Critical Review of Polyphosphate and Polyphosphate Accumulating Organisms for Agricultural Water Quality Management

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ABSTRACT

Despite ongoing management efforts, phosphorus (P) loading from agricultural landscapes continues to impair water quality. Wastewater treatment research has enhanced our knowledge of microbial mechanisms influencing P cycling, especially regarding microbes known as polyphosphate accumulating organisms (PAOs) that store P as polyphosphate (polyP) under oxic conditions and release P under anoxic conditions. However, there is limited application of PAO research to reduce agricultural P loading and improve water quality. Herein, we conducted a meta-analysis to identify articles in Web of Science on polyP and its use by PAOs across five disciplines (i.e., wastewater treatment, terrestrial, freshwater, marine, and agriculture). We also summarized research that provides preliminary support for PAO-mediated P cycling in natural habitats. Terrestrial, freshwater, marine, and agriculture disciplines had fewer polyP and PAO articles compared to wastewater treatment, with agriculture consistently having the least. Most meta-analysis articles did not overlap disciplines. We found preliminary support for PAOs in natural habitats and identified several knowledge gaps and research opportunities. There is an urgent need for interdisciplinary research linking PAOs, polyP, and oxygen availability with existing knowledge of P forms and cycling mechanisms in natural and agricultural environments to improve agricultural P management strategies and achieve water quality goals.

1. INTRODUCTION

Nonpoint phosphorus (P) sources from agricultural landscapes constitute a substantial fraction of diffuse P loading to water bodies around the world due to land application of chemical fertilizer and manure [1–5]. Regional water quality models of the Mississippi River basin estimated that croplands, pasturelands, and rangelands delivered about 80% of P loads to the Gulf of Mexico from 1992 to 2002 [6], and a global study of gray water footprints estimated that agricultural land accounted for 38% of anthropogenic P loads to freshwater from 2002 to 2010 [5]. A recent watershed modeling study estimated that 88% of P inputs into the Great Lakes Basin came from agricultural sources [7], which have contributed to regional eutrophication issues for more than 50 years [8,9]. In addition to various model estimates, long-term monitoring records emphasize the impact of agriculture on P pollution. The majority of stream samples taken near agricultural sites exceeded the United States Environmental Protection Agency's recommendations for P (i.e., 10-1000 μg/L depending on ecoregion) from 1992 to 2004 [3]. In the midwestern United States, agricultural tile drainage contributed nearly 50% of P to Lake Erie's tributaries [10].

There are several known barriers limiting progress toward effective agricultural P water quality management. First, the long-term accumulation of amended P in agricultural soils—termed "legacy P"—consistently contributes to P loading for decades to centuries after P fertilizer application stops [2,11–15]. As a result, more aggressive P management strategies are often required in agricultural soils with legacy P [15,16]. Second, traditional agricultural P management strategies have unintended water quality consequences. For example, no tillage (i.e., the practice of farming without tilling the soil; crop residues are cut and left intact on the field) and tile drains (i.e., the practice of installing perforated pipes in the subsurface to convey ponded surface water off the farm field) were promoted in the mid-1990s for their ability to reduce sediment-bound P, also referred to as particulate P (PP, Table S1), transport from farmland in the midwestern United States to Lake Erie [17]. However, until recently, no tillage and tile drainage management practices ignored the transport of unbound P, also referred to as dissolved P (DP, Table S1; refs 16, 18). Without changes in agricultural P management, tile drains may continue to transport DP and cause water quality issues such as harmful algal blooms [17,19–21]. Last, projected shifts in environmental conditions due to climate change such as increased air and water temperatures, more frequent and intense storms, and prolonged periods of drought [22,23] will further exacerbate P loading from agricultural lands to nearby water bodies, reduce the effectiveness of existing agricultural P water quality management strategies, and lead to more frequent and larger harmful algal blooms [21,24–30].

Without interdisciplinary research that leverages knowledge of microbial P forms and cycling mechanisms, excess P loading due to known (and unknown) barriers may continue to cause freshwater eutrophication and have global ecological and economic impacts. Ecologically, excess P leads to freshwater eutrophication, which causes structural changes to aquatic ecosystems such as decreases in water transparency, potential growth of toxin producing cyanobacteria, hypoxic (i.e., low oxygen) or anoxic (i.e., no oxygen) conditions, and fish die-offs [1,2,11,31,32]. Economically, freshwater eutrophication produces a range of negative outcomes, including increased spending on drinking water treatment and management of threatened and endangered species, recreational space closures, depreciation and/or loss of waterfront real estate, and decreased fish and wildlife production [1,2,33,34]. Furthermore, government support of agricultural water quality management programs can cost taxpayers millions of dollars [35].

Researchers have called for interdisciplinary research to meet global P challenges associated with food and water security [36], yet despite a great deal of research on P in agricultural systems, there remains limited focus on biological (here, microbial) P forms and cycling mechanisms. Research on microbially-mediated P cycling in specialized wastewater treatment plants (Section

5) and in the natural environment (Section 6) may help fill this research gap. Furthermore, we see microbial P forms and cycling mechanisms as rate-limiting steps in our collective knowledge and P water quality management success. Therefore, the objective of this critical review is to inspire a step change in the level of interdisciplinary research that yields more holistic views of biogeochemical P processes, overcomes persistent management challenges to reducing P pollution, and greatly improves water quality.

2. CRITICAL REVIEW OBJECTIVES & METHODS

While remaining grounded in established and effective agricultural P water quality management strategies, we emphasize the need to build on research from other disciplines to examine whether and how microbial P forms and cycling mechanisms impact P water quality goals. Herein, we focused on the role of the microbial P form known as polyphosphate (polyP, Table S1, Section 4) and P cycling mechanisms by a group of microbes known as polyphosphate accumulating organisms (PAOs, Section 5). PAOs are known to store polyP and there is preliminary evidence of their activity outside wastewater treatment plants (Section 6). The objectives of this critical review are to: (1) summarize established research on the role of polyP and PAOs in wastewater treatment plants (Figure 1A), (2) review research on polyP and PAOs across the landscape (e.g., from soils and sediments to lakes and streams and to the ocean; Figure 1B-D) that supports PAO-mediated P cycling observed in wastewater treatment plants, (3) discuss key knowledge gaps with respect to microbial P forms and cycling mechanisms, and (4) illustrate established and emerging diagnostic tools that may encourage interdisciplinary research assessing whether knowledge of PAOs in wastewater treatment plants can be leveraged to benefit agricultural P water quality management.

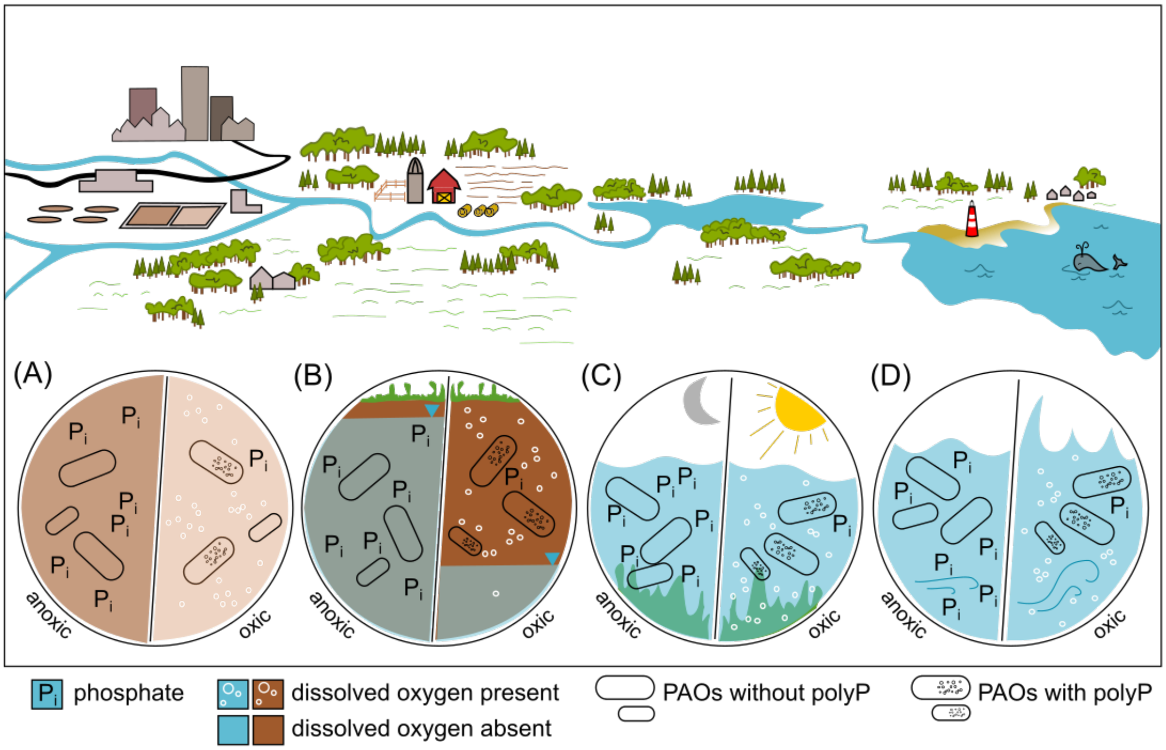

Figure 1. Schematic depicting (A) *known* microbially-mediated phosphate (P_i) cycling in wastewater treatment plants under alternating anoxic/oxic conditions and *hypothesized* (this study) microbially-mediated P cycling in (B) soils (including agricultural and uncultivated soils) and sediments, (C) freshwater (streams and lakes), and (D) marine water (estuaries and oceans). Blue triangles in (B) denote water table depth. The presence and absence of oxygen in the schematic corresponds to more or less bubbles (i.e., open circles), respectively, in the schematic. Abbreviations: polyphosphate accumulating organism (PAO) and polyphosphate (polyP).

We conducted a meta-analysis of articles in Web of Science (https://webofknowledge.com) to (1) quantify the relative importance of PAO and polyP research in five disciplines: wastewater treatment, terrestrial, freshwater, marine, and agriculture, (2) quantify the overlap in PAO and polyP research priorities between these five disciplines, and (3) identify studies of interest in this critical review. We summarized meta-analysis results according to wastewater treatment (Figure 1A), terrestrial (i.e., soils and sediments; Figure 1B), freshwater (i.e., streams and lakes; Figure 1C), and marine (Figure 1D) disciplines. Specifically, we used the `rwos` package (https://github.com/juba/rwos) in R (version 3.6.2; ref 37) to query research articles in the "Science Citation Index Expanded" (SCI) edition of the Web of Science Clarivate Analytics web server. When web server searches resulted in less than 100,000 queries (i.e., the maximum number allowed for download), we downloaded query results and analyzed them using R. We summarized results of the total number of Web of Science searches as well as keyword searches for five disciplinary categories from 1990 to 2019 (see queries in Table S2). For example, we combined soil and sediment searches to form the terrestrial category. We used the total number of articles in Web of Science (Figure S1) to normalize categories that were plotted versus time (e.g., Figure S2). The Web of Science keyword search in includes keywords supplied by authors, text from the article title and abstract, and text from the "keyword plus" field of the Web of Science database [38]. The "keyword plus" field relies on an algorithm to analyze extended terms based on the article's cited references [38]. We acknowledge the meta-analysis was by design constrained by our search terms and may reflect author word choice, discipline-specific terminology, and "keyword plus" algorithm results. For example, authors may have encountered PAO-like behavior in their experiment but did not use "polyphosphate" or "polyphosphate accumulating organisms" when writing about this behavior. When possible, we included multiple versions of a keyword to broaden our search (Table S2). For example, we included "wastewater", "enhanced biological phosphorus removal", and "batch reactor" when searching for articles in the wastewater treatment category.

In addition to the Web of Science meta-analysis, we reviewed research articles that provided preliminary support for hypothesized PAO-mediated P cycling mechanisms outlined in Figure 1B-D (Section 6). Examples of articles providing preliminary support included articles that are relevant to this critical review but (1) did not use "polyphosphate" or "polyphosphate accumulating organisms" in the keywords supplied by authors, text from the article title and abstract, and text from the "keyword plus" field of the Web of Science database or (2) articles that identified the presence of PAO and/or polyP but did not consider their roles in environmental P cycling and/or water quality management. We relied on results from the Web of Science meta-analysis and research article review when summarizing knowledge gaps related to microbial P forms and cycling mechanisms in agricultural water quality management research. We do not summarize research on abiotic and biotic iron and P interactions because these subjects have been thoroughly reviewed elsewhere [39–49]. All data and analysis scripts associated with this publication are available on GitHub at https://github.com/sheilasaia/paper-pao-review.

3. CURRENT STRATEGIES FOR MANAGING PHOSPHORUS IN AGRICULTURAL SETTINGS

The majority of agricultural water quality best management practices for P seek to reduce DP and PP loading to water bodies via human activity (e.g., reduced fertilizer applications) and abiotic environmental mechanisms (i.e., chemical and physical reactions). In terms of human activity, nutrient management approaches encourage farmers to use the "4Rs" when applying inorganic fertilizer or manure to their fields: right source, right timing, right placement, and right rate (Table S3). Chemically, soil mineral amendments increase adsorption of P to soils and soil pH amendments increase soil pH to prevent P leaching (Table S3). For instance, woodchip bioreactors amended with biochar can immobilize DP in surface and subsurface flows [50,51]. Many agricultural water quality best management practices are designed to enhance physical retention of PP alone because over 80% of soil P is bound to organic matter, clay, and minerals [1,16,35,52–54]. For example, vegetated filter strips and constructed wetlands are designed to intercept overland flows and allow sediments along with PP to settle out before they can reach downstream water bodies (Table S3). Other best management practices physically retain P by either minimizing soil erosion during the growing season (e.g., no tillage) and nongrowing season (e.g., cover crops) or increase water infiltration into the soil profile (e.g., tile drainage and soil aeration). Best management practices that combine chemical and physical controls to reduce DP and PP losses (e.g., mineral additions to vegetated buffers) are increasingly favored since agricultural management practices that focus on a single P form can fall short of meeting P load reduction goals and have unintended water quality consequences [10,17,55–58].

Despite these benefits, evidence suggests that agricultural water quality best management practices for P do not always perform as intended (Table S3). Specifically, biochar additions to woodchip bioreactors have a finite adsorption capacity [50] and one study found that, depending on the lumber type, woodchips leached DP during the start-up phase of bioreactor development [51]. Furthermore, PP accumulated in the topsoil of vegetated filter strips and riparian buffers became an unintended P source [59–62]. Use of cover crops led to the accumulation of P in upper layers of the soil profile [17] and leached DP during freeze-thaw conditions [63,64]. No tillage practices enhanced macropore development and increased subsurface transport of DP [17,65]. Moreover, tile drains increased subsurface DP transport [63,66,67]. These examples emphasize the need to adopt agricultural P management strategies that holistically reduce P loading to nearby water bodies by simultaneously considering and relying on physical, chemical, and biological mechanisms to retain P.

Biologically-based strategies are usually production-side centered, thereby focusing on the P needs of the crop. For example, fungal and bacterial amendments increase P availability to crops by mobilizing soil-bound P [53,68–74]. There are only a few agricultural water quality best management practices that facilitate P retention via biological mechanisms (Table S3). This is notable given the widely accepted importance of biological mechanisms on nitrogen (e.g., nitrification and denitrification) and carbon (e.g., respiration) cycling in the environment under alternating anoxic/oxic conditions [60,75,76]. Constructed wetlands are one exception because wetland vegetation and microorganisms may serve as P sinks [77–81]. Thus, constructed wetlands use a combination of physical, chemical, and biological mechanisms to retain P and improve water quality (Table S3). Besides constructed wetlands, research on soil organic P has refocused discussions of P retention in agricultural soils around biological mechanisms (e.g., refs 82, 83). However, the development and implementation of agricultural water quality management practices for soil organic P remains nascent.

In addition to DP and PP forms, a more holistic management focus on multiple P forms may prove beneficial to reducing P loading and improving water quality. As an example, soil organic P—the fraction of the soil PP pool that includes P complexed with or bound to soil organic matter or P within soil macroorganisms and microorganisms (Table S1)—is relevant to this critical review because microbes mobilize a large proportion (i.e., 20%-50%) of soil organic P in

P-limited soils [82,84] and these microbial P pools may increase an additional 30%-240% under implementation of agricultural management strategies such as no tillage and cover crops, among others [85]. Furthermore, studies summarizing data on agricultural soil organic P demonstrate an important knowledge gap. Soil organic P may comprise up to 80% of soil P [53,84,86,87], yet researchers know little about why this pool is so large and how microbial fractions of soil organic P may enhance or inhibit crop nutrient management and water quality management [83,85,86]. Researchers hypothesize that a deeper exploration of mechanisms influencing soil organic P cycling may be key to (1) solving persistent water quality issues due to legacy P and (2) finding a balance between food security and clean water [83,85,86].

4. CONSIDERING POLYPHOSPHATE

In addition to integrating multiple P forms and cycling mechanisms, we argue that agricultural water quality management researchers consider microbial P forms, starting with polyphosphate (polyP). PolyP is a chain of two or more phosphate (PO_4^{3-}) molecules, each bound together by a high-energy phosphoanydride bond (Table S1). PolyP is an inorganic form of P that is commonly included alongside organic P analyses because of its intracellular nature (e.g., ref 88). PolyP can be chemically liberated using common organic extraction procedures (e.g., ref 89). There are several reasons why polyP may be critical for understanding the role of microbial processes on P cycling and transport in agricultural settings. First, polyP is ubiquitous; it is found intracellularly in a wide range of organisms including bacteria, archaea, fungi, plants, and animals [90–95]. Second, polyP plays a role in many basic biological and metabolic functions such as the formation of ATP, RNA, and DNA. Specifically, some microbes use polyP as an energy source, as a P reservoir, for biofilm formation, as a strong ion chelator, as a buffer against alkali conditions, as a regulator of gene expression under periods of stress, and as a regulator of virulence factors [90,92–94,96].

Last, while many research disciplines (individually) have expressed interest in polyP (Sections 5 and 6), our meta-analysis demonstrated that interdisciplinary research (i.e., research engaging multiple disciplines simultaneously) is limited. We identified 9230 articles in Web of Science that included the keyword "polyphosphate" and 22.2% of these articles represented the five categories included in this study (Figure 2C, Table S2). According to journal titles, the remainder of polyP articles came from polymer science and biochemistry fields (e.g., *Polymer Degradation and Stability* and *Journal of Biological Chemistry*; Table S4). Thus, polyP focused research is being conducted independently across multiple disciplines, the majority of which are not directly related to the categories we focused on in this study. Of the five categories included in this study, the wastewater treatment category represented 10.1% (n = 930) of polyP articles (Figure 2C, Table S2) with a consistent number of articles being published each year from 1990 to 2019 (Figure S2A). We expected this meta-analysis result, given that polyP storage and cycling by microbes is especially well-studied in wastewater treatment plants (Section 5). Terrestrial, freshwater, and marine categories combined represented 11.9% (n = 1094) of polyP articles (Figure 2C, Table S2). This research lays a foundation for microbial P cycling in the natural sciences (Section 6). In the agriculture category, we found 14 polyP articles published from 2001 to 2019 and one PAO article published in 2016 (Figure 2C-D, Figure S2B, Table S2). We discuss agricultural discipline results in Section 7. According to comparisons between searches, the majority of polyP articles (n = 838) and PAO articles (n = 601) in the wastewater treatment category did not overlap with articles in another discipline (Figure 3C-D). In combination, these meta-analysis results highlighted an opportunity for interdisciplinary research addressing P forms like polyP in the natural environment.

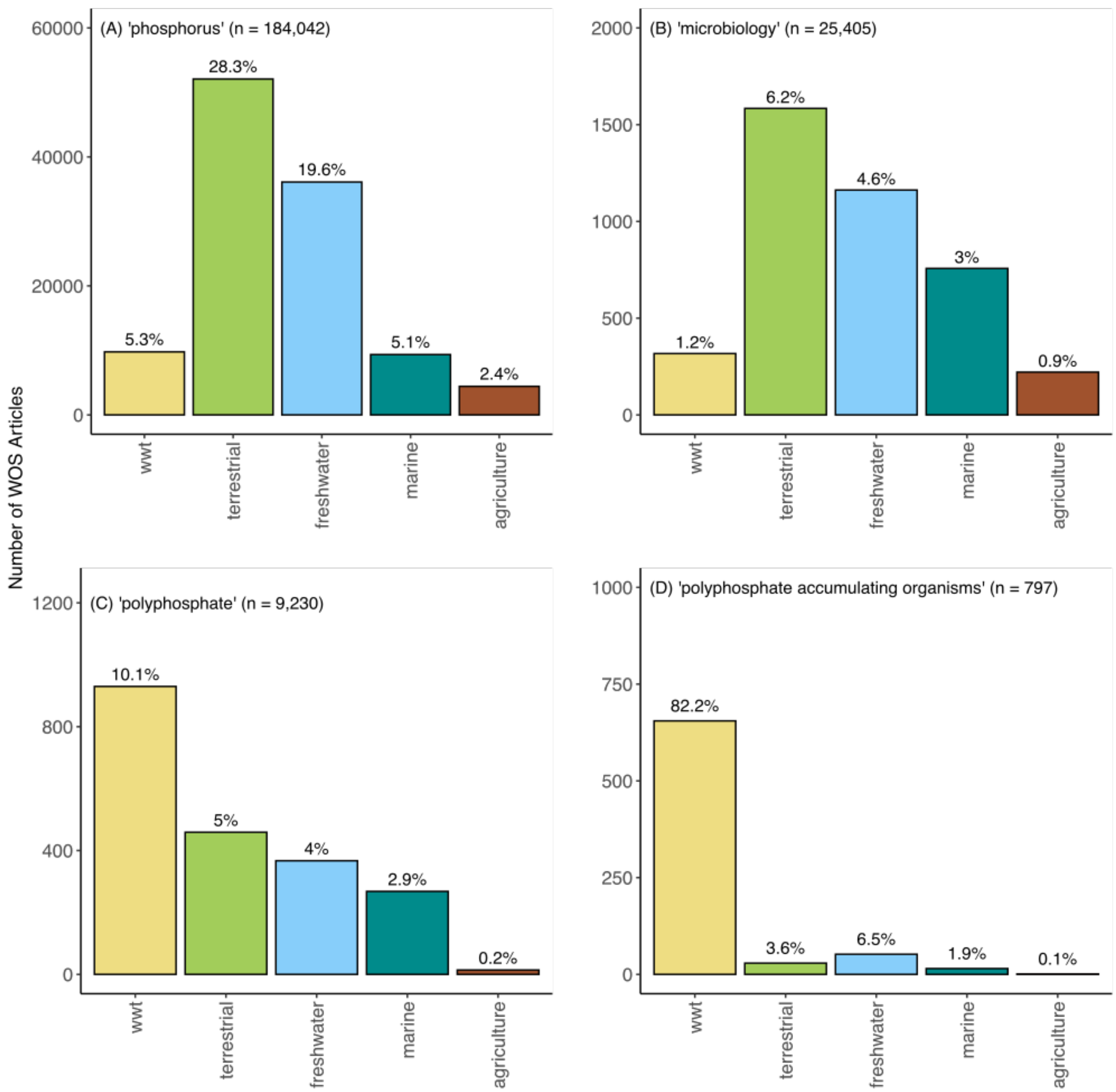

Figure 2. Number and percentage of articles returned from Web of Science (WOS) by category for (A) “phosphorus”, (B) “microbiology”, (C) “polyphosphate”, and (D) “polyphosphate accumulating organisms” keyword searches. Percentages on top of each bar are relative to the number of articles in the full figure subset search (i.e., n = 184,042 for “phosphorus”). Results do not sum to 100% when they are broader than the environments we focused on in this study. Abbreviations: wastewater treatment (wwt). For results by specific habitat (e.g., sediments) see Figure S4.

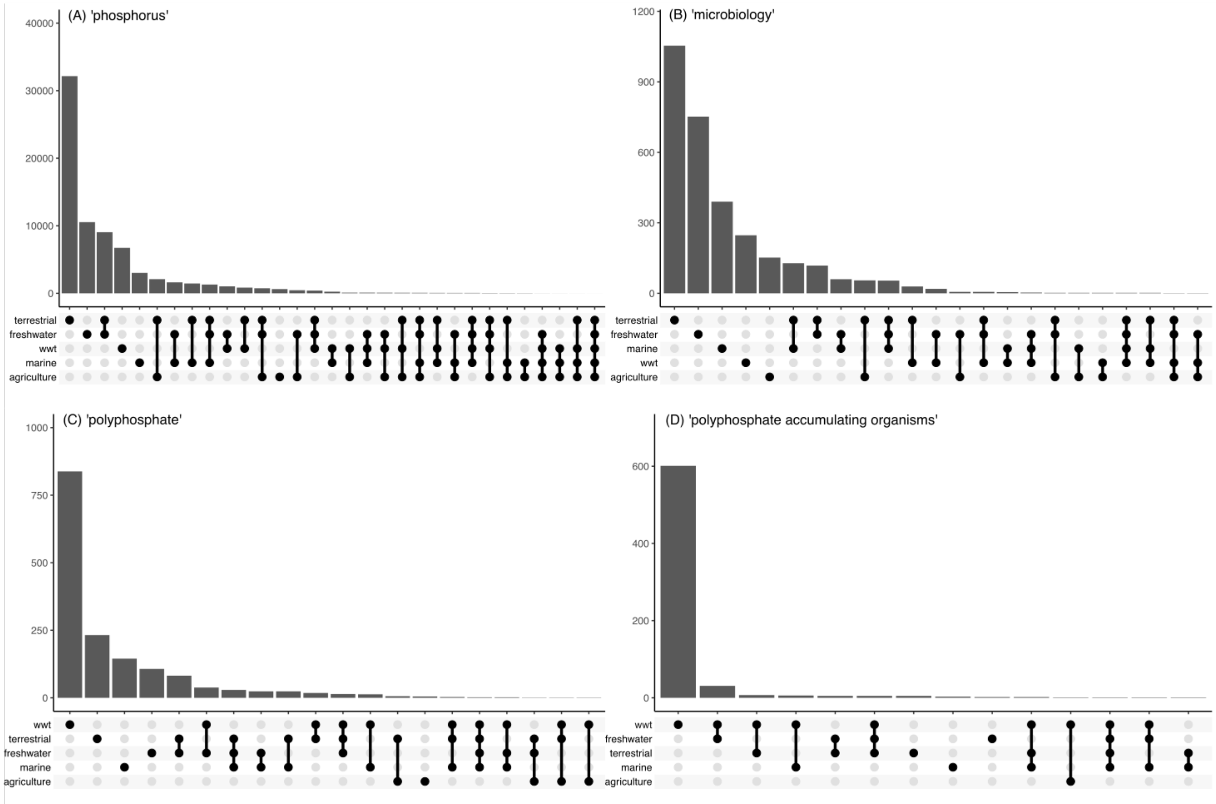

Figure 3. Number of overlapping Web of Science (WOS) articles by category for (A) “phosphorus”, (B) “microbiology”, (C) “polyphosphate”, and (D) “polyphosphate accumulating organisms” keyword searches. Set diagrams under each bar plot show connections between categories. A closed circle represents articles that are not connected to other categories and circles connected with vertical lines represent articles connected to two or more categories. Abbreviations: wastewater treatment (wwt).

5. SPECIALIZED WASTEWATER TREATMENT & POLYPHOSPHATE ACCUMULATING ORGANISMS

In response to growing eutrophication issues in lakes during the 1970s (e.g., ref 97), wastewater treatment plant designs went beyond removing carbon and nitrogen to also removing P (e.g., ref 98). As a result, a specialized wastewater treatment process known as enhanced biological P removal (EBPR) was developed to simultaneously reduce operation costs and remove P from wastewater [99]. EBPR is more economical than conventional wastewater treatment plant P removal because it does not require expensive and logistically complex additions of calcium, aluminum, or iron to chemically precipitate out P [100]. Furthermore, EBPR wastewater treatment does not generate metal-laden waste solids, but instead, P is transferred from solution to waste solids via intracellular storage of P as polyP by microbes [94,98,99,101,102]. EBPR wastewater treatment plant designs generally include three main operating components: (1) an anoxic zone (i.e., oxygen and nitrate molecules are absent; Text S1) with an organic (carbon-containing) energy source such as acetate, (2) an oxic zone (i.e., oxygen molecules are present; Text S1), and (3) a means to recycle a fraction of the settled biomass such that it is subjected to alternating anoxic/oxic conditions (Figure 1A and Figure S3; refs 94, 99, 103). While optimal operating conditions were originally developed empirically rather than based on an understanding of microbial processes, it is now commonly accepted that the characteristic alternating anoxic/oxic conditions of EBPR selects for a group of microbes referred to as polyphosphate accumulating organisms (PAOs). PAOs are capable of taking up phosphate in excess of normal cellular levels coined "luxury uptake" [99,104,105]. Typically, EBPR sludge is 5%-7% P (dry weight) while the P content of conventional sludge ranges from 1-2% [104]. PAOs play a large role in removing P from influent waters of EBPR wastewater treatment plants around the world (e.g., ref 105).

The most frequently studied (model) PAO known by the provisional scientific name *Candidatus* Accumulibacter phosphatis [106], has the ability to synthesize large amounts of polyP under oxic conditions to support the uptake and intracellular storage of organic substrate (Figure 1A and Figure S3; refs 94, 99). This metabolism defines PAOs and enables them to outcompete the majority of other non-PAO heterotrophs with less flexible metabolic capabilities [107]. For a detailed description of *Candidatus* Accumulibacter phosphatis metabolism in EBPR wastewater treatments plants see Text S2 and Figure S3. While we have a better understanding of the PAO metabolism since EBPR was introduced, the metabolic mechanisms separating PAOs from non-PAOs are still debated and studied (e.g., refs 103, 108). Genotypic and phenotypic diversity of *Candidatus* Accumulibacter phosphatis, and PAOs in general, likely explain observed variation in metabolic processes under anoxic conditions.

Rather than a single metabolic model, many markedly different metabolic models may exist [99,101–103,109–117]. *Candidatus* Accumulibacter phosphatis largely relies on the conversion of volatile fatty acids such as acetate to glycogen. *Candidatus* Accumulibacter phosphatis then uses glycogen as an energy reserve during oxic EBPR wastewater treatment conditions (Figure S3B). However, some strains of *Candidatus* Accumulibacter phosphatis can use ethanol and amino acids rather than acetate [103,116]. Non-*Candidatus* Accumulibacter phosphatis PAOs of the *Microlunatus* and *Tetrasphaera* genera can ferment many diverse carbon substrates to make glycogen (Text S2). A growing body of research on PAOs in the *Tetrasphaera* genus has demonstrated their potential importance in EBPR [101]. *Tetrasphaera* PAOs can constitute up to 30%-35% of PAOs in full-scale EBPR wastewater treatment plants [118,119]. Some strains of

Candidatus Accumulibacter phosphatis and several non-*Candidatus* Accumulibacter phosphatis PAOs are called denitrifying PAOs because they can use oxygen, nitrate, and nitrite as electron acceptors. Therefore, they can remove P during oxic EBPR wastewater treatment conditions and also remove oxidized nitrogen molecules (i.e., nitrate and nitrite) during anoxic EBPR wastewater treatment conditions (Text S2). Researchers also demonstrated effective P removal in an EBPR wastewater treatment undergoing alternating anoxic conditions (i.e., oxygen was not present but oxidized nitrogen molecules were present) and oxic conditions. The organisms involved made up 56%-61% of the bacterial community, could not use nitrate, and were neither *Candidatus* Accumulibacter phosphatis nor known denitrifying PAOs [120]. Thus, there are gaps in our knowledge of PAO diversity and metabolisms.

As we learn more about the diversity of PAOs, we may be motivated to revisit metabolic features that distinguish PAOs from non-PAOs as well as how EBPR wastewater treatment plant microbial communities (including PAOs and non-PAOs) contribute to effective P removal. While we focused primarily on P polymers (i.e., polyP) in this study, other polymers (e.g., glycogen and carbon substrates) and flexible PAO metabolisms may prove important in natural habitats where essential nutrients are more limited (Texts S2-S3). Furthermore, PAO survival alongside potential competitor organisms such as glycogen accumulating organisms (Text S2), in various alternating redox condition configurations (i.e., anoxic without oxidized nitrogen molecules present, anoxic with oxidized nitrogen molecules present, and oxic), using diverse carbon substrates, and using oxidized nitrogen molecules (e.g., nitrate) may be of great interest to agricultural professionals looking to manage nitrogen and P simultaneously in the natural environment, where they have less control over environmental conditions (e.g., soil moisture and soil temperature) compared to wastewater treatment plants.

There are several reasons why EBPR PAOs may serve as a starting point for further research on microbially-mediated P management, including in the context of agricultural soils and downstream water bodies. First, PAOs are well studied in the context of EBPR wastewater treatment plants. According to our meta-analysis, we identified 797 articles in Web of Science that included the keyword "polyphosphate accumulating organisms" and 94.3% of these articles represented the five categories included in this study (Figure 2C-D, Table S2). Of the five categories discussed here, the largest number of PAO articles (n = 655, 82.2%) came from the wastewater treatment category (Figure 2D, Table S2). Besides the magnitude of research, we observed that the number of PAO articles in the wastewater treatment category from 1990 to 2019 increased over time; however, this increase was not as dramatic as the number of articles per year in the wastewater treatment category identified using the keyword "phosphorus" from 1990-2019 (Figure S2A). Second, PAOs have been found in many environments around the world (Table S5) and there remains an opportunity for collaborative research between disciplines, especially between EBPR wastewater treatment plant research and research in natural environments. To illustrate this point further, of the 655 PAO articles in the wastewater treatment category, 601 of these articles did not overlap with other categories (Figure 3D). Terrestrial, freshwater, and marine categories combined represented 12% (n = 96) of PAO articles (Figure 2D, Table S2) and it was rare for articles in terrestrial, freshwater, and marine categories to overlap with articles in the wastewater treatment category (Figure 3D). Furthermore, 69 PAO articles in terrestrial, freshwater, or marine categories overlapped with articles in these same categories returned for the keyword "polyphosphate". Third, there is limited research of PAOs in agricultural systems, as evidenced by the one PAO article (i.e., ref 121) that we identified in the agriculture category (Section 7). Last, alternating anoxic/oxic conditions exist in the natural environment and may serve as a means for selecting PAOs; we expand upon this hypothesis in Section 6.

6. LEVERAGING INTERDISCIPLINARY RESEARCH: PROPOSED EVIDENCE & ROLES OF POLYPHOSPHATE CYCLING ACROSS THE LANDSCAPE

Concurrent to EBPR PAO studies in wastewater treatment plants, researchers hypothesized that alterations between anoxic/oxic conditions in several types of natural environments (e.g., soils, sediments, freshwater, and marine waters) may select for microorganisms that have similar phenotypes and genotypes to EBPR PAOs [79,122–132]. From the 1970s to present, research on P cycling in the environment has been motivated by the understanding that anthropogenic P loadings have led to accelerated eutrophication in many freshwater and marine ecosystems worldwide [32,133]. With respect to biological P forms and cycling mechanisms, researchers demonstrated that microorganisms may respond to this increase by storing excess P as polyP [124,132,134]. However, over 30 years later, few studies have addressed this hypothesis and its focus on biological P forms and cycling mechanisms. There is also limited focus on the role of PAOs from both a phenotypic and genotypic perspective. Therefore, in Sections 6.1 to 6.3, we summarized research in terrestrial (Figure 1B), freshwater (Figure 1C), and marine (Figure 1D) environments that provides preliminary evidence for this hypothesis. This evidence draws from our meta-analysis of Web of Science articles and includes discussion of studies identifying known PAOs (i.e., *Candidatus* Accumulibacter phosphatis), identifying new PAOs, identifying and quantifying polyP and P functional genes, and demonstrating relationships between P and oxygen concentrations in terrestrial and aquatic environments.

6.1 Terrestrial Habitats (Soils and Sediments)

In the context of terrestrial environments, wetting and drying events influence the diffusion of oxygen through soil and sediment pores. Soils and sediments tend to be anoxic while saturated, and oxic while unsaturated [135–137]. Therefore, we hypothesize alternating wetting/drying events in soils and sediments appear to be analogs to alternating anoxic/oxic conditions in EBPR wastewater treatment plants. Specifically, we hypothesize soil and sediment PAOs take up P during drying conditions and release P during wetting conditions (Figure 1B). Furthermore, we hypothesize that PAOs release phosphate under wetting soil and sediment conditions, which may negatively impact P management goals for water quality protection. This critical review, which we highlight in further detail below, supported these hypotheses as well as the need for interdisciplinary research on PAOs and polyP in terrestrial habitats such as soils and sediments.

As stated previously, several researchers hypothesized that PAOs in soils and sediment may release P during wet periods and take up P during dry periods, which mirrors the behavior of PAOs like *Candidatus* Accumulibacter phosphatis in EBPR treatment plants [41,122,129,130,138]. We identified several studies documenting the presence of *Candidatus* Accumulibacter phosphatis [42,129,139–14142,128,138–140], its phylogenetically close relatives [130,142], as well as other novel PAOs [143,144] in soils and sediments around the world (Table S5). However, we found no studies that directly addressed the role of *Candidatus* Accumulibacter phosphatis or other (e.g., *Tetrasphaera*) PAOs in soil P cycling under alternating anoxic/oxic conditions. With respect to polyP, there is evidence of polyP accumulation by diverse bacterial species in river sediments contaminated by heavy metals [145] and evidence that microbes accumulated P as polyP under oxic conditions and released P under anoxic conditions in freshwater lake sediments [42,146] and wetland sediments [147,148]. However, only one of these polyP studies (i.e., ref 42) confirmed the presence of *Candidatus* Accumulibacter phosphatis. Consequently, research addressing the role of polyP and PAOs under alternating wetting/drying conditions and their impact on water quality is still needed.

Despite the limited research on the specific role of PAOs in soils under alternating anoxic/oxic conditions, we found several studies exploring the impacts of environmental perturbations such as wetting/drying events on soil organic P. Namely, soil organic P mineralization (after rewetting) was positively correlated with microbial biomass [149] and microbial P [150,151] upon rewetting. Soils undergoing alternating wetting/drying events showed larger increases in microbial P over time compared to soils that did not undergo alternating wetting/drying events [150]. One study estimated that 41% of added phosphate was stored as microbial P upon soil rewetting [152]. One study subjected humid tropical soils to alternating anoxic/oxic conditions and found that biologically-available P pools decreased immediately following anoxic conditions [41]. This finding supports PAO-mediated P release under anoxic conditions. Other studies have observed flushes in P after prolonged drought [25,26] but further research is needed to determine whether this is associated specifically with PAOs or other biological, physical, and chemical P mechanisms. Increases in P release during saturated soil conditions have also been observed at larger landscape [153] and watershed scales [154] but whether and how much microbial P contributes to these patterns, relative to physical and chemical P mechanisms, is still unknown [155].

According to our meta-analysis, polyP research in soils and sediments was more common than PAO research, but fewer than P and microbial research. For the terrestrial category, which included soils and sediments (Table S2), we identified 459 articles in Web of Science that included the keyword “polyphosphate” and 29 articles that included the keyword “polyphosphate accumulating organisms”; this is in contrast to 52,076 and 1,584 articles in the terrestrial category that included the keywords “phosphorus” and “microbiology”, respectively (Figure 2, Figure S4, Table S2). Of the 29 PAO articles that only fell into the terrestrial category, several focused of PAOs commonly found in EBPR wastewater treatment plants. For example, one study looked at the dispersal of *Candidatus* Accumulibacter phosphatis in soils and sediments [129] and a second study applied techniques developed in EBPR wastewater treatment settings to identify a strain of *Candidatus* Accumulibacter phosphatis in lake sediments that preferred nitrate over oxygen [42]. One study sequenced and analyzed a non-*Candidatus* Accumulibacter phosphatis PAO genome (i.e., *Microlunatus* genus); these PAOs were first isolated from soils [110]. Researchers used a soil-based laboratory-scale reactor under alternating anoxic/oxic periods—termed a humus soil activated sludge process—to treat domestic wastewater [156]. While the number of articles remains relatively small, these results demonstrate the collaborative potential for interdisciplinary research. Upon closer inspection of results, most polyP and PAO articles in the terrestrial category addressed soil environments (Figure S4C-D). For example, 3.1% of all polyP articles included “soil” as a keyword while 1.8% included “sediment” as a keyword (Figure S4C). In comparison to all categories studied here with the exception of wastewater treatment, the terrestrial category made up the largest percentage of polyP articles and the second largest percentage of PAO articles (Figure 2C-D, Table S2). Therefore, there are a limited number of studies on polyP and PAOs in terrestrial habitats and none that directly address the role of PAOs in P cycling.

In addition to the limited number of polyP and PAO studies relative to P and microbiology research in terrestrial environments (Figure 2), our meta-analysis identified the limited overlap in research between the terrestrial category and categories other than wastewater treatment. Over half (n = 232, 56.3%) of polyP articles and nearly one-fifth (n = 5, 19.2%) of PAO articles in the terrestrial category did not overlap with any other category (Figure 3C-D). As an example, several of these polyP articles discussed organic P pools (e.g., refs 157, 158). Specifically, one of these demonstrated that the addition of glucose (i.e., an organic substrate) led to accumulation of pyrophosphate [157], which is a polyphosphate polymer with two phosphate molecules (Table S1). A second example demonstrated polyP accumulation and P release in sediment bacteria under oxic and anoxic laboratory conditions, respectively [147]; this behavior supports PAO-mediated P cycling in sediments. This study did not identify the specific organisms involved, but briefly mentioned that the experiment was motivated by research on bacteria in EBPR. In several instances, polyP articles in the terrestrial category overlapped with two or more categories discussed here, these totaled 180 articles. With respect to PAOs, we identified seven PAO articles in the terrestrial category that overlapped with articles in the wastewater treatment category. Several of these studies used soil as a source for developing a sequencing batch reactor that was capable of removing P (e.g., refs 159, 160), which supports the hypothesis that soil microbes may offer some P removal benefits under the right conditions. Like polyP articles, there were several rare instances when PAO articles in the terrestrial category overlapped with multiple other categories discussed here; those totaled 21 articles. We found one article that overlapped with all categories except agriculture (i.e., ref 161). There were 26 polyP and PAO articles that overlapped with one another and were both in the terrestrial category (Figure 3C-D). For example, one of these isolated a new PAO from soils (Table S5; ref 159). Given the nearly 10,000 articles combined on polyP and PAOs in Web of Science, there remains an opportunity for interdisciplinary research addressing the role of PAOs in water quality management between wastewater treatment, terrestrial, freshwater, and marine disciplines.

6.2 Freshwater Habitats (Streams and Lakes)

In streams, rivers, and shallow regions of lakes, alternating anoxic/oxic conditions are often driven by diel cycles in respiration and primary production, respectively. Specifically, oxygen levels in the water column and upper sediment layers increase during the day due to photosynthesis, while during the night the cessation of photosynthesis combined with continued respiration decreases oxygen levels [162,163]. In the case of freshwater tidal wetlands, hydrodynamics (e.g., tides) can also influence alternating anoxic/oxic conditions. For example, the tide brings in oxygen rich water and recedes with water that has a lower concentration of oxygen due to respiration within the wetland [164]. In deeper regions of lakes, alternating anoxic/oxic conditions are often driven by changes in the depths of the oxic epilimnion and anoxic hypolimnion due to either internal waves or wind-induced surface waves [127]. Given the existence of these alternating anoxic/oxic conditions in freshwater habitats, we hypothesize diel changes in oxygen availability driven by either metabolic or wave-driven hydrodynamics causes are analogs to alternating anoxic/oxic conditions in EBPR wastewater treatment plants. Specifically, freshwater PAOs may take up P during the day and/or during windy conditions and release it during the evening and/or during calm conditions (Figure 1C). Our meta-analysis and research article review supported these hypotheses and highlight the need for interdisciplinary research on PAOs and polyP.

A number of studies in freshwater habitats have documented the presence of EBPR PAOs such as *Candidatus* Accumulibacter phosphatis and other microorganisms that can store polyP intracellularly (Table S5). Microscopy-based studies found intracellular polyP granules in freshwater microorganisms [165–167] and stream biofilms [45,168,169]. Consistent with EBPR PAO metabolism, there is evidence that oxygen concentrations influence microbial P cycling in freshwater environments. Microbes accumulated P as polyP under oxic conditions and released P under anoxic conditions in stream biofilms [45]. Other researchers have observed coupled P and oxygen patterns that are generally consistent with EBPR PAO metabolism despite not directly measuring polyP concentrations or known EBPR PAO (i.e., *Candidatus* Accumulibacter phosphatis) genes. As an example, in freshwater streams, diel water column phosphate cycling patterns were inversely related to oxygen availability; when dissolved oxygen was high during the day, phosphate was low and vice versa at night [162,170]. In a productive temperate lake (i.e., Muskegon Lake, Michigan, USA), higher P concentrations have been linked to lower oxygen levels along a seasonal time-series determined using *in situ* sensors [171]. Also, P uptake near the water column-sediment boundary and water column-periphyton boundary coincided with increasing oxygen concentrations while P release near these same interfaces coincided with decreasing oxygen concentrations [45,124,172–176]. While abiotic processes (e.g., redox sensitive dissolution/precipitation of iron and P) cannot be completely ruled out as possible causal mechanisms in the cases described above [45,80], there is consensus that biological processes play a role in coupled P and oxygen patterns, and in some cases, this role is significant. For example, biotic processes accounted for 66% of stream diel water column P uptake and release while the remainder was attributed to abiotic processes, calcium-P precipitation and dilution [162]. In freshwater wetlands, biotic mechanisms accounted for over 83% of short-term (i.e., 12-h) water column P removal [81]. Given examples across several freshwater ecosystems, diel changes in P uptake and release appear coupled with oxygen availability support the behavior of EBPR PAOs in these habitats.

In addition to oxygen availability, there is evidence that polyP storage in freshwater environments depends on P and metal availability. For example, polyP storage by microorganisms in temperate stream biofilms was greatest in nutrient-depleted headwater sites compared to downstream sites that were nutrient-abundant [169]. Another study of temperate stream biofilms demonstrated that increases in polyP storage during natural, P-abundant storm events and controlled pulses of P in mesocosm experiments led to future microbial growth [168]. An *in situ* P stream biofilm enrichment experiment carried out along four streams in Pennsylvania, USA showed that P uptake rates varied predictably along a nutrient gradient [177], such that the development of intracellular polyP granules in stream biofilms was proportional to the degree of nutrient additions to stream ecosystems from the surrounding landscape [178]. A laboratory study of stream biofilms under changing oxygen conditions demonstrated that P uptake and release was correlated with metal (i.e., manganese, calcium, potassium, and magnesium) uptake and release during oxic and anoxic conditions, respectively [45]. Studies of EBPR PAOs have shown similar reliance on metals, likely to balance the negative charge of intracellular polyP (Figure S3; ref 179). Overall, proportional relationships between polyP storage, P, and metal availability support the idea that polyP may be a beneficial polymer for stream microorganisms; it helps them conserve energy and nutrients for future use.

Similar to meta-analysis results for terrestrial habitats, polyP research in streams and lakes was more common than PAO research but more limited than P and microbial research. We found 367 articles in Web of Science that included the keyword "polyphosphate" and 52 articles that included the keyword "polyphosphate accumulating organisms" in the freshwater category (Figure 2, Figure S4, Table S2). Compared to other keyword searches in the freshwater category, there were 36,105 articles that included the keyword "phosphorus" and 1162 articles that included the keyword "microbiology" (Figure 2, Figure S4, Table S2). When compared to terrestrial and marine categories, freshwater had the most PAO articles; 6.5% of all PAO articles fell in the freshwater category while 3.6% and 1.9% of PAO articles came from the terrestrial and marine categories, respectively (Figure 2D, Table S2). A closer look at meta-analysis results within the freshwater category revealed that most polyP articles were associated with lakes while most PAO articles addressed streams (Figure S4C-D).

Besides the limited number of polyP and PAO studies in streams and lakes, meta-analysis results demonstrated limited overlap in research between the freshwater category and categories other than wastewater treatment. Most frequently, polyP articles in the freshwater category did not overlap with any other category (n = 107, Figure 3C). PAO articles most frequently overlapped with the wastewater treatment category (n = 31, Figure 3D). Many of these articles focused on PAO research in EBRP wastewater treatment settings (e.g., refs 114, 180), with several discussing denitrifying PAOs (e.g., ref 181). One study subjected a laboratory-scale EBPR wastewater treatment reactor to P-limited and oxygen-limited conditions for up to a week and found that PAOs adapted to these changes [180]. There were 45 polyP and PAO articles that overlapped with one another and were both in the freshwater category. For example, one of these articles assessed the distribution of *Candidatus* Accumulibacter phosphatis P functional genes in lake water [129]. These results support the flexibility of PAO metabolisms and the need for interdisciplinary research addressing the role of PAOs in water quality management between wastewater treatment, terrestrial, freshwater, and marine disciplines.

6.3 Marine Habitats (Estuaries and Oceans)

Marine habitats discussed here include estuaries, coastal waters, and the open ocean. In coastal estuarine ecosystems, alternating anoxic/oxic conditions are greatly influenced by a combination of anthropogenic nutrient inputs—including nutrient-induced acceleration of primary production [182]—and mixing of stratified layers of the water column [183]. In the open ocean, much like the deeper regions of lakes, alternating anoxic/oxic conditions are driven by the mixing of stratified chemoclines typically caused by waves and wind [183]. Given the potential for alternating anoxic/oxic conditions in marine habitats, we hypothesize wave- and wind-driven oxygen gradients are analogs to alternating anoxic/oxic conditions in EBPR wastewater treatment plants (Figure 1D). Our research article review provided limited support for these hypotheses—especially compared to terrestrial and freshwater habitats—but like Sections 6.1 and 6.2, our meta-analysis highlighted the need for interdisciplinary research on PAOs and polyP in marine environments.

Similar to freshwater, several studies have demonstrated the storage of intracellular polyP and the presence of EBPR PAOs (Table S5). Microscopy- and spectroscopy-based studies have identified intracellular polyP granules in marine sediment bacteria [131,182], and marine microorganisms [184]. For example, one study observed polyP accumulation by marine filamentous cyanobacterial symbionts within sponges and verified the presence of P functional genes such as polyphosphate kinase (*ppk*) and exopolyphosphatase (*ppx*; Text S3, Table S6) associated with polyP cycling using techniques established for EBPR PAOs such as *Candidatus* Accumulibacter phosphatis [185]. Another analyzed the relationships between the abundance of P functional genes in marine microorganism genomes and annual water column phosphate concentrations [186]. Furthermore, we identified two studies that support PAO presence in estuarine waters and sediments. One noted the widespread distribution of PAOs and PAO-related P functional genes (i.e., *ppk*; Text S3, Table S6) in estuarine sediments [187] and the other identified bacteria in the *Rhodobacteraceae* family[70] (*Candidatus* Accumulibacter phosphatis is also a member of this family).

Only a few researchers in marine systems have linked oxygen availability in the water column with P cycling, but these limited studies find support for PAO metabolism in marine environments. Namely, one study found that phosphate concentrations were ~3x greater in the redoxcline—a zone with a strong vertical redox gradient—of a coastal basin compared to the surface [128]. Another found that polyP concentrations in water samples from a coastal inlet decreased as dissolved oxygen concentrations decreased [123]. Elsewhere, researchers identified giant sulfur bacteria (*Thiomargarita namibiensis*) that accumulated polyP under oxic sediment conditions and released phosphate under anoxic sediment conditions, a response that is functionally similar to EBPR PAOs [131]. Therefore, there are a number of studies identifying PAOs in marine sediments but there remain opportunities to assess their role under alternating anoxic/oxic conditions.

In addition to oxygen availability, there is conflicting evidence in marine environments as to whether P availability increases or decreases polyP storage. When taken together, polyP accumulation by marine microorganisms may depend on histories of P availability. For instance, phytoplankton accumulated more polyP in P-depleted regions of the Sargasso Sea compared to regions that were more P-abundant [184]. Similarly, in a metagenomic study of marine environments, the abundance of P functional genes (i.e., *ppk* and *ppx*; Text S3, Table S6) increased when annual P concentrations were lower [186]. In contrast to these findings, one study along an urban to estuarine gradient (i.e., from P-abundant inland to P-depleted open ocean) demonstrated a decrease in the abundance of P functional genes in water column microorganisms [70]. While this study (i.e., ref 70) did not quantify intracellular polyP, their findings are consistent with studies in P-abundant freshwater environments (Section 6.2). Additionally, a laboratory study of marine algal cultures found that intracellular pyrophosphate, which is a form of polyP with only two phosphate molecules (Table S1), accumulation increased under higher water column P concentrations [188]. Therefore, in P-depleted aquatic environments, polyP storage may function as an adaptation to help microorganisms conserve nutrients for later use, while in P-abundant aquatic environments, polyP storage may help microorganisms access alternative energy conservation pathways that enable them to outcompete microorganisms with less metabolic flexibility. More detailed field and laboratory research is needed to elucidate the impact of P availability histories on polyP storage by known and undiscovered PAOs.

According to our meta-analysis results, polyP and PAO articles in marine environments were less common than terrestrial or freshwater categories. For marine environments, we found 268 articles in Web of Science that included the keyword "polyphosphate" and 15 articles that included the keyword "polyphosphate accumulating organisms" (Figure 2, Figure S4, Table S2). Several of the PAO articles discussed the impact of salinity on EBPR processes (e.g., ref 189). As a comparison to other keyword searches in the marine category, there were 9356 articles that included the keyword "phosphorus" and 757 articles that included the keyword "microbiology" (Figure 2, Figure S4, Table S2). By looking more closely at meta-analysis results within the marine category, we found that most polyP and PAO articles were associated with "marine" and "saltwater" keywords rather than "ocean" (Figure S4C-D). This suggests that there is limited polyP and PAO research in the open ocean. Overall, polyP and PAO articles in the marine category were more limited compared to all other categories except for agriculture; 2.9% of all polyP articles and 1.9% of all PAO articles were in the marine category (Figure 2C-D).

Similar to terrestrial and freshwater habitats, we found limited evidence of interdisciplinary PAO research in marine habitats. Nearly two-thirds (n = 145, 59.9%) of polyP articles in the marine category did not overlap with other categories (Figure 3C). Several of these articles discussed polyP and other polymers made by marine sponges, one of which, assessed the benefit of polyP research to disciplines other than those of interest here (i.e., bioengineering/medicine; ref 190). A few of these articles studied cyanobacteria under P-limited conditions and found that cyanobacteria stored P as polyP when P concentrations in marine waters are very low, aiding in their survival [184,191]. Most PAO articles in the marine category overlapped with articles in the wastewater treatment category (n = 6, Figure 3D). As mentioned previously, several articles discussed the impact of salinity on EBPR processes and provide examples of how interdisciplinary research could benefit the marine and wastewater treatment disciplines, as well as coastal agricultural landscapes where saltwater intrusion is becoming increasingly problematic due to sea level rise (e.g., ref 192). There were 14 marine category articles that overlapped with polyP and PAO searches. One of these studies discussed the use of diagnostic tools (Section 8) to study PAO-related bacteria in estuarine sediments [193]. Therefore, there is an opportunity to leverage knowledge of polyP and PAOs in marine environments for the benefit of agricultural P water quality management as well as management of P in natural environments.

7. KNOWLEDGE GAPS IN AGRICULTURAL SETTINGS

Our meta-analysis revealed that despite a large body of research on P in agricultural settings, there remains limited focus on and exploration of the role of polyP and PAOs in agricultural water quality management. We identified 184,042 articles in Web of Science that included the keyword "phosphorus" and 60.7% of these articles represented the categories included in this study (Figure 2A, Table S2). Of the five categories, the largest number of P articles (n = 52,076, 28.3%) came from the terrestrial category (Figure 2A, Table S2) and 32,149 of these did not overlap with any other category (Figure 3A). We identified 4429 P articles in the agriculture category (Table S2). We also observed an increase in P articles in the agricultural category from 1990 to 2019 (Figure S2B). While interdisciplinary research is still limited, there is a large and growing body of P research in each of the five disciplines studied here.

In contrast to P articles, we found only 14 polyP articles (Figure 2C, Table S2) in the agriculture category. Several of these discussed the use of algal polyP as a P fertilizer source (e.g., refs 194, 195). One study used diagnostic tools highlighted in Section 8 to characterize the P pools of marine sediments that were impacted by industrial and agricultural P loads [196]. We found only one PAO article in the agriculture category (Figure 2D, Table S2), which used transmission electron microscopy (Section 8) to analyze green algae cells with and without polyP and demonstrated that electron-dense bodies in algal cells were sites of polyP accumulation when algae were kept under sulfur-depleted conditions [121]. These authors highlighted the importance of P in agriculture and were interested in determining whether intracellular polyP from algal biomass could be used as a renewable biologically-based fertilizer [121]. Other studies have also suggested that PAOs could be used to concentrate P [197]. In addition to other proposed organic P forms (e.g., ref 198), studies identified by our meta-analysis provide preliminary evidence that polyP accumulated by microorganisms can be recycled [199–201] and serve as an alternative organic P source for crops, but the efficacy of this has yet to be tested.

In the agriculture category, we observed an increase in "phosphorus" articles from 1990 to 2019 but did not observe the same marked increase in "polyphosphate", "polyphosphate accumulating organisms", or "microbiology" articles over the same time period (Figure S2B). This finding highlights the limited focus of biological P forms and cycling mechanisms in the agricultural literature (Section 3). Overlapping meta-analysis articles between "phosphorus", "polyphosphate", and "polyphosphate accumulating organisms" keyword searches demonstrate a need for interdisciplinary collaboration that leverages knowledge of polyP and PAOs in wastewater treatments as well as existing and emerging diagnostic tools to reduce nonpoint source pollution from agricultural landscapes. For example, one PAO article we identified that overlapped with all categories except agriculture (Figure 3D) isolated bacteria capable of accumulating P from eutrophic lake water and forest soil samples [161]. This same study offered that these bacteria isolates may be useful in remediating P contaminated environments. We identified another PAO article that isolated fungi capable of accumulating P from soybean plants and surrounding soil [202]. Furthermore, we found one study that quantified polyP in overland flows [203] and several studies characterizing soil organic P [88,204] but no studies directly addressing the role of PAOs under alternating anoxic/oxic conditions. In the next decade, interdisciplinary soil microbiome research is positioned to increase crop yield and resilience [205], but these advancements may also be extended to improve and protect water quality.

According to our meta-analysis and research article review, we identified three additional knowledge gaps that span all five major habitat categories discussed here (i.e., wastewater treatment, terrestrial, freshwater, marine, and agricultural). First, across these categories there is limited identification and quantification of non-*Candidatus* Accumulibacter phosphatis PAOs including those of the *Tetasphaera* and *Microlunatus genera,* denitrifying PAOs, and others (Section 5, Text S2 and S3). For example, studies used established molecular biology tools to assess the presence and/or quantity of *Candidatus* Accumulibacter phosphatis P functional genes in wastewater treatment plants (e.g., refs 206, 207), freshwater, sediments, and soils (e.g., refs 129, 140) but we found only a few studies surveying natural environments for *Candidatus* Accumulibacter phosphatis and non-*Candidatus* Accumulibacter phosphatis PAOs (Table S5). We know polyP use is ubiquitous across the tree of life (Section 4) and PAOs exhibit phenotypic and genetic diversity (Section 5). However, there remains limited analysis of PAOs and P functional genes across bacterial, eukaryotic, and archaeal domains. With regard to P functional genes, more research needs to be done to characterize their abundance, diversity, and role in P cycling in natural habitats. Furthermore, research on nitrogen, glycogen, and other functional genes may uncover insights into PAO metabolism and roles in natural habitats (Text S3). Since it is likely that *Candidatus* Accumulibacter phosphatis is not the only PAO in agricultural soils, additional research—including the application of diagnostic tools (Section 8)—is needed to explore PAO phenotypic and genetic diversity. Furthermore, soil microorganisms are diverse and rich [208–214]. Therefore, new discoveries in agricultural soils may benefit existing PAO research in EBPR wastewater treatment as well as other natural environments by revealing additional genetic and metabolic microbial diversity.

Second, there is limited identification and quantification of P functional genes (i.e., *ppk* and *ppx*; Text S3, Table S6) across the five major categories. Researchers identified *Candidatus* Accumulibacter phosphatis P functional genes (i.e., *ppk*) in EBPR wastewater treatment plants around the world [105,140,206]. However, we found only a few studies quantifying the abundance and diversity of non-*Candidatus* Accumulibacter phosphatis PAO *ppk* in EBPR wastewater treatment plants (e.g., ref 207). We found two studies that quantified PAO-related P functional genes (i.e., *ppk*; Text S3, Table S6) in marine habitats [186,187], but no other studies quantifying *ppk* abundance in agricultural, terrestrial, or freshwater environments. Furthermore, we found only a few *ppx* studies (Text S3, Table S6). With the exception of one marine study [186], none quantified *ppx* abundance and diversity in the remainder of categories discussed here. Due to their role in polyP formation and breakdown—an important defining metabolic characteristic of potential PAOs—further study of *ppk* and *ppx* genes is needed, regardless of discipline. With respect to agriculture, analysis of known P functional genes in agricultural soils may lead to the isolation of novel PAOs that can then be studied in wastewater treatment plants and other natural environments.

Third, there are very few studies that go beyond identification to assess the ecological role of PAOs in categories other than wastewater treatment (Section 6). We summarized many studies in natural systems that either (1) identified PAOs directly (e.g., ref 129) or indirectly (e.g., ref 147) or (2) assessed the role of biologically-mediated P uptake and release under changing environmental conditions (e.g., ref 162). There were a few that did both (e.g., [42]). In agricultural systems, most studies identified PAOs (e.g., ref 143), but did not go beyond this step. Agricultural soils undergo alternating wetting/drying conditions that may facilitate PAO-mediated P cycling (Section 6.1). Therefore, there remains an opportunity to study how PAO presence and quantity relate to the frequency and duration of anoxic/oxic cycling and P availability in agricultural soils, nearby waterbodies, and agricultural management practices such as vegetated buffers and bioreactors. Furthermore, given the known diversity of PAO metabolisms in EBPR wastewater treatment (Section 5, Text S2), there is a need for research on the impact of available electron acceptors (e.g., oxygen and others), carbon substrates, and redox cycling regimes on PAO-mediated P cycling in agricultural soils. For example, carbon substrate availability likely depends on the agricultural crop, soil properties, and composition of soil microorganisms—including bacteria, fungi, and others [215]. This work may be extended to couple P and nitrogen cycling, as denitrifying PAO have been identified in EBPR wastewater treatment (Section 5, Text S2). We also know very little about whether we can actively manage PAO-mediated P cycling in agricultural settings to simultaneously achieve desired water quality goals and crop production goals.

8. PROMISING DIAGNOSTIC TOOLS & RESEARCH APPLICATIONS FOR AGRICULTURAL SETTINGS AND BEYOND

Opportunities exist to apply established and emerging interdisciplinary diagnostic tools such as microscopy, molecular biology techniques, and other measurements to overcome barriers and knowledge gaps presented previously. See Table S7 for a full description of these tools. Microscopy tools can be used to identify the size, location, and amount of intracellular polyP granules and can also be used to label known PAOs in agricultural soils and downstream environments. For example, the 4',6-diamidino-2-phenylindole (DAPI) stain can be used in combination with an epi-fluorescence microscope (e.g., refs 89, 216) or a fluorescence spectrophotometer (e.g., [217]) to identify and quantify polyP storage in microbial cells. When used along with the DAPI stain, fluorescence *in-situ* hybridization (FISH) probes (Table S8) fluorescently label PAOs storing intracellular polyP (e.g., ref 46). Molecular biology tools such as quantitative real-time polymerase chain reaction (qPCR, Table S7 and S9) and next generation sequencing—including shotgun and amplicon metagenomics—can be used to quantify P functional genes and identify undiscovered PAOs in agricultural fields and best management practices such as vegetated buffers. For example, one study developed and used qPCR probes to quantify different genetically similar subgroups of *Candidatus* Accumulibacter phosphatis in nine wastewater treatment plants [218]. Shotgun metagenomics data from a global ocean data set were used to quantify P functional genes [186]. Metabolomics can be used to identify bacterial metabolites in environmental samples (Table S7), which may be important for exploring the metabolisms of PAOs in soils. In addition to microscopy and molecular biology techniques, tools like ^{31}P-nuclear magnetic resonance (NMR) spectroscopy can be used to measure the concentration of polyP and other organic P forms in agricultural soils and sediments (e.g., refs 88, 134). Finally, high-frequency sensors can be used to measure oxygen concentrations in soils and phosphate concentrations in the water column or in tile drainage. These sensors can help capture environmental variables at time scales that are more closely aligned with microbial processes.

When using the tools discussed above, care must be taken to ensure that microbial and environmental measurement time and spatial scales are compatible [155,219–221], measurement bias for/against active microbial community members is understood [222–225], and environmental variables (e.g., pH and temperature) that may influence microbial communities are accounted for in the experimental design [149,210,219,220,225–228]. Researchers must also take care to design controls that consider abiotic processes that may mimic PAO-mediated P cycling (e.g., reductive dissolution of iron-P).

Beyond specific tools, several general frameworks exist to link microbial with ecosystem-scale—or potentially, watershed-scale—processes [220,221,229–233]. These frameworks have been applied to research on microbially-mediated nitrogen and carbon cycling but have not been applied to study microbially-mediated P cycling in natural and agricultural settings. As researchers establish studies to explore microbial P cycling in new habitats, they can refer to previous work for guidance on microbial-scale ecological theories [234,235], statistical approaches and considerations [75,220,225,236–239], method overviews [240–247], bioinformatics [242,248–251], reproducible research [250–254], and modeling [255–261].

9. RESEARCH NEEDS FOR AGRICULTURAL SETTINGS & BEYOND

Our meta-analysis demonstrated a need for research assessing the degree to which polyP and PAOs impact P agricultural water quality management efforts, and ultimately, whether and how these impacts influence the achievement of short- and long-term water quality goals. Research on polyP and PAOs in agricultural landscapes can be combined with established physical and chemical P controls to initiate the development and testing of agricultural P water quality management strategies that overcome what we highlight as persistent barriers to reducing P pollution (Section 1): legacy P, unintended consequences of existing management strategies, and shifts in environmental conditions due to climate change. Meta-analysis results revealed important opportunities for interdisciplinary research on polyP and PAO research in the agricultural sciences and beyond. In addition to advancing agricultural water quality management, studies of polyP and PAOs in agricultural settings may benefit the treatment of wastewater and knowledge of P cycling in natural environments. Specifically, we see expanding the known diversity of PAOs and refining diagnostic tools for characterizing PAO community composition and function as important first steps in this effort. Below, we summarize specific research priorities that we believe will fill key polyP and PAO knowledge gaps while alleviating some of the challenges stymying meaningful reductions in P pollution.

- *Quantify Abiotic versus Biotic Phosphorus Pools* – Comparisons between the relative magnitude of abiotic and biotic P pools is a necessary first-order data need. These comparisons could be made along gradients of legacy P in soils and sediments that capture shorter (years) to longer (decades) histories of legacy P. In addition, P pool comparisons (e.g., inorganic P forms versus organic P forms) between undisturbed soils, cultivated soils, and soils associated with agricultural management practices (e.g., riparian buffers and bioreactors) are needed. Last, P pool comparisons along temperature and soil moisture gradients are needed to address whether and how biotic P retains external and internal P sources compared to abiotic P retention. These studies may also address how abiotic and biotic P retention will be influenced by projected shifts in environmental conditions due to climate change.

- *Identify and Quantify PAOs and P Functional Genes* – Studies identifying and quantifying known PAOs including *Candidatus* Accumulibacter phosphatis, *Tetrasphaera* and *Microlunatus* PAOs, denitrifying PAOs, and other microbes exhibiting PAO metabolism where P release and uptake corresponds with anoxic and oxic conditions, respectively, as well as P functional genes in agricultural soils and agricultural management practices are needed.
- *Describe the Role of PAOs* – Assessment of statistically significant relationships between PAO abundance, P functional gene abundance, and soil P forms (e.g., microbial P) in agricultural soils and management practices undergoing alternating wetting/drying conditions are needed. Furthermore, there is a need for these studies to also examine how the intensity, duration, and frequency of temperature changes and alternating wetting/drying cycles affect PAO-mediated P uptake and release. Last, studies are needed to assess whether agriculture soils contain adequate volatile fatty acid substrates (for *Candidatus* Accumulibacter phosphatis), carbohydrate substrates (e.g., for *Tetrasphaera*),

and electron acceptors (e.g., oxygen and others) to support diverse PAO metabolisms in the face of potential competitors such as glycogen accumulating organisms (Text S2).

- *Implement Next Generation Management Practices* – Studies that design and test the performance and feasibility of in-field or edge-of-field, EBPR-inspired, PAO-friendly agricultural management practices are needed. Furthermore, there is an opportunity to apply lessons learned from EBPR wastewater treatment to augment physical and chemical P retention mechanisms of existing agricultural management strategies with biological P retention mechanisms. For example, an edge-of-field bioreactor intercepting tile drainage for nitrate removal could be retrofitted according to EBPR wastewater treatment design (Section 5) to also treat DP. Hypothetically, the retrofitted bioreactor could promote PAO community growth and removal of DP from tile drain effluent while establishing new methods to recycle P from settled microbial biomass. Additionally, research is needed to assess whether various agricultural soils have adequate resources—including carbon substrate and electron acceptor availability—to support one or many of the diverse PAO metabolisms discussed in Section 5 and Text S2. For agricultural management practices that rely more on rainfall events to drive alternating anoxic/oxic conditions (i.e., saturation of riparian buffer soils), feasibility testing will likely be necessary to address whether the frequency of alternating anoxic/oxic conditions is adequate to ensure PAO community stability and P retention. Feasibility testing may also consider whether oxidized nitrogen molecules (i.e., nitrate and nitrite) are present and whether alternating anoxic/oxic conditions are realistic for a particular locale. Furthermore, it may be important to address whether edge-of-field management practices that use PAOs to remove P will require regular biomass removal and if this can be implemented in a way that simultaneously meets farm and environmental goals in a changing climate.

- *Interdisciplinary Research* – There is a need for interdisciplinary studies that test and leverage diagnostic tools (Section 8) as well as results of research needs listed above (e.g., identification of new PAOs) from agricultural settings to address broader questions concerning the origin and role of polyP and PAOs in wastewater treatment plants and natural environments undergoing alternating anoxic/oxic conditions (Figure 1). Research collaborations between EBPR experts and agricultural scientists may be especially well positioned to address feasibility issues discussed above regarding implementation of next-generation management practices as well as the resiliency of management practices in the face of future changes in climate.

SUPPORTING INFORMATION

The Supporting Information is available online free of charge at https://doi.org/10.1021/acs.est.0c03566 and contains supplementary text, figures, and tables for this study as referred to in the main text of the article, including: Text S1-S3, Figures S1-S4, Tables S1-S9.

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