Vol. 17 (2008): 376–393.

Field-scale spatial variation in yields and nitrogen fixation of clover-grass leys and in soil nutrients

Arja Nykänen[1], Lauri Jauhiainen[2], Jukka Kemppainen[3] and Kristina Lindström[4]

[1] MTT Agrifood Research Finland, Plant Production, Lönnrotinkatu 3, FI-50100 Mikkeli, Finland*

[2] MTT Agrifood Research Finland, Information Management, FI-31600 Jokioinen, Finland

[3] MTT Agrifood Research Finland, Milk and Beef Production, Kipinäntie 16, FI-88600 Sotkamo, Finland

[4] University of Helsinki, Applied Chemistry and Microbiology, PO Box 56, FI-00014 University of Helsinki, Finland

* email: firstname.lastname@mtt.fi

Biological nitrogen fixation (BNF) plays a crucial role in organic farming and red clover (*Trifolium pratense*) is cultivated widely in boreal grasslands for BNF. A geostatistical method, model-based kriging, was used to determine the spatial variation in yield, clover content and BNF of clover-grass leys as well as soil chemical properties throughout two fields in 2004−2006. Based on this variation, principal component analysis (PCA) was used to determine the similar patterns of variation. On one location, total dry matter yields of the leys decreased over three production years from 9 700 to 4 100 kg ha^{-1}, clover content from 53 to 26% and BNF from 150 to 40 kg N ha^{-1}, whereas on the other location the yields increased from 6 500 to 7 100 kg ha^{-1}, clover content from 52 to 62% and BNF from 100 to 120 kg N ha^{-1}. Nutrient concentrations in soil also varied greatly within the fields, although this depended on the nutrient species. Kriging combined with PCA described the spatial variation of ley parameters very informatively, but was not as powerful for describing the pattern of nutrients. Based on the spatial dependence determined in the two fields investigated, it seems that the sampling distance should be 80 m for soil nutrients, 100 m for yield and 60 m for clover content and BNF determination, respectively.

Key-words: Biological nitrogen fixation, geostatistics, kriging, pH, red clover, leys, spatial variation, soil nutrients, *Trifolium pratense*

Manuscript received December 2007

Vol. 17 (2008): 376–393.

Introduction

Biological nitrogen fixation (BNF) of legumes is important in organic plant production, since it forms the basis for the N supply in organic production method. BNF could be utilised in conventional farming, too, to reduce the fossil energy consumption for N fertiliser manufacturing and transport.

Perennial grasslands are cultivated for fodder production in areas where agriculture is based on livestock production. Red clover (*Trifolium pratense*) is one of the most common forage legumes in temperate grasslands. It is cultivated in a mixture with grasses and cut two or three times during the growing season. In organic farming, perennial red clover -based leys are also used as green manure in crop rotations.

The amounts of BNF in red clover have been measured in several studies (Nesheim and Øyen 1994, Kristensen et al. 1995, Väisänen et al. 2000, Huss-Danell et al. 2007). In these studies, measured variation in BNF ranged from 30 to 300 kg ha^{-1} year^{-1}, but the focus was not on spatial variation within the fields. The red clover content of ley yields varies as well (Nykänen et al. 2000) resulting in high variation in clover dry matter yields (3−14 Mg ha^{-1} year^{-1}). This variation should be considered when sampling BNF and calculating N balances.

The ^{15}N abundance is different in soil N and N_2 in the atmosphere. In ^{15}N enrichment technique the differencies expanded by incorporation of ^{15}N enriched nitrogenous compounds into the soil. This method is considered one of the most accurate to measure BNF in field conditions (Witty et al. 1988). It is suitable for experiments such as the ones described in this report, where the natural variation in the field is measured and red clover (N-fixing plant) and grasses (non-N-fixing reference plant) grow as a mixture. According to Ledgard et al. (1985), the ^{15}N -enrichment technique allows for estimation of BNF using the companion grass as reference crop. Clover content of the ley yield is an important factor for BNF calculations, because BNF is highly connected to clover yield (Väisänen et al. 2000, Carlsson and Huss-Danell 2003, Huss-Danell et al. 2007). Near infrared reflectance spectroscopy (NIRS) has been proved to be quick and good method for legume content analyses, if the calibration is done properly and the calibration set includes more than 200 samples (Locher et al. 2005 a, b).

Knowing the variation in parameters affecting growth in fields is important when establishing agricultural experiments and in precision farming. Geostatistical methods are useful for describing and understanding the spatial distribution of measured variables, especially on a large scale. Geostatistics has also been applied to agricultural research (Sutherland and Van Kessel 1991, Wallace and Hawkins 1994, Paz et al. 1996, Stenger et al. 2002, Talkkari et al. 2002, Jauhiainen et al. 2008). Few studies have been done, however, to examine relationships between spatial variability of different parameters (Samra et al. 1992, Shahandeh et al. 2005).

Usually, geostatistics results in a map for each of the characteristics studied. The number of maps can increase proportional to the number of the characteristics studied so that conclusions are difficult to draw. Similarities between maps or between the variables studied have traditionally been measured using correlation coefficients. Principal component analysis (PCA) can be used to determine the relationship between two or more variables and the factor structure underlying a set of variables. There are only a few efforts in soil science to utilise PCA to combine information from several maps of variables (Odlare et al. 2005, Borůvka et al. 2007), although it is a fully valid approach for geostatistical data (Bailey and Catrell 1995). Combination of PCA and geostatistics in analysis of several parameters can give useful information about the interactions between those parameters. It will also assist in choosing locations and assessing the number of replicates needed for field experiments because the range and pattern of the variation of selected parameters is known better. This knowledge is especially important in organic farming research, where the natural variation of nutrients in fields often plays an important role.

The aim of this study was to determine the spatial variation in yield, clover content, BNF and soil nutrients of leys within two selected field sites in eastern Finland and during three production years. A combination of geostatistics and PCA was applied for determining the relationships between measured variables, to decrease the number of maps and to clarify the overall picture of the variation. The results of these case studies were used to discuss the appropriateness of the advice given on soil sampling density in Finland and to suggest a sampling density needed to assess parameters related to ley production and BNF.

Materials and methods

Experimental sites

The experiment was conducted in two-hectare fields located in eastern Finland, in Juva (60°53'N 27°53'E) and in Sotkamo (64°07'N, 28°20'E). The field in Juva had been under organic farming since 1986 and crop rotation consisted of two or three years old red clover – grass leys, cereals with or without peas, and vetches. The soil type of the topsoil (0−25 cm), according to the Finnish soil classification system, was fine sand moraine. According to FAO taxonomy, the soil was tentatively classified as Dystric Regosol (FAO 2006). The Sotkamo field had been under conventional farming with mineral fertilisers and no clovers in the leys. The soil type of the topsoil, according to the Finnish soil classification system, was loamy sand. In the upper part of the field the soil was classified as Haplic Podzol (Yli-Halla et al. 2000) and in the lower part as Dystric Regosol (FAO 2006). Organic C content of both soils was 3% and the N_{tot} content was 1.5%. The slopes of the fields varied from 0% to 8%, being highest in the centre of the field in Juva and in the lower end of the field in Sotkamo (Fig. 1).

In May 2003, leys were established with barley (*Hordeum vulgare*) as a nurse crop. The seed mixture of the leys consisted of 5 kg ha^{-1} red clover (*Trifolium pratense* cv. Bjursele), 10 kg ha^{-1} timothy (*Phleum pratense* cv. Iki) and 6 kg ha^{-1} tall fescue (*Festuca arundinacea* cv. Retu). In Juva, 20 Mg ha^{-1} of aerated cow slurry containing 49 kg N_{tot} ha^{-1}, 35 K kg ha^{-1} and 95 kg P ha^{-1} was applied as a fertiliser before seeding. In Sotkamo, cow manure was applied 26 Mg ha^{-1}, containig 85 kg N_{tot} ha^{-1}, 38 kg K ha^{-1} and 144 kg P ha^{-1}.

In 2004, the spring was cooler and wetter, but the autumn was warmer than average. In July, there were thunderstorms and precipitation was high. The summer of 2005 was hot and there were heavy rainfalls in August. September 2005 was very dry. The growing season 2006 was extremely dry and warm (Fig. 2). All winters in both locations had a thick (30−70 cm) snow cover over the fields, so overwintering caused by physical damage, i.e. low

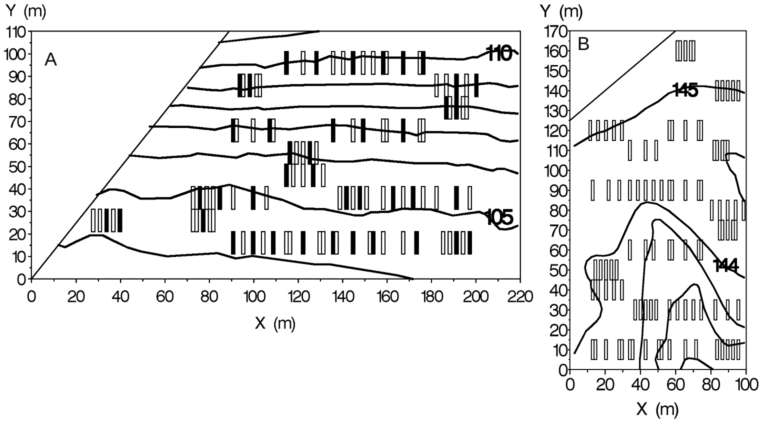

Fig. 1. The experimental fields of Juva (A) and Sotkamo (B) with 105 and 100 plots of 10 m × 1.5 m. The 42 black plots in Juva were used for BNF measurements with the ^{15}N enrichment technique. Lines and numbers inside the maps describe the altitude contours of the fields.

Vol. 17 (2008): 376–393.

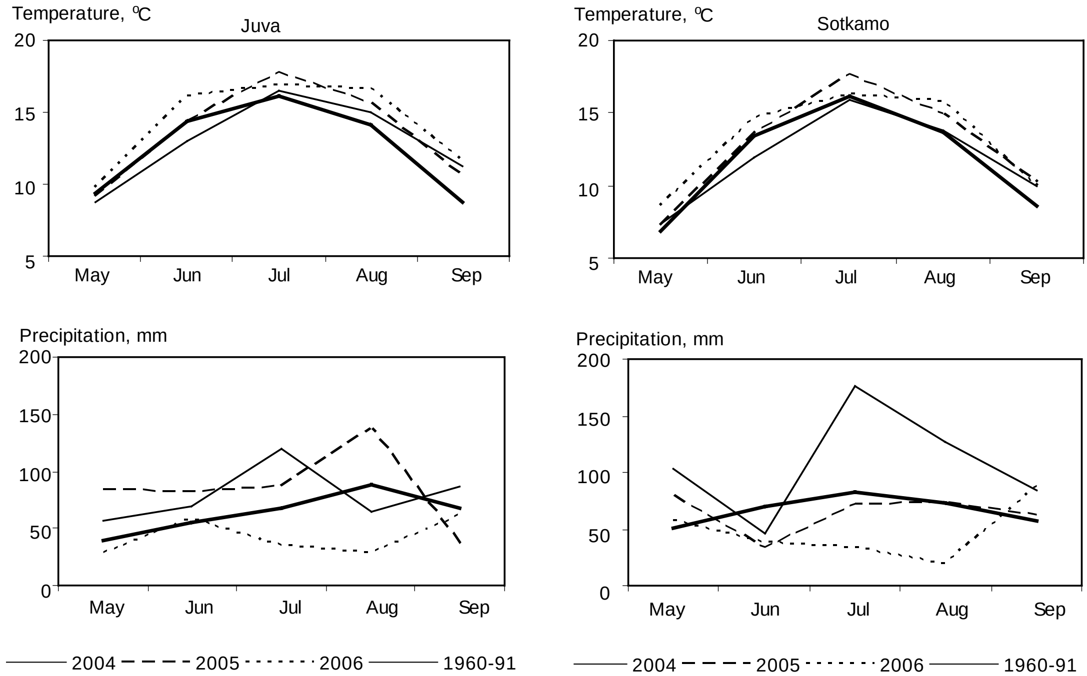

Fig. 2. Monthly precipitation and mean temperature at the nearby weather station of the experimental fields of Juva and Sotkamo during the 2004–2006 growing seasons and long-term means 1961–90.

temperatures and ice cover, might not have been a problem. Plant diseases were not investigated, but at least severe damages caused by clover rot (*Sclerotinia trifoliorum*) were not noticed.

Experimental design

In Juva, 105 plots and in Sotkamo, 100 plots, sized 1.5 × 10 m, were arranged on the experimental fields (Fig. 1). The location of the plots was designed so that there were different distances between plots, which made it possible to calculate spatial correlation more accurately compared to the experimental design with same distances between plots. The plots were in straight rows to ease the harvesting. In Juva, 42 of the plots were chosen for BNF measurements with the ^{15}N enrichment method to limit the expences. These plots were evenly distributed within the field (Fig. 1).

Nitrogen fixation

The BNF was estimated by the ^{15}N enrichment method in the Juva field from 42 plots. The plots were the same each year, because we wanted to follow the yield, clover content and BNF of the ley exactly at the same points of the field each year to exclude the influence of the spatial variation of the field on our results. The fertilisation effect of extra ^{15}N added to the field was considered negligible, because of the very small amount of N added only once a year.

The permanent 1 m^2 micro-plots, which were placed at the upper end of the chosen 42 experimental plots at the beginning of the experiment, were enriched in the early spring (beginning of May) of each of the three years with a ^{15}N (20 atoms %) -double labelled NH_4NO_3 solution at a rate of 5 g N m^{-2}. ^{15}N enriched NH_4NO_3 was dissolved in deionised water and pipetted onto the surface of the soil. The micro-plots were divided into 400 grids (5 cm × 5 cm each) to get as uniform a distribution of the fertiliser as possible over the surface. Two ml of fertiliser solution was pipetted over each grid, followed immediately by 5 ml of deionised water.

The proportion of clover N derived from the atmosphere (Ndfa) was determined as described by Peoples et al. (1989). The atoms % ^{15}N of the atmosphere is 0.3663 and it is subtracted from the atoms % ^{15}N of grasses and legumes to find out the excess of atoms % ^{15}N. The Ndfa was calculated as in equation (1) using grasses in the mixture as a reference plant representing the soil-derived N:

$$\text{Ndfa} = (1- (\text{atoms } \%_{\text{clover}} - 0.3663 \ / \ \text{atoms } \%_{\text{grasses}} - 0.3663)) \times 100 \quad (1)$$

The amount of $B^{15}NF$ was then calculated:

$$B^{15}NF \ (\text{kg N ha}^{-1}) = (\text{clover dry matter yield } (\text{kg ha}^{-1}) \times \text{N\%} \times \text{Ndfa}) / 100. \quad (2)$$

The amounts of measured $B^{15}NF$ for two cuts in three years were regressed on clover dry matter yields, giving the following relationship (R^2=0.93):

$$B^{15}NF \ (\text{kg N ha}^{-1} \text{ y}^{-1}) = \text{clover dry matter yield } (\text{kg ha}^{-1} \text{ y}^{-1}) \times 0.027 + 4 \quad (3)$$

Carlsson and Huss-Danell (2003) have published an equation based on a wider range of data i.e. 13 different experiments for BNF in red clover-grass mixtures (R^2=0.91):

$$\text{BNF } (\text{kg N ha}^{-1} \text{ y}^{-1}) = \text{clover dry mater yield } (\text{kg ha}^{-1} \text{ y}^{-1}) \times 0.026 + 7. \quad (4)$$

The correlation coefficient was high when BNF calculated by equations (3) and (4) were regressed (r=0.99) and the correlation coefficient of determination was 0.92 when measured $B^{15}NF$ was regressed with BNF predicted by equation (4). Therefore we decided to use their formula to calculate BNF for all plots of both fields in Juva and Sotkamo.

Sampling and analysis of plant material

The leys were harvested by a Haldrup plot harvester twice during each growing season. The first cuts of the leys were done at the early beginning of flowering of red clover in Juva on 29 June 2004, 21 June 2005 and 20 June 2006. In Sotkamo, the leys were harvested about three weeks later than in Juva on 15, 7 and 3 July, respectively. The second cuts were done at the generally recommended time in Finland (Pulli 1980) for good overwintering on 24, 30 and 29 August in Juva and on 25, 19 and 28 August in Sotkamo, respectively. Dry matter was determined from samples dried at 105 ºC.

Red clover content was determined by NIRS using wavelenghts of 1100–2500 nm with 2 nm intervals (InfraAnalyzer 500, Bran and Luebbe GmbH, Germany) and multivariate calibration was performed with PLSR (partial least squares regression). Totally 1359 samples were used for calibration: 28 artificial mixtures of red clover and grasses, 28 pure red clover and pure grass samples from other locations and about 50 pure red clover and pure grass from each of six cuts in both experimental locations. Calibration was validated with 28 natural mixtures resulting in R^2 to be 0.95.

In all cuttings, the central 0.25 m^2 of the 1 m^2 micro-plots for $B^{15}NF$ measurements were cut by hand with scissors at a stubble height of 5 cm. The plants were separated into red clover, grasses and weeds. The fractions were weighed and dried at 60 °C for 20 hours. The dry matter yield and clover content were calculated. The samples were milled and passed through a 1 mm sieve and stored away from light and humidity at room temperature. The dried plant samples were ground to fine powder in a

Vol. 17 (2008): 376–393.

ball mill and analysed for ^{15}N (atom-%) and total N (N_{tot}, %) with an atom mass spectrometer (Isotope Ratio Mass Spectrometer, SerCon Ltd, England).

Sampling and analysis of the soil

Soil samples for chemical analyses were taken in the plough layer (the top 0−25 cm) from all plots in May 2003, before fertilization and seeding of the field. Soil samples were air-dried at room temperature and sieved to 2−5 mm prior to analyses. Soil acidity was determined from 1:2.5 soil:water suspensions. Plant-available P, K, Mg, and Ca were extracted by acid ammonium acetate (AAAc, 0.5M CH_3COONH_4 + 0.5M CH_3COOH, pH 4.65, 1:10, 1 h, Vuorinen and Mäkitie 1955) and concentrations determined by spectrophotometry (P) or inductively coupled plasma optic emission spectrometry (ICP-OES) (K, Mg, Ca). The micronutrients Co, Mo, Cu, Fe, Mn and Zn were extracted by AAAc-EDTA (AAAc + 0.02M Na_2-ethylenediaminetetraacetic acid, pH 4.65, 1:10, 1 h) and B by boiling water (1:2 5 min, 1:4 30 min) (Lakanen and Erviö 1971), and amounts were determined by ICP-OES, except Mo, which was determined by atomic absorption spectrometry (AAS). N_{tot} and C_{tot} were measured by a LECO® autoanalyser, in which the soil sample, dried at 60 °C overnight, was ashed at 950 °C. N was determined from the heat conductivity of the gases and C was measured with infra-red spectrometry.

Statistical analysis

The data on dry matter yield and BNF of leys as well as on soil characteristics were analysed by using geostatistical methods to describe and interpret the spatial variation in the field. A variogram measures the correlation between pairs of observations as a function of the displacement between the pairs (Brooker 2001). First, a semivariogram g(h) was calculated as:

$$g(h) = \frac{1}{2N(h)} \sum_{d_{ij}=h} \left(y_i - y_j\right)^2$$

where y_i and y_j are observations from field plots i and j, respectively, d_{ij} is the distance between these plots, and N(h) is the number of field plots separated by the same distance h. Spatial dependence disappears at the distance where the variogram reaches its maximum. At the same time, this means that the value of a measured soil characteristic cannot give a prognosis further than this distance.

The next step was to define a model variogram. A model variogram, *γ(h)*, is a simple mathematical function that models the trend in the semivariogram. A spherical model with a nugget effect had the best fit for all characteristics studied, and the model used was:

$$\gamma(h) = \begin{cases} c_0 + c\left\{\dfrac{3h}{2a} - \dfrac{1}{2}\left(\dfrac{h}{a}\right)^3\right\} & \text{for } h \le a \\ c_0 + c & \text{for } h > a \end{cases}$$

where h is the distance, c_0 is the nugget, *c* is the sill, and *a* is the range. Range is the distance at which the spatial dependence disappears.

Kriging is one technique among many for interpolating a variable from sample points. It has the advantage that the estimated points are obtained with minimum variance (Lark 2000). The estimated values of the parameters of the spherical model were therefore used to predict values at the nodes of a 1 m grid by ordinary punctual kriging. A yield map was created using these kriging estimates.

PCA is a multivariate statistical analysis converting the variables into so-called principal components (PC). It was used to classify the variables, i.e. to find out which of the six cuts or soil nutrients followed similar spatial patterns in the field. PCA was done separately for dry matter yields, clover contents, BNF amounts and soil nutrients.

In PCA the data set consisting of several objects (field plots) and variables (cuts or soil nutrients) was mathematically summarised as a sum of products of pairs of score vectors (components) and loading vectors. Geometrically, a component can be seen to present a linear model of the multidimensional data. The scores can be plotted giving a two- or three-dimensional view of the objects and their relative positions. The corresponding loading vectors give the contribution (loading) of each variable to the corresponding component. Therefore, it was possible to use them for interpretation of groups of objects (plots) with similar characteristics as PCs. The identified PCs were described in the same way as the original variables using kriging.

The geostatistical analyses were done with the VARIOGRAM, NLIN and KRIGE2D procedures of the SAS program, version 9.1. (SAS 2004).

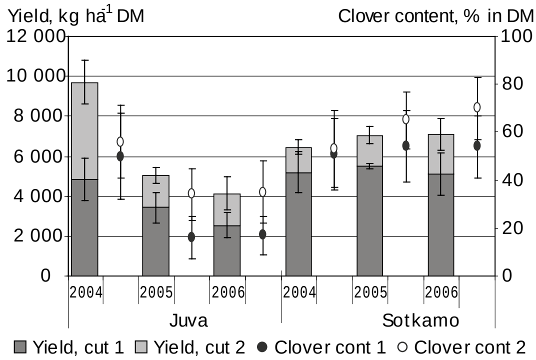

Fig. 3. Dry matter (DM) yields and clover contents of the leys in 2004–2006 in Juva and Sotkamo. (1= first cut, 2=second cut). Vertical lines indicate standard deviations.

Results

Yields and clover contents of leys

Total dry matter yields of the leys in Juva diminished from almost 10 000 kg ha^{-1} in 2004 to less than half of that in 2006, which indicates a decrease in yield with increasing age of the ley. In Sotkamo, the trend was quite the opposite, as yields increased slightly beeing around 7 000 kg ha^{-1}. The yields of the second cuts were always lower than those of the first cuts, except in 2004 in Juva, when they were equal. In Sotkamo, especially, the shorter time for growth of the second cut (45 days, calculated from the beginning of thermal growing season) compared to the first cut (66 days) explained the lower yield (Fig. 3). Variation in yields within the fields was very high. The difference between the highest and lowest yields was roughly as high as the mean yield itself. The only exception was the yield of the second cut in 2006, where the variation was even higher as a result of the very dry July and August (Fig. 2).

The red clover content of the leys was 50% in the first year for both cuts in both fields. In the next two years, the clover content was 17% in the first cut and 35% in the second cut in Juva, but in Sotkamo it stayed at 50% for the first cut and increased to 65 – 70% for the second cut (Fig. 3). Variation in clover contents within the field was high, ranging from 20% to 90% per cut in Sotkamo. In Juva the minimum values were about 10% in all cuts and maximum values ranged from 40% to 90%.

Although the yields and clover contents of leys seem to show a highly positive correlation, based on means presented in Fig. 3, statistically significant correlations were found only in the first cuts in 2005 and 2006 in both fields, and these were negative (data not shown).

On both locations, each cut showed a quite different spatial variation pattern in ley yields, as indicated by PCA. Four PCs were found in the analysis of the six cuts (Fig. 4a and 4b). The PCs seemed to follow the age of the ley. In Juva, PC1 included both cuts in 2004 with loadings of 0.73 and 0.86 for the first and second cuts, respectively. The first cut in 2005 formed PC2 with a loading of 0.82. The second cut in 2005 and the first cut in 2006 formed PC3, with loadings of 0.82 and 0.52. The second cut in 2006 was alone in PC4 with a loading of 0.97. In addition, the first cut in 2006 had some similarities with both cuts in 2004 (PC1) with loading of 0.77. In Sotkamo, the four PCs were formed according to the age of the ley as well. PC1 included the first cut in 2004 (with a loading of 0.94), PC2 included the second cut in 2004 and the first cut in 2005 (with loadings of 0.95 and 0.65), PC3 included the second cut in 2005 (with a load-

Vol. 17 (2008): 376–393.

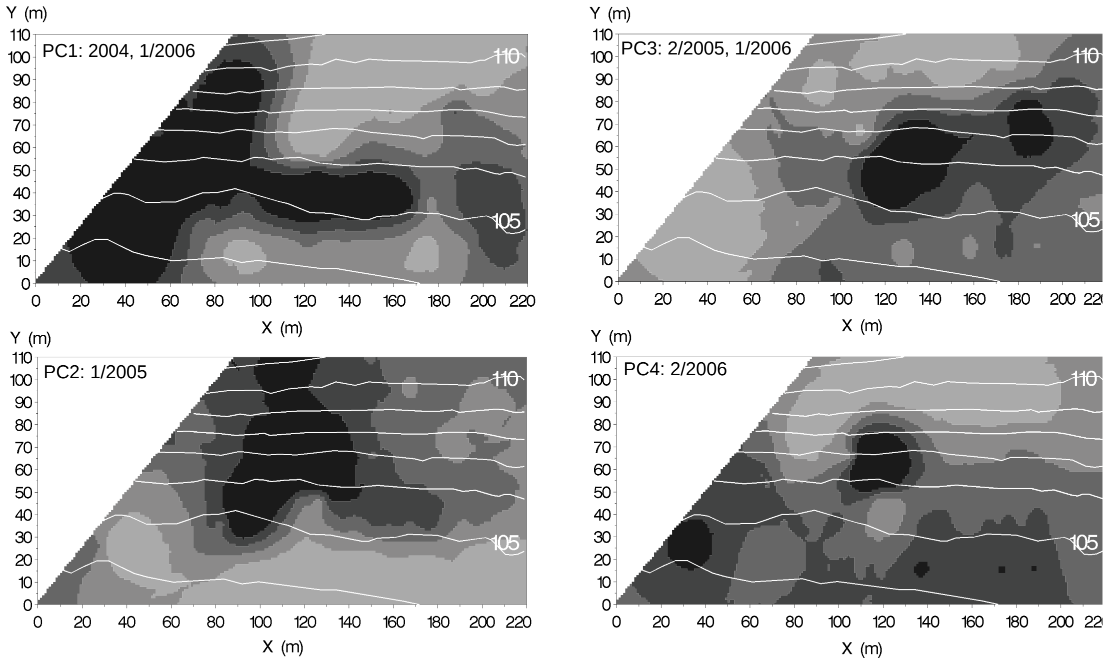

Fig. 4a. Relative yields of clover-grass leys in four principal components (PC) found in Juva in six cuts in 2004–2006. Grey tone density indicates the continuum from high (black) to low (light grey) relative yields. White lines and numbers inside the maps describe the altitude contours of the fields.

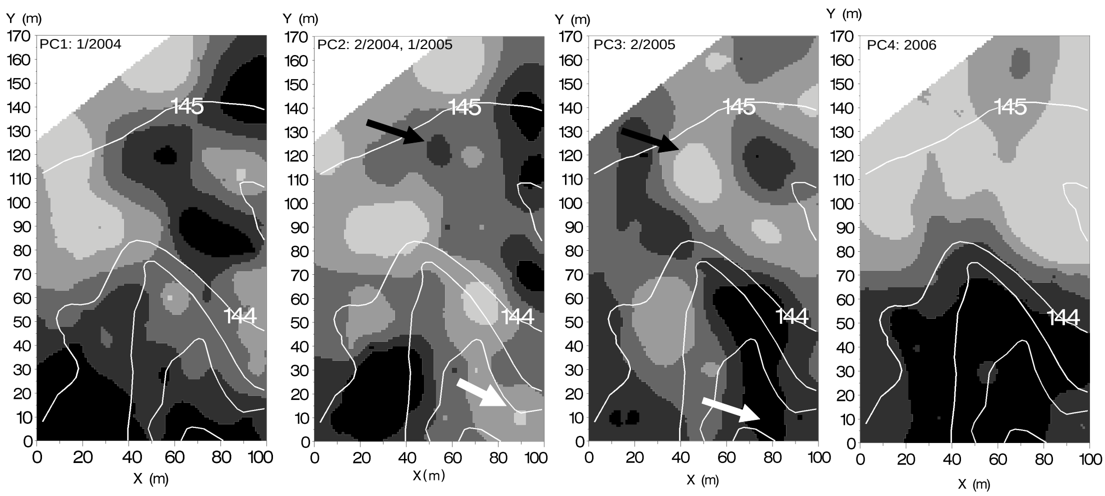

Fig. 4b. Relative yields of clover-grass leys in four principal components (PC) found in Sotkamo in six cuts in 2004–2006. Grey tone density indicates the continuum from high (black) to low (light grey) relative yields. The arrows point out the spots of compensatory yields in PC2 compared to PC3. White lines and numbers inside the maps describe the altitude contours of the fields.

ing of 0.92) and PC4 included the both cuts in 2006 (with loadings of 0.85 and 0.86).

In Juva, the highest yields were always found in the middle of the field, where the slope was almost the steepest (Fig 4a). PCs reflect also quite well the influence of precipitation, because the yields were lower in the lower parts of the field in PC2 in the rainy year 2005 and in the upper end of the field in PC4 in the dry second growth period in 2006. In both locations, but especially in Sotkamo, the PCs also seemed to change along with the age of the ley. In Sotkamo, the changes within the field were more rapid and it is more difficult to draw a clear picture for the yield variations (Fig 4b). An interesting phenomenon appeared in some spots in the Sotkamo field in 2005 in PC2 and PC3, where high first-cut yields were followed by low second-cut yields and vice versa (pointed out by arrows in Fig. 4b).

Spatial dependence disappeared in the analysis of dry matter yields when the distances between plots were 50−60 m in Juva and 40−60 m in Sotkamo. The corresponding range of spatial dependence was much shorter for clover content, 28−44 m and 31−61 m in Juva and Sotkamo, respectively.

Nitrogen fixation

The Ndfa, measured with the ^{15}N enrichment technique from micro-plots in Juva, was relatively high: the mean values of each cut during production years varied from 92% to 96%. The lowest values were found in some plots from the second cut in 2004 and from both cuts in 2006 (Table 1).

The calculated amounts of BNF of the red clover based leys in Juva decreased from 150 kg N ha^{-1} in 2004 to about 40 kg N ha^{-1} for next two years, calculated as means of all plots. In Sotkamo, the amounts increased with increasing age of the leys from 100 to 120 kg N ha^{-1} in three years of ley production. The BNF was always higher in the first than in the second cut in Sotkamo, but not in Juva, reflecting the higher yields in the first cut in Sotkamo (Fig. 5). The variation of BNF within the fields was high, with up to about 200 kg N ha^{-1} y^{-1} between the lowest and the highest values in the plots in 2004. In general, the highest values were twice the mean value and the lowest values were half of the mean value, except in 2004 in Juva.

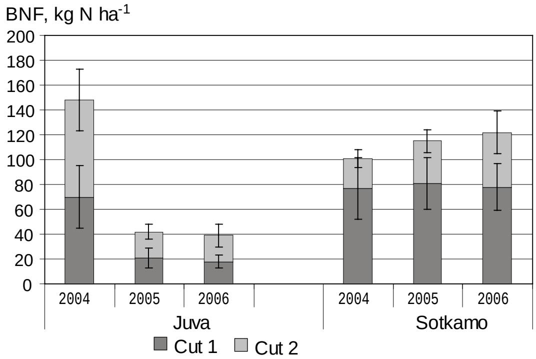

Fig. 5. The amounts of biological nitrogen fixation (BNF) of the leys in 2004–2006 in Juva and Sotkamo. Vertical lines indicate standard deviations.

Despite PCA not being able to combine the yield and clover content maps very effectively, BNF was described by two PCs in both locations (Fig. 6a and 6b). PCA maps of BNF described the pattern of spatial variation of ley yields quite well, as PC1 was quite the same for both ley yield and BNF as well as PC3 and PC4 for ley yield com-

Table 1. Mean, minimum and maximum values (%) of N derived from air (Ndfa) in red clover of six cuts in the Juva field measured by ^{15}N enrichment technique.

	2004		2005		2006	
	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
Mean	0.96	0.92	0.96	0.96	0.94	0.95
Min	0.93	0.56	0.91	0.92	0.83	0.73
Max	0.99	0.98	0.99	0.99	0.99	0.99

Vol. 17 (2008): 376–393.

pared to PC2 for BNF in Juva. In Sotkamo, PC4 for ley yields could be defined by the PC2 for BNF as well (Fig. 4b).

PCA showed that in Juva both cuts in 2004 and the first cut in 2005 followed the same pattern in the field, with loadings of 0.89, 0.80 and 0.75 for the three cuts, respectively. The rest of the BNF amounts formed the second component, with loadings of 0.82, 0.57 and 0.78 in the second cut in 2005 and the first and the second cut in 2006, respectively. The highest BNF amounts were obtained in the left hand side of the field for PC1 and in the centre of the field for PC2. On the other hand, the lowest BNF amounts were always situated on the right hand side of the field (Fig. 6a)

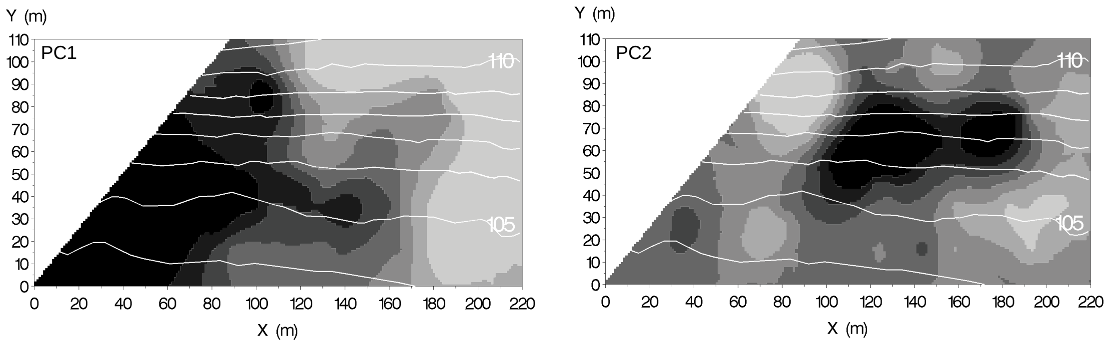

Fig. 6a. Relative amounts of BNF in two principal components (PC) found in PCA in Juva. PC1 included both cuts in 2004 and first cut in 2005. PC2 included the second cut in 2005 and both cuts in 2006. Grey tone density indicates the continuum from high (black) to low (light grey) relative amounts of BNF. White lines and numbers inside the maps describe the altitude contours of the fields.

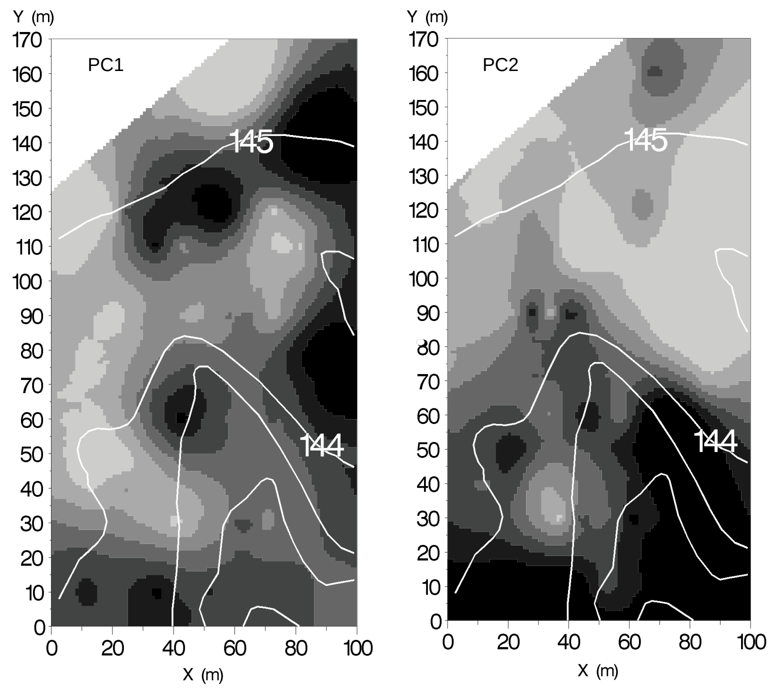

Fig. 6b. Relative amounts of BNF in two principal components (PC) found in PCA in Sotkamo. PC1 included both cuts in 2004 and first cut in 2005. PC2 included the second cut in 2005 and both cuts in 2006. Grey tone density indicates the continuum from high (black) to low (light grey) relative amounts of BNF. White lines and numbers inside the maps describe the altitude contours of the fields.

In Sotkamo two PCs were also found connecting the different cuts in the same way as in Juva. The loadings of PC1 were 0.54, 0.84 and 0.75 for the first and second cuts in 2004 and the first cut in 2005, and loadings of PC2 were 0.86, 0.57 and 0.88 for the second cut in 2005 and the first and second cuts in 2006. The lowest BNF amounts were first observed in the left hand side of the field in PC1, but then moved to the right hand side of the upper end of the field for PC2, where also the biggest difference between two PCs was found (Fig. 6b).

Spatial dependence of BNF disappeared at a range of 31−40 m both in Juva and in Sotkamo. This was at the same level as the spatial dependence of the clover content in Juva and even lower than the spatial dependence of the clover content in Sotkamo reflecting the high variability of the BNF inside the fields.

Soil nutrients

The nutrient status of the soil in the plough layer (0−25 cm) of the fields varied from soil classes 'poor' to 'excessive' according to the seven-class Finnish soil classification system depending on the nutrient under investigation (Viljavuuspalvelu 2000, Table 2). The mean values were in the classes 'acceptable', 'moderately good' and 'good'. The range of most nutrients was higher in Sotkamo than in Juva, since in Sotkamo, the nutrients fell into a range of three to to six classes and in Juva they fell into a range of two to five classes. The level of micronutrients, expressed as overall means, was lower in Sotkamo than in Juva.

In the PCA, two PCs were found in Juva and three in Sotkamo (Table 3, Fig. 7a and 7b). In Juva, the appearance of PC1 included all nutrients

Table 2. Mean, minimum and maximum values of soil nutrients (N_{tot} and C_{tot} %, others mg l^{-1} soil), standard deviation (Sd), soil classes, where the values fall in according to Finnish soil classification (Viljavuuspalvelu 2000), and the range of their spatial dependence (Range, m) in plough layer (0–25 cm) in spring 2003 before ley establishment in Juva and Sotkamo.

	pH	N_{tot}	Ctot	Ca	K	Mg	P	B	Mo	Cu	Fe	Mn	Zn
Juva													
Mean	5.9	0.14	2.9	790	105	95	14	0.46	0.08	5.6	204	40	5.3
Min	5.3	0.09	2.0	419	69	44	4	0.33	0.05	3.6	99	15	2.5
Max	6.8	0.23	4.6	1629	156	250	46	0.60	0.11	8.6	353	101	10.6
Sd	0.3	0.03	0.6	271	23	49	10	0.05	0.02	1.1	55	16	2.3
Classes[a]	2–6			2–4	2–4	1–5	2–6	2–3	4–5	4–5		3–5	4–5
Range	40	40	50	80	80	80	30	15	40	30	50	40	80
Sotkamo													
Mean	6.2	0.15	3.0	1203	74	140	12	0.69	0.02	4.1	558	33	2.0
Min	5.4	0.07	1.1	378	37	26	4	0.27	0.01	1.8	149	9	0.6
Max	6.8	0.42	7.7	2766	158	240	35	1.31	0.05	6.9	1161	78	6.3
Sd	0.4	0.07	1.4	383	26	48	7	0.24	0.01	1.2	225	17	1.3
Classes[a]	3–6			1–6	1–4	1–5	2–5	2–6	1–4	3–5		2–5	1–5
Range	50	40	30	45	75	30	45	45	45	70	40	40	70

[a] Classes in a seven–class Finnish soil classification system: 1=poor, 2=fairly poor 3=acceptable, 4=moderately good, 5=good, 6=high, 7=excessive.

Vol. 17 (2008): 376–393.

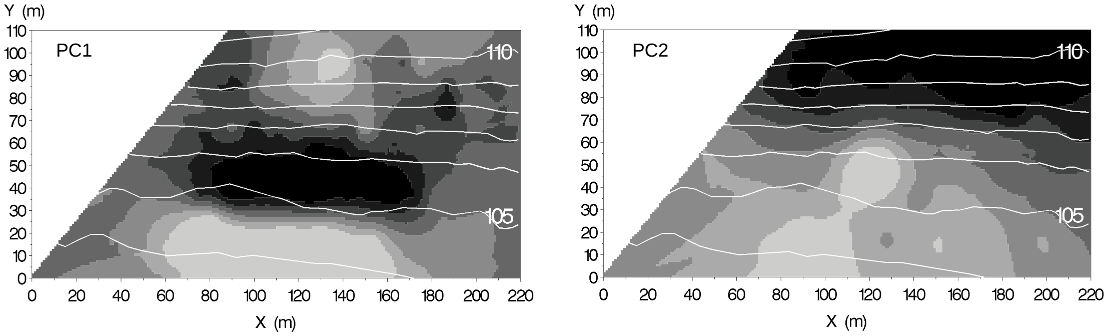

Fig. 7a. Relative nutrient concentrations in two principal components (PC) found in PCA in Juva. Grey tone density indicates the continuum from high (black) to low (light grey) relative nutrient concentrations. White lines and numbers inside the maps describe the altitude contours of the fields.

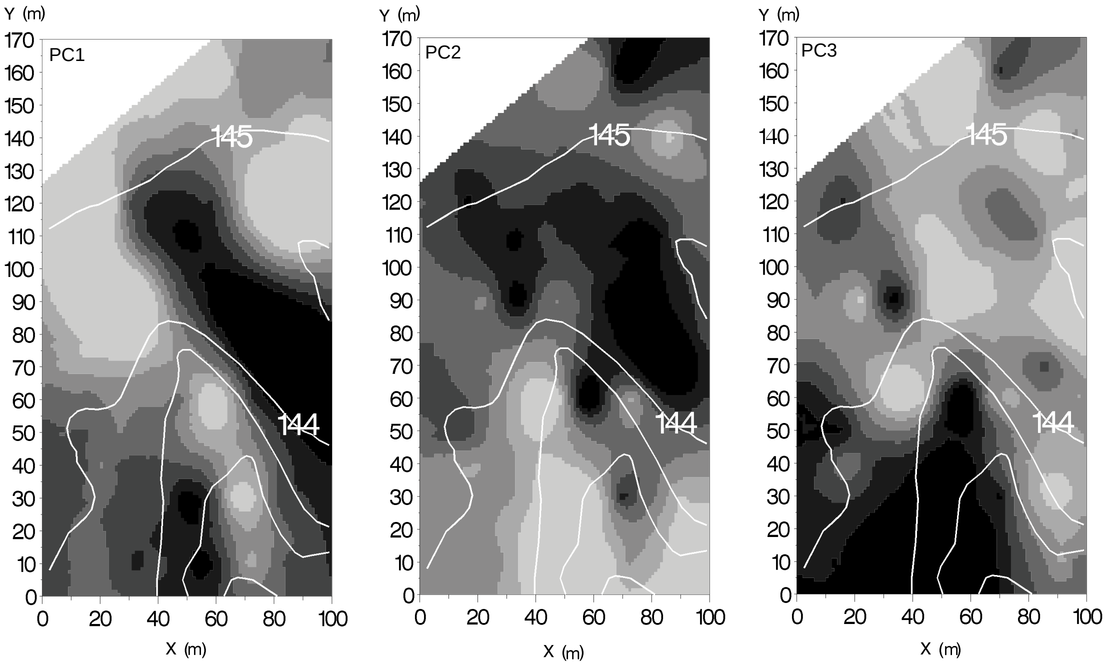

Fig. 7b. Relative nutrient concentrations in three principal components (PC) found in PCA in Sotkamo. Grey tone density indicates the continuum from high (black) to low (light grey) relative nutrient concentrations. White lines and numbers inside the maps describe the altitude contours of the fields.

measured except B. On the other hand, PC2 described the appearance of pH and Ca, Mg, P and Zn. N_{tot} was the only nutrient having a negative loading in Juva in relation to the nutrients. Analysis showed that all the nutrients have low concentrations in the lower part of the field, but nutrients in PC1 have high concentrations in the middle of the field while the nutrients in PC2 have high concentrations in the upper end of the field (Fig. 7a).

In Sotkamo, PC1 included the same nutrients as did PC2 in Juva, but also included B and Mn with positive loadings as well as C_{tot} and Fe with

Table 3. Principal component (PC) loadings for soil nutrients in Juva and Sotkamo. Loadings below 0.50 are omitted for clarity.

	Juva		Sotkamo		
Variable	PC 1	PC 2	PC 1	PC 2	PC 3
Ctot	0.74		–0.68	0.67	
Ntot	0.58	–0.70	–0.61	0.65	
pH		0.89	0.95		
Ca	0.51	0.79	0.81		
K	0.63				
Mg	0.51	0.78	0.70		0.51
P	0.61	0.64	0.73		
B			0.70		
Mo	0.74				0.61
Cu	0.81			0.74	
Fe	0.86		–0.73		
Mn	0.79		0.52		0.64
Zn	0.53	0.51	0.50	0.76	

negative loadings (Table 3). On the other hand, N_{tot}, C_{tot}, Cu and Zn had high positive loadings in PC2 in Sotkamo. PC3 described the appearance of Mg, Mo and Mn. In the Sotkamo field, there were spots in the upper and lower parts of the field where the nutrients had low concentrations in all PCs (Fig. 7b).

Most of the nutrients as well as pH showed short-range spatial dependence at a range of 30–50 m. The spatial dependence of K and Zn in both locations, and Ca and Mg in Juva and Cu in Sotkamo, disappeared at a range of 70–80 m. The range for B was only 15 m in Juva.

Discussion

In this study the dry matter production of the red clover-grass ley diminished by 2 500 kg ha^{-1} in the three years of production in Juva. In Sotkamo, the yields increased slightly as the ley aged, in spite of the very dry growing season in 2006 when the ley was three years old. Nykänen et al. (2000), Väisänen et al. (2000) and Mela (2003) found two-year-old leys to have the highest production and clover content of the red clover based leys in Finland and Mela (2003) concluded that soil type and red clover variety are important factors for clover percistence.

In our experiments, there might be two explanations for the different results between the fields of Juva and Sotkamo. Firstly, this may be a result of the different cultivation histories of the fields. The Juva field had been under organic farming with red clover in crop rotation for 20 years and manure has been used regularly. In Sotkamo, the field had been fertilised with mineral fertilisers and no red clover had been grown for at least 20 years. Especially the cultivation of red clover in Juva may have resulted in more clover pathogens of plant diseases in soil. Secondly, in Juva the first cut of the ley was done quite early in 2005 and 2006 (53 growing days, i.e. 320 degrees as a cumulative temperature) compared to Sotkamo (64 days, 450 degrees) and even the first cut in 2004 in Juva (73 days, 360 degrees). Red clover grows and develops more slowly than grasses in spring. It might be reasonable to wait until red clover is at the beginning of flowering for

Vol. 17 (2008): 376–393.

doing the first cut, because in Finland, red clover is said to be a two-cut-system plant (Pulli 1980).

The Ndfa of red clover was fairly high in all our measurements in the Juva field, which is most likely a result of the strong competition for soil mineral N by grasses grown in the same soil as has been shown earlier (Carlsson and Huss-Danel 2003). Some lower Ndfa values occasionally occurred in the second cut clover in 2004 and in clover from both cuts in 2006. The reason for this might have been higher soil mineral N, which induces higher N uptake from soil in red clover. The soil mineral N might have been higher after N release from manure application in ley establishment for the second cut in 2004. It is also known that drought influences the Ndfa if it continues for an extended period because dry conditions decrease the activity of the nitrogenase enzyme while plant N-uptake from soil continues (Wery et al. 1986, Ledgard et al. 1987). This was the situation in dry growing season in 2006. Generally, BNF and nitrogenase activity are highly correlated with plant growth (Lindström 1984). In those cuts with low Ndfa, a negative correlation was found between Ndfa and N_{tot} content of grasses, which is in line with the findings of Hansen and Vinther (2001) and might indicate higher soil N.

The higher amounts of BNF have been explained in many cases by higher clover dry matter production, not by higher Ndfa (Väisänen et al. 2000, Huss-Danell et al. 2007). Our results from measurements with the ^{15}N enrichment technique in Juva field support these findings, and thus it is reasonable to estimate BNF as a function of clover dry matter yield, for example with the formula developed by Carlsson and Huss-Danell (2003). Certainly, it has to be taken into consideration that this formula was not validated in the Sotkamo field and uncertainties are quite possible in Ndfa values, since the field was converted to organic farming practices in the establishment of the ley. N_{tot} of soil was same in both fields as a mean value, but the maximum values were higher in the Sotkamo field (Table 2). This might affect Ndfa, although it was not proved in the Juva field. In addition, clover content determination by NIRS has a source of error, but according to our experience, higher error is most probably caused by taking of representative sample from the plot and field.

In the Juva field, BNF was highest in the first production year of the ley as could be expected on the basis of high clover dry matter production and is a common finding (Heichel and Henjum 1991, Farnham and George 1993, 1994, Nesheim and Øyen 1994). There might be two explanations for that. Firstly, plant diseases may have reduced the amount of clover in the ley after the first production year. Plant diseases were not determined from the fields. Severe damages of clover root were not observed, but diseases caused by *Fusarium spp*. (root rot) are not easily detected in the field and therefore, root rot might have weakened the clover plants. Secondly, the application of manure may have influenced the clover production in the first year, because amounts of N in soil might have been higher and red clover grown better after manure application.

In Sotkamo, the BNF increased from the first year onwards, which is in agreement in studies where more BNF has been found in the second production year (Kristensen et al. 1995, Väisänen et al. 2000). Väisänen et al. (2000) measured the highest amounts of BNF in the yields of the second production year also on organic farms. The BNF was always higher in the first than in the second cut.

The mean concentrations of nutrients for both fields were mostly similar as in the measurements of Mäkelä-Kurtto and Sippola (2002), who monitored the Finnish soils in 1998 from 705 sites for the same nutrients as we measured. In Juva, Mg and Fe were lower and Cu higher, and in Sotkamo, pH and B were higher than in the data of Mäkelä-Kurtto and Sippola (2002). This result is quite interesting, as it shows that the nutrient levels in a field with a long cultivation history of organic farming can still be sufficiently good.

The spatial dependence was quite similar in the two fields under investigation. This is different from the results of Jauhiainen et al. (2008), as they found the extent of variation in soil properties and associated spatial dependences to vary from field to field. This is most probably explained by the fact that our soils were both mineral soils while Jauhiainen et al. (2008) had several soil types included in their investigations.

The overall variation in nutrients within the fields was quite high as the nutrients fell into two to six out of seven soil classes of the Finnish soil classification system. This suggests that the spatial analysis combined with PCA will work well in these fields and there is higher possibility to find different interactions. In Juva, almost all nutrients had high loadings in the same PC, showing high concentrations in the middle of the field, which also had high ley yields. On the other hand, there are some doubts in combining PCA with spatial analysis in the analysis of soil nutrients, although PCA described similar patterns for several nutrients. In Sotkamo, a positive loading for pH was connected to positive loadings for B, Mn and Zn indicating higher concentrations with higher pH. These results are not likely to be true, because the availability of B, Mn and Zn should be lower with higher pH (Viljavuuspalvelu 2000). Behind this inconsistence there can be factors, which were not measured in our study. In addition, the number of analysed nutrients was high compared to the number of plots. This means that PCA combined different nutrients in a crude way and some of the findings might not be relevant. In studies by other authors, only a few parameters have been included in the same analysis giving fairly good results (Odlare et al. 2005, Borůvka et al. 2007). The experimental design might be improved by taking even more samples from the field. Analysis of other soil properties like biological and physical factors might give us more relevant information about the relationships between soil nutrients, as they are all connected to each other in the soil processes.

Based on the spatial dependence of these two fields, the sampling interval should be 100 m and 60–120 m for yield and clover content determination, respectively. This is important knowledge when BNF of the field should be determined, as BNF is calculated based on the clover yield, i.e. multiplying ley yield by its clover content. This sampling interval corresponds to about 2–3 samples per hectare, which might be too many for farmers during the very busy time of harvesting silage. In addition, these distances might not be valid in all other fields. Farmers can also estimate the yield based on volume weights in storage, as some estimates (700–800 kg m^{-3}, fresh weight) are given in professional magazines. Rinne et al. (2008) have developed a method for red clover content estimation of red clover-grass silage based on the Ca-content of silage, as the Ca-content of grasses and red clovers is different (4 g kg^{-1} vs. 14 g kg^{-1}). In the future, it might be possible to develop the harvesting machines for yield and clover content measurements by NIRS as this technique has developed substantially.

Sampling intervals for nutrient analyses depends on the nutrient under investigation, ranging from 60 to 150 m. On average, macronutrients, except P, have a spatial dependence about 75 m and micronutrients plus P, have a spatial dependence of 30 to 50 m (average 40 m) resulting in sampling intervals of 150 and 80 m (two times the spatial dependence). Our findings in Juva and Sotkamo support the current recommendation for soil sampling in Finland of 1–3 samples per hectare (Viljavuuspalvelu 2000). Actually, the number of soil samples should be decided according to the most variable property, which in our fields means four samples per hectare, based on a spatial dependency of 30 m. This is very well in line with the results of Jokinen (1983), who reported that four samples per hectare describe sufficiently the following soil properties: pH, P, K, Mg, Ca, Al, organic C, particle size distribution and cation exchange capacity. In the Juva field, the spatial dependence of B was only 15 m, which is very low and reflects the very rapid changes in the concentrations of B in the field.

Conclusions

The temporal and spatial variation of yield, clover content and BNF of red clover-grass leys is very high in our Nordic conditions in organic farming. It is difficult to take a representative sample for the detemination of these variables on a field level and new techniques should be developed for this for example in the harvesting machines.

Vol. 17 (2008): 376–393.

The amount of BNF can be promoted, if the clover proportion of the ley, i.e. clover yield, is high. This remains a challenging task.

Acknowledgements. We thank the Ministry of Agriculture and Forestry of Finland for the financing of the experimental work of this study. Acknowledgments for the financial support for the writing of this article belong to the Finnish Cultural Foundation. We also thank the personnel of the MTT Agrifood Research Finland for the valuable field and laboratory work.

References

Bailey, T.C. & Gatrell, A.C. 1995. *Interactive spatial data analysis*. New York, USA: Longman Scientific & Technical. 413 p.

Borůvka, L., Mládková, L., Penížek, V., Drábek, O. & Vašát, R. 2007. Forest soil acidification assessment using principal component analysis and geostatistics. *Geoderma* 140: 374−382.

Brooker, P. 2001. Modelling spatial variablility using soil profiles in the Riverland of South Australia. *Environmental International* 27: 121−126.

Carlsson, G. & Huss-Danell, K. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* 253: 353−372.

FAO 2006. World reference base for soil resources 2006. *World soil resources reports* 103. Rome: Food and Agriculture Organization of the United Nations. 128 p. Available on the Internet: http://www.fao.org/AG/AGL/agll/wrb/doc/wrb2006final.pdf.

Farnham, D.E. & George, J.R. 1993. Dinitrogen fixation and nitrogen transfer among red clover cultivars. *Canadian Journal of Plant Science* 73: 1047−1054.

Farnham, D.E. & George, J.R. 1994. Harvest management effects on dinitrogen fixation and nitrogen transfer in red clover-orchardgrass mixtures. *Journal of Production Agriculture* 7: 360−364.

Hansen, J.P. & Vinther, F.P. 2001. Spatial variability of symbiotic N2 fixation in grass-white clover pastures estimated by the ^{15}N isotope dilution method and the natural ^{15}N abundance method. *Plant and Soil* 230: 257−266.

Heichel, G.H. & Henjum, K.I. 1991. Dinitrogen fixation, nitrogen transfer, and productivity of forage legume-grass communities. *Crop Science* 31: 202−208.

Huss-Danell, K., Chaia, E. & Carlsson, G. 2007. N_2 fixation and nitrogen allocation to above and below ground plant parts in red clover-grasslands. *Plant and Soil* 299: 215−226.

Jauhiainen, L., Öfversten, J. & Kangas, A. 2008. Recommendable block sizes: a case study on Finnish official variety trials of barley cultivars. *Agricultural and Food Science* 17: 63–72.

Jokinen, R. 1983. Variability of topsoil properties at the southern coast of Finland and the number of soil samples needed for the estimation of soil properties. *Journal of the Scientific Agricultural Society of Finland* 55: 109−117.

Kristensen, E.S., Høgh-Jensen, H. & Kristensen, I.S. 1995. A simple model for for estimation of atmospherically-derived nitrogen in grass-clover systems. *Biological Agriculture & Horticulture* 12: 263−276.

Lakanen, E. & Erviö, R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. *Acta Agralia Fennica* 123: 223−232.

Lark, R. 2000. Designing sampling grids from imprecise information on soil variability, an approach based on the fuzzy kriging variance. *Geoderma* 98: 35−59.

Ledgard, S.F., Simpson, J.R., Freney, J.R. & Bergersen, F.J. 1985. Field evaluation of 15N techniques for estimating nitrogen fixation in legume-grass associations. *Australian Journal of Agricultural Research* 36: 247−258.

Ledgard, S.F., Brier, G.J. & Littler, R.A. 1987. Legume Production and nitrogen fixation in hill pasture communities. *New Zealand Journal of Agricultural Research* 30: 413−421.

Lindström, K. 1984. Analyses of factors affecting *in situ* nitrogenase (C_2H_2) activity of *Galega orientalis, Trifolium pratense* and *Medicago sativa* in temperate conditions. *Plant and Soil* 79: 329−341.

Locher, F., Heuwinkel, H., Gutser, R. & Schmidhalter, U. 2005. Development of Near Infrared Reflectance Spectroscopy Calibrations to Estimate Legume Content of Multispecies Legume–Grass Mixtures. *Agronomy Journal* 97:11−17.

Locher, F., Heuwinkel, H., Gutser, R. & Schmidhalter, U. 2005. The Legume Content in Multispecies Mixtures as Estimated with Near Infrared Reflectance Spectroscopy: Method Validation. *Agronomy Journal* 97: 18−25.

Mela, T. 2003. Red clover grown in a mixture with grasses: yield, persistence and dynamics of quality characteristics. *Agricultural and Food Science* 12: 195−212.

Mäkelä-Kurtto, R. & Sippola, J. 2002. Monitoring of Finnish arable land: changes in soil quality between 1987 and 1998. *Agricultural and Food Science in Finland* 11: 273−284.

Nesheim, L. & Øyen, J. 1994. Nitrogen fixation by red clover (*Trifolium pratense* L.) grown in mixtures with timothy (*Phleum pratense* L.) at different levels of nitrogen fertilization. *Acta Agriculturæ Scandinavica* 44: 28−34.

Nykänen, A., Granstedt, A., Laine, A. & Kunttu, S. 2000. Yields and clover contents of leys of different ages in organic farming in Finland. *Biological Agriculture & Horticulture* 18: 55−66.

Odlare, M., Svensson, K. & Pell, M. 2005. Near infrared reflectance spectroscopy for assessment of spatial soil variation in agricultural field. *Geoderma* 126: 193−202.

Paz, A., Taboada, M.T. & Gomez, M.J. 1996. Spatial variability in topsoil micronutrient contents in a one-hectare cropland plot. *Communications in Soil Science and Plant Analysis* 27: 479−503.

Peoples, M.B., Faizah, A.W., Rerkasem, B. & Heeridge, D.F. 1989. *Methods for evaluating nitrogen fixation by nodulated legumes in the field.* Canberra, Australia. Australian Centre for International Agricultural Research. 76 p.

Pulli, S. 1980. Growth factors and management technique used in relation to the developmental rhythm and yield formation pattern of a clover-grass stand. *The Journal of the Scientific Agricultural Society of Finland* 52: 215−280.

Rinne, M., Nykänen, A. Kemppainen, J., Nyholm, L. & Nousiainen, J. 2008. Nurmikasvuston puna-apilapitoisuuden voi ennustaa kalsiumpitoisuudesta (Red clover content of sward can be estimated by its' Ca content). Maataloustieteen Päivät 2008. Available in Internet: http://www.smts.fi/mpol2008/index_tiedostot/Posterit/ps078.pdf. (in Finnish)

Samra, J.S., Rajput, R.K. & Katyal, V. 1992. Structural heterogeneity of soil pH and grain yield of rice and wheat grown in a sodic soil. *Agronomy Journal* 84: 877−881.

SAS. 2004. SAS/STAT 9.1 User's Guide. Cary, NC: SAS Institute Inc.

Shahandeh, H., Wright, A.L., Hons, F.M. & Lascano, R.J. 2005. Spatial and temporal variation of soil nitrogen parameters related to soil texture and corn yield. *Agronomy Journal* 97: 772−782.

Stenger, R., Priesack, E. & Beese, F. 2002. Spatial variation of nitrate-N and related soil properties at the plot scale. *Geoderma* 105: 259−275.

Sutherland, R.A. & Van Kessel, M.T. 1991. Spatial variation of nitrogen-15 natural abundance. *Soil Science Society of American Journal* 55: 1339−1347.

Talkkari, A., Jauhiainen, L. & Yli-Halla, M. 2002. Geostatistical prediction of clay percentage based on soil survey data. *Agricultural and Food Science* 11: 381−390

Väisänen, J., Nykänen, A. & Granstedt, A. 2000. Estimation of biological nitrogen fixation in Finnish organic grasslands. *Grassland Science in Europe* 5: 530−532.

Viljavuuspalvelu 2000. *Viljavuustutkimuksen tulkinta peltoviljelyssä* (Interpretation of analytical results of soil testing for field cultivation). Viljavuuspalvelu Ltd. 31 p. (in Finnish)

Vuorinen, J. & Mäkitie, O. 1955. The method of soil testing in use in Finland. *Agrogeological Publications* 63: 1−44.

Wallace, M. K. & Hawkins, D. M. 1994. Applications of geostatistics in plant nematology. *Journal of Nematology* 26: 626−634.

Wery, J., Turc, O. & Salsac, L. 1986. Relationship between growth, nitrogen fixation and assimilation in a legume (*Medicago sativa* L.). *Plant and Soil* 96: 17−29.

Witty, J.F, Rennie, R.J. & Atkins, C.A. 1988. ^{15}N addition methods for assessing N_2 fixation under field conditions. In: Summerfield, R.J. (ed.). *World crops: Cool season food legumes*. London, UK, Kluwer Academic Publishers. p. 715–730.

Yli-Halla, M., Mokma, L., Peltovuori, T. & Sippola, J. 2000. Suomalaisia maaprofiileja. (Agricultural soil profiles in Finland and their classification. In Finnish). *Publications of Agricultural Research centre of Finland, Serie A* 78. Jokioinen. Agricultural Research Centre of Finland 32 p. + 5 app. Available on the Internet: http://www.mtt.fi/asarja/pdf/asarja78.pdf

Vol. 17 (2008): 376–393.

SELOSTUS

Apilanurmen, typensidonnan ja maan ravinnepitoisuuksien spatiaalinen vaihtelu pellolla

Arja Nykänen, Lauri Jauhiainen, Jukka Kemppainen ja Kristina Lindström

Maa- ja elintarviketalouden tutkimuskeskus ja Helsingin yliopisto

Luonnonmukaisen viljelyn perustan muodostaa palkokasvien biologinen typensidonta, johon tarkoitukseen Suomessa viljellään eniten puna-apilaa (*Trifolium pratense*) monivuotisissa nurmissa heinien kanssa seoksena. Tässä tutkimuksessa käytettiin mallipohjaista paikkatietoon perustuvaa tilastoanalyysiä, jotta saataisiin selville puna-apilanurmien satojen, apilapitoisuuksien ja typensidonnan sekä pellon ravinteiden pinnanmyötäinen (spatiaalinen) vaihtelu. Tämä tieto on hyödyllinen, kun halutaan tietää sopivat etäisyydet näytteiden ottamiseksi em. muuttujien analysoimiseksi. Tieto auttaa myös valitsemaan pelloilta sopivat paikat kenttäkokeille. Kaksi kenttäkoetta tehtiin Juvalla ja Sotkamossa 2004 − 2006. Biologinen typensidonta määritettiin ensin osalta Juvan kokeen ruutuja ^{15}N -rikastusmenetelmällä ja sen jälkeen molemmille pelloille apilasatoon pohjautuvalla laskentakaavalla. Tämä oli perusteltua, koska apilan typestä yli 85 % oli peräisin ilmasta luomupelloilla, joilla liukoisen typen määrä maassa on alhainen. Nurmien kuiva-ainesadot (9 700 → 4 100 kg ha^{-1}), apilapitoisuudet (53 → 26 %) ja ilmasta sidotun typen määrät (150 → 40 kg N ha^{-1}) laskivat Juvalla nurmien vanhetessa. Sotkamossa ne puolestaan nousivat hiukan nurmien vanhetessa (6 500 → 7 100 kg ha^{-1}, 52 → 62 %, 100 → 120 kg N ha^{-1}). Peltojen sisäinen vaihtelu oli minimissään puolet ja maksimissaan kaksinkertainen verrattuna koko pellon keskiarvoon kunakin vuonna. Spatiaalianalyysi, mallipohjainen kriging, ja siihen yhdistetty pääkomponenttianalyysi tuottivat karttoja, jotka kuvasivat peltojen pinnanmyötäistä vaihtelua erittäin informatiivisella tavalla. Maan ravinnepitoisuudet vaihtelivat myös runsaasti pellon sisällä vaikkakin vaihtelu oli eri ravinteilla erisuuruista. PCA ei vaikuttanut tehokkaalta analyysiltä yhdistämään maan ravinnepitoisuuskarttojen informaatiota verrattuna sen toimivuuteen nurmiparametrien osalta. Tilastollisen paikkatietoanalyysin perusteella näytteet nurmisadon määrittämiseksi pitäisi ottaa vähintään 100 metrin välein, apilapitoisuuden määrittämiseksi 60 metrin välein. Pellon ravinnepitoisuuksien määritykseen näytteet tulisi ottaa 80 metrin välein. Kaikkien mitattujen muuttujien vaihtelun muoto oli erilainen eri pelloilla, mutta spatiaalinen riippuvuus niiden välillä oli kohtuullisen samanlaista, sillä molemmat pellot olivat kivennäismaita.