ABSTRACT

Title of Thesis: QUANTIFICATION OF WATER
EXTRACTABLE PHOSPHORUS POOLS IN
SOILS AND MANURES TO PREDICT
PHOSPHORUS LOSS

Lauren Taylor Roswall, Master of Science,
2021

Thesis Directed By: Professor Gurpal Toor, Environmental Science
& Technology Department

Phosphorus (P) is an essential nutrient for sustaining plant, animal, and human health, but can be a pollutant in water bodies. In intensive animal agriculture regions globally, long-term manure application has led to an abundance of P in soils. Exploitation of finite reserves of P is concerning for the future of agriculture and water quality. This research investigates how better understanding the most labile pool of P in soils and manures, i.e., water-extractable P (WEP), can lead to better quantification and risk-assessment of P loss. High P soils were extracted using varying methodology to improve long-term P loss risk assessment. Poultry litter and products were added to a soil to understand how WEP-based application (WEP) affects the loss of dissolved P forms in runoff. We conclude that an accurate quantification of WEP in both soils and manures is needed to improve manure management and mitigate P loss to waterways.

QUANTIFICATION OF WATER EXTRACTABLE PHOSPHORUS POOLS IN SOILS AND MANURES TO PREDICT PHOSPHORUS LOSS

by

Lauren Taylor Roswall

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2021

Advisory Committee:
  Professor Gurpal Toor, Chair
  Professor Robert Hill
  Lecturer Candice Duncan

Dedication

To my loving and supportive parents, without whom I would not have been lucky enough to pursue my passion for the environment.

Acknowledgements

I am amazed at what a team effort it took to accomplish this degree and feel lucky to be surrounded by the ones who accompanied me on this journey. I am forever grateful for the advice, encouragement, and direction from my advisor Gurpal Toor who pushed me to devote two years studying phosphorus. I am lucky to have worked with dedicated lab mates Emileigh Lucas and Yun-Ya Yang who accompanied me while sampling drought-hardened soils, which engulfed two soil corers during the process. And to all the other colleagues and committee members who provided opinions and further insight on my findings, thank you. Of course, I would have not survived the COVID-19 MS degree without the support from UMD friends and loved ones. Thank you for providing a patient listening ear and lightening my mood with your clever jokes, especially when I felt consumed by this thesis. I can only hope to have the same team by my side as I begin the next chapter of life.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Introduction to the Problem

The global depletion of limited phosphorus (P) reserves makes sustainable application and reduction of P loss from working agricultural lands imperative for the future of agriculture and humanity. Before World War II, there was a tight coupling of nutrient cycles in United States agriculture [1] as most agricultural and livestock operations occurred concurrently. Most farms at that time were established to feed families or local communities but rarely went beyond state borders. It was not uncommon to have animals such as horses, cows, and chickens nearby for meat and transportation. As fertilizers were not mass-produced at this time, farmers relied on organic manures generated on their farms to produce crops. This integration of local crop-livestock agriculture resulted in tight nutrient coupling and a closed local P cycle from feed to manure to the soil and back. In other words, P was mined from the soil to grow crops and feed animals and then returned to the same soil as manure.

After World War II, civilization reached an era of mass transportation and fertilizer production, which resulted in the decoupling of crop and livestock operations, a decline in small-scale farming, and a monumental increase in commercial fertilizers use [2]. With cheap fertilizer readily available and chosen over organic amendments like manure to obtain higher yields, farming operations shifted from a necessity for local food production to a profitable enterprise [3, 4]. The United States entered the Green Revolution, where farms began to expand and separate into crop and livestock operations. The increased scale of crop production created two contrasting types of P concern areas: P depleted areas and P saturated areas. Due to the high crop yields and availability of cheap fertilizers in agricultural lands under long-term crop production, such as corn and soybean, some soils are P deficient and need continuous nutrients addition to sustain crop productivity. On the other hand, in the intensive animal production areas, plenty of manure is generated and locally land applied. This long-term application of manure has caused P saturated soils, leaving P vulnerable to loss pathways.

In the United States and globally, the P cycle is broken [1] as P is mined from areas like Florida and North Carolina or overseas like in Morocco. Then P is used in the mid-west region of the US to grow grain crops (corn and soybean) for human and animal use. The grains are shipped to areas where intensive livestock operations exist, such as in the Delmarva Peninsula, to feed animals. The P is returned to the soil, not from where it was mined or used to grow crops, but where the livestock operations exist. Unfortunately, not all this P remains in the soils but is lost to streams, lakes, and oceans. Therefore, to feed the growing Green Revolution, P flowed in a unilateral direction from rocks to farms to waterways [5]. Over the decades, this broken P cycle has resulted in soils both P deprived and P saturated. With Earth’s reserves of P projected to peak by 2030 and deplete in as little as 20 years after that, we must work towards restoring and closing P cycles locally, regionally, and globally [6].

1.2 Justification of Research

Phosphorus is an essential nutrient for human health and plant growth. However, P can be detrimental to the health of aquatic systems as it directly accelerates eutrophication [7]. Eutrophication impacts recreation, water-use, and fisheries as the growth of harmful algal blooms deplete oxygen levels in the water during decomposition and can even enter drinking water supplies leading to livestock death and human illness [8, 9]. In the Chesapeake Bay watershed, P loss from agricultural fields is the leading cause of eutrophication and negatively affects water quality, and thus is the target for many water quality initiatives [10, 11].

In many regions, including Maryland, legislature measures have regulated P applications to farm fields to reduce P loss and promote best management practices (BMPs) on P saturated soils. In 2010, the US Environmental Protection Agency (USEPA) recognized the need for a drastic drawdown of P in high P soils and set goals for water bodies like the Chesapeake Bay. To achieve the USEPA 2025 Total Maximum Daily Loads (TMDLs) for the Chesapeake Bay watershed, P loads need to be reduced by 4 million pounds per year [12]. While some sub-watersheds have successfully responded to the measures and are experiencing a reduction in P losses, other sub-watersheds, such as in the lower Eastern Shore of Maryland, have experienced increased P loads despite the best efforts of regulators and farmers [13]. One reason for the continued P loss is that consecutive fertilization and manure inputs over many years have created what is known as legacy P soils. Legacy P soils are problematic due to P saturation levels, and therefore, accumulation of highly labile P that easily leaves fields in runoff and leaching [14, 15]. Soil P saturation occurs when an excess of P is applied to the soil over many years without much drawdown, rendering all available P-fixing sites occupied. Even though the transfer of P from the labile (soil solution, active pool) to the fixed pool creates recalcitrant P forms, the consecutive P inputs have even enriched all soil P pools. Therefore, any additional P can remain in the labile pools, at least on agronomic time scales. Since legacy P soils do not favorably respond to P drawdown and BMPs, further exploration into the mechanisms controlling P release is needed. Further, it is crucial to accurately quantify the extent of the labile P pool to predict the timescale and severity of P loss.

Current methods for measuring P pools in soils were developed long before the recognition of legacy P soils. Two of the most commonly used methods to determine P levels in soils are (i) Mehlich 3–P (M3–P, the active pool), which measures the amount of available P to crops, and (ii) water-extractable P (WEP) (that includes the soil solution pool), which has been correlated to dissolved reactive P (DRP) in runoff and leaching, the P that is most labile and available to primary producers [14, 16]. As M3–P along with M3– Fe and Al can be used to estimate the P saturation in acidic soils, when combined with WEP, can also be used to determine a field’s level of risk towards P accumulation and P loss. Based on this risk analysis, guidelines can limit and even ban further P inputs from various sources.

In Maryland, a M3–P value of ~500 mg kg^{-1} is used as an environmental threshold, above which no further P can be applied to fields [17]. In 2019, these high P risk fields comprised 6,354 hectares and 11% of the agricultural land in the lower Eastern Shore of Maryland, the state’s most P saturated area [18]. These fields are especially prone to P losses because high values of M3–P indicate a certain level of P saturation in soils resulting in increased susceptibility of P loss to receiving waters. In soils with M3–P values above environmental thresholds, the WEP pool is substantial and highly labile [19]. Reducing P levels in legacy P soils is complex as plants do not uptake large amounts of P, thus, it can take decades to reduce soil P levels with crop removal [20]. While the definition of legacy P soils is broad, for this thesis, legacy P soils are defined as those containing M3–P levels above 500 mg kg^{-1} as these soils are considered to be the highest risk of P loss to water bodies.

In regions such as the Eastern shore of Maryland, where livestock operations exist close to crop production, abundant manure is usually directly applied to fields. Manure disposal is often resource-intensive and financially demanding on farmers. Therefore, when there is a high density of poultry and other livestock operations, the animal waste is usually recycled through direct application to fields as nutrient supplements. This recycling of manure rids livestock farmers of excess manure and allows crop farmers to forgo fertilizer purchases. While manure can be a viable and economically feasible means of nutrients addition, this application can increase the risk of P losses to waterways, especially in soils previously saturated with P [21, 22].

Manure impacts on water quality deterioration are well understood, and decades of literature have been devoted to quantifying the P loss from manure applications, discovering relationships between P loss and manure P levels, application rate and method, and manure type. Currently, many modeling simulations are used to predict P transport from a field-scale or watershed-scale based on EPIC, GLEAMS, ANSWERS, or SWAT models [23-26]. However, research has shown these models often underrepresent P loss as they do not simulate manure application and transfer of P to runoff [27, 28]. While efforts were made to develop simple models to predict P release from manure to rainfall water at field-plot scales, incorporation into larger scales such as field or watershed is needed [29, 30].

Most research on manure application and subsequent P losses stems from the National Phosphorus Project Protocol for simulated rainfall-surface runoff studies [31]. This project was initiated to standardize the way simulated packed soil box and field plot runoff studies were conducted. From this initiative, much has been learned about the incidental loss of P from different types of manure application and rates. Dissolved reactive P (DRP) in runoff was found to be proportional to the WEP content of manure [32]; however, in some studies, this relationship was only the case for manure application rates above 75 kg of total P ha^{-1} [33]. DRP and M3–P in runoff were the same in packed soil box and field-plot studies, rendering packed soil boxes representative of in-field conditions [34]. Most of the WEP in manure and fertilizers is immediately lost upon first rainfall, and all subsequent storm events produce far less P loss [35, 36]. Therefore, incidental P loss constitutes the majority of P loss risk from manure application, which may only occur with 1-2 storm events per year; however, it can encompass a majority of the annual loss [37, 38]. The timing between manure application and the first rain event is also a factor to consider for P loss risk assessment, as more timing allows equilibria between the soil solution, active, and fixed P pools to establish. However, storm hydrology may diminish this effect as even 30 days after application, a significant storm event can mimic the P loss from that of a one-day after application, mainly due to increases in sediment loss [39].

Regulators have considered the impacts consecutive manure applications to farm fields have on eutrophication and the overall health of aquatic ecosystems. Currently, almost all states in the United States have adopted P-indices to help identify critical source areas where P loss risk is high [40]. In these areas, if soil test P

(STP) values still allow for manure application, it is advocated to be done on a P- not an N-basis, as N-based manure application results in the application of 2-3 times the amount of P needed for crop growth [41-43]. In non-critical source areas where manure can be applied unchecked, the soils may still approach P saturation over time, and manure application will no longer be a viable option. Although there are many political, economic, and social aspects tied to manure regulations, many of the agricultural fields could benefit from careful and controlled manure application even if they are currently under environmental threshold P levels, so that water quality will not be threatened and manure application will remain feasible for many years to come.

1.3 Research Approach, Objectives, and Hypotheses

The specific research objectives of this thesis were to gain insights into WEP pools in legacy P soils and different poultry litters and litter products. Chapter 2 investigates if current WEP methods adequately extract the entirety of the WEP pool in legacy P soils and if a different WEP extraction method could better predict long-term P loss risk. Chapter 3 investigates the runoff P losses from a WEP-based approach of poultry litter and poultry litter products application to soils. The hypotheses are that (1) the current WEP method is not extracting the entirety of the pool in legacy P soils due to equilibrium constraints with the low soil to water ratios used, and thus the risk of P loss from these soils is not accurately estimated, and (2) using WEP as a basis for manure application may reduce P loss from agricultural fields to waterways and may be a viable option for continued manure application to fields with optimum P levels.

To accomplish the Chapter 2 objective, a set of soil samples collected from legacy P agricultural fields on the Eastern Shore of Maryland were analyzed and extracted for WEP using different soil to water (w:v) ratios. Analysis of samples included organic and inorganic components of the WEP pools, basic soil properties, total P, M3–P, Al, and Fe. Results were analyzed to address the following research questions: (i) are current methods for extracting WEP viable in legacy P soils? and (ii) which soil to water (w:v) ratio best quantifies the WEP pool in legacy P soils?

To accomplish the Chapter 3 objective, data from previous research using packed soil columns amended with treated and untreated poultry litter and products for rainfall simulations were utilized. Results were analyzed to address the following research questions: (i) do poultry litter and products applied on a WEP-basis result in less accumulation of P in soil and less dissolved P loss in the runoff?, (ii) how does the soil to water (w:v) ratios used during WEP extractions for determining manure application rate affect runoff loss of dissolved P forms?, and (iii) can poultry litter be processed into sustainable products that are desirable for non-agricultural markets?

Chapter 2: Hot-spots of Legacy Phosphorus in Agricultural Landscapes: *Revisiting Water-Extractable Phosphorus Pools in Soils*[1]

2.1 Abstract

Phosphorus (P) losses from intensive agricultural watersheds are an ongoing challenge in protecting and improving water quality. A critical component of mitigating P losses lies in accurately predicting dissolved P loss from soils, which often includes estimating the amount of soluble P extracted with a laboratory-based extraction, i.e., water-extractable P (WEP). A standard extraction method to quantify the WEP pool in soils is critical to accurately quantify and assess the risk of P loss from soils to receiving waters. We hypothesized that narrower soil to water ratios used in current standard methods underestimate the pool of WEP in high or legacy P soils due to the equilibrium constraints that limit further extraction of P from solid to solution phase. Soils from eight legacy P fields (Mehlich 3–P of 502 to 1127 mg kg^{-1}; total P of 692 to 2235 mg kg^{-1}) were used for WEP extractions by varying soil to water ratios from 1:10 to 1:100 (weight:volume) and in eight sequential extractions (equivalent to 1:800) to investigate P release and develop a more exhaustive and robust way for measuring WEP. Extracts were analyzed for total (WEPt) and inorganic (WEPi) pools, and organic (WEPo) pool was calculated. As the ratios

---

[1] This chapter has been published as: *Roswall, T., E. Lucas, Y. Yang, C. Burgis, Isis SPC Scott, & G.S. Toor. 2021. Hotspots of legacy phosphorus in agricultural landscapes: Revisiting water-extractable phosphorus pools in soils. Water,* ***13****, 1006.* *https://doi.org/10.3390/w13081006*

widened, mean WEPi increased from 23.7 mg kg^{-1} (at 1:10) to 58.5 mg kg^{-1} (at 1:100). Further, WEPi became the dominant form, encompassing 92.9% of WEPt at 1:100 in comparison to 79.0% of WEPt at 1:10. Four of the eight selected soils were further extracted using a 1:100 ratio in eight sequential extractions to fully exhaust WEP, which removed a cumulative WEPt of 125 to 549 mg kg^{-1} (276–416% increase) from the first 1:100 extraction. Although WEP concentrations significantly declined after the first extraction, WEP was not exhausted during these sequential extractions, indicating a sizeable pool of soluble P in these legacy P soils. We conclude that (i) legacy P soils are long-term sources of soluble P in agricultural landscapes and (ii) the use of a 1:100 soil to water ratio can improve quantification and risk assessment of WEP loss in legacy P soils

2.1.1 Keywords

Water-extractable phosphorus, extraction ratios, phosphorus saturation, agriculture, legacy soils, water quality

2.2 Introduction

Long-term application of manures and fertilizers in agricultural watersheds has resulted in the accumulation of phosphorus (P) in many soils beyond crop needs. This application has created legacy P fields in agricultural landscapes, which pose a long-term risk of P loss and a threat to water quality.[13, 15, 44, 45] Legacy P soils contribute P to water bodies through leaching and surface runoff. The effect of high P levels in receiving water bodies can be catastrophic, as excessive dissolved P is one of the key drivers of eutrophication.[46-48] The effects on eutrophication is especially concerning in areas with high hydrologic connectivity from land to receiving waters, e.g. areas where seasonally-high water tables become flush with the soil surface, making both leaching and runoff a probable mechanism for P loss. One area with this hydrological connectivity is the lower Eastern Shore of Maryland, which is part of the Chesapeake Bay watershed (165,760 km^2, ~19 million people). Many agricultural soils in this watershed have received historical inputs of manure and fertilizers beyond plant needs, resulting in legacy P soils.[44, 49, 50] We define fields with M3–P >500 mg kg^{-1} as legacy P soils in this paper. Even with the adoption of best management practices (BMPs) and limitations on further P applications, P levels in soil can remain above environmental thresholds for decades.[19, 51] An accurate estimation of P pools (e.g., water-extractable, soil test, recalcitrant P) in legacy P soils is fundamental for assessing the long-term P loss risk to water bodies.

Tools used for risk assessment in fields saturated with P, such as the P site index (PSI) in the United States, are commonly based on soil P tests such as Mehlich 3–P (M3–P). In Maryland, fields with M3–P levels above approximately 150 mg kg^{-1} require the use of the PSI to determine the P application rate. [19, 52] In high P loss risk fields (M3–P: >500 mg kg^{-1}), P applications are banned. Currently, Maryland has about 92,673 ha of agricultural land with M3–P >150 mg kg^{-1}, including 6,354 ha or 11% of total agricultural land in the Lower Eastern Shore with M3–P >500 mg kg^{-1}.[18] Despite M3–P is used as an indicator of soil P content for management purposes, attempts to characterize the relationship between M3–P and P loss have produced ambiguous results. For instance, Sharpley (1995) found a significant relationship between M3–P and dissolved P in surface flows but argued that this relationship is soil-specific and related to the soil P sorption saturation rather than traditional soil test P measures. [53] Other researchers suggested that environmental soil tests, e.g., WEP, were better at predicting P loss in comparison to M3–P in surface and subsurface flows. [14, 16, 54]

Water-extractable P (WEP) in soils represents the pool of immediately available P to both plants and loss pathways. However, inconsistencies in WEP extraction methods and analyses are problematic for comparisons across the studies. Different soil to water ratios, extraction time, and analyses methods have been used in the scientific literature. For example, the most common method for extracting WEP in soils include using a 1:10 or 1:20 soil to water ratio.[55] Other variants of the method include the Hedley fractionation scheme, where the first extractant used is water, typically at a 1:60 soil to water ratio.[56-58] A standardized protocol would facilitate comparisons among WEP measurements, which are required to set environmental thresholds at regional and national scales. Furthermore, protocols for WEP extraction were established before recognition of legacy P soils, and these soils may not have the capacity to adequately exhaust the WEP pool due to the equilibrium constraints at narrow soil to water ratios. These constraints were important for estimating readily available P in manures and biosolids, which resulted in developing a method to extract WEP using a 1:100 soil to water ratio.[59-61] The same attention must be given to legacy P soils to accurately assess the risk and develop BMPs to mitigate P loss from soils.

Conventional methods must effectively extract desired P pools or be modified if not. We hypothesized that the conventional WEP procedures aimed to extract P between 1:10 and 1:60 soil to water ratios do not adequately exhaust the WEP pool in soils and therefore may underestimate the P loss risk in legacy P soils. This inadequacy stems from legacy P soils having a large reservoir of soluble P, which on extraction at narrower ratios reach an equilibrium between soil solution and the solid phase. This equilibrium prevents any further release of P from the solid to solution pool, resulting in underestimating the WEP pool. In fields saturated with P, the equilibrium is controlled by the soil solution P pool, which after runoff and leaching losses is replenished from the soil solids pool. Whereas in low P soils, narrow extraction ratios work well due to the lower amount of soluble P, which is sufficiently extracted. In fact, the use of higher extraction ratios in low P soils can result in concentrations in soil solution below method detection limits.

This paper investigates the relationship of soil to water ratios and the WEP pool across a wide range of extraction ratios to determine whether standard procedures are acceptable for legacy P soils. We then propose a modification to standard protocols to extract most of the WEP from legacy P soils. Specifically, the research objectives were to (i) investigate the impact of varying the soil to solution extraction ratios on the total, inorganic, and organic WEP pools, and (ii) examine the use of larger extraction ratios through sequential extractions to completely exhaust the WEP pool in the legacy P soils.

2.3 Materials and Methods

2.3.1 Site Characteristics

In consultation with the University of Maryland Extension Agents, we identified eight agricultural fields with soil test P (M3–P) values above 500 mg kg–1 for field sampling. These fields are located in the Eastern Shore of Maryland in Caroline (1 field), Dorchester (5 fields), and Somerset (2 fields) counties. A detailed description of field sites and soil properties is included in Table 1. In brief, the soil texture across the eight fields ranged from loamy sand to silt loam, pH from 6.14 to 7.25, organic matter from 0.6 to 2.9%, sand from 51.3 to 86.1%, silt from 11.4 to 40.6%, and clay from 2.5 to 10.4%. Total P in eight fields varied from 692 to 2235 mg kg–1, and Mehlich 3–P ranged from 502 to 1127 mg kg–1 (Table 1). All fields are under long-term crop production, with four fields under commodity crops, i.e., corn-soybean rotation (sites 3, 4, 5, 6) and four fields under vegetable production (sites 1, 2, 7, 8). Although a detailed history of manure and fertilizer application for field sites was not available because of changes in land ownership over the years; these fields have received a combination of poultry manure and fertilizer P. As such, these fields are currently under the State of Maryland regulations that restrict any P applications to fields exceeding the environmental soil P (M3–P) threshold value of 500 mg kg–1.

2.3.2 Soil Collection

For soil sample collection, a uniform transact (middle of the fields) consisting of an area of 4 m x 7 m was identified in each field, from where ten samples were randomly collected with a soil corer at 0–5 cm depth, and then a composite soil sample was taken from each field in October 2019. This depth was chosen because the surface layer is most prone to surface runoff P loss in legacy P soils.[62, 63] Samples were air-dried at 35°C, ground to pass 2-mm sieve, and stored in sealed containers at room temperature until analyses.

2.3.3 Sample Extractions

The WEP extractions were conducted in duplicates with varying soil to water (deionized water, DI) ratios. The current methods of WEP extraction include using DI water to ensure similar water composition across various studies. For the first experiment, 2.50, 1.25, 0.50, and 0.25 g of each soil was weighed and placed in 50 mL centrifuge tubes, and then 25 mL of DI water was added to create soil to water extraction ratios of 1:10, 1:20, 1:50, and 1:100 (w:v). We varied the amount of soil instead of water to ensure consistent headspace in centrifuge tubes during the extraction procedure. For the second experiment, soil samples from four sites (2, 4, 6, and 7) were randomly selected and extracted in eight sequential extractions using soil to water ratios of 1:100 (0.25 g soil: 25 mL DI). Each sequential extraction consisted of filtering the supernatant and adding 25 mL of DI water to the same 0.25 g of soil. A total of eight sequential extractions were repeated to obtain a cumulative soil to water ratio of 1:800. After each addition of DI water, all centrifuge tubes were shaken on an end-over-end shaker for 1 h, centrifuged at 5000 rpm for 5 min, and filtered through a 0.45 µm glass filter paper (Pall Corporation, Ann Arbor, MI, USA) before analysis.

2.3.4 Sample Analysis

The filtered aliquots from both experiments were analyzed for inorganic WEP (WEPi) on a Lachat QuikChem 8500 Automated Ion Analyzer (Hach, Loveland, CO) using the phosphomolybdate blue method of Murphy and Riley.[64] Note that although phosphomolybdate method can hydrolyze a small fraction of labile organic P, this method is typically used to determine inorganic P in water samples. Total WEP (WEPt) in the filtered aliquots was analyzed on an ICAP 7600 Duo View Inductively Coupled Plasma–Optical Emission Spectrometer (ICP–OES) (Thermo Fisher Scientific, Waltham, MA), which converts organic P to inorganic P. Thus, the difference between WEPt and WEPi was determined as organic WEP (WEPo).

Soil particle analysis to determine the percentage of sand, silt, and clay was conducted using a modified pipette method.[65] Mehlich 3–P in soils was determined using the method reported by Wolf and Beegle [66], followed by analysis on ICP–OES. Total P (TP) in soils was determined by the EPA 3050B method.[67]

2.3.5 Statistical Analysis

Statistical analysis was performed using the software R (version 4.0.3). Analysis of variance (ANOVA) was used to assess the effect of extraction ratios on the WEP in the first experiment. The experimental design was a randomized complete block design with replicates. Soil was considered a blocking factor (i = 8 soils), and extraction ratios (j = 4; 1:10, 1:20, 1:50, 1:100) the treatment of interest. The duplicated combinations of soil and extraction ratios constituted a sample size (n) of 64. The interaction term between block and treatment was examined as well as the assumptions of normality, constant variance and independence of the residuals. Whenever violations of the ANOVA assumptions were identified, a Box-Cox transformation was performed, and a new ANOVA was conducted using the transformed data. Once a significant effect of extraction ratios was identified, Tukey's range test, a multiple comparison test ($p = 0.05$), was conducted. For the second experiment, one-way ANOVA was used to analyze the effect of the eight sequential extractions on WEP ($i = 8$ factor levels; $n = 8$ samples per factor level). Multiple comparisons (Tukey's range test; $p = 0.05$) were also conducted as a post-hoc analysis.

2.4 Results and Discussion

2.4.1 Water-Extractable Phosphorus Across Extraction Ratios

The amount of WEPi extracted per unit of soil depended on the soil to water ratio, where increasing the ratio significantly ($p < 0.05$) extracted more WEPi in legacy P soils, averaging from 23.7 mg kg^{-1} (at 1:10) to 58.5 mg kg^{-1} (at 1:100) (Figure 1). As the ratio widened from 1:10 to 1:100, WEPi became more dominant, from 78.6 to 92.9% of WEPt. The overall increase in WEPi is attributed to a large WEP pool in the legacy P soils. We suggest that an equilibrium between the solid phase and soil solution P was reached at narrower soil to solution ratios (1:10 to 1:20), which prevented a further release of WEPi from the solid phase. However, as the ratios widened, a shift in the P equilibrium from the solid phase to soil solution resulted in greater extraction of WEPi. These results imply that soils saturated with legacy inputs of P have a large reservoir of WEP and greater potential for P loss than would be predicted with a standard 1:10 extraction.

Although few published research exist for comparison, Furhman et al.[68] observed an increase of 12.2 mg kg^{-1} in WEPi from 1:10 to 1:50, compared to the increase of 21.8 mg kg^{-1} from 1:10 to 1:50 in our study. Although it is established that soil P release increases as soil to solution ratios widen [69], the increases in WEPi are not always significant at higher ratios (>1:50), as there is not always more WEP available to be extracted. Therefore, these legacy soils warrant the use of larger ratios, while WEP in low P soils may be adequately quantified with standard methods. The variability in absolute and relative amounts of WEP at different ratios limits simplified conversions used in the literature to convert WEP between extraction ratios. For example, Sharpley et al.[58] converted WEP values from 1:10 to 1:25 by using a factor of 2.5, which our data suggests would lead to overestimation of the WEP concentrations.

Mean WEPo showed an initial non-significant increase from 6.3 mg kg^{-1} (at 1:10) to 7.5 mg kg^{-1} (at 1:20), followed by a non-significant decrease to 5.7 mg kg^{-1} (at 1:50) and 4.6 mg kg^{-1} (at 1:100) (Figure 1). Of WEPt, WEPo was 21.4% at 1:10, 17.8% at 1:20, 10.8% at 1:50, and decreased to 7.1% at 1:100 ratio. The ratio at which WEPo pool declined was different for individual sites. For example, sites 1 and 8 with the lowest soil TP (691–749 mg kg^{-1}) showed WEPo decline at 1:20, five sites (3, 4, 5, 6, 7) in the middle range of TP (832–1461 mg kg^{-1}) showed WEPo decline at 1:50, and site 2 with the highest TP (2235 mg kg^{-1}) showed no WEPo decline until 1:100 (Table 2).

We hypothesize that the decrease in WEPo at higher extraction ratios could be due to preferential sorption of WEPo on the newly vacated sites on the soil surface where WEPi was initially present.[70] Since the decline in WEPo was observed at different extraction ratios related to the TP of soils, we argue that the decreasing amount of WEPo is largely attributed to the preferential sorption of WEPo. For example, in soils with the lower TP, an equilibrium between the soil solution and solid phase WEPi was reached at lower extraction ratios. This equilibrium resulted in vacant P fixing sites where WEPi was once weakly sorbed and allowed a part of the WEPo present in the soil solution to sorb back onto the solid phase. Further, overtime significant WEPo mineralization into WEPi may occur on behalf of microbial life. Therefore, analysis methods such as colorimetry, commonly coupled with standard extraction methods disregard the WEPo pool and are more problematic at lower soil to water ratios, as both pools could impact the assessment risk of dissolved P in runoff.

The overall significant increase in mean WEPt as the soil to water ratio widened from 1:10 (30.04 mg kg^{-1}) to 1:100 (63.16 mg kg^{-1}) suggests that these legacy soils contains a large amount of WEP and thus raises concern for using low extraction ratios as this could lead to underestimating the risk of WEP in legacy P soils, especially those with high hydrological connectivity. The difference in mean WEPt values between the 1:50 and 1:100 soil to water ratios was statistically significant (Figure 1). WEPt in individual sites increased between 5 and 44% at the 1:100 ratio, warranting the adoption of the 1:100 ratio over the 1:50 ratio. It is possible that even more WEPt could be extracted using wider ratios beyond 1:100. While these ratios were not included in the first experiment due to equipment limitations, the second experiment was performed to address this possibility.

2.4.2 Water-Extractable Phosphorus as a Proportion of Mehilich-3 Phosphorus and Total Phosphorus

Mehlich 3–P in eight soils ranged from 502 to 1127 mg kg^{-1} (Table 1). The proportion of WEP as M3–P was examined at each of the four extraction ratios (Figure 2A, Table 3). As the extraction ratios widened, the mean %WEPi of M3–P increased from 3.3 to 8.2%, whereas the mean %WEPo of M3–P remained constant around 1% (range: 0.63–1.03%). The overall trend in %WEPt of M3–P showed a consistent significant increase of 1.6% by doubling the solution per unit of soil (Figure 2A). This steady increase further exemplifies the constraints on soluble P release at low soil to solution ratios. A study of WEP as a percentage of M3–P at the 1:10 ratio reported a 16% extraction in untreated soils and a 3% extraction in heavy manure-treated soils.[71] These results suggest that the legacy P soils analyzed in the present study possess WEP pools more characteristic of manure amended than untreated soils. Further, the low percentage of M3–P extracted as WEP may complicate the predictiveness and use of M3–P to determine the risk of P loss from legacy soils. We suggest that the extractions at wider ratios and a fractionation scheme to separate P pools of various lability would provide more insights into the inter-connectedness of P pools in legacy P soils.

Interestingly, the high variability in extracted WEP values (mg kg^{-1}) among sites dissipated when taken as a percent of total P (Figure 2B). For example, even though WEPt at the 1:10 extraction ratio ranged from 18.4 to 54.0 mg kg^{-1}, %WEPt of TP only varied from 1.8 to 2.9% (Table 4). Mean %WEPi of TP also exhibited low variability throughout the extraction ratios and steadily increased from 1.9 to 4.6%, while %WEPo of TP ranged from 0.35 to 0.57%. These results are significant for three reasons: (i) the consistent WEP pools as a percent of TP across eight sites at each extraction ratio, given the wide range in WEP (mg kg$^{-1)}$, indicates an equilibrium induced barrier to further exhausting the WEP pool at low ratios; (ii) the low values of %WEPt of TP (<6%) extracted by these ratios raise concerns about using WEP in legacy P soils to estimate the long-term P loss potential; and (iii) if an equilibrium is reached before exhausting the WEP pool at the conventionally narrower extraction ratios, as our results alluded, this limitation on further P release from the WEP pool will not allow an adequate comparison with other low P or non-legacy P soils.

2.4.3 Sequential Water Extractions to Exhaust Water-Extractable Phosphorus

The increase in WEP as the extraction ratios widened prompted an investigation to determine if the WEP pool can be fully exhausted and quantified using larger extraction ratios. As such, four sites (2, 4, 6, 7) with TP of 832–2235 mg kg^{-1} and M3–P of 602–1127 mg kg^{-1} (Table 1) were randomly selected for further investigation, beginning with a 1:100 ratio and conducting eight sequential extractions to achieve a final 1:800 soil to water ratio.

Concentrations of WEP in the four sites during the eight sequential extractions were highest in the first extraction and began declining before stabilizing (Figure 3). As detectable WEP was present after each of the eight sequential extractions, these extractions did not fully exhaust the WEP pool. In the later extractions (2nd onwards), WEP reached a non-zero equilibrium, i.e., non-significant decrease. A Tukey test for significant differences was conducted to determine at which extraction ratio this equilibrium was reached. For sites 2 and 4 with TP of 2235 and 1401 mg kg^{-1}, an average WEP of 0.56±0.09 and 0.23±0.06 mg L^{-1} was continuously extracted after the second (equivalent to 1:200 for site 2) and first (equivalent to 1:100 for site 4) ratios. Site 6 has a similar TP (to site 4) of 1405 mg kg^{-1} but reached a lower equilibrium value of 0.16±0.02 mg L^{-1} at the third extraction (equivalent to 1:300 ratio). Site 7, which contained the least TP of 832 mg kg^{-1}, extracted an average of 0.09±0.02 mg L^{-1} at the fourth extraction (equivalent to 1:400 ratio). In all sites, WEP extracted in the first extraction was significantly higher than subsequent extractions (2nd to 8th). These results build off the previous experiment to show that a 1:100 ratio is a sufficiently wide WEP extraction ratio to extract most of the WEP in legacy P soils since widening the ratio even further would result in non-significant WEP increases.

The cumulative WEPt (at 1:800) ranged from 125 to 549 mg kg^{-1} across the four sites, with an increase of 276 to 416% from the first 1:100 extraction (Figure 4). WEPo decreased after the first 1:100 extraction for most sites, and a few later extractions had negative values of WEPo (Tables 5 and 6); thus, the cumulative WEP$_i$ at the 1:800 ratio was 93 to >100% of WEPt. The decline and disappearance of WEPo during the later sequential extractions supports our previous assertion of either sorption to vacated P sites or mineralization of organic P. A study on manure treated non-calcareous soils in the Netherlands found that organic P in the form of orthophosphate monoesters was preferentially bound to Al and Fe oxides over orthophosphate in the 0-5 cm layer.[72] Therefore, if sorption is the underlying mechanism, undetectable WEPo throughout the 1:800 ratio would indicate low lability of the resorbed organic P. A study in Texas using a modified Hedley fractionation scheme found a decrease in labile organic P extracted with water and sodium bicarbonate and a 217% increase in HCl extractable organic P forms in legacy P soils compared with unamended soils.[73] Although conducted on calcareous soils and may only apply to our site 2, this suggests that repeated additions of manure and other organic amendments which resulted in legacy P reserves in soil increase the more recalcitrant organic P pools, causing a shift in equilibrium between pools that leads to more inorganic labile P. Regardless of the mechanism, our results suggest that water extractions could not accurately quantify WEPo in legacy P soils, further mystifying its extent and fate in P loss.

The equilibrium WEPt concentrations in the last (8th) sequential extraction were 0.49, 0.18, 0.14, and 0.08 mg L^{-1} for sites 2, 4, 6, and 7, respectively (Figure 3, Table 5). These values represent the soil solution pool that is still present after an equilibrium shift has occurred despite significant WEP depletion during the previous seven sequential extractions. These WEP concentrations (0.08 to 0.49 mg L^{-1}) were 2- to 15-times higher than the US EPA recommended limit of 0.03125 mg L^{-1} total P for Eastern Coastal Plains (Ecoregion XIV) streams and rivers.[74] These results suggest that these fields are sources of P in the landscape even after significant depletion and contain appreciable amounts of WEP that will continue to be released and lost to waterways. As an example, considering average soil bulk densities between 1.3 and 1.5 g kg^{-1} (taken from the USGS web soil survey) in these soils and an average rainfall of ~1100 mm in our study region, the upper surface soil layer could experience yearly soil to solution ratio equivalents to 1:20. Therefore, after 40 years of curbed P input in these legacy P soils, soils could be depleted to the degree the sequential extractions achieved (1:800 ratio) and still be detrimental to losing dissolved P to waterways. Thus, in legacy P fields, approaches to transform the chemical nature of labile P in soils to recalcitrant forms using amendments containing Fe and Al can be effective to reduce WEP in soils, reduce P losses, and meet load reductions in the water bodies.

2.4.4 Impact of Phosphorus Saturation on the Magnitude of Water-Extractable Phosphorus

Knowledge of the P saturation in soils can be used to understand the magnitude of the WEP pool and the risk of P loss in legacy soils. The threshold P saturation ratio (PSR) based on the M3 extract (i.e., M3–PSR), has been used to evaluate P risk in many studies across the eastern United States.[75-77] The relationship of M3–PSR with WEP exhibits a changepoint or a value above which WEP begins to desorb into the solution at an expedited rate. These changepoints are thought to be the cusp of environmentally stable levels of PSR and WEP in soils, and thus these relationships are usually evaluated when making management decisions. Common M3–PSR values reported in literature range between 0.0 and 0.5, with corresponding WEP from ~0 to 25 mg kg^{-1} at 1:10 soil to water extraction ratio.[77] Their associated M3–PSR changepoints range between 0.10 to 0.15. Our legacy P soils had M3–PSR beyond these reported values and ranged between 0.42 and 0.81 (excluding site 2 that had 7.25 pH and the highest amount of M3–Ca of 2662 mg kg^{-1}). Corresponding mean WEPt values were 26.6±6.36 mg kg^{-1} (range: 18.4–34.4 mg kg^{-1}) at 1:10, which increased to 35.7±6.63 mg kg^{-1} (range: 24.3–43.6 mg kg^{-1}) at 1:20, 45.3±9.23 mg kg^{-1} (range: 33.7–56.6 mg kg^{-1}) at 1:50, and 53.3±12.60 mg kg^{-1} (range: 36.5–68.0 mg kg^{-1}) at 1:100 soil to water extraction ratios (Figure 1, Table 2). In other words, WEPt values could be upwards of two times as much (26.6 to 53.3 mg kg^{-1}) as reported in previous studies, depending on the extraction ratio used. Further, our legacy soils M3-PSR were between 4 and 8 times greater than the established environmental changepoints. These results suggest a need to reevaluate the relationship between M3-PSR and WEP (at various extraction ratios) for legacy P soils.

2.5 Conclusions

We investigated the extent of WEP pools in legacy P soils (M3–P >500 mg kg^{-1}) by employing various soil to water ratios. Our analysis was focused on the WEP pool as it is the soluble P pool that is susceptible to loss in soils saturated with historical applications of manures and fertilizers. Due to the large percentages of labile P in soils (i.e., WEP), our results suggest using a wider extraction ratio of 1:100 to adequately quantify most of the WEP pool in soils with M3–P above 500 mg kg^{-1}. This ratio is already recommended to determine WEP in high P sources such as manure and biosolids, and therefore would not be an excessive burden to research and state soil testing laboratories. Furthermore, using a ratio of 1:100 would eliminate the significant discrepancies between using colorimetry (WEPi measurement) and spectroscopy (WEPt measurement) to determine WEP. As the sequential extractions equivalent to 1:800 soil water ratios could not fully exhaust the WEP pool, a modified Hedley fractionation scheme could be used to investigate other less labile and recalcitrant P pools in legacy P soils. A fractionation coupled with advanced synchrotron techniques will help understand the composition of solid phase P minerals that control P equilibria in legacy P soils. By altering the extraction method in legacy P soils, we will be equipped to better assess the risk of P loss and implement effective strategies to reduce P loss to waterways. Future research is suggested in runoff and leachate simulations and field monitoring, aiming to relate the WEP estimated through the proposed methodology and observed P loss through surface and subsurface transport pathways.

Table 1. Physical and chemical characteristics of eight field sites.

	Site 1[1]	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**
pH	6.55	7.25	6.56	6.36	6.14	6.22	6.35	6.38
Organic Matter (%)	0.56	1.87	1.87	2.11	2.89	2.08	1.00	0.99
CEC (meq/100g)	6.40	15.10	11.70	12.50	13.20	10.20	5.90	8.30
% Sand	77.7	53.9	67.7	51.3	56.9	53.6	85.0	86.1
% Silt	17.5	40.6	23.7	39.2	32.7	38.6	12.1	11.4
% Clay	4.8	5.5	8.6	9.5	10.4	7.8	3.0	2.5
Texture	Loamy sand	Sandy loam	Sandy loam	Loam	Sandy loam	Sandy loam	Loamy sand	Loamy sand
Acidity (meq/100g)	2.0	0.0	2.8	2.8	3.9	2.8	2.0	2.0
Total P (mg/kg)	691.9	2235.2	1460.7	1401.1	1446.4	1405.4	832.4	749.4
M3-P (mg/kg)	534.0	1127.0	806.0	692.0	502.0	785.0	602.0	620.0
M3-Fe (mg/kg)	281.6	270.5	357.8	316.7	275.6	183.0	185.4	164.1
M3-Al (mg/kg)	789.1	586.40	784.40	586.67	905.02	1005.99	767.32	615.07
M3-PSR	0.50	1.37	0.73	0.81	0.42	0.62	0.61	0.78
M3-P:Total P	0.77	0.50	0.55	0.49	0.35	0.56	0.72	0.83
M3-Ca (mg/kg)	721.4	2662.4	1375.1	1605.3	1495.1	1203.7	591.7	995.3
M3-Mg (mg/kg)	58.0	167.0	180.0	162.0	158.0	104.0	89.0	111.0

[1] Sites 1, 2, 7, and 8 are under long-term vegetable production. Sites 3, 4, 5, and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Table 2. Inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P in eight field sites.

	Extraction ratios	Site 1[1]	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
		mg kg^{-1}							
WEPt	1:10	18.4	54.0	34.4	34.7	25.4	29.1	23.0	21.4
	1:20	24.3	70.4	43.6	40.8	38.2	38.9	32.6	31.3
	1:50	33.7	91.7	56.6	52.1	51.1	50.0	35.3	38.4
	1:100	41.7	132.2	68.0	63.0	66.1	52.7	45.3	36.5
WEPi	1:10	14.8	44.7	26.8	27.2	19.8	22.1	17.6	16.8
	1:20	22.9	56.5	35.5	33.4	29.9	30.1	24.2	28.2
	1:50	28.4	76.5	52.1	47.9	47.2	44.2	31.9	34.9
	1:100	38.5	121.1	62.9	57.8	61.5	49.2	43.1	34.2
WEPo	1:10	3.5	9.2	7.6	7.5	5.6	7.0	5.3	4.6
	1:20	1.4	13.9	8.2	7.4	8.3	8.8	8.5	3.1
	1:50	5.3	15.2	4.4	4.3	3.9	5.8	3.4	3.5
	1:100	3.2	11.0	5.0	5.2	4.5	3.5	2.2	2.4

[1] Sites 1, 2, 7, and 8 are under long-term vegetable production. Sites 3, 4, 5, and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Table 3. Inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P as percent of Mehlich 3–P in eight field sites.

	Extraction ratio	Site 1[1]	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
			———	———	———	% of Mehlich 3–P	———	———	
WEPt:M3-P	1:10	3.4	4.8	4.3	5.0	5.1	3.7	3.8	3.4
	1:20	4.6	6.2	5.4	5.9	7.6	5.0	5.4	5.0
	1:50	6.3	8.1	7.0	7.5	10.2	6.4	5.9	6.2
	1:100	7.8	11.7	8.4	9.1	13.2	6.7	7.5	5.9
WEPi:M3-P	1:10	2.8	4.0	3.3	3.9	4.0	2.8	2.9	2.7
	1:20	4.3	5.0	4.4	4.8	6.0	3.8	4.0	4.6
	1:50	5.3	6.8	6.5	6.9	9.4	5.6	5.3	5.6
	1:100	7.2	10.7	7.8	8.3	12.3	6.3	7.2	5.5
WEPo:M3-P	1:10	0.7	0.8	0.9	1.1	1.1	0.9	0.9	0.7
	1:20	0.3	1.2	1.0	1.1	1.6	1.1	1.4	0.5
	1:50	1.0	1.3	0.6	0.6	0.8	0.7	0.6	0.6
	1:100	0.6	1.0	0.6	0.8	0.9	0.4	0.4	0.4

[1] Sites 1, 2, 7, and 8 are under long-term vegetable production. Sites 3, 4, 5, and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Table 4. Inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P as percent of total P in eight field sites.

	Extraction ratio	Site 1[1]	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
			% of total P						
WEPt:TP	1:10	2.7	2.4	2.4	2.5	1.8	2.1	2.8	2.9
	1:20	3.5	3.2	3.0	2.9	2.6	2.8	3.9	4.2
	1:50	4.9	4.1	3.9	3.7	3.5	3.6	4.2	5.1
	1:100	6.0	5.9	4.7	4.5	4.6	3.7	5.4	4.9
WEPi:TP	1:10	2.1	2.0	1.8	1.9	1.4	1.6	2.1	2.2
	1:20	3.3	2.5	2.4	2.4	2.1	2.1	2.9	3.8
	1:50	4.1	3.4	3.6	3.4	3.3	3.1	3.8	4.7
	1:100	5.6	5.4	4.3	4.1	4.3	3.5	5.2	4.6
WEPo:TP	1:10	0.5	0.4	0.5	0.5	0.4	0.5	0.6	0.6
	1:20	0.2	0.6	0.6	0.5	0.6	0.6	1.0	0.4
	1:50	0.8	0.7	0.3	0.3	0.3	0.4	0.4	0.5
	1:100	0.5	0.5	0.3	0.4	0.3	0.2	0.3	0.3

[1] Sites 1, 2, 7, and 8 are under long-term vegetable production. Sites 3, 4, 5, and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Table 5. Concentrations of inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P in eight sequential extracts at 1:100 soil to water ratio in four field sites.

Sequential extraction number	Site 2[1]			Site 4			Site 6			Site 7		
	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo
	mg L^{-1}											
1	1.34	1.23	0.11	0.65	0.59	0.05	0.53	0.50	0.03	0.46	0.44	0.02
2	0.85	0.79	0.06	0.32	0.29	0.03	0.25	0.24	0.01	0.22	0.21	0.01
3	0.68	0.63	0.05	0.26	0.25	0.01	0.18	0.18	0.00	0.15	0.15	0.01
4	0.59	0.56	0.03	0.21	0.21	0.00	0.18	0.18	0.01	0.12	0.11	0.01
5	0.52	0.47	0.05	0.22	0.21	0.01	0.17	0.17	0.00	0.10	0.09	0.00
6	0.55	0.53	0.02	0.23	0.23	0.00	0.13	0.14	0.00	0.07	0.07	-0.01
7	0.54	0.51	0.03	0.20	0.19	0.01	0.15	0.14	0.00	0.07	0.07	0.00
8	0.49	0.46	0.03	0.18	0.18	0.00	0.14	0.14	0.00	0.08	0.08	0.00

[1] Sites 2 and 7 are under long-term vegetable production. Sites 4 and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Table 6. Amounts of inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P in eight sequential extracts at 1:100 soil to water ratio in four field sites.

Sequential extraction number	Site 2[1]			Site 4			Site 6			Site 7		
	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo	WEPt	WEPi	WEPo
	mg kg^{-1}											
1	132.2	121.1	11.0	63.0	57.8	5.2	52.7	49.2	3.5	45.3	43.1	2.2
2	84.0	77.9	6.2	32.0	29.3	2.6	24.7	23.9	0.8	21.5	20.9	0.7
3	67.5	62.7	4.8	25.5	24.7	0.8	17.8	17.4	0.3	15.2	14.4	0.7
4	58.3	54.9	3.4	20.9	20.4	0.5	18.0	17.5	0.5	12.1	11.2	0.9
5	51.0	46.1	4.9	21.4	20.3	1.2	17.1	16.7	0.4	9.5	9.4	0.2
6	54.7	52.3	2.4	22.8	22.9	-0.1	13.3	13.5	-0.2	6.6	7.2	-0.6
7	53.2	50.1	3.1	19.9	18.9	1.0	14.5	14.1	0.3	7.0	7.0	0.0
8	48.4	45.0	3.4	17.6	17.8	-0.2	13.8	14.2	-0.4	7.6	7.7	0.0
Cumulative (1-8)	549.3	510.1	39.3	223.1	212.1	11.0	171.8	166.6	5.2	124.9	120.8	4.1

[1] Sites 2 and 7 are under long-term vegetable production. Sites 4 and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

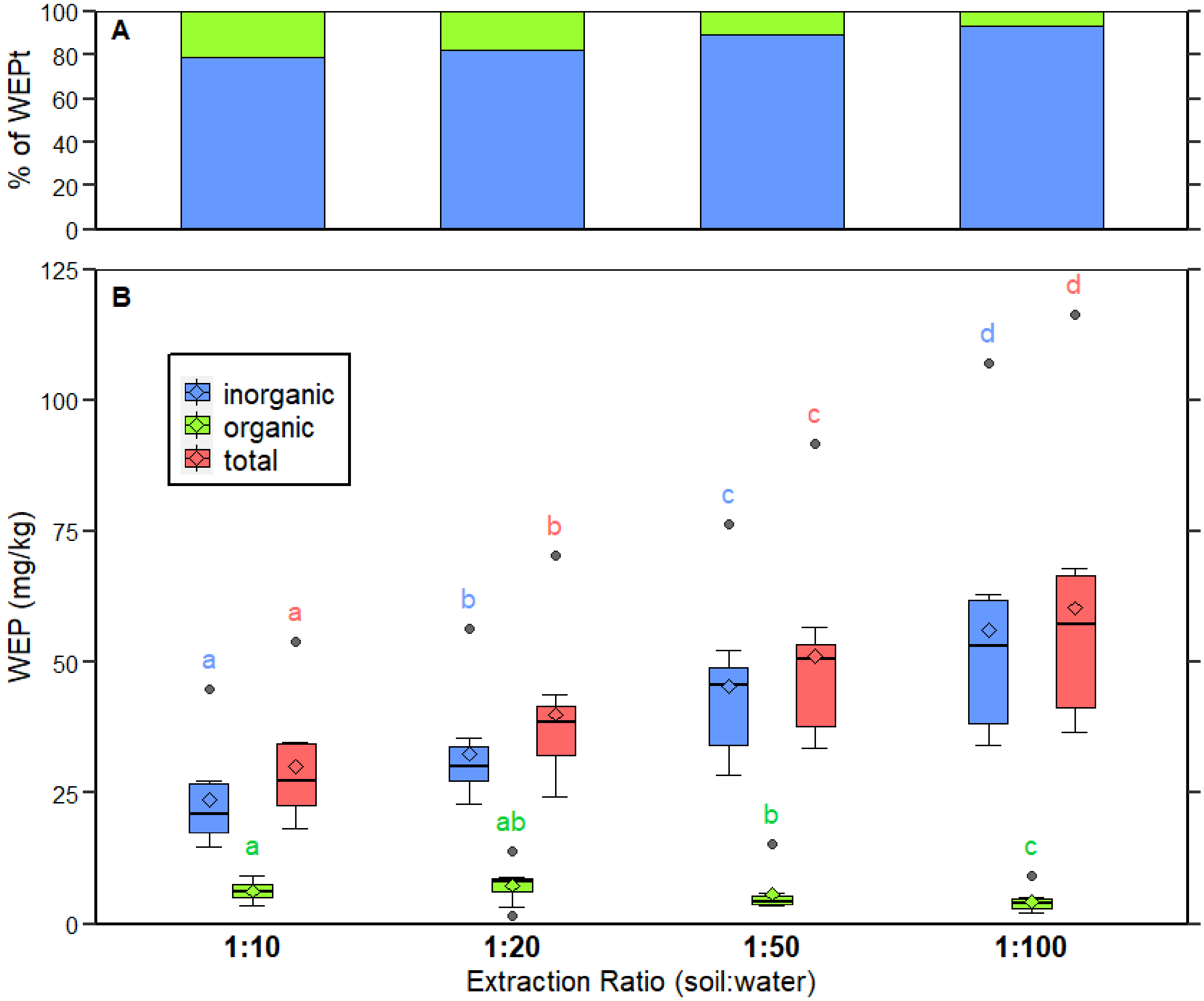

Figure 1. The proportion (A) and amount (B) of inorganic (WEPi (inorganic water-extractable P)), organic (WEPo (organic water-extractable P)), and total (WEPt (total water-extractable P)) pools at four soil-to-water ratios in legacy P soils. Each value represents data from eight fields with two replicate samples (n = 16). Boxplots depict median values (thick black line), mean values (diamond), 25th to 75th percentiles (colored boxes), 1.5 times the interquartile range (whiskers), and outlier values (points). Letters (a, b, c, and d) indicate statistically significant values for each of the water-extractable P (WEP) pools across extraction ratios at $p < 0.05$.

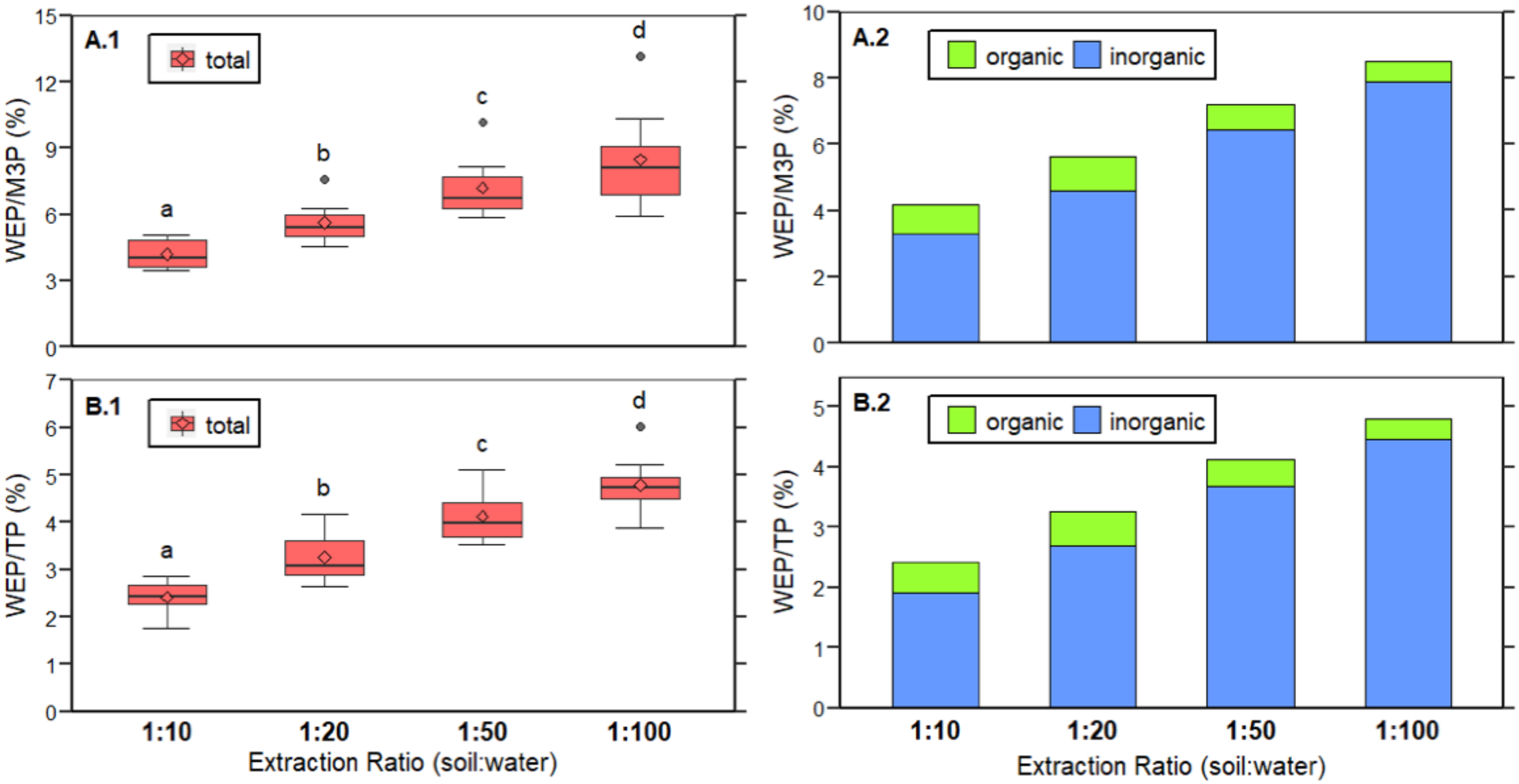

Figure 2. The proportion of inorganic (WEPi), organic (WEPo), and total (WEPt) pools at four soil-to-water ratios in legacy P soils as (A.1,A.2) percent of Mehlich 3–P and (B.1,B.2) percent of total P. Each value represents data from eight fields with two replicate samples (n = 16). Boxplots depict median values (thick black line), mean values (diamond), 25th to 75th percentiles (colored boxes), 1.5 times the interquartile range (whiskers), and outlier values (points). Letters (a, b, c, and d) indicate statistically significant values for WEPt across extraction ratios at *p* <0.05.

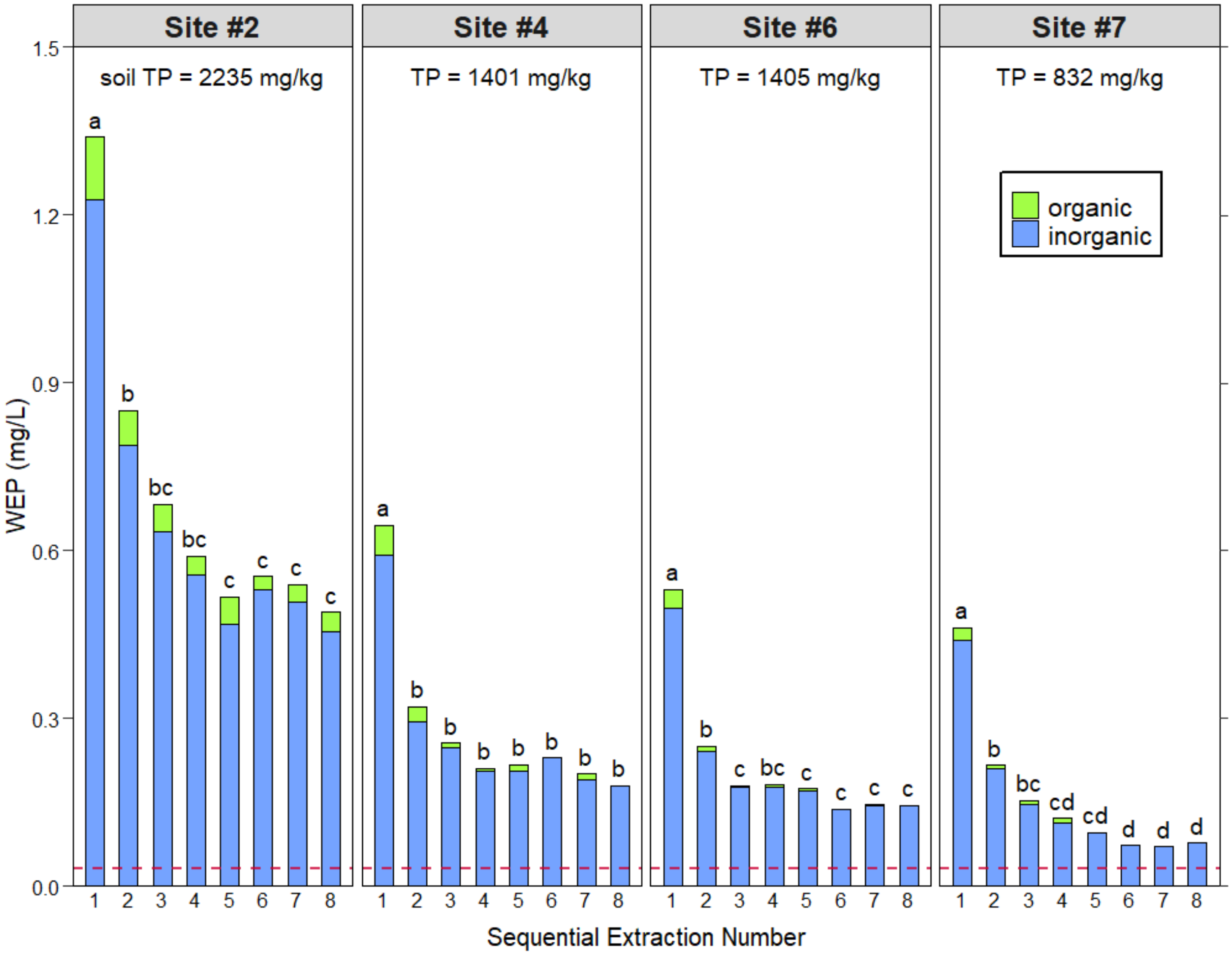

Figure 3. Inorganic (WEPi) and organic (WEPo) water-extractable P concentrations (mg L^{-1}) in eight sequential extractions at 1:100 soil-to-water ratio for four selected sites. Letters (a, b, c, and d) indicate statistically significant values for WEPt (WEPi + WEPo) across sequential extraction numbers for each site at $p < 0.05$. Sites 2 and 7 are under long-term vegetable production. Sites 4 and 6 are under long-term commodity crops (corn–soybean rotation, with and without cover crops) production. The dotted red line indicates the EPA total P limit (0.03125 mg L^{-1}) for surface waters. [74]

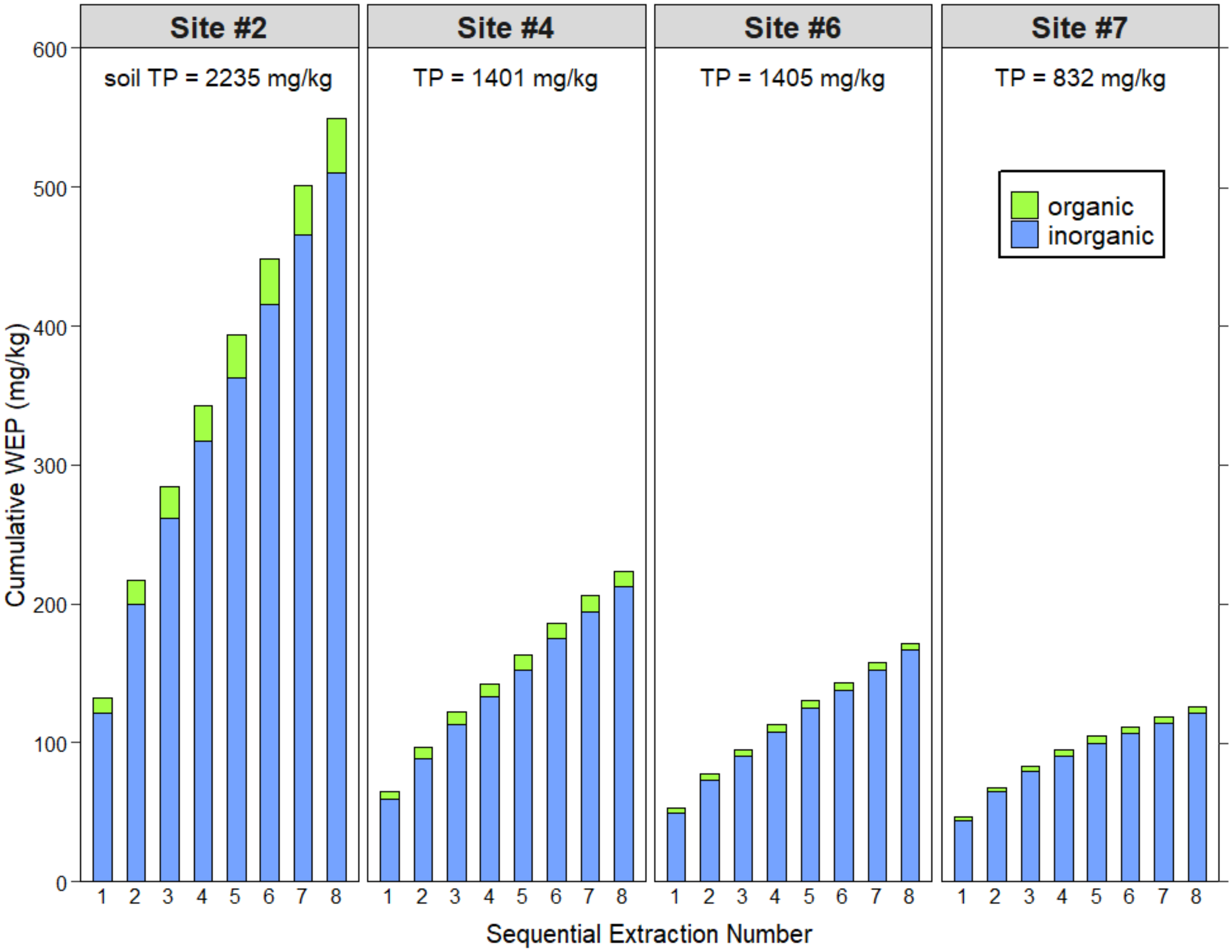

Figure 4. Cumulative amounts of inorganic (WEPi), organic (WEPo), and total (WEPt) water extractable P in eight sequential extracts at 1:100 soil to water ratio in four field sites. Sites 2 and 7 are under long-term vegetable production. Sites 4 and 6 are under long-term commodity crops (corn-soybean rotation, with and without cover crops) production.

Chapter 3: Dissolved Phosphorus Forms in Runoff and Effects on Soil P from a Soil Amended with Poultry Litter and Products

3.1 Abstract

Phosphorus (P) loss from soils amended with manures remains a threat to water quality. Although in many intensive animal production areas, most manure application has shifted from a nitrogen (N)-based to a P-based, P levels in soil can slowly continue to increase if P inputs are not carefully managed. The objective of this study was to investigate how the application of poultry litter and products based on a water-based extraction (i.e., water-extractable P, WEP) affects dissolved reactive P (DRP) and dissolved unreactive P (DUP) loss in runoff in packed soil boxes. Rainfall simulations were conducted to determine the effect of litter type and application rate on soil P and amount and forms of dissolved P in runoff. Three types of litter and litter products were used: (1) raw, unprocessed litter, (2) granulated litter with the addition of urea, and (3) heated, raw litter. Three extraction ratios (litter to water) for determining the WEP in litters, 1:10, 1:100, and 1:200, were used as the basis for WEP application at 3 kg WEP ha^{-1}. There were no significant differences in DUP in runoff among the raw, granulated, and heated litters (0.38 ± 0.07 kg ha^{-1}). However, the DRP was significantly higher from the packed soil boxes receiving granulated litters (1.09 ± 0.02 kg ha^{-1}) than raw (0.81 kg ha^{-1}) and heated (0.58 kg ha^{-1}) litters. The addition of litter products resulted in increased soil M3–P levels from 6.9 mg kg^{-1} in the control to 10.4–11.6 mg kg^{-1}, which was lower than the raw litters (14.1–42.6 mg kg^{-1}); however, the litter application did not affect the soils WEP (0.26

± 0.03 mg kg^{-1}) after three rainfall simulation events. Our results indicate (1) a WEP-based application is dependent on the litter to water extraction ratio used, and thus the use of accurate litter to water ratio (such as 1:250) in raw litters to determine WEP amount is critical to ensure similar amounts of WEP application, (2) runoff losses of P in litter and litter products amended soils were similar as a similar amount of WEP was applied, and (3) granulation and heating created a product that could enhance the use of poultry litter, especially in non-agricultural markets.

3.1.1 Keywords

Phosphorus; dissolved forms; poultry litter; runoff; extraction ratios; water-extractable P

3.2 Introduction

Surface runoff of phosphorus (P) from agricultural soils receiving manure generated from intensive livestock operations continues to be a major source of P to receiving waterways. In these regions, P losses from soils remain above environmental thresholds despite the best management practices (BMPs) to guide manure applications [13, 78, 79]. While manure application to crop fields is a viable and economically feasible means of applying nutrients, it can increase the risk of P losses, especially in P saturated soils due to the long-term addition of manures [22, 80].

In the United States, many states use P-site indices to identify fields where excessive P loss to water bodies may pose high risk [19]. Per local and state regulations to protect water quality, manure application guidelines have been developed to reduce the risk of P loss [81]. Many P saturated soils occur with intensive animal production regions such as northwestern Arkansas and the eastern shore of Maryland. In these areas manure application to fields is limited or even banned by state regulations. Therefore, alternative uses of manure are needed as manure disposal is often resource-intensive and financially demanding on farmers. Importantly, without manure application to fields, farmers may need to purchase commercial fertilizers to meet nutrient requirements of crops to have profitable yields. Therefore, techniques to either transform or reduce high soluble P in manures are imperative to sustain manure application as a long-term option for nutrient additions.

In areas where manure application is still available to farmers, manure can also be composted or treated before field application to reduce moisture content, decrease harmful pathogens, and achieve favorable physical properties [82, 83]. Composting or treating allow manures to be transported to areas where P deficient soils exist, and crops can benefit from manure application. Both raw and treated manures have unfavorable nitrogen to P (N:P) ratios (<3:1) as compared to crop needs, which vary but are consistently above 8:1 [84-86]. Thus, the application of manures to meet all of N needs results in an over-application of P than crops can utilize [41-43, 87, 88]. In many soils with optimal P levels, it is recommended to apply manure on a P-basis, which means applying manure to meet crop P needs (such as at 50 kg of total P ha^{-1}, which is equivalent to the amount of crop P removal in a two-year crop rotation), with the remainder of N supplemented through commercial fertilizers. The P-based application scenario works well in soils with optimum P levels for crop needs, as just enough P would be added for crops to use during the growing season. However, in intensive animal agriculture areas, soils exceed agronomic optimum P levels by several orders of magnitude. In these soils, application of manure, even on a P-basis is prohibited or, if allowed, can exacerbate P loss due to limited P sorption capacity of the soil [89]. Therefore, when manure is applied onto the surface of these soils, the highly labile P present in the manures saturates what is left of the P sorption capacity and hence P is prone to runoff in rain events, causing water quality deterioration [29, 90].

Other than surface broadcasting, knifing, injection, and tilling are other methods of manure application to fields. A decrease in P loss risk is associated with these alternative methods due to the depth of manure application below the effective depth of interaction, i.e., 1.3 to 34.7 mm [91]. While these methods can decrease the amount of dissolved P forms in the runoff, tillage can increase the erosion in soils and thus particulate bound P loss [92, 93]. These alternative methods often require specialized machinery, which often is cost-prohibitive, especially for small farmers. Therefore, either cost-effective alternative methods or a new approach to the surface application of manure is needed for these farmers to sustainably utilize manure.

One of the most commonly used methods to determine the amount of P in manure is water-extractable P (WEP), which has been correlated with dissolved reactive P (DRP) in runoff and leaching because it is the pool of P that is most labile [14, 16, 33]. While most of the P in manure sources is considered available to crops, unlike in soils where more recalcitrant P forms exist, WEP extractions can further differentiate a pool that is prone to immediate loss. In many states in the United

States, WEP is considered as the gold standard indicator of P loss potential [40]. For example, WEP extractions coupled with rainfall simulations have previously investigated the amounts of labile P prone to loss during manure applications [61]. Further, when packed soil runoff box simulations were compared with field plots studies, dissolved P losses between the two were not different, making packed soil box studies comparable to field runoff losses [34]. Studies have reported that WEP desorbed from the solid phase is influenced by the manure to water extraction ratio [94] as the equilibrium constraints cause less P to be released at narrower ratios and more P to be released at wider ratios, especially in manure rich in labile P forms. Therefore, the substantial pool of WEP is underestimated when WEP extractions use a narrower manure to water ratio. In the early 2000s, scientists recognized the importance of standardizing the extraction procedure to accurately determine the amount of WEP in manure. This standardization resulted in a WEP procedure using a 1:100 manure to water extraction ratio (w:v) [60]. Although a manure to water ratio of 1:250 is a more accurate estimate of the total WEP pool in manure [29], the 1:100 ratio was selected due to laboratory constraints and remains as the standard today.

Poultry litter is one type of manure that is prevalent in areas with intensive poultry operations such as the Delmarva Peninsula or southwestern Ozarks. In these areas, nearby soils are increasingly saturated with P, and therefore, a majority of the soils are restricted from receiving further P inputs. Poultry litter consists of feces, urine, and bedding material and has high soluble P content (~30% of total P). The large soluble P pool is problematic as research has consistently shown that as manure or fertilizer WEP increases, so does dissolved P in runoff [29, 33, 36, 95, 96].

Research has investigated the effects of poultry litter application rates, and found that as more litter is applied to the soils, significantly more total P runoff losses occurs and that DRP proportion also increase [33]. However, at application rates less than 75 kg of total P ha^{-1}, DRP did not encompass a majority of the total P loss, which is attributed to high intensity rainfall rates and subsequent large amounts of sediment loss. However, since BMPs and other soil-conservation techniques have aimed to reduce soil erosion from farm fields, actual DRP proportions of total P may be higher in field settings even at low application rates.

The effect of timing between litter application and first storm is important in predicting DRP loss from soil. Most research has shown that as timing between application and a rainfall event increases, the amount of DRP in runoff decreases [36, 97], and that significant differences in DRP loss from manure and unamended soils diminish within one year after application [98]. However, in some cases, a storm with high intensity and duration long after manure application can result in similar or more dissolved runoff P and loads as a storm occurring one day after manure application [39]. Further, since weather is unpredictable and most farmers are constrained by a limited time window to add nutrients to crop fields, controlling the time between application and first storm is difficult. Therefore, it is important to consider worst-case scenarios for manure application as a large enough storm within one week of application can cause as much as 60% of annual DRP losses [97].

Research has aimed to reduce the amount of P in poultry diets through amendments with phytase and aluminum chloride ($AlCl_3$) and found promising reductions in runoff DRP concentrations of 17% and 73%, respectively [99].

However, this does not address the excess manure transportation issue. In areas where the soils are already saturated with P, manure application is prohibited and therefore, off-site transportation to a low-risk field or processing facility is required and is costly. Further, due to the odors, microbes, and other unfavorable properties of unprocessed manure, the use of manure beyond field applications is limited.

Two options for processing poultry litter aimed to eliminate undesirable properties and make a product for other markets, e.g., urban landscapes, are granulation and heating. Granulation involves using water as a binding agent to form granules, which reduce moisture and odor. Other nutrients such as N can be added to create a product with favorable N:P ratio. Heating is another option and while there is no opportunity for increasing other nutrients, it does remove the water, microbes, and odors, and make the manure safer and more favorable to transport. However, both heating and granulation result in a higher proportion of soluble P in the litter products than the unprocessed (raw) litter [59, 100]. Therefore, there may be a concern of increased P loss with application of the processed products.

The application of manure to soil on a P-basis to meet crop needs of a two-year rotation (e.g., ~50 kg total P ha^{-1}) can significantly decrease P export compared with N-based application. However, even long-term P-based application can result in soil P saturation and P surplus if P inputs to match crop P uptake are not closely monitored. While in soils with below optimum P levels, manure can be applied without any restrictions, it is important to understand how even P-based manure application can alter P reserves in these soils. Our research goal was to investigate if P-based manure applications can be further enhanced, i.e., a reduction in P losses, by changing from a total P-based application to a WEP-based application (3 kg WEP ha^{-1}). A poultry litter and two products (granulated with urea and heated raw litter) were used for surface application to packed soil boxes based on WEP amounts extracted at three ratios of manure to water (1:10, 1:100, and 1:200). Our objective was to investigate the effect of manure application (raw, granulated litter, heated litter) based on three WEP extraction ratios on dissolved P losses in runoff. A second objective was to relate laboratory-based manure to water extraction ratios with rainfall simulated manure to water ratios to identify the best ratios that accurately estimate the dissolved P loss. If a WEP-based approach for P application reduces runoff P losses and the P saturation in soils, its adoption into manure guidelines, even in soils close to optimum P levels, can be explored.

3.3 Materials and Methods

3.3.1 Poultry Litter Processing

Poultry litter collected from a poultry house in Arkansas [59] was used for the rainfall simulations. In brief, raw litter was collected from a poultry operation in Decatur, Arkansas. The litter was ground to pass a 5.8 mm mesh screen and mixed using a New Holland 352 feed mill mixer. It was then shipped to Mars Mineral, Inc. (Mars, PA) to produce granulated products with the addition of urea, which increased the N content and balanced the N to P ratio to 8:1 in the final product to match with crop nutrient needs. Water was used to bind the granulated poultry litter together in a bench-scale granulator (12D54L pin mixer, Mars Mineral, inc., Mars, PA) with vibrating screw feeders (series 101 and 1015 volumetric screw feeders, Acrison, Inc., Moonachie, NJ). Granulates were placed on a vibrating fluid bed dryer kept at 232 °C until a final drying at 121 ^oC. A sub-sample of the raw litter was heated at 180 ^oC for 2 h to reduce the moisture content and kill microorganisms. As a result, two litters were produced from litter processing (1) granulated litter with urea [hereafter referred to as granulated litter] and (2) heated raw litter (Table 1). For a detailed description of litter generation, refer to Toor et al. (2007) [59].

3.3.2 Poultry Litter Analysis

Total P in the litter and litter products was determined with a concentrated HNO_3 and H_2O_2 digestion followed by analysis with inductively coupled plasma – optical emission spectroscopy (ICP-OES, SPECTRO Analytical Institute, Kleve, Germany) [101, 102]. Total N in the litter and litter products was determined using the dry combustion method (Model 2000, Leco Corp., St. Joseph, MI). WEP was measured using dry litter to deionized water ratios (w:v) of 1:10, 1:100, and 1:200 [59]. For all water extractions, 200 mL of deionized water was used to ensure equivalent headspace in the centrifuge tubes during extraction. All extracts were completed in triplicate. The extracts were shaken for 1 h in a reciprocating shaker and then centrifuged for 20 min at 2900 rpm before filtering through a 0.45 μm filter. The extracts were analyzed using the ascorbic acid reduction method [103] for inorganic WEP (WEPi) and ICP-OES for total WEP (WEPt = organic + inorganic WEP). Organic WEP (WEPo) was determined as the difference between WEPt and WEPi.

3.3.3 Poultry Litter Characteristics

Dry matter content ranged from 69.5% in the raw litter to 100% in the heated litter (Table 7). Total N:P ratios were 1.35:1 in the raw and heated litters and 7.98:1

in the granulated litter. The addition of urea to the granulated litter resulted in a 22% reduction in total P (17.7 g kg^{-1}) and a 462% increase in total N (141.3 g kg^{-1}) compared to the raw litter. Total WEP in litters depended on the extraction ratio used. At the 1:10 litter to water ratio, total WEP was 1277 mg kg^{-1} in the raw litter, 4326 mg kg^{-1} in the granulated litter, and 6860 mg kg^{-1} in the heated raw litter. Total WEP in all litters increased to 4183–7696 mg kg^{-1} at 1:100 and 4934–8029 mg kg^{-1} at 1:200 ratio. The extraction ratio had a greater effect on WEP in the raw litter than other processed litter products. For a more detailed discussion, refer to Toor et al. (2007) [59].

3.3.4 Litter and Product Treatments

Many studies have been conducted where researchers applied manure on a total N or total P basis. We applied litter and products based on WEP content as this is the P fraction that is most prone to runoff losses. The goal of applying litters on a WEP basis was to keep the amount of WEP application the same across all treatments, thus any differences in dissolved P losses in runoff could be attributed to inherent differences in litters and products processing. Further, previous research has shown that WEP in manures is strongly correlated with runoff dissolved P loss. Litter treatments were applied at 3 kg WEPt ha^{-1} based on extracted WEPt levels at 1:10, 1:100, and 1:200 litter to water (w:v) extraction ratios. The experimental design was completely randomized with two litter sources (raw litter and granulated litter with urea) applied at three extraction ratios (1:10, 1:100, 1:200), whereas the heated raw litter was applied at the 1:100 extraction ratio as WEP amounts were not statistically different among extraction ratios. See Toor et al. (2007) [59] for a deeper discussion of differences in WEP amounts. The control soil runoff box contained no added litter. All treatments were replicated in triplicate, resulting in 24 runoff soil boxes (3 control, 3 heated raw litter, 9 raw litter, and 9 granulated litter).

3.3.5 Runoff Box Preparation

The soil used in the rainfall simulations was Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudult) collected from a field in Northwest Arkansas that was under pasture production for several years. This soil is representative of the soils in the region where poultry litter is used to meet crop needs. Runoff soil boxes were packed according to the National Phosphorus Project Protocol for simulated rainfall-runoff studies [31]. The soil was packed into wooden boxes, which were 100-cm long, 20-cm wide, and 7.5-cm deep (total volume: 15,000 cm^3 or 0.015 m^3). Nine 5-mm holes were drilled in the bottom of each of the boxes to simulate field drainage, and the boxes were sealed with spar urethane. A small vertical flume was made and fixed to the downslope end of the box for runoff collection. A predetermined weight of the soil (12 kg) was packed into each of the boxes. The soil was packed with a wooden tamper to a depth of 5-cm in each of the runoff boxes to achieve a target field bulk density of 1.2 g cm^{-3}. Runoff boxes were covered with porous fabric (cheesecloth) to avoid direct contact of applied poultry litter with the soil. The cheesecloth physically separated the litter and soil while still allowing water to infiltrate and interact with the soil. Furthermore, the separation allowed for litter and soil analysis after the culmination of rainfall simulations.

3.3.6 Rainfall Simulations

Rainfall simulations were conducted over a three-week period in December 2006 and early January 2007 according to the National Phosphorus Project Protocol for simulated rainfall-surface runoff studies [31]. To increase the antecedent soil moisture in the boxes, packed soil boxes were carefully placed in a large container of water and allowed to soak via the bottom drainage holes for 24 h. The boxes were then removed and allowed to drain for 24 h to achieve field moisture capacity before rainfall simulations were conducted. Increasing antecedent soil moisture was repeated before each simulation event (1, 7, and 21 days after litter application) to achieve similar conditions across all soil boxes and ensure soils were not dry enough for cracking. Although a rainfall simulation was not conducted at 14 days, the packed boxes were placed in water and drained, as done before other simulation events, so that the soil would not dry and develop cracks before the final simulation event.

Following the 24 h drainage, each of the runoff soil boxes was artificially rained on at an intensity of 7.0 cm h^{-1} until runoff had occurred to uniformly settle the surface of each of the packed soil boxes. Following this preliminary rainfall event, the boxes were allowed to drain for another 24 h, at which time the actual rainfall simulations commenced. A rainfall simulator equipped with a nozzle (Spraying Systems Fulljet HH50WSQ, Wheaton, IL) was placed 3-m above the soil surface to simulate 7.0 cm h^{-1} rainfall [104]. This rainfall intensity was equivalent to a storm with a 5- to 10-year return period in eastern Arkansas [105]. Water used for rainfall simulations was obtained from a local municipal source and passed through a series of exchange-resin filters to simulate natural rainfall chemistry (low pH and buffering capacity). Immediately before the rainfall simulations, volumetric water content

(VWC) was measured in the packed boxes using a theta probe (ML2x) to ensure similar field capacity conditions. The VWC was 0.354 ± 0.004, 0.356 ± 0.003, and 0.353 ± 0.004 on day 1, day 7, and day 21, respectively. The simulated rainfall was applied for 10 min as this was enough to generate continuous runoff from the boxes. The rainfall volume onto each box was 2333 mL, and the average rainfall to runoff ratio was 0.83±0.03. Runoff from the boxes was collected, volumes were recorded, and a 1-L composite sample was taken for analyses after each of the three simulation events.

3.3.7 Runoff Sample Analysis

A sub-sample of the composite sample was filtered through a 0.45 µm filter, acidified, stored, and analyzed for dissolved reactive P (DRP) and total dissolved P (TDP). The DRP was analyzed using the ascorbic acid reduction method [103], and TDP was analyzed using ICP-OES (ICP-OES, SPECTRO Analytical Institute, Kleve, Germany). Dissolved unreactive P (DUP) was determined as the difference between TDP and DRP. Dissolved P loads were calculated by multiplying the concentrations with the total runoff volume.

3.3.8 Post-simulation Soil and Manure Analysis

Following the last rainfall simulation, packed boxes were allowed to drain for 48 h, and then soil samples were collected from the 0-5-cm depth within each box using a 6-cm in diameter stainless-steel corer. Samples were subsequently air-dried and sieved prior to analysis. A subsample was analyzed for WEP (1:10 soil to water ratio) and Mehlich-3 [106]. The leftover poultry litter and products were removed from the cheesecloth and analyzed for WEPt using a 1:100 (w:v) extraction ratio, following the method described in the Poultry Litter Analysis section.

3.3.9 Statistical Analysis

Statistical analyses were conducted using the JMP statistical software package (JMP Pro 13, SAS Institute). Analysis of variance (ANOVA) was used to assess the significance of litter treatment and the extraction ratio used for application on dissolved P loss during each rainfall simulation and dissolved P forms in the post-simulation soil analysis. Once a significant effect of litter types was identified, Tukey’s posthoc test for multiple comparisons (significance level [p] = 0.05) was conducted to determine significant differences among litter type. Once a significant effect of extraction ratios were identified within each litter type, a student’s t-test for multiple comparisons (significance level [p] = 0.05) was conducted to determine significant differences among extraction ratios. A student’s t-test (significance level [p] = 0.05) was also used to determine significance among soils WEP content after the conclusion of the simulations.

3.4 Results and Discussion

3.4.1 Amount of Litters and Products Added to Packed Soil Boxes

Litter application at 3 kg of WEPt ha^{-1} resulted in varying amounts of litter addition ranging from 7.8 to 47.0 g per runoff box or 0.2 m^2 (Table 8). Although the amount of litter added varied across treatments (equivalent to 467–2348 kg ha^{-1}), the WEPt addition was kept constant (60.00 ± 0.15 mg per box, equivalent to 3 kg WEP ha^{-1}) to allow for a controlled comparison of dissolved P losses in runoff across litter types during the rainfall simulation events. Since WEPt was lowest (1278 mg kg^{-1}) in the raw litter (at 1:10 ratio), the amount of litter added was highest at 47.0 g per runoff box, which is 3-5 times greater than 13.9 g per box for the granulated litter (at 1:10 ratio). When the litters were added based on the 1:100 ratio, the least amount of litter was added in the heated litter treatment (7.8 g per box) because of its high WEPt content (7696 mg kg^{-1}). Overall, the amount applied was more variable when litters were added based on WEPt extracted at a 1:10 ratio (13.9–47.0 g per box) compared to 1:100 (7.8–14.3 g per box) and 1:200 (9.3–12.2 g per box). The amounts of raw litter added decreased from 1:10 (47.0 g per box) to 1:100 (14.3 g per box) and 1:200 (9.3 g per box) treatments because of a greater extraction of WEPt at higher ratios. In contrast, the amount of litter added was similar for granulated litter across all WEPt ratios (12.2 to 13.9 g per box) due to similar levels of extracted WEPt in the granulated litter (Table 7).

3.4.2 Concentrations and Proportions of Dissolved Phosphorus Forms in Runoff Over Three Simulation Events

During the rainfall simulations, concentrations of DRP in the control treatment were similar at days 1 and 7 (0.0146 mg L^{-1}) and slightly decreased to 0.0098 mg L^{-1} at day 21. Concentrations of DUP in the control treatment were 0.535 mg L^{-1} at day 1 and decreased to 0.454 mg L^{-1} at day 7 and 0.435 mg L^{-1} at day 21. In contrast, runoff losses of dissolved P forms were consistently highest in the raw litter treatments and depended on the WEP extraction ratio used for litter application (Figure 5). On day 1, concentrations of DRP were greatest when the raw litter was applied based on the 1:10 ratio (24.8 mg L^{-1}), which decreased in the 1:100 (13.8 mg

L^{-1}) and 1:200 (7.62 mg L^{-1}) treatments. Similarly, DUP decreased as the extraction ratios widened from 1:10 (11.9 mg L^{-1}) to 1:100 (4.45 mg L^{-1}) and 1:200 (2.92 mg L^{-1}). Overall, concentrations of TDP (DRP+DUP) in the raw litter treatments were reduced by 50% in the 1:100 and an additional 41% in the 1:200 treatments compared with the 1:10 treatment.

In the second simulation event on day 7, the runoff of dissolved P forms in the raw litter treatment was significantly lower than day 1. For example, in the 1:10 treatment, DRP was lower by 85% (3.72 mg L^{-1}), and DUP was lower by 38% (7.33 mg L^{-1}). Similarly, DRP was lower by 92% (1.09 mg L^{-1}) in the 1:100 and 93% (0.50 mg L^{-1}) in the 1:200. However, DUP did not decrease as much as DRP on day 7 but was 55% lower (2.00 mg L^{-1}) in the 1:100 and 64% lower (1.06 mg L^{-1}) in the 1:200. In contrast to day 1 where DRP was the dominant form of TDP (68.0–75.8%), DUP became the dominant form on day 7 (64.7–67.3%) across all extraction ratio treatments. On day 7, widening the extraction ratio from 1:10 to 1:100 resulted in a 72% reduction in TDP with an additional 50% reduction when the ratio further widened from 1:100 to 1:200.

By the third simulation event (day 21), runoff from the raw litter was still significantly higher in the 1:10 (DRP: 1.29 mg L^{-1}; DUP: 2.91 mg L^{-1}) than the 1:100 (DRP: 0.42 mg L^{-1}; DUP: 1.20 mg L^{-1}) and 1:200 (DRP: 0.22 mg L^{-1}; DUP: 0.94 mg L^{-1}) treatments. Concentrations of DRP were significantly higher in the raw litter in the 1:100 than 1:200; however, DUP was statistically similar. Like in day 7, DUP was again the dominant form (69.2–81.1%). Although TDP in runoff was lower on day 21 as the ratios widened, the reductions were not as apparent as with the earlier simulations; a 30% reduction in the 1:100 and an additional 29% in the 1:200 compared to the 1:10 treatment.

The granulated litter treatments resulted in similar DRP and DUP concentrations in runoff across the three extraction ratios during the three simulation events (Figure 5). On day 1, DRP concentrations were between 9.22 and 10.00 mg L^{-1} in granulated litter treatments, which exceeded the raw litter 1:200 treatment, though not statistically different at $p < 0.05$. DUP concentrations were much lower and ranged from 1.47 to 1.89 mg L^{-1} across the extraction ratios. Similar to the raw litter runoff losses on day 1, DRP was the dominant form of TDP (84.0–87.1%) in the granulated litter treatments. During the day 7 simulation, concentrations of DRP (0.70–0.74 mg L^{-1}) and DUP (1.10–1.11 mg L^{-1}) were still similar in the 1:100 and 1:200 treatments; however, in the 1:10 treatment, DRP was two-times higher (1.63 mg L^{-1}) while DUP (1.23 mg L^{-1}) was not statistically different from the wider ratios. Just as with raw litter, TDP composition switched from DRP dominance on day 1 to DUP dominance on day 7 (60.0–60.9%), except for in the 1:10 treatment where DRP dominated (56.9%). By day 21, runoff from the granulated 1:10 application was once again similar to other extraction ratios and the DRP was 0.29–0.42 mg L^{-1} and DUP was 0.85–1.10 mg L^{-1} across three ratios. The proportion of DUP increased from day 7 to 72.4–74.3% of TDP on day 21.

Heating the raw litter resulted in less DRP and DUP in runoff over the three simulation events compared with raw litter at 1:100 (Figure 5). On day 1, DRP was 80% lower (5.11 mg L^{-1}), and DUP was 87% lower (1.53 mg L^{-1}) than the raw litter treatment. By day 7, the reduction in P loss in heated litter treatment from the raw litter was 54% DRP (0.50 mg L^{-1}) and 52% DUP (0.96 mg L^{-1}). In the final simulation event, DRP and DUP were 0.26 mg L^{-1} and 0.64 mg L^{-1} in the heated litter, which translates to 38% and 47% lower values than the raw litter, respectively.

3.4.3 Cumulative Dissolved Phosphorus Loss Across Litter Treatments

Across all treatments, the cumulative TDP loss over the three rainfall simulation events was lowest (0.89 kg ha^{-1}) in the heated litter (1:100) and highest (5.02 kg ha^{-1}) in the raw litter (1:10) application treatments (Figure 6). In the control treatment, TDP loss was 0.14 kg ha^{-1} with DUP encompassing 97.3%. Cumulative losses were highest in the raw litter treatment at 1:10 application for both DRP (2.88 kg ha^{-1}) and DUP (2.13 kg ha^{-1}). The 1:100 application of raw litter had the second highest DRP (1.54 kg ha^{-1}) and DUP (0.76 kg ha^{-1}) losses; however, combined, TDP was 54% lower than the 1:10 application. TDP loss in the 1:200 raw litter application was further reduced by 44% (DRP: 0.8 kg ha^{-1}; DUP: 0.48 g ha^{-1}) from the 1:100 application. While the extraction ratios significantly affected the TDP loss from the raw litter, they did not affect TDP composition to the same extent. As a percent of TDP, DRP was 58% in the 1:10 application, 67% in the 1:100 application, and 63% in the 1:200 application.

Losses from the granulated litters did not vary across the extraction ratios as the raw litter as the cumulative DRP loss was 1.10, 1.09, and 1.07 kg ha^{-1} for the 1:10, 1:100, and 1:200 applications, respectively. The average proportion of DUP loss was 25.5% of TDP and was 0.40 kg ha^{-1} (1:10), 0.33 kg ha^{-1} (1:100), and 0.38 kg ha^{-1} (1:200). The heated litter treatment had the lowest TDP loss (DRP: 0.58 kg ha^{-1}; DUP: 0.31 kg ha^{-1}). Compared to the raw litter treatment loss (1:100), heating the litter resulted in a 61.4% reduction in TDP loss. Although heating the litter affected the amount, it did not significantly affect the proportion of P forms (DRP: 65%; DUP: 35%).

3.4.4 Effects of Extraction Ratio, Litter Type, and Time Since Litter Application on Dissolved Phosphorus Loss in Runoff

Vadas et al. [30] found that a litter to water ratio of 1:250 was a good estimate of the total amount of WEP present in litter. Therefore, WEP amounts based on a 1:250 extraction from Toor et al. (2007) [59] were used to calculate the total WEP added to the boxes and used for comparisons (Table 3). Although litter was added to the packed soil boxes to reach 3 kg ha^{-1} of WEPt based on three extraction ratios, the actual amounts of WEPt added (based on all WEP that can be extracted at 1:250 ratio) ranged from 3.08–17.02 kg ha^{-1}. Recalculating the actual amounts of WEP added only affected the raw litters (1:10 and 1:100). The underestimation of WEP added in these two raw litter treatments is because of an equilibrium barrier between the litter solid phase P and solution P that prohibits further P to be released at narrower litter to water ratios in laboratory extractions [69]. The extraction ratios used for application did not affect the amount of WEP added in the granulated litters, as the processing resulted in high solubility of litter P, even at narrow ratios.

3.4.4.1 Effect of Litter Extraction Ratio

The raw litter (1:10) application resulted in the addition of 53 kg total P ha^{-1} and 17 kg WEPt ha^{-1} (based on the extracted amount of WEP at 1:250) to the packed soil runoff boxes (Table 9). This application scenario serves as a typical P-based manure application to crops in a two-year crop rotation and showcases the expected

TDP concentrations in runoff after application of raw litter. Cumulatively, over the three simulation events, the raw litter (1:10) amended soil lost 5.02 kg ha^{-1} of TDP (28.7% of WEPt added). The raw litter (1:100) had 70% less WEPt added (5.18 kg ha^{-1}) but only lost 54% less TDP (2.30 kg ha^{-1}) in the runoff simulations than the raw litter (1:10). We suggest that adding less litter in higher extraction ratio treatments (such as 1:100 and 1:200) resulted in a proportionately larger TDP loss due to the higher litter to water ratios achieved during the rainfall simulations. Since the amount of rainfall water was constant across the treatments (2333 mL), only the amount of litter added affected the simulated litter to water ratios during the rainfall simulations. For example, in the raw litter (1:10), a narrower ratio of 1:50 litter to water occurred during each simulation compared to the raw litter (1:100) where a 1:163 simulated ratio occurred. Therefore, due to equilibrium constraints at narrower litter to water ratios, WEP release from the litter was inhibited in the raw litter (1:10). Although a 1:163 ratio does not allow full release of the WEP pool from the solid phase (compared to a 1:250 ratio), it allows an 74% release as compared with the 36% release in a 1:50 ratio. Therefore, to truly understand how adding larger amounts of litter proportionately affect TDP loss, a similar ratio between litter and water during the simulations would need to occur. Similarly, in the raw litter (1:200), 80% less WEP was added (3.37 kg ha^{-1}) and lost 75% less TDP (1.30 kg ha^{-1}) in the runoff simulations than the raw litter (1:10). Since the raw litter (1:200) resulted in a simulated litter to water ratio of 1:250, we can assume the entire WEP pool had the potential to be released during the simulation on day 1. We hypothesize this was exactly the case as in the subsequent simulations, TDP concentrations were not significantly different from one another, suggesting an equilibrium was reached between the now depleted WEP reserves in the litter and the rainfall water. We would expect this equilibrium concentration to remain constant over any additional rainfall simulations until P reserves in the litter are fully depleted.

In the granulated litters, the extraction ratios did not affect TDP loss during the rainfall simulations because the extraction ratio did not have a significant effect on the extracted amounts of WEP, and therefore, WEP amounts added to the box. While narrower ratios restricted WEP release in the raw litter, they did not in the granulated and heated litters due to the high solubility of P resulting from processing. During heating and granulation, Ca and Mg present in the litter were most likely precipitated and drove the equilibrium between the solid phase P and solution P towards dissolution of Ca−P and Mg−P compounds [59]. This allowed most of the WEP pool to be released at narrow ratios. There was a slight trend in the granulated litters where a decrease in litter added across the ratios led to proportionately smaller TDP losses, although this was not significant at $p < 0.05$. Again, this is most likely due to the increasingly wider simulated manure to water ratios (1:168, 1:184, and 1:191) for the granulated litters (1:10, 1:100, and 1:200, respectively), supporting our assertion about the effect of simulated events extraction ratios. These effects of simulated litter to water ratios will be further explored below (section 3.4.6).

While WEP is an important environmental index for measuring the amount of highly labile P in soils and manure, its effectiveness for P-based manure application is limited. Firstly, a WEP-based approach is highly dependent on the total amounts of WEP added to the soil. Since ratios narrower than 1:250 do not adequately quantify the WEP pool in raw litters, application based on these narrower ratios will not be useful for predicting P loss as more WEP will be added than calculated. Secondly, adding less WEP to the soil does decrease incidental P loss in runoff, but not proportionally to what was added. It is more important to consider the manure to rainfall ratios when predicting how much P added will be lost in subsequent rain events. Overall, even low rates of litter application (e.g. ~ 11 kg of total P ha^{-1}) will produce significant amounts of runoff losses of TDP, between 24% and 43% of WEP added and 10% and 13% of total P added in the litter.

3.4.4.2 Effect of Litters Treatment

The total amounts of WEP added (based on 1:250 lab extraction) to the packed soil boxes was similar among the raw litter (1:200), all granulated litters, and the heated litter (3.27 ± 0.17 kg ha^{-1}) (Table 9). Therefore, these treatments can be compared to determine how litter type affected dissolved P loss in runoff. Cumulative TDP loss from the three rainfall simulations in these treatments was similar (1.31 ± 0.25 kg ha^{-1}), only significantly lower in the heated than the granulated (1:10 and 1:200) litters. However, the granulated litters lost significantly more DRP (1.09 ± 0.02 kg ha^{-1}) than the raw litter (1:200) (0.81 kg ha^{-1}) and the heated litter (0.58 kg ha^{-1}). Even though, TDP loss from these treatments were not highly variable, there were higher DRP losses from the granulated litters. Comparison of these treatments highlights the similarity in TDP loss across these litter and litter products. Losses were more dependent on the total amounts of WEP added to the packed soil boxes than the type or amount of litter. The similarity between poultry litter and products at low WEP application rates means the high solubility of P in the litter products did not result in more dissolved P loss, which was an initial concern.

While the overall loss between the treatments was not different, there were differences in loss as a percent of what was added (Table 9). The granulated litters lost higher percentages of WEP added (1:10: 38.7%, 1:100: 39.8%, and 1:200: 42.7%) than the raw litter (1:200: 34.2%) and the heated litter (23.6%). Heating the raw litter at 180 °C for 2 h resulted in 100% dry matter with potential loss of the organic matter and release of associated cations such as Ca and Mg. Upon rainfall simulations, we hypothesize the freely available Ca and Mg leached into the soil along with labile P species and upon contact, formed Ca–P and Mg–P associations in the soil. Although weakly adsorbed, this P was no longer readily available for runoff loss. Therefore, while the heated litter had the largest WEP pool of all litter treatments, it had the smallest proportional WEP loss.

While the granulation process also went through a heating phase, albeit at a lower temperature (121 °C) than in the heated litter, resulting in 90% dry matter, it did not result in the same amounts of freely available Ca and Mg in the litter. During granulation, the litter is heated on a moving belt where water is continuously added to bind together granules; therefore, any release of Ca and Mg that was caused by heating was bound within the granule upon entering the soil. Compared to the heated litter, the release of the bound but soluble Ca and Mg into the soil would be slower. Based on the pre-simulation water-extraction of the litters, there was 964 mg kg^{-1} soluble Ca and 2222 mg kg^{-1} soluble Mg in the heated litter compared to 629 mg kg^{-1} soluble Ca and 1047 mg kg^{-1} soluble Mg in the granulated littler (1:100). Therefore, the granulation process resulted in one-third and half the amount of soluble Ca and Mg, respectively. Further, the addition of urea likely resulted in the litter encompassing about ~20% less of the total mass in the granulated product. Therefore, upon rainfall simulation the litter to water ratios achieved in granulated litters were greater than reported, and in turn allowed greater dissolved P loss. These two factors likely contributed to higher loss in granulated litters than heated litter treatment.

3.4.4.3 Effect of Days Since Litters Application

Concentrations of TDP from the litter treatments decreased over the three rainfall simulations as has been reported in previous studies [34, 36, 107]. This is because as WEP applied in the manure has more time to interact with the soil, it is converted to less soluble forms until a new equilibrium between soil P pools is established [108]. However, the significance of these decreases only occurred between day 1 and day 7. By day 21, mean TDP concentrations were not significantly different from day 7. This may be due to the low total P application of the litters as other research has indicated statistically significant declines were only observed in application rates of total P above 50 kg ha^{-1} [33]. Further, there were still significant differences between the litter treatments on day 21, unlike some research that found by the third simulation event, different litter types had insignificant differences in dissolved P loss [36]. The raw litter (1:10) was still losing significantly more TDP than the other raw litters (1:100 and 1:200) and granulated and heated litters. Whereas the heated litter was losing significantly less TDP than the raw litters (1:10 and 1:100) and the granulated litter (1:10). This suggests that the heating and granulation processes can alter the P chemistry in raw litter and affect P release during rainfall even after significant depletion of the WEP pool. For example, in raw litter, mineralization will continually release more P that can be subjected to loss with rainfall events. Whereas in the granulated and heated litters, processing resulted in converting organic P into inorganic P (which is why these litters had more WEPi) and thus there is relatively less pool of organic P forms to be mineralized. These results indicate that runoff concentrations of dissolved P forms can significantly differ between these litter types even after successive rainfall simulations and should be considered when determining the best litter types to meet crop requirements and minimize P loss risk.

On day 1, there was a large flush of DRP from all litter types and application rates, which significantly decreased during day 7 and even more on day 21. While DUP also decreased as the rainfall simulations progressed, it did so not nearly to the extent as DRP. In fact, DUP loss remained somewhat constant over the three simulation events, especially in the raw litter (1:200), granulated litters, and heated litter. We suppose that the equilibria reactions controlling DUP release in these litters were not as powerful as those controlling DRP release. As more water was added to the litter during each rainfall simulation, similar amounts of DUP were released, and even surpassed DRP release in the later simulation events. Therefore, as litter is flushed of a majority of its DRP during the first rainfall post-application, subsequent losses were dominated by DUP. Therefore, any analysis techniques such as colorimetry, which disregard the unreactive dissolved P forms, may not adequately quantify the long-term dissolved P losses in litter amended soils.

3.4.5 Post-simulation Litters and Soil Analyses

3.4.5.1 Post-simulation Water-Extractable Phosphorus in Litters

After the last simulation event, the remainder of the litters left on the cheesecloth in the packed runoff soil boxes were removed and extracted at a 1:100 ratio to determine WEP and compare with pre-simulation 1:100 WEP values to obtain percent change (Table 10). After the simulations, the raw litter treatment (1:10) had 740 mg kg^{-1} of WEPi and 102 mg kg^{-1} of WEPo. WEPi concentrations in the raw litter (1:10) were higher than 1:100 (586 mg kg^{-1}) and 1:200 (593 mg kg^{-1}). Even though the raw litter (1:10) lost more DRP during the rainfall simulations than the raw litters (1:100 and 1:200), it still had more WEPi. This is most likely due to equilibrium restraints during in the simulated litter to water ratios during the simulations which inhibited a proportional DRP loss to occur between the raw litter (1:10) and raw litters (1:100 and 1:200). While only the raw litter (1:10) had significantly different WEPi concentrations, all raw litter treatments had different WEPo concentrations (1:100: 156 mg kg^{-1}; 1:200: 230 mg kg^{-1}). WEPo concentrations left in the raw litters reflected the amounts of DUP lost during the rainfall simulations. For example, raw litter (1:10) lost the highest amount of DUP (2.13 kg ha^{-1}) and had the least amount of WEPo left in the litter after the simulations. Therefore, while equilibrium may have affected the amount of WEPi left in the litters, it did not impact the unreactive dissolved P forms to the same extent. While there were some differences in WEPi and WEPo in the raw litter treatments after the culmination of the rainfall simulations, WEPt was not statistically different.

The granulated litters had similar WEPi (530 ± 60 mg kg^{-1}) after the three simulations, while WEPo was slightly lower in the 1:10 (167 mg kg^{-1}) than the wider ratios (1:100: 224 mg kg^{-1} and 1:200: 252 mg kg^{-1}). As in the raw litters, WEPt did not vary between the granulated litters. The heated litter had 657 mg kg^{-1} of WEPi, 278 mg kg^{-1} of WEPo, and the highest WEPt (935 mg kg^{-1}) after the simulations, though not statistically different from the raw litters (1:10 and 1:200) and the granulated litter (1:200).

Although prior to the simulations there were differences in WEPt across litter types, after the simulations WEPt was similar. Therefore, after just three rainfall events (each with a 5- to 10-year return period), these litters were depleted of excess WEP reserves regardless of the type and amount of litter added and the simulated litter to water ratios achieved during simulation. The remaining WEP and release are most likely controlled by chemical properties of the P binding mechanisms in the litters, which appear to be similar amongst the litter and litter products. This suggests that any effect heating and granulated had on the WEP content, i.e. P solubility, prior to the rainfall simulations diminished by the culmination of the study.

Across all treatments, mean %WEPi change from before and after simulations was 86.2 ±3.7%, ranging from 79.9% in the raw litter (1:10) to 89.7% in the granulated litter (1:200). This means that on average 86.2% of the WEPi concentrations in litter was reduced over the course of the three simulations. The reduction in WEPi for the raw litter treatments were slightly lower than the granulated and heated litters and was significantly lower in the 1:10 than the 1:100 and 1:200 applications. Percent WEPo change varied more widely than the %WEPi change. As the ratio used for raw litter application widened from 1:10 to 1:200, less of a percent change in WEPo occurred. For instance, 79.3% of WEPo was reduced in the raw litter (1:10), 68.4% reduced in the raw litter (1:100), and 53.3% reduced in the raw litter (1:200), although it was only significant between the 1:10 and 1:200 application. We hypothesize the reason less WEPo was released in the raw litter (1:200) than the raw litter (1:10) was because the larger simulated litter to water rainfall ratios during the simulations allowed preferential binding of WEPo over WEPi to the newly vacated P sorption sites in the manure [70]. Therefore, when these litters went through a post-simulation WEP extraction, there was proportionately more WEPo retained in the manure and available for release via a more extensive extraction (shaking, 1 h). The granulated litters percent WEPo changes were all negative, meaning WEPo increased in the litters during the simulations. Although the granulation process involves heating the litter and thereby killing microbes, the application of litter to soil boxes and presence of moisture over 21 days in runoff boxes may have allowed a microbial population to grow and immobilize a small amount of WEPi. Just like the raw litter, the percent WEPo change was dependent on the extraction ratio used for the granulated litters. As the ratio used for application widened from 1:10 to 1:200, WEPo increased from 53.2% to 131.2%, where all differences were significant. Therefore, as more water per unit litter was added to the packed soil boxes, significantly more WEPo was added to the litter, most likely due to moisture induced growth of microbial populations. The WEPo in the heated litter was reduced by 80.1%, similar to the raw litter (1:10). Compared to the processing of the granulated litter, the heated litter was brought to 60 ^{o}C higher temperature, resulting in a powder-like litter amendment. Therefore, the initial WEPo content of the soil was higher than in the raw and granulated litters most likely due to contribution of more inert organic P forms that were released at high temperatures. The percent WEPt change was not affected by the ratio used for application but differed across the treatments. In the raw litters, WEPt was reduced on average by 80.9 ± 1.6%, which was significantly lower than the granulated litters (average: 84.3 ± 1.3%) and heated litter (87.9%).

3.4.5.2 Changes in Water-Extractable Phosphorus Content in Soils After Litters Applications

After the simulations, the WEPi content of the soil increased only in the raw litter (1:10 and 1:100) amended soils (Table 11). The raw litter (1:10) increased the WEPi by 656.9% to 2.01 mg kg^{-1}, while the raw litter (1:100) increased the WEPi by 257.7% to 0.732 mg kg^{-1}. This increase was correlated with the amount of litter added to the packed soil boxes in these treatments. Roughly three times the amount of raw litter was added at 1:10 than 1:100, and the increase in soil WEPi was also roughly three times. All other treatments resulted in similar WEPi as the control. We hypothesize in the raw litter treatments (1:10 and 1:100), the decomposition of organic matter resulted in a release of organic acids such as citrate, malate, malonate, oxalate, and tartare [109]. These compounds compete for P binding sites in the soil, rendering more of the litter P in the soil solution [110], thus increasing the WEP content of the soil. In the raw litter (1:200), the overall input of organic matter was low due to the low amount of total litter added, and thus the binding sites were not saturated with the organic acids, allowing more released labile P to sorb to the soil. In the granulated and heated litters, the organic matter was lost during the heating process. Therefore, when the labile P entered the soil during the rainfall simulations, it was quickly adsorbed to the soil keeping it from entering the soil solution.

3.4.5.3 Increase in Mehlich 3–Phosphorus in Soils After Litters Application

After the three simulations, the soil M3–P in the control treatment was 6.9 mg kg^{-1}, and was used to compare against the litter treatments. Overall, the litter additions to the soil increased the M3–P content between 146.9% and 616.4% (Table 11). The extraction ratio used for application of raw litters affected the increase in M3–P. As the raw litter ratios narrowed from 1:200 to 1:10 and more litter was added to the soil, M3–P in the soil increased. In the raw litter (1:10) the highest increase in M3–P occurred, 616.4% (42.6 mg kg^{-1}). The raw litter (1:100) application increased the M3–P by 257.7% (18.1 mg kg^{-1}), while the raw litter (1:200) had a slightly less impact, 202.3% (14.1 mg kg^{-1}). Although the percent increase in M3–P was affected by the amounts of litter added to the packed soil boxes, the increase in M3–P per kg of litter total P added was not affected. In the raw litter (1:10), M3–P increased by 0.674 mg kg per 1 kg of litter total P added. Similarly, M3–P increased by 0.700 mg kg per 1 kg of litter total P in the raw litter (1:100) and 0.655 mg kg per 1 kg of litter total P in the raw litter (1:200).

In the granulated litter applications, the soil M3–P increased to 10.7, 11.6, and 10.4 mg kg^{-1} for the 1:10, 1:100, and 1:200 ratios, respectively. On average, this was a 157.2 ± 20.7% increase from the control. The addition of granulated litter increased the M3–P in the soil on average by 0.354 ± 0.061 mg kg per 1 kg of litter total P, about half as much as the raw litter. The heated litter increased the M3–P by 149.3%, reaching 10.5 mg kg^{-1}, almost the exact same as in the granulated litter (1:200). Overall, the addition of raw litter increased the soils M3–P to a greater extent than both the granulated and heated litters.

A related study identified the effects of dairy manure and P fertilizer (KH_2PO_4) application on the increase in M3–P per kg of total P added [111]. They found that dairy manure increased soil M3–P between 0.172 and 0.193 mg kg^{-1} per kg of total P added. However, with fertilizer, they discovered a larger per unit increase (0.300 and 0.444 mg kg^{-1}). Our results suggest that the granulated and heated litters acted similarly to P fertilizer in terms of increasing M3–P content of soils. This makes sense as fertilizer P is completely soluble and available with very large WEP contents released upon contact with water. Since granulation and heating increased the solubility of the WEP pools, the resultant litter products acted like a source of fertilizer once encountering the soil. The reason poultry litter increased the M3–P content more drastically than the dairy manure is most likely on account of dairy manure's low WEP content (averages: 478 and 487 mg kg^{-1}), which is three times less than the raw poultry litter used in this study. The larger increase in M3–P per unit of total P added in poultry litter than dairy manure gives insight into the high density of critical source areas near poultry operations such as in the southwestern Ozarks.

We hypothesize the reason the granulated and heated litters did not increase the M3–P content of the soil to the same extent as raw litter was because the large influx of labile P and organic matter from the raw litter to the soil caused a shift in the equilibria among P pools in the soil. The M3–P content of the control was 6.9 mg kg^{-1}, which is in the low category for crop growth. Therefore, in an unsaturated soil like in this study, P binding sites on the soil are readily available to sorb additional P inputs. Therefore, when a large flush of available P entered the soil from the raw litter, adsorption reactions occurred, increasing M3–P.

This is important because while the runoff losses across the litter treatments receiving similar amounts of total P (raw litter, 1:200), all granulated litters, and heated litter) were similar, the increase in M3–P relative to the addition of total P was not. The addition of raw litter significantly increased the soil M3–P more than other litter products. Therefore, when applying litter and products to soils that already have high M3–P levels, it is better to use litter products than raw litter, even though losses will be similar.

3.4.6 Effect of Laboratory vs. Simulated Litter to Water Ratios on Incidental Dissolved Phosphorus Loss in the First Rainfall Simulation Event

Laboratory WEP extractions mimic a scenario that is rarely reached during field conditions. Instead of the entirety of manure shaken and in complete contact with water, litter in the field is rained on and, if not saturated and for an adequate period of time, may not allow enough water to come in contact with the litter to establish an equilibrium between solid and solution phase that control release of P. Further, during laboratory extractions, WEP which is physically secluded within manure aggregates may be released when these aggregates are dismantled during the shaking process. We compared the litter to water ratios achieved during the rainfall simulations with laboratory extractions to see how they compare. Since the amount of rainfall was constant across all treatments (2333 mL), only the amount of litter impacted the simulated litter to water ratios.

In the raw litter (1:10), 47 g of litter was added per runoff box (0.2 m^2), reaching a simulated litter to water ratio of ~1:50. In the raw litter (1:100), 14.3 g of litter was added, reaching 1:163, and in the raw litter (1:200), 9.3 g of litter was added, reaching a 1:251 ratio. An equation was generated from laboratory extracted WEPt at varying litter to water ratios (1;10, 1:50, 1:100, 1:200, 1:250) to estimate the percent of litter WEPt released as incidental loss on day 1: Litter WEPt release (%) = 0.3376*water to litter ratio (mL g^{-1}) + 19.276 (Figure 7). Based on this equation, a 1:50 simulated ratio achieved during rainfall simulation would result in a 36% release of WEPt, a 1:163 ratio allows for a 74% release, and a 1:250 ratio should allow for a 100% release of WEPt. Vadas (2005) generated a similar equation by combining poultry and swine manure from multiple studies and obtained the equation: $y = 2.2 [x / (x + 300.1)]$, where y is the Relative Manure WEP Release (based on total release at 1:250 manure to water ratio) and x is the water to manure ratio in mL g^{-1} [30]. Based on this equation, the expected P release would be 31% at a 1:50 ratio, 77% at a 1:163 ratio, and 100% at 1:250, highlighting the similarities in the P release for raw litters using these equations.

On day 1, incidental P loss of TDP from the raw litter (1:10) was 3.57 kg ha^{-1} or 20.7% of the WEPt added (17.02 kg ha^{-1}) to the packed soil boxes ((TDP lost on day 1 – control TDP)/WEPt added based on the 1:250 extraction), while laboratory extractions predict a 31–36% WEPt release. This means that simulated field conditions restricted the WEPt release by 1.7 times lower than the amount expected with laboratory extractions. Incidental P loss from the raw litter (1:100) was 34.5% of the WEPt added, which is 2.2 times less than the expected 74–77% release. In the raw litter (1:200), the simulated litter to water ratio (1:250) should have allowed the total release of the WEPt pool; however, the actual release was 3.6 times less than expected. Therefore, in the raw litters, as the simulated litter to water ratios widened, less of a percent WEP release occurred, limiting the predictability of wider extraction ratios during laboratory WEP extractions to estimate dissolved P loss. Incidental P loss in the raw litters was more representative of laboratory extraction ratios between 1:10 and 1:50, regardless of the simulated ratios achieved during the rainfall simulations. Since the soil used in the study was not saturated with P and had a large proportion of available P binding sites, more DRP or WEP release could have occurred and quickly sorbed to the soil. Therefore, this concept should be explored in soils with higher P saturation to understand how much WEP release occurs at different simulated litter to water ratios.

In the granulated litters, the simulated litter to water ratios widened from 1:168 (1:10) to 1:184 (1:100) to 1:191 (1:200) as the amount of granulated litter added to the packed soil boxes decreased. Unlike in the raw litter, the granulated litter released between 86 and 100% of WEPt regardless of the ratio used during laboratory extraction (Figure 7). During the simulations, the percent WEP release was 29.2–33.6%, similar to the raw litters at 1:100 and 1:200 (29–34%). Therefore, while the percent release did increase as the ratios widened, it was still 2.4 to 2.6 times lower than that predicted with a laboratory WEP extraction. Further, since laboratory extractions in the granulated litter extracted a minimum of 85% of the WEPt, they would not be useful for predicting P loss. Similarly, in the heated litter, widening the extraction ratio during laboratory extraction did not significantly affect the WEP release [59]. Since only 19% of WEPt was released during the simulated litter to water ratio of 1:250 during the rainfall simulations, laboratory WEP extractions of heated litter would also not be useful at predicting dissolved P loss. One reason the percent WEP release was lower in the simulated ratios than in laboratory extractions is because most of the WEP released with rainfall may have been adsorbed to the soil upon contact. This argument is strengthened by our previous results that showed an increase in M3–P content of the soil after the simulations concluded. However, given the wide range of simulated litter to water ratios achieved and the similar percent WEP releases, it is more likely other factors more strongly impacted incidental P loss from these litters.

Overall, the simulated litter to water ratios released between 1.7 and 6.3 times less WEPt than what a laboratory extraction of the same ratio would predict. Our results indicate that while a laboratory WEP extraction is useful for quantifying the WEP pools and predicting P loss in raw litter sources, they may not be as useful in litter sources that are treated, either through granulation or heating, and applied at rates lower than 50 kg of total P ha^{-1}.

3.5 Conclusions

This research investigated the effect of litter type and application rate (based on WEP) on dissolved P forms in runoff and on P levels in soil. While the intent was to add litters based on 3 kg WEP ha^{-1}, equilibrium constraints between the litter solid phase P and solution (water) P led to underestimating the amount of WEP added in the raw litters (1:10 and 1:100). The underestimation on WEP added in the raw litters

(1:10 and 1:100) highlights the importance of correct WEP extraction methodology when determining total amounts of WEP applied to soils. While TDP loss from the comparable litter treatments were similar, only lower in the heated than the granulated (1:10 and 1:200). Compared to the WEP added, the heated litter treatment lost a significantly less amount of TDP than the raw and granulated litters, even though it had the highest WEP solubility and content because the dissociated Mg and Ca were able to rebind with P upon contact with the soil, preventing P from entering runoff. The litter to water ratios during the simulations ranged from 1:50 to 1:300 and did not match the expected WEP release during laboratory extractions. In the raw litters, TDP loss reflected laboratory extraction ratios between 1:10 and 1:50, whereas in the granulated and heated litters, TDP loss did not reflect laboratory extractions at any ratio, due to the high solubility of WEP in the litter products, making WEP extractions less effective at predicting P loss from these litter products. The post-simulation litter analyses revealed similar WEPt contents among the litters indicating similar equilibria between litter solid phase P and solution P, and therefore, similar P loss dynamic from litters after the rainfall simulations. The addition of raw litter increased the soil M3–P between 1.5 and 2 times the amounts of heated and granulated litters, making the litter products more ideal to field applications in P saturated soils. The heated and granulated litters increased the soil M3–P content similar to a P fertilizer [111], making it an alternative to commercial fertilizers that could have more favorable properties, such as additional nutrients like Ca and Mg that bind labile P and reduce dissolved P in runoff. Although there has been concern for increased runoff P due to the high solubility of WEP in litter products, this research proves they are a viable option for the continued and enhanced use of poultry litter in non-agricultural areas.

Table 7. Characteristics of Poultry Litter and Products (Adapted from Toor et al., 2007).

	Raw Litter	**Granulated Litter**	**Heated Litter**
Litter Description	Unprocessed	Raw litter sieved though a 5.8mm sieve, granulated at 232 ^{o}C, dried at 121 ^{o}C, urea added	Raw litter heated at 180 ^{o}C for 2 hours
Dry Matter (%)	69.5	90	100
Total N (g kg^{-1})	30.6	141.3	30.6
Total P (g kg^{-1})	22.7	17.7	22.7
Total N:P	1.35:1	7.98:1	1.35:1
WEP @ 1:10 (mg kg^{-1})	1277 ± 163	4326 ± 471	6860 ± 222
WEP @ 1:100 (mg kg^{-1})	4183 ± 389	4723 ± 178	7696 ± 454
WEP @ 1:200 (mg kg^{-1})	6429 ± 874	4934 ± 167	8029 ± 486

Table 8. **Amount of litter and water-extractable P (inorganic (WEPi), organic (WEPo), and total (WEPt)) added to runoff simulation soil boxes based on 3 kg ha^{-1} of WEPt at various litter to water ratios in the raw, granulated, and heated litter treatments.**

Treatment	WEP ratio	Litter WEPt	Litter added	Litter added	WEPi added	WEPo added	WEPt added
		mg kg^{-1}	*g box^{-1}*	*kg ha^{-1}*	*mg box^{-1}*		
Raw	1:10	1278	47.0	2348	45.1	14.9	60.1
Raw	1:100	4183	14.3	717	52.8	7.0	59.8
Raw	1:200	6429	9.3	467	52.0	7.8	59.8
Granulated	1:10	4327	13.9	693	50.7	9.5	60.1
Granulated	1:100	4732	12.7	635	59.0	1.0	60.0
Granulated	1:200	4934	12.2	608	57.2	3.0	60.2
Heated	1:100	7696	7.8	390	50.0	10.0	60.0

Notes: WEP values are an average of 3 replicates. Each runoff box was 0.2 m^2

Table 9. Total amounts of water extractable P inorganic (WEPi), organic (WEPo), and total (WEPt) forms lost and added to runoff simulation boxes based on the 1:250 extraction (Toor et al., 2007) of WEP in raw, granulated, and heated litters.

				Added			Lost	
Treatment	**WEP ratio**	**Litter WEPt**	**WEPi**	**WEPo**	**WEPt**	**DRP**	**DUP**	**TDP**
		mg kg^{-1}		***kg ha^{-1}***			***kg ha^{-1}***	
Raw	1:10	7241	15.13	1.88	17.02	2.88	2.13	5.02 (28.7%)
Raw	1:100	7241	4.60	0.57	5.18	1.54	0.76	2.30 (41.7%
Raw	1:200	7241	2.99	0.37	3.37	0.81	0.48	1.30 (34.2%)
Granulated	1:10	5053	3.31	0.20	3.51	1.10	0.40	1.50 (38.7%)
Granulated	1:100	5053	3.03	0.18	3.21	1.09	0.33	1.42 (39.8%)
Granulated	1:200	5053	2.91	0.17	3.08	1.07	0.38	1.46 (42.7%)
Heated	1:100	8158	2.51	0.68	3.18	0.58	0.31	0.89 (23.6%)

WEP added and lost values are an average of 3 replicates. WEP lost = cumulatively across the 3 rainfall simulations. Values in parentheses represent %WEPt lost taken by subtracting control TDP lost (0.14 kg ha^{-1}) from TDP lost in the litter treatments and dividing by WEPt.

Table 10. Concentrations and percent reductions in water extractable P inorganic (WEPi), organic (WEPo), and total (WEPt) forms determined in litter and litter products in laboratory extractions before and after three runoff simulations.

Treatment	**WEP ratio**	**WEPi before**	**WEPi after**	**%WEPi reduction**	**WEPo before**	**WEPo after**	**%WEPo reduction**	**WEPt before**	**WEPt after**	**%WEPt reduction**
		——***mg kg^{-1}***	——		——***mg kg^{-1}***	——		——***mg kg^{-1}***	——	
Raw	1:10	3690	740*a*	79.9*a*	493	102*a*	79.3*a*	4183	842*ab*	79.9*a*
Raw	1:100	3690	586*bc*	84.1*b*	493	156*b*	68.4*ab*	4183	742*b*	82.3*ab*
Raw	1:200	3690	593*bc*	83.9*b*	493	230*cd*	53.3*b*	4183	823*ab*	80.3*a*
Granulated	1:10	4614	544*bc*	88.1*c*	109	167*b*	-53.2*c*	4723	711*b*	84.9*b*
Granulated	1:100	4614	556*bc*	88.2*c*	109	224*c*	-105.5*d*	4723	780*ab*	83.5*b*
Granulated	1:200	4614	473*c*	89.7*c*	109	252*cd*	-131.2*e*	4723	725*b*	84.6*b*
Heated	1:100	6301	657*ab*	89.6c	1395	278*d*	80.1*a*	7696	935*a*	87.9*c*

Letters indicate statistically different concentrations within each column. All WEP values are an average of 3 replicates.

Table 11. Post-simulation analysis (WEPi and M3-P) on the soil from the packed soil boxes receiving litter treatments.

Treatment	WEP ratio	WEPi	M3-P	%WEPi	%M3-P	M3-P increase
		——mg kg^{-1}——		*——% increase——*		*mg kg^{-1} (per 1 kg of TP added)*
Control	-	0.306	6.9	-	-	-
Raw	1:10	2.01	42.6	656.9	616.4	0.674
Raw	1:100	0.732	18.1	239.2	257.7	0.700
Raw	1:200	0.225	14.1	-26.5	202.3	0.655
Granulated	1:10	0.253	10.7	-17.3	152.1	0.317
Granulated	1:100	0.258	11.6	-15.7	165.3	0.428
Granulated	1:200	0.285	10.4	-6.9	146.9	0.318
Heated	1:100	0.301	10.5	-1.6	149.3	0.400

Percent increases were taken from the difference between the control treatment and the litter treatments. M3-P increase was taken as the net increase in the soil's M3-P divided by the amount of Total P in the litters

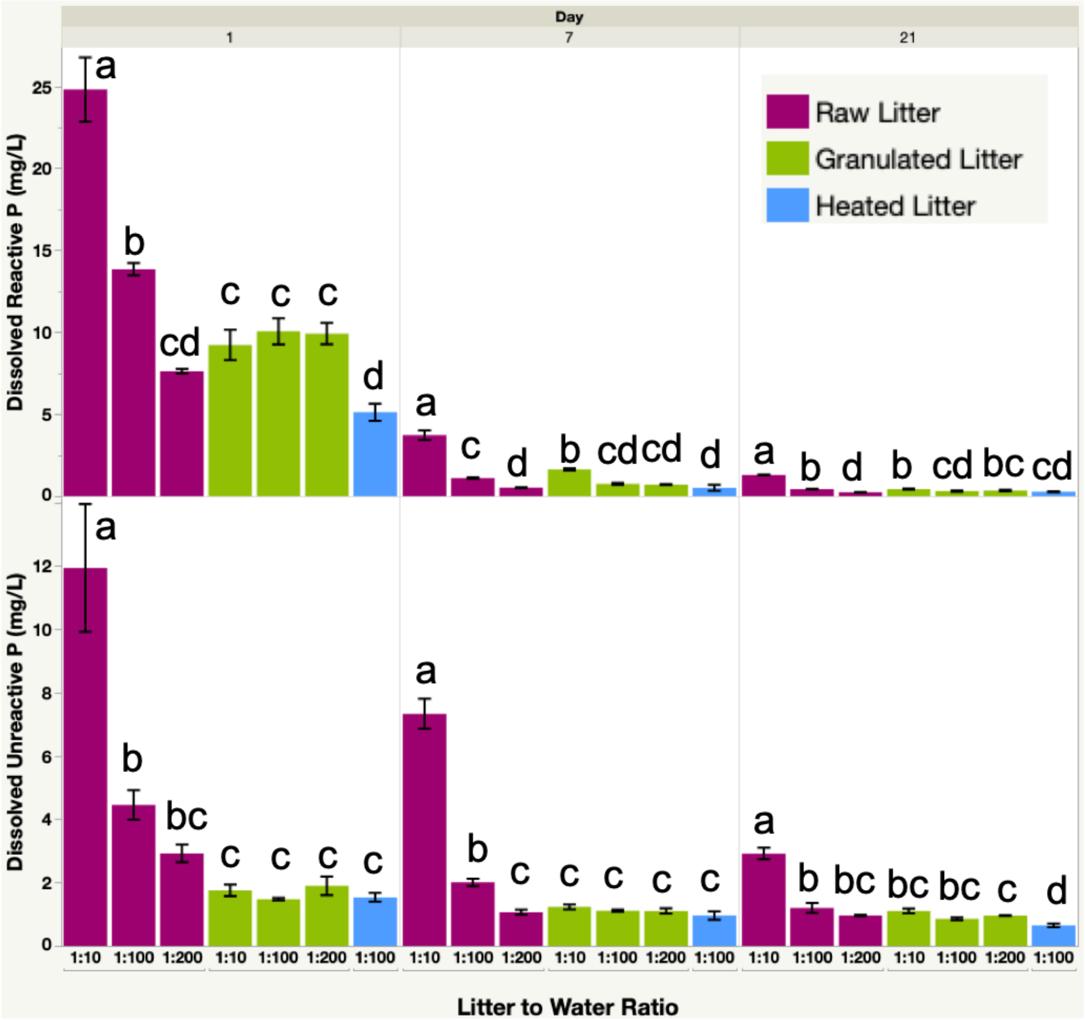

Figure 5. Concentrations of Dissolved Reactive P (DRP) and Dissolved Unreactive P (DUP) during three rainfall simulation events (1, 7, 21 days). Each bar for raw litter (purple), granulated litter (green) and heated litter (blue) represents the mean and standard error of three replicates. Letters within each simulation event (1, 7, or 21 days) for DRP or DUP indicate statistically different concentrations among three litter and extraction ratio treatments at $p < 0.05$.

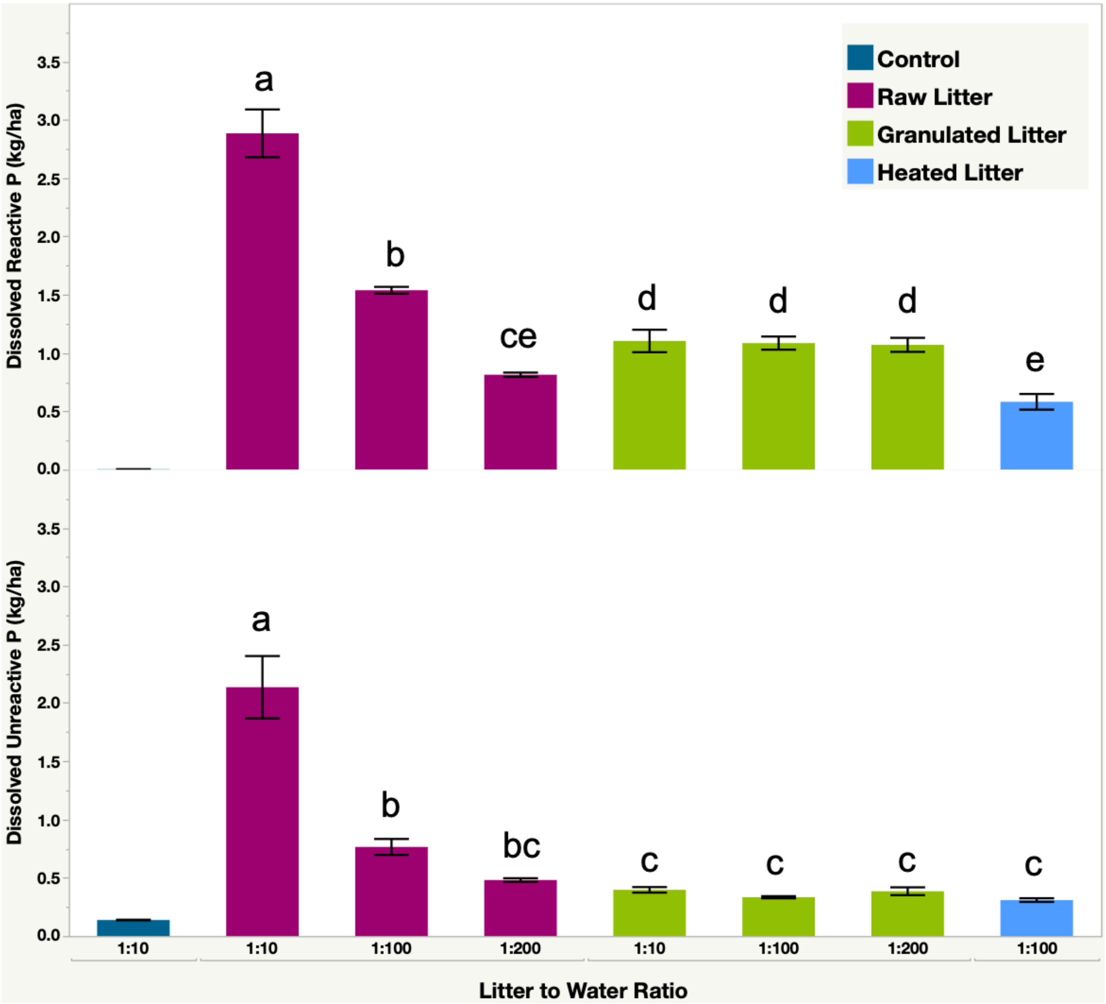

Figure 6. Cumulative Dissolved Reactive P (DRP) and Dissolved Unreactive P (DUP) losses from the three simulation events. Each bar for control (teal), raw litter (purple), granulated litter (green), and heated litter (blue), represents the mean and standard error of the three replicates. Letters indicate statistically different DRP and DUP losses among the litter and extraction ratio treatments at *p* < 0.05. The control treatment had significantly lower DRP and DUP losses than litter treatments.

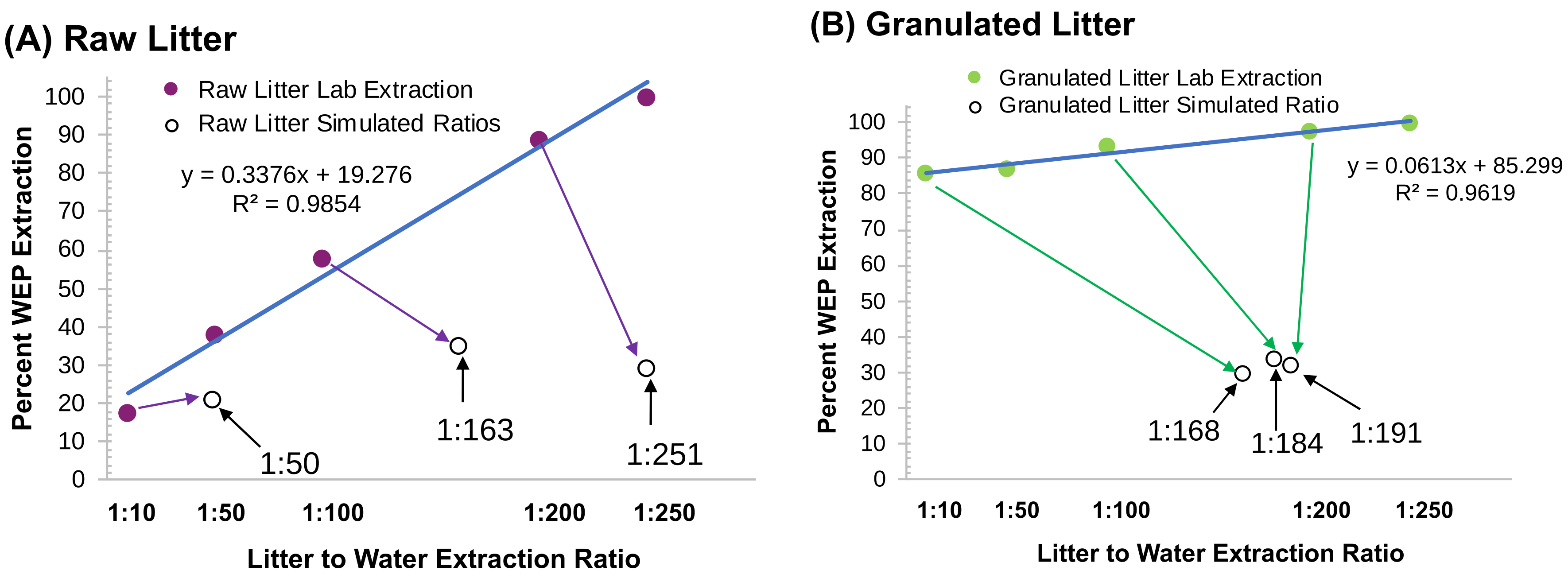

Figure 7. Percent WEP release as a function of the litter to water ratio assuming 1:250 ratio extracted 100 percent of WEP for (A) raw litter and (B) granulated litter. In the raw litter treatment, simulated litter to water ratio was 1:50, 1:163, and 1:251 corresponding to 1:10, 1:100, and 1:200 lab extraction ratios, respectively. In the granulated litter treatment, simulated litter to water ratio was 1:168, 1:184, and 1:191 corresponding to 1:10, 1:100, and 1:200 lab extraction ratios, respectively. Laboratory extractions of WEP data are from Toor et al. [59].

Chapter 4: Final Conclusions and Remarks

This thesis aimed to expand our understanding of an important P pool in soils and manures, the WEP pool. WEP is the most labile and easily lost P pool, therefore, its accurate quantification is critical to reduce P loss from land to water bodies and mitigate eutrophication. In Chapter 2, exhaustive laboratory extractions of WEP in high P soils in Maryland revealed that conventional methodology is underestimating the WEP pool in soils that have already exceeded environmental thresholds. A proposed and enhanced method for laboratory WEP extractions (1:100 soil to water) allows for a larger WEP pool release due to an increased equilibrium between soil solid phase P and solution P and eliminates significant discrepancies between colorimetry and spectroscopy analysis techniques. While the scope of this research was limited to laboratory extractions, further research should investigate how long-term P release from legacy P soils affect the WEP pool and relates to WEP content in the soil. While conventional extraction ratios are useful for assessing incidental P loss from soils due to the lower soil to rainfall ratios reached in natural conditions, they may not be directly related to longer-term P loss as the WEP pool will be continuously replenished from other P pools in the soils.

Manure application to agricultural lands is one of the reasons soils like those studied in Chapter 2 are saturated with P. While many states have implemented manure guidelines which limit or restrict additional manure input to soils, the timescale on which P drawdown is occurring is not meeting USEPA goals. Since manure is a rich source of P and global reserves of P are facing depletion in the next

50 years, it may be the only source of P available in the future. Therefore, we must stabilize P levels in soils below environmental thresholds and enhance manure management so that manure application can be a viable option for nutrient addition. Chapter 3 showed how accurate WEP extraction methodology is also critical for understanding P loss in manure-treated soils. Although there has been concern about the high solubility of poultry litter products created with granulation and heating, this research showed that TDP losses are comparable to unprocessed poultry litter when applied at the same WEP application rate. Further, the heated and granulated litters resulted in a 1.5 to 2 times less of an increase in soil M3–P than unprocessed litter. Therefore, these litter products may be beneficial over raw litter, in soils where adequate levels of M3–P exist, as they would not saturate the P-binding sites as much. Overall, a WEP based application (if WEP is added based on a wider extraction ratio such as 1:250) of poultry litter and products can be useful for predicting dissolved P runoff losses. However, using the extracted WEP content of processed poultry litters to predict P loss in runoff is not as useful as in raw litter due to the high solubility of WEP at low extraction ratios.

Although BMPs and manure management guidelines have aimed to reduce P loss from agricultural lands negatively impacting water quality, they are not working on a timescale necessary to meet nutrient loading goals. This research highlighted one of the reasons legacy P soils are highly problematic, a large WEP pool, and demonstrated through sequential extractions that P loss will continue for a considerable amount of time. Further, it showed that conventional drawdown techniques such as P mining by crops, will not successfully drawdown P levels in these legacy soils to meet nutrient loading goals. If we are to successfully mitigate P loss from these soils, innovative techniques that aim to transform labile P forms into more recalcitrant forms in necessary. However, it is imperative that future research takes a cost-analysis approach for farmers. As further P additions are prohibited on these fields, these techniques should ensure optimal crop growth so not to impact farmer profit. One interesting option could be strictly controlling irrigation frequency (in irrigated fields) and magnitude to induce redox oscillations through rapid wet-dry cycles. These cycles could synthesize reactive, poorly crystalline/amorphous, Fe(III)-(oxyhydr)oxides in the soil itself to remove P. However, short term trade-offs should be considered, as well as the cost and impact these cycles would have on optimal crop growth and overall stability of soil P, soil organic matter, minerals, and greenhouse gases like CO_2, CH_4, and N_2O.

With the global depletion of P nearing, it is important to not only control further P loss from legacy soils but also to properly manage manure on low P soils so that farmers can continue to utilize its rich source of nutrients. Further, the increase in soil organic matter through manure addition promotes favorable properties such as soil aggregation and increased moisture holding capacity, both of which increase crop growth and stabilize soil against erosion. As most farmers concentrated near intensive livestock operations are no longer able to provide these benefits to their soils through manure addition, we may see a decrease in the overall stability of high P soils overtime as decomposition occurs. This would mean both labile P and particulate P forms, which are primarily lost during soil erosion, could be more prone to loss. Manure processing through granulation and heating could be an exciting alternative fertilizer for urban markets. As there was still significant dissolved P loss from the rainfall simulations in this research, field application should be carefully explored and managed. However, as low P soils were used in this research, manure P is expected to dominate P loss. It would be useful to add processed manure to legacy P soils at low WEP application rates to investigate if dissolved P losses and the impact on soil P levels between a control were still significant.

Overall, this research shows how imperative it is to have regulations and guidelines to prevent additional soils from reaching P levels above environmental thresholds. Periodic evaluations on how current management guidelines are affecting soil P levels and runoff P losses should be conducted and guidelines adjusted as needed. As cost-effective drawdown techniques are not working on legacy P soils and manure addition that would provide soils favorable properties to combat soil erosion and promote optimal crop growth is prohibited, we must press forward to find new solutions to meet nutrient loading goals. However, for any innovative solution to be effective, negative impacts on farmers such as decreased crop yields or financial obligations must be considered.

Bibliography

1. Ashley, K., D. Cordell, and D. Mavinic, *A Brief History of Phosphorus: From the Philosopher's Stone to Nutrient Recovery and Reuse.* Chemosphere, 2011. **84**(6): p. 737-746.
2. Falkowski, P., et al., *The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System.* Science, 2000. **290**(5490): p. 291-296.
3. Brinck, J.W., *World Resources of Phosphorus.* Ciba Foundation Symposium Sept 13–15, 1977: p. 23-48.
4. Smil, V., *Phosphorus in the Environment: Natural Flows and Human Interferences.* Annual Review of Environment and Resources, 2000. **25**: p. 53-88.
5. Elser, J. and E. Bennett, *A Broken Biogeochemical Cycle.* Nature, 2011. **478**(7367): p. 29-31.
6. Cordell, D., J.-O. Drangert, and S. White, *The Story of Phosphorus: Global Food Security and Food for Thought.* Global Environmental Change, 2009. **19**(2): p. 292-305.
7. USEPA, *Environmental Indicators of Water Quality in the United States.* EPA 841-R-96-002. USEPA, Office of Water (4503F), 1996.
8. Lawton, L. and G. Codd, *Cyanobacterial (Blue-Green Algal) Toxins and Their Significance in UK and European Waters.* Water and Environment Journal, 2007. **5**: p. 460-465.
9. Martin, A.a.C., G.D., *Health Risks in Eutrophic Water Supplies.* LakeLine, 1994. **14**(1): p. 24-25.

10. Boesch, D., R. Brinsfield, and R. Magnien, *Chesapeake Bay Eutrophication: Scientific Understanding, Ecosystem Restoration, and Challenges for Agriculture.* Journal of environmental quality, 2000. **30**: p. 303-20.

11. Sharpley, A., B. Foy, and P. Withers, *Practical and Innovative Measures for the Control of Agricultural Phosphorus Losses to Water: An Overview.* Journal of Environmental Quality, 2000. **29**(1): p. 1-9.

12. USEPA, *Chesapeake Bay Total Maximum Daily Load for Nitrogen, Phosphorus, and Sediment.* U.S. Environmental Protection Agency 2010: p. 1-93. https://www.epa.gov/sites/production/files/2014-12/documents/cbay_final_tmdl_exec_sum_section_1_through_3_final_0.pdf.

13. Kleinman, P.J.A., et al., *Phosphorus and the Chesapeake Bay: Lingering Issues and Emerging Concerns for Agriculture.* Journal of Environmental Quality, 2019. **48**(5): p. 1191-1203.

14. Pote, D., et al., *Relating Extractable Soil Phosphorus to Phosphorus Losses in Runoff.* Soil Science Society of America Journal, 1996. **60**: p. 855-859.

15. Kusmer, A.S., et al., *Watershed Buffering of Legacy Phosphorus Pressure at a Regional Scale: A Comparison Across Space and Time.* Ecosystems, 2019. **22**(1): p. 91-109.

16. Maguire, R.O. and J.T. Sims, *Soil Testing to Predict Phosphorus Leaching.* Journal of Environmental Quality, 2002. **31**(5): p. 1601-1609.

17. UME, *Soil Fertility Management: Converting Among Soil Test Analyses Frequently Used in Maryland.* Extension Publication SFM-4, University of Maryland Extension, 2001.

https://mda.maryland.gov/resource_conservation/counties/SoilConversions.pdf.

18. MDA, *Soils Data Collection and Verification as of January 2019.* Maryland Department of Agriculture, 2019. https://mda.maryland.gov/Pages/PMT.aspx.
19. Sharpley, A.N., et al., *Development of phosphorus indices for nutrient management planning strategies in the United States.* Journal of Soil and Water Conservation, 2003. **58**(3): p. 137-152.
20. Fiorellino, N., R. Kratochvil, and F. Coale, *Long-Term Agronomic Drawdown of Soil Phosphorus in Mid-Atlantic Coastal Plain Soils.* Agronomy Journal, 2017. **109**(2): p. 455-461.
21. Sharpley, A., P. Kleinman, and J. Weld, *Assessment of Best Management Practices to Minimise the Runoff of Manure-borne Phosphorus in the United States.* New Zealand Journal of Agricultural Research, 2004. **47**(4): p. 461-477.
22. Moore, P.A., et al., *Poultry Manure Management: Environmentally Sound Options.* Journal of Soil and Water Conservation, 1995. **50**(3): p. 321-327.
23. Williams, J.R., *The Erosion-Productivity Impact Calculator (EPIC) Model: A Case History.* Philosophical Transactions: Biological Sciences, 1990. **329**(1255): p. 421-428.
24. Leonard, A.R., Knisel, W.G. and D. A. Still, *GLEAMS: Groundwater Loading Effects of Agricultural Management Systems.* Transactions of the ASAE, 1987. **30**(5): p. 1403-1418.

25. Bouraoui, F. and T.A. Dillaha, *ANSWERS-2000: Runoff and Sediment Transport Model.* Journal of Environmental Engineering, 1996. **122**(6): p. 493-502.

26. Arnold, J.G., et al., *Large Area Hydrologic Modeling and Assessment Part i: Model Development.* Journal of the American Water Resources Association, 1998. **34**: p. 73-89.

27. Pierson, S.T., et al., *Phosphorus Losses from Grasslands Fertilized with Broiler Litter: EPIC Simulations.* Journal of Environmental Quality, 2001. **30**(5): p. 1790-1795.

28. Sharpley, A.N., et al., *Modeling Phosphorus Transport in Agricultural Watersheds: Processes and Possibilities.* Journal of Soil and Water Conservation, 2002. **57**(6): p. 425-439.

29. Vadas, P.A., P.J.A. Kleinman, and A.N. Sharpley, *A Simple Method to Predict Dissolved Phosphorus in Runoff from Surface-Applied Manures.* Journal of Environmental Quality, 2004. **33**(2): p. 749-756.

30. Vadas, P.A., B.E. Haggard, and W.J. Gburek, *Predicting Dissolved Phosphorus in Runoff from Manured Field Plots.* Journal of Environmental Quality, 2005. **34**(4): p. 1347-1353.

31. SERA-17, *National Research Project for Simulated Rainfall – Surface Runoff Studies: Protocol.* 2002. http://www.sera17.ext.vt.edu/Documents/National_P_Protocals.pdf.

32. Delaune, P.B., et al., *Evaluation of the Phosphorus Source Component in the Phosphorus Index for Pastures.* Journal of Environmental Quality, 2004. **33**(6): p. 2192-2200.

33. Kleinman, P.J.A. and A.N. Sharpley, *Effect of Broadcast Manure on Runoff Phosphorus Concentrations over Successive Rainfall Events.* Journal of Environmental Quality, 2003. **32**(3): p. 1072-1081.

34. Kleinman, P.J.A., et al., *Evaluation of Phosphorus Transport in Surface Runoff from Packed Soil Boxes.* Journal of Environmental Quality, 2004. **33**(4): p. 1413-1423.

35. McDowell, R.W., R.M. Monaghan, and P.L. Carey, *Potential Phosphorus Losses in Overland Flow from Pastoral Soils Receiving Long-term Applications of Either Superphosphate or Reactive Phosphate Rock.* New Zealand Journal of Agricultural Research, 2003. **46**(4): p. 329-337.

36. Shigaki, F., A. Sharpley, and L.I. Prochnow, *Source-Related Transport of Phosphorus in Surface Runoff.* Journal of Environmental Quality, 2006. **35**(6): p. 2229-2235.

37. Nash, J.E. and J.V. Sutcliffe, *River Flow Forecasting Through Conceptual Models Part I — A Discussion of Principles.* Journal of Hydrology, 1970. **10**(3): p. 282-290.

38. Owens, L.B. and M.J. Shipitalo, *Surface and Subsurface Phosphorus Losses from Fertilized Pasture Systems in Ohio.* Journal of Environmental Quality, 2006. **35**(4): p. 1101-1109.

39. Vadas, P.A., et al., *The Effect of Rain and Runoff When Assessing Timing of Manure Application and Dissolved Phosphorus Loss in Runoff.* JAWRA Journal of the American Water Resources Association, 2011. **47**(4): p. 877-886.

40. Weld, J.L., D.B Beegle, J.T. Sims, J.L. Lemunyon, A.N. Sharpley, W.J. Gburek, F.C. Coale, and A.B. Leytem, *Comparison of State Approaches to the Development of Phosphorus Indices.* In 2000 Annual meetings abstracts. ASA, CSSA, and SSSA, Madison, WI., 2000: p. 329.

41. Reddy, K.R., et al., *Phosphorus Adsorption-Desorption Characteristics of Two Soils Utilized for Disposal of Animal Wastes.* Journal of Environmental Quality, 1980. **9**(1): p. 86-92.

42. Sharpley, A.N., J.K. Syers, and R.W. Tillman, *An Improved Soil-Sampling Procedure for the Prediction of Dissolved Inorganic Phosphate Concentrations in Surface Runoff from Pasture.* Journal of Environmental Quality, 1978. **7**(3): p. 455-456.

43. Simard, R.R., et al., *Phosphorus Status of Forest and Agricultural Soils from a Watershed of High Animal Density.* Journal of Environmental Quality, 1995. **24**(5): p. 1010-1017.

44. Toor, G.S. and J.T. Sims, *Managing Phosphorus Leaching in Mid-Atlantic Soils: Importance of Legacy Sources.* Vadose Zone Journal, 2015. **14**(12): p. 1-12.

45. Toor, G.S., et al., *Phosphorus Pools in Soils under Rotational and Continuous Grazed Pastures.* Agrosystems, Geosciences & Environment, 2020. **3**(1): p. e20103.

46. Daniel, T.C., et al., *Minimizing Surface Water Eutrophication from Agriculture by Phosphorus Management.* Journal of Soil and Water Conservation, 1994. **49**(2): p. 30.

47. Carpenter, S.R., *Phosphorus Control is Critical to Mitigating Eutrophication.* Proceedings of the National Academy of Sciences, 2008. **105**(32): p. 11039-11040.

48. Osterholz, W.R., B.R. Hanrahan, and K.W. King, *Legacy Phosphorus Concentration–discharge Relationships in Surface Runoff and Tile Drainage from Ohio Crop Fields.* Journal of Environmental Quality, 2020. **49**(3): p. 675-687.

49. Toor, G.S. and J.T. Sims, *Phosphorus Leaching in Soils Amended with Animal Manures Generated from Modified Diets.* Journal of Environmental Quality, 2016. **45**(4): p. 1385-1391.

50. Toor, G.S. and J.T. Sims, *Managing Legacy and New Sources of Phosphorus to Reduce Leaching in Mid-Atlantic Soils.* Crops & Soils, 2016. **49**(5): p. 40-47.

51. Kleinman, P.J.A., et al., *Dynamics of Phosphorus Transfers from Heavily Manured Coastal Plain Soils to Drainage Ditches.* Journal of Soil and Water Conservation, 2007. **62**(4): p. 225-235.

52. Osmond, D.L., et al., *Comparing Ratings of the Southern Phosphorus Indices.* Journal of Soil and Water Conservation, 2006. **61**(6): p. 325-337.

53. Sharpley, A.N., *Dependence of Runoff Phosphorus on Extractable Soil Phosphorus.* Journal of Environmental Quality, 1995. **24**(5): p. 920-926.

54. Hooda, P.S., et al., *Relating Soil Phosphorus Indices to Potential Phosphorus Release to Water.* Journal of Environmental Quality, 2000. **29**(4): p. 1166-1171.

55. Self-Davis, M.L., Moore Jr., P.A., Joern, B.C., *Determination of Water- and/or Dilute Salt-extractable Phosphorus.* Methods of Phosphorous Analysis for Soils, Sediments, Residuals, and Waters, Southern Cooperative Series Bulletin, 2000. **396**: p. 24-26.

56. Beauchemin, S., et al., *Speciation of Phosphorus in Phosphorus-enriched Agricultural Soils Using X-ray Absorption Near-edge Structure Spectroscopy and Chemical Fractionation.* Journal of Environmental Quality, 2003. **32**(5): p. 1809-1819.

57. Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan, *Changes in Inorganic and Organic Soil-phosphorus Fractions Induced by Cultivation Practices and Laboratory Inclubations.* Soil Science Society of America Journal, 1982. **46**(5): p. 970-976.

58. Sharpley, A.N., R.W. McDowell, and P.J.A. Kleinman, *Amounts, Forms, and Solubility of Phosphorus in Soils Receiving Manure.* Soil Science Society of America Journal, 2004. **68**(6): p. 2048-2057.

59. Toor, G.S., et al., *Phosphorus Solubility in Poultry Litters and Granulates: Influence of Litter Treatments and Extraction Ratios.* Transactions of the Asabe, 2007. **50**(2): p. 533-542.

60. Kleinman, P., et al., *Selection of a Water-Extractable Phosphorus Test for Manures and Biosolids as an Indicator of Runoff Loss Potential.* Journal of Environmental Quality, 2007. **36**(5): p. 1357-1367.

61. Kleinman, P.J.A., et al., *Effect of Mineral and Manure Phosphorus Sources on Runoff Phosphorus.* Journal of Environmental Quality, 2002. **31**(6): p. 2026-2033.

62. Sharpley, A.N., *Depth of surface soil-runoff interaction as affected by rainfall, soil slope, and management* Soil Science Society of America Journal, 1985. **49**(4): p. 1010-1015.

63. Vadas, P.A., Mallarino, A. P., McFarland, A., *The Importance of Sampling Depth when Testing Soils for their Potential to Supply Phosphorus to Surface Runoff.* SERA17/IEG, 2005: p. 14.

64. Murphy, J. and J.P. Riley, *A Modified Single Solution Method for the Determination of Phosphate in Natural Waters.* Analytica Chimica Acta, 1962. **27**: p. 31-36.

65. Gee, G.W., Orr, D., *2.4 Particle-Size Analysis*, in *Methods of Soil Analysis*. 2002. p. 255-293.

66. Wolf, A.M., Beegle, D.B., *Recommended Soil Tests for Macronutrients.* Recommended Soil Testing Procedures for the Northeastern United States, 2011. **493**(3): p. 39-47.

67. USEPA, *U.S. EPA Method 3050B Acid Digestion of Sediments, Sludges, and Soils. Revision 2. Washington, DC.* 1996: p. 1-12. http://xenco.com/mobile/pdf/tech/SW846/SW846-3000series/SW846-3050b.pdf.

68. Fuhrman, J.K., et al., *Water-soluble Phosphorus as Affected by Soil to Extractant Ratios, Extraction Times, and Electrolyte.* Communications in Soil Science and Plant Analysis, 2005. **36**(7-8): p. 925-935.

69. Sharpley, A.N., *Effect of Soil Properties on the Kinetics of Phosphorus Desorption.* Soil Science Society of America Journal, 1983. **47**(3): p. 462-467.

70. Leytem, A.B., R.L. Mikkelsen, and J.W. Gilliam, *Sorption of Phosphorus Compounds in Atlantic Coastal Plain Soils.* Soil Science, 2002. **167**(10): p. 652-658.

71. Waldrip, H.M., et al., *Legacy Phosphorus in Calcareous Soils: Effects of Long-Term Poultry Litter Application.* Soil Science Society of America Journal, 2015. **79**(6): p. 1601-1614.

72. Koopmans, G.F., W.J. Chardon, and R.W. McDowell, *Phosphorus Movement and Speciation in a Sandy Soil Profile After Long-term Animal Manure Applications.* Journal of Environmental Quality, 2007. **36**(1): p. 305-315.

73. Dodd, R.J. and A.N. Sharpley, *Recognizing the Role of Soil Organic Phosphorus in Soil Fertility and Water Quality.* Resources Conservation and Recycling, 2015. **105**: p. 282-293.

74. USEPA, *Ambient Water Quality Criteria Recommendations Information Supporting the Development of State and Tribal Nutrient Criteria Rivers and Streams in Nutrient Ecoregion XIV.* EPA 822-B-00-022, 2000.

75. Nair, V.D., et al., *An Environmental Threshold for Degree of Phosphorus Saturation in Sandy Soils.* Journal of Environmental Quality, 2004. **33**(1): p. 107-113.

76. Sims, J.T., et al., *Evaluation of Mehlich 3 as an Agri-environmental Soil Phosphorus Test for the Mid-Atlantic United States of America.* Soil Science Society of America Journal, 2002. **66**(6): p. 2016-2032.

77. Dari, B., et al., *Consistency of the Threshold Phosphorus Saturation Ratio across a Wide Geographic Range of Acid Soils.* Agrosystems, Geosciences & Environment, 2018. **1**(1): p. 180028.

78. Dubrovsky, N.M., Burow, K.R., Clark, G.M., Gronberg, J.M., Hamilton, P.A., and Hitt, K.J. et al., *The Quality of our Nation's Waters—Nutrients in the Nation's Streams and Groundwater, 1992–2004. USGS Circ. 1350. USGS.* 2010.

79. USEPA, *Watershed Assessment, Tracking & Environmental Results. National Probable Sources Contribution to Impairments. USEPA.* 2015. https://iaspub.epa.gov/waters10/attains_nation_cy.control#prob_source.

80. Sharpley, A., *Managing Agricultural Phosphorus to Minimize Water Quality Impacts.* Scientia Agricola, 2016. **73**(1): p. 1-8.

81. USEPA, *USDA and USEPA Unified National Strategy for Animal Feeding Operations.* Washington, DC., 1999: p. 1-34. www.epa.gov/npdes/pubs/finafost.pdf.

82. Bustamante, M., et al., *Co-composting of the Solid Fraction of Anaerobic Digestates, to Obtain Added-value Materials for Use in Agriculture.* Biomass & Bioenergy, 2012. **43**: p. 26-35.

83. Vandecasteele, B., et al., *Composting for Increasing the Fertilizer Value of Chicken Manure: Effects of Feedstock on P Availability.* Waste and Biomass Valorization, 2014. **5**(3): p. 491-503.

84. Greenwood, D.J., et al., *A Unifying Concept for the Dependence of Whole-crop N : P Ratio on Biomass: Theory and Experiment.* Annals of Botany, 2008. **102**(6): p. 967-977.

85. Beauchamp, E.G. and H.A. Hamilton, *Optimum Ratios of Nitrogen and Phosphorus Fertilizers for Corn Determined by Homes Method of Systematic Variations* Canadian Journal of Plant Science, 1970. **50**(2): p. 141-150.

86. Veneklaas, E.J., et al., *Opportunities for Improving Phosphorus-use Efficiency in Crop Plants.* New Phytologist, 2012. **195**(2): p. 306-320.

87. Sims, J.T., et al., *Integrating Soil Phosphorus Testing into Environmentally Based Agricultural Management Practices.* Journal of Environmental Quality, 2000. **29**(1): p. 60-71.

88. Eghball, B., *Soil Properties as Influenced by Phosphorus- and Nitrogen-Based Manure and Compost Applications.* Agronomy Journal, 2002. **94**(1): p. 128-135.

89. Kleinman, P.J.A., et al., *Survey of Water-Extractable Phosphorus in Livestock Manures.* Soil Science Society of America Journal, 2005. **69**(3): p. 701-708.

90. Maltais-Landry, G., et al., *Long-Term Effects of Compost and Cover Crops on Soil Phosphorus in Two California Agroecosystems.* Soil Science Society of America Journal, 2015. **79**(2): p. 688-697.

91. Sharpley, A.N., *Depth of Surface Soil-runoff Interaction as Affected by Rainfall, Soil Slope, and Management.* Soil Science Society of America Journal, 1985. **49**(4): p. 1010-1015.

92. Römkens, M.J.M., D.W. Nelson, and J.V. Mannering, *Nitrogen and Phosphorus Composition of Surface Runoff as Affected by Tillage Method.* Journal of Environmental Quality, 1973. **2**(2): p. 292-295.

93. Andraski, B.J., D.H. Mueller, and T.C. Daniel, *Phosphorus Losses in Runoff As Affected by Tillage.* Soil Science Society of America Journal, 1985. **49**(6): p. 1523-1527.

94. Kleinman, P.J.A., et al., *Measuring Water-Extractable Phosphorus in Manure as an Indicator of Phosphorus in Runoff.* Soil Science Society of America Journal, 2002. **66**(6): p. 2009-2015.

95. Eghball, B., et al., *Long-Term Manure and Fertilizer Application Effects on Phosphorus and Nitrogen in Runoff.* Transactions of the American Society of Agricultural Engineers, 2002. **45**(3): p. 687-694.

96. Haggard, B.E., et al., *Nutrient and β17-Estradiol Loss in Runoff Water from Poultry Litters* JAWRA Journal of the American Water Resources Association, 2005. **41**(2): p. 245-256.

97. Pierson, S.T., et al., *Phosphorus and Ammonium Concentrations in Surface Runoff from Grasslands Fertilized with Broiler Litter.* Journal of Environmental Quality, 2001. **30**(5): p. 1784-1789.

98. Volf, C.A., et al., *Phosphorus Losses in Simulated Rainfall Runoff from Manured Soils of Alberta.* Journal of Environmental Quality, 2007. **36**(3): p. 730-741.

99. Smith, D.R., et al., *Reducing Phosphorus Runoff from Swine Manure with Dietary Phytase and Aluminum Chloride.* Journal of Environment Quality, 2004. **33**(3): p. 1048-1054.

100. Haggard, B., et al., *Effect of Poultry Litter to Water Ratios on Extractable Phosphorus Content and its Relation to Runoff Phosphorus Concentrations.* Biosystems Engineering, 2005. **92**: p. 409-417.

101. Zarcinas, B.A., B. Cartwright, and L.R. Spouncer, *Nitric Acid Digestion and Multi-element Analysis of Plant Material by Inductively Coupled Plasma Spectrometry.* Communications in Soil Science and Plant Analysis, 1987. **18**(1): p. 131-146.

102. Campbell, C.R., and Plank, C.O., *Sample Preperation.* Plant Analysis References for the Southern Region of the United States. Southern Cooperative Series., 1991. **Bulletin 368**.

103. APHA, *Standard Methods for the Examination of Water and Wastewater.* American Public Heallth Association, Washington D.C., 1998. **20th ed**: p. 2-56.

104. Humphry, J.B., et al., *A Portable Rainfall Simulator for Plot-scale Runoff Studies* Applied Engineering in Agriculture, 2002. **18**(2): p. 199-204.

105. USDC, *Rainfall Frequency Atlas of the United States. Technical Paper No. 40.* United States Department of Commerce, 1963.

106. Mehlich, A., *Mehlich 3 Soil Test Extractant: A Modification of Mehlich 2 Extractant.* Communications in Soil Science and Plant Analysis, 1984. **15**(12): p. 1409-1416.

107. Walter, M.T., et al., *Evaluation of Soluble Phosphorus Loading from Manure-applied Fields under Various Spreading Strategies.* Journal of Soil and Water Conservation, 2001. **56**(4): p. 329-335.

108. R. Edwards, D. and T. C. Daniel, *Drying Interval Effects on Runoff From Fescue Plots Receiving Swine Manure.* Transactions of the ASAE, 1993. **36**(6): p. 1673-1678.

109. Dalton, J.D., G.C. Russell, and D.H. Sieling, *Effect of Organic Matter on Phosphate Availability.* Soil Science, 1952. **73**(3): p. 173-182.

110. Toor, G.S. and G.S. Bahl, *Effect of Solitary and Integrated Use of Poultry Manure and Fertilizer Phosphorus on the Dynamics of P Availability in Different Soils.* Bioresource Technology, 1997. **62**(1-2): p. 25-28.

111. Toor, G.S., J.T. Sims, and Z. Dou, *Reducing Phosphorus in Dairy Diets Improves Farm Nutrient Balances and Decreases the Risk of Nonpoint Pollution of Surface and Ground Waters.* Agriculture, Ecosystems & Environment, 2005. **105**(1-2): p. 401-411.