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Comparison of Chemical Methods of Assessing Potentially Available Organic Nitrogen from Organic Residues Applied to a Sandy Soil

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Abstract: More than 90% of the nitrogen (N) in soils is bond as organic N compounds. The available N can be estimated on the mineral N released during time-consuming incubations of soil. Several chemical methods have been developed as substitutes for incubations. On the other hand, there has been an increase in waste production. Residues could potentially offset the need for mineral fertilizers, being both an economic and environmental benefit. Thus, the development of a routine method for prediction of N supply both from soil organic matter (SOM) and the application of organic residues is of great interest. An incubation experiment was performed in a Cambic Arenosol to evaluate different chemical methods. Air-dried soil was mixed with increasing amounts of composted solid municipal waste, secondary pulp-mill sludge, hornmeal, poultry manure, the solid phase from pig slurry, and composted pig manure. Samples were incubated for 244 days under a controlled environment. Among the chemical extractants studied, hot 2 M potassium chloride (KCl) and hot 0.01 M calcium chloride (CaCl_2) showed promise in indicating values of N_0 (potentially available nitrogen), and these simple methods are suitable for use in routine laboratory conditions.

Keywords: Aerobic incubation, chemical methods, nitrogen mineralization, organic residues, sandy soil

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INTRODUCTION

There has long been a need for satisfactory laboratory methods to obtain reliable estimates of potentially available nitrogen (N) resulting from mineralization of soil organic matter (Chae and Tabatabae 1986; Cordovil 2003). Several chemical methods have been proposed (Douglas and Magdoff 1991; Gianello and Bremner 1986; Smith and Li 1993; Wang et al. 2001). It is generally accepted that the most reliable methods currently available are those involving the determination of the mineral N produced during the aerobic or anaerobic incubation of soil over various times, but these biological methods are not simple and rapid enough for use in routine testing. Therefore, there is an urgent need for a rapid chemical method of assessing potentially available soil organic N that is suitable for commercial laboratories.

A further need has emerged because production of on- and off-farm organic residues has increased at a considerable rate, making it urgent to find an environmentally acceptable alternative use. If such residues can be recycled to agricultural land, they can provide sources of both organic matter, to compensate the losses of organic matter that occur in many sandy soils (Appel and Mengel 1990), and mineral nutrients, such as nitrogen (Cordovil 2004). Effective utilization of these on-and off-farm residues as valuable resources in agricultural rotations can enhance sustainable production, including economic competitiveness.

The study reported here further evaluates several chemical methods by applying them to several mixtures of a sandy soil and six different organic residues, and compares these results with those obtained by a biological incubation method, considered to be of particular relevance in assessing potentially available organic N in both soils and residues.

MATERIALS AND METHODS

To provide a rapid estimate of potentially available N (PAN) from the mineralization of different organic residues, a series of chemical extractant procedures were tested on several residues mixed with a sandy soil to give different concentrations.

Materials

The soil used was collected from the upper layer (0–25 cm) of a Cambic Arenosol (FAO 1998). The soil, from an experimental farm in Pegões, Portugal, at 38°04'0 N and 8°37'0 W, with a particle-size distribution of 872 g kg⁻¹ sand, 99 g kg⁻¹ silt, and 29 g kg⁻¹ clay, was air dried at room temperature and sieved to pass a 2-mm mesh. Six organic residues, previously ground to pass a 0.75-mm sieve and dried at 35°C for 48 h, were individually

mixed with the soil. The organic residues were composted solid municipal waste (M), secondary pulp-mill sludge (S), hornmeal (H), poultry manure (P), the solid phase extracted from pig slurry (SP), and composted pig manure (C). The soil and each residue were characterized chemically (Table 1).

Methods of Assessing Potentially Available Nitrogen

Biological Method

A laboratory aerobic incubation experiment was carried out. Batches of 1.5 kg of soil were mixed with variable amounts of each one of the six organic residues tested. The weight of each residue applied was selected to supply 0, 40, 80, 120, 160, or 200 kg ha⁻¹ of its total Kjeldahl N content. Soil and residue mixtures were placed in black plastic bags, wetted with distilled water to 60% water-holding capacity, and maintained at 24°C. Treatments were replicated four times. The bags were opened weekly to maintain aerobic conditions.

Stanford and Smith's (1972) first-order kinetic model was fitted to the results:

$$N_m = N_0(1 - e^{-kt})$$

This model was chosen because it has frequently been used to predict N mineralization from SOM. N_m represents accumulated mineralized N at time t , k is the mineralization constant, and the amount of potentially mineralizable nitrogen is given by N_0 . The term mineralization is in reality the apparent net mineralization (Cordovil et al. 2005).

Chemical Methods

To provide a rapid estimate of the potentially mineralizable N, from the residues applied to the soil, 12 chemical extraction methods were tested. The methods evaluated are quick and easily performed and allow the extraction of N compounds potentially available to the plants. At time zero for the biological method, a 300-g sample was collected from each soil-residue mixture, after it had been wetted, and stored at 4°C until it was subject to chemical analysis (Nicholson, Chambers, and Smith 1996). Subsamples were also taken (Øien and Selmer-Olsen 1980), and treated according to the procedures required by each extraction method tested.

Method 1

This method was used as reference method along with the biological aerobic incubation. After 0, 7, 14, 21, 35, 67, 102, 175, and 244 days, a soil sample was taken from each bag and shaken for 1 h with 100 mL of 2 M potassium chloride (KCl) solution (Mulvaney 1996) at room temperature, centrifuged, and analyzed for mineral N.

Table 1. Characteristics of the soil and organic residues used in the experiment

Property	Soil	Organic residue					
		M	S	H	P	SP	C
OM (g kg ⁻¹)	5.9	482	875	845	766	714	812
TOC (g kg ⁻¹)	—	280	507	490	444	414	471
pH (H ₂ O)	6.1	8.0	6.8	6.5	8.4	6.6	7.5
N _{Kj} (g kg ⁻¹)	0.34	17.8	42.4	116.7	35.5	17.4	21.7
NH ₄ -N (g kg ⁻¹)	13.0	0.4	1.0	6.5	10.5	0.9	4.5
NO ₃ -N (g kg ⁻¹)	5.5	0.2	0.4	0.8	0.4	3.5	0.2
ANF (% of N _{Kj})	—	21.6	24.2	32.5	36.9	25.9	25.0
N _{rec} (% of N _{Kj})	—	74.6	72.4	47.1	31.3	52.5	53.7
C/N ratio	—	15.7	12.7	4.2	12.5	23.8	21.7
Fat and waxes (g kg ⁻¹)	—	8.2	2.6	7.6	7.8	4.1	4.8
Resins (g kg ⁻¹)	—	5.9	3.6	8.3	7.7	12.1	5.8
Lignin (g kg ⁻¹)	—	94	446	269	49	237	287
Cellulose (g kg ⁻¹)	—	426	82	223	135	123	271
Soluble phenolics (g kg ⁻¹)	—	1.31	0.62	0.53	0.74	0.78	0.74
Total fibre (g kg ⁻¹)	—	118	529	749	373	470	458

Notes. OM, organic matter; TOC, total organic carbon; N_{Kj}, total Kjeldahl nitrogen; ANF, active N fraction; N_{rec}, recalcitrant N; C/N, carbon/nitrogen ratio; composted solid municipal waste; secondary pulp-mill sludge; hornmeal; poultry manure; solid phase from pig slurry; composted pig manure.

The following procedures were performed on subsamples of the 300-g soil samples taken at zero time in the incubation study.

Method 2

This method was described by Hong, Fox, and Piekielek (1990). A 3-g wet sample was added to 20 mL of 2 M KCl. After a short manual shaking, mixtures were centrifuged and the supernatant analyzed for mineral N.

Method 3

This was a modification of the previous method, in which the sample was left in the solution for 4 h, instead of being analyzed at once. Every half hour, the mixtures were shaken manually, and after the 4 h, they were centrifuged and analyzed for mineral N content (Jalil et al. 1996).

Method 4

This involved determination of mineral N produced from organic N in soil–residue mixtures, when a 3-g wet sample was heated with 20 mL of 2 M KCl for 4 h in a stoppered tube and placed in a Skalar 5620/40 block digester maintained at 100°C (Campbell et al. 1997). The soil–KCl mixture was then centrifuged and analyzed for nitrate and ammonium ions (Smith and Li, 1993).

Method 5

In this procedure, 10 g of wet soil–residue sample were treated with 50 mL of 2 M KCl, in glass cups covered with a watch glass, and the mixture boiled gently at 100°C on an electric plate for 1 h. The cups were then removed from the hot plate, cooled at room temperature, and shaken manually for a few seconds. The soil–KCl mixture was then centrifuged and analyzed for nitrate and ammonium ions (Smith and Li 1993).

Method 6

This was essentially the KCl method proposed by Øien and Selmer-Olsen (1980). In the procedure used, 4.0 g of wet soil were placed in round-bottom glass bottles and treated with 40 mL of 2 M KCl. The bottles were sealed with rubber stoppers and placed for 20 h in a water bath maintained at 80°C. The bottles were then removed from the water bath, cooled at room temperature, and shaken manually for a few seconds. The soil–KCl mixture was then centrifuged and analyzed for nitrate and ammonium ions (Gianello and Bremner 1986).

Method 7

This was the CaCl₂ method proposed by Houba et al. (1986). In this procedure, a wet 10-g sample of soil/residue mixture was added to 100 mL of 0.01 M CaCl₂ and left to rest for 2 h. Afterward, it was centrifuged and analyzed for nitrate and ammonium ions.

Method 8

This was a modification of method 7, as proposed by Appel and Mengel (1990). A fresh 10-g sample of soil/residue mixture was added to 100 mL of 0.01 M CaCl_2 and left for 2 h. After this time, the mixture was filtered with ashless SandS 589² filter paper. Filtrated solution was used for N determination, and the residue left in the filter paper was treated with an additional 100 mL of 0.01 M CaCl_2 . This mixture was then heated in an oven at 80°C for 20 min and then centrifuged and analyzed.

Method 9

Twenty g of wet soil/residue mixture were treated with 50 mL of 0.5M K_2SO_4 solution. The suspension was shaken for 1 h and then, centrifuged and analyzed for nitrate and ammonium ions (Serrasolsas and Khanna 1995).

Method 10

A 20-g wet sample was shaken for 1 h with 100 mL of distilled water and then centrifuged before analysis for nitrate and ammonium ions (ADAS 1986).

All the extracts obtained after treating the samples with the respective solutions, as referred in the previous 10 methods, were centrifuged at 3500 rpm for 10 min, and the supernatants were collected for N analysis. Contents of ammonium-N ($\text{NH}_4\text{-N}$) and nitrate-N ($\text{NO}_3\text{-N}$) were determined by molecular absorption spectrophotometry using a continuous flow autoanalyzer (Searle 1984; Kempers and Luft 1988).

Method 11

Ten g of wet sample and 60 mL of 0.01M calcium chloride (CaCl_2) were refluxed for 1 h and cooled to room temperature, and 40 mL of 10% potassium sulfate (K_2SO_4) solution were added. Afterward, the mixture was centrifuged at 3500 rpm for 10 min, and an aliquot of 2 mL was taken from the supernatant and digested with 2 mL of concentrated sulfuric acid (H_2SO_4) for 1 h. After cooling, 40 mL of distilled water and another 10 mL of 10 N NaOH solution were added and N was determined (Fox and Piekielek 1978).

With this method, only the ammonium N in the extract was determined by molecular absorption spectrophotometry using a continuous flow autoanalyzer (Searle 1984).

Method 12

This includes the phosphate–borate buffer procedure. It involves the determination of the ammonium N produced from organic compounds, when the mixture of soil/residue is steam distilled with phosphate–borate buffer. In this method, 4 g of wet sample were steam distilled with 40 mL of pH 11.2 phosphate–borate buffer solution for 8 min, and the ammonium N released was collected in boric acid (H_3BO_3). The dihydrogenborate ion (H_2BO_3^-) was then titrated with chloridric acid of known concentration (Gianello and Bremner 1986).

Table 2. Chemical methods tested, temperature used, extraction times, and extracting solution

Method	Extracting solution	Temperature (°C)	Time	Determination
1	2 M KCl	20	1 h	MAS
2	2 M KCl	20	Immediate	MAS
3	2 M KCl	20	4 h	MAS
4	2 M KCl	100	4 h	MAS
5	2 M KCl	100	1 h	MAS
6	2 M KCl	80	20 h	MAS
7	0.01 M CaCl ₂	20	2 h	MAS
8	0.01 M CaCl ₂	20 + 80	2 h + 20 min	MAS
9	0.5 M K ₂ SO ₄	20	1 h	MAS
10	H ₂ O	20	1 h	MAS
11	0.01 M CaCl ₂	100	1 h	MAS
12	Phosphate–borate buffer solution	20	8 min	D

Note. MAS, molecular absorption spectrophotometry; D, distillation.

Table 2 summarizes key features of these procedures.

Statistical Analyzes

Statistical analysis involved one-way and two-way ANOVA and significant differences were based on a LSD test at 95% confidence (Zar 1996).

RESULTS AND DISCUSSION

Figure 1 shows the different amounts of N extracted from the six residues by all the methods studied. In the figure, it is clear that the amounts of N extracted from samples containing municipal solid waste or secondary sludge were always less than those from other residues. In fact, these two residues are the ones containing the greater proportions of recalcitrant N compounds, which are simultaneously more resistant to mineralization and to hydrolysis by chemical extractants. On the other hand, hornmeal (H) produced the largest proportion of mineral N ($N_{\min}$) extracted by the majority of the procedures tested.

Some procedures resulted in the extraction of greater amounts of N than others. For example, procedures 4 and 8 were more efficient at extracting N compared with 3 and 7, apparently because of the higher temperature used. In all the cases where a high temperature was used (methods 4–6, 8, and 11), the amount of N extracted was calculated by subtracting the $N_{\min}$ determined using the same extractant solution but under ambient temperatures (method 2 for KCl and 7 for CaCl₂) (Gianello and Bremner 1986).

Figure 1. Amounts of $N_{\min}$ in each of the residues studied and determined by each one of the 12 chemical methods tested.

Several chemical extraction procedures resulted in a strong correlation with the PAN calculated from the values of N_0 resulting from the fitting of the Stanford and Smith (1972) model to the data from the incubation method performed (Table 3). Whenever potentially mineralizable nitrogen (PMN) was considered instead of PAN, all correlations indicated a poorer fit to the data.

Methods 1 to 6

Method 1, used as reference method, was the one using cold 2 M KCl as extractant solution. This was the chemical method that best correlated with the calculated value of PAN. Mulvaney (1996) claimed that this extracting solution removes all exchangeable NH_4^+ from the soil, as long as this ratio is at least 1:10 and agitated for a minimum of 1 h.

Instead of using this extracting solution, some authors use 1 M KCl or 0.5 M K_2SO_4 for the same purpose. However, Mulvaney (1996) points out that the 2 M KCl, being a more concentrated solution and thus having stronger ionic characteristics, facilitates separation between solid and liquid phases, allowing a clearer solution for analysis, and it is even possible to store the material for months.

As observed in the previous method, the amounts of $N_{\min}$ in all the residues tested obtained by procedure 2 were well correlated with the laboratory incubation data (Table 3). Although Mulvaney (1996) believes that extraction time was not enough to take all the exchangeable NH_4^+ , Hong, Fox, and Piekielek (1990) have a different opinion. In fact, this study shows that this procedure and procedure 3 generated similar results (Table 3) ($P < 0.05$).

Table 3. Correlation coefficients (*r*) between the Results of chemical methods and those of the biological incubation: $N_{\min} + N_0$ = values referring to PAN, and $N_0 - N_{\min}$ = PMN

Chemical methods	Biological incubation vs. PAN ($N_0 + N_{\min}$ initial)	Biological incubation vs. PMN ($N_0 - N_{\min}$ initial)
1	0.95	—
2	0.79	—
3	0.66	—
4	0.85	—
5	0.43	—
6	0.68	—
7	0.80	—
8	0.80	—
9	0.76	—
10	0.47	—
11	0.90	—
12	0.60	—
4–2	—	0.23
5–2	—	0.06
6–2	—	0.31
8–7	—	0.61
11–7	—	0.84

Note. Confidence $p < 0.05$. PAN, potentially available N; PMN, potentially mineralizable N.

Jalil et al. (1996) incubated 42 different soils, 65% of which contained more than 500 g kg^{-1} sand, and concluded that raising extraction time to 4 h but maintaining the temperature of the soil led to higher amounts of N extracted, as well as higher correlation with N_0 ($r^2 = 0.65$). Their results are not consistent with the ones obtained in the present study, for the correlation with method 3 and N_0 are worse than those of method 2 (Table 3). Thus, it was concluded that extraction time alone is not enough to obtain PMN and that raising the temperature is also necessary.

The same authors cited previously tested the same solution but using a higher temperature (method 4) and observed a better correlation with N_0 ($r^2 = 0.78$). In fact, the present study also shows us that method 4 was more effective in predicting apparent net N mineralization during aerobic incubation (Table 3). This was true when the initial $N_{\min}$ present was not subtracted from the total N value determined and had been already observed by other authors (Campbell et al. 1997; Jalil et al. 1996; Rodrigues 2000).

Gianello and Bremner (1986) had also considered this method as the most suited for predicting N mineralization from SOM, when compared to biological methods. In fact, among 12 methods studied by those authors, this was the one that correlated the best with data from aerobic and anaerobic

incubations. They also state that there is no need to further extend the extraction beyond the 4 h.

Campbell et al. (1997) also found a good correlation between this method and SOM mineralization during the aerobic incubation of 42 different soils and reported that it was a good predictor of N_0 for the soils studied. This statement was also confirmed by Gouveia and Coutinho (2001), who compared the same method with results from an aerobic incubation of 26 different soils during 16 weeks ($r = 0.86$). The same was observed by Rodrigues (2000) after making an in situ incubation ($r^2 = 0.78$).

Method 4 seems to allow the extraction of the exchangeable N but also of N hydrolyzed from labile organic forms, which is a good index of PMN prediction in the long term.

Furthermore, results from method 4 are well correlated with N_0k (initial mineralization potential index) (Campbell et al. 1991) and was considered by Campbell et al. (1991, 1996, 1997) to be a good index of soil capacity to supply nitrogen (Campbell et al. 1991; Curtin and Wen 1999). According to these authors, the efficiency of this chemical method in predicting N_0 (PMN) from soil OM is limited to those soils with a stable mineralization index k .

In contrast, Curtin and Wen (1999) tested the same method with 61 different soils mixed with organic manures and concluded that it was less efficient than the method using phosphate–borate buffer solution as extractant. Method 4 was not considered by these authors to be a good predictor of the net apparent mineralization during the 24 weeks of incubation. However, it was well correlated to the amounts of N mineralized during the first 2 weeks. Therefore, they considered that this method seems to extract mainly the low molecular fraction of N compounds. Moreover, Gianello and Brenner (1986) state that among 50 compounds previously identified in the soils, only 6 seem to be extracted by this method. Curtin and Wen (1999) also add that some of the N determined may come from microbial cells of dead microorganisms during the boiling process.

Figure 2 shows the amounts of N determined by method 4, for each one of the residues tested, compared to the respective mineralization kinetics. Concerning municipal solid waste, pulp-mill sludge, and the hornmeal, the amounts of N determined by this method correspond to the mineralization potential during the first 3 weeks of incubation. If the residue considered is poultry manure, this method only extracts the amount of N corresponding to the potential N mineralization during the first 38 days of incubation. Finally, for swine-originated residues in particular (SP and C), method 4 allowed the extraction of sufficient N to surpass the potentially mineralizable N during all the period of incubation (Figure 2).

Therefore, this method seems to be a reliable way of evaluating residue N availability, as long as the basal $N_{\min}$ amount is not subtracted, meaning that potentially available N is to be considered instead of potentially mineralizable N. Thus, it can be a promising method of predicting N availability from the soil as well as from the residues applied to the soil. In fact, Rodrigues (2000) had

Figure 2. Amount of $N_{\min}$ determined by method 4, in each kg of residue tested, and respective net apparent mineralization kinetics curves.

reached the same conclusion after testing this method to a soil supplemented with poultry manure, cattle manure, or composted MSW to potato crop.

Whenever the samples were boiled for 1 h with the same solution (method 5), the correlation of the results obtained with PAN was weak (Table 3). Although the temperature was the same as in method 4, extraction time was lower. This findings agree with those of Gianello and Bremner (1986). In contrast, Hatch, Jarris and Reynolds (1991) as well as Whitehead (2000) have considered this methodology an interesting tool in net N mineralization during a ryegrass crop cycle. Also Smith and Li (1993), after testing several combinations of extraction time, temperature, and solution concentration, came to the conclusion that 1 h of boiling 2 M KCl was the best combination to predict PMN.

By raising extraction time to 20 h and lowering temperature to 80°C (method 6), the correlation obtained was greater than for method 5 (Table 3), although not as good as the one previously obtained by Øien and Selmer-Olsen (1980) and Selmer-Olsen et al. (1981). These authors found a good correlation ($r^2 = 0.96$) between the value of N extracted by this method from 43 different soils and the respective incubation during 14 days at 30°C.

In contrast, this extraction method did not correlate with the amounts of N mineralized during an aerobic incubation of 33 soils during 2 weeks (González-Prieto et al. 1994).

In fact, in this work, the best method for N mineralization prediction was method 4. Moreover, it is a simple and quick method suitable for routine use.

Methods 7, 8, and 11

When N was extracted with a cold solution of 0.01 M CaCl_2 for 2 h (method 7) (Houba et al. 1986), correlation was poor (Table 3) when data was correlated

to N_0 obtained by model fitting. This was probably because the suspensions were not digested and thus did not extract the organic N present in the soil. However, Houba et al. (1986) and Appel and Mengel (1990) have pointed out the extraction with 0.01 M CaCl_2 as an effective one. Houba et al. (1986) have referred to this cold extraction as one very well suited for determination of PMN. In addition to $N_{\min}$ fractions, and when digestion is performed, this method also allows the extraction of soluble organic N present in SOM, which is potentially available for plant nutrition.

However, Velthof et al. (1998) tested the same method on the prediction of N mineralization from different organic residues and concluded that it can only be used as a measure of estimating the PAN from residues with a high $N_{\min}$ content. Also, Appel and Mengel (1990, 1992) and Goss, Forkes, and Smith (2002) point out this method is incapable of predicting the PMN, because only the mineral N is extracted during the procedure used.

For this reason, Appel and Mengel (1990) proposed a modification to this method that consists in a second extraction at 80°C during 20 min (method 8). After comparing it to the N uptake by oilseed (*Brassica napus* cv. Akella), grown in pots in a sandy soil, they concluded that it correlated well and therefore was a good predictor of net N mineralization in sandy soils. The authors consider that the N fraction extracted at 80°C corresponds to the exchange between the saline solution and polypeptides adsorbed to clay minerals. In fact, later the same authors (Appel and Mengel 1998), after analyzing the extracts obtained with this solution, concluded that 50% of the N present was easily mineralizable amino- NH_2 . Among all the studies carried out by these two authors, they claim that this methodology is the best correlated to PAN from soils that have been dried prior to analysis (Appel and Mengel 1998). The same was reported by Goss, Forkes, and Smith (2002).

Although Appel and Mengel (1990, 1992, 1993, 1998) have pointed out several times that method 8 was a good tool for predicting N mineralization from SOM, that was not the case in the present experiment. In fact, results obtained with method 8 and N_0 calculated by model fitting were only fairly correlated and similar to those of method 7 (cold extraction) ($P < 0.95$) (Table 3). Moreover, like observations before for method 4, the subtraction of the initial $N_{\min}$ value lowered the correlation value (Table 3).

This method was also tested by Goss, Forkes, and Smith (2002) to study the mineralization of N from four different manures, with little success. However, the comparison of a 28-day aerobic incubation data and this method, to predict N mineralization from mixtures of three soils with three different manures, was promising ($r^2 = 0.93$).

The present work and the findings of Goss, Forkes, and Smith (2002), lead us to the conclusion that method 8 is not reliable for routine prediction of N mineralization from sandy soil and organic residues mixtures. Because it did not involve digestion, this method only allowed the extraction of initial $N_{\min}$ and N hydrolized during heating of the sample.

Figure 3. Relation between the amount of N extracted by method 11 and potentially available N from the residues tested.

Finally, if the results obtained by performing a boiling 0.01 M CaCl_2 reflux extraction of the N in the samples (method 11) were examined, a good correlation was obtained between this data and N_0 value obtained by fitting the Stanford and Smith model to the incubation results (Figure 3; Table 3). In fact, this procedure allowed the extraction of soluble N_{org} from the extract, because of the digestion of the samples, thus permitting the determination of greater amounts of N than method 8.

Fox and Piekielek (1978), who had obtained promising results after testing this method, did find better correlations with a refluxed CaCl_2 extraction than with an autoclaved procedure using the same solution. Method 11 was tested in this work to try to find an alternative to the popular autoclave procedure. According to the results obtained, it was determined that this procedure, opposite to the autoclave method, can be used in routine analysis for N mineralization prediction.

Method 9

The chemical method involving the extraction of N with a 0.5 M K_2SO_4 solution could not be considered a good mean of predicting N mineralization from the residues tested, as the correlation with N_0 calculated in model fitting was only fair (Table 3), probably because digestion was not performed.

Serrasolsas and Khanna (1995) have associated this chemical extraction with the labile N fraction present in the SOM. Also, Mulvaney (1996) pointed out that this solution allows only the extraction of the exchangeable NH_4^+ , as well as method 1. In contrast, Goss, Forkes, and Smith (2002) concluded that this chemical method extracts only 68.5% of the total amount of N extracted by method 1.

Method 10

The correlation obtained between the data from the incubation and the amounts of N extracted with cold distilled water was very poor (Table 3). Again, the N fraction extracted by this method was the mineral fraction. It

has been stated before that the extract digestion is the only means of obtaining N_{org} from the soil, thus allowing a better prediction of PMN.

Water-soluble N fraction, consisting of low-molecular-weight compounds, is usually considered the most reactive fraction in the SOM, more easily and rapidly available to microorganisms. Jandl and Sollins (1997) state that this fraction of the dissolved OM is rapidly degraded, whereas the high-weight compounds are resistant to microbial attack and to digestion. This is a scarce OM fraction and is buffered by adsorption, dissolution, and root exudation mechanisms (McGill et al. 1986; Curtin and Wen 1999). It is able to mineralize according to the agricultural practices used. Although Groot and Houba (1995) consider this fraction as a valuable indicator of N mineralization, not all agree with this statement. Because of the high C/N ratio of this low-molecular-weight fraction, mineralization can be rapidly followed by immobilization, thus having a poor contribution to net mineralization. Boone (1994) even believes that this type of compound only contributes to 2 to 13% of total mineralized N.

In contrast, Qafoku et al. (2001) concluded that N_0 obtained by fitting the Stanford and Smith (1972) model to N mineralization data obtained in a 112-day and $25 \pm 2^\circ\text{C}$ aerobic incubation of a sandy soil mixed with poultry manure was well correlated to the value of water-soluble N_{org} ($r^2 = 0.87$). Therefore, these authors concluded that the determination of N after extraction with water can be a useful tool for PMN prediction from poultry manure.

Method 12

The phosphate-borate method was only fairly correlated with biological method data (N_0) (Table 3).

Curtin and Wen (1999), however, concluded that this method was a bad indicator of the N_0 of 61 different soils but was well correlated with the amounts of N mineralized during the first 2 weeks of an aerobic incubation. Moreover, they claim that phosphate–borate solution is a powerful SOM extractant, considering that this solution extracts labile organic N from minerals' surface as well as soluble N_{org} . Other authors have also evaluated the present method, concluding that the amounts of N_{min} correlated with N mineralized during the aerobic incubation of 63 soils during 24 weeks ($r^2 = 0.77$) and with canola uptake during a greenhouse experiment (Ila'ava and Waring 1992; Jalil et al. 1996). However, others have found this method to be only fairly correlated to results obtained with maize or potato crops (Hong, Fox, and Piekielek 1990; Rodrigues 2000) or several different crops grown in 123 different soils (Xu et al., 1996). The latter authors also performed aerobic incubations of the same 123 soils and once again phosphate–borate solution did not prove to be efficient in PAN prediction. Rodrigues (2000) even points out this method as incapable of hydrolizing urea, although it extracts biologically active N from organic fertilizers.

On the contrary, Gianello and Bremner (1986, 1988) found good correlations ($0.81 \leq r^2 \leq 0.96$) between this chemical method and N mineralized during anaerobic and aerobic incubation of 30 different soils.

Based on the results presented, it is possible to draw the following conclusions:

Among the 12 methods tested, only 2 of them were well correlated with the biological incubation performed. These were methods 4 and 11, corresponding respectively to hot KCl and CaCl_2 extractions.

Moreover, cold saline solutions, although presenting good correlations in the present work, only allowed the extraction of $N_{\min}$ present in the organic residues at the beginning of the experiment, as well as $N_{\min}$ in the soil solution and exchangeable N immediately available for plant nutrition. In fact, except for method 4, all the other methods where digestion is not performed are not promising in terms of PMN prediction, because they do not include N_{org} in the extracts obtained.

Whenever the same solutions were heated, and depending on the temperature and time of extraction, variable amounts of organic N were hydrolyzed and determined. This fraction corresponds to more labile mineralizable organic N.

For 2 M KCl solution, the most efficient temperature to determine PMN seemed to have been 100°C , with an extraction time of 4 h. In fact, method 4 seems to allow the prediction of N mineralized during the first 3 weeks of incubation of the organic residues studied.

Methods 4 and 11 appeared to be the most efficient ones and seemed to adapt well to PMN/PAN prediction from organic residues and not only from SOM, as has been suggested by several authors in the past. It can also be concluded from the results that it is not useful to subtract the value of initial $N_{\min}$ in the residues, but PAN should be considered instead of PMN when using the referred methods to predict N mineralization.

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