



# **Kol och kväve i mark och grödor i försök med monokulturer och växtföljder. En delstudie inom projektet Climate CAFE**

**Soil and crop carbon and nitrogen in an experiment with monocultures and crop rotations. A study within the project Climate CAFE**



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**Nyckelord: Kolhalt i marken, kvävehalt i marken, kvävefixering, proteinhalt i vall, monokulturer, korn, potatis rajgräs, foderraps, havre, ärt, vall, växtföljd**

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## Sammanfattning

De senaste årens extremväder har visat att vi riskerar att få ett mer varierat klimat i framtiden. EU-projektet Climate CAFE har arbetat med frågor om jordbrukets anpassning till ett framtida mer variabelt klimat. I den svenska delen av projektet har vi undersökt om varierade växtföljder, med baljväxter, kan vara ett bra sätt att tackla dessa variationer. I norra Sverige har vi provtagit tre långliggande försök i försöksserien R8-74 (startad 1965) där monokulturer av olika grödor har jämförts med samma grödor i en växtföljd. Försöken har också jämfört tre olika gödslingsbehandlingsmetoder; hög NPK, låg NPK och stallgödsel kompletterat med NPK. Kol- och kvävehalten i jorden och kvävehalten i vall och ettårigt rajgräs analyserades i prover från försöket i Röbbäcksdalen i Västerbotten. Kvävefixeringen i klöver från vall, och ärt samodlat med havre från Ås i Jämtland, Öjebyn i Norrbotten, och Röbbäcksdalen bestämdes genom att analysera den naturliga abundansen av kväveisotopen  $^{15}\text{N}$  i både baljväxter och gräs, havre och ogräs. Den naturliga abundansen av  $^{15}\text{N}$  analyserades också i jordproverna och proverna av vall och rajgräs.

Kol- och kvävehalten i marken hade minskat i alla växtföljder. I växtföljden med femårig vall var minskningen dock bara 26 % jämfört med 50 % i växtföljder med bara spannmål eller potatis och 40-44 % i växtföljder med varierande inslag av ettårigt gräs eller vall. Kolhalten i jorden är viktig för markens struktur och vattenhållande egenskaper, förutom att den är viktig för atmosfärens koldioxidhalt. Kvävehalten i jorden följde också samma mönster. De olika gödslingsmetoderna hade däremot likvärdig påverkan på kolet och kvävet i jorden. Hög NPK gödsling gav generellt högre vallskörd och högre proteinhalt i vallarna vissa år men detta påverkades inte av växtföljden. Hög NPK gav också lägre andel kvävefixering i både klöver och ärt. I klövern var kvävefixeringen lägst i den blandade växtföljden särskilt i den första vallskörden. Den totala  $^{15}\text{N}$ -abundansen i vallarna var däremot inte påverkad av vare sig gödsling eller växtföljd. Rajgräsets proteinhalt eller  $^{15}\text{N}$ -abundans påverkades inte signifikant av de olika gödslingsbehandlingarna.

Projektet har finansierats av Formas. Fältförsöken i R8-74 finansieras av SLUs NJ-fakultet och SITES (Swedish Infrastructure for Ecosystem Sciences). Jag tackar Ann-Sofi Hahlin, Miriam Larsson och Shakhawat Hossain som gjort mycket av arbetet.

Fotot på förstasidan är från försöket R8-74 i Ås i Jämtland och är taget av David Parsons.

## **Background**

At three sites in northern Sweden, Umeå, Ås and Öjebyn, experiments (R8-74) with monocultures of annual crops are compared with different crop rotations. In addition to the different crop rotation treatments, the experiment also includes fertilization with animal manure complemented with mineral fertilizer compared to only mineral fertilizers at two levels. These experiments have been conducted since 1965 and are still ongoing at the three sites.

Cultivation of perennial crops such as grass/clover ley and use of animal manure have been suggested as measures to enhance carbon sequestration in soil. However in most Swedish long-term experiments the use of animal manure is linked to the use of perennial ley. In R8-74 they are separate factors which makes it possible to test both fertilization and crop rotation effects on soil C and N storage and plant N.

## **Description of the experiment**



The R8-74 experiment in Rönö, Umeå. Photo Cecilia Palmberg

The Umeå site has silty soil. It is a former wetland that was drained in the 1950's and the C concentration was high at the start, 5.3%. The Ås experiment has clay morain with 2.5-3.1 %C at the start and the Öjebyn experiment has sandy soil with 1.5-1.9 %C at the start. From 1965 to 2010 six crops: grass/clover ley, barley, fodder rape, potatoes, annual ryegrass and oats, were either grown in monocultures or in crop rotations (Table 1). There were two six year rotations: Mixed rotation: barley undersown with ley- two years ley- fodder rape- potatoes- annual ryegrass and Long ley rotation: barley undersown with ley- five years ley. There were also two three year rotations: Short grain rotation: barley- barley- oats and Short ley rotation: barley undersown with ley-two years of ley. Mixed rotation was considered to be the reference, containing all monoculture crops. There were two replicate plots of each treatment and six replicate plots of Mixed rotation where each crop was represented in one plot every year. The ley was harvested twice a year, annual ryegrass three times a year and the other crops once a year.

Table 1: Crop order in the crop rotations in the long term monoculture experiment R8-74.

Barley=b, Barley undersown with ley= bl, Grass/clover ley=l, fodder rape=f, annual ryegrass=r, potatoes= p, whole crop pea/oats=w, oats=o

Ordningen på grödorna i de olika växtföljderna i det långliggande försöket R8-74. Korn=b, Korn med vallinsådd= bl, Gräs/klövervall =l, foderraps=f, ettårigt rajgräs=r, potatis= p, helsäd av ärt/havre=w, havre=o

Crop rotations	1965-2010	After 2010
Mixed rotation	bl-l-l-f-p-r	bl-l-l-w-p-r
Long ley rotation	bl-l-l-l-l-l	bl-l-l-l-l-l
Barley undersown with ley or Short ley rotation	bl-bl-bl-bl-bl-bl	bl-l-l-bl-l-l
Short grain rotation	b-b-o-b-b-o	b-b-w-b-b-w
Potatoes	p-p-p-p-p-p	p-p-p-p-p-p
Annual ryegrass	r-r-r-r-r-r-r	r-r-r-r-r-r-r
Barley	b-b-b-b-b-b	b-b-b-b-b-b
Fodder rape or Pea oats	f-f-f-f-f	w-w-w-w-w-w

There was a change in crops in 2010 (Table 1). Fodder rape was replaced by pea/oats harvested as a whole crop and barley undersown with grass clover was replaced by a three year crop rotation with barley undersown with ley and two years of ley.

There were also three different fertilization treatments in a split plot design (Table 2): Low NPK- low dose of NPK that replaced what is removed in the harvests, High NPK- double dose of NPK in Low NPK and Cattle manure+NPK- 10 tonnes per ha of cattle manure annually, complemented with NPK to the same level as Low NPK. From 2010 and still ongoing, the Low NPK treatment was omitted and the NPK fertilisation levels were changed to current recommendations (Table 2).

Table 2: Fertilization treatments: Examples of mineral N-fertilization to different crops.

Fertilization after 2010 in parentheses. Cattle manure was applied as 10 tonnes/ha of solid manure 1965-2010 and as 20 tonnes/ha of cattle slurry after 2010.

Exempel på olika mineralgödslingsgivor till grödor. Gödslingen efter 2010 inom parentes. Tio ton nötfastgödsel per ha spreds varje höst före 2010 och 20 ton nötflytgödsel per ha efter 2010.

Fertilization treatment	N to ley kg/ha	N to barley kg/ha	N to annual ryegrass kg/ha	N to pea/oats kg/ha
Low NPK	75	40	90	0
High NPK	150 (150)	80 (80)	180 (100)	(40)
Cattle manure + NPK	65 (120)	30 (50)	80 (70)	(10)

## Sampling and analysis

Dry stored soil samples from the Umeå experiment from years 2000 (sub soil 40-60 cm) and 2010 (top soil 0-20 cm) were milled in rolling bottles with steel rods after removal of visible roots. Four randomly selected samples of top soil from 1965 were also analysed since another analysis method for soil C was used at that time. The homogenised samples were analysed for % C,  $\delta^{13}\text{C}$ , % N and  $\delta^{15}\text{N}$  on a isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany). Database soil C data from 1988 are also presented as a comparison.

Dry stored plant samples from the first harvest 2001-2006 at the Umeå experiment of grass/clover and annual ryegrass were ball milled to a fine powder and analysed for % N and  $\delta^{15}\text{N}$  on the same mass spectrometer as above.

At all three experimental sites an extra sampling of 50 \* 50 cm plots was made before each harvest 2015 to quantify the percentage of nitrogen fixation in red clover and peas. The plant biomass was sorted into sown species and weeds and % N and  $\delta^{15}\text{N}$  was analysed. The  $^{15}\text{N}$  natural abundance method (Högberg 1997) was used to calculate the %N derived from the atmosphere, %Ndfa, as a measure of how much the legumes in the different treatments depended on nitrogen fixation. Timothy, meadow fescue and weeds were used as reference species for red clover and oats and weeds were used as reference species for peas.

All statistical analyses were made in NCSS 8 (Hintze 2012) and details are given in the results section.

## Results

### *Soil from the Umeå experiment*

All treatments had lower top soil C and N concentrations in 2010 than at the experiment start in 1965 (figures 1 and 2). The soil data were analysed by General Linear Model ANOVA. Fertilization was considered a random factor and Crop rotation was considered a fixed factor and their interaction was included in the model. There were no significant differences between the fertilization treatments in the top soil C or N data. Because of this and to avoid pseudo-replication the means of the three fertilisation treatments were calculated and used for further analysis with one way ANOVA. Long term ley had significantly higher C% ( $p=0.0375$ ) and N% ( $p=0.0222$ ) than all other crop rotations. It was noted that the monoculture barley and Short grain rotation had amongst the lowest treatment means although they were not significantly different due to the poor replication (figure 1). Data from these treatments were pooled to get four replicate “pure grain” plots and they were compared with treatments that were plowed most years but included some kind of forage grass (Mixed crop rotation, monoculture annual ryegrass and barley undersown with grass/clover each year). In this comparison the pure grain treatments had significantly lower soil C% ( $p=0.0328$ ) than the treatments with forage grasses and the N% was approaching significance  $p=0,0510$ ).

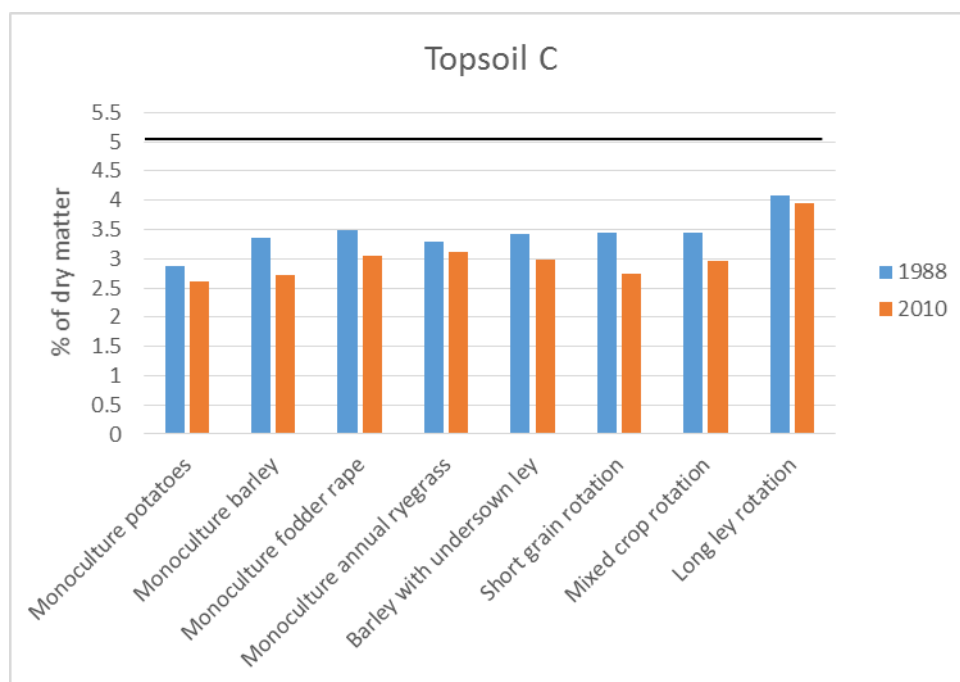


Figure 1. Remaining topsoil carbon after 23 and 45 years of monocultures or mixed crop rotations. Means of all fertilization treatments. The line at the top represents the mean C% in 1965. Kolhalter i matjorden efter 23 och 45 år av monokultur eller växtföljd. Medelvärden av alla gödslingsbehandlingar. Linjen visar medelvärdet av kolhalten 1965.



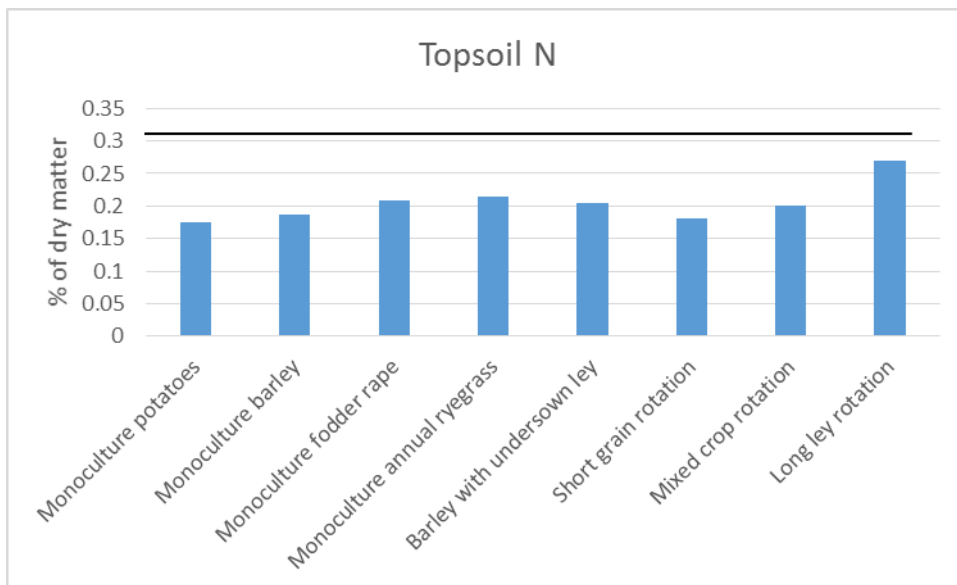


Figure 2. Remaining topsoil nitrogen after 45 years of monocultures or mixed crop rotations. The line at the top represents the N% mean in 1965. Means of all fertilization treatments. Kvävehalten i matjorden efter 45 år av monokultur eller växtföljd. Medelvärden av alla gödslingsbehandlingar. Linjen visar medelvärdet av kvävehalten 1965.

Thus, according to the first ANOVA, NPK fertilization above the level removed with the crops or fertilization with manure did not prevent carbon and nitrogen loss significantly. However the crop rotation with long term ley only had a decrease in top soil carbon concentration of only 26% while the decrease in pure grain rotations or potatoes was 50% and in rotations with both grain crops and forage crops 40-44%. Not all of this decrease in C concentration is due to carbon loss to the atmosphere, since a decrease of the carbon concentration also means an increased soil density that has to be taken into account (Bolinder, Katterer et al. 2010). The large decrease in soil C occurred between 1965 and 1988 (Ericson and Mattsson 2000). Compared to data from soil sampling 1988 the top soil C concentration was only slightly lower in 2010 for the Long ley rotation, while the decrease was large for Monoculture barley and the Short grain rotation (figure 1).

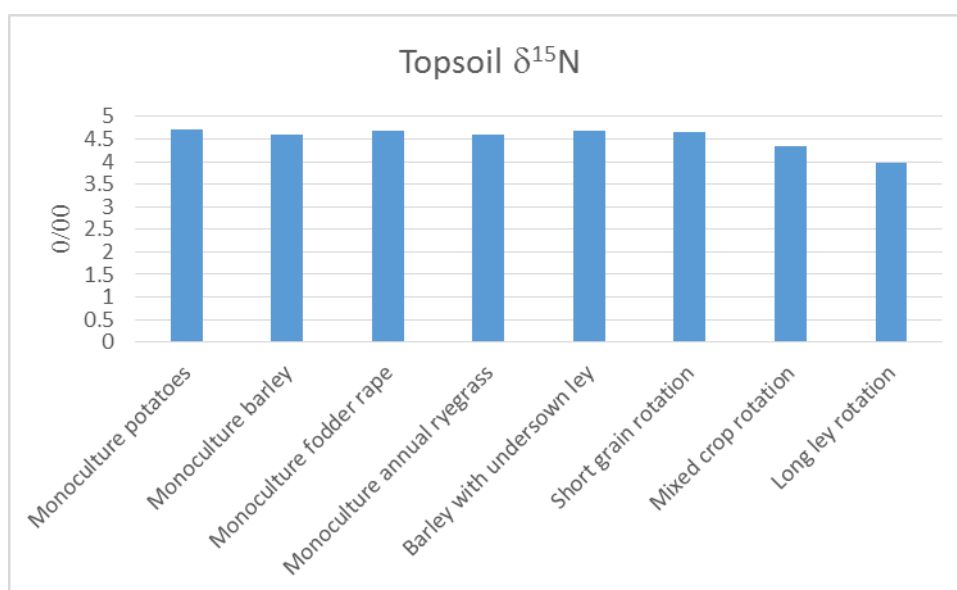


Figure 3. δ¹⁵N of topsoil 2010. Means of all fertilization treatments. δ¹⁵N i matjorden 2010. Medelvärden av alla gödslingsbehandlingar.



There were also significant differences between the treatments in the  $\delta^{15}\text{N}$ , with lower values in treatments with perennial grass/clover ley than in all monocultures (figure 3). This was probably due to the input of atmospheric N low in  $\delta^{15}\text{N}$  via the nitrogen fixation of clovers. It could also indicate that these systems have lower nitrogen losses since nitrogen losses are associated with high  $\delta^{15}\text{N}$  (Högberg 1997). Plots fertilized with cattle manure also had lower  $\delta^{13}\text{C}$  than the plots fertilized with NPK and monoculture fodder rape and potato had higher  $\delta^{13}\text{C}$  than the mixed crop rotation (data not shown). These differences are more difficult to explain.

There were no significant differences between any treatments in the sub soil. Sub soil had 0.69% C concentration and 0.057% N.

#### *N and $\delta^{15}\text{N}$ in stored plant samples from the first harvest 2001-2006*

Ley samples from both crop rotations with ley were analysed using Repeated measures ANOVA, since 2-5 years of data were used for each plot. Crop rotation and fertilisation were fixed between subject factors and year was a random factor within subject factor. For 2002-2006 there were no significant differences in N concentrations between crop rotations or fertilization treatments. However there was a tendency ( $p=0.076$ ) towards higher N% in the high NPK fertilisation treatment (Figure 5).

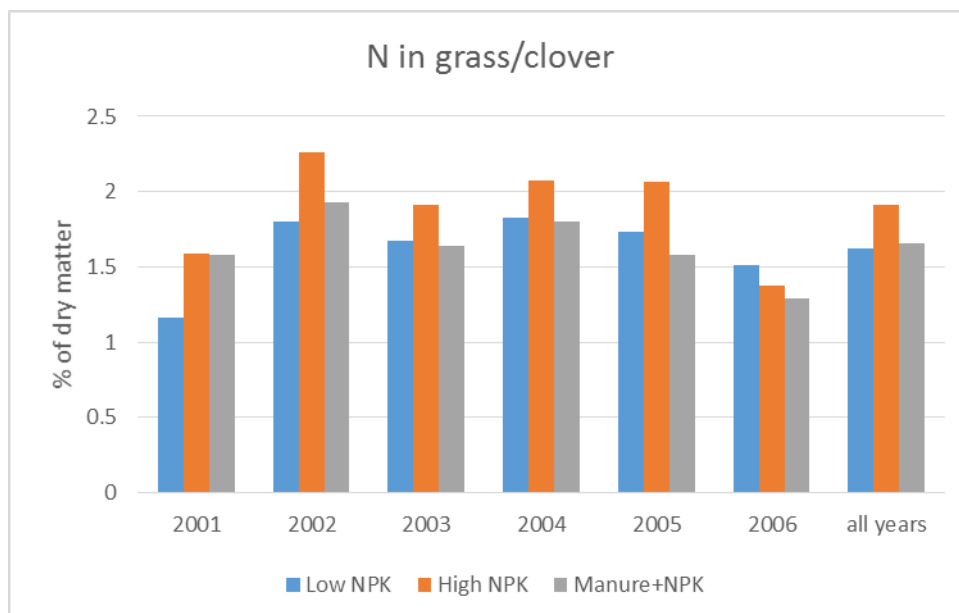


Figure 5. Means of nitrogen concentrations in the harvest of all ley plots with the same fertilization treatment (two mixed crop rotation plots all years and two long term ley plots 2002-2006).

Medelvärden av kvävekoncentrationerna i alla ytor med vall som fått samma gödslingsbehandling (två ytor i den blandade växtföljden varje år och två ytor i den långliggande vallen 2002-2006)

The ley in the mixed crop rotation and the long term ley was only the same age, and thus directly comparable, in 2002 and 2003. A separate analysis was made for these years. There was a significant effect of fertilization ( $p=0.0036$ ) and year but no significant differences between crop rotations. The High NPK treatment gave higher N% in the crop than the low NPK treatment. The High NPK treatment also resulted in 30-50% and the Manure+NPK treatment in 8-15% higher ley yields than the low NPK treatment 1965-1995 (Unpublished data). The  $\delta^{15}\text{N}$  data in both ryegrass and ley only had significant differences between years (data not shown).

For monoculture annual ryegrass the difference between fertilization treatments was tested. There were no significant differences in N% between fertilization treatments. However there were significant differences between the years (figure 6).

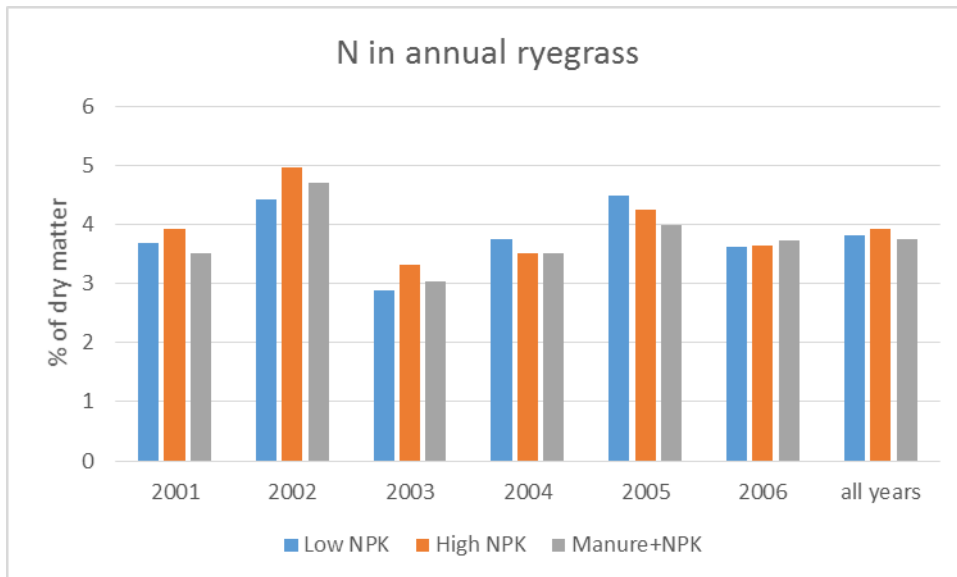


Figure 6. N concentration in annual ryegrass grown in monoculture for 35-40 years. Kvävekoncentrationer i ettårigt rajgräs som odlats år efter år på samma ytor 35-40 år.

#### Nitrogen fixation 2015

Nitrogen derived from atmosphere data from 2015 were analysed using Repeated measures ANOVA for red clover in the two ley harvests. Fertilization treatment and Crop rotation were fixed factors, plots at each site were the subject variable, site was a random factor and harvest occasion was the within subject factor.

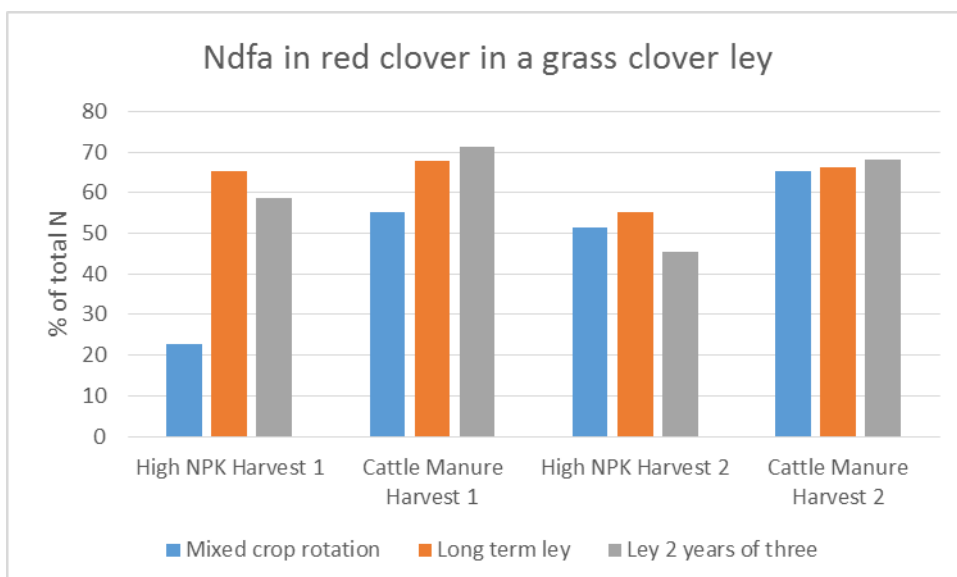


Figure 7. Means of %Ndfa in red clover of 1-2 plots at each of 3 different sites. Medelvärden av andelen fixerat kväve i rödklövern från 1-2 ytor vardera på 3 försökslokaler.

Both fertilisation treatment ( $p=0.00025$ ) and crop rotation ( $p=0.00545$ ) significantly affected nitrogen fixation in leys. %Ndfa was lower in the first harvest in the Mixed crop rotation (Figure 7). The lowest values were seen in the first year ley at all three sites. The high NPK fertilization had lower %Ndfa than the Cattle manure treatment.

General linear model ANOVA was used for peas in the pea/oats whole crop harvest. Fertilization treatment and Crop rotation were fixed factors and site was a random factor. There were significant differences between Fertilization treatments ( $p=0.021$ ) but not between Crop rotations ( $p=0.085$ ). Peas had lower %Ndfa with the high NPK fertilization than with the Cattle manure treatment (figure 8).

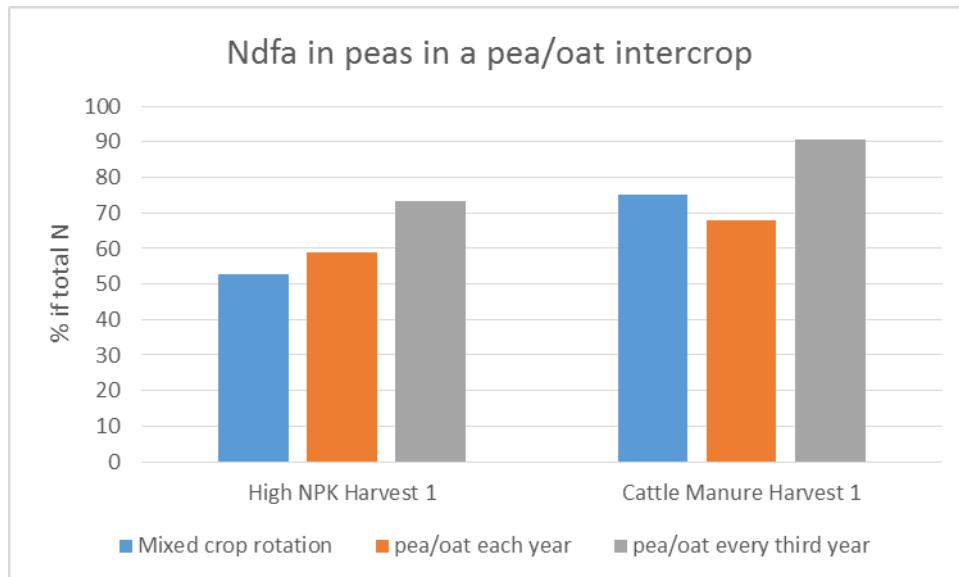


Figure 8. Means of %Ndfa in peas of 1-2 plots at each of 3 different sites. Medelvärden av andelen fixerat kväve i ärt från 1-2 ytor vardera på 3 försökslokaler.

## Conclusion

At the Umeå site, that used to be a wetland and thus had high soil C and N concentration at the start of the experiment, a Long ley rotation with barley undersown with ley that was renewed every six years prevented about half of the decrease of the C and N concentration in the soil compared to pure grain crop rotations. Soil C and N concentration were also somewhat higher in a mixed crop rotation including two years of ley, monoculture annual ryegrass and barley undersown with grass/clover each year than in pure grain rotations. High NPK-fertilization or cattle manure addition did not have any significant effect on soil C and N. The higher soil N under the long term ley compared to the mixed crop rotation did not affect the N concentration of the ley crop. The N concentration was increased by the High NPK-fertilization treatment though, but only significantly in the grass/clover leys, not in annual ryegrass. The doubled NPK fertilisation gave higher ley yields, but not twice as high. A study made in three sites in northern Sweden found negative effect of high NPK fertilization on nitrogen fixation as %Ndfa in red clover, especially in the first harvest of the Mixed rotation, compared to Cattle manure + NPK. A negative effect of high NPK fertilization was also seen in %Ndfa of peas in pea/oat intercrops regardless of crop rotation treatment.

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