

# Seasonal change and distribution of grass nutritive values and minerals in an open pasture surrounded by forest

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**Abstract** The forage quality of pasture species is spatially heterogeneous. In this study, we evaluated the dynamics and spatial structure of forage quality and attempted to relate the observed variation to differences in light availability and soil properties. We compared grass forage quality in open grasslands with forest edge environments and established plots in open Japanese grasslands surrounded by secondary forests. We sampled *Anthoxanthum odoratum* L. and soil from each plot in May and September. These samples were analyzed to determine the crude protein (CP), neutral detergent fiber, and mineral concentrations. We calculated Moran's *I* coefficients across discrete lag distance classes to construct autocorrelograms. The CP model showed a non-random pattern in May at a lag distance of 40 m, but a clustered pattern in September at a small scale. The mineral models produced similar clustered spatial patterns. We found no strong correlation between aboveground (plant) and belowground (soil) mineral contents. CP and some mineral element concentrations in the plants collected in September were significantly greater at the forest edge than in open grasslands. The forest edge therefore appears to be a valuable place to provide grazing

animals with adequate protein and mineral intake during the later part of the growing season.

**Keywords** Forest edge · Open grassland · Spatial structure · Japanese pastures

## Introduction

Estimation of forage quality in a pasture is important so that managers can ensure that grazing animals receive adequate nutrition. The spatial evaluation of pasture quality is also important because it allows managers to determine appropriate stocking rates, which thereby supports responsible pasture and grazing management.

The forage quality of plants in natural environments varies in response to extrinsic factors such as light and soil nutrient conditions, in addition to intrinsic factors such as the species and growth stage of the plant. The CP concentration of herbage is greater at lower levels of solar radiation or lower fertilizer inputs (Neel et al. 2008; Cop et al. 2009). At low soil nitrogen levels, shade increased the lignin, protein, and mineral (P, Ca, Mg) concentrations of plants, but the effects disappeared at high nitrogen levels (Burton 1959). Heavy application of fertilizer (at levels above the reference value) decreased the digestibility and sugar contents of forage, but increased the fiber and P contents (Burton 1959; Cop et al. 2009; Okamoto et al. 2011).

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The availability of light and soil nutrient resources in an open pasture surrounded by secondary forest were found to be spatially heterogeneous due to the presence of the surrounding trees (Peri et al. 2007), which induced a spatial structure in forage quality. For example, the shade created by secondary forest may affect the nutritive values of grass adjacent to the secondary forest by decreasing the light intensity. In addition, the forest soil may have different mineral concentrations from those in the adjacent grassland, and may improve soil nutrient levels if the grassland receives a flow of minerals from the secondary forest. Although the spatial pattern of biomass or productivity in pastures has been investigated, surprisingly few studies have investigated the spatial structure of forage quality in grasslands.

The objectives of this paper are to address the spatial effect of trees in grass quality and to clarify the mechanism(s) underlying the effects in order to develop management recommendations for agroforestry system. We specifically evaluated (1) the dynamics and spatial structure of forage quality, (2) the correlation between forage quality and soil properties, (3) whether nutritive values of herbage in grassland adjacent to a secondary forest (i.e., at the forest edge) increased during the growing season, and (4) whether grass at the forest edge had a higher mineral concentration.

The spatial pattern of forage quality was evaluated along a topographic gradient (Corona et al. 1998), Vasquez de Aldana et al. (2000), but this spatial pattern simultaneously included the effect of species differences along that gradient, making it impossible to determine the extrinsic effect independent of intrinsic effects. We thus used a single grass species (*A. odoratum* L.) to evaluate forage quality in the present study.

## Materials and methods

### Site description

Our study area was at the Kawatabi Research Station of Tohoku University, 60 km north of Sendai, Japan (38°44'N, 140°45'E, 300–600 m above sea level). The climate in this region is temperate, with a mean annual temperature of 10.2 °C and a mean annual rainfall of 1,660 mm based on records from 1979 to 2000. Most

of the forest areas were previously utilized as fuel wood coppices or hay meadows, but some were abandoned in the 1950s and have regenerated naturally as secondary forests of deciduous, broad-leaved tree species such as *Quercus serrata* Murray and *Castanea crenata* Sieb et Zucc. The current landscape pattern of the study site is characterized by semi-natural pastureland surrounded by secondary forests on a moderate slope. The soils are derived from acidic volcanic ash and have adequate supplies of organic matter. The semi-natural pasture has not received fertilization recently, has no exotic species sown in the pasture with no renovation treatments.

### Field survey and chemical analysis

In early May 2011, we randomly established 22 plots (each 1 m<sup>2</sup>) in a 1 ha open pasture surrounded by secondary forest. The mean density of hardwood tree species in the forest area was 2,375 ha<sup>-1</sup>. The open pasture is dominated by the grass *A. odoratum* L. without trees. We took a sample of ~100 g dry mass of ungrazed *A. odoratum* L. from each plot after establishing the plots, and obtained the same amount again in early September.

The plant samples collected in May were analyzed to determine the CP and neutral detergent fiber (NDF) contents, and samples collected in September were also analyzed to determine the mineral content. CP concentrations in the plant samples were measured by determining N using the Kjeldahl method, then multiplying the result by 6.25 to convert the N content into crude protein. NDF was measured using the method of Van Soest et al. (1991). The phosphorus (P) concentration in the collected samples was determined by colorimetric analysis. The sulfur (S) concentration was determined using an X-ray fluorescence spectrometer. Atomic absorption spectroscopy was used to determine other mineral element concentrations (Ca, Mg, K, Na, Fe, Cu, Co, Mn, Zn, Cl, and Se).

To investigate the soil properties, we extracted soil core samples (10 cm diameter × 10 cm depth) from each plot in May and September. Before obtaining the samples, all surface organic matter above the inorganic soil horizons was removed. At each plot, we extracted three samples and homogenized the samples before analysis. The soil samples were dried in an oven at 70 °C for 48 h and then weighed. Exchangeable soil Ca, K, Mg, Mn, Zn, and Cu were measured

using atomic absorption spectrophotometry (Gallaher et al. 1975) after Schollenberger extraction. Available soil phosphorus (P) was measured using the Truog–Bray method.

We used an LI-250 handheld light sensor (LI-COR Inc., Lincoln, NE, USA) to measure the photosynthetic photon flux density (PPFD) at 09:00, 13:00, and 17:00 on 27 June, 23 July, and 8 September at each plot.

### Data analysis

We input the georeferenced CP and mineral data into version 10 of the ArcGIS software and calculated Moran's  $I$ , a coefficient of autocorrelation, to measure the strength of the spatial homogeneity or heterogeneity in the distribution of forage quality. Moran's  $I$  is expressed as:

$$I = \frac{n \sum_{i=1}^n \sum_{j=1}^n w_{ij} (z_i - \bar{z})(z_j - \bar{z})}{2a \sum_{i=1}^n (z_i - \bar{z})^2}$$

where  $n$  is the number of plots in the pasture,  $a$  is the total number of neighboring plots,  $z_i$  and  $z_j$  are the values of a given parameter in plots  $i$  and  $j$ , and  $w_{ij}$  is a weight that represents the spatial relationship (e.g., contiguity) between plots  $i$  and  $j$  (1 adjacent, 0 otherwise). Values of Moran's  $I$  near zero indicate randomness, or spatial homogeneity, while values significantly greater than zero (positive spatial autocorrelation) or less than zero (negative spatial autocorrelation) indicate spatial heterogeneity. Higher absolute values of  $I$  indicate that nearby individuals have a higher likelihood of having similar levels of the parameter value. Lower absolute values of  $I$  indicate that nearby individuals have a higher likelihood of having different levels of values. We calculated Moran's  $I$  coefficients across discrete lag distances of 10, 40, 70, 100, and 130 m to construct autocorrelograms. We judged the resulting spatial pattern to be non-random if the observed Moran's  $I$  coefficient was unusually large or small (i.e., if it fell within the top or bottom 5 % of the spatially randomized distribution of the plots).

We calculated Spearman's correlation coefficient for the mineral values (Ca, P, Mg, K, Zn, Cu, and Mn) in the 22 plots between plants collected in September and soil collected in May and September to clarify the

relationship between aboveground and belowground mineral concentrations.

To compare forage quality and soil properties between open grassland and the forest edge, we divided the 22 plots into open grassland (>10 m from the forest edge) or forest edge (<10 m).

We analyzed the CP and NDF concentrations of the plant samples and the mineral concentrations in the soil using repeated-measures ANOVA to examine the main effects of sampling location (open grassland or forest edge) and season (May or September), as well the interaction between the location and the season.

To determine whether the mean mineral concentrations in the plants differed between sampling locations, we compared the measurements of each mineral element (Ca, P, Mg, K, Na, S, Fe, Zn, Cu Mn, Cl, Co, and Se) using  $t$ -tests. When necessary, data were log-transformed to meet the assumptions of normality and similarities of variance.

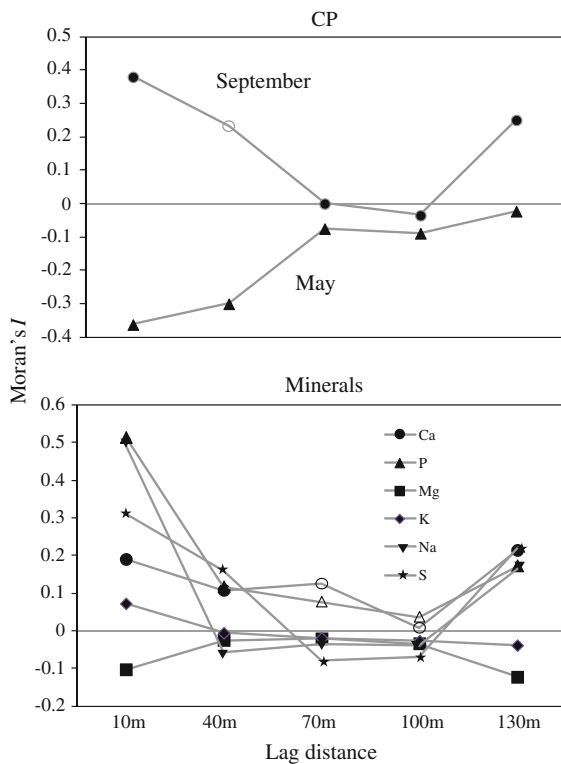
## Results

### Spatial structures of forage quality

The crude protein model generated mirror-image spatial patterns for the two seasons at a small scale ( $\leq 70$  m; Fig. 1). The model showed a negative Moran's  $I$  in May at all lag distances, but a positive value in September at a lag distance of <70 or >100 m, which indicated a non-random pattern and a clustered pattern, respectively, although significant spatial autocorrelation was seen only at the 40 m scale in September. The main mineral models produced similar clustered spatial patterns for all minerals except Mg. There were significant positive spatial autocorrelations for Ca and P at lag scales of 70 and 100 m, respectively.

### Correlations between aboveground and belowground mineral values

We found no strong correlation between the aboveground (plant) and belowground (soil) mineral values within the pasture, except for a significant positive correlation between aboveground K in September and belowground K in May (Table 1).



**Fig. 1** Spatial autocorrelograms generated by models simulating CP and the major minerals in May and September. Positive values of Moran's *I* indicate having similar levels of CP or mineral values, whereas negative values indicate differing levels of CP or mineral values at the spatial scale. White symbols indicate a statistically significant value of Moran's *I*

Comparison of forage quality between the open grassland and forest edge locations

The mean light intensity (PPFD) values reaching plants in the open grassland were 1,410, 609, and 485  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the end of June and July and in early September, respectively. The total daily PPFD received by plants at the forest edge in late June and July and in early September were 26.2, 22.2 and 22.2 % of the corresponding values in open grassland.

**Table 1** Spearman's correlation coefficient values between aboveground (plants in September) and belowground (soil in May and September) exchangeable mineral values in the 22 plots

Soil sampling season	Aboveground concentration (September)						
	Ca	P	Mg	K	Zn	Cu	Mn
May	-0.11	0.13	-0.04	<b>0.57</b>	0.30	0.10	0.14
September	-0.14	0.17	-0.06	0.08	0.34	-0.28	0.22

The boldfaced number indicates a statistically significant relationship

The CP concentrations (mean  $\pm$  SD) of *A. odoratum* L. in the open grassland were  $9.48 \pm 0.68$  and  $20.26 \pm 0.98$  in May and September, respectively, and the corresponding values at the forest edge locations were  $8.69 \pm 0.96$  and  $21.52 \pm 2.07$ , respectively. However, the values only differed significantly between the seasons, not between the locations, though there was a significant location  $\times$  season interaction (Table 2). NDF concentrations of *A. odoratum* L. in the open grassland were  $52.56 \pm 2.01$  and  $55.08 \pm 3.27$  in May and September, respectively, and the corresponding values at the forest edge locations were  $54.61 \pm 4.48$  and  $53.64 \pm 2.17$ , respectively. For NDF, there were no significant differences between locations or seasons, and their interaction (Table 2).

The Ca, P, S, Zn, and Cl concentrations were significantly greater in the plant samples from the forest edge location in September (Table 3), whereas no mineral elements had significantly higher concentrations in the open grassland.

In May, the mean soil mineral concentrations of six of the seven elements (excluding Cu) were higher at the forest edge locations than in open grassland (Fig. 2). However, in September, soil concentrations were higher in the open grassland for all elements except Mg and Cu. In addition, we found no significant interaction between location and season because of the high variation among the plots.

Discussion

We were surprised by the spatial structure observed for CP. Covelo et al. (2008) found that the spatial pattern and scale of nitrogen in senesced leaves differed from that in green leaves, with senesced leaves having a higher patch size. At our study site, *A. odoratum* are senesced in the September and we therefore expected the same phenomenon to occur.

**Table 2** Repeated-measures ANOVA results for the crude protein (CP) concentration and the neutral detergent fiber (NDF) content of *Anthoxanthum odoratum* L

Factor	d.f.	MS	F	p
<b>CP</b>				
Location	1	3.68	1.74	0.20
Season	1	1391.30	1685.58	0.00
Location × Season	1	4.71	5.71	0.03
Error	20	0.83		
<b>NDF</b>				
Location	1	1.0	0.10	0.76
Season	1	6.4	0.75	0.40
Location × Season	1	32.4	3.82	0.06
Error	20	8.5		

Location (open grassland or forest edge) and season (May or September) and their interactions were included in the model as factors

**Table 3** Mineral concentrations (mean ± SD) in *Anthoxanthum odoratum* L

	Open grassland	Forest edge	t value
Ca (%)	0.31 ± 0.05	<b>0.38 ± 0.09</b>	-2.19
P (%)	0.36 ± 0.04	<b>0.43 ± 0.08</b>	-2.19
Mg (%)	0.21 ± 0.02	0.23 ± 0.05	-1.53
K (%)	2.04 ± 0.27	2.24 ± 0.24	-1.73
Na (%)	0.24 ± 0.11	0.20 ± 0.08	0.75
S (%)	0.25 ± 0.02	<b>0.28 ± 0.02</b>	-2.41
Fe (ppm)	108.06 ± 17.03	110.75 ± 13.48	-0.39
Zn (ppm)	36.78 ± 4.64	<b>40.65 ± 3.85</b>	-2.05
Cu (ppm)	8.40 ± 1.40	9.10 ± 1.21	-1.22
Mn (ppm)	487.86 ± 118.67	448.27 ± 155.23	0.68
Cl (ppm)	1.15 ± 0.18	<b>1.42 ± 0.32</b>	-2.53
Co (ppm)	0.80 ± 0.25	0.74 ± 0.12	0.67
Se (ppm)	0.05 ± 0.03	0.05 ± 0.029	0.10

Boldfaced numbers indicate significantly different values ( $p < 0.05$ )

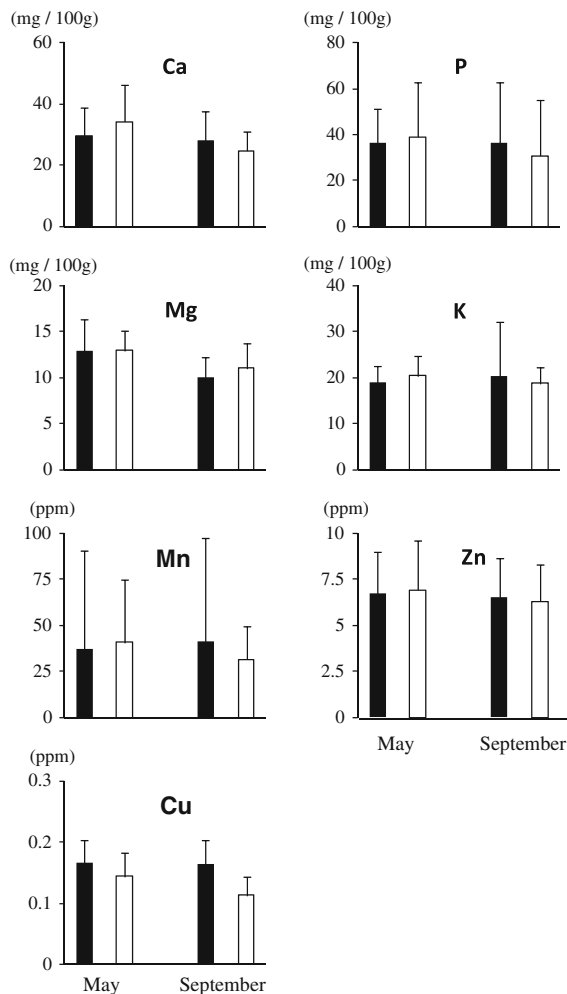
However, the clustered spatial pattern for the mineral concentrations may have reflected autocorrelation in the spatial pattern of soil minerals because plants take up minerals from the soil. Inferred from the results of spatial structure, we can assess the spatial forage qualities in autumn by smaller sample size for analysis because nearby individuals have a higher likelihood of having similar levels and qualities.

The correlation between soil and aboveground plant properties is generally unclear (Epstein et al. 1963; Covelo et al. 2008). Because minerals are

mobile in the soil, they may be leached away from the plants by the percolation of rainfall and may therefore be more difficult for plants to take up shortly after a rainfall. Another possible reason for the lack of a strong correlation is that plants may plastically regulate their nutrient uptake efficiency in response to changes in the soil nutrient availability, and this regulation may be limited by the creation of low-solubility compounds in the soil. This is often the case for andosols, whose high P-fixing capacity results from their high Al and Fe oxide concentrations (Kumagai et al. 1991).

The seasonal increase in the CP concentration at the forest edge during the observed season produced a significant interaction between location and season. This is likely associated with the greater shade at the forest edge under strong summer sunlight, which is supported by previous results (Neel et al. 2008; Peri et al. 2007). However, the lack of a difference for NDF contradicts the results of Senanayake (1995). Shade usually decreases secondary cell wall development in plants, thereby leading to morphological changes in herbage grown under reduced light, and this would likely increase the nutritive value of herbage (Kephart and Buxton 1993).

The mineral concentrations were greater in plant samples from the forest edge in September. Peri et al. (2007) noted that the mean annual macronutrient concentrations in leaves increased as PPFD decreased. Concentration effects (i.e., due to changes in the dry matter or water contents, thus “dilution” or “concentration” of the minerals) are a plausible mechanism that accounts for this phenomenon (Burton 1959).



**Fig. 2** Mineral concentrations in soil samples taken from the open grassland (*black bars*) and forest edge (*white bars*) locations in May and September. Values are mean  $\pm$  SD

However, the higher mineral contents in the forest edge location in our study is more likely attributable to the nearby forest, because higher mineral contents were found in the forest soil and tree leaves than in the grassland vegetation at this study site (Mizuno et al. unpublished data).

A common concern with pastures throughout much of northern Japan that contain C3 cool-season grasses and that are mainly used for pasture is that forage quality and quantity may decline during the summer due to high-temperature injury associated with recent global warming (Sasaki et al. 2003). Our results suggest that the forest edge location, which was more protected from strong direct sunlight than the open grassland, did not suffer from a summer depression,

resulting in higher nutritive values than in the open grassland.

Mineral concentrations are also a concern. The incidence of mineral deficiencies in grazed animals in Japan is more widespread than was previously recognized (National Agriculture and Food Research Organization 2008). At the forest edge, the grass had a higher mineral concentration. The Zn concentration in the forest-edge grass exceeded the requirements (40 ppm) of beef cattle, but the concentration in open grassland was below this requirement. The grass sampled at the forest edge is thus an important source of fodder for grazing animals because of the potentially higher nutrient quality. Because shaded pastures would limit animal production primarily due to lower biomass productivity of the vegetation, planting trees in a pasture may not improve nutrition. However, planting trees at an appropriate level would be valuable if doing so provides a “summer retreat” for both the animals and the plants they feed on.

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