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Mineralizable phosphorus, nitrogen, and carbon relationships in dairy manure at various carbon-to-phosphorus ratios

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ABSTRACT

Phosphorus (P) in animal manure can be an important nutrient for crops or an environmental contaminant if in excess. Organic P in dairy manure may add to the environmentally bioactive P pools upon mineralization. A 353 d incubation study of manures containing C:P between 83 and 130:1 was conducted to determine linkages between C and P transformations and the effects of C:P on the immobilization–mineralization of manure P. As C:P widened from 83:1 to 130:1, P mineralization increased and phosphate accumulated at rates between 0.013 and 0.021 mg kg^{-1} d^{-1}. Water-extractable C was positively correlated with N:P, particularly at narrow C:P ($P < 0.001$). Absence of a negative feedback by phosphate suggested that P mineralization occurred with degradation of organic P-containing C substrates and appeared incidental to microbial P needs. Carbon content in manure may be managed to lower risks of elevated soluble P and C losses under non-limiting N conditions.

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1. Introduction

Animal manure is a valuable nutrient resource on the farm. Determining the fertilizer value and optimal amount of manure to apply to a crop requires accurate knowledge of manure nutrient composition and mineralization potential under the environmental conditions existing at the site of storage prior to field application. Since organic nutrient mineralization is carried out by microorganisms and extracellular enzymes, manure rate of mineralization is influenced by environmental factors such as temperature, pH, water content, and to a great extent the characteristics of the manure itself. Particular to phosphorus, its availability is also controlled by the chemistry of liquid and solid phases of the manure matrix and the surface chemistry of inorganic and organic particulates in that environment (Lindsay, 1979; Dao et al., 2006; Dao, in press). Manure has been used as source of a particular nutrient in crop production, but it contains all major elements required for crop growth, namely, N, P, potassium, and trace minerals. Diets vary greatly across animal species and stages of animal growth, which results in highly heterogeneous composition of manure. Numerous studies have been conducted to quantify manure nutrient contents by animal species, production types, and manure management practices (Peperzak et al., 1959; van Horn et al., 1994; Eck and Stewart, 1995; Kerr et al., 2006; Dao et al., 2006; Dao and Zhang, 2007).

Animal manures are major sources of organic P (Gerritse and Eksteen, 1978; Dao et al., 2005, 2006; Dao and Hoang, 2008). The organic P fraction generally consists of phosphomonoesters and diesters of inositol, phospholipids, and nucleic acids (Peperzak et al., 1959; Koopmans et al., 2003; Dao, 2003). Peperzak et al. (1959) surveyed P forms in manures from different classes of livestock and categorized the forms as alcohol-soluble (phospholipids), alcohol-insoluble (nucleic acid-P) and acid-soluble (inositol phosphates) fractions. Dissolved organic P forms in swine manure have been associated with large molecular-weight phosphomono- and diesters and phospholipids. Spectrometric studies re-validated the identities of these P forms, using ^{31}P nuclear-magnetic resonance spectroscopy, mass spectrometry, or high-performance liquid chromatography (Florentz et al., 1983; Lehrfeld, 1994; Llewelyn et al., 2002).

Adding a biological significance and availability dimension to these chemical P species, Dao (2003) and Dao and Hoang (2008) reported that animal manure has a large organic P fraction that is mineralized by extracellular phosphohydrolases to release inorganic phosphate. The production of extracellular phosphohydrolases occurs in response to the organisms' needs for inorganic phosphate, yet at a large expenditure of cellular resources and energy. Recent development of a ligand-based *in situ* enzymatic assay has allowed in-depth studies of the mineralization of organic P and the turnover of P pools in manure and manure-amended soils. Dao (2003) first reported the approach of using polycarboxylate ligands in combination with extracellular phosphohydrolases to simultaneously extract, hydrolyze, and categorize P species, without

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separate steps of an extraction and isolation of the extract for enzymatic assay. The reaction is preferentially driven toward the dephosphorylation of organic P. The experimental approach differs from enzymatic methods that required separate steps of extraction and subsequent analysis of the extracted organic P (e.g., Hedley et al., 1982; He and Honeycutt, 2001; Turner et al., 2002; Bunemann, 2008).

Nitrogen in the forms of amino acids and nucleic acids is also needed for protein synthesis and the production of phosphohydrolases and other oxidative enzymes that catalyze the mineralization of C substrates and the mineralization–immobilization of phosphate in cells of living plants and microorganisms. Such linkages between nutrient transformations and the interdependence of catabolic and synthetic processes often have been observed (Gressel et al., 1996; Olander and Vitousek, 2000; Dao and Cavigelli, 2003). Gressel et al. (1996) reported that inorganic P derived from the mineralization of mono-, diesters, and pyrophosphate was correlated with carbonyl and aromatic C levels derived from the decomposition of structural components of the forest floor litter of a mixed conifer stand. Dao and Cavigelli (2003) found significant nonlinear correlation between cumulative CO_2–C and both inorganic N and WEP released from stockpiled and composted cattle manure.

Information about interactions between other nutrient transformations and the turnover of organic P in dairy manure is limited but essential to the understanding of nutrient release to plants, soils, and the environment. Based on our preliminary knowledge of manure constituents and individual nutrient mineralization and release behavior, this study was conducted to determine (1) the effects of C:P ratios on the equilibrium between P mineralization and immobilization and (2) the relationships between C, N, and P transformations to derive manure composition characteristics favorable to the conservation of nutrients under aerobic fermentation conditions commonly found in on-farm liquid manure storage structures.

2. Methods

2.1. Manure preparation and incubation

Bulk batches of reconstituted dairy manure with a range of C:P ratios were prepared from freshly collected feces and urine (in a ratio of 1.6–1) from Holstein cows at the US Department of Agriculture, Agricultural Research Service Beltsville Agricultural Research Center dairy facility, in Prince George's County, Maryland (39.03° N and 76.85° W). The mature fresh Holstein herd was fed a total mixed ration based on corn silage to attain milk production goal of 10 Mg cow^{-1} y^{-1}. The urine was acidified with concentrated hydrochloric acid (pH 4) immediately after collection to avoid loss of ammonia. The feces-urine mix was blended in 20-L plastic containers, subdivided into packages of 5 kg, and kept frozen until used in the incubation study. Selected manure properties are presented in Table 1.

Phosphorus-depleted manure solids were prepared by incubating freshly reconstituted manure amended with exogenous phosphohydrolases for 36 h at 22 °C. Under these conditions, organic P forms were solubilized and dephosphorylated, releasing soluble phosphate to the manure liquid phase (Dao, 2003). The suspension was filtered through a sieve with 840 um openings and the P-depleted solids retained on the sieve were stored for later use. A range of C:P and N:P ratios was established in five batches of modified reconstituted manure, by either using the manure suspension as is, or by filtering out and replacing selected amounts of untreated manure solids with P-depleted manure solids in the suspension. The C:P and N:P ratios were measured and found to be between 83–130 and 4.6–5.2, respectively. The C:N ratio remained constant across all reconstituted manure batches at 20 ± 0.5, as only small amounts of soluble N were lost during the substitution of manure solids with P-depleted solids in the modified C:P manure batches.

For each manure batches, triplicate samples for each sampling date (1 g dry weight equivalent) were weighed into plastic vials (28 by 61 mm, height and diameter). A total of 225 vials were segregated into five groups of similar C:P ratios and were placed in 15 glass containers (3.7 L) that were capped to avoid evaporation and loss of water/liquid phase. The containers were arranged in a randomized complete block design, and incubated at 22 °C. The containers were uncapped and flushed with compressed moist air stream to re-establish aerobic headspace conditions every day for the first week of incubation, and twice a week thereafter. At specific times (1, 2, 5, 7, 14, 21, 28, 42, 56, 84, 112, 140, 177, 231, and 353 d), triplicate vials were removed to measure the distribution of manure P forms, water-extractable and total C, NH_4^+ and total N concentrations.

2.2. Manure analysis

Bioactive P fractions in manure samples were determined according to Dao (2003), Dao et al. (2006). The fractions included water-extractable P (WEP), ligand-exchangeable inorganic phosphate-P (EEP_i), organic ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate (EDTA)-exchangeable phosphohydrolase-labile P (EDTA-PHP), and the all-inclusive total bioactive P (WEP + EEP_i + EDTA-PHP), which were determined as follows.

The WEP in manure was measured in duplicate subsamples of each of the three replicate vials (equivalent to 0.1 g dry wt) equilibrated with deionized water for 1 h (1–100, w/v). An identical wet manure subsample was equilibrated with a 0.005 M EDTA (1–100, w/v) for 1 h on a gyratory shaker (250 rpm) to measure EEP_i. Total bioactive P was determined following EEP_i measurements. An aliquot of a stock solution of *Aspergillus ficuum* phosphohydrolases that were prepared in our laboratory (Dao and Hoang, 2008), containing 0.5 unit mL^{-1} of enzyme and deionized water were added to replace the 5-mL volume removed for the EEP_i measurement. The manure-enzyme mixture was shaken at 250 rpm for 22 h. The sample tubes were immersed in boiling

Table 1
Selected chemical characteristics of component fractions used in producing reconstituted manure at selected manure carbon-to-phosphorus ratios.

	Dry matter (g kg^{-1})	WEP[a] (mg kg^{-1})	TP[a] (mg kg^{-1})	N (g kg^{-1})	C (g kg^{-1})
Reconstituted manure[b]	1.30	1073 ± 74	4545 ± 426	23.8 ± 2.4	420 ± 67
Fresh manure solids	1.52	1090 ± 64	3318 ± 76	n.d[c]	n.d
Filtered manure liquid[d]	0.59	1566 ± 20	6255 ± 334	n.d	n.d
P-depleted solids	1.50	270 ± 19	802 ± 41	n.d	n.d

[a] Phosphorus fractions: WEP = water-extractable P, TP = total acid-digest P.
[b] Initial batch of dairy manure.
[c] Not determined.
[d] Fraction passing through sieve with 840-μm openings.

water for 10 min, centrifuged at 10,000×g, and aliquots of the supernatant were used for P analysis. This measurement yielded the all-inclusive total bioactive P fraction, which is the sum of WEP, EEP_i, and organic EDTA-PHP forms. The EDTA-PHP fraction, hence, was calculated as the difference between total bioactive P and EEP_i fractions.

A subsample of the extract used for the measurement of WEP was analyzed for carbonate-C and total water-extractable C (WEC), which were determined by UV-persulfate oxidation using a Phoenix 8000 (Teledyne Instruments, Mason, OH) and the APHA standard method number 5310C (APHA, 1998). In addition, total C and N concentrations of all manures were determined according to method APHA 5310B using a vario Max CNS elemental analyzer (Elementar Americas, Inc., Mt Laurel, NJ). Total P concentrations of the manure suspensions were determined by acid-digestion using a modified potassium persulfate digestion procedure (Dao et al., 2006). Reagent-grade $K_2S_2O_8$ (1 g) and 12 ml of 5.5 M H_2SO_4 were added to manure subsamples (equivalent to 0.1 g dry wt). The mixture was brought to a boil at 180 °C for 0.5 h in a heating block and the digestion temperature was raised and maintained at 350 °C for an additional 0.5 h. The digest was diluted to a final known volume for P analysis. Extracted and total P concentrations were determined colorimetrically using the phosphomolybdate-ascorbic acid method (method number APHA 4500P) and an auto-analyzer (Bran-Luebbe, Buffalo Grove, IL). Absorbance was recorded at 660 nm. Although small quantities of soluble organic P species such phosphate monoesters and phosphorylated nucleotides might be present in the aqueous extracts, the measurement of WEP and EEP_i fractions by the phosphomolybdate-ascorbic acid method yielded primarily soluble inorganic phosphate species, when compared to high-performance ion-chromatographic technique (Dao, 2003). Therefore, in the present study, WEP and EEP_i represented pools of inorganic P species, and the EDTA-PHP fraction consisted of organic P forms, though quantified as phosphate following an enzymatic incubation-hydrolysis analytical procedure (Dao, 2003).

As the solid matter content of the manures perceptibly declined over time, all P, N, and C concentrations in the dairy manures were accordingly adjusted. The changes in manure solid matter content will be further discussed in Section 3.

2.3. Statistical analysis and numerical methods

The triplicate chambers containing groups of modified manure suspensions of identical C:P ratio (5) were arranged in a constant temperature room maintained at 22 °C according to a randomized complete block design. Random effects of C:P ratio on nutrient redistributions repeatedly measured in time were detected following analysis of variance using PROC MIXED and computation of least-squares means (SAS Institute, 2004). Significant differences in treatment means were detected using the Tukey adjustment of least-squares means at the 0.05 probability level. The increase or decrease in mineralizable C, N, and P concentrations as function of time of incubation was also fitted to kinetic rate equations to describe their accumulation or disappearance from manure suspensions. Comparisons of parameters of linearized forms of the kinetic models were made using PROC GLM and a CLASS statement of the Statistical Analysis System (SAS, Cary, NC) as outlined in Dao et al. (1982). In summary, individual and common regression analyses were calculated to evaluate the homogeneity of residual variances and detect significant differences in slope and intercept of relative concentrations (C/C_o) vs. time plots for the C:P treatments at the 0.05 level of probability. Concentration distributions of WEC and NH_4–N were also fitted to the log-normal distribution function as described in Dao and Cavigelli (2003). Briefly, the flux density distribution was described by four parameters that included (i) the amplitude or $concentration_{max}$, (ii) time of occurrence of $concentration_{max}$, (iii) the distribution width at half-$concentration_{max}$, and (iv) the asymmetry at half-$concentration_{max}$. The root mean-squared error (RMSE) for the fitted distribution was computed in addition to the coefficient of determination (r^2) to determine the goodness of fit of the log-normal equation to the concentration data.

3. Results and discussion

3.1. Kinetics of P mineralization

An enrichment of manure P was apparent as the ratio of total C to total P in the manure suspensions declined and narrowed over time (Fig. 1). A loss in mass of manure solids due to C mineralization resulted in the enrichment of P in the manure suspensions and increased concentration on a dry weight basis. The decline approximately followed an exponential first-order decay reaction kinetic model.

Water-extractable P concentrations, unadjusted (not shown) or adjusted for the loss of dry matter, reflected the increased mineralization of manure organic P and the accumulation of WEP in all dairy manure suspensions over time (Fig. 2). The concentrations of WEP increased in all C:P treatments up to 180 d, after which the accumulation occurred at a slower rate for the next 170 d. The mineralization of organic P forms occurred in all manure C:P treatments, in spite of inorganic phosphate levels present at the initiation of the incubation that ranged from 1200 to 2250 mg kg^{-1} (Fig. 2). The accumulation of WEP forms in the manure suspensions data was described by the first-order reaction rate model, for all C:P treatments (Table 2). The pattern was indicative of a biological process rather than a chemical desorption or dissolution of inorganic P substrates. The latter process would have maintained a leveled concentration of EEP_i in the manure suspension, which was not observed as discussed in a subsequent section.

Each C:P treatment had a different initial WEP concentration as the result of the substitution of the original manure solids with P-depleted solids. The increase of WEP concentrations occurred at different rates of accumulation, with higher rates at the wider C:P treatments than in treatments with C:P < 105:1 (Fig. 2 and Table 2). This mineralization trend did not appear to be just a function of total organic P content in the suspensions. The wider C:P treatments had less organic P (i.e., more P-depleted solids substituted for the original manure solids) but whatever amount was available, the organic P pool was mineralized rapidly to support the decomposition of the extra load of water-insoluble C substrates.

The accumulation of water-extractable and exchangeable inorganic P forms (TEP_i) (data not shown) paralleled that of WEP forms, indicating that little or no inorganic P was further sequestered to add to the pool of insoluble EEP_i. The chemistry of the liquid phase was such that the additional mineralized P was maintained in soluble state, as part of the WEP pool. In a dissolved C-rich medium, many possible macromolecules could act as complexing agents of counterions to keep phosphate in solution (Ma et al., 2001; Dao, 2003).

Overall, the accumulation of the all-inclusive total bioactive P (WEP + EEP_i + EDTA-PHP) followed first-order reaction kinetics (Table 3). Rates of total bioactive P accumulation as a function of C:P of the manure suspensions increased as the ratio widened, a trend similar to that observed with the WEP fraction. The contribution of potentially mineralizable EDTA-PHP to the total bioactive P increase was significant as the size of this enzyme-labile pool increased significantly at times greater than 180 d (Fig. 3). Initially, a net immobilization of P was observed for the first 30 d. Net

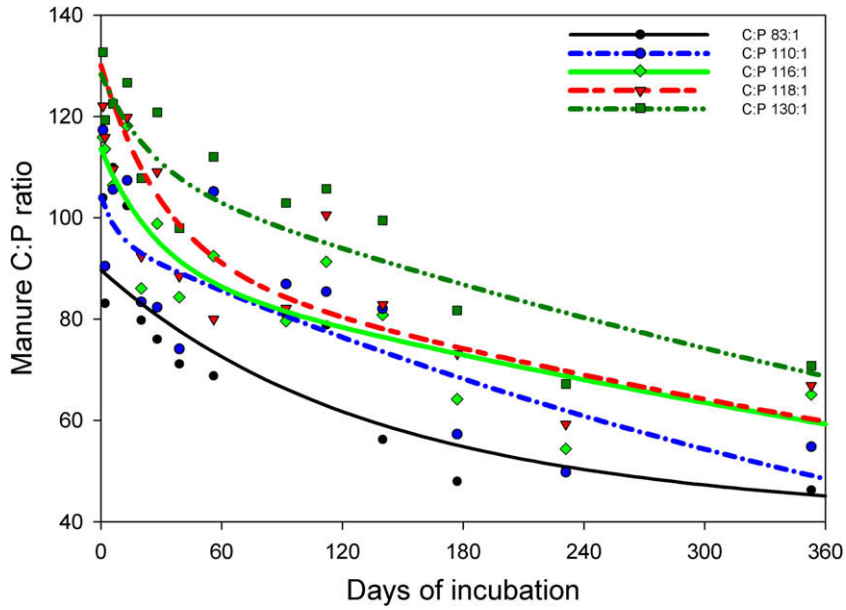

Fig. 1. Changes in carbon-to-phosphorus ratios in selected dairy manure suspensions as the result of mass loss over 353 days of incubation at 22 °C. The manure suspensions had varying initial C:P ratios ranging from 83: to 130:1.

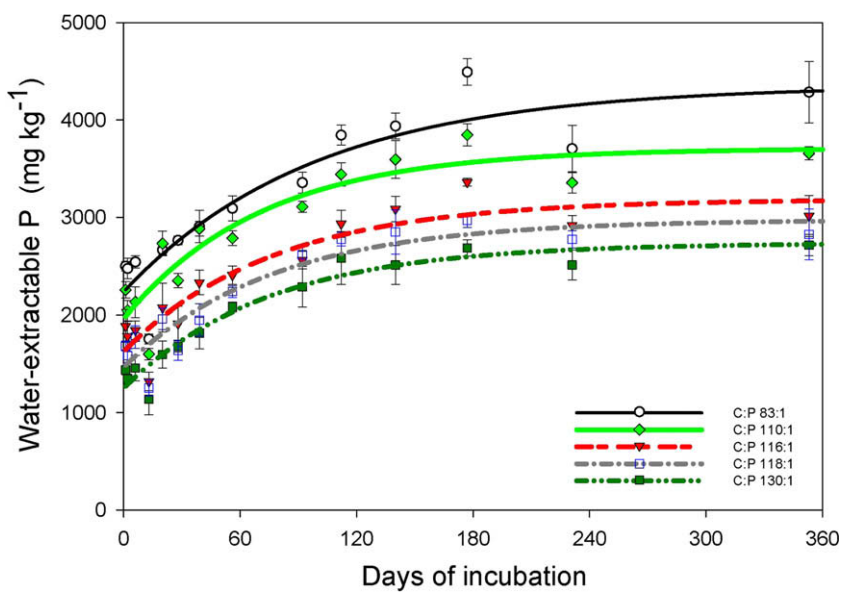

Fig. 2. Temporal changes in concentrations of water-extractable P (WEP) in selected reconstituted dairy manure suspensions as affected by manure carbon-to-phosphorus ratio.

mineralization then appeared subdued for the next 90 d. During that period, it was postulated that active mineralization of organic P attempted to keep up with the initially high rate of C mineralization as WEP was accumulating at an exponential rate, according to first-order reaction kinetic model (Table 4 and Fig. 2). The immobilization of P (i.e., as EDTA-PHP forms) increased significant by $t = 180$ d, as the rate of manure C mineralization began to subside. Although no microbial numbers were obtained during the incubation period, it is postulated that temporal trends in EDTA-PHP mirrored the fluctuations of the manure microbial populations. In addition, unlike the relationship between C and N where a wide C:N ratio is needed to conserve N via N immobilization into microbial biomass, wide C:P induced increased organic P mineralization and at a higher kinetic rate than for treatments with C:P < 105:1.

3.2. Manure nitrogen transformation

Nitrogen existed in the manure suspensions as NH_4–N, with a concentration profile described by a log-normal distribution function (Dao and Cavigelli, 2003; Green et al., 2007). No difference was observed in NH_4–N distribution over time between any of the C:P treatments because initial N content of the various manure suspensions was similar across all suspensions of the various C:P treatments. Fitting a common log–normal distribution function to all C:P treatments yielded a general asymmetrical peak shape of NH_4–N release and re-immobilization with parameters as follows: maximal NH_4–N concentration (C_{max}) or amplitude of the distribution ($a = 22{,}300$ mg kg^{-1}), time of occurrence of C_{max} ($t = 6$ d), and distribution width at half-maximal concentration

Table 2
Effect of carbon-to-phosphorus ratio in dairy manure suspension on the rate of water-extractable P accumulation at 22 °C.

C:P	k^a (mg kg^{-1} d^{-1})	Standard error	r^{2b}	RMSE[b]
83:1	0.0132 c	0.0011	0.832	356
105:1	0.0147 bc	0.0016	0.831	325
110:1	0.0162 b	0.0017	0.820	295
116:1	0.0178 ab	0.0016	0.836	278
118:1	0.0161 b	0.0017	0.821	259
124:1	0.0206 a	0.0015	0.849	227
130:1	0.0154 b	0.0011	0.890	187

[a] Kinetic rate coefficients, followed by same letter are not significantly different at the 0.05 level of probability.
[b] Measures of goodness of fit of the first-order reaction model ($C = a + C_o(1 - \exp[-kt])$: r^2 = coefficient of determination; RMSE = residual mean-squared error.

Table 3
Effect of carbon-to-phosphorus ratio in dairy manure suspension on the rate of total bioactive P accumulation at 22 °C.

C:P	k^a (mg kg^{-1} d^{-1})	Standard error	r^{2b}	RMSE[b]
83:1	0.00315 bc	0.00012	0.939	280
105:1	0.00296 c	0.00016	0.837	449
110:1	0.00324 bc	0.00016	0.934	231
116:1	0.00372 b	0.00016	0.885	286
118:1	0.00354 b	0.00016	0.886	259
124:1	0.00444 a	0.00016	0.848	294
130:1	0.00350 b	0.00016	0.903	223

[a] Kinetic rate coefficients, followed by same letter are not significantly different at the 0.05 level of probability.
[b] Measures of goodness of fit of the first-order reaction model ($C = C_o + b(1 - \exp[-kt])$: r^2 = coefficient of determination; RMSE = residual mean-squared error.

(c_{FWHC} = 63 d). Therefore, there was a rapid release via the deamination of organic N substrates. Ammonium–N was re-immobilized as liquid-phase concentrations returned to 50% of C_{max} by Day 63 and C:N remained at about 20:1 over the remaining period of incubation.

Within each of the five C:P manures, WEC accumulation declined with decreasing N:P (Fig. 4). A loss in manure mass, which resulted in N and P enrichment in the manures, was observed throughout the 353-d incubation period. Gaseous N losses from the manures and incubation chambers yielded smaller enrichment of N and a decline in the manure N:P ratio. Thus, for a certain level of P in the various C:P manures, WEC accumulation or C mineralization was correlated with manure N.

The goodness of fit of the regression between N:P and WEC markedly improved as C:P decreased and narrowed in manures. At C:P of 83:1, the manure was responsive to changes in N:P or directly to N concentration, degrading complex forms of manure C, i.e., an N-limited system. Conversely, manure of C:P of 130:1 was not as responsive to changes in N:P or N concentration, in effecting C mineralization. That is, N did not significantly alter C mineralization and WEC accumulation in a P-limited environment. The intermediary conditions and C mineralization dependence on N:P between the two extreme manure states are also shown on Fig. 4. Therefore, a high catabolic activity to degrade complex C substrates occurred most favorably at wide N:P and narrow C:P ratios. Both P and N must be available for microbes to sustain high rates of mineralization of complex manure C solids. These results are in agreement with nutrient requirements and enzyme induction relationship observed in studies of litter decomposition in wetland and stream ecosystems (Elwood et al., 1981; Qualls and Richardson, 2000; Gusewell and Freeman, 2005) and soil (Olander and Vitousek, 2000; Dao and Cavigelli, 2003). The experimental results suggested that these linkages were also apparent in high-carbon media as dairy manure suspensions.

3.3. Manure carbon mineralization

Concentrations of WEC declined over the entire period of incubation while WEP and EDTA-PHP accumulated in the manure suspensions for all C:P treatments. Concentrations of WEC declined rapidly during the first three weeks of incubation, with rates of disappearance following a double exponential kinetic model (Table 4). Two periods were distinguished (i.e., 1–39 d, and 56–353 d) to quantify and facilitate the statistical comparison of C:P effects on the rapid disappearance of WEC from the manure suspensions and the slower disappearance rate for the remainder of the incubation period. These results suggested a composite of reactions taking place in a mixture of simple and complex structures of C substrates. Comparisons of the reaction rate coefficients (i.e.,

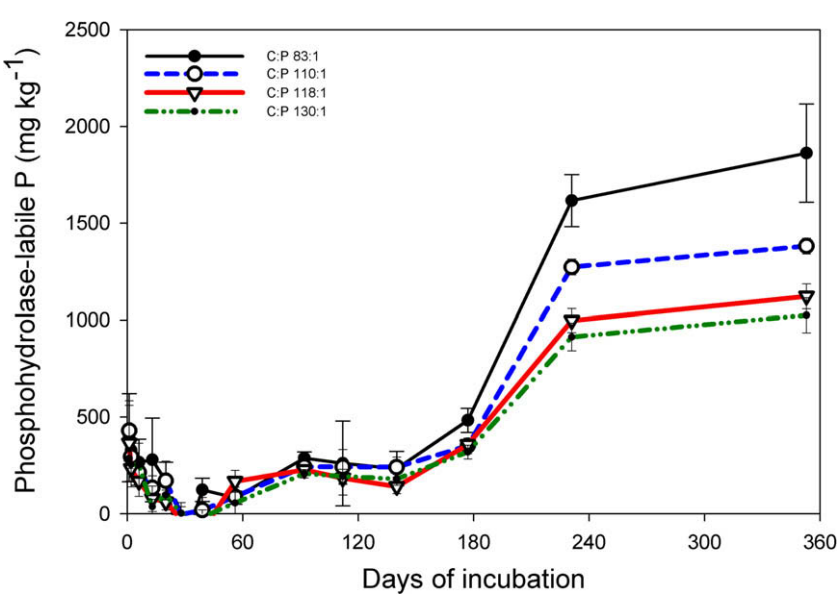

Fig. 3. Effect of carbon-to-phosphorus ratios of selected dairy manure suspensions on the accumulation of EDTA-exchangeable phosphohydrolase-labile P (EDTA-PHP) during incubation for 353 days at 22 °C.

Table 4
Effect of carbon-to-phosphorus ratio in dairy manure suspensions on water-extractable carbon disappearance[a] at 22 °C.

C:P	Double first-order – first-order reaction parameters[a]			Time $t < 39$ d	Time $t > 56$ d
	k_1 (mg kg^{-1} d^{-1})	k_2 (mg kg^{-1} d^{-1})	RMSE[a] (mg kg^{-1})	k_{1-39d} [b] (mg kg^{-1} d^{-1})	$k_{56-353d}$ [b] (mg kg^{-1} d^{-1})
83:1	0.114	0.0005	876	0.0157 a[c]	0.0017 a[c]
105:1	0.128	0.0017	1059	0.0117 b	0.0016 ab
110:1	0.087	0.0007	879	0.0125 ab	0.0006 b
116:1	0.106	0.0023	739	0.0086 bc	0.0010 ab
118:1	0.129	0.0003	648	0.0103 b	0.0003 bc
124:1	0.087	0.0002	542	0.0096 b	0.0002 c
130:1	0.109	0.0088	457	0.0055 c	0.0002 c

[a] Concentration distributions fitted to the double first-order–first-order reaction model ($C = C_1 \times \exp(-k_1 t) + C_2 \times \exp(-k_2 t)$). RMSE = Root mean-squared error.
[b] Concentration distributions fitted to the first-order reaction model ($C = C_o \times \exp(-kt)$).
[c] Kinetic rate coefficients, followed by same letter are not significantly different at the 0.05 level of probability.

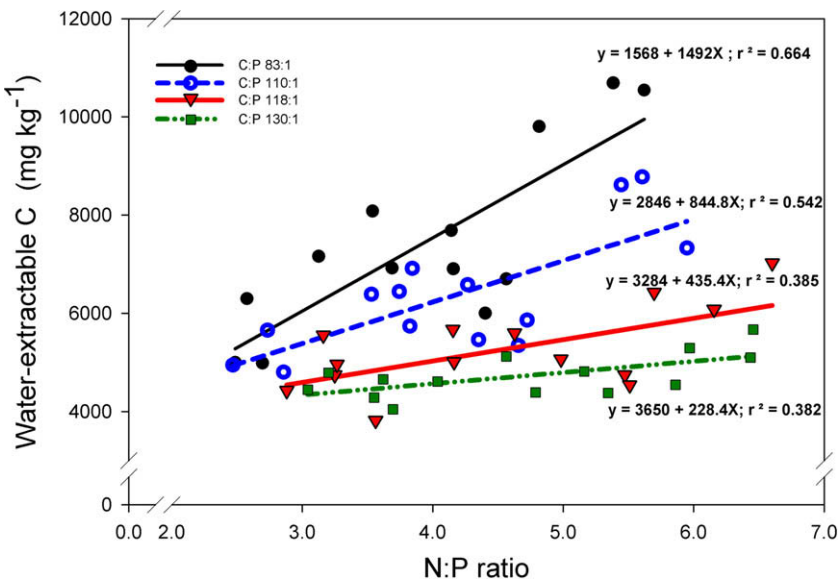

Fig. 4. Effect of nitrogen-to-phosphorus ratios of selected dairy manure suspensions on the accumulation of water-extractable carbon during their incubation for 353 days at 22 °C.

slopes of time vs. ln (C/C_o) plots) indicated that the rate of disappearance or re-immobilization of WEC from the manure suspensions was highest for narrow C:P treatments and lower for wide C:P treatments. These results suggested that the turnover of C involved WEC as an intermediary stage but no clear indication of how this pool contributed to the loss of C and the observed narrowing of all suspensions' C:P was detectable. It was simply noted that the WEC fraction was dynamic and was likely associated with the transformation of fine manure particulates as there were more of this size fraction (<840 um) in narrow C:P treatments.

Many studies have highlighted the stimulatory effects of inorganic P on C mineralization and the decomposition of plant litter (Gressel et al., 1996; Gusewell and Freeman, 2005). In P-limited ecosystems, explanations for enhanced C mineralization with nutrient availability included, either a deficiency in P that limited microbial activity and organic matter decomposition; or decomposers have unique requirements for nutrients, enzyme co-factors, or energy. In this study, inorganic WEP and organic EDTA-PHP were accumulating in the manure suspensions in all C:P treatments throughout the incubation period and thus there was not a negative feedback mechanism to inhibit the mineralization of organic P substrates. A plausible explanation for the absence of a feedback effect might include the following scenario. Phosphohydrolases were induced and released in response to the need to degrade a branched complex organic structure; removing the phosphate moiety gave other catabolic enzymes, oxidases, proteases, etc. access to the C backbone of the molecule, and in the process gaining energy from the cleavage of the C–O–P ester bond. Therefore, the dephosphorylation and release of phosphate still occurred in spite of the presence of readily available dietary inorganic P in the manure suspensions. In other words, the results suggested that organic P mineralization occurred with the mineralization of P-containing C substrates, regardless of whether a need for inorganic P existed, or moreover, that dephosphorylation appeared incidental to microbial needs for precursors of energetic biomolecules. In the manure suspensions, phosphorylated organic C substrates were readily available. In previous studies, dairy manure collected from more than 100 farms across five states of the US Northeast had a large pool of PHP, accounting for more than 36% of manure total P (Lugo-Ospina et al., 2005; Dao et al., 2006).

3.4. Implications for management of dairy manure

These findings have a critical implication for dairy manure management. In confined animal production operations, manure is collected and stored in a central location, undergoing biological transformations before land disposal. Manure handling and storage conditions should be managed to minimize the conversion of insoluble P forms to soluble bioactive ones. The manure contains microbial and fungal populations in sufficient numbers to secrete

phosphohydrolases and oxidative enzymes to degrade organic C and N substrates. Given the biological need to assimilate C substrates for growth and development, wide C:P enhances organic P hydrolysis and N immobilization. The absence of an inhibitory effect of inorganic P results in the continued mineralization and protracted release of inorganic P during storage or on agricultural fields or in an unintended receiving aquatic environment. Soluble phosphate accumulates and is readily available P for plant uptake when land applied. However, should growing plants not be present or ready to assimilate the mineralized phosphate, risks of transport and discharge could be significant (Kleinman et al., 2006; Green et al., 2007; Dao et al., 2008).

The increased mineralization and accumulation of WEP may also be beneficial in scenarios of treatment of animal manure to reduce soluble P. Manure P remediation approaches based on P-immobilizing manure additives would benefit from the increased mineralization of particulate organic P forms (Dao and Daniel, 2002; Zhang et al., 2006). The increased mineralization would also be helpful in the generation and precipitation of insoluble magnesium–ammonium phosphate (i.e., struvite). The process has been proposed as an economical mechanism for phosphate removal in manure suspensions (Greaves et al., 1999; Burns et al., 2001; Celen et al., 2007). Therefore, modifying the manure suspension's composition to widen its C:P would enhance the efficiency of the P removal stage.

4. Conclusions

Manure handling, collection, and storage conditions may have to be managed to minimize gaseous losses, reduce enrichment of nutrients in manures, and moderate the transformation of insoluble P forms to soluble bioactive ones. As a proof of concept, our experimental results suggest that the C load of dairy manure suspensions could be used to control the rate of mineralization of manure C and organic P under non-limiting N conditions. In dairy manure suspensions with a C:P of 83 to 105:1, there was a slower rate of WEP accumulation than in manures with C:P exceeding 105:1. Maintaining a C:P in that range in manure storage structures such as pits or lagoons also conserved manure C. The practice may enhance manure nutrient value to recycling manure P in production agriculture or in emergent P removal and recovery technologies. Furthermore, the results indicate that the methodology we used can describe P transformations in manure suspensions, regardless of manure source. The characterization of P dynamics was solely based on manure C, N, and P composition. Moreover, the process did not rely upon the knowledge of animal management factors such as feed composition or manure handling and storage factors that had been deemed crucial to predicting P speciation and environmental fate.

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