MINERALIZATION OF N, P AND S IN SOILS AMENDED WITH POULTRY LITTER OR INORGANIC FERTILIZERS: A COMPARISON OF METHODS

by

NICOLAS WYNGAARD

(Under the Direction of Miguel Cabrera)

ABSTRACT

Estimating soil N, S, and P mineralization (conversion from organic to plant-available, inorganic nutrients) potential is necessary to improve the determination of fertilizer rates for crop production. The purpose of this study was to measure the N, S, and P mineralization potentials of soils amended with inorganic fertilizer (IF) or poultry litter (PL), and to evaluate different methods to estimate mineralization. To this end, soil samples from four long-term (> 10 yr) field trials with PL- and IF-amended plots were analyzed. The N mineralization potential (N_o) measured by aerobic incubations (24 wk) was 1.8 to 233.1 g N kg^{-1} greater in PL- than in IF-amended soils, depending on the type of soil. Different chemical and biological methods to estimate N_o were evaluated, but only the 7-d anaerobic incubation (N_{an}) presented a strong association with $N_{o,}$ which was similar for both fertilization treatments (r^2= 0.96). The S mineralization potential (S_o) was 0 to 21.2 g S kg^{-1} greater in PL-amended soils than in IF-amended soils, depending on the type of soil. The 7-d aerobic incubation, the extraction with $NaHCO_3$, and N_{an} were good estimators of S_o (r^2=0.85, 0.85 and 0.99, respectively). The increase in N_o and S_o observed in PL- relative to IF-amended soils was closely associated with the soil clay content ($r^2 > 0.90$). The cumulative organic P mineralized in a 13-d incubation experiment, measured by the isotopic dilution method, varied between 9.7 and 90.7 mg P kg^{-1}, and was greater or equal in PL- than in IF-amended soils, depending on the type of soil. The mineralized P was strongly associated with the organic P content in the coarse fraction of the soil (> 53 μm) ($r^2=0.92$), but not with any other measured labile P pool in the soil. Additionally a model was developed to predict P mineralization based on soil respiration during an aerobic incubation and the C:P ratio in the coarse fraction of the soil. Our results suggest that the quantification of P_o in the CF as well as the measurement of respiration corrected by the C_t:P_o ratio of the CF are promising non-isotopic indicators of P_o mineralization rates.

INDEX WORDS: mineralization, nitrogen, sulfur, phosphorus, poultry litter, inorganic fertilizer, incubations, isotopic dilution, coarse fraction

MINERALIZATION OF N, P AND S IN SOILS AMENDED WITH POULTRY LITTER OR INORGANIC FERTILIZERS: A COMPARISON OF METHODS

by

NICOLAS WYNGAARD

B.S., Universidad Nacional de Mar del Plata, Argentina, 2008

Mg. Sc., Universidad Nacional de Mar del Plata, Argentina, 2011

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2015

MINERALIZATION OF N, P AND S IN SOILS AMENDED WITH POULTRY LITTER OR INORGANIC FERTILIZERS: A COMPARISON OF METHODS

by

NICOLAS WYNGAARD

Major Professor:	Miguel Cabrera
Committee:	Dorcas H. Franklin
	Leticia S. Sonon
	David E. Kissel
	Aaron Thompson

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
December 2015

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Poultry industry operations in the United States are concentrated in the southeastern states (Alabama, Arkansas, Georgia, Mississippi and North Carolina) where almost 5 billion broilers (*Gallus gallus domesticus*) are produced annually (National Agricultural Statistics Service, 2014). This value represents 60% of the total broilers produced in the country. Considering that each broiler generates about 1.5 kg of poultry litter (PL) annually (a mixture of excreta, organic bedding material and feed) (Watts et al., 2010), then there is a regional production of 7.5 million Mg PL year^{-1}. This material is commonly used to fertilize crops and pastures, because it is rich in available macro and micro nutrients (Eghball et al., 2002).

Due to the high cost of transporting PL, farmers in regions of high PL production tend to over-apply this product in a limited zone close to the poultry production facilities. As a result, the PL generated in areas of high broiler production surpasses the capacity of local farmlands to assimilate the nutrients. This situation leads to an over application and consequent accumulation in soil of macronutrients such as N, P, Ca, K, Mg, and micronutrients like Cu and Zn (He et al., 2009a; Schomberg et al., 2009a). This excess can be harmful for both the crops and the environment. For example, N can be exported from the system by gas emissions or leaching, negatively affecting the atmosphere and ground water quality, respectively (Chang and Janzen, 1996; Zebarth et al., 1999), while P can migrate to surface waters increasing the risk of eutrophication (Sharpley et al., 1994).

Regardless of the negative consequences of over-applying PL, this product has the potential to improve soil quality (soil capacity to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health) in the long term when it is applied at adequate rates. This is because the use of repeated applications of organic fertilizers can enhance the soil's labile C pool (Sommerfeldt et al., 1988) and also augment microbial biomass and microbial activity (Witter et al., 1993). Watts et al. (2010) determined an increase in total organic carbon (TOC) of 7.3 g kg^{-1} in the upper 5 cm of a fine sandy loam soil after 14 years of annual applications of PL; while Kingery et al. (1994) observed an increase of 4 g TOC kg^{-1} at a 0 to 15 cm depth after more than 20 years of PL application on a fine loamy soil. Also, Daigh et al. (2009) reported an accumulation of 8.7 g TOC kg^{-1} in the upper 10 cm of a silt-loam soil after a 10 - year period.

Considering that the organic fraction of soils represents up to 98 % of total N, 80 % of total P (Kellogg et al., 2006), and at least 90% of total S (Zhou et al., 1999), then it is evident that if the application of PL increases organic matter, it may also enhance the amount of organic nutrients in soil, leading to an increase in the mineralization (conversion from organic to plant-available, inorganic nutrients) potential. This process is vital for crops mineral nutrition, and must be quantified in order to determine the adequate fertilization rate to be used in some soils (Zebarth et al., 1999). However, there is limited information regarding N mineralization in soils amended with PL, and there is no information on P and S mineralization rates.

There are several methods to estimate nutrient mineralization in soil, based on either chemical or biological principles. Chemical methods measure the amount of specific organic fractions that can be easily mineralized (Mulvaney et al., 2001). They are based on empirical approximations and their use is limited. On the other hand, biological methods quantify the amount of nutrients released by microbial activity under controlled temperature and moisture conditions. These methods require lengthful incubations. The techniques available to estimate mineralization and their precision varies depending on the considered nutrient.

1. NITROGEN

1.1 Changes in soil N caused by repeated PL applications

One of the ways by which the N mineralization potential of a soil can be increased is through the accumulation of active (easily mineralizable) and passive (recalcitrant) organic fractions (Franzluebbers et al., 1994). However, not only the quantity but the quality of the accumulated fractions will finally determine the resulting mineralization rate (Paustian et al., 1992).

Many studies have demonstrated that the long-term application of PL has the potential to increase soil total N concentration. For example, He et al. (2009b) determined, on a sandy loam Typic Hapludult from Alabama, that total N concentration in the upper 20 cm of soil was 300, 440, 1200, and 1280 mg kg^{-1} after 0, 5, 10, and 15 years of fertilization with PL, respectively, at a 2.4 Mg ha^{-1} rate. In the same study site, and after 14 years of PL fertilization treatment, Watts et al. (2010) determined an increase in total N concentration of 0.3 and 0.5 mg N kg^{-1} soil in soybean or corn cropping systems, respectively. This increase was observed in the 0 to 5 cm layer from soils under no-tillage system, but not in those conventionally tilled. No difference in the soil C:N ratio was generated by PL application. On the other hand, Kingery et al. (1994) determined that a long-term PL application (>15 years) to pastures not only increased soil total N but reduced the C:N ratio in the upper 15 cm of fine-loam and clayey soils from Alabama. Adeli et al. (2007) observed that soil total N content was 0.61, 0.69 and 0.77 g N kg^{-1} when the soil was amended for three years with 0, 4.5, and 6.7 Mg of PL, respectively, on a silt loam soil from Mississippi. This increase of total N content with increasing PL applications was also described by Adeli et al. (2010).

The previously described results demonstrate that the rate and the years under treatment with PL increase total N, and that this increase is affected by the cropping and tillage systems. Even though some studies examined this response in coarse soils (He et al., 2009b) or in fine-textured soils (Adeli et al., 2010; Kingery et al., 1994; Watts et al., 2010), there are no available studies analyzing the effect of soil texture on the accumulation of total N in soil after repeated PL applications. This is important because part of the total N from PL is accumulated as organic N compounds (He et al., 2014; Xiao-Tang et al., 2006), and the clay content determines in part the capacity of a soil to protect organic compounds against decomposition (Six et al., 2002).

It has been shown that long-term fertilization with PL can affect not only the quantity of organic N compounds in the soil, but also their relative composition. Xiao-Tang et al. (2006) demonstrated that 15 years of PL amendment at a rate of 7.5 Mg ha^{-1} on a calcareous alluvial soil from China increased the concentration of aminoacid-N, ammonium-N, and hydrolysable-unknown-N but did not modify amino-sugar-N. He et al.

(2014) also determined that the concentration of amino-compounds in the 0 to 20 cm layer of a sandy loam soil from Alabama amended with PL was ten times greater than in the un-amended control.

1.2. Methods to measure or estimate N mineralization

1.2.1 .Long term aerobic incubations

The use of long-term incubations (150 days or more) to determine mineralizable N was initially described by Stanford and Smith (1972), but many modifications were proposed regarding the incubation time and the water content during the process (Keeney and Nelson, 1982). Even though this technique is time-consuming, and therefore inadequate for routine analysis, it is considered the standard method to determine N mineralization potential (Schomberg et al., 2009b).

Long-term incubations are used to determine the potentially mineralizable N (N_0), which is the fraction of soil N mineralized according to first-order kinetics under optimal conditions of temperature, moisture, and aeration:

$$N_t = No\ (1 - e^{-kt})$$

Where t = time, k = mineralization constant and N_t = N mineralized at time t.

Long-term incubations tend to overestimate the amount of N mineralized on the field, probably because of the disturbance generated by drying and sieving the soil samples (Cabrera, 1993).

1.2.2. Short anaerobic incubation (Nan)

In this method, the N mineralization potential is determined by measuring the amount of NH_4^+ released by a water-saturated soil sample during a 7- or 14-d incubation at 40^{o}C (Waring and Bremner, 1964).

The advantage of Nan relative to other methods to estimate N mineralization is that it is a simple method, which is quicker than long-term incubations and has a low requirement of reactants and equipment. Part of its simplicity lies on the fact that the saturation can be easily reached by using an excess of water, no matter the texture of the sample; while in other incubations the water content is set at 60% of water-holding capacity, and this condition must be determined for each specific soil analyzed.

1.2.3.. Extraction with hot KCl, phosphate-borate or NaOH

These methods are based on the use of different chemical fractions to extract and quantify specific N compounds that are related to the N mineralization potential of the soil.

The hot KCl method (Gianello and Bremner, 1986) consists of heating the sample with 2 mol L^{-1} KCl in a 100^{o}C water bath for 4 h. After this, the NH_4^+-N content is measured in the extract. This method quantifies the mineral forms of N and some of the hydrolysable NH_4^+-N. The results from this method are presented with or without subtracting the initial NH_4^+-N concentration (Schomberg et al., 2009b).

The extraction with phosphate-borate consists on the steam distillation of a soil sample with phosphate-borate (pH = 11.2) for 8 min (Gianello and Bremner, 1988). This procedure extracts a greater N fraction than hot KCl, due to its capacity to extract more soluble organic N and labile N associated with mineral surfaces (Curtin and Wen, 1999).

The principal extracted compounds are NH_4^+-N and some amino-acids (like glucosamine, galactosamine, aspargines, and glutamine) (Stanford, 1978).

The extraction with NaOH also involves the use of steam distillation, but 50% NaOH is used instead of phosphate-borate. Even though the principle is the same in both extractions, NaOH is a stronger extractant due to its high alkalinity, which results in a greater N extracted fraction (Sharifi et al., 2007). The extracted N compounds are basically NH_4^+-N, amino sugars, amides, and some aminoacids (Stanford, 1978).

1.2.4. Amino-sugars analysis (ISNT, Illinois Soil N Test)

This method is based on the estimation of an alkali-extractable fraction of soil called amino-sugar (Mulvaney et al., 2006). These compounds are structural components of a wide group of substances called mucopolisaccharides. The most common ones in soil are deoxy-2-amino-D-glucose (D-glucosamine) and 2-deoxy-2-amino-D-galactose (D-galactosamine).

Some authors state that the concentration of N-amino-sugars in soil is related to the response of crops to N fertilization (Khan et al., 2001; Mulvaney et al., 2001). Consequently, they propose to use the concentration of these compounds in the soil as an index to recommend N fertilization rates. However, the direct measurement of these compounds is not simple, and this is why other techniques to estimate the amino-sugar content have been developed. One of these methods quantifies the amount of NH_3 released when a sample is heated with an alkali (Khan et al., 2001; Mulvaney et al., 2001), which yield results closely related to the amino-sugar content (R^2=0.82) (Khan et al., 2001).

The advantages of this method are that it has a low requirement of reactants and it does not demand incubation, which allows results to be determined within a day. However, other authors consider that this technique is not efficient for estimating the N mineralization rate (Genovese et al., 2009; Marriott and Wander, 2006; Osterhaus et al., 2008), as it measures just one constant component (amino-sugars) of soil organic matter that is potentially mineralizable.

1.2.5. Nitrogen in the soil coarse fraction

The soil coarse fraction (CF) of soil is composed of those organic and mineral particles whose size is greater than 53 μm (Cambardella and Elliott, 1992). This fraction has been also named "particulate organic matter" (Cambardella and Elliott, 1992) or "particulate fraction" (Wyngaard et al., 2013). It is greatly affected by management practices and its organic pool is very labile (Janzen et al., 1998). Franzluebbers et al. (2000) showed that there is a strong relationship between the particulate fraction and the rate of C mineralization.

1.2.6. Comparison of methods

Schomberg et al. (2009b) performed a detailed comparison of methods to estimate N_0, and determined that there is a high correlation (range of r^2 values from 0.76 to 0.88) between the evaluated methods (phosphate-borate, Nan, N in the CF and hot KCl) and the long-term incubations. However, Sharifi et al. (2007) found much lower regression coefficients between the same variables: 0.46 for hot KCl, 0.39 for N-CF, 0.13 for phosphate-borate, and 0.51 for ISNT. This disparity can be a consequence of differences in the pools of mineralizable N among soils. For example, Schomberg et al. (2009b), while evaluating the amount of N measured by different methods, determined in most of the soils the following trend: N-CF > Nan > phosphate-borate > KCl. However, in some soils there was a change in this sequence. This indicates that the relative composition of N fractions measured by each of the methods can vary.

1.3. Nitrogen mineralization in soils amended with PL

Previous studies on a sandy loam soil from Alabama found that the long-term application of PL increased the N mineralized by up to 33 mg N kg^{-1}soil, in a 90-day experiment (Watts, et al., 2010). Even though the use of a short-term, closed incubation system in their study allowed them to distinguish between treatments, it did not allow them to estimate the mineralization potential of the soil (N_o). Kingery et al. (1996) using a 254-day open incubation system (where the products of mineralization are periodically removed), determined a 10.5 and 4.8 mg N kg^{-1} increase in mineralization in PL-amended soil under conservation or conventional tillage, respectively. The data from this study could not be fit into a first-order single pool or a two-pool model, and could only be fit to a simple quadratic model. Therefore, N_o could not be determined.

Considering that long-term PL fertilization modifies the relative composition of organic N compounds in the soil (section 1.1) and that the chemical methods to estimate N mineralization rely on quantifying specific organic N pools (section 1.2), it is important to evaluate if the association between the real mineralization potential of the soil (N_o) and the estimated one is similar or not in PL- amended compared to IF-amended soils.

2. SULFUR

2. 1. Changes in soil organic S caused by repeated PL applications

There are no published studies analyzing the effect of long-term PL applications on soil organic S accumulation. However, previous studies have evaluated the effect of other organic fertilizers.

Knights et al. (2000) demonstrated that the long-term amendment of soils with farmyard manure (7 Mg ha^{-1} in dry basis) increases organic sulfur content as compared to soils fertilized with inorganic fertilizers. Organic S increased from 220 mg kg^{-1} in 1860 to 500 mg kg^{-1} in 1999. Eriksen and Mortensen (1999) using long-term animal manure applications demonstrated that the accumulation of organic S depends on the soil particle size distribution, but they did not quantify the effect of texture on S accumulation.

A study in a clayey soil from India amended for 22 years with farmyard manure (15 Mg ha^{-1} year^{-1} on dry basis) showed not only a 71.6 mg kg^{-1} increase in soil organic S, but also in the organic S extracted by NaOH and $NaHCO_3$ (Reddy et al., 2001). These two fractions are considered to account not only for immediately plant-available S, but also for potentially mineralizable S. Therefore, long-term fertilization likely increases the S mineralization potential of the soil.

2.2. Methods to measure or estimate S mineralization

2.2.1. Incubations

Tabatabai and Al-Khafaji (1980) used the same long-term, open incubation method described by Stanford and Hanway (1955) for N mineralization, but quantifying the amount of SO_4^{2-}-S in the leachate. This approach, where soils are leached periodically to remove

SO_4^{2-}-S released during a 24-wk incubation, has been used extensively (Ghani et al., 1991; Maynard et al., 1983; Reddy et al., 2001; Tanikawa et al., 2014) and it is considered the standard method to estimate S mineralization potential.

In contrast to the case of N, a closed incubation system (were the sulfate released by mineralization is not periodically removed and it accumulates) cannot be used for S because the accumulation of the sulfate released from the organic fraction inhibits the mineralization process (Maynard et al., 1983). Despite this fact, some authors have proposed the use of short-term, closed incubations (less than 14 d) as a S mineralization estimator, assuming that the concentration of sulfate released in less than 14 d is not enough to inhibit the mineralization (Ghani, 1994; Jones and Tabatabai, 1986).

It has also been proposed to use the results from N incubations as estimators of S mineralization, taking into account the N:S ratio of the soil organic matter (Walker, 1957). However, Maynard et al., (1983) have shown that the relationship between NO_3^- and SO_4^{2-} released during incubation can be greater or smaller than the N:S of the soil organic matter, depending on the type of incubation (open or closed system) and the type of soil.

2.2.2. Chemical extractions

The S organic pool has two major sub-pools: ester sulfates (C-O-S, C-N-S, C-S-S) and carbon-bonded S. The first sub-pool is more labile (Barrow, 1961), and most of the chemical methods are based on extracting and quantifying part of it. The methods that showed higher correlation with S uptake by crops in unfertilized plots are the extraction with 0.25 M KCl heated at 40ºC for 3 h (Blair et al., 1991), 0.01 M $Ca(H_2PO_4)_2$ at 20ºC for 1 h (Bao et al., 1999), and NaCl after a wetting-drying cycle (Williams and Steinbergs, 1959).

2.2.3. Sulfur in the soil coarse fraction

The particle-size separation of soil samples is frequently used to distinguish fractions with different turnover rates (Christensen, 1992). The determination of S in these fractions has been previously used to compare between soils (Kirkby et al., 2011) and soil management practices (Galantini and Rosell, 1997). However, the use of S in the CF has not been evaluated as an estimator of S mineralization.

2.2.4. Comparison of methods

Even though the use of chemical extractants has been compared to plant S uptake (Blair et al., 1991), no comparison has been made between the S pools extracted by chemical methods and the potentially mineralizable S determined in long-term aerobic incubations (S_o). Thus, the use of chemical extractants as estimators of S mineralization is based on empirical results, and it should be therefore confirmed by comparison with S_o. Also, this comparison would allow the evaluation of which extractant is more efficient as a S mineralization estimator.

2.3. S mineralization in soils amended with PL

There is limited research regarding S mineralization from soil, basically due to two factors: the relatively low concentration of S in soil compared to N and the lack of precise and economical methods to measure S concentration in soil extracts (Ketterings et al., 2011). There have been studies involving the long-term effect of organic fertilizers on S mineralization, but none of them used PL as fertilizer.

Knights et al. (2001) using a long-term open incubation system determined an increase of 10 mg S kg^{-1} in the cumulative mineralized S from soil samples with a history of farmyard manure application, as compared to samples that never received organic fertilizers. The mineralization rates were constant through time, resulting in a linear increase of the cumulative mineralized S, which do not allow one the calculation of the total potentially mineralizable S. Also, when evaluating the long-term effect of farmyard manure in a clayey soil from India, Reddy et al. (2001) determined an increase of 3.4 mg S kg^{-1} in cumulative S mineralized when compared to un-amended soils.

3. PHOSPHORUS

3. 1. Changes in soil organic P caused by repeated PL applications

Repeated application of PL generates an accumulation of labile organic P in the soil in the form of *myo*-inositol hexakisphosphate (phytate). This has been shown using a variety of techniques: P fractionation (Xue et al., 2013), enzyme additions to derive enzyme-labile organic P fractions (He et al., 2008), and nuclear magnetic resonance spectroscopy (Hill and Cade-Menun, 2009). Phytate-P is the major component of the hydrolysable organic P present in PL (He et al., 2008) because birds have a low capacity to digest phytate-like P compounds present in the feed, which are then released through their feces (Taylor, 1965). Phytate-P can be stabilized by adsorption to clays (Anderson and Arlidge, 1962) or by binding to metal ions and oxides (Evans and Martin, 1988, 1991; Ognalaga et al., 1994), and to organic molecules (Borie et al., 1989). This interaction prevents the decomposition of phytate by hydrolytic enzymes and leads to its accumulation in soil. On the other hand, other P-containing compounds as simple monoesters and DNA are weakly adsorbed by the soil matrix, due to the low number of P groups in comparison to phytate-like P, and remain more vulnerable to microbial decomposition (Greaves and Wilson, 1970).

3.2. Methods to measure or estimate P mineralization

There are few methods to determine the mineralization of P. This is because of the methodological inconvenience caused by the high reactivity of the PO_4^{3-} ions, which are adsorbed by the soil matrix once they are released from the organic pools to the soil solution (Frossard et al., 1996).

3.2.1. Incubations

Dossa et al. (2009) used the incubation method proposed by Stanford and Smith (1972), and measured the P content in the leached water by a colorimetric method. However, they worked with samples from semiarid soils of Senegal with more than 95% sand content and low P sorption capacity. In other soils with a higher sorption capacity the use of this method is not possible, as the mineralized P is adsorbed by the soil matrix instead of remaining in solution.

3.2.2. Isotopic dilution methods

The use of isotopic dilution experiments to determine P mineralization has been widely used (Achat et al., 2009; Bünemann et al., 2012; Randriamanantsoa et al., 2015), and it is considered the only reliable method to quantify this process. However, this method is expensive and labor-consuming, and consequently it cannot be used as a routine analysis.

The underlying principle of isotopic dilution studies is that after labeling the soil solution with an isotope with low abundance (such as ^{32}P), its concentration will be diluted in time by the release of the more abundant isotope from the organic matter by mineralization (Kirkham and Bartholomew, 1954, 1955). However, the dilution of isotopically-labeled P can also be caused by ^{31}P fluxes from desorption or dissolution, or by the adsorption or immobilization of the labeled P (Di et al., 2000).

The microbial tracer uptake by microorganisms during the incubation experiment can be accounted for by using fumigation-extraction methods (Bünemann et al., 2012), while diverse methodologies listed below have been developed to overcome the inconvenience of the interaction of phosphate with the soil matrix:

a. Incorporation of a second treatment where the incubated soil is previously sterilized so that no mineralization occurs and all the isotopic dilution is caused by soil adsorption (Walbridge and Vitousek, 1987). However, the use of sterilization can solubilize unavailable P forms, leading to an overestimation of the results (Kellogg et al., 2006)

b. Incubation of a non-sterile sample for a short time (100 minutes). This methodology assumes that this period is insufficient for biological processes to occur, and then only soil adsorption will generate isotopic dilution. After this, the reduction in ^{32}P is modeled as a function of time, and the results are extrapolated to the incubation time of the main sample (days or weeks) (Lopez-Hernandez et al., 1998).

c. Theoretical correction by the use of equations based on the mass balance of the system (Kirkham and Bartholomew, 1954).

3.2.3. Zou Method

This technique is based on distinguishing the processes that take place during a one-day incubation of a soil sample subject to two different treatments: sterilization (using radiation or NaN_3) and sterilization + enzyme denaturation (by autoclaving) (Zou et al., 1992). In the sterilization treatment two processes will occur: the solubilization of inorganic P and the mineralization of organic P (by the action of the extracellular phosphatase enzyme present in the soil), while in the sterilization + enzyme denaturation the only process that will supposedly take place is the solubilization of inorganic P. Then, the net mineralization is calculated as the difference between both treatments.

A correction factor in the autoclaving treatment must be used to consider the effect of the high temperature on P solubilization. This is the main source of variation in the results of this method (Kellogg et al., 2006). Another important consideration regarding this technique is that the sorption of P by the soil matrix is controlled by the use of anion exchange resins during the incubation, to which the P is adsorbed and then quantified.

The underlying assumptions of this method are that: 1) the sterilization treatment affects inorganic P dynamics equally in both treatments, 2) gross organic P mineralization is not affected by sterilization, and 3) all the mineralized organic P is sequestered by the resins. However, Kellogg et al. (2006) demonstrated that the Zou method greatly over-estimates the mineralization as compared to isotopic dilution studies, suggesting the violation of one or more of the assumptions.

3.2.4. P in the coarse fraction

The P concentration in the soil fraction > 53 µm has been proposed as an estimator of P mineralization (Ciampitti et al., 2011). Ciampitti et al. (2011) showed that the concentration of total P in the CF (mg P in the CF kg^{-1} soil) is positively related to the P accumulation in corn plants growing in unfertilized plots on a Mollisol in Argentina. The amount of plant-available inorganic P in the CF was negligible in similar soils (Wyngaard et al., 2013) and thus cannot explain observed differences in plant P uptake. A possible explanation is that the mineralization of organic P in the CF supplied plant available P during the growing season. As the determination of total or organic P in the CF is simple and economical, it could be a promising tool to estimate potential P_o mineralization.

3.3. P mineralization in soils amended with PL

Despite the widely proven effect of long-term PL amendments over soil labile organic P pools, there is no information regarding the effect of this practice over P mineralization. However, a previous study has demonstrated that repeated applications of organic fertilizers can increase organic P mineralization rates: Oehl et al. (2004) determined an increase in the P mineralization rate of 1 mg P kg^{-1} d^{-1} after 20 years of fertilization with anaerobically composted farmyard manure in an Haplic Luvisol from Switzerland.

4. GENERAL HYPOTHESIS

The long-term application (more than 10 years) of PL increases the concentration of organic N, P, and S, as well as the mineralization rate of these nutrients, as compared to soils fertilized with mineral fertilizers, and this increase is affected by soil texture.

5. SPECIFIC HYPOTHESIS

5.1. Nitrogen

5.1. 1. The long-term application of PL (more than 10 years) increases soil N mineralization potential.

5.1.2. The accumulation of N in PL-amended soils is greater in clayey soils.

5.1.3. The association between N mineralization potential (N_o) and N mineralization estimated by chemical methods is affected by the history of fertilization with mineral or organic fertilizer.

5.2. Sulfur

5.2.1. The long-term application of PL (more than 10 years) increases soil S mineralization potential.

5.2.2. The accumulation of S in PL-amended soils is greater in clayey soils.

5.2.3. The S fractions extracted by chemical methods are associated with those measured by long-term incubations.

5.3. Phosphorus

5.3.1. The long-term application of PL (more than 10 years) increases soil P mineralization potential.

5.3.2. The content of P in the CF is associated with the organic phosphorus mineralization rate.

6. OBJECTIVE

The aim of this research was to compare methods to estimate N, P, and S mineralization in soils amended with PL as compared to soils amended with mineral fertilizers.

REFERENCES

Achat, D.L., Bakker, M.R., Augusto, L., Saur, E., Dousseron, L., Morel, C., 2009. Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: combining isotopic dilution and extraction methods. Biogeochemistry 92, 183-200.

Adeli, A., Sistani, K., Rowe, D., Tewolde, H., 2007. Effects of broiler litter applied to no-till and tillage cotton on selected soil properties. Soil Science Society of America Journal 71, 974-983.

Adeli, A., Tewolde, H., Sistani, K., Rowe, D., 2010. Comparison of broiler litter and commercial fertilizer at equivalent N rates on soil properties. Communications in Soil Science and Plant Analysis 41, 2432-2447.

Anderson, G., Arlidge, E., 1962. The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals, and hydrated sesquioxides in acid media. Journal of soil science 13, 216-224.

Bao, L., Ei, Z.W., Shutian, L., Hong, W., 1999. Indices of Sulfur-Supplying North China. Pedosphere 9, 25-34.

Barrow, N., 1961. Studies on mineralization of sulphur from soil organic matter. Crop and Pasture Science 12, 306-319.

Blair, G.J., Chinoim, N., Lefroy, R.D.B., Anderson, G., Crocker, G., 1991. A soil sulfur test for pastures and crops. Soil Research 29, 619-626.

Borie, F., Zunino, H., Martínez, L., 1989. Macromolecule-P associations and inositol phosphates in some Chilean volcanic soils of temperate regions. Communications in Soil Science & Plant Analysis 20, 1881-1894.

Bünemann, E., Oberson, A., Liebisch, F., Keller, F., Annaheim, K., Huguenin-Elie, O., Frossard, E., 2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. Soil Biology and Biochemistry 51, 84-95.

Cabrera, M., 1993. Modeling the flush of nitrogen mineralization caused by drying and rewetting soils. Soil Science Society of America Journal 57, 63-66.

Cambardella, C., Elliott, E., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777-783.

Chang, C., Janzen, H., 1996. Long-term fate of nitrogen from annual feedlot manure applications. Journal of Environmental Quality 25, 785-790.

Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates, Advances in soil science. Springer, pp. 1-90.

Ciampitti, I.A., García, F.O., Picone, L.I., Rubio, G., 2011. Soil carbon and phosphorus pools in field crop rotations in Pampean soils of Argentina. Soil Science Society of America Journal 75, 616-625.

Curtin, D., Wen, G., 1999. Organic Matter Fractions Contributing to Soil Nitrogen Mineralization Potential. Soil Sci. Soc. Am. J. 63, 410-415.

Daigh, A.L., Brye, K.R., Sharpley, A.N., Miller, D.M., West, C.P., Brahana, V.J., 2009. Five-Year Change in Soil Profile Chemical Properties as Affected by Broiler Litter Application Rate. Soil Science 174, 531-542.

Di, H., Cameron, K., McLaren, R., 2000. Isotopic dilution methods to determine the gross transformation rates of nitrogen, phosphorus, and sulfur in soil: a review of the theory, methodologies, and limitations. Soil Research 38, 213-230.

Dossa, E., Khouma, M., Diedhiou, I., Sene, M., Kizito, F., Badiane, A., Samba, S., Dick, R., 2009. Carbon, nitrogen and phosphorus mineralization potential of semiarid Sahelian soils amended with native shrub residues. Geoderma 148, 251-260.

Eghball, B., Wienhold, B.J., Gilley, J.E., Eigenberg, R.A., 2002. Mineralization of manure nutrients. Journal of Soil and Water Conservation 57, 470-473.

Eriksen, J., Mortensen, J.V., 1999. Soil sulphur status following long-term annual application of animal manure and mineral fertilizers. Biology and Fertility of Soils 28, 416-421.

Evans, W., Martin, C., 1988. Interactions of Mg (II), Co (II), Ni (II), and Zn (II) with phytic acid. VIII. A calorimetric study. Journal of inorganic biochemistry 32, 259-268.

Evans, W., Martin, C., 1991. The interactions of inositol hexaphosphate with Fe (III) and Cr (III). A calorimetric investigation. XV. Journal of inorganic biochemistry 41, 245-252.

Franzluebbers, A., Haney, R., Honeycutt, C., Schomberg, H., Hons, F., 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Science Society of America Journal 64, 613-623.

Franzluebbers, A., Hons, F., Zuberer, D., 1994. Long-term changes in soil carbon and nitrogen pools in wheat management systems. Soil Science Society of America Journal 58, 1639-1645.

Frossard, E., López-Hernández, D., Brossard, M., 1996. Can isotopic exchange kinetics give valuable information on the rate of mineralization of organic phosphorus in soils? Soil Biology and Biochemistry 28, 857-864.

Galantini, J.A., Rosell, R.A., 1997. Organic fractions, N, P and S changes in an Argentine semiarid Haplustoll under different crop sequences. Soil and Tillage Research 42, 221-228.

Genovese, M., Echeverría, H., Studdert, G., Rozas, H.S., 2009. Amino-sugar nitrogen in soils: calibration and relationship with anaerobically incubated soil nitrogen. Ciencia del Suelo 27, 225-236.

Ghani, A., 1994. An overview of organic sulphur levels in New Zealand pastoral soils and methods for measuring the mineralizable pool of organic sulphur. Sulphur in Agric 18, 13-17.

Ghani, A., McLaren, R., Swift, R., 1991. Sulphur mineralisation in some New Zealand soils. Biology and Fertility of Soils 11, 68-74.

Gianello, C., Bremner, J., 1986. Comparison of chemical methods of assessing potentially available organic nitrogen in soil. Communications in Soil Science & Plant Analysis 17, 215-236.

Gianello, C., Bremner, J., 1988. A rapid steam distillation method of assessing potentially available organic nitrogen in soil. Communications in Soil Science & Plant Analysis 19, 1551-1568.

Greaves, M., Wilson, M., 1970. The degradation of nucleic acids and montmorillonite-nucleic-acid complexes by soil microorganisms. Soil Biology and Biochemistry 2, 257-268.

He, Z., Endale, D.M., Schomberg, H.H., Jenkins, M.B., 2009a. Total Phosphorus, Zinc, Copper, and Manganese Concentrations in Cecil Soil Through 10 Years of Poultry Litter Application. Soil Science 174, 687-695.

He, Z., Honeycutt, C.W., Cade-Menun, B.J., Senwo, Z.N., Tazisong, I.A., 2008. Phosphorus in poultry litter and soil: Enzymatic and nuclear magnetic resonance characterization. Soil Science Society of America Journal 72, 1425-1433.

He, Z., Honeycutt, C.W., Tazisong, I.A., Senwo, Z.N., Zhang, D., 2009b. Nitrogen and phosphorus accumulation in pasture soil from repeated poultry litter application. Communications in Soil Science and Plant Analysis 40, 587-598.

He, Z., Senwo, Z., Zou, H., Tazisong, I., Martens, D., 2014. Amino compounds in poultry litter, litter-amended pasture soils and grass shoots. Pedosphere 24, 178-185.

Hill, J.E., Cade-Menun, B.J., 2009. Phosphorus-31 nuclear magnetic resonance spectroscopy transect study of poultry operations on the Delmarva Peninsula. Journal of Environmental Quality 38, 130-138.

Janzen, H., Campbell, C., Izaurralde, R., Ellert, B., Juma, N., McGill, W., Zentner, R., 1998. Management effects on soil C storage on the Canadian prairies. Soil and Tillage Research 47, 181-195.

Jones, M.B., Tabatabai, M., 1986. Sulfur availability indexes, Sulfur in agriculture. Agronomy Monograph 27., Madison, WC, pp. 549-566.

Keeney, D.R., Nelson, D., 1982. Nitrogen—inorganic forms. Methods of soil analysis. Part 2. Chemical and microbiological properties, 643-698.

Kellogg, L.E., Bridgham, S.D., López-Hernández, D., 2006. A Comparison of Four Methods of Measuring Gross Phosphorus Mineralization. Soil Sci. Soc. Am. J. 70, 1349-1358.

Ketterings, Q., Miyamoto, C., Mathur, R.R., Dietzel, K., Gami, S., 2011. A comparison of soil sulfur extraction methods. Soil Science Society of America Journal 75, 1578-1583.

Khan, S., Mulvaney, R., Hoeft, R., 2001. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Science Society of America Journal 65, 1751-1760.

Kingery, W., Wood, C., Delaney, D., Williams, J., Mullins, G., 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. Journal of Environmental Quality 23, 139-147.

Kingery, W., Wood, C., Williams, J., 1996. Tillage and amendment effects on soil carbon and nitrogen mineralization and phosphorus release. Soil and Tillage Research 37, 239-250.

Kirkby, C., Kirkegaard, J., Richardson, A., Wade, L., Blanchard, C., Batten, G., 2011. Stable soil organic matter: a comparison of C: N: P: S ratios in Australian and other world soils. Geoderma 163, 197-208.

Kirkham, D., Bartholomew, W.V., 1954. Equations for Following Nutrient Transformations in Soil, Utilizing Tracer Data1. Soil Sci. Soc. Am. J. 18, 33-34.

Kirkham, D., Bartholomew, W.V., 1955. Equations for Following Nutrient Transformations in Soil, Utilizing Tracer Data: II.1. Soil Sci. Soc. Am. J. 19, 189-192.

Knights, J., Zhao, F., McGrath, S., Magan, N., 2001. Long-term effects of land use and fertiliser treatments on sulphur transformations in soils from the Broadbalk experiment. Soil Biology and Biochemistry 33, 1797-1804.

Knights, J., Zhao, F., Spiro, B., McGrath, S., 2000. Long-term effects of land use and fertilizer treatments on sulfur cycling. Journal of Environmental Quality 29, 1867-1874.

Lopez-Hernandez, D., Brossard, M., Frossard, E., 1998. P-isotopic exchange values in relation to Po mineralisation in soils with very low P-sorbing capacities. Soil Biology and Biochemistry 30, 1663-1670.

Marriott, E.E., Wander, M.M., 2006. Total and Labile Soil Organic Matter in Organic and Conventional Farming Systems. Soil Science Society of America Journal 70, 950.

Maynard, D., Stewart, J., Bettany, J., 1983. Sulfur and nitrogen mineralization in soils compared using two incubation techniques. Soil Biology and Biochemistry 15, 251-256.

Mulvaney, R.L., Khan, S.A., Ellsworth, T.R., 2006. Need for a Soil-Based Approach in Managing Nitrogen Fertilizers for Profitable Corn Production. Soil Science Society of America Journal 70, 172.

Mulvaney, R.L., Khan, S.A., Hoeft, R.G., Brown, H.M., 2001. A Soil Organic Nitrogen Fraction that Reduces the Need for Nitrogen Fertilization Soil Sci. Soc. Am. J. 65, 1164-1172.

National Agricultural Statistics Service, 2014. Poultry production and value: 2013 summary. 14.

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., Oberson, A., 2004. Basal organic phosphorus mineralization in soils under different farming systems. Soil Biology and Biochemistry 36, 667-675.

Ognalaga, M., Frossard, E., Thomas, F., 1994. Glucose-1-phosphate and myo-inositol hexaphosphate adsorption mechanisms on goethite. Soil Science Society of America Journal 58, 332-337.

Osterhaus, J.T., Bundy, L.G., Andraski, T.W., 2008. Evaluation of the Illinois soil nitrogen test for predicting corn nitrogen needs. Soil Science Society of America Journal 72, 143-150.

Paustian, K., Parton, W.J., Persson, J., 1992. Modeling soil organic matter in organic-amended and nitrogen-fertilized long-term plots. Soil Science Society of America Journal 56, 476-488.

Randriamanantsoa, L., Frossard, E., Oberson, A., Bünemann, E.K., 2015. Gross organic phosphorus mineralization rates can be assessed in a Ferralsol using an isotopic dilution method. Geoderma.

Reddy, K.S., Singh, M., Tripathi, A.K., Swarup, A., Dwivedi, A.K., 2001. Changes in organic and inorganic sulfur fractions and S mineralisation in a Typic Haplustert after long-term cropping with different fertiliser and organic manure inputs. Soil Research 39, 737-748.

Schomberg, H.H., Endale, D.M., Jenkins, M.B., Sharpe, R.R., Fisher, D.S., Cabrera, M.L., McCracken, D.V., 2009a. Soil Test Nutrient Changes Induced by Poultry Litter under Conventional Tillage and No-Tillage. Soil Science Society of America Journal 73, 154.

Schomberg, H.H., Wietholter, S., Griffin, T.S., Reeves, D.W., Cabrera, M.L., Fisher, D.S., Endale, D.M., Novak, J.M., Balkcom, K.S., Raper, R.L., Kitchen, N.R., Locke, M.A., Potter, K.N., Schwartz, R.C., Truman, C.C., Tyler, D.D., 2009b. Assessing Indices for Predicting Potential Nitrogen Mineralization in Soils under Different Management Systems. Soil Science Society of America Journal 73, 1575.

Sharifi, M., Zebarth, B.J., Burton, D.L., Grant, C.A., Cooper, J.M., 2007. Evaluation of Some Indices of Potentially Mineralizable Nitrogen in Soil. Soil Science Society of America Journal 71, 1233.

Sharpley, A.N., Chapra, S., Wedepohl, R., Sims, J., Daniel, T.C., Reddy, K., 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. Journal of Environmental Quality, 437-451.

Six, J., Conant, R., Paul, E., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155-176.

Sommerfeldt, T., Chang, C., Entz, T., 1988. Long-term annual manure applications increase soil organic matter and nitrogen, and decrease carbon to nitrogen ratio. Soil Science Society of America Journal 52, 1668-1672.

Stanford, G., 1978. Evaluation of ammonium release by alkaline permanganate extraction as an index of soil nitrogen availability. Soil Science 126, 244-253.

Stanford, G., Hanway, J., 1955. Predicting Nitrogen Fertilizer Needs of Iowa Soils: II. A Simplified Technique for Determining Relative Nitrate Production in Soils1. Soil Sci. Soc. Am. J. 19, 74-77.

Stanford, G., Smith, S.J., 1972. Nitrogen Mineralization Potentials of Soils1. Soil Sci. Soc. Am. J. 36, 465-472.

Tabatabai, M.A., Al-Khafaji, A.A., 1980. Comparison of Nitrogen and Sulfur Mineralization in Soils1. Soil Sci. Soc. Am. J. 44, 1000-1006.

Tanikawa, T., Noguchi, K., Nakanishi, K., Shigenaga, H., Nagakura, J., Sakai, H., Akama, A., Takahashi, M., 2014. Sequential transformation rates of soil organic sulfur fractions in two-step mineralization process. Biology and Fertility of Soils 50, 225-237.

Walbridge, M.R., Vitousek, P.M., 1987. Phosphorus mineralization potentials in acid organic soils: Processes affecting 32PO43− isotope dilution measurements. Soil Biology and Biochemistry 19, 709-717.

Walker T. W. (1957) The sulphur cycle in grassland soils. Journal of the British Grassland Society 12, 10-18.

Waring, S., Bremner, J., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature 201, 951-952.

Watts, D.B., Torbert, H.A., Prior, S.A., Huluka, G., 2010. Long-Term Tillage and Poultry Litter Impacts Soil Carbon and Nitrogen Mineralization and Fertility. Soil Science Society of America Journal 74, 1239-1247.

Williams, C., Steinbergs, A., 1959. Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. Crop and Pasture Science 10, 340-352.

Witter, E., Mårtensson, A., Garcia, F., 1993. Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biology and Biochemistry 25, 659-669.

Wyngaard, N., Vidaurreta, A., Echeverría, H.E., Picone, L.I., 2013. Dynamics of Phosphorus and Carbon in the Soil Particulate Fraction Under Different Management Practices. Soil Science Society of America Journal 77, 1584-1590.

Xiao-Tang, J., Xue-Jun, L., Zhang, F.-S., Christie, P., 2006. Effect of long-term fertilization on organic nitrogen forms in a calcareous alluvial soil on the North China Plain. Pedosphere 16, 224-229.

Xue, Q.Y., Shamsi, I.H., Sun, D.S., Ostermann, A., Zhang, Q.C., Zhang, Y.S., Lin, X.Y., 2013. Impact of manure application on forms and quantities of phosphorus in a Chinese Cambisol under different land use. Journal of Soils and Sediments 13, 837-845.

Zebarth, B., Paul, J., Van Kleeck, R., 1999. The effect of nitrogen management in agricultural production on water and air quality: evaluation on a regional scale. Agriculture, ecosystems & environment 72, 35-52.

Zhou, W., Li, S.T., Wang, H., He, P., Lin, B., 1999. Mineralization of organic sulfur and its importance as a reservoir of plant-available sulfur in upland soils of north China. Biology and Fertility of Soils 30, 245-250.

Zou, X., Binkley, D., Doxtader, K.G., 1992. A new method for estimating gross phosphorus mineralization and immobilization rates in soils. Plant and Soil 147, 243-250.

CHAPTER 2

LONG-TERM FERTILIZATION WITH POULTRY LITTER OR INORGANIC FERTILIZER AFFECTS METHODS TO ESTIMATE NITROGEN MINERALIZATION POTENTIAL

---

[1] Wyngaard, N., M.L. Cabrera; A. Shober, and R. Kanwar. Submitted to Soil Science Society of America Journal. 07/27/2015.

ABSTRACT

Estimation of soil nitrogen (N) mineralization can improve the determination of N fertilizer rates for crop production. This study evaluated the change in total N (N_t) and N mineralization potential (N_o) after long-term applications of poultry litter (PL) or inorganic fertilizer (IF) to soils with contrasting texture. The capacity of different laboratory methods to estimate N_o under both fertilization strategies was also assessed. To this end, samples from four field sites that were fertilized with PL or IF for more than ten years were aerobically incubated for 24 wk to determine N_o, which was related to the following quick methods for N mineralization: Illinois soil test analysis (ISNT); extraction with hot KCl, NaOH, or phosphate-borate (PB); and anaerobic incubation (N_{an}). Soil N_t concentrations of PL-amended soils were 0.08 to 0.63 g kg^{-1} higher than IF-amended soils, this difference was closely correlated with the clay content and resulted in an increase in N_o of 1.8 to 233.1 mg kg^{-1}. Of the chemical methods evaluated, PB extraction showed a weak correlation with N_o (R^2= 0.55). However, N released by ISNT, NaOH, and KCl extractions was highly correlated with N_o (R^2> 0.94), but each of these methods resulted in separate correlation models for IF- and PL-amended soils. In contrast, the association between N_{an} and N_o resulted in one model for both fertilizer treatments (R^2= 0.96). Due to its simplicity, biological basis, and capacity to predict N_o in PL- and IF-amended soils, we recommend the use of N_{an} as an estimator of soil N mineralization potential.

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; N_{an}, anaerobic N mineralization; N_o; potentially mineralizable N; NaOH-N, NaOH distillable N; N_t, total nitrogen; PB, phosphate-borate; PB-N, phosphate-borate distillable N; PL, poultry litter; UAN, urea-ammonium nitrate fertilizer;

1. INTRODUCTION

Nitrogen (N) is the most frequently deficient nutrient in cropping systems (Havlin et al., 2005). The importance of the mineralization process (conversion from organic N to inorganic N) becomes evident when considering that more than 95% of soil N is present in organic compounds, and that plants only take up inorganic N forms (NO_3^- and NH_4^+). Due to this contribution of N mineralization to crop nutrition, quantifying the soil N mineralization is helpful to determine the adequate N fertilizer rate to be used (Zebarth et al., 1999).

There are several available methods to estimate N mineralization potential in soils, and these methods are based on chemical or biological principles. Chemical extraction methods, such as extractions with hot KCl (Gianello and Bremner, 1986), NaOH (Sharifi et al., 2007) or phosphate-borate (PB) (Gianello and Bremner, 1988), as well as the Illinois soil N test analysis (ISNT) (Khan et al., 2001), measure the amount of specific organic N fractions that can be easily mineralized (Mulvaney et al., 2001). These chemical extractions are based on empirical approximations and their use for determining N_o is limited.

Biological N mineralization methods quantify the amount of inorganic N released due to microbial activity under controlled temperature and moisture conditions. Among these methods, the long-term aerobic incubation method (24 wk at 80% of field capacity) described by Stanford and Smith (1972) is considered the standard method to measure the potentially mineralizable N (N_o); results obtained from other biological or chemical methods are usually evaluated by comparison to N_o (Jalil et al., 1996, Schomberg et al., 2009, Sharifi et al., 2007). However, the Stanford and Smith (1972) technique is time-consuming due to the long incubation time required. Another biological method to estimate N mineralization potential is the short-term (1 wk) incubation under waterlogged conditions at 40 ^{o}C described by Waring and Bremner (1964).

Besides chemical extractions and biological methods, soil total N (N_t) concentration and the total carbon (C_t) to organic N ratio 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) of the soil, as obtained by physical fractionation of the soil (Cambardella and Elliott, 1992), was also compared previously with N mineralization (Franzluebbers et al., 2000, Schomberg et al., 2009).

As previously stated, chemical extraction methods to estimate N mineralization rely on measuring a labile N pool that is assumed to be correlated with N_o. Therefore, any management practice that modifies the relative composition of this labile N pool may affect the results of a chemical extraction method, leading to an over or under estimation of the N mineralization potential. The long-term application of poultry litter (PL) as fertilizer is a management practice that can affect the relative composition of organic N compounds in the soil (He et al., 2014, Xiao-Tang et al., 2006). Poultry litter is a mixture of excreta, feathers, feed, and organic bedding material that is generated at an annual rate of approximately 13 million Mg in the United States (National Agricultural Statistics Service, 2012). Poultry litter is a common source of plant nutrients and organic matter in areas of intensive poultry production. However, there is no information regarding how the long-term application of PL may affect the capacity of chemical methods to accurately predict soil N_o.

Previous studies conducted on a sandy loam soil found that long-term application of PL can increase the soil N mineralization potential due to the accumulation of N_t (Watts et al., 2010). Considering that the accumulation of organic compounds derives mainly from the capacity of the soil mineral matrix to protect those compounds against microbial decomposition (Six et al., 2002), it is important to evaluate how the long-term application of PL affects the accumulation of N_t and, therefore, the N mineralization potential of soils with contrasting particle size distribution.

The objectives of this study were to 1) evaluate the accumulation of C_t and N_t in soils with different textures following long-term (>10 yr) fertilization with PL or IF, 2) compare the N mineralization potential of soils amended with PL to soils amended with inorganic fertilizer (IF), and 3) determine if the relationships between N_o and mineralizable N determined following various chemical extraction methods is similar in soils fertilized with PL or IF.

2. MATERIALS AND METHODS

In the fall of 2012, composite soil samples (0 to 20 cm) were taken from four different sites located in Delaware (39°40'13"N 75°45'10"W), Maryland (39°10'42"N 76°10'58"W), Iowa (42°01'22"N 93°46'35"W) and Georgia (33°24'04"N 83°29'33"W). These sites were selected based on their contrasting particle size distribution (Table 2.1). At each site, long-term (> 10 yr) effects of PL and IF soil treatments were evaluated. The

IF treatments were applied in the form of urea-ammonium nitrate (UAN) or ammonium nitrate at a similar plant available N rate as applied with the PL treatments (Table 2.1). The experimental arrangement at all sites was a randomized complete block design with four replications. Initially, the Delaware and Maryland sites were managed under corn (*Zea maize* L.) monoculture (2000 – 2002) and were later switched to a corn - soybean (*Glycine max* L.) rotation (post 2003), whereas the Iowa site was always managed under a corn - soybean rotation. The Georgia site was managed as a tall fescue (*Festuca arundinacea* Schreb.) - bermudagrass (*Cynodon dactylon* L.) pasture. All sites were managed under conventional tillage except from the Georgia pasture which was grazed and sometimes hayed. Composite soil samples were taken from each site (in three of four blocks) almost one year after the last fertilizer application. Soil samples were air-dried, ground to pass a 2-mm sieve, and all recognizable plant tissue was removed.

Soil C_t and N_t concentrations in the soil samples were measured by dry combustion (LECO, 2014). To facilitate this analysis, the values for C_t and N_t concentrations (g kg^{-1}) 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 mol L^{-1} 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 as previously described for non-fractionated samples.

Four different soil N chemical extraction methods were evaluated. The first method evaluated was the hot KCl method proposed by Gianello and Bremner (1986). Briefly, 3 g soil was heated in a closed tube with 20 mL of 2 mol L^{-1} KCl solution in a water bath at

100^{o}C for 4 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). The resulting NH_4^+-N concentration in the extract was expressed before and after subtracting the initial NH_4^+-N concentration (Cold_KCl-N, determined in unheated samples), rendering the variables Hot_KCl-N or Hyd_KCl-N, respectively. Second, the ISNT method (Khan et al., 2001) was evaluated. To this end, 6.2 g soil was treated with 10 mL of 2 mol L^{-1} NaOH and heated on a hot plate at 50^{o}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. Finally, two distillation methods were evaluated. In each of the distillation methods, 5 g of soil was placed in a steam-distiller with one of two alkaline extractant: 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 PB solution (PB-N) (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.

A long-term aerobic incubation was performed as described by Stanford and Smith (1972). To this end, 20 g of soil was mixed with 20 g of 1-mm diameter acid-washed sand; the mixture was transferred to a 60-mL polypropylene syringe with glass wool at the bottom. The soil-sand mixture was moistened with deionized water to a water content of 80% of field capacity (estimated as the water content in mixed soil-sand samples after saturating them and letting them drain for two days) and then leached with 200 mL of 0.01 mol L^{-1} $CaCl_2$ in 5-mL increments to remove the initial NH_4^+ and NO_3^-. The water content was then adjusted by removing the excess water by vacuum filtration at 6 kPa. After this, the polypropylene syringes were covered with a porous membrane and incubated at 30^{o}C for 24 weeks, adjusting the water content every 3 d. Samples were bi-weekly leached with a 0.01 mol L^{-1} $CaCl_2$ solution. Concentrations of NO_2^--N and NO_3^--N in the leachate were determined together 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 each soil, three incubation replications were performed. For estimating N_o, data were fit to a first-order model: $N_m = N_o \text{ x } (1\text{-}e^{-kt})$, where N_m is the cumulative amount of N mineralized ($NH_4^+ + NO_2^- + NO_3^-$) at a specific time (t, d), and k (d^{-1}) is the first-order rate constant.

Additionally, a short-term anaerobic incubation in waterlogged (anoxic) conditions was evaluated as a method to estimate N_o (Waring and Bremner, 1964). This method consisted of incubating 10 g soil in a stoppered tube filled with water for 7 days at 40°C. After the incubation period, the N in resulting slurry was extracted with 30 mL of a 4 mol L^{-1} KCl solution and steam-distillated as described previously. The ammonia in the distillate was trapped in a mixed boric acid and indicator solution, which was then titrated with sulfuric acid. Mineralized N was then determined by subtracting the initial NH_4^+ content in the soil samples from the concentration of NH_4^+ determined after the incubation.

The effects of research site and fertilization treatments over the studied variables were evaluated 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, quadratic, exponential, and logarithmic models were evaluated, compared, and selected based on their significance and r^2 (simple linear model) or R^2 (non-linear models). Only selected models are presented. A residual sum of squares analysis (Milliken and Debruin, 1978) was used to determine if, for each soil, the models used to predict N_o in IF and PL treatments were different from a single, common model incorporating both IF and PL treatments.

3. RESULTS

3.1. C and N as affected by long-term PL or IF fertilization

The long-term amendment with PL increased soil C_t concentration (relative to IF plots) in Iowa and Georgia (+2.2 and +4.5 g kg^{-1}, respectively); no difference was observed between treatments in Delaware and Maryland (Table 2.2). We observed a greater accumulation of N_t in PL-amended soils compared to soils receiving IF in Maryland (+0.22 g kg^{-1}), Iowa (+0.38 g kg^{-1}) and Georgia (+0.63 g kg^{-1}). Considering the results on an area basis, the accumulation of C_t in PL-amended soils relative to soils receiving IF at the 0-20 cm depth was -0.2, 0.41, 5.4 and 10.98 Mg ha^{-1} in Delaware, Maryland, Iowa and Georgia, respectively. These values represent -0.5, 1, 11 and 25%, respectively, of the C_t applied as PL during the experiment (calculated based on the annual PL application rate and the duration of the experiment, Table 2.1). The capacity of the soil to accumulate C_t was closely associated with soil clay content: ΔC_t (PL - IF, g g^{-1}) = 0.0495 × Clay content (g g^{-1}) + 0.167 ($r^2 = 0.94$; $p = 0.031$).

The accumulation of N_t in the soil was 1, 22, 33 and 60 % of the PL- applied N_t in Delaware, Maryland, Iowa and Georgia, respectively. We determined a strong association between N_t and soil texture: ΔN_t (PL - IF, g g $^{-1}$) = 0.4591 × clay content (g g $^{-1}$) + 0.1131 ($r^2 = 0.99$; $p = 0.005$). Also, we observed a significant reduction in the C:N ratio following long-term PL application for the Delaware and Iowa only (Table 2.2).

The C_t and N_t concentrations and the C:N ratio in the CF of the soil were also affected by fertilization treatments and the research sites. We determined an interaction between sites and treatments for C_t, but not for N_t or the C:N ratio in the CF. Mean N_t concentrations in the CF were as follows: Georgia (381.8 mg kg^{-1}) ≥ Iowa (264.5 mg kg^{-1}) ≥ Maryland (106.7 mg kg^{-1}) = Delaware (101.8 mg kg^{-1}). Also, the N_t concentration in PL-amended soils (282.6 mg N kg^{-1}) was greater than soils receiving IF (144.8 mg N kg^{-1}) across all sites. Regarding the C:N ratio in the coarse fraction, only Delaware soils (33.3) had a C:N ratio that was statistically greater than that from the other soils; the C:N ratio averaged 21.7, 17.7 and 14.9 for Maryland, Georgia and Iowa, respectively. Also, on average, the C:N ratio in the CF was greater in IF-amended soils (25.4) than in PL-amended soils (18.5).

3.2. N mineralization measurement in soils amended with IF or PL

The cumulative amount of N mineralized during a 24-week incubation experiment differed among sites and treatments (Figure 2.1). The difference in N_o concentration (calculated by fitting the mineralization data to a first-order model) between PL and IF treatments was: 1.8 mg kg^{-1} for Delaware, 33.6 mg kg^{-1} for Maryland, 125.7 mg kg^{-1} for Iowa and 233.1 mg kg^{-1} for Georgia (Table 2.3). However, this difference was only significant for soils from Iowa and Georgia. We observed a strong quadratic association between N_o and C_t or N_t ($R^2 = 0.96$ and 0.95, respectively; $p < 0.001$ in both cases) and an exponential relationship between N_o and the C:N ratio ($R^2 = 0.63$). A similar association between C_t, N_t or C:N ratio and N_o was observed for the CF of the soil (data not shown).

3.3. N mineralization estimation in soils amended with IF or PL

The range of soil N concentrations determined using selected chemical extraction or biological methods to estimate N mineralization was 2 to 10 mg kg^{-1} for Hot_KCl-N, 2 to 6 mg kg^{-1} for Hyd_KCl-N, 33 to 391 mg mg^{-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 N kg^{-1} for N_{an} (Table 2.3). 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 of the evaluated methods were capable of detecting differences in soil N pools between research sites and fertilizer treatments, with the exception of Hyd_KCl and ISNT, which only identified differences in N concentration between sites.

When analyzing the association between the methods to estimate N mineralization and No, all of the chemical methods (Hot_KCl-N, Hyd_KCl-N, NaOH-N and ISNT) except for PB-N presented two different models depending on the fertilizer treatment (Figure 2.3). In the case of PB-N, even though a significant single model was determined ($p = 0.042$), the association with N_o was low ($r^2 = 0.55$). Contrarily, a strong linear association between N_{an} and N_o was determined ($r^2 = 0.96$).

4. DISCUSSION

The increase in C_t and N_t concentration in soils following repeated applications of PL is thoroughly described in the literature (Adeli et al., 2007, Daigh et al., 2009, He et al., 2009, Kingery et al., 1994, Sharpley et al., 1993, Watts et al., 2010). However, these previous studies either used one soil in their study or multiple soils with the same textural class. The use of soils with contrasting texture in our study allowed for further investigation of the interaction between clay content and C_t and N_t accumulation from PL, which was not analyzed previously. The strong relationship we observed between C_t and soil clay content is likely explained by the greater capacity of soil fines to physically protect the organic matter against decomposition through micro-aggregation or formation of intimate clay-organic matter associations (Six et al., 2002).

The greater magnitude of the difference in C_t or N_t between PL- and IF- amended soils in the CF than in non-fractionated samples (Table 2.2) supports the idea of the CF being having a faster decomposition rate and being more sensitive to changes in management practices than the whole soil (Janzen et al., 1998). For example, the C_t and N_t concentrations in the CF were 39.6 and 97.2% greater in PL- than in IF-amended soils in Georgia, respectively. In contrast, in the whole soil this difference was only 17.0% for C_t and 25.4% for N_t. However, even though the difference between treatments was numerically greater, the high variability in C_t and N_t measurements in the soil CF (coefficient of variation 23.0 and 47.3%, respectively) limited the statistical significance of this difference. As a consequence, the determination of C_t and N_t was statistically more sensitive to differences between treatments in the whole soil than in the CF.

The increase in N_t in PL-amended soils translated to an increase in N mineralization. Previous studies, working with 10 or more contrasting soil samples have determined a similar relationship between N_t and N mineralization (Camargo et al., 2004, Schomberg et al., 2009, Sharifi et al., 2007); whereas other studies observed only a weak association (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 is important to determine the mineralization rate, but also the quality of soil N. This is why the C:N ratio of the soil was a better predictor of N mineralization than N_t in some cases (Springob and Kirchmann, 2003). The weak relationship between the C:N ratio and N_o in our study ($R^2 = 0.63$), as compared to that of Springob and Kirchmann (2003), may be a consequence of the narrower range of C:N values of the soils we used in this experiment (C:N from 10 to 16 in our study compared to C:N from 10 to 30, respectively). Considering that the relative accumulation of N_t from PL was greater than reported for C_t in our study, a reduction in the soil C:N ratio was expected in PL-amended plots. However, this reduction in C:N ratio was only significant in the soils from Delaware and Iowa (Table 2.2).

Other studies quantified N mineralization in PL-amended soils. In a two-year study on a fine-sandy loam soil, 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-d incubation of 0 to 10 cm samples. In a 90-d study using a closed incubation system, Watts et al. (2010) described differences in N mineralized between PL- and IF-amended sandy loam soils collected from the top 0 to 5 cm that ranged from 4.1 to 49.4 mg kg^{-1}, depending on the tillage system and crop rotation. Our study also confirmed an increase in N mineralization in soils amended with PL, but fitting the data to a first-order model in our study 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 values obtained using different N mineralization methods.

Among the chemical methods to estimate N mineralization, Hot_KCl-N and Hyd_KCl-N values (Table 2.3) were in the range of those determined by Schomberg et al. (2009), Sharifi et al. (2007) and Curtin and Wen (1999), but were lower than those determined by Jalil et al. (1996) and Wang et al. (2001). All of these studies worked with more than 19 contrasting soil samples from different sites, with different properties and management history. Therefore, it is difficult to identify the reason of these contrasting results among the previous experiments.

The values we reported for ISNT (Table 2.3) 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. Finally, the N_{an} values we reported were similar to those obtained by Schomberg et al. (2009), with exception of the Georgia soils, which exhibited greater N_{an} and N_o concentrations. Schomberg et al. (2009) evaluated 11 soils, but only one under a pasture system (N_{an}: 115.7 mg kg^{-1}) similar to the pasture system in Georgia (N_{an}: 126 and 172 mg kg^{-1} in IF- and PL- amended soils, respectively). The greater values for N_{an} in our study may result from the combination of the pasture system and the PL fertilization treatment.

The correlation between all methods to estimate N mineralization and N_o was significant ($p < 0.05$) (Figure 2.3). However, the models to describe the association between the evaluated chemical methods and N_o were different depending on the fertilizer treatment, except with PB-N (which presented a weak correlation with N_o). The PL-amended soil presented greater values of N_o at same values of several chemical estimators. For example, for a ISNT value of 375 mg kg^{-1}, the N_o value estimated using the IF model was 244 mg kg^{-1}, whereas it was 450 mg kg^{-1} for the PL model. Consequently, the use of chemical extractions models to estimate N mineralization calibrated in soils with a mineral fertilization history can result in the underestimation of the real mineralization potential when used in organically-amended soils.

This difference in N_o concentration but not in the N extracted by several chemical extractions for PL-amended soils can be explained by the change in soil labile organic N pools caused by repeated PL applications. Long-term PL fertilization increases total hydrolyzable N, acid-insoluble N, amino-acid N and hydrolyzable unknown N, but it does not modify amino-sugar N concentration (Xiao-Tang et al., 2006). 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 known compounds; three of those compounds 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 N compounds that accumulated in PL-amended soil (Xiao-Tang et al., 2006).

In contrast to all chemical extraction methods, which only account for a specific fraction of the organic N, N_{an} is a biological method that quantifies the actual amount of N mineralized under specific conditions. The anaerobic biological method to estimate N mineralization (N_{an}) presented was strongly associated with soil N_o (Figure 2.3), and this relationship was not affected by fertilization treatments. Wang et al. (2001) 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.

5. CONCLUSION

Due to its simplicity, biological basis, and capacity to predict N_o we recommend the use of N_{an} as an estimator of N mineralization potential. This index can be used to evaluate soil health or the effect of different management practices on N cycling (Dominguez et al., 2009), or to determine adequate N fertilizer rates for crop production (Reussi Calvo et al., 2013; Sainz Rozas et al., 2008). Additionally, the results of this study demonstrate that, contrasting to chemical extractions, the estimation of N_o by N_{an} is not modified by the long-term use of an organic fertilizer. Considering the increase in surface under organic farming in the past decade (Willer and Lernoud, 2015), the use of chemical extractions by soil testing laboratories can lead to an underestimation of the soil N mineralization potential when these methods are calibrated in inorganically amended soils. This underestimation could result in an over-application of N fertilizers, increasing the economic and environmental cost of crops production. The use of N_{an} by soil testing laboratories is a reliable alternative to estimate soil N mineralization capacity independently of the field’s fertilization history.

REFERENCES

Adeli, A., K. Sistani, D. Rowe and H. Tewolde. 2007. Effects of broiler litter applied to no-till and tillage cotton on selected soil properties. Soil Sci. Soc. Am. J. 71: 974-983.

Bengtsson, G., P. Bengtson and K.F. Månsson. 2003. Gross nitrogen mineralization, immobilization, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biol. Biochem. 35: 143-154.

Camargo, F., C. Gianello and M. Tedesco. 2004. Soil nitrogen availability evaluated by kinetic mineralization parameters. Commun. Soil Sci. Plan. 35: 1293-1307.

Cambardella, C. and E. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56: 777-783.

Crooke, W. and W. Simpson. 1971. Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J. Sci. Food Ag. 22: 9-10.

Curtin, D. and G. Wen. 1999. Organic Matter Fractions Contributing to Soil Nitrogen Mineralization Potential. Soil Sci. Soc. Am. J. 63: 410-415.

Daigh, A.L., K.R. Brye, A.N. Sharpley, D.M. Miller, C.P. West and V.J. Brahana. 2009. Five-Year Change in Soil Profile Chemical Properties as Affected by Broiler Litter Application Rate. Soil Sci. 174: 531-542.

Domínguez, G.F., N.V. Diovisalvi, G.A. Studdert and M.G. Monterubbianesi. 2009. Soil organic C and N fractions under continuous cropping with contrasting tillage systems on mollisols of the southeastern Pampas. Soil Till. Res. 102: 93-100.

Franzluebbers, A., R. Haney, C. Honeycutt, H. Schomberg and F. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64: 613-623.

Franzluebbers, A., F. Hons and D. Zuberer. 1994. Long-term changes in soil carbon and nitrogen pools in wheat management systems. Soil Sci. Soc. Am. J. 58: 1639-1645.

Gianello, C. and J. Bremner. 1988. A rapid steam distillation method of assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plan. 19: 1551-1568.

Gianello, C. and J.M. Bremner. 1986. A simple chemical method of assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plan. 17: 195-214.

Havlin, J., J.D. Beaton, S.L. Tisdale and W.L. Nelson. 2005. Soil fertility and fertilizers: An introduction to nutrient managementPearson Prentice Hall Upper Saddle River, New Jersey, USA.

He, Z., C.W. Honeycutt, I.A. Tazisong, Z.N. Senwo and D. Zhang. 2009. Nitrogen and phosphorus accumulation in pasture soil from repeated poultry litter application. Commun. Soil Sci. Plan. 40: 587-598.

He, Z., Z. Senwo, H. Zou, I. Tazisong and D. Martens. 2014. Amino compounds in poultry litter, litter-amended pasture soils and grass shoots. Pedosphere 24: 178-185.

Jalil, A., C. Campbell, Y. Jame, J. Schoenau, J. Henry and G. Lafond. 1996. Assessment of two chemical extraction methods as indices of available nitrogen. Soil Sci. Soc. Am. J. 60: 1954-1960.

Janzen, H., C. Campbell, R. Izaurralde, B. Ellert, N. Juma, W. McGill, et al. 1998. Management effects on soil C storage on the Canadian prairies. Soil Till. Res. 47: 181-195.

Keeney, D.R. and D. Nelson. 1982. Nitrogen-inorganic forms. p. 643-698. *In* A.L. Page *et al*. (ed.). Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Khan, S., R. Mulvaney and R. Hoeft. 2001. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. Soil Sci. Soc. Am. J. 65: 1751-1760.

Kingery, W., C. Wood, D. Delaney, J. Williams and G. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23: 139-147.

Kingery, W., C. Wood and J. Williams. 1996. Tillage and amendment effects on soil carbon and nitrogen mineralization and phosphorus release. Soil Till. Res. 37: 239-250.

LECO. 2014. Organic application notes. www.leco.com.

Levene, H., 1960. Robust tests for equality of variances. Contributions to probability and statistics: Essays in honor of Harold Hotelling 2, 278-292.

Milliken, G.A. and R.L. Debruin. 1978. A procedure to test hypotheses for nonlinear models: A procedure to test hypotheses. Commun. Stat. Theor. M. 7: 65-79.

Mulvaney, R.L., S.A. Khan, R.G. Hoeft and H.M. Brown. 2001. A Soil Organic Nitrogen Fraction that Reduces the Need for Nitrogen Fertilization. Soil Sci. Soc. Am. J. 65: 1164-1172.

National Agricultural Statistics Service. 2012. Poultry production and value: 2011 summary. 14.

Reussi Calvo, N.I., H. Sainz Rozas, H. Echeverría and A. Berardo. 2013. Contribution of Anaerobically Incubated Nitrogen to the Diagnosis of Nitrogen Status in Spring Wheat. Agron. J. 105: 321-328.

Sainz Rozas, H., P.A. Calviño, H.E. Echeverría, P.A. Barbieri and M. Redolatti. 2008. Contribution of anaerobically mineralized nitrogen to the reliability of planting or presidedress soil nitrogen test in maize. Agron. J. 100: 1020-1025.

SAS Institute Inc. 2013. SAS® 9.4 In-Database Products: User's Guide, Third Edition. Cary, NC: SAS Institute Inc.

Schomberg, H.H., S. Wietholter, T.S. Griffin, D.W. Reeves, M.L. Cabrera, D.S. Fisher, et al. 2009. Assessing Indices for Predicting Potential Nitrogen Mineralization in Soils under Different Management Systems. Soil Sci. Soc. Am. J. 73: 1575.

Selles, F., C. Campbell, B. McConkey, S. Brandt and D. Messer. 1999. Relationships between biological and chemical measures of N supplying power and total soil N at field scale. Can. J. Soil Sci. 79: 353-366.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 591-611.

Sharifi, M., B.J. Zebarth, D.L. Burton, C.A. Grant and J.M. Cooper. 2007. Evaluation of Some Indices of Potentially Mineralizable Nitrogen in Soil. Soil Sci. Soc. Am. J. 71: 1233.

Sharpley, A.N., S.J. Smith and W.R. Bain. 1993. Nitrogen and Phosphorus Fate from Long-Term Poultry Litter Applications to Oklahoma Soils. Soil Sci. Soc. Am. J. 57: 1131-1137.

Six, J., R. Conant, E. Paul and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241: 155-176.

Springob, G. and H. Kirchmann. 2003. Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. Soil Biol. Biochem. 35: 629-632.

Stanford, G. 1978. Evaluation of ammonium release by alkaline permanganate extraction as an index of soil nitrogen availability. Soil Sci. 126: 244-253.

Stanford, G. and S.J. Smith. 1972. Nitrogen Mineralization Potentials of Soils. Soil Sci. Soc. Am. J. 36: 465-472.

Wang, W., C.J. Smith, P.M. Chalk and D. Chen. 2001. Evaluating chemical and physical indices of nitrogen mineralization capacity with an unequivocal reference. Soil Sci. Soc. Am. J. 65: 368-376.

Waring, S. and J. Bremner. 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature 201: 951-952.

Watts, D.B., H.A. Torbert, S.A. Prior and G. Huluka. 2010. Long-Term Tillage and Poultry Litter Impacts Soil Carbon and Nitrogen Mineralization and Fertility. Soil Sci. Soc. Am. J. 74: 1239-1247.

Willer, H. and Lernoud, J. 2015. The world of organic agriculture. Statistics and emerging trends 2015. Research institure of organic agriculture, Frick and IFOAM-Organics International, Bonn.

Wyngaard, N. and M.L. Cabrera. 2015. Measuring and estimating sulfur mineralization potential in soils amended with poultry litter or inorganic fertilizer. Biol. Fert. Soils 51: 545-552.

Xiao-Tang, J., L. Xue-Jun, F.-S. Zhang and P. Christie. 2006. Effect of long-term fertilization on organic nitrogen forms in a calcareous alluvial soil on the North China Plain. Pedosphere 16: 224-229.

Zebarth, B., J. Paul and R. Van Kleeck. 1999. The effect of nitrogen management in agricultural production on water and air quality: evaluation on a regional scale. Agric. Ecosyst. Environ. 72: 35-52.

TABLES AND FIGURES

Table 2.1. Selected soil properties (0 to 20 cm) and fertilizer rates of soils from four experimental sites that were fertilized with inorganic fertilizer (IF) or poultry litter (PL) for more than 10 years

Site	Clay	Sand	pH	Years	IF annual rate	PL annual rate	C rate as PL	N rate as PL
	g 100 g^{-1}	g 100 g^{-1}			kg ha^{-1} N	Mg ha^{-1} $year^{-1}$ PL	Mg ha^{-1} $year^{-1}$ C	kg ha^{-1} $year^{-1}$ N
Delaware	14.5	82.0	6.1	11	270	9.9	3.75	210
Maryland	21.8	16.1	6.0	11	270	9.9	3.75	210
Iowa	29.2	42.0	5.5	14	150	9.4	3.56	200
Georgia	39.9	43.6	5.5	15	92	7.8	2.96	170

Table 2.2. Total carbon (C_t), total nitrogen (N_t) and C:N ratio of whole soil samples and coarse fraction (>53 µm) samples from four soils after more than 10 years of fertilization with poultry litter (PL) or inorganic fertilizers (IF). Different letters indicate significant ($p < 0.05$) differences between treatments and sites as indicated by the Tukey-Kramer test. CV% = standard deviation (%).

		Soil			Coarse fraction		
Site	Treatment	C_t	N_t	C:N	C_t	N_t	C:N
		g kg^{-1}	g kg^{-1}		g kg^{-1}	mg kg^{-1}	
Delaware	IF	12.4 d	0.76 f	16.3 a	3.6 bc	89.4	40.6
	PL	11.7 d	0.84 f	14.0 b	3.0 bc	114.2	26.0
Maryland	IF	11.6 d	1.10 e	10.5 d	2.0 c	80.9	24.6
	PL	13.4 d	1.32 d	10.1 d	2.5 c	132.6	18.8
Iowa	IF	23.0 c	1.98 c	11.6 c	2.3 c	151.9	15.4
	PL	25.2 b	2.36 b	10.7 d	5.5 ab	377.0	14.5
Georgia	IF	26.4 b	2.48 b	10.6 d	5.3 ab	256.9	20.8
	PL	30.9 a	3.11 a	9.9 d	7.4 a	506.7	14.7

(Continues in the next page)

(Continued from previous page)

	ANOVA					
Site (S)	< 0.001	< 0.001	< 0.001	< 0.001	0.002†	< 0.001†
Treatment (T)	< 0.001	< 0.001	< 0.001	< 0.001	0.010†	0.003†
S x T	< 0.001	< 0.001	< 0.001	0.004	0.213	0.127
CV (%)	3.05	4.51	2.67	23.0	47.3	21.4

† means comparison results analyzed in the text

Table 2.3. 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) or inorganic fertilizers (IF). Different letters indicate significant ($p < 0.05$) differences between treatments and soils as indicated by the Tukey-Kramer test. CV% = standard deviation (%).

Soil	Treatment	N_o	*k*	Cold_KCl-N	Hot_KCl-N	Hyd_KCl-N	ISNT	NaOH-N	PB-N	N_{an}
		mg kg^{-1}	day^{-1}	------------------------------------ mg kg^{-1} ------------------------------------						
Delaware	IF	60.3 d	0.012	0.5 e	2.2 d	1.7	33.3	88.3 g	16.1 f	41.0 f
	PL	62.1 d	0.014	0.6 e	2.4 d	1.8	67.7	87.2 g	14.4 f	34.5 f
Maryland	IF	107.7 cd	0.014	0.6 e	2.4 d	1.8	111.8	121.8 f	19.5 e	60.6 e
	PL	141.3 c	0.014	0.6 e	2.4 d	1.8	129.3	144.7 e	23.5 d	70.1 de
Iowa	IF	145.5 c	0.010	1.2 c	4.0 c	2.8	228.7	223.5 d	61.9 a	73.0 d
	PL	244.2 b	0.010	0.8 d	3.4 c	2.6	233.0	247.8 c	50.3 c	107.5 c
Georgia	IF	248.2 b	0.012	4.4 a	10.4 b	6.0	364.8	301.5 b	57.3 b	126.6 b
	PL	481.3 a	0.010	1.9 b	6.9 a	5.1	390.8	352.3 a	58.9 ab	172.2 a

(Continues in next page)

(Continued from previous page)

					ANOVA				
Site (S)	< 0.001	0.092	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment (T)	< 0.001	0.362	< 0.001	< 0.001	0.052	0.410	< 0.001	0.001	< 0.001
S x T	< 0.001	0.908	< 0.001	< 0.001	0.058	0.700	< 0.001	< 0.001	< 0.001
CV (%)	12.6	23.6	3.8	7.5	10.9	11.6	2.6	3.2	4.4
					Averages				
Delaware	61.2	0.013	0.6	2.3	1.7 c	50.5 d	87.7	15.3	37.8
Maryland	124.5	0.014	0.6	2.4	1.8 c	120.6 c	133.3	21.5	65.3
Iowa	208.4	0.010	1.0	3.7	2.7 b	230.8 b	235.6	56.1	90.2
Georgia	364.7	0.011	3.1	8.7	5.5 a	377.8 a	326.9	58.1	149.7
IF	140.4	0.012	1.7	4.8	3.1	198.8	183.8	38.7 a	75.3
PL	239.0	0.012	1.0	3.8	2.8	191.0	208.0	36.8 b	96.2

Abbreviations: N_o: potentially mineralizable N; *k*: N mineralization constant; Cold_KCl-N: NH_4^+-N extracted with 2M KCl; Hot_KCl-N: NH_4^+-N extracted with 100 ^{o}C 2M 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 condition.

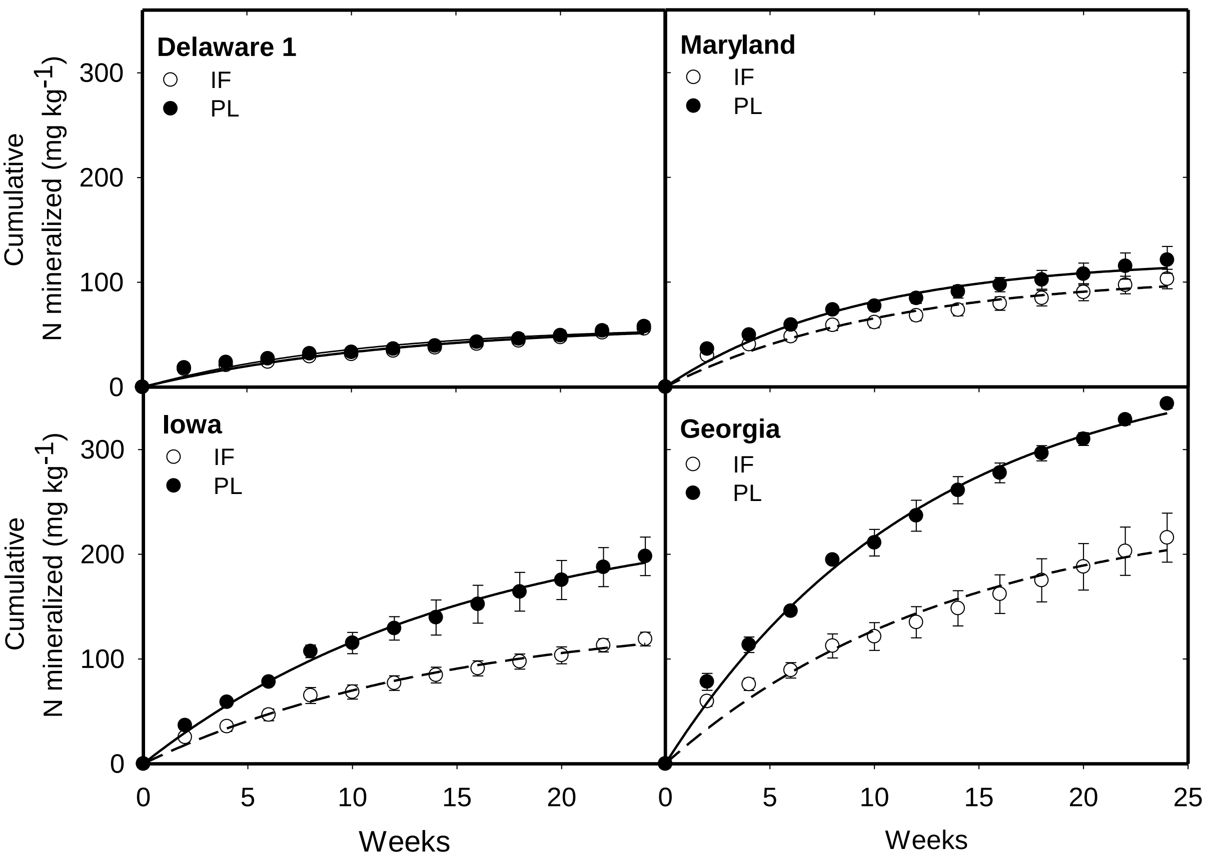

Figure 2.1. Cumulative amount of N mineralized in four soils that were fertilized with poultry litter (PL) or inorganic fertilizer (IF) for more than 10 years and were incubated at 30±1 °C for 24 weeks. Vertical bars represent the standard deviation among replications.

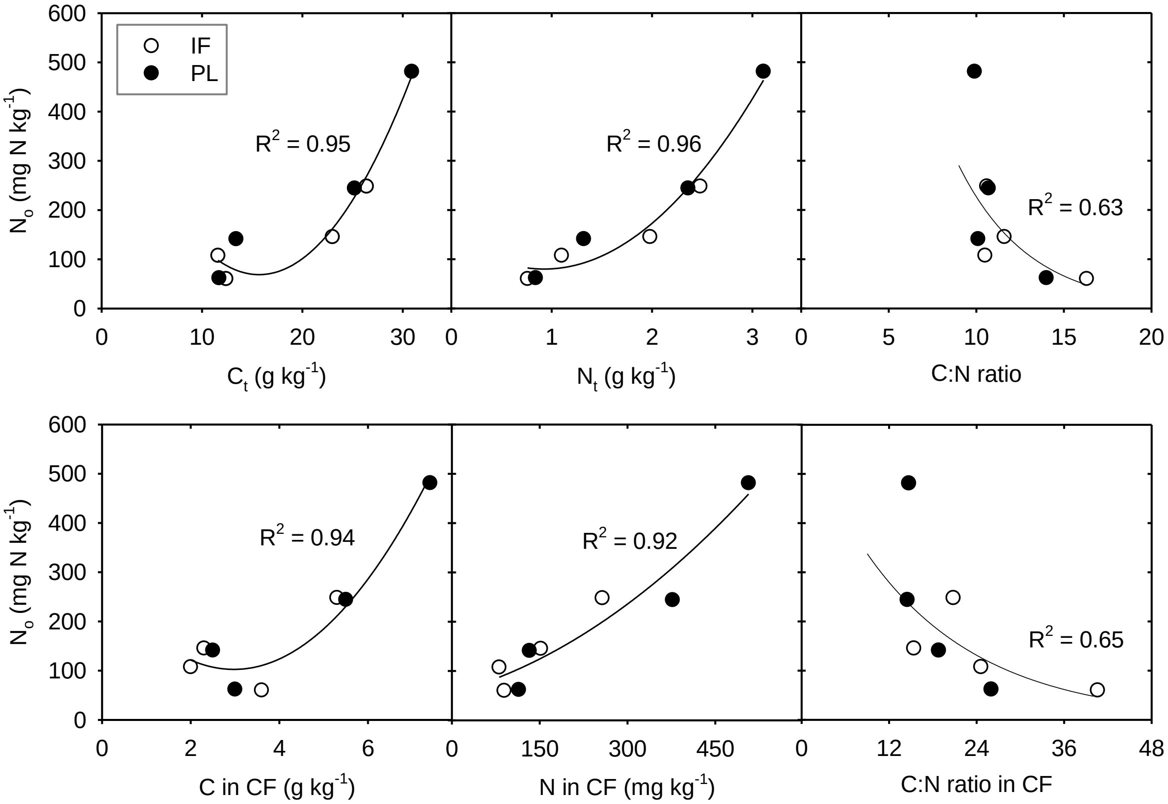

Figure 2.2. Association between total carbon (C_t), total N (N_t) or C:N ratio in the whole soil and the coarse fraction (>53 µm) and the potentially mineralizable nitrogen (N_o) determined by long-term incubation (24 weeks) in four soils that had been fertilized with poultry litter (PL) or inorganic fertilizer (IF) for more than 10 years.

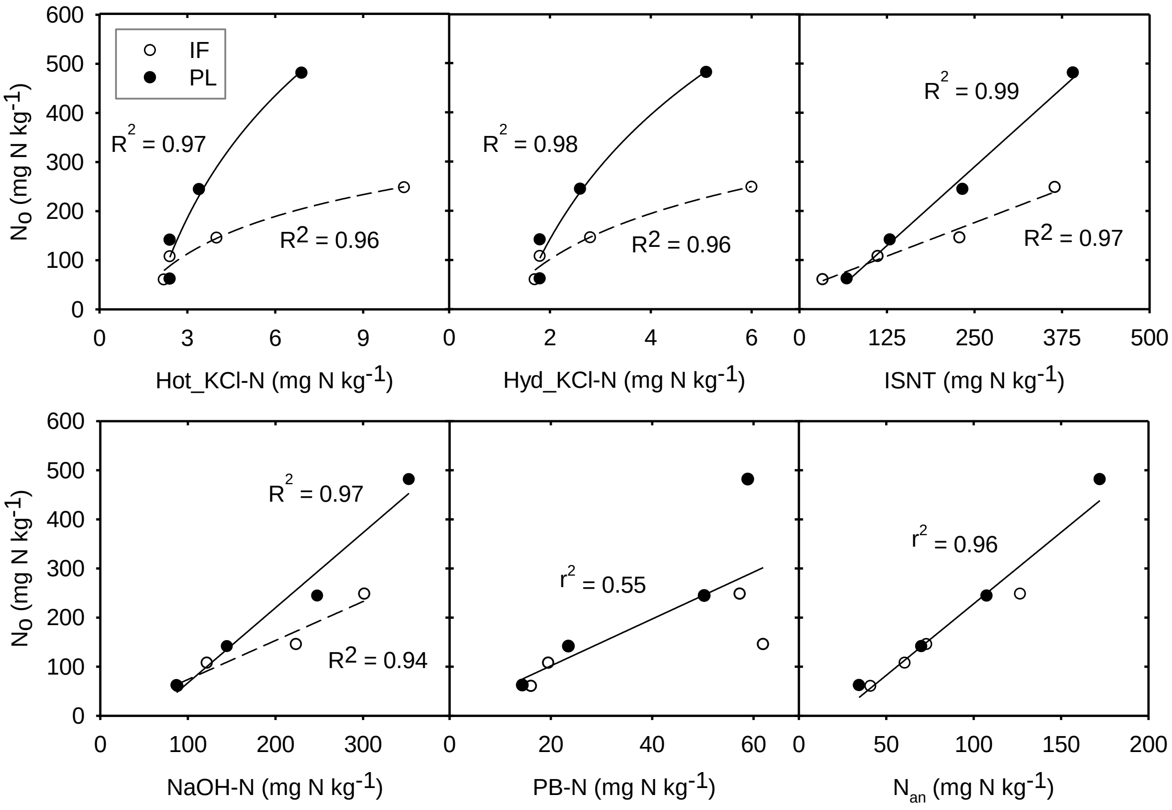

Figure 2.3. Association between different methods to estimate N mineralization and the potentially mineralizable nitrogen (N_o) determined by long-term incubation (24 weeks) in four soils that had been fertilized with poultry litter (PL) or inorganic fertilizer (IF) for more than 10 years. Each point represents the average of three replications. Abbreviations: Hot_KCl-N: NH_4^+-N extracted with 100°C 2M 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.

CHAPTER 3

MEASURING AND ESTIMATING SULFUR MINERALIZATION POTENTIAL IN SOILS AMENDED WITH POULTRY LITTER OR INORGANIC FERTILIZER[1]

---

[1] Wyngaard, N., M.L. Cabrera. 2015. Biol. Fert. Soils 51:545-552. Reprinted here with permission of publisher.

ABSTRACT

The effect of long-term (more than 10 years) application of poultry litter (PL) or inorganic fertilizer (IF) on sulfur (S) mineralization potential (S_o) was evaluated using four different soils (Delaware 1, Delaware 2, Iowa and Georgia). Also, different methods to estimate S availability (extraction with KCl, $NaHCO_3$ and KCl after a wetting-drying cycle, aerobic incubations for 7 and 14 days, and NH_4^+ quantification after an anaerobic incubation) were compared to S_o. When compared to long-term application of IF, long term-application of PL generated an increase in total soil S ranging from 0 to 0.14 g kg^{-1}, which was positively associated with the soil clay content (R^2=0.98), and resulted in an increase in S_o ranging from 0 to 21.2 g kg^{-1}. From the evaluated methods to estimate S_o only three of them were significantly ($p<0.05$) correlated to S_o: aerobic incubation for 7 days (r=0.85), extraction with $NaHCO_3$ (r=0.85), and nitrogen anaerobic incubation (r=0.99). Based on the reduced time and labor involved and in their capacity to distinguish between treatments, we recommend further use and further evaluation of the last two methods as estimators of S_o.

1. INTRODUCTION

Sulfur (S) is an essential element for plant growth that increasingly limits crops production in several regions of the world. One of the main reasons for the increased frequency of S deficiencies is the depletion of soil organic matter (Tisdale et al. 1986), which contains more than 90% of the total S in soil (Zhou et al. 1999). This organic S can be converted to SO_4^{2-} through the process of mineralization, either via biochemical (hydrolysis of sulfate esters by sulfatase enzymes) or biological processes (release of S bonded to carbon by microorganisms) (McGill and Cole 1981). Due to the significant contribution of S mineralization to plant S uptake (Eriksen et al. 1995), this process must be accounted for in order to accurately predict plant-available S during the growing season of crops.

Another reason for the increased frequency of S deficiencies is the increased use of inorganic fertilizers (IF) with reduced S content (Tisdale et al. 1986). In contrast, the long-term use of organic fertilizers can increase the organic S pool of the soil (Eriksen and Mortensen 1999; Knights et al. 2001), leading to an increase in the S mineralization potential (Knights et al. 2001; Reddy et al. 2001). Among the organic fertilizers, poultry litter (PL) (a mixture of excreta, organic bedding material and feed) is highly important in the United States where almost 8.6 billion broilers (*Gallus gallus domesticus*) are produced annually (National Agricultural Statistics Service 2012) with the generation of about 13 million Mg of poultry litter. Despite the significant use of PL as an organic amendment in the United States,, there is no information regarding the accumulation of S in soil and the change in S mineralization potential after its long-term application (>10 years).

Several methods have been proposed to estimate the labile S fraction that is plant available throughout the season. Some of these methods are based on the treatment of a soil sample with an extractant as KCl (Blair et al. 1991), $NaHCO_3$ (Kilmer and Nearpass 1960), or NaCl after a wetting-drying cycle (Williams and Steinbergs 1959). Other methods are based in the incubation of soil samples under aerobic conditions. The use of closed incubation systems is not valid for long periods of time, as the accumulation of sulfate inhibits the mineralization process (Maynard et al. 1983). However, the use of long-term incubations (24 weeks) in open systems, where soils are leached of SO_4^{2-} periodically during the incubation, has been extensively used (Ghani et al. 1991; Maynard et al. 1983; Reddy et al. 2001; Tanikawa et al. 2014) and it is considered the standard method to estimate S mineralization potential. Even though the use of chemical extractants has been compared to plant S uptake, no comparison has been ever made between these methods and long-term aerobic incubations.

It must also be considered that S mineralization is closely associated with nitrogen (N) mineralization, as both nutrients are present in the organic pool of most soils (Kellogg et al. 2006; Zhou et al. 1999). Because of this, it has been suggested that the methods to estimate N mineralization can potentially be used to estimate S mineralization (Gharmakher et al. 2009). Among the N methods, the 7-days anaerobic incubation described by Waring and Bremner (1964) (N_{an}) has been widely used to determine the effect of management practices over N mineralization and to improve N diagnosis in crops (Reussi Calvo et al. 2013; Sainz Rozas et al. 2008; Schomberg et al. 2009). However, this index has not been evaluated as an indicator of S mineralization.

The particle-size separation of soil samples is also frequently used to distinguish fractions with different turnover rates (Christensen 1992). The determination of S in these fractions has been previously used to compare between soils (Kirkby et al. 2011) and soil management practices (Galantini and Rosell 1997). A specific type of soil fractioning is the one proposed by Cambardella and Elliott (1992), which distinguishes between the particulate fraction (PF, > 53 µm) and the fraction associated with minerals (AF, < 53 µm), PF being more dynamic and sensitive to changes in management practices (Janzen et al. 1998). This is why the content of N in PF has been evaluated as an estimator of N mineralization potential (Schomberg et al. 2009). However, the use of S in PF (S-PF) has never been evaluated as an estimator of S mineralization.

Given the lack of information on S accumulation and mineralization potential in soils receiving PL, as well as the limited data available on methods to estimate S mineralization potential, the objectives of this study were to: 1) determine the long-term (>10 years) effect of fertilizer treatment (PL or IF) on S content and S mineralization potential of four soils, and 2) evaluate several methods to estimate S mineralization potential in soil.

2. MATERIALS AND METHODS

In the Fall of 2012, composite soil samples (0 to 20 cm) were collected from long-term research plots at four different locations in the states of Delaware, Iowa, and Georgia (USA). These locations, identified as Delaware 1, Delaware 2, Iowa and Georgia, were chosen based on their contrasting soil properties and on the presence of plots with the fertilization treatments of interest for this study. Some selected soil properties and management practices in each site are described in Table 3.1. In every location, two different fertilization treatments were sampled (PL and IF). The IF treatments received the same rate of N as PL, but in the form of urea-ammonium nitrate (UAN) or ammonium nitrate; however, these treatments did not receive any S input. The S content in the applied PL was considered to be 1.6 g kg^{-1} as described by Tabatabai and Chae (1991). The collected samples were air dried and ground to pass through a 2 mm-sieve, and all recognizable crop residues were removed.

Total S (S_t) in the samples was determined by wet digestion with HNO_3 and $HClO_4$ (Zhao et al. 1994) followed by quantification using inductively coupled plasma emission spectrometry (ICP-AES) (Thermo Fisher 61E). Sulfate concentration (S_i) was determined by ion chromatography (IC) (Met Rohm IC 820 separation system, 819 conductivity detector with carbonate and cation suppression) after the extraction of soil samples with 0.01 M NH_4Cl at a 10:1 soil:solution ratio (Maynard et al. 1987). This extractant was used instead of the usual 0.01M $Ca(H_2PO_4)_2$ because the high concentration of orthophosphate ions interfered with the IC quantification of sulfate. Organic S content (S_{org}) was calculated as the difference between S_t and S_i. Additionally, total carbon (C_t) was measured by dry combustion using a TruSpec CN analyzer (LECO Corporation, St. Joseph, MI).

In order to quantify S-PF, soil samples were dispersed with 2 N sodium hexametaphosphate and sieved through a 53-µm sieve. The slurry that passed through this sieve was then dried and considered as AF, where total S was quantified by ICP-AES. The difference between S_t and S-AF was considered to be S-PF, which is the S content in the fraction that remained on top of the sieve (Cambardella and Elliott 1992).

Three chemical methods to estimate S availability by crops were evaluated. The first one was the extraction of soil with 0.25 M KCl at 40 ^oC for 3 hours, followed by filtration and S_t quantification in the extract by ICP-AES (Blair et al. 1991) (KCl method). Secondly, the method proposed by Williams and Steinbergs (1959) was utilized. This technique consists in rewetting of samples, which are then dried in a boiling water bath, placed in an oven at 102 ^oC for one hour and extracted with 0.1% NaCl before the quantification of S_t in the extract by ICP-AES (Heat method). The third method was the extraction of the soil samples with 0.3 M $NaHCO_3$ and subsequent measurement of S_t

(Kilmer and Nearpass 1960) ($NaHCO_3$ method). The KCl, Heat, and $NaHCO_3$ methods were evaluated with and without subtracting the initial S_i present in the samples, and three laboratory replications per sample were performed.

Additionally, two short-term destructive incubations were evaluated. To this end, 10 g of soil was thoroughly mixed with 20 g of 1-mm acid-washed sand and transferred to a 50-ml plastic container. After this, the soil was moistened to achieve a water content equivalent to 80% of field capacity (Maynard et al. 1983), covered with a porous plastic film, and incubated at 30 ^oC. The samples were weighed every three days to correct for water content. Six replications were used, with three of them used to measure sulfate content as previously described after seven days of incubation (7d method) and the other three used to measure sulfate content after 14 days (14d method). The initial value of S_i was subtracted from the value determined at the end of the incubation period. The short-term N anaerobic incubation (N_{an}) was performed by quantifying the amount of NH_4^+ released after the incubation of 10 g of soil samples saturated with water at 40 ^oC for 7 days (Waring and Bremner 1964).

The long-term aerobic incubation was executed following the procedure described by Stanford and Smith (1972) as modified by Pirela and Tabatabai (1988). In detail, 20 g of soil was mixed with the same amount of 1-mm acid-washed sand and transferred to a 60-ml Monoject® polypropylene syringe with glass wool at the bottom. The soil-sand mixture was moistened to a water content of 80% of field capacity and then leached with 200 ml of 0.01 M $CaCl_2$ in 5-ml increments to remove the initial sulfate. The excess water was removed by vacuum filtration at 6 kPa. After this, the samples were covered with a porous membrane and incubated at 30±1 ^oC for 24 weeks, gravimetrically correcting the water content every three days. Samples were leached every two weeks with 0.01 M $CaCl_2$ and sulfate concentration was measured in the leachate by IC. Three replicates were carried out for each soil. For estimating the potentially mineralizable S (S_o), data were fit to a first-order model: $S_m=S_o$ [1-exp(-kt)], where S_m is the cumulative amount of S mineralized at a specific time (t) and *k* is the first-order rate constant.

The effect of soil type and fertilization treatments on the analyzed variables was evaluated using the SAS PROC MIXED procedure (SAS Institute Inc 2013). Effects were considered statistically significant at $p < 0.05$, and means were compared using Tukey-Kramer test. The exponential model for S mineralization was fit using SAS PROC NLIN. The correlation between variables of interest was determined using the SAS PROC CORR procedure (SAS Institute Inc 2013).

3. RESULTS AND DISCUSSION

3.1. S mineralization in soils amended with PL or IF

The S_t content was closely associated with S_{org} , which represented an average of 97.5% of the total S content. Both S_t and S_{org} varied among soils and were modified by fertilization treatments (Table 3.2). Soils from Iowa and Georgia, with a greater clay content, more years under treatment, and a greater S input, showed a greater accumulation of S_t in PL plots relative to IF: 0.14 g kg^{-1} (61%) and 0.08 g kg^{-1} (27%), respectively. On the other hand, the soils from Delaware showed no increase in Delaware 1 but a small increment of 0.03 g kg^{-1} (17%) in Delaware 2. The accumulation of S_t was associated with that observed for C_t (Table 3.2) in Delaware 1 (0 g C_t kg^{-1}), Delaware 2 (1.7 g C_t kg^{-1}) and Iowa (2.3 g C_t kg^{-1}). However, Georgia showed a greater C_t accumulation (4.5 g C_t kg^{-1})

than the one observed in Iowa, which does not follow the same trend observed for S_t. It has been previously observed that even though C content is linearly related to S_t content, the relationship is weaker than the one commonly found between C and N (Gharmakher et al. 2009). Therefore, an increase in C_t content does not necessarily imply a similar increment in S_t (Eriksen and Mortensen 1999).

In order to generate a basic S balance in PL plots, S_t concentration must be converted from g kg^{-1} to kg ha^{-1} using the soil bulk density. However, information about this variable was not available. To obtain an approximation of bulk density, the computation described by Rawls (1983), which uses particle size distribution and organic matter content as predicting variables, was performed. The estimated bulk densities were 1.45, 1.15, 1.22 and 1.22 g cm^3 for Delaware 1, Delaware 2, Iowa and Georgia, respectively. This led to a difference in S_t content between PL and IF plots of 0, 69, 340 and 195 kg ha^{-1} respectively, which represents an accumulation of 0, 40, 89 and 98% of the S applied as PL. The capacity of the soil to accumulate S was closely associated with the clay content (Figure 3.1). The reason for this relationship is the larger organic matter protection capacity of soils with a greater clay content (Ladd et al.; Six et al. 2002). The effect of texture on S_t is clear in the Delaware soils, where the management practices and climate were similar. However, it should be considered that other factors may have affected the accumulation of S in the other sites where climate and management practices were different. For example, the different crops used in each one of the sites can generate a different input of plant residues, which in turn would affect the S_{org} input. Consequently, the high S_t content in Georgia may not be only a consequence of the soil texture, but also of the high volume of residues returned to the soil by the established pasture.

Regarding S content in the PF of the soil, the differences between fertilization treatments were only observed in soils from Iowa and Georgia (Table 3.2). The overall value of S-PF in the evaluated soils was similar to the one observed by Kirkby et al. (2011) in the light fraction of the soil, which is equivalent to PF but involves a different fractionation method. These authors determined an average value for S in the light fraction of 0.012 mg kg^{-1} in long-term cropped plots and 0.022 mg kg^{-1} in a pasture, while in the present study these values were 0.012 mg kg^{-1} and 0.026 mg kg^{-1} (pasture in Georgia), respectively, in soils not amended with PL. A high variability between replications was also determined. Those samples with a S_t content below 0.23 g kg^{-1} presented an average variation coefficient of 246%, whereas those samples above this value averaged only 15% (data not shown). It should be kept in mind that three steps are required to estimate S-PF, involving the fractioning of the soil together with the measurement of S in two fractions (Total and AF). Therefore, the final result accumulates the error of these three steps, and the weight of this error is greater in samples with a low S_t content. Some replications from soils with low S_t even showed negative values of S-PF, which is irrational and proves that the variability of the method can be greater than the actual value of S-PF. Even though the association between S-PF and mineralization (Table 3.3) was significant, we do not recommend the use of this technique as an estimator of S_o, based on its high variability and complexity.

The potentially mineralizable S pool (S_o) varied between 13.8 and 71.3 mg kg^{-1} which is within the values previously reported by Pirela and Tabatabai (1988) (Table 3.2). Also, S_o represented from 9.4 to 17.1% of S_t. This is in line with the findings of Tanikawa et al. (2014), who determined that after 280 days of incubation at 35 ^{o}C the evaluated soils mineralized between 7.1 and 15.8% of the initial S. Also, Pirela and Tabatabai (1988) determined that between 2.4 and 17.5% of S_t was mineralized when soil samples were incubated at 30 ^oC for 98 days. The response of S_o to PL application varied between sites (Table 3.2, Figure 3.2). There was no difference in S_o between PL and IF in Delaware 1, whereas in Delaware 2, Iowa, and Georgia this difference was 3.6, 16.8 and 21.2 mg kg^{-1}, respectively. Previous research has determined that the long-term application of organic amendments has the capacity to increase the soil S mineralization potential (Eriksen and Mortensen 1999; Knights et al. 2001). However, these authors used cattle manure instead of PL. The present study proves that even though PL has a greater mineralization rate relative to other organic waste materials derived from hog, horses, and cows (Tabatabai and Chae 1991), there is still an annual accumulation of S_{org} in the soil, which leads to an increase in its S mineralization potential. But this accumulation, as the pattern of S mineralization from organic wastes, is highly dependent not only on the type of amendment but also on the characteristics of the soil where it is applied.

The sulfur mineralization potential (S_o) was positively correlated to S_t, S_{org}, S-PF and C_t, but not to S_i or the C:S ratio (Table 3.3). Riffaldi et al. (2006) also described a high correlation between S_o and S_{org} or C_t. This seems logical as the size of the mineralizable S pool increases with S_{org}, which is closely associated to C_t in the organic matter. However, other authors did not find a significant relationship between these variables (Pirela and Tabatabai 1988; Tabatabai and Al-Khafaji 1980).

The first-order rate constant k ranged between 0.025 and 0.075 $week^{-1}$, and was affected by the soil type and fertilization treatment, with no interaction between them (Table 3.2). The Georgia soil showed an average k value 50% smaller than the others soils, but the PL treatment presented *k* values 27% greater than IF. There was no relationship between *k* and S_t, S_{org}, S-PF, C_t, S_i or C:S ratio (Table 3.3), which is in agreement with previous findings from Riffaldi et al. (2006). There was also no correlation between S_o and *k*, indicating that the differences observed in *k* values were not derived from the size of the mineralizable S pool. In summary, PL application increased the S_{org} pool consequently increasing S_o, and also increasing the rate of mineralization *k*. However, the increase in *k* could not be explained based on the measured soil properties.

3.2. Evaluation of methods to estimate S mineralization potential in soil

The results of different methods to estimate S mineralization and availability are presented in Table 3.5. The only chemical method that was significantly associated with S_o was $NaHCO_3$ (r=0.85) and this association was not improved by subtracting the initial S_i content. The values of S extracted by $NaHCO_3$ ranged between 8.8 and 51.2 mg kg^{-1}, and were similar to the ones estimated by Kilmer and Nearpass (1960) in Delaware and Iowa soils. However, the soil from Georgia exceeded these values. Even though the forms of S extracted by this method are unknown, between 10 to 46% of the extracted S was present as S_i. On the other hand, the Heat and KCl methods, which have been previously associated with plant S uptake, were not correlated with S_o; although they were strongly associated with each other (Table 3.5). This indicates that the S fraction quantified by both extractions is probably the same, but is not associated with the S pool mineralized in long-term aerobic incubations.

Regarding the incubation methods, both 7d and 14d were correlated with S_o (r=0.85 and 0.70 respectively, Table 3.5). On average, 70% of the mineralized S in 14 days was released during the first week of incubation. This can be explained by the greater rate of S mineralization in this period (Figure 3.2), and also by the fact that in closed incubation systems the mineralization process is limited by the accumulation of sulfate, which represses the activity of the arylsulfatase enzyme (Maynard et al. 1983). As 7d and 14d had a similar performance as predictors of S_o, the first one is recommended due to its reduced incubation time. However, it must be noticed that even though 7d was correlated to S_o, the high coefficient of variation, which averaged 37% between replications (data not shown), did not allow to make a clear distinction between soils and treatments (Table 3.4). Therefore, even though 7d followed the same trends observed in S_o, differences in 7d were not always statistically significant. This high variability was mostly observed in the Delaware soils, where the concentration of S_i released in a week was low and close to the detection limit of the IC.

Finally, the method to estimate N mineralization (N_{an}) was closely related to S_o, with a Pearson correlation coefficient of 0.99. The values of N_{an} ranged between 41 and 172 mg kg^{-1}, and those in Georgia soils were greater than the ones observed by Schomberg et al. (2009) in a wide range of soils. It should be pointed out, however, that Schomberg et al. (2009) evaluated soils under continuous cropping, whereas our soil from Georgia was under a pasture system. The variability in N_{an} between replicates was low, with an average coefficient of variation of 3.7% (data not shown), and the observed differences between soils and treatments were the same as the ones determined for S_o. The close relationship between N and S mineralization has been previously described (Gharmakher et al. 2009; Tabatabai and Al-Khafaji 1980). However, the results from the present study demonstrate that a method used to estimate N mineralization can also be used in estimating S mineralization. Also, N_{an} was related to $NaHCO_3$ and 7d, which are the other methods that were significantly correlated to S_o. This indicates that the labile fractions mineralized during 7 days in aerobic or waterlogged conditions are the same or strongly related to the ones chemically extracted by $NaHCO_3$.

Based on the observed relationship between S_o and different chemical extractions and incubations, only three of the evaluated methods can be recommended to predict S_o: $NaHCO_3$, 7d and Nan. However, 7d takes the same amount of time as N_{an} to be performed but is more labor consuming. Moreover, 7d requires the measurement of S_i before and after the incubation, which not only adds to the total economic cost of the technique but increases the variability between replications, reducing the capability of the method to statistically distinguish between soils and treatments. On the other hand, $NaHCO_3$ involves only one measurement and can be performed in one day, as no incubation is required. Finally, although the use of N_{an} requires an incubation of 7 days, the close relationship to S_o makes it feasible to use this method to simultaneously estimate N and S mineralization, which represents an advantage for soil testing laboratories. Further studies should be performed to relate N_{an} not only to S_o, but to S uptake by crops under field conditions.

4. CONCLUSIONS

Our results provided previously unavailable information regarding the long-term effect of fertilizer treatment (PL or IF) on the S mineralization potential of four soils. The effect of long-term PL application on S_t and S_{org} was closely associated with the accumulation of C_t and with the clay content of the soil. The increase in S_{org} observed in PL plots with a clay content greater than 20% resulted in an increase in S_o and k. We also evaluated seven methods that had not been previously used to estimate the S mineralization potential of soil ($NaHCO_3$, Heat, KCl, short-term incubations, N_{an} and S-PF). Our results showed that $NaHCO_3$, 7d and N_{an} were well correlated to $S_{o.}$ However, based on their reduced time and labor involved, and on their capability to distinguish between treatments, we recommend the use and further evaluation of $NaHCO_3$ and N_{an} for estimating the S mineralization potential of soils.

REFERENCES

Blair GJ, Chinoim N, Lefroy RDB, Anderson G, Crocker G (1991) A soil sulfur test for pastures and crops Soil Res 29:619-626

Cambardella C, Elliott E (1992) Particulate soil organic-matter changes across a grassland cultivation sequence Soil Sci Soc Am J 56:777-783

Christensen BT (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. In Stewart BA (Ed.) Advances in soil science. Springer, Heidelberg, pp 1-90

Eriksen J, Mortensen JV (1999) Soil sulphur status following long-term annual application of animal manure and mineral fertilizers Biol Fertil Soils 28:416-421

Eriksen J, Mortensen JV, Nielsen JD, Nielsen NE (1995) Sulphur mineralisation in five Danish soils as measured by plant uptake in a pot experiment Agriculture, ecosystems & environment 56:43-51

Galantini JA, Rosell RA (1997) Organic fractions, N, P and S changes in an Argentine semiarid Haplustoll under different crop sequences Soil Till Res 42:221-228

Ghani A, McLaren R, Swift R (1991) Sulphur mineralisation in some New Zealand soils Biol Fert Soils 11:68-74

Gharmakher HN, Machet J, Beaudoin N, Recous S (2009) Estimation of sulfur mineralization and relationships with nitrogen and carbon in soils Biol Fert Soils 45:297-304

Janzen H, Campbell C, Izaurralde R, Ellert B, Juma N, McGill W, Zentner R (1998) Management effects on soil C storage on the Canadian prairies Soil Till Res 47:181-195

Kellogg LE, Bridgham SD, López-Hernández D (2006) A Comparison of Four Methods of Measuring Gross Phosphorus Mineralization Soil Sci Soc Am J 70:1349-1358

Kilmer VJ, Nearpass D (1960) The determination of available sulfur in soils Soil Sci Soc Am J 24:337-340

Kirkby C, Kirkegaard J, Richardson A, Wade L, Blanchard C, Batten G (2011) Stable soil organic matter: a comparison of C: N: P: S ratios in Australian and other world soils Geoderma 163:197-208

Knights J, Zhao F, McGrath S, Magan N (2001) Long-term effects of land use and fertiliser treatments on sulphur transformations in soils from the Broadbalk experiment Soil Biol Biochem 33:1797-1804

Ladd JN, Foster R, Nannipieri P, Oades JM (1996) Soil structure and biological activity. In Stotzky G and Bollag JM (Eds.) Soil Biochemistry, vol 9. Marcel Dekker, New York, pp 23-78

Maynard D, Kalra Y, Radford F (1987) Extraction and determination of sulfur in organic horizons of forest soils Soil Sci Soc Am J 51:801-806

Maynard D, Stewart J, Bettany J (1983) Sulfur and nitrogen mineralization in soils compared using two incubation techniques Soil Biol Biochem 15:251-256

McGill W, Cole C (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter Geoderma 26:267-286

National Agricultural Statistics Service (2012) Poultry production and value: 2011 summary:14

Pirela HJ, Tabatabai MA (1988) Sulfur mineralization rates and potentials of soils Biol Fert Soils 6:26-32

Rawls Wj (1983) Estimating soil bulk density from particle size analysis and organic matter content Soil Sci 135:123-125

Reddy KS, Singh M, Tripathi AK, Swarup A, Dwivedi AK (2001) Changes in organic and inorganic sulfur fractions and S mineralisation in a Typic Haplustert after long-term cropping with different fertiliser and organic manure inputs Soil Res 39:737-748

Reussi Calvo NI, Sainz Rozas H, Echeverría H, Berardo A (2013) Contribution of anaerobically incubated nitrogen to the diagnosis of nitrogen status in spring wheat Agron J 105:321-328

Riffaldi R, Saviozzi A, Cardelli R, Cipolli S, Levi-Minzi R (2006) Sulphur mineralization kinetics as influenced by soil properties Biol Fert Soils 43:209-214

Sainz Rozas H, Calviño PA, Echeverría HE, Barbieri PA, Redolatti M (2008) Contribution of anaerobically mineralized nitrogen to the reliability of planting or presidedress soil nitrogen test in maize Agron J 100:1020-1025

SAS Institute Inc (2013) SAS® 9.4 In-Database Products: User's Guide, Third Edition. Cary, NC: SAS Institute Inc.

Schomberg HH, Wietholter S, Griffin TS, Reeves DW, Cabrera ML, Fisher DS, Novak JM, Balkcom KS, Raper RL, Kitchen NR, Locke MA, Potter KN, Schwartz RC, Truman CC, Tyler DD (2009) Assessing indices for predicting potential nitrogen mineralization in soils under different management systems Soil Sci Soc Am J 73:1575

Six J, Conant R, Paul E, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils Plant Soil 241:155-176

Stanford G, Smith SJ (1972) Nitrogen Mineralization Potentials of Soils Soil Sci Soc Am J 36:465-472

Tabatabai M, Chae Y (1991) Mineralization of sulfur in soils amended with organic wastes J Environ Qual 20:684-690

Tabatabai MA, Al-Khafaji AA (1980) Comparison of Nitrogen and Sulfur Mineralization in Soils Soil Sci Soc Am J 44:1000-1006

Tanikawa T, Noguchi K, Nakanishi K, Shigenaga H, Nagakura J, Sakai H, Akama A, Takahashi M (2014) Sequential transformation rates of soil organic sulfur fractions in two-step mineralization process Biol Fert Soils 50:225-237

Tisdale S, Reneau R, Platou J (1986) Atlas of sulfur deficiencies Sulfur in agriculture. In Tabatabai MA (Ed.) Sulfur in Agriculture, Agronomy, Madison, WI, pp 295-322

Waring S, Bremner J (1964) Ammonium production in soil under waterlogged conditions as an index of nitrogen availability Nature 201: 951-952

Williams C, Steinbergs A (1959) Soil sulphur fractions as chemical indices of available sulphur in some Australian soils Crop Pasture Sci 10:340-352

Zhao F, McGrath S, Crosland A (1994) Comparison of three wet digestion methods for the determination of plant sulphur by inductively coupled plasma atomic emission spectroscopy (ICP-AES) Commun Soil Sci Plan 25:407-418

Zhou W, Li ST, Wang H, He P, Lin B (1999) Mineralization of organic sulfur and its importance as a reservoir of plant-available sulfur in upland soils of north China Biol Fert Soils 30:245-250.

TABLES AND FIGURES

Table 3.1. Selected soil properties and management practices in plots that were fertilized with poultry litter or inorganic fertilizer for more than 10 years.

Site	Serie	Clay (%)	Sand (%)	pH	Years of treatment	Soil use	PL Annual rate
Delaware 1	Woodstown	14.5	82.0	6.1	11	Corn (*Zea maiz* L.)	9.9 Mg PL ha^{-1} (15.8 kg S ha^{-1})
Delaware 2	Mattapex	21.8	16.1	6.0	11	Corn	9.9 Mg PL ha^{-1} (15.8 kg S ha^{-1})
Iowa	Nicollet/ Webster	29.2	42.0	5.5	14	Corn - Soybean (*Glicine max* L.)	17 Mg ha^{-1} (27.2 kg S ha^{-1})
Georgia	Cecil	39.9	43.6	5.5	15	Tall fescue (*Festuca arundinacea* Schreb.) - Bermudagrass (*Cynodon dactylon* L.)	8 Mg PL ha^{-1} (12.8 kg S ha^{-1})

Table 3.2. Concentration of total, inorganic and organic S (S_T, S_i and S_{org} respectively), S in the particulate fraction (S-PF), C:S ratio and parameters for first-order S mineralization model in four soil after more than ten years of treatment with poultry litter (PL) or inorganic fertilizers (IF).

Soil	**Fertilizer**	**S_t** g kg^{-1}	**S_i** mg kg^{-1}	**S_{org}** g kg^{-1}	**S-PF** g kg^{-1}	**C_t** g kg^{-1}	**C:S** -	**So** mg kg^{-1}	***k*** week^{-1}
Delaware 1	IF	0.12 f	1.5 g	0.12 f	0.013 b	12.4 g	104.9 a	15.1 f	0.052
Delaware 1	PL	0.12 f	5.5 c	0.12 f	0.017 b	11.7 g	96.4 b	13.8 f	0.065
Delaware 2	IF	0.17 e	3.2 f	0.16 e	0.017 b	11.6 f	69.2 e	23.8 e	0.047
Delaware 2	PL	0.20 d	4.1 e	0.19 d	0.014 b	13.3 e	67.4 e	27.4 d	0.068
Iowa	IF	0.23 c	4.8 d	0.23 c	0.001 b	23.0 d	97.8 b	22.0 e	0.050
Iowa	PL	0.37 a	16.3 a	0.35 a	0.053 a	25.2 c	68.2 e	38.8 c	0.075
Georgia	IF	0.29 b	5.1 d	0.29 b	0.026 b	26.4 b	90.2 c	50.1 b	0.025
Georgia	PL	0.37 a	6.6 b	0.36 a	0.067 a	30.9 a	83.3 d	71.3 a	0.032
ANOVA	Soil (S)	<0.0001	<0.0001	<0.0001	0.0055	<0.0001	<0.0001	<0.0001	<0.0001
	Fertilizer (F)	<0.0001	<0.0001	<0.0001	0.0009	<0.0001	<0.0001	<0.0001	0.0002
	S x F	<0.0001	<0.0001	<0.0001	0.0064	<0.0001	0.0006	<0.0001	0.2639

Table 3.3. Pearson correlation coefficients and significance between parameters for the first-order S mineralization model (S_o and *k*) and total, inorganic and organic S (S_T, S_i and S_{org} respectively), S in the particulate fraction (S-PF) and C:S ratio.

	S_t	**S_i**	**S_{org}**	**C_t**	**C:S**	***k***	**S-PF**
S_i	0.71*						
S_{org}	0.99***	0.68					
C_t	0.92**	0.50	0.94**				
C:S	-0.40	-0.45	-0.35	-0.04			
k	-0.23	0.41	-0.27	-0.48	-0.32		
S-PF	0.83*	0.64	0.82*	0.71*	-0.35	-0.17	
S_o	0.86**	0.34	0.87**	0.86**	-0.26	-0.58	0.84**

*** Significant at $p<0.0001$; ** $p<0.01$; * <0.05

Table 3.4. Results from different methods to estimate S mineralization and availability in four different soils amended for more than ten years with poultry litter (PL) or inorganic fertilizers (IF).

Soil	**Fertilizer**	**$NaHCO_3$**	**$NaHCO_3$-Si**	**Heat**	**Heat-Si**	**KCl**	**KCl-Si**	**Nan**	**7 d**	**14 d**
		-- mg kg^{-1} --								
Delaware 1	IF	8.8 f	7.3 e	9.0 e	7.5 b	5.2 g	3.7 g	41.0 f	1.7 bc	2.3 de
Delaware 1	PL	14.3 ef	8.7 e	10.3 de	4.8 c	9.7 e	4.1 g	34.5 g	0.5 c	1.8 e
Delaware 2	IF	18.8 e	15.6 d	11.5 cd	8.3 b	9.0 f	5.8 f	60.6 e	1.4 bc	3.3 c
Delaware 2	PL	19.7 e	15.6 d	12.3 c	8.2 b	11.7 d	7.6 e	70.1 d	2.3 b	5.0 b
Iowa	IF	28.7 d	23.9 c	13.1 c	8.3 b	17.1 b	12.3 b	73.0 d	2.3 b	2.2 de
Iowa	PL	35.5 c	19.2 d	24.1 a	7.7 b	34.0 a	17.7 a	107.5 c	5.3 a	7.7 a
Georgia	IF	51.2 b	46.1 b	13.0 c	7.9 b	13.4 c	8.3 d	126.6 a	2.5 b	2.7 cd
Georgia	PL	42.3 a	35.7 a	17.1 b	10.5 a	16.8 b	10.2 c	172.7 b	6.8 a	7.8 a
ANOVA	Soil (S)	<0.0001	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001	0.0049	<0.0001
	Fertilizer (F)	0.4255	0.0102	<0.0001	0.9094	<0.0001	<0.0001	<0.0001	0.0226	<0.0001
	S x F	0.0021	0.0118	<0.0001	0.0237	<0.0001	<0.0001	<0.0001	0.0507	<0.0001

Table 3.5. Pearson correlation coefficients and significance between S mineralization potential (S_o) and the results from different methods to estimate S mineralization and availability.

	S_o	**$NaHCO_3$**	**Heat**	**KCl**	**N_{an}**	**$NaHCO_3$-S_i**	**Heat-S_i**	**KCl-S_i**	**7d**
$NaHCO_3$	0.85^{**}								
Heat	0.55	0.57							
KCl	0.39	0.52	0.97^{***}						
N_{an}	0.99^{***}	0.89^{**}	0.61	0.48					
$NaHCO_3$-S_i	0.82^{*}	0.95^{**}	0.31	0.25	0.84^{**}				
Heat-S_i	0.67	0.50	0.34	0.2	0.74^{*}	0.54			
KCl-S_i	0.43	0.58	0.92^{**}	0.96^{***}	0.53	0.35	0.36		
7d	0.85^{**}	0.64	0.80^{*}	0.67	0.87^{**}	0.5	0.72^{*}	0.69	
14d	0.70^{*}	0.44	0.84^{**}	0.69	0.71^{*}	0.26	0.6	0.66	0.91^{**}

*** Significant at $p<0.0001$; ** $p<0.01$; * <0.05

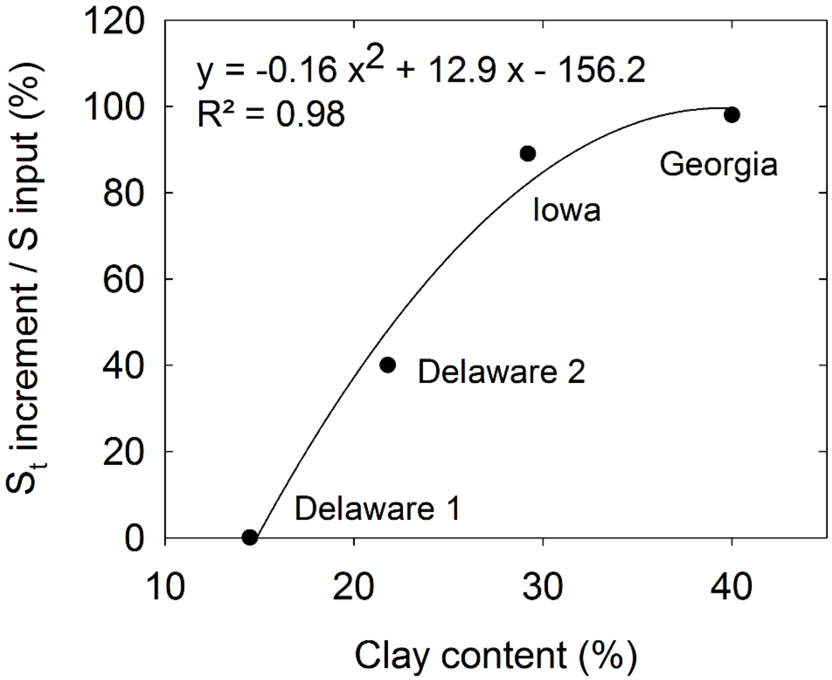

Figure 3.1. Percentage of S_t accumulated in soils amended with poultry litter (PL) relative to S input from PL as a function of soil clay content after more than ten years of yearly application

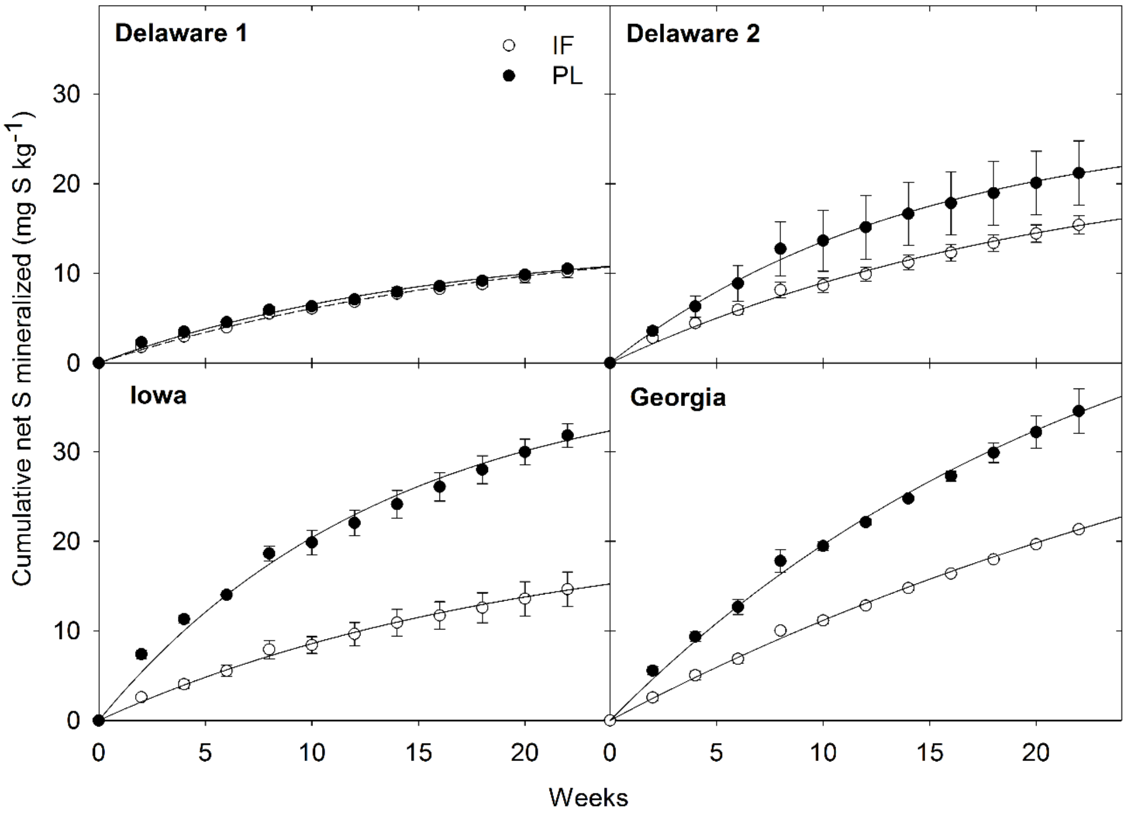

Figure 3.2. Cumulative amount of S mineralized in four soils that had been treated with poultry litter (PL) or inorganic fertilizer (IF) for more than ten years and were incubated at 30±1 ^{o}C for 24 weeks . Vertical bars represent the standard deviation among replications

CHAPTER 4

PHOSPHORUS IN THE COARSE SOIL FRACTION IS RELATED TO SOIL ORGANIC PHOSPHORUS MINERALIZATION MEASURED BY ISOTOPIC DILUTION

---

[1] Wyngaard, N., M.L. Cabrera, K.A. Jarosh, E.K. Bunemann. Submitted to the journal Soil Biology and Biochemistry. 07/13/2015.

ABSTRACT

A sound prediction of soil organic phosphorus (P_o) mineralization would be helpful to improve fertilizer recommendations. However, the only method to measure basal P_o mineralization is based on expensive and time consuming isotopic dilution experiments. A proposed method to estimate P_o mineralization is the quantification of P in the coarse fraction (CF, >53 µm) of the soil. The aim of this study was to evaluate if P in the CF can effectively predict P_o mineralization. Soil samples from three North American long-term arable field experiments (>10 years) with different textures (sand content 15 to 82%) and different fertilization treatments (non-P-fertilized, mineral P fertilizer, poultry litter) were analyzed. Soil samples were physically fractioned into CF and fine fraction (FF, <53 µm). Total P (P_t), P_o, and total carbon (C_t) were determined in each fraction. Gross and net P_o mineralization rates as well as soil respiration were determined in a 13-day-incubation experiment. The cumulative gross P_o mineralization over 13 days ranged from 7.3 to 38.6 mg P kg^{-1}, while P_t in CF varied between 9.7 and 90.7 mg P kg^{-1} and P_o in CF between 7.2 and 17.7 mg kg^{-1}. Mineralization of P_o followed the general trend: poultry litter ≥ mineral fertilizer > non-P-fertilized. No association between P_t in CF and mineralized P_o was observed. However, a significant linear relationship between P_o in CF and net mineralized P_o was observed after 6 days (R^2: 0.80, p=0.001) and a quadratic one after 13 days of incubation (R^2: 0.92, p<0.001). Additionally, gross and net mineralized P_o could also be predicted from C respiration, and these models were significantly improved by incorporating the C_t:P_o ratio of the CF. Our results suggest that the quantification of P_o in the CF is a promising non-isotopic alternative to estimate basal P_o mineralization.

1. INTRODUCTION

Phosphorus (P) is a macronutrient involved in the energy metabolism and in the synthesis of cellular structures of plants (Raghothama et al., 2005). Consequently, the availability of P controls the growth and development of all crops. It is estimated that 5,700 million hectares of soils worldwide do not have the adequate available P content required to achieve optimum crop production (Batjes, 1997). To alleviate this deficiency, usually mineral P fertilizers are used at rates based on availability tests such as those developed by Bray and Kurtz (1945), Mehlich (1984) and Olsen et al. (1954). However, all of these soil P tests consider only the available inorganic P pool, without taking into account organic P (P_o) which can represent up to 80% of total P (P_t) (Harrison, 1987) and can become plant available through the process of mineralization. Therefore, the measurement or estimation of P_o mineralization rates is highly important to improve P fertilizer recommendations.

The quantification of P_o mineralization is complicated by the high reactivity of phosphate ions, which are rapidly adsorbed by the soil mineral matrix after being released from the organic pool to the soil solution. This reactivity makes it impossible to quantify P_o mineralization by incubation-extraction methods as the ones used to measure net mineralization rates of other nutrients such as nitrogen (N) and sulfur (S). Currently, the only method to quantify P_o mineralization is based on isotopic dilution experiments under steady-state conditions, rendering basal gross and net P_o mineralization rates (Bünemann, 2015; Oehl et al., 2001). However, such experiments are expensive and time consuming (Bünemann, 2015). Thus, there is a need to develop and evaluate non-isotopic methods to estimate the P_o mineralization potential of soils.

The P_t concentration in the soil fraction > 53 μm has been proposed as an estimator of P mineralization (Ciampitti et al., 2011). This fraction has been previously named "particulate organic matter" (Cambardella and Elliott, 1992) or "particulate fraction" (Wyngaard et al., 2013). Here, we will refer to it as "coarse fraction" (CF), since this fraction is not only composed by organic matter or mineral particles, but by both components. Ciampitti et al. (2011) showed that the concentration of P_t in the CF (mg P_t-CF kg^{-1} soil) is positively related to the P accumulation in corn plants growing in unfertilized plots on a Mollisol in Argentina. The amount of plant-available inorganic P (P_i) in the CF was negligible in other Mollisol (Wyngaard et al., 2013) and thus cannot explain observed differences in plant P uptake. A possible explanation is that the mineralization of P_o in the CF supplied plant available P during the growing season. As the determination of P_t or P_o in the CF is simple and economical, it could be a promising tool to estimate potential P_o mineralization.

Others have suggested using soil respiration to estimate P_o mineralization, assuming that all net P_o mineralization derives from microbial decomposition of organic matter driven by the need of energy (Bünemann et al., 2012; Achat et al., 2010). The empirical evidence that P_o in CF as well as soil respiration are associated with P_o mineralization should be validated by comparison with results from isotopic dilution experiments.

In a long-term study on a Mollisol in Argentina, P_t and P_o in the CF were modified by long-term cultivation but not by the application of inorganic fertilizers (Wyngaard et al., 2013). However, it has not been evaluated if these P pools are affected by the use of organic P fertilizers. A widely used organic fertilizer in the southeastern states of the United

States is the litter derived from the poultry industry (PL, a mixture of excreta, organic bedding material, feathers and wasted feed). Different studies using sequential P fractionation (Xue et al., 2013), enzyme additions to derive enzyme-labile P_o fractions (He et al., 2008) or nuclear magnetic resonance spectroscopy (Hill and Cade-Menun, 2009) demonstrated that the repeated application of PL generates an accumulation of P_o in the soil in the form of *myo*-inositol hexakisphosphate (phytate). It would be important to evaluate if PL application also increases P_t and P_o in the CF and the soil P_o mineralization potential measured by isotopic dilution.

Our objectives were to 1) determine the effects of long-term application of mineral P fertilizer or PL on enzyme-labile P_o fractions, concentrations of P_t and P_o in the CF, and gross and net P_o mineralization rates measured by isotopic dilution, 2) evaluate the potential association between P_o mineralization measured by isotopic dilution and enzyme-labile P_o fractions as well as the concentrations of P_t and P_o in the CF, and 3) evaluate the use of respiration as a predictor of P_o mineralization.

2. MATERIALS AND METHODS

2.1. Field experiments and soil sampling

Composite soil samples were collected from three long-term agricultural field experiments in the United States in August 2014 at 0 to 20 cm depth. These sites were located in Delaware (39°40'13"N 75°45'10"W, on a Hapludult, Woodstown series, according to the Natural Resources Conservation Service (1999)), Maryland (39°10'42"N 76°10'58"W, Hapludult, Mattapex series) and Iowa (42°01'22"N 93°46'35"W, Hapludoll, Nicollet/Webster series). These soils were classified by their particle size distribution as sandy loam (14.5 % clay and 82.0 % sand), silt loam (21.8 % clay and 16.1 % sand) and clay loam (29.2 % clay and 42.0 % sand), respectively.

The experimental design in these long-term experiments was a complete randomized plot arrangement with six replications in Delaware and Maryland (55 m^2 per plot), and four replications in Iowa (400 m^2 per plot). In Delaware and Maryland, three different treatments have been compared since 2000: 1) no P fertilizer application (Control), 2) inorganic P fertilizer application (IF) (30 kg P ha^{-1} $year^{-1}$, as 0-46-0) and 3) PL application (PL) (30 kg P ha^{-1} $year^{-1}$). In Iowa, the evaluated treatments were: 1) Control, 2) inorganic N fertilization without P (Control+N) (90 kg N ha^{-1}, as 32-0-0), and 3) PL (90 kg P ha^{-1} $year^{-1}$), all maintained since 1998. Since their establishment, Delaware and Maryland field trials were managed under corn (*Zea mays* L.) monoculture while the Iowa study was managed under a corn/soybean (*Glycine max* L.) rotation.

All soil samples were taken almost one year after the last fertilizer application. Separate composite soil samples were collected from three replications in each study to obtain three samples for each treatment and site. After sampling, soils were sieved (4 mm), mixed, and kept at 4°C for up to 3 months until the laboratory experiments were performed. A portion of the soil was air-dried, sieved through a 2-mm sieve and used for soil analysis (Section 2.2). Replicates from each soil and treatment combination were analyzed separately, except for the isotopic dilution experiment (Section 2.4), for which equal portions of all replicates of a given soil and treatment combination were mixed into a composite soil sample.

2.2. Soil analysis

Total C (C_t) and N (N_t) concentrations in soil were determined by dry-combustion. Soil P_o was determined by the ignition method (Saunders and Williams, 1955), P_t by wet digestion with H_2O_2 and H_2SO_4 (Anderson and Ingram, 1989), and P_i was derived from P_t minus P_o. Soil pH was measured in a 1:2.5 soil-to-water suspension, and particle size distribution was determined by the pipette method (Robinson, 1922). In all soil analyses three to four analytical replicates, were performed.

Additionally, the soil was physically separated into two fractions: the CF and the fine fraction (FF) following a modification of the procedure described by Cambardella and Elliott (1992). Briefly, 10 g of dry soil sample was shaken overnight with 30 ml of 1 M NaCl instead of 0.05 M $(NaPO_3)_6$ to avoid the addition of P (Salas et al., 2003). After this, the suspension was wet-sieved through a 53-µm sieve. The fraction that remained on top of the sieve was designated the CF, while the one that passed through the sieve was called the FF. After drying and milling both fractions, the concentrations of P_i, P_o, P_t and C_t were measured in CF and FF as detailed above. These concentrations were always expressed on a whole-soil basis ((mg P or C)/(kg CF or FF) x (kg CF or FF)/kg soil).

2.3. Enzyme addition assay

To quantify enzyme-labile P_o fractions in NaOH-EDTA extracts, the protocol described by Keller et al. (2012) was performed. To this end, soil samples were shaken for 16 h with a 0.25 M NaOH-0.05 M EDTA solution in a 1:10 ratio. After this, filtered aliquots of the extract were transferred to a microplate, buffered with a MES buffer (final molarity 0.2, pH 5.2) and submitted to three separate enzyme treatments: acid phosphatase (from *Solanum tuberosum*, Sigma chemicals, product No. P1146), phytase (from *Peniophora* *lycii*, RONOZYME® NP, DSM Nutritional Products, Denmark) and acid phosphatase + nuclease (from *Penicillium citrinum*, Sigma chemicals, product N8630). The samples were agitated and incubated for 24 h in the dark at 37ºC. Then, the P_i concentration in the incubated extracts was quantified colorimetrically in a microplate reader (EL 800, Biotek) using malachite green (Ohno and Zibilske, 1991).

The same procedure was simultaneously repeated on another plate, in which the activity of the enzymes was evaluated by their reaction with *myo*-inositol phosphate (Sigma chemicals, product P8810), glycerol phosphate (Sigma chemicals, product G7879) and deoxyribonucleic acid (DNA) (Sigma chemicals, product D3159). The P release by the enzymes from the model substrates in water averaged 100%, 94% and 98% for acid phosphatase, acid phosphatase + nuclease and phytase, respectively. The model substrates together with the respective enzymes were also added to the NaOH-EDTA soil extracts in MES buffer to verify that the extracts did not affect the enzyme activity. The recovery of a 10 µg ml^{-1} orthophosphate solution was used as a correction factor for P sorption or precipitation. This recovery averaged 96%, 99% and 100% in the presence of acid phosphatase, acid phosphatase + nuclease and phytase, respectively.

Three enzyme-labile P_o pools were calculated: simple monoester-like P (P released by phosphatase), DNA-like P (P released by nuclease + phosphatase minus P released by phosphatase alone) and phytate-like P (P released by phytase minus P released by phosphatase). Also, the non-hydrolysable P_o pool was calculated as the difference between P_o in the NaOH-EDTA extract and the sum of all three enzyme-labile pools. The P_o concentration was estimated as the difference between P_t (determined by digestion with ammonium persulfate and sulfuric acid in an autoclave) and P_i in the extract, both measured colorimetrically (Ohno and Zibilske, 1991).

2.4. Isotopic dilution experiment

This experiment was performed as described by Oehl et al. (2001), incorporating the estimation of microbial P immobilization suggested by Bünemann et al. (2007). The protocol consists of two separate and complementary studies: 1) a batch experiment to determine the baseline of isotopic dilution caused by physicochemical processes (2.4.1) and 2) an incubation experiment with ^{33}P-labeled soils to determine the isotopic dilution caused by both biological and physicochemical processes (2.4.2). This second part of the experiment was accompanied by the measurement of soil respiration.

2.4.1. Batch experiment: isotopic exchange kinetics (IEK)

The aim of this part of the experiment was to assess changes in the specific activity (SA, i.e. $^{33}P/^{31}P$) of phosphate in the soil solution caused by physicochemical processes (Fardeau et al., 1991). Soil samples were pre-incubated at 30°C for two weeks at 55% water-holding capacity. This procedure was performed to reach a constant soil respiration rate and constant parameters of isotopic exchange after the disturbance generated by sieving and mixing the soil. After this, an amount of pre-incubated soil equivalent to 10 g dry weight was shaken for 16 hours in a 250-ml Nalgene bottle with 99 ml of water (including the water contained in the soil sample). At time zero, 1 ml of carrier-free $^{33}PO_4^{3-}$ tracer was added to the soil-water suspension while stirring. The introduced activity (R) ranged between 24-206 kBq, depending on the soil. The suspension was sampled with a syringe after 1, 4, 10, 30, 60 and 90 min and immediately filtered through a membrane filter (0.2 µm pore size). At each time point, the radioactivity in the filtrate (r) was quantified by liquid scintillation counting in a betacounter (TRI-CARB, 2500 TR). Additionally, at the end of the experiment, the suspension was sampled and filtered as above to determine the phosphate concentration in solution (C_p in mg l^{-1}) using malachite green (Ohno and Zibilske, 1991).

The temporal development of radioactivity remaining in the soil solution ($r_{(t)}/R$) is described by the following formula (Fardeau et al., 1985):

$$r_{(t)}/R = m*(t + (m)^{1/n})^{-n} + r_{\infty}/R \quad (1)$$

where t is time, m and n are model fitting parameters, and r_{∞}/R is the maximum possible dilution of the isotope. This last value was estimated by the ratio between the phosphate concentration (in mg kg^{-1}) in the soil solution ($10*C_p$) and total P_i (estimated by the difference between P_t determined by digestion and P_o measured by ignition).

Finally, the quantity of isotopically exchangeable P at each time point, the so-called E-value ($E_{(t)}$) (mg P kg^{-1}), was estimated as:

$$E_{(t)est} = 10*C_p/(r_{(t)}/R) \quad (2)$$

using the $r_{(t)}/R$ calculated according to equation 1.

The approach of using IEK experiments to estimate changes in the SA of phosphate in the soil solution (SA_{H2O}) caused only by physicochemical processes is based on the assumption that during the short time of the experiment there is no incorporation of ^{33}P into microorganisms. In order to confirm this assumption, the experiment was repeated in the presence of a microbial inhibitor (10 mg l^{-1} $HgCl_2$ solution added before labeling).

Since C_p, r_1/R and *n* were unaffected by the presence of the inhibitor (data not shown), it was concluded that no inhibitor was required during the short-term IEK experiments.

Six analytical replicates of the IEK experiment were performed for each soil and treatment. Three of these replicates were performed at the beginning and three at the end of the incubation experiment (2.3.2) to verify that there was no change of the IEK parameters over time. As no change over time was observed (data not shown), the six replicates were analyzed jointly.

2.4.2. Incubation experiment

The aim of this part of the experiment was to assess changes in the SA of phosphate in the soil solution caused by both physicochemical and biological processes. Additionally, soil respiration and microbial P were quantified. The incubation experiment was performed in three separate sets, one for each soil. In detail, soil samples were pre-incubated at 30°C for two weeks at 40% water-holding capacity. After this, a fraction of the pre-incubated soil equivalent to 220 g dry soil was labeled with 10 ml of a ^{33}P solution (from 468 to 894 kBq ml^{-1}, depending on the soil) and additional water was added to achieve a moisture level corresponding to 55% water-holding capacity. The resulting label was 21.7, 27.1 and 41.4 kBq g^{-1} for Delaware, Maryland and Iowa, respectively. The labeled soil was mixed thoroughly for 10 min and then divided into three sub-samples, which were placed in plastic bags and incubated at 30°C in the dark, keeping a constant water content.

Three different time-points were considered: 1, 6 and 13 days. These time-points represent the moment at which the samples were filtered or the resins were removed from the soil suspension, even though the extraction procedure started 16 hours before. For each time-point, one of the incubated sub-samples was used to measure the SA of soil solution phosphate and microbial P.

Soil solution phosphate was extracted by shaking moist soil equivalent to 10 g dry soil with 100 ml water (including the water in the sample) for 16 h. After this, the soil-water suspension was stirred for 10 min to generate conditions similar to those in the IEK experiment (2.4.1), sampled with a plastic syringe and filtered through a 0.2-µm membrane. The *r* and *Cp* values were quantified as described in 2.4.1, with four analytical replicates. Using the *r* and *Cp* obtained at each incubation time, the measured E-value (E_{meas}) was calculated according to equation 2. At a given time point, the difference between E_{meas} and E_{est} (determined as described in 2.4.1) represents the cumulative gross P_o mineralization (ΔE).

The fumigation-extraction procedure described by Kouno et al. (1995) was used to quantify microbial P. This method is based on shaking a soil sample with water and an anion exchange resin membrane (BDH #55164, 6 cm x 2 cm) saturated with CO_3^{2-} for 16 h, with and without hexanol. The membranes are then eluted with a 0.1 M NaCl/HCl solution, and the radioactivity and phosphate content in the eluates are quantified as described in 2.4.1. The difference in phosphate concentration (in mg kg^{-1}) between fumigated and non-fumigated samples after correction for adsorption of the P released during fumigation is referred to as hexanol-labile P ($^{31}P_{hex}$). The sorption correction factor was obtained from the recovery of a 7.5 mg P kg^{-1} spike, evaluated for each soil and treatment at all sampling dates. This factor averaged 0.89 ± 0.13 for Delaware, 0.92 ± 0.10 for Maryland and 0.88 ± 0.23 for Iowa. No conversion factor (kp) was applied.

The radioactivity recovered in fumigated samples may partly originate from ^{33}P liberated from the solid phase upon release of ^{31}P from the lysed microbial cells. This effect was estimated by measuring the recovery of ^{33}P in samples receiving the ^{31}P spike mentioned above. The ^{33}P in fumigated subsamples was then corrected based on the linear relationship of ^{31}P added (0, 7.5 mg P kg^{-1}) and ^{33}P recovered, inserting the measured $^{31}P_{hex}$ as ^{31}P added. Finally, the potential sorption of ^{33}P released during fumigation was corrected for based on the recovery of a ^{33}P spike, rendering $^{33}P_{hex}$. The recovery of the ^{33}P spike averaged 0.88 ± 0.17 for Delaware, 0.88 ± 0.09 for Maryland and 0.97 ± 0.22 for Iowa. No significant difference was found between the recovery of ^{31}P and ^{33}P spikes.

Immobilized P was calculated as suggested by Bünemann et al. (2007):

$$\text{P immobilized (mg P kg}^{-1}) = ((^{33}P_{hex}/^{31}P_{hex})/(^{33}P_{H2O}/^{31}P_{H2O}))*^{31}P_{hex} \quad (3)$$

where the term $^{33}P_{hex}/^{31}P_{hex}$ represents the SA of the hexanol-labile phosphate and $^{33}P_{H2O}/^{31}P_{H2O}$ represents the SA of phosphate in the soil solution. Finally, the immobilized P was subtracted from the cumulative gross P_o mineralization to derive the cumulative net P_o mineralization. The gross and net P_o mineralization rates were calculated by dividing the cumulative gross and net P_o mineralization, respectively, by the number of incubation days.

Simultaneously to the incubation experiment, soil respiration was measured according to Alef (1995). To this end, pre-incubated soil samples were thoroughly mixed with water instead of ^{33}P labeling solution for 10 min to emulate the physical disturbance generated during soil labeling. After this, soil at 55% water-holding capacity equivalent to 20 g dry soil was placed in a plastic vial inside a 1-l Mason jar together with a 50-ml Erlenmeyer beaker containing 10 ml of 0.5 M NaOH. Blanks without soil were also included. At day 5, traps were exchanged and the NaOH was back-titrated with HCl after the addition of 4 ml of 1.5 M $BaCl_2$ and phenolphthalein. The respiration measurement was finished with a second titration at day 12.

The C:P ratio of net mineralization was calculated as the ratio between measured C respiration rate and the net P_o mineralization rate. On the other hand, the C:P ratio of gross mineralization was calculated as the ratio between gross C respiration rate and gross P mineralization. The gross C respiration rate was calculated from the measured C respiration rate, assuming that 60% of the C mineralized is released as CO_2 while 40% is assimilated into the microbial biomass (Murphy et al., 2003).

2.5. Statistical analysis

Due to the different fertilizer treatments and rates, each experimental site was analyzed separately. 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. Differences between treatments were tested with a one-way ANOVA using the SAS PROC MIXED procedure (SAS Institute Inc, 2013). Effects were considered significant at $p < 0.05$, and the Tukey-Kramer test was used to compare fertilizer treatment means if analysis of variance indicated significant treatment effects. The full model in the IEK experiment (Equation 1) was fitted using R in order to determine the parameters m and n (R Core Team, 2013). The linear and quadratic models to associate P_o in the CF and net and gross mineralized P were fitted using PROC REG (SAS Institute Inc, 2013). Multiple linear regression (PROC REG) was used to estimate P_o mineralization rates using respiration and C:P ratios of the soil and soil fractions as regressors.

3. RESULTS

3.1. Soil analysis

Soil pH was moderately acidic in Delaware and Maryland, ranging from 5.1 to 6.1 (Table 4.1). In Delaware, pH was greater in the PL amended treatments, while no significant difference between treatments was observed in Maryland. In Iowa, soil pH was moderately acidic in the Control + N plot and neutral in Control and PL. An increase in C_t relative to the Control was observed in PL plots in Delaware (+12.7%) and Maryland (+7.8%) but not in Iowa. Concentrations of P_i ranged between 91 and 481 mg kg^{-1}, while P_o ranged between 119 and 370 mg kg $^{-1}$ (Table 4.1). The repeated application of IF or PL increased P_i in all soils relative to the Control, while P_o concentration was increased by PL in Delaware and Maryland, and by IF in Delaware. The use of N fertilizers without P addition in Iowa (Control + N treatment) decreased the concentration of P_i compared to the Control, but lead to an increase in P_o.

After the physical fractionation of the soil, P_t in the CF ranged from 10 to 91 mg kg^{-1}, with P_o representing 20 to 88% of it (Table 4.2). In all soils, P_t and P_o in the CF were greater in PL amended soils than in the Control. An increase in C_t concentration in the CF of PL treatments was observed only in the Iowa soil where C_t was increased by a factor of 1.7 compared to the Control. Even though the application of this organic amendment tended to reduce the C_t:P_o ratio in the CF, this difference was significant only in the Delaware soil. The long-term use of mineral fertilizers did not modify P_t nor P_o in the CF relative to the Control. Likewise, all measured properties in the CF and the FF of Iowa soils were similar in control and Control + N. In the FF, P_t ranged between 162 and 698 mg P kg^{-1}, with P_o representing 47 to 78 % of it. In most soils, C_t in the FF was greatest in PL, followed by IF. However, no treatment effects on P_o nor the C:P_o ratio in the FF were found. The recovery of P_t and P_o in the CF plus the FF (Table 4.2) represented 90 ± 7 and 109 ± 9% of the P_o measured in the non-fractioned samples, respectively (Table 4.1).

3.2. Enzyme addition assay

The concentration of P_i in NaOH-EDTA extracts of all soils was greater in P fertilized treatments than in the Control, irrespective of the P source (Table 4.3). Enzyme-labile P_o fractions showed a high variability, and no differences between fertilizer treatments were observed for simple monoester-like P, DNA-like P and non-hydrolysable P_o. However, the concentration of phytate-like P was increased by PL application in Maryland and Iowa and additionally by IF fertilization in Maryland.

3.3. Isotopic dilution experiment

The concentration of water-extractable phosphate during the soil incubation ranged between 0.2 and 10.7 mg kg^{-1} and was not always constant between sampling times (Figures 4.1 a-c), with values on day 1 typically being greater than later values. In all soils, the concentration of water-extractable phosphate was highest in PL and lowest in the Control, while IF was intermediate (Delaware, Maryland) or not different from the Control (Iowa). The analytical variability was low, with an average coefficient of variation of 4.4%. The recovery of water-extractable ^{33}P varied in the order PL > IF ≥ Control = Control + N, and it decreased over time in all soils and treatments (Figures 4.1 d-f). Therefore, also the SA in water-extractable phosphate decreased over time, and it was typically greater in non-P-fertilized than in P-fertilized treatments (Figure 4.1 g-i).

Hexanol-labile P ranged between 1.2 and 11.9 mg P kg^{-1} (Figures 4.2 a-c). Consistent treatment differences were observed only in Maryland and Iowa, where hexanol-labile P was greater in P-fertilized than in non-P-fertilized treatments. Temporal trends were significant mainly in Delaware (all treatments), with an increase on the last sampling date. The recovery of ^{33}P and the SA in the hexanol-labile P pool showed a high analytical variability (average variation coefficients of 29% and 27%, respectively), and for most soils and treatments did not change significantly over time (Figures 4.2 d-i).

The fitting parameter *m* of the IEK experiment ranged between 0.20 and 0.64 and for a given soil varied in the order PL > IF > Control = Control + N (Table 4.4). In the case of the *n* parameter, the values ranged between 0.18 and 0.37, with the greatest values generally determined in the Control, followed by IF. Finally, the water extractable phosphate measured in the IEK experiment ranged between 0.2 and 10.4 mg P kg^{-1} (Table 4.4) and was similar to the concentrations of water-extractable phosphate observed during the incubation experiment (Figure 4.1).

Soil respiration rates (Table 4.5) during the first 6 days varied between 6.0 and 14.6 µg C g^{-1} d^{-1}. Averaged across soils and treatments, rates were 37% lower for the second measurement period (day 6 - 13). An increase in the respiration rate relative to the Control was observed for IF in Maryland and for PL in Maryland and Iowa, while in Delaware respiration rates were similar in all treatments.

The difference between extrapolated E-values (E_{est}) and measured E-values (E_{meas}) was always significant (Table 4.5). The resulting cumulative gross P_o mineralization in PL (Table 4.5) was always greater than in the Control, and in Delaware additionally greater than in IF. In Iowa, no difference between Control and Control + N was observed. The gross P_o mineralization rates ranged from 0.6 to 4.5 mg P kg^{-1} day^{-1} (Table 6).

The analytical variability of microbial P immobilization (P_{imm}) was high (Table 4.5), with average variation coefficients of 27, 23 and 21% for Delaware, Maryland and Iowa, respectively. A significant effect of long-term PL amendment on microbial P immobilization was observed only in Delaware. The resulting net P_o mineralization (Table 4.5) followed a similar trend as gross P_o mineralization, being always greater in IF and PL than in the Control. Also, no difference in net P_o mineralization was observed between Control and Control + N. The resulting net mineralization rate ranged from 0.3 to 4.1 mg P kg^{-1} day^{-1} (Table 6).

The C:P ratios of gross mineralization ranged between 5 and 22, while for net mineralization they ranged between 3 and 16 (Table 4.6). There was a significant interaction between fertilization treatments and incubation date, but in general the C:P ratio of both gross and net mineralization was greater in the Control than in the fertilized treatments.

4. DISCUSSION

4.1. Changes in soil P pools caused by long-term application of fertilizers

Fertilization with IF or PL generally increased the concentrations of P_i and P_o in the soil (Table 4.1). The increase in P_i is primarily explained by adsorption and precipitation processes (Pierzynski et al., 2005). On the other hand, the increase in P_o concentration can be explained by variations in the amount and quality of plant residues (Zheng et al., 2002) and/or by the accumulation of organic P compounds already present in PL (Cade-Menun et al., 2015; He et al., 2008). Even though there is not enough information to calculate a P balance for the studied long-term experiments, the increments in P_i and P_o pools in the P-fertilized treatments indicate that the P-balance is highly negative in the Control and less negative, even or positive in the P-fertilized treatments.

On average, 56%, 95% and 64% of the increase in P_i generated by P fertilization in soils of Delaware, Maryland and Iowa was located in the FF, respectively (Tables 1 and 2). This trend coincides with the clay + silt content of these soils, which was 18, 84 and 58% for Delaware, Maryland and Iowa, respectively, and is in line with the greater P sorption capacity of the FF compared to the CF (Wyngaard et al., 2013). The change in P_t and P_o caused by PL was significant in the CF but not in the FF (Table 4.2), which agrees with the findings of Ciampitti et al. (2011) that P_t in the CF is more sensitive to the long-term application of fertilizers than P_t in whole soil samples. Consequently, the accumulation of P_o derived from the long-term application of fertilizers was mostly associated with particulate organic residues, while the accumulation of P_i was dominated by sorption to the FF. The observed values of P_t and P_o in the CF were in a similar range as in previous studies (Ciampitti et al., 2011; Wyngaard et al., 2013), except for the PL treatment in Iowa, which exceeded all other values.

The concentrations of DNA-like P, phytate-like P, simple monoester-like P and non-hydrolysable P_o (Table 4.3) were generally lower than in other studies (Annaheim et al., 2015; Requejo and Eichler-Löbermann, 2014; Keller et al., 2012; Koopmans et al., 2007). This difference between our study and others can be a consequence of an incomplete extraction of the total P_o by the NaOH-EDTA extract, which is supported by the fact that the sum of all organic fractions extracted by NaOH-EDTA (Table 4.3) represented on average only 19% of the P_o measured by calcination (Table 4.1). While the calcination technique may overestimate P_o, such an overestimation has been primarily observed in strongly weathered soil (Condron et al., 1990), which does not apply to the soils used in this study.

The observed accumulation of phytate-like P in PL amended treatments (Table 4.3) can be a consequence of a direct and/or an indirect process. The direct process is the accumulation of phytate-like P already present in PL. Phytate-like P is the major component of the hydrolysable P_o present in PL (He et al., 2008) because birds have a low capacity to digest phytate-like P compounds present in their feed, which are then released through their feces (Taylor, 1965). On the other hand, an indirect phytate accumulation can originate from an increase in plant-derived phytate inputs (Anderson and Arlidge, 1962) caused by fertilization. In Maryland, the long-term IF fertilization generated an increase in phytate-like P (Table 4.3), pointing to an indirect accumulation effect. Consequently, the observed increase in phytate-like P in PL plots in Maryland (and also in Iowa where the IF treatment was not evaluated) can be a combination of both direct and indirect processes. However, in Delaware no difference in phytate-like P between treatments was observed, although P inputs were the same as in Maryland. Phytate-like P can be stabilized by adsorption to clays (Anderson and Arlidge, 1962) or by binding to metal ions and oxides (Ognalaga et al., 1994; Evans and Martin, 1991, 1988;) and organic molecules (Borie et al., 1989). This interaction prevents the decomposition of phytate by hydrolytic enzymes and leads to its accumulation in soil. Because Delaware is a sandy loam soil (82% sand), its capacity to stabilize and accumulate plant and PL derived phytate-like P is low, reducing the effect of long-term PL applications on phytate accumulation in soil.

Although phytate-like P can be protected by clay-sized particles, no P_o accumulation was observed in the FF (Table 4.2). However, the magnitude of the change in phytate-like P between PL and Control treatments (1.4 to 4.9 mg kg^{-1}) is very low when compared to the size of the P_o pool in the FF (110 to 380 mg kg^{-1}) and to the variability of the measurement of P_o in the FF (standard deviation ranging between 7 and 50 mg kg^{-1}).

The range of values for water-extractable phosphate was broader in this study (Figure 4.1 a-c) than in other studies (Bünemann et al., 2012; Achat et al., 2009b; Bünemann, 2008; Oehl et al., 2004). This is a consequence of the high concentrations of water-extractable phosphate in soils amended with PL. Both water-extractable and hexanol-labile P changed over time (Figures 4.1 and 4.2), due to greater values in day 1. This demonstrates that steady conditions were not fully met on day 1 and the measured mineralization rates derived from this day were not truly basal. Consequently, this sampling point will not be used to calculate mineralization rates.

4.2. Changes in P_o mineralization rates caused by long-term application of fertilizers

Soil respiration rates (Table 4.5) were similar to those observed by Oehl et al. (2004) in arable fields. As in other P_o mineralization studies, in spite of the pre-incubation of the soil to achieve stable conditions, soil labeling generated a transient increase in soil respiration (Achat et al., 2009a; Bünemann et al., 2007; Bünemann et al., 2012). More importantly, the observed trends in soil respiration between soils and fertilization treatments coincided, in general, with those observed for soil C_t accumulation (Table 4.1).

Gross and net cumulative P_o mineralization decreased in the order PL $\geq$ IF > Control = Control + N (Table 4.5). The difference between organic and mineral fertilization can be explained by the increase in microbial biomass and P_o in treatments with organic amendment, as previously observed by Oehl et al. (2004). The rates of gross P_o mineralization were in a similar range as those determined by Oehl et al. (2004) and Bünemann et al. (2012) for arable and grassland soils in Switzerland, respectively. The decrease of these rates over time can be explained by the disturbance generated during the soil labeling and also by the recycling of ^{33}P labelled microbially bound P compounds during the incubation (remineralization *sensu strictu*) (Bünemann, 2015).

4.3. Association between P pools and P_o mineralization

Neither gross nor net P_o mineralization were significantly associated with P_t, P_o, P_t in FF, P_o in FF, P_t in CF, simple monoester-like P, phytate-like P, DNA-like P, non-hydrolysable P_o, nor hexanol-labile P at any sampling date (data not shown). In contrast, a highly significant association between P_o in CF and gross and net P_o mineralization was found both for Day 6 and Day 13 (Figure 4.3). The fact that P_o mineralization was not related to the concentrations or forms of organic P in the bulk soil but to its concentrations in a soil physical fraction reinforces the importance of physical protection for the stabilization of organic compounds (Six et al., 2002). Consequently, even though 89% to 98% of the P_o was located in the FF, P_o in the CF determined the mineralization rate of the soil during the incubation.

The association between gross or net P_o mineralization and P_o in the CF was above the 1:1 regression line both on Day 6 and 13. This indicates that the cumulative P_o mineralized is greater than the amount of P_o in the CF of the soil, suggesting that other sources of P_o are also actively mineralized during the incubation. Moreover, the difference between the regression line and the 1:1 line was increased in Day 13 in comparison to Day 6, indicating that the relative contribution of P_o in the CF to mineralization decreased with time. In the closed incubation system used, there is no new input of P_o to the CF, which is apparently depleted at a faster rate than P_o in the FF. Previous studies conceptualized two-pool models to describe P_o mineralization, dividing the mineralizable pool between living and non-living soil organic matter (Müller and Bünemann, 2014; Achat et al., 2010). Our study demonstrates that the dynamics of non-living organic matter can also be potentially divided into two pools of different turnover, as it was proposed for nitrogen (Deans et al., 1986).

It is interesting that the relationship between P_o in the CF and cumulative mineralized P_o was quadratic on Day 13, which may indicate that above a certain threshold of P_o in the CF the capacity of the soil to mineralize P_o tends to stabilize. A similar trend was observed by Ciampitti et al. (2011) while analyzing the relationship between P uptake from corn and P_t concentration in the CF. The inflection point of the function determined by these authors was 13.2 mg P_t kg^{-1} while in the present study it was 16.2 and 15.0 mg P_o kg^{-1} for gross and net mineralization, respectively.

4.4. Prediction of P_o mineralization from soil respiration

The association between respiration rates and gross and net P_o mineralization rates was statistically significant (Table 4.7) but presented a low regression coefficient (R^2=0.52 and 0.50, respectively). It was observed that this low association between C and P_o mineralization was caused by the relatively small P_o mineralization rate in some treatments such as Delaware + PL, as compared to other treatments with similar respiration rates. This trend coincided with a greater C_t:P_o ratio in the soil and in the CF of these treatments (Tables 1 and 2). Therefore, the C_t:P_o ratio was incorporated as a regressor to account not only for the organic matter decomposition rate, but also for its composition. The incorporation of the C_t:P_o ratio of the soil or of the FF did not improve the model significantly. However, the incorporation of the C_t:P_o ratio of the CF to the model significantly improved the regression coefficient to 0.77 and 0.59 for gross and net P_o mineralization, respectively. This suggests that during the incubation experiment the relative importance of P_o mineralization from the CF was greater than that from the FF, and supports the hypothesis of a greater turnover rate of the P_o accumulated in the CF. The P_{hex} concentration was also evaluated as a regressor to improve the estimation of P_o mineralization from respiration, but it did not significantly improve the model (data not shown).

The observed C:P ratios of gross mineralization (Table 4.6) were similar to those observed in other arable soils (Randriamanantsoa et al., 2015; Oehl et al., 2004). Kirkby et al. (2011) suggested to compare the C_t:P_o ratio of stable organic matter versus the C:P ratio of mineralization. Along these lines, the C:P ratio of gross and net mineralization in our study was not associated with the C_t:P_o ratio of the whole soil nor of the FF. However, a significant linear association between the C_t:P_o ratio of the CF and the C:P ratio of net mineralization was observed at day 13 ($R^2 = 0.67$, $p < 0.001$), and a quadratic one when evaluating the C:P ratio of gross mineralization ($R^2 = 0.92$, $p < 0.001$ for day 6 and $R^2 = 0.98$, $p < 0.001$ for day 13) (data not shown). The fact that the C_t:P_o ratio of the CF is associated with the P_o mineralization rate and with the C:P ratio of mineralization, while the C_t:P_o of the soil and the FF are not, supports the hypothesis of the CF having a relative greater contribution to P_o mineralization than the FF. It would be important to confirm these findings in future studies by measuring C_t and P_o concentrations in CF and FF at the end of the incubation period, to determine the relative depletion of these pools.

Even though there is a relationship between P_o in the CF and P_o mineralization, the C_t:P_o ratio of the CF averaged 253 (Table 4.2), which is greater than the C:P ratio of gross and net mineralization (average 8 and 6, respectively). These last values are most similar to those described for the C:P ratio of the microbial biomass in arable soils, which ranges between 10 and 26 (Brookes et al., 1984; Oehl et al., 2004). This would suggest that gross and net mineralization rates originate entirely from remineralization (*sensu latu*) (Bünemann, 2015), and that the contribution of the P_o in the CF to the P_o mineralization rate is indirect: P_o in CF influences the microbial biomass, which in turn controls the gross and net P_o mineralization rates. A link between P in the CF and microorganisms has been indicated before: Salas et al. 2003 showed that plant residues added to soil are quickly colonized by fungi, and the P in the resulting particulate organic matter may originate mainly from fungal P immobilization.

5. CONCLUSIONS

Repeated additions of poultry litter as a fertilizer in three long-term field experiments in the United States increased the concentrations of P_t and P_o in the coarse fraction of the soil (>53 µm), and of phytate-like P in the whole soil, and resulted in an increase in P_o mineralization measured in an isotopic dilution experiment compared to other treatments. Mineralization of P_o followed the general trend: poultry litter ≥ inorganic fertilizer > control = control + nitrogen fertilizer. The concentration of P_o in the CF was significantly associated with P_o mineralization. The quantification of P_o in the coarse fraction therefore is a promising non-isotopic alternative to estimate basal P_o mineralization. However, further research is required to verify the association between P_o in the coarse fraction and P_o mineralization in a broader range of soils and land-use types, and to assess if changes in P_o in the coarse fraction indeed influence plant P uptake.

Acknowledgments

This work was partly supported by the Swiss National Science Foundation (grant no. 3100A0-122141). We thank Arne Korsbak from Novozyme (DSM Nutritional Products) for the supply of Ronozyme NP (M). We would also like to acknowledge the International Plant Nutrition Institute (IPNI) for the financial aid provided to carry on this project, Laurie Schönholzer-Mauclaire for her assistance in the lab, and the keepers of the field trials Tom Sims, Antonio Mallarino, Ramesh Kanwar, Amy Shober and Shawn Tingle for facilitating information and the soil samples.

REFERENCES

Achat, D.L., Bakker, M.R., Augusto, L., Saur, E., Dousseron, L., Morel, C., 2009a. Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: combining isotopic dilution and extraction methods. Biogeochemistry 92, 183-200.

Achat, D.L., Bakker, M.R., Morel, C., 2009b. Process-based assessment of phosphorus availability in a low phosphorus sorbing forest soil using isotopic dilution methods. Soil Science Society of America Journal 73, 2131-2142.

Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C., 2010. Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. Soil Biology and Biochemistry 42, 1479-1490.

Alef, K., 1995. Soil respiration, In: Nannipieri, P. (Ed.), Methods in applied soil microbiology and biochemistry. Academic press, London, pp. 214-219.

Anderson, G., Arlidge, E., 1962. The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals, and hydrated sesquioxides in acid media. Journal of Soil Science 13, 216-224.

Anderson, J.M., Ingram, J., 1989. Tropical soil biology and fertility. A handbook of methods. CAB international Wallingford, Oxon, UK, 221 pp.

Annaheim, K., Doolette, A., Smernik, R., Mayer, J., Oberson, A., Frossard, E., Bünemann, E., 2015. Long-term addition of organic fertilizers has little effect on soil organic phosphorus as characterized by 31 P NMR spectroscopy and enzyme additions. Geoderma 257-258, 67-77.

Batjes, N., 1997. A world dataset of derived soil properties by FAO–UNESCO soil unit for global modelling. Soil Use and Management 13, 9-16.

Borie, F., Zunino, H., Martínez, L., 1989. Macromolecule-P associations and inositol phosphates in some Chilean volcanic soils of temperate regions. Communications in Soil Science & Plant Analysis 20, 1881-1894.

Bray, R.H., Kurtz, L., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59, 39-46.

Brookes, P., Powlson, D., Jenkinson, D., 1984. Phosphorus in the soil microbial biomass. Soil Biology and Biochemistry 16, 169-175.

Bünemann, E., 2008. Enzyme additions as a tool to assess the potential bioavailability of organically bound nutrients. Soil Biology and Biochemistry 40, 2116-2129.

Bünemann, E., 2015. Assessment of gross and net mineralization rates of soil organic phosphorus - a review. Soil Biology & Biochemistry. dx.doi.org/10.1016/j.soilbio.2015.06.026.

Bünemann, E., Marschner, P., McNeill, A.M., McLaughlin, M.J., 2007. Measuring rates of gross and net mineralisation of organic phosphorus in soils. Soil Biology and Biochemistry 39, 900-913.

Bünemann, E., Oberson, A., Liebisch, F., Keller, F., Annaheim, K., Huguenin-Elie, O., Frossard, E., 2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. Soil Biology and Biochemistry 51, 84-95.

Cade-Menun, B.J., He, Z., Zhang, H., Endale, D.M., Schomberg, H.H., Liu, C.W., 2015. Stratification of Phosphorus Forms from Long-Term Conservation Tillage and Poultry Litter Application. Soil Science Society of America Journal 79, 504-516.

Cambardella, C., Elliott, E., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777-783.

Ciampitti, I.A., García, F.O., Picone, L.I., Rubio, G., 2011. Soil carbon and phosphorus pools in field crop rotations in Pampean soils of Argentina. Soil Science Society of America Journal 75, 616-625.

Condron, L.M., Moir, J., Tiessen, H., Stewart, J., 1990. Critical evaluation of methods for determining total organic phosphorus in tropical soils. Soil Science Society of America Journal 54, 1261-1266.

Deans, J., Molina, J., Clapp, C., 1986. Models for predicting potentially mineralizable nitrogen and decomposition rate constants. Soil Science Society of America Journal 50, 323-326.

Evans, W., Martin, C., 1988. Interactions of Mg (II), Co (II), Ni (II), and Zn (II) with phytic acid. VIII. A calorimetric study. Journal of Inorganic Biochemistry 32, 259-268.

Evans, W., Martin, C., 1991. The interactions of inositol hexaphosphate with Fe (III) and Cr (III). A calorimetric investigation. XV. Journal of Inorganic Biochemistry 41, 245-252.

Fardeau, J.-C., Morel, C., Jappe, J., 1985. Cinétique d'échange des ions phosphate dans les systèmes sol-solution: vérification expérimentale de l'équation théorique. Comptes rendus des séances de l'Académie des sciences. Série 3, Sciences de la vie 300, 371-376.

Fardeau, J., Morel, C., Boniface, R., 1991. Cinétiques de transfert des ions phosphate du sol vers la solution du sol: paramètres caractéristiques. Agronomie 11, 787-797.

Harrison, A.F., 1987. Soil organic phosphorus: a review of world literature. CAB International Wallingford.

He, Z., Honeycutt, C.W., Cade-Menun, B.J., Senwo, Z.N., Tazisong, I.A., 2008. Phosphorus in poultry litter and soil: Enzymatic and nuclear magnetic resonance characterization. Soil Science Society of America Journal 72, 1425-1433.

Hill, J.E., Cade-Menun, B.J., 2009. Phosphorus-31 nuclear magnetic resonance spectroscopy transect study of poultry operations on the Delmarva Peninsula. Journal of Environmental Quality 38, 130-138.

Keller, M., Oberson, A., Annaheim, K.E., Tamburini, F., Mäder, P., Mayer, J., Frossard, E., Bünemann, E.K., 2012. Phosphorus forms and enzymatic hydrolyzability of organic phosphorus in soils after 30 years of organic and conventional farming. Journal of Plant Nutrition and Soil Science 175, 385-393.

Kirkby, C., Kirkegaard, J., Richardson, A., Wade, L., Blanchard, C., Batten, G., 2011. Stable soil organic matter: a comparison of C: N: P: S ratios in Australian and other world soils. Geoderma 163, 197-208.

Koopmans, G., Chardon, W., McDowell, R., 2007. Phosphorus movement and speciation in a sandy soil profile after long-term animal manure applications. Journal of Environmental Quality 36, 305-315.

Kouno, K., Tuchiya, Y., Ando, T., 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry 27, 1353-1357.

Levene, H., 1960. Robust tests for equality of variances. Contributions to probability and statistics: Essays in honor of Harold Hotelling 2, 278-292.

Mehlich, A., 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science & Plant Analysis 15, 1409-1416.

Müller, C., Bünemann, E.K., 2014. A ^{33}P tracing model for quantifying gross P transformation rates in soil. Soil Biology and Biochemistry 76, 218-226.

Murphy, D., Recous, S., Stockdale, E., Fillery, I., Jensen, L., Hatch, D., Goulding, K., 2003. Gross nitrogen fluxes in soil: theory, measurement and application of 15 N pool dilution techniques. Advances in Agronomy 79, 69-118.

Natural Resources Conservation Service, 1999. Soil Taxonomy: A basic system of soil classification for making and interpreting soil surveys. US Department of Agriculture.

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., Oberson, A., 2004. Basal organic phosphorus mineralization in soils under different farming systems. Soil Biology and Biochemistry 36, 667-675.

Oehl, F., Oberson, A., Sinaj, S., Frossard, E., 2001. Organic phosphorus mineralization studies using isotopic dilution techniques. Soil Science Society of America Journal 65, 780-787.

Ognalaga, M., Frossard, E., Thomas, F., 1994. Glucose-1-phosphate and myo-inositol hexaphosphate adsorption mechanisms on goethite. Soil Science Society of America Journal 58, 332-337.

Ohno, T., Zibilske, L.M., 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Science Society of America Journal 55, 892-895.

Olsen, S.R., Cole, C., Watanabe, F.S., Dean, L., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture Washington, DC.

Pierzynski, G.M., McDowell, R.W., Sims, J., Sharpley, A., 2005. Chemistry, cycling, and potential movement of inorganic phosphorus in soils, In: Sims, J.T., Sharpley, A.N.

(Eds.), Phosphorus: agriculture and the environment, Madison, WI, USA, pp. 53-86.

R Core Team, 2013. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. URL http://www. R-project. org.

Raghothama, K.G., Sims, J., Sharpley, A., 2005. Phosphorus and plant nutrition: an overview. Phosphorus: agriculture and the environment, 355-378.

Randriamanantsoa, L., Frossard, E., Oberson, A., Bünemann, E.K., 2015. Gross organic phosphorus mineralization rates can be assessed in a Ferralsol using an isotopic dilution method. Geoderma 257-258, 86-93.

Requejo, M.I., Eichler-Löbermann, B., 2014. Organic and inorganic phosphorus forms in soil as affected by long-term application of organic amendments. Nutrient Cycling in Agroecosystems 100, 245-255.

Robinson, G.W., 1922. A new method for the mechanical analysis of soils and other dispersions. The Journal of Agricultural Science 12, 306-321.

Salas, A., Elliott, E., Westfall, D., Cole, C., Six, J., 2003. The role of particulate organic matter in phosphorus cycling. Soil Science Society of America Journal 67, 181-189.

SAS Institute Inc, 2013. SAS® 9.4 In-Database Products: User's Guide, Third Edition. Cary, NC: SAS Institute Inc.

Saunders, W., Williams, E., 1955. Observations on the determination of total organic phosphorus in soils. Journal of Soil Science 6, 254-267.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 591-611.

Six, J., Conant, R., Paul, E., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155-176.

Taylor, T., 1965. The availability of the calcium and phosphorus of plant materials for animals. Proceedings of the Nutrition Society 24, 105-112.

Wyngaard, N., Vidaurreta, A., Echeverría, H.E., Picone, L.I., 2013. Dynamics of Phosphorus and Carbon in the Soil Particulate Fraction Under Different Management Practices. Soil Science Society of America Journal 77, 1584-1590.

Xue, Q.Y., Shamsi, I.H., Sun, D.S., Ostermann, A., Zhang, Q.C., Zhang, Y.S., Lin, X.Y., 2013. Impact of manure application on forms and quantities of phosphorus in a Chinese Cambisol under different land use. Journal of Soils and Sediments 13, 837-845.

Zheng, Z., Simard, R.R., Lafond, J., Parent, L.E., 2002. Pathways of soil phosphorus transformations after 8 years of cultivation under contrasting cropping practices. Soil Science Society of America Journal 66, 999-1007.

TABLES AND FIGURES

Table 4.1. General soil properties (pH, total carbon (C_t), total nitrogen (N_t), organic P (P_o) and inorganic P (P_i) in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means ± standard deviation of three field replicates. Different letters indicate significant ($p < 0.05$) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treatment	pH	C_t g kg^{-1}	N_t g kg^{-1}	P_i ----------------------mg kg^{-1} ------ ---------	P_o	C_t:Po
Delaware	Control	5.1 ± 0.0 b	12.6 ± 0.2 b	0.68 ± 0.02 b	90.9 ± 0.9 b	118.5 ± 0.9 b	106.1 ± 2.0 a
	IF	5.2 ± 0.1 b	13.1 ± 0.3 b	0.81 ± 0.02 a	145.2 ± 3.8 a	140.7 ± 3.8 a	93.3 ± 0.3 b
	PL	5.5 ± 0.0 a	14.2 ± 0.3 a	0.86 ± 0.01 a	155.6 ± 7.8 a	141.8 ± 7.8 a	100.0 ± 4.6 ab
Maryland	Control	5.5 ± 0.3	10.2 ± 0.1 c	0.90 ± 0.02 b	349.7 ± 3.4 c	345.5 ± 3.4 b	29.6 ± 0.2 a
	IF	6.1 ± 0.4	10.5 ± 0.1 b	0.95 ± 0.02 ab	413.4 ± 8.8 b	352.3 ± 8.8 b	29.7 ± 0.7 a
	PL	5.9 ± 0.2	11.0 ± 0.1 a	1.01 ± 0.03 a	443.3 ± 2.3 a	369.6 ± 2.3 a	29.7 ± 0.3 a
Iowa	Control	6.6 ± 0.1 b	17.2 ± 0.3 b	1.27 ± 0.04 b	137.0 ± 4.7 b	245.5 ± 4.7 b	69.9 ± 1.2 ab
	Control + N	5.8 ± 0.1 c	18.7 ± 0.2 a	1.41 ± 0.02 a	119.5± 4.0 c	281.3 ± 4.0 a	66.4 ± 1.5 b
	PL	7.2 ± 0.1a	17.0 ± 0.1 b	1.34 ± 0.01 ab	481.4 ± 3.8 a	230.6 ± 3.8 c	73.6 ± 1.0 a
Significance level (*p*)	Delaware	< 0.001	0.003	< 0.001	< 0.001	0.006	0.013
	Maryland	0.274	< 0.001	0.012	< 0.001	0.013	0.933
	Iowa	< 0.001	< 0.001	0.003	< 0.001	< 0.001	0.003

Table 4.2. Concentrations of total C (C_t), total P (P_t), organic P (P_o) and C:P_o ratio after physical fractionation into two fractions (coarse and fine) of three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). All concentrations are expressed on a whole soil basis. Means ± standard deviation of three field replicates. Different letters within columns indicate significant ($p < 0.05$) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treat.	Coarse fraction (>53µm)				Fine fraction (<53 µm)			
		C_t	P_t†	P_o†	C_t:P_o	C_t	P_t	P_o	C_t:P_o
		g kg^{-1}	----------- mg kg^{-1}--------			g kg^{-1}	---------- mg kg^{-1} ---------		
Delaware	Control	3.8 ± 0.4	9.7 ± 1.2 b	8.5 ± 0.4 b	478.0 ± 25.5 a	8.8 ± 0.3 b	161.6 ± 23.1	110.2 ± 15.5	81.1 ± 10.5
	IF	3.3 ± 0.5	19.9 ± 5.0 ab	11.3 ± 0.7 b	293.7 ± 30.7 b	9.6 ± 0.3 ab	208.5 ± 36.4	120.9 ± 22.4	90.1 ± 21.0
	PL	4.0 ± 0.6	25.9 ± 6.7 a	14.9 ± 1.0 a	302.2 ± 18.7 b	10.3 ± 0.3 a	219.4 ± 38.7	138.6 ± 27.4	86.2 ± 6.6
Maryland	Control	1.9 ± 0.2	19.9 ± 4.2 b	11.3 ± 0.4 b	156.7 ± 13.8	8.3 ± 0.2 b	613.4 ± 31.0	379.6 ± 50.1	22.2 ± 2.7
	IF	1.8 ± 0.2	31.0 ± 0.5 ab	12.2 ± 0.7 ab	132.3 ± 7.4	8.6 ± 0.2 ab	664.9 ± 30.1	366.7 ± 26.1	23.7 ± 2.1
	PL	1.9 ± 0.1	42.5 ± 1.0 a	14.7 ± 0.7 a	133.5 ± 9.8	9.0 ± 0.1 a	697.5 ± 51.0	379.3 ± 33.6	24.0 ± 2.3

(continues in next page)

Table 4.2. (continued from previous page)

Iowa	Control	1.7 ± 0.4 b	25.0 ± 1.5 b	6.1 ± 0.9 b	285.4 ± 91.0	15.5 ± 0.6 a	354.9 ± 21.8 a	261.2 ± 7.4	59.3 ± 3.7
	Control + N	2.2 ± 0.3 ab	25.2 ± 1.5 b	7.2 ± 0.4 b	335.8 ± 122.5	16.4 ± 0.3 a	375.8 ± 31.5 a	294.8 ± 24.2	56.2 ± 5.0
	PL	2.9 ± 0.3a	90.7 ± 8.3 a	17.7 ± 2.3 a	164.1 ± 13.8	14.1 ± 0.2 b	592.4 ± 35.5 b	278.0 ± 17.9	50.8 ± 2.6
Significance level (*p*)	Delaware	0.364	0.041	0.003	0.003	0.020	0.262	0.481	0.864
	Maryland	0.897	0.010	0.008	0.107	0.020	0.170	0.929	0.745
	Iowa	0.025	0.000	0.001	0.070	0.004	0.000	0.251	0.171

†P_t and P_o determined by the ignition method (Saunders and Williams, 1955)

Table 4.3. Concentrations of different phosphorus forms in NaOH-EDTA-extracts determined by enzyme additions in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means ± standard deviation of three field replicates. Within columns, different letters indicate significant ($p < 0.05$) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treatment	Inorganic P	Organic P			
			Simple monoester P	DNA-like P	Phytate-like P	Non-hydrolysable Po
		-- mg P kg^{-1} --				
Delaware	Control	34.0 ± 3.0 b	2.5 ± 1.8	0.3 ± 0.5	9.9 ± 0.6	3.4 ± 3.7
	IF	79.9 ± 3.8 a	2.8 ± 1.0	0.9 ± 0.6	12.6 ± 1.3	10.7 ± 5.4
	PL	86.2 ± 6.4 a	0.6 ± 1.0	0.5 ± 0.6	11.3 ± 2.9	5.8 ± 1.6
Maryland	Control	52.7 ± 4.9 b	6.2 ± 3.5	1.5 ± 1.8	2.9 ± 0.9 c	40.0 ± 4.2 ab
	IF	97.7 ± 0.2 a	5.3 ± 4.0	0.5 ± 0.0	7.2 ± 0.5 b	59.9 ± 16.3 a
	PL	106.0 ± 6.6 a	1.9 ± 2.3	0.8 ± 1.2	12.6 ± 1.5 a	29.1 ± 7.8 b
Iowa	Control	57.3 ± 2.5 b	2.6 ± 0.3	0.0 ± 0.0	14.9 ± 1.2 b	38.1 ± 2.3 ab
	Control + N	43.2 ± 5.4 b	3.9 ± 2.5	0.5 ± 0.5	14.1 ± 1.0 b	62.2 ± 15.7 a
	PL	265.9 ± 5.6 a	13.7 ± 18.7	1.3 ± 2.2	19.8 ± 0.8 a	29.4 ± 4.3 b
Significance level (*p*)	Delaware	< 0.001	0.177	0.656	0.326	0.302
	Maryland	< 0.001	0.293	0.679	< 0.001	0.024
	Iowa	< 0.001	0.495	0.635	0.002	0.016

Table 4.4. Parameter of isotopic exchange kinetics during an incubation experiment in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means ± standard deviation of six analytical replicates. Within each soil different letter in the same column indicate significant differences ($p < 0.05$) according to the Tukey-Kramer test.

Soil	Treatment	10*Cp (mg P kg^{-1})	*m*	*n*
Delaware	Control	0.20 ± 0.01c†	0.39 ± 0.04 b	0.32 ± 0.02 a
	IF	1.22 ± 0.08 b	0.60 ± 0.03 a	0.25 ± 0.00 b
	PL	1.75 ± 0.12 a	0.64 ± 0.03 a	0.22 ± 0.01 c
Maryland	Control	0.20 ± 0.01 c	0.11 ± 0.01 c	0.37 ± 0.01 a
	IF	0.95 ± 0.08 b	0.23 ± 0.01 b	0.28 ± 0.01 b
	PL	1.18 ± 0.05 a	0.27 ± 0.01 a	0.27 ± 0.00 b
Iowa	Control	0.45 ± 0.04 b	0.20 ± 0.01 b	0.30 ± 0.01 b
	Control + N	0.27 ± 0.04 b	0.21 ± 0.03 b	0.35 ± 0.01 a
	PL	10.35 ± 0.27 a	0.37 ± 0.00 a	0.18 ± 0.00 c

Table 4.5. Cumulative P_o mineralization ($\Delta E_{(t)}$), P immobilization (P_{imm}) determined as the difference between the isotopic exchangeable P estimated in an IEK batch experiment ($E_{(t)est}$) and the one measured at 6 and 13 days in an incubation experiment with three soils subjected to long-term fertilizer treatments (Control, inorganic P fertilization (IF), Control + N and poultry litter (PL)). Means ± standard deviation of four analytical replicates. Different letters within columns indicate significant ($p < 0.05$) differences between treatments for a given soil according to the Tukey-Kramer test.

Soil	Treatment	Days	Respiration rate	$E_{(t)est}$	$E_{(t)meas}$	Gross P_{min} $\Delta E_{(t)}$	Gross P_{imm}	Net P_{min}
			mg C kg^{-1} d^{-1}	--	--	mg P kg^{-1}	--------------------------------	--------------------------------
Delaware	Control	6	12.0 ± 0.2 a	10.0 ± 1.9 c	17.2 ± 0.5 f	$7.3 \pm 0.5^{***}$† d	1.1 ± 0.7 c	6.2 ± 0.5 d
		13	9.2 ± 0.5 b	12.4 ± 2.4 c	21.4 ± 0.8 e	$9.0 \pm 0.4^{***}$ d	1.6 ± 0.3 bc	7.4 ± 0.4 d
	IF	6	12.3 ± 0.2 a	17.2 ± 1.2 b	38.1 ± 1.7 d	$20.9 \pm 1.7^{***}$ c	1.9 ± 0.3 abc	19.0 ± 1.7 c
		13	9.0 ± 0.1 b	20.3 ± 1.4 ab	48.3 ± 1.0 b	$28.0 \pm 1.0^{***}$ b	1.5 ± 0.5 bc	26.5± 1.0 b
	PL	6	13.5 ± 0.2 a	18.0 ± 1.6 ab	44.8 ± 0.8 c	$26.9 \pm 0.8^{***}$ b	2.5 ± 0.5 ab	24.4 ± 0.8 b
		13	8.3 ± 0.2 b	20.8 ± 1.9 a	55.3 ± 2.3 a	$34.5 \pm 2.3^{***}$ a	3.3 ± 0.3 a	31.2 ± 2.3 a

(continues in next page)

Table 4.5. (continued from previous page)

Maryland	Control	6	11.6 ± 0.2 c	38.6 ± 4.8 c	52.5 ± 3.6 d	13.9 ± 3.6** d	3.0 ± 0.5 a	10.9 ± 3.6 d
		13	7.1 ± 0.4 e	49.5 ± 6.1 b	77.6 ± 2.1 b	28.1 ± 2.1*** b	3.8 ± 1.1 a	26.5 ± 2.1 b
	IF	6	13.5 ± 0.1b	44.4 ± 2.0 bc	65.4 ± 2.7 c	21.0 ± 2.7*** c	2.8 ± 0.4 a	18.2 ± 2.7 c
		13	8.2 ± 0.1 d	53.6 ± 2.5 a	91.0 ± 2.0 a	37.4 ± 2.0*** a	2.4 ± 0.1 a	35.0 ± 2.0 a
	PL	6	14.6 ± 0.4 a	46.7 ± 1.8 b	67.8 ± 0.9 c	21.1 ± 0.9*** c	2.7 ± 1.4 a	18.4 ± 0.9 c
		13	8.3 ± 0.4 d	56.2 ± 2.2 a	94.9 ± 2.9 a	38.6 ± 2.9*** a	3.1 ± 0.4 a	35.6 ± 2.9 a
Iowa	Control	6	6.4 ± 0.4 cb	26.0 ± 1.3 cd	37.5 ± 5.0 cd	8.6 ± 0.5*** c	3.0 ± 0.5 a	3.5 ± 3.7 c
		13	3.6 ± 0.6 d	31.2 ± 1.6 c	39.3 ± 2.1 cd	8.3 ± 1.0*** c	3.8 ± 1.1 a	4.5 ± 1.0 c
	Control + N	6	6.0 ± 0.1 c	20.7 ±2.1 d	31.2 ± 3.4 d	10.5 ± 3.4** c	2.8 ± 0.4 a	7.8 ± 3.4 c
		13	4.1 ± 0.1 d	25.6 ±2.5 d	36.1 ± 1.5 c	10.5 ± 1.5*** c	2.4 ± 0.1 a	8.1 ± 1.5 c
	PL	6	12.9 ± 0.5 a	112.0 ±3.6 b	133.6 ± 2.3 b	21.6 ± 2.3*** b	2.7 ± 1.4 a	18.9 ± 2.3 b
		13	7.1 ± 0.2 c	124.4 ±4.1 a	155.9 ± 1.7 a	31.5 ± 1.7*** a	3.1 ± 0.4 a	28.4 ± 1.7 a

† Differences $\Delta E_{(t)}$ between $E_{(t)est}$ and $E_{(t)meas}$ are non-significant (NS) or significant at a 0.001 (***), 0.01 (**) or 0.05 (*) probability level.

Table 4.6. Gross and net daily P mineralization rates (P_{min} rate) and C:P ratios at 6 and 13 days in an incubation experiment with three soils subjected to long-term fertilizer treatments (Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means ± standard deviation of four analytical replicates.

Soil	Treatment	Days	Gross P_{min} rate mg P kg^{-1} d^{-1}	Net P_{min} rate mg P kg^{-1} d^{-1}	Gross P_{min} C:P	Net P_{min} C:P
Delaware	Control	6	1.2 ± 0.1 e	1.0 ± 0.1 d	16.7 ± 1.2 b	11.7 ± 1.0 b
		13	0.7 ± 0.0 f	0.6 ± 0.0 e	22.1 ± 1.1 a	16.1 ± 1.0 a
	IF	6	3.5 ± 0.3 b	3.2 ± 0.3 b	5.9 ± 0.5 cd	3.9 ± 0.4 c
		13	2.2 ± 0.1 d	2.0 ± 0.1 c	7.0 ± 0.3 c	4.4 ± 0.2 c
	PL	6	4.5 ± 0.1 a	4.1 ± 0.1 a	5.0 ± 0.2 d	3.3 ± 0.1 c
		13	2.7 ± 0.2 c	2.4 ± 0.2 c	5.2 ± 0.3 cd	3.5 ± 0.3 c
Maryland	Control	6	2.3 ± 0.6 b	1.8 ± 0.6 c	9.0 ± 2.6 a	7.2 ± 2.9 a
		13	2.2 ± 0.2 b	2.0 ± 0.2 bc	5.1 ± 0.4 bb	3.5 ± 0.3 b
	IF	6	3.5 ± 0.5 a	3.0 ± 0.5 a	6.5 ± 0.8 ab	4.5 ± 0.7 ab
		13	2.9 ± 0.2 ab	2.7 ± 0.2 ab	4.8 ± 0.3 b	3.1 ± 0.2 b
	PL	6	3.5 ± 0.2 a	3.1 ± 0.2 a	6.9 ± 0.3 ab	4.8 ± 0.2 ab
		13	3.0 ± 0.2 ab	2.7 ± 0.2 ab	4.7 ± 0.3 b	3.0 ± 0.2 b
Iowa	Control	6	1.4 ± 0.1 cd	0.6 ± 0.8 cd	5.6 ± 0.3 b	6.9 ± 0.6 ab
		13	0.6 ± 0.1 d	0.3 ± 0.1 d	7.2 ± 1.0 ab	11.1 ± 2.9 a
	Control + N	6	1.7 ± 0.6 bc	1.3 ± 0.6 c	6.3 ± 1.8 ab	5.6 ± 2.2 b
		13	0.8 ± 0.1 d	0.6 ± 0.1 cd	8.7 ± 1.4 a	6.8 ± 1.5 ab
	PL	6	3.6 ± 0.4 a	3.2 ± 0.4 a	6.0 ± 0.7 ab	4.1 ± 0.5 b
		13	2.4 ± 0.1 b	2.2 ± 0.1 b	4.9 ± 0.3 b	3.3 ± 0.2 b

Table 4.7. Linear models to estimate gross or net P_o mineralization (P_{min}) using respiration (Resp) and the C:P ratio in the non-fractioned soil, in the coarse fraction and in the fine fraction ($C{:}P_{Total}$, $C{:}P_{CF}$ and $C{:}P_{FF}$, respectively) as regressors.

Variables	Model	Significance level (*p*)	R^2
Resp	Gross $P_{min} = 0.09 + 0.024\ Resp^{\dagger}$	< 0.001	0.52
Resp + $C{:}P_{Total}$	Gross $P_{min} = 0.62 + 0.24\ Resp^{\dagger} – 0.01\ C{:}P_{Total}$	< 0.001	0.56
Resp + $C{:}P_{CF}$	Gross $P_{min} = 1.52 + 0.22\ Resp^{\dagger} – 0.0046\ C{:}P_{CF}^{\dagger}$	< 0.001	0.77
Resp + $C{:}P_{FF}$	Gross $P_{min} = 0.45 + 0.24\ Resp^{\dagger} – 0.01\ C{:}P_{FF}$	0.003	0.54
Resp	Net $P_{min} = - 0.15 + 0.24\ Resp^{\dagger}$	0.001	0.50
Resp + $C{:}P_{Total}$	Net $P_{min} = 0.28 + 0.24\ Resp^{\dagger} – 0.01\ C{:}P_{Total}$	0.003	0.54
Resp + $C{:}P_{CF}$	Net $P_{min} = 1.17 + 0.21\ Resp^{\dagger} – 0.0043\ C{:}P_{CF}^{\dagger}$	< 0.001	0.69
Resp + $C{:}P_{FF}$	Net $P_{min} = 0.13 + 0.24\ Resp^{\dagger} – 0.01\ C{:}P_{FF}$	0.004	0.52

[†] Indicates significant contribution ($p < 0.05$) of the variable to the model.

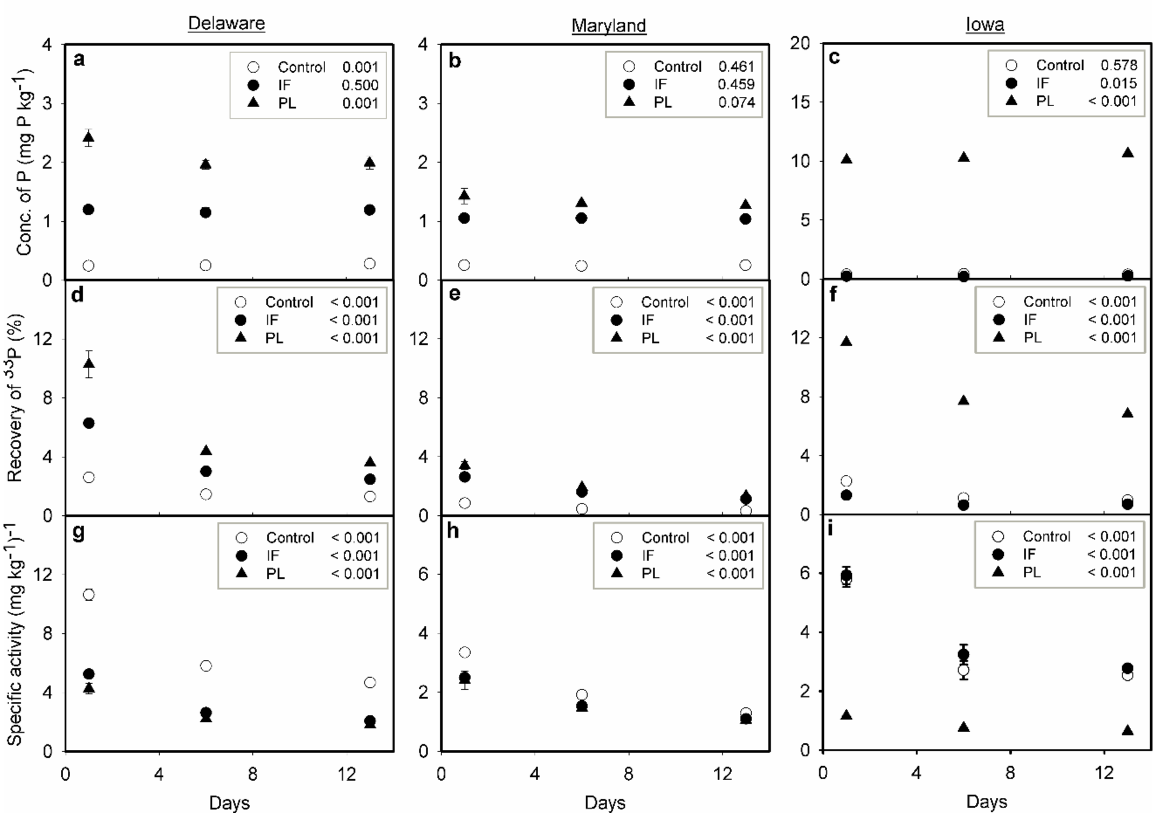

Figure 4.1. Concentration of phosphate (a, b and c), recovery of ^{33}P (d, e and f) and specific activity (g, h and i) in water-extractable phosphate during an incubation experiment in three different soils subject to different fertilizers treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Time-points: 1, 6 and 13 days after labeling. Significance values in the legend indicate the effect of time for each treatment separately in a one-way ANOVA. Error bars represent the standard deviation (n=4).

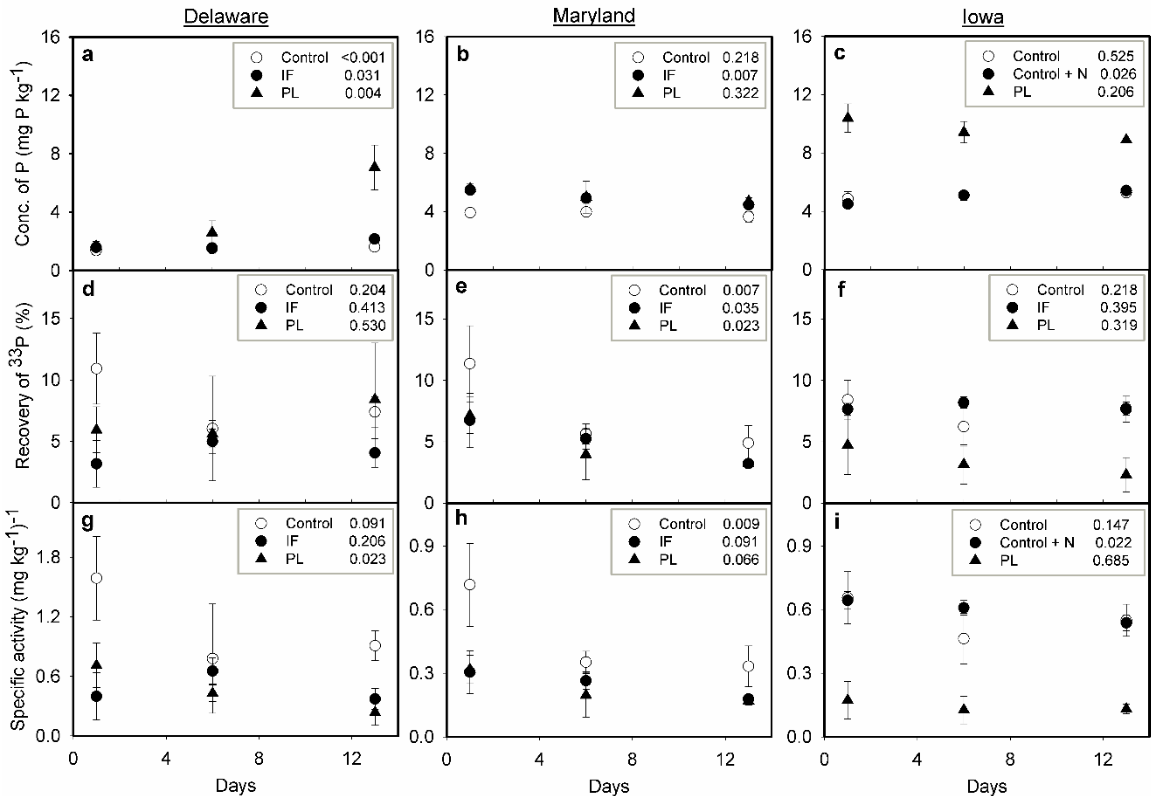

Figure 4.2. Concentration of phosphate (a, b and c), recovery of ^{33}P (d, e and f) and specific activity (g, h and i) in hexanol-labile P during an incubation experiment with three different soils subject to different long-term fertilizers treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Time-points: 1, 6 and 13 days after labeling. Significance values in the legend indicate the effect of time for each treatment separately in a one-way ANOVA. Error bars represent the standard deviation (n=4).

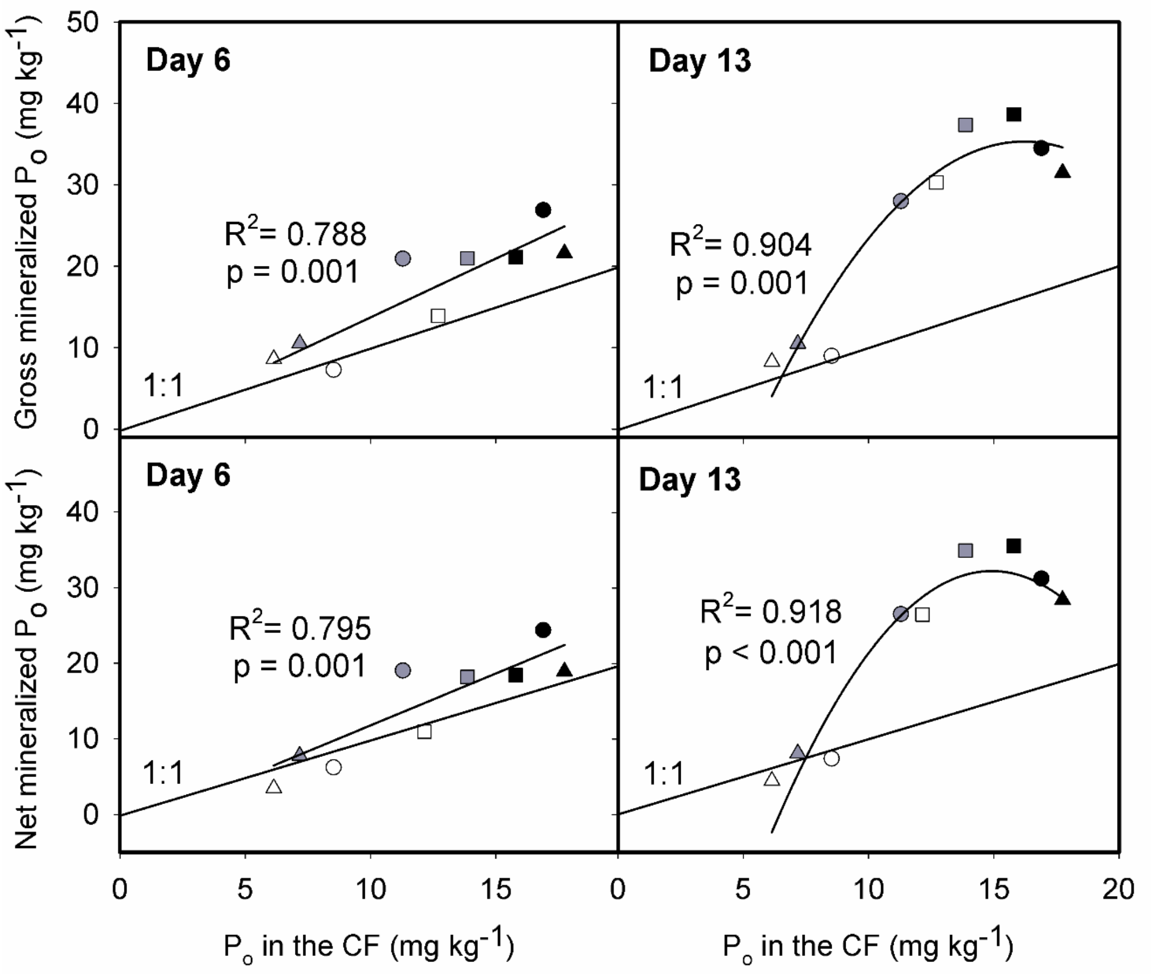

Figure 4.3. Association between organic phosphorus in the coarse fraction (CF) of the soil and the cumulative gross and net P mineralization at 1, 6 and 13 days of incubation of three soils that had received poultry litter or inorganic fertilizer for more than 10 yr. Different shapes represent different soils: circle (Delaware), square (Maryland) and triangle (Iowa). Different colors represent different fertilizer treatments: white (control), grey (inorganic fertilizer) and black (poultry litter).

CONCLUSION

The use of PL as a fertilizer can lead to an accumulation of labile N, P and S organic pools in the soil, which in turn results in an increase in the N, P and S mineralization potential. However, this accumulation process is not significant in all soils. The texture of the soil seems to explain this difference. Soils with a greater clay and silt content, and consequently a greater capacity to protect organic matter against decomposition, present a greater accumulation of mineralizable nutrients.

Even though there are many simple and quick methods to estimate the N mineralization potential of the soil, our study demonstrated that the use of chemical extractions can lead to an underestimation of the N mineralization potential in PL-amended soils when these methods are calibrated in inorganically amended soils. The quantification of the NH_4^+ released after a 7-d anaerobic incubation proved to be a good predictor for both N and S mineralization potential independently of the fertilization history. The fact that a N mineralization estimator can be used to predict S mineralization highlights the strong correlation between the mineralization rates of both nutrients. This finding will allow soil testing laboratories to estimate simultaneously the N and S mineralization potential by using a single cheap and simple method. The chemical extraction with $NaHCO_3$, which has been previously associated with S uptake from pastures, also proved to be a strong indicator of S mineralization independently of the fertilization history.

While further research is required in a broader range of soils and land-use types, our data provides evidence that there is an association between the of P_o content of the soil and its mineralization potential. This is important, as there is no other non-isotopic method to estimate P_o mineralization. Thus, the concentration of P_o in the CF surges as a promising indicator to differentiate soils according to their P_o mineralization potential, without focusing on the actual rate of mineralization which will be affected by other factors such as temperature and water content. In order to estimate P_o mineralization rates, we recommend to measure soil respiration and correct it by the C_t:P_o ratio of the CF.