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Residual Soil Nitrate: A Comparison between Air-Dried and Field-Moist Soil Samples

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In the framework of the European nitrate directive (91/676/EEG), losses of nitrate (NO_3)– nitrogen (N) to both surface and groundwater are limited to 50 mg/l. Because the residual NO_3-N in the soil profile after harvest is considered the main determinant of nitrate leaching during wintertime, the Flemish government imposed a limit value of 90 kg NO_3-N ha^{-1} up to a soil depth of 90 cm between 1 October and 15 November. This study compared two different soil sample preparation methodologies. When samples were analyzed immediately upon arrival, no differences in NO_3-N concentration were observed. However, although field-moist samples are maintained at 4 °C, nitrification is not completely stopped, as indicated by the increased NO_3-N concentration in field-moist samples 10 days after storage at 4 °C . In contrast, nitrification in air–dried samples is stopped during the oven drying when 40 °C is reached. Moreover, the reproducibility was significantly greater in air-dried samples as compared to field-moist samples.

Keywords Mineralization, nitrogen, testing methodology

Introduction

In 1991, the European Commission (EC) issued a nitrate directive (91/676/EEC) concerning the protection of water against contamination by nitrates from agricultural sources. A limit of 50 mg L^{-1} for nitrate (NO_3)–nitrogen (N) in both surface water and groundwater was laid down in this directive (EC 1991).

Because of its high solubility, nitrate can enter groundwater. Where groundwater recharges stream flow, nitrate-enriched groundwater can contribute to eutrophication, a process leading to high algal populations, especially blue-green algae, and the death of aquatic life as a result of its excessive demand for oxygen. Moreover, elevated nitrate in groundwater is a concern for drinking water use because nitrate can cause health problems to humans and animals (WHO 1985). In the past, the agricultural sector has been identified as the major contributor to the nitrate contamination of the groundwater and surface

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water, mainly resulting from overfertilization (McLay et al. 2001; VLM 2001). The residual NO_3-N in the soil profile after harvest is considered the main determinant of nitrate leaching during wintertime (Bogaert et al. 2000).

To meet the EC directive, the application of manure and fertilizers has been restricted in Flanders. Since 2000, the Flemish government declared that the residual nitrate in the 0- to 90-cm soil layer should not exceed 90 kg NO_3-N ha^{-1} from 1 October until 15 November (Anonymous 1999; Bries et al. 2000). If the amount of nitrate exceeds 90 kg ha^{-1}, attendant measures would apply. When the limit is severely exceeded (i.e., 150 kg NO_3-N ha^{-1}), the farmer is fined. Therefore, intensive soil sampling in agricultural fields is carried out by officially recognized laboratories during this period.

Because nitrogen in the soil is susceptible to rapid changes (ammonification, nitrification, fixation, denitrification, and volatilization), it is important to determine nitrate and ammonium N levels as quickly as possible after sampling (Mengel and Kirkby 2001). In the soil samples stored for some time, ammonification and nitrification can continue, depending on the storage conditions of the samples. Several sample preparations have been applied over the past few years. However, until now, no study was published on the impact of soil sample preparations on the NO_3-N concentration in the sample. Therefore the objective of this study was to determine the impact of sample preparation on the nitrate content in field-moist and air-dried soil samples. In addition, it is of primary importance to farmers that soil NO_3-N determinations are precise and reliable. Therefore, the second objective is to validate the reproducibility of the different sampling methods over time.

Materials and Methods

In March–June 2009, the Soil Service of Belgium analyzed NO_3-N concentrations in soil samples from farmers' fields that represented the different soil textures commonly occurring in Flanders, that is, sand, sandy loam, loam, and clay. Three experiments were carried out. In a first experiment the NO_3-N content in air-dried and field-moist samples was compared in 80 soil samples (based on the detection limit, 54 soil samples were included in the statistical analysis). In a second experiment, the reproducibility of using air-dried and field-moist samples was determined for four soil textures. In this experiment, 143 soil samples were taken, and based on the detection limits, 98 were included in the statistical analysis. Finally, in a third experiment, the effect of time on the mineralization in field-moist samples was studied. Out of 41 soil samples, 21 were considered based on the detection limit. The NO_3-N and ammonium (NH_4)-N content was determined at three time points: after 1, 2, and 10 days at 4 °C.

The practical soil samples were taken at depths of 0–30, 30–60, and 60–90 cm, using soil coring tubes (2.0 cm outer diameter). Each sample was composed of 15 borings. At sampling, the soil samples were packed and transported in cotton bags at 4 °C. The samples were gently pressed to preserve the original compaction state (as in the soil). Upon arrival, the soil samples were stored at 4 °C for a maximum period of 2 days. Prior to further processing, each individual sample was homogenized in the cotton bag at 4 °C. Next, each sample was divided into two subsamples: The first subsample was removed from the bag and labeled as the field-moist subsample, while the second subsample remained in the cotton bag and was labeled as the air-dried subsample.

The methodology used for the determination of N in air-dried soil samples is BELAC accredited (BDBMET047), while the methodology described in ISO 14256-2 is used for field-moist soil samples (BELAC 2009; ISO 2005). In Table 1, the performance characteristics in the validation reports are represented for both methodologies. To guarantee the

Table 1
Performance characteristics of the determination of NO_3-N in air-dried and field-moist soil samples as carried out at the Soil Service of Belgium

Parameter	Air-dried samples	Field-moist samples
Determination limit (6 × S)	1.6 mg N kg^{-1}	4.0 mg N kg^{-1}
Trueness	106.5%	106.5%
Bias	6.5%	6.5%
$RSD_{R,pool}$	5.8%	12.5%
Measurement uncertainty (quadratic summation)	24.9%	33.4%

Note. RSD, relative standard deviation (%) = 100 × s / X with s as standard deviation.

quality of analysis, the Soil Service of Belgium has participated 10 times a year since 1990 in the international proficiency test Bipea (Bipea 2009). At the national level, it also participates in the Flemish proficiency test Vlarisub organized by ILVO (Institute for Agricultural and Fisheries Research) and the proficiency test organized by VITO (Flemish Institute for Technological Research) (Vandecasteele 2009).

The subsamples to be air-dried were placed on grids in a forced-air oven at 45 °C (Weiss Technik, UK; WT/VTU100/150) in the original cotton bag for 24 h. The height of the soil samples did not exceed 5 cm. Next, the air-dried samples were sieved with a 2-mm sieve. On the same day, 20 g air-dried soil of a well-homogenized air-dried sample were weighed and 40 ml 1% potassium chloride (KCl) was added (1:2 ratio). Next, the samples were shaken (50 rpm) in an end-over-end shaker for 30 min, followed by centrifugation for 5 min (Jouan C312 2000 rpm). Subsequently, the NO_3-N content was determined colorimetrically (at 543 nm) after reduction to nitrogen dioxide (NO_2)-N as α-napthylamine-paradiazobenzene-parasulphonic acid by using a continuous flow analyzer (Skalar, the Netherlands; SA4000). The next day, a second air-dried subsample of 20 g was analyzed following the previously described procedure to check the reproducibility.

The field-moist samples were manually sieved using a 5 mm sieve at 4 °C. For the fresh field-moist samples, 40 g soil was weighed and immediately extracted with 200 mL 1 M KCl (i.e., 1:5 ratio) in an end-over-end shaker (50 rpm) for 1 h. Subsequently, the same procedure as described previously was applied. The same day, a second field-moist subsample of 40 g (to check reproducibility) and a third subsample (to determine impact of storage time on mineralization) were weighed and kept at 4 °C until analysis. Immediately after sieving (using a 5-mm sieve), the dry matter was determined at 105 °C.

The experimental results were statistically analyzed using the statistical package Statistica 8 (Statsoft, Inc. 2007). Analysis of variance (ANOVA) was performed after verifying the conditions, that is, normality and homogeneity of variances. When the ANOVA showed significant ($P \leq 0.05$) differences, differences between the treatments were identified by Tukey's test ($P \leq 0.05$). Both one-way and two-way ANOVAs were carried out depending on the data set.

Results

In 54 soil samples, the NO_3-N contents were compared in air-dried and field-moist samples (Figure 1). The 54 soil samples included loam (34), sandy loam (5), and sand (15) soil

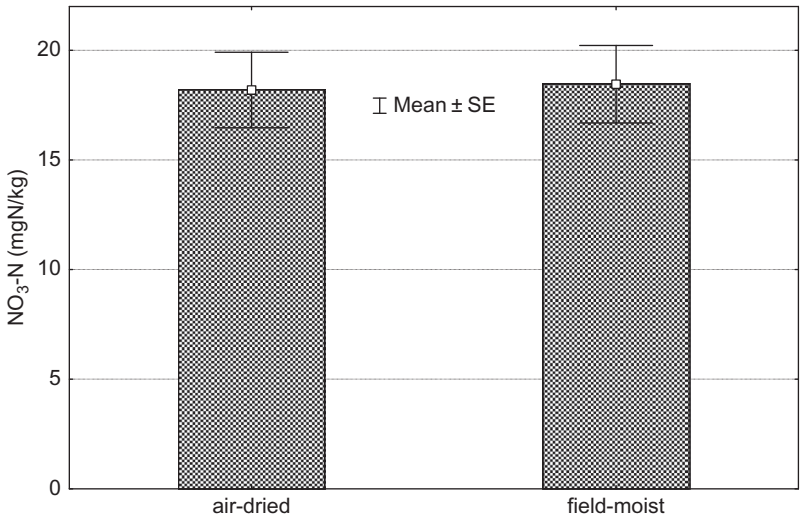

Figure 1. Impact of soil preparation on NO_3-N concentration in soil samples (n = 54) classed as loam (n = 34), sandy loam (n = 5), or sand (n = 15) in texture.

textures. Nitrate-N concentration in field-moist samples (18.5 mg N kg^{-1}) did not differ significantly ($P \leq 0.05$) from NO_3-N concentration in air-dried samples (18.2 mg N kg^{-1}).

In a second experiment, a total of 143 soil samples were analyzed, representing the different soil types commonly occurring in Flanders. However, based on the determination limit, which is 1.6 mg N kg^{-1} for air-dried samples and 4.0 mg N kg^{-1} for field-moist samples (Table 1), and by pairwise deletion, only 98 soil samples were included for the statistical analysis. Globally, the NO_3-N content in air-dried soil samples did not differ significantly ($P \leq 0.05$) from the NO_3-N content in field-moist soil samples (Table 2), both at day 1 and day 2, confirming the observations from the first experiment. No significant effect of time could be observed. However, the reproducibility, expressed as the absolute deviation, was significantly ($P \leq 0.05$) better (i.e., a lower absolute deviation) in the air-dried samples compared to the field-moist samples.

Table 2
NO_3-N content in soils determined in air-dried and field-moist samples at different times of analysis

		NO_3-N (mg N kg^{-1})		
Treatment	N	Day 1	Day 2 (duplo)	Absolute deviation (%)
Air-dried	98	15.12 n.s.	15.82 n.s.	7.95 a
Field-moist	98	15.22 n.s.	16.36 n.s.	15.27 b

Notes. n.s., not significantly different according to Tukey's HSD test ($P \leq 0.05$). For each soil type, capital letters indicate a significant main effect of treatment according to Tukey's HSD test (one-way ANOVA) ($P \leq 0.05$). Small letters indicate significant differences according to Tukey's HSD test (one-way ANOVA) ($P \leq 0.05$).

Table 3
NO_3-N content in different soils determined in air-dried and field-moist samples at different times of analysis

Soil type	Treatment	N	NO_3-N (mg N kg^{-1}) Day 1	NO_3-N (mg N kg^{-1}) Day 2 (duplo)	Absolute deviation (%)
Sand	Air-dried	15	18.45 n.s.	17.46 n.s.	5.34 n.s.
	Field-moist	15	17.91 n.s.	17.86 n.s.	12.20 n.s.
Sandy loam	Air-dried	11	14.60 n.s.	15.84 n.s.	10.53 n.s.
	Field-moist	11	13.71 n.s.	16.14 n.s.	20.12 n.s.
Loam	Air-dried	38	17.87 n.s.	19.42 n.s.	8.76 n.s.
	Field-moist	38	18.79 n.s.	19.08 n.s.	12.15 n.s.
Clay	Air-dried	34	10.76 n.s.	11.07 n.s.	7.37 a
	Field-moist	34	10.54 n.s.	12.72 n.s.	18.54 b

Notes. n.s., not significantly different according to Tukey's HSD test ($P \leq 0.05$). For each soil type, capital letters indicate a significant main effect of treatment according to Tukey's HSD test (one-way ANOVA) ($P \leq 0.05$). Small letters indicate significant differences according to Tukey's HSD test (one-way ANOVA) ($P \leq 0.05$).

Table 4
Intralaboratory reproducibility (RSD_R based on duplos), of the determination of NO_3-N in air-dried and field-moist soil samples

Soil	RSD_R (%) Air-dried samples	RSD_R (%) Field-moist samples
All soil	8.5	12.5
Sand	8.0	15.0
Sandy loam	10.3	17.2
Loam	9.0	7.9
Clay	6.7	13.9

Notes. RSD, relative standard deviation (%) = 100 × s / X with s as standard deviation.

In sand, sandy loam, loam, and clay, no significant ($P \leq 0.05$) difference in NO_3-N content was observed in air-dried and field-moist samples, regardless the day of analysis (Table 3). Concerning the reproducibility, in all soil types greater absolute deviation (indicating lower reproducibility) was observed for field-moist soil samples. However, only in clay, the absolute deviation of field-moist samples was significantly ($P \leq 0.05$) greater than the absolute deviation of air-dried samples.

Linked to the differences in absolute deviation, the intralaboratory reproducibility is given in Table 4 as the RSD_R, the relative standard deviation based on duplo measures (i.e., extractions and measures at day 1 and day 2). For all soils analyzed, the RSD_R was 8.5% for air-dried samples and 12.5% for field-moist samples. For most individual soil textures, the RSD_R calculated for air-dried samples was always lower compared to the RSD_R calculated for field-moist samples. Only for loam, the RSD_R was slightly greater for air-dried samples than for field-moist samples.

Finally, in a third experiment, the effect of storage time on the mineralization was studied in field-moist samples (Figure 2). For sand, sandy loam, and loam, mineralization,

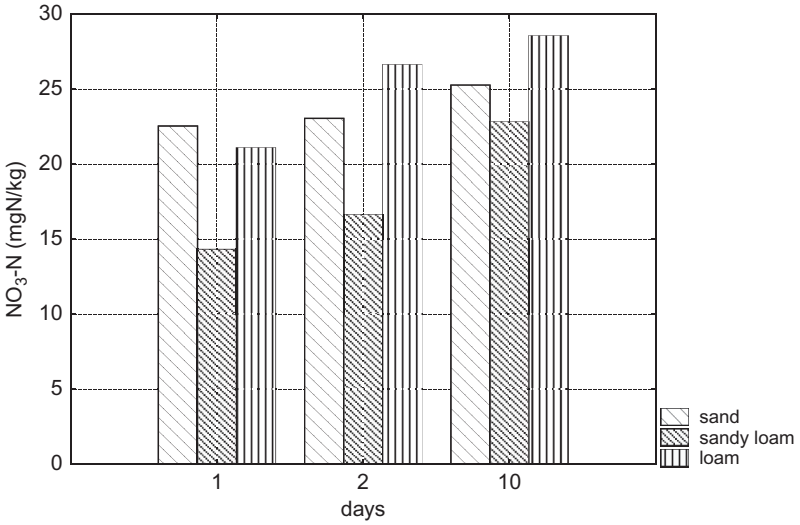

Figure 2. Mineralization at 4 °C in field-moist soil as function of time.

measured as NO_3-N content, increased over time during 10 days, even when soil samples were stored at 4 °C. The NH_4-N concentration was less than the detection limit and did not increase during this 10-day period.

Discussion

As shown by the experiments, the sample preparation (air-dried versus field-moist samples) will not influence the NO_3-N determination when soil samples are analyzed immediately upon arrival, when the field-moist samples are manipulated as much as possible at 4 °C, and when 40-g subsamples are taken. However, when field-moist samples are stored for some time, even at 4 °C, the nitrification (i.e., the biological oxidation of ammonia to nitrate) will continue. In contrast, in air-dried soil samples nitrification is stopped during the air drying.

Nitrification is a two-step process; in a first step ammonia is oxidized to NO_2^-, which then is further oxidized to NO_3^- (Mengel and Kirkby 2001). The nitrification in the soil is mediated by autotrophic nitrifying bacteria. *Nitrosomonas, Nitrosolobus,* and *Nitrosospira* are genera that oxidize ammonia to NO_2^-. The nitrite produced is then rapidly oxidized to nitrate by *Nitrobacter* species. Both ammonium oxidizers and nitrite oxidizers are obligately aerobic. Besides the presence of O_2, humidity and temperature are crucial parameters for a successful mineralization (Mengel and Kirkby 2001; Schweigert, Pinter, and van der Ploeg. 2004).

During the preparation of the field-moist samples, additional oxygen is added to the soil samples by sieving and by spreading the sieved field-moist soil in an aluminum dish during storage. The extra aeration stimulates the aerobic nitrifying bacteria in the presence of water. Consequently, this will result in an increasing NO_3-N content during storage. Extrapolated to a field situation, plowing or tillage, which also mean extra aeration, is known to result in a greater NO_3-N content compared to no tillage (Alvarez and Steinbach 2009). In contrast to field-moist samples, air-dried soil samples are dried in their original

compacted state (i.e., without sieving and thus without additional aeration). The compaction that takes place during field sampling when the soil sample is gently pressed in the cotton bag will have no influence on nitrification (De Neve and Hofman 2000). The air-dried samples are only sieved after drying. In this case, the additional oxygen during sieving will not stimulate nitrification, because the humidity levels after drying are inhibitory (Soulides and Allison 1961; Paul and Clark 1996).

In relation to the temperature, the cool storage (at 4 °C) of field-moist samples will still allow bacterial activity, although at a significantly reduced rate (Grunditz and Dalhammar 2001; Avrahami, Liesack, and Conrad 2003). During air-drying on grids in a forced-air oven (45 °C), the temperature in the soil sample will rise above 40 °C within 30 min (data not shown). At drying temperatures greater than 40 °C, soil microbial activity decreases drastically. Moreover, most soil microbes die off because of the thermal denaturation of proteins and alterations in the permeability of membranes (Soulides and Allison 1961). To illustrate, Grunditz and Dalhammar (2001) showed that 40 °C inhibited *Nitrosomonas* activity.

Finally, the humidity of the soil samples during drying in a forced-air oven (45 °C) is rapidly reduced to levels that are inhibitory for the nitrifying bacteria. In contrast, the field-moist samples will maintain their humidity.

Next to a precise determination of the NO_3-N content in the soil, the farmer expects legal certainty. Analytical methods (including the sample preparation) used for the determination of NO_3-N in the soil should allow the farmer to request a counteranalysis at any time. Therefore, the reproducibility should be as high as possible. When using field-moist samples, this legal certainty cannot be guaranteed, because the nitrification continues during storage even at 4 °C (as shown in our third experiment). Therefore, it is important that when NO_3-N concentrations are determined in field-moist soil samples, this is executed as soon as possible upon arrival. Even when field-moist samples are immediately frozen upon arrival, the thawing will enhance nitrification (Esala 1996). Hence, counteranalysis will result in a greater NO_3-N content. Moreover, the intralaboratory reproducibility was lower for field-moist soil samples compared with air-dried soil samples, indicated as a greater absolute deviation in NO_3-N content after 1 and 2 days of storage in field-moist samples. In addition, fresh field-moist soil samples cannot be stored for longer periods, while air-dried soil samples can be stored for an indefinite period of time.

To summarize, the soil sample preparation will not influence the NO_3-N determination if NO_3-N levels are determined immediately upon arrival. However, the reproducibility of the NO_3-N determination in air-dried samples is significantly greater compared to field-moist soil samples, also because in field-moist samples the measurement of the soil water content introduces another source of variation. Moreover, homogeneous subsampling after using a 5-mm sieve (when using field-moist samples) is more difficult compared to subsampling after using a 2-mm sieve (when using air-dried samples), even when 40 g is taken instead of 20 g. Therefore, to deliver to farmers a precise and reliable/reproducible NO_3-N content, air-dried samples are preferable to the field-moist samples.

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