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Pretreatment of soil samples before NO_3-N analysis

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Summary

Soil samples (sand, loam, clay) were stored for either 2 or 3 days or for 2 months at varying temperatures (−20 °C, +4 °C, room temperature, 30-40 °C and 70 °C). As references field moist samples were used. Oven drying caused significant decreases in extractable NO_3-N for the three soils and for the short as well as for the long storage period. The other treatments (−20 °C and +4 °C) and drying at room temperature gave results that equalled those obtained on reference samples except for the samples at room temperature and at the 2-month storage period.

In that case, a substantial increase in NO_3-N was measured, especially in the sandy soil with a relatively high organic matter content.

Introduction

Before analysis is carried out, soil samples are often stored for varying length of time under varying conditions. Quite often they are air-dried or oven-dried to facilitate handling as grinding and weighing (Molloy & Lockman, 1979). In the Netherlands, air drying is used at the Institute of Soil Fertility, Haren (Gr) and the Laboratory of Soils and Plant Analysis at Oosterbeek for soil samples analysed for e.g. P and K fertilizer recommendations; in the case of mineral nitrogen the samples are handled field-moist as is also done with glasshouse soil samples for all determinations (Sonneveld & van den Ende, 1971).

Other individuals or institutions prefer to cool or freeze the field-moist samples (Scharpf & Wehrmann, 1976; Gehlen, 1978). This treatment is recommended (Gorin et al., 1978) if micro-organisms and their activities have to be studied. All the practices mentioned may cause marked changes in physical and chemical properties of soils and affect biological transformations as well (Griffiths & Birch, 1961; Hesse, 1971). A major change that may occur is the transformation of organic N into available (NH_4 + NO_3) forms.

According to Munro & Mackay (1964) and Jaszcolt (1974), air drying of moist samples increases nitrate production. Westfall et al. (1978) observed a substantial

increase in NO_3 contents even in refrigerated samples within seven days. This procedure and others used by these authors led to errors in N dressings ranging from 52 to 147 kg ha^{-1} and brought them to recommend air drying within 12 hours after collection of the samples. This technique is also used for making nitrogen, phosphorus and potassium fertilizer recommendations in several Great Plains States of the USA (Ward, 1971; Keogh & Maples, 1973). The effect of incubation after drying or heating or wetting-drying cycles on soil N transformation has been studied intensively (Birch; 1959, 1960, 1964; van Schreven; 1967, 1968) and in most cases a considerable increase in plant available N was found.

The above-mentioned increases in mineral N, especially NO_3-N, are brought about by ammonification or nitrification of nitrogen or both. The drying temperatures mostly used lie below 50 to 60 °C so that even the relatively heat-susceptible *Nitrosomonas* and *Nitrobacter* bacteria are not killed (Gorin et al., 1978). Besides that, Clark (1950) and Nevo & Hagin (1966) postulate an NO_3 increase caused by physical changes in the structure of the organic matter in sterilized samples.

On the other hand Melo & Suzuki (1976) observed a decrease in NO_3-N contents and an increase in NH_4-N contents in dried compared with field-moist samples and therefore recommend storing at low temperature (refrigerator or freezing) even for periods up to 6 months. They did not offer an explanation of the unexpected decrease in NO_3 contents, but it is likely that some soluble organic carbon (and nitrogen) fractions are volatilized.

After the initial drying and subsequent resulting in an increase of NO_3 contents, Munro & Mackay (1964) stored samples at -20 and -15 °C for up to 15 weeks without finding any further changes in the NO_3 contents. Under these conditions samples with 10 and 20 % moisture could be stored without any changes too, but at $+5$ °C and increasing length of storage, the NO_3 contents decreased.

According to Munro & Mackay, drying to a moisture content remaining above the wilting point can be done safely. The sharp increase in NO_3-N was found in samples dried to below the wilting point.

Other techniques used to restrict biological activity, such as adding inhibitors like toluene, chloroform, mercuric chloride and others (Storrier, 1966; Robinson, 1967) or micro wave drying (Thien et al., 1978) have not proved to be effective since ammonification was found to continue in spite of the inhibitors and the effects of drying proved to be inconsistent.

In the present paper the effects of storage and of some treatments, i.e. freezing and drying (see 'Materials and methods') on the NO_3 contents of three soils will be described. As standards the results obtained on field-moist soils without further treatment are used.

Materials and methods

Samples of air-dry soils as described in Table 1 and pretreated as discussed by Schuffelen et al. (1952) were mixed with varying amounts of NO_3-N (0, 14 and 28 mg kg^{-1} dry soil) as $Ca(NO_3)_2$ and incubated on 6 December 1978 in portions of 5 kg dry soil in 6-litre pots. Moisture was added to 50 % of the maximum water-

Table 1. Characteristics of soils used in an incubation experiment for measuring NO_3-N contents.

	Clay[1]	Loam[2]	Sand[3]
< 2 μm (%)	35	20	–
organic matter; C (%)	1.4	1.4	11.2
CEC (meq/100 g)	18.6	13.7	17.5
$CaCO_3$ (%)	1.4	7.7	0.0
pH-KCl	6.95	7.20	4.79
N_{tot} (g kg^{-1})	1.7	1.2	–
Pw; P_2O_5 (mg l^{-1})[4]	86	18	34
K-HCl; K_2O (mg kg^{-1})[5]	230	203	210
Mg-NaCl; MgO (mg kg^{-1})[6]	28	88	–
Max. water capacity (ml kg^{-1})	380	400	460

[1] Surface layer of a clay soil, from the Unilever Experimental Station, Duiven.
[2] Surface layer of a 'polder' vague soil (de Bekker, 1979; de Bakker & Schelling, 1966) from the Research Static for Arable Farming and Field Production of Vegetables, Lelystad.
[3] Topsoil of a 'goor' earth soil from the area of Veenendaal.
[4] Extraction with water according to Sissingh (1971).
[5] Extraction with 0.1 *M* HCl.
[6] Extraction with 1 *M* NaCl.

holding capacity.

The pots were stored in an unheated glasshouse with an average temperature of about 12 °C until January 1979, afterwards decreasing to −10 °C at the time of subsampling on 18 January 1979. As the soils were frozen at that date, the pots to be sampled stood at room temperature the night before sampling. The soils chosen belong to types used in the Netherlands for open-air spinach growing. They did not receive any organic amendments such as farmyard manure, compost or sewage sludge in the last five years.

The experiment includes 27 units (3 soils × 3 levels of nitrogen × 3 replicates). Since it was impossible to handle such a large number of samples in the laboratory within an acceptable period of time (one day) the replicates were used as 'blocks' and per week the samples (= pots) of one 'block' were subsampled as follows. Per pot nine 500-g samples were taken and treated as follows (Nos 1-9):

- Freezing, −20 °C (Nos 1 and 7).
- Refrigerator, +4 °C (Nos 2 and 8).
- Room temperature, +20 °C (Nos 3 and 9).
- No treatment (No 4 = standard).
- Drying at 30 - 40 °C (air-dry) (No 5).
- Drying at 70 °C (No 6).

The samples were put in plastic bags; the Nos 5 and 6 after drying.

Samples Nos 1, 2, 3, 5 and 6 were stored for a short period (2 - 3 days) and Nos 7, 8 and 9 for a long period (2 months). The No 4 samples, without any treatment, were extracted for NO_3-N in field-moist state on the same day the Nos 1, 2, 3, 5 and 6 were extracted.

Even if direct analysis (No 4), without drying and storage, is preferred the number

of samples and the facilities and internal organization of a laboratory quite often necessitate such pre-treatments. As samples for inorganic nitrogen analysis must be taken as short as possible before the start of the growth of the crop, small laboratories might need storage and pre-treatment facilities, and larger ones a reliable working programme for an efficient handling of large numbers of field-moist samples.

Of the pre-treatments mentioned freezing, drying at 70 °C, and probably storage in a refrigerator are the most reliable ones; air drying and storage at room temperature beyond 2 or 3 days will probably exert unpredictable effects on nitrate contents (Eagle & Matthews, 1958).

After the pre-treatments and the appropriate storage period, about 20 g of the samples (in duplicate) of one 'block' were weighed and extracted with 50 ml 0.01 *M* $CuSO_4$ after 15 min shaking. The ratio soil weight : extraction solution does not affect the quantity of NO_3 extracted over a wide range (Guiot, 1975). In the filtered extracts, the NO_3 concentrations were measured with a 'Orion' ion specific electrode and a Beckman mV meter. The moisture contents of the samples were determined simultaneously by drying at 105 °C. The results of the NO_3 analysis (Table 2) were expressed as mg NO_3-N per kg dried (105 °C) soil.

An analysis of variance was worked out for the treatments Nos 1 - 6 (short storage) and Nos 7, 8 and 9 (long storage). This analysis includes a complete factorial schema with 3 ('blocks') 3 (NO_3-N levels including ON) 6 or 3 (treatments) units. The data of the two 'storage' periods could not be analysed together since the chemical analyses were not be carried out simultaneously. With a 'Dunnett test' (see Miller, 1966) the results of the treatment combinaties within the first group (short storage period) were compared with those of the untreated samples (No 4).

Results and discussion

The data are listed in Table 2. In this table, the results of the NO_3 contents of the samples are corrected for the NO_3 amounts added, i.e. 14 and 28 mg kg^{-1} N for level 1 and level 2, respectively.

From the statistical tests it must be concluded that a 'block' effect exists for the data of the long storage periods. The differences between the data in the three different weeks can only be explained by postulating unknown differences in the handling of the samples, the extraction procedure included. Also Guiot (1975) pointed out that small differences in the way samples are treated, i.e. extraction speed, extraction time, etc., interacting with the particle size of the soils may lead to variations in the final results. For the short as well as for the long storage period, the interaction NO_3 level × treatments was not significant for any of the soils. Hence the mean values, as given in Table 2, represent the best estimates of the effects of NO_3 levels and of the treatments Nos 1 - 9.

The addition of NO_3 salts to the soil samples had a marked effect on the NO_3 amounts of soil-NO_3 extracted. This result can be compared with the 'priming action' due to enhanced breakdown of native soil organic matter after addition of fresh organic material as described by Sauerbeck (1963) and others.

Since in practical situations, i.e. for advices on dressings, mostly a momentary

Table 2. No_3 contents (mg kg^{-1} dried soil) in extracts of samples of three soils handled in various ways before extraction. Means of three 'blocks' (see text), two determinations per 'block'.

Treatments[1]		Clay				Loam				Sand			
	NO_3 level →	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$	0	1	2	$\bar{x}$
Freezing	1	48.7	48.9	53.3	50.2	18.3	22.0	20.3	20.2	48.0	49.0	50.3	49.1
Refrigerator	2	43.7	45.0	50.0	46.2	18.3	22.7	17.3	19.4	39.7	47.3	45.3	44.1
Room temp.	3	45.0	44.0	52.7	47.2	17.7	18.0	19.0	18.2	42.7	44.3	48.3	45.1
'Untreated'	4	47.3	47.7	50.7	48.6	19.0	20.3	22.3	20.6	46.3	51.7	56.7	51.6
Drying (35 °C)	5	38.3	37.3	39.7	38.4	18.7	15.3	18.7	17.6	31.0	39.7	34.7	35.1
Drying (70 °C)	6	38.3	43.3	44.7	42.1	15.7	15.0	16.3	15.7	33.3	44.0	34.3	37.2
Average ($\bar{x}$)		43.6	44.3	48.5	45.5	17.9	18.9	19.0	18.6	40.2	46.0	44.9	43.7
$E(S_{\bar{x}})$		4.14	–	–	–	2.87	–	–	–	6.58	–	–	–
'Dunnett test'[2]		–	–	–	4.56	–	–	–	3.15	–	–	–	7.23
Freezing	7	49.0	61.0	64.0	58.0	19.0	19.7	25.7	21.4	28.7	10.0	39.7	36.1
Refrigerator	8	57.0	56.7	65.3	59.7	22.3	21.7	27.7	23.9	37.0	41.0	44.7	40.9
Room temp.	9	64.0	69.7	72.0	68.6	26.0	30.0	35.0	30.3	250.3	258.7	272.3	260.4
Average ($\bar{x}$)		56.7	62.4	67.1	–	22.4	23.8	29.4	–	105.3	113.2	118.9	–
$E(S_{\bar{x}})$		3.70	–	–	–	2.31	–	–	–	6.99	–	–	–

[1] 1 - 6: short storage period; 7, 8 and 9: long storage period.

[2] Calculated with percentage of a 5-'one tail' statistic (α = 0.05 and 34 degrees of freedom for the rest variance; see Miller, 1966).

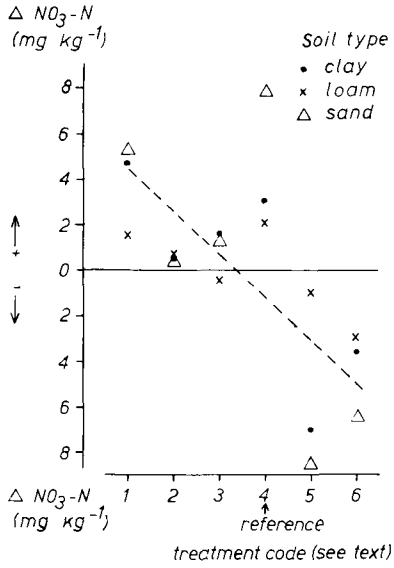

Fig. 1. Effects of various treatments on the quantities of extractable NO_3-N expressed as deviations from the mean.

status is measured, such priming effects will not be recognized in the results of the NO_3 analysis.

The effects of the treatments (Nos 1 - 9) are significant for the three soils used and for both storage periods. The mean values (Table 2 and Fig. 1) show an overall decrease in NO_3-N contents with rising temperatures during the short storage period (treatments Nos 1 - 6). The 'Dunnett test' shows significantly lower NO_3-N contents for the drying treatments (air drying and drying at 70 °C) than for the reference samples (treatment No 4).

Volatilization after reduction of some of the nitrate might have caused these differences but such an assumption does not explain the opposite results obtained by other authors (Munro & Mackay, 1964; Westfall et al., 1978, and others) who found higher NO_3 contents with one or another drying procedure. A slower way of drying possibly promotes the formation of NO_3. In our experiment the drying was performed in stoves with a noticeable air circulation. From the same group of data it can be concluded that a short storage is acceptable even if the samples are stored at room temperature. As the estimates of the standard deviation – $E(S_x)$ in Table 2 – are nearly equal for samples subjected to short and long storage periods, a comparison is possible without further statistical treatment. Nevertheless, except for the sandy soil treatments 7 and 8, the mean values of the treatments Nos 7, 8 en 9 are higher than those of the reference treatment (No 4) and in fact also higher than for the other treatments in the 'short storage' group. With the longer storage period also the specific differences between the soils are more visible than when the soil samples are stored only for 2 or 3 days. Aside from being not very practical, storage at room temperature for a long period leads to enhance mineralization and nitrification, especially in the sandy soil (Table 2), as is emphasized by other authors too. The rela-

tively high organic matter content of this sandy soil ensures a high microbial activity, and this does not hold for the recently reclaimed loam and for the clay soil. Long storage below room temperature (treatments Nos 7 and 8) suppresses nitrification but nevertheless, in accordance with Westfall et al. (1978), substantially higher nitrate contents than after a short storage periode are found. It should be emphasized here that a difference in the analysis of 5 mg kg^{-1} NO_3-N makes a difference of 45 kg ha^{-1} in an advice for N dressings (soil profile 0.6 m; density 1.5). This means that the differences between dried (mean of Nos 5 and 6) and field-moist (No 4) samples, for the short storage period of 3 days, leads to reductions in quantities adviced of 35, 75 and 139 kg ha^{-1} N for loam, clay and sand, respectively.

The conclusion from this work is that direct handling of field-moist samples is preferable and that, if storage is necessary, a period of up to 3 days in a freezer, a refrigerator, or at room temperature is acceptable. This conclusion justifies once more the handling of samples on which total inorganic N is determined in the Netherlands (the '$N_{min.}$ method')[1]. Oven drying of samples or storing them for a long (2-month) period affects the nitrate contents in an unacceptable way, and these effects are closely related to soil type.

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