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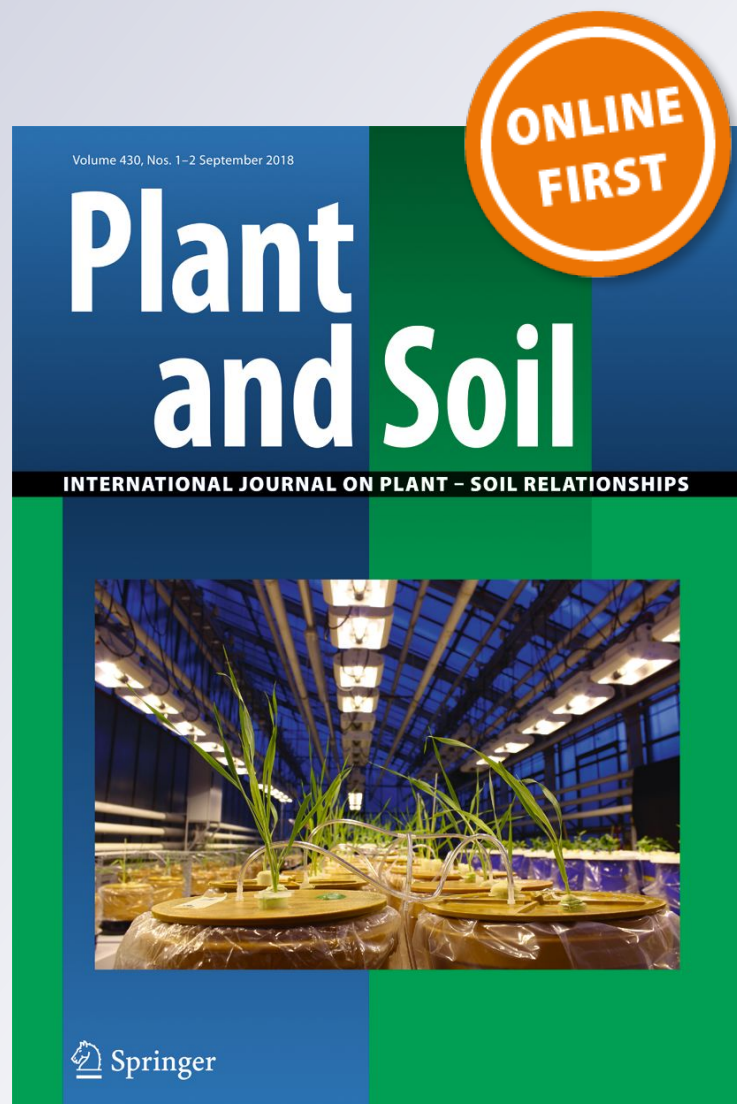
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Fertilization strategy can affect the estimation of soil nitrogen mineralization potential with chemical methods

Nicolas Wyngaard [iD] · **M. L. Cabrera** · **A. Shober** ·
R. Kanwar

Abstract
Aims Our objective was to evaluate if the relationship between quick indices of mineralizable N and soil N mineralization potential (N_o) is affected by long-term fertilization with inorganic fertilizers (IF) and/or poultry litter (PL).
Methods Samples from four long-term research sites that were fertilized with IF, IF+PL, or PL (>10 years) were aerobically incubated for 24 weeks to determine N_o. Soil N_o was then related to the results from the Illinois soil test analysis (ISNT), extractions with hot KCl, NaOH, or phosphate-borate (PB), and an anaerobic incubation (N_{an}).

Results All evaluated chemical methods were correlated with N_o ($R^2 > 0.59$). However, the models to describe the association between the chemical methods and N_o differed depending on the fertilizer treatment, due to the inability of chemical indices to estimate the N_o increase observed in some PL-amended soils. In contrast, the relationship between N_{an} and N_o was not affected by fertilization strategy.
Conclusions Chemical indices can lead to the underestimation of soil N mineralization potential in PL-treated soils when the indices are calibrated in IF-amended soils, whereas the biological method N_{an} predicted N_o independently from the field's fertilization history.

N. Wyngaard (✉)
Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Ruta 226 km 73.5, C.C. 276, 7620 Balcarce, Argentina
e-mail: nicowyngaard@hotmail.com

N. Wyngaard
CONICET, Moreno 3527, Mar del Plata, 7600 Buenos Aires, Argentina

M. L. Cabrera
Crop and Soil Sciences Department, University of Georgia, 120 Carlton St, Athens, GA, USA

A. Shober
Plant and Soil Sciences Department, University of Delaware, 531 South College Ave, Newark, DE, USA

R. Kanwar
Department of Agricultural and Bio-systems Engineering, Iowa State University, Ames, IA, USA

Abbreviations

CF	Coarse fraction
Cold_KCl-N	Initial KCl-extractable ammonium
C_t	Total carbon
Hot_KCl-N	Hot KCl-extractable ammonium
Hyd_KCl-N	Hydrolyzable N
IF	Inorganic fertilizer
ISNT	Illinois soil nitrogen test
N_{an}	Anaerobic N mineralization
N_o	Potentially mineralizable N
NaOH-N	NaOH distillable N
N_t	Total nitrogen
PB	Phosphate-borate extractable N
PB-N	Phosphate-borate distillable N

| PL | Poultry litter |
| UAN | Urea-ammonium nitrate fertilizer |

Introduction

The application of fertilizer nitrogen (N) is frequently needed to increase yields because N is a commonly deficient nutrient in cropping systems (Havlin et al. 2005). However, to avoid over- or under-application of N, the rate of fertilizer N should be adjusted to account for the soil's capacity to supply N through mineralization (Zebarth et al. 1999; St. Luce et al. 2011; Hirzel and Rodríguez 2017). Stanford and Smith (1972) recommended the use of a long-term incubation to determine the N mineralization potential of the soil (N_o) as well as its first-order rate constant of mineralization (k). These parameters (N_o and k) are then used to estimate N mineralization under field conditions, with consideration of soil temperature and water content (Stanford and Smith 1972). This incubation based estimation of N_o is considered to be the standard method to measure the soil's potential to mineralize N (St. Luce et al. 2011). Plus, interest in adopting this incubation method is increasing, especially in areas with strong regulations to control groundwater contamination with nitrate derived from excess fertilizer N applications (Heumann et al. 2011a, 2011b). As such, several researchers have evaluated this incubation method under field conditions. While some researchers observed promising results (Delphi 2000; Zinati et al. 2007; Heumann et al. 2011a), other evaluations of N_o as a predictor of field N mineralization resulted in overestimation (Cabrera and Kissel 1988) or underestimation of mineralized N (Campbell et al. 1988; Egelkraut et al. 2003).

One of the difficulties in implementing the N_o approach for nutrient management is the requirement of a long-term incubation (180–240 days) of soil samples, which is unsuitable for routine analysis in soil testing labs. Consequently, many studies have been conducted to identify quick indices (based on biological or chemical principles) that can accurately estimate N_o (Table 1; Jalil et al. 1996; Schomberg et al. 2009; Sharifi et al. 2007). Biological indices quantify the amount of inorganic N released due to microbial activity under controlled temperature and moisture conditions, whereas chemical indices quantify a soil fraction that is related to N mineralization. One commonly used biological index is a short-term (1 week) soil incubation that is conducted under waterlogged conditions at 40 °C (N_{an}) (Waring and Bremner 1964).

Soil total-N (N_t) and total organic carbon (C_t) concentrations have been used as indicators of N mineralization potential with mixed results (Bengtsson et al. 2003; Franzluebbers et al. 1994; Selles et al. 1999). Also, N_t concentration in the coarse fraction (CF) (>53 μm) of the soil, as obtained by physical fractionation (Cambardella and Elliott 1992), was related previously to N_o (Schomberg et al. 2009). These authors observed that even if the CF is meant to be more labile than the whole soil, the association between N_o and N_t-CF was lower than that observed with N_t ($r^2 = 0.77$ and 0.88, respectively).

Chemical extractions including hot KCl (Gianello and Bremner 1986), NaOH (Sharifi et al. 2007), phosphate-borate (PB) (Gianello and Bremner 1988), and the Illinois soil N test (ISNT) (Khan et al. 2001) measure the amount of specific soil organic N fractions that can be easily mineralized (Mulvaney et al. 2001). These chemical indices are based on empirical approximations, i.e., they rely on the measurement of a labile N

Table 1 Description of methods used to measure or estimate nitrogen mineralization potential

Method	Abbreviation	Type	Reference
Long-term aerobic incubation	N_o	Biological	Stanford and Smith (1972)
Anaerobic incubation	N_{an}	Biological	Waring and Bremner (1964)
Hot KCl extractable N	Hot_KCl-N	Chemical (Extraction)	Gianello and Bremner (1986)
Hydrolysable N	Hyd_KCl-N	Chemical (Extraction)	Gianello and Bremner (1986)
Illinois soil nitrogen test	ISNT	Chemical (Extraction)	Khan et al. (2001)
NaOH distillable N	NaOH-N	Chemical (Distillation)	Sharifi et al. (2007)
Phosphate-borate distillable N	PB-N	Chemical (Distillation)	Gianello and Bremner (1988)

Plant Soil

pool, which is assumed to be linearly associated with N_o. Therefore, any management practice that modifies the relative composition of the labile N pool may affect the results of a chemical extraction method, leading to an over- or under-estimation of N_o.

Poultry litter (PL), which is a mixture of excreta, feathers, feed, and organic bedding material, is commonly applied to pastures and crops in areas with intensive poultry production. The long-term land application of PL can affect the relative composition of organic-N compounds in the soil (He et al. 2014; Ju et al. 2006). Previously, researchers found that long-term application of PL can increase the soil N mineralization rate (as measured by 90-d aerobic incubations) of a sandy loam soil due to the accumulation of N_t (Watts et al. 2010). However, it remains unknown how long-term application of PL affects quick N mineralization indices, especially as compared to soils with a history of commercial inorganic fertilizer (IF) application. The objective of this study was to determine if the relationships between quick N mineralization indices and N_o is affected in soils with different N fertilization strategies (e.g., PL and/or IF).

Materials and methods

Sample collection

In 2012, composite soil samples (0 to 20 cm) were collected from four long-term experimental sites located in Delaware (39°40′13″N 75°45′10″W, Hapludult, Woodstown series) (Soil Survey Staff 2017), Maryland (39°10′42″N 76°10′58″W, Hapludult, Mattapex series), Iowa (42°01′22″N 93°46′35″W, Hapludoll, Nicollet/Webster series), and Georgia (33°24′04″N 83°29′33″W, Hapludult, Cecil series). At each site, there was a study that evaluated long-term (>10 years) effects of PL and/or IF treatments on soil properties. The IF treatments were applied in the form of urea-ammonium nitrate solution (UAN) or ammonium nitrate (Table 2). The rate of N applied as IF was calculated on the basis of soil tests to obtain the maximum yield in each crop. At Iowa and Georgia, the PL rate (Table 2) was calculated based on crops N requirements, N soil tests, and PL analysis. Plant available N in PL was estimated as the mineral N plus the easily mineralizable N (i.e., 50% of the total organic N) based on manure analysis (Qafoku et al. 2001). At Delaware and Maryland, PL rate was determined on a phosphorus (P) – based strategy, this is to say, to satisfy crops P demand. The PL used in our study was obtained from commercial broiler houses located near each of the study sites. When necessary, all other nutrients (P, sulphur (S), and potassium (K)) were applied at rates sufficient to ensure an optimum availability for crops.

At all sites, the experimental arrangement was a randomized complete block design with three replications and two treatments: IF and PL (Iowa and Georgia) or IF and IF+PL (Delaware and Maryland). Plot size was 55 m^2 in Delaware and Maryland, 400 m^2 in Iowa, and 7600 m^2 in Georgia. Delaware and Maryland sites

Table 2 Inorganic fertilizer (IF) and poultry litter (PL) application rates at four long-term experimental sites, including the characterization of nitrogen (N) and carbon (C) concentration in the PL, and the cumulative amount of N and C applied as IF or PL throughout the experiment

Site	Treatment	IF fertilizer	IF rate kg ha^{-1} year^{-1}	Cumulative N as IF* kg ha^{-1} N	PL rate Mg ha^{-1} year^{-1}	C in PL g kg^{-1}	N in PL g kg^{-1}	Cumulative N as PL* kg ha^{-1} N	Cumulative C as PL* Mg ha^{-1} C
Maryland	IF	UAN	181	2356	–	–	–	–	–
	IF+PL	UAN	181	2356	3.1	491.3	27.4	1108	19.8
Delaware	IF	UAN	138	1796	–	–	–	–	–
	IF+PL	UAN	138	1796	3.3	545.5	27.4	1177	23.4
Iowa	IF	UAN	168	2352	–	–	–	–	–
	PL	–	–	–	9.2	386.6	22.4	2884	49.8
Georgia	IF	AN	152	2280	–	–	–	–	–
	PL	–	–	–	7.8	379.5	21.8	2550	44.4

*Cumulative amount of N as IF was calculated from the yearly N rate and the total of years of IF application (Table 3)

*Cumulative amount of N and C as PL was calculated using the yearly amount of PL applied, the N and C concentration in PL, and the total of years of PL application (Table 3)

were initially managed under corn (*Zea mays* L.) mono-culture (2000–2005) and were later switched to a corn - soybean (*Glycine max* L.) rotation (2005–2012); ground was fallow between crops. The preceding crop to soil sampling was soybean in Delaware and corn in Maryland. The treatments (IF or IF+PL) were applied to these field plots by surface broadcast and then incorporated into the soil within 24 h by tilling (disc harrow) to a depth of approximately 20 cm; IF and IF+PL rates are described in Table 2. The Iowa site was always managed under a corn - soybean rotation with the soil remaining fallow between crops. The preceding crop to soil sampling was corn. The fertilizers (IF or PL) were surface broadcasted at rates as described in Table 2, and then incorporated into the soil within 24 h by tilling to a depth of approximately 20 cm (disc harrow). The Georgia site was managed as a tall fescue (*Festuca arundinacea* Schreb.) - bermudagrass (*Cynodon dactylon* L.) pasture. Each year, two steers of approximately 400 kg grazed each plot (2.6 steer ha^{-1}) for 6 months, while the rest of the year the pasture was used to produce hay. The treatments at this site (IF or PL) were applied by surface broadcast. A summary of the soil characteristics and management practices at each site can be found in Table 3.

Three composite soil samples were taken from the upper 20 cm of each plot almost 1 year after the last fertilizer application. These samples were composed of at least ten random soil cores (5 cm diameter, 20 cm long). Soil samples were air-dried, ground to pass a 2-mm sieve, and all recognizable plant tissue was removed. The soil sampling depth is representative of

the average soil depth used for various quick N mineralization methods used to estimate N availability for crops, as described by Lawrence et al. 2009 (0–20 cm), Mulvaney et al. 2006 (0–30 cm), Reussi Calvo et al. 2018 (0–20 cm), Vanotti et al. 1995 (0–30 cm), Nayyar et al. 2006 (0–15 cm), and Hong et al. 1990 (0–20 cm).

Sample analyses

Soil pH (1:2 soil/water) and particle size distribution (Bouyoucos 1962, as described by Gee and Bauder 1986) were determined at soil samples. Soil C_t and N_t concentrations were measured by dry combustion (LECO 2014). To facilitate comparison of results, changes in C_t and N_t concentrations (g kg^{-1}) between fertilization treatments were also expressed on an area basis (kg ha^{-1}) using bulk density values previously described for these soils (Wyngaard and Cabrera 2015). Additionally, soil samples were physically fractionated by wet sieving (53-μm sieve) after shaking overnight with 0.33 M sodium hexametaphosphate (Cambardella and Elliott 1992). The fraction that remained on top of the sieve was considered the CF. After drying and milling, total C_t and N_t in the CF were determined (C_t-CF and N_t-CF, respectively) as previously described for non-fractionated samples.

A long-term aerobic incubation was conducted on each soil sample in triplicate to determine N_o as described by Stanford and Smith (1972). Briefly, 20 g of soil was mixed with 20 g of 1-mm diameter acid-washed sand and the mixture was transferred to a 60-mL

Table 3 Selected soil properties (0–20 cm depth) at four experimental sites that received long-term applications (>10 years) with inorganic fertilizer (IF) and/or poultry litter (PL)

Site	Treatment	Clay g kg^{-1}	Silt g kg^{-1}	Sand g kg^{-1}	pH	Years	Soil use
Delaware	IF	145	35	820	6.1	13	Corn - Soybean
	IF+PL	145	35	820	6.1	13	Corn - Soybean
Maryland	IF	218	621	161	6.0	13	Corn - Soybean
	IF+PL	218	621	161	6.3	13	Corn - Soybean
Iowa	IF	292	288	420	5.5 b	14	Corn - Soybean
	PL	292	288	420	7.2 a	14	Corn - Soybean
Georgia	IF	399	165	436	5.5 b	15	Pasture
	PL	399	165	436	6.1 a	15	Pasture

Different letters indicate significant ($p < 0.05$) differences between treatments for each site, as indicated by the Tukey-Kramer test

CV% = variation coefficient (%)

polypropylene syringe with glass wool at the bottom. The soil-sand mixture was moistened with deionized water to 80% field capacity (estimated as the water content in mixed soil-sand samples after saturating them and letting them drain for 2 days) and then leached with 200 mL of 0.01 M $CaCl_2$, applied in 5-mL increments, to remove the initial NH_4^+ and NO_3^-. Excess water was then removed from the soil-sand mixture by vacuum filtration at 6 kPa. After vacuum filtration, the polypropylene syringes were covered with a porous membrane and incubated at 30 °C for 24 weeks, adjusting the water content to 80% of field capacity every 3 days. Samples were leached with a 0.01 M $CaCl_2$ solution biweekly. The concentration of $NO_2 + NO_3^-$-N in the leachate was determined by the Griess-Ilosvay technique (Keeney and Nelson 1982) and the concentration of NH_4^+-N was determined by the salicylate-hypochlorite method (Crooke and Simpson 1971). For estimating N_o, inorganic N concentrations from each leaching event were fitted to a first-order model: $N_m = N_o$ x $(1-e^{-kt})$, where N_m is the cumulative amount of N mineralized ($NH_4^+ + NO_2^- + NO_3^-$, mg N kg^{-1}) at a specific time (t, d), and k (d^{-1}) is the first-order rate constant.

Additionally, we evaluated the short-term anaerobic soil incubation (N_{an}) as a method to estimate N_o (Waring and Bremner 1964 as described by Keeney 1982). In brief, 10 g soil was incubated in a stoppered tube filled with water for 7 days at 40 °C. After the incubation period, N in the resulting slurry was extracted with 30 mL of a 4 M KCl solution and steam-distilled as described previously. Ammonia in the distillate was trapped in a mixed boric acid and indicator solution, which was then titrated with sulfuric acid. The N_{an} was determined by subtracting the initial NH_4^+-N content in the soil samples from the concentration of NH_4^+ determined after the incubation.

Four different chemical indices for estimating potentially mineralizable N (N_o) were evaluated: KCl extractable N (Cold_KCl-N; Keeney 1982), hot KCl extractable N (Hot_KCl-N, Gianello and Bremner 1986), hyrdolyzable N (Hyd_N; Gianello and Bremner 1986), and ISNT (Khan et al. 2001). The Cold_KCl-N was extracted by shaking 3 g soil with 20 mL of 2 M KCl for 1 h. After centrifugation, NH_4^+-N concentration in the extract was determined by the salicylate-hypochlorite method using a rapid flow analyzer (ALPKEM RFA 300-series) (Crooke and Simpson 1971). To determine Hot_KCl-N, 3 g soil was heated in a closed tube with 20 mL of 2 M KCl solution in a water bath at 100 °C for 4 h. The NH_4^+-N concentration in the extract was determined as for Cold_KCl-N. Hydrolyzable N was determined by subtraction, where Hyd_KCl-N = Hot_KCl-N – Cold_KCl-N. For the ISNT method (Khan et al. 2001), 1 g soil was treated with 10 mL of 2 M NaOH and heated on a hot plate at 50 °C for 5 h in a wide-mouth, 0.9-L Mason jar with a H_3BO_3-indicator trap. The NH_3 collected in the trap was then quantified by acidimetric titration.

Two distillation methods were also evaluated: NaOH (NaOH-N; Sharifi et al. 2007) and phosphate borate (PB-N; Gianello and Bremner 1988). In each of the distillation methods, 5 g of soil was placed in a steam-distiller with one of two alkaline extractants: a) 50 mL of a 50 g 100 mL^{-1} NaOH solution (NaOH-N) (Sharifi et al. 2007) or b) 50 mL of a pH 11.2 phosphate-borate solution (PB-N; composed of 0.02 M Na_3PO_4 and 0.005 M $Na_2B_4O_7$; Gianello and Bremner 1988). Ammonia in the distillate was trapped in a mixed boric acid and indicator solution, which was then titrated with sulfuric acid.

Statistical analyses

As fertilization treatments were different depending on the site, the effects of fertilization over the studied variables were evaluated separately for each site using the SAS PROC GLM procedure (SAS Institute Inc. 2013). The normality of distribution was confirmed using the Shapiro and Wilk (1965) procedure, while the homogeneity of variances was confirmed using the Levene (1960) test. Effects were considered statistically significant at $p < 0.05$ and means were compared using the Tukey-Kramer test. The exponential model to determine N_o was fit using SAS PROC NLIN. To determine the association between soil variables or methods to estimate N mineralization and N_o, linear and quadratic models were evaluated, and selected based on the significance of the quadratic term ($p < 0.05$). A residual sum of squares analysis (Milliken and Debruin 1978) was used to determine if, for each method, the models used to predict N_o in soils receiving IF, IF+PL, or PL treatments were different from a single, common model incorporating all treatments. When a single model was not significant, all combinations of treatments were tested against each other by the same statistical procedure.

Results

Soil pH, C_t, and N_t concentrations in soil

The PL-amended soils from Iowa and Georgia had higher soil pH than the IF amended soils (+31 and 11%, respectively) (Table 3). In contrast, the pH of soils receiving the IF + PL treatments as part of the Delaware and Maryland studies did not differ from soils receiving IF.

The PL-amended soils had greater C_t concentration relative to IF plots in samples collected from Iowa and Georgia (+2.2 and + 4.5 g kg^{-1}, respectively), but no difference was observed between soils receiving PL + IF or IF treatments from Delaware and Maryland (Table 4). Considering the results on an area basis, the difference in C_t between PL- (with or without IF) and IF-amended soils at treatments at the 0–20 cm depth was −0.2, 0.4, 5.4, and 11.0 Mg ha^{-1} in Delaware, Maryland, Iowa, and Georgia, respectively (data not shown). These values represent −1.0, 1.7, 10.8, and 24.7%, respectively, of the C_t applied as PL during the experiment. The C_t difference between treatments at each site was closely associated with soil clay content (Table 3): ΔC_t (PL - IF, g kg^{-1}) = 0.0495 × clay content (g g^{-1}) + 0.167 (r^2 = 0.94; p = 0.031; n = 12).

We also observed a greater N_t concentration in PL-amended soils compared to soils receiving only IF in Maryland (+0.22 g kg^{-1}), Iowa (+0.38 g kg^{-1}), and Georgia (+0.63 g kg^{-1}). The N_t difference between PL- (with or without IF) and IF-amended soils was 1, 22, 33, and 60% of the N_t applied as PL in Delaware, Maryland, Iowa, and Georgia, respectively. These values were also strongly associated with soil texture (Table 3): ΔN_t (PL - IF, g g^{-1}) = 0.4591 × clay content (g g^{-1}) + 0.1131 (r^2 = 0.99; p = 0.005; n = 12). The C_t-CF and N_t-CF were also affected by fertilization treatments (Table 4). While no difference between treatments was observed in Delaware and Maryland, the C_t-CF and N_t-CF contents were greater in PL-amended soils as compared to IF-amended soils from Iowa (+139% for C_t-CF and + 148% for N_t-CF) and Georgia (+39% for C_t-CF and + 97% for N_t-CF).

Nitrogen mineralization potential (N_o) in soils amended with IF, IF+PL, or PL

The cumulative amount of N mineralized during a 24-wk incubation experiment differed among sites and treatments (Fig. 1). The difference in N_o concentration (calculated by fitting the mineralization data to a first-order model) between soils receiving PL or IF+PL and soils receiving IF only was 2, 34, 126, and 233 mg kg^{-1} for Delaware, Maryland, Iowa, and Georgia, respectively (Table 5). However, this difference was only significant for soils from Iowa and Georgia. We observed a significant association between N_o and C_t or N_t across all sites (r^2 = 0.87 and 0.86 respectively; $p < 0.05$ in all cases) (Fig. 2). A similar, but weaker, association between C_t-CF or N_t-CF and N_o was observed across all sites (Fig. 2).

Quick indices of mineralizable N in soils amended with IF, IF+PL, or PL

Soil N concentrations, as determined using selected chemical or biological indices to estimate N_o, ranged from 2 to 10 mg kg^{-1} for Hot_KCl-N, 2 to 6 mg kg^{-1} for Hyd_KCl-N, 33 to 391 mg kg^{-1} for ISNT, 88 to 352 mg kg^{-1} for NaOH-N, 14 to 62 mg kg^{-1} for PB-N, and 35 and 172 mg kg^{-1} for N_{an} (Table 5). The proportion (%) of N_t extracted by these methods followed the trend: NaOH-N (11.2 ± 0.5) = ISNT (10.1 ± 2.9) > N_{an} (4.9 ± 0.7) > PB-N (2.1 ± 0.4) > Hot_KCl-N (0.25 ± 0.08) = Hyd_KCl-N (0.17 ± 0.04). All evaluated methods were somewhat sensitive to past fertilizer management, except for Hyd_KCl-N and ISNT (Table 5).

Table 4 Total carbon (Ct) and total nitrogen (Nt) of whole soil samples and coarse fraction (CF; >53 μm) samples from four soils after more than 10 years of fertilization with poultry litter (PL) and/or inorganic fertilizers (IF)

| Site | Treatment | Soil | | Coarse Fraction | |
		C_t g kg^{-1}	N_t g kg^{-1}	C_t-CF g kg^{-1}	N_t-CF mg kg^{-1}
Delaware	IF	12.4	0.76	3.6	89.4
	IF+PL	11.7	0.84	3.0	114.2
Maryland	IF	11.6	1.10 b	2.0	80.9
	IF+PL	13.4	1.32 a	2.5	132.6
Iowa	IF	23.0 b	1.98 b	2.3 b	151.9 b
	PL	25.2 a	2.36 a	5.5 a	377.0 a
Georgia	IF	26.4 b	2.48 b	5.3 b	256.9 b
	PL	30.9 a	3.11 a	7.4 a	506.7 a
	CV (%)	3.05	4.51	23.0	47.3

Different letters indicate significant ($p < 0.05$) differences between treatments for each site, as indicated by the Tukey-Kramer test. CV% = variation coefficient (%)
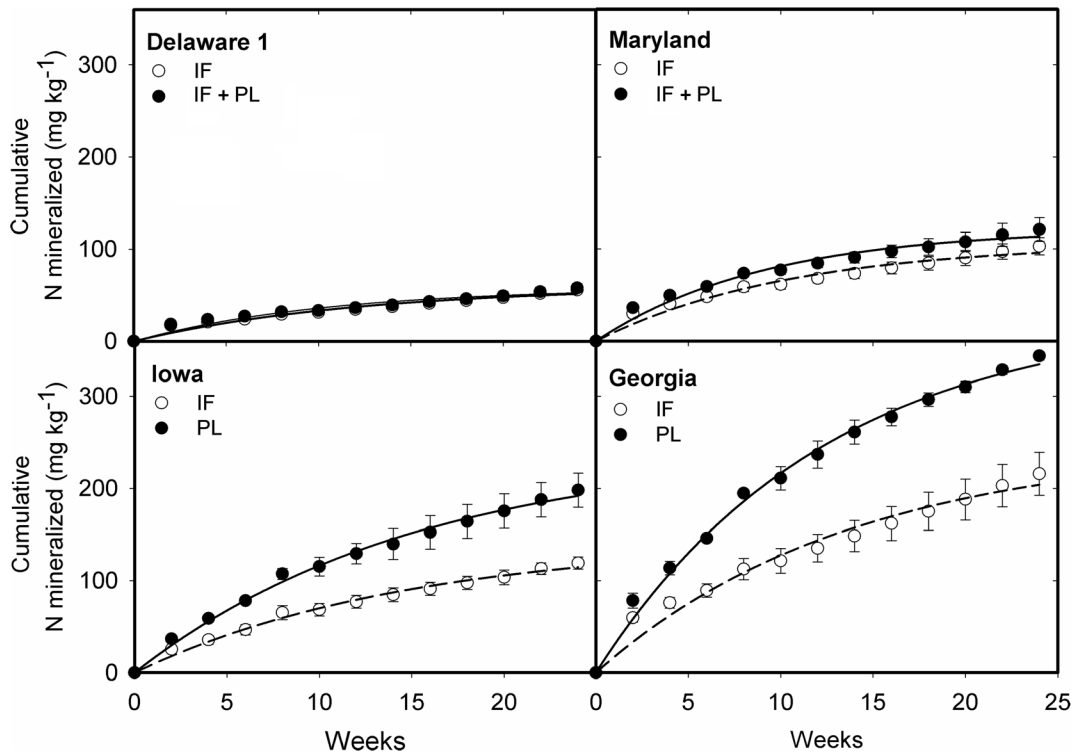
Fig. 1 Cumulative amount of N mineralized (N_m) in soils from four sites that were fertilized with poultry litter (PL) and/or inorganic fertilizer (IF) for more than 10 years and were incubated at $30 \pm 1\ °C$ for 24 weeks. Vertical bars represent the standard deviation among replications

However, only results of the N_{an} method followed the same trend to N_o, where the PL-amended soils from Iowa and Georgia had significantly greater N concentrations than soils receiving only IF (Table 5).

Table 5 Parameters for the first-order N mineralization model (N_o and k) and results from methods to estimate N mineralization in four soils after more than 10 years of fertilization with poultry litter (PL) and/or inorganic fertilizers (IF)

Site	Treatment	N_o mg kg^{-1}	k day^{-1}	Cold_KCl-N mg kg^{-1}	Hot_KCl-N	Hyd_KCl-N	ISNT	NaOH-N	PB-N	N_{an}
Delaware	IF	60.3	0.012	0.5	2.2	1.7	33.3	88.3	16.1	41.0
	IF+PL	62.1	0.014	0.6	2.4	1.8	67.7	87.2	14.4	34.5
Maryland	IF	107.7	0.014	0.6	2.4	1.8	111.8	121.8 b	19.5 b	60.6
	IF+PL	141.3	0.014	0.6	2.4	1.8	129.3	144.7 a	23.5 a	70.1
Iowa	IF	145.5 b	0.010	1.2 a	4.0	2.8	228.7	223.5 b	61.9 a	73.0 b
	PL	244.2 a	0.010	0.8 b	3.4	2.6	233.0	247.8 a	50.3 b	107.5 a
Georgia	IF	248.2 b	0.012	4.4 a	10.4 a	6.0	364.8	301.5 b	57.3	126.6 b
	PL	481.3 a	0.010	1.9 b	6.9 b	5.1	390.8	352.3 a	58.9	172.2 a
	CV (%)	12.6	23.6	3.8	7.5	10.9	11.6	2.6	3.2	4.4

Different letters indicate significant ($p < 0.05$) differences between treatments for each site as indicated by the Tukey-Kramer test. CV% = variation coefficient (%)

Abbreviations: N_o: potentially mineralizable N; k: N mineralization constant; Cold_KCl-N: NH_4^+-N extracted with 2 M KCl; Hot_KCl-N: NH_4^+-N extracted with 100 °C 2 M KCl; Hyd_KCl-N: (Hot_KCl-N) minus (Cold_KCl-N); ISNT: Illinois Soil Test Analysis; NaOH-N: NH_4^+-N extracted by distillation with NaOH (50%); PB-N: NH_4^+-N extracted by distillation with phosphate-borate buffer (pH = 11.2); N_{an}: NH_4^+-N measured after 7 days of incubations under waterlogged conditions

Fig. 2 Association between total carbon (C_t) or total nitrogen (N_t) in the whole soil and the coarse fraction (>53 µm) (CF) and the potentially mineralizable nitrogen (N_o) determined by long-term incubation (24 weeks) in four soils that had been fertilized with poultry litter (PL) and/or inorganic fertilizer (IF) for more than 10 years
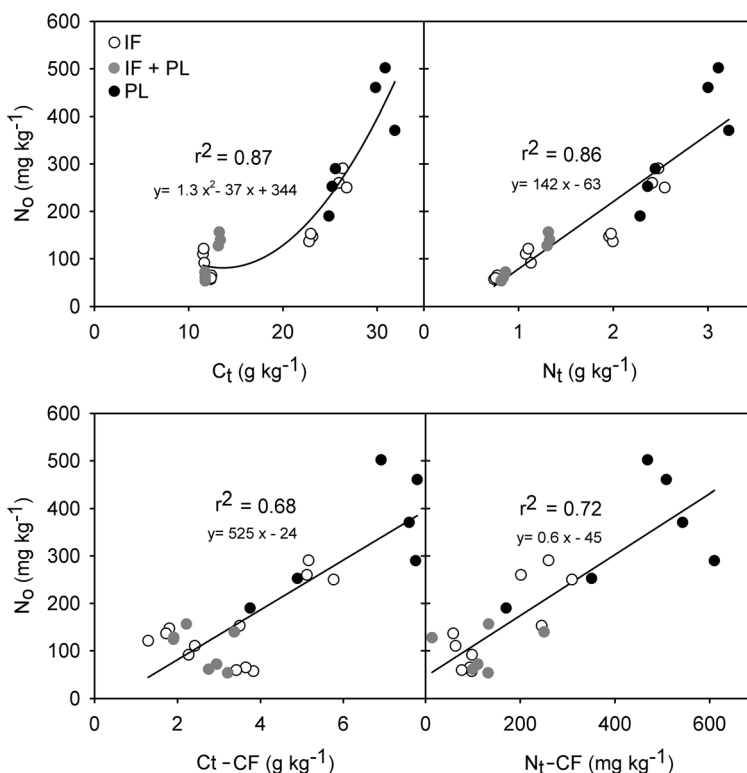
Across all sites, the percentage of N extracted relative to N_o averaged 1.9% for Hot_KCl-N, 1.1% for Hyd_KCl-N, 106% for ISNT, 120% for NaOH, and 52% for N_{an} (Table 6). The proportion of N_o accounted for by each specific index was also affected by fertilization history. In general, the proportion of N_o extracted

by chemical methods (extractions and distillations) from the Iowa and Georgia soils was smaller for soils receiving IF than for PL-amended soils. On the contrary, the proportion of N_o accounted for by N_{an} was not affected by fertilization history for any of the soils evaluated.

Table 6 Proportion of the potentially mineralizable N (N_o) extracted by different methods to estimate N mineralization in four soils after more than 10 years of fertilization with poultry litter (PL) and/or inorganic fertilizers (IF)

Site	Treatment	Cold_KCl-N	Hot_KCl-N	Hyd_KCl-N	ISNT	NaOH-N	PB-N	N_{an}
Delaware	IF	0.009	0.029	0.020	0.81	1.47	0.27	0.68
	IF+PL	0.010	0.028	0.018	0.86	1.42	0.24	0.57
Maryland	IF	0.006	0.017 a	0.011	1.14 a	1.14	0.18	0.57
	IF+PL	0.004	0.013 b	0.009	0.85 b	1.03	0.17	0.50
Iowa	IF	0.008 a	0.019 a	0.011	1.61 a	1.54 a	0.43 a	0.50
	PL	0.003 b	0.011 b	0.008	0.96 b	1.05 b	0.21 b	0.47
Georgia	IF	0.017 a	0.023 a	0.006 b	1.47 a	1.13 a	0.22 a	0.48
	PL	0.004 b	0.012 b	0.007 a	0.83 b	0.80 b	0.13 b	0.41

Mean comparison performed at each site

Abbreviations: Cold_KCl-N: NH_4^+-N extracted with 2 M KCl; Hot_KCl-N: NH_4^+-N extracted with 100 °C 2 M KCl; Hyd_KCl-N: (Hot_KCl-N) minus (Cold_KCl-N); ISNT: Illinois Soil Test Analysis; NaOH-N: NH_4^+-N extracted by distillation with NaOH (50%); PB-N: NH_4^+-N extracted by distillation with phosphate-borate buffer (pH = 11.2); N_{an}: NH_4^+-N measured after 7 days of incubations under waterlogged conditions

Residual sum of squares analysis for chemical methods resulted in the development of two separate empirical models to predict N_o depending on fertilization history (Table 7). For most of the chemical methods, two separate models were developed: IF and IF+PL or PL and IF+PL (Fig. 3). The determination coefficient for the association between chemical indices and N_o ranged from 0.59 to 0.97. On the contrary, residual sum of squares analysis indicated that a single empirical model was appropriate to predict N_o at IF-, PL-, or IF+PL-amended soils by the biological method, N_{an} ($R^2 = 0.94$).

Discussion

The increase in soil pH caused by animal manure application has been previously described (Hue 1992; Whalen et al. 2000; Wyngaard et al. 2016). Hue (1992) determined that 5 g PL per kg soil is equivalent to 3.4 $cmol_c$ kg^{-1} of Ca (OH)$_2$. In our study, the change in pH between IF and PL treatments was greater at greater rates of PL (Table 3). Also, at Delaware and Maryland the PL treatments also received IF. Consequently, at these sites the increase in soil pH caused by PL could have been tempered by the acidifying effect of

Table 7 Results from the residual sum of squares (SS) analysis to determine if N mineralization potential (N_o) can be estimated from laboratory methods, using a single regression model (independently from fertilization history) or not

Method	Comparison	Single model		Separate models			
		SS	df	SS	df	F-value	*p* value
Hot_KCl-N	All treatments	196,105	22	27,744	18	27.3	<0.001
	IF vs IF+PL	1633	16	16,017	14	0.1	0.905
	IF vs PL	180,719	16	17,900.5	14	63.7	<0.001
	PL vs IF+PL	31,088	10	21,571	8	1.8	0.215
Hyd_KCl-N	All treatments	140,122	22	27,934	18	18.1	<0.001
	IF vs IF+PL	1662	16	16,145	14	0.2	0.821
	IF vs PL	129,737	16	18,499	14	42.1	<0.001
	PL vs IF+PL	27,740	10	21,223	8	1.2	0.341
ISNT	All treatments	64,586	22	11,980	18	19.8	<0.001
	IF vs IF+PL	7510	16	5307	14	2.9	0.084
	IF vs PL	63,041	16	10,946	14	33.3	<0.001
	PL vs IF+PL	8453	10	7707	8	0.4	0.681
NaOH-N	All treatments	52,680	22	18,621	18	8.2	0.003
	IF vs IF+PL	9300	16	7008	14	2.3	0.132
	IF vs PL	49,867	16	18,310	14	12.1	0.006
	PL vs IF+PL	17,515	10	11,924	8	1.9	0.200
PB-N	All treatments	166,505	22	42,388	18	13.2	<0.001
	IF vs IF+PL	36,513	16	32,011	14	1.0	0.390
	IF vs PL	163,628	16	41,445	14	20.6	<0.001
	PL vs IF+PL	42,316	10	11,320	8	11.0	0.003
N_{an}	All treatments	22,236	22	17,273	18	1.3	0.307
	IF vs IF+PL	2740	16	2209	14	1.7	0.214
	IF vs PL	19,791	16	16,445	14	1.4	0.275
	PL vs IF+PL	16,939	10	15,892	8	0.3	0.747

For each comparison, a significant difference between the single model and the cumulative, individual models ($p < 0.05$) indicates differences in N_o prediction between fertilization strategies

Abbreviations: df: degrees of freedom; Hot_KCl-N: NH_4^+-N extracted with 100 °C 2 M KCl; Hyd_KCl-N: (Hot_KCl-N) minus NH_4^+-N extracted with 2 M KCl; ISNT: Illinois Soil Test Analysis; NaOH-N: NH_4^+-N extracted by distillation with NaOH (50%); PB-N: NH_4^+-N extracted by distillation with phosphate-borate buffer (pH = 11.2); N_{an}: NH_4^+-N measured after 7 days of incubations under waterlogged conditions
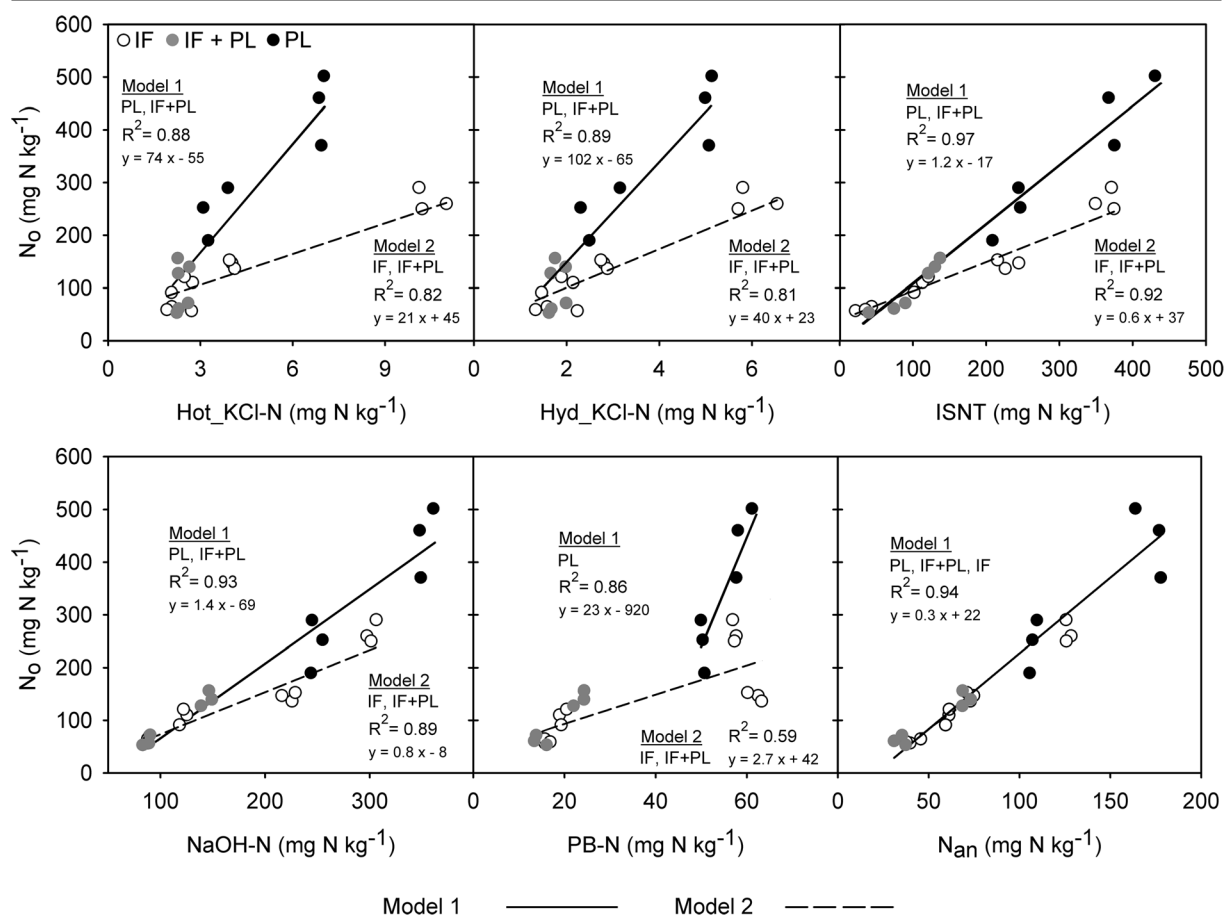
Fig. 3 Relationship between different quick methods to estimate N mineralization and the potentially mineralizable nitrogen (N_o) determined by long-term incubation (24 weeks) in soils that had been fertilized with poultry litter (PL) and/or inorganic fertilizer (IF) for more than 10 years. All shown regressions are significant at $p < 0.05$. Different models are presented based on the results from the residual sum of squares analysis (Table 7). Abbreviations:

Hot_KCl-N: NH_4^+-N extracted with 100 °C 2 M KCl; Hyd_KCl-N: hydrolysable N; ISNT: Illinois Soil Test Analysis; NaOH-N: NH_4^+-N extracted by distillation with NaOH (50%); PB-N: NH_4^+-N extracted by distillation with phosphate-borate buffer (pH = 11.2); N_{an}: NH_4^+-N measured after 7 days of incubations under waterlogged conditions

N applied as IF (Mahler et al. 1985; Barak et al. 1997; Liebig et al. 2002).

Differences in C_t and N_t concentration between soils receiving PL and IF applications were previously described (Adeli et al. 2007; Daigh et al. 2009; He et al. 2009; Kingery et al. 1994; Sharpley et al. 1993; Watts et al. 2010). These management-based differences in C_t and N_t are often explained by the reduced availability of the N in PL, where only approximately 55% of the N in PL is mineralized into a plant available form during the first year; and the remaining N is potentially lost or accumulates in the soil (Eghball et al. 2002). However, our study lacks baseline data to confirm that the greater C_t and N_t in PL-amended soils is caused by an accumulation of PL-derived organic matter. Therefore, we

speculate that the differences in C_t and N_t between soils receiving IF and PL treatments can be a consequence of: a) an accumulation of PL derived C_t and N_t in PL-amended soils (Eghball et al. 2002), or b) a decrease in C_t and N_t concentration in IF-fertilized soils caused by a priming effect (IF increasing the decomposition rate of the native soil organic matter) (Kuzyakov et al. 2000). The fact that C_t and N_t concentrations differed between fertilization treatments at some sites (Iowa and Georgia) but not at others (Delaware and Maryland for C_t and Delaware for N_t) can be a consequence of several factors. For example, 1) The unresponsive Delaware and Maryland sites received less cumulative C and N as PL than the Iowa and Georgia sites; 2) The PL-amended soils at the Delaware and Maryland were also fertilized

with IF, which may have accelerated the PL organic matter turnover by N priming effect (Kuzyakov et al. 2000), thereby limiting C_t and N_t accumulation; 3) The relationship we observed between clay content and the C_t or N_t difference between fertilization treatments may also help explain the contrasting results from the various sites, as soil fines play a key role in protecting organic matter against decomposition (Six et al. 2002). Therefore, the capacity of the soils from Delaware or Maryland (145 and 218 g kg^{-1} clay, respectively) to accumulate organic compounds derived from PL was inherently lower than for the soils from Iowa and Georgia (292 and 399 g kg^{-1} clay, respectively); 4) It should also be noted that the Georgia site, which exhibited the greatest C_t and N_t difference between fertilization treatments, was managed as an undisturbed pasture, whereas the other sites were managed under grain crops with some amount of tillage. Lower soil disturbance in the pasture system may have led to a lower decomposition rate and greater surface accumulation of the organic C and N applied as PL (Nyakatawa et al. 2001; Wyngaard et al. 2016); 5) The Georgia site received cattle manure. Considering a yearly animal excreta of 47 kg N (Smith and Frost 2000) and a C:N ratio of 18.6 (Whitehead 1995), the total amount of C and N deposited to plots managed under both fertilization treatments (IF and PL) in cattle manure was 17 Mg ha^{-1} and 915 kg ha^{-1}, respectively. However, it must be considered that nutrients in manure were recycled within the pasture, whereas the PL represents an input of new C and N to the system. Even if soil C and N inputs were not modified by the presence of animals, the distribution of N in labile organic pools may have been affected by cattle manure inputs. Along this line, researchers have reported increased concentrations of total hydrolysable N, amino-sugars, amino-acids, and acid insoluble-N in soils receiving long-term applications of pig manure (Huang et al. 2009; Zhang et al. 2014).

The greater relative difference between PL- and IF-amended soils in C_t-CF or N_t-CF than in C_t or N_t (non-fractionated samples) (Table 4) supports the idea of the CF being more dynamic and having a greater response to management practices than the whole soil (Janzen et al. 1998). For example, C_t-CF and N_t-CF were 39 and 97% greater in PL-amended Georgia soils than in IF-amended soils, respectively. In contrast, this difference was only 17% for C_t and 25% for N_t in the whole soil. However, even though the relative difference between treatments was numerically greater, the high variability of C_t-CF and

N_t-CF measurements (coefficient of variation 23 and 47%, respectively) limited the statistical significance of this difference. Consequently, the determination of N_t was statistically more sensitive to differences between treatments in the whole soil than in the CF.

The greater N_t content in PL-amended soils from Iowa and Georgia translated into a greater N mineralization potential (Fig. 2). Previously, researchers determined a similar relationship between N_t and N mineralization for 10 or more contrasting soils samples (Camargo et al. 2004; Schomberg et al. 2009; Sharifi et al. 2007); whereas other researchers observed only a weak association between N_t an N mineralization (Jalil et al. 1996; Selles et al. 1999; Springob and Kirchmann 2003). These contradictory results are often explained by considering that not only the quantity of soil N important to determine the mineralization rate, but also the quality of soil N (Springob and Kirchmann 2003).

In a two-years study, Kingery et al. (1996) determined a 4.8 mg kg^{-1} difference in the cumulative N mineralized between PL- and IF-amended soils after a 254-day incubation of a fine-sandy loam soil (0 to 10 cm samples). In a 90-day closed incubation study, Watts et al. (2010) also described differences in mineralized N from PL- and IF-amended sandy loam surface soils (0 to 5 cm), ranging from 4.1 to 49.4 mg kg^{-1}, depending on the tillage system and crop rotation. Our results confirmed that PL-amended soils from Iowa and Georgia had a greater N mineralization potential than soils receiving IF. In addition, fitting our data to a first-order model allowed for the estimation of the long-term effect of PL on N_o, which is a novel aspect of our work. As the determination of N_o concentration is considered the standard to describe N mineralization potential (Schomberg et al. 2009), its calculation in our study allowed the development of relationships between N_o and different methods to estimate N mineralization potential.

Among the chemical indices used to estimate N_o, Hot_KCl-N and Hyd_KCl-N values (Table 5) were within the range of N extracted determined by Curtin and Wen (1999), Sharifi et al. (2007), and Schomberg et al. (2009), but were lower than values from Jalil et al. (1996) and Wang et al. (2001) for soils from Canada and Autralia. The values we reported for ISNT (Table 5) were within the range of those determined by Khan et al. (2001) for a wide range of sites with contrasting soil texture, cropping, and tillage systems. Similarly, the proportion of N_t extracted by the two distillation methods agreed with the findings of Sharifi et al.

(2007), who determined an extraction percentage of N_t ranging from 12 to 21% using NaOH and from 2 to 9% using PB, on 153 contrasting soil samples from agricultural sites in Canada and United States. The greater N extraction capacity of NaOH over PB is explained by the higher pH of the former extractant. Finally, the N_{an} values we reported were similar to those obtained by Schomberg et al. (2009), with the exception of the Georgia soil, which exhibited greater N_{an} and N_o values. Schomberg et al. (2009) evaluated 11 soils, but only one was a pasture, with a N_{an} value (N_{an}: 115.7 mg kg^{-1}) similar to that in Georgia (N_{an}: 126 and 172 mg kg^{-1} in IF- and PL- amended soils, respectively). The greater value for N_{an} in the PL-amended soil from Georgia may result from the combination of the pasture system, which promotes organic matter accumulation, and the organic fertilization treatment.

The relationship between all chemical and biological methods to estimate N mineralization and N_o was significant (Fig. 3). However, the relationship between N_o and chemical estimators was different for PL-amended soils when compared to soils receiving only IF. For example, for an ISNT value of 375 mg kg^{-1}, the N_o value estimated using Model 2 (IF and IF+PL) was 300 mg kg^{-1}, whereas it was 460 mg kg^{-1} for Model 1 (PL and IF+PL). Consequently, predicting N_o for manure amended soils using a model calibrated for soils with a mineral fertilization history may result in the underestimation of the real mineralization potential of the manure-amended soils. This underestimation can be significant in areas with intensive animal production, where manure is commonly used as a nutrient source, or in organic farming systems, whose adoption has considerably increased during the past decade (Willer and Lernoud 2015). Contrary to the trend observed at Iowa and Georgia, soils collected from field trials in Delaware and Maryland had low N_o, and the magnitude of the difference between IF and IF+PL treatments was negligible; these results coincide with the trends observed for C_t and N_t values in these sites.

The fact that the ratio between the N extracted by chemical indices and N_o was smaller in PL-amended soils from Iowa and Georgia as compared to IF (Table 6) suggests that there is a change in N labile pools in PL-amended soils that is not accounted for during chemical extractions. Long-term PL fertilization increases total hydrolyzable N, acid-insoluble N, amino-acid N, and hydrolyzable unknown N concentrations in soils, but it does not affect soil amino-sugar N concentration (Ju

et al. 2006). On the contrary, long-term IF fertilization increases soil amino-acid N, but it does not modify other organic-N fractions (Huang et al. 2009). The use of NaOH extractants, as in NaOH-N and ISNT, hydrolyses mostly amino-sugars, together with some amides and amino-acids (Stanford 1978). Similarly, the use of KCl extractions at boiling temperature hydrolyses only eight compounds, three of which are amino-sugars (Gianello and Bremner 1986). Consequently, these chemical extractions rely on estimating N mineralization based on quantifying labile N compounds (amino-sugars), which are not the main N compounds that accumulate in PL-amended soils. It should be noted that in Georgia the soil use was a pasture, and that cattle manure may affect the soil organic-N composition (Huang et al. 2009; Zhang et al. 2014). However, as both IF and PL treatments had the same yearly animal charge, any difference between treatments is due to the external N inputs.

While chemical extraction indices only account for a specific fraction of the organic N, N_{an} is a biological index that quantifies the actual amount of N mineralized under specific conditions. The N_{an} was strongly associated with soil N_o in soils receiving IF, IF+PL, or PL (Fig. 3) and the proportion of N_o accounted for by N_{an} was not affected by fertilization history (Table 6). Because the relationship between N_{an} and N_o was independent from fertilization history, we suggest that N_{an} accounts for the labile N pool that dominate some PL-amended soils; such forms of N cannot be fully accounted for by chemical extraction methods. The advantages of biological methods over chemical mineralization indices was described by Wang et al. (2001), who compared a short-term incubation under waterlogged conditions to chemical extraction methods and determined that chemical methods were ineffective at extracting biologically labile organic N compounds.

Our results demonstrate that the evaluated chemical indicators are unable to fully account for the increase in N mineralization potential caused by long-term PL amendments in some soils. Consequently, using these indicators to predict N availability may result in an underestimation of the real mineralization potential, and a consequent over-application of external N inputs.

Conclusion

Estimating the N mineralization potential of soils with a history of PL application using a chemical index that

was calibrated for soils with a mineral fertilization history may result in the underestimation of the real N mineralization potential. This underestimation could result in an over-application of N fertilizers, which could increase the economic and environmental costs of crop production. In contrast, the use of N_{an} is promising as an alternative to estimate soil N mineralization potential regardless of fertilization history. However, the length of the N_{an} incubation (7 days) may be a limitation for some soil testing labs, indicating a necessity of developing faster strategies to predict N_o. Also, it would be necessary to compare these quick chemical and biological methods with N_o on additional soils to cover a wider range of fertilization history (i.e., other sources of manure or inorganic fertilizers) and soil properties (i.e., varying physical and chemical properties).

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