Impact of grazing on carbon and nutrient cycling in a grass-encroached Scots pine forest
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Citation for published version (APA):
Smit, A. (2000). Impact of grazing on carbon and nutrient cycling in a grass-encroached Scots pine forest
Amsterdam: Universiteit van Amsterdam

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Download date: 27 Jan 2019
2 The impact of grazing on spatial variability of humus profile properties in a grass-encroached Scots pine ecosystem.

Published in Catena 36 (1999) 85–98 (the term 'plot-scale' has been substituted by 'site-scale')

Abstract
Grazing is often used in management of grass-encroached Scots pine (Pinus sylvestris) forests in The Netherlands, because it is thought to increase diversity of vegetation and humus profiles. To test this hypothesis, two 1 ha sites in a primary pine stand on drift sand were grazed for 7 years at high and moderate intensities. The humus profiles of these two sites and of a control site were sampled at various scales to assess changes in grass biomass, thickness of ectorganic horizons, organic matter stocks, root stocks, and pH. Whether grazed or not, site factors such as slope angle and aspect, distance to trees and parent material did not explain the spatial variability of the sites. Spatial variability could be linked to the tussock structure of the grass layer. With continued grazing, ectorganic horizons became more compact (particularly the F horizon), stocks of organic matter increased, grass biomass decreased, variances tended to decrease and ranges tended to increase. These results suggest that at the landscape scale, where much variation in grazing intensity can be expected, spatial variability of humus profile properties and thus site diversity will increase. However, grazing intensities in the experiment were far greater than those normally employed in management of grass-encroached forests. Therefore grazing will scarcely affect site diversity, unless grazing variability is enhanced by special treatments, such as temporary fencing to create locally high grazing intensities.

Keywords: spatial variability, humus form, grazing, organic matter, sampling strategy
**Introduction**

Grazing by large herbivores has become popular in forest management in The Netherlands, for example to counteract grass encroachment resulting from atmospheric deposition (Aerts, 1989; Heij and Schneider, 1991). Grazing usually leads to an increase in biodiversity, because it results in: (a) a more open vegetation structure and greater availability of light which favours shade-intolerant species (Kooijman and De Haan, 1995; Kobayashi et al., 1997) and (b) increased pattern diversity through interaction between spatial patterns initially present in the landscape and variation in livestock density and/or grazing intensity (Bakker, 1989).

One feature largely ignored in grazing studies is the humus profile. This is a serious omission, since this part of the soil profile forms an important link between the vegetation and abiotic properties of the ecosystem and regulates the availability of nutrients and moisture (Kimmins, 1987; Schaap et al., 1997). Grazing affects the humus profile through changes in litter input and composition (Pastor et al., 1993), but it may also influence soil temperature and moisture regime, which determine the rate of decomposition. Changes in the humus profile caused by grazing also exhibit spatial patterns, resulting from initial differences and variation in grazing intensity.

Spatial variability in humus profile properties can be considerable (Arp and Krause, 1984; Grigal et al., 1991). Emmer (1997) demonstrated that this even holds for mono-specific, even-aged stands of *Pinus sylvestris*. The causes of spatial variation are diverse and are related partly to spatial variability in litter input and decomposition. They act at various scales: geomorphic factors, such as slope angle and aspect, induce large scale variation; tree distribution and associated litter input act on a scale of several metres; grass tussocks may affect the humus profile at the centimetre scale. Effects of grazing intensity on humus profile properties are also complex because grazing, trampling and manuring are localised. Also, the implications of such spatial variability for sampling schemes and strategies have not yet been studied in detail.

The purpose of this study was to evaluate the impact of grazing on spatial patterns in humus profile properties. Fenced 1 ha areas with moderate and high intensity grazing and a control site were located in a fairly homogeneous 70 years old primary pine stand with a uniform undergrowth of *Deschampsia flexuosa* on drift sand. The uniformity of the herb layer eliminated selective feeding on preferred species or parts of the sites. Questions to be answered were: i) does grazing induce changes in spatial variability of humus profile properties? and, if so, at what scale? and ii) do these changes depend on grazing intensity? It was necessary to know the initial spatial variability of humus profiles of the sites studied and its causes. I also considered the implications of spatial variability for optimal sampling strategies.

**Methods**

**Study area**

The study area was in a drift sand area north of Kootwijk (5°46'F, 52°10'N, 30 m above sea level) in The Netherlands. In 1928, Scots pines (*Pinus sylvestris* L.) were planted to stabilise the drift sand, which is highly quartzitic and well sorted, with a median particle size of about 175 μm (Koster, 1978). Two types of drift sand were distinguished, one with 0.75%-1.5% organic matter (low OM) and the other with < 0.75% (very low OM) (Mekkink and Kemmers, 1993). The soils are classified as Haplic.
Arenosols (FAO, 1998). On the acidic sand, a Mormoder humus form (Green et al., 1993) has developed. pH(H₂O) values are in the Al-buffering range, around 4 in the subsoil and below (3.3–3.7) in the topsoil. The herb layer was dominated by Deschampsia flexuosa (L) Trin., with some patches of Vaccinium myrtillus L. Soil and vegetation development are similar to other forest stands of similar age on drift sand as described by Emmer (1995a).

**Experimental design and sampling**

In the studied stand, three rectangular sites of 1 ha were fenced. The first site was used as a control and was not grazed. The 'heavily' grazed site was grazed every summer for a period of 20 days by five cows. After this period all edible grass had been consumed. The 'moderately' grazed site was grazed for half of this period (10 days) by the same five cows. The first grazing period was in 1990, the last in 1996.

Before the first grazing period in 1990, all sites were sampled to document their initial composition and test whether initial differences existed between them. In each site, 50 humus profiles were sampled (Sevink et al., 1993) in a rectangular grid with a spacing of 15 m (Figure 2.1). Differences were negligible (Kemmers et al., 1996). Because the 1990 sampling method differed slightly from that of later years, the 1990 data were used only to determine initial differences between sites and not for further comparisons. Sampling was repeated in 1992, 1994 and 1996 (after 3, 5 and 7 grazing periods, respectively), immediately after removal of the cattle. At each sampling point, site factors such as slope angle and aspect, distance to the nearest tree trunk, soil type class (low or very low OM) and the presence of a buried podzol profile in the subsoil (within 180 cm) were recorded.

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**Figure 2.1.** Sampling locations of the site-scale experiment (left) and the detailed-scale experiment (right). Each dot represents a sampling point.

Site-scale

- **Control**
- **Heavily grazed**
- **Moderately grazed**

**Detailed-scale**

At each sampling point two adjacent samples of the humus profile were taken with a monolith sampler (area of sampling surface: 42 cm², Wardenaar, 1987). The first served to describe morphological characteristics and the thickness of L, F, H and EAh horizons (definitions according to Green et al., 1993). These horizons were sampled, and samples from the remaining part of the upper 5 cm of the
mineral soil profile (the thickness of the EA\textsubscript{h} horizon was always less than 5 cm). The second sample was divided into three parts: the L and F horizon combined, the H horizon if present, and the upper 5 cm of the mineral soil. Samples from each part were used to measure pH. Results for all these samples will be referred to below as 'site scale'.

In 1996, short distance variability was assessed by digging trenches 2.5–3 m long and 40 cm deep. In each of the three sites seven trenches were dug at random. Descriptions of horizon thickness and morphological characteristics of the humus profile were made at 10 cm intervals along one side of each trench.

In 1997, spatial variability was assessed again. Two plots (A and B in Figure 2.1) of 10m x 10m with a herb layer dominated by Deschampsia flexuosa were selected. The first (A) was located in the heavily grazed field, about 5 m from the fence, the second (B) was placed just outside the same fence in the non-grazed part of the stand. In both plots, 180 sample points were located at irregularly spaced sites (Figure 2.1). At each sample point the above ground biomass was sampled from a 25cm x 25cm surface area, after measuring the height of the grass. While cutting the grass, the locations and sizes of Deschampsia tussocks were mapped. Next, at each point, the humus profile was sampled with the monolith sampler; it was described and divided into an ectorganic part and a mineral part (0–5 cm depth). The results of these measurements will be referred to below as 'detailed scale'.

**Laboratory analyses**

Because of the large number of samples involved at the site scale, analyses were limited to organic matter content, root content and pH (Sevink et al., 1993). The soil samples of the site scale experiment were dried at 70°C for 48 hours, and roots, bark, and large woody particles were removed by hand-picking and weighed separately. For loss on ignition (LOI%), as a measure of the organic matter content, samples were heated to 550°C for 16 hours. pH was measured potentiometrically in suspensions of soil material in either distilled water or 1 M KCI; weight/volume ratios (soil/liquid) were 1:10 for organic samples and 1:2.5 for mineral samples. To calculate stocks of organic matter (kg,OM m\textsuperscript{-2}) sample weight was multiplied by the fraction of the organic matter content and then divided by the sampling surface area. Samples at the detailed scale (soil and biomass) were dried at 70°C for 48 hours and weighed. Half (randomly selected) were analysed for LOI. Stocks of organic matter were calculated for the ectorganic horizons and for the upper part of the mineral soil as before.

**Statistical analyses**

To find the cause of spatial variability in the humus profile, four site factors were divided into equally sized classes as follows: slope angle (0–5°; 6–10°; >10°), slope aspect (NW–NE, E, W and flat, SW–SE), parent material (low OM, very low OM or presence of a buried podzol within 180 cm) and distance to nearest tree trunk (< 3 m; ≥ 3 m). A two-way analysis of variance (ANOVA) was then used to see whether any of these factors or pairs of factors influenced the variability in thickness of horizons and stocks of organic matter. Sites and sampling years were analysed separately. Part of the ANOVA was a Levene's homogeneity of variance test to judge whether variances were different. If they were, the nonparametric Kruskal-Wallis one-way ANOVA was used (Norusis, 1993).
All properties (thickness, pH, organic matter stocks and roots) were tested for all horizons individually, and stocks of organic matter and roots were summed over all horizons. Thickness of the L, F and H horizon were also summed, as the thickness of the ectorganic profile is often used in humus profile classification keys.

Semivariograms were constructed, using the software Variowin release 2.2 (Panatier, 1996), to estimate spatial correlation of measured properties. Semivariograms are calculated by the following formula:

$$\hat{\gamma}(h) = \frac{1}{2n(h)} \sum_{i=1}^{n(h)} [z(x_i) - z(x_i + h)]^2$$

(2.1)

Where $h$ is the distance vector (the lag), $z$ is the value at a certain point $(x_i)$ and $n$ is the number of pairs having the same lag. Then $\gamma(h)$ is plotted against $h$ and a model is fitted to this variogram (Burrough and McDonnell, 1998). The fitted curve shows three features; first, at large values of $h$ the curve levels off and this horizontal part, called the sill is equal to the overall variance; second, the distance at which the sill is reached is called the range, this is the limit of spatial dependence; third the variance at lag zero is called the nugget variance, which is the variance at a lag smaller than the sampling interval (Davis, 1986; Webster and Oliver, 1990).

Figure 2.2. Means (A) and variances (B) of horizon thickness and organic matter stocks after 7 annual periods of grazing (1996) (error bars: 95% confidence interval). ●: not grazed; ○: moderately grazed; ●: heavily grazed;
thick F = thickness of F horizon, thick H = thickness of H horizon, thick E = thickness of E horizon, OM-ect = organic matter stocks in ectorganic horizons, OM-end = organic matter stocks in endorganic horizons, total OM = organic matter stocks in total humus form.

The site scale data were used to see whether a spatial correlation could be demonstrated at distances larger than 15 m. The detailed scale data were used to compare semivariograms on a smaller scale. The influence of above ground biomass or tussocks on humus profiles was tested in two ways. Bivariate correlation analysis was used to identify a relationship between the measured humus factors and the biomass, and a t-test was used to test for significant differences between the humus profiles on and between tussocks. Changes in the spatial distribution of aboveground biomass were examined using frequency distributions.
Results

Humus profile variability in the non-grazed area

Statistical analyses for the non-grazed sites, which include all sites in 1990 and the control site in 1996, showed that the relations between humus profile properties and site factors were on the whole not significant. Properties tested were thicknesses of the ectorganic profile and the F and H horizons separately, organic matter stocks and root stocks of the complete humus profile. Site factors included slope angle and aspect, distance to trees, parent material and the presence of a buried podzol profile in the subsoil. Interaction between pairs of factors also showed no effect at the site scale. However, there were larger variances in both thickness and organic matter stocks of the F horizon than in underlying horizons (Figure 2.2B). In spite of this, no spatial correlation was found at this scale. At all distances the variance was comparable to the overall variance. Since the minimal distance between sample points was 15 m, any spatial correlation was probably on a smaller scale than the site scale (Webster and Oliver, 1990).

Figure 2.3. Soil profiles showing thicknesses of ectorganic and endorganic horizons, measured in trenches in non-grazed (A) and heavily grazed (B) sites (1996).
Figure 2.4: Fitted models of semivariograms of soil properties measured at the detailed scale (1997). The x-axes show the distance between samples, the y-axes the variance.

At the detailed scale, there was also a large variance as can be seen in Figure 2.3A, which gives an example of one of the trench profiles. This non-grazed profile showed large differences in horizon thickness at short distances, which was also evident from the variogram models of the detailed scale sampling in the non-grazed and heavily grazed sites (Figure 2.4). In that Figure, the modelled variance was plotted against the distance between sample points (i.e. the lag). For the thicknesses of both the F and H horizons, the variances increased sharply until the level of the sill (the variance
at which the graph flattens). For the F horizon the distance at which the variogram reached the sill (i.e. the range) is less than 0.5 m, and the thickness of the H horizon had a range of 1.5 m. The stocks of organic matter showed only a slight difference between nugget and sill, and the thickness of the E horizons was a pure nugget model (no increase in variance over distance). The sill of organic matter stocks in the ectorganic horizons was lower than the variance at the site scale (Figure 2.2).

Variograms of this variable with a lag size of more than 5 m (not presented) showed an increase of variance up to the site scale level at a distance of between 6 and 9 m.

The variogram of the above ground biomass had a range of 0.6 m, which was about the same as that of the F horizon. This short range was in line with the frequency distribution of the biomass (Figure 2.5A), which shows an evenly spread amount of biomass and only a small number of patches with little or no biomass. There was no correlation between above ground biomass and thickness of the F horizon. However, there was a significant difference ($p < 0.001$) between the thicknesses of the F horizon within tussocks ($\bar{x}_{(\text{on tussock, not grazed})} = 7.5$) and between tussocks ($\bar{x}_{(\text{between tussocks, not grazed})} = 5.5$).

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Figure 2.5. Frequency distributions of above ground biomass 1997: A: not grazed; B: heavily grazed.

The impact of grazing on humus profile variability

After seven years of grazing the impact of site factors, such as slope angle and aspect, distance to tree trunks or presence of a buried podzol profile, on the properties studied (see previous section) was mostly not significant, and where the impact was significant it was not consistent over the years. The years 1992 and 1994 and the pairs of factors gave comparable results. As expected, the pH was not changed by grazing, because it is strongly buffered at the low range (data not shown).

At the site scale, seven periods of grazing not only induced changes in mean thickness of horizons and stocks of organic matter, but also changed the variances (Figure 2.2). The impact of grazing was most distinctive in the thickness and organic matter stocks of the F horizon (Figure 2.2A). The increase of total organic matter stocks was strongly related to the stocks in the ectorganic horizons. Considering mean values, the impact of grazing in the moderately grazed site was less pronounced than in the heavily grazed site, but the variances (Figure 2.2B) showed a different trend.
In the heavily grazed site there was a decrease of variance for all properties, whereas variances in the moderately grazed site showed increases in the thickness of the F and E horizons and in the organic matter stocks in the E horizon. Differences in variance did not result in changes in the spatial correlation. As in the non-grazed area, in the grazed site there was no spatial correlation at site scale.

At the detailed scale not only did the means and overall variance change, but also the spatial correlation. An example from one of the trenches (Figure 2.3B) shows thinner humus profile and lower variances indicate that irregularities were smoothed. This agrees with the results of the detailed scale experiment shown as modelled variograms. The thickness of both F (Figure 2.4A) and H (Figure 2.4B) horizons showed a decreased sill and an increased range. In contrast to these results, variograms of the thickness and organic matter stocks of the E horizon (Figures 2.4C and 2.4F) changed in another way: variances increased and there was a distinctive difference between nugget and sill. The range of thickness of the E was of the same order as that of the F horizon. The range of total organic matter stocks (Figure 2.4C) was comparable to that of organic matter in the ectorganic horizons (Figure 2.4E).

In the latter variogram there was a strong increase of the sill. For the above ground biomass (Figure 2.4D) the range was larger. This change in spatial pattern can also be seen in the frequency distribution (Figure 2.5B) where the number of patches without vegetation increased. Field observations showed that the tussock structure of the grass has disappeared.

**Discussion and conclusions**

**Humus profile variability in the non-grazed site**

Although there was a large variation at the site scale, no spatial correlation was found. This implies that the measured site factors do not explain the variance in the non-grazed stand and also that there was no other site factor causing significant spatial variability over larger distances. According to Emmer (1997), these results are opposite to those of Fantä (1986), who described a higher accumulation rate of soil organic matter stocks on the north- and north-east-facing slopes; however, it is possible that this kind of site was not captured in the 15 m sampling grid because of its low frequency of occurrence.

For most properties variance at the detailed scale equals the variance at the site scale. This means that variation does not increase at distances larger than 5 m and that variances observed at the site scale are the result of the detailed scale spatial variability. The thickness and stocks of organic matter in the E horizons are exceptions, in that they show much lower variances at the detailed scale than on the site scale. This could result from discrepancies in soil profile descriptions by different persons (Federer, 1982). This is particularly relevant for the E horizon, which can only be identified by its slightly bleached nature. However, the means for both thickness and organic matter stocks show no differences between the years.

At the detailed scale, variances of morphologic humus profile properties are probably generated by the tussock structure of *Deschampsia flexuosa*. The lack of correlation between aboveground biomass and humus profile properties can be explained by the difference in sampling size. The aboveground biomass was sampled over an area of 625 cm², whereas the sampling area of the humus profile was only 42 cm².
The system studied is monospecific in both the tree and herb layers, but a more varied species composition of these layers may induce spatial variability, the scale of which will vary according to that of the layers.

**Impact of grazing on humus profile variability**

The impact of grazing occurred at different scale levels. At the site scale variances of horizon thickness and organic matter stocks changed, but the site factors did not explain them. On steep slopes humus profiles were often changed by trampling, but the area affected was less than 1% of the grazed sites and the phenomenon was missed by the 50 site scale sampling points. Therefore it is not important in spatial terms.

The impact of grazing on variances differed between the moderately grazed and the heavily grazed sites. In the moderately grazed site, variances of thickness and stocks of organic matter increased. These probably resulted from trampling and grazing at different locations, so that patches where humus profiles were affected by trampling and compaction occurred next to patches where the profiles were changed by grazing. The tussock structure of the grass was preserved. The impact of trampling appeared first in the upper horizons. In the heavily grazed site, trampling was spread more evenly and was more intense, resulting in more compact upper horizons with less spatial variability.

At the detailed scale the sill decreased and the range increased with heavy grazing, meaning that the gradients in the spatial patterns became less abrupt. The structure of the grass also changed; the tussocks disappeared and there were many bare patches without vegetation. A pattern was created with steep gradients of humus profile properties between patches with small litter inputs and high light intensities next to patches with large litter inputs and lower light intensities. This pattern, however, did not result in steep gradients in thickness of horizons, probably because compaction smoothed the humus profile. Moreover, variance in ectorganic stocks increased, possibly because rootstocks decreased and belowground inputs of organic matter from grass roots increased as a result of grazing.

**Implications of grazing on the variability at landscape scale**

When large-scale grazing is introduced, even in areas with a uniform herb layer, grazing intensities will not be evenly spread (Wallis de Vries, 1994) and a mosaic of patches with different grazing intensities develops. Grazing at moderate and heavy intensities also affected the vegetation structure. Oosterveld (1978) showed that the boundaries between grazed and non-grazed patches became more pronounced with increasing grazing intensities, but when the grazing intensity exceeded a certain level, the patches disappeared and a homogeneous grazed vegetation without spatial patterns emerged. My results are similar and suggest that, at the landscape scale, spatial variability in humus profile properties increases, giving a larger variability in plant growth conditions. However, in practice grazing intensities are usually much less than in my experiments (Van Wieren et al., 1997), reaching the experimental levels only locally. At lower intensities only minor changes in spatial variability can be expected. Nevertheless, spatial variability can be enhanced by management aimed at short term and locally high grazing pressures, for example by temporary fencing.
Implications for sampling strategies and sample sizes

It is well known that spatial variability and spatial correlation are important aspects in the design of an optimal sampling strategy. However, in forest ecosystems little is known about factors controlling spatial variability, other than the site factors such as slope angle and aspect, distance to tree trunks or presence of a buried podzol profile. The consequence is that the sampling strategy employed was based on a 'best guess', which can now be evaluated for its efficiency.

Optimal sample spacing is larger than the range of the semivariograms (Webster and Oliver, 1990; Marriott et al., 1997), because only outside this range are observations spatially independent. The tussock structure of the herbal layer seemed to be the main factor controlling the short-range spatial variability of the humus profiles. This explains why the 15 m sample grid was effective in providing reliable estimates for mean values of the properties investigated. At the detailed scale the range increased for both the aboveground biomass and the thickness of the F horizon, leading to an increase in the optimal distance between sample locations. At this scale grazing is a regulating factor in selecting a sampling design, but the primary factor determining the optimal sampling scheme still seems to be the tussock structure of the herbal layer.

As can be seen in Figures 2.3 and 2.4, variances of several properties strongly increase at very small distances. Because of this short-range variability the small sample surface area of a monolith sampler can give different results from larger sample sizes (e.g. 25cm x 25cm) in the assessment of mean values or variance in humus profile properties. When the purpose is to obtain reliable estimates for the population means, a larger sample surface will probably give lower variances, but since it is more difficult to separate the organic horizons, the sampling error will increase. A monolith sampler allows more accurate sampling and is less time consuming, so more samples can be taken per time unit.

If the purpose of the study is to assess the spatial variability of soil and humus profile properties, sampling of small surfaces as by the monolith sampler thus provides more reliable and accurate data.

In sampling humus profiles, a proper strategy with regard to both sampling area and sampling distances should be applied. This is particularly relevant for comparative and temporal studies on ecosystem properties and processes in systems with more complex vegetation. Moreover, this study illustrates that, although mean values may be identical, variances and ranges in variability may differ, which ecologically may be more relevant parameters than mean values.

Acknowledgements

I thank Prof. dr. J. Sevink and Dr. A.M. Kooijman for the many stimulating discussions and for comments on earlier drafts of this manuscript. I also thank E. Berkhout, J. Steenbeek, G. Schuurmans and R. Winteraeken for assistance in the field. K. Klinka gave many valuable comments when reviewing an earlier version of this paper.