Acta Agric. Scand. 41: 243-252, 1991

Carbon and Nitrogen Mineralization and Crop Uptake of Nitrogen from Six Green Manure Legumes Decomposing in Soil

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Marstorp, H. and Kirchmann, H. (Swedish University of Agricultural Sciences, Department of Soil Sciences, Box 7014, S-75007 Uppsala, Sweden). Carbon and nitrogen mineralization and crop uptake of nitrogen from six green manure legumes decomposing in soil. Received Febr. 20, 1991. Acta Agric. Scand. 41: 243-252, 1991.

Carbon and nitrogen mineralization from six green-manure legumes was studied in an incubation experiment. The values measured were fitted to a two-component and a one-component first-order model, respectively. Estimated parameters were compared. During 115 days of incubation approximately 30-35% of total N in white clover, black medic and subterranean clover was mineralized. Red clover and persian clover mineralized only 20% and egyptian clover 17%. White clover, black medic and subterranean clover showed a net mineralization during the whole incubation period. Red clover, persian clover and egyptian clover showed apparently an initial immobilization followed by a net mineralization after approximately 2 weeks. In a pot experiment, N uptake in rye grass from the six legumes was measured and comparisons were made with the incubation experiment after corrections for temperature differences. The amount of N harvested in the pot experiment was approximately 90% of the net N mineralization during decomposition. *Key words: Trifolium pratense* L., *T. repens* L., *T. resupinatum* L., *T. alexandrinum* L., *T. subterraneum* L., *Medicago lupulina L., mineralization rate, k-value.*

INTRODUCTION

Efficient use of green-manure legumes requires knowledge of plant growth and decomposition dynamics. Some growth characteristics of six legume species were earlier published in this journal (Kirchmann, 1988).

Decomposition and N dynamics of green manures incorporated into soil are complex. Energy requirement of the soil microbial population is considered to be the driving force behind decomposition and N mineralization (McGill & Cole, 1981). It is well known that the carbohydrate composition and the N content of green manures varies with species and growth stage. This influences both the decomposition rate and the N dynamics in soil during decomposition and hence the N nutrition of a green manured crop.

The use of controlled conditions for decomposition and N mineralization studies of green manures makes it possible to estimate kinetic parameter values. These parameter values can then be used for comparisons of different green manures and for predictions of the kinetics under changing environmental conditions.

The aim of this investigation was to study the kinetics of C and N mineralization of six green manure legumes in soil, to determine N uptake from decomposing legume material by subsequent crops and to compare the decomposition and uptake kinetics.

Nitrogen mineralization is the transformation of N from the organic state into the inorganic forms of NH_4^+ or NH_3 and immobilization is the transformation of inorganic N into the organic state (Jansson & Persson, 1982). The net mineralization or net immobilization will then be the net result of the mineralization/immobilization turnover. In the literature, however, the term "nitrogen mineralization" or "nitrogen immobilization" is often used for this net result. Sometimes, any gaseous losses are also included. In this paper, we use the terms "net mineralization" and "net immobilization" for the net result of the mineralization/immobilization turnover, including any gaseous losses.

MATERIAL AND METHODS

Plant material

Above-ground plant material of six different legumes (red clover *Trifolium pratense* L., white clover *T. repens* L., black medic *Medicago lupulina* L., persian clover *T. resupinatum* L., egyptian clover *T. alexandrinum* L. and subterranean clover *T. subterraneum* L.) harvested from a field experiment (Kirchmann, 1988) 100 days after emergence were used for this investigation. The plant material was dried at 40°C and milled (2 mm mesh-size) and analyzed for Kjeldahl nitrogen (Bremner & Mulvaney, 1982), total carbon by combustion (Ströhlein Instruments) and "Klason" lignin (Bethge et al., 1971) (Table 1). The total nitrogen content of the plant materials ranged from 2.2 to 3.1% and the total carbon content ranged from 39.5 and 46.1%. The Klason lignin-C ranged between 9.1 and 12.5%.

Incubation experiment

Shoots of the above-mentioned plant materials were incubated in a sand-soil mixture for 115 days at 25±2°C. Ten grams of acid washed silica sand (0.3–0.5 mm) and 10 g of soil (Säby soil, A_p horizon of a Typic Haplaquept; 16% clay (<0.002 mm), 83% silt (0.2–0.002), 1% sand (0.2–2 mm), pH = 6.8, organic carbon = 3.4%, total N = 0.28%) were mixed with 0.1 g of plant material to which 4 ml of deionized water were added. The resulting moisture content amounted to 40% of the water holding capacity of the sand-soil mixture measured according to Jansson (1958). The mixtures were placed into 100 ml centrifuge tubes closed with rubber stoppers. Evolved carbon dioxide was trapped in sodium hydroxide contained in small vials placed inside the tubes. Tubes were opened at intervals to prevent lack of oxygen, vials were changed and the amount of CO_2 determined according to Stotzky (1965). Net N mineralization was measured by removing samples for extraction with 50 ml 2 M KCl during the incubation period. Amounts of nitrate (including nitrite) and ammonium were analyzed on a Technicon autoanalyzer. All measurements were made in triplicates.

Table 1. *Chemical composition of the leguminous crops (stems and leaves) used for the incubation and pot experiment*

Species	% of dry matter Total C	Total N	C/N	Lignin C
Red clover	45.9	2.61	17.6	9.08
White clover	39.5	3.10	12.7	10.26
Black medic	42.9	3.12	13.8	10.88
Persian clover	46.1	2.20	20.9	11.38
Egyptian clover	45.3	2.52	18.0	12.47
Subterranean clover	42.6	3.02	14.1	9.69

Pot experiment

Uptake of N from decomposing legume material was determined in a pot experiment using rye grass (*Lolium multiflorum* L.). Pots (Kick-Brauckmann) contained 7 kg of the same soil as used for the incubation study. To each pot 500 mg of N in the form of legume material was added. Unfertilized pots were used as standards for comparison. Three replicates were cropped from May to September taking 4 harvests of above-ground material. All pots were uniformly treated with 5 g of superphosphate, 2 g of potassium sulfate and micronutrients. Pots were regularly irrigated with deionized water and the soil moisture content was not a limiting environmental factor. Air temperature was recorded during crop growth.

Statistical techniques

Cumulative curves on CO_2–C evolution and accumulated amounts of inorganic N were subjected to non-linear regression analyses using the NLIN procedure of SAS (SAS Institute Inc., 1985). Mean values for each samling time were used. Curves were fitted to one- and two-component first-order models. Regression models with highest adjusted R^2-values (R^2_a), lowest residual variances (S^2_r) and uncorrelated residuals were chosen.

By using dummy variables the asymptotic standard errors of estimated parameters were derived and probable differences between parameters could be tested by a *t*-test.

Correlations were performed with the SAS procedure CORR and simple linear regression with the SAS procedure REG (SAS Inst. Inc., 1985).

Net N mineralization during incubation and amounts harvested per pot were subjected to analysis of variance using the GLM procedure computing least-squares means (SAS Inst. Inc., 1985).

Comparison of the pot and incubation experiments

Temperature differed between the pot and the incubation experiments, whereas moisture was kept at an optimal level for decomposition in both experiments. To be able to compare the results, temperature differences had to be eliminated.

Two concepts of temperature correction were used, the straight temperature-sum method, and the Q_{10}-method. According to the temperature-sum method recorded mean daily temperature values (T>0°C) were summed over the plant growth period.

$$T_{sum} = \Sigma T_i$$

where T_i is the mean temperature of the *i*th day in °C. From a Q_{10} relationship a control function ranging in value from 0 to 1 was derived modifying the number of days in the pot experiment. Cropping days were adjusted with respect to the constant incubation temperature of 25°C using the relationship

$$f = Q_{10}^{(T_i - 25)/10}$$

where T_i is the mean daily temperature of the *i*th day and Q_{10}=1.8 (Andrén & Paustian, 1987).

RESULTS

Carbon mineralization from decomposing legumes during incubation

At each sampling time accumulated amounts of CO_2–C from soil were subtracted from amounts evolved from soil with plant materials. The difference gives the amounts of

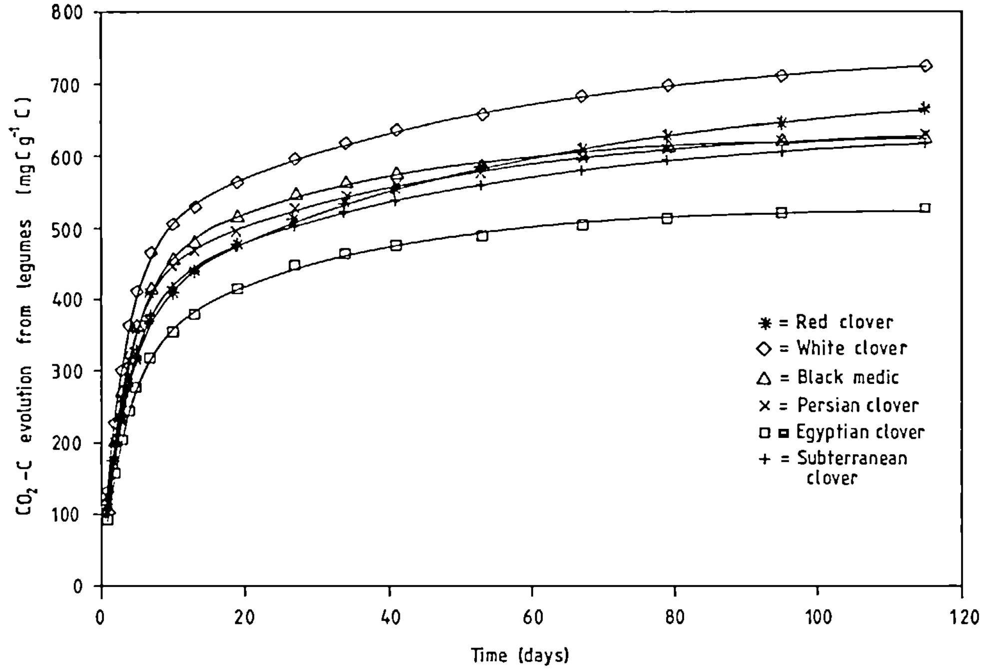

Fig. 1. Cumulative CO_2–C mineralization from six legumes during aerobic decomposition in soil (symbols illustrate experimental data, lines represent the curve fitting result).

CO_2–C evolved from decomposing plant materials assuming no priming effect. White clover had the highest release of CO_2–C during the 115 days of incubation and 72% of the total carbon was lost. Red clover lost 67% and black medic, persian clover and subterranean clover lost around 62%. Egyptian clover had the lowest CO_2–C releast, amounting to 53%.

Accumulated amounts of CO_2–C were fitted to the two-component first-order model $CO_2\text{–C}=C_1(1-e^{-k_1t})+C_2(1-e^{-k_2t})$ where C_1 and C_2 are different pools of plant carbon subject to mineralization, k_1 and k_2 are rate constants, and t is time. Fig. 1 shows the measured amounts of CO_2–C evolved expressed as mg CO_2–C per gram plant C added and

Table 2. *Parameter values for C mineralization in the incubation experiment, R^2=0.9996*
Values within the same column with the same letter are not significantly different (p=0.01)

Species	k_1 (day^{-1})	k_2 (day^{-1})	C_1 (mg C g^{-1} legume C)	C_2 (mg C g^{-1} legume C)
Red clover	0.28^{a}	0.019^{cd}	379^{a}	318^{a}
White clover	0.31^{a}	0.021^{cde}	476^{b}	272^{b}
Black medic	0.30^{a}	0.032^{ab}	424^{cf}	207^{c}
Persian clover	0.31^{a}	0.024^{bc}	419^{d}	226^{c}
Egyptian clover	0.31^{a}	0.036^{a}	305^{e}	226^{cd}
Subterranean clover	0.28^{a}	0.021^{cde}	395^{af}	243^{d}

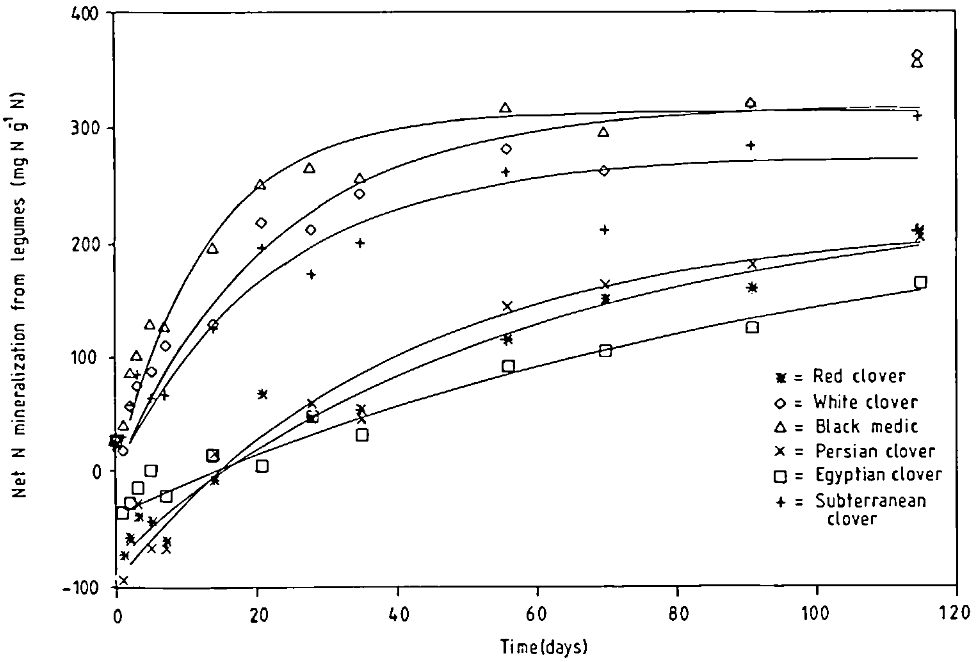

Fig. 2. Cumulative net N mineralization from six legumes during aerobic decomposition in soil (symbols illustrate experimental data, lines represent the curve fitting result).

the derived regression lines. Table 2 shows the estimated model parameters, i.e. the rate constants and pool sizes. Significant differences between plant materials were obtained for the rate constant k_2 but not for the rate constant k_1. However, the rate constants and the pool sizes were strongly correlated.

Nitrogen mineralization from decomposing legumes during incubation

The accumulated amounts of NH_4^+ and NO_3^- in untreated soil at each sampling time were subtracted from amonts in soil treated with plant materials. The difference gives the net N mineralization from decomposing plant material, assuming no priming effect.

White clover, black medic and subterranean clover showed a net mineralization during the whole incubation period (Fig. 2). In treatments with red clover, persian clover and egyptian clover the amounts of inorganic N decreased during the first day and did not reach the level of the control until after approximately 2 weeks. The initial decrease correlated well with the C/N-ratio of the legumes (r=0.91) and a turning point of approximately 15 was estimated.

Significant differences were obtained between the net N mineralization from the different legumes (Table 3). During the 115 days of incubation, approximately 30–35% of total N in white clover, black medic and subterranean clover was mineralized. In contrast, red clover and persian clover mineralized only about 20% and Egyptian clover 17%. The net N mineralization correlated well with the C/N-ratio of the legumes (r=−0.93).

Although the net N mineralization is the net result of mineralization, immobilization and any gaseous losses, it may be useful to fit the obtained values to a regression equation in order to compare the different plant materials. Accumulated amounts of inorganic N were fitted to a one-component first-order regression model $N=N_0(1-e^{-kt})-I$, where N_0 is the mineralizable amount of plant nitrogen, k is a rate constant and t is time. I is the lowest inorganic N level relative to the control. For red clover, persian clover and egyptian clover this level was reached after 1 day. For white clover, black medic and subterranean clover I was put to 0. Values before day 2 were omitted from the regression analysis. Subtraction of the lowest inorganic N level (I) from a first-order equation offers a possibility to fit both negative and positive values for net N mineralization to a common equation. A minor error will, however, occur in that $Y=I$ at time $t=0$ and not at time $t=1$ for curves showing an initial decrease. All derived pool sizes were around 300 mgN g^{-1} plant N originally added (Table 4). Egyptian clover had the lowest rate constant ($k=0.009$ day^{-1}) and black medic the highest ($k=0.076$ day^{-1}). The variance between the legumes differed to such an extent that it was doubtful whether a common variance could be assumed and hence it was not possible to make a common regression with dummy variables.

Nitrogen uptake from decomposing legumes by rye grass

N yields from white clover, black medic and subterranean clover were throughout higher during the whole growth period compared to red clover, persian and egyptian clover. At the end of the growing season the N-uptake percentages were 31% and 27% of added N for black medic and white clover, respectively. Utilization of N from red clover, persian clover and egyptian clover amounted to about 10% during the cropping period.

Variance analysis of N yields showed that N-yields in pots fertilized with red clover, persian clover and egyptian clover were not significantly different (Table 5).

Table 3. *Net N mineralization from legumes incubated in soil for 115 days*

Values with the same letter are not significantly different ($p=0.01$)

	Net N mineralization	
Species	mg N $treatment^{-1}$	mg N g^{-1} legume N
Soil	0.936^{a}	-
Red clover	1.452^{b}	210
White clover	1.992^{c}	362
Black medic	1.985^{c}	357
Persian clover	1.360^{bd}	207
Egyptian clover	1.329^{d}	166
Subterranean clover	1.818^{e}	310

Table 4. *Parameter values for net nitrogen mineralization in the incubation experiment*

Species	k (day^{-1})	N_0 (mg N g^{-1} legume N)	R^2
Red clover	0.017	317	0.962
White clover	0.045	320	0.933
Black medic	0.076	315	0.924
Persian clover	0.024	316	0.938
Egyptian clover	0.009	298	0.975
Subterranean clover	0.045	276	0.890

Comparison between legume N mineralization during incubation and legume N taken up by rye grass during cropping

Experimental data on N uptake from legumes by rye grass and net N mineralization from legumes during incubation (derived from the regression equations) were compared with the aim to find out whether there were any differences between the net mineralization of legume N in the incubation study and the uptake of N in the pot study. Only regression models with temperature corrected values gave good agreements (Table 6). No temperature correction of the *I* parameter was made. Best agreement was achieved with Q_{10}-adjusted values although the differences between Q_{10} and temperature-sum adjusted models were small. Addition of a time variable did not improve the agreement.

Models with no corrections for the initial decrease (*I*) in inorganic nitrogen level showed lower R_a^2-values and higher S_e^2 values than models with these corrections. Fig. 3 shows a plot of harvested N as a function of N mineralized during incubation and the regression line for a model with Q_{10}-adjusted values without time component, N Harvest= $0.87N_{incubation\ Q10}-5.79$. The intercept is not significantly different from zero and the harvested amount of N is approximately 10% the amount mineralized during incubation.

Table 5. *Effect of soil incorporated above-ground legume material on N yields of rye grass in a pot experiment*

500 mg N added per pot. Values with the same letter in the same column are not significantly different (p=0.05)

	Cumulative N yield							
	mg N pot^{-1}				mg N g^{-1} legume N			
	Days after sowing				Days after sowing			
Species	41	52	74	113	41	52	74	113
Soil	230^a	295^a	319^a	352^a	–	–	–	–
Red clover	248^a	324^a	360^b	409^b	36	58	82	114
White clover	303^b	396^b	436^d	488^{cd}	146	202	234	272
Black medic	317^b	415^b	457^d	508^{cd}	174	240	276	312
Persian clover	238^a	315^a	351^b	403^b	16	40	64	102
Egyptian clover	240^a	312^a	347^b	396^b	20	34	56	88
Subterranean clover	288^c	376^c	414^c	463^c	116	162	190	222

Table 6. *Regression models use to compare N harvested in the pot experiment and net N mineralization in the incubation experiment*

Regression model	R_a^2	S_e^2
N Harvest $N=\alpha+\beta N_{inc.}$	0.887	925
N Harvest $N=\alpha+\beta N_{inc.\ Q10}$	0.953	388
N Harvest $N=\alpha+\beta N_{inc.\ tempsum}$	0.947	434
N Harvest $N=\alpha+\beta N_{inc.\ Q10}+time_{Q10}$	0.956	363
N Harvest $N=\alpha+\beta N_{inc.\ Q10\ (without\ I)}$	0.8244	1 442

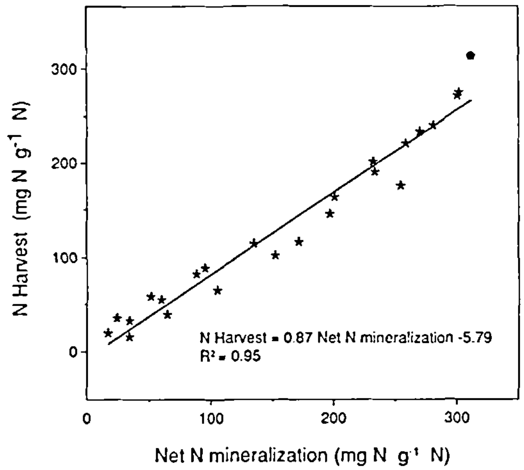

Fig. 3. Comparison of net N mineralization from legumes during incubation and legume N harvest in rye grass in pot experiment (symbols illustrate experimental data, lines represent the curve fitting result).

DISCUSSION

The use of a difference method to calculate the CO_2-C and net N mineralization from decomposing legumes assumes the priming effect to be very small. Reviews on the priming effect by Jenkinson (1966) and Sauerbeck (1966) indicate positive or negative but usually small effects after addition of plant materials, which is confirmed by more recent studies (Martin & Haider, 1979; Dalenberg & Jager, 1989).

A two-component first-order regression model (Jenkinson, 1977) has been widely used to describe the decomposition kinetics of organic materials. The model assumes that the organic material can be approximated to two different pools of constant but different decomposability. The pools are supposed to maintain their initial decomposability throughout the decomposition process. However, the decomposability may not be constant during the decomposition period and thus the estimated parameters may not have biological significance. Therefore regression models taking into account a change of the decomposability of the organic material have been used by Janssen (1984) and Cheshire (1988). A theory in which the quality of the substrate changes during the decomposition process has been developed by Bosatta & Ågren (1991).

Although a two-component first-order model is an oversimplification of the decomposition process it may, however, be reasonable to regard the initial decomposition as a decomposition of a substrate of constant decomposability. In this experiment the rate constant k_1 may be interpreted as representing the decomposability of labile C compounds in the plant material and C_1 as the labile amount available for mineralization. Significant differences were obtained between estimated C_1 values for the different plant materials but not for the k_1 values. However, the strong correlation obtained between estimated parameter values makes an interpretation difficult. Estimated parameter values of the second component showed significant differences. They may be regarded as representing an average of the decomposability and the amount of mineralizable C respectively of more or less stabile plant compounds and products formed during the decomposition. The sum of estimated pool sizes varies from 53 to 75% of the total C. The rest may be regarded as stable or stabilized C not subject to mineralization during the period measured.

The net N mineralization rates and the amounts of N mineralized were of the same order as reported by Kirchmann & Bergqvist (1989) for white clover using the same soil. Breland (1989), who used fresh white clover tops and lower incubation temperature (15°C), obtained somewhat higher values, i.e. 40–50% of applied N. The net N mineralization from black medic and white clover was higher than from the other legumes tested. This is in accordance with results from field trials (Müller & Sundman, 1988) with ^{15}N-labelled legumes where N from white clover was more available for plant uptake and losses than N from red clover and subterranean clover.

The inorganic N content in the soil of the incubation study, and probably also in the pot study decreased initially in treatments with red clover, persian clover and egyptian clover. The reduction might indicate an immobilization of N or a denitrification. As respiration rates were only slightly different between legumes during the first 24 hours, denitrification is unlikely and immobilization more probable. The close correlation between the C/N ratios of the plant materials and the initial reduction of inorganic N in soil supports this view. The initial reduction occurred during the first 24 hours and ought to be related to the N content and turnover of the easily decomposable compounds.

The comparison of net N mineralization in the incubation experiment with N uptake in the pot study showed good agreement between the experiments. Leaving out the *I* parameter in the regression equation gave poorer agreement. This indicates that whatever the nature of the process, it is going on both in the incubation and in the pot study. The intercept of the regression line (Fig. 3) is small and not significantly different form zero. This means that the harvested amount of N is a constant fraction of incubation N (approximately 90%). But as only the shoots were harvested and root N is not considered in the figures, the total N harvest may be closer to the net mineralization during incubation.

The differences between species obtained both in the incubation study and in the pot study show the importance of choosing appropriate green manure crops for agricultural purpose.

ACKNOWLEDGEMENTS

H. Marstorp performed the incubation experiment and H. Kirchmann the pot experiment. H. Marstorp received financial support from the Swedish Council for Forestry and Agricultural Research. The authors thank Jan Persson, Gerd Johansson and Erick Zagal for helpful comments on the manuscript and Morgan Zaar and Pär Hillström for laboratory assistance.

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