

ΕΔΑΦΟΛΟΓΙΑ.— **Evaluation of methods for assessment available soil nitrogen**, by A. D. Simonis, S. B. Bladenopoulou, P. G. Koukoulakis*,
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ABSTRACT

Twenty one soil samples representative of the main soil groups of Northern Greece were cropped with ryegrass plants, and various biological and chemical methods and technics were used for the determination of the available N of soils. The best index of the soil available N, which was accounted for more than 54% of total uptake by ryegrass plants, was obtained with the potentiometric method-leaching of soil with a solution of 0,5 N KCl and measurement of N with the specific nitrate electrode.

INTRODUCTION

Under the present energy crisis, and the problems of environmental protection, the need for the improvement of the low N fertilizer efficiency is given top priority, in the research programs and the belief for its importance is being established more firmly with the time (Simonis, 1987). The immense quantities of energy which are needed for the production of Nitrogenous fertilizers and the high losses of N in the soils due to leaching, denitrification and volatilization, constitute an important economic problem as well as a menace to the environment.

A successful approach to the problem of rational Nitrogenous fertilizer use may be achieved by taking into account the mineral soil N ($\text{NO}_3\text{-N}$) in addition to the N needs for the maximum crop yields. This quantity depends on the organic N which is nitrified during the period of crop growth and the residual quantity of inorganic N (min-N) ($\text{NH}_4^+ + \text{NO}_3^-$) which is initially present in the soil (Standford, 1977).

The need for a method which will give a sufficient index of the available N of soil and will allow the accurate prediction of the N quantity needed

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for optimum yield, has for years been the subject of objective discussion amongst research workers.

Numerous Laboratory methods and techniques for the assessment of the available soil N, differing in their general principles and technical details, have at various times, been proposed. These methods include: aerobic-anaerobic biological incubation procedures, chemical techniques, water, inorganic acids, bases, oxidizing agents as well as tracer techniques and electro-ultra-filtration. Most of these methods have been critically analyzed and reviewed by various research workers (Hansen and van Schreven, 1955, Bremner, 1965, Stanford and Legg, 1968, Dahnke and Vasey, 1973, Stanford, 1982, Keeney, 1982, Meisinger, 1984).

None of the proposed methods for available N assessment has been universally accepted. All of them are characterized by limitations and vices which are basically due to the fact that almost all soil N is basically found in organic form, which must be mineralized (NH_4^+ , NO_3^-) before being taken up by plants. Furthermore the processes of NO_3 leaching, denitrification, biological fixation and NH_3 volatilization, complicate furthermore the problem of N availability and make more difficult its solution (Dahnke and Vasey, 1973). Our knowledge about the relationships between the chemical composition of the organic matter and N availability in various soils, is very limited, and the available information concerning the complex biochemical processes which determine the various transformation of soil N is very scarce. It is impossible to simulate in the laboratory the biochemical effect of microorganisms, and the only method that can give a close simulation is the one of incubation.

During the last decade very encouraging results have been obtained in the USA and W. Europe with respect to the determination of min-N in the profile (Smith, 1980, Henkens 1980, Ris et al, 1981, Becker and Aufhammer, 1982, Keeney, 1982, Stanford, 1982, Koukoulakis, 1984). Soil sampling is done up to a depth of one m. during the first stages of plant growth and the soil samples are either analyzed immediately or placed in freezer for analysis in due time.

The use of models seems to be rather promising in the study of soil N transformation where the inclusion of submodels may be very helpful in describing the various phases or processes involved in N cycle (Frissel, and van Veen, 1982· Greenwood, 1982· Tinker and Addiscott, 1984· Rachhpal-Singh and Nye, 1986).

The quantity of soil N taken up by plants in being ususally used as the standard criterium for the avaluation of laboratory methods, while the experiments with ryegrass in pots give in relatively short time biological data with respect to soils capacity to provide plants with N (Simonis, 1985).

The additive N uptake by a consecutive crops in the greenhouse, may be evaluated as a function of the initial N content of soil, and the relative potential availability of organic N. In these sense, the present work aims at, determining, by means of a large number of laboratory techniques, the available soil N, and evaluating these methods by correlating their values with the N quantities taken up by plants.

MATERIALS AND METHODS

1. Soils used and their characteristics.

Twenty one soil samples (25 cm depth) were taken selectively from various regions of N. Greece. The soils had been chozen on the basis of preliminary soil tests, so that they were representing the main Soil Groups of N. Greece. In Table 1 the parent material, Soil Group, as well as some basic characteristics determined by the universaly accepted methods (Jackson, 1958) are given.

2. Laboratory methods used for soil N.

The methods used and their characteristics are given in Table 2.

3. The technique of pot experiments.

To obtain standard biological criteria for the evaluation of analytical methods used for soil N determination, the soils were cropped with ryegrass according to the following experimental technique, About 1000 g soil (particle size $<2\text{mm}$) and 400 g coarse sand ($>2\text{mm}$), which had previously been washed five times with concentrated solution of HCl, were mixed by means of a mechanical shaker along with the nutrient solution which contained 100 mg P and 100 mg K in the form of K_2HPO_4 and K_2SO_4 , respectively, and adequate, quantity of water. 1.5 g of ryegrass seeds was broadcasted uniformly over the soil of each pot. Plants were cut consecutively each 30 days. Four cuts were made in total. The grass was dried at 75°C , washed for the determination of dry matter, and total N was determined by Kjeldahl method.

RESULTS

The range and the average values of soil N, determined by various methods, are given in Table 3.

The average quantities of N taken up by ryegrass plants during the experimental period, were calculated on the basis of the N quantity taken up by each consecutive crop or cut, depicted in Fig. 1.

The exponential Cobb-Douglas equations curves for the N uptake ($y = ax^b$) are given in Table 4.

To evaluate each soil N method used, the available N values were correlated with the total N uptake of crops, which was considered as the standard biological criterium. In Tabl. 5 the correlation coefficients which were obtained, as well as the corresponding coefficients based on the total cumulative yield of ryegrass, are given.

In Tabl. 6 the correlation coefficients realting the available soil N values and the values of the methods used, are given, while the coefficients correlation between total plant N taken up and available soil N, for soil groups with different pH, texture, total CEC and percent base saturation, are given in Table 7.

DISCUSSION - CONCLUSION

Various quantities of N, differing significantly, were extracted from the soils studied by the extracting agents/methods, used (Table 3). Relatively, small quantities of N were extracted with the weak extractants i.e. 0.01 Ba(OH)₂, 0.5M NaHCO₃, 1N KCl-MgO, Ca(OH)₂ + 0.2% GTA and anaerobic incubation, while large quantities were extracted by means of the acid hydrolysis with HCl. In practical advisory work regarding fertilization, the quantity extracted is not what counts most, but whether this quantity is a reliable index of soils capacity to provide plants with N. The soils studied, differed significantly with respect to their capacity to provide plants with N. The total N quantity taken up by ryegrass varied between 4,1 and 38.4 mg/100g soil (Fig. 1). Largest quantities of N were extracted from soils 1 and 17 as shown by the values of the a coefficients of the exponential regression equation ($y = ax^b$), obtained for the cumulative N uptake curves (Table 4).

The correlation of the available soil N (N-values) with the total yield of ryegrass, were generally small than the corresponding N-uptake values (Table 5). Many factors influence the yield and the crop response to the added N, but at the same time they affect slightly the N uptake (Simonis, 1985). That is in the present work the methods are being evaluated on the basis on plant N uptake.

The correlations between N taken up by plants and the available N which was determined by the various methods used, with the exception of acid hydrolysis (0.1 and 5.0 N HCl), were statistically significant. The highest correlations with the total uptake of four cuts, were obtained with the following methods, Electrometric ($r=0.742^{***}$), the anaerobic method of 14 and 21 days ($r=0.712^{***}$ and $r=0.720^{***}$), respectively, and the method of total N ($r=0.710^{***}$) (Table 5). Only the methods which accounted for over 50% of the variability in N uptake ($r=0.50$), are considered that they give relatively satisfactory evaluation of the available soil N (Cornforth and Walmsley, 1971). The methods of aerobic incubation and of the 1N KCl-MgO extractant, gave about the same results ($r^2=0.48$).

The method of total N was very closely correlated with the values of the aerobic incubation ($r=0.894^{***}$), with anaerobic incubation of 7, 14 and 21 days ($r=0.902^{***}$, $r=0.907^{***}$ and $r=0.888^{***}$, respectively). Also close correlation were attained with the methods of anaerobic incubation of 21 days, with 0.5 M Na_2CO_3 extraction of N ($r=0.892^{***}$), as well as with acid hydrolysis 0.1 and 5.0 N HCl ($r=0.901^{***}$ and $r=0.890^{***}$, respectively). Also close correlations were obtained between the methods of anaerobic incubation for 21 days and the 0.5 M NaHCO_3 -N ($r=0.892^{***}$), as well as the method of basic hydrolysis with $\text{KMnO}_4(\text{c})$ and the electrometric method ($r=0.890^{***}$) (Table 6).

The correlations between the total N uptake by ryegrass plants, and the available N values, were generally better in the acid soils ($\text{pH}<5.5$) rather than in the basic ones ($\text{pH}>7.0$), as well as in soils with total CEC >30 me/100g rather than in those with $\text{CEC}<15$ me/100g (Table 7). The method used were least affected by mechanical analysis and the percent saturation of soils (Table 7).

Among the methods tested, in electrometric was proved to be the best one. In order to be used in practical work, it must be calibrated, so that its

result be satisfactory for each crop and under definite soil climatic factors. Such calibration is currently conducted by the Soil Science Institute, Thessaloniki, Greece.

TABLE 1

Parent material, Soil Group and general characteristics of the soils used

No soil samples	Parent material	Soil Group	Clay %	Silt %	C/N %	pH	CaCO ₃ %	CEC me/100g soil
1	sch. micaceous	Lithic Dystrochrept	17,2	40,8	11,60	4,18	-	35,10
2	sch. micaceous	Typic Dystrochrept	9,2	29,6	11,71	5,45	-	25,17
3	granite	Ultic Haploxeralf	17,2	24,8	11,33	5,45	-	15,33
4	dep. cal. schis.	Ultic Haploxeralf	8,8	17,6	11,53	4,45	-	12,08
5	dep. cal. schis.	Mollic Xerofluvent	24,8	35,6	11,46	4,95	-	35,20
6	alluvium	Typic Xerofluvent	17,2	45,2	11,75	6,60	1,2	36,37
7	alluvium	Typic Haploxeralf	7,2	43,6	11,41	7,12	1,2	35,91
8	alluvium	Typic Haploxeralf	35,3	26,9	11,48	6,34	0,6	35,33
9	dep. cal. mar.	Typic Haploxeralf	32,8	28,8	11,42	7,15	0,6	31,33
10	dep. cal. mat.	Vertic Haploxeralf	34,3	27,5	11,26	6,02	0,6	35,23
11	dep. cal. sch.	Vertic Palexeralf	10,8	27,6	11,48	5,05	-	13,05
12	dep. cal. sch.	Typic Haploxeralf	17,2	17,2	11,23	4,85	-	36,10
13	dep. cal. sch.	Ultic Haploxeralf	29,2	17,2	9,67	5,95	-	19,83
14	dep. cal. mat.	Typic Haploxeroll	33,2	27,2	11,52	6,95	1,8	42,50
15	dep. cal. mat.	Typic Haploxeroll	36,8	27,6	10,88	7,03	3,0	34,98
16	dep. cal. mat.	Vertic Haploxeroll	32,8	33,2	11,47	7,05	13,7	34,58
17	dep. cal. mat.	Lithic Haploxeropl	20,8	51,6	11,00	6,95	1,2	19,33
18	Gneiss	Lithic Dystrocrept	6,8	23,6	10,28	4,18	-	12,70
19	Gneiss	Lithic Dystrocrept	5,2	15,2	11,64	4,35	-	12,21
20	limestone	Typic Eutrochrept	48,8	43,2	12,00	6,65	1,4	50,00
21	dep. cal. mat.	Calcic Chromoxerert	31,2	18,8	10,25	6,05	1,4	19,91

TABLE 2
Extraction methods for «available» soil N.

	Methods	Temperature Co		Time	Forms of N determined		Bibliography
1	Total N Kjeldahl	375°		1 day	Oliko N		Bremner (1960)
2	Aerobic incubation	35°		21 days	NH ₄ -N, NO ₃ -N		Gasser (1964)
3	Anaerobic »	35°		7 »	NH ₄ -N		Waring and Bremner (1964)
4	» »	35°		14 »	NH ₄ -N		» »
5	» »	35°		21 »	NH ₄ -N		» »
6	Basic oxidation with KMnO ₄ α	100°		15'	NH ₄ -N		Truog (1951)
7	» » KMnO ₄ β	100°		10'	NH ₄ -N		Zaccariah (1964)
8	» » KMnO ₄ γ	100°		10'	NH ₄ -N		Stanford (1978)
9	Acid hydrolysis 0,1 N HCl	25°		24 h	NH ₄ -N		Cornfield (1971)
10	» » 1,0 N HCl	25°		24 h	NH ₄ -N		»
11	» » 5,0 N HCl	25°		24 h	NH ₄ -N		»
12	0,1 N Ba(OH) ₂	25°		30'	γλυκόζη		Jenkinson (1968)
13	0,1 N Ba(OH) ₂ distillation	400°		25'	NH ₄ -N		»
14	0,2 M NaHCO ₃ distillation	100°		30'	NH ₄ -N, NO ₃ -N		Richard et al (1960)
15	1 N KCl	100°		15'	NH ₄ -N		Cottenie (1980)
16	1 N KCl Devarda	100°		15'	NH ₄ -N, NO ₃ -N		»
17	Ca(OH) ₂ 0,2% GTA			15'	NO ₃ -N		Sims and Jackson (1971)
18	0,25 N H ₂ SO ₄ ashing	100°		30'	NH ₄ -N, NO ₂ -N		Stanford and Smith (1978)
19	Electrometric - 0,5 N KCl	25°		30'	NH ₄ -N, NO ₃ -N		Stanford and Smith (1978)
20	Electro-Ultra-filt. (EUF)	20°, 80°		30', 35'	EUF-Norg		Nemeth et al (1979)

TABLE 3

Ranges and mean values of available N soils studied

a/a	Methods	Ranges of values mg /100g soil	Average values mg /100g soil
1	total N	60 - 468	169,2
2	Aerobic incubation	2,10 - 8,45	4,3
3	» » 7 days	0,60 - 2,20	1,0
4	» » 14 days	0,80 - 2,70	1,2
5	» » 2 days	0,80 - 3,00	1,4
6	Basic oxidation α	5,1 - 14,2	9,9
7	» » β	4,5 - 14,0	8,5
8	» » γ	5,6 - 14,9	8,6
9	Acid hydrolysis 0.1 N HCl	10,0 - 37,0	16,1
10	» » 1,0 N HCl	11,0 - 42,0	21,5
11	» » 5,0 N HCl	20,0 - 65,0	27,6
12	0,1 N Ba(OH) ₂	1,8 - 4,6	2,4
13	0,1 N Ba(OH) ₂ -distillation	0,8 - 3,6	1,3
14	0,5 M NaHCO ₃	1,9 - 7,5	3,1
15	1 N KCl-MgO	1,45 - 3,88	2,2
16	1 N KCl-Devarda	2,27 - 8,92	4,7
17	Ca(OH) ₂ 0,2% GTA	1,92 - 4,22	2,4
18	0,25 N H ₂ SO ₄	5,0 - 14,8	4,7
19	Electrometric	6,2 - 11,9	8,8
20	EUF	2,15 - 8,4	4,4

TABLE 4

Cobb-Douglas exponential equations ($x=ax^b$) for the curves obtained

	axb	
1	5,559 × 0,714	0,977
2	3,522 × 0,723	0,981
3	2,324 × 0,836	0,997
4	2,040 × 0,925	0,999
5	2,550 × 0 805	0,993
6	2,601 × 0,829	0,997
7	3,294 × 0,733	0,985
8	2,381 × 1,006	0,980
9	2,368 × 0,838	0,998
10	2,046 × 0,878	0,998
11	2,458 × 0,831	0,995
12	2,718 × 0,855	0,998
13	2,854 × 0,836	0,995
14	2,961 × 0,779	0,989
15	1,297 × 0,894	0,998
16	3,814 × 0,842	0,994
17	5,379 × 0,693	0,972
18	2,991 × 0,709	0,991
19	3,212 × 0,803	0,991
20	3,150 × 0,864	0,997

TABLE 5

Correlation coefficients of N-values with the total yield and N uptake
by ryegrass

a/a	Availability index	N	Correlation coefficient (r)	
			Total yield sum of 4 cuts	Total N uptake ryegrass
1	Total N		0,625**	0,710***
2	Aerobic incubation		0,555**	0,692***
3	Anaerobic incubat	7 days	0,450*	0,612**
4	»	» 14 »	0,632**	0,712***
5	»	» 21 »	0,690***	0,720***
6	Basic oxidation	α	0,421ns	0,640**
7	»	» β	0,371ns	0,655**
8	»	» γ	0,455*	0,555**
9	Acid hydrolysis	0,1 HCl	0,202ns	0,225ns
10	»	» 1,0 HCl	0,318ns	0,540*
11	»	» 5,0 HCl	0,376ns	0,425ns
12	0,1 Ba(OH) ₂		0,321ns	0,541*
13	0,1 Ba(OH) ₂ -distillation		0,376ns	0,545*
14	0,5 M NaHCO ₃		0,352ns	0,525*
15	1 N KCl-MgO		0,542*	0,692***
16	1 N KCl-Derards		0,612**	0,620**
17	Ca(OH) ₂ 0,2% GTA		0,610**	0,635**
18	0,25NH ₂ SO ₄		0,376ns	0,525*
19	Electrometric		0,620**	0,742***

*P<0,05

**P<0,01

***P<0,001

[illegible]

TABLE 6 (Continued)

[illegible]

TABLE 7

Coefficients of correlation between soil N-values and cumulative N uptake by ryegrass. The soils grouped on the basis of pH, texture, CEC and percent base saturation

	Total uptake				
	Texture		pH		
	C, SCL n=10,	L, SL, LS n=11	< 5,5 n=9	5, 5-7, 0 n=8	7, 0< n=4
Total N	0,921***	0,923***	0,915***	0,843**	0,954*
Aerobic incubation	0,902***	0,913***	0,943***	0,911**	0,981*
Anaerobic Incubat. 7 days	0,931***	0,915***	0,977***	0,955***	0,990**
» » 14 days	0,917***	0,932***	0,895***	0,870**	0,991**
» » 21 days	0,913***	0,913***	0,793**	0,813**	0,993**
Basic oxidation a	0,922***	0,918***	0,877**	0,833**	0,997**
» » b	0,983***	0,922***	0,855**	0,910**	0,997**
» » g	0,911***	0,984***	0,973***	0,945***	0,992**
Acid hydrolysis 0,1 N HCl	0,944***	0,925***	0,934***	0,901**	0,997**
» » 1,0 N HCl	0,953***	0,944***	0,945***	0,911**	0,992**
» » 5,0 N HCl	0,812**	0,932***	0,933***	0,907**	0,993**
0,1 Ba(OH) ₂	0,905***	0,896***	0,962***	0,923***	0,993**
0,1 Ba(OH) ₂ distillation	0,933***	0,925***	0,963***	0,956***	0,996**
0,5 M NaHCO ₃	0,914***	0,911***	0,955***	0,958***	0,995**
1 N KCl-MgO	0,967***	0,925***	0,991***	0,934***	0,981*
1 N KCl Devarda	0,971***	0,932***	0,963***	0,947***	0,990**
Ca(OH) ₂ 0,2% GTA	0,897***	0,945***	0,972***	0,955***	0,990**
0,25 N H ₂ SO ₄	0,951***	0,967***	0,945***	0,911**	0,996**
Electrometric	0,936***	0,923***	0,955***	0,910**	0,993**

TABLE 7 (Continued)

	N				
	CEC (me/100g Soil)			Percent Basis saturation %	
	<15 n=4	15-30 n=5	30< n=12	<60 n=10	60< n=11
Total N	0,990**	0,952**	0,963***	0,992***	0,934***
Aerobic incubation	0,992**	0,954**	0,956***	0,972***	0,962***
Anaerob. incub. 7 days	0,994**	0,954**	0,943***	0,972***	0,980***
» » 14 days	0,992**	0,995***	0,990***	0,941***	0,931***
» » 21 days	0,982*	0,990**	0,987***	0,882***	0,942***
Basic oxidation a	0,983*	0,990**	0,956***	0,872***	0,876***
» » b	0,973*	0,996***	0,962***	0,832**	0,890***
» » g	0,997**	0,956***	0,943***	0,990***	0,793**
Acid Hydrolysis 0,1 N HCl	0,993**	0,997***	0,933***	0,843**	0,945***
» » 1,0 N HCl	0,956*	0,990**	0,942***	0,945***	0,903***
» » 5,0 N HCl	0,967*	0,996***	0,974***	0,943***	0,984***
0,1 N Ba(OH) ₂	0,956*	0,995***	0,975***	0,955***	0,945***
0,1 N Ba(OH) ₂ distillation	0,983*	0,980**	0,964***	0,945***	0,936***
0,5 M NaHCO ₃	0,998**	0,990**	0,998***	0,946***	0,902***
1 N KCl-MgO	0,997**	0,995**	0,980***	0,911***	0,942***
1 N KCl Devarda	0,998**	0,981**	0,952***	0,900***	0,932***
Ca(OH) ₂ 0,2% GTA	0,995**	0,978**	0,934***	0,943***	0,923***
0,25 N H ₂ SO ₄	0,992**	0,990**	0,932***	0,965***	0,923***
Electrometric	0,992**	0,990**	0,975***	0,968***	0,980***

*P < 0,05 **P < 0,01, ***P < 0,001

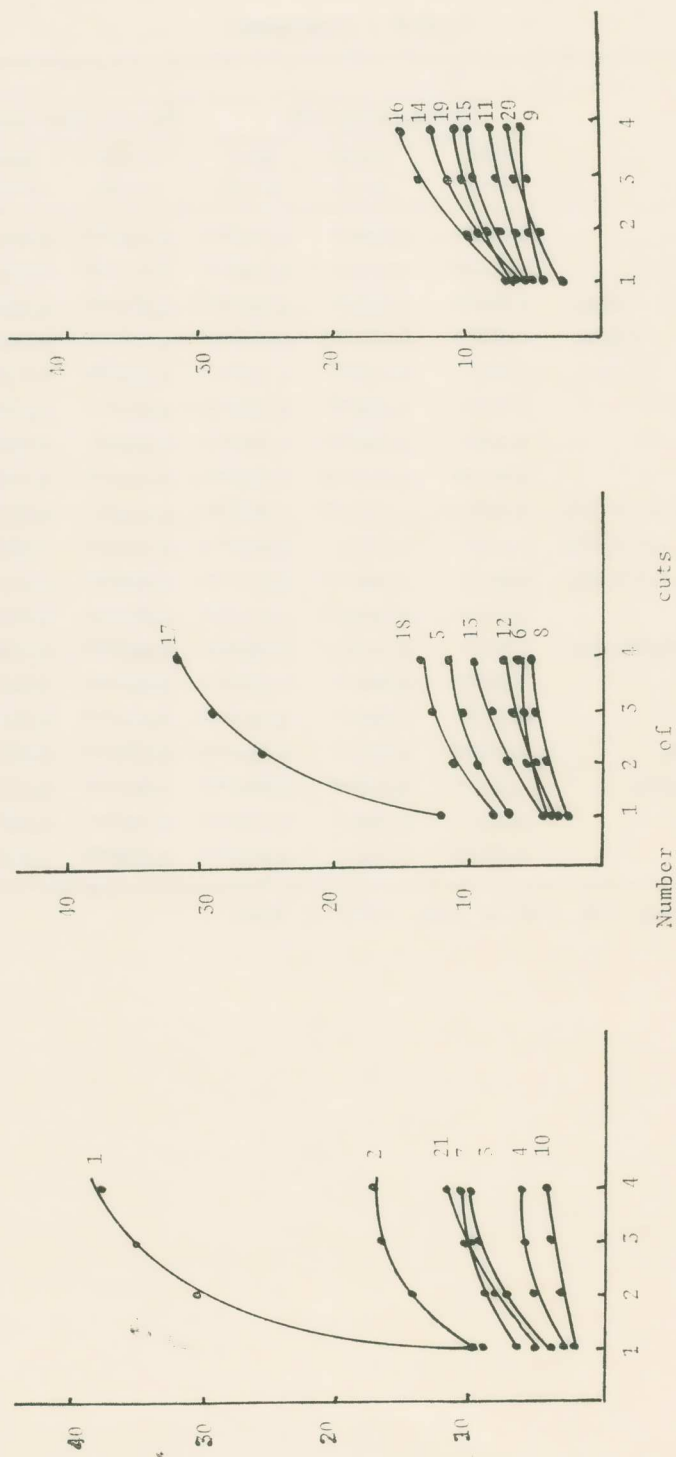


Fig. 1. Cumulative N uptake by ryegrass by successive cropping from the analysed soils (No 1-21).

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Π Ε Ρ Ι Λ Η Ψ Η

Έκτίμηση τών μεθόδων προσδιορισμού του διαθεσίμου άζώτου του έδάφους

Είκοσι ένα δείγματα έδάφους, αντιπροσωπευτικά τών κυρίων έδαφικων ομάδων τής Βορ. Ελλάδας καλλιεργήθηκαν με φυτά ryegrass και διάφορες βιολογικές και χημικές μέθοδοι - τεχνικές χρησιμοποιήθηκαν για τόν προσδιορισμό του διαθεσίμου N του έδάφους. Ο καλύτερος δείκτης διαθεσίμου άζώτου του έδάφους που προσδιόρισε περισσότερο από τò 54% τής όλικης πρόσληψης από τά φυτά ryegrass ήταν αυτός που πάρθηκε με την ηλεκτρομετρική μέθοδο - έκπλυσης του έδάφους με διάλυμα 0,5 N KCl και μέτρηση του N με ειδικò ήλεκτροδίο νιτρικων.