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The uptake and translocation of fertilizer N^{15} in young trees of Scots pine and Norway spruce

Upptagning och translokation av N^{15}-märkt handelsgödselkväve hos tall och gran

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According to the available information, nitrogen is the nutrient element which is most often the limiting factor for the growth of forest trees in Scandinavia (TAMM & CARBONNIER, 1961; BRANTSEG, 1962). Investigations are in progress to discover the extent of nitrogen fertilizer requirement of forest stands, the most suitable form of nitrogen, and the most suitable time and method for supplying it. The tracer technique, which has been of great use in the study of the nutritional problems of field crops, has only been used occasionally as an analytical aid to the solution of these problems in forest crops. Thus, no references seem to be available which deal with the use of the tracer technique in connection with the nutrient element nitrogen. There are many possible reasons for this, amongst which are the high cost of N^{15}-enriched materials, the costliness of the equipment for stable isotope separation, and perhaps also the difficulty of interpreting the results obtained. This last difficulty is to a large extent connected with the existence of a microbial immobilization-remineralization cycle in the soil (cf. JANSSON, 1958).

This paper reports a preliminary investigation, carried out with the intention of studying the uptake and translocation of fertilizer nitrogen labelled with N^{15} in young trees of Scots pine (*Pinus silvestris* L.) and Norway spruce (*Picea abies* Karst.). It was expected that the data obtained about the distribution of N^{15} in the plants might give valuable information about the extent and rate of dynamic exchange (breakdown v. resynthesis) of cellular proteins in tissues of various ages, as well as from different plant organs. The investigation was not intended to obtain information on the fertilizer-nitrogen balance in the soil-plant system, nor was it expected to elucidate the quantitative relationships of the uptake of nitrogen by forest trees in a natural stand.

Materials and methods

Experimental area and site. The experiments, which commenced in the summer of 1962, were carried out partly in an 11-year-old stand of self-sown pine, and partly in a similar stand of 12-year-old spruce. The pine area was situated in Vifors Forest District, 50 km north of Gävle (province of Gästrikland). The site was a young pine stand of low or moderately low site quality, whose vegetation consisted mainly of *Calluna* and *Vaccinium vitis-idaea*. The soil was podzolic, with a mor layer 2—3 cm thick, and a thin, but clearly distinguishable leached layer (A_2 horizon). The mineral soil was a fine sand, almost completely free from clay. The pH in the mor layer varied between 4.2 and 4.6.

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The Norway-spruce area was situated at Dalkarlsnäs, 5 km west of Säter (province of Dalarna). The soil was a blocky moraine, the block frequency on the surface being about 90 %; the finer material could be classified as a silt loam. The soil was covered with a mor layer 5—6 cm thick. The gorund vegetation was dominated by grasses.

The precipitation for the months July to December for the year in which the experiments began, was 241 mm for Gävle and 304 mm for Säter.

Experimental technique. In both experimental areas, a number of plants was selected which were of similar age and which stood relatively isolated, each at a distance of at least 3 m from its nearest neighbour. At the time of lay-out of the experiment the Scots pine plants were 11 years old. The experiment included 24 trees, of which 6 were controls. The other 18 received 10 g of nitrogen in the form of one or other of the following nitrogenous fertilizers: ammonium sulphate (20.8 % N), calcium nitrate (15.4 % N) and calcium cyanamide (18.8 % N). In each treatment, 6 trees were selected at random in the experimental area, which measured about 2000 m^2. The nitrogen in the fertilizers used was labelled with N^{15}; thus the ammonium sulphate contained 0.949, the calcium nitrate 2.096, and the calcium cyanamide 0.886 atomic % of N^{15} in excess. The relatively low atomic percentage of N^{15} in the fertilizers used was dictated by the high cost of isotopically enriched materials. In consequence of this, the requirement of precision in the mass-spectrometer analyses was increased. The nitrogenous materials were supplied as topdressings on a circular area with the tree stem as its centre and a radius of 80 cm. The date of the application was 29 June 1962.

In the experiment with Norway spruce, six 12-year-old plants were chosen, of which three received 20 g of nitrogen in the form of N^{15}-labelled ammonium sulphate. The three remaining trees were used as controls. The fertilizer was spread on 7 July 1962, but in other respects the experiment was identical with that carried out on pine.

In both pine and spruce, the longitudinal growth of the needles was not complete at the time when the fertilizer were applied.

Sampling and analysis. Samples of various plant organs of various ages were taken in the autumn of the year in which the experiments were laid out (1962), and in the following two years. The samples consisted mainly of needles and their corresponding twigs. In the case of pine, two branches were removed from opposite sides of the stem in the third whorl from the top. Samples were taken only from the main shoot of each branch, which was separated by cutting into the current year's, the second year's and the third year's growth. The needles were removed and dried at 60° C, after which they were ground up. In the autumn of 1962, the samples were taken only from the current and the previous year's growth.

In spruce, the sampling and preparation of the needles and twigs was done in the same way as that described for pine, except that the samples were taken from the fifth whorl from the top. The needles were removed after the samples had been dried. In 1962, the samples were taken only for the last three year's growth, but in the following autumn they were taken for the last four, and after the third growing season, for the last five years' growth.

The dried and ground-up samples were investigated for their content of total nitrogen by the Kjeldahl digestion procedure, and for the atomic

percentage of N^{15} in this fraction by means of the Consolidated-Nier isotope-ratio mass spectrometer, model 21-201. In mass spectrometric determinations of atomic percentage of N^{15} in excess the average deviation from the arithmectical mean did not exceed 0.0005 %.

Results

Experiment with Scots pine

Because a limited number of plants was included in each treatment, the investigation could not suitably be used to study the influence of nitrogen fertilizers on the height and diameter increment of trees. However, the data obtained on atomic percentages of N^{15} were expected to give information about the rate and extent of continuous breakdown and resynthesis of cellular proteins in various plant organs, depending on their age. Such data for the samples taken after the first, second and third growing seasons, respectively, have been compiled in Tables 1—3. They include no information about the total nitrogen content in the control trees. This has been omitted, partly to save space and partly because the range of variation in the sample material was too wide to show clearly whether there were significant differences in the total nitrogen content between the controls and the fertilized trees.

The data concerning the proportion of the added labelled nitrogen in the total nitrogen fraction in the needle and twig samples taken at the end of the first growing season (1962) show, *inter alia*, that this proportion was higher on the average for the trees treated with ammonium sulphate and calcium nitrate than for those treated with calcium cyanamide. This was true both of the current year's and of the previous year's increment. However, the scatter of values was considerable in any one treatment. Nevertheless, it was possible to establish that the atomic percentage of N^{15} was significantly higher in the current year's needles than in those of the previous year. In the case of the twigs (defoliated), the relationship was almost opposite, though the differences themselves were small. If the atomic percentages of N^{15} in the needles and twigs of the same year were compared, they were found to be different for the current year's and the previous year's issue. In the current issue, the proportion of labelled nitrogen was higher in the needles than in the twigs, whereas in the previous year's issue the relationship was reversed. This last difference was clear and statistically significant.

In the samples collected in the autumn of 1963 (after two growing seasons) the content of labelled nitrogen expressed as a percentage of total nitrogen, was throughout higher than that in the samples taken after the first growing season (see Table 2). This indicates that there had been an additional uptake of residual fertilizer nitrogen from the soil or a translocation from the root system. It was noticeable that the figures for atomic percentage of N^{15} showed a tendency to decrease as the age of the needles and twigs increased. With the exception of the increment for 1961, the atomic percentage of N^{15} was significantly higher in the needles than in the corresponding twigs.

Table 1. Nitrogen content and fertilizer-N^{15} distribution in needle and twig issues of Scots pine. First growing season.

Date of N^{15} application June 29, 1962 (10 g N per tree)
Date of sampling October 14, 1962.

Treatments and tree number, resp.	Current issue (1962)						Issue of 1961					
	Needles			Twigs			Needles			Twigs		
	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N
Ammonium sulphate (7)	1.46	0.045	4.7	0.90	0.038	4.0	1.39	0.024	2.5	0.51	0.032	3.4
(8)	1.22	0.019	2.0	0.75	0.016	1.7	1.18	0.013	1.4	0.45	0.023	2.4
(9)	1.50	0.038	4.0	0.81	0.030	3.2	1.32	0.017	1.8	0.51	0.029	3.0
(10)	1.46	0.012	1.3	0.81	0.011	1.2	1.41	0.010	1.1	0.63	0.023	2.4
(11)	1.55	0.023	2.4	0.86	0.022	2.3	1.52	0.018	1.9	0.56	0.024	2.5
(12)	1.37	0.019	2.0	0.83	0.018	1.9	1.49	0.016	1.7	0.51	0.017	1.8
Mean	1.43	0.026	2.7	0.83	0.023	2.4	1.39	0.016	1.7	0.53	0.025	2.6
Calcium nitrate (13)	1.28	0.025	1.2	0.81	0.022	1.0	1.36	0.014	0.7	0.45	0.023	1.1
(14)	1.48	0.039	1.9	0.81	0.033	1.6	1.50	0.017	0.8	0.49	0.028	1.3
(15)	1.40	0.114	5.4	0.79	0.094	4.5	1.40	0.035	1.7	0.54	0.074	3.5
(16)	1.28	0.033	1.6	0.73	0.031	1.5	1.28	0.031	1.5	0.45	0.028	1.3
(17)	1.16	0.075	3.6	0.74	0.065	3.1	1.25	0.055	2.6	0.47	0.062	3.0
(18)	1.23	0.040	1.9	0.67	0.037	1.8	1.25	0.034	1.6	0.41	0.036	1.7
Mean	1.31	0.054	2.6	0.76	0.047	2.3	1.34	0.031	1.3	0.47	0.042	2.0
Calcium cyanamide (19)	1.35	0.008	0.9	0.82	0.011	1.2	1.39	0.005	0.6	0.54	0.011	1.2
(20)	1.26	0.019	2.1	0.75	0.014	1.6	1.17	0.007	0.8	0.55	0.015	1.7
(21)	1.41	0.020	2.3	0.79	0.018	2.0	1.30	0.010	1.1	0.47	0.016	1.8
(22)	1.30	0.008	0.9	0.77	0.003	0.3	1.31	0.003	0.3	0.49	0.003	0.3
(23)	1.38	0.003	0.3	0.78	0.002	0.2	1.35	0.003	0.3	0.49	0.009	1.0
(24)	1.11	0.014	1.6	0.73	0.011	1.2	1.16	0.011	1.2	0.45	0.013	1.5
Mean	1.30	0.012	1.4	0.77	0.010	1.1	1.28	0.007	0.7	0.50	0.011	1.3
Mean (total), 7—24	1.344	—	2.23	0.786	—	1.91	1.335	—	1.31	0.498	—	1.94
Standard deviation of mean	±0.029	—	±0.32	±0.013	—	±0.27	±0.026	—	±0.16	±0.022	—	±0. 21

Table 2. Nitrogen content and fertilizer-N^{15} distribution in needle and twig issues of Scots pine. The second growing season.

Date of N^{15} application June 29, 1962 (10 g N per tree)
Date of sampling September 29, 1963.

Treatments and tree No., respectively	Current issue (1963)						Issue of 1962						Issue of 1961					
	Needles			Twigs			Needles			Twigs			Needles			Twigs		
	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N
Ammonium sulphate (7)	1.34	0.033	3.5	0.68	0.031	3.3	1.26	0.033	3.5	0.46	0.031	3.3	1.23	0.017	1.8	0.34	0.023	2.4
(8)	1.09	0.024	2.5	0.62	0.022	2.3	1.11	0.019	2.0	0.38	0.020	2.1	1.03	0.013	1.4	0.27	0.017	1.8
(9)	1.51	0.029	3.1	0.79	0.028	3.0	1.46	0.034	3.6	0.54	0.025	2.6	1.39	0.017	1.8	0.43	0.023	2.4
(10)	1.31	0.040	4.2	0.71	0.040	4.2	1.28	0.037	3.9	0.56	0.032	3.4	1.19	0.029	3.1	0.41	0.028	3.0
(11)	1.50	0.049	5.2	0.78	0.045	4.7	1.51	0.034	3.6	0.54	0.037	3.9	1.40	0.027	2.8	0.39	0.037	3.9
(12)	1.33	0.060	6.3	0.70	0.056	5.9	1.30	0.049	5.2	0.52	0.046	4.8	1.21	0.046	4.8	0.40	0.036	3.8
Mean	1.35	0.039	4.1	0.71	0.037	3.9	1.32	0.034	3.6	0.50	0.032	3.4	1.24	0.025	2.6	0.37	0.027	2.9
Calcium nitrate (13)	1.32	0.022	1.1	0.69	0.022	1.1	1.37	0.023	1.1	0.49	0.019	0.9	1.26	0.015	0.7	0.37	0.019	0.9
(14)	1.39	0.049	2.3	0.65	0.048	2.3	1.40	0.044	2.1	0.45	0.040	1.9	1.37	0.020	1.0	0.36	0.033	1.6
(15)	1.44	0.092	4.4	0.70	0.084	4.0	1.31	0.070	3.3	0.45	0.070	3.3	1.29	0.069	3.3	0.36	0.074	3.5
(16)	1.18	0.131	6.3	0.65	0.132	6.3	1.19	0.086	4.1	0.49	0.099	4.7	1.23	0.073	3.5	0.33	0.098	4.7
(17)	1.37	0.139	6.6	0.71	0.137	6.5	1.34	0.125	6.0	0.50	0.122	5.8	1.22	0.090	4.3	0.38	0.095	4.5
(18)	1.26	0.163	7.8	0.65	0.149	7.1	1.19	0.130	6.2	0.40	0.136	6.5	1.19	0.130	6.2	0.33	0.138	6.6
Mean	1.33	0.099	4.8	0.68	0.095	4.6	1.30	0.080	3.8	0.46	0.081	3.9	1.26	0.066	3.2	0.36	0.076	3.6
Calcium cyanamide (19)	1.43	0.014	1.6	0.73	0.012	1.4	1.35	0.012	1.4	0.49	0.012	1.4	1.33	0.099	1.0	0.36	0.011	1.2
(20)	1.15	0.020	2.3	0.75	0.020	2.3	1.03	0.023	2.6	0.48	0.017	1.9	0.97	0.012	1.4	0.42	0.015	1.7
(21)	1.41	0.015	1.7	0.72	0.014	1.6	1.27	0.014	1.6	0.50	0.012	1.4	1.24	0.013	1.5	0.40	0.015	1.7
(22)	1.39	0.018	2.0	0.70	0.018	2.0	1.30	0.016	1.8	0.47	0.014	1.6	1.27	0.010	1.1	0.38	0.010	1.1
(23)	1.50	0.007	0.8	0.75	0.009	0.5	1.37	0.004	0.5	0.49	0.005	0.6	1.35	0.004	0.5	0.38	0.005	0.6
(24)	1.23	0.029	3.3	0.69	0.023	2.6	1.13	0.022	2.5	0.44	0.023	2.6	1.14	0.017	1.9	0.33	0.020	2.3
Mean	1.35	0.017	2.0	0.72	0.016	1.7	1.24	0.015	1.7	0.48	0.014	1.6	1.22	0.011	1.2	0.38	0.013	1.4
Mean (total), 7—24	1.342	—	3.61	0.704	—	3.39	1.287	—	3.06	0.481	—	2.93	1.295	—	1.99	0.369	—	2.44
Standard deviation of mean	±0.028	—	±0.49	±0.011	—	±0.47	±0.049	—	±0.24	±0.012	—	±0.40	±0.045	—	±0.47	±0.009	—	±0.44

Table 3. Nitrogen content and fertilizer-N^{15} distribution in needle and twig issues of Scots pine. The third growing season.

Date of N^{15} application June 29, 1962 (10 g N per tree)
Date of sampling September 25, 1964

Treatments and tree No., respectively	Current issue (1964)						Issue of 1963						Issssue of 1962					
	Needles			Twigs			Needles			Twigs			Needles			Twigs		
	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N	N %	Atomic % N^{15} in exc.	Labelled N as % of total N
Ammonium sulphate (7)	1.25	0.026	2.7	0.61	0.025	2.6	1.07	0.031	3.3	0.38	0.024	2.5	1.08	0.031	3.3	0.33	0.025	2.6
(8)	1.02	0.018	1.9	0.63	0.017	1.8	1.08	0.020	2.1	0.40	0.016	1.7	0.97	0.021	2.2	0.35	0.018	1.9
(11)	1.39	0.038	4.0	0.65	0.038	4.0	1.25	0.045	4.7	0.45	0.038	4.0	1.21	0.050	5.3	0.34	0.031	3.3
(12)	1.19	0.048	5.1	0.63	0.045	4.7	1.15	0.053	5.6	0.43	0.044	4.6	1.16	0.049	5.2	0.34	0.040	4.2
Mean	1.21		3.4	0.63		3.3	1.14		3.9	0.42		3.2	1.11		4.0	0.34		3.0
Calcium nitrate (13)	1.20	0.019	0.9	0.61	0.017	0.8	1.18	0.020	1.0	0.42	0.016	0.8	1.10	0.015	0.7	0.34	0.015	0.7
(14)	1.33	0.029	1.4	0.66	0.021	1.5	1.07	0.041	2.0	0.44	0.032	1.5	1.13	0.048	2.3	0.33	0.029	1.4
(15)	1.22	0.053	2.5	0.63	0.055	2.6	1.06	0.060	2.9	0.39	0.053	2.5	1.04	0.043	2.1	0.30	0.044	2.1
(16)	1.23	0.135	6.4	0.50	0.130	6.2	1.02	0.131	6.3	0.45	0.117	5.6	1.01	0.074	3.5	0.33	0.092	4.4
(18)	1.07	0.156	7.4	0.56	0.148	7.1	1.03	0.159	7.6	0.37	0.146	7.0	1.03	0.105	5.0	0.30	0.124	5.9
Mean	1.21		3.7	0.59		3.6	1.07		4.0	0.41		2.2	1.06		2.7	0.32		2.9
Calcium cyanamide (19)	1.21	0.012	1.4	0.71	0.009	1.0	1.19	0.009	1.0	0.46	0.009	1.0	1.02	0.011	1.2	0.35	0.009	1.0
(21)	1.20	0.013	1.5	0.62	0.012	1.4	1.22	0.013	1.5	0.43	0.010	1.1	1.13	0.012	1.4	0.32	0.012	1.4
(22)	1.29	0.015	1.7	0.64	0.015	1.7	1.26	0.016	1.8	0.47	0.015	1.7	1.20	0.014	1.6	0.31	0.013	1.5
(23)	1.27	0.005	0.6	0.73	0.003	0.3	1.22	0.009	1.0	0.42	0.005	0.6	1.12	0.004	0.5	0.33	0.004	0.5
(24)	1.18	0.020	2.3	0.64	0.018	2.0	1.12	0.020	2.3	0.40	0.015	1.7	1.13	0.015	1.7	0.31	0.014	1.6
Mean	1.23		1.5	0.67		1.3	1.20		1.5	0.44		1.2	1.12		1.3	0.32		1.2
Mean (total), 7—24	1.218		2.84	0.630		2.69	1.137		3.08	0.422		2.29	1.095		2.57	0.327		2.32
Standard deviation of mean	± 0.035		± 0.56	± 0.015		± 0.53	± 0.021		± 0.57	± 0.008		± 0.60	± 0.027		± 0.44	± 0.004		± 0.42

After the third growing season (in the autumn of 1964), both needles and twigs again showed a clear decline in the atomic percentage of N^{15} (see Table 3). By this time, the number of sample trees had been reduced from 18 to 14; therefore the mean values presented are not directly comparable with those for earlier years.

The analytical data indicate that the total nitrogen content tends to decrease with the increasing age of the needles (cf. WHITE, 1954; TAMM, 1955a; LEYTON & ARMSON, 1955; WILL, 1957).

Experiment with Norway spruce

Three 12-year-old trees were supplied with 20 g of nitrogen in the form of N^{15}-labelled ammonium sulphate on 7 July 1962. At the end of the first growing season, needle and corresponding twig samples were taken only from the three most recent years' increments, in the second year from the last four years' increments, and in the third year from the last five years' increments. The results are shown in Table 4.

As in the pine investigation, the distribution of the atomic percentage of N^{15} was the primary object of interest. It was noticeable that the content of N^{15} decreased with increasing age in both needles and twigs sampled in each of the three years in question. It appeared also that the atomic percentage of N^{15} was of a similar order of magnitude in needles as in twigs of the same physiological age. It may be of interest to mention that, as in the case of Scots pine, the samples taken in spruce after the first growing season showed a rather higher atomic percentage of N^{15} for the current year's needles than for the current year's twigs (defoliated), whereas for the needles and twigs from the second and third years' increments, this relationship was reversed, the atomic percentage of N^{15} being significantly higher in twigs than in needles. This difference was not clearly visible in samples taken two and three growing seasons after the nitrogen application. In spruce the recovery of labelled fertilizer nitrogen was generally considerably higher than in pine. This cannot be explained solely by the spruce's having been given a double dose of nitrogen.

Recovery of labelled nitrogen in the whole tree

In the autumn of 1963, after two growing seasons, four Scots pines and one Norway spruce were felled for the investigation of the labelled nitrogen recovery in the above-ground parts of the tree and for the investigation of the distribution of N^{15} in the various organs. In each tree the trunk (bark + wood), the branches and the needles were separated. The samples were dried and weighed, after which their total nitrogen content and atomic percentage of N^{15} were determined.

Table 5 shows that in Scots pine, the highest atomic percentage of N^{15} was found in the needles, after which came the wood, and finally the bark and defoliated branches. Of the labelled nitrogen supplied, not more than 3—9 % was recovered in the above-ground parts of the tree. But in Norway spruce the corresponding figure was 23 %.

Table 4. Nitrogen content and fertilizer-N^{15} distribution in needle and twig issues of Norway spruce.
Age of the plants 13 year
20 g N* in form of ammonium sulphate added per tree
Date of nitrogen application July 7, 1962

Tree No.	Issue	Date of sampling								
		14.10.1962			29.9.1963			25.9.1964		
		Total N %	Atomic % N^{15} in exc.	Labelled N as % of total N	Total N %	Atomic % N^{15} in exc.	Labelled N as % of total N	Total N %	Atomic % N^{15} in exc.	Labelled as % of total N
4	Needles, 1964							1.09	0.042	4.4
	1963				1.18	0.073	7.7	0.99	0.059	6.2
	1962	1.22	0.072	7.6	1.12	0.060	6.3	1.01	0.040	4.2
	1961	1.17	0.054	5.7	1.08	0.045	4.7	0.91	0.032	3.4
	1960	1.08	0.045	4.7	1.00	0.036	3.8	0.84	0.028	3.0
	Twigs, 1964							0.69	0.046	4.8
	1963				0.69	0.068	7.2	0.55	0.045	4.7
	1962	0.76	0.071	7.5	0.65	0.045	4.7	0.49	0.032	3.4
	1961	0.58	0.060	6.3	0.60	0.037	3.9	0.43	0.036	3.8
	1960	0.45	0.060	6.3	0.47	0.038	4.0	0.46	0.026	2.7
6	Needles, 1963				1.45	0.115	12.1			
	1962	1.49	0.108	11.4	1.26	0.079	8.3			
	1961	1.37	0.098	10.3	1.15	0.077	8.1			
	1960	1.17	0.084	8.8	1.10	0.070	7.4			
	Twigs, 1963				0.80	0.113	11.9			
	1962	0.82	0.089	9.4	0.74	0.076	8.0			
	1961	0.68	0.085	9.0	0.66	0.067	7.0			
	1960	0.55	0.090	9.5	0.57	0.065	6.8			
7	Needles, 1964							1.04	0.073	7.7
	1963				1.16	0.081	8.5	1.00	0.078	8.2
	1962	1.21	0.067	7.0	1.08	0.050	5.3	0.91	0.060	6.3
	1961	1.12	0.062	6.5	1.03	0.054	5.7	0.88	0.048	5.1
	1960	1.01	0.046	4.8	1.02	0.042	4.4	0.94	0.048	5.1
	Twigs, 1964							0.60	0.075	7.9
	1963				0.84	0.076	8.0	0.51	0.076	8.0
	1962	0.95	0.066	6.9	0.69	0.045	4.7	0.55	0.060	6.3
	1961	0.75	0.069	7.2	0.62	0.050	5.3	0.46	0.058	6.1
	1960	0.48	0.073	7.7	0.43	0.048	5.1	0.45	0.056	5.9

* 0.949 atomic-% N^{15} in excess

Distribution of total nitrogen and labelled nitrogen in the bark and in the stem wood from different annual rings

The stem segments, taken at 20—40 cm above ground from both a Scots pine and a Norway spruce plant (after two growing seasons), the bark and wood were separated from each other, and subsequently the wood was separated into its various annual rings. The samples were ground up and analysed for their total nitrogen content and their atomic percentage of N^{15}. The analysis results in Table 6 show that the total nitrogen content was

Table 5. Amounts of total nitrogen and fertilizer-N^{15} recovery in different parts of the experimental trees.

The date of sampling — September 29, 1963 (two growing seasons after the addition of N^{15}-tagged nitrogen materials).

Tree species and treatment, respectively	Part of the trees	Dry weight per tree, g	N %	Amount of N per tree, g	Atomic-% N^{15} in exc.	Amount of labelled N recovered, g
Scots pine (9) (ammonium sulphate, 10 g N* per tree)	Wood	1097	0.123	1.35	0.022	0.031
	Bark	228	0.392	0.89	0.020	0.019
	Branches	980	0.543	5.32	0.022	0.123
	Needles	755	1.489	11.24	0.030	0.355
	Total	3060	—	18.80	—	0.528
Scots pine (10) (ammonium sulphate, 10 g N* per tree)	Wood	822	0.123	1.01	0.035	0.037
	Bark	155	0.416	0.64	0.034	0.023
	Branches	865	0.515	4.45	0.033	0.155
	Needles	665	1.285	8.54	0.038	0.342
	Total	2507	—	14.64	—	0.557
Scots pine (17) (calcium nitrate, 10 g N** per tree)	Wood	944	0.108	1.02	0.107	0.052
	Bark	217	0.426	0.92	0.098	0.043
	Branches	1046	0.470	4.92	0.093	0.218
	Needles	772	1.330	10.27	0.124	0.608
	Total	2979	—	17.13	—	0.921
Scots pine (20) (calcium cyanamide, 10 g N*** per tree)	Wood	1081	0.125	1.35	0.019	0.029
	Bark	240	0.344	0.83	0.013	0.012
	Branches	1253	0.459	5.75	0.015	0.097
	Needles	921	1.110	10.22	0.018	0.208
	Total	3495	—	18.15	—	0.346
Norway spruce (6) (ammonium sulphate, 20 g N* per tree)	Wood	1450	0.142	2.06	0.105	0.228
	Bark	237	0.618	1.46	0.098	0.151
	Branches	2028	0.562	11.40	0.087	1.045
	Needles	2115	1.226	25.93	0.113	3.088
	Total	5830	—	40.85	—	4.512

* 0.949 atomic-% N^{15} in excess
** 2.096 atomic-% N^{15} in excess
*** 0.886 atomic-% N^{15} in excess

highest in the wood from the current year's ring, and that it decreased with the increasing age of the annual ring, though the decrease was not regular. The atomic percentage of N^{15} decreased in the same way, but the trend in this case was still more marked. It was noticeable that the atomic percentage of N^{15} was higher in the wood of the current ring than in the bark. For Norway spruce the atomic percentage of N^{15} was highest in the wood of the ring formed in the year in which the fertilizer was supplied.

Discussion

The most interesting point which emerged from this investigation was that the labelled nitrogen was unevenly distributed in the various organs,

Table 6. Content of total N and distribution of tagged N in bark and in wood of annual rings of different age from two trees of Scots pine and Norway spruce, respectively.

Date of sampling September 29—30, 1963.
Fertilizer N^{15} added June 29 and July 2, 1962, respectively.
The stem segments taken 10 to 20 cm above the ground.

Tree species and treatment, respectively	Issue	Total N %	Atomic % N^{15} in excess	Labelled N as % of total N
Scots pine (17) (calcium nitrate, 10 g N* added per tree)	Bark	0.336	0.101	4.8
	Wood, current ring (1963)	0.143	0.143	6.8
	Wood, 2nd annual ring (1962)	0.092	0.090	4.3
	» 3rd annual ring (1961)	0.075	0.078	3.7
	» 4th annual ring (1960)	0.076	0.065	3.1
	» 5th annual ring (1959)	0.079	0.055	2.6
	» 6th annual ring (1958)	0.086	0.076	3.6
	» 7th annual ring (1957)	0.093	0.068	3.2
	» 8-11th annual rings (1953-56)	0.101	0.057	2.7
Norway spruce (6) (ammonium sulphate, 20 g N** added per tree)	Bark	0.580	0.109	11.5
	Wood, current ring (1963)	0.142	0.130	13.7
	» 2nd annual ring (1962)	0.120	0.147	15.5
	» 3rd annual ring (1961)	0.097	0.076	8.0
	» 4th annual ring (1960)	0.088	0.075	7.9
	» 5th annual ring (1959)	0.084	0.084	8.9
	» 6th annual ring (1958)	0.069	0.074	7.8
	» 7-8th annual rings (1956—57)	0.079	0.082	8.6
	» 9-10th annual rings (1954—55)	0.080	0.061	6.4
	» 11-13th annual rings (1951—53)	0.074	0.045	4.7

* Calcium nitrate, 2.096 atomic % N^{15} in excess
** Ammonium sulphate, 0.949 atomic % N^{15} in excess

and in organs of different ages. As was shown in Tables 1—4, the atomic percentage of N^{15} in the needles had a clear tendency to decrease with their increasing age, both in the year in which the experiment was started and in each of the two following years. This suggests that the stationary state, as regards the nitrogen turnover in the entire plant had not been reached even three growing seasons after the application of the labelled nitrogen. This may indicate either a relatively slow rate of continuous breakdown and resynthesis of cellular proteins or a slow rate of transport and redistribution of mobile nitrogen within the plant. In this connection, it may be mentioned that, according to VICKERY *et al.* (1939) and HEVESY *et al.* (1940), in whose work the tracer technique was used, the cytoplasmic proteins of the leaf are continuously re-hydrolysed and resynthesized. Thus, according to Hevesy and his colleagues, 12 % of the protein in sunflower leaves was renewed within 12 days of the addition of N^{15}-labelled $(NH_4)_2SO_4$ to the culture solution. Using orange cuttings, WALLACE *et al.* 1954) showed that a uniform distribution of N^{15}-labelled nitrogen was not reached in all parts of the plants within 75 days. The labelled nitrogen tended to accumulate in young leaves and fruit. The same authors found that the highest concentration of

N^{15} was in the top leaves, and that amongst these it was higher in newly flushed than in old leaves. As regards coniferous trees, TAMM (1955b) found that the activity of P^{32}, two months after the application of P^{32}-labelled potassium dihydrophosphate, was noticeably higher in current needles of Norway spruce than in those of the second and third years' issue.

The uneven distribution of the atomic percentage of N^{15} in tissues of different origin and age is probably best explained by the findlings of BURR and TAKAHASHI (1955), who demonstrated that the highest N^{15} accumulation occurred in leaves which were at the stage of maximum activity. They concluded that nitrogen flows to places where there is metabolic demand and not to those where there is a nutritional vacuum. This accords with the observations reported by BIDDULPH (1951), according to which the highest relative activity of P^{32} invariably occurred in the regions of highest growth-rate. WILLIAMS (1955) propounded the view that the redistribution of nitrogen in the plant is the result of competition between meristems and other tissues of differing metabolic activity. It seems likely that, in systems to which N^{15}-labelled nitrogen has been added, the time required for the various organic nitrogen compounds to reach their final N^{15} activity depends on the role which they play in the metabolism. The differing N^{15} distributions found in leaves of different ages are probably due to the fact that the leaves differ in their basic metabolism according to age (BIDDULPH, 1951). In young leaves, the predominant process is the synthesis of new protoplasm, which results in growth towards maturity. In older, mature leaves, photosynthesis is the dominant function, and little growth takes place. The possibility cannot be excluded that in older tissues some form of inactivation of proteins have been occurred, for example by reaction with lignin derivatives.

The fact that the current needles showed a higher N^{15} activity than older needles, even after the third growing season, is puzzling; it may perhaps indicate that there was a new uptake of residual labelled nitrogen from the soil or from root tissues.

Another result which merits particular mention is the fact that the atomic percentage of N^{15} in samples of the second and third years' growths, collected after the first growing season, was higher in twigs than in needles of same physiological age. For the current needles and twigs, a reverse relationship was reported (cf. Tables 1 and 4). In samples taken two and three growing seasons after fertilizer application, no similar trend could be established. It appears possible that in the period July to September (1962), when the main uptake of the added fertilizer nitrogen probably occurred, the metabolic activity of the leaves of second and third years' growths was low relative to that of the cambium and adjacent tissues. However, no experimental data are available to confirm the correctness of this suggestion.

An analysis of the stems of two sample trees which were felled about 15 months after the nitrogen fertilizer had been applied showed that the proportion of labelled nitrogen in the total nitrogen fraction in the wood of different annual rings decreased as the age of the rings increased. This seems to indicate a decrease in metabolic activity in the wood tissues as their age increases, a result which is in accordance with the findings of other workers (GOODWIN and GODDARD, 1940). It is often stated that cells from wood tissue

are metabolically inactive. The results obtained here do not necessarily contradict this statement, since metabolic activity in wood most likely can be ascribed to the cells of the wood rays, which are certainly living. The proportion of the wood volume occupied by these living cells may be considered to decrease as the age of the annual rings increases. Thus, in the pith, a large proportion of the total nitrogen is probably found in tissues which are already dead and which are therefore completely excluded from the cycle of continuous breakdown and resynthesis of cellular proteins.

Summary

The uptake and translocation of labelled fertilizer nitrogen was investigated in 11-year-old and 12-year-old trees of Scots pine and Norway spruce growing in different self-sown stands. The fertilizer was applied as a top-dressing to the soil 0—80 cm around the stem. The experiment area with Scots pine was situated at Vifors in the province of Gästrikland, and that with Norway spruce at Dalkarlsnäs, S.W. of Säter, in the province of Dalarna. The pine plants received 10 g of nitrogen in the form of either calcium nitrate. ammonium sulphate or calcium cyanamide during the summer of 1962. The nitrogen materials added had been enriched with the isotope N^{15}. The spruce plants were fertilized with isotope-labelled ammonium sulphate in a dose corresponding to 20 g of nitrogen per tree. In the autumns of the years 1962, 1963 and 1964, samples were taken of needles and of corresponding defoliated twigs from the issues of different years and were investigated in respect of their total content of nitrogen and of N^{15}. Some trees which were felled in the autumn of 1963 were analysed to determine the total recovery of added labelled nitrogen in the above-ground portion of the tree. The distribution of the atomic percentage of N^{15} was investigated in the bark and wood from annual rings of different years by the use of stem segments.

The analysis showed that at the end of the first growing season the added labelled nitrogen could be traced in all the parts of the trees analysed. The distribution of atomic percentage of N^{15} was not completely uniform, indicating that the stationary state of the breakdown-resynthesis turnover of nitrogen had still not been reached. It was found that the current needles usually showed a higher atomic percentage of N^{15} than the needles of previous years. The same tendency was also visible in samples taken after the second and third growing seasons. No completely satisfactory explanation could be found for this, but it was suggested to depend on different turnover rates for cell proteins in tissues of different ages or perhaps also the slow translocation and redistribution of mobile nitrogen in the tree. A possible inactivation of cellular proteins, for example through their reaction with lignin derivatives, has also been suggested.

It was established that the atomic percentage of N^{15} in the current year's increment, in samples taken after the first growing season, was on the average insignificantly higher in needles than in corresponding twigs. This relationship was reversed in samples from the second and third years' increments, the atomic percentage of N^{15} being significantly higher in twigs than in needles. This possibly indicates a high metabolic activity of the

cambium, relative to that of the leave tissue of second and third years' growths (turing the period of July to September).

In the samples taken after the second and third growing seasons, the distribution of N^{15} was still ununiform. The atomic percentage of N^{15} was invariably highest in the current year's needles, and decreased as their age increased. The content of labelled nitrogen was only insignificantly lower in twigs than in needles of the same age.

The quantitative recovery of the labelled nitrogen was determined for the entire above-ground portion of a number of trees. It appeared from the data obtained that in Scots pine the recovery of added fertilizer nitrogen varied between 3 and 8 %. For a single investigated Norway spruce, the recovery was 23 %. The relatively low figures could be attributed partly to leaching, and partly to the root competition of nearby trees, and not least to the activity of the microbial population in the soil.

Data on the N^{15} content of the wood from different annual rings showed that the added fertilizer nitrogen was recovered even in samples taken in the centre of the stem. However, the atomic percentage N^{15} decreased with the increasing age of the annual ring. The metabolic activity in the stem wood was ascribed chiefly to the cells of the wood rays.

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Sammanfattning

Upptagning och translokation av N^{15}-märkt handelsgödselkväve hos tall och gran

I 11—12-åriga plantor av tall resp. gran i självföryngrade bestånd undersöktes upptagningen och translokationen av tillfört N^{15}-märkt gödselkväve. Försöket med tallplantor var förlagt till Vifors, Gävleborgs län, och med gran till Dalkarlsnäs, SV om Säter, Kopparbergs län. Som kvävekällor användes ammoniumsulfat, kalciumnitrat och kalkkväve. Tallplantorna tillfördes 10 g N (den 29 juni 1962), vilket applicerades på markens yta, 0—80 cm från stammen. Granplantorna erhöll 20 g N, som tillfördes i form av ammoniumsulfat den 7 juli 1962. På hösten 1962, 1963 och 1964 uttogs prover på barr och kvistar från olika års tillväxt och undersöktes med avseende på halten av total-N samt atom-% N^{15} i denna kvävefraktion. I ett mindre antal träd, vilka fälldes hösten 1963, bestämdes det totala innehållet av märkt kväve, som kunde återfinnas i de ovanjordiska delarna av trädet. I 2 trädstammar undersöktes det märkta kvävets förekomst i ved från olika årsringar.

Analysresultaten gav vid handen att i slutet av den första vegetationsperioden (hösten 1962) det tillförda, märkta kvävet kunde spåras i alla undersökta delar av plantan. Distributionen av atom-% N^{15} var dock ojämn, tydande på att jämvikten i N^{15}-fördelningen mellan olika växtorgan och växtorgan av olika ålder ännu ej hade uppnåtts. Man fann bl. a. att årets barr i regel uppvisade en högre atom-% N^{15} än barren av äldre årsgångar. Detta förmodades tyda på olika »turnover» hastigheter för cellproteiner från vävnader av olika ålder. Möjligheter till en viss inaktivering av cellproteinerna, t. ex. genom reaktionen med ligninderivat, har likaledes antytts.

I prover tagna på hösten av anläggningsåret kunde likaledes fastställas att medan i årets tillväxt atom-% N^{15} var något högre i barren än i resp. kvistar, var förhållandet det motsatta i motsvarande prover från andra och tredje årets tillväxt. I sistnämnda fall var atom-% N^{15} högre i kvistar än i barr. Hög metabolisk aktivitet hos kambiecellerna under den senare hälften av vegetationsperioden ansågs kunna vara en tänkbar förklaring till detta.

Prover analyserade efter den andra och tredje vegetationsperioden visade att fördelningen av atom-% N^{15} i träden fortfarande var ojämn. Atom-% N^{15} var genomgående högst i årets barr och avtog sedan med stigande ålder hos barren. I kvistar var relativa halten av märkt kväve endast obetydligt lägre än i barr av samma ålder.

I ett mindre antal träd bestämdes den totala mängden märkt gödselkväve, som kunde återfinnas i de ovanjordiska delarna av plantan. Det framgick att återvinningen hos tallplantorna varierade mellan 3 och 8 %. Motsvarande siffra för granen var 23 %. Den relativt låga N^{15} »recovery» ansågs bero på dels utlakningsförlusterna och dels rotkonkurrensen från närstående träd, men kanske icke minst på aktiviteten av markens mikrobflora.

Atom-% N^{15} i ved från olika årsringar visade att det tillförda kvävet åter-

fanns även i vedprover tagna mitt inne i stammens kärna. Värden på atom-% N^{15} avtog dock med stigande ålder på årsringen. Resultatet antydde förekomsten av levande, metaboliskt aktiva celler även i ved, som bildats för 8—10 år sedan. Dessa celler ansågs i huvudsak vara lokaliserade till märgstrålarna.