The balance of uptake, utilization and accumulation of the major elements in grass

W. DIJKSHOORN

Institute for Biological and Chemical Research on Field Crops and Herbage,
Wageningen, the Netherlands

Introductory

Extensive use has been made of analysis on mineral constituents in grass for the purpose of attaining more definiteness on their effect upon growth and quality for the grazing cattle. For this branch of grassland research that involves questions as to the uptake and utilization of soil elements by grass and their distribution over different plant species, a programme of greenhouse experiments was started with the idea of strengthening the approach to field problems by furnishing the fundamentals for generalizations in a more rapid and less expensive manner than could be attained by means of field experiments.

It is the purpose of the present paper to review the main results with regard to the uptake, utilization and accumulation of ions of the major elements and to arrange these data in a manner consistent with the electrochemical and biochemical aspects of ion translocation into and ion utilization by the growing plant. Much use is made of a domain of plant physiology concerning the role of organic acid synthesis and breakdown in response to ion accumulation that can be traced back to the days of Pfeffer and has been reinstated by the work of *Pucher (26), Ulrich (31), Burström (6), Chouteau (8), Dijkshoorn (16)* and others. It will be shown that balance obtained is useful to further considerations on the particular role of potassium when account is taken of the relative rates of uptake and redistribution of the cations by grass and other plant species.

A simplified balance of uptake, utilization and accumulation of potassium nitrate

Merely as an introduction to the more complex balance of uptake, utilization and accumulation of the different elements involved in normal growth, consider the comparatively simple case of a plant growing on a solution of the neutral salt potassium nitrate. Let the amount of available salt be 10 equivalents and let the nitrate be absorbed to complete exhaustion. The plant therefore contains 10 equivalents NO^-_3. It is known that NO^-_3 is absorbed by grass in excess over K^+. When the uptake has established, let the amount of absorbed K^+ be 5 equivalents, so that the plant contains 10 equivalents of NO^-_3 and 5 equivalents of K^+.

Since the prerequisite for movement and uptake of ions is electroneutrality, the uptake of 10 equivalents nitrate and 5 equivalents potassium is associated with an uptake of 5 equivalents H^+ ions. This uptake of H^+ ions together with the salt

ions means that the exhaustion of the solution also concerns H+ supplied by the water. Its counter ion OH− remains behind in the solution in association with the excess K+ over NO−$_3$.

Therefore, the residual salt in the solution contains 5 equivalents of titratable alkali and this is the physiological alkaline effect, associated with excess salt anion uptake over salt cation uptake and typical for nitrate nutrition of Gramineous and many other plants. Its presence is indicated by an increased pH of the sufficiently exhausted solution. In the presence of the soil system the increase of pH is far less pronounced because the residual OH− becomes neutralized by H+ desorbed by the adsorption of the excess salt cations over salt anions remaining in the soil solution *(Walker, 36)*.

The balance of uptake concerns 5 equivalents K+, 5 equivalents H+ (= the acidity of the uptake = the external alkaline effect) and 10 equivalents NO−$_3$.

Assume that 8 equivalents of the nitrate absorbed are used up in the formation of organic N. This means a reduction of NO−$_3$ to the reduction stage of ammonia and the ionic equation:

$$(N^{5+}O_3) - + \ 8\ H+ + \ 8\ e \ \longrightarrow \ (N^{3-}H_3) + 2\ H_2O + OH-$$
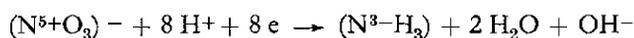
shows that in the metabolic reduction of nitrate its equivalent is transformed into a strongly basic OH− ion. This means, here, that the utilization of nitrate liberates 8 equivalents of internal alkali.

The balance of uptake showed that the acidity of the uptake was 5 equivalents H+. It is clear that this acidity neutralizes 5 equivalents of the internal alkali under the formation of water.

Thus, the balance of uptake and utilization indicates that what remains in the plant is 5 equivalents K+, 3 equivalents OH− and 2 equivalents NO−$_3$. Instead of internal acidification by the acidic uptake, utilization of the nitrate in excess of the acidity absorbed tends to render the plant tissue more alkaline.

The following step is to convert the strongly basic OH− anion into a less basic anion. The formation of HCO−$_3$ by CO_2 addition reduces the alkalinity considerably, but the bicarbonate anion is still too basic to allow a common degree of accumulation without affecting the pH of the tissue too much. A second transformation into an anion which is less basic than bicarbonate is required and this is accomplished by metabolic carboxylation of bicarbonate leading to its transformation into an only slightly basic carboxyl function of one of the common organic anions, in grass mainly malate and citrate.

The result is that the internal alkali produced in nitrate utilization in excess of the acidity of the uptake is rendered harmless to the plant by its conversion into the practically neutral salts of the organic acids.

The final balance of ion accumulation shows the presence of 5 equivalents K+, 2 equivalents NO−$_3$ and 3 equivalents organic anions. This means that in ion accumulation the excess of salt cations over unchanged inorganic salt anions equals the amount of organic anions formed. In the present example, the amount of organic anions can be found by plant analysis on K and on NO_3 and subtracting the NO_3 equivalents from the K equivalents.

Plant analysis has shown that the major proportion of the total uptake by grass concerns the elements K, Na, Mg, Ca, Cl, P, S, N and Si. With a single exception, these elements are absorbed in the ionic state and for balance studies it is necessary to express the quantities involved as electrochemical equivalents.

Some difficulty may arise in choosing the correct valence for P and Si. However, this can be simply solved by taking into account the pH of the plant tissues. For *Gramineae* this is slightly on the acid side (pH 5 to 6) and therefore P should be expressed as $H_2PO^-_4$. Although on a weight basis there is considerable Si in grass, Si must be omitted from the balance, because silicic acid is a very weak acid with pK values of 10 and 12 so that silicate anions only occur in a medium more alkaline than pH about 9*. Apparently, Si passes into the plant in the non-ionic state, probably as a polyacid, which might be connected with its typical location in the peripheric tissues of the plant body.

The balance of uptake can therefore be made by conversion of plant analytical data on the cations, Cl, total N, total P and total S into the equivalents of their common ionic state at pH 5 to 6: K^+, NA^+, Mg^{++}, Ca^{++}, Cl^-, NO^-_3, $H_2PO^-_4$, $SO^=_4$, if the balance concerns the more common case of nitrate as the only source of nitrogen. Plant analysis has shown that, apart from artefacts, this balance covers 97 per cent of the total uptake of salt ions.

The next step is the addition of the equivalents of K, Na, Mg and Ca and considering the sum obtained as a single salt cation. Herewith, we have agreed to neglect the specific value of each of the cation species K, Na, etc. and have appreciated them all by one single property of being a salt cation. In the more simple case of potassium nitrate uptake it was pointed out that the deficit of K in the uptake is occupied by H^+ absorption. The common function of the above cations in the balance of uptake is also to prevent excessive H^+ uptake (compare also *Wadleigh, 35*). In this respect they are comparable because they form practically neutral salts with the inorganic and organic anions resulting from uptake and utilization.

To obtain the total of anion equivalents absorbed it is sufficient to add the equivalents of the anions mentioned above as representative for the ionic forms of uptake of Cl, N, P and S. Their common property is that they are the anions of neutral salts at the given conditions of uptake.

To show graphically the aspects of the ionic balance of uptake by *Lolium perenne* during its growth at two nitrate levels of supply consider figure 1. As the age increases, total nitrogen (N) becomes lower in both treatments in a similar fashion. In short, the line thus defined is the line of the effect of age on the utilization of nitrogen in growth and is independent of the treatment. It is not until the 28th day of growth that nitrate exhaustion is reached in the lower treatment (a) as evident from the sudden onset of a decline of NO_3 in the herbage. From there on total N declines at a more rapid rate because at exhaustion the uptake ceases and the amount absorbed is thenceforth diluted by further growth.

The proportion of salt cations (C) over salt anions (A + N) absorbed is shown in the lower graphs. It is seen that when the relative proportion of nitrate in the total

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* Its inclusion by *Bear (4)* and others rests upon a misconception of the properties of silicic acid and silicates. *Pucher et al. (26)* correctly omitted Si in the balance of ion accumulation.

uptake declines at the advance of age (log N declines more steeply than log C and log A) the proportion of salt cations relative to salt anions absorbed increases, which indicates that the acidity of the uptake becomes gradually lower. When nitrate uptake ceases, the further uptake exhibits a slight excess of salt cations over salt anions (log C declines less rapidly than log A) and their proportion increases more rapidly in the herbage.

It is seen, however, that the total uptake remains acid during the entire period and its acidity is given by the difference between absorbed anions $(A+N)$ and absorbed salt cations (C). During early growth the balance shows an H+ uptake of about 2100 mEq/kg dry weight and its lowest value of about 600 mEq/kg dry weight is found at the latest date of harvesting with exhaustion of nitrate (fig. 1a). At the standard time of 28 days, used for re-growth in most of our experiments, N corresponds to 2700 mEq/kg and the H+ uptake to 1400 mEq/kg dry matter, in the absence of nitrate exhaustion and at moderate Cl supply.

The balance of utilization

In terms of the balance-sheet, utilization should be regarded as the conversion of a salt ion into some form of chemical combination which is non-ionic.

A portion of the cations absorbed is subject to complex formation (e.g. Mg in chlorophyll), adsorption by structural configurations (Ca in phosphatides, pectates, etc.) or formation of insoluble salts (e.g. Ca oxalate). This binding may concern their specific physiological functions for growth, but the plants' use of the cations is not associated with a change of their ionic valance. Therefore, there is no utilization of salt cations in the present sense. There is, however, utilization of the H+ ions because they loose their ionic state during neutralization by the internal alkali production.

Among the anions, phosphate becomes involved in the metabolism by partial esterification. Although at normal phosphate levels there is an appreciable proportion present as free orthophosphate in the herbage, at low levels more than 50 per cent is involved in organic linkage (fig. 2). However, this form of combination does not affect its ionic state, because the first acidic function of phosphoric acid deter mines the ionic state of phosphate at the pH of the tissues and this is not involved in the esterification, when the small change in pK^1 is neglected. Therefore, there is no utilization of phosphate.

Utilization in the present sense occurs only with nitrate and sulphate. As long as growth proceeds, a considerable portion of these anions is reduced by the metabolism to organic N and organic S, and they cease to exist as anions. Electroneutrality requires that this process liberates their equivalents as other anions and the ionic equations of their reduction:

$$(N^5+O_3)- + 8\,H \rightarrow (N^3-H_3) + 2\,H_2O + OH- \text{ and}$$
$$(S^6+O_4) + 8\,H \rightarrow (S^2-H_2) + 2\,H_2O + 2OH-$$
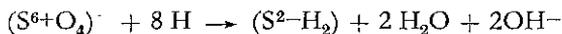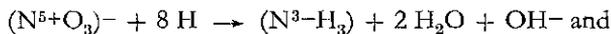
show that their utilization in the metabolism liberates the equivalent amount of the strongly basic OH- anion *(Dijkshoorn, 13)*.

mg eq/kg

a

b

$\frac{C}{A+N}$

075
070
065
060
055
050
045
040

0 10 20 30 40 days   0 10 20 30 40 days
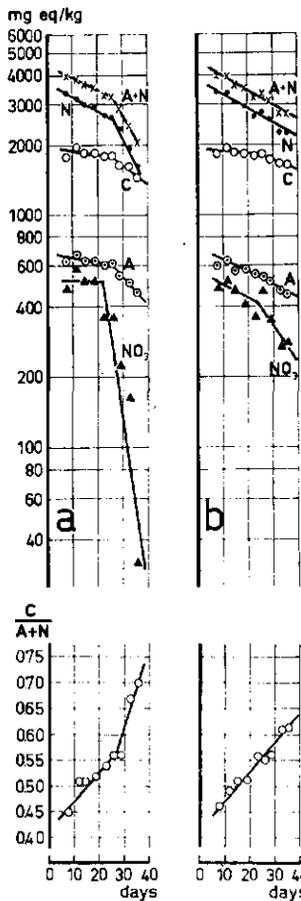
Figure 1

Figure 1 Salt cations ($C = K + Na + Mg + Ca$), elements absorbed as anions ($A = Cl + P + S$, and N and $A + N$) and of accumulated NO^{-3}, in me. per kg dry matter produced, plotted against the time in days of re-growth at lower (a) and at higher (b) nitrate supply. Pot experiment with *Lolium perenne (Dijkshoorn, 10)*

Figure 2 Total phosphorus (P_t), TCA-soluble phosphorus (P_s), TCA- insoluble phosphorus (P_p) and free inorganic orthophosphate (P_i) in microgramatoms P per gram dry matter of herbage of *Lolium perenne*, grown at varying P levels *(Dijkshoorn* and *Lampe, 15)*
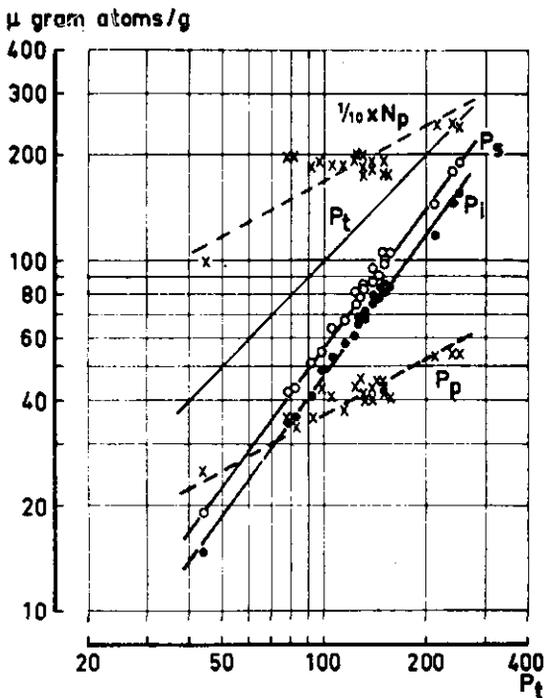
μ gram atoms/g

Figure 2

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This means that the internal alkali produced equals organic N + organic S expressed as their equivalents of NO_3^- and SO_4^{--}.

For assessing the balance of utilization we have investigated the utilization of nitrate and sulphate, by growing *Lolium perenne* on solutions of various equivalent proportions of nitrate and sulphate and otherwise constant ionic composition. Analysis of the protein fraction showed that proteinization of sulphate and nitrate occurs in a constant proportion of 0.027 moles S per mole N. Herbage analysis showed that the same proportion holds for total organic S and total organic N (fig. 3 and 4).

The conversion of this relationship to equivalents gives that organic S is found by multiplying organic N by 0.054. The excess of total sulphur over organic sulphur, as found by herbage analysis on total S, is unchanged inorganic sulphate. Its absence indicates sulphur shortage.

Other investigations have shown that the non-protein N is made up of NO_3^- and of organic nitrogenous compounds. Only when nitrate exhaustion occurred at some stage of previous growth, NO_3^- is low or absent.

For balance work the ion utilizaton and internal alkali production is computed from data on total Kjeldahl N, obtained by the use of a method which includes all nitrate nitrogen, and on nitrate. Organic N is found by difference and organic S by the use of the proportionality factor* and the values are added to calculate the internal alkali production.

Hitherto, uptake and utilization have been regarded as discrete steps. Actually, of course, there is a ceaseless interplay between the acidity of the uptake and the alkaline utilization and it can be assumed that the latter is a condition to the progress of acid uptake with excess salt anions over salt cations. In the absence of utilization the acidic uptake cannot proceed without excessive internal acidification or breakdown of carboxylates. It is of interest to note in this connection that the acidity of the uptake declines when at the advance of age less N is utilized per each kg of dry matter produced. Also it was found that after nitrate exhaustion uptake continues with an excess of salt cations over salt anions (fig. 1) which means that the uptake has become alkaline and the external effect reversed from alkaline to acid (compare e.g. *Wander* and *Sites, 37*).

The balance of accumulation

A complete balance for herbage of 4 weeks re-growth is given in figure 5.

It is very similar to the balance found after 28 days in the time of re-growth series of figure 1, made under comparable conditions of season (summer in greenhouse) and nutrition (adequate at low Cl). It is seen that salt cations and salt anions were absorbed with an acidity of the uptake of 1.4 equivalents H^+ per kg dry matter, while anion utilization indicates the liberation of 2.5 equivalents internal alkali. Accordingly, the excess internal alkali was 1.1 equivalents per kg dry matter.

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* Errors due to lack of precision of this factor 0,054 are small relative to the errors involved in the balance. Its use is preferable to neglecting S-assimilation (*Pierce* and *Appleman, 25*) or to the arbitrary assumption that 0,7 of total S remains unutilized (*Pucher et al., 26*).
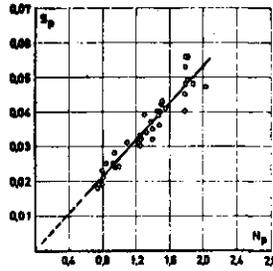
Figure 3 Protein-sulphur and protein-nitrogen in gramatoms per kg dry weight of herbage of *Lolium perenne*, grown at varying sulphate and nitrate supply *(Dijkshoorn et al., 17)*
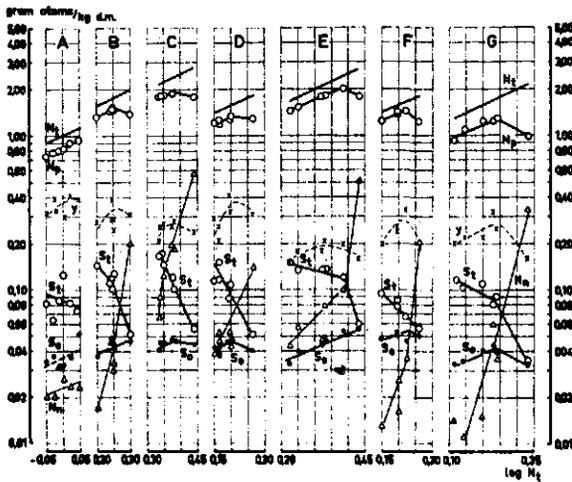

Figure 4 Total nitrogen (N_t), protein-nitrogen (N_p), nitrate-nitrogen (N_n), total sulphur (S_t) and organic sulphur (S_0) in gramatoms N or S per kg dry weight of herbage of *Lolium perenne* grown on solutions of varying nitrate-sulphate proportions and otherwise constant ionic supply *(Dijkshoorn et al., 17)*

The final balance of accumulation is 1.8 equivalents salt cations, $3.2—2.5 = 0.7$ salt anions (Cl^- + total P as $H_2PO^-_4$ or $RHPO^-_4$ + inorganic $SO^=_4$ + inorganic NO^-_3) and the difference $1.8—0.7 = 1.1$ equivalents of organic anions per kg dry matter.

This implies the complete neutralization of the excess internal alkalinity by its transformation into organic anions, either by carboxylation reactions or by its complete neutralization by organic acid molecules formed in the metabolism. Neutralization is here used in the loose sense that each equivalent of the excess internal alkalinity is destroyed by an equivalent H^+ resulting from the synthesis of an equivalent of organic acid molecules. This concept is more simple than that of carboxylation reactions (direct transformation of HCO^-_3 into $RCOO^-$) and the numerical result for the balance is the same. For the present purpose, the choice between these mechanisms is what suits the convenience.

At this point it is advisable to review briefly the evidence for considering this complete neutralization as a condition to normal growth.

From experiments of *Martin (22, 23)*, *Leuthart (21)*, *Hurd-Karrer (18)* and others it can be inferred that the buffer capacity (equivalents of H^+ or OH^- required to change the pH by one unit) is only 0.02 per litre of sap which is less than 0.2 equivalents per kg dry matter. It is only in the more acid range of pH 3 to 5 that the buffer capacity increases considerably due to the presence of organic acids capable of buffering in this region *(Böning* and *Böning-Seubert, 2; Rombeck, 28)*. It was found that the actual pH of the plant sap is little dependent on the treatment *(Arnon, 1)*.

Also the buffer capacity in the region pH 5 to 7 is unaffected by the treatment, but in the more acid range there is a large effect of treatment on the buffer capacity *(Böning* and *Böning-Seubert, 2)*, showing alterations which agree with changes in the organic anions to be expected from the shifts of the balance of accumulation. This, and much other evidence, shows that the organic anions do act as a regulatory unit in a way better defined as neutralization than as buffering. They serve to the neutralization of in the order of 1 equivalent excess internal alkali at a buffer capacity of only about 0.2 equivalents per kg dry material.

Hurd-Karrer (18) found that proteins play little or no part in the buffer system. Compare also *Chibnall* and *Grover (7)*. This can be expected, because their density of ionic charge is low and, with the acidic formulation of ionization, pK's are about 2.4 and 9.

According to *Martin (22, 23)* the buffer capacity in the range pH 5 to 7 is mainly due to the phosphates. As an illustration figure 6 shows titration curves of the ash of grass grown at ample P supply. This result shows that the buffer capacity due to phosphate is only 0.02 equivalents per kg dry matter between pH 5 and 6, and 0.08 between 6 and 7.

As shown above, there must be practically complete transformation of the excess internal alkalinity into the salts of organic anions, to prevent any excessive rise of the internal pH. The number of equivalents of organic anions formed follows directly from the balance of accumulation, if the internal pH remains unchanged. There is often little point in attempts to apply a separate determination of the organic acids, because difficulties are sometimes involved in their complete

Balance-sheet of uptake and utilization by Lolium perenne L, eq./kg d.m.	
uptake	
cations $K+Na+Mg+Ca$	1.8
anions $NO_3+Cl+H_2PO_4+SO_4$	3.2
H^+	1.4
utilization	
cations $K+Na+Mg+Ca$	0
anions <u>nitrate</u>	<u>2.4</u>
<u>sulphate</u>	<u>0.1</u>
<u>chloride</u>	<u>0.</u>
phosphate	0
final state	
metabolic alkali $\underline{HCO_3^-}$	<u>2.5</u>
acidity uptake H^+	1.4
excess internal alkalinity	1.1
organic anions (malate etc.)	1.1
cations-inorganic anions	$1.1 = C^+ A^-$

Figure 5 Balance sheet of herbage of *Lolium perenne* of 4 weeks re-growth at adequate nutrition low Cl level in the soil, natural light under greenhouse conditions in the summer, as a closed crop in pots *(Dijkshoorn, 13)*
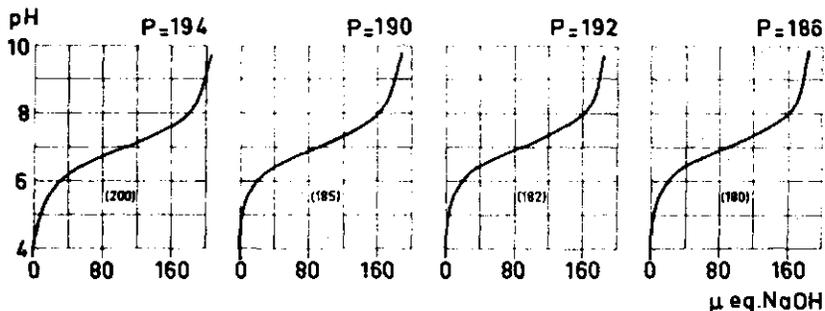

Figure 6 Titration curves of the ash of 1 gram dry matter of *Lolium perenne* grown at ample phosphorus supply *(Dijkshoorn* and *Lampe, 15)*

recovery by extraction. For *Lolium perenne* we were able to recover the complete fraction as the water-soluble, non-volatile organic acids and to achieve their separation by partition chromatography on silica gel (fig. 7). *Pierce* and *Appleman (25)* were able to recover the acids from a number of plant species by ether extraction of the acidified material in amounts very near to the quantities predicted by the balance of ion accumulation.

The above examples on the balance of *Lolium perenne* all yielded an organic anion content very near to 1 equivalent per kg dry material, independent of age (fig. 1) or treatment (fig. 8). A further examination of the results indicated that the nutrition was always adequate with the only exception that nitrogen was somewhat short at the lower fertilization levels.

Thus, in spite of a larger variation in the acidity of the uptake and in utilization of nitrate and sulphate (internal alkali production) the plants appeared to maintain their organic anion content at the same level.

From this we have acquired a certain affection for considering this value as essential to normal, optimal growth. It was found that lower values occurred only when there was more severe N shortage or K shortage, when the plants accumulated excessive amounts of Cl or when NH_4 was utilized instead of nitrate, all conditions leading to less vigorous growth. Before continuing on this line we have to consider briefly the selectivity of the plant in ion uptake.

The effect of ion selectivity of the plant

The balance of accumulation indicates the presence of inorganic cations $K^+ + Na^+ + Mg^{++} + Ca^{++} = C^+$ and of inorganic anions $Cl^- + H_2PO^-_4 + SO_4 + NO_3^- = A^-$ and the difference $C^+—A^- =$ organic anions. They were only considered as the ions of neutral salts, which can accumulate without affecting the internal pH. Further, experience has shown a tendency to maintain the organic anion value approximately constant under varying conditions of supply and age when nutrition remains adequate.

Given a certain anion uptake and accumulation and varying proportions of cation supply there must be a tendency toward a constant total salt cation content which reminds of the older theorem of "cation constancy" of *Van Itallie (33)*. Further, at varying anion uptake and accumulation, there must be a variation of total salt cations in a similar sense, which recalls Bear's postulate on "constancy of the cation-anion ratio" *(Kretschmer et al., 19)*.

The ability of the plant to substitute K for Na, Mg, Ca or Mg for K, Na, Ca, etc., if each of these cations is replaced by another cation in the supply, for their common function to reduce the acidity of the uptake is seen in figure 8, which shows that at the increase of nitrate uptake and accumulation, total cations C increases in a similar fashion independent of the varying proportions in which the individual cations are absorbed.

It is seen that application of the nitrates of K and Na makes those cations increase in the herbage in proportion to the extra demand for total cations. However,when Mg or Ca is supplied, there is also an increase in Na and in the divalent cation not supplied of which both are available in constant amount. Apparently the divalents
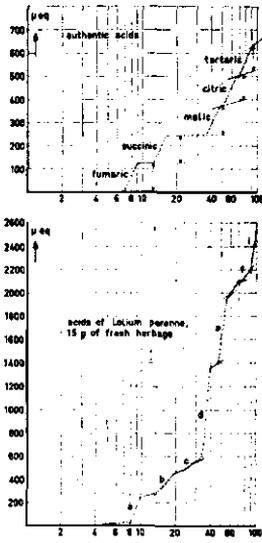
Figure 7 Partition chromatogram of the water-soluble, non-volatile organic acids of *Lolium perenne,* after previous isolation and separation from ozalic acid on exchange columns *(Dijkshoorn* and *Lampe 16)*

Figure 8 Pot experiment with *Lolium perenne* and *Dactylis glomerata,* conducted under the conditions of figure 5. The nitrates of K, Na, Mg and Ca were applied to the soil at rates from nil to 40 mEq per pot *(abscissae),* nitrogen fully adequate at the highest rate of application (compare fig. 1). For explanation of symbols see figure 1 *(Said 29)*
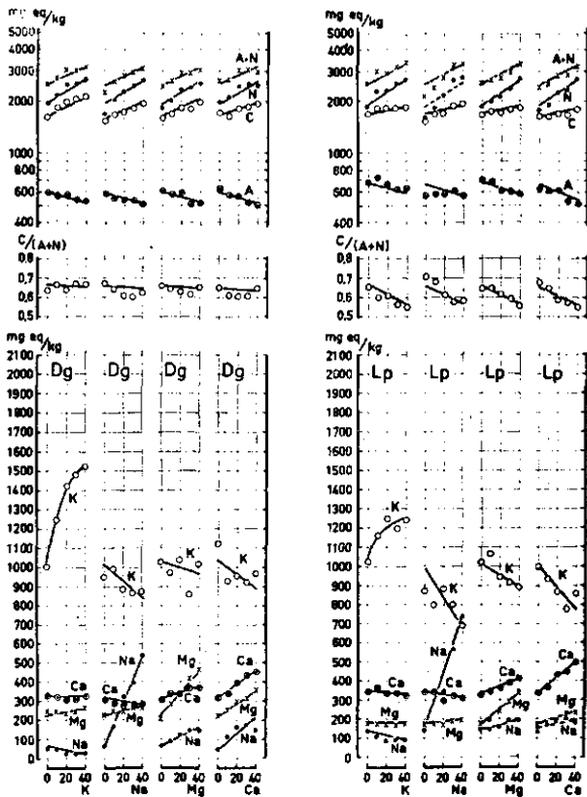
Figure 7

Figure 8

supplied are not absorbed at a sufficient rate relative to growth and the increase of the cation demand so that more of the other cations of constant supply are taken up to prevent an increase of the deficit in salt cation relative to salt anion accumulation. This reflects the selectivity of the grasses in cation uptake.

For both grass species and for *Plantago lanceolata* the selectivity for the cations was examined by re-growth on solutions of varying proportions of two cations and otherwise constant ionic supply (fig. 9). It is seen that replacement in the solution leads to substitution in the herbage, but its degree of completeness depends on the plant species and on the two cations compared. In the dicotylodenous herb *Plantago lanceolata* there is practically complete substitution and, within the experimental error, it is concluded that this species does not discriminate between the four cations studied, there is no selectivity. The grasses show a pronounced preference for the monovalent cations as apparent from the much steeper gradients and within the monovalents *Lolium perenne* is not selective while *Dactylis glomerata* absorbs Na with more difficulty than K. Also for the divalent cations the latter species shows selectivity while *Lolium perenne* absorbs Mg and Ca at a comparable rate. This is also reflected in the cationic composition of the two species when grown side by side in one experiment. *Dactylis* is lower in Ca and Na but higher in Mg and mostly it is higher in K because Ca and Na are absorbed with relatively more difficulty and, therefore, the cation demand is completed by the uptake of more K which is the most easily absorbed.

In the foregoing sections the salt cations were considered as operative in a similar way: the prevention of excessive H+ uptake and the formation of neutral salts with the inorganic and organic anions resulting from uptake and utilization.

Here, plant selectivity comes to the fore making the plants discriminate between the cations according to their relative rate of uptake. The prevention of H+ uptake required that uptake and translocation of the salt cations proceed at a rate sufficiently large to keep up with the growth and requirements for H+ exclusion.

The selectivity of the grasses indicates that K is mostly superior in this respect, because it is absorbed more rapidly than Na and the divalents.

If sufficient K is replaced by a less mobile cation, e.g. Ca, in the supply, salt cation uptake may fall short, excessive H+ uptake may result and, when the balance of utilization and accumulation of anions remains unchanged, the organic anion content may become depressed. This condition prevails in the experiment of figure 10 which shows reduced growth, increased H+ uptake and (as calculated from the balance of accumulation) reduced organic anion contents when K is replaced by Ca in the fertilizer. It is seen that K drops to lower values when Ca-nitrate is supplied (which is due to dilution by the increased growth), but K remains above the level of specific K shortage associated with ill-conditioned plants, which is 200 mEq K per kg dry weight. There was reduced growth but the appearance remained healthy.

This is a common aspect of the effect of increasing the K supply beyond the specific requirements for growth. Although at moderate K supply the plant is able to substitute other cations for K, sufficient exclusion of H+ uptake for optimal growth may be attained and the plants, although healthy in appearance, show a further response in growth when additional K supply relieves the stress on the balance of accumulation by a further exclusion of excessive H+ uptake and raising the organic anion content to a more normal level for optimal growth.
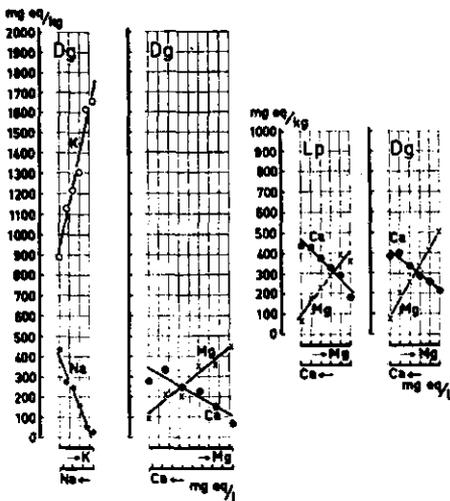
Figure 9 Effect of equivalent replacement of cations in the nutrient solution on their equivalent substitution in the herbage of *Lolium perenne* (L$_p$), *Dactylis glomerata* (D$_g$) and *Plantago Lanceolata* (L$_p$). *Abscissae:* subdivision in mEq per litre, increase of supply indicated by arrows *(Said 29)*

Figure 10 Effect of nitrates of *K* and of Ca (*abscissae:* mEq per pot of 5 kg soil) on per cent d. m. in fresh material (% d.m.), yield (g dm per pot) and composition of herbage of *Dactylis glomerata*, re-growth at initially low K level *(Said 29)*
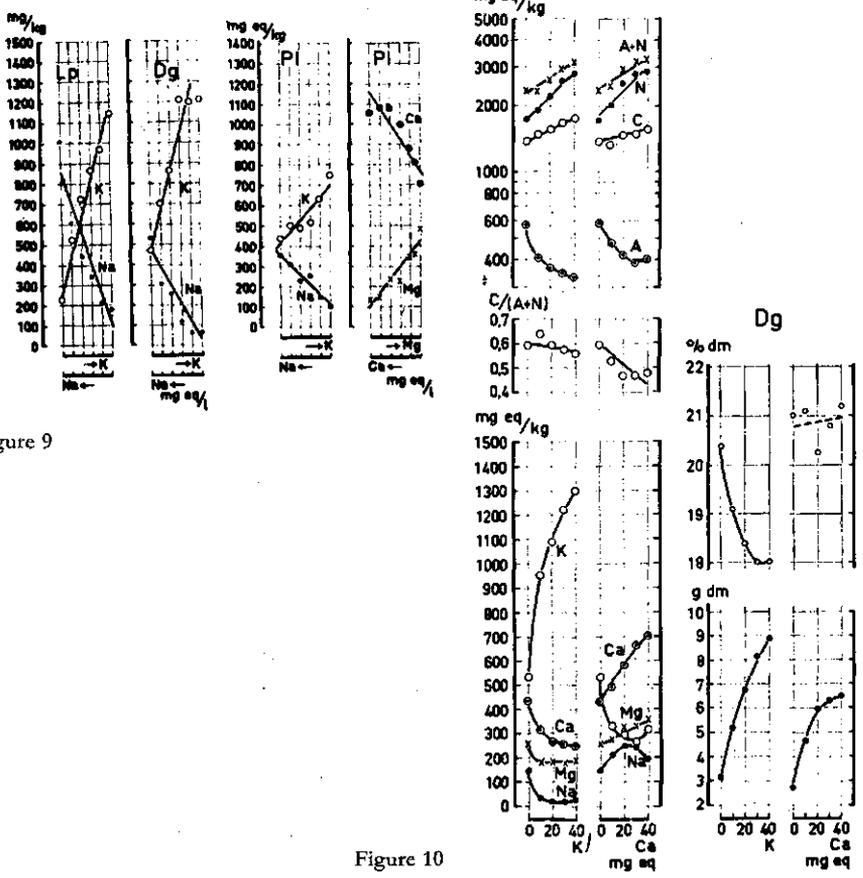
Figure 9

Figure 10

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Substituting other cations for K

It has been shown before that, when K is moderately reduced in the supply, the plant · can maintain its balance by increasing the uptake of other cations of constant supply. At low K, *Dactylis glomerata* was apparently unable to substitute Ca for K at a sufficient rate and the uptake became more acid at reduced growth and low organic anions content. The question remains, what happens if K is replaced by a cation with the same rate of uptake, that is, complete equivalent substitution in the herbage, as e.g. Na in *Lolium perenne* or any other cation in *Plantago lanceolata*. We might expect that in this case H+ uptake and organic anion content remains unaffected, even when K falls to lower levels in the plant.

Actually, it is found that in this case the balance of accumulation is often shifted to an increased salt cation content relative to inorganic anions and the organic anion content is apparently increased to above the normal value with a reduction of the growth.

This aspect came also to our attention through the work of *Böning* and *Böning-Seubert (2)*, *Vladirimov (34)*, *Chouteau (8)* and others, who invariably found that K shortage induces a higher organic anion content in tobacco. The data of *Chouteau* make it clear that Ca substitutes for K in excess so that at K shortage the salt cations are relatively increased. A similar effect for barley of substituting Na for K is shown in figure 11, the data are from *Lehr* and *Wybenga (20)*. Here the situation is very similar to our finding on the effect of Na for K substitution at low K levels in *Lolium perenne*. It should be stressed that in these cases the yield was always markedly reduced by replacing K in the supply, although its substitution in the herbage by the other cation occurred in excess.

Apparently, these cases refer to conditions where the selectivity of the plant allows a complete internal substitution, but there results a congestion of alkalinity or organic anions in the tops, because the downward translocation of excess alkalinity or organic salts cannot proceed at an adequate rate, probably because the cation used for substituting K, although sufficiently translocated upward, cannot be transported downward at an adequate rate for accompanying excess alkalinity produced in the shoots and removed by subsequent downward transport through the phloem, back to roots. At present it seems that the interpretation of these effects and the particular role of K for regulation of the balance of accumulation involves the possible rate of internal circulation in the plant. Although Ca in tobacco and Na in barley may substitute K very effectively in the total plant material, data on their distribution over different plant parts indicate their lower mobility in translocation when compared with K.

Anion induced cation shortage

The accumulation balance relates the difference between salt cations and accumulated inorganic salt anions, including total phosphorus: $C^+ - A^-$, to the accumulation of organic anions.

The effect of replacement of anions in the supply in *Lolium perenne* is shown in figure 12. It is seen that when Cl is replaced by H_2PO_4 in the supply, there is only partial substitution of Cl by H_2PO_4 in the herbage and if H_2PO_4 is replaced by $SO_4^=$
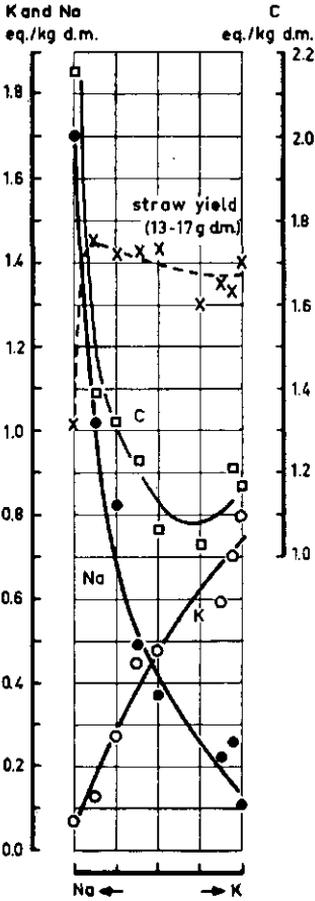
Figure 11
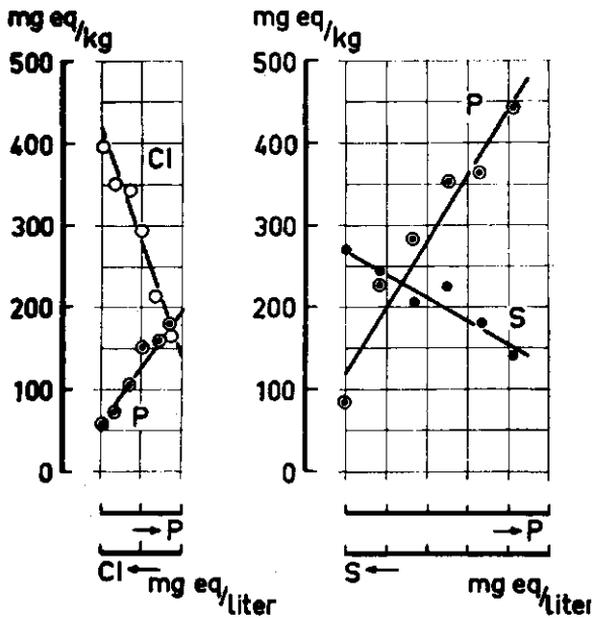
Figure 12

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in the supply $SO^=_4$ substitutes H_2PO_4 only partially in the herbage. The order of decreasing relative rate of uptake is $Cl^->H_2PO^-_4>SO^=_4$. Further, it is known that NO^-_3 may accumulate to about 0.5 equivalents (fig. 1) prior to exhaustion and Cl may increase to about 1 equivalent per kg dry material when supplied in pot experiments without leaching.

Therefore, the greatest increase in A^- occurs when Cl is supplied in the fertilizer or when nitrate-exhausted plants are made to accumulate NO^-_3 subsequent application of nitrate.

A typical example of nitrate-induced cation demand is shown in figure 13. The experiment was made in connection with observations on an increased grass tetany frequency in grazing cattle following a rise of temperature in the spring. It is seen that transference of the plants during the re-growth from 10 to 20° C induced at first a rapid decline of nitrate in the herbage due to increased utilization. There is, however, a gradual building up of the nitrate accumulation level during the next ten days and this covers 0.3 equivalents of accumulated nitrate. Associated with this increase total salt cations C also increases by 0.3 equivalents per kg dry herbage. An examination of the data showed that the organic anions $(C+—A-)$ remained unchanged for all temperature treatments and times of re-growth, at about 1 equivalent, that is the normal value. Here, the response to the increase of accumulated anions is a corresponding increase in cations at constant organic anion content. Due to plant selectivity the absolute increase was greatest for K which was high in the supply, and the herbage became higher in K relative to the other cations when compared with the constant temperature treatments or with the treatment with transfer to the lower temperature. *(Dijkshoorn* and *'t Hart, 14.)*

Generally the application of Cl in the fertilizer tends to increase the level of accumulated anions and this is mostly associated with an increased cation content *(Dijkshoorn* and *'t Hart, 14; Dijkshoorn, 11, 12)*. However, the balance of accumulation showed that increased Cl was not completely compensated by the increase in cations, so that the organic anions $(C+—A^÷)$ declined more or less to below the normal value. Hitherto, we were unable to directly relate these findings with reduced growth, probably because the total yield of herbage is not so sensitive to more moderate changes in the actual rate of growth following unbalanced nutrition as indicated by sampling at the date of harvesting.

The lowering of organic anions in intact plants by Cl supply has been demonstrated by *Ratner* and *Akimochkina (27)* with sugar beets fed with either a K-clay or with KCl. The effect was also observed in tobacco by *Böning* and *Böning-Seubert (2)*.

An increased demand for K has been found to be associated with higher Cl levels. *Boresch (3)* mentioned that the chlorine toxicity symptoms in strawberry are difficult to distinguish from K deficiency and that its occurence depends on K relative to Cl rather than on the absolute K content. Very similar conditions were reported for citrus by *Cooper* and *Gorton (9)* and for beans by *Buchner (5)*. *Buchner* also mentions increased internal acidity due to high Cl in potato and states that the inability to neutralize organic acids inhibits starch breakdown in the leaves and the translocation of the intermediates to the tubers.

Of course, internal acidification is not a condition sine qua non for excessive anion accumulation. The pathway of synthesis of organic acids by carboxylation in the presence of excess internal alkalinity becomes reversed by excess internal acidity resulting from accumulation of excess unutilized salt anions over salt
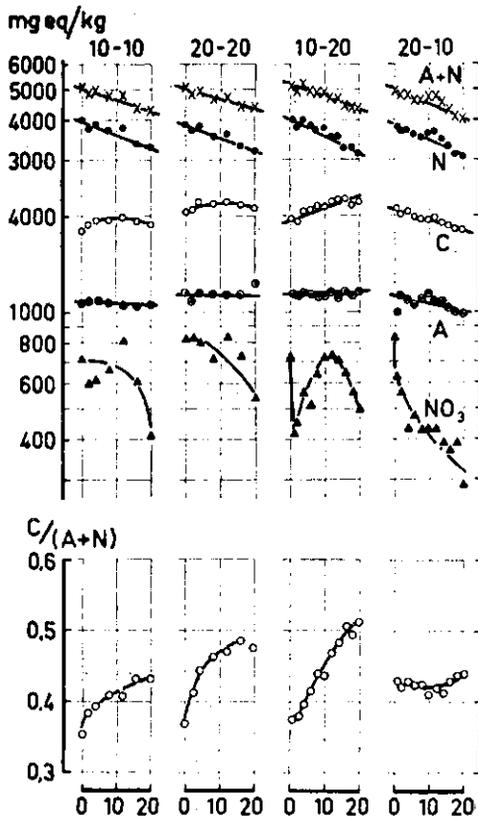
Figure 13 Composition of *Lolium perenne* grown at 10°C, at 20°C, after transference from 10 to 20°C and after transference from 20 to 10°C. *Abscissae:* time in days after transference. Periodic harvesting, first harvest occurred 11 days after the beginning of re-growth and is indicated as zero time of age. Records are given of total cations (C = K + Na + Mg + Ca), total nitrogen (N), non-nitrogenous anions (A = Cl + P + S), total anions (A + N), nitrate (NO$_3$) and the ratio of salt cations to salt anions in the uptake – C/(A+N) – in mEq per kg of dry herbage of the ionic forms supplied *(Said 29)*
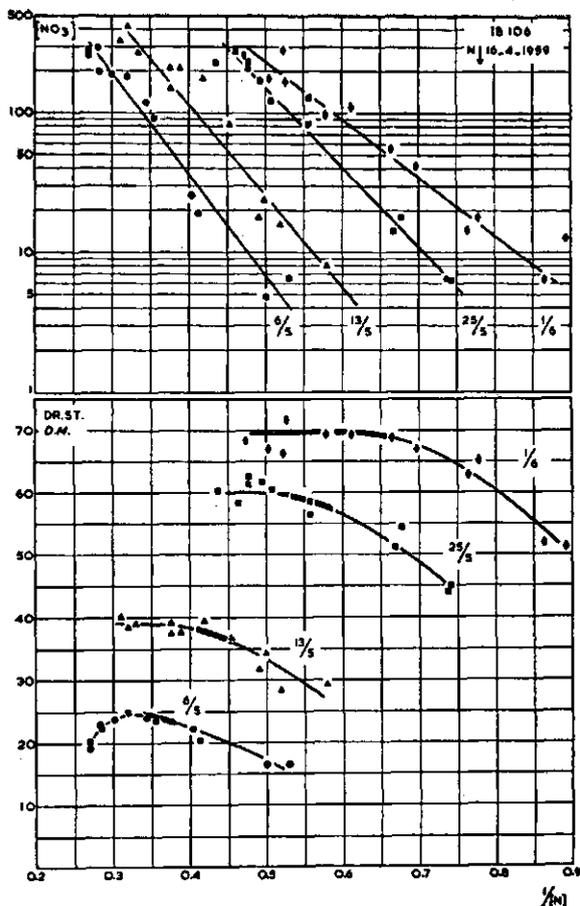
Figure 14 Upper graph: relation between mEq NO_3 per kg of dry herbage (ordinates) and kg dry herbage produced per gramatom of N absorbed (*abscissae*, reciprocals of N content in gramatoms of N per kg dry matter) in grass of different cutting dates grown after the application of varying rates of N on April 16. Lower graph: the corresponding yields in units of 100 kg dry matter per ha.

For each cutting date $1/N$ decreases and NO_3 in the herbage increases as a result of increasing the rate of N application (direction of the lines). Cutting at a later date shifts the line to higher $1/N$ levels and to higher yields (position of the lines), because at more advanced age less N is utilized per kg dry matter and growth has continued over a longer period. The highest yields occur if NO_3 is present beyond a level of about 50 mEq per kg dry herbage, indicating adequate supply up to the date of cutting.

Field experiment of the Institute for Soil Fertility, Groningen, the Netherlands *(Van Burg 32)*.

cations, and the decarboxylation liberates alkalinity in the form of HCO^-_3 that neutralizes the internal acidity. When this neutralization proceeds to completion, the internal pH remains the same, the only effect is that organic anions are used up for the production of sufficient internal alkali (compare also *Ulrich, 31* and *Burström, 6*).

Unbalanced salt ion accumulation with reduced organic anion contents may also be expected at higher internal levels of unutilized nitrate at ample nitrate supply, because in the accumulated state nitrate and chloride must act in a similar way. The only difference is that nitrate exhaustion, when reached in a further stage of growth, leads to complete metabolic consumption so that nitrate disappears rapidly and the stress on the organic anion content is relieved. In the case of chloride exhaustion there is only dilution by growth and the stress on the balance of accumulation disappears less rapidly.

In pot experiments with grass we failed to detect growth inhibition at high internal nitrate levels. Improved growth was always obtained through the effect of better nitrate supply associated with this condition. To this comes the fact that sufficient utilization of nitrate favours the synthesis of organic anions by the metabolism *(Böning* and *Böning-Seubert, 2; Chouteau, 8; Dijkshoorn, 16,* and *others)*. However, the pot experiments of *Sorensen* (1959) with oats show that reduced growth occurred at the highest N level applied while this effect disappeared in a later stage of growth when nitrate had declined from 0.5 to 0.04 equivalents per kg dry material.

The predominance of the improving effect of nitrate utilization over the inhibiting effect of nitrate accumulation due to unbalance of ion accumulation in grass is also shown by the results of *Van Burg (32)* on the effect on time of growth at different N levels in the field. Figure 14 shows that growth reduction due to excessive N application only occured in the youngest cut, but this effect may have originated from fertilizer salt damage to the sward.

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