COMPARISON OF CARBON AND NITROGEN STOCKS UNDER GRAZED AND UN-GRAZED AREAS

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ABSTRACT

Grazing is an important part of the grassland ecosystem dynamics and plays a key role in carbon and nitrogen storage in the system. The exclusion from grazing is considered one of the major practical implications to restore the ecosystem and prevent grassland degradation. The study focused on the effects of grazing on vegetation and soil properties in subarctic grassland in Iceland, comparing a grazed and an un-grazed area. The aim of this pilot study was to examine the effects of exclusion from grazing on the main compartments (soil, vegetation and sward/litter) of carbon and nitrogen storage in the system. The area excluded from grazing had less total carbon and nitrogen storage in the system down to 30 cm depth in soil and the distribution of the carbon and nitrogen stocks among the system compartments was slightly different. The results showed that the carbon and nitrogen stocks in vegetation and sward/litter were slightly higher in the un-grazed area as compared to the grazed area, which indicated the recovery of the ecosystem after exclusion from grazing, whereas the carbon and nitrogen in soil was significantly higher in the grazed area as compared to the un-grazed area. The study did not answer whether the difference observed in soil carbon and nitrogen stocks was due to the effects of grazing or environmental factors such as soil moisture. In addition, the soil was the largest reservoir of carbon and nitrogen in both study areas. Therefore, further research to investigate the effects of exclusion from grazing are needed not only to understand the ecology of this fragile ecosystem but also to enhance sustainability of grazing management.

This paper should be cited as:

Amgaa B (2012) Comparison of carbon and nitrogen stocks under grazed and un-grazed areas. United Nations University Land Restoration Training Programme [final project] http://www.unulrt.is/static/fellows/document/Amgaa2012.pdf

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1. INTRODUCTION

Arctic and alpine plants have adaptive mechanisms which enable them to survive and function in environments with a cool, short growing season and low temperatures (Bliss 1962; Bliss 1971). The rates of biogeochemical processes in these regions are often limited by the summer temperatures (Raynolds et al. 2008), which results in lower plant productivity than in other ecosystems (Billings & Mooney 1968; Körner 2003). Nitrogen (N) is one of the common limiting nutrients for plant growth and net primary productivity in the terrestrial ecosystems as well as many marine ecosystems (Vitousek & Howarth 1991; Verhoeven et al. 1996), and particularly in alpine, arctic and subarctic ecosystems where N is the most constraining factor to both plant growth (Bliss 1962; Martinsen et al. 2012) and net primary production (Melillo et al. 1993; LeBauer & Treseder 2008).

Grazing is an important part of grassland ecosystem dynamics and plays a key role in storage and cycling of soil C and N and other nutrients (Wei et al. 2011; Martinsen et al. 2012). According to Piñeiro et al. (2010), grazing can affect soil C and N stocks through three major pathways: 1) changes in net primary production (NPP pathway), 2) changes in the nitrogen stock (N pathway), and 3) changes in organic matter (decomposition pathway, Fig. 1). Herbivores affect directly NPP either positively (below-ground NPP) or negatively (above-ground NPP), which in turn controls the amount of NPP that reaches into the soil (Fig. 1). Grazing also may affect soil organic carbon formation by altering the proportion of NPP that is allocated to below- and aboveground organs. Piñeiro et al. (2010) also found that the proportion of C allocation to belowground organs is increased with grazing, which in turn increases soil organic matter formation (Fig. 1). On the other hand, grazing may influence NPP indirectly by changing species composition or soil nutrients (especially N), or soil water availability (Fig. 1), but it depends on the grazing history and moisture gradient (Milchunas & Lauenroth 1993).

Soil organic matter also plays an important role in C and N cycling in soil (Knicker 2011), whereas the topographic soil moisture gradient is one of the main controlling factors of the N turnover in alpine tundra ecosystems (Fisk et al. 1998). Soil organic matter is also a primary variable that influences the soil's water holding capacity and water infiltration, while soil moisture controls the decomposition rate of soil organic matter (Hudson 1994; Saxton & Rawls 2006), the largest reservoir of C and N in the plant-soil system (Follett 2001; Knicker 2011) (Fig. 1).

Furthermore, Throop et al. (2004) also showed that insect herbivores could have a substantial impact on ecosystem processes such as soil organic carbon and nitrogen mineralization. McNaughton et al. (1988) also indicated that the large African mammals are one of the major organizers for ecosystem processes and structure, and their effects are also important for nutrient cycling (C and N) in African ecosystems. But the required level of grazing which influences soil C and N storage in northern, alpine and arctic ecosystems is not well understood (Martinsen et al. 2012).

The effects of grazing on soil C and N storage and cycling have been studied predominantly in the temperate grasslands of North America and in the semi-arid grasslands of Asia (mostly in China) (Wu et al. 2011). However, there has been almost no research about the effects of grazing on soil C and N storage, neither in Mongolia nor Iceland. The evaluation of the effects of grazing on ecosystem C and N storage can be a useful indicator on sustainability of grazing management in Mongolia and Iceland. The importance of such an indicator is high, considering that livestock production is the largest enterprise in the agricultural sectors of both countries and, consequently, most of the land in Mongolia and Iceland is utilized as rangeland (Johnson et al. 2006; Barkarson & Jóhannsson 2009).

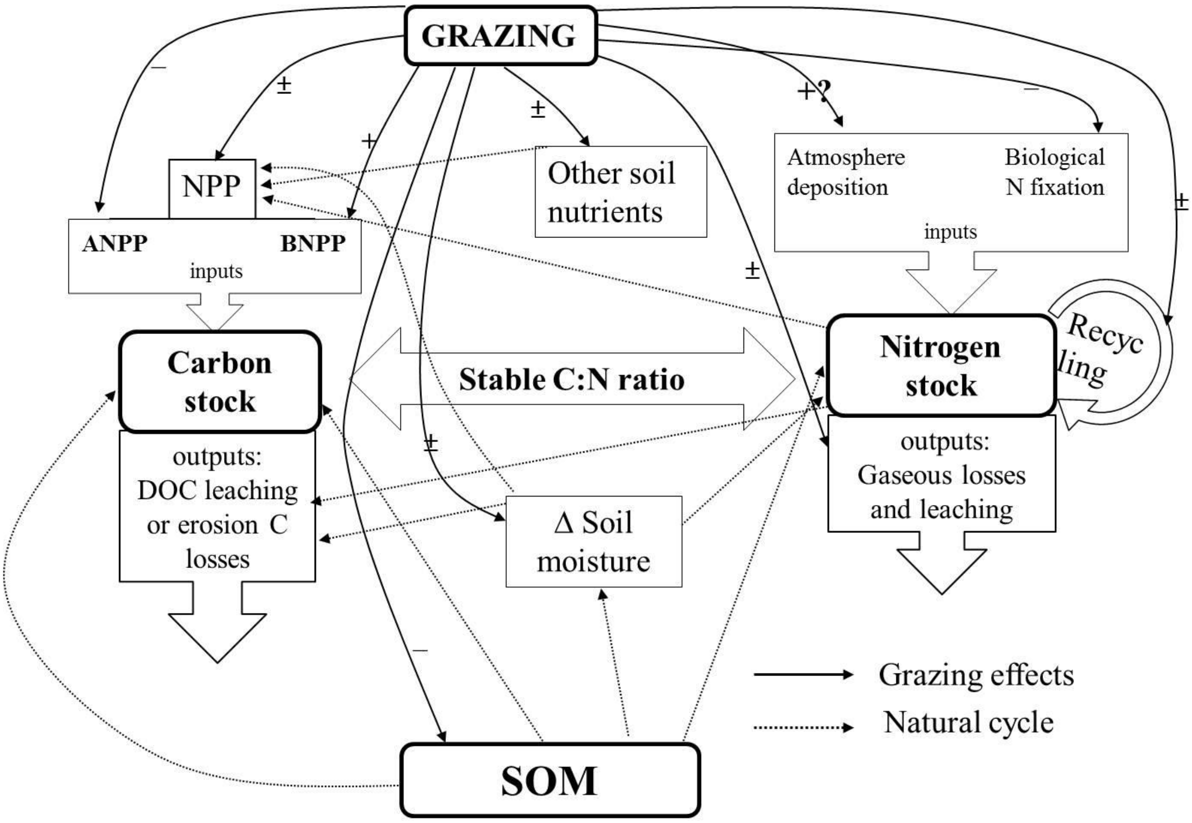

Fig. 1. *Grazing effects on carbon and nitrogen stocks in soil: re-drawn and modified from Piñeiro et al. (2009). NPP, net primary production; ANPP, aboveground NPP; BNPP, belowground NPP; SOM, soil organic matter; and DOC, dissolved organic carbon.*

Overgrazing, accelerated soil erosion and continuous rangeland degradation are serious environmental problems both in Mongolia and Iceland; hence, Mongolian and Icelandic rangeland managers are faced with a challenge to promote and advance sustainable land management (Arnalds 1987; Lise et al. 2006; Barkarson & Jóhannsson 2009). Thus, understanding the effects of grazing on soil C and N storage is important for fragile ecosystems, where livestock grazing plays a major role for the nutrient cycles.

Although a number of studies have addressed the response of soil C and N to grazing, the response to exclusion from grazing is still not well studied. Our experiment was therefore conducted to investigate the effects of grazing on vegetation and soil properties in subarctic grassland in Iceland, comparing a grazed area and an un-grazed area. The aim of this pilot study was to examine the effects of exclusion from grazing on the main compartments of C and N in the system. The compartments included were soil, vegetation and sward/litter in our study. The hypotheses were that: (1) grazing would decrease the total amount of nitrogen in the system, because nitrogen is removed from the system through growth of livestock and the N invested in that growth is not returned back into the system; and (2) exclusion from grazing would increase the carbon stocks of the system because of no removal of carbon through grazing from the system. Understanding the changes in C and N stocks of ecosystem compartments under grazing is also essential for recovery of ecosystems, restoration and sustainable land use.

2. LITERATURE REVIEW

Grasslands cover approximately 40.5 percent of the Earth's land surface (excluding Greenland and Antarctica) (White et al. 2000), most of which are used for managed grazing (Asner et al. 2004). Most of the land in Iceland has been utilized for livestock grazing since man arrived in the late ninth century. Due to various kinds of human activities such as grazing, charcoal production and deforestation, the rangelands of Iceland have been subjected to various degrees of degradation (Arnalds 1987; Arnalds & Barkarson 2003; Barkarson & Jóhannsson 2009).

The parent material of all Icelandic soil is of volcanic origin (Arnalds 2004). Andosol is the dominant soil in Iceland and it has specific physical properties such as low bulk density, high porosity and high water retention, and the vegetation has evolved without large herbivores (Arnalds 1987; Arnalds & Barkarson 2003; Arnalds 2004; Óskarsson et al. 2004). Due to their special characteristics, Andosols are very susceptible to erosion (by wind and water) when the surface coverage is weakened, and the vegetation structure and composition in Iceland have changed and decreased as a result of livestock grazing (Arnalds 1987; Arnalds & Barkarson 2003). Thus, Iceland is an example of a fragile northern ecosystem where soil erosion, and extensive vegetation degradation have been taking place more actively there since the settlement in A.D. 874 than in any other European country (Arnalds 1987; Arnalds & Barkarson 2003).

Continuous or heavy grazing can result in decreased above-ground biomass and changes in community composition and structure (Milchunas et al. 1988; Wei et al. 2011), and in turn, result in the soil being more susceptible to wind erosion, loss of soil organic carbon and nitrogen as well as loss of soil biological properties (Yong-Zhong et al. 2005). Yong-Zhong et al. (2005) also found that soil organic carbon and total nitrogen concentration and soil biological properties were improved following a 10-year of exclusion from livestock grazing. In addition, the impact of livestock grazing and plant-microbe interactions are also important regulators of plant-soil C and N cycling in terrestrial ecosystems (Knops et al. 2002; Yong-Zhong et al. 2005; Wei et al. 2011; Wu et al. 2011; Wu et al. 2012). However, such plant-microbe interactions have been excluded in most soil C and N studies (Wu et al. 2011) as well as in this pilot study.

Milchunas and Lauenroth (1993) and Piñeiro et al. (2010) reported that soil N and organic C had positive, negative or no responses to grazing, after reviewing 236 grasslands data around the world and 20 scientific papers which analysed the effects of grazing on soil organic matter stocks by comparing grazed vs. un-grazed sites containing 67 comparisons. Among the reported studies, the response of soil C and N to grazing differs according to vegetation (Verhoeven et al. 1996), community composition and structure, soil properties, climate, seasonal variations (Liu et al. 2011), interaction between plant and microbe (Wu et al. 2011), evolutionary history of grazing (Milchunas & Lauenroth 1993) and its intensity (Li et al. 2008; He et al. 2011; Liu et al. 2011). Due to a number of these factors, it is still controversial whether the response of soil carbon and nitrogen storage to grazing is increase, decrease or remains unchanged (Milchunas & Lauenroth 1993; Wei et al. 2011).

3. MATERIAL AND METHODS

3.1 Site description

The research reported here was conducted at Hvítársíða, North-west Iceland, on a hillside along the glacial river Hvítá. The area is divided up into sections by fairly deep canyons running up and down the hillside formed by melting water from the edge of the retreating glacier during the last Ice Age (Anna Guðrún Thórhallsdóttir, personal communication, 28 August, 2012). Farms are located at the bottom of the hillside, mostly separated by the natural landmarks of the canyons. The size of the farms varies according to the distance between the canyons and how far beyond the hill each farm owns land. There are 14 farms located along the hillside and of these, two farms were chosen for data collection: the grazed "Sámsstaðir-Hvítársíðu" and un-grazed "Hvammur-Hvítársíðu" (Fig. 2). All weather data were obtained from the three nearest meteorological stations: Stafholtsey, Húsafell and Augastaðir (Fig. 2). The Húsafell station measured only the temperature while Augastaðir station measured precipitation close to Húsafell, so the data of Húsafell and Augastaðir stations are complementary. The Stafholtsey station measured both temperature and precipitation. According to recent five-year (2007-2011) meteorological observations of the stations, the average annual air temperature has been 4.0°C, with the coldest average monthly temperature of -2.3°C and -2.0°C in February and warmest 12.2°C and 11.9°C in July for Stafholtsey and Húsafell, respectively. The annual precipitation has been 854.7 mm and 996.6 mm for Stafholtsey and Augastaðir, respectively, of which 50% occurs from September until December (data from the Icelandic Meteorological Office).

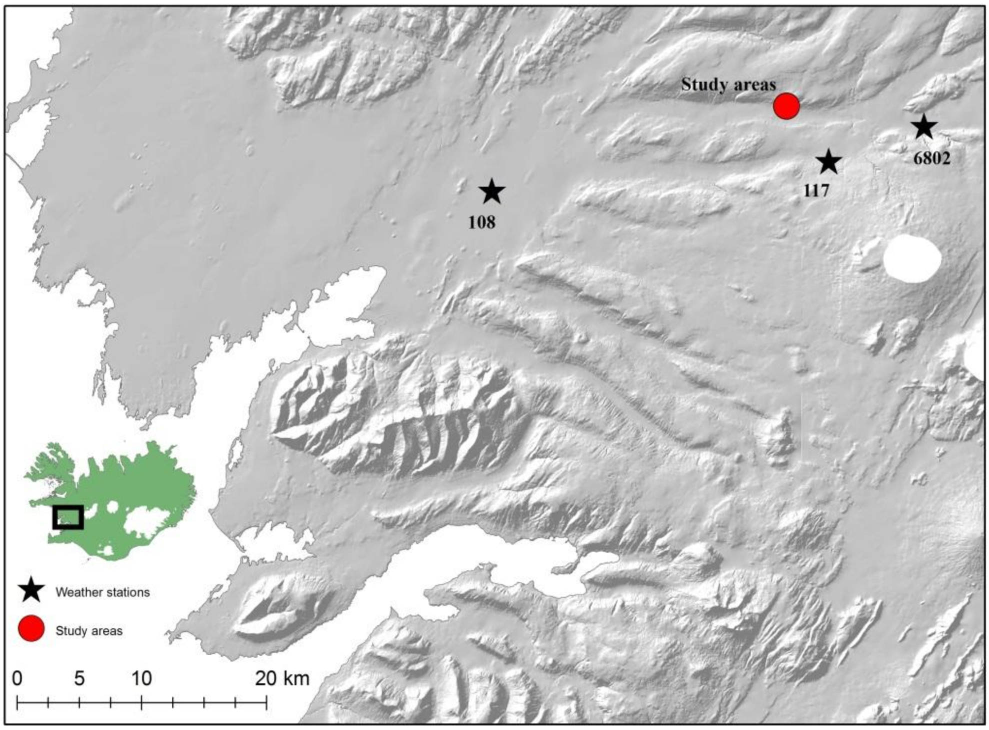

Fig. 2. *Location of the study area and the nearest weather stations in Iceland. The study area includes both grazed and ungrazed areas. The weather stations are: 108 Stafholtsey; 6802 Húsafell; 117 Augastaðir.*

The soils of the study areas are classified into a complex of Brown Andosols, Histic Andosols and Histosols according to the soil map of Iceland (Arnalds & Óskarsson 2009). The precise soil type of the experimental site could thus not be determined from the soil map. The carbon content of the soils at the study areas is in the range of Brown Andosols. The vegetation of the study areas is a mixture of sedges (e.g., *Carex nigra*), grasses (e.g., *Agrostis capillaris, Agrostis vinealis, Festuca rubra, Festuca vivipara*), and short forbs (e.g., *Thymus arcticus, Galium boreale, Vaccinium uliginosum, Empetrum nigrum*), with the un-grazed area dominated by *Festuca vivipara*, whereas *Agrostis vinealis* is the most abundant species in the grazed area (Jargalsaikhan 2012). All plant and soil samples were randomly collected along a horizontal area (west to east) at each study area. The study areas were closely comparable to each other by altitude and steepness of the hillside (see 3.1.1). The grazed area was around 500-800 m to the west from the un-grazed area.

3.1.1 Grazing history

The grazed area; Sámsstaðir-Hvítársíðu (64°42.7240' N; 021°09.6710' E; elevation 97 m a.s.l.) is a sheep farm, with 500 winter-fed sheep. The sheep are housed during the winter and let out to graze the hillside after lambing in late May. Most of the flock is driven to mountain pastures in early July, and only a small part of the flock (around 50-60 sheep that are either old or young lambs) remains grazing on the hillside throughout the summer. In the middle of September, the sheep are gathered from the mountains and the whole flock is let out to graze the hillside until middle or late November, when winter sets in.

The un-grazed area; Hvammur-Hvítársíðu (64°42.6050' N; 021°06.0940' E; elevation 101 m a.s.l.) is a dairy farm. The farm had sheep earlier that grazed the hillside comparable to the grazing on the grazed area during that time. In 1980 all sheep were removed from the farm and the hillside fenced off. Only a few heifers grazed part of the fenced area in summers until around year 2000, and from that time the hillside has been totally excluded from livestock grazing within the fence, except for an occasional sheep that managed to go through the fence and was subsequently removed from the site.

3.2 Methodology

In case of studying the effects of grazing by comparing grazed and un-grazed areas, the ideal would have been to have two un-grazed areas, and then start grazing one of them. The approach of this study was to study the effects of grazing on the main compartments of carbon and nitrogen stocks by looking at two “comparable areas” where grazing has been excluded from one of them. Before the 1980s, livestock used to graze in the un-grazed area of our research, similar to the present management of the grazed area. Similarity in elevation, slope and vegetation type was used as criteria when sampling spots were being selected. It was also assumed that the study areas were comparable to each other in soil properties. The assumption was that the only important difference between the study areas was the exclusion of grazing from the un-grazed area.

3.3 Field sampling and experimental design

The sampling within each area was on a horizontal area (50 m long and 20 m wide) along the hillside. Sampling spots within the area were selected randomly. Soil and plant samples were collected to measure the C and N content in the various compartments of the plant-soil system.

Soil samples were collected within a depth of 0-30 cm by soil auger (2 cm diameter) with 5 replications at each experimental area. Each soil sample was randomly collected and included 5 soil cores mixed into a single composite sample to minimize variation in soil properties (Fig. 3). Additionally, soil moisture was randomly measured to a depth of 6 cm (the length of the prongs) by an impedance probe (Theta Probe Delta-T Devices, Cambridge, UK) with 10 replications at each area on 16 June 2012. The Theta Probe generates a 100 MHz sinusoidal signal and measures the impedance of the sampling volume, which is approximately a cylinder 4 cm in diameter and 6 cm long surrounding the centre prong of the probe. More detailed information on the Theta Probe was described by Miller and Gaskin (1997) and Kaleita et al. (2005).

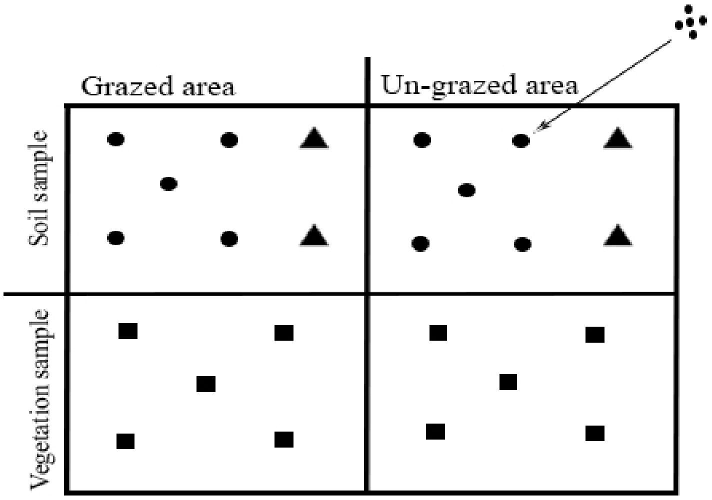

Fig. 3. The sampling design. Symbols representing different samples with replications; ● soil samples; ▲ soil bulk density; ■ plant samples (vegetation and SL)

Bulk density samples were randomly collected horizontally at both areas in separate tubes below 5 cm from the soil surface with 2 replications using the core method as described by Blake and Hartge (1986). The diameter and height of the tubes were 7.4 cm and 7.7 cm, respectively.

Vegetation was clipped to ground level in 25 x 25 cm frames with 5 replications in each study area along the horizontal area. Sward and litter layer samples (SL) were collected down to the soil surface within each clipped frame using 10 x 10 cm frames (Fig. 3).

Soil and plant samples were placed in sealed plastic and paper bags, respectively, and transported to the laboratory immediately for further analysis.

3.4 Laboratory analysis

In the laboratory, all collected soil and plant samples (vegetation and SL) were dried at 60°C in an oven for over 48 hours.

3.4.1 Soil samples

After drying, soil samples intended for C and N analysis were passed through two different mesh size sieves (2 mm and 4.75 mm) to remove plant crowns, roots and determine the gravel content (mineral particles >2 mm). Each soil fraction (<2 mm and >2 mm) was weighed with a high precision electron balance (10^{-2} g). A subsample from each soil sample <2 mm was taken and milled to be homogeneous before C and N analysis. The subsamples were divided into two sub-subsamples; one for C and N analysis using dry combustion (Vario Max CN analyzer) and the other to determine water content of the sub-subsamples, which were dried at 105°C in an oven for 24 hours and weighed again. The water content was calculated as the difference between weights before and after drying. The calculated water content of each sample was used to correct the C and N content of the samples. The volume of the particles >2 mm in each soil sample was determined by the water displacement method as described in Soil Survey Staff (2009).

The bulk density samples were weighed and then dried at 105°C for over 48 hours and weighed again to measure the bulk density. Bulk density samples were also passed through a 2 mm size sieve to determine the density of particles larger than 2 mm and less than 2 mm, which were weighed, respectively. The volume of the particles >2mm in bulk density samples was also determined separately, using the same procedures applied on the soil samples. Bulk density was calculated as the oven-dry mass of soil collected within the tube divided by the volume (calculated from the tube diameter and its height). The bulk density data were also used to convert soil C and N concentrations (%) to C and N mass (gram per m^2) into a depth of 30 cm soil.

3.4.2 Vegetation samples

All plant (vegetation and SL) samples were dried at 60°C in an oven for over 48 hours and weighed with a high precision electronic balance (10^{-2} g). Soil and the smallest organic matter particles of the SL samples were separated from the larger particles using a 2 mm sieve and the two parts were weighed. The organic matter of SL less than 2 mm was analysed with loss on ignition as described by Heiri et al. (2001). The data on loss on ignition were also used to calculate the organic content of SL less than 2 mm. The total amount of organic matter in the SL samples was calculated by the sum of the weight of SL larger than 2 mm and the organic content in SL less than 2 mm.

Two subsamples of each category (vegetation and SL) >2 were taken randomly to measure C and N content. The subsamples were milled to be homogeneous and divided into two sub-subsamples; one for C and N analysis using dry combustion (Varian CN analyzer), and the other to determine water content of that sub-subsamples, which were dried at 105°C in an oven for 24 hours and weighed again. The water content was calculated as the difference between weights before and after drying. The value of the water content of each sample was used to correct the C and N results for the vegetation and sward/litter samples. The average of two subsamples of each category was used to calculate the amount of C and N in the vegetation and SL per m^2 at each study area.

3.4.3 Wetness index

The wetness index of each area was calculated based on vegetation data from Jargalsaikhan (2012). The index was calculated from the coverage estimate of individual vascular plant species, estimated through relative frequency, and the wetness coefficient of that species. A list of wetness coefficients of Icelandic vascular plants was obtained from Hlynur Óskarsson (Hlynur Óskarsson, personal communication, 13 September 2012). The wetness index of each area was calculated in two steps. First the impact of each wetness coefficient was calculated as the sum of the frequencies of all species with that coefficient divided by the sum of the frequencies of all species identified at the site, and multiplied by the wetness coefficient. Then the impact of all wetness coefficients was summed to give the combined wetness index of the area (Carter et al. 1988).

3.4.4 Calculation of total amount of C and N in soil

Before calculation of the amount of carbon and nitrogen per m^2, variables that are necessary to calculate the amount of C and N in soil were measured; these are also described and abbreviated as follows:

1. Bulk density samples (**Bd**) from each site
2. Weight of particles <2mm in the Bd samples: $\mathbf{W_{<2mm}}$
3. Weight of particles >2mm in the Bd samples: $\mathbf{W_{>2mm}}$
4. Volume of the Bd samples: $\mathbf{V_c}$
5. Volume of particles >2mm in Bd samples: $\mathbf{V_{>2mm}}$
6. %C in soil particles <2mm
7. %N in soil particles <2mm
8. Weight of particles <2mm in the soil samples
9. Weight of particles >2mm in the soil samples

The amounts of C and N in the soil (0-30 cm) per m^2 were calculated by examination of their concentrations and soil bulk density as described below:

1. Calculation of the Bd of particles <2mm in the Bd samples as:
   $Bd_{<2mm} = W_{<2mm} / (V_c\text{-}V_{>2mm})$
2. Calculation of the Bd of particles >2mm in the Bd samples as:
   $Bd_{>2mm} = W_{>2mm} / V_{>2mm}$
3. The volume of particles <2mm and particles >2mm in the soil samples were calculated from the weight of these size categories in each soil sample by using the average bulk density of the areas.
   $V(\text{soil sample})_{<2mm} = W_{<2mm} / Bd_{<2mm}$
   $V(\text{soil sample})_{>2mm} = W_{>2mm} / Bd_{>2mm}$
4. Calculation of the ratio of the volume of particles <2mm in each soil sample as:
   $R = V_{<2mm} / (V_{<2mm} + V_{>2mm})$
5. Calculation of the volume of particles <2mm in soil in 1 m^2 down to 30 cm depth as:
   $V(\text{soil})_{<2mm}$ = Volume of (particles <2mm) = Soil volume x R
   Soil volume = 100 cm x 100 cm x 30 cm = 30000 cm^3
6. Calculation of the weight of soil particles <2mm in soil in 1 m^2 down to 30 cm depth as:
   $W(\text{soil m}^{-2})_{<2mm} = V(\text{soil})_{<2mm} \times Bd_{<2mm}$
7. Calculation of the amount of C in soil in 1 m^2 down to 30 cm depth for each soil sample as:
   $C\ m^{-2} = W(\text{soil m}^{-2})_{<2mm} \times \%C / 100$
8. Calculation of the amount of N in soil in 1 m^2 down to 30 cm depth as:
   $N\ m^{-2} = W(\text{soil m}^{-2})_{<2mm} \times \%N / 100$

3.4.5 Calculation of total amount of C and N in vegetation and sward/litter

The variables that are necessary to calculate the total amount of C and N in vegetation and SL were measured before calculation; these are described as follows:

1. The weight of each vegetation sample: $\mathbf{W_B}$
2. The weight of total organic matter in each SL sample: $\mathbf{W_{SL}}$
3. %C in vegetation
4. %N in vegetation
5. %C in SL
6. %N in SL

The amounts of C and N in vegetation and SL per m^2 were calculated by their C and N concentration and the weight of each category as described below:

1. Calculation of the weight of the vegetation biomass and SL organic matter per m^2 as:
   $W_{(B\,m^{-2})} = W_B \times 16$
   $W_{(SL\,m^{-2})} = W_{SL} \times 100$
2. Calculation of amount of C and N in vegetation per m^2 for each vegetation sample as:
   $C\ m^{-2} = W_{(B\,m^{-2})} \times \%C / 100$
   $N\ m^{-2} = W_{(B\,m^{-2})} \times \%N / 100$
3. Calculation of the amount of C and N in SL per m^2 for each SL sample as:
   $C\ m^{-2} = W_{(SL\,m^{-2})} \times \%C / 100$
   $N\ m^{-2} = W_{(SL\,m^{-2})} \times \%N / 100$

Finally, the carbon and nitrogen of the compartments were added directly to get the total carbon and nitrogen per square meter to a depth of 30 cm soil at each study area.

3.5 Statistical analysis

One-way analysis of variance (ANOVA) was conducted for the vegetation and soil properties (C, N and moisture) between the grazed and un-grazed areas. The sampling size of the study was not enough to evaluate the differences at a level of $p \leq 0.05$. Therefore, significant differences for all of the statistical tests were evaluated at the level of $p \leq 0.10$. All statistical analyses were performed using the JMP 8.0 (SAS Institute 2008).

4. RESULTS

4.1 Soil moisture, bulk density and wetness index

Soil moisture was significantly higher in the grazed area as compared to the un-grazed area, with 36.0% and 28.1%, respectively (Fig. 4). The water content determined in the bulk density samples was comparable to the soil moisture measured by the Theta Probe (data not shown). But the average bulk density was higher in the un-grazed area than in the grazed area with 0.8 g cm^{-3} and 0.6 g cm^{-3}, respectively, (Table 1). There was, however, no statistically significant difference in the bulk density between the two study areas ($p = 0.54$), which may have been related to insufficient sample size. The wetness index was 2.0 and 1.8 for the grazed and the un-grazed areas, respectively (plant frequency data not shown).

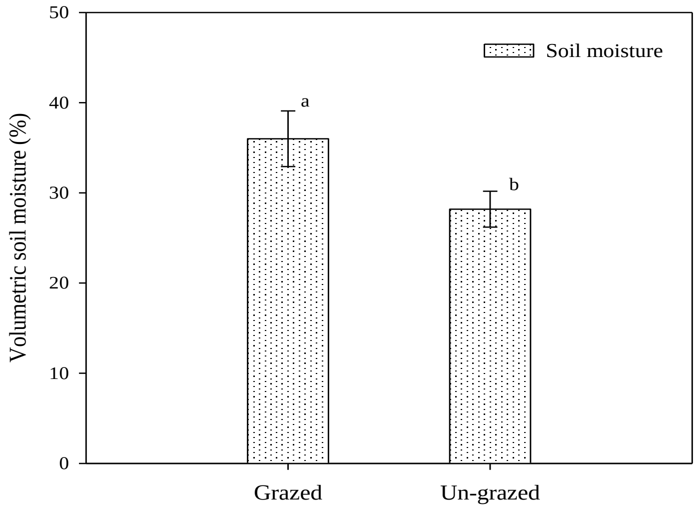

Fig. 4. *Soil moisture (mean ± SE) measured by Theta Probe in the study areas. Bars not sharing the same letter differ significantly at* $p \leq 0.10$.

4.2 Vegetation and sward/litter

Grazing did not significantly affect vegetation biomass nor SL organic matter (p = 0.36; p = 0.16, respectively), even though vegetation biomass and SL organic matter were larger in the un-grazed area (Fig. 5). The vegetation biomass averaged 436.5 g m^{-2} and 291.9 g m^{-2} for the un-grazed and grazed areas, respectively, while the SL organic matter averaged 1626.7 g m^{-2} and 1231.8 g m^{-2}.

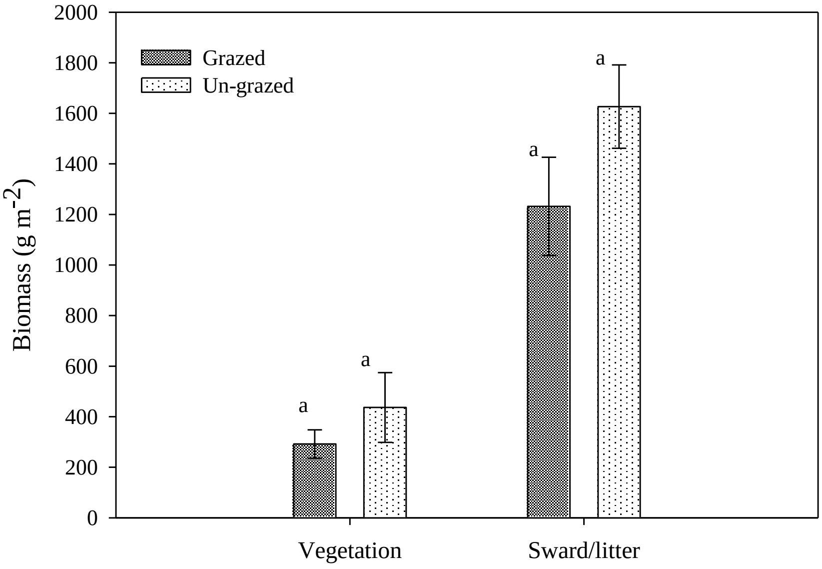

Fig. 5. *Vegetation biomass and sward/litter organic matter (mean ± SE) in the grazed and un-grazed areas. Bars not sharing the same letter differ significantly at* $p \leq 0.10$.

4.3 Carbon and nitrogen content

4.3.1 In soil

ANOVA results showed that the amount of carbon in the 0 to 30 cm profile was significantly different between the two study areas ($p = 0.07$). Soil carbon stock averaged 13.2 kg m^{-2} and 16.9 kg m^{-2} for the un-grazed and grazed areas, respectively (Fig. 6). Similarly, in the grazed area, the amount of nitrogen in the soil (0-30 cm) was significantly higher as compared to the un-grazed area ($p = 0.05$). Soil nitrogen stocks averaged 1.5 kg m^{-2} and 1.1 kg m^{-2} for the grazed and the un-grazed areas, respectively (Fig. 6).

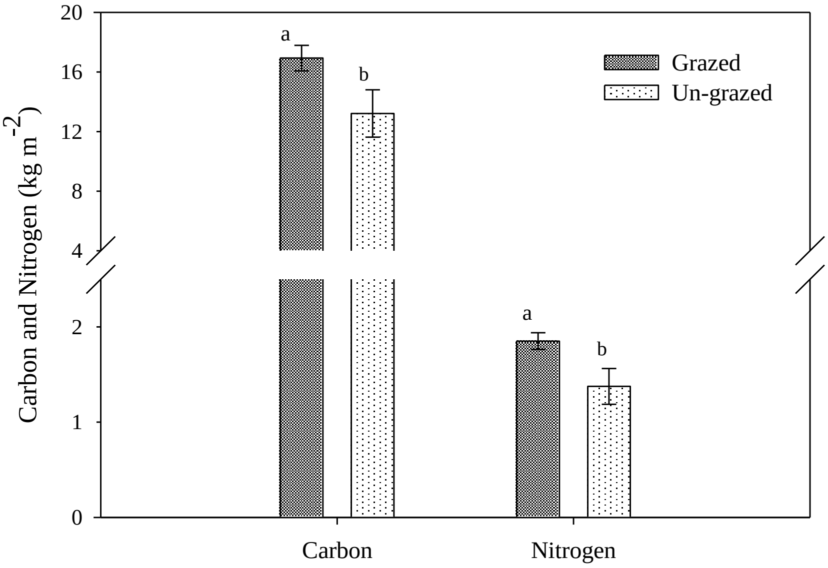

Fig. 6. *The amount of soil carbon and nitrogen stocks (mean ± SE) from the soil surface to 30 cm depth. Bars not sharing the same letter differ significantly at* $p \leq 0.10$.

The concentration of soil C was slightly higher in the grazed area as compared to the un-grazed area, whereas the concentration of soil N was significantly higher (Table 1). The C:N ratio in soil was higher in the un-grazed area; there was, however, no statistically significant difference between the grazed and the un-grazed areas ($p = 0.12$) (Fig. 7).

In the un-grazed area, the vegetation and SL had larger amounts of carbon and nitrogen stocks as compared to the grazed area. But in both categories, the results were not significantly different between the two study areas ($p = 0.39$ and $p = 0.12$, respectively; Fig. 8). In contrast, in the grazed area, the concentrations of C and N in vegetation were higher as compared to the un-grazed area, whereas the concentrations in the SL (>2 mm) were higher in the un-grazed area than in the grazed area. But the results were not statistically significant (Table 1). The carbon stock in vegetation averaged 186.1 g m^{-2} and 128.4 g m^{-2} for the un-grazed and the grazed, respectively, while the carbon stock in the SL averaged 723.8 g m^{-2} and 529.2 g m^{-2} (Fig. 8). Similarly, the amount of nitrogen stock in vegetation and SL samples was higher in the un-grazed area than in the grazed area; however, in both categories, the amount of nitrogen also did not differ significantly ($p = 0.79$ and $p = 0.11$, respectively; Fig. 9).

Table 1. *Summary of the results of calculated C m^{-2} and N m^{-2} stocks in the three ecosystem compartments of the study areas. Measured %C, %N and C:N;↑ means that C, N and C:N ratio increase in the un-grazed area; * means significantly different at level of $p \leq 0.10$; NS means not significantly different.*

	Grazed	Un-grazed	Difference	Significance
Soil moisture (%)	36 ± 9.76	28.2 ± 6.31	↓	*
Bulk density (g cm^{-3})	0.62 ± 0.09	0.8 ± 0.20	↑	NS
Soil				
%C	7.2 ± 0.23	5.9 ± 0.38	↓	NS
C kg m^{-2}	16.9 ± 0.85	13.2 ± 1.58	↓	*
%N	0.6 ± 0.08	0.5 ± 0.12	↓	*
N kg m^{-2}	1.5 ± 0.07	1.1 ± 0.15	↓	*
C:N ratio	11.4 ± 0.15	12.3 ± 0.28	↑	NS
Sward/Litter				
%C	43.0 ± 1.66	44.5 ± 0.51	↑	NS
C g m^{-2}	529.2 ± 83.47	723.8 ± 73.34	↑	NS
%N	1.0 ± 0.07	1.2 ± 0.27	↑	NS
N g m^{-2}	15.5 ± 1.56	20.0 ± 2.02	↑	NS
C:N ratio	35.8 ± 6.01	36.2 ± 0	↑	NS
Vegetation				
%C	44.0 ± 0.41	42.6 ± 1.06	↓	NS
C g m^{-2}	128.4 ± 24.61	186.1 ± 58.91	↑	NS
%N	1.6 ± 0.16	1.2 ± 0.04	↓	NS
N g m^{-2}	4.5 ± 0.86	5.0 ± 1.59	↑	NS
C:N ratio	28.3 ± 0	37.0 ± 0	↑	NS
Total carbon and nitrogen storage				
C kg m^{-2} (0-30 cm)	87.9 ± 0.89	69.7 ± 1.61	↓	*
N kg m^{-2} (0-30 cm)	7.6 ± 0.07	5.6 ± 0.15	↓	*

4.3.2 In vegetation and sward/litter

The nitrogen stock in vegetation averaged 5.0 g m^{-2} and 4.5 g m^{-2} for the un-grazed area and the grazed area, respectively, whereas the nitrogen stock in the SL sample averaged 20.0 g m^{-2} and 15.5 g m^{-2} (Fig. 9). In the un-grazed area, the vegetation and SL samples had a higher C:N ratio as compared to the grazed area, but the difference was not significant (Table 1). The amounts of carbon and nitrogen stocks were higher in the SL organic matter as compared to the vegetation biomass (Fig. 8 and Fig. 9).

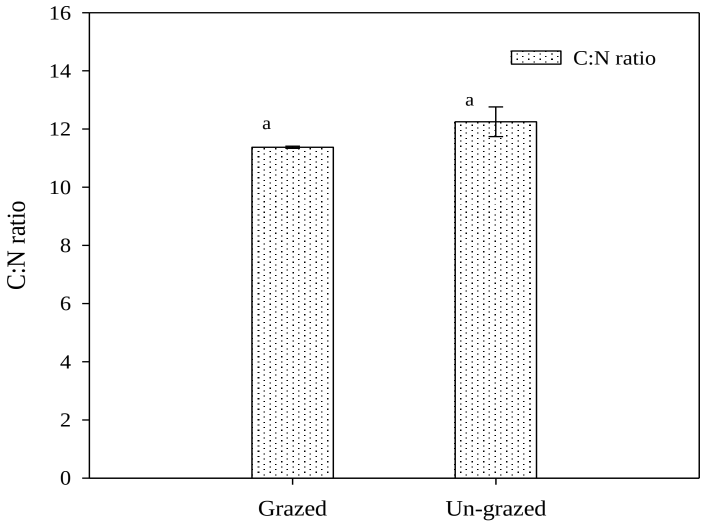

Fig. 7. *The soil C:N ratio (mean ± SE) of the study areas. Bars not sharing the same letter differ significantly at p ≤ 0.10.*

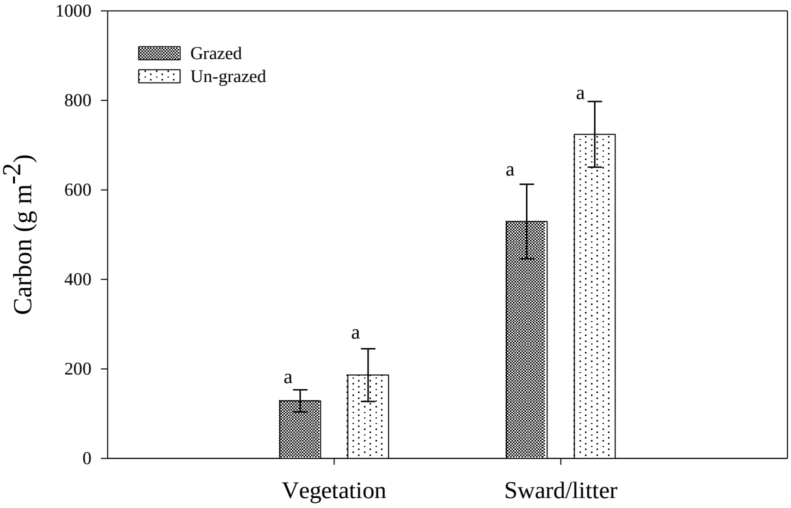

Fig 8. *The amount of carbon stock per m*^{2} *(mean ± SE) in the vegetation biomass and sward/litter organic matter. Bars not sharing the same letter differ significantly at p ≤ 0.10.*

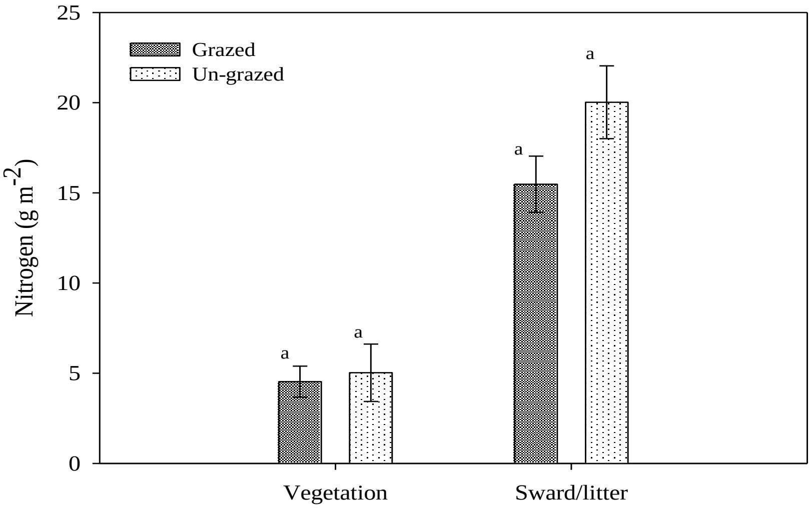

Fig. 9. *The amount of nitrogen stock per m*^{2} *(mean ± SE) in the vegetation biomass and sward/litter organic matter. Bars not sharing the same letter differ significantly at* $p \leq 0.10$.

4.4 Total carbon and nitrogen

Total carbon storage was significantly higher in the grazed area than in the un-grazed area (p = 0.09), with 87.9 kg m^{-2} and 69.7 kg m^{-2}, respectively (Fig. 10). The carbon stock of soil, SL and vegetation accounted for approximately 96%, 3% and 1% of the total carbon storage in the grazed area, respectively, in comparison to 94%, 5% and 1% of the total carbon storage in the un-grazed area.

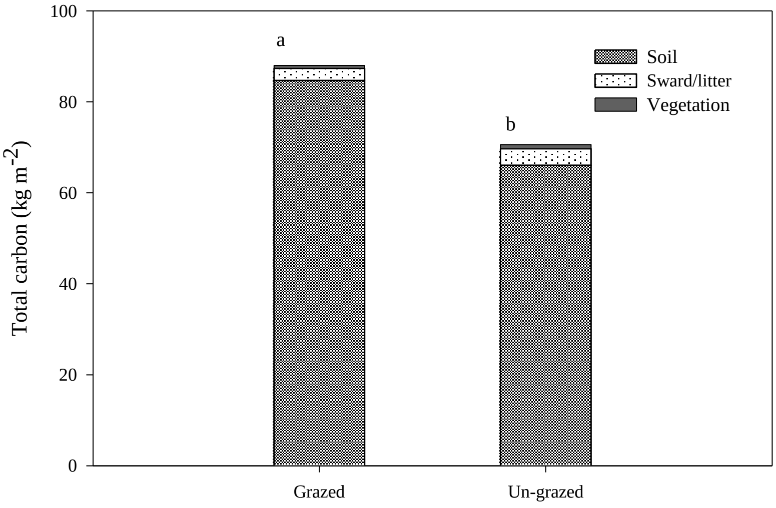

Fig. 10. *Total carbon storage (0-30 cm depth) at the study areas. The bars not sharing the same letter differ significantly at* $p \leq 0.10$.

Similarly, in the grazed area total nitrogen storage was significantly higher as compared to the un-grazed area (p = 0.05), with 7.6 kg m^{-2} and 5.6 kg m^{-2}, respectively (Fig. 11). The nitrogen stock of soil, SL and vegetation accounted for approximately 98.7%, 1% and 0.3% of the total nitrogen storage in the grazed area, respectively, in comparison to 98%, 1.6% and 0.4% of the total nitrogen storage in the un-grazed area. Furthermore, in both study areas the soils had higher amounts of carbon and nitrogen stocks as compared to the other compartments. In addition, in the grazed area, the soil had a higher amount of carbon and nitrogen stocks as compared to the un-grazed area. In contrast, in the un-grazed area, the vegetation biomass and SL organic matter had amounts of carbon and nitrogen stocks as compared to the grazed area (Fig. 10 and Fig. 11). The results of the carbon and nitrogen stocks in the compartments are summarized in Table 1.

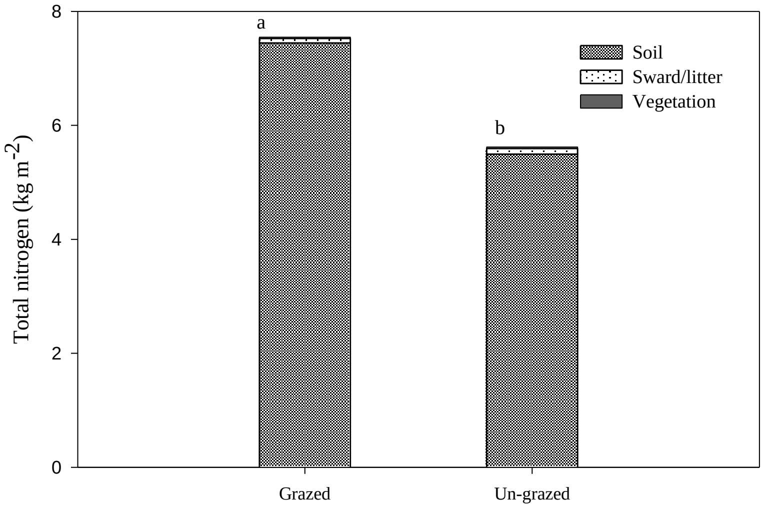

Fig 11. *Total nitrogen storage (0-30 cm depth) at study areas. Bars not sharing the same letter differ significantly at* $p \leq 0.10$.

5. DISCUSSION

The exclusion from grazing did not increase soil carbon and nitrogen storage of the system, which is in contrast with the initial hypotheses, but for the vegetation and sward/litter (SL) compartments it did. The results showed that the carbon and nitrogen stocks in the vegetation and SL were slightly higher on average in the un-grazed area as compared to the grazed area, while the opposite was the case for the carbon and nitrogen stocks in the soil (Table 1). The grazed area had significantly higher amounts of carbon and nitrogen stocks in the soil (0-30 cm depth) (Table 1). The soil at depths of 0-30 cm had 3.7 kg m^{-2} more carbon and 0.4 kg m^{-2} more nitrogen in the grazed area than in the un-grazed area (Table 1). The results of this study were consistent with the findings of Manley et al. (1995) and Schuman et al. (1999) who reported that the soil carbon and nitrogen stocks at a depth of 0-30 cm were higher in the grazed treatment as compared to the exclosure treatment in northern mixed-grass rangeland, Wyoming, USA. Nosetto et al. (2006) also reported that the exclusion from grazing did not increase the carbon storage of the system, and their results suggested two possible explanations: (1) low grazing intensity in the initial condition and (2) slow ecosystem recovery in the time frame of the study. In contrast, some studies reported lower carbon and nitrogen storage in the grazed site than in the grazing excluded site (Pei et al. 2008; Jeddi & Chaieb 2010).

These higher amounts of C and N in soil at the grazed area are possibly due to environmental factors such as soil moisture and soil type. The results indicate that the grazed area had significantly higher soil moisture than that of the un-grazed area (Fig. 4), which is consistent with the findings of Bremer et al. (2001) and Gao et al. (2009) who found that soil moisture was higher in the grazed area than in the un-grazed area. In an alpine meadow on the eastern Tibetan Plateau, Gao et al. (2009) also found that soil C and N concentrations were significantly higher in the heavy grazing site than in the light and moderate grazing site, which was consistent with the results of this study. The results of this study show that the concentrations of C and N in soil were slightly and significantly higher, respectively, in the grazed area as compared to the un-grazed area (Table 1). Soil moisture could increase an accrual of organic matter in soil (Hudson 1994; Saxton & Rawls 2006) if anaerobic conditions are frequent in the grazed area, which would lead to an increase the carbon and nitrogen stocks in the soil (Straková et al. 2012). According to the calculation of the wetness index, the index was higher for the grazed as compared to the un-grazed area, which indicates that the grazed area had more wetland characteristics than the un-grazed area. In our study, the bulk density was also higher in the un-grazed area as compared to the grazed area, which is also consistent with the lower carbon and nitrogen stocks found in the un-grazed area (Table 1).

The soils of the area are not homogeneous but defined as a mixture of Brown Andosols, Histic Andosols and Histosols (Arnalds & Óskarsson 2009). Brown Andosols have less carbon content than Histosols (Arnalds 2004; Óskarsson et al. 2004). Due to the low decomposition rate of organic matter under water saturated conditions, Histosols have higher carbon and nitrogen content, particularly when soil temperature is low (Batjes 1996). The difference in soil moisture observed at the study areas could explain the higher carbon and nitrogen content in the grazed area compared to the un-grazed area if anaerobic conditions were more common in the grazed area. On the other hand, the difference in soil moisture could possibly be explained by different hydrological preconditions, e.g. vertical soil water flow of the areas or changes in hydrological parameters such as water holding capacities, infiltration rate, and interception or runoff introduced by grazing.

Therefore, more research is needed on the hydrological parameters of the study areas to determine the interaction of environmental factors (soil moisture), soil C and N stocks and grazing. Another lesson from this study is that it is important to ensure that study areas have the same preconditions prior to grazing exclusion.

The results presented in the summary table indicate that exclusion from grazing has a positive effect on vegetation biomass and SL organic matter (Fig. 5) but differences were not significant. This effect has important consequences for ecosystem recovery and soil properties (Jeddi & Chaieb 2010). The increases in C and N stocks in vegetation and SL in the un-grazed area are explained by the higher amount of SL and vegetation registered in the un-grazed area (Fig. 5). In addition, the %C and %N in SL contributed to this difference, but for the vegetation the concentration of C and N was less in the un-grazed area (Table 1). Reeder and Schuman (2002) reported that grazing exclusion can limit cycling of aboveground C and N, and thus carbon and nitrogen are immobilized in plant compartments accumulating on the soil surface, a result also reported by Manley et al. (1995) and Schuman et al. (1999). The accumulation of SL and vegetation on the soil surface can affect soil temperature and soil moisture, which in turn influences decomposition and the formation rate of the soil organic matter as well as carbon and nutrient cycling (Reeder et al. 2001; Straková et al. 2012). The results showed that the vegetation biomass and SL organic matter were higher in the un-grazed area than in the grazed area (Fig. 5), which was consistent with Li et al. (2012) who reported that the carbon and nitrogen storage in biomass was increased significantly after 7-25 years exclusion from grazing in the Horqin Sandy Grassland, Inner Mongolia, China.

The results of this study also indicate that in the grazed area, the surface coverage is weakened as compared to the un-grazed area, and thus the grazed area could have less resistance to eroding forces such as wind and water.

In addition, the C:N ratio was consistently higher in the un-grazed area as compared to the grazed area in all compartments (Table 1). The difference in C:N ratio between the study areas indicates that exclusion from grazing had affected more total carbon storage than total nitrogen storage in the system, which is in agreement with the results of Pei et al. (2008).

6. CONCLUSIONS

The area excluded from grazing had larger C and N stocks in the vegetation and sward/litter compartments, but for the soil it was the opposite. More than 90% of the C and N of the system were in the soil, whereas less than 10% of the C and N were in the vegetation (Fig. 10 and Fig. 11). Therefore the soils were the largest reservoir of the carbon and nitrogen storage of the system as compared to other compartments. The C and N stocks in the soil were significantly higher in the grazed area than in the un-grazed area (Table 1) thus rejecting the initial hypothesis. Although the increases in C and N stocks in the vegetation and sward/litter indicated the recovery of the ecosystem after exclusion from grazing, we failed to detect significant changes in carbon and nitrogen in those compartments. The observed difference in soil carbon and nitrogen stocks may have been the result of grazing or different soil types in the study area, insufficient sampling and environmental factors (soil moisture) or pre-conditions before exclusion from grazing. Hence, further studies to investigate the effects of exclusion from grazing are required, not only to understand the ecology of this fragile ecosystem but also to enhance sustainable grazing management.

ACKNOWLEDGEMENTS

I would like to thank the directors of the UNU-LRT Programme, Hafdís Hanna Ægisdóttir and Berglind Orradóttir, giving me the opportunity to be one of the UNU-LRT fellows, 2012, and programme assistants, Þorbjörg Valdís Kristjánsdóttir, Rannveig Ólafsdóttir and Muhammad Azfar Karim for taking care of us during the training period.

My special and deepest thanks go to my supervisor, Jón Guðmundsson, for his guidance and encouragement throughout the entire period of the individual project and writing.

I want to acknowledge all lecturers of the Agricultural University of Iceland and Soil Conservation Service for giving interesting talks and sharing their knowledge and experience with us as well as the laboratory assistance of Anne Bau and Brita Berglund for their guidance in laboratory analysis.

I would like thank Sigmundur Helgi Brink for his help with the ArcGis programme.

I am grateful to the director and staff of the Soil Conversation Service in Gunnarsholt for hosting and all the different kinds of support that you have provided.

I would also like to thank the UNU-LRT fellows, 2012, for their support and sharing their knowledge and experience and spending time together.

For those whom I have not mentioned, I say “thank you”.

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