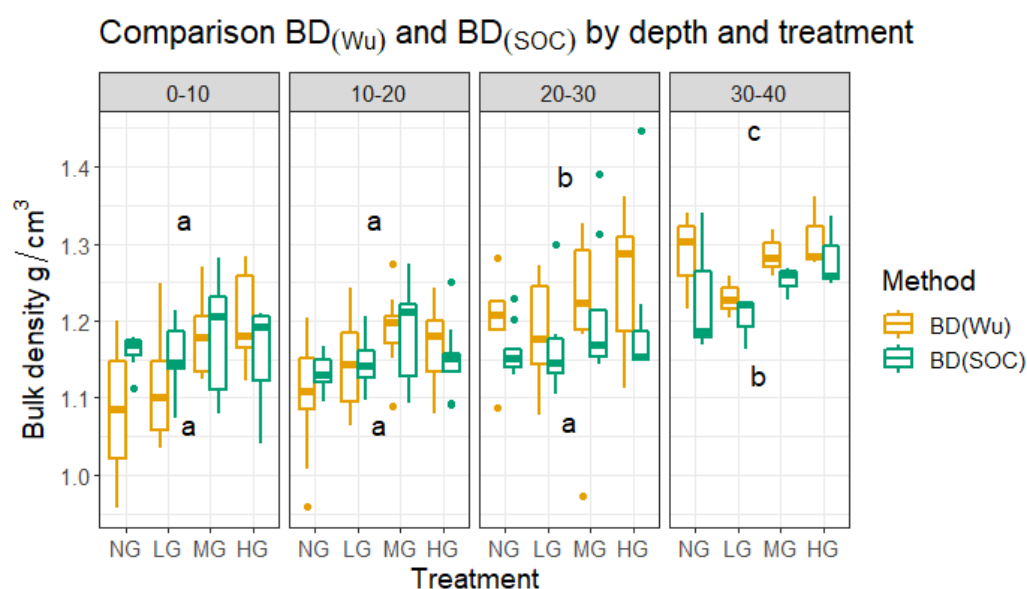


Appx 5: Grain size distribution by depth. The grain size fractions are shown as a percentage of bulk soil, in the colours green, yellow and grey for the clay, silt and sand fractions respectively. $n = 2$ for each depth, see Appx 3 for sample selection.

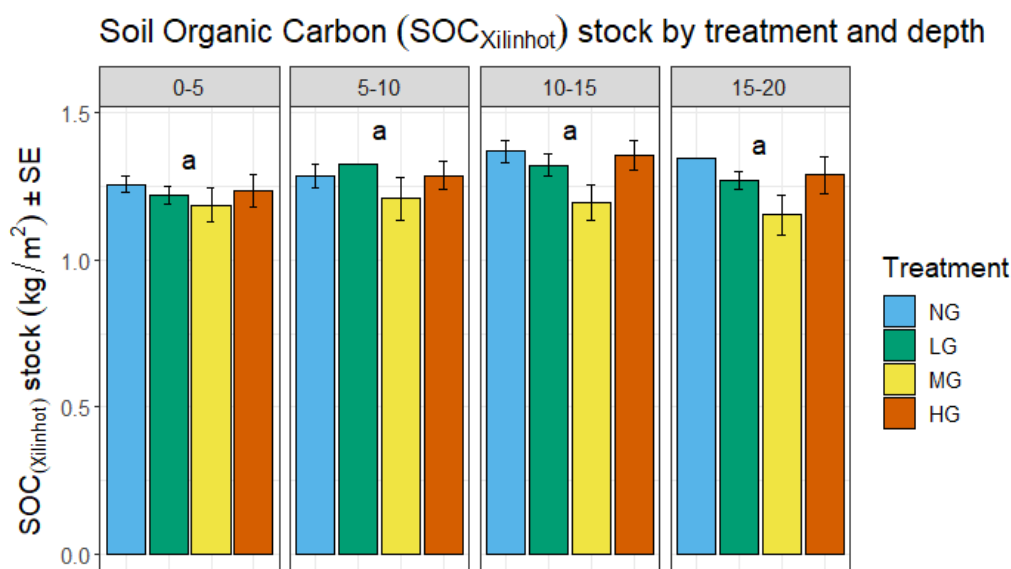


Appx 6: Hot water extractable carbon (HWECE) in mg/kg by depth (grids) when centrifuged at 1690 RCF and 3803 RCF, for 10 and 15 minutes respectively. Different letters indicate significant differences between samples of different RCF ($p < 0.05$). $n = 5$ for each set. See also Appx 3 for sample selection.

7.2.2| Soil Organic Carbon (SOC) stocks, appendix

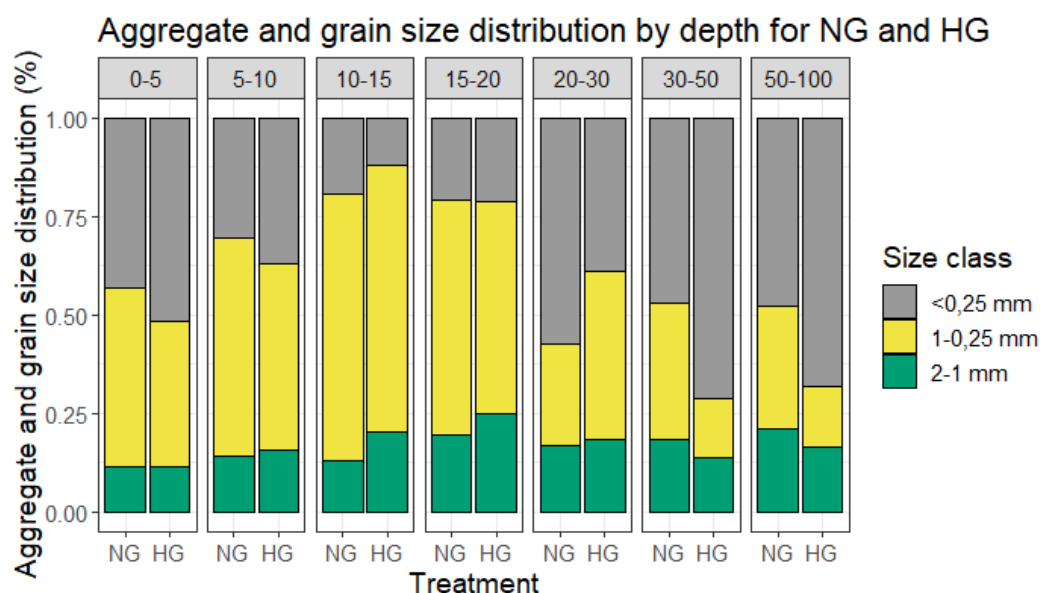


Appx 7: Comparison by depth and treatment between bulk density as measured by Wu Yantao ($BD_{(Wu)}$) and calculated based on SOC ($BD_{(SOC)}$). Different letters indicate significant differences between depths for $BD_{(Wu)}$ (upper letters) and $BD_{(SOC)}$ (lower letters). Note: $BD_{(Wu)}$ is available for bigger depth intervals than $BD_{(SOC)}$, and $BD_{(SOC)}$ was thus averaged to match the $BD_{(Wu)}$ dataset. For the deepest depth interval, $BD_{(SOC)}$ -values represent 30 to 50 cm depth. See Appx 3 for sample comparison.



Appx 8: Average soil organic carbon (SOC) stocks \pm standard error (SE) in kg/m² by treatment and depth in the upper 20 cm, calculated from SOC_(Xilinhot) and BD_(SOC). Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the reference, and the three treatments low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. Depth intervals are separated in grids. Different letters indicate significant differences between depths ($p < 0.05$). For each sampling depth between 0 to 30 cm, $n = 47$ or 48. For each sampling depth between 30 and 100, $n = 12$. See also Appx 3 for sample selection.

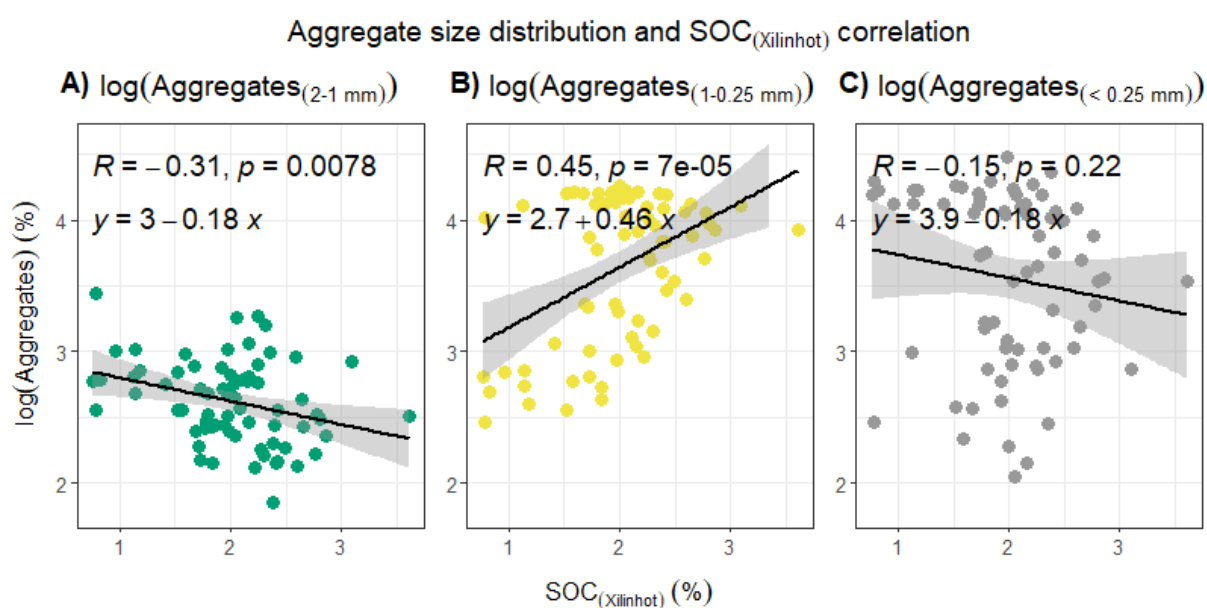
7.2.3| Aggregate size distribution and stability, appendix



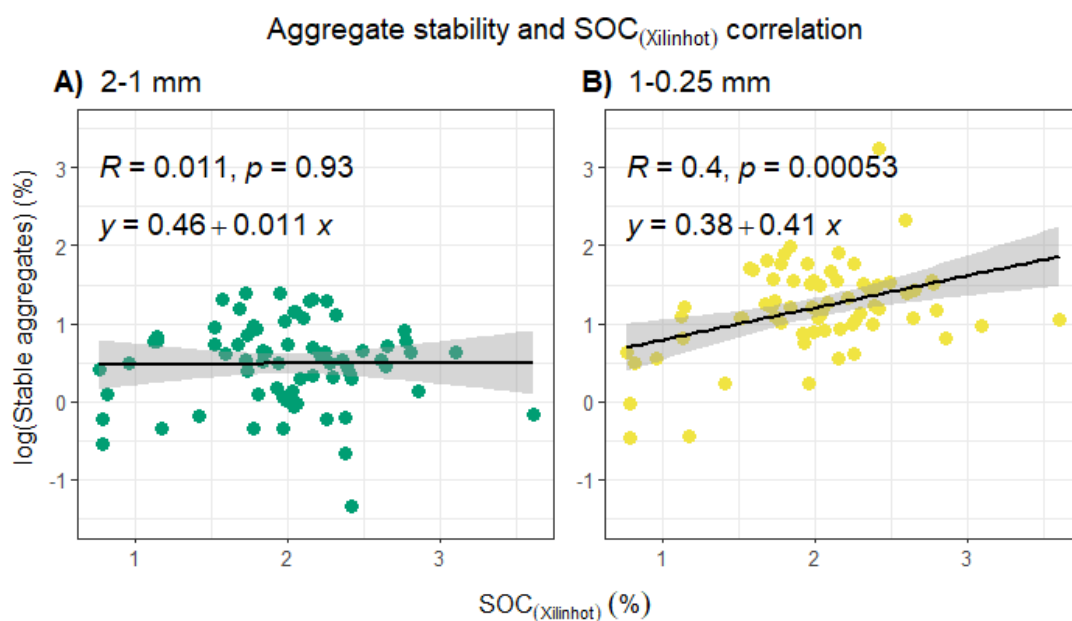
Appx 9: Average aggregate and grain size distribution by depth for the non-grazing (NG, reference) and high grazing (HG) treatment shown on the x-axis. Aggregates of size 2-1 mm is shown in green, aggregates of size 1-0.25 mm is shown in yellow and anything beneath 0.25 mm is shown in gray. For 0 - 5 cm, $n=24$. For 5 - 10 cm, $n=23$. For the three intervals between 10 to 30 cm, $n = 2$. For the two intervals 30 to 100 cm, $n = 9$. See Appx 3 for sample selection.

Appx 10: Average aggregate size distribution (ASD) \pm standard error (SE) for the various size fractions, both uncorrected and corrected for grain content. All values are presented as percentages of total bulk soil. n represents number of observations, see Appx 3 for sample selection.

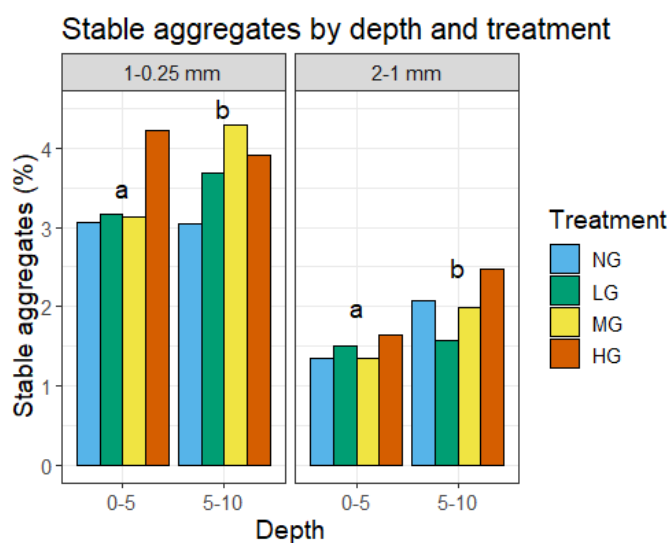
Depth (n)	ASD _{2-1 mm} (%) \pm SE	ASD _{(corr) 2-1 mm} (%) \pm SE	Difference ASD _{2-1 mm}
0-5 (24)	11.75 \pm 0.75	11.74 \pm 0.75	0.02 \pm 1.06
5-10 (23)	14.32 \pm 0.99	14.33 \pm 0.99	-0.01 \pm 1.40
10-15 (2)	16.39 \pm 3.57	16.42 \pm 3.52	-0.03 \pm 5.01
15-20 (2)	21.91 \pm 2.61	21.60 \pm 2.53	0.31 \pm 3.63
20-30 (2)	17.43 \pm 0.83	17.05 \pm 0.81	0.38 \pm 1.16
30-50 (9)	15.54 \pm 0.76	15.39 \pm 0.74	0.15 \pm 1.06
50-100 (9)	18.25 \pm 1.80	18.22 \pm 1.80	0.03 \pm 2.55
Average			0.13 \pm 2.27
Depth (n)	ASD _{1-0.25 mm} (%) \pm SE	ASD _{(corr) 1-0.25 mm} (%) \pm SE	Difference ASD _{1-0.25 mm}
0-5 (24)	45.13 \pm 3.74	44.69 \pm 3.74	0.44 \pm 5.29
5-10 (23)	54.82 \pm 2.51	54.18 \pm 2.56	0.64 \pm 3.59
10-15 (2)	66.54 \pm 0.37	66.04 \pm 0.56	0.50 \pm 0.67
15-20 (2)	55.53 \pm 3.08	55.03 \pm 2.96	0.50 \pm 4.27
20-30 (2)	33.69 \pm 8.19	33.15 \pm 8.27	0.54 \pm 11.64
30-50 (9)	22.69 \pm 4.88	22.56 \pm 4.87	0.13 \pm 6.90
50-100 (9)	26.10 \pm 6.76	26.02 \pm 6.73	0.08 \pm 9.54
Average			0.40 \pm 5.98



Appx 11: Correlation and linear relationships between between SOC_(Xilinhot) and amount of aggregates in the 1-2mm (A, green), 0.25-1 mm (B, yellow) and < 0.25 mm (C, grey) size class. For 0 - 5 cm, n=24. For 5 - 10 cm, n=23. For the three intervals between 10 to 30 cm, n = 2. For the two intervals 30 to 100 cm, n = 9. See Appx 3 for sample selection.

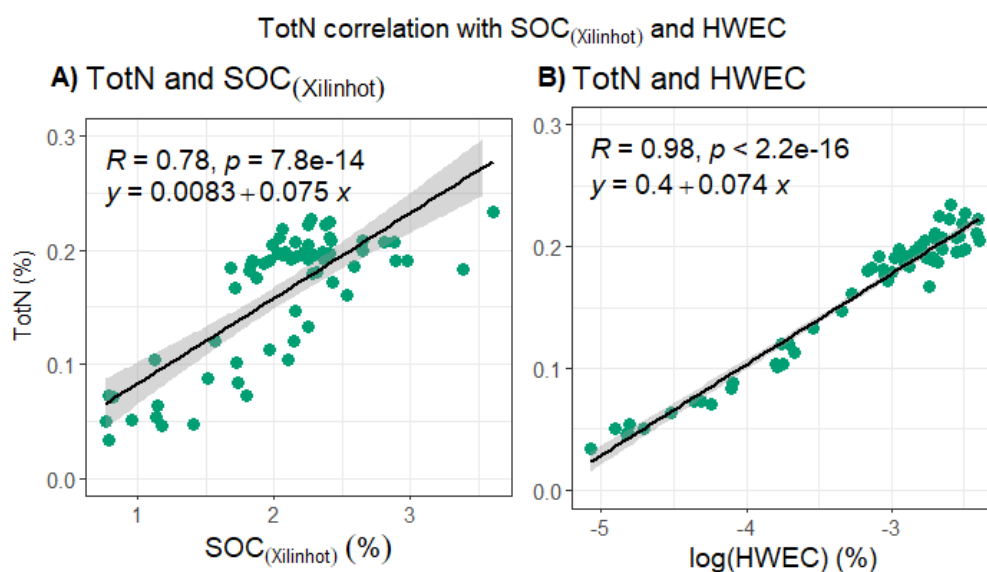


Appx 12: Correlation and lack thereof between SOC_(Xilinhot) and amount of stable aggregates in the 1-2mm (A, green) and 0.25-1 mm (B, yellow) size class. For 0 - 5 cm, n=24. For 5 - 10 cm, n=23. For the three intervals between 10 to 30 cm, n = 2. For the two intervals 30 to 100 cm, n = 9. See Appx 3 for sample selection.

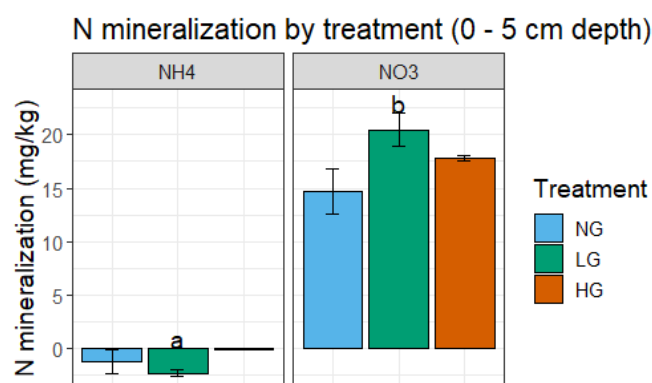


Appx 13: Average amount of stable aggregates of the two size fractions 1-0.25 mm and 2-1 mm \pm standard error (SE), separated by grids and shown by treatment as percent of bulk soil within the upper 10 cm. Treatment is shown in blue, green, yellow and red respectively for non-grazing (NG) as the control, low grazing (LG), moderate grazing (MG) and high grazing (HG) intensity. n = 6 for each treatment and depth combination. See also Appx 3 for sample selection.

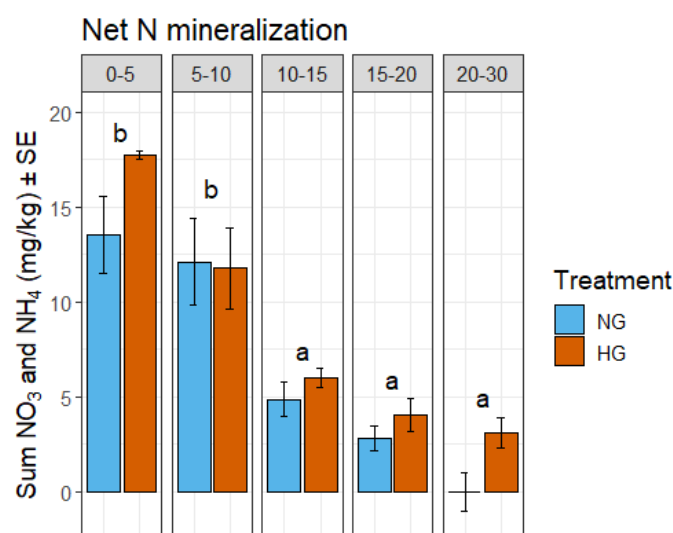
7.2.4| Total nitrogen (TotN) content and N mineralization, appendix



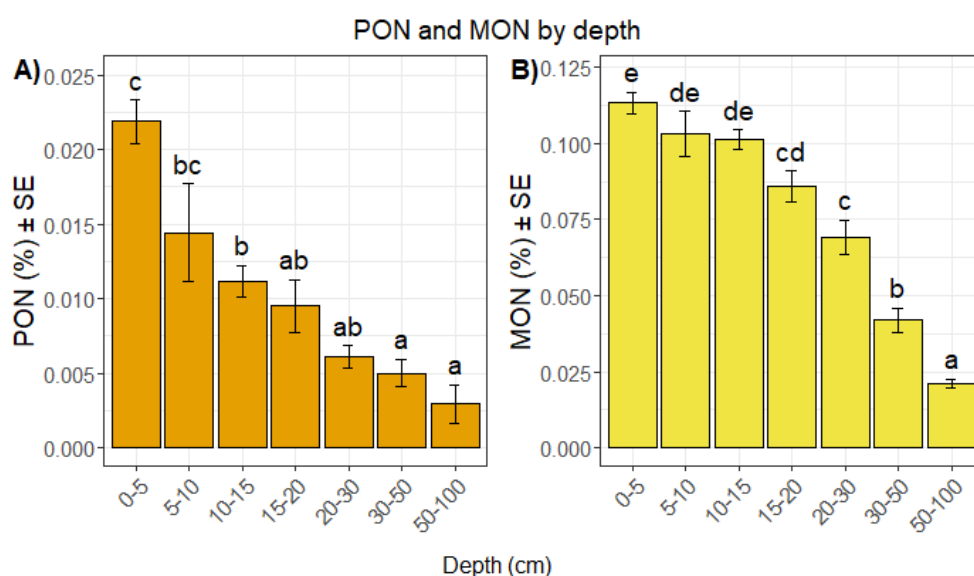
Appx 14: Correlation and linear relationship between totN and SOC_(Xilinhot) (A), and between totN and the logarithm of Hot Water Extractable Carbon (HWEC, B), both given as percentages of bulk soil.



Appx 15: Average NO₃ and NH₄ mineralization in mg/kg \pm standard error (SE) separated by grids and shown by treatment within the upper 5 cm. Treatment is shown in blue, green and red respectively for non-grazing (NG) as the reference, low grazing (LG) and high grazing (HG) intensity. Negative NH₄-mineralization indicates immobilization. Different letters indicate significant differences in inorganic N compound ($p < 0.05$). $n = 9$. See also Appx 3 for sample selection.



Appx 16: Average net N mineralization in mg/kg \pm standard error (SE) by treatment and depth. Treatment is shown in blue and red respectively for non-grazing (NG) as the reference and high grazing (HG) intensity. Depth intervals are separated in grids. Letters show significance between depths ($p < 0.05$). For each sampling depth, $n = 6$. See also Appx 3 for sample selection.



Appx 17: Average particular organic nitrogen (PON, A, orange) and mineral associated organic carbon (MON, B, yellow) \pm standard error (SE) as a percentage of bulk soil by depth on the x-axis. Note different values on y-axis. Different letters indicate significant differences between depths ($p < 0.05$). For depth interval 0 - 5 and 10 - 15 cm, $n = 18$. For 5 - 10, 15 - 20 and 20 - 30 cm, $n = 3$. For 30 - 50 and 50 - 100, $n = 9$. See also Appx 3 for sample selection.



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