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Harvesting strategy and N fertilization influence ¹³⁴Cs uptake by forage plants

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The root uptake of ¹³⁴Cs by forage plants was studied as a function of growth stage and N fertilization with biotite supplementation. The study was conducted by means of pot experiments with peat soil. In the growth stage studies, ryegrass, white clover and yellow-flowered lucerne were cut once 30, 60 or 90 days after sowing or three times at intervals of 30 days. In the one-cut system, at 90 d, the activity concentration of ¹³⁴Cs in ryegrass and clover was higher and that in lucerne lower than in the three-cut system. In both treatments, the activity concentration in ryegrass decreased and that in legumes, generally, tended to increase with time. In the N fertilization studies, ryegrass was grown at different levels of ammonium nitrate (100, 200 and 400 mg N 1^{-1}) and biotite (0, 10, 20 and 40 g 1^{-1}) application. The addition of N to soil increased and that of biotite decreased the ¹³⁴Cs activity concentration in ryegrass.

The differences in forage ¹³⁴Cs between the two harvesting systems were small. Although ammonium nitrate increased the ¹³⁴Cs uptake by ryegrass, in the event of fallout, moderate rates of ammonium fertilizer could be used provided that biotite or K are applied at adequate levels.

Key words: Cs, nitrogen, biotite, peat, Lolium, legumes, seasonal variation

Introduction

Forages are the main source of feed for ruminants. The most important factors affecting the yield and quality of forages are growth stage and N fertilization. To obtain high quality forage it is recommended that herbage be harvested at an early stage of growth. However, owing to seasonal growth, harvesting at the time of highest quality does not usually coincide with harvesting at the time of maximum dry matter yield (Rinne and Nykänen 2000). In the event of radioactive fallout, farmers may be forced to relinquish their claims to nutritive value and amount of feeds. Little information is available on the effect of growth stage on uptake of radiocaesium by legumes.

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An earlier study (Paasikallio 1999) showed that the ¹³⁴Cs activity concentration in ryegrass on peat soil was reduced by biotite low in soluble potassium. Ammonium and potassium salts are known to be the fertilizers most effective in influencing plant uptake of radiocaesium. Potassium decreases and ammonium, in contrast to potassium, usually enhances caesium uptake (Evans and Dekker 1969, Haak and Eriksson 1973, Cawse 1990, Belli et al. 1995). It was hypothesized that the rate of ammonium fertilizer might play an important role in counteracting the ability of biotite to reduce radiocaesium uptake by plants.

Peat was chosen as a growth medium because the radiocaesium uptake by plants from peat soil may present a serious problem due to the low caesium fixing capacity of peat. The addition of biotite made the peat substrate more like cultivated peatland, and a high rate of biotite addition was intended to simulate the effect of soil amendment application.

The objectives of this study were to compare the uptake of radiocaesium by grass and legumes harvested at three different times during the growing season and, further, to investigate the effect of various rates of ammonium nitrate application on ¹³⁴Cs uptake by grass grown on peat soil treated with different levels of biotite.

Material and methods

Pot experiments

Outdoor pot experiments were conducted in Jokioinen, in 1997 (Exp. 1, growth stage) and in 1998 (Exp. 2, N-fertilization). Plastic pots, capacity 3.5 litres, were filled with peat soil in four replicates. Different rates of biotite (Kemira Agro Oy, Siilinjärvi) were added to the soil, which consisted of slightly decomposed, sieved *Sphagnum fuscum* moss peat and was originally unfertilized and unlimed (pH 3.5–4.5 and 99% organic matter according to the producer, Kekkilä Oy, Parkano). The clay fraction (< 0.002 mm) of biotite was < 1% (Paasikallio 1999). The pots were placed at random and protected from rain. The soils were kept at constant moisture (about 60% of the water holding capacity). In 1997, mean temperature in June, July and August (16.1, 17.8 and 17.8°C, respectively) was higher than that for a 30 year period (14.3, 15.8 and 14.2°C). In 1998, mean monthly temperature during the growing season was a little lower (13.7, 15.2 and 13.0°C) than normal. Meteorological data from Jokioinen were obtained from Monthly Reports of Finnish Meteorological Institute.

Growth stage

Biotite was mixed in peat soil at a rate of 20 g l⁻¹. Italian ryegrass (Lolium multiflorum Lam., var. Tetraploid Turgo) was fertilized with 200 mg N (NH₄NO₃) and 1.4 g Ca (CaCO₃) per litre of soil and white clover (Trifolium repens L., var. Jögeva) and yellow-flowered lucerne (Medicago falcata L., var. Karlu) with 50 mg N and 2.8 g Ca 1⁻¹. Other fertilizers added were 150 mg K (KCl), 150 mg Mg (MgSO₄·7H₂O), 50 mg P $(NaH_2PO_4 \cdot 2H_2O)$, 40 mg Fe (FeSO₄ · 7H₂O), 20 mg Mn (MnSO₄·H₂O), 5 mg Cu (CuSO₄·5H₂O), 5 mg Zn (ZnSO₄·H₂O), 1 mg B (H₃BO₃) and 1 mg Mo (Na₂MoO₄) per litre of soil. Caesium 134 (CsCl in aqueous solution) was mixed in the fertilized soils at a rate of 370 kBq per pot. About 0.6 litre of the soil per pot was left uncontaminated. A half of it was put at the bottom of the pot and after filling the pot with contaminated soil another half was put at the soil surface. The depth of each uncontaminated soil layer was about 15 mm. Before they were sown, the seeds of legumes were treated with inoculant (Rhizobium bacteria). Seeds were sown on 12 June. After each cut, the soils of harvested and unharvested plants were fertilized with half a dose of N and P. In treatment 1, the plants were cut once about 30, 60 or 90 days after sowing (11 July, 8 August and 8 September). In treatment 2, the

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plants were cut three times at intervals of about 30 days (dates as above). The first cut was common for both treatments.

The comparison of forage dry matter production of a single harvest to 3-cut system was achieved by cumulating the individual harvest of 3-cut system. The corresponding ¹³⁴Cs concentrations in 3-cut system were calculated by means of the weighed mean concerning also previous cuts.

N-fertilization with biotite addition

Italian ryegrass was grown on peat soil with biotite addition at a rate of 0 (control), 10, 20 and 40 g l⁻¹. Each of the soil-biotite mixtures was fertilized with 100, 200 and 400 mg N (as NH₄NO₃) and with 1.4 g Ca (as CaCO₃) l⁻¹. Caesium 134 (CsCl in aqueous solution) was added at a rate of about 240 kBq per pot. Seeds were sown on 29 May. The plants were cut three times at intervals of about 30 days (3 July, 3 August and 2 September). Otherwise fertilization and the experiment were conducted in much the same way as with ryegrass in the growth stage experiment.

Sample analysis

Ashed plant material was dissolved in HCl, and the plant K concentration was determined by plasma emission spectroscopy. Cesium 134 was measured in dry plant material using a gamma counter with a NaI(Tl) well-type crystal detector. The activity concentration of ¹³⁴Cs, the concentration of K, and yield are expressed as dry matter.

Statistical methods

In the growth stage experiment, the effect of the two cutting systems on the ¹³⁴Cs activity con-

centration and yield and the differences in these factors between plant species were evaluated by analysis of variance. In the fertilization experiment, the effect of N and biotite on the ¹³⁴Cs activity concentration, yield and K content of plants was evaluated by repeated measurements analysis of variance. Completely randomized design was used in both experiments.

In the N fertilization experiment, three cuts were taken from each pot and the cuts of one pot were correlated. This correlation was taken into account in the statistical models. The covariance structure of the three repeated measurements was obtained by comparing all biologically sensible structures using Akaike's and Schwartz's Bayesian information criterion (Wolfinger 1996). The unstructured covariance structure proved useful in each analysis of variance. For the fertilization experiment, the statistical model was as follows:

$$\begin{split} Y_{ijk} &= \mu + treatment_i + pot_k \left(treatment_i \right) + cut_j + \\ \left(treatment \times cut \right)_{ij} + \epsilon_{ijk} \end{split}$$

where μ is the intercept, and treatment_i represents the fixed effect associated with the ith treatment. Treatments are factorial combinations of the biotite and N levels in the fertilization experiment. Pot_k (treatment_i) is the normally distributed random effect of the pots. Cut_j and (treatment × cut)_{ij} represent the fixed effect associated with the jth cut and treatment × cut interaction, respectively. ε_{ijk} are correlated residual errors with covariance structures as defined above. In the growth stage experiment, the cuts were analysed separately. Thus, the model included only intercept, treatment and residual effects from the model of the fertilization experiment.

The assumptions of statistical models were checked by graphical methods: boxplot for normality of errors and plots of residuals for constancy of error variance (Neter et al. 1996). The constancy of error variance was achieved by log or square-root transformation. The parameters of the models were estimated by the restricted maximum likelihood (REML) estimation method using the SAS system and the MIXED procedure.

Results

Growth stage

Sixty days after the sowing, the activity concentration of ¹³⁴Cs was higher in one-cut (treatment 1) than in three-cut (treatment 2) ryegrass (P < 0.001), whereas legumes had equal ¹³⁴Cs concentrations in both cut systems (Fig. 1a). At 90 d, the activity concentration was higher in one-cut ryegrass and clover, but lower in onecut lucerne than in the corresponding three-cut plants (P < 0.001). The plant species \times cut interaction was significant for both cut systems after 60 and 90 days from sowing ($P_{60,90} < 0.001$). In early growth, the ¹³⁴Cs activity concentration in ryegrass was clearly higher and in later growth lower than that in legumes. In contrast to legumes, ryegrass ¹³⁴Cs decreased with time and number of cuts. In later growth, in the one-cut system, clover ¹³⁴Cs was significantly higher than the ¹³⁴Cs of other species and in the three-cut system, legume ¹³⁴Cs was higher than the ¹³⁴Cs of ryegrass (Table 1).

The yield of one-cut plants was significantly higher than the cumulative yield of three-cut plants ($P_{60} < 0.001$ and P_{90} d 0.01) (Fig. 1b). The plant species × cut interaction was significant for both cut systems ($P_{60} = 0.02$, $P_{90} < 0.001$). In early growth, the yield of ryegrass was higher than that of legumes. The yield and cumulative yield of all plant species increased considerably with time and number of cuts. At 60 d, the yield of all one-cut plants was equal, whereas in threecut plants, the yield of lucerne was lower than that of the other species. At 90 d, in both cutting systems, the yield of clover was significantly higher than that of the other plants (Table 1). Soil pH, determined at the end of the experiment, was 6.3 for grass, 6.8 for clover and 7.0 for lucerne.

N fertilization with biotite addition

In general, the ¹³⁴Cs activity concentration of ryegrass increased significantly (P < 0.001) with increasing N fertilization. It was highest on control soils (no biotite) and decreased significantly (P < 0.001) with increasing biotite level of the soil (Fig. 2, Table 2). At lower biotite levels, the ¹³⁴Cs uptake by plants increased with increasing rates of N relatively less than it did at higher biotite levels. The N × biotite interaction was significant for each cut (P < 0.001). On biotite soils, plant ¹³⁴Cs decreased markedly with number of cuts, especially at higher soil N levels.

Cutting	Cutting	Significance				
system	time					
		Ryegrass	Ryegrass	Clover		
	(d)	vs. clover	vs. lucerne	vs. lucerne		
Activity concentrat	ion of ¹³⁴ Cs					
One-cut	60	0.01	< 0.001	0.01		
Three-cut	60	< 0.001	n.s.	< 0.01		
One-cut	90	< 0.001	n.s.	< 0.001		
Three-cut	90	< 0.001	< 0.001	n.s.		
Yield						
One-cut	60	n.s.	n.s.	n.s.		
Three-cut*	60	n.s.	< 0.005	< 0.005		
One-cut	90	< 0.001	< 0.001	< 0.005		
Three-cut*	90	< 0.001	n.s.	< 0.001		

Table 1. Significance between plant species for data of ¹³⁴Cs activity concentration and yield (Exp. 1).

* = cumulative yield, n.s. = non-significant.

Vol. 11 (2002): 143-152. a) b) Ryegrass Ryegrass ¹³⁴Cs in plant (cpm g⁻¹ x 10⁻²) 1 cut - 1 cut - - 3 cuts(i) - - 3 cuts(i) 3 cuts(ii) 3 cuts(ii) Yield (g/pot) ∽ ¹³⁴Cs in plant (cpm g⁻¹ x 10⁻²) Clover Clover Yield (g/pot) Δ Φ ¹³⁴Cs in plant (cpm g⁻¹ x 10⁻²) Lucerne Lucerne Yield (g/pot)

Fig. 1. Activity concentration of 134 Cs (a) and yield (b) for ryegrass, clover and lucerne at different times after sowing. In treatment 1(1 cut), plants were cut once at the age of 30, 60 or 90 days. In treatment 2 (3 cuts), plants were cut three times at intervals of 30 d. In treatment 2, both cumulative (3 cuts-i) and individual (3 cuts-ii) harvest and corresponding 134 Cs concentrations are presented. Data points denote means and SD of four (* = three) replicates. (Exp. 1).

Time (d)

Time (d)

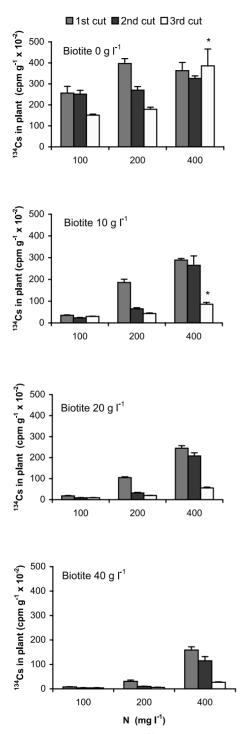


Fig. 2. Activity concentration of 134 Cs for ryegrass in three cuts at different rates of N and biotite application to peat soil. Means and SD of four (* = three) replicates. (Exp. 2).

Some of the N (mg l⁻¹) – biotite (g l⁻¹) combinations had an equal effect on the ¹³⁴Cs activity concentration in plants: N-biotite combination rates of 100-0 and 400-20 gave about 250×10^{-2} cpm, those of 200-10 and 400-40 about 170×10^{-2} cpm and those of 100-10 and 200-40 about 33×10^{-2} cpm ¹³⁴Cs g⁻¹.

The yield was lowest in the first cut and on all soils without biotite. In later cuts, the yield increased with increasing biotite and N fertilization (Table 3). In the first cut, K was taken up effectively, but in later cuts, on soils without biotite, the K content of plants was very low. With an increase in the biotite level from 0 to 40 g l⁻¹, the soil pH, determined at the end of the experiment, rose from about 5 to 7 (Paasikallio 1999).

Discussion

The radiocaesium activity concentration in pasture plants has been reported to be higher in spring than in autumn (Bunzl and Kracke 1989, Schechtner and Henrich 1990, Salt et al. 1992, Ehlken and Kirchner 1996), which is in accordance with the data on ryegrass in our growth stage experiment. Late harvest time has been recommended by Schechtner and Henrich (1990) as a means to reduce the ¹³⁷Cs concentration in grassland forage, provided that N and K fertilization is sufficient. On the other hand, late harvest reduces the nutritional quality of forage. Besides the season, the slow-releasing K in biotite probably accounted for the decrease in ryegrass ¹³⁴Cs with time. On the other hand, the tendency of the ¹³⁴Cs concentration in legumes to increase during the growing season was suggested to be due to their symbiotic bacterial fixation of N and hence, at least at the later stage of the growth, more ammonium was available to them than to ryegrass. Because the ammonium ions are known to increase the radiocaesium concentration of plants, their effect on legume ¹³⁴Cs might have surpassed the decreasing effect of K ions.

	Degree	of freedom		
Source of variation	Numerator	Denominator	F-value	P-value
Ν	2	36	3629	< 0.001
Biotite	3 6 2	36	4000	< 0.001 < 0.001 < 0.001
Biotite × N		36	270	
Cut		71	1085	
N × cut	4	71	211	< 0.001
Biotite × cut	6	71	52	< 0.001
Biotite \times N \times cut	12	71	45	< 0.001

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Table 2. Results of analysis of variance for ¹³⁴Cs activity concentration data (Exp. 2).

It was assumed that for pure peat soil of this study the caesium fixation by small amounts of biotite was not of major importance, although the clays and also the micas are known to fix Cs (Sawhney 1964, Zachara et al. 2002). The evidence supporting this assumption is e.g., the observed long-term plant availability of Chernobyl radiocaesium in British upland areas without showing significant ageing effects (Howard et al. 1990, Rigol et al. 1998) and the findings

of Ehlken and Kirchner (1996) who did not observe any ageing process of radiocaesium in peat soil. Furthermore, the presence of organic matter is known to reduce the fixation of caesium by clay minerals (Dumat and Staunton 1999, Staunton et al. 2002). Another factor, which is reported to decrease plant uptake of radiocaesium with time, is its migration in the rooting zone. However, in this study, ¹³⁴Cs was mixed in the soil and hence its migration was unlikely.

Biotite (g l ⁻¹)		Yield (g/pot)		K (g kg ⁻¹)			
	N (mg l ⁻¹)						Significance	
	100	200	400	100	200	400	Yield	К
1st cut								
0	9	10	10	37	38	38	n.s.	n.s.
0 10	10	10	10	50	54	42	< 0.005	< 0.001
20		12	11					
	10			48	53	45	n.s.	< 0.001
40	10	11	11	49	55	46	< 0.005	< 0.001
2nd cut								
0	9	14	17	6	3	5	< 0.001	n.s.
10	13	22	24	24	26	28	< 0.001	n.s.
20	13	25	27	25	25	34	< 0.001	< 0.001
40	12	24	31	24	26	40	< 0.001	< 0.001
3rd cut								
0	8	8	*2	5	5	*8	< 0.001	0.05
10	13	20	*26	30	25	*23	< 0.001	< 0.001
20	14	24	31	31	36	35	< 0.001	< 0.001
40	15	24	35	30	36	38	< 0.001	< 0.001

Table 3. Yield and K content of ryegrass in three cuts at different rates of N and biotite application to peat soil.

Means of four (*= three) replicates, n.s. = non-significant.

At the end of the growing season, in both cutting systems, the ¹³⁴Cs concentration in grass was lower than that in clover, which is consistent with earlier findings (Garrett et al. 1971, Schechtner and Henrich 1990, Salt et al. 1992, Veresglou et al. 1995). The radiocaesium concentration in white clover has been reported to be higher than that in lucerne (Evans and Dekker 1968, Schechtner and Henrich 1990); here, however, this was not always the case. Belli et al. (1995) reported that the ¹³⁷Cs concentration in grass (Phleum pratense) was higher than that of a legume (Lotus corniculatus). Their results corroborate the findings of our study at the early growth stage, when ¹³⁴Cs uptake by ryegrass was clearly higher than that by legumes.

In the N fertilization experiment, ammonium nitrate substantially promoted ¹³⁴Cs transfer to plants, thus supporting the findings of several earlier reports (Jackson et al. 1965, Evans and Dekker 1969, Cawse 1990, Schechtner and Henrich 1990, Lasat et al. 1997), according to which ammonium ions markedly increase the radiocaesium concentration in plants, particularly when the soil K is low. Ammonium ions decrease the fixation of caesium ions in soil and, consequently, the caesium concentration increases in soil solution and in plants. Haak and Eriksson (1973) reported that ammonium and urea fertilization increased the ¹³⁷Cs concentration in wheat straw and timothy more than did nitrate fertilization. The effect of N fertilization depended on the N and K rates and soil type. According to them, the increase in the ¹³⁷Cs concentration in plants due to an ammonium fertilizer was most effective in coarse mineral and organic soils. Grauby et al. (1990) recommended that ammonium fertilizers not be used in the event of a nuclear accident. On the other hand, according to Schechtner and Henrich (1990) and Belli et al. (1995), ammonium or urea fertilization did not increase the radiocaesium concentration in plants, which was attributed to the diluting effect of the growth of plant biomass on the radiocaesium concentration.

In our study, biotite application considerably decreased the ¹³⁴Cs concentration in plants,

as was reported by Paasikallio (1999). The effect of biotite was attributed mainly to its lowsoluble K. Potassium salts are known to reduce the transfer of radiocaesium to plants, especially in peat soils of low K status (Haak and Eriksson 1973, Jackson and Nisbet 1990, Rosén 1991). Potassium ions dilute the radiocaesium concentration in soil solution, thus decreasing its transfer to plants. The K content of the plants in soil without biotite was rather high in the first cut, but in later cuts, the plants suffered from K deficiency and had poor growth, because K-fertilization was given only once at the beginning of the experiment.

Potassium and ammonium ions compete with radiocaesium for uptake and they are reported to be equally effective in releasing soil-fixed caesium in organic soils having non-specific exchange sites (Schulz 1965). That ammonium increased caesium uptake relatively less at lower than at higher biotite levels was attributed to interactions between K and ammonium, which are known to be complicated in plant nutrition (Wang et al. 1996).

Conclusions

The study indicates that the differences in forage ¹³⁴Cs between the harvest systems were small although statistically significant. The differences might have been caused as well by other factors, e.g. climatic conditions, the effects of which were not studied. The study also indicates that, although ammonium nitrate markedly increased the plant radiocaesium concentration, in the event of nuclear fallout, moderate rates of ammonium fertilizers could be used on peat soils, provided that adequate rates of biotite or potassium fertilizer are applied to reduce radiocaesium transfer from soil to herbage.

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SELOSTUS

Korjuuaika ja typpilannoitus vaikuttavat rehukasvien radiocesiumpitoisuuteen

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Kasvuvaihe ja typpilannoitus ovat tärkeimpiä rehukasvien laatuun ja satoon vaikuttavia tekijöitä. Laadukkaan rehun tuottamiseksi sato on korjattava varhaisessa kasvuvaiheessa. Ydinlaskeumatilanteessa kasvin mahdollisimman matalasta aktiivisuuspitoisuudesta tulee rehun ravitsemuksellista laatua tärkeämpi tekijä. Koska kasvien radiocesiumpitoisuus vaihtelee kasvukauden aikana, on oikean korjuuajan valitseminen tärkeää. N-lannoituksen määrällä on myös merkitystä, sillä sen tiedetään toisinaan lisäävän kasvin radiocesiumpitoisuutta varsinkin kun lannoitteena käytetään ammonium-typpeä. Tutkimuksen tarkoituksena oli verrata niitettyjen ja niittämättä jätettyjen rehukasvien radiocesiumpitoisuutta kasvukauden aikana sekä selvittää ammoniumnitraatti-lannoituksen vaikutusta radiocesiumin kulkeutumiseen heinään.

Korjuuajan ja -kertojen vaikutusta raiheinän, valkoapilan ja sirppimailasen radiocesiumpitoisuuteen tutkittiin astiakokeiden avulla. Kasvualustana oli ¹³⁴Cs:lla käsitelty turvemaa, johon oli lisätty biotiittia 20 g l⁻¹. Radiocesiumpitoisuus määritettiin kolmesti niitetyistä ja niittämättömistä kasveista kolme kertaa 30 päivän välein kylvöstä laskettuna. N-lannoitustutkimuksessa raiheinää kasvatettiin ¹³⁴Cs:a sisältävässä turvemaassa, johon oli lisätty typpeä ammoniumnitraattina 100, 200 ja 400 mg l⁻¹ ja biotiittia 0, 10, 20 ja 40 g l⁻¹. Heinä niitettiin kolme kertaa 30 päivän välein.

Niittämättömän, 90 päivän ikäisenä korjatun heinän ja apilan radiocesiumpitoisuus oli suurempi ja mailasen pienempi kuin vastaavien kolmesti niitettyjen kasvien viimeisen niiton aktiivisuuspitoisuus. Heinän aktiivisuuspitoisuus pieneni ja palkokasvien yleensä suureni jonkin verran aikaa myöten, mikä saattoi johtua siitä, että palkokasvit saivat heinää enemmän ammonium-typpeä symbioottisen N₂-sidonnan kautta. N-lannoitustutkimuksessa ammoniumnitraatti lisäsi huomattavasti heinän ¹³⁴Cs-pitoisuutta, kun taas biotiitti pienensi sitä. Aktiivisuuspitoisuus oli pienempi heinällä, joka oli kasvanut runsaasti typpeä ja biotiittia sisältävässä maassa kuin vähän typpeä ja kokonaan ilman biotiittia kasvaneella heinällä.

Tulokset osoittivat, että eri korjuutapojen väliset erot kasvin radiocesiumpitoisuudessa olivat yleensä pieniä. Erot saattoivat johtua myös muista tekijöistä, kuten esim. sääoloista, joiden vaikutusta ei tutkittu. Vaikka ammoniumnitraatti lisäsi heinän radiocesiumpitoisuutta, sitä voidaan käyttää kohtuullisesti laskeumatilanteessa, kunhan maahan samalla lisätään riittävästi biotiittia tai K-lannoitetta.