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Philosophiae Doctor (PhD) Thesis 2011:10

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Summary

Grazing in outlying fields has a long history and is important in local communities worldwide. Alpine ecosystems, representing a large proportion of the land area in Europe, are under pressure from changes in climate and land-use. During the last decades grazing pressure has both decreased and increased in alpine ecosystems, but little is known about the effects on soil carbon (C) storage and nitrogen (N) cycling. As part of a sheep grazing experiment in a low alpine system at Hol, southern Norway, with three density levels of sheep; no sheep (control), 25 sheep km^{-2} and 80 sheep km^{-2}, we determined effects of grazing on C and N in organic horizons of grassland soils. In addition, effects of grazing on leaching of nutrients and coliform bacteria were assessed.

After 7 years of grazing, high sheep density reduced soil carbon concentration and carbon stocks at equivalent soil mass as compared to control. By contrast, the opposite was observed at the low sheep density. The form of soil organic matter (SOM), expressed as ratios of particulate organic C to soil organic C, and the lability of SOM (potential carbon mineralization) were only slightly affected by grazing. The lability of SOM was not affected by grazing directly, but significantly related to the mineral content of the O-horizons. In general, there were large differences between plant communities (i.e. snowbed and grasslands with willow-shrub) for several soil attributes.

The grasslands were characterized by a tight N-cycling with low concentrations of inorganic N in soil water and surface runoff, and small rates of potential N mineralization. There was a strong seasonal effect on inorganic N of the soil water, with decreasing values in the course of the growing season, probably due to increasing nutrient demand. Effects of grazing on bioavailable N (as determined by buried PRS^{TM} exchange resins) were greater at low as compared to high altitudes. At low altitudes, low sheep density reduced amounts of bioavailable N. In turn, significantly greater rates of potential N mineralization were found at sites with high sheep density compared to those with low density or no grazing. However, effects of grazing on distribution and cycling of N as determined using $\delta^{15}N$ natural abundance and added $^{15}NH_4$-N were small.

Sheep grazing significantly reduced infiltration rates and increased concentrations of coliform bacteria in stream water indicating risks for drinking water quality in areas affected by grazing. There was, however, no indication of grazing induced risk in losses of nutrients.

A low grazing pressure in this system is positive for soil carbon storage. In terms of N, these findings suggest that grazing may stimulate N-cycling, but the availability of N remains

low. Nitrogen removal through grazing is small compared to the total soil N pool. This suggests that grazing in this low alpine ecosystem is sustainable from a nutrient point of view.

Sammendrag

Beite i utmark har lange tradisjoner og er viktig i lokalsamfunn over hele verden. Alpine økosystemer, som representerer en stor andel av landarealet i Europa, er under press med tanke på endringer i klima og arealbruk. I løpet av de siste tiårene har beitetrykket både gått ned og økt i alpine systemer, men det er lite kunnskap om effekter på karbon (C) lagring og nitrogen (N) omsetning i jord. Som en del av et sauebeiteprosjekt i et lavalpint område i Hol, sør Norge, med tre tetthetsnivåer av sau; ikke beitet (kontroll), 25 sau km^{-2} and 80 sau km^{-2}, var formålet med studien å undersøke effekter av beite på C og N i organiske sjikt i grasmark. I tillegg ble effekter av beite på avrenning av næringsstoffer og koliforme bakterier undersøkt.

Etter 7 års beiting var karbonkonsentrasjonen og karbonlageret basert på ekvivalent jordmasse redusert ved høy tetthet av sau sammenlignet med kontroll, i motstetning til for lav sauetetthet hvor det økte. Formen av organisk materiale (SOM) i jord, uttrykt som forholdet mellom partikulært C og totalt jord organisk C, samt labiliteten av SOM (potensiell karbonmineralisering) var i liten grad påvirket av beite. Labiliteten av SOM var ikke påvirket av beite direkte, men signifikant relatert til mineralinnholdet i O-sjiktene. Det var generelt store forskjeller mellom plantesamfunn (snøleier og grasmark med vier) for flere jordattributter.

Grasmarkene var karakterisert med en lukket N sirkulering, med lave konsentrasjoner av uorganisk N i jordvann og overflateavrenning, og lav potensiell N mineralisering. Det var en sterk sesongeffekt på uorganisk N i jordvann med nedadgående konsentrasjoner gjennom vekstsesongen, antakeligvis på grunn av et høyt næringsbehov. Effekter av beiting på biologisk tilgjengelig N (undersøkt ved hjelp av nedgravde PRS^{TM} ionebyttere) økte med høyde over havet. Ved lavere høyder ble biologisk tilgjengelig N redusert ved lav sauetetthet. Videre ble det funnet signifikant høyere rater av potensiell nitrogenmineralisering ved høy sauetetthet i forhold til lav tetthet og kontroll. Effekter av beiting var imidlertid små når det ble undersøkt basert på δ^{15}N natural abundance og tilført ^{15}NH$_4$-N.

Infiltrasjonskapasiteten ble signifikant redusert ved sauebeite. I tillegg økte konsentrasjonene av koliforme bakterier, noe som indikerer en risiko for drikkevannskvaliteten i områder berørt av beite. Det ble imidlertid ikke funnet noen indikasjon på økt risiko for tap av næringsstoffer som følge av sauebeite.

Et lavt beitetrykk er positivt for dette systemet med tanke på karbonlagring. Med tanke på N tyder disse funnene på at beite kan stimulere N omsetning, men at tilgjengeligheten allikevel forblir lav. Nitrogenuttak med sau er liten sammenlignet med det totale lageret av N

i jorden. Dette tyder på at beiting i dette lavalpine økosystemet er bærekraftig med tanke på næringsstoffer.

List of papers

Paper I

Martinsen, V., Mulder, J., Austrheim, G. and Mysterud, A., 2010. **Carbon storage in low-alpine grassland soils: effects of different grazing intensities of sheep**. *Submitted to European Journal of Soil Science.*

Paper II

Martinsen, V., Mulder, J., Austrheim, G., Hessen, D.O. and Mysterud, A., 2010. **Effects of sheep grazing on availability and leaching of soil nitrogen: controlled experiment in low alpine grassland soils**. *Submitted to Arctic, Antarctic and Alpine Research.*

Paper III

Martinsen, V., Austrheim, G., Mysterud, A. and Mulder, J., 2010. **Effects of herbivory on N-cycling and distribution of added $^{15}NH_4^+$ in N-limited low-alpine grasslands**. *Submitted to Plant & Soil.*

Paper IV

Martinsen, V., Grund, F., Ness, M., de Wit, H.A., Austrheim, G., Mysterud, A. and Mulder, J., 2010. **Variations in surface water quality caused by plant community and grazing at an alpine site in Hol, Southern Norway**. *Submitted to Boreal Environment Research.*

Introduction

Low intensity livestock farming produce about half of the world's food (Herrero *et al.* 2010). Managed grazing systems, defined as "any geographically extensive operation designed for the production of animals for consumption, including for meat, milk, and any major animal products", covers more than 25 % of the global land surface (Asner *et al.* 2004). These systems occupy bioclimatically and edaphically marginal lands throughout much of the world (Asner *et al.* 2004). This is also the case in Norway, where there is a long history of using marginal fields for low intensity grazing (Austrheim *et al.* 2008a; Olsson *et al.* 2000; Warren 1998). Although sheep grazing pressure has remained relatively stable in Norway since 1950, the proportion of sheep grazing in mountain areas has increased. In 1999, 75 % of all sheep in Norway grazed in the northern boreal and alpine region (Austrheim *et al.* 2008c). Despite the ecological and economic importance of these unfertilized, semi natural ecosystems, detailed investigations about effects of different grazing intensities on belowground C and N dynamics with implications for surface water quality are rare. Most studies assessing effects of grazing on these processes are either conducted in intensively managed systems or by means of grazing exclosures (i.e. "grazed" vs. "not grazed"). In terms of sustainable management, being important for ecosystem functioning and thus potentials for mitigating climate change (Laca *et al.* 2010), more information on belowground processes and properties as affected by grazing in these marginal outlying fields is required.

Grazing by large herbivores may have large effects on ecosystem structure and processes (Bowns & Bagley 1986; Hobbs 1996; van der Wal & Brooker 2004; vandeKoppel *et al.* 1997; Wardle & Bardgett 2004; Wardle *et al.* 2004). Several mechanisms controlling ecosystem productivity and diversity may simultaneously be altered by herbivory. These mechanisms include changes in the quantity and quality of resources and changes in successional trajectory (Wardle & Bardgett 2004), all of which are important for linkages and feedback processes between aboveground and belowground compartments. Herbivory may drive systems between alternate states (Briske *et al.* 2005; vandeKoppel *et al.* 1997). The direction and magnitude of change largely depends on the idiosyncratic nature (e.g. forest or grassland, dry or wet) of different habitats within ecosystems in addition to the herbivore density, selectivity and size, as well as the time scale of grazing treatment (Augustine & McNaughton 1998; Beaulieu *et al.* 1996; Pastor *et al.* 1993; Pastor *et al.* 1997; Wardle & Bardgett 2004; Whitehead 2000).

The current knowledge of how indirect and direct impacts of herbivory affect belowground and aboveground properties and processes of grassland habitats is outlined in Figure 1. Plant community composition and productivity and the dynamics of soil organic matter (SOM) are strongly controlled by the context factors which are not directly related to herbivory (Figure 1; nitrogen (N)-deposition, climate, land use history, topography and parent material). These factors have direct and indirect impacts on nutrient cycling and productivity by controlling temperature, moisture, and supply of N (Piñeiro *et al.* 2010; Schlesinger 1997; Torn *et al.* 2009). Thus, by constituting a great influence on edaphic properties and processes, the context factors are important determinants of storage and potential losses of carbon (C) and nutrients from a system (Piñeiro *et al.* 2010; Post *et al.* 1982; Post *et al.* 1985; Schlesinger 1997; Torn *et al.* 2009). Furthermore, superimposed differences associated with location, grazing by herbivores may modify and affect the above mentioned processes and properties (Figure 1). Impact of grazing will be described in more details in the section "specific background and hypothesis".

Arctic and alpine ecosystems, with their short growing season, low temperatures and heterogenic vegetation structure (Körner 2003; Nagy & Grabherr 2009), differ considerable from lowland systems. Biogeochemical processes are limited by temperature (Hagedorn *et al.* 2010) and the biomass production is smaller than in lowland systems (Nagy & Grabherr 2009). Despite a small input of plant biomass, there is evidence for greater pools of labile SOM at higher altitudes (Christensen 2001; Leifeld *et al.* 2009). Mountainous regions are subject to a number of drivers of ecosystem change, such as climate (Beniston 2009), land use (Austrheim *et al.* 2008c) and N deposition (Hole & Engardt 2008). These changes may affect the storage of carbon (Kammer *et al.* 2009) and the nutrient retention capacity of these regions.

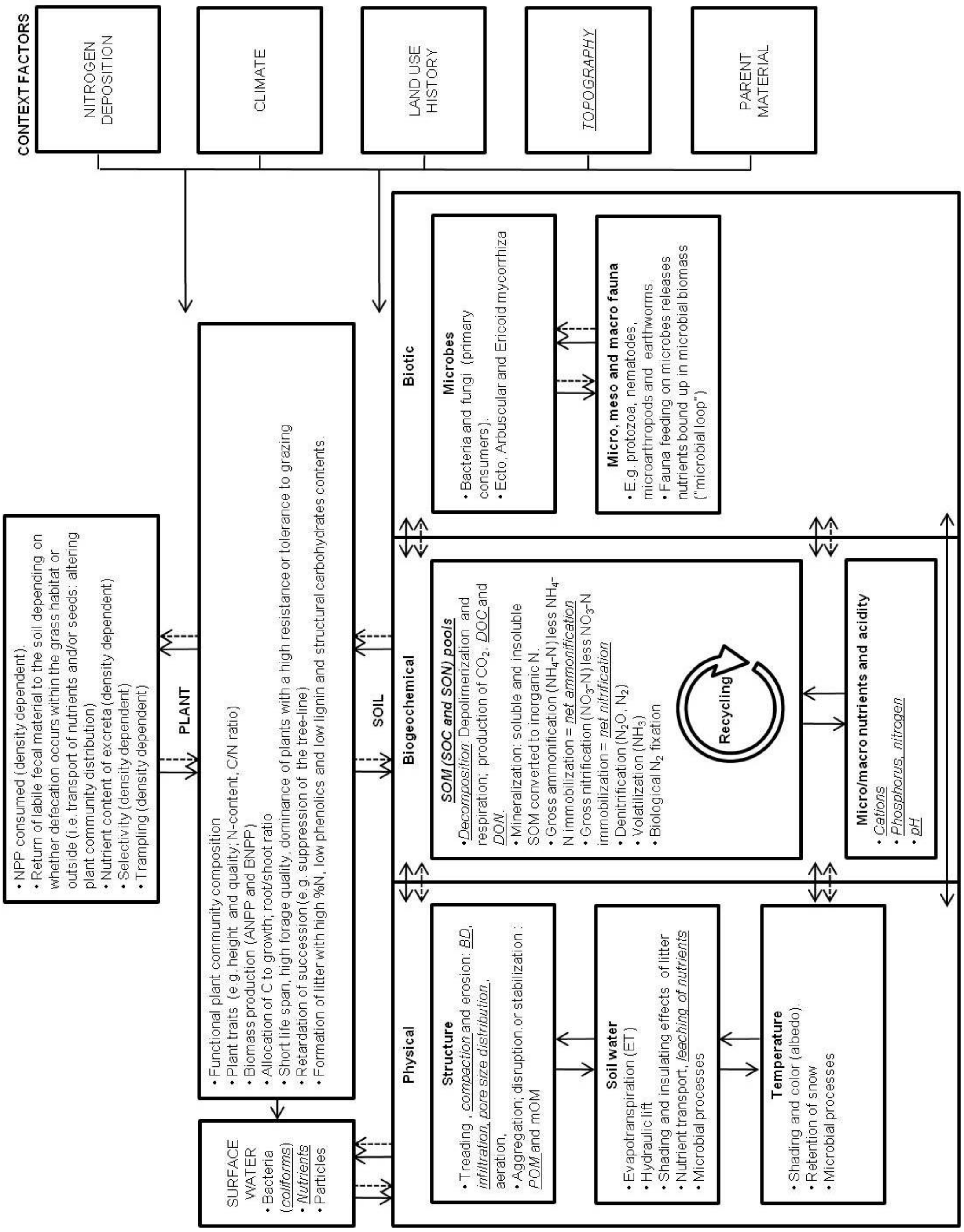


Figure 1. Linkages between aboveground and belowground properties and processes as affected by context factors and herbivory in productive (grazing preferred) grassland habitats. Grazing in these habitats generally impose positive feedbacks (Bardgett & Wardle 2003; Wardle *et al.* 2004). Direct and indirect linkages (controls) between compartments are described by solid and dashed arrows, respectively. Factors or processes in *italics with an underscore* are *directly or indirectly assessed in this study*. The figure is modified after figure 1 in Wardle *et al.* (2004), Piñeiro *et al.* (2010) and Evju *et al.* (2009), and figure 2 in Bardgett & Wardle (2003), Ehrenfeld *et al.* (2005) and Piñeiro *et al.* (2010). NPP = net primary production, ANPP and BNPP = above and belowground NPP, C = carbon, N = nitrogen, SOM = soil organic matter, SOC and SON = soil organic C and N, DOC and DON = dissolved organic C and N, BD = bulk density, POM = particulate organic matter, mOM = mineral associated organic material.

Objectives

The main objectives of this study were to determine effects of different grazing intensities of sheep on soil carbon (C) and nitrogen (N), and leaching of nutrients and coliform bacteria at a semi natural low alpine experimental site in Hol, southern Norway.

Sub-goal 1: Determine effects of grazing, taking into account site heterogeneity associated with plant community, on C storage, form (particulate organic carbon to total soil organic carbon ratio; POC to SOC ratio) and lability (potential C mineralization) in organic horizons (Paper I).

Sub-goal 2: Determine effects of grazing superimposed differences associated with plant community and seasonal changes on *in situ* availability of inorganic N (buried PRS^{TM} exchange resins and N content of plants), potential N mineralization and leaching of N (Paper II). In addition, N cycling was assessed by means of N content in plants, $\delta^{15}N$ natural abundance and distribution of added $^{15}NH_4$-N (Paper III).

Sub-goal 3: Compare (1) surface runoff water quality between three alpine plant communities of increasing productivity (snowbed, dwarf shrub heath and tall herb meadow) and (2) determine effects of two density levels of sheep (no sheep and high sheep density) on infiltration capacity and pore size distribution within snowbeds and tall herb meadows. In addition, we assessed effects of the two density levels of sheep on concentrations of nutrients and potential of grazing induced fecal contamination of stream water (Paper IV).

Specific background and hypothesis

In the next sections I will give a more detailed description of some factors and processes potentially being affected by grazing which have been studied in this thesis (highlighted in Figure 1). These include storage of carbon (C) and nitrogen (N), lability and form of soil organic matter (SOM), and cycling and availability of N. In the last section, I highlight some factors important for surface water quality. I will use this background to formulate main hypothesis on effects of grazing on the factors and processes mentioned above.

Soil organic matter (SOM)

Soil organic matter (SOM) is an important soil fraction affecting soil attributes and processes known to influence ecosystem functioning and productivity (Dawson & Smith 2007; Schlesinger 1997; Whitehead 2000). Organic matter in soils is the largest terrestrial carbon (C) reservoir (Schlesinger 1997) and may be a potential source or sink of C to the atmosphere (Torn *et al.* 2009). Mineralization of SOM controls the input of nitrogen (N) to plants (Bardgett 2005; Whitehead 2000). The amount of organic matter stored in soils at different stages of succession is determined by the equilibrium between inputs of plant derived material and losses by decomposition and leaching (Cebrian & Duarte 1995; Dawson & Smith 2007; Ehrenfeld *et al.* 2005). Furthermore, accumulation and losses of SOM is controlled by natural, site specific factors like parent material, climate, topography and land cover (Piñeiro *et al.* 2010; Post *et al.* 1982; Schils *et al.* 2008; Torn *et al.* 2009). These factors may indirectly affect SOM due to a change in e.g. temperature, moisture and acidity (Darmody *et al.* 2004; Dawson & Smith 2007), or cause changes in primary production and decomposition that directly affect SOM (Cebrian & Duarte 1995; Dawson & Smith 2007; Piñeiro *et al.* 2010). Differences in site specific factors result in a high variability in SOM quantity and quality which may be substantial even on small spatial scales (Burke *et al.* 1999; Hiller *et al.* 2005; Ostler *et al.* 1982; Stanton *et al.* 1994).

Carbon (C)

According to Piñeiro *et al.* (2010) grazing may alter the content of soil organic carbon (SOC) through, (1) affecting the fraction of net primary production (NPP) entering the soil (i.e. the net primary production pathway), (2) affecting the soil nitrogen storage (i.e. the nitrogen pathway) and (3) affecting the decomposition of soil organic matter (i.e. the decomposition pathway) (Piñeiro *et al.* 2010). Grazing has been reported to increase (Leifeld & Fuhrer 2009;

Manley *et al.* 1995), decrease (Steffens *et al.* 2008) or have little or no effect (Steffens *et al.* 2008; Tracy & Frank 1998) on storage of soil organic carbon (SOC) in surface soils. The direction of grazing induced change in SOC storage depends on ecosystem productivity (Wardle *et al.* 2004) and grazing intensity (Steffens *et al.* 2008). A high grazing pressure may decrease the input of above (Tracy & Frank 1998) and below ground (root) biomass (Johnson & Matchett 2001), thereby reducing the storage of soil carbon (He *et al.* 2008). Moreover, stimulation of N-mineralization by herbivory may increase the N content of plants (Tracy & Frank 1998) and thus the decomposability. As plant turnover rates control the size and turnover of detrital C pools (Cebrian & Duarte 1995), indirect effects of grazing on N-cycling may also have impact on SOC storage of soils.

Grazing can affect the form of SOC including the free light particulate organic material (POM) fraction that is uncomplexed organic matter neither recognizable as litter nor associated with organomineral complexes (Christensen 2001). Particulate organic matter consists mainly of root fragments and aboveground plant residues (Golchin *et al.* 1994). As reported by Leifeld et al. (2009), this fraction is relatively young, with a mean residence time in the order of years to decades (Leifeld *et al.* 2009). Leifeld & Fuhrer (2009) found an increased ratio of particulate organic carbon (POC) to soil organic carbon (SOC) in top-soils of a frequently grazed pasture compared to a meadow grazed for short periods in the Swiss Alps, which was suggested to be due to incorporation of plant materials by treading (Leifeld & Fuhrer 2009). In contrast, Steffens *et al.* (2009) observed a larger contribution of free POM to the total SOM within grazing exclosures due to enhanced litter inputs (Steffens *et al.* 2009). POM is a potential source of readily available C for decomposers (Christensen 2001) and is more mineralizable than heavy SOM fractions (Whalen *et al.* 2000). Short-term shifts in SOC storage and turnover are associated with the dynamic nature of POM (Post & Kwon 2000).Thus changes in the POC to SOC ratio due to grazing induced effects on physical or chemical mechanisms of stabilization (i.e. effects on decomposition) (Dawson & Smith 2007; Torn *et al.* 2009) and/or removal of biomass may potentially affect the lability of SOM.

Nitrogen (N)

Nitrogen (N) is a limiting nutrient for plant growth in terrestrial ecosystems (Vitousek & Howarth 1991). Particularly, alpine and arctic ecosystems with their short growing season and low temperatures are strongly N limited (Bowman *et al.* 1993; Frank 2008; Grellmann 2002; Shaver & Chapin 1980). Nitrogen mineralization in these systems is small (Chapin *et al.* 1988; Giblin *et al.* 1991; Nadelhoffer *et al.* 1991), differs between plant communities (Bjork *et al.* 2007; Makarov *et al.* 2003), and varies during the growing season (Bardgett *et al.* 2002; Frank 2008; Morecroft *et al.* 1992a). Grazing by large herbivores may alter the availability of N in these systems through direct and indirect impacts on ecosystem processes and properties important for N cycling (Ewing *et al.* 2010; Frank 1998; Frank & Evans 1997; Frank *et al.* 2000; van der Wal & Brooker 2004). Direct impacts of grazing include trampling, plant defoliation and nutrient return in form of urea and faeces (McNeill & Unkovich 2007; van der Wal *et al.* 2004). In turn, this may affect soil properties (e.g. temperature, moisture and bulk density), vegetation cover (e.g. composition, biomass and nutritional status) and recycling of N (McNeill & Unkovich 2007).

Herbivory may enhance (Frank 1998; Hobbs 1996; Tracy & Frank 1998) or retard (Harrison & Bardgett 2004; Pastor *et al.* 1993) N mineralization. Effects of grazing on N mineralization (i.e. positive or negative) depends on ecosystem productivity (Bardgett & Wardle 2003; Wardle *et al.* 2004) and herbivore selectivity (Pastor *et al.* 1997). The extent to which recycling of nutrients through the excreta is affected by grazing, depends on the herbivore density and the concentration of nutrients in the forage consumed (Whitehead 2000). Mobæk *et al.* (2009) reported herbivore density-dependent selection for productive habitats. This pattern has later been associated with a larger proportion of high quality forage ingested by sheep at small densities than at great densities (Mysterud *et al.* 2010). Christianson & Creel (2009) found a positive relationship between N content in faeces of elk (*Cervus elaphus*) and the quality of forage grasses (expressed as faecal chlorophyll). In turn, Kyvsgaard *et al.* (2000) showed a positive correlation between percentage N in faeces (from sheep) and net mineralization of faeces N. Thus, impacts of grazing on soil N-mineralization may differ between grazing regimes (Figure 1).

Changes in decomposition and mineralization rates may induce positive feedbacks (i.e. increased uptake) of N in plants (Ehrenfeld *et al.* 2005). Increased N availability may increase N concentration and/or plant tissue production (Nagy & Grabherr 2009). Hobbie & Gough (2002) found positive relationships between foliar N content and net N mineralization rates at two tundra sites dominated by moist tussock tundra in northern Alaska. Furthermore, plants take up primarily NO_3^- , NH_4^+ and organic N-compounds (Näsholm *et al.* 1998; Whitehead 2000). The acquisition and content of nutrients in plants is related to nutrient concentrations in the soil solution (Chapin 1980; McNeill & Unkovich 2007) and phenological stage (Mysterud *et al.* 2010). Soil solution concentrations of N and plant N contents may vary with season (Arndal *et al.* 2009; Elberling *et al.* 2008; Morecroft *et al.* 1992b; Mysterud *et al.* 2010), species specific adaptations to N uptake (Bradshaw *et al.* 1964; Gigon & Rorison 1972) and rates of N-mineralization (Detling 1998; Ehrenfeld *et al.* 2005; Hobbie & Gough 2002). Hence, grazing induced increase in N-cycling (e.g. ammonification, nitrification and/or denitrification) and physical influence (e.g. trampling and plant removal) may change the quantity and form of N taken up by plants, and therefore the $\delta^{15}N$ (natural abundance) in plants (Högberg 1997; Hyodo & Wardle 2009; Marshall *et al.* 2007; Nadelhoffer *et al.* 1996).

The $\delta^{15}N$ of a system will change only if N-pools with different $\delta^{15}N$ are mixed or if a N sink resulting from N-transformations associated with isotope fractionation is lost from the system (Robinson 2001). Since several key processes in N-cycling involve isotope fractionation [e.g. NH_3 volatilization, nitrification and denitrification (Robinson 2001)] the natural abundance of ^{15}N has been used as an indicator of the impact of various drivers on turnover and availability of N (Frank & Evans 1997; Frank *et al.* 2000; Garten *et al.* 2007; Högberg *et al.* 1996; Hyodo & Wardle 2009; Makarov *et al.* 2008; Menezes 2009). Isotopic enrichment (increased $\delta^{15}N$ of total soil-N) with soil depth is found in forests (Högberg *et al.* 1996) and in alpine and arctic tundra (Makarov *et al.* 2008). This is probably caused by accumulation of recalcitrant (more decomposed) organic matter in deeper horizons (Högberg 1997). In Yellowstone National Park, Frank & Evans (1997) found an increased soil $\delta^{15}N$ in ungulate urine and dung patches, which was related to enhanced N-loss via leaching, ammonia volatilization and/or denitrification. By contrast, due to a grazing induced stimulation of microbial productivity, Frank *et al.* (2000) report decreased soil $\delta^{15}N$ in areas being grazed vs. not grazed, despite herbivore induced stimulation of net N mineralization and nitrification at grazed sites.

In addition to $\delta^{15}N$ natural abundance, applications of ^{15}N-enriched tracers are used to study N-cycling in different environments (Näsholm *et al.* 1998; Providoli *et al.* 2005; Rütting *et al.* 2010). Ewing *et al.* (2010) clearly show the importance of detritus and soil moisture controlling movement of N in Yellowstone grasslands. Despite a lack of grazing induced differences in plant ^{15}N, they found a greater retention of added ^{15}N in litter from historically ungrazed compared to grazed sites, due to greater litter pools at ungrazed sites. A great tracer recovery in soils has been reported by e.g. Gerzabek *et al.* (2004), Näsholm *et al.* (1998) and Hagedorn *et al.* (2005). Hagedorn *et al.* (2005) found inorganic inputs of N to be stabilized more effectively (in stable mineral associated humus pools) than C.

Despite a tight N-cycling in arctic and alpine areas due to strong plant and microbial demand for nutrients (Gerzabek *et al.* 2004; Jaeger *et al.* 1999; Nadelhoffer *et al.* 1991), herbivore induced increase in N cycling, can increase the risk of N loss from the system (Dahlin *et al.* 2005; Frank & Groffman 1998; Haynes & Williams 1992; McNeill & Unkovich 2007; Whitehead 2000). High concentrations of nitrate in soil solutions under urine and faeces patches was reported by Haynes & Williams (1992) and Shand & Coutts (2006), respectively. Depending on environmental conditions, losses through volatilization (e.g. NH_3, N_2O and N_2) (Frank & Groffman 1998) and leaching of N into the subsoil or into a drainage system can be significant (Whitehead 2000). Grazing may thus have severe implications for surface water quality.

Surface water quality

In a small catchment of Dartmoor, UK, Meyles *et al.* (2006) found clear effects of sheep grazing on bulk density (increased), porosity (reduced), and soil water content at standard matric pressures (reduced). They argue that grazing, even without removing the vegetation completely, may enhance soil water movement as observed by a reduced wetness threshold (i.e. field capacity is reached more rapidly) at intensively grazed sites. A more rapid soil water movement increases the flow of water to streams (Meyles *et al.* 2006). Furthermore, input of nutrient in form of urea and faeces in addition to changed nutrient dynamics and physical impact by grazing can increase loads of fecal bacteria, nutrients and sediments to surface runoff due to removal of vegetation and reduced soil infiltration (Derlet *et al.* 2008; Elliott & Carlson 2004; Meyles *et al.* 2006; Muirhead *et al.* 2006).

Not only may grazing affect surface runoff quality. Structural and functional differences in addition to edaphic factors associated with plant community (Nielsen *et al.* 2009; Strand *et al.* 2008; Vinton & Burke 1997) also may have impact on the quality of water leaching from different plant communities (Palmer *et al.* 2001; Sjøeng *et al.* 2007; Strand *et al.* 2008). The vegetation cover in alpine areas consists of a mosaic of different plant communities determined by climatic, topographical, lithological and edaphic factors as well as land use (Körner 2003; Nagy & Grabherr 2009). This heterogeneity could result in a great variation in stream water quality in mountain areas.

Dissolved organic matter (DOM) was reported to be smaller in surface water from non-forested than from forested catchments (Hood *et al.* 2005; Hood *et al.* 2003; Skjelkvåle & Wright 1998) and was positively related to carbon (C) and N contents, and CN ratios of the soil (Hood *et al.* 2003; Strand *et al.* 2008). By contrast, concentrations of NO_3-N and NH_4-N were negatively related to organic matter content and CN ratio of the soil (Hood *et al.* 2003; Strand *et al.* 2008). Nitrate (NO_3-N) in Norwegian lakes was positively related to N deposition and negatively to vegetation density (Hessen *et al.* 2009). Furthermore, Sjøeng *et al.* (2007) found a significantly positive correlation between percentage bare rock and amount of NO_3-N leached in 12 headwater catchments with a high N-deposition (1.63 – 2.75 g m^{-2} yr^{-1}) of southwest Norway. Together, these studies clearly highlight the important linkage between vegetation and soil in controlling leaching of DOM and nutrients to surface waters.

Hypothesis

The main hypotheses are listed below. More specific hypotheses are listed in the papers. Increasing densities of sheep at this low N deposition, un-fertilized low alpine site were hypothesized to

(1) reduce C storage, POC to SOC ratio and the lability of C due to removal of plant biomass, trampling (i.e. physical impacts) and input of urea and feces which may increase *in situ* decomposition (Paper I)

(2) increase rates of N-cycling due to physical impacts by trampling and input of urea and faces (Paper II and III)

(3) increase loads of inorganic N and coliform bacteria to runoff water due to physical impact by trampling (i.e. reduced infiltration) and input of fecal matter (Paper IV).

Methods

Study site and experimental design

The study site is located in the low alpine region (1050 - 1320 m a.s.l.) in Hol municipality, Buskerud county, southern Norway (7°55′ - 8°00′ E, 60°40′ - 60°45′ N) (Mysterud & Austrheim 2005). Vegetation is dominated by dwarf shrub heaths with smaller patches of lichen heaths, snow beds and alpine meadow communities in lee-sides (Rekdal 2001b). A total of 104 vascular plants was recorded in 2001 (Austrheim *et al.* 2005). The bedrock consists of meta-arkose and quaternary deposits of till and colluvium (Kristiansen & Sollid 1985; Sigmond 1998). Soils vary spatially, from peaty deposits in poorly drained pockets to freely drained soils with shallow (restricted by bedrock) and acidic organic horizons. Soils were classified as leptic podsols and dystric haplic gleysols (IIUSS Working Group WRB 2006) in snowbeds and willow-shrubs, respectively (Paper I). In willow-shrub areas, soils may also be classified as dystric haplic cambisols, depending on topographical position and thus moisture content. Mean annual temperature (MAT) is - 1.5 ^{O}C and mean annual precipitation (MAP) is about 1000 mm (Evju *et al.* 2009), approximately 75% of which falls as snow. Temperature and precipitation varies considerable during the growing season (from mid June to mid September) and between years (Table S1 in supporting information Paper IV). The average wet N deposition is estimated at 0.416 g m^{-2} yr^{-1} (Aas *et al.* 2008).

In 2001, an experimental manipulation started with the aim at determining ecological effects of various grazing intensities by sheep. A large enclosure (2.7 km^{2}) was fenced and divided in three blocks, each replicated with three sub-enclosures (approximately 0.3 km^{2}) with three treatments; no sheep (control), low grazing density (25 sheep km^{-2}) and high grazing density (80 sheep km^{-2}) of domestic sheep (*Ovis aries*) (Mysterud & Austrheim 2005; Mysterud *et al.* 2005). The study is set up as a randomized block design (Figure 2). Sheep grazing occurs from the end of June to the beginning of September (since 2002). The grazing pressure prior fencing in 2001 was low (< 10 sheep per km^{2}) with occasional visits of *Rangifer tarandus* and *Alces alces* assumed to have only negligible effect on the system (Mysterud *et al.* 2005).

Previous findings at the study site clearly document effects of grazing on selected aboveground plant properties listed in Figure 1. These findings include sheep density dependent effects on quality of forage (Mysterud *et al.* 2010), recruitment of birch (Speed *et al.* 2010), plant traits and abundance (Austrheim *et al.* 2008b; Evju *et al.* 2009; Evju *et al.* 2006) and habitat selectivity (Mobæk *et al.* 2009). Mysterud *et al.* (2010), documenting a marked decline in N-content of plants in course of the growing season, found the effect of growing season to interact with grazing (most strongly for *Avenella flexuosa*) with a smaller decline and greater N-contents of plants towards the end of the season at high sheep density. Speed *et al.* (2010) found an increase in birch recruitment after eight years of grazing at non grazed areas as compared to sites being grazed even at low densities. Furthermore, the shrubs *Juniperus communis* and *Betula nana* increased at low sheep densities vs. non-grazed sites after four years of grazing whereas three species of graminoids increased at the high grazing treatment (Austrheim *et al.* 2008b). At the plant community level Austrheim *et al.* (2008b) found reduced vascular plant height and cover after four years of grazing. Moreover, direct indications of an overall higher biomass removal in high density treatments as compared to low and no grazing observed by a reduction in lamb weights (Mysterud & Austrheim 2005) and a lower vascular plant biomass production (Austrheim, unpublished material) at high sheep density. Together, these density dependent effects of grazing on above ground properties may result in different responses and feedbacks in belowground compartments (Figure 1). In addition, sheep density affected other ecosystem components such as rodents (Steen *et al.* 2005), birds (Loe *et al.* 2007), invertebrates (Mysterud & Austrheim 2005; Mysterud *et al.* 2005) which were expected to be mediated by grazing effects on plants.

Sampling locations used in the PhD research

Twenty-seven soil plots (each 0.25 m^2, at 3 different altitudinal levels in 3 replicates within the 3 enclosures in the eastern block) were established in 2007 ("**Location A**"; Figure 2) within grassland habitats. Soil was sampled by horizon (18/06/2007-10/08/2007) and macrorhizons (type 19.21.35, Eijkelkamp, the Netherlands) installed just below or within the O-horizon and in the mineral soil (O-horizon 4.5 cm, mineral soil 18.3 cm) for soil water sampling. Precipitation and soil moisture and temperature at two depths (6 cm and 22 cm) was measured at location A (Table S1 in supporting information Paper IV). Data on soil water and N-stock (O-horizon and mineral soil) were included in paper II (Figure 5) and referred to in paper IV. Chemical and physical soil attributes from location A is reported in Table 1 (thesis).

Fifty four soil plots [each 1 m^2, within all 9 enclosures at 2 different altitudinal levels (1168 and 1259 m a.s.l., respectively) and in 3 replicates] were established in 2008 ("**Location B**"; Figure 2). The sites were selected based on criteria of similar altitude and plant community (grassland habitats partly covered with willow-shrubs).

Plant root simulator probes (PRS^{TM}; Western Ag Innovations Inc., Saskatoon, Canada) were used to assess available inorganic nitrogen (NH_4-N and NO_3-N) in soils. The plots received $^{15}NH_4Cl$ in early July 2008 (30.56 mg N m^{-2}) to study retention and cycling of N. Soil plots of location B, occurring within the grazed enclosures were fenced in 2008 to prevent damage to the PRS^{TM}-probes. Data on soil water from all 54 plots are included in paper II, and 27 plots at the lowest altitude level are included in paper III (N-content, $\delta^{15}N$ natural abundance and ^{15}N tracer recovery in soils and plants).

Eighty-nine plots were selected for soil and vegetation sampling in 2008 ("**Location C**"; Figure 2). The plots were selected from 180 plots (0.25m^2), established 2001, in each enclosure using a balanced stratified procedure among altitudinal levels and habitats (Austrheim *et al.* 2005). All 89 plots are located within grassland habitats. O-horizon and vegetation samples from location C, were further divided in two different grassland plant communities; snowbed (n= 56) and grassland with scattered willow-shrub (n=33). O-horizon samples were used to determine potential C (Paper I) and N (Paper II) mineralization. Six soil plots [1 from control snowbed (%SOC = 11.61), 3 from control willow-shrub (%SOC = 8.71, 45.8 and 50.75), 1 from low density snowbed (%SOC = 11.52) and 1 from high density snowbed (%SOC = 10.41)] differed greatly in O-horizon soil attributes compared to the other 83 grassland habitat plots. Thus, the plots were excluded from the analysis in paper I and II. Selected physical and chemical soil attributes of these plots are reported in Table 1, Paper I and in Table 2 (thesis).

In September 2006, fifty four spots with standing water located in topographic depressions within the plant communities snowbed (SNOWB, n= 18), dwarf shrub heath (DSH, n= 21) and tall herb meadow (THM, n= 15) were selected for water sampling ("**Surface runoff**", Figure 2). Infiltration rates and pore size distribution were determined at 6 different sites in block 3, each consisting of 3 plots ("**Infiltration**", Figure 2 and 3). Two sites were located in the non-grazed enclosure (G) in THM (n= 3) and SNOWB (n=3). Four sites were located in the grazed enclosure (H) in THM and SNOWB outside sheep tracks ($n_{\text{THM no track}}$ = 3, $n_{\text{SNOWB no track}}$ = 3) and on well defined sheep tracks ($n_{\text{THM track}}$ = 3, $n_{\text{SNOWB track}}$ = 3), respectively. Two headwater catchments were defined using GPS (Oregon 300, Garmin) in the enclosures G (~5.3 ha) and H (~8.9 ha) ("**Catchment boarder**", Figure 2; thesis, and Table S2 in paper IV).

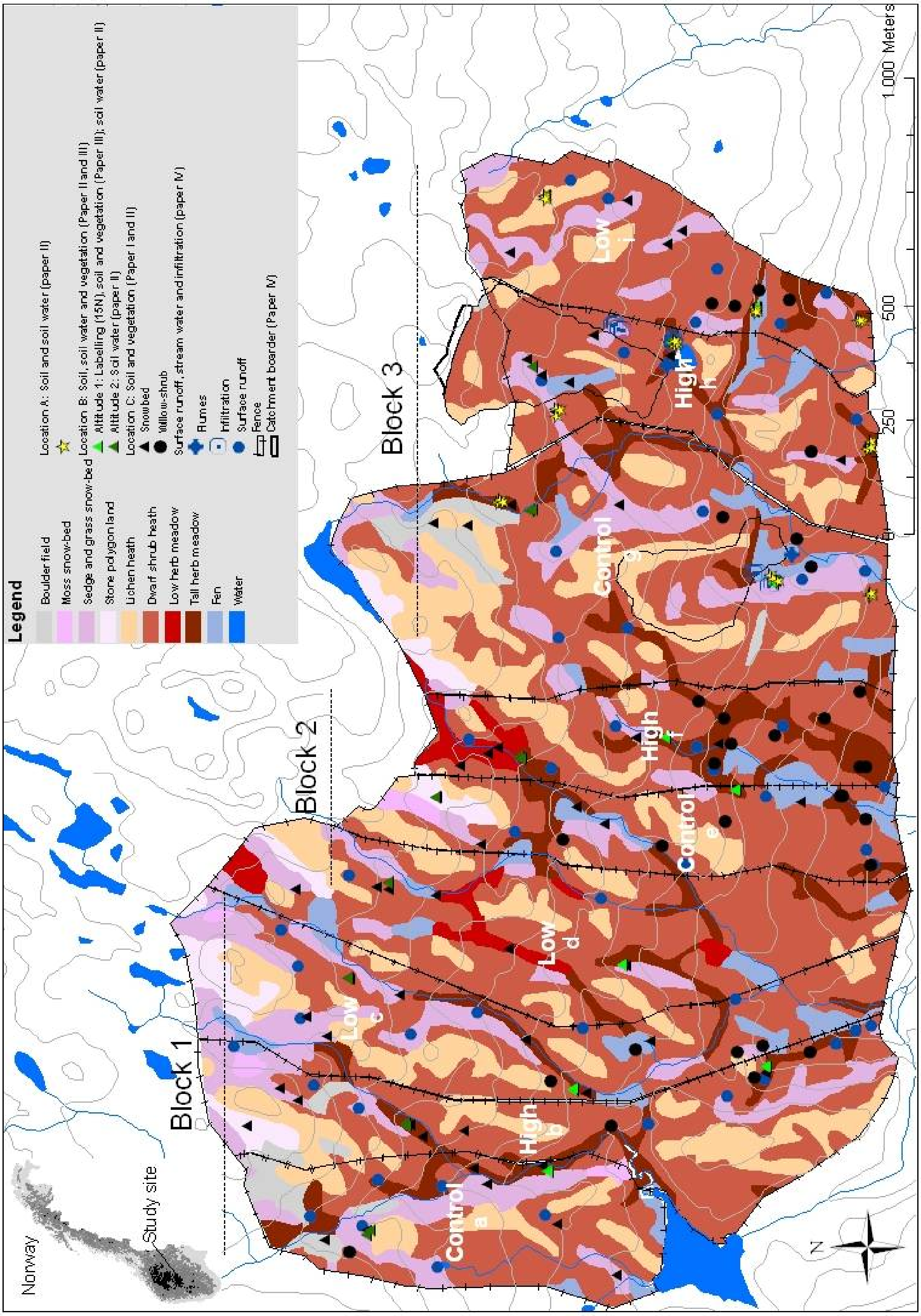


Figure 2. Experimental design Hol, southern Norway. The enclosure (~2.7 km^{-2}) is divided in three blocks, each replicated with three sub-enclosures (~0.3 km^{-2}, labelled a,b..i) with three grazing treatments [no sheep (control), low density (25 sheep km^{-2}) and high density (80 sheep km^{-2})]. Different plant communities and sites used for sample collection (described in the papers I-IV) are listed in the legend. The map is modified after Rekdal (2001b) using ESRI® ArcMap^{TM} 9.3.

In June 2007, flumes [RBC flume (0.16 l s^{-1} - 9 l s^{-1}), type 13.17.02, (Eijkelkamp 2001)] were installed in two small streams draining the two catchments for water flux measurements ("**Flumes**", Figure 2 and 3; thesis, and Figure S1 in paper IV). Data on surface runoff quality, infiltration and pore size distribution in addition to stream water quality are reported in paper IV.

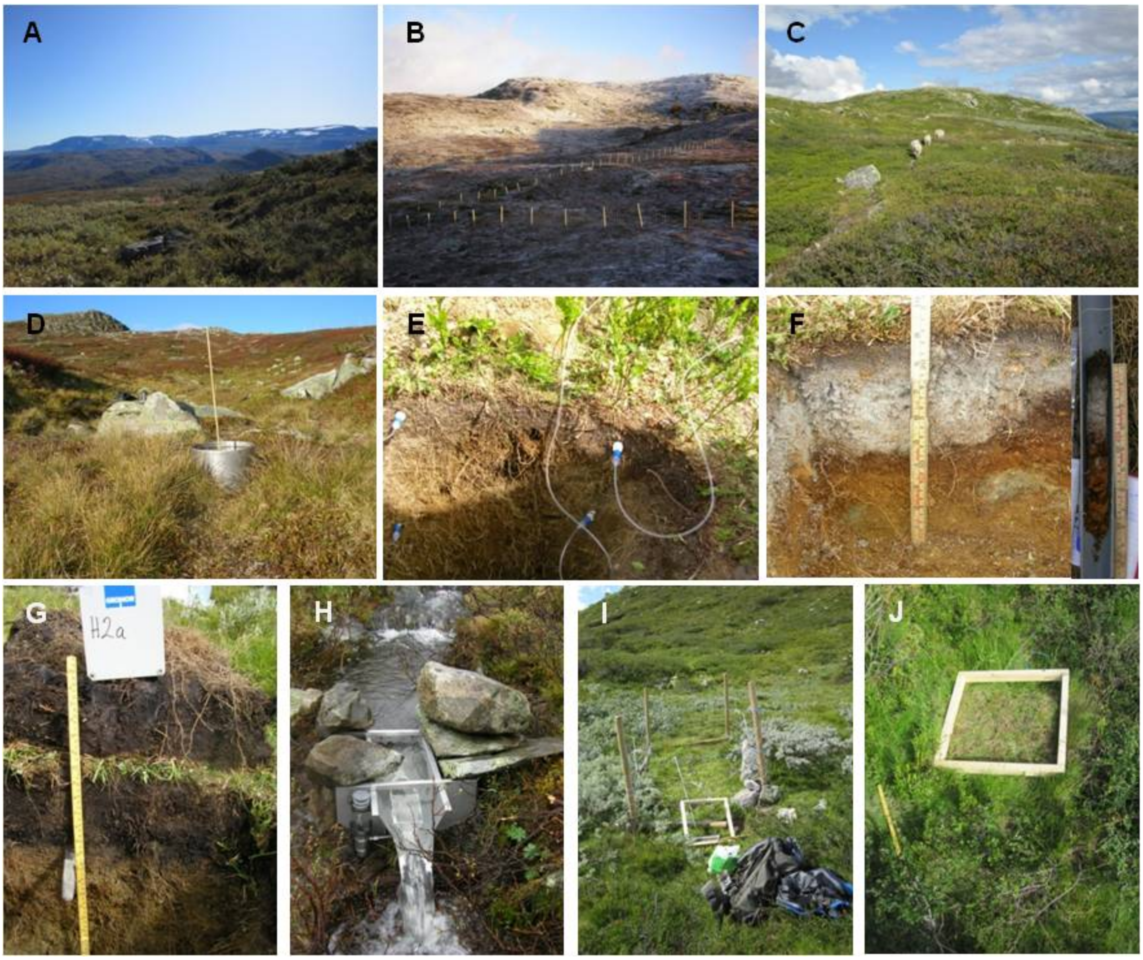


Figure 3. Hol, southern Norway. A) Landscape in summer. B) Landscape in autumn. C) Sheep on a sheep track in dwarf shrub heath. D) Infiltration measurements. E) Soil water sampling with macrorhizons. F) Soil profile from a grassland habitat in snowbed. G) Soil profile from a grassland habitat in willow-shrub. H) Flume for stream water sampling and water flux determination. I) Site for ^{15}N-application, biomass harvest and soil water sampling. J) Biomass harvest (photos Vegard Martinsen).

Sampling, laboratory processing and analysis

Detailed descriptions of sampling and laboratory processing (including references for procedures and calculations) are given in the material and method section of the papers I to IV. All statistical analyses were conducted using the statistical package R (version 2.10.1) (http://www.r-project.org/). In general, we used Linear mixed effects models (lmer) with random effects reflecting the block-wise randomization design, i.e. enclosure (n=9) nested within block (n=3). However, due to uneven sampling procedure, missing values or temporal variations in the sampling/analysis procedure, the random effects varied depending on the model of interest (specified in the papers). The fixed factors also varied depending on the dependent variables and factors of interest in the different models. Student's t-tests (normally distributed variables) and Wilcoxcon's rank-sum tests (variables not normally distributed) were used to test for differences between plant communities (snowbed and willow-shrub) for selected soil parameters (Table 1; Paper I and Table 2; thesis).

Soil

Dry matter (DM) corrected weight of soils (sieved at 2 mm; corrected for roots and gravels) was used to determine bulk density (BD; g cm^{-3}), thus representing BD of the fine earth fraction. All soil (and plant) samples were analyzed for total C and total N. In addition, we determined pH, potential cation exchange capacity (CEC; extractions with 1 M ammonium acetate adjusted to pH 7) and loss on ignition (LOI; POM samples only). Phosphorous and base cations (not presented in the papers) were determined by extractions with ammonium lactate (pH = 3.75, soil:AL ratio; 4g: 80 ml), which gives a measure of plant available nutrients (Krogstad 1992). Stocks of C and N were calculated based on soil volume (equation 1, Paper I) and based on equivalent soil mass, as decribed by Ellert & Bettany (1995) and outlined in equation 2, Paper I. Equvialent soil mass was calculated based on the horizon with lowest soil mass per m^{-2}, thus avoiding the need of deeper soil depths to obtain the equivalent mass. The selected O-horizon was within willow-shrub with no sheep grazing at 2.13 cm depth (BD = 0.14) with a calculated equivalent soil mass of 2.982 kg m^{-2}. The equivalent soil mass represents an average sampling depth of 1.13 cm, 0.87 cm and 1.20 cm within snowbeds and 1.45 cm, 1.25 cm and 1.59 cm within willow-shrubs for no sheep, high grazing density and low grazing density, respectively.

Particle size and density fractionation was done [modified, based on Leifeld & Kögel-Knabner (2005)] to obtain a free, light (density < 1.8 g cm^{-3}) POM fraction (20 μm < POM <2000 μm) of O-horizon soils (location C, Paper I). The remaining mineral associated organic material fraction (mOM) was used to calculate percentage recovery, but not further analyzed. The POC to SOC ratio was calculated to assess grazing induced differences in the form of SOM (Paper I). Furthermore, closed flasks (12 ml) with field moist soil (equivalent to 1 g of dry soil) from each sample location (location C; n = 89) were incubated in the dark (15 °C) and, at 6 different time periods (period 1 = 0 days (11 hours), period 2 = 4 days, period 3 = 8 days, period 4 = 15 days, period 5 = 20 days and period 6 = 27 days) placed in a robotized incubation system (Molstad *et al.* 2007) at 15 °C and connected to a gas chromatograph (GL system 7890, Agilent Technologies) to measure CO_2 concentration in head space. The potential C mineralization was used to assess the lability of SOM (Paper I).

Potential nitrogen mineralization (Paper II) was determined in incubation experiments conducted on O-horizon samples from location C. The soils were extracted in 25 ml 2M KCl and filtered prior to analysis and determination of NH_4-N and NO_3-N [after 0 days (i.e. initial extractable N), 15 days and 63 days of incubation, respectively]. Rates of net ammonification and net nitrification were determined by subtracting initial extractable soil NH_4-N and NO_3-N (μg g soil^{-1}) from final amounts (after 15 and 63 days, respectively) of extracted NH_4-N and NO_3-N, respectively. The sum of produced NH_4-N and NO_3-N represents net mineralization (Vestgarden & Kjonaas 2003).

To assess in situ availability of inorganic nitrogen (NH_4-N and NO_3-N) (location B, Paper II), PRS^{TM}-probes were inserted directly in the O horizon. The amount of adsorbed N represents nutrient surpluss rather than net mineralization (Western AG Innovations Inc. 2009). Furthermore, to assess effects of grazing on cycling and distribution of added ^{15}N-tracer, we determined δ^{15}N natural abundance and recoveries of added ^{15}N (30.56 mg N m^{-2} as NH_4Cl 99.98 atom % ^{15}N; IN 5037, Icon Isotopes, USA), respectively in soil and plant components (*Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa*, *Nardus stricta*, surface layer, O-horizon soil and roots) at location B (Paper III).

Water

Soil water was collected using macrorhizons installed just below or within the O-horizon (location A; 4.5 cm, location B; 3.6 cm). Soil water collected on 10 occasions from June to August in 2008 and 2009 at location A and B were included in Paper II. Soil water samples from location A are included in Table 1 (thesis). Surface runoff collected in the topographical depressions (107 grab samples of standing water; paper IV) and water collected in the streams of enclosure G and H (136 samples; paper IV) were collected using PVC bottles for chemical analysis and sterile Polysterol bottles (IDEXX Laboratories) for fecal bacteria determination. The samples were stored cold (< 4 °C) and filtered prior to analysis. Samples used for bacteria determination were analyzed short time after sampling. The water samples were analyzed for selected chemical attributes and the dissolved inorganic N to dissolved organic N ratio (i.e. DIN:DON ratio; see paper II) and the DOC:DON ratio (paper IV) calculated. The determination and quantification of waterborne bacteria, i.e. total coliforms and *E.coli*, were conducted on un-filtered water samples using Colilert®-18 and Quanty-Tray®/2000 (IDEXX Laboratories Inc. 2010) according to the procedure described by Eckner (1998).

Infiltration and pore size distribution

In paper IV, methods and results of infiltration rates and pore size distribution are presented ("**Infiltration**", Figure 2 and 3; thesis). Infiltration rates (cm hour^{-1}) were determined as the amount of water per surface area and time unit penetrating the soils using double (outer ring: Φ 55-58, inner ring: Φ 30-33 cm) and single ring (Φ 30-33 cm) infiltrometers. The soils were pre-wetted for 1 hour before the rate measurements in order to saturate the soil pores (i.e. obtain steady infiltration rates), thereby reducing the risk of horizontal flow. The measured infiltration rates thus represent approximate differences in saturated hydraulic conductivity between the soils. Readings of the decreasing water level within the inner rings were taken every 10 minutes between 1 and 2 hours after start of the experiment (i.e. 7 records for each plot). At each site, two samples of the OA-horizon were sampled using 100 cm^3 steel rings to a maximum depth of 3.7 cm (n=36). The undisturbed soil samples were used to determine the distribution of the pore size fractions > 200 μm (i.e. macropores), 30-200 μm (i.e. mesopores) and 3-30 μm (i.e. micropores) using ceramic pressure plates (Richards 1948).

Discussion of the main results

Characteristics of the study site

An overview of selected chemical and physical soil attributes of organic and mineral horizons, and of O-horizons is given in Table 1 and Table 2, respectively. General differences between the grassland communities snowbed and grassland with willow-shrub are described in Paper I. Phosphorous (P) and base cations extracted with ammonium lactate gives a measure of plant available nutrients (Krogstad 1992). Based on the amount of P (~14 mg 100 g soil^{-1}) and calcium (~110 mg 100 g soil^{-1}) in O-horizons (Table 1), the system may be classified as having an intermediate to high availability of the important plant available nutrients P and calcium (Krogstad 1992). The amount of P and base cations decreases significantly from the O-horizon to the mineral soil, probably due to accumulation of nutrients in the O-horizon due to plant uptake and re-deposition in the litter layer. Also, the greater content of SOM in O-horizons (~ 43%, i.e. twice the %C; Table 1) as compared to the mineral soil (~ 3.8% i.e. twice the %C; Table 1) suggests a greater adsorption of base cations on cation exchange binding sites in O-horizons (McBride 1994).

The system has previously been classified as "base-rich" [especially in landscape depressions with seepage water (Austrheim *et al.* 2005)] based on soil analysis of the upper 5 cm of 181 soils (Evju *et al.* 2006) and a high proportion of herbs. Acidity and the presence of calcium are important factors controlling species distribution (Austrheim *et al.* 2010; Nagy & Grabherr 2009). Comparing Hol (i.e. "base-rich" site) with an acidic site in Setesdal, Austrheim *et al.* (2005) found a greater species pool of herbs (55 species) at Hol as compared to Setesdal (13 species). The amount of extractable P and base cations reported by Austrheim *et al.* (2005) is somewhat between that of the O-horizon and the mineral soil reported in Table 1. This is probably due to differences in sampling strategy (consequently 5 cm vs. sampling by horizon), as the O-horizon depth at several sites is < 5 cm (Table 2).

Base saturation and soil base cation concentrations are relatively high compared with acid-sensitive sites included in the national monitoring programme for effects of acid deposition. For example Storgama, receiving high loads of S and N [1.10 g N m^{-2} yr^{-1} and 0.63 g S m^{-2} yr^{-1}, respectively (Stuanes *et al.* 2008)] has a base saturation of ca 1.1 to 8.6% (Strand *et al.* 2008). Despite an intermediate to high availability of base cations at Hol, O-horizons (pH$_{\text{h2o}}$ ~4.7; Table 1 and 2) and the mineral soil (pH$_{\text{h2o}}$ ~4.9;

Table 1) were acidic, with base saturation between 22 and 44 % (Table 2). The low pH in both the soil extracts and in the soil water suggest absence of calcium carbonates (McBride 1994). Total soil carbon concentrations (%) are thus representing concentrations of total organic carbon (reported as both % total C and % SOC). However, the pH of runoff water in topographic depressions and of stream water were close to 7 (Table 1 and 3; Paper IV), indicating sources of sub-soil alkalinity which buffers the water. The high base saturation of O-horizons suggests a relatively high buffer capacity of the soil. In addition, Hol receives low loads of S deposition [~ 0.191 g S m^{-2} yr^{-1} (Aas *et al.* 2008)]. Relatively high soil base saturation, low S deposition and production of alkalinity in the sub-soils probably explain the near-neutral pH of surface water. Furthermore, pH and base saturation in O-horizons at location C were significantly greater in grasslands with willow-shrubs than grasslands in snowbeds (Table 2), probably due to recharge of ground water rich in base cations.

Differences in surface water chemistry between plant communities of increasing productivity (i.e. snowbed, dwarf shrub heath and tall herb meadow) were assessed in Paper IV (the plant communities is described by Rekdal (2001a) and in Paper IV). Conductivity (µS cm^{-1}) was significantly greater in tall herb meadow (16.9 µS cm^{-1}) as compared to dwarf shrub heath (13.7 µS cm^{-1}) and snowbed (11.9 µS cm^{-1}) communities (Table 1; Paper IV). Dissolved organic carbon (DOC), total-N and DON concentrations (Table 1; Paper IV) were significantly smaller in snowbed (~1.09, 0.048 and 0.047 mg l^{-1}, respectively) as compared to dwarf shrub heath (~1.96, 0.081 and 0.073 mg l^{-1}, respectively) and tall herb meadow (~1.49, 0.061 and 0.061 mg l^{-1}, respectively). The greater DOC and DON concentrations of the productive plant community (i.e. tall herb meadow) as compared to dwarf shrub heath and snowbed were related to a higher biomass production and greater C pools of the former (Paper IV). Furthermore, the low CN ratio of the O-horizons (~17.9; Table 1 and 2) suggests a high availability of N and potentials for leaching of inorganic N (Hood *et al.* 2003; Sjøeng *et al.* 2007). However, concentrations of inorganic N in soil water were low and dominated by DON (Table 1; thesis, Table 1; Paper II), the same as observed in surface runoff (Table 1 and 3; Paper IV). This will be discussed further in the section describing N dynamics.

Table 1. Mean physical and chemical soil and soil water[a] attributes of O-horizons and mineral-horizons from location A (grassland habitats, eastern block), Hol, southern Norway. Standard error (se) and number of samples (n) is shown.

	Soil				Soil water			
	Variable	Mean	se	n	Variable	Mean	se	n
O-horizon	BD/g cm^{-3}	0.37	0.03	27	Conductivity/µS cm^{-1}	12.43	0.79	73
	Total depth/cm	8.44	1.16	27	pH	5.64	0.07	72
	pH	4.50	0.08	27	NO_3-N/mg l^{-1}	0.07	0.02	13
	Total C/% of fine earth	21.45	2.14	27	NH_4-N/mg l^{-1}	0.06	0.00	160
	Total N/% of fine earth	1.21	0.11	27	Tot-N/mg l^{-1}	0.64	0.04	218
	C stock/kg m^{-2}	5.50	0.83	27	DON/mg l^{-1}	0.59	0.04	220
	N stock/kg m^{-2}	0.33	0.05	27	DOC/mg l^{-1}	10.93	0.67	156
	CN	17.53	0.53	27	DOC:DON	21.08	0.63	154
	P-Al/mg 100 g soil^{-1}	13.96	1.03	27				
	Ca-Al/mg 100 g soil^{-1}	109.86	24.18	27				
	Mg-Al/mg 100 g soil^{-1}	18.11	1.85	27				
	K-Al/mg 100 g soil^{-1}	45.54	4.91	27				
	Na-Al/mg 100 g soil^{-1}	2.38	0.24	27				
Mineral-horizon	BD/g cm^{-3}	0.75	0.04	27	Conductivity/µS cm^{-1}	11.35	0.78	84
	Total depth/cm	17.81	1.38	27	pH	5.97	0.06	84
	pH	4.94	0.06	27	NO_3-N/mg l^{-1}	0.05	0.01	25
	Total C/% of fine earth	1.89	0.14	27	NH_4-N/mg l^{-1}	0.09	0.02	119
	Total N/% of fine earth	0.11	0.01	27	Tot-N/mg l^{-1}	0.37	0.02	216
	C stock/kg m^{-2}	2.36	0.23	27	DON/mg l^{-1}	0.31	0.02	217
	N stock/kg m^{-2}	0.13	0.02	27	DOC/mg l^{-1}	3.37	0.15	162
	CN	19.65	1.22	27	DOC:DON	15.59	1.05	159
	P-Al/mg 100 g soil^{-1}	2.23	0.24	27				
	Ca-Al/mg 100 g soil^{-1}	10.49	3.11	27				
	Mg-Al/mg 100 g soil^{-1}	0.88	0.11	27				
	K-Al/mg 100 g soil^{-1}	2.36	0.30	27				
	Na-Al/mg 100 g soil^{-1}	0.73	0.04	27				

[a] Soil water was sampled in September 2007 (n=54), June (n= 23), July (n= 27) and August (n=26) 2008, and June (n= 25), July (n= 23), August (n= 23) and September (n= 25) 2009. Note: N-stock and depth of the mineral soil are the same as those reported in Figure 5, Paper II. However, N stock, depth and total N concentration of the O-horizons differ from Figure 5, Paper II, because the average of location A and location B were used in Paper II. C and N stocks are based on entire depths of horizons, thus assuming that % SOC is the same for the entire O-horion as for the sample depth.

Due to differences in O-horizon depth, BD and/or C concentration, C and N stocks (based on soil volume) varied between sites (i.e. location A and location C; Table 1 and Table 2) and between plant communities (i.e. grassland habitat within snowbed and willow-shrub, respectively; Table 2). Despite no significant difference between grasslands in snowbed and grasslands with willow-shrub in C and N stocks of the total O-horizon, C and N stocks per cm soil depth (i.e. per unit volume of soil) were significantly larger in snowbeds than in willow-shrubs (Table 2). As concentrations of C and N only slightly differed between the two plant communities, this is probably due to the greater bulk density in snowbeds (Table 2). A high mineral content in surface soils of snowbeds is reported by Hiller *et al.* (2005) and Virtanen *et al.* (2008). Our results are partly in accordance with these findings, as bulk density (BD) was significantly greater in snowbeds than grasslands with willow-shrubs (i.e. increased bulk density with increased amount of mineral matter) (Table 2). Furthermore, grasslands in snowbeds have a greater and a more long lasting snow cover than grasslands with willow-shrubs. Compaction due to heavy snowpack was assumed to be the cause of the smaller infiltration rates and the smaller fraction of macro pores (and greater BD) in the ungrazed snowbed as compared to the ungrazed tall herb meadow (comparable to willow-shrub) reported in Paper IV.

The contribution of particulate organic carbon (POC) to bulk soil organic carbon (SOC) in the O-horizons was high both in snowbeds and willow-shrubs with only minor differences between the plant communities (82% and 79 %; Table 2). The large POC to SOC ratio found at our study site is similar to what was found by Leifeld *et al.* (2009) in the Swiss Alps (> 80% in 0-5 cm soils at the highest elevations; 2200 m a.s.l.). Despite minor differences in the POC to SOC ratio between the two plant communities, the great variability in several physical and chemical attributes described above illustrates the heterogenic nature of this system.

Table 2 (modified after Table 1, Paper I). Mean O-horizon soil attributes (±se) from 83[a] grassland habitats (location C) within two plant communities (snowbed and willow-shrub) at three density levels of sheep, Hol, southern Norway. Abbrevetions not included in Paper I: C and N stock tot. O-hor. depth[b] = C and N stock of the total O-horion depth (volume based), Inorganic N stock per cm and of the total O-hor. depth is the sum of NH_4-N and NO_3-N[c] per cm soil depth and of the total O-horion depth, respectively. Tests of difference between habitats are based on Student's t-tests (latin letters) and Wilcoxon's rank-sum tests (greek letters); different letters[d] indicate difference at the level of significance $p < 0.05$.

Factor	Snowbed								Willow-shrub							
	High		Low		No sheep		All treatments		High		Low		No sheep		All treatments	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Altitude/m.a.s.l	1248	13	1251	10	1216	12	1239 $^{\alpha}$	7	1141	7	1170	10	1162	22	1154 $^{\beta}$	7
Slope/%	14.44	2.06	11.11	1.59	14.41	2.18	13.30 $^{\alpha}$	1.13	15.36	1.61	16.88	3.77	13.75	3.10	15.33 $^{\alpha}$	1.46
Total O-hor depth/cm	3.82	0.63	5.30	1.11	4.07	0.63	4.40 $^{\alpha}$	0.48	6.80	1.06	6.06	1.81	7.85	1.81	6.88 $^{\beta}$	0.82
Sample depth/cm	2.74	0.27	3.20	0.28	3.26	0.28	3.06 $^{\alpha}$	0.16	4.00	0.30	3.38	0.45	3.74	0.51	3.76 $^{\beta}$	0.22
BD/g cm^{-3}	0.35	0.01	0.26	0.01	0.28	0.02	0.30 ^{a}	0.01	0.27	0.03	0.20	0.02	0.23	0.03	0.24 ^{b}	0.02
CEC/cmol$_c$ kg soil^{-1}	40.67	2.08	47.75	2.34	45.80	2.58	44.80 ^{a}	1.40	45.63	3.81	50.63	12.50	43.21	5.31	45.65 ^{a}	2.99
Base saturation/%	33.28	3.96	26.30	5.14	22.87	2.97	27.46 $^{\alpha}$	2.44	43.41	3.62	43.89	14.54	37.25	5.24	41.87 $^{\beta}$	3.03
pH	4.63	0.08	4.56	0.11	4.42	0.08	4.54 ^{a}	0.05	4.91	0.07	4.64	0.26	4.84	0.11	4.84 ^{b}	0.07
SOC/% of finearth	22.01	1.08	25.79	1.32	25.56	1.44	24.43 ^{a}	0.76	20.94	1.66	29.25	2.69	25.09	2.48	24.26 ^{a}	1.36
SON/% of finearth	1.37	0.07	1.47	0.08	1.44	0.09	1.42 $^{-}$	0.04	1.21	0.11	1.45	0.16	1.40	0.18	1.32 $^{-}$	0.08
C:N ratio bulk soil	16.13	0.34	17.72	0.49	18.03	0.66	17.28 $^{\alpha}$	0.31	17.73	0.60	20.71	1.45	18.52	0.96	18.74 $^{\beta}$	0.57
C stock per cm/kg m^{-2}	0.75	0.02	0.65	0.03	0.69	0.03	0.70 ^{a}	0.02	0.51	0.02	0.56	0.03	0.54	0.04	0.53 ^{b}	0.02
C stock tot. O-hor depth /kg m^{-2}	2.94	0.52	3.46	0.72	2.79	0.42	3.06 $^{\alpha}$	0.32	3.58	0.60	3.16	0.86	4.00	0.84	3.58 $^{\alpha}$	0.41
N stock per cm/kg m^{-2}	0.05	0.00	0.04	0.00	0.04	0.00	0.04 ^{a}	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03 ^{b}	0.00
N stock tot. O-hor depth /kg m^{-2}	0.19	0.03	0.20	0.04	0.16	0.03	0.18 $^{\alpha}$	0.02	0.22	0.04	0.17	0.05	0.23	0.05	0.21 $^{\alpha}$	0.03
Inorganic N stock per cm/g m^{-2}	0.19	0.03	0.11	0.02	0.07	0.01	0.13 ^{a}	0.01	0.14	0.02	0.07	0.01	0.11	0.04	0.12 ^{a}	0.02
Inorganic N stock tot. O-hor depth /g m^{-2}	0.62	0.10	0.55	0.13	0.26	0.03	0.48 ^{a}	0.06	0.84	0.14	0.44	0.16	0.51	0.07	0.64 ^{b}	0.08
POM/% of bulk soil	59.56	3.02	73.03	3.04	67.38	3.73	66.64 $^{\alpha}$	2.00	60.08	4.95	73.84	4.24	66.85	6.42	65.55 $^{\alpha}$	3.17
POC/% C of POM	28.15	0.72	30.60	0.95	31.61	0.92	30.09 ^{a}	0.53	27.09	0.98	31.38	1.95	29.45	1.51	28.86 ^{a}	0.84
PON/% N of POM	1.75	0.05	1.84	0.06	1.76	0.06	1.78 ^{a}	0.03	1.54	0.08	1.65	0.13	1.65	0.13	1.60 ^{b}	0.06
C:N ratio POM	16.21	0.39	16.84	0.59	18.21	0.64	17.07 ^{a}	0.33	17.88	0.66	19.40	1.31	18.32	1.09	18.40 ^{b}	0.54
POC to SOC ratio	0.76	0.02	0.87	0.02	0.83	0.02	0.82 $^{\alpha}$	0.01	0.77	0.03	0.81	0.05	0.79	0.04	0.79 $^{\alpha}$	0.02
PON to SON ratio	0.76	0.02	0.92	0.03	0.83	0.03	0.83 $^{\alpha}$	0.02	0.77	0.04	0.85	0.04	0.80	0.05	0.80 $^{\alpha}$	0.02
Ash content POM/%	45.20	1.35	40.74	1.59	39.42	1.60	41.83 ^{a}	0.92	47.41	1.93	39.94	3.68	42.71	2.79	44.16 ^{a}	1.58

[a]Six soil plots are removed from this summary table (see material and methods). [b]Assuming that % SOC is the same for the entire O-horion as for the sample depth. [c]The sum of NH_4-N and NO_3-N is based on the initial extractions (i.e. at day 0) of inorganic N presented in Figure 3, Paper II. The inorganc N stocks (g m^{-2}) was caluclated as [µg N g soil^{-1} * BD (g cm^{-3}) * soil depth (cm) * 10]/1000. [d]Not tested is marked “-“. Total O-hor depth, Inorganic N stock per cm, Inorganic N stock tot. O-hor depth, POC and C:N ratio of POM were ln transformed prior to analysis. Note: SOC % = Total C % as reported in Table 1.

Physical impacts of grazing

As illustrated in Figure 1, grazing by large herbivores may have direct effects on physical soil properties important for biogeochemical processes and feedbacks within the soil-plant system. At Hol, grazing significantly increased bulk density (BD, g cm^{-3}) of O-horizons (Paper I and IV) and ash content (%) of the POM fraction (Paper I). In addition, infiltration rates (cm $hour^{-1}$) and the fraction of macro pores (> 200 µm; volume %) decreased at sites with heavy sheep traffic (Paper IV). Effects of the three density levels of sheep within snowbeds and willow-shrubs and effects of high grazing density (enclosure H) vs. no grazing (enclosure G) in snowbed and tall herb meadow were assessed in Paper I and Paper IV, respectively. Moreover, differences between well defined sheep tracks vs. no sheep tracks were assessed in Paper IV.

High sheep density caused significantly greater BD (both in snowbeds and willow-shrubs) as compared to low sheep density and control (Table 3, Paper I). Within the tall herb meadow assessed in Paper IV, BD was significantly greater at the grazed as compared to the non grazed site (Table 2, Paper IV). These findings are in accordance with e.g. Steffens *et al.* (2008), reporting soil compaction of surface horizons caused by grazing. However, there was no effect of grazing on BD in the snowbed assessed in Paper IV (Table 2, Paper IV). But, as reported by Mobæk *et al.* (2009) tall herb meadow is classified with a higher grazing value compared to snowbed which indicates a larger impact of the sheep in the grazing preferred habitat. By contrast, the comparison of BD on sheep tracks vs. outside sheep tracks, revealed (both in the snowbed and in the tall herb meadow) significantly greater BD on tracks as compared to no tracks (Table 2, Paper IV).

Infiltration rates (cm $hour^{-1}$) were significantly affected by plant community and grazing, with smaller infiltration rates in snowbed as compared to tall herb meadow and significantly smaller rates in tall herb meadow at the grazed compared to non grazed site (Figure 2a and Table 2, Paper IV). The difference in infiltration rates were greater between snowbed and tall herb meadow outside the sheep tracks (~9 cm $hour^{-1}$) as compared to the tracks (~2 cm $hour^{-1}$), associated with a larger difference between no tracks vs. tracks in tall herb meadow as compared to snowbeds (Figure 2b and Table 2, Paper IV). Furthermore, the percentage of macropores (> 200 µm; volume %) was significantly greater in tall herb meadow as compared to snowbed at the non-grazed site (~20 % vs. ~10 %) but did only slightly differ at the grazed site (~10 % vs. ~8.5 %) (Figure 2c and Table 2, Paper IV). Comparing percentage of macropores on no tracks vs. tracks revealed no effect of plant community, but a significantly smaller share of macropores on tracks vs. no tracks (Figure 2d and Table 2, Paper IV). This is in accordance with the lower infiltration rates and greater BD on sheep tracks. Previously, increased BD and reduced porosity caused by sheep grazing has been reported by Meyles *et al.* (2006).

Similar to the effects of grazing on BD, we found a significantly greater ash content of the POM fraction (which did not differ significantly between willow-shrubs and snowbeds) in the high grazing treatment as compared to the low grazing treatment and control sites (Table 3, Paper I). As the ash content of the POM fraction was used as an indication of grazing induced mixing of organic matter and minerals, these findings suggest (1) compaction is caused by a heavy snowpack (i.e. greater BD and lower infiltration rates in snowbeds vs. willow-shrubs and tall herb meadow; Paper I and IV) and a high grazing density (Paper I, particularly on sheep tracks and in grazing preferred habitats; Paper IV) and (2) mixing of organic and mineral matter is primarily a consequence of trampling (ash content of POM; Paper I). Overall, however, both compaction and mixing of organic and mineral matter clearly depends on herbivore density.

The combination of natural differences between the plant communities and grazing affecting soil physical attributes may indeed have impact on storage and calculations of stocks of SOM. As reported in Figure S1 of the supporting information to Paper I, BD and bulk soil carbon concentration (% SOC) were significantly and negatively correlated (rho = -0.74, $p<0.05$). This is similar to what has been reported by e.g. De Vos *et al.* (2005) and Steffens *et al.* (2008). In the next section, our findings on grazing induced effects on C and N storage in addition to form and lability of SOM are reported.

Carbon and nitrogen storage, form and lability of SOM

Grazing by large herbivores may strongly affect storage of SOM (He *et al.* 2008; Piñeiro *et al.* 2010). Due to its importance in affecting soil attributes and processes known to influence ecosystem functioning and productivity (Dawson & Smith 2007; Schlesinger 1997; Whitehead 2000), more knowledge about factors controlling SOM storage is required. In this study, assessing effects of three density levels of sheep on C and N storage, form and lability of SOM, we aimed at identifying positive or negative impacts of grazing on SOM. Assessing effects of different grazing densities is important in a management point of view, both for the sustainability of grazing in outlying lands and in terms of potentials for short term mitigating of climate change (Laca *et al.* 2010). As summarized in Paper I, we found evidence for herbivory-dependent effects on carbon storage (expressed either as concentrations or stocks) and POC to SOC ratios of SOM interacting with site (i.e. plant community). However, these effects were strongly related to differences in soil physical attributes (i.e. BD and ash content of the POM fraction), which are also associated with grazing and plant community (previous section).

Bulk density and ash content of the POM fraction were included as covariates for the different models presented in Table 2, Paper I. Due to differences in physical soil attributes between the habitat and grazing treatments (previous section), we assessed differences between the treatments and habitats based on the mean [either BD (%SOC, C-stock per cm) or ash content of the POM fraction (POC to SOC ratio)] of the covariate within each community and treatment (Table 2; thesis and Table 1; Paper I). These predications were included in Figure 2 of Paper I and are included as Figure 4 in the thesis. Predicted % SOC was lower at high sheep density compared to low sheep density and control both within snowbeds and willow-shrubs (Figure 4a). The two grazing treatments showed the highest (at low grazing pressure) and lowest (at high grazing pressure) predicted SOC within both plant communities, with SOC at control sites intermediate. The differences between the treatments were more pronounced within willow-shrubs than within snowbeds with predicted SOC differing as much as ~8.5% between high and low sheep densities (Figure 4a).

The minimum adequate model for carbon stock per cm soil (C-stock volume based/ kg m^{-2}) included a significant interaction effect of treatment and plant community in addition to a significant increase in C-stock per unit increase in bulk density (Table 2, Paper I). These are the same predictors as for SOC (Table 2, Paper I). The predicted C-stock at high sheep density in snowbeds was greater than at low sheep density and control, thus the opposite of what was observed for % SOC (Figure 4a and 4b). In contrast, in willow-shrubs (with significantly lower BD than snowbeds; Table 2), there were only minor differences in predicted volume based C-stocks between the treatments (Figure 4b).

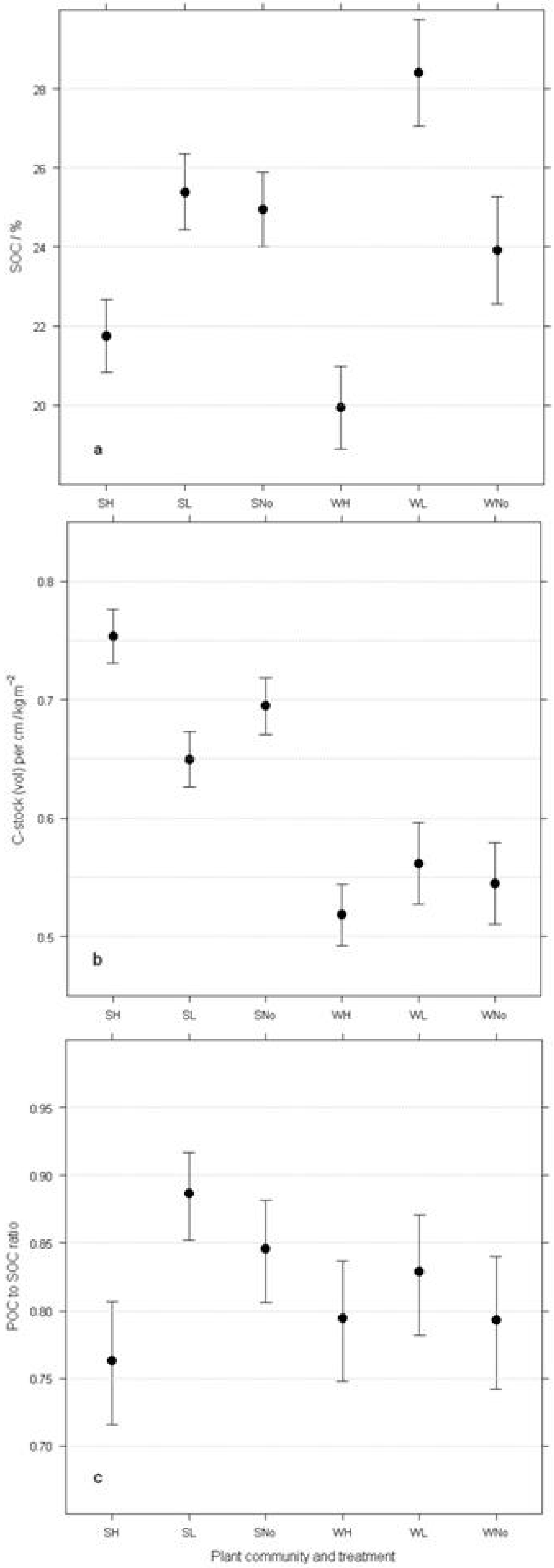


Figure 4 (the same as Figure 2, Paper I). Predicted **a** total bulk soil carbon content (SOC/% of fine earth; n = 82), **b** Carbon stock (volume based) per cm soil (C-stock/kg m^{-2}; n = 83) and **c** particulate organic carbon to soil organic carbon ratio (POC:SOC; n= 83) ± SE in O-horizon soil samples from grassland habitats within two different plant communities (snowbed and willow-shrub) at three levels of grazing, Hol, southern Norway. Predictions are based on fixed effect estimates derived from linear mixed effect models (Table 2; Paper I) at mean bulk density (g cm^{-3}) (Figure 4a and 4b) and mean ash content (Figure 4c) within each treatment combination (Table 2; thesis and Table 1; Paper I). SH = Snowbed-high, SL = Snowbed-low, SNo= Snowbed-no sheep, WH = Willow-shrub-high, WL = Willow-shrub-low and WNo = Willow-shrub-no sheep.

Carbon stocks based on equivalent mass (C stock eqv.mass; kg m^{-2}; cf. Ellert & Bettany 1995), was significantly affected by grazing, but not plant community (Table 2; Paper I). The estimated C-stocks eqv. mass (Figure 5) were lower for the high sheep density (0.64 ± 0.04) compared to low (0.80 ± 0.04) and control (0.76 ± 0.04), indicating greater loss of soil carbon and/or lower biomass input in areas with high sheep densities. Similar effects of grazing on C stock based on equivalent mass were reported by Steffens *et al.* (2008). As earlier noted by Ellert & Bettany (1995), C stocks based on soil equivalent mass are more sensitive to detect management induced changes in carbon storage than estimates based on soil volume. In contrast to SOC and C-stock eqv.mass, carbon stocks calculated per soil volume revealed greater predicted C-stocks in snowbeds for the high sheep density compared to the low density and control (Figure 4a,b; thesis and Table 2; Paper I). However, within willow-shrubs, there were only minor differences in predicted volume based C-stocks between the treatments (Figure 4b). These findings illustrate the importance of the reporting unit when assessing effects of land use on carbon storage. Because volume based C-stocks are based on an unequal mass of soil, it was suggested in Paper I to use C concentrations or C stock eqv.mass for comparisons of C stores.

Carbon and nitrogen concentrations were significantly correlated (rho = 0.85, $p<0.05$, Figure S1, Paper I). C stock eqv.mass was reduced at high grazing (see above). By contrast, estimated N stock eqv.mass (~42 g m^{-2}) was neither related to plant community nor to grazing (Figure 5). According to the N budget in Figure 5 (Paper II), a rough estimate of expected N removal in sheep biomass per growing season is approximately 0.36 g N m^{-2}. After 7 years of grazing, about 2.5 g N m^{-2} would thus have been removed as sheep biomass from the system. This is ~6% of the N stock eqv.mass. Apparently, this amount is too small for detection with the methods used in this study. Nevertheless, these findings indicate that C loss to a greater extent than N loss is enhanced at high sheep density as compared to low sheep density and non grazed sites.

Particulate organic matter (POM) consists mainly of root fragments and aboveground plant residues (Golchin *et al.* 1994).Hence, any reduction in biomass input or increased decomposition would reduce the POC to SOC ratio within the soil. The predicted POC to SOC ratio in snowbed was lower at high sheep density compared to low density and control, the latter two only slightly differing (Figure 4c). In grassland with willow-shrubs, the predicted POC to SOC ratios revealed only minor differences between the grazing treatments. Not surprisingly, given the high share of POC of the total SOC (Table 2) and thus the high correlation between POC and SOC (rho = 0.77, $p<0.05$, Figure S1, Paper I), the predicted POC to SOC ratios showed a similar pattern (though not as pronounced) as bulk soil carbon concentration (% SOC). Furthermore, the POC to SOC ratio was negatively correlated with ash content of the POM fraction (rho = 0.57, $p<0.05$, Figure S1, Paper I). In addition, there was a significantly greater ash content of the POM fraction at high sheep density (Table 3, Paper I). Thus, it was suggested that the somewhat lower POC to SOC ratios at the high grazing treatment is induced by physical disruption of POM (treading) causing increased decomposition. In addition, the sites with high sheep density have lower inputs of plant biomass (Austrheim, unpublished material).

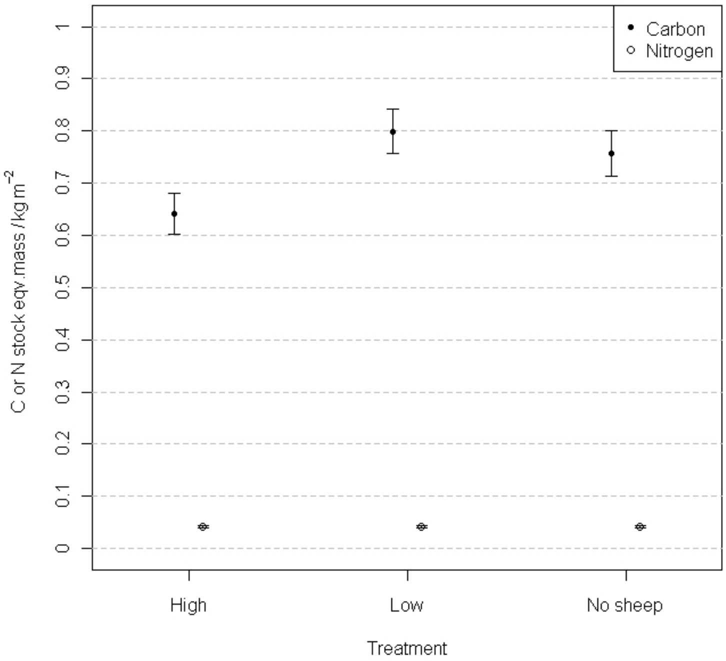


Figure 5. Estimated mean (± se) carbon (C) and nitrogen (N) stock at equivalent mass[a] (kg m^{-2}) of O-horizon soil from 83 grassland habitats, at three density levels of sheep grazing, location C, Hol, southern Norway. Estimates are based on linear mixed effect models[b].

[a] The equivalent soil mass represents an average sampling depth of 1.13 cm, 0.87 cm and 1.20 cm within snowbeds and 1.45 cm, 1.25 cm and 1.59 cm within willow-shrubs for no sheep, high grazing density and low grazing density, respectively (see the section "Sampling, laboratory processing and analysis; soil").

[b] Random effects were enclosure (n=9) nested within block (n=3).The fixed effect parameter estimates for C stocks are presented in Table 2, Paper I. The minimum adequate model for the N stocks did not include grazing treatment or plant community (i.e. willow-shrub and snowbed).

In a parallel study at Hol (results not shown) the ^{14}C content of POM suggests that the POM fraction has a relatively low mean residence time. Recently, similar observations were reported by Leifeld & Fuhrer (2009) and Leifeld *et al.* (2009). Uncomplexed organic matter being a source of readily available C and energy to the decomposers was reported by Christensen (2001). Therefore we expected samples with a high POC to SOC ratio to have higher potential carbon mineralization rates [measured as CO_2-C fluxes (µg g soil^{-1} hour^{-1})]. However, despite differences in the POC to SOC ratios (Figure 4c) potential carbon mineralization, measured as the mean CO_2-C flux (µg g soil^{-1} hour^{-1}) during 27 days of laboratory incubation, was neither affected by treatment nor by plant community (Table 2, Paper I). The only significant factor decreasing the decomposability of SOM was BD (Table 2, Paper I). This may be an effect of less available substrate for microbial decomposition at higher BD. Furthermore, normalizing CO_2-C fluxes on amount of soil particulate organic carbon (µg g POC^{-1} hour^{-1}), revealed greater CO_2-C fluxes from soils in willow-shrub than snowbeds, but no effect of grazing treatment (Table 2, Paper I). In addition, there was a significant increase in CO_2-C flux with increased ash content of POM. The positive response in carbon mineralization per g POC^{-1} to increased ash content of POM is indicative of sites exposed to more disturbances (i.e. POM disruption and mixing of mineral and organic matter). However, greater amounts of mineral matter could also have resulted in a greater stabilization in organomineral complexes (Christensen 2001) and thus reduced the decomposability of SOM. As the quality of SOM may be distinguished from quantity by identifying the fraction of SOC found in the mineralizable fraction (Gregorich *et al.* 1994), our findings suggest that there might be minor differences in the quality of the organic matter between the habitats, but that 7 years of grazing at the densities provided at our study site, is not sufficient to affect the decomposability of SOM.

Our findings indicate herbivore density dependent effects on C storage and the POC to SOC ratio (i.e. form of SOM), both being reduced at high grazing and somewhat enhanced at low grazing density as compared to no grazing. By contrast, there was no evidence for grazing induced effects on the lability of SOM or on N storage. These findings thus suggest that low sheep density is sustainable in terms of storage of C, but that even a high sheep density has minor effects on the total N storage. Nevertheless, sheep density may affect the pool of inorganic N, which is important for plant availability and thus uptake and growth of plants. Grazing induced effects on N dynamics will be discussed in the next section.

Nitrogen dynamics: Availability, cycling and leaching of N

Nitrogen being an essential nutrient for microbes and plants constitutes an important control on ecosystem productivity (Figure 1). Great accumulations of SOM caused by constraints on biogeochemical processes controlling turnover and availability of N (Figure 1) increases the storage of organic N in cold and wet regions (Post *et al.* 1985). Experimental addition of N in arctic and alpine regions increased biomass production of plants (Bowman *et al.* 1993; Shaver & Chapin 1980) indicating a potential N limitation in these systems. At Hol, comparing the pool of inorganic N (i.e. the exchangeable pool potential available for plants) with the total N pool of O-horizons (Table 2) clearly shows that the amount of readily available N constitutes only a minor (~ 0.4 %) fraction of the total N pool. Small changes in the mineralization-immobilization-turnover would potentially have a great impact on the pool of inorganic N and thus the amount of plant available inorganic N. In Paper II and Paper IV we assessed effects of grazing on N availability, cycling and leaching (i.e. mainly dynamics associated with the pool of inorganic N).

The availability of inorganic nitrogen (expressed as PRS^{TM}-adsorbed surplus N; NH_4-N+NO_3-N) was determined during the growing season in 2008 at two different altitudinal levels (Figure 2 and Table 2; Paper II). As a further indication of availability and levels of excess inorganic N, we determined dissolved inorganic nitrogen to organic nitrogen ratios (DIN:DON) in O-horizons water (Figure 4, Table 1 and 5; Paper II). In addition, we determined effects of plant community and grazing (Paper II), and grazing and seasonal differences (Paper III) on N content of plants as an indirect measure of the availability of N in soils. In general, the amount of PRS^{TM}- adsorbed N and concentrations of inorganic N in O-horizon soil water were low and dominated by NH_4-N (Table 1; Paper II). The low concentrations of inorganic N in soil water resulting in low DIN:DON ratios, illustrate the predominace of organic nitrogen in this system (Figure 4, Table 5; Paper II and Table 1; thesis). Furthermore, at the two studied altitude levels, PRS^{TM}- adsorbed N was significantly lower in the middle of the growing season as compared to the start and end. A significant decline during the entire season (i.e. from June to August) was observed for the DIN:DON ratios in soil water of the O-horizons. The decline was less pronounced at low and high grazing density as compared to the non grazed sites (Figure 4, Table 5; Paper II). Changes in availability of soil N throughout the growing season due to seasonal changes in microbial and plant demand has been reported by e.g. Lipson *et al.* (Lipson *et al.* 1999) and Weintraub & Schimel (2005).

Low sheep density caused a significant reduction in surplus (PRS^{TM}-adsorbed) N compared to high sheep density and control at the lowest altitude level in the early period of the growing season (Figure 2; Paper II). However, this difference diminished throughout the growing season due to an increase and decrease in PRS^{TM}- adsorbed N at the low sheep density and control, respectively (Figure 2; Paper II). We found no significant effects of grazing at the highest altitude level. The smaller amounts of PRS^{TM}- adsorbed N at the low density treatment could have been related to a greater biomass demand at these sites. However, as shown in Table S1 in Paper III, the harvested biomass (at location A where we determined PRS^{TM}- adsorbed N) was smallest at the low sheep density. The grazing induced difference in PRS^{TM}- adsorbed N is therefore probably related to other factors or processes as indicated in Figure 1.

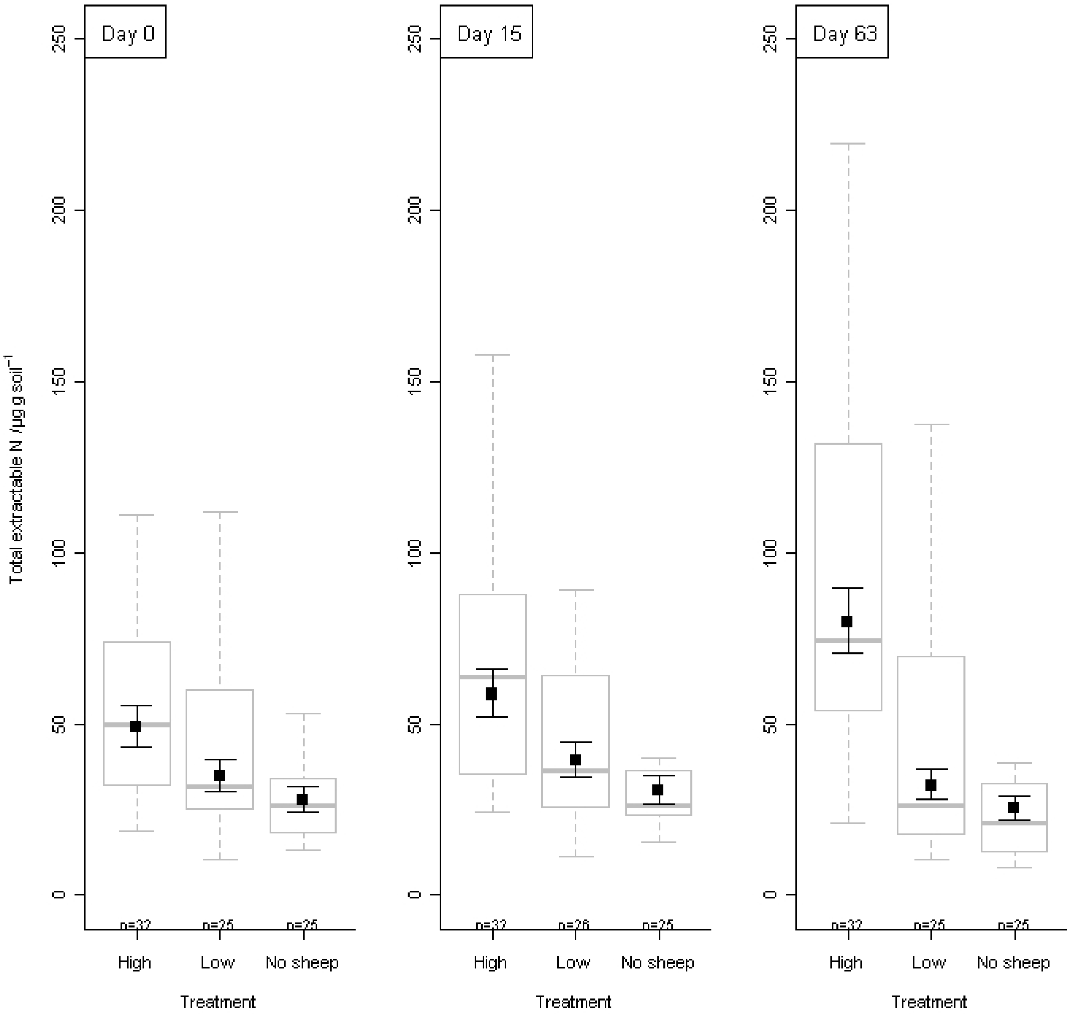
The nitrogen content (% of total N) differed between the plant functional (growth form) groups studied in Paper II [i.e. grasses (*Avenella flexuosa* and *Anthoxantum odoratum*), herbs (*Alchemilla alpina* and *Rumex acetosa*) and woody species (*Vaccinium myrtillus* and *Salix lapponum)*] but was significantly lower for plants sampled in grassland with willow-shrubs than in snowbeds (Table 3; Paper II). Grasses had a lower N content as compared to herbs and woody species, with the latter two only slightly differing (Table 3; Paper II). However, the N content of the functional plant groups was not affected by grazing (Table A1; Paper II).

There was a significant effect of season in the N content of three out of the four species (i.e. *A. alpina*, *A. flexuosa* and *N. stricta,* but not *V. myrtillus*) assessed in Paper III, with significantly lower N contents at late stages of the growing season (Figure 1; Paper III). This is in accordance with previous findings at Hol (Mysterud *et al.* 2010) and what was reported by e.g. (Morecroft *et al.* 1992b). The increased amounts of surplus (PRS^{TM}-adsorbed) N (Figure 2; Paper II) towards the end of the season, suggest less uptake of N by the plants. Furthermore, we found little evidence for grazing induced differences in N-content of the plants, with the exception of somewhat greater N contents in *N. stricta* at high sheep density as compared to low density and the non-grazed sites (Fig. 1; Paper III). In this study, the plants were protected from grazing. The impact of grazing on the N content of plants, which was related to plant phenology (Mysterud *et al.* 2010), would thus be eliminated. As we found only minor effects of grazing on the N content, this suggest that direct impacts of grazing (i.e. plant tissue removal preventing plant aging) is a better predictor of the total N content of plants than potential differences in the availability of N in soils (which was affected by grazing and season, cf. PRS^{TM}-adsorbed N).

The pool of inorganic N in the O-horizon was significantly greater in grassland with willow-shrubs than in snowbeds (Table 2), probably due to greater thickness of the O-horizon in the former. However, the inorganic N stock per cm soil depth did not differ significantly between the plant communities. Plant community was not included as a significant explanatory variable in determining amounts of extractable N (i.e. potential net N mineralization; Table 4 and Table A1, Paper II). The amount of soil extractable inorganic nitrogen (NH_4-N + NO_3-N; µg g^{-1}soil) in the O-horizon was significantly higher at high sheep density as compared to low sheep density and no grazing. However, the amount of initial extractable N varied in all grazing treatments with a large variability between the repeated extractions at each soil sample (Figure 6). Pools of inorganic N which are calculated based on the initial extractions were also somewhat greater at the high sheep density (Table 2). There was a significant net mineralization throughout the incubation experiment at high sheep density, but O-horizons from the low sheep density and control enclosures showed an immobilization even after 63 days of incubation (Figure 6; thesis, Table 4; Paper II). About 85 % of the total extractable inorganic N was in the form of NH_4-N (Table 1; Paper II).

Across 6 arctic ecosystems in Alaska, Nadelhoffer *et al.* (1991) point at the importance of the quality of SOM for N mineralization in surface soils, as differences associated with ecosystems were more pronounced than temperature on N mineralization (Nadelhoffer *et al.* 1991). Our data indicate that grazing is more important for the amount of extractable N per gram soil than plant community (Table A1, Paper II). Net N mineralization and net immobilization occur when microbes are predominately limited by C and N, respectively (Bardgett 2005). As % C and C stock eqv.mass were both reduced at the high sheep density as compared to low sheep density and no grazing (see previous section), it suggests that microbes at the high sheep density are limited by C (hence a net mobilization of N) whereas microbes at low sheep density and no grazing are somewhat more limited by N (hence a net immobilization of N). In addition, the POC to SOC ratio was somewhat smaller at the high sheep density as compared to the low density and the non grazed sites. As turnover of uncomplexed organic matter may be coupled with immobilization of N (Christensen 2001) and act as a sink for plant available N (Whalen *et al.* 2000), our findings on potential net N mineralization also agree with the grazing induced effects of the form of SOM (i.e. the POC to SOC ratio).

Figure 6 (the same as Figure 3; Paper II). Total inorganic nitrogen (NH_4-N + NO_3-N; µg g^{-1} soil) from O-horizon samples of grassland habitats at tree different grazing treatments (location C) during 63 days of incuabtion (day 0 = start of incubation; initial extractable N, day 15 = extractable N after 15 days of incubation and day 63 = extractable N after 63 days of incubation). The figure shows total nitrogen (± se) based on fixed effect estimates derived from a linear mixed effect model (Table 3; Paper II) superimposed on Box-whisker plots (medians, 25th and 75th quartile and minimum and maximum values, i.e. whiskers) based on the original data. n=247[a]. An increase or decrease in extracted N from day 0 indicates net mineralization or immobilization, respectively.



[a] Six plots are omitted from the analysis. One outlier was removed in addition to one missing value.

In Paper III, we assessed effects of grazing on ecosystem N-cycling by measuring $\delta^{15}N$ natural abundance and the system's distribution of added tracer N (applied as $^{15}NH_4Cl$) in different soil and plant components [*Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa*, *Nardus stricta*, surface layer (a mixture of cryptogams, litter, roots and O_i), O-horizon soil and roots]. For details regarding calculations and analysis, see Paper III. The natural abundance of ^{15}N differed significantly between the ecosystem components with O-horizons being significantly enriched ($\delta^{15}N$ ~3.5 ‰) and plants significantly depleted ($\delta^{15}N$ ~ -1.6 ‰) as compared to $\delta^{15}N$ of the atmospheric standard (Figure 2, Paper III). Despite a tendency for greater $\delta^{15}N$ in the soil and plant components at the high grazing treatment (Table 2; Paper III), grazing had no significant effect on $\delta^{15}N$, neither in the above-ground nor in the belowground components.

Recoveries of added tracer ^{15}N were greatest in the litter layer and O-horizon (Table 3, Paper III). This is in accordance with other labelling experiments, indicating great retentions of N in these layers (Ewing *et al.* 2010; Gerzabek *et al.* 2004). The proportional tracer recoveries (i.e. the total tracer recovery at each plot rescaled to 100%) of the litter layer and O-horizon did not differ between the grazing treatments (Table 1; Paper III). However, we found evidence for greater proportional recoveries per gram plant N (*V. myrtillus* and *A. flexuosa*) at non-grazed as compared to grazed sites (Figure 3; Paper III). The proportional recovery per gram plant N was smallest at the low grazing treatment for *V. myrtillus*, *A. flexuosa* and *N. stricta* (Figure 3, Table 1; Paper III), indicating a greater N-cycling (high immobilization-mineralization turnover) and thus ^{15}N dilution at low grazing density as compared to high grazing density and control.

Due to lack of biomass data for 2008, we used ^{15}N atom% excess to determine differences in ^{15}N enrichment short term (1 month) and long term (13 months) after tracer addition (Online Resource 2 and 3; Paper III). The difference in ^{15}N atom% excess between the grazing treatments decreased with time after tracer addition, but for *A. flexuosa* only (Figure 4, Table 1; Paper III)). As described in Paper III, this suggests reduced effects of grazing on ^{15}N dilution with increased time since tracer application probably due to stabilization in the soil organic N pool.

Surface water quality

The low rates of PRS^{TM}-adsorbed N, low concentrations of DIN and DIN:DON ratios in soil water reported in the previous section, indicate that N is effectively re-cycled and remains in the plant-soil system (Gerzabek *et al.* 2004). However, due to observed surface runoff (especially in sheep tracks) during heavy rain, it was assumed that there would be losses of nutrients (i.e. smaller potentials for nutrient retention in the soil matrix). In Paper IV, we assed stream water quality of a grazed (H) and an ungrazed (G) catchment in addition to differences associated with plant communities of different productivity.

The chemical composition of streamwater draining the grazed and non-grazed catchment differed in several aspects not related to grazing (pH, conductivity and concentrations of base cations; Table 3; Paper IV) but more likely related to lithological differences between the catchments and the location of the fen close to the flume in the non-grazed catchment (discussion; Paper IV). Furthermore, as was observed for soil water (Table 1; thesis, Table 1; Paper II); DON was the dominant form of N both in stream water and in standing water (i.e. surface runoff) of the topographic depressions (Table 1 and 3; Paper IV). Stream water concentrations of NH_4-N (0.02 - 0.03 mg l^{-1}) and NO_3-N (0.02 - 0.03 mg l^{-1}) were low, with no significant differences between the catchments. As compared to other systems these concentration levels are small. In the Green Lakes Valley (Colorado Front Range), with a similar N deposition as in Hol (~0.4 g m^{-2} yr^{-1}), concentrations in stream water of alpine sites ranged between 0.17 and 0.37 mg l^{-1} NO_3-N (Hood *et al.* 2003). Furthermore, at two sites of southwest Norway receiving a high N deposition (1.63 – 2.75 g m^{-2} yr^{-1}), Sjøeng *et al.* (2007) measured NO_3-N concentrations between 0.013 and 0.567 mg l^{-1}. Nitrate contributed between 9 and 59 % of the total N concentrations in runoff from these catchments (Sjøeng *et al.* 2007).

Concentrations of total coliforms and *E.coli* were significantly greater in the grazed as compared to the non grazed catchment (Table 3; Paper IV). At the grazed site, concentrations of *E. coli* were enhanced with increasing water flux (Table 4; Paper IV). Increased concentrations of bacteria with increased discharge is also reported by Collins *et al.* (2005) and Muirhead *et al.* (2006). The greater concentrations of total coliforms and *E. coli* in stream water at high discharge suggest an increase in the contribution of surface runoff relatively high in these bacteria. *E. coli* is effectively retained (filtration and adsorption) when infiltrating the soil (Muirhead *et al.* 2006). This is in accordance with our findings with smaller infiltration rates at the grazed site and in particular in the tracks. Despite a strong indication of increased surface runoff in response to grazing, we observed no difference in concentrations of inorganic N and P in stream water. This suggests a relatively strong retention of these nutrients, even in sheep tracks.

Conclusions

Soil carbon concentration (%SOC) and carbon stocks at equivalent soil mass in O-horizons decreased at high sheep density but increased at low sheep density compared non grazed sites after 7 years of grazing. By contrast, there was no effect of grazing on nitrogen stocks at equivalent soil mass. Bulk density (BD) and ash content of the particulate organic matter (POM) fraction were significantly increased at the high sheep density as compared to low sheep density and no grazing. In addition, bulk density was strongly affected by plant community with grassland in snowbeds having significantly greater BD than grassland with willow-shrubs. POC to SOC ratios were only slightly affected by grazing, with a small decrease and a moderate increase at the high and low sheep density, respectively. The lability of SOM (measured as potential carbon mineralization rates) was significantly related to the mineral content of the O-horizons, but there was no significant effect of grazing.

The system is characterized by low concentrations of inorganic N in soil water associated with a large demand for N (as determined by buried PRS^{TM} exchange resins). There was a strong seasonal effect on inorganic N and DIN:DON ratios of the soil water, with decreasing values in the course of the growing season, probably due to increasing nutrient demand. Effects of grazing on bioavailable N (PRS^{TM} exchange resins s) were greater at low as compared to high altitudes. At low altitudes, low sheep density reduced amounts of bioavailable N. Furthermore, potential net N mineralization rates were significantly greater at the high sheep density as compared to low sheep density and no grazing. Despite a pronounced seasonal decline in the N content of plants, there were small effects of grazing. The δ^{15}N natural abundance of selected plant and soil components, revealed little evidence for grazing induced effects on N cycling. By contrast, based on proportional tracer recoveries and tracer enrichments there was an indication of somewhat greater N cycling at grazed as compared to non-grazed areas.

Plant communities affect surface runoff quality with greater concentrations of DOC and DON in tall herb meadow (productive) as compared to snowbed (less productive). Furthermore, plant community and grazing level are associated with significantly different soil physical properties. Infiltration rates and the fractions of macro pores were smaller in snowbeds as compared to tall herb meadows, and a further decline occurs due to heavy sheep traffic. The effect of trampling on soil compaction, particularly pronounced in sheep tracks, results in increased surface runoff to the stream. This gives rise to significantly greater amounts of coliform bacteria in the grazed vs. the non grazed catchment, indicating a grazing induced potential for surface water contamination.

Overall this study has shown:

- Sheep grazing at low densities in this system increases soil C storage. However, soil C stores decline even at relatively moderate increases in sheep density (H1), illustrating a density dependent effects of grazing on C storage. Seven years of grazing is however, too short to have severe impacts on the form and lability of SOM (H1).

- Grazing significantly affects potential mineralization rates and the bioavailability of N (H2). However, there was no clear pattern of density dependent effects of grazing on cycling and availability of N. Concentrations of inorganic N in soil water were low with no indication of increased risk in N loss due to grazing. Losses associated with weight gain of the sheep are relatively small compared to the total N-pool in the system. Thus, the sheep densities used in this experiment are likely to have minor impacts on the system in terms of long-term N losses.

- Sheep grazing significantly reduces infiltration rates, in particular in areas with heavy sheep traffic (H3). Concentrations of coliform bacteria in stream water are enhanced at grazed vs. non-grazed sites (H3). However, there is no effect of grazing on inorganic N and P concentrations. Overall, the results suggest a strong retention of N and P in this low-alpine ecosystem.

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PAPER I

Martinsen, V., Mulder, J., Austrheim, G. and Mysterud, A., 2010. **Carbon storage in low-alpine grassland soils: effects of different grazing intensities of sheep**.

Carbon storage in low-alpine grassland soils: effects of different grazing intensities of sheep.

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Summary

Grazing in outlying fields has a long history and is important in local communities worldwide. During the last decades grazing pressure has both decreased and increased in alpine ecosystems, but little is known about the effects on soil carbon storage. As part of a sheep grazing experiment with three levels of sheep densities; control, 25 sheep km^{-2} and 80 sheep km^{-2}, we tested effects of grazing on soil organic carbon storage, form of soil organic matter (SOM) and lability (potential carbon mineralization) in organic horizons of low-alpine grasslands in southern Norway. After 7 years of grazing, high sheep density reduced soil carbon concentration (%SOC) and carbon stocks at equivalent soil mass as compared to control. By contrast, the opposite was observed at the low sheep density. The form of SOM, expressed as ratios of particulate organic carbon to soil organic carbon, were only slightly affected by grazing, with a small decrease and moderate increase at the high and low density, respectively. The lability of SOM was not affected by grazing directly, but significantly related to the mineral content of the O-horizons. In general, there were large differences between the plant communities (i.e. snowbed and willow-shrub) for several soil attributes. We conclude that a low grazing pressure in this system is positive for soil carbon storage, but that 7 years of grazing had limited impacts on the form and lability of SOM. Low sheep density thus has the potential to partly buffer effects of climate change.

Introduction

Land use may be an important factor mitigating climate change, as it may impact soil organic matter (SOM) storage (Schils *et al.* 2008). Soil organic matter is a crucial soil fraction affecting soil attributes and processes known to influence ecosystem functioning and productivity. The amount of organic matter stored in soils is controlled by natural, site specific factors like parent material, climate, topography and land cover, and human-induced factors associated with land use (Piñeiro *et al.* 2010; Schils *et al.* 2008). These factors may indirectly affect SOM due to a change in e.g. temperature, moisture and acidity (Darmody *et al.* 2004), or cause changes in primary production and decomposition that directly affect SOM (Piñeiro *et al.* 2010). Differences in site specific factors result in a high variability in SOM quantity and quality and may be pronounced even at small spatial scales (Burke *et al.* 1999; Hiller *et al.* 2005; Ostler *et al.* 1982; Stanton *et al.* 1994).

Grazing may have severe impacts on ecosystem functioning (Wardle *et al.* 2004) thereby potentially modifying soil organic carbon storage (SOC) (Piñeiro *et al.* 2010). Grazing has been reported to increase (e.g. Leifeld & Fuhrer 2009; Manley *et al.* 1995), decrease (e.g. He *et al.* 2008; Steffens *et al.* 2008) or have little or no effect (Tracy & Frank 1998) on SOC. A high grazing pressure may decrease the input of above and below ground (root) biomass (Johnson & Matchett 2001), thereby reducing the storage of soil carbon (He *et al.* 2008). According to Piñeiro *et al.* (2010) grazing may alter the content of SOC through 1) affecting the fraction of net primary production (NPP) entering the soil (i.e. the net primary production pathway), 2) affecting the soil nitrogen storage (i.e. the nitrogen pathway) and 3) affecting the decomposition of soil organic matter (i.e. the decomposition pathway) (Piñeiro *et al.* 2010).

Grazing also may affect the form of SOC including the particulate organic material (POM) fraction. POM, representing uncomplexed organic matter [i.e. neither recognizable as litter nor associated with organomineral complexes (Christensen 2001)], consists mainly of root fragments and aboveground plant residues (Golchin *et al.* 1994). As reported by Leifeld et al. (2009), this fraction is relatively young, with a mean residence time in the order of years to decades (Leifeld *et al.* 2009). Leifeld & Fuhrer (2009) found an increased ratio of particulate organic carbon to soil organic carbon in top-soils of a frequently grazed pasture compared to a meadow grazed for short periods in the Swiss Alps, which was suggested to be due to incorporation of plant materials by treading (Leifeld & Fuhrer 2009). In contrast, Steffens *et al.* (2009) observed a larger contribution of free POM to the total SOM within grazing exclosures due to enhanced litter inputs (Steffens *et al.* 2009). POM is a potential source of readily available C for decomposers (Christensen 2001) and is more mineralizable than heavy SOM fractions (Whalen *et al.* 2000). Thus changes in the POC to SOC ratio due to grazing may potentially affect the lability of SOM.

Low intensity livestock farming produce about half of the world's food (Herrero *et al.* 2010). Norway consists mostly of outlying fields, with a long history of grazing (Austrheim *et al.* 2008a). During the last decades, sheep densities have both decreased and increased in alpine ranges (Austrheim *et al.* 2008b), but little is known about the effects on soil carbon storage. Here we test effects of different grazing intensities of sheep on SOC storage, form (POC to SOC ratio) and lability in organic horizons of low-alpine grassland soils. The study was done as part of a sheep grazing experiment with three levels of sheep densities (Mysterud & Austrheim 2005). Previous findings from this grazing experiment indicate a reduction in lamb weights (Mysterud, unpublished material) and a lower vascular plant biomass production (Austrheim, unpublished material) with increased grazing pressure. Both factors indicate an overall higher biomass removal in high grazing treatments compared to low and control. We predicted: (1) that increasing densities of sheep decreases the potential for soil C storage as compared to non grazed sites, and (2) a decreased fraction of POC to SOC with increasing densities of sheep associated with a decline in lability (i.e. lower potential carbon mineralization) of SOM.

Material and methods

Site description

The study site is located in the low alpine region (1050 - 1320 m.a.s.l.) in Hol municipality, Buskerud county, southern Norway (7°55′ - 8°00′ E, 60°40′ - 60°45′ N) (Mysterud & Austrheim 2005). Vegetation is dominated by dwarf shrub heaths with smaller patches of lichen heaths, snow beds and alpine meadow communities in lee-sides (Rekdal 2001). The bedrock consists of meta-arkose and quaternary deposits of till and colluvium (Kristiansen & Sollid 1985; Sigmond 1998). Soils are leptic podsols (snowbeds) and dystric haplic gleysols (willow-shrubs) (IIUSS Working Group WRB 2006). Mean annual temperature (MAT) is -1.5 ^{O}C and mean annual precipitation (MAP) is about 1000 mm (Evju *et al.* 2009), approximately 75% of which falls as snow. In 2001 a large enclosure (2.7 km^2) was fenced and divided in three blocks, each replicated with three sub-enclosures (approximately 0.3 km^2) with three treatments; no sheep (control), low grazing density (25 sheep km^{-2}) and high grazing density (80 sheep km^{-2}) of domestic sheep (*Ovis aries*) (Mysterud & Austrheim 2005; Mysterud *et al.* 2005). The study is set up as a randomized block design (Figure 1). Sheep grazing occurs from the end of June to the beginning of September.

Twenty plots (0.5 x 0.5 m) were established in each enclosure (a total of 180 plots) in 2001 using a balanced stratified procedure among altitudinal levels and habitats (Austrheim *et al.* 2005). Soil was sampled (summer 2008) at 89 plots within two different grassland plant communities; snowbed (n= 56) and willow-shrub (n=33) (Figure 1). Willow-shrubs are associated with leeside vegetation, less snow cover and a longer growing season than snowbeds (Moen *et al*. 1999). Further description of experimental design, selection of vegetation plots and plant species composition is given by Austrheim *et al.* (2005).

Soil samples

Soil was sampled (cylinder, diameter 5.2 cm) to a maximum depth of 5 cm within the O-horizon during the period 05.08.08-08.08.08. Four soil samples were taken adjacent to the vegetation plots (at all four sides). To obtain enough soil material for analysis, more than four samples were taken if the O-horizon was <5 cm. The vegetation was cut at the soil surface and the litter (i.e. O_i) was removed. Soil samples at each plot were bulked and stored dark and cold (< 4 °C) prior to analysis. The (field moist) soil samples were homogenized and divided into two subsamples, one for chemical analysis (air-dried at 40 °C for 4-5 days) and one for determination of potential carbon mineralization (kept cold and moist). The air-dried samples were sieved at 2 mm and the weight of dry roots and gravels (> 2 mm) determined. Sub samples of the air-dried and sieved samples were dried at 60 °C to determine dry matter content (DM) and additionally milled prior to determination of total carbon (C) and total nitrogen (N).

Total C and N was analyzed by dry combustion (Leco CHN-1000; Leco corporation, Sweden) (Nelson & Sommers 1982) and the Dumas method (Bremmer & Mulvaney 1982), respectively. Dry matter corrected weight of soils (corrected for amount of roots and gravels) was used to determine bulk density (BD; g cm^{-3}), thus representing bulk density of the fine earth fraction. pH was determined electrometrically (Orion, model 720) in a soil solution with distilled water (volume soil : volume water ratio 0.4) (Krogstad 1992). Soil samples (air-dried and sieved at 2 mm) were extracted with 1 M ammonium acetate (adjusted to pH 7) and base cation concentrations determined in the extracts. Extractable acidity was determined by back-titration with sodium hydroxide (to pH 7). The sum of exchangeable base cations and acidity were used to determine cation exchange capacity (CEC) according to (Schollenberger & Simon 1945). Exchangeable cation concentrations were determined using ICP-OES.

Carbon and nitrogen stock calculations

Volume based C and N stocks were calculated as

$$\text{E-stock} = \text{BD (g cm}^{-3}\text{) x Depth (cm) x E concentration (\%) x 0. 1}, \qquad (1)$$

where E-stock is the volume based elemental stock (density) (kg m^{-2}), BD is bulk density of the fine earth, Depth is the soil depth and E concentration is the elemental concentration of C or N (% by weight).

As grazing and habitat may affect bulk density and depth of the organic horizon, this also may affect elemental stock calculations. Therefore, C and N stocks were also calculated per equivalent soil mass as, described by (Ellert & Bettany 1995):

$$\text{C-stock (eqv. mass)} = \text{Equivalent mass (kg m}^{-2}\text{) x C concentration (\%) x } 10^{-2}, \qquad (2)$$

where C-stock eqv. mass is the carbon stock (kg m^{-2}) per equivalent mass of soil.

Density fractionation

Particle size and density fractionation was done [modified, based on Leifeld & Kögel-Knabner (2005)] to obtain a free, light (density < 1.8 g cm^{-3}) particulate organic matter (POM) fraction (20 µm < POM <2000 µm). The remaining mineral associated organic material fraction (mOM) was used to calculate percentage recovery, but not further analyzed. 10 g of air dry and sieved (2mm) soil was weighted in to a 100 mL centrifuge tube. 70 ml of sodium polytungstate, $Na_6(H_2W_{12}O_{40})$ *H_2O (Sometu, Berlin, Germany), adjusted to a density of 1.8 g cm^{-3}, was added to the tube and gently inverted 5 times. The suspension was left for 10 minutes prior to 10 minutes of centrifugation at 1500 relative centrifugation force (= 1.5 times gravity). The supernatant with floating particles was poured on a 20 μm sieve and washed with distilled water (until conductivity <100 μS/cm; all material <20 μm was washed away) to retrieve the POM fraction. The remaining suspension in the tube was gently stirred and the steps repeated (with addition of sodium polytungstate) once to retrieve the mOM (i.e. sediment material). The POM and mOM fractions were dried (60 °C) to determine recovery rate (~95 %) and to calculate percentage POM in the samples (w:w). The samples were milled prior to determination of total C and total N. In some cases with a high percentage POM, the calculated share of particulate organic carbon to soil organic carbon (POC:SOC) resulted in values >1. As the share POC of SOC cannot make up more than 100%, these values were set to 1 (i.e. 100 %). Samples of POM were combusted at 550 ºC to determine loss on ignition (LOI), and the ash content of the POM fraction.

Potential carbon mineralization

Closed flasks (12 ml) with field moist soil (equivalent to 1 g of dry soil) from each sample location (n = 89) were incubated in the dark (15 °C; aerated every second day to prevent anoxia) and, at 6 different time periods (period 1 = 0 days (11 hours), period 2 = 4 days, period 3 = 8 days, period 4 = 15 days, period 5 = 20 days and period 6 = 27 days) placed in a robotized incubation system (Molstad *et al.* 2007) at 15 °C and connected to a gas chromatograph (GL system 7890, Agilent Technologies) to measure CO_2 concentration in head space. When measuring the rates of gaseous release (determined as the increase in elemental concentration per unit of time) a significant proportion of air was removed (i.e. transferred to the gas chromatograph) from the headspace of each flask. This diluting effect on measured CO_2-C was accounted and corrected for using elemental nitrogen (N_2). The measured headspace CO_2-C concentrations were retrieved in ppm and converted to mol assuming a molar gas volume at 15°C of 23.64 l mol^{-1}.

Statistical analysis

Statistical analyses were conducted using the libraries lme4, multcomp, ltm and lattice in the statistical package R (version 2.10.1) (http://www.r-project.org/).

Student's t-tests (normally distributed variables) and Wilcoxcon's rank-sum tests (variables not normally distributed) were used to test for differences between plant communities (snowbed and willow-shrub) for selected soil parameters (Table 1).

We used Linear mixed effects models (lmer) with random effects reflecting the block-wise randomization design, i.e. enclosure (n=9) nested within block (n=3). The fixed factors included grazing treatment (3 levels; control, high and low), plant community (snowbed and willow-shrub) and two-way interactions between treatment and plant community (i.e. categorical explanatory variables). Bulk density and ash content of POM (i.e. continuous explanatory variables) were included as covariates to account for differences in these variables associated with treatment and plant community. We assumed no treatment or plant community specific differences in the response of the covariates (i.e. no difference in slopes). For CO_2-C fluxes (i.e. potential carbon mineralization) we used the average of the measured fluxes (measured at 6 different time periods) to avoid temporal pseudoreplication (repeated measurements on the same soil sample). Some variables were transformed (ln or arcsine) prior to analysis to avoid violations of the model assumptions.

Backward selection was used (models fitted by maximum likelihood (ML)) and models compared based on AIC ("smaller is better") and likelihood ratio tests (Chi squared) to obtain the minimum adequate model. The best model was re-fitted based on restricted maximum likelihood (REML) and the estimated effects (including se) were calculated using general linear hypothesis testing (glht in multcomp). Adjustments of p-values in multiple comparisons were conducted using a single-step method (Hothorn *et al.* 2008). Residuals and predicted random effects were plotted (histograms and QQ normal plots) to assess normality and potential outliers.

Predictions of different treatment combinations for given levels of the continuous explanatory variables (i.e. bulk density or ash content of POM) were calculated using glht() in multcomp and reported with standard error (se). Multiple correlations between selected soil parameters (including p-values obtained by package ltm ()) were calculated using spearman rank correlation (because not all the variables were normally distributed). These are presented in supporting information (Figure S1). Six soil plots [1 from control snowbed (%SOC = 11.61), 3 from control willow-shrub (%SOC = 8.71, 45.8 and 50.75), 1 from low density snowbed (%SOC = 11.52) and 1 from high density snowbed (%SOC = 10.41)] differed greatly in O-horizon soil attributes compared to the other 83 grassland habitat plots. Thus, the plots were excluded from the analysis.

Results

Differences between the plant communities

An overview of selected soil chemical and physical data (means ± s.e.) is given in Table 1. The mean altitudinal level was lower for sampling plots in willow-shrubs as compared to snowbeds. Willow shrubs were associated with deeper O-horizons, lower bulk densities (BD) and higher base saturations than snowbeds. The carbon concentration in the O-horizon bulk soil (% SOC) did not differ significantly between the plant communities. However, carbon stock per cm soil depth (i.e. per unit volume of soil) was significantly larger in snowbeds than in willow-shrubs, due to the greater bulk density in snowbeds (Table 1). The form of organic material (expressed as fraction of particulate organic C to the total amount of bulk soil organic

C; POC to SOC ratio) and the percentage POM in the bulk soil, did not differ significantly between the plant communities. However, C:N ratios of the bulk soil and of the POM fraction were significantly greater in willow-shrubs than snowbeds (Table 1).

Effects of grazing and plant community on quantity, form and lability of SOM

Bulk soil carbon concentration

The fitted linear mixed effect model for bulk soil carbon concentration (% SOC) revealed a significant interaction between plant communities and treatments and a significant decrease in percent SOC per unit increase in BD (Table 2). At the mean bulk density within each community and treatment (Table 1), predicted bulk soil carbon concentrations were lower at high sheep density compared to low sheep density and control both within snowbeds and willow-shrubs (Figure 2a). The two grazing treatments showed the highest (at low grazing pressure) and lowest (at high grazing pressure) predicted SOC within both plant communities, with SOC at control sites intermediate. The differences between the treatments were more pronounced within willow-shrubs than within snowbeds with predicted SOC differing as much as ~8.5% between high and low sheep densities (Figure 2a). For high grazing pressure and control the SOC was somewhat lower in willow-shrubs than snowbeds. By contrast, at low grazing pressure the predicted bulk SOC was greater in willow-shrubs than snowbeds, illustrating the interaction between habitat and treatment (Figure 2a; Table 2).

Carbon stocks

The fitted model for carbon stocks based on equivalent mass (C-stock eqv. mass/ kg m^{-2}) after model reduction included grazing as the only significant factor (Table 2). Estimated C-stocks eqv. mass (Table 2) were lower for the high density treatment (0.64 ± 0.04) compared to low

(0.80 ± 0.04) and control (0.76 ± 0.04), indicating greater loss of soil carbon and/or lower biomass input in areas with high sheep densities.

The fitted model for carbon stock per cm soil (C-stock volume based/ kg m^{-2}) included a significant interaction effect of treatment and plant community in addition to a significant increase in C-stock per unit increase in bulk density (Table 2). These are the same predictors as for SOC (Table 2). The predicted C-stock at high sheep density in snowbeds was (at the mean bulk density of each treatment; Table 1) greater than at low grazing density and control, thus the opposite of what was observed for bulk soil carbon concentration (Figure 2a and 2b). In contrast, in willow-shrubs (with significantly lower BD than snowbeds; Table 1), there were only minor differences in predicted volume based C-stocks between the treatments (Figure 2b).

Particulate organic carbon to soil organic carbon ratio (POC to SOC ratio)

The fraction of particulate organic carbon of total soil organic carbon (POC to SOC ratio) was significantly explained by grazing, plant community, interaction between grazing and plant community and ash content of the POM-fraction (Table 2), with a significant reduction in the POC to SOC ratio per unit increase in the ash content of the POM fraction. At the mean POM ash content within each treatment and plant community (Table 1), the predicted POC to SOC ratio in snowbed was lower at high sheep density compared to low density and control, the latter two only slightly differing (Figure 2c). In willow-shrubs, the predicted POC to SOC ratios revealed only minor differences between the grazing treatments. For all plant community and treatment combinations, the predicted POC to SOC ratios showed a similar pattern (though not as pronounced) as bulk soil carbon concentration (% SOC).

Potential carbon mineralization

Potential carbon mineralization, measured as the mean CO_2-C flux (µg g soil^{-1} hour^{-1}) during 27 days of laboratory incubation, was neither affected by treatment nor by plant community (Table 2). Normalizing CO_2-C fluxes on amount of soil particulate organic carbon (µg g POC^{-1} hour^{-1}), which is believed to be the most labile soil C fraction, revealed greater CO_2-C fluxes from soils in willow-shrub than snowbeds, but no effect of grazing treatment (Table 2).

Discussion

Grazing lands have a significant potential for short term mitigating of climate change (Laca *et al.* 2010). Several studies have shown that grazing may strongly affect C storage (He *et al.* 2008; Piñeiro *et al.* 2010; Steffens *et al.* 2008), but grazing induced effects on SOC concentrations and stocks, fraction POM and lability of SOM, in addition to physical parameters like BD, are expected to depend on herbivore densities as well as plant community characteristics. In this study we found evidence for herbivory-dependent effects on carbon storage (expressed either as concentrations or stocks) and POC to SOC ratios of SOM interacting with site (i.e. plant community). However, these effects were strongly related to differences in soil physical attributes (i.e. BD and ash content of the POM fraction), which are also associated with grazing and plant community.

Within each of the two plant communities (snowbed and willow-shrub) there was a significant effect of grazing on O-horizon bulk density (BD/g cm^{-3}). High sheep density caused significantly greater BD compared to low sheep density and control (Table 3). Previously, soil compaction caused by grazing has been reported by Steffens *et al.* (2008). Similar to the effects of grazing on BD, we found a significantly greater ash content of the POM fraction (which did not differ significantly between the plant communities) in the high grazing treatment compared to low and control sites (Table 3). Together, these findings suggest that there are two different mechanisms controlling incorporation of mineral matter in these O-horizons; viz. compaction (caused by snow cover and trampling) and mixing of organic matter and minerals (caused by the sheep).

Bulk density (BD) and bulk soil carbon concentration (%SOC) were significantly negatively correlated (rho = -0.74, $p<0.05$; Figure S1), similar to what has been reported by e.g. De Vos *et al.* (2005) and Steffens *et al.* (2008). Others have used this relationship to develop pedotransfer functions to predict bulk density based on organic matter content (De Vos *et al.* 2005; Perie & Ouimet 2008). However, the fitted model for SOC has in addition to BD, other explanatory variables including site (i.e. plant community) and grazing regime (Table 2). In accordance with our hypotheses, SOC concentrations were (at the mean bulk density within each plant community and treatment combination) smaller in areas with high sheep density compared to low and control (Figure 2a). This suggests different pedotransfer functions depending on location and management practice.

Calculating C-stocks based on equivalent mass (C-stock eqv.mass; kg m^{-2}; cf. Ellert & Bettany 1995), revealed lower C-stock estimates for the high sheep density compared to low and control, the latter two only slightly differing (Table 2). Similar effects of grazing on C-stock based on equivalent mass were reported by Steffens *et al.* (2008). As earlier noted by Ellert & Bettany (1995), C-stocks based on soil equivalent mass are more sensitive to detect management induced changes in carbon storage than estimates based on soil volume (Table 2, Figure 2b). In contrast to SOC and C-stock eqv.mass, carbon stocks calculated per soil volume revealed greater predicted C-stocks in snowbeds for the high sheep density compared to the low density and control (Figure 2a,b; Table 2). However, within willow-shrubs, there were only minor differences in predicted volume based C-stocks between the treatments (Figure 2b). These findings illustrate the importance of the reporting unit when assessing effects of land use on carbon storage. Because volume based C-stocks are based on an unequal mass of soil, we suggest using C-concentrations (SOC) or C-stock eqv.mass for comparisons of carbon stores.

Particulate organic matter (POM) consists mainly of root fragments and aboveground plant residues (Golchin *et al.* 1994) hence; any reduction in biomass input or increased decomposition would reduce the POC to SOC ratio within the soil. Based on the fitted model (Table 2), the predicted POC to SOC ratio (at mean ash content within each treatment and plant community; Table 1) was lowest at high sheep density both in particular in snowbeds but also in willow-shrubs (Figure 2c). By contrast, Leifeld & Fuhrer (2009) found significantly greater SOC with a greater fraction of POC (0-4 cm soil depth) in a frequently grazed pasture (dairy cows grazing daily from mid June to mid September) compared to a meadow grazed for short periods in the autumn in the Swiss Alps. They suggest that the greater fraction of POC in the pasture compared to the meadow is caused by incorporation of plant material by treading (Leifeld & Fuhrer 2009). At Hol, the POC to SOC ratio was negatively correlated with ash content of the POM fraction (Figure S1). In addition, there was a significantly greater ash content of the POM fraction at high sheep density (Table 3). Thus, we suggest that the somewhat lower POC to SOC ratios at the high grazing treatment at Hol is induced by physical disruption of POM (treading) causing increased decomposition. In addition, the sites with high sheep density have lower inputs of plant biomass (Austrheim, unpublished material).

In a parallel study at Hol (results not shown) the ^{14}C content of POM suggests that the POM fraction has a relatively low mean residence time. Recently, similar observations were reported by Leifeld & Fuhrer (2009) and Leifeld *et al.* (2009). Therefore we expected samples with a high POC to SOC ratio to have higher potential carbon mineralization rates [measured as CO_2-C fluxes (μg g soil^{-1} hour^{-1})]. However, our results revealed no effects of treatment or plant community (Table 2), despite differences in the POC to SOC ratios (Figure

2c). The only significant factor decreasing the decomposability of SOM was BD (Table 2). This may be an effect of less available substrate for microbial decomposition at higher BD.

The fitted model for CO_2-C fluxes normalized to POC (µg CO_2-C g POC^{-1} $hour^{-1}$), revealed greater flux estimates from willow-shrubs than from snowbeds, with a significant increase in CO_2-C flux with increased ash content of POM (Table 2). The positive response in carbon mineralization per g POC^{-1} to increased ash content of POM is indicative of sites exposed to more disturbances (i.e. POM disruption and mixing of mineral and organic matter). As the quality of SOM may be distinguished from quantity by identifying the fraction of SOC found in the mineralizable fraction (Gregorich *et al.* 1994), these findings suggest that there might be minor differences in the quality of the organic matter between the habitats, but that 7 years of grazing at the densities provided in Hol, is not sufficient to affect the decomposability of SOM.

Plant community and soil properties affecting C storage

The mineral content in surface soils (organic horizons) of snowbeds is found to be high (Hiller *et al.* 2005; Virtanen *et al.* 2008). Our results are partly in accordance with these findings, as bulk density (BD) was significantly greater in snowbeds than willow-shrubs (i.e. increased bulk density with increased amount of mineral matter) (Table 1).

The contribution of particulate organic carbon (POC) to bulk soil organic carbon (SOC) in the O-horizons was high both in snowbeds and willow-shrubs with only minor differences between the plant communities (82% and 79 %; Table 1). The large POC to SOC ratio found at Hol is similar to what was found by (Leifeld *et al.* 2009) in the Swiss Alps (> 80% in 0-5 cm soils at the highest elevations; 2200 m.a.s.l.).

O-horizons were significantly deeper and pH and base saturation significantly greater in willow-shrubs (intermediate snow cover) than snowbeds (heavy snow cover) (Table 1).

Ostler *et al.* (1982) reported a decrease in total soil depth with increasing snow cover on a sub-alpine meadow in the Uinta Mountains (USA). Snow cover may also affect other soil attributes. A decrease in snow cover correlated with higher SOM content (Ostler *et al.* 1982; Stanton *et al.* 1994), nutrient levels and acidity (Stanton *et al.* 1994). Our results revealed no difference in SOC concentrations between the two plant communities and, in contrast to Stanton *et al.* (1994), a significantly higher pH (i.e. lower acidity) and greater base saturation in willow-shrubs (early melting) than snowbeds (late melting). The high pH and base saturation combined with the deeper O-horizon soils in willow-shrubs than snowbeds in the Hol system may be due to return-flow of ground water rich in base cations.

Conclusions

Soil carbon concentration (%SOC) and carbon stocks at equivalent soil mass decreased at high sheep density but increased at low density compared to controls with no sheep after 7 years of grazing. High sheep density significantly increased bulk density (BD). In addition, bulk density was strongly affected by plant community with snowbeds having significantly greater BD than willow-shrubs.

POC to SOC ratios were only slightly affected by grazing, with a small decrease and a moderate increase at the high and low sheep density, respectively. The lability of SOM (measured as potential carbon mineralization rates) was significantly related to the mineral content of the O-horizons.

In general, there were large differences between the plant communities (i.e. snowbed and willow-shrub) for several soil attributes. We conclude that sheep grazing at low densities in this system increases soil C storage. However, soil C stores decline even at relatively moderate increases in sheep density. Seven years of grazing is too short to have severe impacts on the form and lability of SOM.

Acknowledgements

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Figure legends

Figure 1. Experimental design (3 enclosures with different grazing pressure of sheep in 3 blocks, Hol, southern Norway. The area of each enclosure is ~0.3 km^{-2}, total area is ~2.7 km^{-2}. White enclosures = no sheep (control), light grey enclosures = low (25 sheep km^{-2}) and dark grey enclosures = high (80 sheep km^{-2}) sheep density. Points represent locations (n=89) for soil sampling within grassland habitats in two plant communities [snowbed (triangles) and willow-shrub (circles)].

Figure 2. Predicted **a** total bulk soil carbon content (SOC/% of fine earth; n = 82), **b** Carbon stock (volume based) per cm soil (C-stock/kg m^{-2}; n = 83) and **c** particulate organic carbon to soil organic carbon ratio (POC:SOC; n= 83) ± SE in O-horizon soil samples from grassland habitats within two different plant communities (snowbed and willow-shrub) at three levels of grazing (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep), Hol, southern Norway. Predictions are based on fiexed effect estimates derived from linear mixed effect models (Table 2) at mean bulk density (g cm^{-3}) (Figure 2a and 2b) and mean ash content (Figure 2c) within each treatment combination (Table 1). SH = Snowbed-high, SL = Snowbed-low, SNo= Snowbed-no sheep, WH = Willow-shrub-high, WL = Willow-shrub-low and WNo = Willow-shrub-no sheep.

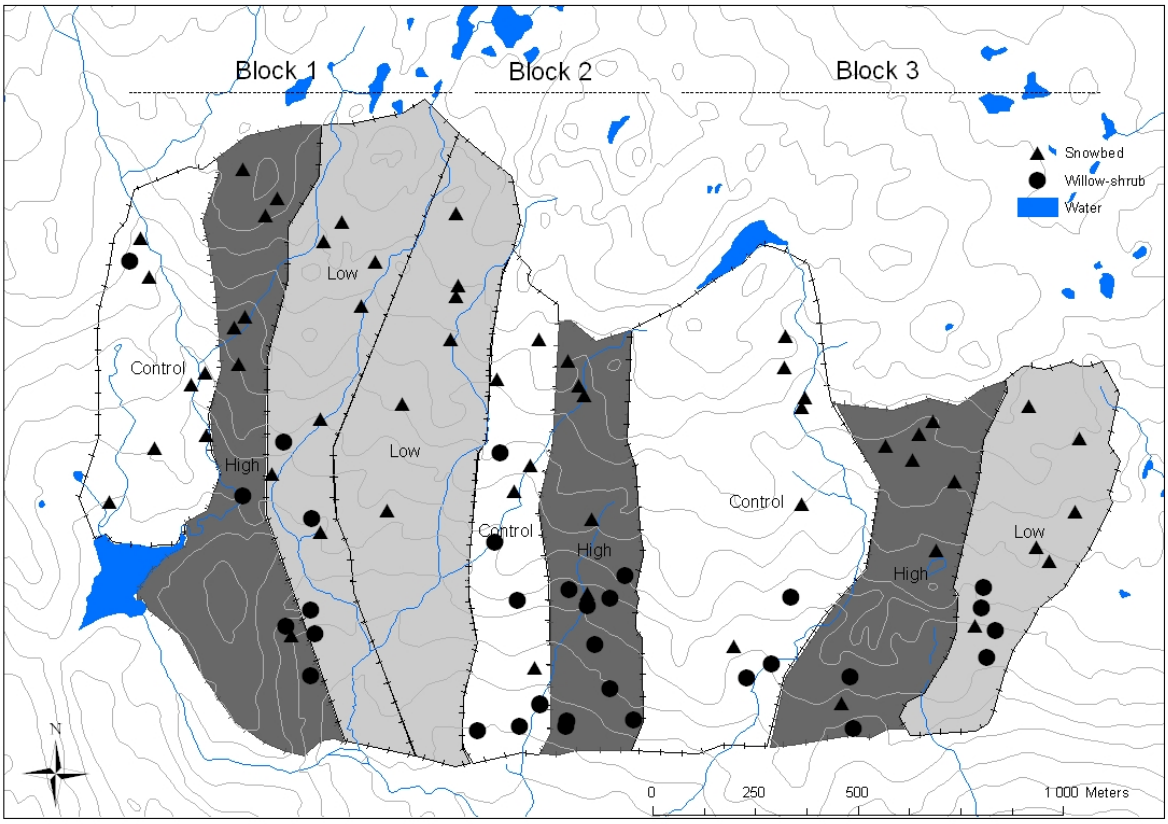
Block 1
Block 2
Block 3
Snowbed
Willow-shrub
Water
Control
High
Low
Low
Control
High
Control
High
Low
N
0
250
500
1 000 Meters

Figure 1.

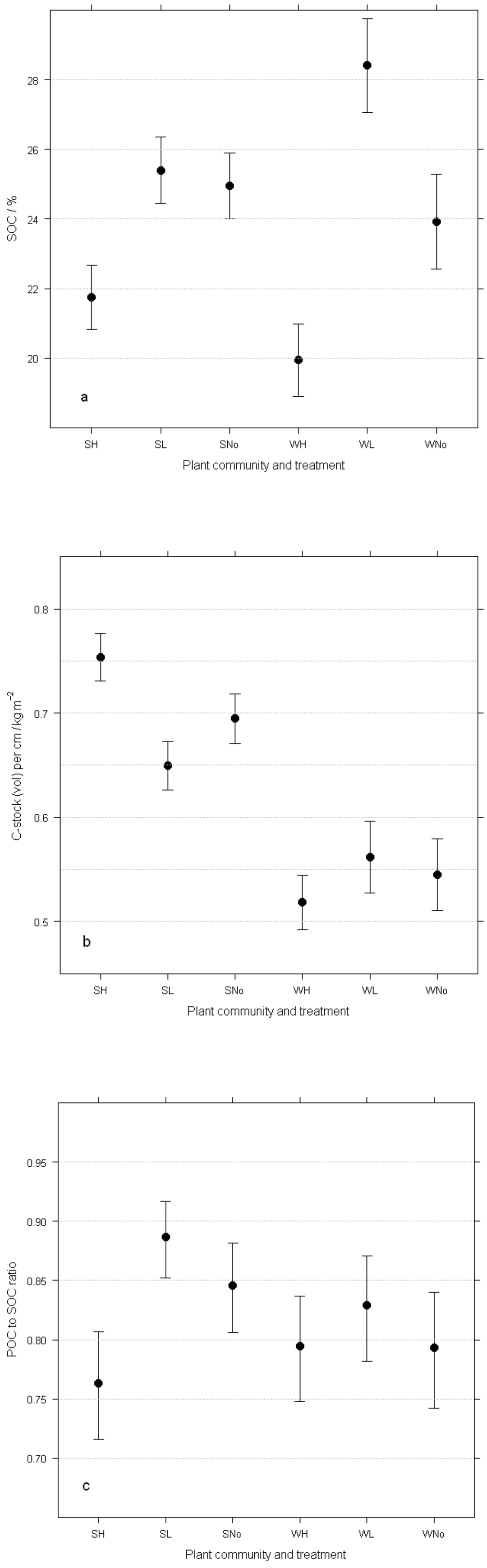
a
SOC / %
20
22
24
26
28
SH
SL
SNo
WH
WL
WNo
Plant community and treatment
b
C-stock (vol) per cm / kg m^{-2}
0.5
0.6
0.7
0.8
SH
SL
SNo
WH
WL
WNo
Plant community and treatment
c
POC to SOC ratio
0.70
0.75
0.80
0.85
0.90
0.95
SH
SL
SNo
WH
WL
WNo
Plant community and treatment

Figure 2

Table 1: Mean O-horizon soil attributes from 83[a] grassland habitats (sampled 2008) within two plant communities (snowbed and willow-shrub) at different grazing densities of sheep (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep = control), Hol, Norway. S.e. = standard error. Abbrevetions: BD = bulk density of the fine earth, CEC = cation exchange capacity, SOC = bulk soil organic carbon, SON = bulk soil organic nitrogen, CN ratio bulk soil = carbon to nitrogen ratio in bulk soil, C stock per cm= carbon stock (density) of bulk soil per cm soil depth (i.e. volume based), N stock per cm= nitrogen stock (density) of bulk soil per cm soil depth (i.e. volume based), POM = particulate organic matter, POC = carbon content in particulate organic matter, PON = nitrogen content in particulate organic matter, CN ratio POM = carbon to nitrogen ratio of POM, POC to SOC ratio = carbon in particulate organic matter to bulk soil organic carbon ratio and PON to SON ratio = nitrogen in particulate organic matter to bulk soil organic nitrogen ratio. Tests of difference between habitats are based on Student's t-tests (latin letters)and Wilcoxon's rank-sum tests (greek letters); different letters[b] indicate difference at the level of significance p <0.05.

Factor	Snowbed								Willow-shrub							
	High		Low		No sheep		All treatments		High		Low		No sheep		All	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Altitude/m.a.s.l	1248	13	1251	10	1216	12	1239 $^{\alpha}$	7	1141	7	1170	10	1162	22	1154 $^{\beta}$	7
Slope/%	14.44	2.06	11.11	1.59	14.41	2.18	13.30 $^{\alpha}$	1.13	15.36	1.61	16.88	3.77	13.75	3.10	15.33 $^{\alpha}$	1.46
Total O-hor depth/cm	3.82	0.63	5.30	1.11	4.07	0.63	4.40 $^{\alpha}$	0.48	6.80	1.06	6.06	1.81	7.85	1.81	6.88 $^{\beta}$	0.82
Sample depth/cm	2.74	0.27	3.20	0.28	3.26	0.28	3.06 $^{\alpha}$	0.16	4.00	0.30	3.38	0.45	3.74	0.51	3.76 $^{\beta}$	0.22
BD/g cm^{-3}	0.35	0.01	0.26	0.01	0.28	0.02	0.30 ^{a}	0.01	0.27	0.03	0.20	0.02	0.23	0.03	0.24 ^{b}	0.02
CEC/cmol$_c$ kg soil^{-1}	40.67	2.08	47.75	2.34	45.80	2.58	44.80 ^{a}	1.40	45.63	3.81	50.63	12.50	43.21	5.31	45.65 ^{a}	2.99
Base saturation/%	33.28	3.96	26.30	5.14	22.87	2.97	27.46 $^{\alpha}$	2.44	43.41	3.62	43.89	14.54	37.25	5.24	41.87 $^{\beta}$	3.03
pH	4.63	0.08	4.56	0.11	4.42	0.08	4.54 ^{a}	0.05	4.91	0.07	4.64	0.26	4.84	0.11	4.84 ^{b}	0.07
SOC/% of finearth	22.01	1.08	25.79	1.32	25.56	1.44	24.43 ^{a}	0.76	20.94	1.66	29.25	2.69	25.09	2.48	24.26 ^{a}	1.36
SON/% of finearth	1.37	0.07	1.47	0.08	1.44	0.09	1.42 $^{-}$	0.04	1.21	0.11	1.45	0.16	1.40	0.18	1.32 $^{-}$	0.08
C:N ratio bulk soil	16.13	0.34	17.72	0.49	18.03	0.66	17.28 $^{\alpha}$	0.31	17.73	0.60	20.71	1.45	18.52	0.96	18.74 $^{\beta}$	0.57
C stock per cm/kg m^{-2}	0.75	0.02	0.65	0.03	0.69	0.03	0.70 ^{a}	0.02	0.51	0.02	0.56	0.03	0.54	0.04	0.53 ^{b}	0.02
N stock per cm/kg m-^{2}	0.05	0.00	0.04	0.00	0.04	0.00	0.04 ^{a}	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03 ^{b}	0.00
POM/% of bulk soil	59.56	3.02	73.03	3.04	67.38	3.73	66.64 $^{\alpha}$	2.00	60.08	4.95	73.84	4.24	66.85	6.42	65.55 $^{\alpha}$	3.17
POC/% C of POM	28.15	0.72	30.60	0.95	31.61	0.92	30.09 ^{a}	0.53	27.09	0.98	31.38	1.95	29.45	1.51	28.86 ^{a}	0.84
PON/% N of POM	1.75	0.05	1.84	0.06	1.76	0.06	1.78 ^{a}	0.03	1.54	0.08	1.65	0.13	1.65	0.13	1.60 ^{b}	0.06
C:N ratio POM	16.21	0.39	16.84	0.59	18.21	0.64	17.07 ^{a}	0.33	17.88	0.66	19.40	1.31	18.32	1.09	18.40 ^{b}	0.54
POC to SOC ratio	0.76	0.02	0.87	0.02	0.83	0.02	0.82 $^{\alpha}$	0.01	0.77	0.03	0.81	0.05	0.79	0.04	0.79 $^{\alpha}$	0.02
PON to SON ratio	0.76	0.02	0.92	0.03	0.83	0.03	0.83 $^{\alpha}$	0.02	0.77	0.04	0.85	0.04	0.80	0.05	0.80 $^{\alpha}$	0.02
Ash content POM/%	45.20	1.35	40.74	1.59	39.42	1.60	41.83 ^{a}	0.92	47.41	1.93	39.94	3.68	42.71	2.79	44.16 ^{a}	1.58

[a]6 soil plots are removed from this summary table (see material and methods). [b]Not tested is marked “-“. A simular summary table including all 89 soil plots is presented in the supporting material (Table S2). Total O-hor depth, POC and C:N ratio POM were ln transformed prior to analysis.

Table 2. Linear mixed models and parameter estimates (based on REML-estimation) for total bulk soil carbon content (SOC/% of fine earth); carbon stock (volume based) per cm soil (C-stock per cm/kg m^{-2}); carbon stock based on equvialent mass mass[a] (C-stock eqv.mass//kg m^{-2}); particulate organic carbon to bulk soil organic carbon ratio (POC to SOC ratio) (arcsine transformed), carbon flux[b] (μg CO_2-C g $soil^{-1}$ $hour^{-1}$) and carbon flux[b] (μg CO_2-C g POC^{-1} $hour^{-1}$) (ln transfomred) within two different plant communities (snowbed and willow shrub; factor "pl.comm") at three levels of grazing (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep; factor "treatment") within enclosures (n=9; factor enclosure; random effect) nested in blocks (n=3; factor "block"; random effect) in grassland habitats (Hol, Norway). Continous variables are: bulk density of the fine earth (g cm^{-3}) (BD; ln transfomred) and ash content of POM (%). Mean effects of different factor combinations and effects of continous variables (including standard error and t-values) are shown. P -values are based on simultaneous tests for general linear hypotheses (indicating wheater estimates are significantly different from 0). The model reduction steps (based on ML-estimation) are presented in supporting infromation (Table S1). Not relevant is marked "-".

Model	Parameter	Estimate	se	t	p
SOC ~ treatment x pl.comm + ln(BD) +(1 | enclosure) + (1 | block). n = $82^{c,d}$	***SOC/% of finearth***				
	Control-snowbed	2.744	2.227	1.232	-
	High-snowbed	3.342	1.892	1.767	-
	Low-snowbed	1.657	2.329	0.711	-
	Control-willow	-1.892	2.733	-0.692	-
	High-willow	-3.392	2.384	-1.423	-
	Low-willow	0.358	2.885	0.124	-
	ln(BD)	-17.569	1.552	-11.320	<0.001
C-stock per cm ~ treatment x pl.comm + ln (BD) +(1 | enclosure) + (1 | block). n = $82^{c,d}$	***C-stock per cm/kg m^{-2}***				
	Control-snowbed	0.888	0.059	15.130	-
	High-snowbed	0.914	0.050	18.440	-
	Low-snowbed	0.856	0.062	13.910	-
	Control-willow	0.770	0.072	10.690	-
	High-willow	0.722	0.063	11.470	-
	Low-willow	0.807	0.076	10.630	-
	ln(BD)	0.153	0.041	3.698	<0.01
C-stock eqv.mass ~ treatment +(1 | enclosure) + (1 | block). n = 83^{c}	***C-stock eqv.mas/kg m^{-2}***				
	Control	0.757	0.043	17.540	-
	High	0.642	0.040	16.030	-
	Low	0.799	0.043	18.770	-
Arcsine (POC to SOC) ~ treatment x pl.comm + Ash content POM (1 | enclosure) + (1 | block). n = 83^{c}	***Arcsine (POC to SOC)***				
	Control-snowbed	1.699	0.111	15.350	-
	High-snowbed	1.660	0.120	13.820	-
	Low-snowbed	1.804	0.113	16.030	-
	Control-willow	1.665	0.122	13.680	-
	High-willow	1.749	0.126	13.930	-
	Low-willow	1.677	0.119	14.130	-
	Ash content POM	-0.018	0.002	-8.125	<0.001
CO_2-C flux/g $soil^{-1}$~ ln (BD) + (1 | enclosure) + (1 | block). n = $82^{c,d,e}$	***CO_2-C flux/µg g $soil^{-1}$ $hour^{-1}$***				
	Intercept	1.107	0.548	2.021	-
	ln(BD)	-1.701	0.372	-4.568	<0.001
ln(CO2-C flux/g POC^{-1}) ~ pl.comm + Ash content POM + (1 | enclosure) + (1 | block). n = $83^{c,e}$	***ln(CO_2-C flux/µg g POC^{-1} $hour^{-1}$)***				
	Snowbed	3.781	0.203	18.597	-
	Willow-shrub	3.962	0.216	18.305	-
	Ash content POM	0.036	0.005	7.745	<0.001

[a] Equivalent soil mass was calculated based on the horizon with lowest soil mass per m^{-2}, thus avoiding the need of deeper soil depths to obtain the equivalent mass. The selected O-horizon was within willow-shrub with no sheep grazing at 2.13 cm depth (BD = 0.14) with a calculated equivalent soil mass of 2.982 kg m^{-2}. The equivalent soil mass represents an average sampling depth of 1.13 cm, 0.87 cm and 1.20 cm within snowbeds and 1.45 cm, 1.25 cm and 1.59 cm within willow-shrubs for no sheep, high grazing density and low grazing density, respectively.

[b] The head space volume of each flask was calculated by means of Boyle's law (average uncertainty of ± 0.13 ml). The dilution when measuring rates of gaseous release (accounted for using N_2) differed between the flasks (different headspace volumes) and was not constant throughout the experiment. Thus, an average dilution factor (based on N_2) for each flask was used (CV = 12.2 %). Using the average dilution factor resulted in some negative calculated fluxes of CO_2-C (underestimation of the dilution). These values were removed from the dataset.

[c] Six soil plots are omitted from the analysis (see material and methods).

[d] One soil plot omitted due to lack of bulk density.

[e] Based average measured CO_2-C fluxes at 6 different time intervals during 27 days of incubation. One value was omitted (unrealistic large flux) prior to calulation of the mean CO_2-C fluxes.

Table 3. Linear mixed models and parameter estimates (based on REML-estimation) for bulk denstiy of the fine earth (g cm^{-3}) (BD; ln-transfomred) and Ash content of POM (%) within two different plant communities (snowbed and willow shrub; factor “pl.comm”) at three levels of grazing (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep; factor “treatment”) within enclosures (n=9; factor enclosure; random effect) nested in blocks (n=3; factor “block”; random effect) in grassland habitats (Hol, Norway). Estimates, standard error and t-values of mean effects of different factor combinations are shown. P -values are based on simultaneous tests for general linear hypotheses (indicating wheater estimates are significantly different from 0). The model reduction steps are presented in the supporting information (Table S1). Not relevant is marked “-“.

Model	Parameter	Estimate	se	t	p
	ln(BD)/g cm^{-3}				
ln(BD) ~ treatment + pl.comm +(1 | enclosure) + (1 | block). n = 82[a,b]	Control-snowbed	-1.284	0.072	-17.946	-
	High-snowbed	-1.077	0.070	-15.465	-
	Low-snowbed	-1.371	0.072	-19.156	-
	Willow-shrub	-0.281	0.062	-4.535	<0.001
	Ash content POM/%				
Ash content POM ~ treatment+(1 | enclosure) + (1 | block). n = 83[a]	Control	40.368	1.898	21.270	-
	High	46.091	1.787	25.790	-
	Low	40.645	1.880	21.620	-

[a] 6 soil plots are omitted from the analysis (see material and methods). [b] 1 soil plot omitted due to lack of bulk density.

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Supporting information

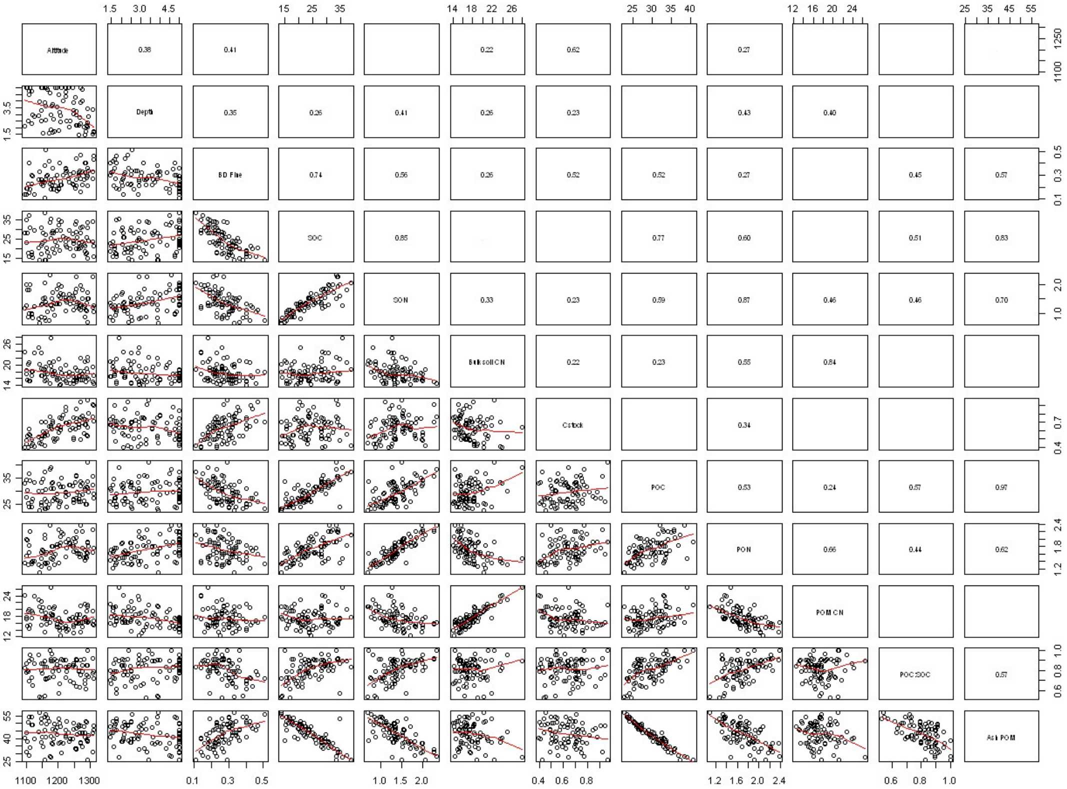

Figure S1. Spearman rank correlation coefficients (abs.) and smoothing lines between altitude; sampling depth (Depth); bulk density of fine earth (BD Fine); bulk soil organic carbon (SOC); bulk soil organic nitrogen (SON); carbon to nitrogen ratio of the bulk soil (Bulk soil CN); bulk soil carbon stock per cm soil (C stock); carbon in particulate organic matter (POC); nitrogen in particulate organic matter (PON); CN ratio of the particulate organic matter fraction (POM CN); particulate organic carbon to bulk soil organic carbon ratio (POC to SOC ratio) and Ash content of POM from O horizon soil samples within 82[a] grassland habitates, Hol, Noway. Units of factors are listed in table 1. All correlation coefficients (rho) are significant at the level of significance $p<0.05$. Empty cells in the upper panel indicate no significant correlation. [a]One soil plot is not included due to missing data on bulk density.

Table S1: Model selection for the fixed effect structure of linear mixed models (based on ML-estimation) for percentage carbon of the bulk soil (% SOC); carbon stock per cm soil depth (kg m^{-2}) ; carbon stock based on equvialent mass (kg m^{-2}); Particulate organic carbon to bulk soil organic carbon ratio (POC to SOC ratio) (arcsine-transformed), carbon flux (CO_2-C g $soil^{-1}$ $hour^{-1}$) carbon flux (CO_2-C g POC^{-1} $hour^{-1}$) (ln-transfomred), bulk density of the fine earth (g cm^{-3}) (ln transformed) and Ash content of the POM fraction (%) within two different plant communities (snowbed and willow shrub; factor “pl.comm”) at three levels of grazing (high = 80 sheep km^{-2}, how = 25 sheep km^{-2} and no sheep; factor “treatment”) in grassland habitats (Hol, Norway). Continous variables are BD and ash content of POM.The models alwas included enclosures (n=9) nested in blocks (n=3) as random effects. Step indicate models with a different fixed effect structure for each dependent variable. AIC= Akaike’s information criterion. ΔAIC = change in AIC betwen the models (negative values indicate improved fit). P is the p-value based on likelihood ratio tests (Chi squared) between two models. P-values < - 0.05 indicate significantly lower explanatory power. Step comp. indicates the models compared.

Dependent variable	**Step**	**Model (fixed effects)**	**AIC**	**Δ AIC**	**P**	**Step comp.**
SOC/%	1	***~ treatment*pl.comm + ln (BD)***	456.90			
	2	~ treatment + pl.comm + ln (BD)	460.87	3.97	0.02	2 vs. 1
	3	~ treatment*pl.comm	537.17	80.27	<0.001	3 vs. 1
C-stock per cm/kg m^{-2}	1	***~ treatment*pl.comm + ln (BD)***	-136.92			
	2	~ treatment + pl.comm + ln (BD)	-133.59	3.33	0.03	2 vs. 1
	3	~ treatment*pl.comm	-125.19	11.73	<0.001	3 vs. 1
C stock eqv.mass/kg m^{-2}	1	~ treatment*pl.comm	-40.59			
	2	~ treatment + pl.comm	-42.33	-1.74	0.32	2 vs. 1
	3	***~ treatment***	-44.22	-1.88	0.73	3 vs. 2
	4	~ pl.comm	-38.75	3.59	0.02	4 vs. 2
	5	~ 1	-40.75	3.47	0.02	5 vs. 3
arcsine (POC to SOC)	1	***~ treatment*pl.comm + Ash content POM***	-78.44			
	2	~ treatment + pl.comm + Ash content POM	-74.47	3.97	0.02	2 vs. 1
	3	~ treatment*pl.comm	-33.06	41.41	<0.001	3 vs. 1
CO_2-C flux/µg g soil^{-1} hour^{-1}	1	~ treatment*pl.comm + ln (BD)	257.64			
	2	~ treatment + pl.comm + ln (BD)	254.82	-2.82	0.55	2 vs. 1
	3	~ pl.comm + ln (BD)	251.24	-3.58	0.81	3 vs. 2
	4	~ treatment + ln (BD)	253.62	-1.20	0.37	4 vs. 2
	5	~ treatment + pl.comm	266.92	12.10	<0.001	5 vs. 2
	6	***~ ln (BD)***	250.29	-0.95	0.30	6 vs. 3
	7	~1	268.00	17.71	<0.001	7 vs. 6
ln(CO_2-C flux)/µg g POC^{-1} hour^{-1}	1	~ treatment*pl.comm + Ash content POM	54.59			
	2	~ treatment + pl.comm + Ash content POM	51.74	-2.85	0.56	2 vs. 1
	3	***~ pl.comm +Ash content POM***	49.75	-1.99	0.37	3 vs. 2
	4	~ treatment + Ash content POM	55.16	3.42	0.02	4 vs. 2
	5	~ treatment + pl.comm	91.19	39.45	<0.001	5 vs. 2
	6	~Ash content POM	93.59	43.83	<0.001	6 vs. 3
	7	~pl.comm	53.11	3.36	0.02	7 vs. 3
ln (BD)/g cm^{3}	1	~treatment*pl.comm	29.35			
	2	***~treatment + pl.comm***	25.72	-3.63	0.83	2 vs. 1
	3	~pl.comm	30.88	1.54	0.01	3 vs. 2
	4	~treatment	42.41	16.69	<0.001	4 vs. 2
Ash content POM/%	1	~ treatment*pl.comm	572.87			
	2	~ treatment + pl.comm	569.82	-3.05	0.62	2 vs. 1
	3	~pl.comm	571.82	2.00	0.05	3 vs. 2
	4	***~ treatment***	568.90	-0.92	0.30	4 vs. 2
	5	~1	571.30	2.40	0.04	5 vs. 6

Table S2: Mean chemical and physical O-horizon soil attributes from 89 grassland habitats (sampled 2008) within two vegetatioin types (snowbed and willow-shrub) at different grazing densities of sheep (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep = control), Hol, Norway. S.e. = standard error. Abbrevetions: BD = bulk density of the fine earth, CEC = cation exchange capacity, SOC = bulk soil organic carbon, SON = bulk soil organic nitrogen, C:N ratio bulk soil = carbon to nitrogen ratio in bulk soil, C stock per cm= carbon stock (density) of bulk soil per cm soil depth (i.e. volume based), N stock per cm= nitrogen stock (density) of bulk soil per cm soil depth (i.e. volume based), POM = particulate organic matter, POC = carbon content in particulate organic matter, PON = nitrogen content in particulate organic matter, C:N ratio POM = carbon to nitrogen ratio of POM, POC to SOC ratio = carbon in particulate organic matter to bulk soil organic carbon ratio and PON to SON ratio = nitrogen in particulate organic matter to bulk soil organic nitrogen ratio.

Factor	Snowbed								Willow-shrub							
	High		Low		No sheep		All treatments		High		Low		No sheep		All treatments	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Altitude/m.a.s.l	1242	14	1248	10	1213	12	1235	7	1141	7	1170	10	1154	17	1152	7
Slope/%	14.74	1.97	11.58	1.58	13.89	2.12	13.39	1.09	15.36	1.61	16.88	3.77	12.27	2.37	14.70	1.38
Total O-hor depth/cm	3.73	0.60	5.08	1.08	4.02	0.59	4.28	0.46	6.80	1.06	6.06	1.81	8.63	1.46	7.23	0.79
Sample depth/cm	2.71	0.26	3.09	0.28	3.25	0.27	3.01	0.16	4.00	0.30	3.38	0.45	4.09	0.40	3.88	0.21
BD/g cm^{-3}	0.36	0.01	0.27	0.02	0.29	0.02	0.31	0.01	0.27	0.03	0.20	0.02	0.24	0.04	0.24	0.02
CEC/$cmol_c$ kg $soil^{-1}$	39.37	2.34	46.34	2.61	44.30	2.84	43.40	1.53	45.63	3.81	50.63	12.50	45.14	6.70	46.07	3.21
Base saturation/%	33.71	3.73	26.10	4.83	23.33	2.81	27.68	2.31	43.41	3.62	43.89	14.54	36.05	4.68	41.11	2.94
pH	4.64	0.08	4.56	0.10	4.43	0.07	4.55	0.05	4.91	0.07	4.64	0.26	4.80	0.13	4.82	0.07
SOC/% of finearth	21.40	1.19	25.04	1.46	24.78	1.56	23.72	0.83	20.94	1.66	29.25	2.69	27.82	3.84	25.25	1.68
SON/% of finearth	1.33	0.07	1.43	0.08	1.40	0.09	1.39	0.05	1.21	0.11	1.45	0.16	1.43	0.18	1.34	0.08
C:N ratio bulk soil	16.16	0.32	17.69	0.46	17.83	0.65	17.21	0.30	17.73	0.60	20.71	1.45	19.44	0.94	19.02	0.56
C stock per cm/kg m^{-2}	0.74	0.03	0.64	0.03	0.68	0.03	0.69	0.02	0.51	0.02	0.56	0.03	0.54	0.03	0.53	0.02
N stock per cm/kg m-^{2}	0.05	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.03	0.00	0.03	0.00	0.03	0.00	0.03	0.00
POM/% of bulk soil	57.39	3.59	71.00	3.52	65.12	4.17	64.49	2.26	60.08	4.95	73.84	4.24	66.26	8.06	65.48	3.59
POC/% C of POM	28.06	0.69	31.16	1.06	31.26	0.94	30.14	0.55	27.09	0.98	31.38	1.95	32.66	2.32	29.99	1.06
PON/% N of POM	1.72	0.05	1.84	0.06	1.74	0.06	1.77	0.03	1.54	0.08	1.65	0.13	1.67	0.11	1.61	0.06
C:N ratio POM	16.42	0.42	17.13	0.63	18.20	0.60	17.23	0.33	17.88	0.66	19.40	1.31	19.89	1.15	18.92	0.57
POC to SOC ratio	0.74	0.02	0.88	0.02	0.82	0.02	0.81	0.02	0.77	0.03	0.81	0.05	0.76	0.06	0.78	0.03
PON to SON ratio	0.74	0.03	0.92	0.02	0.81	0.03	0.82	0.02	0.77	0.04	0.85	0.04	0.76	0.07	0.79	0.03
Ash content POM/%	45.42	1.30	40.05	1.65	40.12	1.66	41.90	0.94	47.41	1.93	39.94	3.68	37.49	4.03	42.29	1.92

PAPER II

Martinsen, V., Mulder, J., Austrheim, G., Hessen, D.O. and Mysterud, A., 2010. **Effects of sheep grazing on availability and leaching of soil nitrogen: controlled experiment in low alpine grassland soils**.

Effects of sheep grazing on availability and leaching of soil nitrogen: controlled experiment in low alpine grassland soils.

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Summary

Alpine ecosystems are generally nitrogen (N) limited with low rates of N mineralization. Herbivory may affect N cycling and N losses and thus long-term productivity of ecosystems.

Using a controlled grazing experiment in a low-alpine region at Hol, Southern Norway with three density levels of sheep, we determined effects of grazing on i*n situ* availability of inorganic N, potential N mineralization and mobility of dissolved inorganic N (DIN) and dissolved organic N (DON) in soil water of O-horizons. In addition, we studied the temporal and spatial variation of these processes.

The low alpine systems at Hol were characterized by small rates of N mineralization and relatively large plant demands for N. Significantly greater rates of potential N mineralization were found at sites with high sheep density compared to those with low density or no grazing. There was a strong seasonal effect on inorganic N and DIN:DON ratios of the soil water, with decreasing values in the course of the growing season, probably due to increasing nutrient demand. Effects of grazing on bioavailable N (as determined by buried PRS^{TM} exchange resins) were greater at low as compared to high altitudes. At low altitudes, low sheep density reduced amounts of bioavailable N.

We conclude that grazing may significantly stimulate N-cycling, but the availability of N remains small in this N limited system. Despite the herbivore induced effects on N cycling, we found no evidence for short-term increased risk in N loss via soil water. Nitrogen removal through grazing is small compared to the total soil N pool. This suggests that grazing in this low alpine ecosystem is sustainable from a nutrient point of view.

Introduction

Nitrogen (N) is a limiting nutrient for plant growth in terrestrial ecosystems (Vitousek & Howarth 1991). Particularly, alpine and arctic ecosystems with their short growing season and low temperatures are strongly N limited (Bowman *et al.* 1993; Frank 2008; Grellmann 2002; Shaver & Chapin 1980). Nitrogen mineralization in these systems is small (Chapin *et al.* 1988; Giblin *et al.* 1991; Nadelhoffer *et al.* 1991) and differs between plant communities (Bjork *et al.* 2007; Makarov *et al.* 2003) and varies during the growing season (Bardgett *et al.* 2002; Frank 2008; Morecroft *et al.* 1992). Absorption of nutrients by plants is related to nutrient concentrations in soil solution (Chapin 1980). Hobbie & Gough (2002) found positive relationships between foliar N content and net N mineralization rates at two tundra sites dominated by moist tussock tundra in northern Alaska. Thus, factors affecting the form and lability of N (and hence N availability) are likely reflected in the N content of plants.

Ungulates are important agents of ecosystem change (Hobbs 1996). In addition to direct grazing and plant removal, thus altering vegetation structure and quality, impacts of grazing include trampling and recycling of nutrients in the form of faces and urea (Hester & Baillie 1998; van der Wal *et al.* 2004; van der Wal & Brooker 2004). This may induce productivity since the ratio of available nutrients per unit of plant biomass will increase. In turn, grazing may affect physical and chemical soil properties [e.g. temperature (Olofsson *et al.* 2004), bulk density (Steffens *et al.* 2008) and soil solution chemistry (Haynes & Williams 1992; Shand & Coutts 2006)] and thus organic matter (OM) stocks and mineralization rates (Piñeiro *et al.* 2010; Stark *et al.* 2002).

Mineralization of OM by heterotrophic microbes produces ammonium (NH_4^+), which may be oxidized to nitrate (NO_3^-) (Jackson *et al.* 2008). Herbivory may enhance (Frank & Groffman 1998a; Hobbs 1996; Tracy & Frank 1998) or retard (Harrison & Bardgett 2004) N mineralization, depending on ecosystem productivity (Wardle *et al.* 2004). Mobæk *et al.*

(2009) reported herbivore density dependent selection for productive habitats, associated with a larger proportion of high quality forage ingested by sheep at small densities than at great densities (Mysterud *et al.* 2010). Christianson & Creel (2009) found a positive relationship between N content in faeces of elk (*Cervus elaphus*) and the quality of forage grasses (expressed as faecal chlorophyll). Correspondingly, Kyvsgaard *et al.* (2000) showed a positive correlation between percentage N in faeces (from sheep) and net mineralization of faeces N. Thus, impacts of grazing on soil N-mineralization may differ between grazing regimes.

Despite a tight N-cycling due to strong plant and microbial demand for nutrients (Jaeger *et al.* 1999; Nadelhoffer *et al.* 1991), associated with little loss of N in alpine areas (Gerzabek *et al.* 2004), herbivore -induced increase in N-cycling, may increase the risk of N-loss from the system (Dahlin *et al.* 2005; Frank & Groffman 1998b; Haynes & Williams 1992). High concentrations of nitrate in soil solutions under urine and faeces patches were reported by Haynes & Williams (1992) and Shand & Coutts (2006), respectively. Frank & Groffman (1998b) found a significant herbivore induced increase in denitrification at mesic sites in grasslands of Yellowstone National Park, with rates about double that of atmospheric N deposition (Frank & Groffman 1998b).

Most studies determining effects of grazing on availability and leaching of N are conducted using grazing exclosures where animals are excluded from a fenced area. By contrast, experimental designs with a fixed number of animals in enclosures are rare. Here, we use a controlled grazing experiment in a low-alpine region of Southern Norway with three levels of grazing intensity. Our objectives were to determine effects of different sheep densities on i*n situ* availability of inorganic N, potential N mineralization rates and mobility of DIN and DON in soil water of O-horizons as a proxy of runoff export on N. In addition, we studied the temporal and plant community related variation of these processes. We hypothesize that i*n situ* availability of inorganic N (expressed as PRS^{TM}- adsorbed surplus N) (**H1**), N content of plants (% total N) (**H2**) and potential nitrogen mineralization rate (**H3**) is in order high sheep density > low sheep density > no sheep. In turn, if availability of N increases with grazing densities, we expect greater dissolved inorganic to organic ratios of N (DIN:DON) in soil water from areas being heavily grazed compared too moderately or not grazed (**H4**). The amount of mobile inorganic N (adsorbed on PRS^{TM}-probes or dissolved in soil water) is expected to change throughout the growing season, with lowest values at high primary production and thus high N uptake, even if the effect of grazing (H1 and H4) remains constant (**H5**).

Material and methods

Site description and plot locations

The study site is located in the low alpine region (1050 - 1320 m.a.s.l.) in Hol municipality, Buskerud county, southern Norway (7°55′ - 8°00′ E, 60°40′ - 60°45′ N). Vegetation is dominated by dwarf shrub heaths with patches of snow beds and alpine meadow communities and lichen heaths (Austrheim *et al.* 2008; Rekdal 2001). The bedrock consists of meta-arkose and quaternary deposits of till and colluviums (Kristiansen & Sollid 1985; Sigmond 1998). Soils are spatially variable, including peaty deposits in poorly drained pockets and freely drained soils with shallow and acidic organic horizons (~ 5,4 cm; pH_{h2o} ~4.7). Organic horizons have a large percentage (~65 %) of free particulate organic matter (POM) (Martinsen *et al.* 2010). Mean annual temperature (MAT) is -1,5 ^{O}C and mean annual precipitation (MAP) is about 1000 mm (Evju *et al.* 2009), approximately 75% of which falls as snow.

In 2001 a large enclosure (2.7 km^2) was fenced and divided in tree blocks each with tree sub-enclosures (approximately 0.3 km^2) with no sheep (control), low density (25 sheep km^{-2}) and high density (85 sheep km^{-2}) of domestic sheep (*Ovis aries*) (Mysterud &

Austrheim 2005; Mysterud *et al.* 2005). The experiment is set up as a randomized block design (Figure 1). Sheep grazing occurs from the end of June to the beginning of September (approximately 10 weeks).

Twenty-seven soil plots (each 0.25 m^2, at 3 different altitudinal levels in 3 replicates within the 3 enclosures in the eastern block) were established in 2007 ("Location A"; Figure 1). In addition, 54 soil plots [each 1 m^2, within all 9 enclosures at 2 different altitudinal levels (1168 and 1259 m.a.s.l., respectively) and in 3 replicates] were established in 2008 ("Location B"; Figure 1). Soil plots of location B, occurring within the grazed enclosures were fenced in 2008 to prevent damage to the PRS^{TM}-probes and macrorhizons. In a separate study, all plots of location B received a small amount of $^{15}NH_4Cl$ (N input about 7% of the annual input in wet deposition) in early July, 2008, which did not affect the relative difference in inorganic N between grazing treatments.

Eighty-nine plots were selected for soil and vegetation sampling in 2008 ("Location C"; Figure 1). The plots were selected from 180 plots (0.25m^2), established 2001, in each enclosure using a balanced stratified procedure among altitudinal levels and habitats (Austrheim *et al.* 2005). All 89 plots are located within grassland habitats. Soil and vegetation samples from location C, were further divided in two different grassland plant communities; snowbed (n= 54) and grassland with scattered willow-shrub (n=33). In a related study, differences associated with these grassland plant communities were assessed (Martinsen *et al.* 2010). Willow-shrubs are typically located at lower altitudes than snowbeds, and have thicker O-horizons, lower bulk densities, higher pH and C:N ratios than snowbeds (Martinsen *et al.* 2010).

Soil sampling

O-horizons were sampled during the periods 18/06/07-11/08/07 (location A), 26/06/08-01/07/08 (location B) and 05/08/08-08/08/08 (location C). The soil sampling strategy differed slightly between the periods: At location A (n= 27) the soil was sampled using 100 cm^3 steel rings to a maximum depth of 3.7 cm in the corner of each plot. At location B, the O-horizon was sampled (2 samples per plot) using a cylinder-shaped auger (diameter 2.5 cm) to a maximum depth of 10 cm in the corners of each of the 54 plots. Samples from the 3 plots within each enclosure and altitudinal level were bulked prior to analysis, leaving 18 O-horizon samples for analysis. At location C (n=89) the O-horizon was sampled using a cylinder-shaped auger (diameter 5.2 cm) to a maximum depth of 5 cm. Four soil samples were taken at most one meter from the vegetation plots. To obtain enough soil material for analysis, more than 4 samples were taken if the O-horizon was <5 cm. The soil samples at each of the 89 plots were bulked per plot prior analysis. Field moist soil samples (~ 60 % water) were homogenized and divided into two subsamples, one for chemical analysis and one for determination of potential nitrogen mineralization rate.

Prior to sampling the O-horizon the vegetation was cut at the soil surface and the O_i removed. All soil samples were stored dark and cold (< 4 °C) prior to analysis. A sub-sample of each soil was air-dried (40 °C for 4-5 days), sieved at 2 mm and the weight of dry roots and gravel (> 2 mm) determined. Sub samples of the air-dried fine earth fractions were dried at 105 °C (location A and B) and 60 °C (location C) to determine dry matter content (DM) and subsequently milled prior to determination of total organic carbon (C) and total nitrogen (N).

Total C and N was analyzed by dry combustion (Leco CHN-1000; Leco corporation, Sweden) (Nelson & Sommers 1982) and the Dumas method (Bremmer & Mulvaney 1982), respectively. Bulk density (BD; g cm^{-3}) was based on the fine earth fraction of the soil.

Potential nitrogen mineralization (location C):

Potential nitrogen mineralization rate was determined in incubation experiments conducted between 17/10/08 and 19/12/08. At the start of the experiment (day 0), three field moist subsamples from each of the 89 soil plots were placed in PVC tubes. The amount of soil used was equivalent to 5 g of dry soil for 82 of the soil plots. Due to lack of soil, between 2.5 and 3.6 g dry equivalent soil was placed in PVC tubes from the remaining 7 soil plots. One of the sub-samples was immediately frozen (background level), while the remaining two were incubated (dark) in an incubation cabinet (Termaks series 6000) at 15 °C. After 15 days and 63 days of incubation, respectively, the two remaining samples were removed and frozen. To prevent water loss during incubation, a lid was placed on top of the sample tubes and a container with water was placed among the samples.

After thawing, the soils were extracted in 25 ml 2M KCl and filtered prior to analysis. Nitrate-N (sum of NO_3^- and NO_2^-) was determined photometrically (flow injection analysis; FIA star 5020 analyzer, Tecator) according to the Norwegian standard NS 4745 (NSF 1975a). Ammonium-N was determined photometrically (Photometer, Gilford Instrument) according to NS 4746 (NSF 1975b). In both cases the detection limit was 0.02 mg l^{-1}. Rates of net ammonification and net nitrification were determined by subtracting initial extractable soil NH_4-N and NO_3-N (µg g $soil^{-1}$) from final amounts (after 15 and 63 days, respectively) of extracted NH_4-N and NO_3-N, respectively. The sum of produced NH_4-N and NO_3-N represents net mineralization (Vestgarden & Kjonaas 2003).

In situ availability of inorganic nitrogen (location B)

Plant root simulator probes (PRS^{TM}; Western Ag Innovations Inc., Saskatoon, Canada) were used to assess available inorganic nitrogen (NH_4-N and NO_3-N) in soils. Four cation and 4 anion PRS^{TM} –probes were inserted (~5 cm) in the soil within each of the 54 plots during 3 periods [03/07/08-23/07/08 (n=432); 23/07/08-11/08/08 (n=432);11/08/08/-31/08/08 (n=432)]. The four cation and 4 anion probes at each plot and period, were bulked prior to analysis (n_{cation}=162; n_{anion}=162). After each period, the PRS^{TM}-probes were shiped to

Western Ag Innovations for analysis. The PRS^{TM}-probes were eluted using a 0.5N HCl solution for 1 hour and analyzed for NH_4-N and NO_3-N colourimetrically (Western AG Innovations Inc. 2009). As the ion adsorption is not linear in time, we used the recommended reporting unit "amount adsorbed N cm^{-2} PRSsurface time of $burrial^{-1}$" (Western AG Innovations Inc. 2009). The method detection limit was 0.2 µg N cm^{-2} 20 days of $burrial^{-1}$. The PRS^{TM}-probes were inserted directly in the O horizon and the amount of adsorbed N represents nutrient surpluss rather than net mineralization (Western AG Innovations Inc. 2009).

Soil water sampling and analysis (location A and B)

Soil water was collected using macrorhizons (type 19.21.35, Eijkelkamp, the Netherlands) installed just below or within the O-horizon (location A; 4.5 cm, location B; 3.6 cm). A syringe was used to collect the water. Soil water was collected on 10 occasions from June to August in 2008 (18/06/08-22/08/08) and 2009 (09/06/09-25/08/09). The samples were stored cold (< 4 °C) and filtered prior to analysis. NH_4-N and NO_3-N were determined as described above. Total N was determined photometrically (flow injection analysis; FIA star 5020 analyzer, Tecator) after oxidation by peroxodisulphate according to the Norwegian standard NS 4743 (NSF 1993). Dissolved organic N (DON) was calculated as total N less the sum of NH_4-N and NO_3-N. Dissolved organic carbon (DOC) was determined using a total organic carbon analyzer (TOC-V CPN, Shimadzu) according to NS 1484 (NSF 1997).

Plant sampling (location C)

Six different plant species representing 3 plant functional groups were sampled. Plant tissue was sampled from grasses (*Avenella flexuosa* and *Anthoxantum odoratum*) and herbs (*Alchemilla alpina* and *Rumex acetosa*) and leaves was sampled from woody species

(*Vaccinium myrtillus* and *Salix lapponum)* in the period 29/07/08-07/08/08 (Mysterud *et al.* 2010). The plants were milled (1 mm sieve size; Culatti, type DFH48) and dried (60ºC) prior analyis. Total carbon and nitrogen was determined by combustion in Flash EA^{TM} 1112 automatic elemental analyzer (Thermo Finnegan, Milan, Italy).

Statistical analysis

Statistical analyses were conducted using the libraries lme4, multcomp and gplots in the statistical package R (version 2.10.1) (http://www.r-project.org/).

We used Linear mixed effects models (lmer) with random effects reflecting the block-wise randomization design. The random effects were not the same for all models, as the sampling strategy (i.e. repeated measurements on the same plots and factors included in the analysis) differed depending on the data sets (i.e. location A, B and/or C) and the dependent variable for which the models were fitted. The random factors are listed in table 1 to 4 and in the Appendix (Table A1).

Grazing treatment (3 levels; no sheep, high and low density) was always included as a fixed factor. In addition, altitude level (2 levels; 1168 or 1259 m.a.s.l., respectively) and period (3 levels; early, middle or late summer) were included as fixed effects for the PRS^{TM} - adsorbed N model (Table 1). Plant community (two levels; grassland in snowbed or willow-shrub) and plant functional group (3 levels; grasses, herbs or woody species) were included for the plant N-content model (Table 2). Day of incubation (3 levels; day 0, day 15 and day 63) and plant community (two levels) were included for the total extractable N model (Table 3). Finally, month (3 levels; June, July and August) and altitude (2 levels) were included as fixed effects for the DIN:DON ratio model (Table 4). All full models were fitted with relevant interactions. Some variables were transformed (ln or sqrt) prior to analysis to avoid violations of the model assumptions. Estimated parameters were back-transformed to the original scale before inclusion in the figures.

Backward selection was used [models fitted by maximum likelihood (ML)] and models were compared based on AIC ("smaller is better") and likelihood ratio tests (Chi squared) to obtain the minimum adequate model. The best model was re-fitted based on restricted maximum likelihood (REML) and the estimated effects (including standard error) were calculated using general linear hypothesis testing [glht in multcomp]. Only adjusted p-values [single-step method (Hothorn *et al.* 2008)] are reported. We report differences between fixed effect factor levels based on "treatment contrasts". Treatment contrasts is the default in R, with factor levels arranged in alphabetical sequence [Crawley (2007), pg. 377]. Thus the intercept will be the factor level that comes first in the alphabet. The only exception is the plant N-content model, where differences between months are based on differences with June (intercept) and not August. Residuals and predicted random effects were plotted (histograms and QQ normal plots) to assess normality and potential outliers.

Six soil plots from location C were excluded from the analysis, due to large deviations compared to the rest of the grassland plots (see Martinsen *et al.,* 2010). Missing values and values below the detection limit resulted in non balanced datasets. Number of samples for each figure and model are reported in figure legends and in the tables, respectively.

Results

In situ availability of inorganic nitrogen

The availability of inorganic nitrogen (expressed as PRS^{TM}-adsorbed surplus N; NH_4-N+NO_3-N) was in general low and significantly affected by grazing treatment, altitude and time of the growing season (Figure 2, Table 2). At both altitudes PRS^{TM}- adsorbed N was significantly lower in the middle of the growing season as compared to the start and end, indicating a high plant and microbial demand for N in the middle of the growing season (Figure 2). At the lowest altitude in the early period of the growing season, low sheep density caused a significant reduction in surplus N compared to high sheep density and control. However, this difference diminished throughout the growing season due to an increase and decrease in PRS^{TM}- adsorbed N at the low sheep density and no sheep, respectively. At altitude 2, values for PRS^{TM}-adsorbed N were similar in size as at altitude level 1, but there was no significant difference in surplus N between the grazing treatments (Figure 2, Table 2). The average of PRS^{TM}- adsorbed N during the growing season and the two altitudes was dominated by NH_4-N (Table 1).

N content of plants

The nitrogen content differed between the plant functional groups and was significantly lower in grassland in willow-shrubs than snowbeds (Table 3). Grasses had a lower N-content as compared to herbs and woody species, with the latter two only slightly differing (Table 3). The N-content of the functional plant groups was not affected by grazing (Table A1).

Potential nitrogen mineralization

The amount of soil extractable inorganic nitrogen (NH_4-N + NO_3-N; µg g^{-1}soil) was highly variable within the treatments with a large variability between the repeated extractions at each soil sample (Figure 3, Table 4). Soils from the enclosures with high sheep density had significantly greater amounts of initial extractable N (reflecting the amount of exchangeable N at the time of sampling) compared to the O horizons from the low sheep density and control. There was a significant net mineralization throughout the incubation experiment at high sheep density (Table 4). By contrast, soils originating from the low sheep density and enclosures with no sheep, showed an immobilization even after 63 days of incubation (Figure 3). About 85 percent of the total extractable inorganic N was in the form of NH_4-N (Table 1).

Inorganic nitrogen in soil water

The concentrations of inorganic N in O-horizon soil water were low compared the total N, with a greater fraction of NH_4-N than NO_3-N (Table 1). Thus, the dissolved inorganic nitrogen to organic nitrogen ratios (DIN:DON) in the O-horizon soil water were in general low, illustrating the predominace of organic nitrogen in this system (Figure 4, Table 5). The fitted model for the DIN:DON ratios included an interaction between sheep density and growing season (i.e. month). DIN:DON ratios declined significantly from June to August. However, the decline was less pronounced at low and high sheep density as compared to the non grazed sites (Figure 4, Table 5).

Discussion

It is well documented that herbivory may affect N cycling (Hobbs 1996). However, effects of herbivores on availability of N are expected to depend on herbivore densities as well as differences associated with habitat characteristics and seasonal variations. In this study we found clear effects of grazing on rates of PRS^{TM}-adsorbed N (a measure of surplus N; H1) and potential N mineralization rate (H3). Soils at high sheep density had the highest amounts of initial exchangeable N and showed a significant net N mineralization after 63 days of incubation (Table 4, Figure 3). In all grazing treatments, there was a strong seasonal effect on PRS^{TM}-adsorbed N, with a significant decline in the middle of the growing season (Table 2, Figure 2). The effect of grazing on PRS^{TM}-adsorbed N was most pronounced early in the growing season at the lowest altitude level (in contrast to H5), with less PRS^{TM}-adsorbed N at low sheep density as compared to the non grazed sites. There were only small differences between the high density and control (Table 2, Figure 2).

Despite grazing induced differences in potential N mineralization and surplus N, the N content of plants was strongly related to plant community and not (as predicted; H2) affected by grazing. However, dissolved inorganic to dissolved organic N ratios (DIN:DON) in soil water were significantly affected by growing season (strong seasonal decline) and sheep density (H4). DIN:DON ratios declined less throughout the growing season at low and high sheep density as compared to the non grazed sites (Table 5, Figure 4). Together, these findings illustrate that effects of grazing on N-cycling and availability strongly depend on herbivore density interacting with temporal and spatial variations in nutrient supply.

Nitrogen mineralization rates in alpine and arctic soils are low (Giblin *et al.* 1991; Nadelhoffer *et al.* 1991). At our study site, assuming that SOM consists of ~50% carbon, the average amounts of net mineralized N (after 9 weeks of incubation) were 7.5 (±12), 13 (±16) and 101 (±19.7) µg N g SOM^{-1} for the non grazed sites, low density and high density sites, respectively. A comparable range of mineralized N is reported by Nadelhoffer *et al.* (1991). Across 6 arctic ecosystems in Alaska, Nadelhoffer *et al.* (1991) found a great variability in amount of mineralized N (13 weeks of incubation) with average values ranging from 6 to 129 µg N g SOM^{-1}. The amount of mineralized N was enhanced with increased incubation temperature (9 to 15 ºC), indicating a temperature control on N mineralization. In addition, these authors also point at the importance of the quality of SOM for N mineralization in surface soils, as differences associated with ecosystems were more pronounced than temperature on N mineralisation (Nadelhoffer *et al.* 1991). Our data indicate that grazing (in addition to number of incubation days) is more important for the amount of extractable N than the plant community (Table 4, Figure 3). Thus, direct impacts of grazing activity like trampling and input of nutrients in the form of faces and urea (Martinsen *et al.* 2010; van der

Wal *et al.* 2004; van der Wal *et al.* 2001) are likely more important in controlling N mineralization than inherent habitat characteristics at our study site.

Many of the PRS^{TM}-adsorbed N values and inorganic N concentrations in soil water (DIN) were at or even below the detection limit (Table 1). By reinvestigating a ^{15}N-tracer experiment in a alpine grassland in Austria (^{15}N introduced by single pulse labelling in 1974/1975), Gerzabek *et al.* (2004) report a N loss of only 55% during 27-28 years. The low rates of PRS^{TM}-adsorbed N (Figure 2, Table 2), low concentrations of DIN and DIN:DON ratios in soil water (Figure 4, Table 1 and 5) at our study site, thereby confirms that N in alpine ecosystems is effectively re-cycled and remains in the plant-soil system (Gerzabek *et al.* 2004). Furthermore, we found a strong seasonal variation in PRS^{TM}-adsorbed N and DIN:DON ratios of the soil water with a decline in the middle of the growing season. Changes in availability of soil N throughout the growing season due to seasonal changes in microbial and plant demand has been reported by e.g. Lipson et al. (1999) and Weintraub & Schimel (2005). Factors like N deposition and catchment productivity may also affect DIN:DON ratios, as reported by Hessen et al. (2009). They found a significant positive relationship between NO_3-N to organic N ratios and N deposition. However, the N deposition is likely the same within the experimental area at Hol, and will therefore not affect the relative differences in soil water DIN:DON ratios between the grazing treatments.

Grazing may also promote nitrification. Frank *et al.* (2000) found a grazing induced increase in nitrification rate of 68% in the Yellowstone National Park and Gao et al. (2009) report significantly greater gross nitrification and denitrification at low grazing intensity as compared to heavy grazing in the Tibetain Platau, China. We found evidence for increased net N mineralization at the sites with high sheep density compared to low density and controls (Figure 3, Table 4). However, the fraction of NO_3-N to the sum of inorganic N (NH_4-N + NO_3-N) was in general very low (irrespective of grazing) (Table 1). The fraction NH_4-N of the total initial extractable inorganic N ranged from about 80% to 100%. NH_4-N being the dominant form of inorganic N in mountain areas is also reported by Mullen *et al.* (1998). Furthermore, inorganic N adsorbed on the PRS^{TM}-probes was dominated by NH_4-N and ranked from about 70% to 75% at the low and high sheep density, respectively (Table 1). But, we also found NH_4-N to be the dominant form of inorganic N in soil water. Only 8% of the 225 water samples (Figure 4,Table 1 and 5) had NO_3-N values above the detection limit. Together, these findings clearly indicate low nitrification potentials in this system, thereby little risk in N loss through leaching.

Ungulates are important agents of change in ecosystems (Hobbs 1996) and may have severe impacts on carbon and nitrogen storage (Martinsen *et al.* 2010; Pineiro *et al.* 2009). However, impacts of grazing depend on herbivore density and the productivity of the system (Wardle *et al.* 2004). Our results clearly show that grazing may affect N cycling. A N budget for our system (Figure 5) shows that losses of N associated with grazing are smaller and about the same as N leaching in the soil water for both low and high sheep density sites, respectively. At high sheep density, the estimated N removal associated with weight gain is slightly smaller than the N input in deposition and is very small compared to the total N-pool of the system (Figure 5). Furthermore, removal of N by the sheep as compared to the total input of N is probably even smaller, as N-fixation was not included and has been found to be significant in high elevation systems (Hobara et al. 2006). Losses of N associated with denitrification (Frank & Groffman 1998b) and volatilization from faeces and urea may be significant (Dahlin *et al.* 2005). Gaseous fluxes of N were not assessed in our study, but are likely small due to the low amounts of NO_3-N.

Grazing may affect the spatial distribution of N as sheep returns a great share of ingested N in form of urea and faeces (Dahlin *et al.* 2005). Assuming a retention of ingested N of ~30%, an estimated removal of 0.36 g N m^{-2} growing $season^{-1}$ at high sheep density implies a biomass removal of ~1.2 g N m^{-2} growing $season^{-1}$ [about half of the biomass production (2.2 g N m^{-2}); Figure 5]. Depending on location of excretal return, this may locally enhance substrate availability (and quality), stimulating microbial activity and nutrient cycling (Hobbs 1996).

We conclude that grazing significantly affects N mineralization rates and the bioavailability of N. However, despite grazing induced effects on N cycling, we found no evidence for increased risk in N loss in soil water. Losses associated with weight gain of the sheep are relatively small compared to the total N-pool in the system. Thus, the sheep densities used in this experiment are likely to have minor impacts on the system in terms of long-term N losses.

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Figure legends

Figure 1. Sampling locations and experimental design (3 enclosures with different sheep densities in 3 blocks) Hol, southern Norway. The area of each enclosure is ~0.3 km^{-2}, total area is ~2.7 km^{-2}. White enclosures = no sheep (control), light grey enclosures = low (25 sheep km^{-2}) and dark grey enclosures = high (80 sheep km^{-2}) sheep densities. Points represent three different sampling locations for soil, soil water and vegetation. At location A (27 soil plots; 3 altitudinal levels), soil and soil water was sampled. At location B (54 soil plots; 2 altitudinal levels) soil and soil water was sampled in addition to determination of *in situ* PRS^{TM}- adsorbed inorganic nitrogen. At location C (89 soil and vegetation plots) soil (sub samples used to determine potential N minerlaization) and vegetation was sampled.

Figure 2. *In situ* PRS^{TM} adsorbed inorganic nitrogen (sum NH_4-N and NO_3-N; µg N cm^{-2} 20 days burial^{-1}) in organic soils within grassland habitats (location B, Hol, Norway) at two altitudinal levels (Altitude 1 ~ 1168 m.a.s.l. and altitude 2 ~ 1259 m.a.s.l.), tree burial periods (03.07-23.07, 23.07-11.08 and 11.08-31.08 2008) and tree grazing treatments (high; 80 sheep km^{-2}, low ; 25 sheep km^{-2} and control (=no sheep)). The figure shows PRS^{TM}-adsorbed N (± se) based on fixed effect estimates derived from a linear mixed effect model (Table 1) superomposed on Box-whisker plots (medians, 25th and 75th quartile and minimum and maximum values, i.e. whiskers) based on the original data. n = 156[a].

Figure 3. Total inorganic nitrogen (NH_4-N + NO_3-N; µg g^{-1} soil) from O-horizon samples of grassland habitats (location C, Hol, Norway) at tree different grazing treatments [high; 80 sheep km^{-2}, low ; 25 sheep km^{-2} and control (= no sheep)] during 63 days of incuabtion (day 0 = start of incubation; initial extractable N, day 15 = extractable N after 15 days of incubation and day 63 = extractable N after 63 days of incubation). The figure shows total nitrogen (± se) based on fixed effect estimates derived from a linear mixed effect model (Table 3)

superimposed on Box-whisker plots (medians, 25th and 75th quartile and minimum and maximum values, i.e. whiskers) based on the original data. n=247[a]. An increase or decrease in extracted N from day 0 indicates net mineralization or immobilization, respectively.

Figure 4. Dissolved inorganic nitrogen (NH_4-N + NO_3-N) to organic nitrogen ratio (DIN:DON) in O-horizon soil water from grassland habitats (location A and B, Hol, Norway) at three different grazing treatments (high; 80 sheep km^{-2}, low ; 25 sheep km^{-2} and control (= no sheep)) througout the growing season. The figure shows estimated DIN:DON ratios (± se) derived from a linear mixed effect model (Table 4) superimposed on Box-whisker plots (medians, 25th and 75th quartile and minimum and maximum values, i.e. whiskers) based on the original data. n=225[a].

Figure 5. Nitrogen budget [nitrogen pools (closed boxes) and fluxes (stippled boxes)] for grassland habitats at Hol, Norway. Assumptions for the nitrogen budget are listed in the footnote[a] .

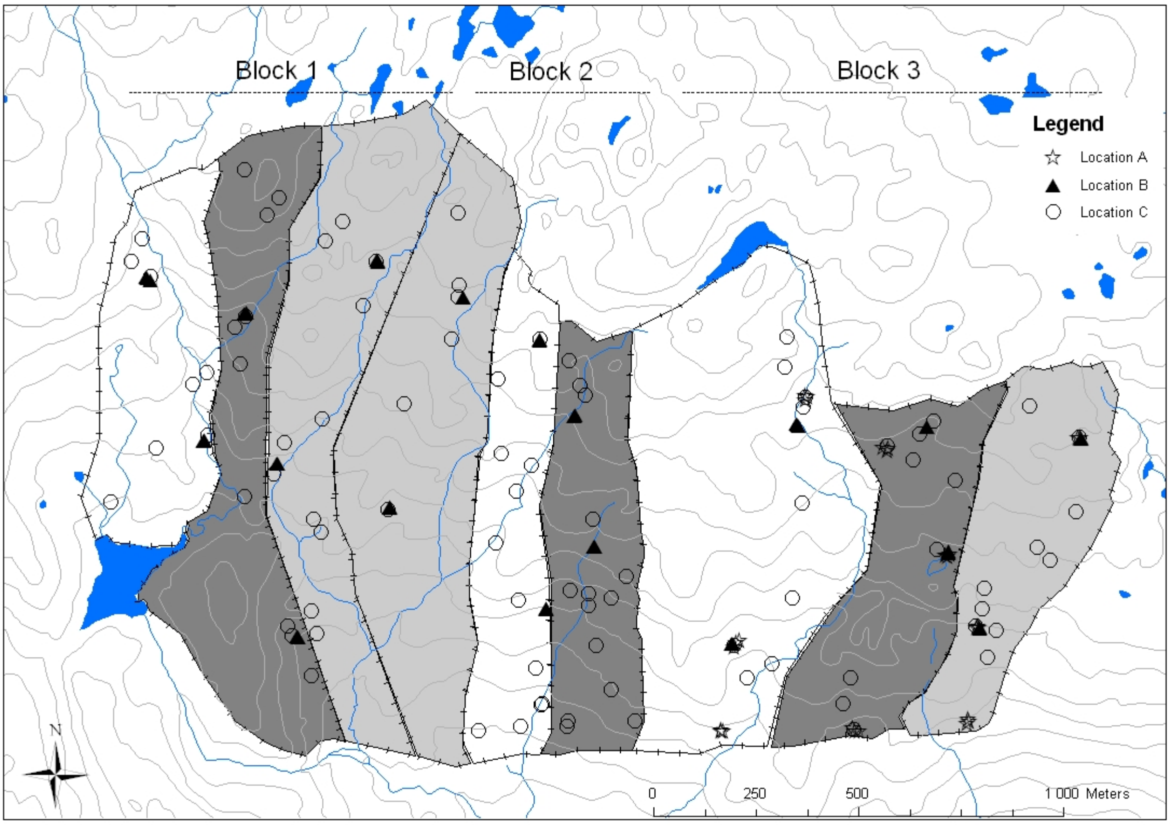
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Figure 1.

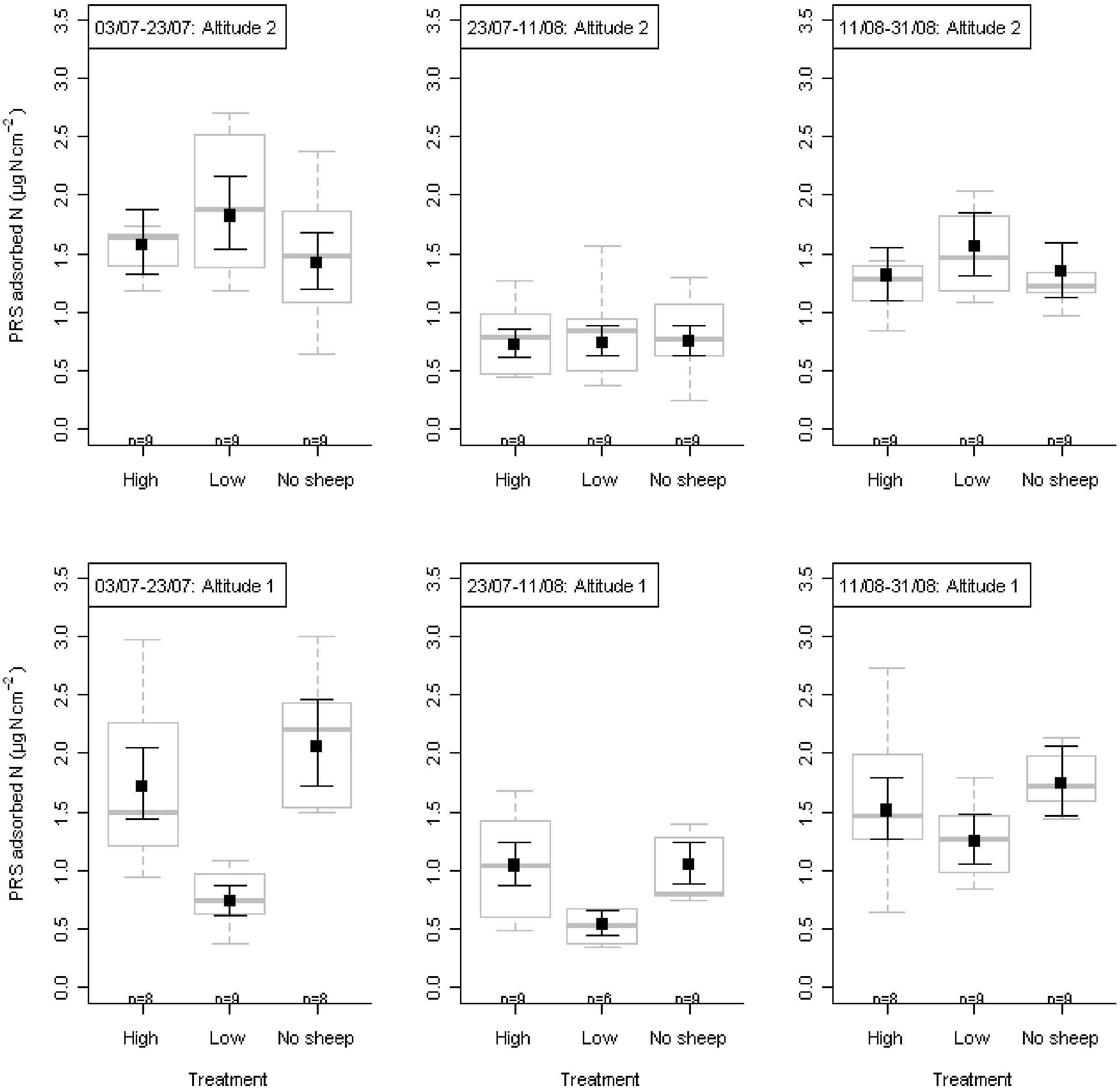

Figure 2

[a]Six values omitted from the analysis [3 were below the detection limit and 3 considered as outliers (23.5, 7.5 and 6.7 μg N cm^{-2} 20 days of burrial^{-1})].

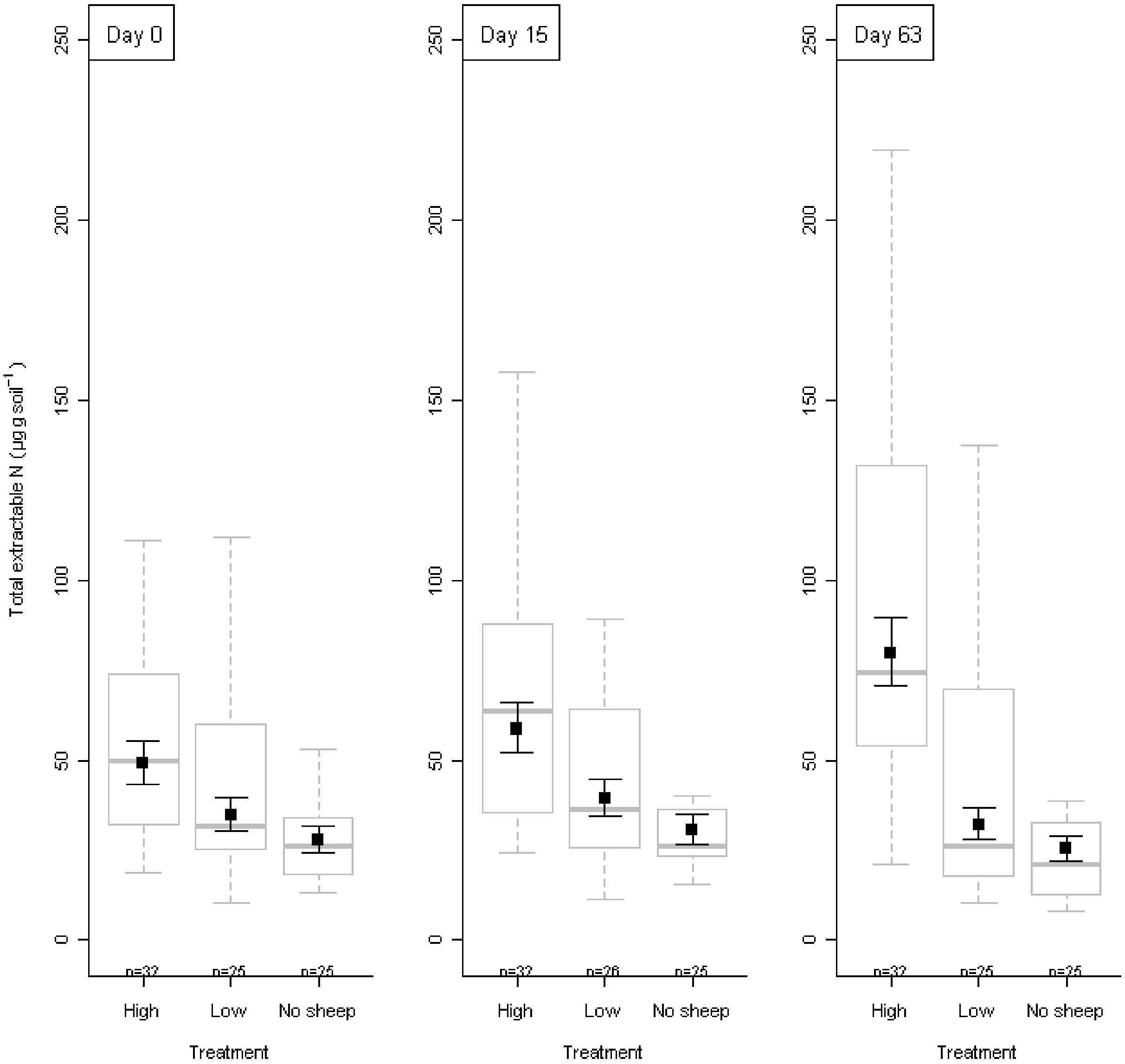

Figure 3.

[a] Six plots are omitted from the analysis (see material and methods). One outlier was removed in addition to one missing value.

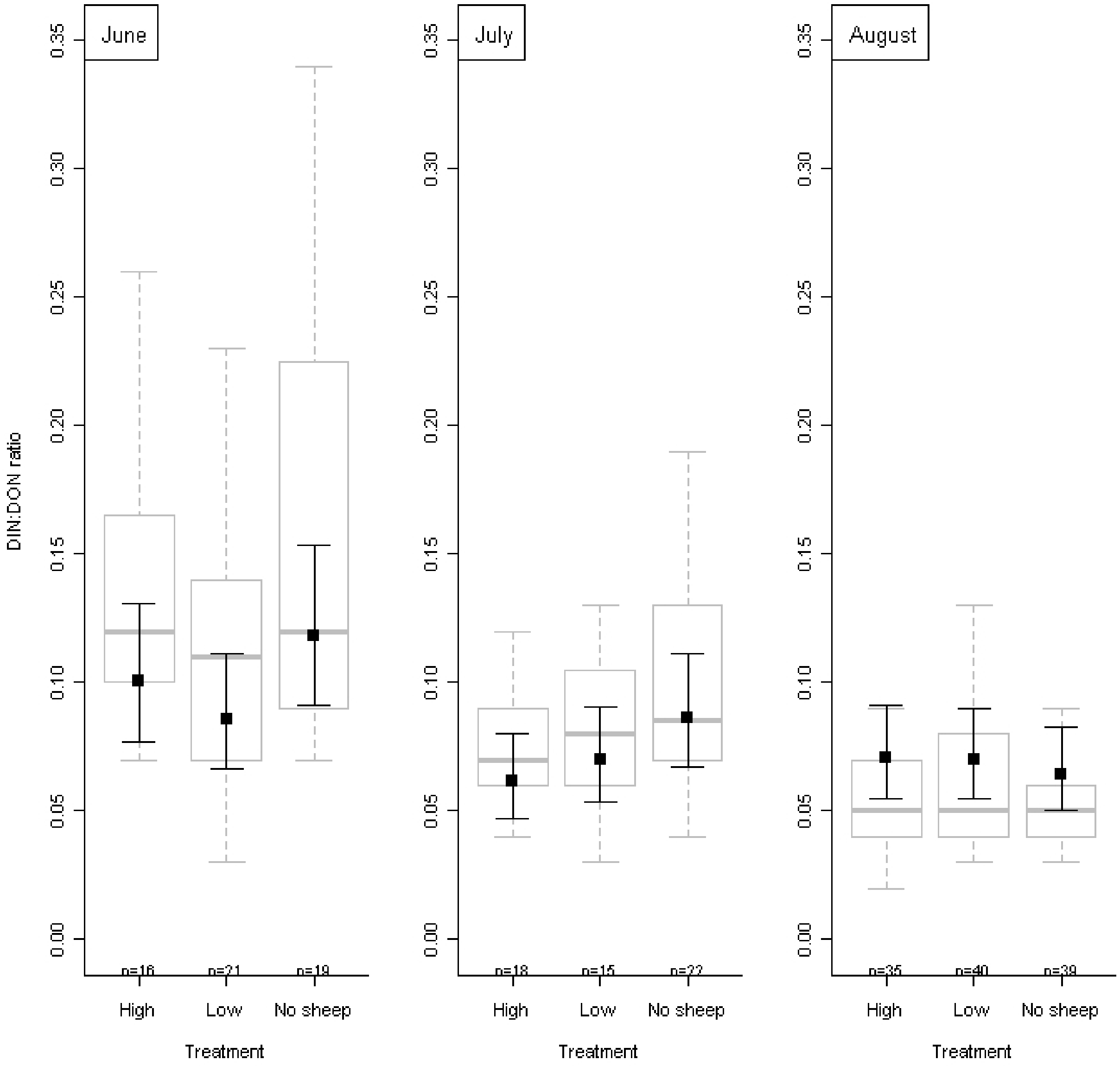

Figure 4.

^{a}Based on the datasets from location A (altitude level 2 and 3) and location B. One value deleted prior to analysis (DIN:DON ratio > 0.6).

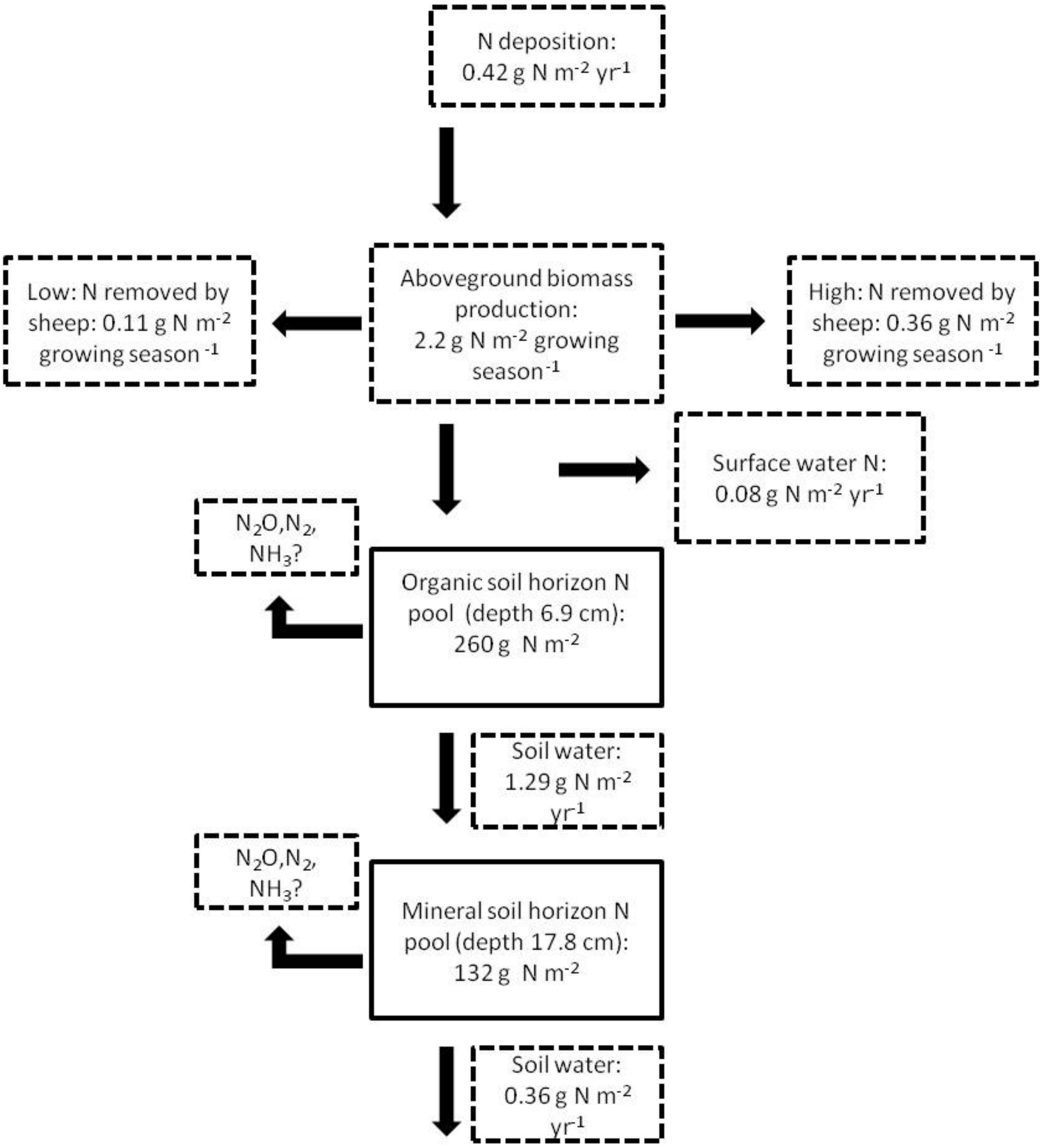

Figure 5.

[a] Input data and assumptions:

1. Average N deposition is 0.416 g m^2 year (Aas *et al.* 2008).
2. Yearly precipitation; 1000 mm (Evju *et al.* 2009) .
3. Aboveground biomass is based on average biomass production in 2008 on plots close to "location C plots" (Austrheim, unpublished material).
4. N-content of plants (% by weight) is based on average values from location C (graminoids; 1.1%, herbs; 2.3 % and woody species; 1.8 %) and unpublished material for cryptogams sampled 2009 (1.1%).
5. 10 % of the net (i.e. excluding impediment) area (Figure 1) consists of grassland habitats.
6. Lambs gain weight and feed in grassland habitats only (corresponding to a net lamb density of 143 and 527 lambs km^{-2} grassland for the low and high grazing densities, respectively.
7. Lambs gain 22.87 kg (low density) and 20.15 kg (high density) weight during the growing season (Mysterud, unpublished material).
8. All weight gain of the lambs is in form of meat, with an average protein content of 216.6 g kg $meat^{-1}$ (Ådnöy *et al.* 2005) corresponding to 216.6/6.25 = 34.7 g N kg $meat^{-1}$ (i.e.3.47 %).
9. Surface water fluxes are based on the mean N-concentration of water samples collected 2006-2009 in Hol (mean 0.08 mg l^{-1}; data not reported).
10. O-horizon N-pool is based on average values of the datasets from location A and C (see material and methods).

11. Mineral soil N-pool is based on average values of the dataset from location A (soil samples from the mineral horizon were in addition to O-horizon samples (see material and methods) sampled in 2007; data not reported).
12. Soil water fluxes from the O-horizon are based on location A (altitude level 2 and 3) and B (see material and methods).
13. Soil water fluxes from the mineral-horizon are based on location A (altitude level 2 and 3) [soil water samples from the mineral horizon were in addition to O-horizon water samples (see material and methods) sampled in 2008-2009; data not reported].
14. We have no data on N_2O, N_2 or NH_3 emissions.

TABLE 1

Mean (± se) NO_3-N, NH_4-N and total N[a] of PRS^{TM}-adsorbed N (µg N cm^{-2} 20 days burial^{-1}; location B), soil extractable N (µg g^{-1} soil; location C) and soil water concentrations (mg l^{-1}; location A and B) from O-horizons of grassland habitats at three density levels of sheep (no sheep = control, low density = 25 sheep km^{-2} and high density = 80 sheep km^{-2}), Hol, Norway. Number of samples above the detection limit is shown (n).

Variable	**Treatment**	**NO_3-N**			**NH_4-N**			**Tot-N[a]**		
		mean	se	n	mean	se	n	mean	se	n
PRS adsorbed N (µg N cm^{-2} 20 days burrial^{-1})	High	0.381	0.017	40	1.126	0.090	49	1.379	0.092	52
	Low	0.410	0.025	32	0.962	0.085	48	1.209	0.091	51
	No sheep	0.419	0.025	41	1.170	0.083	50	1.461	0.088	53
										156
Extractable N (µg N g soil^{-1})	High	13.120	2.910	60	67.884	5.409	96	76.084	5.773	96
	Low	8.969	4.780	24	41.043	3.489	76	43.875	3.768	76
	No sheep	4.840	2.250	21	36.929	5.226	75	38.284	5.332	75
										247
Soil water N (mg l^{-1})	High	0.032	0.004	9	0.105	0.020	69	1.338	0.128	69
	Low	0.061	0.022	5	0.088	0.011	76	1.261	0.098	76
	No sheep	0.026	0.003	5	0.109	0.018	80	1.299	0.114	80

[a] Total N represents the sum of inorganic N (NO_3-N and NH_4-N) for PRS^{TM}-adsorbed N and soil extractable N and total inorganic and organic N for the soil water (see material and methods).

TABLE 2.

Parameter estimates of (ln) PRS^{TM}- adsorbed inorganic nitrogen (µg N cm^{-2} 20 days burial^{-1}) within O-horizons of grassland habitat soils location B, Hol, Norway. The estimates derive from a linear mixed effect model (REML-estimation) with plot, site, enclosure and block as random factors. The fixed effect factors are treatment [different sheep densities (high = 80 sheep km^{-2}, low = 25 sheep km^{-2} and no sheep = control)], altitude [two altitudinal levels (~1168 and1259 m.a.s.l)] and period [three burrial periods (03.07-23.07; 1. 23.07-11.08; 2 and 11.08-31.08; 3]. The table shows estimated differences between factor levels (i.e. “treatment contrasts”; see material and method), se and adjusted p-values. Values in bold indicate significant differences. Estimated parameters for each factor combination (±se) are shown. P1, P2 and P3 = period 1, 2 and 3; A1, A2 = altitude level 1 and 2; C = control (no sheep); H = high sheep density and L= low sheep density.

Parameter estimates based on treatment contrasts					*Estimates for each factor combination*		
Parameter (fixed effects)	**Estimate**	**se**	**z**	**P**	**Parameter**	**Estimate**	**se**
Intercept	**0.7215**	**0.1773**	**4.0690**	**<0.01**	P1A1_C	0.7215	0.1773
Treatment (high vs. control)	-0.1819	0.2440	-0.7460	0.9982	P1A1_H	0.5396	0.1773
Treatment (low vs. control)	**-1.0360**	**0.2405**	**-4.3080**	**<0.01**	P1A1_L	-0.3145	0.1725
Altitude (alt 2 vs. alt 1)	-0.3728	0.2405	-1.5500	0.7144	P1A2_C	0.3487	0.1725
Period (period 2 vs. period 1)	**-0.6801**	**0.1520**	**-4.4730**	**<0.01**	P2A1_C	0.0414	0.1725
Period (period 3 vs. period 1)	-0.1688	0.1520	-1.1100	0.9513	P3A1_C	0.5527	0.1725
Treatment (high vs. control): Altitude (alt 2 vs. alt 1)	0.2882	0.3401	0.8470	0.9941	P1A2_H	0.4551	0.1725
Treatment (low vs. control): Altitude (alt 2 vs. alt 1)	**1.2870**	**0.3377**	**3.8110**	**<0.01**	P1A2_L	0.5996	0.1725
Treatment (high vs. control): Period (period 2 vs. period 1)	0.1765	0.2150	0.8210	0.9956	P2A1_H	0.0360	0.1725
Treatment (low vs. control): Period (period 2 vs. period 1)	0.3669	0.2266	1.6190	0.6633	P2A1_L	-0.6277	0.1912
Treatment (high vs. control): Period (period 3 vs. period 1)	0.0397	0.2188	0.1810	1.0000	P3A1_H	0.4106	0.1773
Treatment (low vs. control): Period (period 3 vs. period 1)	**0.7013**	**0.2111**	**3.3220**	**0.0127**	P3A1_L	0.2180	0.1725
Altitude (alt 2 vs. alt 1) : Period (period 2 vs. period 1)	0.0309	0.2111	0.1460	1.0000	P2A2_C	-0.3005	0.1725
Altitude (alt 2 vs. alt 1) : Period (period 3 vs. period 1)	0.1145	0.2111	0.5420	0.9999	P3A2_C	0.2944	0.1725
Treatment (high vs. control): Altitude (alt 2 vs. alt 1) : Period (period 2 vs. period 1)	-0.3100	0.2985	-1.0380	0.9696	P2A2_H	-0.3276	0.1725
Treatment (low vs. control): Altitude (alt 2 vs. alt 1) : Period (period 2 vs. period 1)	-0.6250	0.3070	-2.0360	0.3592	P2A2_L	-0.3077	0.1725
Treatment (high vs. control): Altitude (alt 2 vs. alt 1) : Period (period 3 vs. period 1)	-0.1717	0.3013	-0.5700	0.9999	P3A2_H	0.2688	0.1725
Treatment (low vs. control): Altitude (alt 2 vs. alt 1) : Period (period 3 vs. period 1)	-0.8050	0.2957	-2.7220	0.0773	P3A2_L	0.4417	0.1725

Random effects:

Groups	**Name**	**Variance**	**% of Var**
Plot	Intercept	0.0356	20.1
Site	Intercept	0.0402	22.7
Enclosure	Intercept	0.0000	0.0
Block	Intercept	0.0050	2.8
Residual		0.0965	54.4

Number of observations: 156. Groups: Plot, 54; Site, 18; Enclosure, 9, Block, 3.

TABLE 3.

Parameter estimates of (sqrt) total nitrogen (%) of plants [tree plant functional groups (PFG): Grasses (*Avenella flexuosa* and *Anthoxantum odoratum*), herbs (*Alchemilla alpina* and *Rumex acetosa*) and woody species (*Vaccinium myrtillus* and *Salix lapponum)*] within grassland plots at two different plant communitites (snowbed and willow-shrub) location C, Hol, Norway. The estimates derive from a linear mixed effect model (REML-estimation) with plot, enclosure, block and plant species as random factors. The table shows estimated differences between factor levels (i.e. "treatment contrasts"; see material and method), se and adjusted p-values. Values in bold indicate significant differences. Estimated parameters for each combination of plant functional group and habitat (±se) are shown.

Parameter estimates based on treatment contrasts					*Estimates for each factor level*		
Parameter (fixed effects)	**Estimate**	**se**	**z**	**P**	**Parameter**	**Estimate**	**se**
Intercept	**1.0542**	**0.1432**	**7.3630**	**<0.001**	Snowbed-grasses	1.0542	0.1432
Pl.comm (willow-shrub vs. snowbed)	**-0.1039**	**0.0200**	**-5.1810**	**<0.001**	Willow-shrub-grasses	0.9504	0.1437
PFG (herbs vs. grasses)	0.4662	0.1915	2.4350	0.0511	Snowbed-herbs	1.5204	0.1432
					Willow-shrub-herbs	1.4166	0.1437
PFG (woody species vs. grasses)	0.3435	0.1915	1.7940	0.2247	Snowbed-woody species	1.3977	0.1432
					Willow-shrub-woody species	1.2939	0.1436

Random effects:

Groups	**Name**	**Variance**	**% of Var**
Plot		0.0051	8.04
Enclosure		0.0053	8.37
Block		0.0045	7.06
Plant species		0.0365	57.80
Residual		0.0118	18.73

Number of observations: 505.
Groups: Plot, 89; Enclosure, 9; Block, 3; Plant species; 6.

TABLE 4.

Parameter estimates of (ln) total nitrogen (NH_4-N + NO_3-N; µg g^{-1} soil) extracted on organic soil samples from grassland habitats (location C, Hol, Norway). The estimates derrive from a linear mixed effect model (REML-estimation) with plot, enclosure and block as random factors. The fixed effect factors are Treatment [different sheep densities (high (H) = 80 sheep km^{-2}, low (L) = 25 sheep km^{-2} and no sheep (C) = control)] and Day [number of incubation days (day 0 (D0) = start of incubation; initial extractable N, day 15 (D15) = extractable N after 15 days of incubation and day 63 (D63) = extractable N after 63 days of incubation)]. The table shows estimated differences between factor levels (i.e. "treatment contrasts"; see material and method), se and adjusted p-values. Values in bold indicate significant differences. Estimated parameters for each factor combination (±se) are shown.

Parameter estimates based on treatment contrasts					*Estimates for each factor combination*		
Parameter (fixed effects)	**Estimate**	**se**	**z**	**P**	**Parameter**	**Estimate**	**se**
Intercept	**3.3179**	**0.1358**	**24.4330**	**< 0.001**	D0_C	3.3179	0.1358
Treatment (high vs. control)	**0.5737**	**0.1756**	**3.2670**	**0.0085**	D0_H	3.8916	0.1211
Treatment (Low vs. control)	0.2253	0.1855	1.2140	0.7768	D0_L	3.5431	0.1344
Day (15 vs. 0)	0.0966	0.1027	0.9410	0.9230	D15_C	3.4145	0.1358
Day (63 vs. 0)	-0.0898	0.1027	-0.8740	0.9463	D63_C	3.2281	0.1358
Treatment (high vs. control): Day (15 vs. 0)	0.0824	0.1371	0.6010	0.9940	D15_H	4.0706	0.1211
Treatment (low vs. control): Day (15 vs. 0)	0.0310	0.1448	0.2140	1.0000	D15_L	3.6707	0.1333
Treatment (high vs. control): Day (63 vs. 0)	**0.5745**	**0.1371**	**4.1910**	**< 0.001**	D63_H	4.3763	0.1211
Treatment (low vs. control): Day (63 vs. 0)	0.0128	0.1459	0.0880	1.0000	D63_L	3.4661	0.1344

Random effects:

Groups	**Name**	**Variance**	**% of Var**
Plot	Intercept	0.3009	69.0
Enclosure	Intercept	0.0000	0.0
Block	Intercept	0.0033	0.7
Residual		0.1319	30.2

Number of observations: 247. Groups: Plot, 83; Enclosure, 9; Block, 3.

TABLE 5.

Parameter estimates of (ln) dissolved inorganic nitrogen to organic nitrogen ratio (DIN:DON) of O-horizon soil water from grassland habitats at location A and B, Hol, Norway. The estimates derive from a linear mixed effect model (REML-estimation) with plot, site, enclosure, block and year as random factors. The fixed effect factors are Treatment [different sheep densities (high (H) = 80 sheep km^{-2}, low (L) = 25 sheep km^{-2} and no sheep (C) = control)] and Month (June, July and August). The table shows estimated differences between factor levels (i.e. "treatment contrasts"; see material and method), se and adjusted p-values. Values in bold indicate significant differences. Estimated parameters for each month and treatment combination (±se) are shown.

Parameter estimates based on treatment contrasts					*Estimates for each factor level*		
Parameter (fixed effects)	**Estimate**	**se**	**z**	**P**	**Parameter**	**Estimate**	**se**
Intercept	**-2.1351**	**0.2597**	**-8.2220**	**<0.001**	June_C	-2.1351	0.2597
Treatment (high vs. control)	-0.1637	0.1593	-1.0280	0.8865	June_H	-2.2988	0.2635
Treatment (low vs. control)	-0.3203	0.1496	-2.1410	0.1886	June_L	-2.4554	0.2572
Month (July. vs. June)	-0.3139	0.1277	-2.4570	0.0906	July_C	-2.4489	0.2559
Month (Aug. vs. June)	**-0.6115**	**0.1250**	**-4.8920**	**<0.001**	Aug_C	-2.7465	0.2510
Treatment (high vs. control): Month (July. vs. June)	-0.1774	0.1900	-0.9340	0.9273	July_H	-2.7901	0.2628
Treatment (low vs. control): Month (July. vs. June)	0.1062	0.1873	0.5670	0.9962	July_L	-2.663	0.2633
Treatment (high vs. control): Month (Aug. vs. June)	0.2607	0.1698	1.5350	0.5460	Aug_H	-2.6496	0.2514
Treatment (low vs. control): Month (Aug. vs. June)	0.4090	0.1585	2.5800	0.0661	Aug_L	-2.6578	0.2502

Random effects:			
Groups	**Name**	**Variance**	**% of Var**
Plot	Intercept	0.0168	5.56
Site	Intercept	0.0161	5.32
Enclosure	Intercept	0.0000	0.00
Block	Intercept	0.0032	1.07
Year	Intercept	0.1081	35.81
Residual		0.1577	52.24

Number of observations: 225. Groups: Plot, 69; Site, 24; Enclosure, 9, Block, 3; Year, 2.

Appendix

Table A1: Model selection for the fixed effect structure of linear mixed effect models (based on ML-estimation) for ln(PRS^{TM}- adsorbed N) (µg N cm^{-2} 20 days burrial^{-1}), sqrt(N-content plants) (%), ln(total extractable N) (µg N g soil^{-1}) and ln(DIN:DON) ratio of soil water. The models always included enclosures (n=9) nested in blocks (n=3) as random effects. In addition, plot and site (each consisting of 3 plots) were included for the PRS^{TM}-adsorbed N model (based on dataset B; see material and methods); plot and plant species (6 different species) was included for the plant N-content model (based on dataset C; see material and methods); plot was included for the total extractable N model (based on dataset C; see material and methods) and plot, site (each consisting of 3 plots) and year was included for the DIN:DON ratio model (based on dataset A and B; see material and methods). Step indicates models with a different fixed effect structure for each dependent variable. AIC= Akaike's information criterion. ΔAIC = change in AIC between the models (negative values indicate improved fit). P is the p-value based on likelihood ratio tests (Chi squared) between two models. P-values < - 0.05 indicate significantly lower explanatory power. Step comp. indicates the models compared.

Dependent variable	Model (random effects)	Step	Model (fixed effects)	Df	AIC	Δ AIC	P	Step comp.
ln(PRS adsorbed N) (µg N cm^{-2} 20 days burrial^{-1})	~(1block)+(1|enclosure)+(1|site) +(1|plot)	1	***~ treatment*altitude*period***	23	162.16			
		2	~treatment*altitude+treatment*period+ altitude*period	19	164.24	2.08	0.042	1 vs. 2
sqrt(N-content plants) (%)	~(1block)+(1|enclosure) +(1|plot)+(1|plant species)	1	~treatment*pl.comm*PFG	23	-619.90			
		2	~treatment*pl.comm+treatment*PFG+pl.comm*PFG	19	-624.55	-4.65	0.501	1 vs. 2
		3	~treatment*pl.comm+treatment*PFG	17	-623.39	1.16	0.076	3 vs. 2
		4	~treatment*PFG+pl.comm*PFG	17	-626.72	-2.17	0.399	4 vs. 2
		5	~treatment*pl.comm+pl.comm*PFG	15	-631.91	-7.36	0.958	5 vs. 2
		6	~treatment+pl.comm+PFG	11	-632.62	-8.07	0.440	6 vs. 2
		7	***~pl.comm+PFG***	9	-633.49	-0.87	0.209	7 vs. 6
		8	~treatment+PFG	10	-611.69	20.93	<0.001	8 vs. 6
		9	~treatment+pl.comm	9	-630.62	2.00	0.038	9 vs. 6
		10	~pl.comm	8	-611.83	21.66	<0.001	10 vs. 7
		11	~PFG	7	-630.94	2.55	0.038	11 vs. 7
ln(total extractable N) (µg N g soil^{-1})	~(1block)+(1|enclosure)+(1|plot)	1	~treatment*day*pl.comm	22	397.46			
		2	~treatment*day+treatment*pl.comm +day*pl.comm	18	391.46	-6.00	0.737	1 vs. 2
		3	~treatment*day+treatment*pl.comm	16	389.21	-2.25	0.419	3 vs. 2
		4	~treatment*day+day*pl.comm	16	390.57	-0.89	0.212	4 vs. 2
		5	~treatment*pl.comm+day*pl.comm	14	408.90	17.44	<0.001	5 vs. 2
		6	~treatment*day+pl.comm	14	388.34	-0.87	0.209	6 vs. 3
		7	***~treatment*day***	13	388.19	-0.15	0.174	7 vs. 6
		8	~treatment+day	9	407.44	19.25	<0.001	8 vd. 7
ln(DIN:DON ratio)	~(1block)+(1|enclosure)+(1|site) +(1|plot) +(1|year)	1	~treatment*altitude*month	24	293.28			
		2	~treatment*altitude+ treatment*month+altitude*month	20	290.22	-3.06	0.293	1 vs. 2
		3	~treatment*altitude+treatment*month	18	286.61	-3.61	0.825	3 vs. 2
		4	~treatment*month+altitude*month	18	288.48	-1.74	0.324	4 vs. 2
		5	~treatment*altitude+altitude*month	16	294.17	3.95	0.018	5 vs. 2
		6	~treatment*month+altitude	16	284.75	-5.47	0.639	6 vs. 2
		7	~treatment+month+altitude	12	288.13	3.38	0.023	7 vs. 6
		8	***~treatment*month***	15	284.72	-0.03	0.161	8 vs. 6
		9	~treatment +month	11	288.48	3.76	0.019	9 vs. 8

PAPER III

Martinsen, V., Austrheim, G., Mysterud, A. and Mulder, J., 2010. **Effects of herbivory on N-cycling and distribution of added $^{15}NH_4^+$ in N-limited low-alpine grasslands**.

Effects of herbivory on N-cycling and distribution of added $^{15}NH_4^+$ in N-limited low-alpine grasslands

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Abstract

Aims:

Many rangelands around the world have been degraded by severe overgrazing with resulting long-lasting loss of nutrients and reduced productivity. In other cases, grazing may increase nutrient cycling and enhance ecosystem productivity. The aim of this study was to determine effects of 3 density levels of sheep (no sheep, 25 km^{-2} and 80 km^{-2}) on availability, cycling and distribution of nitrogen (N) at a low-alpine site of Southern Norway.

Methods:

Availability of N in soils was determined by means of N-content of plants. Indirect effects of herbivory on N-cycling were assessed by $\delta^{15}N$ natural abundance and the system's distribution of added $^{15}NH_4$-N.

Results:

We found no evidence for grazing induced effects on availability or cycling of N based on N content of plants and $\delta^{15}N$ natural abundance. Litter layers and O-horizons were the largest sinks for the added $^{15}NH_4$-N, illustrating the importance of these layers in controlling short-term distribution of inorganic N. Based on proportional tracer recoveries and tracer enrichments we found an indication of somewhat greater N cycling at grazed as compared to non-grazed sites.

Conclusions:

We conclude that the levels of grazing studied have limited impacts on distribution and cycling of N and thus provide a case of sustainable ecosystem management in the long-term.

Key words: Alpine system, Nitrogen cycling, Nitrogen availability, Grazing, ^{15}N tracer, $\delta^{15}N$ natural abundance.

Introduction

Grazing by large herbivores may have severe impacts on ecosystem structure (van der Wal and Brooker 2004) through direct and indirect impacts on ecosystem processes and properties important for N-cycling (Ewing et al. 2010; Frank 1998; Frank et al. 2000; Frank and Evans 1997; van der Wal and Brooker 2004). Direct impacts of herbivory include trampling, plant defoliation and nutrient return in form of urea and faeces (McNeill and Unkovich 2007; van der Wal et al. 2004). In turn, this may affect soil properties (e.g. temperature, moisture and bulk density), vegetation cover (e.g. composition, biomass and nutritional status) and recycling of N (Martinsen et al. 2010a; Martinsen et al. 2010b; McNeill and Unkovich 2007). Together, these factors are important determinants for the N-distribution and internal N-cycling within an ecosystem with potential implications for transformations and losses of N (De Deyn et al. 2009; Ewing et al. 2010; Nadelhoffer et al. 1992). Indeed, these processes may drive systems between alternate stable states (Briske et al. 2005; vandeKoppel et al. 1997) , or they may enhance nutrient cycling depending on ecosystem properties (Wardle et al. 2004) and level of grazing (Martinsen et al. 2010a).

Plants take up primarily NO_3^- , NH_4^+ and organic N-compounds (Michelsen et al. 1996; Sorensen et al. 2008), the two latter N-forms being especially important in N-limited systems with low pH and low nitrification rates (Högberg 1997; Hyodo and Wardle 2009; Näsholm et al. 1998; Schimel and Chapin 1996). Plant uptake reflects N concentrations in the soil solution (McNeill and Unkovich 2007) which may vary with season (Arndal et al. 2009; Elberling et al. 2008; Morecroft et al. 1992), species specific adaptations to N uptake (Bradshaw et al. 1964; Gigon and Rorison 1972) and rates of N-mineralization (Detling 1998; Hobbie and Gough 2002). Thus, grazing induced increase in N-cycling (e.g. ammonification, nitrification and/or denitrification) and physical influence (e.g. trampling and plant removal) may change the quantity and form of N taken up by plants, and hence the δ^{15}N (natural abundance) of plants (Högberg 1997; Hyodo and Wardle 2009; Marshall et al. 2007; Nadelhoffer et al. 1996).

Changes in $\delta^{15}N$ of a system will occur only if N-pools with different $\delta^{15}N$ are mixed or if a N sink resulting from N-transformations associated with isotope fractionation is lost from the system (Robinson 2001). Since several key processes in N-cycling involve isotope fractionation [e.g. NH_3 volatilization, nitrification and denitrification (Robinson 2001)] the natural abundance of ^{15}N has been used as an indicator of the impact of various drivers on turnover and availability of N (Frank et al. 2000; Frank and Evans 1997; Garten et al. 2007; Högberg et al. 1996; Hyodo and Wardle 2009; Makarov et al. 2008; Menezes 2009). Isotopic enrichment (increased $\delta^{15}N$ of total soil-N) with soil depth is found in forests (Högberg et al. 1996) and in alpine and tundra (Makarov et al. 2008). This is probably caused by accumulation of recalcitrant (more decomposed) organic matter in deeper horizons (Högberg 1997). Garten *et al.* (2007) report greater $\delta^{15}N$ in SOM at N-rich sites associated with higher rates of nitrification and lower CN ratios of leaf litter-fall. In Yellowstone National Park, Frank & Evans (1997) found an increased soil $\delta^{15}N$ in ungulate urine and dung patches, which was related to enhanced N-loss via leaching, ammonia volatilization and/or denitrification. By contrast, due to a grazing induced stimulation of microbial productively, Frank *et al.* (2000) report decreased soil $\delta^{15}N$ in areas being grazed vs. not grazed, despite herbivore induced stimulation of net N mineralization and nitrification at grazed sites.

In addition to $\delta^{15}N$ natural abundance, applications of ^{15}N-enriched tracers are commonly used to study N-cycling in different environments (Ewing et al. 2010; Näsholm et al. 1998; Providoli et al. 2005; Rütting et al. 2010). By applying labelled (^{13}C,^{15}N) glycine and labelled $^{15}NH_4^+$, Näsholm *et al.* (1998) found organic N to be an important source of N for plants (e.g. *Avenella flexuosa* and *Vaccinium myrtillus*) in boreal forests. Furthermore, Ewing *et al.* (2010) clearly show the importance of detritus and soil moisture controlling movement of N in

Yellowstone grasslands. Despite a lack of grazing induced differences in plant ^{15}N, they found a greater retention of added ^{15}N in litter from historically ungrazed compared to grazed sites due to greater litter pools at ungrazed sites. The study clearly highlights the importance of litter being a key regulator of N flow (Ewing et al. 2010).

In this study we have investigated long term (8 year) effects of grazing on N-cycling in low alpine grasslands using an experimental design with three density levels of domestic sheep (no sheep, low; 25 km^{-2} and high; 80 km^{-2}). We measured seasonal differences in total N-concentration of four plants (*Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa*, *Nardus stricta*), at each sheep density level as an indirect measure of effects of herbivory on N availability in soils. Furthermore, effects of herbivory on ecosystem N-cycling between 2001 and 2008 were assessed by measuring $\delta^{15}N$ natural abundance and the system's distribution of added tracer N (applied as $^{15}NH_4Cl$) in different soil and plant components (*Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa*, *Nardus stricta*, surface layer, O-horizon soil and roots). Observations were made in small plots protected from grazing to avoid removal of ^{15}N-labelled plant biomass by the sheep.

Previous findings documented a marked decline of plant N in the course of the growing season (Mysterud et al. 2010). Mysterud *et al.* (2010) also found the effect of growing season to interact with grazing (most strongly for *Avenella flexuosa*) with a smaller decline and greater N-contents of plants towards the end of the season at high sheep density. The impacts of grazing on N-content of the plants in late stages of the season was attributed to grazers keeping grasses in a young phenological stage (Mysterud et al. 2010). Furthermore, the system has a strong demand for N as observed by small concentrations of inorganic N and low potential mineralization rates in O-horizons (Martinsen et al. 2010a). However, potential mineralization rates were significantly greater in areas with high sheep density as compared to low sheep density and no sheep. Clear effects of grazing impact on bulk density (BD) and soil organic carbon (C) storage was reported by Martinsen *et al.* (2010b).

We hypothesize total N-concentrations of plants to increase in the order no grazing < low grazing intensity < high grazing intensity (**H1a**) and to be greater early vs. late in the growing season (**H1b**). δ^{15}N natural abundance of all ecosystem components are expected to increase with increasing grazing intensity (**H2a**) and to be greater in belowground than above ground compartments (**H2b**). Assuming no grazing induced difference in species specific physiological N-uptake mechanisms, we predict tracer recovery to increase in order high grazing intensity < low grazing intensity < no grazing (**H3a**) due to greater dilutions of added ^{15}N at grazed sites, which we hypothesize have a greater cycling of N. Differences between grazing levels in ^{15}N enrichment (expressed as ^{15}N atom% excess) are expected to decline with increased time since tracer application (**H3b**).

Material and methods

Site description

The study was conducted in a low alpine region (1050 – 1300 m.a.s.l.) in Hol municipality, Buskerud county, southern Norway (7°55′ - 8°00′ E, 60°40′ - 60°45′ N) (Mysterud and Austrheim 2005). Mean annual temperature (MAT) is - 1.5 ^{O}C and mean annual precipitation (MAP) is about 1000 mm (Evju et al. 2009), approximately 75% of which falls as snow. The bedrock consists of meta-arkose and quaternary deposits of till and colluvium (Kristiansen and Sollid 1985; Sigmond 1998). Soils are freely drained with shallow and acidic organic horizons. Vegetation is dominated by dwarf shrub heaths with smaller patches of lichen heaths, snow beds and alpine meadow communities in lee-sides (Rekdal 2001). Further description of plant species composition is given by Austrheim *et al.* (2005).

In 2001 a grazing experiment (within a large enclosure; ~2.7 km^{-2}) with three treatments of domestic sheep (*Ovis aries*); no sheep (control), low grazing density (25 sheep km^{-2}) and high grazing density (80 sheep km^{-2}) was established (Mysterud et al. 2005; Mysterud and Austrheim 2005). The experiment is set up as a randomized block design with three blocks, each divided in three sub-enclosures (~0.3 km^{-2}) randomly assigned to each of the three grazing treatments (Mysterud et al. 2005; Mysterud and Austrheim 2005). Sheep grazing occurs from the end of June to the beginning of September each year (since 2002).

In June 2008, we established one site, each consisting of three adjacent 1 m x 1 m plots within each of the 9 sub-enclosures (i.e. 9 plots within each of the three grazing treatments; a total of 27 plots). The sites were selected based on criteria of similar altitude (mean altitude 1168 m.a.s.l) and plant community (Martinsen et al. 2010a). All sites were located in grassland habitats partly covered with willow-shrubs. The sites were enclosed during the summer 2008 to enable sampling of plants not affected by direct grazing the given season.

Addition of ^{15}N tracer

The ^{15}N tracer was added to all 27 (1 m x 1 m) plots in July 2008 (9th-11th). Prior to ^{15}N addition, the plots received 2 litres of stream water (applied using a backpack sprinkler) to pre-wet the system. Next, 30.56 mg N m^{-2} (as ammonium chloride (NH_4Cl) 99.98 Atom % ^{15}N; IN 5037, Icon Isotopes, USA) in 5 litre of stream water (6.11 mg N l^{-1}) was slowly added (to prevent surface runoff) using a 1 m long pvc tube (Φ 13 mm) with 10 small holes, each separated by 10 cm (a total of 100 addition points per m^{-2}). The pvc tube with the holes was inserted below the vegetation, on top of the litter layer to prevent contamination of the vegetation. The N concentration of the stream water was low (0.08 mg N l^{-1}, (Martinsen et al. 2010a), and no corrections for the ^{15}N from the stream water were made. After tracer addition, the plots received additional 3 litres of stream water from a back pack sprayer to wash off ^{15}N from the leaves in case of contamination. The added N represents about 7.3% of the annual N deposition (Aas et al. 2008) and was assumed not to affect the N cycling in the system. At some plots, some surface runoff was generated during application, which will have resulted in tracer loss. Thus, the percentage tracer recovery may in some cases be underestimated.

^{15}N natural abundance: Soil and vegetation sampling

Prior to tracer addition (26th of May to 2nd of June 2008), soil from the O-horizon and above ground plant tissue (only fully developed individuals) of *Avenella flexuosa, Nardus stricta and Alchemilla alpina* were sampled adjacent to the 27 plots. Two O-horizon soil cores were sampled per site, using an auger (diameter 2.5 cm) to a maximum depth of 10 cm. The vegetation was cut at the soil surface and the litter (O_i) removed. At each site, both soil cores and vegetation samples collected adjacent to the plots were bulked prior to determination of ^{15}N natural abundance. Thus for each site we have one value for natural abundance for the soil and each of the four plant species, so that the number of analyzed samples for the soil and each of the plant species was $n_{soil} = 9$, $n_{Avenella\ flexuosa} = 9$, $n_{Nardus\ stricta} = 9$ and $n_{Alchemilla\ alpina} = 8$ (absent in one enclosure). In addition, *Vaccinium myrtillus* and an integrated sample of cryptogams, roots and O_i (herafter called surface layer) was sampled outside each of the 27 plots September 10th and September 20-21 2009, respectively. *Vaccinium myrtillus* was sampled and bulked at each site in the same way as the plants in 2008 ($n_{Vaccinium\ myrtillus} = 8$, absent in one enclosure). The average volume of surface layer ($n_{surface\ layer} = 27$) was 75.8 cm^3 (42.1 cm^2 x 1.8 cm).

Tracer addition experiment: Soil and vegetation sampling

After tracer addition, the O-horizon and above ground plant tissue of *Avenella flexuosa, Nardus stricta and Alchemilla alpina* were sampled at two occations (12th-13th of August 2008 and 2nd- 4th of August 2009) inside the 27 plots. In 2008, two randomly located O-horizon soil cores (vegetation and O_i removed) were sampled at each plot using an auger (diameter 5.2 cm) to a maximum depth of 10 cm (and bulked per plot prior to analysis; $n_{soil} = 27$). The number of samples of each of the plant species used for analysis was $n_{Avenella\ flexuosa} = 27$, $n_{Nardus\ stricta} = 21$ and $n_{Alchemilla\ alpina} = 19$).

In 2009, all aboveground vegetation within a sub-area (0.5 m x 0.5 m) in the middle of each of the 27 plots was harvested quantitatively. The harvested plant shoots were sorted at the species level; *Avenella flexuosa* (n= 27)*, Nardus stricta* (n= 18, only 17 were used for chemical analysis)*, Alchemilla alpina* (n=14) and *Vaccinium myrtillus* (n= 21). These species were selected due to their most frequent presence at the plots. The rest of the vegetation was divided into 6 compartments [ferns, rest of herbs, rest of woody species, rest of cryptogams, rest of graminoids and one containing non-recognizable or withered plant material ("litter mix")] to get an esimate of the total aboveground biomass (Online Resource 1). Three O-horizon soil cores (vegetation and O_i removed) randomly located within each sub-area of the 27 plots were sampled using an auger (diameter 2.5 cm; bulked per plot prior to analysis; $n_{soil} = 27$). Finally, three surface layer samples (randomly located within each sub-area) were sampled (the same procedure as before tracer addition) and bulked prior to analysis ($n_{surface\ layer} = 27$). The average volume of surface layer sampled was 162.5 cm^3 (108.3 cm^2 x 1.5 cm).

Analysis and laboratory processing

All soil and surface layer samples were stored dark and cold (< 4 °C) prior to analysis. Soils were air-dried (40 °C for 4-5 days) and sieved at 2 mm to separate the dry roots and gravels from the soil. The weight of dry roots and gravels (> 2 mm) was determined and the roots included for chemical analysis (representing bulked roots of different plant species). Sub samples of the air-dried fine earth fraction were dried at 105 °C to determine dry matter content (DM), while soil samples from August 2008 were dried at 60°C. Vegetation and surface layer samples were air-dried (40 °C for 4-5 days) and homogenized. The aboveground biomass (g) was calculated as the air dry weight of each aboveground vegetation compartment (see above) divided by the area sampled (0.25 m^2). Bulk density ($g\ cm^{-3}$) was based on the fine earth (DM corrected) fraction of the soil. Density of the roots greater than 2 mm ($g\ cm^{-3}$) was calculated as the air dried weight of roots divided by the volume of soil sampled. Furthermore, density ($g\ cm^{-3}$) of the surface layers was calculated as the air dried weight of the surface layers divided by the volume sampled. pH of the soil (~ 4.4, Online Resource 3) was determined electrometrically (Orion, model 720) in a soil solution with distilled water (volume soil : volume water ratio 1:2.5) (Krogstad 1992).

Air dried subsamples of the O-horizon, soil roots, surface layers and plant tissue (4 species) were ground in a ball mill (Fritsch, pulverisette, type 05002) prior to analysis of total C and N content and ^{15}N isotopic composition both for the natural abundance and the ^{15}N labelling experiment. The analyses were conducted at The Macaulay Land Use Research Institute, Aberdeen, UK. The total N contents and the ^{15}N:^{14}N isotope ratios of the milled dried material were determined using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany). The isotope ratios were calculated with respect to N_2 reference gasses injected with every sample and traceable to IAEA reference materials USGS40 and USGS41 (both L-glutamic acid). The N contents of the samples were calculated from the area output of the mass spectrometer calibrated against National Institute of Standards and Technology (NIST) standard reference material 1547 peach leaves which was analysed with every batch of ten samples. Long term precision for quality control standards (milled flour) were: total N = 1.7 ± 0.04 % and ^{15}N:^{14}N = 0.367 ± 0.0002 atom % (mean ± sd, n = 200).

Calculations

The isotopic values of ^{15}N natural abundance are reported as

$$\delta^{15}N = 1000 * \left(\frac{R_{sample} - R_{s\tan dard}}{R_{s\tan dard}} \right) \tag{1}$$

where $\delta^{15}N$ is expressed in ‰, R is the ^{15}N:^{14}N isotope ratio and $R_{standard}$ = 0.0036765 (atmospheric N_2; $\delta^{15}N$ = 0‰) (Robinson 2001). Percentage increase in atom% ^{15}N of enriched samples relative to the background atom% ^{15}N, i.e. ^{15}N atom% excess was calculated as

$$^{15}N_{atom\%excess} = \left(\frac{Atom\%_{enriched} - Atom\%_{background}}{Atom\%_{background}} \right) * 100 \tag{2}$$

We assumed negligible N isotope fractionations during tracer movement in the system (Robinson 2001) and no difference in ^{15}N natural abundance within the pools from 2008 to 2009. The mass of recovered ^{15}N in each pool (mg m^{-2}) was calculated as

$$Mass^{15}N = \left(X_{sample} * N_{content} * Mass \right) * 10^3 \tag{3}$$

where X_{sample} is the tracer fraction in the samples, calculated according to (Providoli et al. 2005):

$$X_{sample} = \frac{\left(F_{sample} - F_{reference} \right)}{\left(F_{tracer} - F_{reference} \right)} \tag{4}$$

$N_{content}$ is the concentration of total N per unit biomass, surface layer or soil (g g^{-1}), mass (g m^{-2}) is the total mass of each pool (biomass = total dry weight (g) per m^{-2}; surface layer, soil and roots = density (g cm^{-3}) * depth (cm) * 10^4) and 10^3 is a conversion factor from g to mg. F_{sample} is the fractional abundance of ^{15}N in the sample (i.e. $[^{15}N/(^{15}N + ^{14}N)]*100$ = atom%

^{15}N) and F_{tracer} and $F_{reference}$ is the fractional abundance of ^{15}N in the applied tracer (99.98 atom % ^{15}N) and in the non-labelled samples (i.e. natural abundance), respectively. The percentage tracer recovered ^{15}N in each pool was calculated as the mass of recovered ^{15}N in each pool divided by the amount of added tracer (30.56 mg N m^{-2}).

To account for differences in tracer recovery between plots (reported as proportional recovery), the total recovery at each plot (n = 27) was rescaled to 100% (Finzi and Berthrong 2005), which gives the proportional partitioning of added ^{15}N in the different ecosystem pools (i.e. vegetation, surface layer, O-horizon and roots) (Clemmensen et al. 2008).

Statistics

Statistical analyses were conducted using the libraries lme4 and multcomp in the statistical package R (version 2.10.1) (http://www.r-project.org/). We used Linear mixed effects models (lmer()) with random effects reflecting the block-wise randomization design. The random effects always included blocks (3 levels) and three sub-enclosures nested within each block (n=9). In some cases, not all sub-enclosures were included due to missing data (specified in the figure legends).

The dependent variables were N-content (Fig. 1, Table 1), δ^{15}N natural abundance (Fig. 2), proportional tracer recovery (Fig. 3, Table 1) and ^{15}N atom% excess (Fig. 4, Table 1). All variables except δ^{15}N were ln transformed to avoid violations of the model assumptions. Grazing treatment (3 levels) was always included as a fixed factor (i.e. explanatory variable). Sampling component (7 levels; A. alpina, V. myrtillus, A. flexuosa, N. stricta, surface layer, O-horizon and roots) was included as fixed factor for the δ^{15}N (‰) natural abundance model (Fig. 2). The remaining models (i.e. for N-content, proportional tracer recovery and ^{15}N atom% excess) were run for each component separately. The fixed factors included month for the N-content models (Fig. 1 and Table 1) and ^{15}N atom% excess models (Fig. 4 and Table 1).

The levels of month are specified in the figure and table legends. The proportional tracer recovery models included one continuous variable ln(N-pool/g m^{-2}) in addition to grazing treatment (Fig. 3, Table 1). All full models were fitted with relevant interactions. Estimated parameters were back-transformed to the original scale before plotting.

Backward selection was used [models fitted by maximum likelihood (ML)] and models compared based on AIC ("smaller is better") and likelihood ratio tests (Chi squared) to obtain the minimum adequate model. The best model was re-fitted based on restricted maximum likelihood (REML) and the estimated effects (including se) were calculated using general linear hypothesis testing [glht() in multcomp]. Only adjusted p-values [single-step method (Hothorn et al. 2008)] are reported. We report differences between fixed effect factor levels based on "treatment contrasts". Treatment contrasts is the default in R, with factor levels arranged in alphabetical sequence [Crawley (2007), pg. 377]. Thus the intercept will be the level that comes first in the alphabet. The only exceptions are in the N-content models and ^{15}N atom% excess models, where differences between months for *V.myrtillus* and surface layer are based differences to September (intercept) and not June (Table 1). Residuals and predicted random effects were plotted (histograms and QQ normal plots) to assess normality and potential outliers. Any missing or omitted values are reported in the figure legends.

Results

Nitrogen concentration

The total N concentrations (% N) of *A. alpina, A. flexuosa* and *N. stricta* were significantly smaller in August 2008 (i.e. 1 month after tracer addition; 1.5-1.7 % N) than in June 2008 (prior tracer addition; 1.8-2.3 % N), illustrating a seasonal decline in %N and the low impact of the added tracer N (30.56 mg N m^{-2}) (Fig. 1, Table 1). A further decline was observed 13 months after tracer addition (i.e. August 2009). There was no effect of grazing except for a tendency of greater N contents for *N. stricta* at high density of sheep as compared to low density and control treatments (Fig. 1, Table 1). By contrast, the N-concentration of the litter layer was significantly greater at sites with high sheep density as compared to low sheep density and non-grazed sites. N-concentrations did not differ between grazing treatments for the O-horizon or the roots (Table 1).

^{15}N natural abundance determined prior to tracer addition (June 08)

The surface layer (a mixture of cryptogams, litter, roots and O_i), *A. flexuosa*, *A. alpina*, *V. myrtillus* and *N. stricta* were depleted (the 3 latter significantly depleted), and the O-horizon soil significantly enriched in ^{15}N relative to the atmospheric N_2 (Fig. 2, Table 2). The bulked plant roots within O-horizons had δ^{15}N values close to 0, i.e. somewhat between the O horizon and the plants (Fig. 2). Despite tendencies of greater δ^{15}N levels in plants, surface layer and O-horizon at the high grazing density (Table 2), the differences were not significant.

Tracer addition experiment: ^{15}N recovery 13 months after tracer addition

The total recovery of added tracer-N 13 months after tracer addition (August 2009) differed greatly between the ecosystem components and the grazing treatments (Table 3). The surface layer and the O-horizon were the largest sinks per unit surface area (>90% of the recovered tracer), illustrating the importance of these components for initial N-retention. Also, the proportional tracer recovery (i.e. percent recovery of each individual component normalized to the total amount of recovered tracer at each plot) revealed greatest recovery in these two components, but no significant effect of grazing (Table 1 and 3).

There was a large variation (0.07-4.6 %) in the proportional tracer recovery for *A. alpina*, *V. myrtillus*, *A. flexuosa* and *N. stricta* 13 months after tracer addition (Table 3). The proportional tracer recovery was positively related to the plant N-pool per unit of surface area and (expressed per gram of plant N m^{-2}) revealed significant effects of grazing for *V. myrtillus*, *A. flexuosa* and *N. stricta* (Fig. 3, Table 1). Proportional tracer recoveries were significantly greater at the sites with no sheep grazing (4.22 and 3.93 %) compared the high (1.39 and 2.22 %) and low (0.75 and 1.17 %) grazing treatments for *V. myrtillus* and *A. flexuosa,* respectively. For *A. flexuosa* and *N. stricta*, proportional recoveries were significantly smaller at the low as compared to the high grazing treatment and the non grazed site (Fig. 3, Table 1).

Tracer addition experiment: Temporal change in distribution of added tracer (1 month vs. 13 months after addition)

The tracer enrichment of *A. alpina* (expressed as ^{15}N atom% excess) did not differ significantly between 1 and 13 months after tracer addition and was not affected by grazing (Fig. 4, Table 1). By contrast, there was a significant interaction between time after tracer application and grazing treatment for ^{15}N atom% excess in *A. flexuosa*, with greater differences between the control as compared to the high and the low grazing treatments in 2008 than 2009 (Fig. 4, Table 1). ^{15}N atom% excess in *N. stricta* was significantly affected by grazing, but did not differ between the years (Fig. 4, Table 1).

Discussion

A key concern for ecosystem management is to establish sustainable levels of grazing. Heavy grazing may potentially deteriorate in particular arid ecosystems in the long term, while moderate grazing by large herbivores in more productive systems may enhance N-cycling (Bardgett and Wardle 2003) and thereby also N-uptake of plants (Hobbie and Gough 2002). We found little evidence of grazing induced differences in N-content of the four plant species

(*A. alpina*, *V. myrtillus*, *A. flexuosa* and *N. stricta*) assessed in this study (H1a), when controlling for the effect induced by delayed phenology at high grazing pressure (Mysterud et al. 2010). However, we found evidence for somewhat greater N contents in *N. stricta* at high sheep density as compared to low density and the non-grazed sites (Fig. 1, after removal of three outliers). As has been reported by e.g. Morecroft *et al.* (1992) and Mysterud *et al.* (2010), N-content of the all species (except *V. myrtillus*) was significantly lower at late stages of the growing season (H1b).

According to Nagy & Grabherr (2009) plants exposed to increased availability of N may either increase N concentration or plant tissue production (i.e. no increase in tissue N-content). The latter may have been the case at the high grazing treatment (Online Resource 1). Calculating N-pools of the standing biomass revealed, however, no significant difference between the grazing treatments (results not shown). Possibly, grazing may affect below-ground N-pools which may be significantly greater than aboveground pools (Fisk et al. 1998), but we have no data for this. Since all the plants investigated in our study were protected from grazing, so the phenological stage between treatments was similar, the treatment-related differences in N-content observed by Mysterud *et al.* (2010) cannot be explained by N availability and must have been related to phenology. By contrast, there was a significantly greater N-concentration in the surface layer at high sheep density as compared to the sites with low sheep density and no sheep (Table 1) which we assume is caused by contamination of urea and faeces. There were no effects of grazing on N-concentrations in the O-horizon or in the roots. The former is in accordance with previous findings at Hol (Martinsen et al. 2010a; Martinsen et al. 2010b) who found a tight N-cycling associated with little loss of N, even in areas with a high sheep density.

The natural abundance of ^{15}N differed significantly between the ecosystem components with O-horizons being significantly enriched ($\delta^{15}N$ ~3.5 ‰) and plants significantly depleted

(δ^{15}N ~ -1.6 ‰) as compared to δ^{15}N of the atmospheric standard (H2b). Levels of δ^{15}N natural abundance in the plants were ~-1.5 ‰, -2.5 ‰, -0.6 ‰ and -1.7 ‰ for *A. alpina*, *V. myrtillus*, *A. flexuosa* and *N. stricta,* respectively. The difference in δ^{15}N natural abundance between the plants is probably caused by species specific traits, as δ^{15}N abundance in plants depends on the source of plant N and the form of N used, both of which are related to mycorrhizal status and rooting depth (Högberg 1997; Michelsen et al. 1996; Nadelhoffer et al. 1996; Weigelt et al. 2005). Due to great seasonal and site specific variations in ^{15}N natural abundance within and between species (Karlsson et al. 2000; Nadelhoffer et al. 1996), it is difficult to make direct comparisons between δ^{15}N levels in plants at Hol and those reported elsewhere.

As suggested by Högberg (1997), under N-limited conditions characterized by low rates of nitrification (and thus little N fractionation), uptake of NH_4-N in plants would result in depleted N at the soil surface due to accumulation of ^{15}N depleted litter. At Hol, plants and litter-layer were depleted in ^{15}N relative to the atmospheric standard (Fig. 2). Low nitrification potentials at Hol were reported by Martinsen *et al.* (2010a). They found small concentrations of NO_3-N in soil water, associated with little available NO_3-N (assessed by PRS^{TM}-adsorption) and low potential nitrification rates in O-horizons. This further supports our earlier findings that N loss is small at Hol.

Levels of ^{15}N in O-horizons (3.5‰) were somewhat greater than what has been reported from comparable systems (Makarov et al. 2008; Michelsen et al. 1996; Michelsen et al. 1998). The δ^{15}N of total N were from -1.4 ‰ to 2.8 ‰ in organic horizons in three mountain areas [NW Caucasus (Russia), the Khibiny Mountains (NW Russia) and Abisko (N Sweden)] (Makarov et al. 2008), close to 0 ‰ in a heath tundra and forest tundra in northern Sweden (Michelsen et al. 1998) and from -0.7 to 0.5 ‰ in a subalpine heath and fellfield (evergreen and deciduous dwarf shrubs and mosses) close to Abisko (N Sweden) (Michelsen et al. 1996).

Possibly, the relatively large $\delta^{15}N$ values in the O horizons at our site are due to contamination with mineral soil. Mineral soil includes more stabilized organic matter generally characterized by larger $\delta^{15}N$ values, and commonly increasing with soil depth (Högberg et al. 1996; Makarov et al. 2008). Contamination of the O horizons with mineral soil at Hol is suggested by the relatively large bulk density (BD), which was reported to be 0.3 and 0.24 g cm^{-3} in snowbeds and willow-shrub, respectively (Martinsen et al. 2010b). In the present study, BD was from 0.4 to 0.5 (Online Resource 3). This illustrates that the sampling procedure is of great importance when determining $\delta^{15}N$ in different soil horizons.

Despite a tendency for greater $\delta^{15}N$ in the soil and plant components at the high grazing treatment (Table 2), grazing had no significant effect on $\delta^{15}N$, neither in the above-ground nor in the belowground components (H2a). Based on previous studies at Hol, indicating mixing of O-horizon and mineral soil [increased BD (Martinsen et al. 2010b)], a greater $\delta^{15}N$ would have been expected at grazed sites due to trampling. In turn, there is evidence for a greater biomass removal at high sheep grazing as compared to low sheep grazing and control, as lamb weights are reduced at high sheep density (Mysterud, unpublished material). Removal of depleted plant biomass by herbivores was expected to increase $\delta^{15}N$ of O-horizons and eventually of plants. As discussed by Evju *et al.* (2006), the high sheep density used in this experiment may correspond to a moderate grazing pressure. As we found only weak evidence for increased $\delta^{15}N$ natural abundance at the intensively grazed site (Table 2), we suggest that the effects of grazing at the levels provided at Hol are too small for detection based on ^{15}N natural abundance. By contrast, the proportional tracer recovery in the species *V. myrtillus*, *A. flexuosa* and *N. stricta* (Fig. 3) and the significant treatment*month interaction (Table 1) for ^{15}N excess in *A. flexuosa* suggest differences in N-cycling between the grazing treatments (H3a).

Recoveries of added tracer ^{15}N were greatest in the litter layer and O-horizon (Table 3). This is in accordance with other labelling experiments, indicating great retentions of N in these layers (Ewing et al. 2010; Gerzabek et al. 2004; Näsholm et al. 1998; Rütting et al. 2010). Tracer recoveries in surface layers and O-horizons were somewhat larger at the low sheep density as compared to high and control (Table 3). However, the proportional tracer recovery did not differ between the grazing treatments (Table 1). Furthermore, we found evidence for greater proportional recoveries per gram plant N (*V. myrtillus* and *A. flexuosa*) at non-grazed as compared to grazed sites (Fig. 3). The proportional recovery per gram plant N was smallest at the low grazing treatment for *V. myrtillus*, *A. flexuosa* and *N. stricta* (Fig. 3, Table 1), indicating a greater N-cycling (high immobilization-mineralization turnover) and thus ^{15}N dilution at low grazing density as compared to high grazing density and control. The same pattern was also observed for ^{15}N uptake (^{15}N atom% excess) in *A. flexuosa* and *N. Stricta* (Fig. 4).

The calculated ^{15}N atom% excess and proportional recoveries were correlated (results not shown). Due to lack of biomass data for 2008, we used ^{15}N atom% excess to assess differences in ^{15}N enrichment short term (1 month) and long term (13 months) after tracer addition (Online Resource 2 and 3). The difference in ^{15}N atom% excess between the grazing treatments decreased with time after tracer addition, but for *A. flexuosa* only (Fig. 4). This suggests reduced effects of grazing on ^{15}N dilution with increased time since tracer application (H3b) probably due to stabilization in the soil organic N pool. The latter is mostly unavailable to plants and substantially greater than the plant-available (mineral) N fraction in the soil (McNeill and Unkovich 2007).

Effects of grazing by large herbivores on ecosystem structure and processes have been described in a wide range of environments (Bowns and Bagley 1986; van der Wal and Brooker 2004; vandeKoppel et al. 1997). At Hol, effects of grazing have been reported on plant traits and abundance (Austrheim et al. 2008; Evju et al. 2006) . The shrubs *Juniperus communis* and *Betula nana* increased at low sheep densities vs. non-grazed sites after four years of grazing whereas three species of graminoids increased at the high grazing treatment (Austrheim et al. 2008). Moreover, at the plant community level Austrheim *et al.* (2008) found reduced vascular plant height and plant cover after four years of grazing. Also, the percent of bare soil decreased less at high sheep density as compared to non grazed sites (Austrheim et al. 2008). Together, these findings clearly indicate effects of sheep grazing in this system. However, as we found only small (indirect) effects of grazing on N-cycling, we cannot discern whether observed or potential future changes in ecosystem structure and processes are driven by indirect effects on N-cycling (Wardle et al. 2004) or direct effects on aboveground vegetation.

Conclusions

Based on N-content of four different plant species (all being protected from grazing) and δ^{15}N of seven different soil and plant components, we found no evidence for grazing induced effects on N-cycling. However, based on added ^{15}N-tracer recoveries and enrichments, there was some indication of greater N-cycling at grazed vs. non grazed sites. We conclude that grazing at the levels provided in this system, has only minor effects on N-cycling. Direct effects of grazing on aboveground vegetation may have greater impacts on ecosystem structure than indirect effects on N-cycling.

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Figure legends

Fig. 1 Estimated mean (± se) N-content (% total-N) of *Alchemilla alpine* (n=41), *Vaccinium myrtillus* (n= 29), *Avenella flexuosa* (n=63) and *Nardus stricta* (n= 44[a]) from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. The figure shows estimated N-contents of plots with no ^{15}N addition (June 2008; J08 and September 2009; S09[b]), 1 month (i.e. August 2008; A08) and 13 months (i.e. August 2009; A09) after ^{15}N addition (July 2008). Estimates are based on linear mixed effect models[c] presented in Table 1.

Fig. 2 Estimated mean δ^{15}N (‰) natural abundance (± se) of above- and below-ground components (sampled prior to tracer addition) from 27 grassland habitats, Hol, Norway. Values derive from a linear mixed effect model with component (7 levels; *Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa*, *Nardus stricta*, surface layer, O-horizon and bulked plant roots >2 mm) as the only fixed effect factor[a]. ** and *** indicate significant difference from $\delta^{15}N = 0$ at the level of significance 0.01 and 0.001, respectively. Positive δ^{15}N values indicate enrichment and negative δ^{15}N values indicate depletion of ^{15}N relative to atmospheric N_2 ($\delta^{15}N = 0$). n=79.

Fig. 3 Estimated mean (± se) proportional tracer recovery as % of recovered tracer per g plant N-pool and m^2, 13 months after ^{15}N addition (August 2009) for *Alchemilla alpina* (n = 14), *Vaccinium myrtillus* (n= 20[a]), *Avenella flexuosa* (n= 26[a]) and *Nardus stricta* (n= 17) from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Values derive from linear mixed effect models[b] presented in Table 1.

Fig. 4 Estimated mean (± se) ^{15}N atom% excess[a] (percentage increase in atom% ^{15}N of enriched samples relative to the background atom% ^{15}N) of *Alchemilla alpina* (n= 33), *Avenella flexuosa* (n= 54) and *Nardus stricta* (n= 38) 1 month (i.e. August 2008; A08) and 13 months (i.e. August 2009; A09) after ^{15}N addition to 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Values derive from linear mixed effect models for each plant species[b] presented in Table 1. **Note**: *Vaccinium myrtillus* is not included because not sampled in August 2008.

Figures

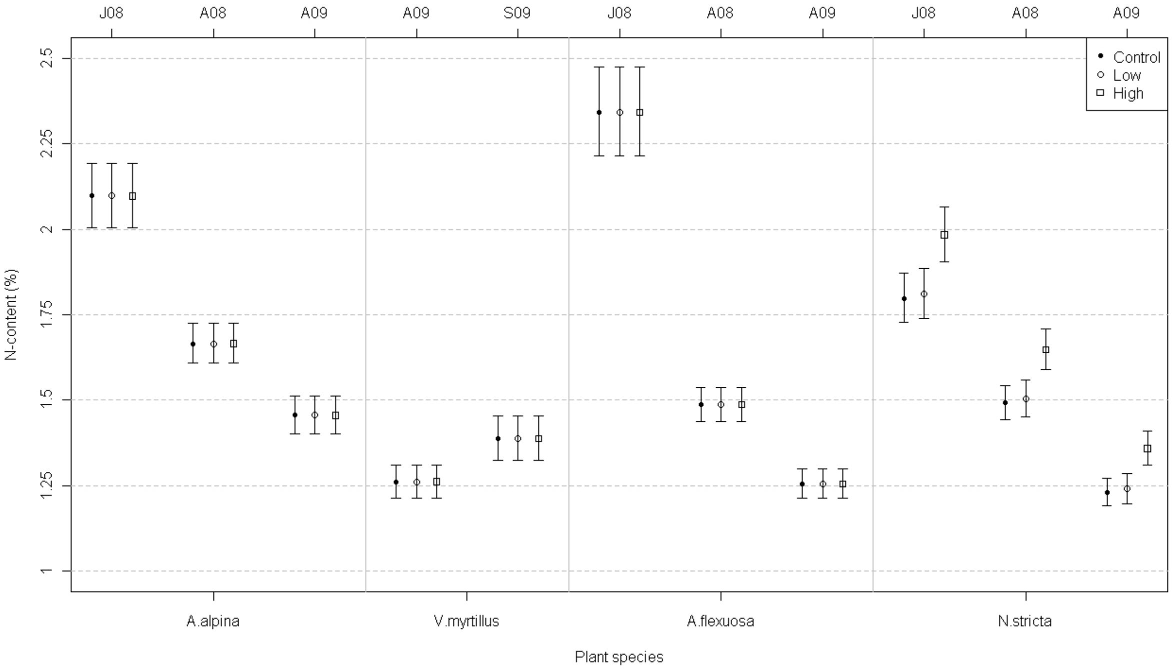

Fig.1.

[a]Three plots (2 from enclosure D; low and 1 from enclosure H; high) with N-contents of 3.36, 2.45 and 2.53 % were omitted from the analysis in order to fulfil the model criteria. This significantly affected the model. When including the 3 plots, treatment was not significantly affecting the N-content of *N. stricta*. [b]*V.myrtillus* and surface layer were sampled in 2009 for determination of ^{15}N natural abundance (see material and methods). [c]Block and enclosure were always included as random factors. Models: ln(N-content)$_{A.alpina}$ ~ month. Var. of rand. effects: Enclosure (8) = 0.0015, Block (3) = 0.0012 and Resid. = 0.0115. ln(N-content)$_{V.myrtillus}$ ~ month. Var. of rand. effects: Enclosure (8) = 0.0036, Block (3) = 0.0017 and Resid. = 0.0093. ln(N-content)$_{A.flexuosa}$ ~ month. Var. of rand. effects: Enclosure (9) = 0.0012, Block (3 blocks) = 0.0000 and Resid. = 0.0264. ln(N-content)$_{N.stricta}$ ~ treatment + month. Var. of rand. effects: Enclosure (9) = 0.0011, Block (3) = 0.0000 and Resid. = 0.0078.

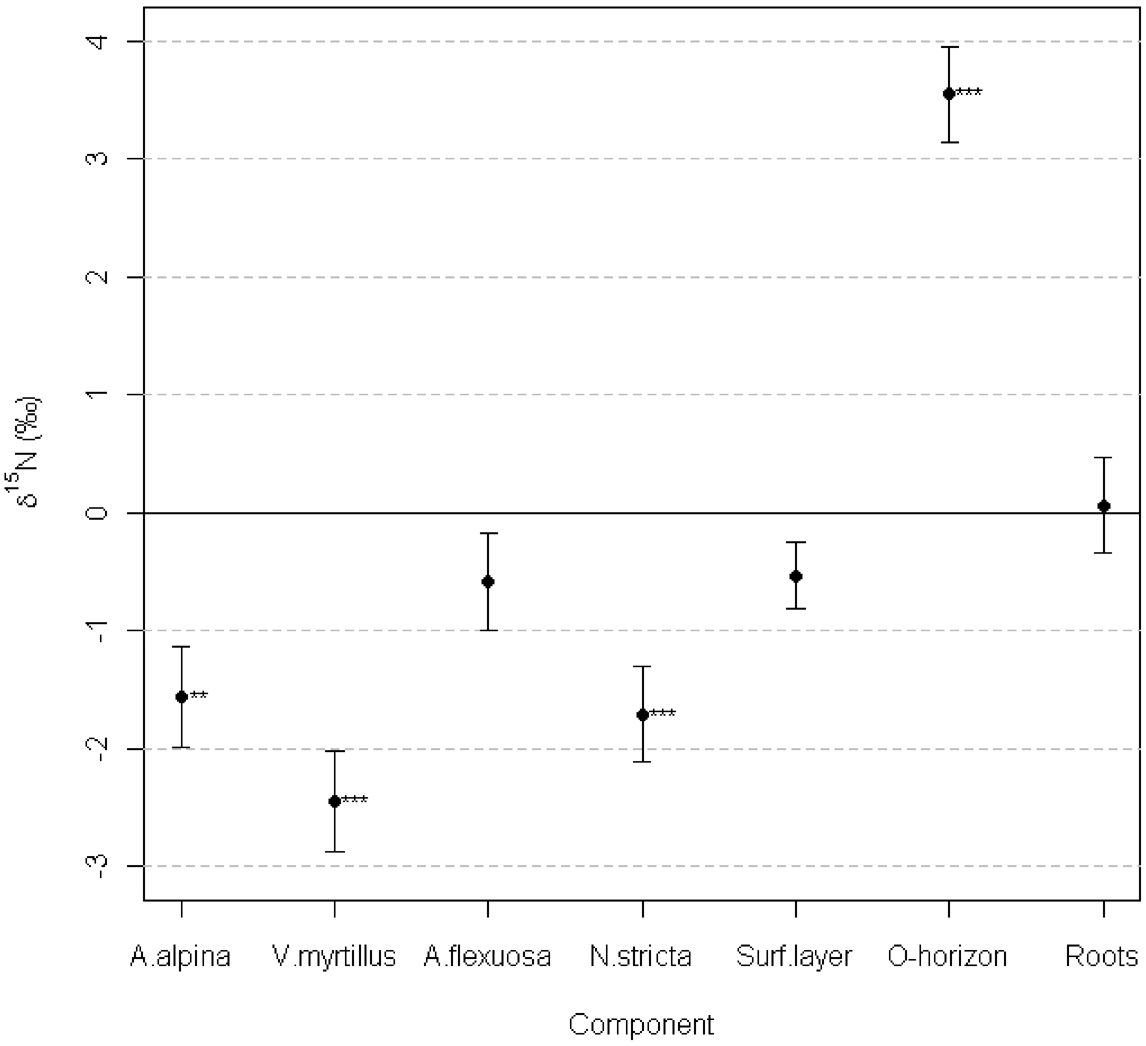

Fig. 2.

[a]Model: $\delta^{15}N$ ~ Component + (1 | Block) + (1 |Enclosure). Variance of the random effects: Enclosure = 0.2006, Block = 0.0423 and Resid. = 1.1559.

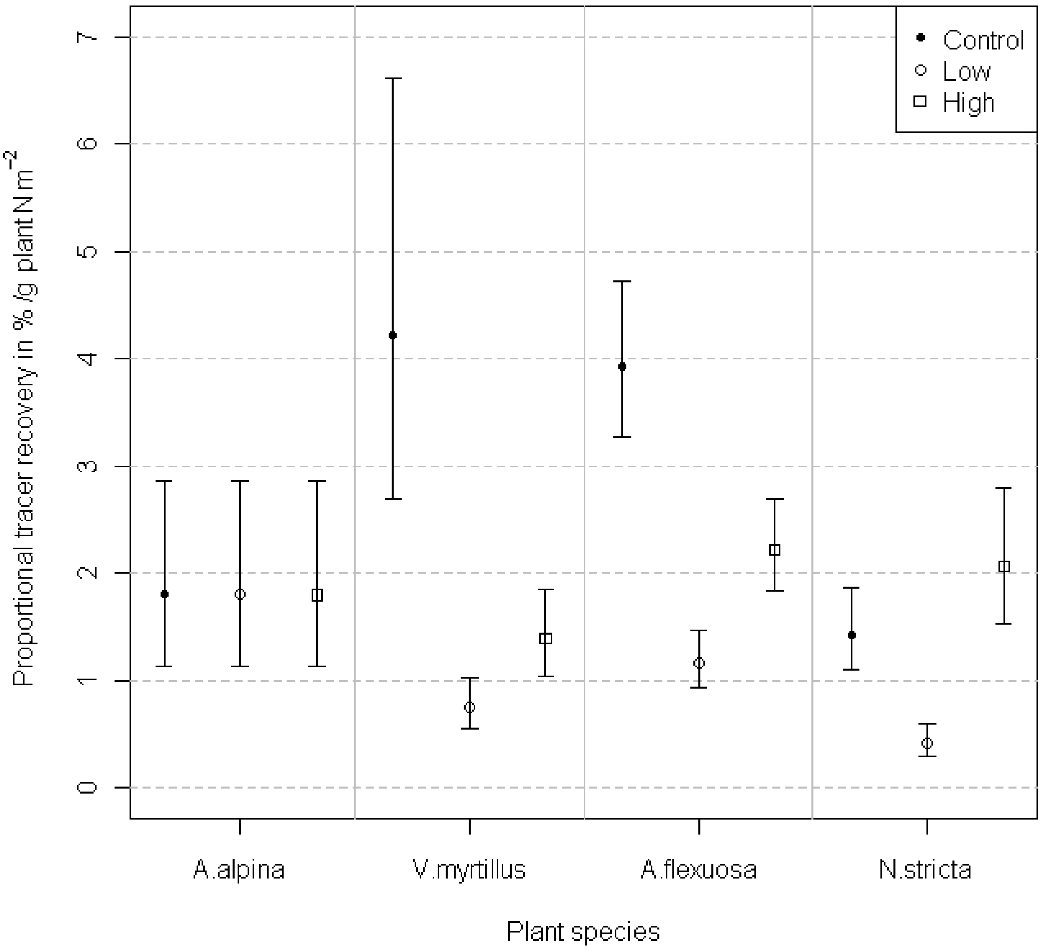

Fig.3.

[a]One plot from enclosure H (high grazing pressure) was omitted from the analysis but did not affect the fixed effect structure or the difference between factor levels in the model. [b]Blocks and enclosures were always included as random factors. Models: $\mathbf{ln(Prop.recov)}_{\mathit{A.alpina}}$ ~ ln(N-pool/g m^{-2}). Var. of rand. effects: Enclosure (6) = 0.704, Block (3) = <0.000 and Residual = 0.227. $\mathbf{ln(Prop.recov)}_{\mathit{V.myrtillus}}$ ~ treatment + ln(N-pool/g m^{-2}). Var. of rand. effects: Enclosure (8) = 0.0725, Block (3) = 0.0542 and Residual = 0.1922. $\mathbf{ln(Prop.recov)}_{\mathit{A.flexuosa}}$ ~ treatment + ln(N-pool/g m^{-2}). Var. of rand. effects: Enclosure (9) = 0.0000, Block (3) = 0.0572 and Residual = 0.1287. $\mathbf{ln(Prop.recov)}_{\mathit{N.stricta}}$ ~ treatment + ln(N-pool/g m^{-2}). Var. of rand. effects: Enclosure (7) = 0.0000, Block (3) = 0.0699 and Residual = 0.2068.

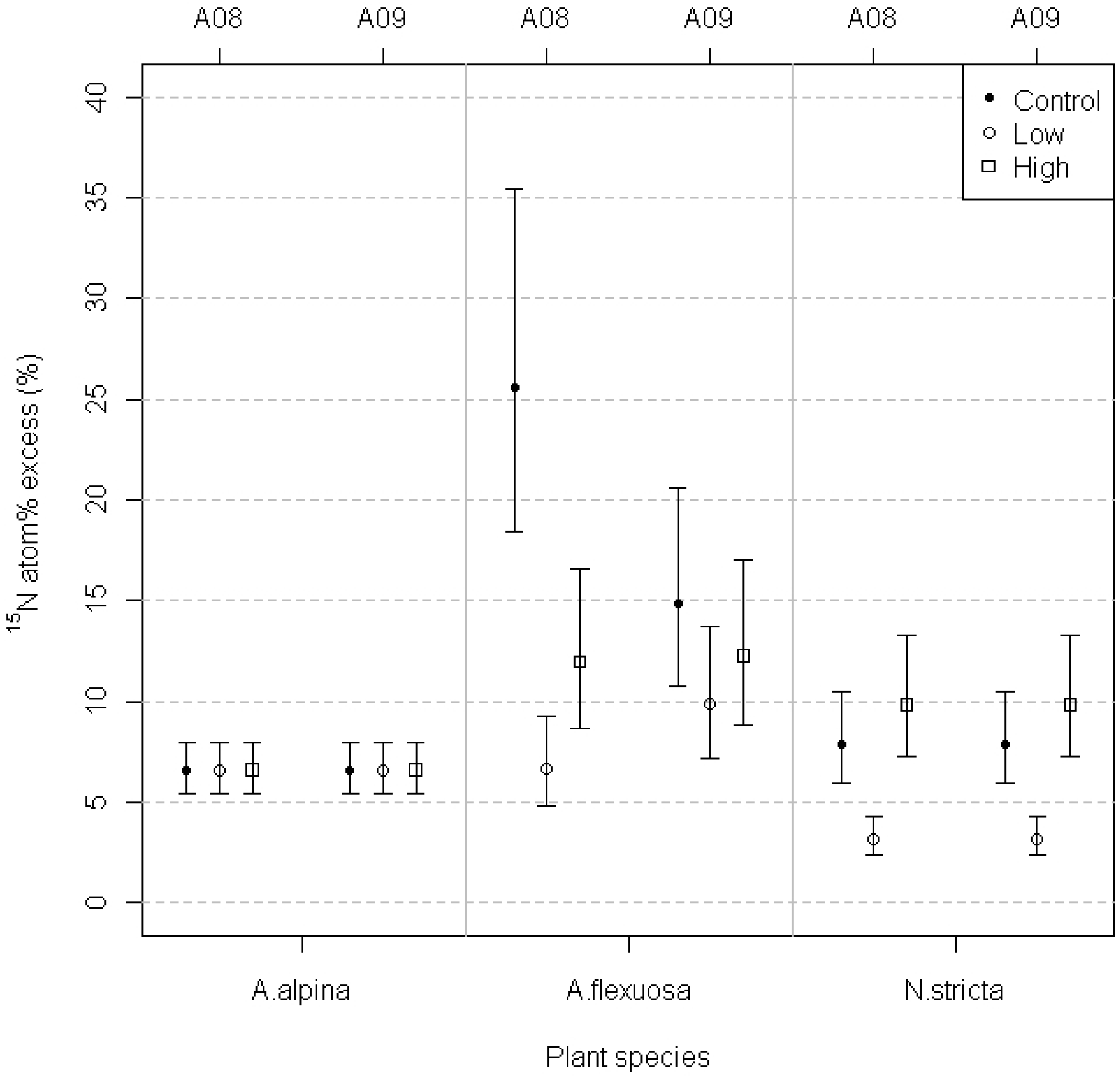

Fig. 4.

[a] ^{15}N atom% excess was used instead of proportional tracer recovery for the comparisons between 2008 and 2009 due to lack of data on plant biomass in 2008.[b] Blocks and enclosures were always included as random factors Models: **ln(^{15}N atom% excess)**$_{A.alpina}$ ~ 1. Var. of rand. effects: Enclosure (8) = 0.116, Block (3) = 0.000 and Residual = 0.696. **ln(^{15}N atom% excess)**$_{A.flexuosa}$ ~ treatment * month. Var. of rand. effects: Enclosure (9) = 0.046, Block (3) = 0.168 and Residual = 0.318. **ln(^{15}N atom% excess)**$_{N.stricta}$ ~ treatment. Var. of rand. effects: Enclosure (9) = 0.109, Block (3) = 0.037 and Residual = 0.403.

Tables

Table 1 Parameter estimates of N-content (% total-N, ln-transformed), proportional tracer recovery (ln-transformed) and ^{15}N atom% excess (percentage increase in atom% ^{15}N of enriched samples relative to the background atom%, ln-transformed) of different plant and soil components (*Alchemilla alpina, Vaccinium myrtillus, Avenella flexuosa*, *Nardus stricta,* surface layer, soil from the O-horizon and bulked plant roots >2 mm) within grassland habitats, Hol, Norway. The estimates derive from linear mixed effect models (REML-estimation; minimum adequate models) with block and enclosure (see material and methods and Fig. 1, 3 and 4) as random factors. The fixed effects of the **N-content models** include month [three levels for *A.alpina, A.flexuosa*, *N.stricta,* soil and roots: June 2008 (J08), August 2008 (A08) and August 2009 (A09); and two levels for *V.myrtillus* and surface layer: September 2009 (S09) and August 2009 (A09)] and treatment (3 levels of grazing; control, low and high). The **proportional tracer recovery models** include treatment and a continuous variable; N-pool of the individual plant species and of the roots [ln(N-pool/g m^{-2})]. The fixed effects for the **^{15}N atom% excess models** are the same as those for the N-content models (*Vaccinium myrtillus* and surface layer are not included because not sampled A08). N-pools were not included due to lack of data in 2008.The table shows estimated differences between factor levels (i.e. "treatment contrasts"; see material and method), se and adjusted p-values (difference from 0). Bolded values indicate significant differences (significantly different from 0). Number of observations (n) is shown.

Dependent variable	Component	Model	n	Parameter (fixed effects)	Estimate	se	z	P
ln(N-content (% total-N))	*Alchemilla alpina*	~ month	41	**Intercept (J08)**	**0.741**	**0.045**	**16.464**	**<0.001**
				Month (A08 vs. J08)	**-0.231**	**0.045**	**-5.101**	**<0.001**
				Month (A09 vs. J08)	**-0.365**	**0.048**	**-7.596**	**<0.001**
	Vaccinium myrtillus	~ month	29	**Intercept (S09)**	**0.327**	**0.047**	**6.980**	**<0.001**
				Month (A09 vs. S09)	**-0.096**	**0.040**	**-2.376**	**0.032**
	Avenella flexuosa	~ month	63	**Intercept (J08)**	**0.851**	**0.055**	**15.351**	**<0.001**
				Month (A08 vs. J08)	**-0.454**	**0.063**	**-7.259**	**<0.001**
				Month (A09 vs. J08)	**-0.624**	**0.063**	**-9.972**	**<0.001**
	Nardus stricta	~treatment + month	44[a]	**Intercept (control J08)**	**0.586**	**0.040**	**14.651**	**<0.001**
				Treatment (high vs. control)	0.099	0.044	2.276	0.092
				Treatment (low vs. control)	0.007	0.043	0.169	1.000
				Month (A08 vs. J08)	**-0.186**	**0.036**	**-5.097**	**<0.001**
				Month (A09 vs. J08)	**-0.379**	**0.037**	**-10.264**	**<0.001**
	Surface layer	~treatment * month	54	**Intercept (control S09)**	**0.399**	**0.040**	**9.921**	**<0.001**
				Treatment (high vs. control)	**0.152**	**0.057**	**2.677**	**0.033**
				Treatment (low vs. control)	0.038	0.057	0.666	0.939
				Month (A09 vs. S09)	-0.006	0.057	-0.098	1.000
				Treatment (high vs. control): Month (A09 vs. S09)	-0.131	0.080	-1.636	0.333
				Treatment (low vs. control): Month (A09 vs. S09)	0.199	0.080	2.473	0.058
	O-horizon	~ month	63	Intercept (J08)	0.149	0.111	1.340	0.343
				Month (A08 vs. J08)	0.142	0.103	1.379	0.322
				Month (A09 vs. J08)	**-0.293**	**0.103**	**-2.850**	**0.011**
	Roots	~ month	63	Intercept (J08)	-0.061	0.077	-0.788	0.675
				Month (A08 vs. J08)	0.002	0.081	0.025	1.000
				Month (A09 vs. J08)	**-0.264**	**0.081**	**-3.247**	**0.003**
ln(Proportional tracer recovery (%))	*Alchemilla alpina*	~ ln(N-pool/g m^{-2})	14	Intercept	0.587	0.465	1.263	0.330
				ln(N-pool (g m^{-2}))	**1.122**	**0.112**	**10.061**	**<0.001**
	Vaccinium myrtillus	~ treatment + ln(N-pool/g m^{-2})	20[b]	**Intercept**	**1.440**	**0.449**	**3.205**	**0.004**
				Treatment (high vs. control)	**-1.108**	**0.403**	**-2.750**	**0.018**
				Treatment (low vs. control)	**-1.731**	**0.457**	**-3.790**	**<0.001**
				ln(N-pool (g m^{-2}))	**0.993**	**0.182**	**5.460**	**<0.001**
	Avenella flexuosa	~ treatment + ln(N-pool/g m^{-2})	26[b]	**Intercept**	**1.369**	**0.183**	**7.480**	**<0.001**
				Treatment (high vs. control)	**-0.572**	**0.178**	**-3.212**	**0.005**
				Treatment (low vs. control)	**-1.215**	**0.211**	**-5.757**	**<0.001**
				ln(N-pool (g m^{-2}))	**0.903**	**0.067**	**13.381**	**<0.001**
	Nardus stricta	~ treatment + ln(N-pool/g m^{-2})	17	Intercept	0.356	0.265	1.342	0.492
				Treatment (high vs. control)	0.368	0.286	1.286	0.530
				Treatment (low vs. control)	**-1.234**	**0.312**	**-3.957**	**<0.001**
				ln(N-pool (g m^{-2}))	**0.805**	**0.076**	**10.543**	**<0.001**
	Surface layer	~1	27	**Intercept**	**4.153**	**0.096**	**43.370**	**<0.001**
	O-horizon	~1	26[c]	**Intercept**	**3.097**	**0.241**	**12.830**	**<0.001**
	Roots	~ ln(N-pool/g m^{-2})	27	Intercept	-0.144	0.258	-0.556	0.812
				ln(N-pool (g m^{-2}))	**0.830**	**0.141**	**5.902**	**<0.001**
ln(^{15}N atom% excess)	*Alchemilla alpina*	~ 1	33	**Intercept**	**1.886**	**0.193**	**9.758**	**<0.001**
	Avenella flexuosa	~ treatment * month	*54*	**Intercept (control A08)**	**3.241**	**0.327**	**9.926**	**<0.001**
				Treatment (high vs. control)	-0.760	0.319	-2.387	0.079
				Treatment (low vs. control)	**-1.344**	**0.319**	**-4.221**	**<0.001**
				Month (A09 vs. A08)	-0.543	0.266	-2.041	0.174
				Treatment (high vs. control): Month (A09 vs. A08)	0.569	0.376	1.513	0.452
				Treatment (low vs. control): Month (A09 vs. A08)	0.940	0.376	2.499	0.059
	Nardus stricta	~ treatment	38	**Intercept (control)**	**2.064**	**0.285**	**7.232**	**<0.001**
				Treatment (high vs. control)	0.220	0.385	0.570	0.881
				Treatment (low vs. control)	**-0.903**	**0.386**	**-2.341**	**0.049**
	O-horizon	~ treatment * month	*52*[d]	**Intercept (control A08)**	**1.163**	**0.342**	**3.403**	**0.004**
				Treatment (high vs. control)	0.062	0.502	0.124	1.000
				Treatment (low vs. control)	**-1.945**	**0.483**	**-4.024**	**< 0.001**
				Month (A09 vs. A08)	-0.566	0.319	-1.776	0.288
				Treatment (high vs. control): Month (A09 vs. A08)	-0.330	0.470	-0.702	0.945
				Treatment (low vs. control): Month (A09 vs. A08)	**1.355**	**0.451**	**3.008**	**0.013**
	Roots	~1	54	**Intercept**	**1.423**	**0.253**	**5.626**	**<0.001**

[a]Three plots (2 from enclosure D; low and 1 from enclosure H; high) with N-contents of 3.36, 2.45 and 2.53 % were omitted from the analysis in order to fulfil the model criteria. [b]One plot from enclosure H (high grazing pressure) was omitted from the analysis but did not affect the fixed effect structure or the difference between factor levels in the model. [c]BD missing. [d]Two outliers were omitted to fulfil the model criteria. This did not affect the model structure.

Table 2 Mean total carbon and nitrogen concentration (%), $\delta^{15}N$ natural abundance (‰) and sampling depth (cm) for different plant and soil components (sampled prior to tracer addition, i.e. May-June 2008) from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Standard error (se) and number of replicates (n) is shown. "-" indicates no value.

		C conc.			N conc.			$\delta^{15}N^{b}$			Depth		
Treatment	**Component**	**(%)**	**se**	**n**	**(%)**	**se**	**n**	**(‰)**	**se**	**n**	**(cm)**	**se**	**n**
Control	*Alchemilla alpina*	43.0	0.2	2	2.2	0.0	2	-1.72	0.36	2	-	-	-
	Vaccinium myrtillus[a]	47.4	0.2	3	1.4	0.0	3	-3.02	1.17	3	-	-	-
	Avenella flexuosa	42.4	0.2	3	2.5	0.1	3	-1.97	0.39	3	-	-	-
	Nardus stricta	42.0	0.5	3	1.9	0.1	3	-1.50	0.60	3	-	-	-
	Surface layer[a]	36.6	2.0	9	1.5	0.1	9	-0.77	0.30	9	1.8	0.2	9
	O-horizon	23.3	6.7	3	1.1	0.2	3	2.91	1.73	3	2.5	0.2	3
	Roots	42.7	2.4	3	1.0	0.2	3	-0.48	0.33	3	2.5	0.2	3
Low	*Alchemilla alpina*	42.8	0.2	3	2.1	0.2	3	-2.20	0.65	3	-	-	-
	Vaccinium myrtillus[a]	47.7	0.6	2	1.3	0.0	2	-2.82	0.53	2	-	-	-
	Avenella flexuosa	42.1	0.1	3	2.4	0.3	3	0.23	0.71	3	-	-	-
	Nardus stricta	41.7	0.2	3	1.7	0.1	3	-2.29	0.15	3	-	-	-
	Surface layer[a]	39.4	1.8	9	1.6	0.1	9	-0.51	0.29	9	2.2	0.6	9
	O-horizon	22.8	2.2	3	1.4	0.0	3	3.80	0.81	3	4.9	2.3	3
	Roots	40.9	1.7	3	0.9	0.1	3	0.49	0.64	3	4.9	2.3	3
High	*Alchemilla alpina*	42.9	0.1	3	2.0	0.0	3	-0.77	0.12	3	-	-	-
	Vaccinium myrtillus[a]	47.4	0.1	3	1.4	0.0	3	-1.69	0.35	3	-	-	-
	Avenella flexuosa	42.4	0.3	3	2.2	0.1	3	-0.01	0.54	3	-	-	-
	Nardus stricta	42.3	0.2	3	2.0	0.1	3	-1.34	0.58	3	-	-	-
	Surface layer[a]	38.0	2.2	9	1.7	0.0	9	-0.33	0.41	9	1.5	0.1	9
	O-horizon	19.9	8.2	3	1.1	0.3	3	3.93	1.07	3	3.2	0.8	3
	Roots	42.7	1.0	3	1.0	0.1	3	0.17	0.22	3	3.2	0.8	3

[a] Sampled 2009 (see material and methods).

[b] The isotope ratio (R_{sample}) may be calculated according to equation (1) prior to determination of

$$Atom\%^{15}N = 100*\left(\frac{R_{sample}}{R_{sample}+1}\right).$$

Table 3 Mean pool of total nitrogen (N pool), percentage fractional abundance of added ^{15}N tracer (X sample), recovered mass of added ^{15}N tracer (mass ^{15}N), mean percentage recovery of added ^{15}N tracer within each treatment and component combination (recovery), mean percentage of the total percentage recovery of added ^{15}N tracer within each treatment (total recovery) and proportional recovery (i.e. percent recovery of each component relative to the total recovery at each plot) of 7 plant and soil components from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Samples are collected 13 months after ^{15}N tracer addition (August 2009). Standard error (se) and number of replicates (n) is shown. "-"indicates no value.

Treatment	Component	N pool (g m^{-2})	se	n	X sample (%)	se	n	Mass ^{15}N (mg m^{-2})	se	n	Recovery (%)	se	n	Total recovery (%)	se	n	Proportional recovery (%)	se	n
Control	*Alchemilla alpina*	0.16	-	1	0.068	-	1	0.111	-	1	0.36	-	1	48.33	6.19	9	0.61	-	1
	Vaccinium myrtillus	0.20	0.05	6	0.063	0.201	6	0.090	0.015	6	0.30	0.05	6				0.74	0.19	6
	Avenella flexuosa	1.23	0.29	9	0.061	0.096	9	0.619	0.156	9	2.03	0.51	9				4.57	0.97	9
	Nardus stricta	0.31	0.12	7	0.033	0.063	7	0.091	0.042	7	0.30	0.14	7				0.55	0.19	7
	Surface layer	57.73	8.08	9	0.019	0.028	9	10.393	1.972	9	34.00	6.43	9				68.49	6.38	9
	O-horizon	50.91	7.81	8	0.009	0.025	9	3.659	0.843	8	11.98	2.75	8				26.17	4.59	8
	Soil roots	2.43	0.87	9	0.018	0.036	9	0.361	0.112	9	1.18	0.37	9				2.69	0.94	9
Low	*Alchemilla alpina*	0.09	0.08	5	0.025	0.071	5	0.035	0.033	5	0.12	0.11	5	89.91	10.10	9	0.13	0.12	5
	Vaccinium myrtillus	0.77	0.20	6	0.023	0.040	6	0.191	0.074	6	0.62	0.24	6				0.67	0.26	6
	Avenella flexuosa	0.24	0.08	9	0.039	0.046	9	0.110	0.045	9	0.36	0.15	9				0.39	0.14	9
	Nardus stricta	0.11	0.05	5	0.019	0.041	5	0.017	0.007	5	0.06	0.02	5				0.07	0.03	5
	Surface layer	66.44	3.79	9	0.031	0.054	9	19.835	3.205	9	64.92	10.49	9				67.65	7.40	9
	O-horizon	195.02	47.15	9	0.004	0.010	9	7.007	1.453	9	22.96	4.73	9				30.04	7.59	9
	Soil roots	2.02	0.31	9	0.017	0.026	9	0.353	0.077	9	1.15	0.25	9				1.37	0.35	9
High	*Alchemilla alpina*	0.62	0.18	8	0.034	0.091	8	0.148	0.053	8	0.48	0.17	8	72.91	11.49	9	0.85	0.36	8
	Vaccinium myrtillus	0.61	0.13	9	0.027	0.053	9	0.216	0.074	9	0.71	0.24	9				1.16	0.42	9
	Avenella flexuosa	0.57	0.10	9	0.048	0.065	9	0.265	0.062	9	0.87	0.20	9				1.33	0.32	9
	Nardus stricta	0.58	0.24	5	0.061	0.174	5	0.285	0.191	5	0.93	0.62	5				1.30	0.71	5
	Surface layer	65.77	5.51	9	0.024	0.046	9	15.173	3.306	9	49.66	10.81	9				65.19	6.78	9
	O-horizon	115.97	35.43	9	0.006	0.011	9	5.871	1.598	9	19.18	5.19	9				28.17	6.37	9
	Soil roots	3.18	0.67	9	0.015	0.022	9	0.472	0.126	9	1.54	0.41	9				2.67	0.88	9

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Online resource 1 Mean above-ground biomass (± se) of vegetation components (see material and methods) from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Total biomass (g m^{-2}) and proportion biomass (%) of each vegetation component for each of the different grazing treatments are shown. n = number replicates. "-" indicates no value. The sampling was conducted 13 months after ^{15}N addition (August 2009).

Treatment	Component	Biomass (g m^{-2})	se	n	Total biomass[a] (g m^{-2})	se	n	Proportion of total biomass[a] (%)	se
Control	*Alchemilla alpina*	11.6	-	1	224.0	22.3	9	6.1	-
	Vaccinium myrtillus	16.5	4.1	6				6.9	2.2
	Avenella flexuosa	104.8	24.7	9				43.5	8.4
	Pteridophyta spp.	3.6	1.2	3				1.5	0.6
	Nardus stricta	24.5	9.3	7				11.3	3.6
	Rest herbs	6.9	4.3	7				4.9	3.5
	Rest woody species	34.2	10.0	9				13.9	4.0
	Rest cryptogames	25.6	16.2	5				13.2	7.1
	Rest graminoids	32.9	9.2	9				17.0	5.6
	Litter mix	66.0	18.4	9					
Low	*Alchemilla alpina*	6.3	5.3	5	188.3	29.4	9	2.8	2.0
	Vaccinium myrtillus	60.7	15.2	6				27.1	5.6
	Avenella flexuosa	18.7	6.0	9				9.7	2.3
	Nardus stricta	9.3	4.2	5				6.9	3.5
	Rest herbs	17.6	4.6	9				11.1	2.5
	Rest woody species	71.2	27.9	8				35.4	8.9
	Rest cryptogames	25.4	4.7	9				15.6	3.8
	Rest graminoids	14.3	2.6	9				8.7	1.6
	Litter mix	90.3	16.0	9					
High	*Alchemilla alpina*	41.3	11.9	8	271.6	36.8	9	20.3	6.7
	Vaccinium myrtillus	49.9	12.9	9				17.3	3.2
	Avenella flexuosa	46.4	10.2	9				17.3	3.1
	Nardus stricta	34.9	15.6	6				9.8	4.3
	Rest herbs	9.2	2.1	9				3.9	1.1
	Rest woody species	71.7	27.8	9				21.9	6.2
	Rest cryptogames	8.1	3.4	9				3.8	1.6
	Rest graminoids	26.5	5.2	9				11.3	2.7
	Litter mix	86.5	17.9	9					

[a] Litter mix (see material and methods) is not included.

Online resource 2 Mean C and N (total carbon and nitrogen concentration), CN (total carbon to total nitrogen ratio), atom% ^{15}N (percentage of the ^{15}N isotope to the total number of ^{14}N + ^{15}N atoms of the enriched samples) and ^{15}N atom% excess (percentage increase in atom% ^{15}N of the enriched relative to the background atom% ^{15}N) of *Alchemilla alpina*, *Vaccinium myrtillus*, *Avenella flexuosa* and *Nardus stricta* from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Samples are collected 1 month (August 2008) and 13 months (August 2009) after ^{15}N tracer addition. Standard error (se) and number of replicates (n) is shown. "-"indicates no value.

Treatment	Year	Component	C (%)	se	n	N (%)	se	n	CN	se	n	Atom% ^{15}N	se	n	^{15}N atom% excess (%)	se
Control	2008	*Alchemilla alpina*	42.1	0.6	3	1.7	0.2	3	26.19	3.81	3	0.419	0.030	3	14.53	8.16
		Avenella flexuosa	42.5	0.1	9	1.5	0.1	9	29.60	1.95	9	0.479	0.025	9	30.89	6.88
		Nardus stricta	42.3	0.2	7	1.5	0.1	7	28.65	0.99	7	0.400	0.007	7	9.29	1.82
Low	2008	*Alchemilla alpina*	43.2	0.2	7	1.6	0.1	7	27.43	0.96	7	0.394	0.009	7	7.62	2.56
		Avenella flexuosa	42.3	0.2	9	1.5	0.1	9	30.15	2.39	9	0.403	0.010	9	9.86	2.73
		Nardus stricta	42.1	0.1	7	2.0	0.3	7	23.50	2.37	7	0.380	0.005	7	4.04	1.26
High	2008	*Alchemilla alpina*	43.3	0.1	9	1.8	0.1	9	24.88	0.87	9	0.402	0.012	9	9.76	3.16
		Avenella flexuosa	42.8	0.1	9	1.6	0.1	9	27.75	1.21	9	0.425	0.013	9	15.99	3.54
		Nardus stricta	42.7	0.3	7	1.7	0.1	7	25.14	1.39	7	0.411	0.013	7	12.26	3.64
Control	2009	*Alchemilla alpina*	43.2	-	1	1.4	-	1	30.89	-	1	0.434	-	1	18.54	-
		Vaccinium myrtillus	47.5	0.2	6	1.2	0.0	6	40.19	1.69	6	0.428	0.020	6	17.27	5.46
		Avenella flexuosa	42.5	0.1	9	1.2	0.0	9	35.95	1.42	9	0.426	0.009	9	16.59	2.61
		Nardus stricta	42.2	0.2	7	1.2	0.1	7	35.23	1.94	7	0.399	0.006	7	9.08	1.72
Low	2009	*Alchemilla alpina*	43.5	0.1	5	1.4	0.1	5	31.99	1.63	5	0.391	0.007	5	6.84	1.93
		Vaccinium myrtillus	48.5	0.2	6	1.2	0.0	6	39.01	1.15	6	0.388	0.004	6	6.17	1.10
		Avenella flexuosa	42.4	0.3	9	1.3	0.1	9	32.92	1.41	9	0.405	0.005	9	10.52	1.24
		Nardus stricta	41.6	0.1	5	1.3	0.1	5	33.59	1.57	5	0.385	0.004	5	5.25	1.13
High	2009	*Alchemilla alpina*	43.1	0.4	8	1.5	0.1	8	28.59	1.25	8	0.400	0.009	8	9.15	2.48
		Vaccinium myrtillus	47.8	0.2	9	1.3	0.1	9	36.25	2.00	9	0.393	0.005	9	7.48	1.44
		Avenella flexuosa	42.9	0.1	9	1.3	0.1	9	33.80	1.64	9	0.415	0.006	9	13.18	1.77
		Nardus stricta	42.5	0.2	5	1.4	0.1	5	30.96	1.27	5	0.427	0.017	5	16.48	4.73

Online resource 3 Mean physical and chemical attributes of the surface layers, O-horizon soils and bulked roots from 27 grassland habitats at different grazing densities of sheep (control = no sheep, low = 25 sheep km^{-2} and high = 80 sheep km^{-2}), Hol, Norway. Samples are collected 1 month (August 2008) and 13 months (August 2009) after ^{15}N tracer addition. Standard error (se) and number of replicates (n) is shown. "-" indicates no value. Depth is depth of the O-horizon soil or surface layer, BD is bulk density of the fine earth fraction (O-horizon soil) and density of bulked roots and surface layer, water cont. is the gravimetric water content (soil and surface layer only), C and N are total carbon and nitrogen concentrations, CN is the total carbon to total nitrogen ratio, atom% ^{15}N is the percentage of the ^{15}N isotope to the total number of ^{14}N + ^{15}N atoms of the enriched samples and ^{15}N atom% excess is the percentage increase in atom% ^{15}N of the enriched relative to the background atom% ^{15}N.

Treatment	Year	Component	Depth (cm)	se	n	BD (g cm^{-3})	se	n	Water cont. (%)	se	n	pH	se	n	C (%)	se	n	N (%)	se	n	CN	se	n	Atom% ^{15}N	se	n	^{15}N atom% excess (%)	se
Control	2008	O-horizon	2.2	0.3	9	-	-	-	58.2	2.5	9	4.2	0.1	9	20.8	2.3	9	1.2	0.2	9	17.6	0.9	9	0.381	0.003	9	3.8	0.7
		Roots	2.2	0.3	9	-	-	-	-	-	-	-	-	-	40.7	1.9	9	1.0	0.1	9	42.8	3.2	9	0.387	0.003	9	5.5	0.8
Low	2008	O-horizon	4.7	1.1	9	-	-	-	57.5	1.4	9	4.8	0.1	9	20.7	1.0	9	1.4	0.1	9	14.3	0.4	9	0.371	0.002	9	0.9	0.4
		Roots	4.7	1.1	9	-	-	-	-	-	-	-	-	-	41.5	1.6	9	1.0	0.1	9	46.8	5.5	9	0.384	0.005	9	4.7	1.3
High	2008	O-horizon	2.2	0.3	9	-	-	-	59.3	2.2	9	4.3	0.1	9	22.7	2.0	9	1.5	0.2	9	15.3	1.0	9	0.381	0.004	9	3.6	1.2
		Roots	2.2	0.3	9	-	-	-	-	-	-	-	-	-	41.5	1.3	9	1.0	0.1	9	46.7	5.7	9	0.393	0.007	9	7.2	1.8
Control	2009	Surface layer	1.6	0.2	9	0.27	0.04	9	66.3	0.9	9	-	-	-	39.5	1.9	9	1.5	0.1	9	26.6	1.2	9	0.385	0.003	9	5.0	0.7
		O-horizon	1.5	0.1	9	0.41	0.05	8	51.5	4.0	9	-	-	-	14.1	1.7	9	0.8	0.1	9	17.4	1.0	9	0.376	0.002	9	2.4	0.7
		Roots	1.5	0.1	9	0.02	0.01	9	-	-	-	-	-	-	42.0	1.5	9	0.8	0.1	9	57.5	4.5	9	0.384	0.004	9	4.8	1.0
Low	2009	Surface layer	1.2	0.1	9	0.29	0.02	9	61.5	1.1	9	-	-	-	38.1	1.4	9	1.9	0.1	9	20.3	0.7	9	0.397	0.005	9	8.5	1.5
		O-horizon	4.6	1.1	9	0.42	0.04	9	52.3	1.2	9	-	-	-	16.1	1.8	9	1.1	0.1	9	14.3	0.3	9	0.372	0.001	9	1.2	0.3
		Roots	4.6	1.1	9	0.01	0.00	9	-	-	-	-	-	-	41.4	1.3	9	0.7	0.0	9	58.5	4.4	9	0.384	0.002	9	4.7	0.7
High	2009	Surface layer	1.6	0.2	9	0.28	0.03	9	64.3	1.5	9	-	-	-	35.3	1.8	9	1.5	0.1	9	23.3	1.0	9	0.390	0.005	9	6.4	1.3
		O-horizon	2.9	0.8	9	0.51	0.06	9	48.7	3.0	9	-	-	-	13.6	2.8	9	0.8	0.1	9	15.3	1.0	9	0.374	0.001	9	1.6	0.3
		Roots	2.9	0.8	9	0.02	0.00	9	-	-	-	-	-	-	42.3	0.6	9	0.7	0.0	9	60.7	3.0	9	0.382	0.002	9	4.2	0.6

PAPER IV

Martinsen, V., Grund, F., Ness, M., de Wit, H.A., Austrheim, G., Mysterud, A. and Mulder, J., 2010. **Variations in surface water quality caused by plant community and grazing at an alpine site in Hol, Southern Norway**.

Differences in surface water quality caused by plant community and grazing at an alpine site in Hol, Southern Norway

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Abstract

Alpine ecosystems, representing a large proportion of the land area in Europe, are under pressure from changes in climate and land-use. This may impact water quality in downstream surface waters. We assess effects of plant communities of increasing productivity (snowbed, dwarf shrub heath and tall herb meadow) on concentrations of dissolved organic carbon and nitrogen (DOC and DON), ammonium (NH_4-N), nitrate (NO_3-N) and phosphorus (tot-P and PO_4-P) in surface runoff. In addition, we investigated effects of two density levels of sheep (no sheep; 80 sheep km^{-2}). The system is characterized by low concentrations of inorganic N in runoff, and there are no differences associated with plant community or grazing. Concentrations of DOC and DON are significantly smaller in snowbeds as compared to tall herb meadow and dwarf shrub heath. Infiltration rates and the fraction of pores > 200 µm are significantly smaller in snowbeds than in tall herb meadow and they are further reduced by grazing in both vegetation types. Despite soil compaction, due to sheep trampling our results indicate no risk of nutrient leaching from areas being grazed. By contrast, the concentration of coliform bacteria in runoff is significantly enhanced at the grazed site indicating risks for drinking water quality in areas affected by grazing.

1. **Introduction**

High elevation upland ecosystems are important mediators of nutrients and pollutants to downstream freshwater and marine ecosystems. They partially retain atmospheric pollutants (Fenn *et al.* 1998) and are a source of dissolved organic matter (DOM) and associated nutrients and pollutants (Battarbee *et al.* 2009; Hessen *et al.* 2009; Hood *et al.* 2003). Mountainous regions are subject to a number of drivers of change, such as climate (Beniston 2009), land use (Austrheim *et al.* 2008) and N deposition (Hole and Engardt 2008) that may affect their nutrient retention capacity. Despite their considerable extension, little is known about the role of alpine ecosystems on surface water quality where land use such as livestock grazing could be a strong environmental factor.

The vegetation cover in alpine areas consists of a mosaic of different plant communities determined by climatic, topographical, lithological and edaphic factors as well as land use (Körner 2003; Nagy and Grabherr 2009). A strong control of edaphic factors on vegetation patch distribution, plant species composition (quality of litter input) and plant cover (biomass) may per se affect chemical properties and processes in soils (Nielsen *et al.* 2009; Strand *et al.* 2008; Vinton and Burke 1997) important for surface water quality (Palmer *et al.* 2001; Sjøeng *et al.* 2007; Strand *et al.* 2008).

Dissolved organic matter (DOM) was reported to be smaller in surface water from non-forested than from forested catchments (Hood *et al.* 2005; Hood *et al.* 2003; Skjelkvåle and Wright 1998) and was positively related to carbon (C) and N contents, and CN ratios of the soil (Hood *et al.* 2003; Strand *et al.* 2008). By contrast, concentrations of NO_3-N and NH_4-N were negatively related to organic matter content and CN ratio of the soil (Hood *et al.* 2003; Strand *et al.* 2008). Lake NO_3-N in Norwegian lakes was positively related to N deposition and negatively to vegetation density (Hessen *et al.* 2009). Furthermore, Sjøeng *et al.* (2007) found a significantly positive correlation between percentage bare rock and amount of NO_3-N leached in 12 headwater catchments with a high N-deposition (1.63 – 2.75 g m^{-2} yr^{-1}) of southwest Norway. These studies clearly highlight the important linkage between vegetation and soil in controlling leaching of DOM and nutrients to surface waters. Thus, any activity influencing vegetation and soil dynamics may be an important driver for a change in surface water quality.

Grazing by large herbivores may alter vegetation structure (Austrheim and Eriksson 2001), which directly and indirectly affects physical and chemical soil properties and processes (Frank and Groffman 1998; van der Wal *et al.* 2001). In addition to changed nutrient dynamics, grazing also may increase loads of sediments, nutrients and fecal bacteria in surface runoff due to removal of vegetation and reduced soil infiltration (Derlet *et al.* 2008; Elliott and Carlson 2004; Meyles *et al.* 2006; Muirhead *et al.* 2006). In a small catchment of Dartmoor, UK, Meyles *et al.* (2006) found clear effects of sheep grazing on bulk density (increased), porosity (reduced) and soil water content at standard matric pressures (reduced). They argue that grazing, even without removing the vegetation completely, may enhance soil water movement as observed by a reduced wetness threshold (i.e. field capacity is reached more rapidly) at intensively grazed sites. A more rapid soil water movement increases the flow of water to streams (Meyles *et al.* 2006).

Although sheep grazing pressure has remained relatively stable in Norway since 1950, the proportion of sheep grazing in mountain areas has increased. In 1999, 75 % of all sheep in Norway grazed in the northern boreal and alpine region (Austrheim *et al.* 2008). Sheep grazing is also an important land use in the north-Atlantic region (UK, Iceland, and Faroe Islands) as well in several Mediterranean countries (Dýrmundsson 2006; Hadjigeorgiou *et al.* 2005). So far most studies of effects of different herbivore pressures on water quality in mountain areas have been done in intensively used, fertilized systems, whereas detailed investigations in un-fertilized semi-natural systems are rare.

Previously, we reported findings from grazing experiments in an unfertilized low alpine system at Hol, which indicate greater bulk density (BD), lower pH and more shallow depths of O-horizons in snowbeds as compared to willow-shrubs (comparable to tall herb meadow) with a significant increase in BD with increased sheep density in both plant communities (Martinsen *et al.* 2010b). Furthermore, we found greater rates of potential N-mineralization in areas being heavily grazed as compared to non-grazed sites. Yet, in general concentration of inorganic N in soil water was small (Martinsen *et al.* 2010a).

The objectives of this study were to assess (1) differences in surface runoff water quality (DOC, DON, NH_4-N, NO_3-N and PO_4-P) between three alpine plant communities of increasing productivity (snowbed, dwarf shrub heath and tall herb meadow) at Hol and (2) effects of two density levels of sheep (no sheep and high sheep density; 80 sheep km^2) on infiltration capacity and pore size distribution within snowbeds and tall herb meadows. In addition, we assessed effects of the two density levels of sheep on concentrations of nutrients and potential of grazing induced fecal contamination of stream water.

We predict concentrations of DOC and DON to increase and inorganic N concentrations to decrease with increased vegetation productivity, due to larger SOM-pools and greater N demands in higher productivity vegetation types (**H1**). We also expect infiltration rates and fraction of macropores to be smaller in snowbeds vs. tall herb meadow and smaller in the grazed compared to not grazed area (**H2**). As a direct measure of sheep impact on surface water quality, we predict concentrations of inorganic N and bacteria [i.e. total coliforms and *Escherichia coli (E.Coli*)] to be greater in the grazed vs. the not grazed catchment (**H3**).

2. Methods

2.1 Site description

The study site is located in the low alpine region (1050 - 1320 m.a.s.l.) in Hol municipality, Buskerud county, southern Norway (7°55′ - 8°00′ E, 60°40′ - 60°45′ N). The site is within a large fenced enclosure (~2.7 km^{-2}, Figure 1), being established in 2001 as part of a controlled grazing experiment (Mysterud and Austrheim 2005). The fenced enclosure is divided into three blocks, each with tree sub-enclosures (approximately 0.3 km^{2} each) with no sheep (control), low density (25 sheep km^{-2}) and high density (80 sheep km^{-2}) of domestic sheep (*Ovis aries*). Sheep grazing occurs from the end of June to the beginning of September each year (Mysterud and Austrheim 2005). The bedrock consists of meta-arkose and quaternary deposits of till and colluvium (Kristiansen and Sollid 1985; Sigmond 1998). Soils are acidic and spatially variable, including peaty deposits in topographical depressions and freely drained soils with shallow organic horizons (Martinsen *et al.* 2010b). Vegetation is dominated by dwarf shrub heaths with smaller patches of lichen heaths, snow beds and alpine meadow communities in lee-sides (Rekdal 2001b). Mean annual temperature (MAT) is -1.5 ^{O}C and mean annual precipitation (MAP) is about 1000 mm (Evju *et al.* 2009). Temperature and precipitation varies considerable during the growing season (from mid June to mid September) and between years (Table S1). The average wet N deposition is estimated at 0.416 g m^{2} year^{-1} (Aas *et al.* 2008).

2.2 Location and sampling procedures

The most frequent form of snowbed at the study site is sedge and grass snowbed (SNOWB), dominated by *Avenella flexuosa* with elements of *Salix herbacea*, *Carex bigelowii*, *Anthoxanthum oduratum* and *Alchemilla alpina* (Rekdal 2001a; Rekdal 2001b). Snowbeds are located in areas with a large and long lasting snow cover, on soils of low to intermediate fertility with great variations in water availability in the course of the growing season (Rekdal 2001a). Dwarf shrub heath (DSH), dominated by low shrubs (*Betula nana, Vaccinium* *myrtillus, Empetrium nigrum* spp.) and *Avenella flexuosa* (Rekdal 2001b), occurs in areas with a stable snow cover on soils of low to intermediate fertility with moderate water availability (Rekdal 2001a). Tall herb meadow (THM) is a species rich vegetation community including *Salix* spp., herbs and ferns typical in topographical depressions along streams and rivers, with a stable, but relatively early melting snow cover. THM is located on sites of high fertility with high water availability either from soil- or stream water (Rekdal 2001a). Biomass production (g dry weight m^{-2} yr^{-1}) increases in order SNOWB (25-75 g m^{-2} yr^{-1}) < DSH (50-100 g m^{-2} yr^{-1}) < THM (100-250 g m^{-2} yr^{-1}) (Rekdal 2001a).

In September 2006, fifty four spots with standing water located in topographic depressions within the plant communities SNOWB (n= 18), DSH (n= 21) and THM (n= 15) were selected for water sampling (Figure 1). Based on topographical features and field observations, surface runoff accumulating in the depressions (i.e. standing water accumulated after rainfall) was assumed generated within the same plant community. Due to difficulties in finding suitable depressions within the different enclosures, the three plant communities were not represented in all enclosures. Thus, grazing effects were not included in the statistical analysis. A total of 107 grab samples of standing water were collected few days after rain events using PVC bottles in August and September 2006 and in the months June to September 2007. Samples were stored cold (4 °C) and dark prior to analysis.

Measurements of infiltration rates and pore size distribution were conducted at 6 different sites in block 3, each consisting of 3 plots (Figure 1). Two sites were located in the non-grazed enclosure (enclosure G) in THM (n= 3) and SNOWB (n=3). Four sites were located in the grazed enclosure (enclosure H) in THM and SNOWB outside sheep tracks ($n_{\text{THM no track}} = 3$, $n_{\text{SNOWB no track}} = 3$) and on well defined sheep tracks ($n_{\text{THM track}} = 3$, $n_{\text{SNOWB track}} = 3$), respectively.

Infiltration rates (cm hour^{-1}) were determined as the amount of water per surface area and time unit penetrating the soils using double (outer ring: Φ 55-58, inner ring: Φ 30-33 cm) and single ring (Φ 30-33 cm) infiltrometers. In most cases, single ring infiltrometers (inserted 7-8 cm into the soil) were used due to presence of stones at the soil surface. The soils were pre-wetted for 1 hour before the rate measurements in order to saturate the soil pores (i.e. obtain steady infiltration rates), thereby reducing the risk of horizontal flow. The measured infiltration rates thus represent approximate differences in saturated hydraulic conductivity between the soils. Readings of the decreasing water level within the inner rings were taken every 10 minutes between 1 and 2 hours after start of the experiment (i.e. 7 records for each plot). The measurements were conducted with a falling water head. In cases with high infiltration rates, water was added (i.e. increased water head) to the inner rings. Difference in the water head between the measurements may have influenced the measured rates as reported by Gregory *et al.* (2005).

At each site, two samples of the OA-horizon were sampled using 100 cm^3 steel rings to a maximum depth of 3.7 cm (n=36). The undisturbed soil samples were used to determine the distribution of the pore size fractions > 200 μm (i.e. macropores), 30-200 μm (i.e. mesopores) and 3-30 μm (i.e. micropores) using ceramic pressure plates (Richards 1948). The different pore size fractions were calculated based on weight of the soil samples at different matric potentials (pF). The macropores were determined as the difference in weight between water saturation and pF 1 (i.e. - 0.02 bar), the mesopores were determined by weight difference between pF 1 and pF 2 (i.e. - 0.1 bar) and the micropores as the difference in weight between pF 2 and pF 3 (i.e. - 1 bar). Bulk density (BD; g cm^{-3}) was determined at pF 3 and not at the wilting point (pF 4.2) due to loss of all data at pF 4.2. Thus the reported values represent a slight over-estimation of the true BD. A further description of the procedure is given by Ness (2008).

Two headwater catchments were defined using GPS (Oregon 300, Garmin) in the enclosures G (~5.3 ha) and H (~8.9 ha) (Figure 1, Table S2). In June 2007, flumes [RBC flume (0.16 l s^{-1} - 9 l s^{-1}), type 13.17.02, (Eijkelkamp 2001)] were installed in two small streams draining the two catchments for water flux measurements (Figure S1). Streamwater samples at each flume were collected from June to September 2007 (n= 46), from June to October 2008 (n=24) and from May to September 2009 (n= 66) using PVC bottles for chemical analysis and sterile Polysterol bottles (IDEXX Laboratories) for fecal bacteria determination. The water samples used for chemical analysis were stored dark and cold (4 °C) prior to analysis. Samples used for bacteria determination were analyzed short time after sampling. Analysis of tot-P, PO_4-P, total coliforms and *E.Coli* were conducted on samples from 2009 only.

2.3 Chemical analysis

Conductivity (712 Conductometer) and pH (Orion, model 720) were determined on unfiltered water samples. All water samples were filtered (0.45 µm) prior to further chemical analysis. Nitrate-N (sum of NO_3^- and NO_2^-) was determined photometrically (flow injection analysis; FIA star 5020 analyzer, Tecator) according to the Norwegian standard NS 4745 (NSF 1975a). Ammonium-N was determined photometrically (Photometer, Gilford Instrument) according to (NSF 1975b). Total N was determined photometrically (flow injection analysis; FIA star 5020 analyzer, Tecator) after oxidation by peroxodisulphate according to the Norwegian standard NS 4743 (NSF 1993). Dissolved organic N (DON) was calculated as total N less the sum of NH_4-N and NO_3-N. Dissolved organic carbon (DOC) was determined using a total organic carbon analyzer (TOC-V CPN, Shimadzu) according to NS 1484 (NSF 1997b). The detection limit was 0.02 mg l^{-1} for NO_3-N, NH_4-N and total-N and 0.2 mg $l^{-1}$for DOC. Values below the detection limit were not included in the statistical analysis. Total P (after oxidation with potassiumperoxodisulphate to orthophosphate) and PO_4-P were determined photometrically (UV-1201 UV-VIS-Spectrophotometer, Shimadzu) after reaction with ammoniummolybdate according to the Norwegian standard NS-EN 1189 (NSF 1997a). The detection limits for phosphorous was 1 $\mu g\ l^{-1}$. Base cations (Ca, Mg, K and Na) were determined by ICP-OES (Optima 5300 DV, Perkin Elmer) using Standard Reference Material® 1643e.

The determination and quantification of waterborne bacteria, i.e. total coliforms and *E.coli*, were conducted on un-filtered water samples using Colilert®-18 and Quanty-Tray®/2000 (IDEXX Laboratories Inc.) according to the procedure described by Eckner (1998). Quanty-Tray®/2000 is a semi-automated quantification method based on the standard most probable number (MPN) of bacteria in a water sample providing counts from 1 $(100\ ml)^{-1}$ to a maximum of 2419.6 $(100\ ml)^{-1}$ without dilution (IDEXX Laboratories Inc. 2010). Water samples collected after 31/07/09 were diluted (streamwater: distilled water 1:1). For statistical purposes, we used 2 times the upper detection limit for samples with counts > 2419.6 MPN 100 ml^{-1} (i.e. 4839.2 for undiluted samples and 2*4839.2 = 9678.4 for diluted samples). Half the detection limit was used for samples with counts < 1 MPN 100 ml^{-1} (i.e. 0.5 for undiluted and 2*0.5 = 1 for diluted samples). A further description of the procedure is given by Grund (2010).

2.4 Statistical analysis

Statistical analyses were conducted using the libraries lme4 and multcomp in the statistical package R (version 2.10.1). We used Linear mixed effects models (lmer) and ordinary models (lm). Dependent variables (response variables) were soil physical and surface water chemical variables specified in the Tables 1 to 4. Some variables were ln or sqrt transformed prior to analysis to avoid violations of the model assumptions. The fixed factors included plant community (DSH, SNOWB and THM) for surface runoff variables (Table 1), grazing treatment (high, low), plant community (THM, SNOWB) and sheep track (track, no track) for infiltration rates and pore size distribution (Table 2) and catchment (G,H) for streamwater variables (Table 3). In addition, water flux (Q) was included as a continuous explanatory variable for NH_4-N and coliforms in streamwater (Table 4). The random effects were not the same for all models, as the sampling strategy differed depending on the data sets and variable of interest. Random effects included in the different models are described in the Tables 1 to 4.

The full models were fitted with relevant interactions. Backward selection was used [models fitted by maximum likelihood (ML)] and models were compared based on AIC ("smaller is better") and likelihood ratio tests (Chi squared) to obtain the minimum adequate model (Table S3). The best model was re-fitted based on restricted maximum likelihood (REML) and the estimated effects (parameter estimates including standard error) calculated using general linear hypothesis testing (glht in multcomp). Only adjusted p-values [single-step method (Hothorn *et al.* 2008)] are reported. Differences between fixed effect factor levels in the tables 2 and 4 are based on "treatment contrasts" [see Crawley pg. 377 (2007)]. Residuals and predicted random effects were plotted (histograms and QQ normal plots) to assess normality and potential outliers. Estimated parameters were back-transformed to the original scale before inclusion in the figures.

3. Results

3.1 Chemistry of surface runoff collected in topographic depressions

Conductivity ($\mu S\ cm^{-1}$) and concentrations ($mg\ l^{-1}$) of total-N, DON and DOC in standing water of the topographic depressions (Figure 1) were significantly affected by plant community (Table 1). Conductivity was significantly greater in THM as compared to DSH and SNOWB communities. DOC, total-N and DON concentrations were significantly smaller in SNOWB (~1.09, 0.048 and 0.047 mg l^{-1}, respectively) as compared to DSH (~1.96, 0.081 and 0.073 mg l^{-1}, respectively) and THM (~1.49, 0.061 and 0.061 mg l^{-1}, respectively). Concentration of NO_3-N was below the detection limit for all the samples and NH_4-N was only present in 10 of the samples, of which 8 were sampled in DSH (Table 1). Due to the low concentrations of inorganic N, total-N largely consisted of DON. The pH and DOC:DON ratio did not differ significantly between the plant communities. We also analyzed for total P and PO_4-P, but most values were at or below the detection limit, and were therefore not included.

3.2 Effects of plant community and grazing on infiltration and pore size distribution

Infiltration rates (cm $hour^{-1}$) were significantly affected by plant community and grazing, with smaller infiltration rates in SNOWB as compared to THM and significantly smaller rates in THM at the grazed compared to non grazed site (Figure 2a, treatment*pl.comm. interaction Table 2). By contrast, bulk density (BD; g cm^{-3}) was significantly greater in SNOWB as compared to THM and significantly greater at the grazed vs. the non grazed site in THM (Table 2), illustrating an inverse relationship between infiltration rate and BD. Infiltration rates in SNOWB and THM were significantly smaller and BD significantly greater on tracks compared to no tracks, both indicating soil compaction on sites with heavy sheep traffic (Figure 2b, Table 2). The difference in infiltration rates were greater between SNOWB and THM on the no tracks (~9 cm $hour^{-1}$) as compared to the tracks (~2 cm $hour^{-1}$), associated with a larger difference between no tracks vs. tracks in THM as compared to snowbeds (Figure 2b, Table 2).

The percentage of macropores (> 200 μm; volume %) was significantly greater in THM as compared to SNOWB at the non-grazed site (~20 % vs. ~10 %) but did only slightly differ at the grazed site (~10 % vs. ~8.5 %) (Figure 2c, Table 2). Comparing percentage of macropores on no tracks vs. tracks revealed no effect of plant community. There was a significantly smaller share of macropores on tracks vs. no tracks (Figure 2d, Table 2). This is in accordance with the lower infiltration rates and greater BD on sheep tracks. The percentage of mesopores (30-200 µm; volume %) was not affected by grazing or tracks vs. no tracks (Figure 2c,d, Table 2). However, it was significantly larger in SNOWB compared to THM (Figure2c, Table 2). The micro pore fraction (3-30 µm; volume %) was significantly smaller on sheep tracks vs. no tracks (Figure 2d, Table 2), but did not differ between plant communities (Figure 2c, Table 2).

3.3 Effects of grazing on chemical and biological streamwater quality parameters

Concentrations of tot-N, DON and DOC were in general larger and associated with a greater variation at the non-grazed (i.e. G) catchment as compared to the grazed (i.e. H) catchment (Table 3). Also conductivity, pH and concentrations of base cations were larger in G as compared to H. As was observed in the topographical depressions (Table 1), also in streamwater DON was the major constituent of total-N. The concentration of NH_4-N and NO_3-N did not differ significantly between the catchments (at G: 0.023 mg 1^{-1} and 0.023 mg 1^{-1}, respectively and at H: 0.026 mg 1^{-1} and 0.030 mg 1^{-1}, respectively) indicating little effects of grazing on loss of inorganic N. By contrast, the concentration of total coliform bacteria and *E. Coli* (expressed as the MPN 100 ml^{-1}) were significantly greater in the grazed (2109 and 81, respectively) vs. the non-grazed (704 and 10, respectively) catchment. Concentrations of PO_4-P and total P were small and did not differ significantly between the catchments (Table 3).

As a direct measure of hydrological response and of the potential difference between the catchments in loss of nutrients and pathogens, concentrations of NH_4-N and total coliforms and *E.coli* were analyzed in relation to water flux (Q) (Table 4). There was no significant effect of Q on NH_4-N and total coliforms. However, the amount of *E.Coli*, being a strong indicator for fecal contamination, was positively related to water flux in the grazed catchment but not in the non grazed catchment (Table 4).

4. Discussion

4.1 Chemistry of surface runoff collected in topographic depressions

Standing water collected in the topographic depressions was characterized by low concentrations of inorganic N and did not differ between the plant communities, despite differences in soil fertility (Rekdal 2001a). No samples had NO_3-N concentration greater than the detection limit (Table 1). Also, concentrations of NO_3-N and NH_4-N in O-horizon water within the grazed and non-grazed enclosures in block 3 (Figure 1) were low (0.06 mg l^{-1} and 0.06 mg l^{-1}, respectively; results not shown). Low concentrations of NH_4-N (~0.005-0.03 mg l^{-1}) in O-horizon water were also reported by Elberling *et al.* (2008) at Zackenberg, Northeast Greenland. The concentrations of inorganic N in the standing water were even smaller than those in soil and stream water at Hol and may be due to in situ removal of N in the former (Peterson *et al.* 2001). Levels of NH_4-N in streamwater (Table 3) were in the same low range as those in standing water in the topographic depressions. Together, this illustrates a strong demand for inorganic N and a low net nitrification potential in this system. This is confirmed by the previously reported in situ net-mineralization and nitrification rates in O-horizons of grassland habitats at Hol (Martinsen *et al.* 2010a).

There were no clear effects of season on the chemistry of the standing water (results not shown) despite previous findings indicating a significant decline in concentrations of inorganic N (expressed as the DIN:DON ratio of O-horizon water) in the course of the growing season (Martinsen *et al.* 2010a). Furthermore, pH and DOC:DON ratios were similar to the streamwater collected in the two catchments (Table 3) and did not differ between the plant communities. Small differences in the DOC:DON ratio suggest similar quality of SOM origin from the three vegetation types (Strand *et al.* 2008).

Concentrations of DOC in the topographic depressions and in the two catchments were similar and somewhat greater than values reported by Skjelkvåle & Wright (1998) in a Norwegian lake survey [total organic carbon (TOC) concentration 0.4 mg l^{-1} to 4.3 mg l^{-1} for mountain lakes and lakes in forested catchments, respectively]. Concentrations of DOC and DON were significantly lower in SNOWB as compared to THM and DSH (Table 1). The difference in DOC and DON concentrations between the plant communities could be biased by influence of water origin from other vegetation types (see section 2.2). However, the large differences (especially between DSH and SNOWB) are in accordance with previous reports on biomass production (Rekdal 2001a) and soil C-pools (Martinsen *et al.* 2010b), both being greater in THM as compared to SNOWB. As DOC is positively related to the content of SOM (Strand *et al.* 2008), we argue that the observed differences most likely reflects differences associated with the plant communities. In terms of fluxes, assuming that yearly runoff equals precipitation (i.e. ca 1000 mm yr^{-1}) and no variation in DOC concentration between the growing season and winter, DOC fluxes are approximately 1.1, 1.5 and 2 g m^{-2} yr^{-1} for SNOWB, THM and DSH, respectively. Despite the large difference between the plant communities, these fluxes are rather small as compared to what has been reported for e.g. forested catchments in Finland [(3-10 g m^{-2} yr^{-1} (Mattsson *et al.* 2003); 2.3-14.8 g m^{-2} yr^{-1} (Rantakari *et al.* 2010)].

4.2 Effects of plant community and grazing on infiltration and pore size distribution

Infiltration rates differed considerably between the plant communities and between the no sheep tracks vs. the well defined tracks (Figure 2). The smallest infiltration rate (0.54 cm $hour^{-1}$) was observed in snowbed on a well defined sheep track (Figure 2, Table 2). The amount of precipitation (sum per hour) exceeded this amount on several occasions during the years from 2007 to 2009 (Figure S1). As infiltration rates determined by ring infiltrometers tend to overestimate the true vertical infiltration capacity (~20% for rings of diameter 30 cm) (Tricker 1978), substantial amounts of surface runoff may have been generated in the sheep tracks.

Bulk density was significantly greater in the SNOWB as compared to the THM (Table 2). This is in accordance with previous findings at the study site (Martinsen *et al.* 2010b) and probably reflects the greater snowpack (hence compaction) in SNOWB. We found no difference in BD between the two grazing treatments in the SNOWB, which suggests a greater effect of snow cover than trampling impacts of sheep in snowbeds. By contrast, within the THM, BD was significantly greater at the grazed as compared to the non grazed site (Table 2). As THM is classified with a higher grazing value compared to snowbed (Mobaek *et al.* 2009) it indicates a larger impact of the sheep, as much time is spent here, while feeding. The grazing induced increase in BD associated with a decrease in infiltration, is in accordance with previous reports [e.g. Steffens *et al.* (2008) and Wheeler *et al.* (2002)]. However, Wheeler *et al.* (2002), reporting effects of one time heavy grazing of cattle, found a significant recovery (i.e. reduced BD and increased infiltration) one year after cessation of grazing.

Plant community and grazing had greater effects on the macropore size fraction (i.e. > 200 μm) than on the fractions of meso (30-200 μm) and micro (3-30 μm) pores (Figure 2, Table 2). The smaller share of macropores in the sheep tracks, were in accordance with the lower infiltration rates and greater BD as compared to outside the tracks. The small effects of plant community and grazing (outside the tracks) on the meso- and micropore size fractions were not surprising, as disruption of these fractions require a substantial pressure, as reported by Jansson & Johansson (1998). They found only minor changes in the pore size fractions 10-

50 µm after compaction by a tracked and wheeled forest machine (about 20 000 kg) (Jansson and Johansson 1998).

4.3 Effects of grazing on chemical and biological streamwater quality parameters

The chemical composition of streamwater draining the grazed (H) and non-grazed (G) catchment differed in several aspects not related to grazing (Table 3). The pH, conductivity and concentrations of base cations were greater in the non-grazed as compared to the grazed catchment. This is probably related to lithological differences between the catchments (amounts of base cations in the soil were greater in G than H, results not shown). Also DOC concentrations in the surface water of the non-grazed catchment were significantly greater than in grazed catchment (Table 3).We suggest that the location of the fen close to the flume in catchment G (Figure 1) explains the greater DOC concentrations in G, as wetlands may contribute significantly to export of TOC (Laudon *et al.* 2004; Rantakari *et al.* 2010).

Total N was dominated by organic N, the same as was observed for runoff water in the topographic depressions (Table 1 and 3). There was no difference in concentrations of NH_4-N and NO_3-N between the catchments, indicating low risk in N loss even at high grazing pressure at the low level of atmospheric N-deposition at Hol. It is well-known that N retention is seasonally controlled with higher N losses during winter and snowmelt (de Wit *et al.* 2008). Unfortunately, we have no data on stream water data for the winter period, due to poor accessibility of the site. Thus, we do not know if increased N concentrations and fluxes occur during winter and snow melt. However, two stream water samples collected in late autumn (October) 2008 and 4 collected during snowmelt in May 2009, had concentrations of NH_4-N and NO_3-N below the detection limit (i.e. 0.02 mg l^{-1}). Thus, grazing induced effects on potential N mineralization as reported by Martinsen (2010a), which may potentially increase leaching of NO_3-N (McNeill and Unkovich 2007), are not reflected in the streamwater chemistry in this system. Moreover, in addition to effective retention of N in the soil and vegetation (Sjøeng *et al.* 2007), in-stream processing of N also may explain the low concentrations observed at our study site. Comparing N dynamics in headwater streams across North America, Peterson *et al*. (2001) found the most rapid uptake and transformation of inorganic N to occur in small streams, with NH_4-N entering streams being removed within a few tens to hundreds of meters (Peterson *et al.* 2001).

Concentrations of total coliforms and *E.coli* were significantly greater in the grazed as compared to the non grazed catchment (Table 3). We also found a significant increase in concentrations of *E.Coli* with increasing water flux (Table 4) indicating a greater potential for surface water contamination at the grazed site. Increased concentrations of bacteria with increased discharge is also reported by Collins *et al.* (2005) and Muirhead *et al.* (2006). The greater concentrations of total coliforms and *E.Coli* in stream water at high discharge suggest an increase in the contribution of surface runoff relatively high in these bacteria. *E.Coli* is effectively retained (filtration and adsorption) when infiltrating the soil (Muirhead *et al.* 2006). This is in accordance with our findings with smaller infiltration rates at the grazed site and in particular in the tracks. Despite a strong indication of increased surface runoff in response to grazing, we observed no difference in concentrations of inorganic N and P in stream water. This suggests a relatively strong retention of these nutrients, even in sheep tracks.

5. Conclusions

Plant communities affect surface runoff quality with greater concentrations of DOC and DON in THM (productive) as compared to SNOWB (less productive). Furthermore, plant community and grazing level are associated with significantly different soil physical properties. Infiltration rates and the fractions of macro pores were smaller in snowbeds as compared to tall herb meadows, and a further decline occurs due to heavy sheep traffic. The effect of trampling on soil compaction, particularly pronounced in sheep tracks, results in increased surface runoff to the stream. This gives rise to significantly greater amounts of coliform bacteria in the grazed vs. the non grazed catchment, indicating a grazing induced potential for surface water contamination. Despite the grazing-induced contamination of stream water with coliform bacteria, there is no effect of grazing on inorganic N and P concentrations in stream water. Overall, our results suggest a strong retention of N and P in this low-alpine ecosystem.

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Figure legends

Figure 1. Sampling locations and experimental design, Hol, Southern Norway. The map shows three different grazing densities of sheep [no sheep (control), low density (25 sheep km^{-2}) and high density (80 sheep km^{-2}) within three blocks, separated by fences. Different plant communities and sites used for water collection and infiltration (pore size determination) measurements are listed in the legend.

Figure 2. Infiltration rates (cm hour^{-1}) **a and b** and pore size distribution (% of the total pore volume) **c and d** of the pore size fractions >200 µm ("macropores"), 30-200 µm ("mesopores") and 3-30 µm ("micropores") of OA-horizon soils in two plant communities (snowbed and tall herb meadow) at two grazing regimes (no sheep = control and high sheep density= 80 sheep km^{-2}), on tracks or no tracks, Hol, Southern Norway. The figure shows estimated values (± se) deriving from linear mixed effect models (Table 2). CSNOWB = no grazing in snowbed, CTHM = no grazing in tall herb meadow, HSNOWB = high grazing density in snowbed, HTHM = high grazing density in tall herb meadow. HSNOWtr and HTMtr is high grazing density on tracks in snowbed and tall herb meadow, respectively. Note the different estimates and se of infiltration rates and pore size fractions for HSNOWB and HTHM between **a and b** and **c and d**, respectively. Differences arise from the different models described in Table 2. Model reduction steps are reported in Table S3.

Tables

Table 1. Chemical characteristics of standing water in topographic depressions within 3 plant communities in an alpine system, Hol, southern Norway. SNOWB = snow bed, DSH = dwarf shrub heath and THM = tall herb meadow. Standard error (se) and number of samples (n) is shown. "-" indicates no value[1]. Different letters indicate difference between plant communities for the selected variables at a level of significance $p<0.05$. The tests are based on parameter estimates deriving from linear mixed effect models[2,3].

Variable	**SNOWB**			**DSH**			**THM**		
	Mean	se	n	Mean	se	n	Mean	se	n
Conductivity/µS cm^{-1}	11.9 ^{a}	1.3	35	13.7 ^{a}	1.1	36	16.9 ^{b}	1.1	34
pH	6.5 ^{a}	0.1	33	6.7 ^{a}	0.1	36	6.7 ^{a}	0.1	35
NO_3-N/mg l^{-1}	- -	-	-	- -	-	-	- -	-	-
NH_4-N/mg l^{-1}	0.03 -	-	1	0.03 -	0.01	8	0.03 -	-	1
Tot-N/mg l^{-1}	0.05 ^{b}	0.005	30	0.08 ^{a}	0.01	35	0.06 ^{a}	0.01	34
DON/mg l^{-1}	0.05 ^{b}	0.005	30	0.07 ^{a}	0.01	35	0.06 ^{a}	0.01	34
DOC/mg l^{-1}	1.09 ^{b}	0.196	35	1.96 ^{a}	0.41	36	1.50 ^{a}	0.22	36
DOC:DON ratio	29.8 ^{a}	5.0	30	28.7 ^{a}	3.1	35	27.7 ^{a}	2.1	34

[1] All samples out of 93 analyzed for NO_3-N and 97 out of 107 samples analyzed for NH_4-N were below the detection limit. [2] The linear mixed effect models for each of the chemical surface water variables included plant community (three levels: DSH, SNOWB and THM) as fixed factor and block (three levels: east, mid, west) and year (two levels: 2006 and 2007) as random effects. Conductivity, DOC and DOC:DON were ln transformed and tot-N and DON sqrt transformed prior to analysis. One value of the measured conductivity (THM, 83 µS cm^{-1}) was considered as outlier and thus omitted from the analysis. This did not affect the outcome of the tests. [3] Var. of the random effects: Cond.: block = 0.0819, year = 0.0083, resid.= 0.1436; pH: block = 0.0089, year = 0.0000, resid.= 0.3070; Tot-N: block = 0.0006, year = 0.0003, resid.= 0.0049; DON: block = 0.0009, year = 0.0004, resid.= 0.0044 ; DOC: block = 0.0635, year = 0.0661, resid.= 0.3186; DOC:DON: block = 0.0041, year = 0.0000, resid.= 0.1298.

Table 2. Parameter estimates (± se) of linear mixed effect models for infiltration rates (cm hour^{-1}), pore size distribution (% of the total pore volume) of the pore size fractions >200 µm ("macropores"), 30-200 µm ("mesopores") and 3-30 µm ("micropores") and bulk density (BD; g cm^{-3}) at pF 3(see section 2.2) of OA-horizon soils in two plant communities (snowbed and tall herb meadow) at two grazing regimes (no sheep = control and high sheep density= 80 sheep km^{-2}), on tracks or no tracks, Hol, Southern Norway. The fixed effects include treatment (no sheep grazing, i.e. control or high sheep density), plant community (Pl.comm.; tall herb meadow or snowbed) and track or no track (track.no.track). The random effects include time (7 levels) and laboratory replication (Lab.run; 2 levels). P-values indicate whether estimates or estimated differences are significantly different from 0. The model reduction steps (based on ML-estimation) are presented in supporting information (Table S3).

Model	Parameter (fixed effects)	Estimate	se	t	p	Random effects	
						Groups	Variance
Infiltration no track ~ treatment * pl.comm. (n=28)	**Infiltration no track/cm hour^{-1}**						
	Intercept	3.286	0.939	3.500	0.002	Time	1.105
	Treatment (high vs. control)	2.143	1.203	1.781	0.200	Residual	5.066
	Pl.comm. (THM vs. SNOWB)	19.114	1.203	15.888	< 0.001		
	Treatment (high vs. control): Pl.comm. (THM vs. SNOWB)	-10.029	1.701	-5.894	< 0.001		
Infiltration track ~ pl.comm. * track.no.track (n=28)	**Infiltration track/cm hour^{-1}**						
	Intercept	5.429	0.464	11.705	< 0.001	Time	0.315
	Pl.comm. (THM vs. SNOWB)	9.086	0.583	15.579	< 0.001	Residual	1.190
	Track.no.track (track vs. no track)	-4.886	0.583	-8.377	< 0.001		
	Pl.comm. (THM vs. SNOWB): Track.no.track (track vs. no track)	-7.086	0.825	-8.591	< 0.001		
Pores > 200 µm no track ~ treatment * pl.comm. (n=24)	**Pores > 200 µm no track/%**						
	Intercept	9.950	2.387	4.168	< 0.001	Lab.run	2.991
	Treatment (high vs. control)	-1.417	2.900	-0.489	0.954	Residual	25.228
	Pl.comm. (THM vs. SNOWB)	10.100	2.900	3.483	0.002		
	Treatment (high vs. control): Pl.comm. (THM vs. SNOWB)	-8.783	4.101	-2.142	0.095		
Ln(Pores 30-200 µm no track) ~pl.comm. (n= 17)[1]	**Ln(Pores 30-200 µm no track/%)**						
	Intercept	2.170	0.114	18.971	< 0.001	Lab.run	0.015
	Pl.comm. (THM vs. SNOWB)	0.274	0.108	2.532	0.022	Residual	0.050
Pores 3-30 µm no track ~1 (n= 24)	**Pores 3-30 µm no track/%**						
	Intercept	17.700	1.109	15.950	< 0.001	Lab.run	0.000
						Residual	29.543
BD at pf 3 no track~treatment * pl.comm. (n=24)	**BD at pf 3 no track/g cm^{-3}**						
	Intercept	1.243	0.058	21.528	<0.001	Lab.run	0.000
	Treatment (high vs. control)	-0.078	0.082	-0.959	0.672	Residual	0.020
	Pl.comm. (THM vs. SNOWB)	-0.475	0.082	-5.816	<0.001		
	Treatment (high vs. control): Pl.comm. (THM vs. SNOWB)	0.302	0.116	2.612	0.028		
Ln(Pores > 200 µm track) ~ track.no.track (n=24)	**Ln(Pores > 200 µm track/%)**						
	Intercept	2.125	0.145	14.677	< 0.001	Lab.run	0.000
	Track.no.track (track vs. no track)	-0.834	0.205	-4.075	0.000	Residual	0.251
Pores 30-200 µm track ~1 (n= 16)[1]	**Pores 30-200 µm track/%**						
	Intercept	12.333	1.408	8.760	< 0.001	Lab.run	1.171
						Residual	22.250
Ln(Pores 3-30 µm track) ~track.no.track (n= 24)	**Ln(Pores 3-30 µm track/%)**						
	Intercept	2.874	0.099	29.034	< 0.001	Lab.run	0.000
	Track.no.track (track vs. no track)	-0.350	0.140	-2.501	0.022	Residual	0.118
BD at pf 3 track~track.no.track (n=24)	**BD at pf 3 track/g cm^{-3}**						
	Intercept	1.078	0.051	21.133	< 0.001	Lab.run	0.000
	Track.no.track (track vs. no track)	0.172	0.072	2.379	0.030	Residual	0.031

[1] Some values were omitted due to invalid measurements (below 0).

Table 3. Mean chemical and biological streamwater quality parameters of a non grazed (G) and grazed (H) catchment in an alpine system, Hol, southern Norway. Standard error (se) and number of samples (n) is shown. Different letters indicate difference between the catchments for selected variables at a level of significance $p<0.05$. The tests are based on parameter estimates deriving from linear mixed effect models[1,2,3] and normal linear models[4]. Some of the factors were transformed prior to analysis (see footnote). "-" indicates no value[5].

Variable	**G**				**H**			
	Mean		se	n	Mean		se	n
[1]Conductivity/µS cm^{-1}	33.0	-	1.9	54	8.3	-	0.2	70
[1]pH	7.3	-	0.1	54	6.7	-	0.0	70
[1]NO_3-N/mg l^{-1}	0.02	-	0.00	3	0.03	-	0.01	3
[2]NH_4-N/mg l^{-1}	0.02	a	0.00	8	0.03	a	0.00	8
[1]Tot-N/mg l^{-1}	0.12	-	0.01	57	0.08	-	0.00	73
[1]DON/mg l^{-1}	0.12	-	0.01	57	0.07	-	0.00	73
[2]DOC/mg l^{-1}	2.80	a	0.13	59	1.88	b	0.05	75
[2]DOC:DON ratio[2]	26.7	a	1.1	56	28.3	a	1.1	72
[3]PO_4-P/µg l^{-1}	1.39	a	0.07	8	1.48	a	0.13	14
[3]Tot-P/µg l^{-1}	1.71	a	0.26	29	2.01	a	0.20	34
[4]Ca/mg l^{-1}	6.57	a	0.45	4	1.09	b	0.06	4
[4]Mg/mg l^{-1}	0.33	a	0.02	4	0.12	b	0.01	4
[4]K/mg l^{-1}	0.65	a	0.06	4	0.15	b	0.03	4
[4]Na/mg l^{-1}	1.05	a	0.04	4	0.54	b	0.01	4
[3]Total coliform/MPN 100 ml^{-1}	704	a	239	16	2109	b	564	19
[3]E. Coli/MPN 100 ml^{-1}	10	a	3	17	81	b	33	20

[1] Not tested due to difference in the variance between G and H and too few observations (NO_3-N).

[2] The linear mixed effect models (LMER) included catchment as fixed factor and year (three levels: 2007, 2008 and 2009) as random effect. Var. of the random effects: NH_4-N: year = 0.0000, resid. = <0.0000; Ln(DOC): year = 0.0251, resid. = 0.0539; DOC:DON: year = 5.8267, resid. = 78.7942.

[3] Sampled in 2009 only. LMER included catchment as fixed factor and month [five levels (May to October) for PO_4-P and Tot-P and 4 levels (July to October) for total coliforms and *E.Coli*] as random effect. Var. of the random effects: Ln(PO_4-P): month = 0.0000, resid. = 0.0588; Ln(Tot-P): month = 0.0412, resid. = 0.1872; Ln(tot.coliforms): month = 1.3911, resid. = 1.5812; Ln(*E.coli*): month = 2.9166, resid = 1.7907.

[4] Sampled in 2009 only, all values ln transformed prior testing.

[5] Eighty two samples out of 88 analyzed for NO_3-N and 114 out of 130 samples analyzed for NH_4-N were below the detection limit.

Table 4. Parameter estimates (±se) of ln (NH_4-N; mg l^{-1}), ln(total coliforms; MPN 100 ml^{-1}) and ln(*E.Coli*; MPN 100 ml^{-1}) of surface water samples from a non grazed (G) and grazed (H) catchment in an alpine system, Hol, southern Norway. The estimates derive from linear mixed effect models (REML-estimation) with month (three levels[1]: July, August and September) as random effects. The fixed effect factors are catchment (G and H) and water flux (Q/l s^{-1}). P -values are based on simultaneous tests for general linear hypotheses (indicating wheater estimates are significantly different from 0). The model reduction steps (based on ML-estimation) are presented in supporting infromation (Table S3).

Model	**Parameter (fixed effects)**	**Estimate**	**se**	**t**	**p**	**Random effects** Groups	 Variance
Ln(NH_4-N) ~ 1 (n=13)	**Ln(NH_4-N/mg l^{-1})**						
	Intercept	-3.727	0.066	-56.840	<0.001	Month	0.000
						Residual	0.056
Ln(Total coliform) ~ Catchment (n=32)	**Ln(Total coliform (MPN 100 ml^{-1})**						
	Intercept	5.499	0.752	7.314	<0.001	Month	1.304
	Catchment (H vs. G)	1.282	0.463	2.770	<0.01	Residual	1.701
Ln(E.Coli) ~ Catchment * Q (n=34)	**Ln(E.Coli/MPN 100 ml^{-1})**						
	Intercept	1.403	0.943	1.488	0.244	Month	2.003
	Catchment (H vs. G)	0.127	0.624	0.204	0.972	Residual	1.202
	Q	0.008	0.346	0.022	0.999		
	Q (H vs. G)	0.805	0.380	2.120	0.050		

[1] All observations except one for NH_4-N (July 2007) are from 2009.

Figures

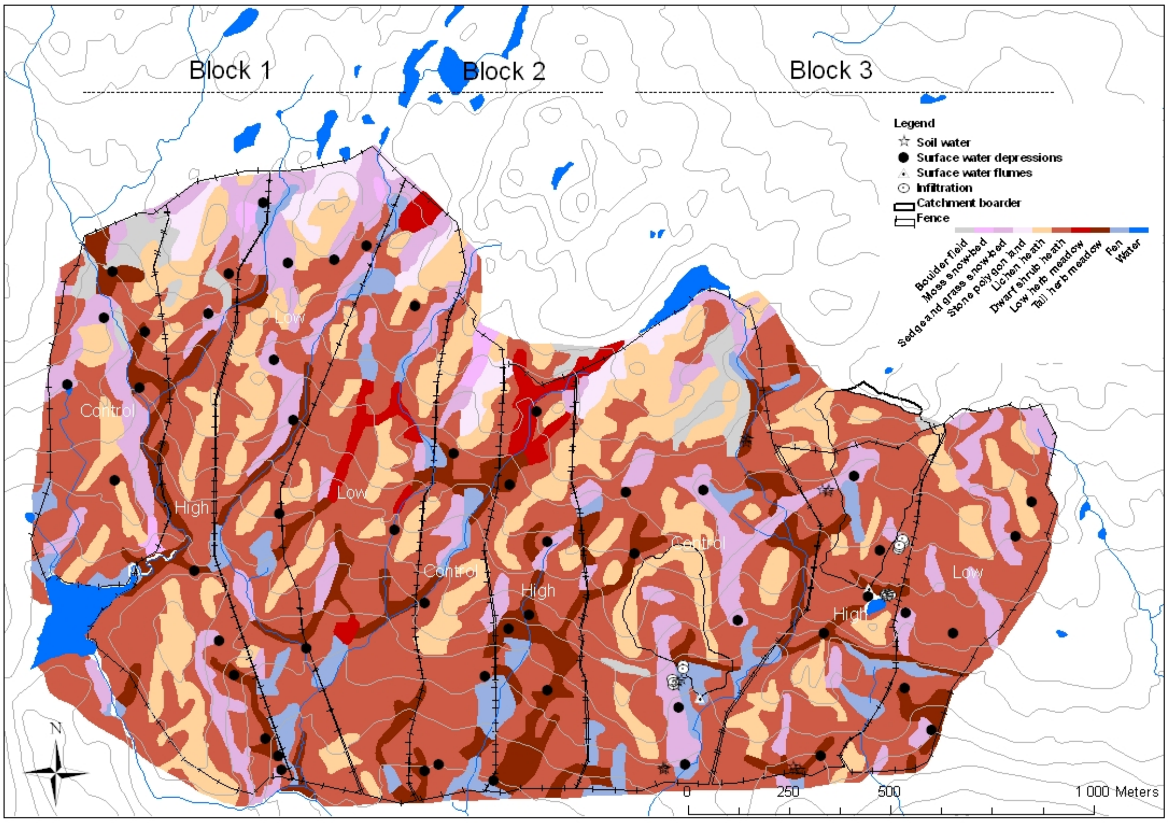

Figure 1

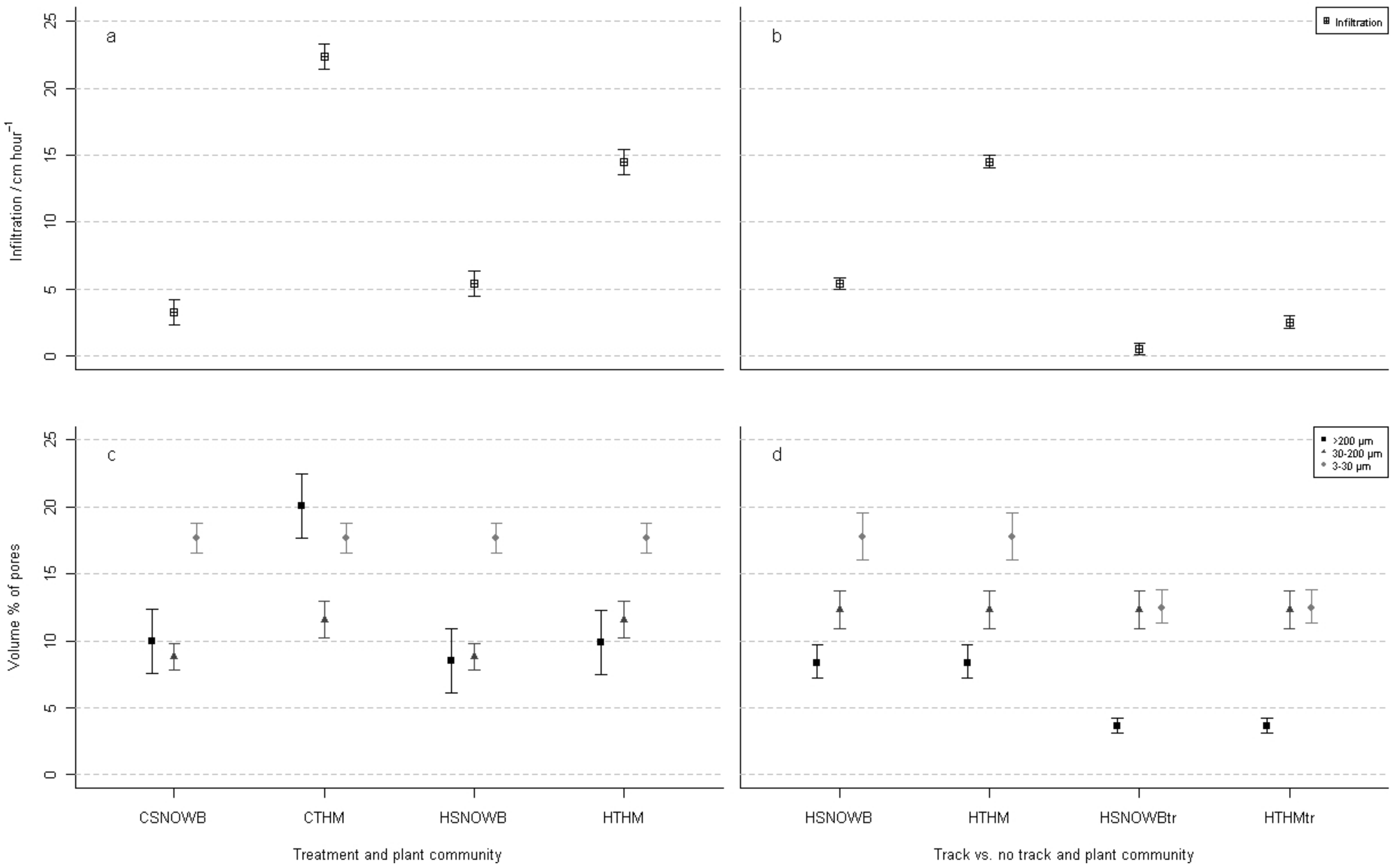
a
b
c
d
Infiltration / cm hour^{-1}
Volume % of pores
0
5
10
15
20
25
Infiltration
>200 µm
30-200 µm
3-30 µm
CSNOWB
CTHM
HSNOWB
HTHM
HSNOWB
HTHM
HSNOWBtr
HTHMtr
Treatment and plant community
Track vs. no track and plant community

Figure 2.

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Supporting information

Table S1. Total precipitation (P/mm), average air temperatures (ºC) (AT) and average temperatures and soil moisture contents (%) of the O-horizon (depth 6 cm; ST1 and SM1) and the mineral soil (depth 22 cm; ST2 and SM2) for the months June (19/06) to September (19/09) in the years 2007 to 2009, Hol, Southern Norway[1]. "-" indicate missing values.

Factor	2007				2008				2009			
	June	July	Aug	Sept	June	July	Aug	Sept	June	July	Aug	Sept
P/mm	96.2	240.4	86.8	30.4	32.6	69.2	98.8	81.4	18.4	141.2	113.4	28.2
AT/°C	9.1	8.9	8.9	4.0	5.6	11.7	8.8	5.2	12.0	10.6	8.9	7.0
ST1/°C	10.3	9.9	8.8	5.7	6.9	10.3	9.6	7.0	10.0	10.5	8.7	6.9
ST2/°C	8.7	8.9	8.1	6.2	6.3	9.1	9.4	7.5	9.8	11.4	10.2	9.0
SM1/%	72.7	71.8	67.1	75.4	55.7	55.8	32.8	25.3	42.0	29.2	20.9	42.9
SM2/%	44.5	44.4	37.5	37.2	-	-	-	-	-	-	-	-

[1] Measurements were conducted at a location close to the flume in enclosure G. Air temperature (107 Temperature Probe, Campbell Scientific Ltd.), precipitation (ARG 100 Tipping Bucket Raingauge, Campbell Scientific Ltd.), soil moisture (TDR, ML 1 ThetaProbe Soil Moisture Sensor, Delta-T Devices Ltd.) and temperature (107 Temperature Probe, Campbell Scientific Ltd) at two depths (6 cm and 22 cm) were logged (CR10, Campbell Scientific Ltd.) in the same periods as the water flux measurements (Figure S1). The logging interval was 1 hour.

Table S2. Area[1] and vegetation distribution of two catchments located in a non grazed area (catchment G) and an area with high (80 sheep km^{-2}) sheep density (catchment H), Hol, Southern Norway.

	G		H	
Vegetation type	Area/m2	% of area	Area/m2	% of area
Boulder field			692	1
Dwarf shrub heath	24584	46	58430	65
Fen	4017	7	4867	5
Lichen heath	13769	26	12076	13
Sedge and grass snow bed	7289	14	7884	9
Tall herb meadow	4133	8	1532	2
Not classified			4224	5
Total	53792	100	89703	100

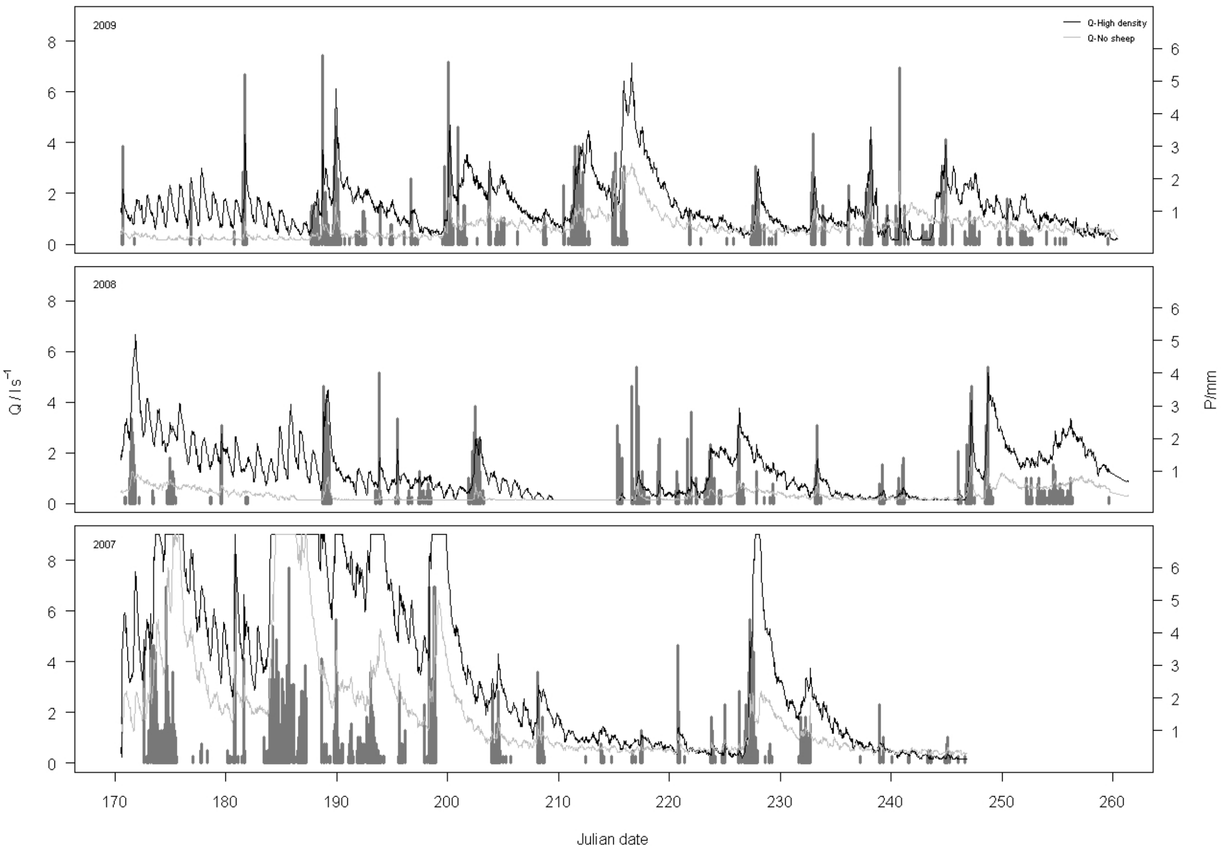
[1] Decription of the areal determination of the two catchments is given by (Grund, 2010).

Table S3. Model selection for the fixed effect structure of linear mixed models (based on ML-estimation) for infiltration rates (cm hour^{-1}), pore size distribution (% of the total pore volume) of the pore size fractions >200 µm ("macropores"), 30-200 µm ("mesopores") and 3-30 µm ("micropores") and bulk density at pf 3(BD; g cm^{-3}), NH_4-N (mg l^{-1}), total coliforms (MPN 100 ml^{-1}) and *E.Coli* (MPN 100 ml^{-1}) at two different treatments (catchments); no sheep (catchment G) or high sheep density (catchment H) and two plant communities (SNOWB and THM) on sheep tracks or no sheep tracks, Hol, Southern Norway. Q (l s^{-1}) is included as a continuous variable. Step indicate models with a different fixed effect structure for each dependent variable. AIC= Akaike's information criterion. P is the p-value based on likelihood ratio tests (Chi squared) between two models. P-values < - 0.05 indicate significantly lower explanatory power. Step comp. indicates the models compared. The selected models are highlighted in bold.

Dependent variable	Step	Model (fixed effects)	AIC	P	Step comp.
Infiltration no track/cm hour^{-1}	1	**~treatment*pl.comm.**	**136.97**		
	2	~treatment + pl.comm.	158.03	0.00	2 vs. 1
Infiltration track/cm hour^{-1}	1	**~track.no.track*pl.comm.**	**97.08**		
	2	~track.no.track + pl.comm.	131.13	0.00	2 vs. 1
Pores > 200 µm no track/%	1	**~treatment*pl.comm.**	**154.74**		
	2	~treatment + pl.comm.	157.51	0.03	2 vs. 1
Ln(Pores 30-200 µm no track/%)	1	~treatment*pl.comm.	6.21		
	2	~treatment + pl.comm.	6.15	0.16	2 vs 1
	3	~treatment	9.79	0.02	3 vs.2
	4	**~pl.comm.**	**5.29**	0.29	4 vs. 2
	5	~1	8.75	0.02	5 vs. 4
Pores 3-30 µm no track/%	1	~treatment*pl.comm.	158.80		
	2	~treatment + pl.comm.	156.94	0.70	2 vs. 1
	3	~treatment	155.08	0.71	3 vs.2
	4	~pl.comm.	156.22	0.26	4 vs. 2
	5	**~1**	**154.35**	0.67	5 vs.1
BD at pf 3 no track/g cm^{-3}	1	**~treatment*pl.comm.**	**-18.14**		
	2	~treatment + pl.comm.	-13.10	0.01	2 vs. 1
Ln(Pores > 200 µm track/%)	1	~track.no.track*pl.comm.	43.11		
	2	~track.no.track + pl.comm.	41.47	0.55	2 vs. 1
	3	**~track.no.track**	**40.89**	0.23	3 vs.2
	4	~pl.comm.	53.59	0.00	4 vs. 2
	5	~1	52.39	0.00	5 vs. 3
Pores 30-200 µm track/%	1	~track.no.track*pl.comm.	100.85		
	2	~track.no.track + pl.comm.	101.34	0.11	2 vs. 1
	3	~track.no.track	99.44	0.75	3 vs.2
	4	~pl.comm.	102.41	0.08	4 vs. 2
	5	~1	100.45	0.13	5 vs. 1
	6	**~1**	**100.45**	0.08	6 vs. 3
Ln(Pores 3-30 µm track/%)	1	~track.no.track*pl.comm.	26.18		
	2	~track.no.track + pl.comm.	24.40	0.64	2 vs. 1
	3	**~track.no.track**	**22.65**	0.62	3 vs.2
	4	~pl.comm.	28.47	0.01	4 vs. 2
	5	~1	26.66	0.01	5 vs. 3
BD at pf 3 track/g cm^{-3}	1	~track.no.track*pl.comm.	-9.08		
	2	~track.no.track + pl.comm.	-7.77	0.07	2 vs. 1
	3	**~track.no.track**	**-9.16**	0.44	3 vs.2
	4	~pl.comm.	-4.15	0.02	4 vs. 2
	5	~1	-5.67	0.02	5 vs. 3
Ln(NH$_4$-N/mg l^{-1})	1	~Catchment * Q	3.80		
	2	~Catchment + Q	5.63	0.05	2 vs. 1
	3	~Q	4.05	0.52	3 vs. 2
	4	~Catchment	4.86	0.27	4 vs. 2
	5	**~1**	**4.36**	0.26	5 vs. 2
Ln(Total coliform (MPN 100 ml^{-1})	1	~Catchment * Q	118.08		
	2	~Catchment + Q	119.71	0.06	2 vs. 1
	3	~Q	123.06	0.02	3 vs. 2
	4	**~Catchment**	**119.80**	0.15	4 vs. 2
	5	~1	124.77	0.01	5 vs. 4
Ln(E.Coli/MPN 100 ml^{-1})	1	**~Catchment * Q**	**118.79**		
	2	~Catchment + Q	121.29	0.03	2 vs. 1

Figure S1.

Mean water flux[1] per hour (Q/ l s^{-1}) and sum precipitation per hour (P/mm) for the periods June to September in the years 2007, 2008 and 2009, Hol, Southern Norway. Q is reported for a catchment with no sheep grazing (catchment G) and high (80 sheep km^{-2}) sheep density (catchment H). Water fluxes between 0.16 l s^{-1} and 9 l s^{-1} are reported. Julian dates: 170 correspond to 19/06/07, 18/06/08 and 19/06/09 and 260 correspond to 17/09/07, 16/09/08 and 17/09/09.



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[1] Water fluxes were measured in flumes [RBC flume (0.16 l s^{-1} - 9 l s^{-1}), type 13.17.02, (Eijkelkamp, 2001)] installed in two small streams draining the two catchments. The sum of air pressure and water column (cm) in each flume was logged (Mini-Diver DI501, Schlumberger Water Services Divers®) in 15 minutes intervals and the water level determined by subtraction of logged air pressure (Baro-Diver DI500, Schlumberger Water Services Divers®). Water fluxes were calculated based on calculated water levels (mm) according to (Eijkelkamp, 2001) for the periods mid of June to mid of September from 2007 to 2009 . A further description of the water flux measurements is given by (Grund, 2010).