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ARTICLE
Disease Ecology

Soil characteristics at artificial salt licks and their potential impacts on occurrence of chronic wasting disease

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Abstract

Salt lick sites, where artificial salt blocks are placed at permanent locations, are common in summer grazing areas for free-ranging sheep in Norwegian mountains. These areas often overlap with areas used by wild reindeer, and reindeer are frequently observed at these salt lick sites. The first cases of chronic wasting disease (CWD) were discovered among Norwegian wild reindeer in 2016, and salt lick sites were presumed to be hotspots for the transmission of CWD. In this study, we compare soil properties at salt lick and nearby control sites not affected by salt blocks and review how salt-induced changes may influence the persistence and transmission of CWD. Three wild reindeer areas were studied: one CWD-affected area, Nordfjella, and two areas without CWD, Knutshø and Forollhogna. The soils at the salt lick sites were strongly influenced by dissolving salt blocks and increased animal activity. The salt lick sites had higher pH and ionic strength and increased levels of sodium (Na), chlorine (Cl), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), and iodine (I), reflecting the composition of the salt blocks. The increased animal activity was reflected in eroded topsoil causing less soil organic matter (SOM), and there were higher amounts of elements related to defecation and urination, giving higher concentrations of inorganic nitrogen (Inorg-N), phosphate (PO_4-P), sulfate (SO_4-S), and potassium (K) as well as high gastrointestinal parasite frequency and diversity. The high salt content in the salt lick soils may stimulate geophagy, and as the soil is heavily contaminated by animal excretions, this may facilitate prion transmission. In addition, the high pH and ionic strength in the salt lick soils increase both the cation attraction and anion diffusion toward the soil particles, thereby facilitating both persistence and transmission of CWD. There was an increase in salinity at the salt lick sites in a gradient from west to east, most likely related to the coinciding decrease in precipitation. This suggests that if the use of permanent salt lick sites is discontinued, the salt lick sites in the east will maintain their attraction for congregating animals and geophagy longer than the western sites.

KEYWORDS
chronic wasting disease (CWD), mineral lick, mountain grazing, parasites, prion disease, prion preservation, reindeer, salt lick, sheep, soil ionic strength, soil pH, transmission hotspots

INTRODUCTION

Since chronic wasting disease (CWD) was first reported in captive mule deer (*Odocoileus hemionus*) in Colorado in the late 1960s (Williams & Young, 1980), CWD has been discovered in both captive and wild cervid populations over large parts of North America (https://www.cdc.gov/prions/cwd/occurrence.html). As this fatal prion disease is almost impossible to eradicate without culling the entire infected population (Uehlinger et al., 2016) and is linked to population declines (DeVivo et al., 2017; Edmunds et al., 2016; Monello et al., 2014), it has been suggested that CWD can cause local extirpation of cervids (Almberg et al., 2011). In addition, this uncontrolled spread of CWD implicates a very low, but not negligible, risk of spillover to humans (EFSA et al., 2019). Consequently, when CWD was discovered in a wild reindeer (*Rangifer tarandus*) in the Nordfjella wild reindeer area, Norway, in 2016 (Benestad et al., 2016), Norwegian authorities responded with strong measures, including culling of the affected subpopulation, to confine and, if possible, eradicate the disease (VKM et al., 2017). Since then, classic CWD has been detected in two of 7310 examined reindeer from a second wild reindeer area, Hardangervidda, right south of Nordfjella, while 12,807 wild and 63,286 semidomesticated reindeer from other parts of the country have tested negative (http://apps.vetinst.no/skrantesykestatistikk/NO/#omrade, visited 3 May 2024). This may indicate that the disease has spread, but that the spread so far is limited.

A long incubation period with preclinical shedding of infectious prions in saliva, urine, and feces (Haley et al., 2009, 2011; Henderson et al., 2015; Hoover et al., 2017; Tennant et al., 2020), combined with the ability of the prions to persist in the environment for several years (Seidel et al., 2007; Somerville et al., 2019) are traits that explain the inexorable increase in distribution and occurrence of CWD in North America (EFSA et al., 2018; EFSA et al., 2019; Haley & Hoover, 2015), and there is no scientific evidence indicating that CWD in reindeer in Norway has traits behaving very differently from the North American (VKM et al., 2021). Sorption to soil components and immobilization in the upper soil layer is suspected to facilitate both environmental persistence and uptake by and infection in susceptible hosts (Johnson et al., 2006, 2007; Kuznetsova et al., 2020; Maddison et al., 2010; Schramm et al., 2006; Wyckoff et al., 2016). Several models of CWD epidemiology highlight the importance of indirect transmission through soil (Almberg et al., 2011; Miller et al., 2006; Sharp & Pastor, 2011).

The fate of prion in soil is strongly dependent on the clay content (Dorak et al., 2017; Walter et al., 2011), clay mineralogy (Revault et al., 2005; Rigou et al., 2006), oxides (Jacobson et al., 2013), soil organic matter (SOM) (Bartelt-Hunt et al., 2012) as well as soil chemical composition (Smith et al., 2011). Most studies point to electrostatic attraction as the most important adsorption mechanism (e.g., Bartelt-Hunt et al., 2012). The numerous positively charged amino acids on the N terminal of the prion may bind to negatively charged surfaces of clay minerals and SOM (Maddison et al., 2010; Saunders et al., 2009). The C terminal of the prion with more hydrophobic properties may also interact with low charge surfaces (quartz, kaolinite, oxides) as well as with organic material (Charlet et al., 2007). The electrostatic forces influencing prion attraction are strongly dependent on pH and ionic strength. These factors are consequently decisive for prion interactions with clay minerals and oxides (Jacobson et al., 2013; Ma et al., 2007; Polano et al., 2008). The cation composition and concentration in the soil may influence the interlayer spacing of swelling clays and thereby the prions' ability to enter the interlayers (Polano et al., 2008). Some soil properties may also degrade or deactivate prions. Manganese oxides have been found to degrade prions, in vitro, with increasing degradation as pH decreased (Russo et al., 2009). Soil with a high content of SOM, particularly well-humified organic matter, may also promote degradation and deactivation of prion (Bartelt-Hunt et al., 2012; Giachin et al., 2014; Kuznetsova et al., 2018; Maddison et al., 2010; Saunders et al., 2008, 2011).

North American CWD response and management plans frequently emphasize the important role artificial point-sources of food, minerals, or water have on the transmission of the disease by causing deer to congregate

(Janousek et al., 2021; Mejía-Salazar et al., 2018). These sites may act as congregation hotspots for both domestic and wild animals, thereby increasing the risk for disease transmission, both within and between species (Lavelle et al., 2014; Plummer et al., 2018; Sorensen et al., 2014). During the risk assessments performed by the Norwegian Scientific Committee on Food and Environment (VKM) after the discovery of CWD in wild reindeer in Norway, artificial permanent salt lick sites were identified as potential hotspots for transmission (VKM et al., 2018). The salt lick sites were considered hotspots both because of the greater risk of direct transfer caused by the concentration of susceptible hosts, but also because of the well-known habits of the animals to ingest soil from salt licks and the corresponding increased probability of transmission of prions bound to soil (e.g., Lavelle et al., 2014; Schramm et al., 2006). At salt lick sites, farmers place salt blocks on poles or in feeders to provide domestic free-ranging sheep with salt, primarily sodium chloride (NaCl) frequently supplemented with other essential elements (see Appendix S1: Table S1 for details on the general chemical composition of commercially available salt blocks used in Norwegian mountain grazing areas). In addition to providing supplemental nutrients, these salt licks facilitate more efficient pasture use and ease herding and surveillance of free-ranging domestic sheep in Norwegian mountain areas. According to public documents, between 50 and 100 kg of salt is supplied to each of these permanent salt lick sites during the grazing season. As the poles or feeders the salt blocks are placed on are anchored in bedrock or large stones, these sites are used for many years, and therefore we refer to them as permanent salt lick sites as opposed to mobile or ambulating salt licks. In Nordfjella, salt lick sites were frequently visited by sheep as well as by other animals, including animals from the CWD-affected wild reindeer subpopulation, red deer (*Cervus elaphus*), and moose (*Alces alces*) (Figure 1; VKM et al., 2017, 2018).

The vegetation surrounding the salt block is typically eroded by animal stomping and trampling, exposing the underlying mineral soil, and the surface of the soil is frequently sprinkled with fecal pellets. Precipitation slowly

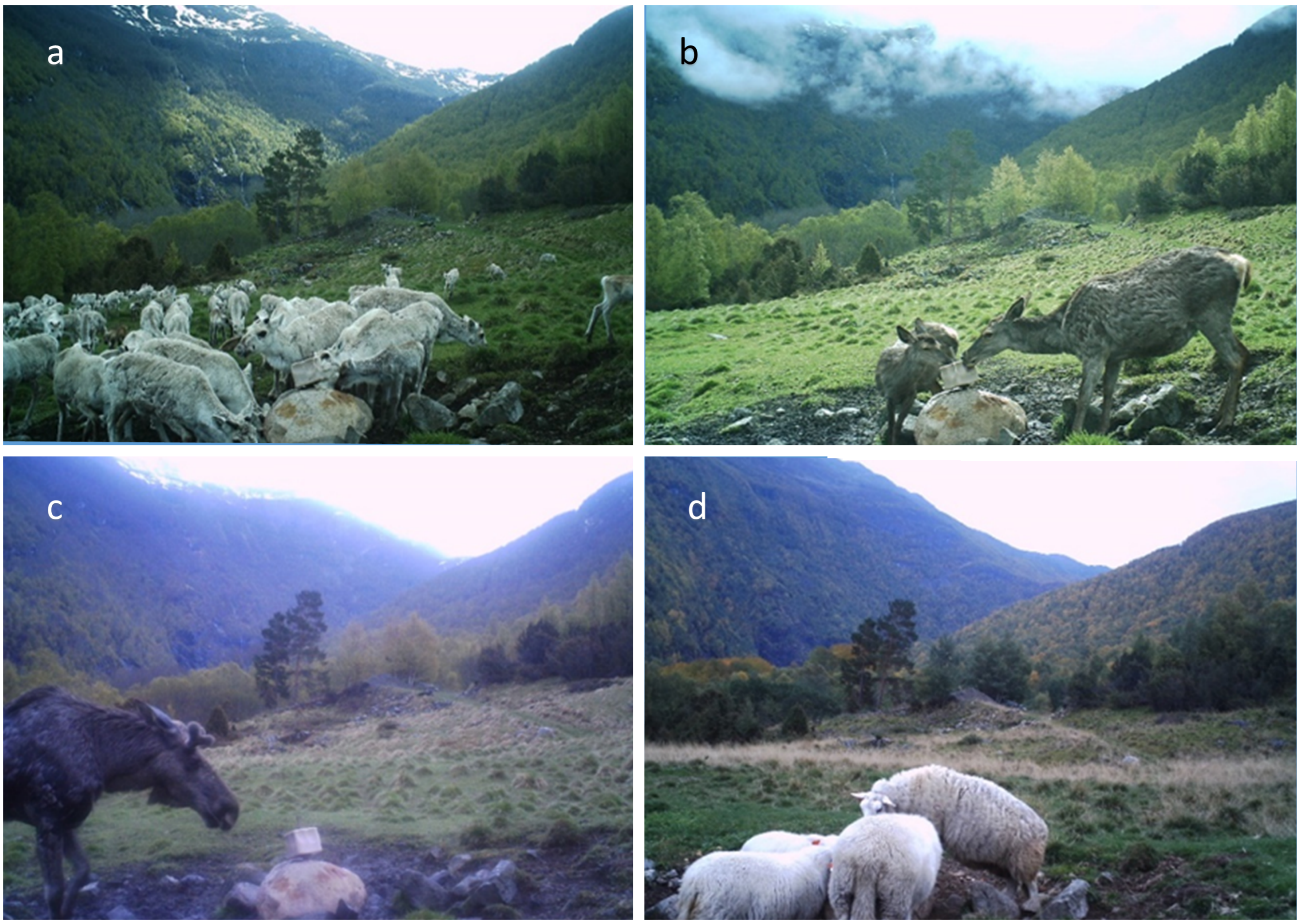

FIGURE 1 (a) Wild reindeer, (b) red deer, (c) moose, and (d) domestic sheep visiting the same salt lick site in the Nordfjella area, not far from the site where the first reindeer with chronic wasting disease was found in 2016. Image credit: Lars Nesse, Lærdal, Norway.

dissolves the salt block and salt seeps into the surrounding soil. This stimulates geophagia; the animals lick soil surfaces and ingest soil, which increases transmission risk (Daszak et al., 2000; Johnson et al., 2007; Klaus & Schmidg, 1998; Kroesen et al., 2020; Panichev et al., 2013; Slabach et al., 2015). It is estimated that deer may consume more than 500 g of soil monthly from areas around salt blocks (Schultz & Johnson, 1992). Animal activity may induce soil compaction, which in turn reduces infiltration and increases surface runoff (Martinsen et al., 2013). The reduced soil pore volume at the salt lick sites may reduce dispersion, dilution, and transport of prions in the soil solution and thereby increase prion availability in the upper soil layers. This may also create local anoxic conditions that facilitate changes in the composition and bioavailability of many elements. Given the possibility of increased geophagy around the salt licks, the local changes in soil element composition may influence the element uptake of the visiting animals. It has been suggested that changes in element balance may influence prion disease development (Ragnarsdottir & Hawkins, 2006). Clinical studies have shown that animals infected with CWD may have element imbalances (Johnson et al., 2013), but whether the imbalance is a cause or a consequence of the prion disease is not yet known (Toni et al., 2017).

Climate may also influence the persistence and distribution of prions in soil. Prions are extremely temperature tolerant (Taylor, 1999), suggesting that natural fluctuations in soil temperatures would have little influence on their persistence. However, precipitation and fluctuations in soil moisture, particularly cycles of wetting and drying (Yuan et al., 2015), as well as freezing and thawing (Yuan et al., 2018), may degrade prions and significantly reduce both prion persistence and bioavailability. High precipitation levels may erode soil particles to which prions are bound diluting the infectious material while spreading it over larger areas and particularly into depressions in the landscape. Precipitation also contributes to the transport of unbound prions to deeper soil layers either through interstitial pores in sandy soils or via cracks and channels in finer textured soils. This dilutes the infectious material and makes it less available for geophagy. Heavy rain may also rapidly leach easily soluble salts from the soils around salt licks, making them less attractive for geophagia.

Our overarching hypothesis is that the use of permanent salt lick sites has changed local soil properties in ways that may influence the persistence and transmission of CWD. In the current study, we describe differences in soil properties between unimpacted control sites and permanent salt lick sites in three wild reindeer areas in Norway. We then review what is known about soil-prion interactions to predict how the use of salt licks is likely to impact the persistence and transmission of CWD prions. Finally, we examine how regional variation in climate and soil composition may interact with salt lick sites to facilitate or impede the emergence and transmission of CWD.

MATERIALS AND METHODS

Study areas

Three wild reindeer areas were studied: Nordfjella, Knutshø, and Forollhogna (Figure 2). Nordfjella was selected because CWD was found in wild reindeer in the northern part of the area (Zone 1) in 2016 (Benestad et al., 2016), while Forollhogna was selected as it is the wild reindeer area closest to the area where CWD was diagnosed in two moose in 2016 (Pirisinu et al., 2018). Knutshø was selected because the reindeer population here is currently experiencing a non-CWD-related decline, and concerns have been raised as to whether it is more vulnerable to the introduction of CWD. All areas have extensive sheep summer grazing (Table 1). The climate in these areas differs little with regard to temperature, but the mean annual precipitation ranges from 400 to 2000 mm (seklima.met.no; Figure 2).

Nordfjella has a rough topography with deep valleys and steep mountains. Large parts of the area lie above 1500 m above sea level (m asl), with peaks reaching 1900 m asl. It is characterized by bare rock and stone screes with only sparse vegetation. The bedrock in the area belongs to the Jotunheim nappe, which consists of nutrient-poor, hard, mostly igneous rock, and some metamorphic sedimentary rock. Remnants of the Cambro-Silurian nappe occur in streaks from west to east in the southern region (Strand et al., 2011). Knutshø consists of rounded mountains up to 1700 m asl that are generally well-covered with soil and vegetation. Less than 10% of the area is without soil and vegetation cover. The bedrock in Knutshø is part of the Trondheim nappe consisting of nutrient-rich, easily weatherable Cambro-Silurian sedimentary rocks (Strand et al., 2015). Forollhogna is also a part of the Trondheim nappe but has patches of intrusive Caledonian bedrock. A large part of Forollhogna is covered by glacial deposits and fluvial sediments, and occasional eskers are prominent in the landscape. Only the highest peaks, 1100–1300 m asl are without soil cover, and only 7% of the area is considered unproductive (Jordhøy et al., 2010). General information on the three regions is given in Table 1.

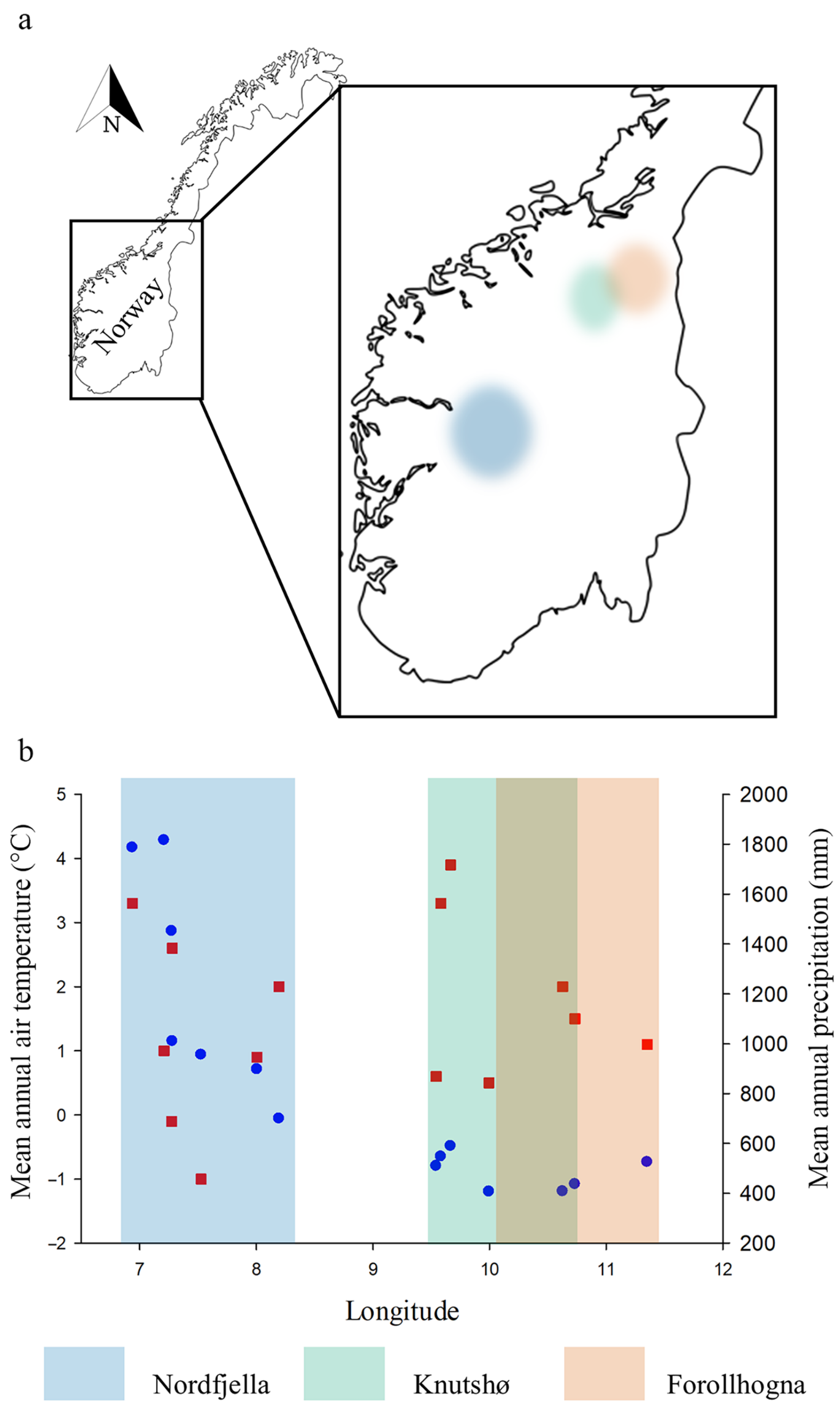

FIGURE 2 (a) The three study areas, Nordfjella, Knutshø, and Forollhogna, investigated in this study. (b) Mean annual air temperature (red squares) and mean annual precipitation (blue circles) by longitude. Meteorological data are from nearby meteorological stations (seklima.met.no). Only those above 480 m, providing both temperature and precipitation, were included.

Selection of salt lick sites

The locations of permanent salt lick sites in Nordfjella, Knutshø, and Forollhogna were provided by The Norwegian Food Safety Authority, Oppdal Bygdealmenning (rural commons), and rangers from The Norwegian Nature Inspectorate, respectively. In Nordfjella and Forollhogna, all permanent salt lick sites were documented, while in Knutshø, only information on the salt licks within Oppdal municipality was available. Not all salt lick sites were sampled; sites were chosen to get representative samples with respect to topography, vegetation, and altitude of the regions. Only salt lick sites in active use and with soil material close (within a 2-m radius) to the salt block were

TABLE 1 Information on the three study areas, location, elevation, number of grazing animals (here sheep and reindeers), and number of sites with permanent artificial salt blocks observed in 2017.

Area information	Nordfjella	Knutshø	Forollhogna
Location	60.5° N–61.0° N 7.2° E–8.7° E	62.2° N–62.7° N 9.6° E–10.7° E	62.4° N–62.9° N 10.0° E–11.3° E
Size (km^2)	3004	1776	1848
Elevation (m asl)	1100–1933	900–1690	800–1332
Reindeer population size in winter 2017	2500	1500	1700
Sheep on pasture in summer 2017	51,000	47,000	43,000
Sheep/reindeer ratio	23	31	25
Approximate no. salt licks in 2017	421	250[a]	195

[a]Based on numbers from pasture areas (in Oppdal municipality), housing 44% of the sheep population, that is, assuming a similar salt lick density relative to sheep density over the whole area.

chosen for the study. Control sites were chosen 200–500 m away from the corresponding salt lick site. The control sites should be edaphically similar to the salt lick site but without visible signs of frequent animal use.

Soil sampling

The soil sampling was done in the summer grazing season (i.e., from June to October) of 2018. For this study, we have 31 locations from Nordfjella, 6 from Knutshø, and 10 from Forollhogna, giving a total of 47 locations. Salt lick and control sites were sampled at each location; some locations were sampled twice during the growing season. The paired sampling of salt lick and control sites gave a total of 53×2, 12×2, and 19×2 soil samples from Nordfjella, Knutshø, and Forollhogna, respectively. Soil samples from the salt lick sites were collected according to the following procedure: a 2-m ruler was placed on the ground with one end against the pole holding the salt block and positioned so that it followed the main water flow direction. Two other rulers were placed at a 45° angle on the first ruler so that they were 90° to each other, with the first in the middle. Loose feces and debris were removed from the soil surface before the upper 2–5 cm of soil (10–15 g) was sampled every 20 cm along the rulers, starting 20 cm from the salt lick and stopping at the 2-m mark. If stones or solid rock prevented sampling at a sampling point, additional sampling points were made every 20 cm until 10 subsamples were obtained. The resulting 30 subsamples were gathered in a plastic container, making up approximately 500 mL of soil. The subsamples were thoroughly mixed, and the container was closed with an airtight lid. The soil samples were either air-dried immediately upon return to the laboratory or kept moist in a fridge or freezer until air-drying. The control sites were sampled in comparable amounts and similar soil depths as at the salt lick sites. All soil samples were sieved through 2-mm sieves and split into three subsamples: one for standard soil analysis, one for parasite analysis, and one was kept for prion analysis (not part of this study).

Soil analysis

All soil analyses followed the procedure of Van Reeuwijk (2002), with the exceptions, modifications, and instruments stated below. Loss on ignition (LoI) at 550 ± 25°C was converted to SOM after correcting for clay-bound water (Van Reeuwijk, 2002), which for most samples did not imply any correction due to very low clay content. The particle size distribution of the mineral soil was determined for all samples with less than 20% LoI using the pipette method; only the three major particle sizes are reported: clay (<2 μm), silt (2 μm–0.06 mm), and sand (0.06–2 mm). Due to limited sample size, electric conductivity (EC) and pH were measured in the same 1:2.5 v/v ratio of soil to distilled water suspension, EC was measured first using a conductometer (Metrohm 712, Metrohm, Herisau, Switzerland), and thereafter pH was measured using a glass membrane combination electrode (ORION SA 720 pH/ISE meter). Water-extractable phosphate (PO_4-P) was measured using a spectrophotometer (Agilent UV–Vis spectrophotometer, model Cary 60) while water-soluble chlorine (Cl), iodine (I), and sulphate (SO_4-S) were measured by ion chromatography (IC) using a Zellweger analytics Lachat IC 5000. Inorganic nitrogen was extracted using a 1 M KCl solution. The nitrate (NO_3-N) and ammonium (NH_4-N) nitrogen in the extracts were analyzed by flow injection analysis using a FIAStar 5000 analyzer. In this study, we refer to inorganic nitrogen (Inorg-N) which is the sum of NO_3-N and NH_4-N. Exchangeable cations were extracted using a buffered ammonia acetate solution (NH_4oAc) at pH 7. Cations, except for acidity, were analyzed using inductively coupled plasma mass spectrometry ICP-MS (Agilent 8800 Triple Quadrupole). Determination of extractable acidity was done by titrating the percolate back to pH 7.00 by use of NaOH. The sum of the calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), sodium (Na^+), and exchangeable acidity (exchangeable H^+) was calculated and represents the soil's potential cation exchange capacity (CEC). These were also used in the calculation of the base saturation (BS%) where the sum of nonacid cations is presented as the percentage of the CEC. Ammonium acetate (NH_4oAc) at pH 7 was also used to extract the more bioavailable copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), and cobalt (Co), analyzed using ICP-MS (Agilent 8800 Triple Quadrupole). Total carbon (Tot-C) and total nitrogen (Tot-N) were measured by dry combustion using a LECO TruSpec CNH analyzer. Active amorphous iron (Fe_{ox}), aluminum (Al_{ox}), and manganese (Mn_{ox}) and oxide-associated phosphate (P_{ox}) were extracted by acid ammonium oxalate (pH 3.5) and measured using ICP-MS (Agilent 8800 Triple Quadrupole). The decrease in phosphorus saturation (DPS) was calculated according to Kleinman (2017) using the following equation: $DPS = [(P_{ox} - PO_4\text{-}P)\ mmol/0.5 \times (Al_{ox}\ mmol + Fe_{ox}\ mmol)] \times 100$. Water-soluble P ($PO_4$-P) was subtracted from P_{ox} to ensure that the P used in the DPS index represented the more stable P.

Parasite analysis

DNA metabarcoding was conducted only on selected soil samples due to limited resources as part of a larger study examining gastrointestinal nematode diversity in the soils surrounding salt licks (Utaaker et al., 2023; NCBI SRA Accession No. PRJNA911771). Briefly, DNA was isolated from 15 mL of sieved soil using the FastDNA Spin Kit for Soil tube (MP Biomedicals, Germany) and the internal transcribed spacer (ITS2) 2 region of ribosomal DNA (rDNA) was amplified using the NC1 and NC2 primer set, which targets the Clade V group of parasitic nematodes (Gasser et al., 1993). Samples were indexed using IDT for Illumina DNA/RNA UD Indexes, normalized using a SequalPrep Normalization Plate, pooled in equal volumes, and sequenced in a paired-end 300-bp run on the Illumina MiSeq sequencing platform at the Genomics Core Facility (GCF), Norwegian University of Science and Technology (NTNU), Trondheim, Norway. Sequences were quality filtered, error corrected, merged, and chimeric sequences were removed using DADA2 (Callahan et al., 2016) and the parameters specified in Utaaker et al. (2023) before taxonomic assignment against the Nemabiome database (www.nemabiome.ca) as described in Utaaker et al. (2023). For each area, parasitic nematode species richness was estimated by taking the sum of nematode species found in all samples of salt lick soil or control sites, respectively, and dividing it by the number of observations.

Statistics

Statistical analyses were conducted using the statistical package R (version 4.3.2) (R Development Core Team, 2022). Linear mixed-effects models were fitted using the R extension package lme4 (Bates et al., 2015) with random effects for each location with paired soil samples from salt lick and control sites to account for edaphic differences and correlations within locations due to repeated measurements. Fixed effects included area (three levels—Nordfjella, Knutshø, and Forollhogna) and site (two levels—salt lick and control) for comparisons of soil properties at salt lick and control sites within and between areas, and longitude and site for assessing area differences in soil properties. Following a model reduction using backward stepwise elimination of nonsignificant terms, comparisons of means with adjustment of *p* values for multiple comparisons were conducted using the R extension package lsmeans (Lenth, 2016). Model checking was based on visual inspection of residual and QQ plots. The data were log-transformed when it did not exhibit a normal distribution.

RESULTS

Comparisons of soil properties at salt lick and control sites

The soils at the salt lick sites were heavily influenced by the dissolving salt block. The EC, Na, Cl, Ca, Mg, I, and Zn concentrations (Table 2) were all significantly higher at the salt licks compared with the control sites. There was significantly more Mn at the salt lick sites at Nordfjella and Forollhogna, but there were no differences between the salt lick and control sites at Knutshø (Table 2). The concentration of cobalt (Co) was generally below the detection limit (85% of the samples) and there were no significant differences between the salt lick and the control sites (results not shown). Control sites had significantly higher Fe content than the salt lick sites in all three areas. An indirect effect of the increased EC and the solution of carbonates from the salt block was a significant increase in soil pH at the salt lick sites (Table 3).

TABLE 2 Summary statistics for paired control (Con) and salt lick (Salt) sites (mean and SE).

Location	Site type	Mean	SE	S	R
EC (mS cm^{-1})[a]					
Nordfjella	Con	0.10	0.01	a	B
	Salt	0.58	0.07	b	*A*
Knutshø	Con	0.09	0.02	a	B
	Salt	2.22	0.53	b	*B*
Forollhogna	Con	0.03	0.01	a	A
	Salt	0.96	0.18	b	*A*
Na (mg kg^{-1})[a]					
Nordfjella	Con	25.7	3.60	a	B
	Salt	308.5	43.30	b	*A*
Knutshø	Con	33.4	9.8	a	B
	Salt	1426.2	419.8	b	*B*
Forollhogna	Con	8.4	2.0	a	A
	Salt	865.7	202.5	b	*A*
Cl (mg kg^{-1})[a]					
Nordfjella	Con	27.2	5.2	a	B
	Salt	149.1	28.2	b	*A*
Knutshø	Con	19.9	7.9	a	AB
	Salt	2101.3	837.1	b	*C*
Forollhogna	Con	6.5	2.1	a	A
	Salt	552.1	174.7	b	*B*
Ca (mg kg^{-1})[a]					
Nordfjella	Con	690.2	93.1	a	B
	Salt	1257.7	169.6	b	*B*
Knutshø	Con	1112.9	324.4	a	B
	Salt	2624.4	764.9	b	*B*
Forollhogna	Con	105.3	24.2	a	A
	Salt	499.5	114.6	b	*A*
Mg (mg kg^{-1})[a]					
Nordfjella	Con	165.2	18.88	a	B
	Salt	414.7	47.4	b	*B*
Knutshø	Con	240.1	54.2	a	B
	Salt	602.7	136.0	b	*B*
Forollhogna	Con	40.2	7.3	a	A
	Salt	101.0	18.2	b	*A*
K (mg kg^{-1})[a]					
Nordfjella	Con	210.4	22.9	a	B
	Salt	1027.5	111.9	b	*B*
Knutshø	Con	289.1	59.7	a	B
	Salt	1411.7	291.6	b	*B*
Forollhogna	Con	87.4	14.6	a	A
	Salt	426.7	71.2	b	*A*
SO_4-S (mg kg^{-1})[a]					
Nordfjella	Con	30.1	3.5	a	B
	Salt	55.0	6.4	b	*A*
Knutshø	Con	22.3	5.6	a	AB
	Salt	113.3	28.2	b	*B*
Forollhogna	Con	11.2	2.2	a	A
	Salt	35.9	7.1	b	*A*
I (μg kg^{-1})[a]					
Nordfjella	Con	51.86	4.92	a	A
	Salt	94.20	8.94	b	*A*
Knutshø	Con	35.84	7.23	a	A
	Salt	351.03	70.80	b	*B*
Forollhogna	Con	46.76	7.46	a	A
	Salt	126.12	20.13	b	*A*
Mn (mg kg^{-1})[a]					
Nordfjella	Con	39.3	6.9	a	B
	Salt	96.2	16.9	b	*B*
Knutshø	Con	61.8	23.6	a	B
	Salt	112.1	42.8	a	*B*
Forollhogna	Con	6.2	1.9	a	A
	Salt	33.6	10.1	b	*A*
Cu (mg kg^{-1})[a]					
Nordfjella	Con	1.17	0.11	a	B
	Salt	1.17	0.11	a	*B*
Knutshø	Con	0.62	0.13	a	A
	Salt	0.62	0.13	a	*A*
Forollhogna	Con	0.35	0.06	a	A
	Salt	0.35	0.06	a	*A*
Fe (mg kg^{-1})[a]					
Nordfjella	Con	8.41	0.83	b	A
	Salt	6.08	0.60	a	*A*
Knutshø	Con	8.41	0.83	b	A
	Salt	6.08	0.60	a	*A*
Forollhogna	Con	8.41	0.83	b	A
	Salt	6.08	0.60	a	*A*
Zn (mg kg^{-1})[a]					
Nordfjella	Con	5.59	0.77	a	B
	Salt	11.10	1.54	b	*B*
Knutshø	Con	2.97	0.88	a	B
	Salt	7.19	2.14	b	*AB*
Forollhogna	Con	0.56	0.13	a	A
	Salt	3.54	0.83	b	*A*

(Continues)

TABLE 2 (Continued)

Note: $n = 53, 12, 19$ for Nordfjella, Knutshø, and Forollhogna, respectively. Lowercase letters indicate differences between control and salt lick sites within each area (S), uppercase non-italic letters indicate differences between areas (R) for the control sites, and uppercase italic letters indicate differences between areas for the salt lick sites. Different letters indicate differences at a level of significance $p < 0.05$.

Abbreviation: EC, electric conductivity.

[a]Statistics were done on log-transformed data.

TABLE 3 Summary statistics for paired control (Con) and salt lick (Salt) sites (mean and SE).

Location	Site type	Mean	SE	S	R
SOM (%)					
Nordfjella	Con	29.26	2.34	b	B
	Salt	20.31	2.34	a	*B*
Knutshø	Con	30.17	5.05	b	B
	Salt	15.88	5.05	a	*AB*
Forollhogna	Con	8.02	3.98	a	A
	Salt	8.50	3.98	a	*A*
pH					
Nordfjella	Con	4.75	0.08	a	A
	Salt	6.22	0.08	b	*A*
Knutshø	Con	5.16	0.18	a	A
	Salt	7.04	0.18	b	*B*
Forollhogna	Con	5.11	0.14	a	A
	Salt	6.18	0.14	b	*A*
CEC ($cmol_{(+)}$ kg^{-1})[a]					
Nordfjella	Con	32.64	2.62	b	B
	Salt	25.16	2.02	a	*B*
Knutshø	Con	21.90	3.78	a	B
	Salt	35.47	6.12	b	*B*
Forollhogna	Con	10.61	1.44	a	A
	Salt	13.66	1.86	a	*A*
BS (%)					
Nordfjella	Con	22.6	2.96	a	A
	Salt	62.5	2.96	b	*A*
Knutshø	Con	47.6	6.27	a	B
	Salt	95.1	6.27	b	*B*
Forollhogna	Con	11.8	4.97	a	A
	Salt	72.0	4.97	b	*A*
Al_{ox} (g kg^{-1})					
Nordfjella	Con	1.84	0.15	a	A
	Salt	1.84	0.15	a	*A*
Knutshø	Con	1.56	0.31	a	A
	Salt	1.56	0.31	a	*A*
Forollhogna	Con	2.62	0.25	a	B
	Salt	2.62	0.25	a	*B*
Fe_{ox} (g kg^{-1})					
Nordfjella	Con	3.86	0.26	a	A
	Salt	3.86	0.26	a	*A*
Knutshø	Con	3.09	0.57	a	A
	Salt	3.09	0.57	a	*A*
Forollhogna	Con	5.46	0.44	a	B
	Salt	5.46	0.44	a	*B*
Mn_{ox} (g kg^{-1})[a]					
Nordfjella	Con	0.08	0.01	a	A
	Salt	0.25	0.05	b	*A*

(Continues)

TABLE 3 (Continued)

Location	Site type	Mean	SE	S	R
Knutshø	Con	0.11	0.05	a	A
	Salt	0.20	0.08	a	*A*
Forollhogna	Con	0.07	0.02	a	A
	Salt	0.11	0.03	a	*A*
P_{ox} (g kg^{-1})[a]					
Nordfjella	Con	0.56	0.05	a	B
	Salt	0.81	0.07	b	*B*
Knutshø	Con	0.45	0.08	a	B
	Salt	0.64	0.12	b	*B*
Forollhogna	Con	0.21	0.03	a	A
	Salt	0.30	0.04	b	*A*
PO_4-P (mg kg^{-1})[a]					
Nordfjella	Con	8.75	2.16	a	B
	Salt	18.05	4.46	b	*B*
Knutshø	Con	6.97	3.68	a	B
	Salt	23.48	12.41	b	*B*
Forollhogna	Con	0.25	0.11	a	A
	Salt	2.68	1.12	b	*A*
DPS (%)†					
Nordfjella	Con	27.46	2.44	a	B
	Salt	40.36	3.60	b	*B*
Knutshø	Con	24.18	4.54	a	B
	Salt	35.54	6.68	b	*B*
Forollhogna	Con	7.32	1.08	a	A
	Salt	10.76	1.59	b	*A*
C/N ratio					
Nordfjella	Con	22.2	0.74	b	A
	Salt	15.9	0.74	a	*A*
Knutshø	Con	22.2	0.74	b	A
	Salt	15.9	0.74	a	*A*
Forollhogna	Con	22.2	0.74	b	A
	Salt	15.9	0.74	a	*A*
Inorg-N (mg kg^{-1})[a]					
Nordfjella	Con	15.78	2.52	a	B
	Salt	144.45	23.07	b	*B*
Knutshø	Con	12.73	4.04	a	AB
	Salt	116.49	36.99	b	*AB*
Forollhogna	Con	6.17	1.56	a	A
	Salt	56.43	14.31	b	*A*

Note: $n = 53, 12, 19$ for Nordfjella, Knutshø, and Forollhogna, respectively. Lowercase letters indicate differences between control and salt lick sites within each area (S), uppercase non-italic letters indicate differences between areas (R) for the control sites, and uppercase italic letters indicate differences between areas for the salt lick sites. Different letters indicate differences at a level of significance $p < 0.05$.

Abbreviations: BS, base saturation; CEC, cation exchange capacity; DPS, degree of phosphorus saturation; SOM, soil organic matter.

[a]Statistics were done on log-transformed data.

This increase in pH, together with the increase in base cations from the salt blocks themselves, contributed to a significant increase in BS at the salt lick sites compared with the control sites (Table 3).

The increase in pH will generally cause an increase in CEC, but only the Knutshø site shows significantly higher CEC at the salt lick sites (Table 3). For Nordfjella, the control site showed significantly higher CEC compared with the salt lick sites, but Nordfjella was also the area with the least difference in EC between salt lick and control sites (Table 3). There was significantly lower SOM content at the salt lick sites in Nordfjella and Knutshø compared with their respective control sites, but this was not the case for Forollhogna (Table 3). Manganese extracted with oxalate (Mn_{ox}) was significantly higher at the salt lick sites compared with the control sites at Nordfjella; this was not observed in Knutshø and Forollhogna. Oxalate extractable phosphorus (P_{ox}), however, shows significantly higher concentrations at the salt lick compared with the control sites for all areas (Table 3).

The salt lick sites were also significantly different from the control sites regarding properties related to more intense animal activity. The most obvious sign of animal activity was the high frequency of nematode parasite DNA in the salt lick soil compared with the control sites (Figure 3). Nematode species DNA analysis showed that nematode species richness was higher in Nordfjella and Knutshø ($n = 10$) than in Forollhogna ($n = 5$) Table 4. The most nematode parasitic DNA per sample was found in Knutshø, as indicated (Figure 3). The chemical properties are also consistent with higher animal activity at the salt lick sites. The salt lick soils had significantly higher concentrations of elements associated with urine and feces such as ammonium acetate extractable K, water-soluble sulphate (SO_4-S), water-soluble phosphate (PO_4-P), and inorganic nitrogen (Inorg-N) (Tables 2 and 3). Salt lick soil C/N ratios were significantly lower than control samples, and the phosphorus saturation index (DPS) was significantly higher at the salt licks (Table 3).

Soil texture did not significantly differ between the salt lick sites and the control sites in any of the regions (Table 5). Texture analysis was only performed on 104 of the 168 samples in this study, since no particle size analyses were done on samples with high SOM (>20%) content. However, the analysis shows that the mineral soils had a low clay content and a high sand content for both salt lick and control sites, with very little difference between the areas (Table 5). The ratio of clay to SOM was also low; only eight samples (seven in Forollhogna and one in Nordfjella) had ratios above 1. There were no significant differences between salt lick and control sites with respect to clay to SOM ratios.

Regional differences in soil properties at salt lick and control sites

The SOM content in the soil showed a significant ($p < 0.001$) decrease with longitude (Figure 4a, Table 3, which did not differ between control and salt lick sites; $p = 0.215$). For pH, we found no significant relationship with longitude ($p = 0.057$; Table 2 Figure 4b). For EC, there was a significant ($p < 0.001$) increase for the salt

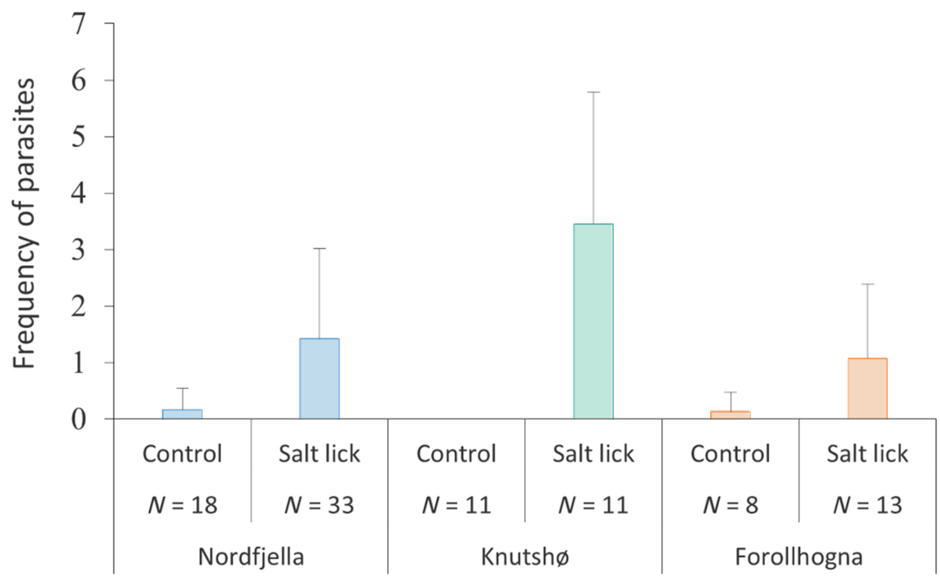

FIGURE 3 Frequency of parasite DNA observations (no./site) at the salt lick and control sites for the three areas: Nordfjella, Knusthø, and Forollhogna (mean + SD).

TABLE 4 Species of nematodes found in the soil at the salt lick and control sites for all three areas: Nordfjella, Knusthø, and Forollhogna.

Nematode species DNA	Nordfjella		Knutshø		Forollhogna	
	Salt lick ($n = 20$)	Control ($n = 19$)	Salt lick ($n = 11$)	Control ($n = 10$)	Salt lick ($n = 8$)	Control ($n = 7$)
Chabertia ovina	1	...	3		1	
Haemonchus contortus	3					
Haemonchus sp. 1	2					
Haemonchus sp. 2	1					
Muellerius capillaris			1			
Nematodirella longissimespiculata			1			
Nematodirus battus	14	2	11		4	1
Nematodirus sp. 1		1				
Oesophagostomum venulosum	1					
Teladorsagia circumcincta	10		11		5	
Teladorsagia sp. 1	1					
Trichostrongylus colubriformis			2			
Trichostrongylus sp. 1	1		3		1	
Trichostrongylus sp. 2			4		1	
Trichostrongylus sp. 3	1		2			
Trichostrongylus sp. 5			1			
No. species	10	2	10	0	5	1
No. observations	35	3	39	0	12	1
Observations/samples	1.7	0.15	3.5	0	1.5	0.14

Note: n = number of samples analyzed. Empty cells indicate species were not detected.

TABLE 5 Median particle size distribution (in percentage) with the range (minimum–maximum) in parentheses for each of the three mountain areas and categorized in salt lick and control sites.

Particle size category	Nordfjella		Knutshø		Forollhogna	
	Salt lick ($N = 34$)	Control ($N = 19$)	Salt lick ($N = 9$)	Control ($N = 6$)	Salt lick ($N = 18$)	Control ($N = 18$)
Clay[a]	3 (1–13)	1 (1–5)	4 (4–5)	3 (2–5)	3 (1–13)	3 (0–13)
Silt[a]	35 (1–65)	37 (19–78)	49 (40–70)	40 (36–53)	33 (6–61)	37 (11–61)
Sand[a]	62 (33–86)	61 (18–80)	46 (26–57)	57 (42–61)	64 (33–81)	58 (36–85)
Clay/SOM[b]	0.3 (0.1–1.0)	0.1 (0.1–0.8)	0.3 (0.2–0.8)	0.3 (0.1–0.5)	0.5 (0.1–1.3)	0.4 (0.1–1.2)

Abbreviation: SOM, soil organic matter.
[a]Percentage in fine earth fraction.
[b]Ratio by mass percentage.

lick sites with longitude, while there was a significant ($p < 0.001$) decrease with longitude for control sites (Figure 4c). Na and Cl showed the same trend with longitude as EC. Zinc (Zn) also showed a significant decrease with longitude for both salt lick and control sites ($p < 0.001$); however, the decrease was significantly ($p < 0.001$) lower for salt lick sites (Figure 4d). Knutshø appeared to be the area most severely influenced by the salt blocks, having significantly higher EC, Na, Cl, I, pH, and BS% compared with Forollhogna and Nordfjella (Tables 2 and 3). For Mn, there was a significant ($p < 0.001$) decrease in Mn concentrations with longitude for both salt lick and control sites. Nordfjella and Knutshø had significantly higher Mn concentrations both

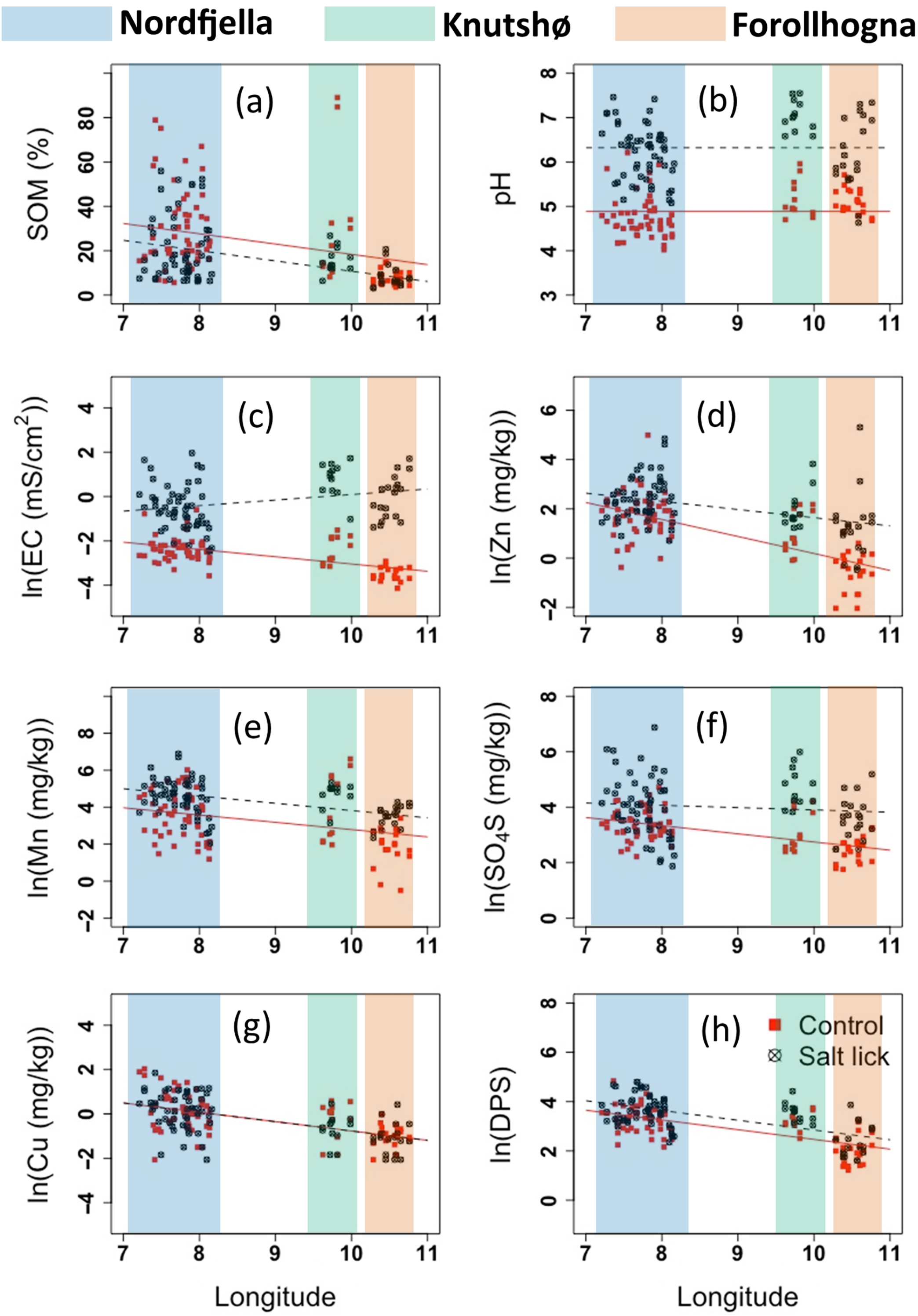

FIGURE 4 Relationship between longitude and soil properties at the salt lick and control sites for Nordfjella, Knutshø, and Forollhogna. Regression lines (dashed blue for salt lick and solid red for control) are based on linear mixed-effects models with random effects reflecting the edaphic differences between the three locations. The regression equations for each model are presented in Appendix S1: Table S2. SOM, soil organic matter. EC, electric conductivity.

in control and salt lick sites compared with Forollhogna (Table 2). For water-soluble SO_4-S, there was a significant ($p < 0.001$) decrease with longitude for control sites, but not for salt lick sites ($p = 0.531$; Figure 4f). Also, here, Knutshø deviated from the two other areas, as the salt lick sites here had significantly higher SO_4-S concentrations than both Nordfjella and Forollhogna (Table 2). Copper (Cu) concentrations were higher at both salt lick and control sites in Nordfjella compared with Knutshø and Forollhogna (Table 2) and there was a significant negative relationship ($p < 0.001$) between Cu concentrations and longitude (Figure 4g), but no differences between salt licks and control sites. Nordfjella and Knutshø exhibited significantly higher K, P_{ox}, PO_4-P, DPS, and Inorg-N at both salt lick and control sites compared with Forollhogna (Tables 2 and 3). There was a significant ($p = 0.0001$) decrease in phosphorus saturation (DPS) with longitude for both salt lick and control (Figure 4h).

Our sampling scheme did not allow for a detailed study of seasonal variation in soil properties, but at Nordfjella, some locations were sampled several times during the season. For Mn, there was a large variation both for control and salt lick sites, spanning more than 100 mg kg^{-1} for control sites and more than 150 mg kg^{-1} for salt licks (Figure 5). The concentrations were lowest in the drier summer months and highest in spring and late autumn. Though the concentrations were lower, there was also seasonal variation in soil Cu concentrations (Figure 5).

DISCUSSION

Soil chemistry and parasite compositions were changed at salt lick sites in all three areas studied. We attribute these changes to two factors: first, an increase in salt-related elements following leaching and fragmentation from the salt block to the soil (Table 2), as the changes in soil composition partly reflect the composition of the dissolving salt block (see Appendix S1: Table S1 for element composition of salt blocks). Second, increased animal activity in the area around the salt block causes erosion, compaction, and additions of parasites and elements originating from urine and feces (Tables 3 and 4, Figure 3). The persistence of CWD prions in soils and the likelihood that the prion will be transmitted to hosts depend on a suite of soil conditions and host presence, each of which we found to be strongly affected by salt lick use across diverse climatic and geographic regions (Figure 4a–h). These regional differences may be important when considering the risk for future transmission of CWD to currently unaffected areas.

How can increased salt in the soil influence the persistence and transmission of prions?

Our analyses demonstrate higher pH in soils sampled from salt lick sites across the studied regions. Several studies (Dorak et al., 2017; Imrie et al., 2009) have indicated that CWD is more prevalent in areas with higher soil pH. In Northern Illinois, Dorak et al. (2017) found a persistent presence of CWD in areas with soils having a pH higher than 6.6 and clay content <18%. A third of the salt lick and none of the paired control soils investigated in our study fit these criteria; all sites have <18% clay, leaving pH >6.6 as the main criteria (Table 3). The distinct increase in pH at the salt lick sites is an indirect effect of the dissolving salt block, the combined effect of dissolving NaCl and the liming effect of the dissolving

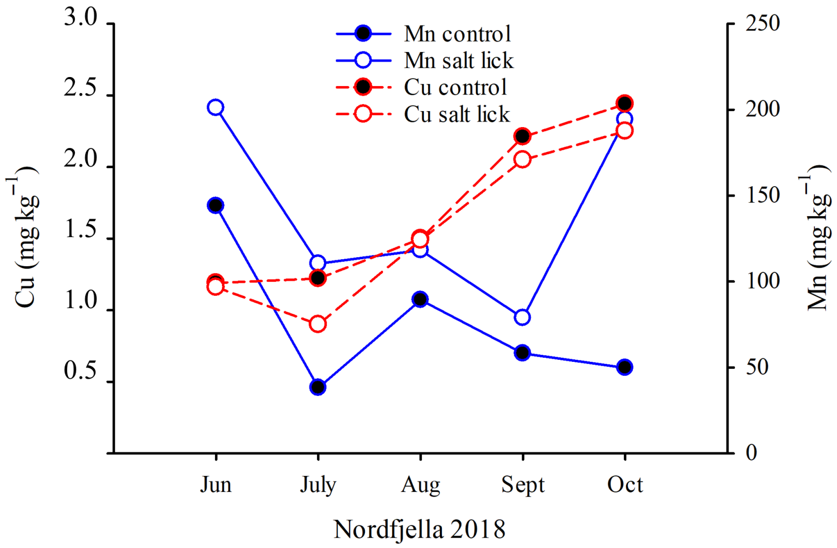

FIGURE 5 Seasonal variation in NH_4OAc extractable copper (Cu) and manganese (Mn) for Nordfjella, presented as the arithmetic mean of the salt lick site and control sites. The number of samples varied between 8 and 21 per site and month.

carbonates. Dorak et al. (2017) suggested that the elevated pH >6.6 represented the isoelectric point (IEP) of the CWD prion based on findings for scrapie and CWD prions (Bolton et al., 1985; Ma et al., 2007). Prions become more negatively charged with increasing pH (e.g., Jacobson et al., 2013). The negative surface charge of clay and SOM also increases with increasing pH, causing generally repulsive conditions between soil and prions. This suggests that there should be more unbound or weakly bound prions at sites with high pH. However, despite an increase in pH, we found no consistent increase in the CEC at the salt lick sites (Table 3). The pH and EC were significantly positively correlated ($r = 0.75$, $p < 0.0001$). It is generally believed that high pH and high ionic strength/EC may alter prion interactions with and persistence in soil by increasing prion aggregate size, hydrophobicity, as well as charge (Jacobson et al., 2013; Ma et al., 2007; Saunders et al., 2011).

Our results also demonstrate greater salt concentrations in soils sampled from salt lick sites across the studied areas. High salt concentration may induce the formation of prions with a more protease-resistant conformation (Bartelt-Hunt et al., 2012; Concha-Marambio et al., 2014; Nishina et al., 2004). The high salt concentration observed at our salt lick sites may also cause more hygroscopic properties in the soil, leading to more constant moist soil conditions which again may promote prion persistence (Bartelt-Hunt et al., 2012; Charlet et al., 2007; Yuan et al., 2015). The increased salt content may also leave the soil with adhering prions more susceptible to erosion and thereby spreading prions with surface runoff (Nichols et al., 2009). This may reduce the prion concentrations at the salt lick sites and thereby reduce the risk of infecting animals congregating here, but it also implies exposing larger areas to prions. The prions following the eroded soil particles may aggregate in nearby landscape depressions, in ponds where animals quench their thirst.

Several elements introduced by the dissolving salt blocks may influence both persistence and likelihood of prion transmission to animals. In particular, high concentrations of Mn and Zn are suggested to influence prion conformation, increase aggregation, increase protease resistance, and affect how the prions interact with and adsorb to soil surfaces (Brown, 2017; Charlet et al., 2007; Polano et al., 2008; Rana et al., 2009). We found high levels of Mn at salt lick sites, levels similar to the concentrations found in areas prone to scrapie (Charlet et al., 2007; Ragnarsdottir & Hawkins, 2006). According to Davies and Brown (2009) high Mn concentrations may increase the persistence of prions in soil by almost 100-fold when compared with soil with low concentrations, but it should be noted that they used recombinant prion proteins in their study, and these could behave very differently from prions (Jacobson et al., 2013). However, if Mn does have the suggested effect, prions may be more likely to persist at the salt lick sites than at the control sites. There are many studies showing anomalies regarding Cu and Mn in the brains and lymph nodes of prion-infected animals (Rivera et al., 2015; White et al., 2010; Wolfe et al., 2010). However, few studies have found any significant connection with soil contents of these trace elements (Chihota et al., 2004; Imrie et al., 2009).

Some of the elements supplied through artificial salt blocks may also alleviate prion disease development. In a study comparing areas with and without CWD, Nichols et al. (2016) found that CWD-positive sites had a significantly lower concentration of Mg and a lower Mg/Cu ratio in the water samples compared with CWD-negative sites, though no such differences were found in corresponding soil samples. Following these results, they fed prion-infected mice with water containing different Mg and Mg/Cu ratios and concluded that a higher Mg/Cu ratio in feed might give significantly longer prion disease survival times (Nichols et al., 2016). Iodine is another element that may contribute to alleviating prion disease development, as Imrie et al. (2009) found scrapie and BSE to be more prevalent in areas with low levels of iodine. The salt lick soils in our study have significantly higher Mg and iodine than the control site soils, and geophagia of salt lick soil could therefore increase Mg and iodine, potentially affecting prion disease progression (Table 2).

How can increased animal activity at the salt lick sites influence the persistence and transmission of prions?

Increased animal activity at salt lick sites was observed through wildlife cameras (Figure 1). Our soil samples confirm increased animal activity at the salt lick sites through loss of SOM in the topsoil, an increase in elements commonly associated with urine and feces, and a higher occurrence of parasites compared with the control sites (Tables 3 and 4, Figure 3).

The lower SOM content at the salt lick sites we ascribe to an increase in erosion caused by trampling; similar erosion has been observed in several studies (Tuomi et al., 2021; Walters & DeLuca, 2007). The role of SOM with regard to prion persistence and transmission is unclear and dependent on SOM quantity, quality, and relationship to microbial activity. SOM may contribute to prion persistence in soil through electrostatic binding and encapsulation; however, humic acids formed by decomposing SOM may degrade prions, and humic substances may hinder the prion replication process and reduce prion infectivity (Bartelt-Hunt et al., 2012; Giachin et al., 2014; Johnson et al., 2011; Kuznetsova et al., 2018; Saunders et al., 2008). The lower C/N ratio of the SOM at salt lick sites suggests more humified SOM than at the control sites (Table 3). Giachin et al. (2017) found that SOM may interact with prions without altering the protein structures, encapsulate them, and thereby prevent degradation while simultaneously reducing bioavailability. The effects of both organic material and soil microorganisms on the fate of prions are, however, understudied. The huge complexity of SOM and the risk of interference with the detection of prions make interpretation difficult (Kuznetsova et al., 2018; Pucci et al., 2008; Smith et al., 2014).

Erosion will also expose mineral soil, allowing it to come in more immediate contact with shed prions. Clay content and clay mineralogy are among the soil properties emphasized as most important for environmental prion persistence and transmissibility (Revault et al., 2005; Wyckoff et al., 2016). Walter et al. (2011) analyzed soil data from two CWD-infected areas in northern Colorado, USA, and found that a 1% increase in clay content increased the odds of CWD infection by between 4% and 9% in areas where the clay content varies from 0% to 38%; the clay in their study was mostly smectite. In our study, the soils were coarse textured; the clay percentage never exceeded 13% (Table 5). The clay mineralogy in Norway is dominated by fine-grained mica with hardly any smectites (Gjems, 1967). This implies that clay content and clay mineralogy may be less important for environmental persistence and transmission of CWD in the Norwegian mountain areas compared with North American studies (Dorak et al., 2017; Kuznetsova et al., 2014; Walter et al., 2011; Wyckoff et al., 2016). Johnson et al. (2007) found that soils with a Clay/SOM ratio higher than >1:1 tended to enhance oral transmission of prions. Our soils generally have low clay/SOM ratios, and though the salt lick soils have higher ratios than the control site soils (Table 5), the difference is not significant. This suggests that none of the soils in this study should enhance oral transmissibility of prions.

There were significantly more parasites in the soils at the salt licks compared with control sites (Figure 3). Higher species richness may indicate multiple host species visiting the salt licks, or more diverse nematode fauna of grazing ruminants in certain areas (Table 4). Many of the parasite species identified can affect both wild and domestic ruminants. The most prevalent nematode DNA belonged to *Nematodirus battus* and *Teladorsagia circumcincta*; these are commonly associated with sheep but have also been found in Norwegian wild reindeer (Bye, 1987; Robertsen, 2020; Utaaker et al., 2023) highlighting the risk of spillover of parasites in all three areas. Most of the other genera identified are commonly found in domestic ruminants, though these parasites are generally promiscuous regarding host specificity (Walker & Morgan, 2014). Given the proportional relation between the number of sheep and reindeer in the areas (Table 1), the dominance of parasites from domestic animals is not surprising, and though this makes host identification elusive, it underscores the potential for disease transmission at salt lick sites. Regardless of host species, the parasitological findings clearly illustrate that animals defecate more frequently at salt lick sites than at control sites, indicating higher activity and longer time spent around salt licks.

The large increases in inorganic N, water-extractable PO_4-P and SO_4-S, and exchangeable K at the salt licks suggest increased addition of urine and feces from the visiting animals (Table 3; Barrow & Lambourne, 1962; Shand & Coutts, 2006). A side effect of the urine is an increase in soil pH resulting from the hydrolysis of urea (Shand & Coutts, 2006) amplifying the pH effect of the dissolving salt block. The low C/N ratios at the salt lick sites are related to the high input of inorganic N, but they also reflect more decomposed SOM following long-term animal activity at the salt lick sites (Table 3). The phosphorus saturation index (DPS) expresses the long-term sorption of P, particularly in acidic soils. The increase in DPS suggests that these salt lick sites have been used by animals over an extended period of time (Table 3). The fertilizing effect of urine and feces at the salt lick site may provide attractive grazing areas even after a site is abandoned as a salt lick site. Plants grown in prion-contaminated soils may accumulate prions, and the plants can act as carriers of infectivity (Pritzkow et al., 2015).

As the salt seeps from the salt block to the soil, the salt-enriched soil may become attractive for geophagia (Dalke et al., 1965; Walters & DeLuca, 2007). Through geophagia, the animals could also ingest soil-bound prions as well as parasites and potentially harmful elements. In an area with CWD infection among elk and mule deer in north-central Colorado, Lavelle et al. (2014) suggested that though the animals' focal intake mineral lick sites were Na, there may also be an incidental abnormally high intake of Mn, which may influence prion disease development negatively. The salt lick sites in both Nordfjella and Forollhogna had significantly higher Mn, suggesting that this may be a risk in the event of geophagia in these areas (Table 2). Deficiency of P has been suggested as one of the possible reasons for the observed antler-gnawing behavior in the Nordfjella wild reindeer population (Mysterud et al., 2020). The higher P content at the salt lick sites may function as an attractant and trigger geophagia among reindeer at Nordfjella (Table 3).

Regional differences that may influence persistence and transmission of prion infectivity

The differences between salt lick and control sites for salt-related properties increased in a gradient from west to east (Figure 4c,d,f). We assume that these differences were mostly related to differences in precipitation, high precipitation in the west and lower precipitation in the east (Figure 2). Higher precipitation indicates a rapid loss of particularly sodium, as found by Thackston and Holbrook (1992) at artificial salt lick sites in Georgia, USA. They also observed that there was little use of the salt lick sites among the deer once the use of salt blocks ceased. A regional variation in precipitation quality may also cause differences in how the animals seek salt licks. Marine Na declines with distance from coastlines; thus, salt deprivation in ruminants and salt-seeking behaviors should increase with distance inland, as found by Maro and Dudley (2022). According to maps by Manders et al. (2010) there is a marked difference in Na deposition from sea salts among the areas in our study, more in Nordfjella and less in Knutshø and Forollhogna. Sulfate may also follow precipitation through both sea salt and anthropogenic deposition, but the amount of S added through these sources in our study areas is generally low and follows the same distribution pattern as Na (Barbu et al., 2009). These deposition gradients coincide with our finding (Figure 4c,f). The amount of SOM decreases from west to east (Figure 4a) also following the decrease in precipitation; this is a well-established relationship for Norwegian soils (e.g., Strand et al., 2016) reflecting wetter conditions in the west and drier in the east. These relationships suggest that salt lick sites in the eastern wild reindeer areas may pose a greater attraction to salt-seeking animals and stay so for a longer period compared with those in western areas.

Surface runoff may wash free, mineral-bound, or SOM-encapsulated prions to landscape depressions or to waterbodies (Nichols et al., 2009). Prions trapped in moist soil, frequently subjected to reducing conditions, may be better preserved (Bartelt-Hunt et al., 2012; Chapron et al., 2014; Charlet et al., 2007; Nichols et al., 2009; Yuan et al., 2015). Waterlogging and reducing conditions may cause increased solubility, changes in bioavailability and toxicity of many elements (e.g., Mn, Fe, Na, Al, and B) following changes in pH and redox potential (Rengasamy, 2010). These moist and wet soils may serve as a reservoir for the preservation and transmission of prions (Ragnarsdottir & Hawkins, 2006). Reducing and oxidizing conditions can change rapidly, and seasonal variation may influence the elements most sensitive to redox processes. Bioavailable Mn shows a temporal, seasonal variability (Figure 5) with higher values early and late in the season and lower values midsummer. The salt lick sites at Nordfjella had significantly higher Mn_{ox} concentrations as well as bioavailable Mn compared with the control sites (Tables 2 and 3) which may suggest that these sites were more prone to alternating redox conditions (Russo et al., 2009). The natural temperature range in the areas studied does not contribute directly to prion degradation since prions are not temperature sensitive (Taylor, 1999). However, cycles of drying and wetting along with freezing and thawing may reduce prion infectivity (Yuan et al., 2015, 2018) and these cycles will occur frequently in all areas.

CONCLUSIONS

The use of permanently placed artificial salt licks in Norwegian mountain grazing areas will likely create hotspots for CWD transmission, primarily because the salt lick sites facilitate the congregation of animals and thereby direct transmission of prions between animals. Elevated salt content at the salt lick sites, particularly high Na concentration, may increase geophagia of soil contaminated with excretions from other animals and thereby increase the probability of indirect transmission of prions. The increase in pH, change in ionic strength, and higher concentrations of Mn and Zn at the salt lick sites may all provide soil conditions that promote the persistence of prions in soil. Increased compaction and high Na concentration may destroy soil aggregates and increase hygroscopic soil properties, contributing to more moist soil conditions at the salt lick sites. Wet, anoxic soils may promote prion persistence. Erosion and surface runoff from the salt lick may disperse and remove the prions, thereby reducing the infection likelihood at the salt licks, but prions following eroded particles and surface runoff to lower landscape positions will also experience moister soil conditions that again promote persistence. Regional differences between the areas studied suggest that the influence of salt is strongest in areas with lower precipitation. This also suggests that the salt lick sites will lose their attraction as salt providers after salt blocks are removed, though how rapidly this will happen is dependent on leaching conditions: precipitation amounts, landscape, and soil properties. The legacy of the fertilizer effect from urine and feces at the salt lick sites will, however, linger, probably maintaining the salt lick sites as congregation spots for grazing animals despite the missing salt attraction.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Strand et al., 2025) are available from the Norwegian University of Life Sciences DataNO: https://doi.org/10.18710/U45OVZ.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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