THE OCCURRENCE OF NITROGEN IN SOIL PROFILES UNDER PINE

by

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ABSTRACT

This paper describes an analysis for nitrogen in the soils of pure coniferous and mixed hardwood and coniferous stands in New England. A discussion of the literature relating to nitrogen and forest growth is given. The methods of making total nitrogen determinations are compared and a conclusion reached as to the best method for this work. Attempts were made to determine nitrates and recommendations are made for procedure in the determination of nitrates in forest soils. Results of the analyses showed larger amounts in the soils under mixed stands.

Because various European investigators have shown that the nitrogen content of soils is more closely correlated with the growth of the stand than any other single chemical factor in the soil, it was thought that a study of the nitrogen in pure and mixed stands might bring some interesting facts to light. Up to the present time there has been very little work of this kind done in this country. This study was undertaken partly as an orientation study of conditions in New England forest soils and partly to determine whether there is any explanation of the better growth of mixed stands in terms of nitrogen content of the soils in these stands.

The growth and development of mixed coniferous and hardwood forests is superior to that of one of pure conifers. The
quality of the softwoods produced in the mixed stand is superior
to that of the pure stand. The conifers in the mixed stand
prune better than those in a pure stand and a larger amount of
clear lumber is secured. An advance growth of hardwoods is
found in the mature mixed type and therefore it is easier and
less expensive to perpetuate it than to work for a pure coniferous growth. The original forests of New England were mixed,
and there is a natural tendency to revert to the climax mixed
type. In the climax type a biological equilibrium is maintained
which apparently results in a better growth due to a better
soil condition.

Nitrogen has been shown by European investigators to be more closely correlated with the growth possibilities of a site than any other single chemical element in the soil. A number

of soil analyses were made in the southern part of Finland by Valmari (1921) and the correlation coefficients between the growth of normally developed Scotch pine stands and the chemical composition of the soil have been worked out from the figures by Ilvessalo (1923) as follows:

Nitrogen..... 0.736 ± 0.056

Lime 0.612 ± 0.069

Potash 0.214 ± 0.091

Phosphoric acid No correlation

It has also been shown by Falckenstein (1912) that in the sandy lands of northern Germany there is a closer correlation between the nitrogen content of the humus and the site class than with any other single chemical factor.

The rate of nitrogen formation depends mainly on the rate of humus decomposition. In the soils of dense spruce and pine stands Nemec and Kvapil (1924-25) have shown that nitrogen fixation is greater in the top stratum containing much humus and organic matter than in the mineral strata and in adjacent mineral soils. Carrying this idea still farther Hesselman (1926) has shown that the rate of nitrogen availability depends on the rate of humus decomposition.

The rate of humus decomposition in turn depends on a number of different factors. The most important of these factors, according to Hesselman, is temperature. He has shown that under conditions which are practically alike in the northern and southern parts of Sweden, except for temperature, the humus decomposition is more rapid in the warmer region. The hydrogention concentration as determined by buffer substances is also of importance in litter decomposition. Since the activity of fungi

and bacteria is influenced by hydrogen-ion concentrations, the amounts of acid and alkaline buffer substances determine the rate of decomposition of the organic matter. Hardwood litter contains a larger amount of basic buffers than softwood litter. Therefore, the admixture of hardwoods helps to determine the nitrogen availability. The lime content of the soil is also of importance, since lime is a basic buffer. In lime-rich regions the admixture of hardwoods in the stand has a negligible effect upon the humus decomposition, since the basic buffers are supplied by the lime in the soil (Hesselman, 1926). That nitrates are present in measurable quantities, however, even in very acid soils, has been shown by Clark (1924). The more acid strata of soils under pine stands are relatively rich in nitrogen and organic matter according to Nemec and Kvapil. In studying the nitrogen forms in different soils, Kudriavtseva (1924) found fixed differences in the forms of nitrogen in soils of different acidities. In the less acid soils they are more stable in character, less easily leached out and tend to accumulate in difficulty hydrolysable forms, while those in the more acid soils on the contrary were more mobile, more easily hydrolyzed and more soluble in acids.

For the most part there is no relation between aeration and litter decomposition (Hesselman, 1926). The main tendency under aerobic conditions is for the stable nitrogenous substances to change gradually into unstable forms with the reverse occurring under anerobic conditions (Kudriavtseva, 1924).

Another factor entering into the rate of nitrogen transformation is the age of the stand. Hesselman (1926) has shown that in young stands there is a lively nitrogen transformation which diminishes as the stand ages. Nemec and Kvapil have also shown that the nitrate content of the humus decreases as the age of the stand increases. The density of the stand also seems to have some effect on the rate of nitrogen transformation. Hesselman has shown that in the case of thinnings there is a correlation between increased growth and the increased rate of nitrogen transformation. Sufficient work has not been done on this point, however, to warrant any definite conclusion. Both of these effects are due to the continued formation of acid buffers which have a cumulative effect in counteracting the alkaline buffers of the soil.

Material

A number of sample plots were laid out in various stands in the north central part of Massachusetts. In the case of the comparison plots in mixed and pure stands, an attempt was made to find stands under as nearly like conditions as possible. Each plot was carefully measured and the stand was mapped.

Notes were taken on the species, age, origin (seed or sprout), height, and D.B.H. Notes were also taken on the aspect, general slope of the land, and the soil profile where the samples were taken. These notes are compiled in Table I.

In the description of the various soil layers, the classification as given by Fisher (1928) is used, except that Fisher's burnt sienna horizon (Fig. I) is subdivided into an upper and lower layer. The upper layer is usually considerably darker than the lower portion and contains a larger amount of organic matter. The dark brown, the upper portion of the burnt sienna, is the dark coffee brown layer of the Bureau of Soils; the light brown, a lower portion of the burnt sienna, is the light coffee brown of the Bureau of Soils. In case the dark

brown layer was missing, the light brown layer is arbitrarily divided into upper and lower levels and designated as upper and lower light brown layers. The subsoil is the same as the yellow horizon in Fisher's description.

The soil samples were collected in paper bags and soon after being brought in from the field were spread out on papers on the floor of a warm attic. They were also covered with papers to prevent dust or other foreign material from collecting while they were being dried. After becoming thoroughly dried out, they were replaced in the paper bags and kept in the attic until they were to be analyzed.

Methods

Before the analyses of the soils were begun, four of the methods of determining total nitrogen were studied. These four methods were (1) Kjeldahl-Gunning-Arnold method; (2) Kjeldahl method modified to include the nitrogen of nitrates using sodium thiosulfate; (3) Kjeldahl method modified to include the nitrogen of nitrates using zinc dust; (4) Gunning method modified to include the nitrogen of nitrates. All four of these methods were used as described in the Official Methods of Analysis of the Association of Official Agricultural Chemists. Of the four methods the second, Kjeldahl method using sodium thiosulfate, proved to be the best for this work. Consistent results were obtained and the method was fairly simple in operation. The Method is given below:

Reagents:

For ordinary work 0.5 N acid is recommended. For work in determining very small quantities of nitrogen, 0.1 N is recommended.

^{*} Taken from Official and Tentative Methods A.O.A.C. with variations.

(a) Standard hydrochloric acid. Determine the absolute strength as follows:

Preliminary test: Place a measured portion of the acid to be standardized in an Erlenmeyer flask and add an excess of calcium carbonate to neutralize free acid and a few drops of a 10 per cent solution of potassium chromate as indicator. Titrate with 0.1 N silver nitrate solution and note the exact quantity required to precipitate the chlorides.

Final determination: To a measured portion of the acid to be standardized, add from a buret 1 drop in excess of the required quantity of silver nitrate solution as determined by the preliminary test. Heat to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a Gooch crucible previously heated to 140° - 150° C. and weighed; wash with hot water, testing the filtrate to verify an excess of silver nitrate. Dry the silver chloride at 140° - 150° C.. cool. and weigh.

- (b) Standard alkali solution. Accurately determine the strength of this solution by titration against the standard acid.

 A O.1 N solution is recommended.
- (c) <u>Sulfuric acid</u>. Contains 93 to 96 per cent sulfuric acid and is free from nitrates and ammonium sulfate.
- (d) <u>Metallic mercury</u>, <u>or mercuric oxide</u>. Mercuric oxide should be prepared in the wet way, but not from mercuric nitrate.
- (e) Granulated zinc nitrogen free. Added to the contents of the distillation flash to prevent bumping.
- (f) <u>Sulfide</u> <u>solution</u>. Dissolve 40 grams of commercial potassium sulfide in 1 liter of water.

- (g) Sodium hydroxide solution. Dissolve approximately 410 grams of commercial sodium hydroxide, free from nitrates, in 1 liter of water. This solution should have a specific gravity of 1.325.
- (h) <u>Cochineal indicator</u>. Digest 3 grams of pulverized cochineal in a mixture of 50 C.C. of 95 per cent alcohol and 200 C.C. of water for 1 or 2 days at ordinary temperature with frequent agitation, and then filter.
- (1) <u>Methyl red indicator</u>. Dissolve 1 gram of methyl red (dimethyl-amino-azo-benzene-ortho-carboxylic acid) in 50 c.c. of 95 per cent alcohol, dilute to 100 c.c. with water, and filter 1f necessary.
 - (j) Sodium thiosulfate. (Na2S2O3. 5H2O)
 - (k) Commercial salicylic acid.

Determination

Place 5 gr. of soil in a Kjeldahl digestion flash. Add 30 c.c. of sulfuric acid containing 1 gr. of salicylic acid, shake until thoroughly mixed, allow to stand for at least 30 minutes with frequent shaking or until complete solution results and then add 5 gr. of crystallized sodium thiosulfate and digest as follows:

Heat over a low flame until all danger from frothing has passed. Then increase the heat until the acid boils briskly, and continue the boiling until white fumes no longer escape from the flask (5-10 minutes). Add approximately .7 gr. of mercuric oxide, or its equivalent in metallic mercury, and continue the boiling until the liquid in the flask is colorless or nearly so. In case the contents of the flask are likely to become solid before this point is reached, add 10 more c.c. of sulfuric acid.

Allow to cool, dilute with about 200 c.c. of water and add a few pieces of zinc to prevent bumping and 25 c.c. of potassium sulfide solution with shaking. Next add sufficient sodium hydroxide solution to make the reaction strongly alkaline (100 c.c.), pouring it down the side of the flask so that it does not mix at once with the acid solution. Connect to the condenser, the tip of which extends below the standard acid in the receiver; mix the contents by shaking and distill until all the ammonia has passed over into a measured quantity of the standard acid. The first 150 c.c. of the distillate generally contain all the ammonia. Titrate with standard alkali solution, using methyl red or cochineal indicator.

A great deal of work has been done and a voluminous literature has been built up on the determination of nitrates. Gill (1894) has said, "No determination requires more care, or occasions more trouble in its execution, or is more unsatisfactory when finished than the one in question". A large number of methods of more or less value have been developed, but three general methods are in use:

- L I. The Zinc-Iron Reduction Method.
 - II. The Tiemann-Schulze Method.
 - III. The Colorimetric Method.

Of these three, the colorimetric method is the most sensitive and the most rapidly carried out. In the determination of nitrates in soils, these are two factors of prime importance.

The principle of the colorimetric method is the nitration of phenoldisulphonic acid, the resulting picric acid being measured colorimetrically. The great difficulty in the use of the method is in obtaining a clear soil extract. The majority

of forest soils contain a large amount of organic matter, and when an extract of such a soil is made, it has a light yellow color which is thought to be a colloidal carbon sol. The literature is replete with descriptions of methods for the clearing of this extract. These methods were all tried in the attempt to find a method which would clear the extract from the dried soil. but it seemed to be impossible. An experiment was performed to determine whether it was easier to obtain a clear extract from a fresh soil than from the same soil after it had been dried. A forest soil containing a large amount of organic matter was obtained and an extract made as soon as the soil was brought in from the field. It was found that this extract could be cleared by the method given below. The soil was then dried in an oven at 98° C. and another extract was made. It was impossible to clear this extract by any of the methods used. Thus it is apparent that the determination of nitrates is best carried out in moist soils.

A method of clearing up the extract was developed which gives consistent results and which seems to be more efficient than any of the other methods tried. The procedure is as follows:

Make a soil extract from 100 grams of soil in 500 c.c. of distilled water and filter - preferably using a Chamberland-Pasteur filter. Take 100 c.c. of the extract and add 20 c.c. of a normal solution of alum (potassium aluminum sulfate). Heat almost to boiling and then add 6 c.c. of 1-1 NH₄OH. Filter; add 2 grams of decolorizing carbon to the filtrate and filter again. A clear solution should be obtained. Care should be taken that the decolorizing carbon is free of nitrates. If nitrates are present in the carbon, they may be removed by repeated washing with hot water.

After the clear solution is obtained, the procedure of the phenoldisulfonic acid method is followed."

Reagents:

- (a) <u>Phenoldisulfonic acid solution</u>. Dissolve 25 grams of pure white phenol in 150 c.c. of concentrated sulfuric acid. Add 75 c.c. of fuming sulfuric acid (13-15 per cent SO₃), and heat at 100° C, for two hours.
- (b) Standard nitrate solution. Dissolve 0.607 gram of pure sodium nitrate in 1 liter of nitrate-free water. Evaporate 50 c.c. of this solution to dryness in a porcelain dish; when cool, treat with 2 c.c. of the phenoldisulfonic acid solution, rubbing with a glass rod to insure intimate contact, and dilute to 500 c.c. One c.c. is equivalent to 0.01 mg. of nitrogen as nitrate. (This solution is permanent). Prepare standards for comparison by adding strong ammonium hydroxide to measured volumes of the standard solution in 100 c.c. Nessler tubes.

Determination:

Take 50 c.c. of the clear soil extract and evaporate to dryness in a porcelain evaporating dish on a steam bath. When cool, treat with 2 c.c. of the phenoldisulfonic acid solution, rubbing with a glass rod to insure intimate contact. Dilute with water and add slowly strong ammonium hydroxide until the maximum color is developed. Transfer to a colorimetric cylinder and compare with the standards in the usual manner. The Duboscq colorimeter proved to be very satisfactory for this work.

^{*} Taken from Official and Tentative Methods of A.O.A.C.

Discussion of Results

A summary of the analyses of the soils studied is given in Table II. The table is divided into two halves, the mixed stands being placed on one side and the pure stands on the other. Comparison plots of mixed and pure stands have been placed on the same line. The column "% N soil" gives the percentage of nitrogen determined in that part of the sample which would pass through a millimeter sieve. The column "% N fine + coarse material" gives the percentage of nitrogen in the sample on the basis of the entire sample, including that which would not pass through a millimeter sieve. The column (F + C)D is equal to the column %N fine + coarse material multiplied by the depth of the layer from which the sample was taken. This gives the amount of nitrogen per unit of area actually available to the stand growing on the soil.

In the case of the comparison plots of mixed larch and pine and pure white pine, the mixed plot shows a decidedly higher value (1.074) than the pure plot (.065). From this it would seem that a mixture of larch is decidedly advantageous in raising the nitrogen content.

In the case of the comparison plots of mixed pine and hardwood stands and pure pine stands, there are five cases where the mixed stands showed a higher percentage of nitrogen than the pure stands and one case in which the values are practically alike, the difference being but .005 per cent in the upper layers of the soil. The amount of nitrogen available in a given growing space depends upon the depth of the layer. When this factor is taken into consideration ((F + C)D), the figures for the mixed stands are consistently higher than they are for the

pure pine stands. These data bear out Hesselman's conclusion, that the admixture of hardwoods helps determine the amount of nitrogen present in the soil. Sufficient work has not been done on the increased yield due to the larger percentage of nitrogen in the mixed stand to show just how much the growth is increased, but very evidently there is some increase. This furnishes a further reason for growing mixed rather than pure pine stands in New England.

In the case of the two comparison plots 11 and 12, it was found that there was considerably more nitrogen in the plot in which Rubus was found growing. From this it would seem that Rubus is an indicator of favorable nitrogen conditions. More samples are necessary, however, to form any definite conclusions.

An interesting fact is shown by plots 24, 25, 26, and 27. The nitrogen content of the upper layers of the plots which have not been burned is much greater than that of the burned plots. From this it would seem that burning is decidedly disadvantageous from the standpoint of the nitrogen content of the soil.

This study is not adequate for quantitative conclusions, but there is sufficient evidence to demonstrate the value to silvicultural practice of a similar but more detailed study of this sort. The differences in the nitrogen contents of the soil under pure pine and the soil under mixed pine and hardwoods is only a single case in the variation of nitrogen content which is undoubtedly controlled to a large extent by silvicultural treatment. There is a need for more "seasonal control" studies before extensive comparative investigations are made. While Clark (1924) did not find a large and significant seasonal variation in nitrates in England, more determinations of this effect

due to local New England conditions of temperature and rainfall are needed. Investigations of the factors which influence the transformation of the organic soil materials is the outstanding problem in forest soil research. The decomposition products of the organic matter have a profound effect on the physical properties of the soil. The availability of nutrients and especially nitrogen is likewise determined to a large extent by the nature of the humus transformation. This study offers more evidence that the condition of the soil under a pure stand of a single species of softwood is improved by the admixture of hardwoods and certain other conifers.

SUMMARY

- 1. Comparative tests of methods for determining total nitrogen including nitrates were made on forest soils. The Kjeldahl method using sodium thiosulfate was found to be simple in operation and gave consistent results.
- 2. In the attempt to determine nitrates by the colorimetric method (phenoldisulfonic acid method), it was found impossible to clear the soil extracts from dried soils. A method for clearing with the Chamberlain-Pasteur filter followed by alum and decolorizing carbon is given for use with extracts from moist soils.
- 3. If the percentage of nitrogen is reduced to an area basis, the values for the mixed stands are consistently higher than for the pure white pine stands.
- 4. The one sample obtained showed that the admixture of larch with white pine is decidedly advantageous in raising the content of total nitrogen.
- 5. The comparison of burned and unburned plots shows that burning diminishes the total nitrogen content of the soil.

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	t	A I	X	E D	S T A N D S	Age of	Cżnys	t õuna			P. U. R. E. S.	T A N I	2 5			r
	Plot No.		As pec	t Slope	Species	Stand Years	per	Hgt.	AV. DBH. In.	Plot	Location	As- pect Slope	Species	Age of Stand Years	Stems per Acre	Av. Het. Ft.
	1	NE corner, Comp. V. Tom Swam block, Harvard Forest.	P W	Gen tl e	White pine, ash, hard maple, oak	8-11	62,920) 5 .5	Q. 4	4	Within 25 ft. of Plot 1	W Gentle	White pine	11	53,240	-
	2	Within 25 ft. of Plot 1	¥	Gentle	White pine, red oak	8-11	67,760	5.5	0.3	3	Within 25 ft. of Plot 2	W Gentle	White pine	11	33 , 880	0 6.0
consist and the second	5	l mile SE of Warwick; bottom of steep slope.	īvl	Level	White pine, larch*	35-38	629	45.0	6.6	6	Within 50 ft. of Plot 5	Lvl Level	White pine	<i>3</i> 5-38	•	48.0
	7	1.5 miles E of Warwick; on side hill	SW	Mod. steep	Pine, red oak	35 - 50	155			8	Within 25 yds. of Plot	7 SW Mod. Steep	White pine	50	140	
	9	4.5 miles NE of Warwick; (Bullock Lot)	N	Mod.	Pine, Birch, ash	12-14	40,443	8.5	0.6	10	Within 25 yds. of Plot 9) NW Gentle	~	12-14		
	11	Within 25 yds of Plot 9	NW	Gentle	Clear cut; no Rubus						,		da.			
	15	Within 25 yds of Plot 11	NT	Gentle	Do ; Rubus pres.							•				
	13	Within 2 mile of Plot 9	Lvl	Level	Clear; Blueberry and grass											
	14	5 miles from Athol (Fratt Lot)	9	Gentle	Pine, oak, ash, maple	10				16	Within 30 yds. Plot 14	S Gentle	White pine	20		
	15	Within 25 yds of Plot 14	g	Gentle	Pine, oak, ash, maple	10				18	Within 25 ft. Plot 17	W Gentle	White pine	35	680	32.0
	17	Comp. IV, Tom Swamp Block, Harvard Forest.	質	Gentle	Pine, beech, birch oak	, 35	450	35.0		20	Within 30 yds. Plot 17	W Gentle	White pine	45		40.0
	19	within 100 yds of Plot 17	W	Gentle	Pine, hemlock, birch, chestnut	5 - 60						:	***************************************	•	<i>y</i> (
	21	Adams Fay Lot, Harvard Forest	Lvl	Level	Pine, hemlock, birch, oak, maple	60				22	Within 25 yds. Plot 21	Lvl Level	White pine and hemlock	60		
	23	Within 50 yds of Plot 22	Lvl	Level	Clear cut; herbace	ous				24	250 yds. N. of Plot 23	Lvl Level	đo	60		
										25	Within 50 ft. Plot 24	Lvl Level	do	60		
	•									26	Within 25 yds. Plot 24	Lvl Level	đo	60		
										27	Within 50 yds. Plot 24	Lvl Level	White pine	60		

TABLE II. SUMMARY OF SOIL ANALYSES

	:					•		; -4					pure Sta	n d s		
Plot		tht of Coarse Ma tl	e		% N Fine + Coarse		(F+C)	% N S o11		(F+C)	Depth of Layer	Fine + Coarse	Wanter and the second s	Soil Type	Weigh C Soil	oarse
1	47.2 281	2	Dark brown	Comp.V, Tom Swamp. Young stand. Plots 1,2,3,4 close together.	Matl .337 .195	1/8	.042	.350 .223	;317 ;187	.037	1/8	Matl .293 .160	THE PROPERTY AND ADDRESS OF THE PARTY OF THE	Dark brown Light brown	187 504	15 83
2	134 312	9	Dark brown		.288 .203	1/8	.036	.308	. 224	.020	1/10	.198		Dark brown Light brown	273	36
5	219 192	16	Dark brown	Bottom of steep slope. Soil moist. Pine-larch mixture.	.358 .231	3	1.074	.384	.278 .190	.065	1/4	.258 .178		Upper lt.br Light brown		24 22
7	291 476	42 159	Dark brown	On fairly steep side hill.	.199 .078	5/8	.125	. 228		.039	1/4	.155 .070		Dark brown Light brown	385 470	55 171
9	243 293	18 39	•	Location - Bullock Lot.	.212 .176	2	.424	. 228	.306 .229		1/2	.287 .206	Plot near 9. Not pure pine. Hdwd litter pres.	Dark brown Light brown	301 405	20 45
11 12	630	58	Unner laver	· Under slash pile.No Rubus pres. · Under slash pile.Rubus present.	.163 .240		×	.178								
13	714	76	Upper layer	Reforestation area. Top of hill	184			.204	!		•					
14 14	700 192	•	Dark brown	Location - Pratt Lot. Stand 7	.106 .057				.179 .067			.171 .055	Pratt Lot. Pure plot old er than mixed. Age 20 yr	- Dark brown s Lght brown	n 395 n 590	
15 15	238 71		:	Pratt Lot. Stand 7 to 10 years	.148 .101			.184 .129	.211 .126	.018	1/10	.176 .092	Location: near Plot 17.	Upper lt.b	r. 397 a 476	73 181
17 17	295 483	61	Upper lt.br	. Tom Swamp. Plots 17 and 18 at top of small local rise.	.171 .111	2	.342	.206 .143		ror.	1/10	.202	Near bottom of long slope near Plots 17,18.	Upper lt.b	r. 264 r. 541	42 112
19	414	44		r. Pine-beech mixture.	.145	3	.435	.161	•	.039	3/8	.103 .101	Location: near Plot 21	Upper lt.b	r. 326 368	4 8 8 2
	≥V0 ∺TO			r. Adams-Fay Lot. Near large kett	_	3/4	.102			2 .186	1	.186	Under burned slash pile.	Dark brown	876	29
51 51	311		Lower 1t.br	r. hole. Pine, hem., better howde	3040		.062	.106	.234	.226		,226	Not burned. Comparable to Plot 24.	Subsoil	745	s 2 8
23 23	603 259		Upper It.Dr Subsoil	r. Fay Lot. Widest N-S strip cut. Coming up to herbaceous growth	n			:103	i	252	3/8	.673	The state of the s	Dark brown	223	3 23
									.182	2.948	4	.158		, Dark brown	614	92