A 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 aerobic conditions and release P under anaerobic 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

Non-point 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 (Carpenter et al., 1998; Carpenter, 2005; Dubrovsky et al., 2010; Jarvie et al., 2013; Mekonnen & Hoekstra, 2018). 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 (Alexander et al., 2008), and a global study of grey water footprints estimated that agricultural land accounted for 38% of anthropogenic P loads to freshwater from 2002 to 2010 (Mekonnen & Hoekstra, 2018). A recent watershed modeling study estimated that 88% of P inputs into the Great Lakes Basin came from agricultural sources (Hamlin et al., 2020), which have contributed to regional eutrophication issues for more than 50 years (Chapra and Dolan, 2012; Dolan and Chapra, 2012). 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 to 1000 μg/L depending on ecoregion) from 1992 to 2004 (Dubrovsky et al., 2010). In the midwestern United States, agricultural tile drainage contributed nearly 50% of P to Lake Erie's tributaries (Smith, King, Johnson, et al., 2015).

There are several known barriers limiting progress towards 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 (Bennett et al., 2001; Carpenter, 2005; Goyette et al., 2018; Haygarth et al., 2014; Powers et al., 2016; Sharpley et al., 2013). As a result, more aggressive P management strategies are often required in agricultural soils with legacy P (Kleinman, Sharpley, Buda, et al., 2011; Sharpley et al., 2013). 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 (Jarvie et al., 2017). 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; Kleinman, Sharpley, Buda, et al., 2011; Kleinman, Sharpley, McDowell, et al., 2011). Without changes in agricultural P management, tile drains may continue to transport DP and cause water quality issues such as harmful algal blooms (Christianson et al., 2016; Jarvie et al., 2017; Scavia et al., 2014; Smith et al., 2015). 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 (IPCC, 2014; USGCRP, 2018) 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 (Bieroza et al., 2019; Kaushal et al., 2014; Lisboa et al., 2020; Maccoux et al., 2016; Markelov et al., 2019; Paerl & Otten, 2013; Smith, King, & Williams, 2015; Williams & King, 2020).

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 (Bennett et al., 2001; Carpenter et al., 1998; Carpenter, 2005; Dodds & Smith, 2016; Schindler, 2012). 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 (Carpenter et al., 1998; Carpenter, 2005; Dodds et al., 2009; Sekaluvu et al., 2018). Furthermore, government support of agricultural water quality management programs can cost taxpayers millions of dollars (Gregory et al., 2007).

Researchers have called for interdisciplinary research to meet global P challenges associated with food and water security (Reitzel et al., 2019), 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 a rate-limiting step 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 focus 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, to the ocean; Figures 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 that assesses whether knowledge of PAOs in wastewater treatment plants can be leveraged to benefit agricultural P water quality management.

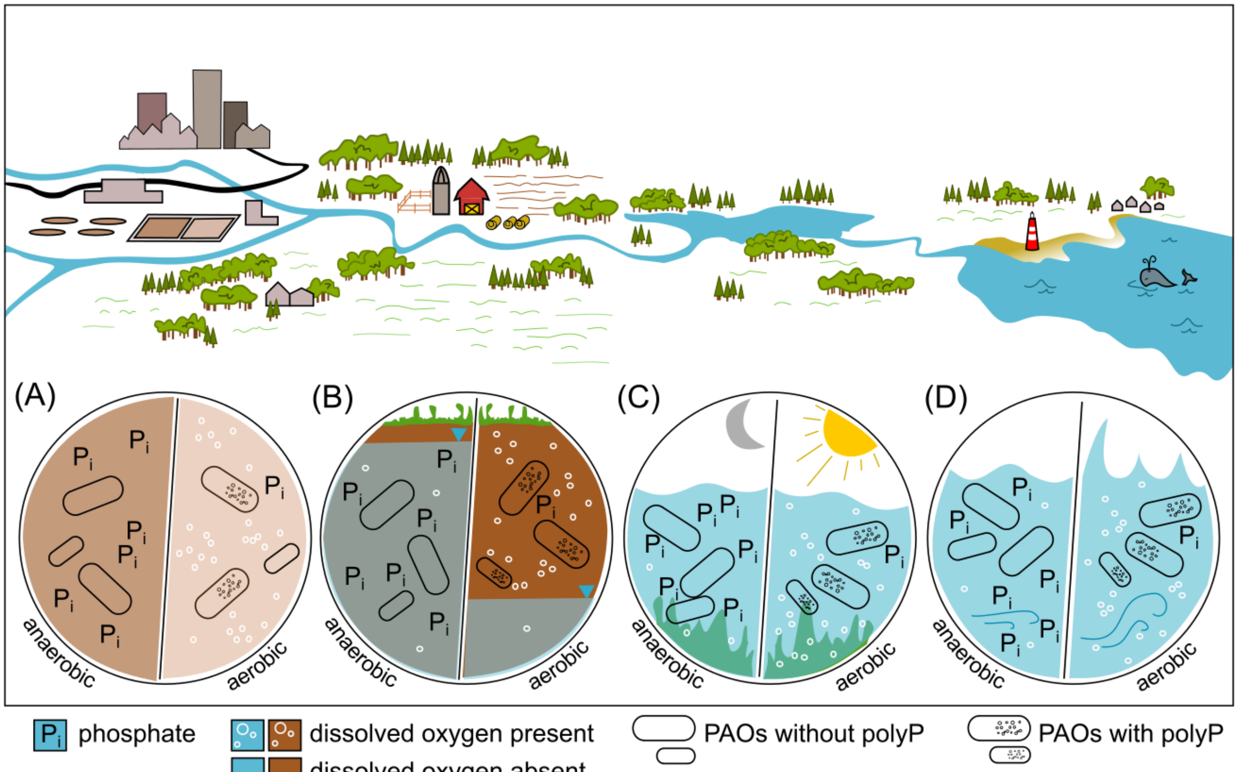

Figure 1. Schematic depicting (A) *known* microbially-mediated phosphate (P_i) cycling in wastewater treatment plants under alternating anaerobic/aerobic conditions and *hypothesized* (this study) microbially-mediated P cycling in (B) soils (including agricultural and uncultivated soils) and sediments, (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; R Core Team 2019) 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 keyword search in Web of Science queries keywords supplied by authors, text from the article title and abstract, and text from the "keyword plus" field of the Web of Science database (Clairvate; 2020). The "keyword plus" field relies on an algorithm to analyze extended terms based on the article's cited references (Clairvate; 2020). 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 Figures 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 will not summarize research on abiotic and biotic Fe and P interactions because these subjects have been thoroughly reviewed elsewhere (Chacon et al., 2006; Gerke, 2010; Lin et al., 2018; Martins et al., 2011; River & Richardson, 2018; Safarzadeh-Amiri et al., 2017; Saia et al., 2017; Sulu-Gambari et al., 2016; Wan et al., 2019; Wilfert et al., 2015; Wu et al., 2019). All data and analysis scripts associated with this publication are available on GitHub at <will fill in upon publication> and Zenodo (DOI: <will fill in upon publication>).

3. CURRENT STRATEGIES FOR MANAGING PHOSPHORUS IN AGRICULTURAL SETTINGS

The majority of agricultural water quality best management practices 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 (Bock et al., 2015; Sharrer et al., 2016). 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 (Brady & Weil, 2008; Carpenter et al., 1998; Gregory et al., 2007; Kleinman, Sharpley, Buda, et al., 2011; Schachtman et al., 1998; Sharpley & Menzel, 1987). 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 non-growing 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; Watts & Torbert, 2009) 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 (Garcia et al., 2016; Iho et al., 2017; Jarvie et al., 2017; Smith, King, Johnson, et al., 2015; Williams et al., 2016).

Despite these benefits, evidence suggests that agricultural water quality best management practices do not always perform as intended (Table S3). Specifically, biochar additions to woodchip bioreactors have a finite adsorption capacity (Bock et al, 2015) and one study found that, depending on the lumber type, woodchips leached DP during the start-up phase of bioreactor development (Sharrer et al., 2016). Furthermore, PP accumulated in the topsoil of vegetated filter strips and riparian buffers became an unintended P source (Lyons et al., 1998; Vidon et al., 2010; Young & Briggs, 2008; Young & Ross, 2016). Use of cover crops led to the accumulation of P in upper layers of the soil profile (Jarvie et al., 2017) and leached DP during freeze-thaw conditions (Jarvie et al., 2015; Liu et al., 2019). No tillage practices enhanced macropore development and increased subsurface transport of DP (Jarvie et al., 2017; Kleinman et al., 2009). Moreover, tile drains increased subsurface DP transport (Jarvie et al., 2015; King et al., 2015; Kleinman et al., 2015). 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 (Hayat et al., 2010; Javot et al., 2007; P. Jeffries et al., 2003; Richardson et al., 2011; Richardson & Simpson, 2011; Rodríguez & Fraga, 1999; Schachtman et al., 1998; Tapia-Torres et al., 2016). 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 anaerobic/aerobic conditions (Bernhardt et al., 2017; McClain et al., 2003; Vidon et al., 2010). Constructed wetlands are one exception because wetland vegetation and microorganisms may serve as P sinks (Kellogg & Bridgham, 2003; Noe et al., 2003; Reddy et al., 1999; Richardson, 1985; Scinto & Reddy, 2003). 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., Bünemann et al., 2011; George et al., 2018). However, the development and implementation of soil organic P-focused agricultural water quality management practices 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 (Bünemann et al., 2011; Cross & Schlesinger, 1995) 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 (Dodd & Sharpley, 2015). 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 (Cross & Schlesinger, 1995; Haygarth et al., 2018; McLaren et al., 2015; Schachtman et al., 1998), 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 (Dodd & Sharpley, 2015; George et al., 2018; Haygarth et al., 2018). 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 (Dodd & Sharpley, 2015; George et al., 2018; Haygarth et al., 2018).

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 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., Cade-Menun, 2017). PolyP can be chemically liberated using common organic extraction procedures (e.g., Eixler et al., 2005). 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 (Brown & Kornberg, 2004; Harold, 1964; Kornberg, 1995; Rao et al., 2009; Seviour & Nielsen, 2010; Zhang et al., 2002). 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 buffers against alkali conditions, as a regulator of gene expression under periods of stress, and as a regulator of virulence factors (Brown & Kornberg, 2004, 2008; Kornberg, 1995; Rao et al., 2009; Seviour & Nielsen, 2010).

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 9,230 articles in Web of Science that included the keyword "polyphosphate" and 22.1% 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 focus 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 = 1,094) 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, which was published in 2016 (Figures 2C, 2D, S2B, Table S2). We discuss agricultural discipline results in Section 7. Based on 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 (Figures 3C and 3D). In combination, these meta-analysis results highlighted an opportunity for interdisciplinary research addressing P forms like polyP in the natural environment.

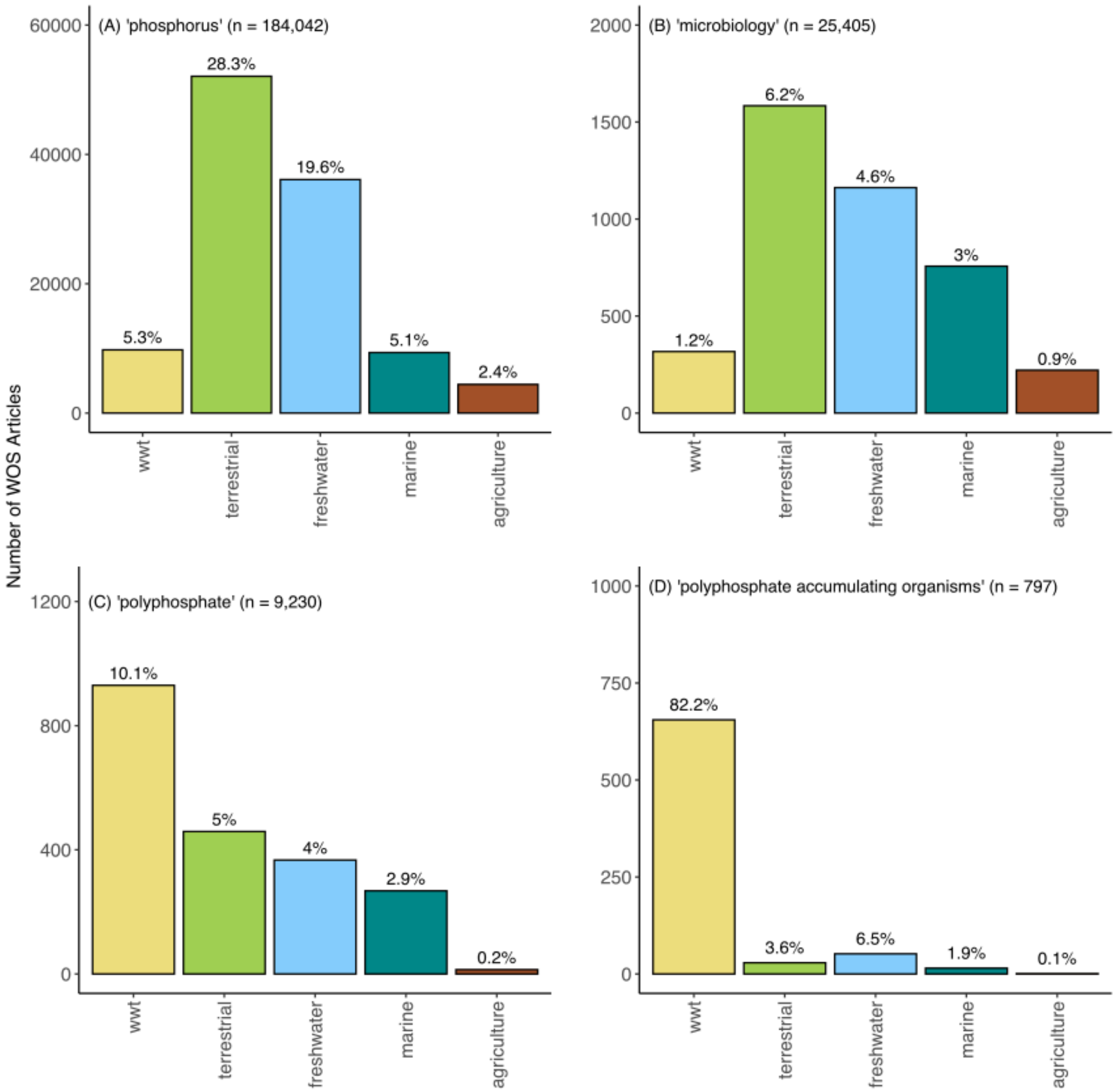

Figure 2. Number and percentage of articles returned from Web of Science (WOS) by category for (A) 'phosphorus', (B) 'microbiology', (C) 'polyphosphate', and (D) 'polyphosphate accumulating organisms' keyword searches. Percentages on top of each bar are relative to the number of articles in the full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

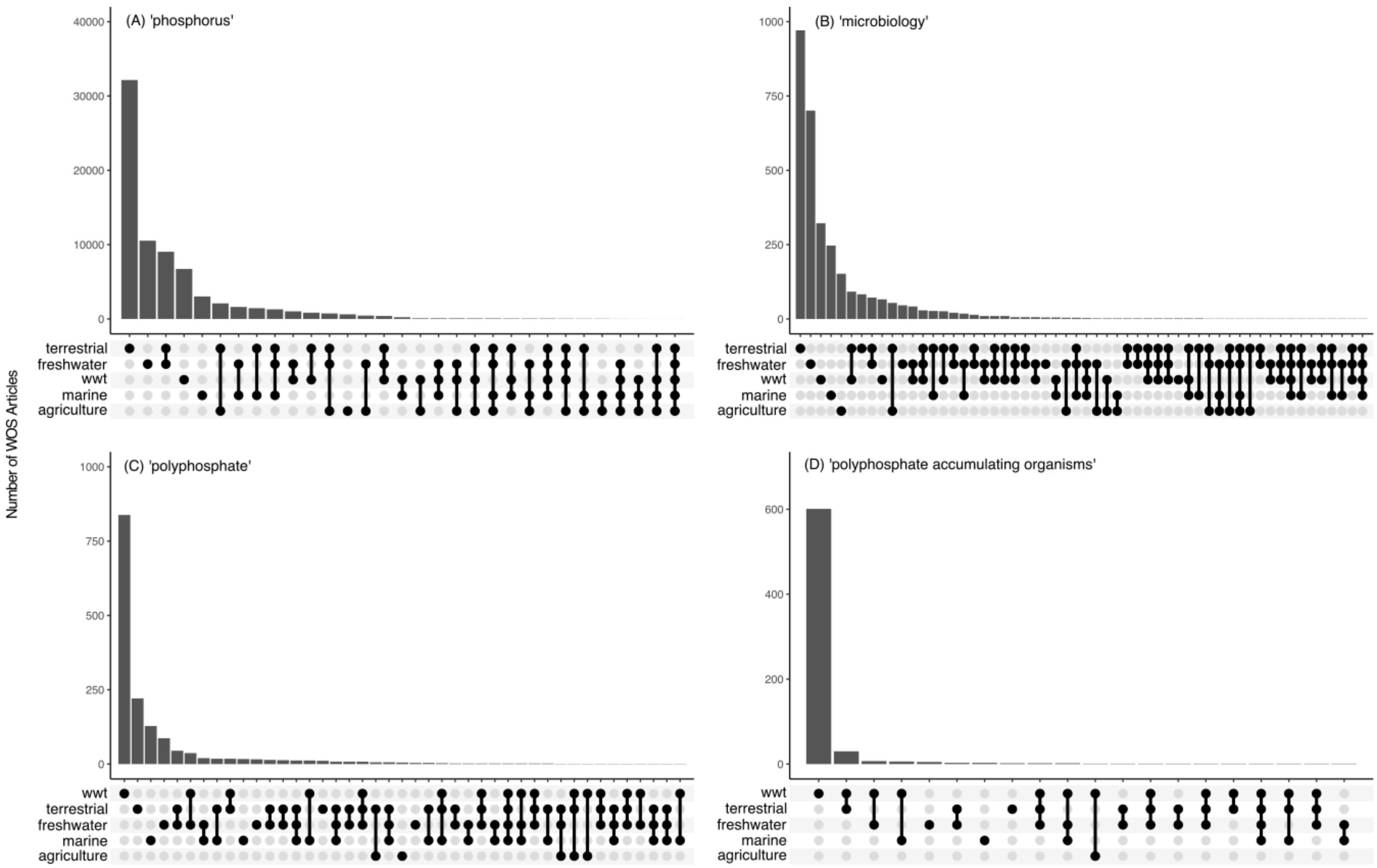

Figure 3. Number of overlapping Web of Science (WOS) articles by category for (A) ‘phosphorus’, (B) ‘microbiology’, (C) ‘polyphosphate’, and (D) ‘polyphosphate accumulating organisms’ keyword searches. Set diagrams under each bar plot show connections between categories; a closed circle represents articles 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., Schindler, 1977), wastewater treatment plant designs went beyond removing carbon and nitrogen to also removing P (e.g., Barnard, 1976). 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 (Seviour et al., 2003). EBPR is more economical than conventional wastewater treatment plant P removal because it does not require expensive and logistically complex additions of Ca, Al, or Fe to chemically precipitate out P (Oehmen et al., 2007). 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 (Barnard, 1976; Nielsen et al., 2019; Seviour et al., 2003; Seviour and McIlroy, 2008; Seviour and Nielsen, 2010). EBPR wastewater treatment plant designs generally include three main operating components: (1) an anaerobic (i.e., no molecular oxygen and generally no nitrate present) reactor with an organic (carbon-containing) energy source such as acetate, (2) an aerobic zone, and (3) a means to recycle a fraction of the settled biomass such that it is subjected to alternating anaerobic/aerobic conditions (Seviour et al., 2003; Seviour and Nielsen, 2010; Skennerton et al., 2014; Figures 1A and S3). 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 anaerobic/aerobic 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” (Gebremariam et al., 2011; Seviour et al., 2003; Yall et al., 1972). Typically, EBPR sludge is 5-7% P (dry weight) while the P content of conventional sludge ranges from 1-2% (Yuan et al., 2012). PAOs play a large role in removing P from influent waters of EBPR wastewater treatment plants around the world (e.g., Mao et al., 2015).

The most frequently studied (model) PAO known by the provisional scientific name *Candidatus* Accumulibacter phosphatis (Hesselmann et al., 1999), has the ability to synthesize large amounts of polyP under aerobic conditions to support the uptake and intracellular storage of organic substrate (Seviour et al., 2003; Seviour and Nelson, 2010; Figures 1A and S3A). This metabolism defines PAOs and enables them to outcompete the majority of other non-PAO heterotrophs with less flexible metabolic capabilities (Gebremariam et al., 2011). For a detailed description of *Candidatus* Accumulibacter phosphatis metabolism in EBPR wastewater treatments plants see Text S1 and Figure S3. While we have a better understanding of PAO metabolism since EBPR was introduced, the metabolic mechanisms separating PAOs from non-PAOs are still debated and studied (e.g., Barnard et al., 2017; Skennerton et al., 2014). Genotypic and phenotypic diversity of *Candidatus* Accumulibacter phosphatis, and PAOs in general, likely explain observed variation in metabolic processes under anaerobic conditions.

Rather than a single metabolic model, many markedly different metabolic models may exist (Crocetti et al., 2000; Kawakoshi et al., 2012; Kristiansen et al., 2013; Mao et al., 2014; Mino et al., 1998; Nielsen et al., 2019; Quang et al., 2019; Qiu et al., 2019; 2020; Rubio-Rincón et al., 2017; Seviour et al., 2003; Seviour and McIlroy, 2008; Skennerton et al., 2014). *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 aerobic EBPR wastewater treatment conditions (Figure S3B). However, some strains of *Candidatus* Accumulibacter phosphatis can use ethanol and amino acids rather than acetate (Qiu et al., 2019; 2020; Skennerton et al., 2014). Non-*Candidatus* Accumulibacter phosphatis PAOs of the *Microlunatus* and *Tetrasphaera* genera can ferment many diverse carbon substrates to make glycogen (Text S1). A growing body of research on PAOs in the *Tetrasphaera* genus has demonstrated their potential importance in EBPR (Nielsen et al., 2019). *Tetrasphaera* PAOs can make up to 30-35% of PAOs in full-scale EBPR wastewater treatment plants (Mielczarek et al., 2013; Nguyen et al., 2011). 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 aerobic EBPR wastewater treatment conditions and also remove nitrogen molecules during anoxic EBPR wastewater treatment conditions (Text S1). Researchers also demonstrated effective P removal in an EBPR wastewater treatment undergoing alternating anoxic (i.e., no oxygen present but nitrate and nitrite molecules are present) and aerobic 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 (Cokro et al., 2017). 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 focus primarily on P polymers (i.e., polyP), other polymers (e.g., glycogen and carbon substrates) and flexible PAO metabolisms may prove important in natural habitats where essential nutrients are more limited (Text S1 and S2). Furthermore, PAO survival alongside potential competitor organisms such as glycogen accumulating organisms (Text S1), in various alternating redox condition configurations (i.e., anaerobic, anoxic, and aerobic), using diverse carbon substrates, and using 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 controls (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. Based on meta-analysis results, 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 (Figures 2C, 2D, 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, and 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., Ota et al., 2016) that we identified in the agriculture category (Section 7). Last, alternating anaerobic/aerobic 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 natural alterations between anaerobic/aerobic conditions in several types of natural environments (e.g., soils, sediments, freshwater, and marine waters) may select for microorganism that had a similar phenotypes and genotypes to EBPR PAOs (Davelaar, 1993; Diaz et al., 2012; Gächter et al., 1988; Hupfer et al., 2007; Hupfer & Lewandowski, 2008; McMahon & Read, 2013; McParland et al., 2015; Peterson et al., 2008; Pett-Ridge & Firestone, 2005; Reddy et al., 1999; Schulz & Schulz, 2005; Uhlmann & Bauer, 1988). 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 world-wide (Schindler, 2012; Conley et al., 2014). With respect to biological P forms and cycling mechanisms, researchers demonstrated that microorganisms may respond to this increase by storing excess P as polyP (Gächter et al., 1988; Uhlmann and Bauer, 1988; Kenney et al., 2001). However, over 30 years later, few studies have addressed this hypothesis and its focus on biological P forms and cycling mechanisms and 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, P functional genes, and demonstrating relationships between P and oxygen concentrations in terrestrial and aquatic environments.

6.1 Terrestrial Habitats (Soils & 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 anaerobic while saturated, and aerobic while unsaturated (Burgin & Groffman, 2012; Silver et al., 1999; Smith & Tiedje, 1979). Therefore, we hypothesize alternating wetting/drying events in soils and sediments appear to be analogs to alternating anaerobic/aerobic 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 PAOs release phosphate under wetting soil and sediment conditions, which may negatively impact P management goals for water quality protection. Our 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 (Davelaar, 1993; Pett-Ridge and Firestone, 2005; Peterson et al., 2008; Lin et al., 2018; 2020). We identified several studies documenting the presence of *Candidatus* Accumulibacter phosphatis (Archibald, 2010; Kunin et al., 2008; Martins et al., 2011; Peterson et al., 2008; Valdivia, 2009), its phylogenetically close relatives (DeAngelis et al., 2010; Pett-Ridge & Firestone, 2005), as well as other novel PAOs (DebRoy et al., 2013; Li et al., 2013) 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 anaerobic/aerobic conditions. With respect to polyP, there is evidence of polyP accumulation by diverse bacterial species in river sediments contaminated by heavy metals (Narancic et al., 2012) and evidence that microbes accumulated P as polyP under aerobic conditions and released P under anaerobic conditions in freshwater lake sediments (Amirbahman et al., 2013; Martins et al., 2011) and wetland sediments (Khoshmanesh et al., 1999, 2002). However, only one of these polyP studies (i.e., Martins et al., 2011) 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 anaerobic/aerobic 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 (Dinh et al., 2016) and microbial P (Grierson et al., 1998; Turner & Haygarth, 2001) 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 (Grierson et al., 1998). One study estimated that 41% of added phosphate was stored as microbial P upon soil rewetting (Yevdokimov et al., 2016). One study subjected humid tropical soils to alternating anoxic (i.e., no oxygen) and aerobic conditions and found that biologically available P pools decreased immediately following anoxic conditions (Lin et al., 2018). This finding supports PAO-mediated P release under anerobic conditions. Other studies have observed flushes in P after prolonged drought (Kaushal et al., 2016; Lisboa et al., 2020) 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 (Dupas et al., 2015) and watershed scales (Franklin et al., 2013) but whether and how much microbial P contributes to these patterns, relative to physical and chemical P mechanisms, is still unknown (Blackwell et al., 2010).

Based on 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 26 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", respectfully (Figures 2, S4, Table S2). Of the 26 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 (Peterson et al., 2008) 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 (Martins et al., 2011). One study sequenced and analyzed a non-*Candidatus* Accumulibacter phosphatis PAO genome (i.e., *Microlunatus* genus); these PAOs were first isolated from soils (Kawakoshi et al., 2012). Researchers used a soil-based laboratory-scale reactor under alternating anaerobic and aerobic periods—termed a humus soil activated sludge process—to treat domestic wastewater (Wu et al., 2013). While the number of articles remains relatively small, these results demonstrate the collaborative potential for interdisciplinary research. Upon closer inspection of results within the terrestrial category, most polyP and PAO articles in the terrestrial category addressed soil environments (Figures S4C, S4D). 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 (Figures 2C, 2D, 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. Nearly half (n = 221, 48.1%) of polyP articles and (n = 29, 4.3%) of PAO articles in the terrestrial category did not overlap with any other category (Figures 3C and 3D). As an example, several of these polyP articles discussed organic P pools (e.g., Bünemann et al., 2008; Darch et al., 2016). Specifically, one of these demonstrated that the addition of glucose (i.e., organic substrate) led to accumulation of pyrophospate (Bünemann et al., 2008), which is a polyphosphate polymer with two phosphate molecules (Table S1). A second example demonstrated polyP accumulation and P release in sediment bacteria under aerobic and anaerobic laboratory conditions, respectively (Khoshmanesh et al. 2002); 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 rare instances, polyP articles in the terrestrial category overlapped with 2 or more categories discussed here, but these summed to a total of 98 articles. With respect to PAOs, we identified 7 PAO articles in the terrestrial category that overlapped with articles in the wastewater treatment category. Several of these used soil as a source for developing a sequencing batch reactor that was capable of removing P (e.g., Zhang et al., 2003 and Zhu et al., 2011), 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 added to a total of 21 articles. We found one article that overlapped with all categories except agriculture (i.e., Krishnaswamy et al., 2011). There were 26 polyP and PAO articles that overlapped with one another and were both in the terrestrial category (Figures 3C, 3D). For example, one of these isolated a new PAO from soils (Zhang et al., 2003; Table S5). 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 & Lakes)

In streams, rivers, and shallow regions of lakes, alternating anaerobic and aerobic 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 (Cohen et al., 2013; Dodds, 2003). In the case of freshwater tidal wetlands, hydrodynamics (e.g., tides) can also influence alternating anaerobic/aerobic 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 (Findlay & Fischer, 2013). In deeper regions of lakes, alternating anaerobic/aerobic conditions are often driven by changes in the depths of the aerobic epilimnion and anoxic hypolimnion due to either internal waves or wind-induced surface waves (McMahon & Read, 2013). Given the existence of these alternating anaerobic/aerobic conditions in freshwater habitats, we hypothesize diel changes in oxygen availability driven by either metabolic or wave-driven hydrodynamics causes are analogs to alternating anaerobic/aerobic 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 (Hupfer et al., 2004; Sicko-Goad & Lazinsky, 1986; Stevenson & Stoermer, 1982) and stream biofilms (Rier et al., 2016; Saia et al., 2017; Taylor, 2016). Consistent with EBPR PAO metabolism, there is evidence that oxygen concentrations influence microbial P cycling in freshwater environments. Microbes accumulated P as polyP under aerobic and released P under anaerobic in stream biofilms (Saia et al., 2017). 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 stream 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 (Cohen et al., 2013; Ford et al., 2018). 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 (Weinke & Biddanda, 2018). 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 (Carlton & Wetzel, 1988; Fleischer, 1978; Gächter et al., 1988; Gächter & Meyer, 1993; Read et al., 2014; Saia et al., 2017; Sherson et al., 2015). While abiotic processes (e.g., redox sensitive dissolution/precipitation of Fe and P) cannot be completely ruled out as a possible causal mechanisms in the cases described above (Richardson, 1985; Saia et al., 2017), 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, Ca-P precipitation and dilution (Cohen et al., 2013). In freshwater wetlands, biotic mechanisms accounted for over 83% of short-term (12-hour) water column P removal (Scinto & Reddy, 2003). 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 (Taylor, 2016). 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 (Rier et al, 2016). 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 (Price & Carrick, 2016), 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 (Price & Carrick, 2011). 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 aerobic and anaerobic conditions, respectively (Saia et al., 2017). Studies of EBPR PAOs have shown similar reliance on metals; likely to balance the negative charge of intracellular polyP (Figure S3; Li et al., 2018; Schönborn et al., 2000). 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" (Figures 2, S4, Table S2). Compared to other keyword searches in the freshwater category, there were 36,105 articles that included the keyword "phosphorus" and 1,162 articles that included the keyword "microbiology" (Figures 2, 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 (Figures S4C, S4D).

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 = 87, Figure 3C). PAO articles most frequently overlapped with the wastewater treatment category (n = 30, Figure 3D). Many of these articles focused on PAO research in EBRP wastewater treatment settings (e.g., Quang et al., 2019; Wong et al., 2018), with several discussing denitrifying PAOs (e.g., Salehi et al., 2019). 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 (Wong et al., 2018). 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 (Peterson et al., 2008). These results support the flexibility of PAO metabolisms and 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 & Oceans)

Marine habitats discussed here include estuaries, coastal waters, and the open ocean. In coastal estuarine ecosystems, alternating anaerobic/aerobic conditions are greatly influenced by a combination of anthropogenic nutrient inputs—including nutrient-induced acceleration of primary production (Diaz & Rosenberg, 2008)—and mixing of stratified layers of the water column (Helm et al., 2011). In the open ocean, much like the deeper regions of lakes, alternating anaerobic/aerobic conditions are driven by the mixing of stratified chemoclines typically caused by waves and wind (Helm et al., 2011). Given the potential for alternating anaerobic/anaerobic conditions in marine habitats, we hypothesize wave- and wind-driven oxygen gradients are analogs to alternating anaerobic/aerobic 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 (Diaz & Rosenberg, 2008; Schulz & Schulz, 2005), and marine microorganisms (Martin et al., 2014). 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 S2, Table S6) associated with polyP cycling using techniques established for EBPR PAOs such as *Candidatus* Accumulibacter phosphatis (Zhang et al., 2015). Another analyzed the relationships between the abundance of P functional genes in marine microorganism genomes and annual water column phosphate concentrations (Temperton et al., 2011). 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 S2, Table S6) in estuarine sediments (Watson et al., 2019) and the other identified bacteria in the *Rhodobacteraceae* family; *Candidatus* Accumulibacter phosphatis is also a member of this family (Jeffries et al., 2016).

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 (McParland et al., 2015). Another found that polyP concentrations in water samples from a coastal inlet decreased as dissolved oxygen concentrations decreased (Diaz et al., 2012). Elsewhere, researchers identified giant sulfur bacteria (*Thiomargarita namibiensis*) that accumulated polyP under aerobic sediment conditions and released phosphate under anoxic (i.e, no oxygen) sediment conditions, a response that is functionally similar to EBPR PAOs (Schulz & Schulz, 2005). Therefore, there are a number of studies identifying PAOs in marine sediments but there remain opportunities to assess their role under alternating anaerobic/aerobic 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 (Martin et al., 2014). Similarly, in a metagenomic study of marine environments, the abundance of P functional genes (i.e., *ppk* and *ppx*; Text S2, Table S6) increased when annual P concentrations were lower (Temperton et al., 2011). 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 (Jeffries et al., 2016). While Jeffries et al. (2016) 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 (Cade-Menun &

Paytan, 2010). 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.

Based on meta-analysis results, polyP and PAO articles in marine environments were less common than terrestrial or freshwater categories. For marine environments, we found 367 articles in Web of Science that included the keyword “polyphosphate” and 15 articles that included the keyword “polyphosphate accumulating organisms” (Figures 2, S4, Table S2). Several of the PAO articles discussed the impact of salinity on EBPR processes (e.g., Wang et al., 2018). As a comparison to other keyword searches in the marine category, there were 9,356 articles that included the keyword “phosphorus” and 757 articles that included the keyword “microbiology” (Figures 2, 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” (Figures S4C, S4D). 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 were in the marine category and 1.9% of all PAO articles were in the marine category (Figures 2C, 2D).

Similar to terrestrial and freshwater habitats, we found limited evidence of interdisciplinary research in marine habitats. About one-third (n = 128, 34.9%) 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 their benefit to disciplines other than those of interest here (i.e., bioengineering/medicine; Wang et al., 2014). 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 (Martin et al., 2014; Li & Dittrich, 2019). 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., Rabbani et al., 2013). 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 (Castle & Kirchman, 2004). 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 55.4% 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 30,817 of these did not overlap with any other category (Figure 3A). We identified 4,429 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., Mukherjee et al., 2015; Siebers et al., 2019). 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 (Shan et al., 2016). 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 (Ota et al., 2016). 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 (Ota et al., 2016). Other studies have also suggested that PAOs could be used to concentrate P (Acevedo et al., 2015). In addition to other proposed organic P forms (e.g., Margenot et al., 2019), studies identified by our meta-analysis provide preliminary evidence that polyP accumulated by microorganisms can be recycled (MacDonald et al., 2016; Metson & Bennett, 2015; Withers et al., 2014) and serve as an alternative organic P source for crops, but the efficacy of this has yet to be tested.

The increase in P articles in the agriculture category from 1990 to 2019 did not demonstrate a similar increasing correlation over time as articles containing “polyphosphate”, “polyphosphate accumulating organisms”, or “microbiology” keywords over the same 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 non-point 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 (Krishnaswamy et al., 2011). 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 (Ye et al., 2015). Furthermore, we found one study that quantified polyP in overland flows (Bourke et al., 2009) and several studies characterizing soil organic P (Cade-Menun, 2017; B. Cade-Menun & Liu, 2013) but no studies directly addressing the role of PAOs under alternating anaerobic/aerobic conditions. In the next decade, interdisciplinary soil microbiome research is positioned to increase crop yield and resilience (NASEM, 2018), but these advancements may also be extended to improve and protect water quality.

Based on 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 S1 and S2). 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., (Albertsen et al., 2012; Mao et al., 2016), freshwater, sediments, and soils (e.g., Kunin et al., 2008; Peterson et al., 2008) 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 S2). 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 (Bardgett & Van Der Putten, 2014; Dunbar et al., 2002; Fierer & Jackson, 2006; Gans et al., 2005; Hug et al., 2016; Schloss & Handelsman, 2006; Tringe et al., 2005). 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 S2, 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 (Kunin et al., 2008; Albertsen et al., 2012; Mao et al., 2015). 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., Mao et al., 2016). We found two studies that quantified PAO-related P functional genes (i.e., *ppk*; Text S2, Table S6) in marine habitats (Temperton et al., 2011; Watson et al. 2019), but no other studies quantifying *ppk* abundance in agricultural, terrestrial, or freshwater environments. Furthermore, we found only a few *ppx* studies (Text S2, Table S6). With the exception of one marine study (Temperton et al., 2011), 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., Peterson et al., 2008) or indirectly (e.g., Khoshmanesh et al. 2002) or (2) assessed the role of biologically-mediated P uptake and release under changing environmental conditions (e.g., Cohen et al., 2013). There were a few that did both (e.g., Martins et al., 2011). In agricultural systems, most studies identified PAOs (e.g., DebRoy et al., 2013), 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 anaerobic/aerobic 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 (Text S1), 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 (Gunina & Kuzyakov, 2015). This work may be extended to couple P and nitrogen cycling, as denitrifying PAO have been identified in EBPR wastewater treatment (Text S1). 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., Aschar-Sobbi et al., 2008; Eixler et al., 2005) or a fluorescence spectrophotometer (e.g., Martin & Van Mooy, 2013) 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. Sulu-Gambari et al., 2016). 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 sub-groups of *Candidatus* Accumulibacter phosphatis in nine wastewater treatment plants (Zhang et al., 2016). Shotgun metagenomics data from a global ocean dataset were used to quantify P functional genes (Temperton et al., 2011). 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., Kenney et al., 2015; Cade-Menun, 2017). 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 (Battin et al., 2016; Bier et al., 2015; Blackwell et al., 2010; Hall et al., 2018), measurement bias for/against active microbial community members is understood (Carini et al., 2016; Jones & Lennon, 2010; Lennon & Jones, 2011; J. Schimel & Gulledge, 1998), and environmental variables (e.g., pH and temperature) that may influence microbial communities are accounted for in the experimental design (Battin et al., 2016; Bier et al., 2015; Dinh et al., 2016; Fierer & Jackson, 2006; Lauber et al., 2009; Oliverio et al., 2016; Rousk et al., 2011; Schimel & Gulledge, 1998). Researchers must also take care to design controls that consider abiotic processes that may mimic PAO-mediated P cycling (e.g., reductive dissolution of Fe-P).

Beyond specific tools, several general frameworks exist to link microbial with ecosystem-scale—or potentially, watershed-scale—processes (Bier et al., 2015; Hall et al., 2018; Martiny et al., 2015; Nemergut et al., 2014; Prosser, 2013; Schimel et al., 1999; Wallenstein & Hall, 2012). 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 (Choudoir et al., 2017; Prosser et al., 2007), statistical approaches and considerations (Bernhardt et al., 2017; Bier et al., 2015; Buttigieg & Ramette, 2014; Rocca et al., 2015; Schimel &

Gulledge, 1998; Willis, 2016; Willis et al., 2017), method overviews (Ekblom & Wolf, 2014; Howe et al., 2014; Howe & Chain, 2015; Kozich et al., 2013; Pallen, 2016; Riesenfeld et al., 2004; Schloss, 2020; Zimmerman et al., 2014), bioinformatics (Cock et al., 2009; Howe et al., 2014; Loman & Watson, 2013; Shade & Teal, 2015; Wilson et al., 2016), reproducible research (da Veiga Leprovost et al., 2014; Perez-Riverol et al., 2016; Schloss, 2017; Shade & Teal, 2015; Wilson et al., 2016), and modelling (Graham et al., 2014, 2016; Manzoni et al., 2014; Powell et al., 2015; Reed et al., 2014; Todd-Brown et al., 2012; Wieder et al., 2013).

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 anaerobic and aerobic 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.

- *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 to 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 S1. For agricultural management practices that rely more on rainfall events to drive alternating anaerobic/aerobic conditions (i.e., saturation of riparian buffer soils), feasibility testing will likely be necessary to address whether the frequency of alternating anaerobic/aerobic conditions is adequate to ensure PAO community stability and P retention. Feasibility testing may also consider whether anerobic/anoxic or anoxic/aerobic conditions are more 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 anaerobic/aerobic 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.

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Supporting Information for

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

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Contents Metadata

This document includes the supplementary figures and tables for this study as referred to in the main text of the article. All data and analysis scripts associated with this publication are available on GitHub at <will fill in upon publication> and Zenodo (DOI: <will fill in upon publication>).

A preprint of this publication is available on the EarthArXiv at https://eartharxiv.org/ge95h/.

TEXT

Text S1. Metabolisms of Polyphosphate Accumulating Organisms and Their Competitors

The metabolism of model polyphosphate accumulating organisms (PAO), *Candidatus* Accumulibacter phosphatis, in operational enhanced biological P removal (EBPR) wastewater treatment plants is described as follows. During anaerobic (i.e., no oxygen, nitrate, or nitrite are present) conditions (Figure S3A), *Candidatus* Accumulibacter phosphatis takes up short chain volatile fatty acids (VFAs) such as acetate and store them as poly-β-hydroxyalkanoates (PHAs) like poly-β-hydroxybutyrate (PHB; Seviour et al. 2003; Seviour and Nielsen 2010). Intracellular polyphosphate (polyP) and glycogen concentrations decrease because they are used to convert VFA to PHA (Seviour et al., 2003; Seviour and Nielsen, 2010). Phosphate cleaved from the terminal end of a polyP chain during this process is exported from the cell, thereby contributing to an increase in the concentration of phosphate (i.e., P_i in Figure S3) in the bulk wastewater (Seviour et al., 2003; Seviour and Nielsen, 2010). *Candidatus* Accumulibacter phosphatis uses the energy released from the respiration of PHAs to replace polyP and glycogen (Seviour et al., 2003; Seviour and Nielsen, 2010) during aerobic (i.e., oxygen is present) periods (Figure S3B). As a result, *Candidatus* Accumulibacter phosphatis uptakes phosphate to build polyP chains, thereby drawing down bulk water phosphate concentrations in the wastewater prior to its discharge from the wastewater treatment plant (Seviour et al., 2003; Seviour and Nielsen, 2010). Recent research has documented the use of diverse carbon substrates such as ethanol and amino acids, rather than acetate, in some strains of *Candidatus* Accumulibacter phosphatis (Qiu et al., 2019; 2020) as well as the ability to use oxygen and nitrate as electron acceptors (Camejo et al., 2016).

There are two other types of PAOs that may be of interest to researchers studying P cycling microbes in natural habitats because these organisms have more flexible metabolisms than *Candidatus* Accumulibacter phosphatis. First, two major genera (i.e., *Tetrasphaera* and *Microlunatus*) of PAOs that can accumulate polyP under aerobic conditions like *Candidatus* Accumulibacter phosphatis (Nielsen et al., 2019), but unlike *Candidatus* Accumulibacter phosphatis, *Tetrasphaera* and *Microlunatus* PAOs can ferment carbon substrates such as glucose (Kristiansen et al., 2013; Nakamura et al., 1995b; Nguyen et al., 2011), sugar alcohols (Nakamura et al., 1995b), and amino acids (Kong et al., 2005; Nakamura et al., 1995b; Nguyen et al., 2011) rather than relying on direct uptake of volatile fatty acids like acetate. Second, denitrifying PAOs—including some strains of *Candidatus* Accumulibacter phosphatis (Camejo et al., 2016; Carvalho et al., 2007; Zeng et al., 2002) and non-*Candidatus* Accumulibacter phosphatis organisms (Li et al., 2011; Vieria et al., 2018)—can accumulate P as polyP under aerobic conditions and can use nitrate, and in some cases nitrite, as an electron accepter under anoxic (i.e., no oxygen is present but nitrate and/or nitrite are present) conditions (Kapagiannidis et al., 2009; Vieira et al., 2018).

Glycogen accumulating organisms (GAOs) use volatile fatty acids like acetate, but do not accumulate P intracellularly as polyP, which puts them in competition with PAOs for acetate (Oehmen et al., 2007). This competition can impact the effectiveness of smaller laboratory-scale EBPR wastewater treatment reactors (Mielczarek et al., 2013; Oehmen et al., 2007). However, there is limited evidence that GAOs complete with PAOs in large, full-scale EBPR wastewater treatment plants (Nielsen et al., 2019). For example, GAOs have been observed to make up anywhere from < 1 to 20% of the bacterial community in full-scale EBPR wastewater treatment plants (Law et al., 2016; Mielczarek et al., 2013; Stokholm-Bjerregaard et al., 2017), yet these plants effectively remove P (Nielsen et al., 2019). In one instance, GAOs and PAOs behaved synergistically in a laboratory sized EBPR wastewater treatment reactor. Specifically, the GAOs reduced nitrate to nitrite then the PAO used the nitrite as an electron acceptor for anoxic P update (Rubio-Ricón et al., 2017a). The two most widely studied GAOs include organisms in the *Dechloromonas* and *Competibacter* genera (Crocetti et al. 2002; Nobu et al., 2014; McIlroy et al., 2014; McIlroy and Seviour, 2009; Oehmen et al., 2007; Seviour and McIlroy, 2008).

Text S2. Phosphorus Cycling Functional Genes

There are several known functional genes associated with PAO-mediated P uptake and release in EBPR (Table S6). For an organism to be classified as a PAO, it must have genes that form polyP (i.e., polyphosphate kinases) as well as genes that break down polyP (i.e., exopolyphosphatases) (Mao et al., 2014; Ohtake et al. 2001; Seviour et al., 2003, Skennerton et al., 2014). Polyphosphate kinases PPK1 and PPK2—coded for by *ppk1* and *ppk2,* respectively—catalyze the reversible reaction of ATP to ADP to form intracellular polyP (Table S6). The nucleotide sequence for *ppk1* was first isolated from *Escherichia coli* (Akiyama et al., 1992) and has since been identified in a wide range of bacterial, archaeal, and eukaryotic organisms (Trelstad et al., 1999; Zhang et al., 2002; Rao et al., 2009; Kawakoshi et al., 2012) and PAOs (McMahon et al., 2002; He et al., 2007; Zhang et al., 2016). PPK1 is likely a membrane-bound protein with four domains concentrated in regions where the inner and outer cell membranes come together (Ahn and Kornberg, 1990). The nucleotide sequence of *ppk2* was first isolated from *Pseudomonas aeruginosa* (Zhang et al., 2002). PPK2 differs from PPK1 in its ability to catalyze the formation of polyP from both GTP and ATP as well as enzyme cofactors. Also, PPK2 has an affinity for Mn^{2+} while PPK1 has an affinity for Mg^{2+} (Zhang et al., 2002; Rao et al., 2009). Because they are highly conserved (Zhang et al., 2002; Rao et al., 2009), *ppk*'s such as *ppk1* and *ppk2* are an ideal gene marker for bacterial strain diversity. Some microbes possess two *ppk*'s (*ppk1* and *ppk2*) while others only have one (Zhang et al., 2002; Rao et al., 2009; Temperton et al., 2011; Kawakoshi et al., 2012). Specific to PAOs, the *Candidatus* Accumulibacter phosphatis genome has a single copy of *ppk1* and it evolves faster than *Candidatus* Accumulibacter phosphatis 16S rRNA genes (Kunin et al., 2008; He and McMahon, 2011a).

Exopolyphosphatase PPX1 and PPX2/GPPA (coded for by *ppx1* and *ppx2/gppA*) catalyze the breakdown of polyP. PPX1 breaks off the terminal phosphate molecules of a polyP chain when excess phosphate is present (Table S6). PPX1 preferentially acts on longer chains of polyP (i.e., 500 phosphate molecules or longer), does not act on ATP, and cannot be inhibited by ADP or ATP (Akiyama et al., 1993). PPX2/GPPA, also referred to as pentaphosphate phosphohydrolase, inhibits polyP accumulation at the enzymatic level by hydrolyzing stress response nucleotides pppGpp to ppGpp or catalyzes the release of phosphate by breaking polyP chains (Table S6). PPX2/GPPA is thought to be less active than PPX1, prefers longer polyP chains (i.e., 1000 residues or longer), and is inhibited by the presence of short- and medium-length polyP chains (Keasling et al., 1993). Some organisms have both *ppx1* and *ppx2/gppA* (Keasling et al., 1993; Alcántara et al., 2014), but this trend is not well characterized for PAOs.

We focus on *ppk* and *ppx* as a starting point; however, other P cycling genes of interest include *pap*, *phoX*, *phoD*, *phoA*, *pit*, *pst*, and *ppn*. Associated protein functionality and key traits are summarized in Table S6. Additionally, glycogen and poly-β-hydroxyalkanoate (PHA) genes may also be of interest (McIlroy et al., 2014; Oyserman et al., 2016). As researchers continue to learn more about the diversity of PAO metabolisms through study of these organisms in natural habitats, other emerging, important genes may be uncovered. For example, denitrifying genes such as *nosZ*, *nirS*, and *nirK* may be important to exploring the functionality of denitrifying PAOs (e.g., Vierira et al., 2018).

FIGURES

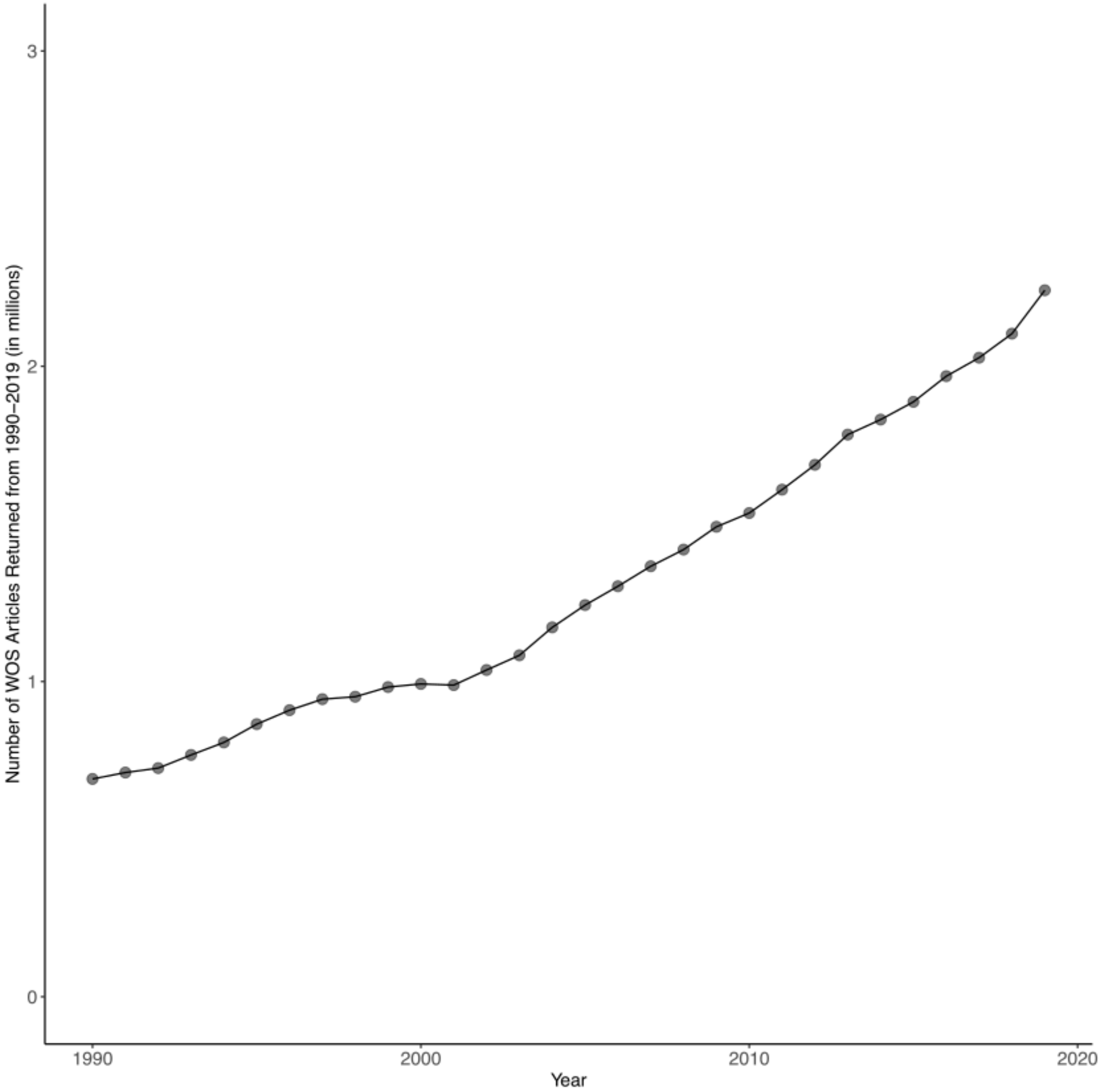

Figure S1. Number of the total number of Web of Science (WOS) articles published per year from 1990 to 2019.

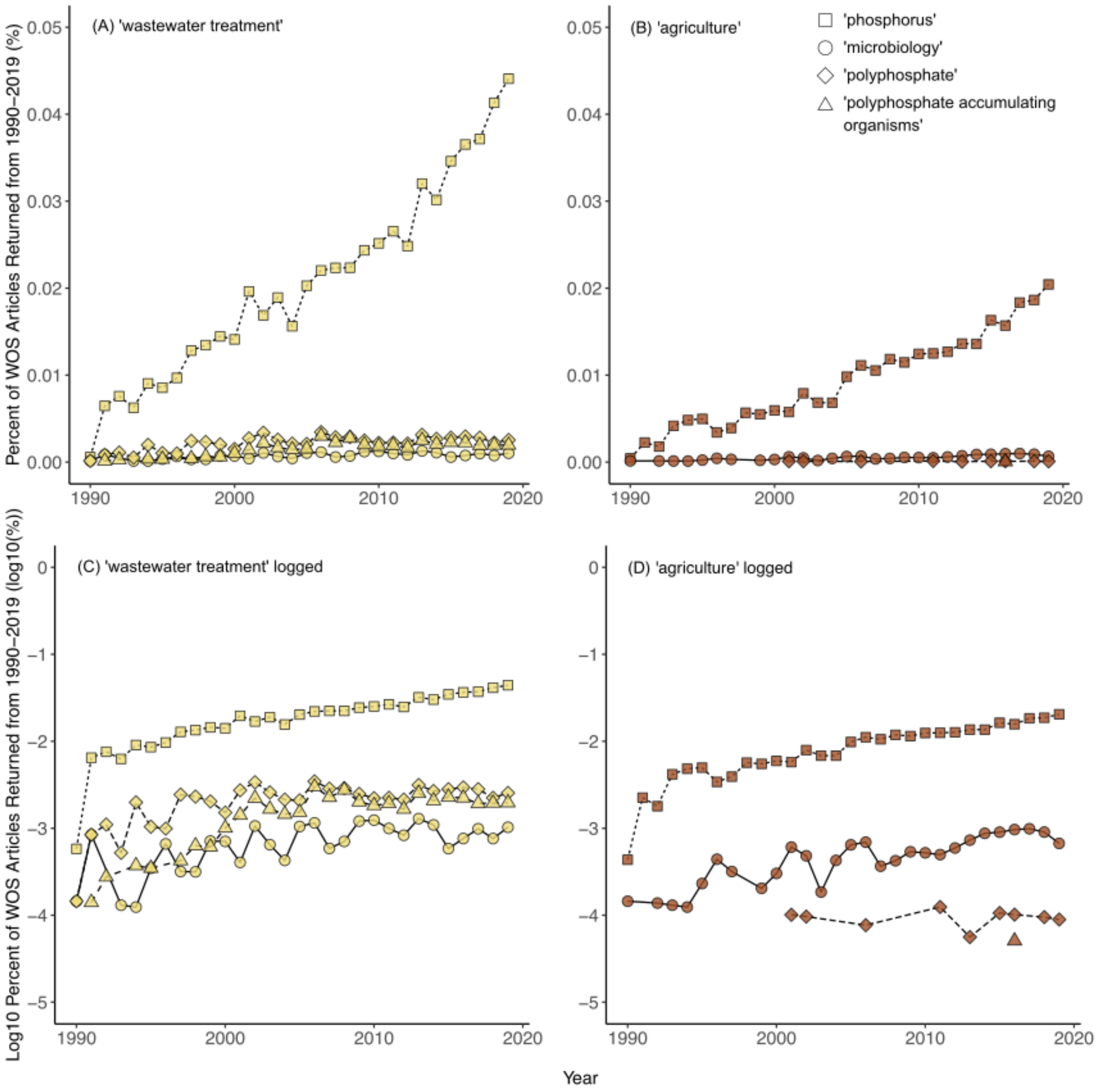

Figure S2. Percent of Web of Science (WOS) articles returned from 1990-2019 for various (A) ‘wastewater treatment’ and (B) ‘agriculture’ keyword searches. Logged (base 10) results as shown in (A) for ‘wastewater treatment’ and in (B) for ‘agriculture’ keyword searches are shown in (C) and (D), respectively. For example, the open box line represents the WOS search “ ’wastewater treatment’ AND ‘phosphorus’ ”. Note: There is only one “ ‘agriculture’ AND ‘polyphosphate accumulating organisms’ ” article in 2016.

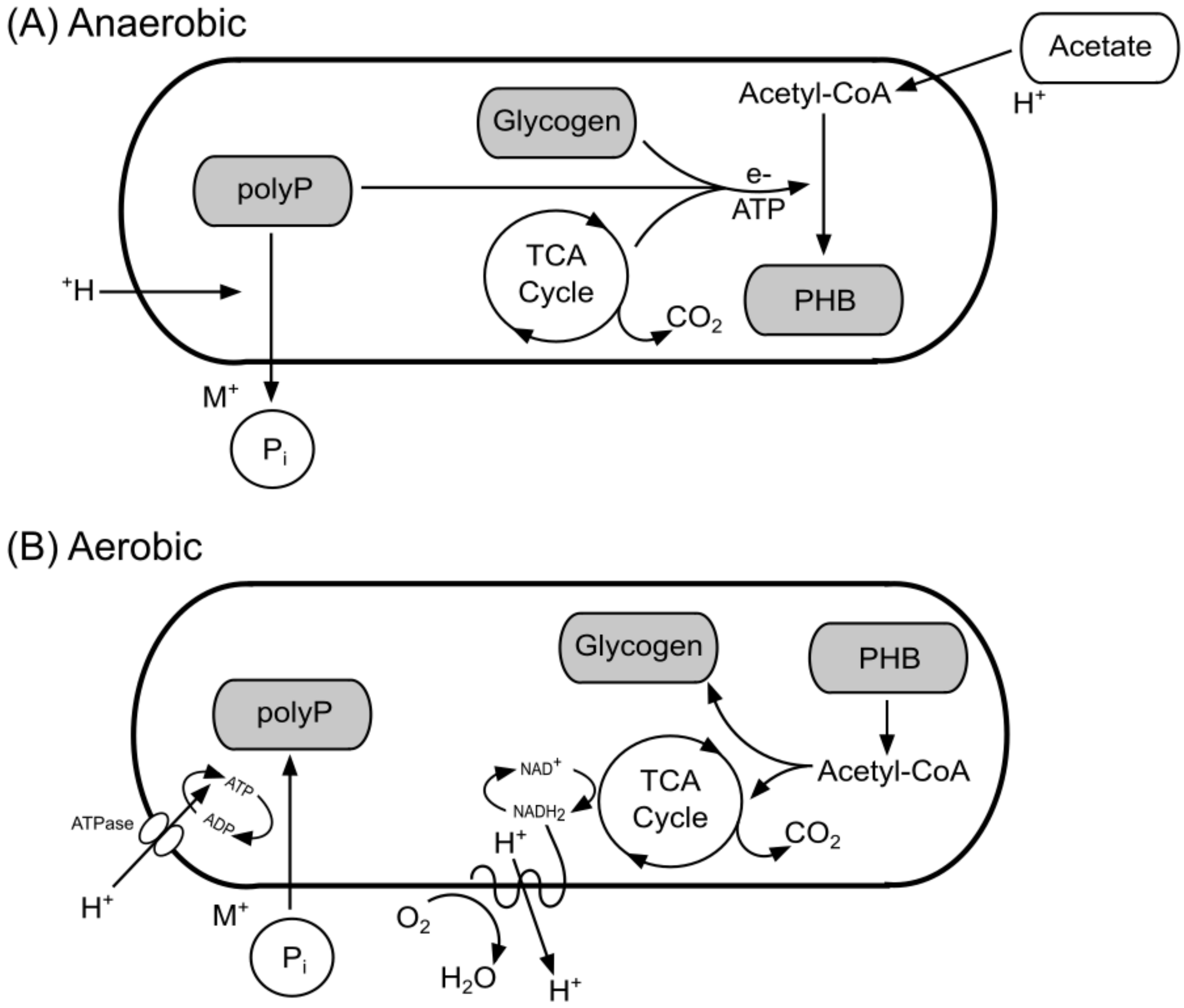

Figure S3. (A) Anaerobic and (B) aerobic metabolism of model polyphosphate accumulating organism (PAO), *Candidatus* Accumulibacter phosphatis. The poly-β-hydroxyalkanoate (PHA) known as poly-β-hydroxybutyrate (PHB) is specific to CAP. Abbreviations: metal cations (M^+), phosphate (P_i). Adapted from Seviour et al. (2003), Seviour and Nielsen (2010), and Skennerton et al. (2014).

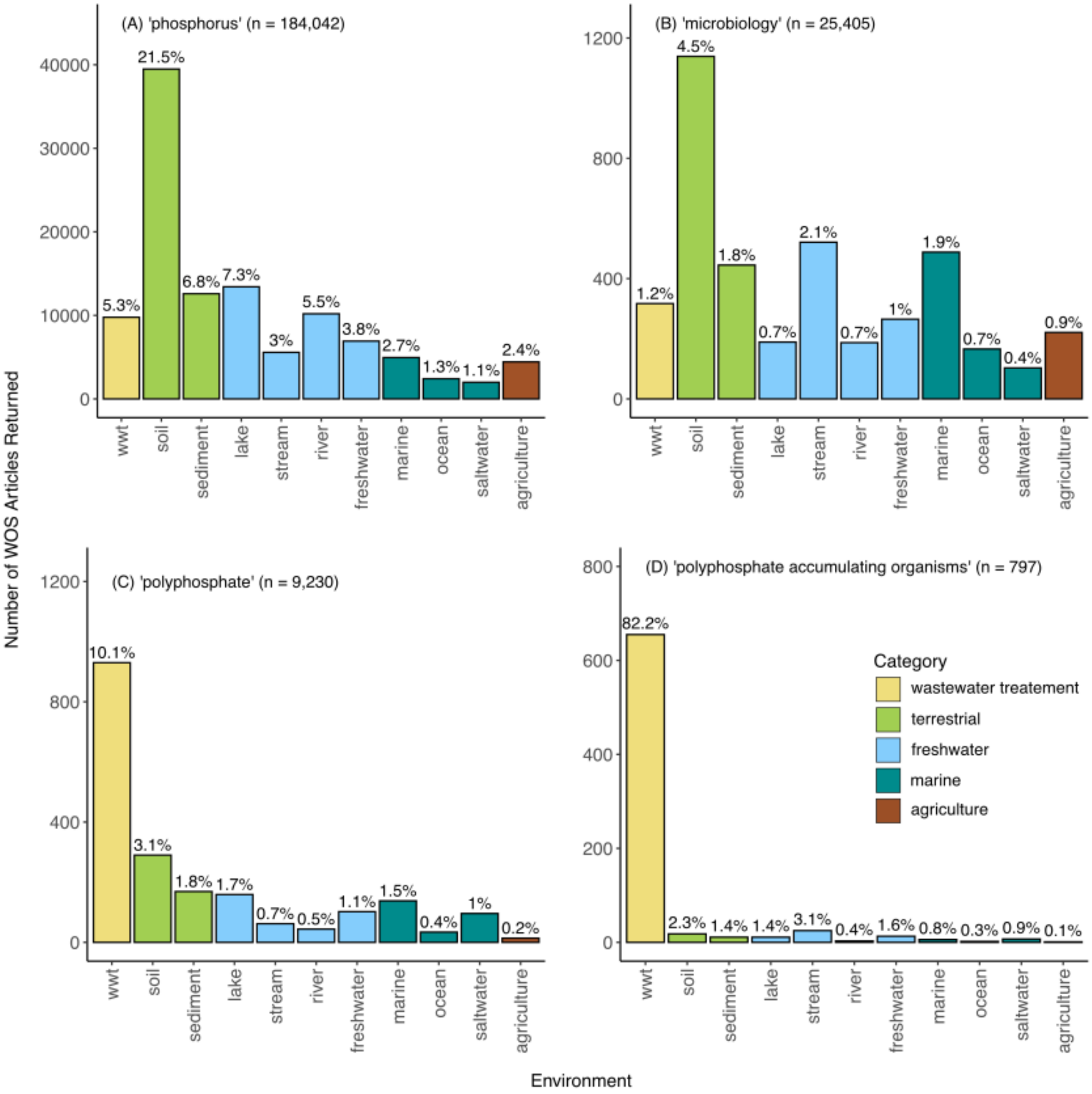

Figure S4. Number and percentage of articles returned from Web of Science (WOS) by environment for (A) ‘phosphorus’, (B) ‘microbiology’, (C) ‘polyphosphate’, and (D) ‘polyphosphate accumulating organisms’ keyword searches. Environments are grouped into five categories: wastewater treatment (wwt), terrestrial, freshwater, marine, and agriculture. 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’); they may not sum to 100% when search results are broader than the environments we focus on in this study.

TABLES

Table S1. Common phosphorus (P) forms and their descriptions.

Form	Common Abbreviation	Description	References
dissolved phosphorus	DP	Operationally defined form of P that passes through 0.45um filter.	USEPA, 1978; Zeckoski et al., 2013
particulate phosphorus	PP	Operationally defined form of P that is obtained by subtracting DP from TP.	Zeckoski et al., 2013
total phosphorus	TP	Total amount of P in a soil or water sample. For water samples TP is measured on an unfiltered sample and for soil samples TP is determined after digestion using strong acids and bases like fluoric acid, hydrogen peroxide, hydrochloric and nitric acids, sodium hydroxide.	USEPA, 1978; Zeckoski et al., 2013
soluble reactive phosphorus, dissolved orthophosphate	SRP	Portion of the DP fraction (i.e., P passing through 0.45 um filter) that can be detected with the molybdenum blue assay. SRP consists of primarily Pi but may also include hydrolyzed Po due to the required acidity of the molybdenum blue assay.	USEPA, 1978; Benitez-Nelson, 2000; Zeckoski et al., 2013
inorganic phosphorus	Pi	P that is not associated with organic (carbon) molecules. Examples include phosphate, polyphosphate, and phosphate bound directly to metals (e.g., apatite - Ca10(PO4)6(OH)2).	Zeckoski et al., 2013
dissolved inorganic phosphorus	DIP	The Pi fraction of DP.	USEPA, 1978; Benitez-Nelson, 2000
phosphate, orthophosphate	PO43-	The most basic form of Pi in the environment.	Zeckoski et al., 2013
organic phosphorus	Po	P associated with organic (carbon containing) material/molecules. This may include phosphate bound to organic matter as well as phosphate inside plants, animals, and microorganisms (e.g., as DNA or polyP).	Zeckoski et al., 2013; Cade-Menun et al., 2005; Cade-Menun, 2015; 2017
polyphosphate	polyP	Two or more phosphate molecules bound together by a high energy phosphoanydride bond (i.e., tetrahedral phosphate groups are linked via O2 bonds). It is a Pi on its own, but because it is typically stored intracellularly, it is often considered a Po.	Harold, 1964; Kornberg, 1995; Cade-Menun et al., 2005; Cade-Menun, 2015; 2017
pyrophosphate	--	A polyP with only two phosphate molecules bound together by a phosphoanydride bond. It is a Pi on its own, but because it is typically stored intracellularly, it is often considered a Po.	Cade-Menun et al., 2005; Cade-Menun, 2015; 2017
dissolved organic phosphorus	DOP	The Po fraction of DP.	USEPA, 1978; Benitez-Nelson, 2000
orthophosphate monoesters	--	Extracted from organic P. Includes sugar phosphates (e.g., glucose 1-phosphate).	Cade-Menun et al., 2005; Cade-Menun 2015

orthophosphate diesters	--	Extracted from organic P and they include DNA, RNA, and phospholipids.	Cade-Menun et al., 2005; Cade-Menun, 2015
phosphonate	--	Extracted from organic P and they are most commonly found as free molecules or membrane phosphonolipids.	Cade-Menun et al., 2005; Cade-Menun, 2015
microbial phosphorus	--	Pi and Po stored intracellularly by microorganisms; therefore, it is considered a form of Po. Calculated based on the difference in P detected before and after fumigation of soil or sediment samples.	Hedley et al., 1982
bound phosphorus	--	A general term that refers to P (usually inorganic P) that is attached to soil or sediment via bonds or associations with organic matter or metals such as Ca, Fe, and Al.	--
labile phosphorus	--	P that is easy converted into other forms.	Zeckoski et al., 2013
bioavailable phosphorus	--	P that can easily be taken up by plants, animals, microorganisms. Determined by summing SRP and what is extracted from PP using NaOH.	Sharpley et al., 1991; Zeckoski et al., 2013

Table S2. Summary of Web of Science queries results. Note: Rows in the table below are not exclusive. For example, if an article is returned for "phosphorus" AND "soil" as well as "phosphorus" and "sediment" then it will be counted once in each corresponding row.

Theme	Category	Query Keywords	Number of Results Returned
Phosphorus			
	all	"phosphorus"	184,042
	wastewater treatment	"phosphorus" AND ("wastewater" OR "enhanced biological phosphorus removal" OR "batch reactor")	9,774
	terrestrial	"phosphorus" AND "soil"	39,481
	terrestrial	"phosphorus" AND "sediment"	12,595
	freshwater	"phosphorus" AND "lake"	13,421
	freshwater	"phosphorus" AND "stream"	5,572
	freshwater	"phosphorus" AND "river"	10,190
	freshwater	"phosphorus" AND ("freshwater" OR "fresh water")	6,922
	marine	"phosphorus" AND "marine"	4,949
	marine	"phosphorus" AND "ocean"	2,419
	marine	"phosphorus" AND ("salt water" OR "saltwater")	1,988
	agriculture	"phosphorus" AND "agriculture"	4,429
Microbiology			
	all	"microbiology"	25,405
	wastewater treatment	"microbiology" AND ("wastewater" OR "enhanced biological phosphorus removal" OR "batch reactor")	317
	terrestrial	"microbiology" AND "soil"	1,139
	terrestrial	"microbiology" AND "sediment"	445
	freshwater	"microbiology" AND "lake"	189
	freshwater	"microbiology" AND "stream"	521
	freshwater	"microbiology" AND "river"	187
	freshwater	"microbiology" AND ("freshwater" OR "fresh water")	265
	marine	"microbiology" AND "marine"	488
	marine	"microbiology" AND "ocean"	166
	marine	"microbiology" AND ("salt water" OR "saltwater")	103
	agriculture	"microbiology" AND "agriculture"	221
Polyphosphate			
	all	"polyphosphate"	9,230
	wastewater treatment	"polyphosphate" AND ("wastewater" OR "enhanced biological phosphorus removal" OR "batch reactor")	930
	terrestrial	"polyphosphate" AND "soil"	290

	terrestrial	"polyphosphate" AND "sediment"	169
	freshwater	"polyphosphate" AND "lake"	159
	freshwater	"polyphosphate" AND "stream"	62
	freshwater	"polyphosphate" AND "river"	44
	freshwater	"polyphosphate" AND ("freshwater" OR "fresh water")	102
	marine	"polyphosphate" AND "marine"	138
	marine	"polyphosphate" AND "ocean"	34
	marine	"polyphosphate" AND ("salt water" OR "saltwater")	96
	agriculture	"polyphosphate" AND "agriculture"	14
PAOs			
	all	"polyphosphate accumulating organisms"	797
	wastewater treatment	"polyphosphate accumulating organisms" AND ("wastewater" OR "enhanced biological phosphorus removal" OR "batch reactor")	655
	terrestrial	"polyphosphate accumulating organisms" AND "soil"	18
	terrestrial	"polyphosphate accumulating organisms" AND "sediment"	11
	freshwater	"polyphosphate accumulating organisms" AND "lake"	11
	freshwater	"polyphosphate accumulating organisms" AND "stream"	25
	freshwater	"polyphosphate accumulating organisms" AND "river"	3
	freshwater	"polyphosphate accumulating organisms" AND ("freshwater" OR "fresh water")	13
	marine	"polyphosphate accumulating organisms" AND "marine"	6
	marine	"polyphosphate accumulating organisms" AND "ocean"	2
	marine	"polyphosphate accumulating organisms" AND ("salt water" OR "saltwater")	7
	agriculture	"polyphosphate accumulating organisms" AND "agriculture"	1

Table S3. Targeted forms, advantages, and disadvantages for agricultural phosphorus (P) best management practices (BMPs).

Abbreviations: particular P (PP), dissolved P (DP).

BMP	Targeted P Form(s)	Advantages	Disadvantages	References
tile drains (including other controlled drainage)	PP, DP	Increases infiltration and reduces DP and PP losses in overland flow. Intercepts lateral, subsurface flows. DP in infiltrating or lateral flows adsorbs to soil. Increases crop yield.	PP builds up in the top layers of the soil profile. Serves as a conduit (i.e., artificial preferential flow path) facilitating subsurface transport of DP to nearby streams and water bodies. Medium cost to operations.	Rittenberg et al., 2015; McDowell et al., 2014b; 2016; King et al., 2015; Williams et al., 2016
soil aeration	PP, DP	Decrease of PP and DP loss by increasing infiltration of overland flows. Increased airflow to plant leads to increases in plant growth and nutrient uptake. Airflow also increases soil carbon respiration and leads to subsequent mineralization of organic P to DP.	Loss of PP to atmosphere through wind erosion. Increases DP and PP loss via overland flow and erosion by disturbing soil structure.	Saif, 1981; Schachtman et al., 1998; Johnson et al., 2011; Baumhardt et al., 2015
soil amendments (e.g., pH- or mineral-based)	DP	Decreases DP loss via overland flow and subsurface leaching. Includes adding lime or red mud to raise soil pH in acidic soils or additions of minerals (e.g., aluminum sulfate) to aid DP adsorption to field soils or other BMPs (e.g., riparian buffer soils).	Amendments cannot remove P once it reaches P adsorption capacity. Limited research on the ecological impacts of mineral accumulation downstream. High cost to operations.	Callahan et al., 2012; Buda et al., 2012; McDowell and Nash, 2012; McDowell et al., 2014b; 2016
woodchip bioreactors with biochar additions	DP	Post-processing of tile drainage that removes DP. Edge-of-field practice that does not take agricultural land out of production.	No longer removes DP when biochar amendments reach P adsorption capacity. Select woodchip types leach P during the start-up phase. High cost to operations.	Christianson et al., 2012; McDowell et al., 2014b; Bock et al., 2015; Sharrer et al, 2016;
vegetated filter strip & riparian buffers	PP, DP	Decreases PP losses by slowing down overland flow. Reduces DP losses by facilitating infiltration and adsorption in soil profile.	Accumulation of P in the top layers of the soil profile. Soil cannot remove P once it reaches P adsorption capacity. Preferential flow paths develop if not properly maintained. Medium cost to operations.	Hoffmann et al., 2009; Tomer et al., 2011; Rittenberg et al., 2015; McDowell et al., 2014b; 2016; Liu et al., 2017
constructed wetlands	PP, DP	Decreases PP losses by collecting and slowing down overland flows. Wetland vegetation and organisms can take up DP. Wetland sediments may adsorb DP.	PP collects in the wetland. High cost to operations.	Hill and Robinson, 2012; Kaushal et al., 2014; Rittenberg et al., 2015; McDowell et al., 2014b; 2016; Liu et al., 2017
reduced tillage (e.g., no till)	PP	Preserves soil structure, which holds PP in place during subsequent overland flow runoff events. Limited tillage decreases mineralization of organic P to DP.	Promotes formation of preferential flow paths, which can serve as DP conduits. May cause vertical stratification of P.	Sharpley et al., 2011; Tomer et al., 2011; Baumhardt et al., 2015; Rittenberg et al., 2015; Christianson et al., 2016;
terracing & contour farming	PP	Decreases PP losses by minimizing soil erosion. May be coupled with tile drainage.	Limited impact on DP. Requires considerable alteration of field slopes.	Gassman et al., 2006; Rittenberg et al., 2015

cover crops (also referred to as catch crops)	PP, DP	Preserves soil structure during non-growing seasons, which holds PP in place during subsequent overland flow runoff events. Increases infiltration capacity, which reduces DP losses.	Freeze and thaw events trigger DP losses from cover crops.	Kleinman et al., 2005; Tomer et al., 2011; Bergström et al. 2015; Rittenberg et al., 2015; Cober et al., 2019; Liu et al., 2019
stream fencing	PP, DP	Decreases DP and PP loads by preventing direct defecation of livestock into the stream and ensuring streambank stability. Low cost to operations.	Alternative supplies are required to water livestock.	Tomer et al., 2011; McDowell et al., 2014b; 2016
land conservation (e.g., conservation reserve program; CRP)	PP, DP	Reduces PP losses and P fertilizer applications by removing erodible land from cropping and pasturing. Crop land is lost but producers enter 10-year rental contract with the US Department of Agriculture; they are compensated for this loss.	High cost to operations. Duration of practice implementation may depend on economic viability for the producer.	Gregory et al., 2007; Tomer et al., 2011; Rittenberg et al., 2015; Smith et al., 2015b
nutrient management (e.g., 4Rs: right source, right timing, right placement, and right rate).	PP, DP	Decreases DP and PP losses by using low solubility P fertilizers, avoiding fertilizer application when probability of precipitation is high, reducing amount of fertilizer applied, encouraging mixing of fertilizer into soil, and balancing crop needs with P available through fertilizer and existing soil P. Low cost to operations.	P is an essential and typically limited nutrient for plant growth; decreases in P availability decrease crop yield.	Schachtman et al., 1998; Good and Beatty, 2011; Tomer et al., 2011; Bergström et al. 2015; Rittenberg et al., 2015; McDowell et al., 2014b; Christianson et al., 2016; McDowell et al., 2016

Table S4. Top twenty journals in Web of Science ranked by article count that included the keyword "polyphosphate" but not keywords associated with the five categories (i.e., wastewater treatment, terrestrial, freshwater, marine, and agriculture).

Rank	Journal Name	Number of Publications
1	Polymer Degradation and Stability	325
2	Journal of Biological Chemistry	258
3	Journal of Applied Polymer Science	215
4	Journal of Thermal Analysis and Calorimetry	119
5	Polymers for Advanced Technologies	118
6	Proceedings of the National Academy of Sciences (PNAS)	106
7	Journal of Bacteriology	81
8	Industrial and Engineering Chemistry Research	80
9	Royal Society of Chemistry (RSC) Advances	77
10	Journal of Fire Sciences	66
10	PLOS ONE	66
11	Biochemical and Biophysical Research Communications	63
12	Biochemical Journal	60
13	Applied and Environmental Microbiology	56
14	Biochemistry	52
15	Composites Part B: Engineering	50
16	Polymer Composites	46
17	Federation of European Biochemical Societies (FEBS) Letters	45
17	Polymers	45
18	Fire and Materials	43
19	Blood	40
20	Biochemistry (Moscow)	39

Table S5. A summary of polyphosphate accumulating organisms (PAOs) identified in engineered and natural environments. Abbreviations: full-scale wastewater treatment plant (WWTP), sequencing batch reactor (SBR), uncharacterized wastewater (Mixed).

Phylum/Class	Genus	Carbon Source	Reactor Type /Environment	Reference
Actinobacteria	--	Mixed	WWTP	Auling et al., 1991; Beer et al., 2006
Actinobacteria	--	Mixed	SBR	Bark et al., 1993
Actinobacteria	*Arthrobacter*	Mixed	Rhizosphere soil	Li et al., 2013
Actinobacteria	*Gordonia*	Mixed	WWTP	Beer et al., 2006
Actinobacteria	*Microlunatus*	Mixed	Pure culture	Kawakoshi et al., 2012
Actinobacteria	*Microlunatus*	Mixed	SBR	Nakamura et al., 1991; 1995a; 1995b; Kawaharasaki et al., 1998
Actinobacteria	*Microlunatus*	Mixed	WWTP	Beer et al., 2006
Actinobacteria	*Tetrasphaera*	Mixed	WWTP	Maszenan et al., 2000; Nguyen et al., 2011; Kong et al., 2005; Albertsen et al., 2012; Kristiansen et al., 2013
Actinobacteria	*Tetrasphaera*	Mixed	SBR	Günther et al., 2009
Alphaproteobacteria	--	Mixed	WWTP	Zilles et al., 2002a
Alphaproteobacteria	--	Mixed	SBR	Kawaharasaki et al., 1998
Alphaproteobacteria	*Defluviicoccus**	Acetate	SBR	Nobu et al., 2014
Bacilli	*Bacillus*	Mixed	Rhizosphere soil	DebRoy et al., 2013
Betaproteobacteria	--	Mixed	WWTP	Zilles et al., 2002a
Betaproteobacteria	--	Acetate	SBR	Stante et al., 1997
Betaproteobacteria	--	Mixed	SBR	Ge et al., 2015
Betaproteobacteria	Accumulibacter	Mixed	WWTP	Beer et al., 2006; Albertsen et al., 2012; Nguyen et al., 2012; Mao et al., 2015
Betaproteobacteria	Accumulibacter	Mixed	SBR	Liu et al., 2001; Günther et al., 2009; Mao et al., 2014; Skennerton et al., 2014
Betaproteobacteria	Accumulibacter	Acetate	SBR	Hesselmann et al., 1999; Crocetti et al., 2000; McMahon et al., 2002; García Martin et al., 2006; Kim et al., 2010
Betaproteobacteria	Accumulibacter	Propionate	SBR	García Martin et al., 2006
Betaproteobacteria	Accumulibacter	Mixed	Estuary	Kunin et al., 2008; Peterson et al., 2008
Betaproteobacteria	Accumulibacter	Mixed	Freshwater	Kunin et al., 2008; Peterson et al., 2008
Betaproteobacteria	Accumulibacter	Mixed	Soil	Kunin et al., 2008; Valdivia, 2009; Archibald, 2010
Betaproteobacteria	Accumulibacter	Mixed	Sediment	Kunin et al., 2008; Watson et al., 2019
Betaproteobacteria	*Dechloromonas*	Mixed	WWTP	Zilles et al., 2002b; Kong et al., 2007
Betaproteobacteria	*Propinoibacter*	Acetate	SBR	Crocetti et al., 2000
Betaproteobacteria	*Rhodocyclus*	Mixed	WWTP	Zilles et al., 2002a; 2002b; Kong et al., 2004; 2005
Betaproteobacteria	*Rhodocyclus*	Acetate	SBR	Hesselmann et al., 1999; Crocetti et al., 2000; McMahon et al., 2002

Cyanobacteria	*Leptolyngbya*	--	Marine, SBR	Zhang et al., 2015, Oyserman et al., 2017
Gammaproteobacteria	--	Mixed	SBR	Liu et al., 2001
Gammaproteobacteria	*Acinetobacter*	Mixed	WWTP	Wagner et al., 1994; Streichan et al., 1990
Gammaproteobacteria	*Halomonas*	Mixed	WWTP	Nguyen et al., 2012
Gammaproteobacteria	*Pseudomonas*	Mixed	WWTP	Nguyen et al., 2012
Gammaproteobacteria	*Pseudomonas*	Mixed	SBR	Günther et al., 2009
Gammaproteobacteria	*Thiomargarita*	Mixed	Sediment pore water	Schulz and Schulz, 2005
Gammaproteobacteria	*Beggiatoa*	Acetate	Pure culture	Brock and Schulz-Vogt, 2011
Gammaproteobacteria	*Thiothrix*	Acetate	SBR	Rubio-Ricón et al., 2017b
Gemmatimonadetes	*Gemmatimonas*	Mixed	SBR	Zhang et al., 2003
Melainabacteria		Mixed	WWTP	Soo et al., 2014
Chlorophyta	*Parachlorella*	--	Pure culture	Ota et al., 2016

*This organism was described as a competitor to PAOs, a glycogen accumulating organism (GAO), but had genes coding for intracellular polyphosphate storage.

Table S6. Functional genes and associated proteins related to phosphorus (P) cycling. Abbreviations: polyphosphate (polyP).

Gene	Protein (Protein Abbreviation)	Function and Key Traits	References
ppk1	polyphosphate kinase 1 (PPK1)	Catalyzes the de-phosphorylation of ATP to make polyP. Has an affinity for Mg^{2+}.	Ahn and Kornberg, 1990; Akiyama et al., 1992; Trelstad et al., 1999; Zhang et al., 2002; Zhu et al., 2005; Rao et al., 2009; Kawakoshi et al., 2012
ppk2	polyphosphate kinase 2 (PPK2)	Catalyzes the de-phosphorylation of ATP and GTP to make polyP. Has an affinity for Mn^{2+}.	Ishige et al., 1998; 2002; Zhang et al., 2002; Nocek et al., 2008; Rao et al. 2009; Kawakoshi et al. 2012
ppx1	exopolyphosphatase (PPX1)	Liberates the terminal phosphate molecule in longer (>500) polyP chain. Requires Mg^{2+} and KCl. Does not act on ATP and cannot be inhibited by ADP or ATP.	Reizer et al., 1992; Akiyama et al., 1993; Wurst and Kornberg, 1994; Kornberg, 1995; Zago et al., 1999; Ohtake et al., 2001; Rangarajan et al., 2006; Lichko et al., 2006; Rao et al., 2009; Kawakoshi et al., 2012
ppx2/gppA	exopolyphosphatase/ pentaphosphate phosphohydrolase (PPX/GPPA)	Liberates the terminal phosphate in a polyP chain and important in cellular stress response by hydrolyzing pppGpp to ppGpp.	Keasling et al., 1993; Reizer et al., 1993; Rao et al., 2009; Kawakoshi et al., 2012; Alcántara et al., 2014
ppn	endopolyphosphatase (PPN)	Cleaves phosphate from polyP chains (not selective to terminal phosphate). Has only been found in archaea, fungi, and mammals.	Kumble and Kornberg, 1996; Shi and Kornberg, 2005; Lichko et al., 2006; Rao et al., 2009
pap	AMP phosphotransferase (PAP)	Catalyzes reaction of AMP to ADP using energy from breakdown of polyP.	Rao et al., 2009; Mao et al., 2014; Skennerton et al., 2014
phoX, *phoD,* *phoA*	alkaline phosphatase (APase)	Under P limited conditions, catalyzed the breakdown of Po to Pi.	Temperton et al., 2011; von Sperber et al., 2014; Morrison et al., 2016; Ragot et al., 2015; 2016; George et al., 2018; Margenot et al., 2018
pit	low-affinity phosphate transporter (PIT)	Binds phosphate and brings it into the cell.	Mao et al., 2014
pst	high-affinity phosphate transporter (PST)	Binds phosphate and brings it into the cell.	Mao et al., 2014

Table S7. Descriptions, references, and examples of tools that may benefit the study of polyphosphate (polyP) and polyphosphate accumulating organisms (PAOs) in agricultural landscapes and beyond.

Tool	Description/Purpose	Use Category	Use References	Example in Wastewater Treatment	Example in the Environment
Microscopy					
TEM	Transmission electron microscopy (TEM) is used with energy dispersive x-ray analysis to identify intracellular polyP granules.	metabolism	Sick-Goad and Lazinsky, 1986	Günther et al., 2009	Uhlmann and Bauer, 1988
NanoSIMS	Nanometer-scale second ion mass spectrometry (NanoSIMS) is a microscopy-based spectroscopy that is used to identify intracellular polyP granules as well as the 2-D and 3-D spatial distribution of P and various cations such as Fe, Al, Ca, and Mg.	metabolism	Herrmann et al., 2007; Hoppe et al., 2013; Mueller et al., 2013; Kruse et al., 2015; Gao et al., 2016	--	Sulu-Gambari et al., 2016
Light Microscopy					
Lead staining	Light microscopy technique used to identify intracellular polyP granules	metabolism	Stevenson and Stoermer, 1982	--	Stevenson and Stoermer, 1982
Neisser's (methylene) blue	Light microscopy stain used to identify intracellular polyP granules.	metabolism	Crocetti et al., 2000	Crocetti et al., 2000	Schulz and Schultz, 2005
Toludine blue	Light microscopy stain used to identify intracellular polyP granules.	metabolism	Schulz and Schulz, 2005	Streichan et al., 1990	Schulz and Schultz, 2005
Epi-fluorescence Microscopy					
Tetracycline	Fluorescent stain used to identify intracellular polyP granules.	metabolism	Günther et al., 2009	Günther et al., 2009	--
DAPI	4',6-diamidino-2-phenylindole (DAPI) is a fluorescent stain used to identify and quantify intracellular polyP granules.	metabolism	Hung et al., 2002; Aschar-Sobbi et al., 2008; Diaz and Ingall, 2010, Martin and Van Mooy, 2013	Hung et al., 2002	Rier et al., 2016
FISH/qFISH	Fluorescence in-situ hybridization (FISH) is a fluorescent oligonucleotide probe that hybridizes to 16S rRNA or 23S rRNA sequences. Quantitative FISH (qFISH) is used to quantify PAOs and study the diversity of PAO communities. FISH is	microbial community, metabolism	Amann et al., 1990; 2001; Seviour et al., 2003; Seviour and Nielsen, 2010	metabolism: Hesselman et al., 1999; diversity: Beer et al., 2006; quantification: Albertsen et al., 2012	Castle and Kirchman, 2004; Sulu-Gambari et al., 2016

	used in combination with epi-fluorescence microscopy or fluorescence spectroscopy. Can also be used with DAPI to co-locate polyP in PAO cells. See Table S8.				
Molecular Biology					
PCR/qPCR	Polymerase chain reaction (PCR) is used to amplify and identify PAO 16S rRNA and functional genes (e.g., *ppk*). Quantitative real-time PCR (qPCR) is used to amplify and quantify PAO 16S rRNA and functional genes. Both can be sued to determine PAO strain diversity and PAO community functional gene diversity. See Table S9.	metabolism, functional genes, microbial community	He et al., 2010; Seviour and Nielsen, 2010	Zhang et al., 2016	Peterson et al., 2008
Shotgun metagenomics	Non-targeted next generation sequencing technique used to identify potential PAOs, study the metabolic potential of potential PAOs, and quantify the relative abundance of PAO functional genes.	metabolism, functional genes, microbial community	Riesenfeld et al., 2004; Howe et al., 2014; Zimmerman et al., 2014; Howe and Chain, 2015; Wang et al., 2015; Choi et al., 2016, Menzel and Krogh, 2016	Skennerton et al., 2014	Temperton et al., 2011
Amplicon metagenomics	Targeted (16S rRNA, 23S rRNA, internal transcribed spacer region) next generation sequencing technique used to quantify known PAOs and study PAO communities.	microbial community	Riesenfeld et al., 2004, Kozich et al., 2013, Zimmerman et al., 2014	Oyserman et al., 2017	Locke, 2015
Metabolomics	A technique that relies on mass spectrometry to identify all metabolites in liquid and solid samples.	metabolism	Aguiar-Pulido et al., 2016	Herbst et al., 2019	--
Flow cytometry	Cell sorting technique often used in conjunction with DAPI to identify potential PAOs.	metabolism, microbial community	Zilles et al., 2002a; 2002b; Hung et al., 2002	Kim et al., 2010	Locke, 2015
Other					
^{31}P-NMR	31P-nuclear magnetic resonance (NMR) spectroscopy is used to measure the concentration of various P forms (e.g., polyphosphate) in water and soil/sediments.	metabolism	Cade-Menun, 2015	Peng et al., 2010	Bourke et al., 2009; Read et al., 2014, McDowell et al., 2015; Cade-Menun, 2017
Sensors	Used to measure environmental variables (e.g., P concentration, dissolved oxygen concentration) along a range of time scales.	metabolism	Pellerin et al., 2016; Rode et al., 2016; Fares et al., 2016	Lanham et al., 2013	Cohen et al., 2013

Table S8. A summary of fluorescence *in-situ* hybridization (FISH) probes used to identify polyphosphate accumulating organism (PAO)-related organisms (updated from Seviour and Nielsen 2010). Abbreviations: not determined (ND).

Probe Name	Sequence (5'-3')	Formamide (%)	Target	Reference
ALF1b	GCTGCCTCCCGTAGGAGT	20	Alphaproteobacteria	Manz et al., 1992
BET42[a]	GCCTTCCCACTTCGTTT	35	Betaproteobacteria	Manz et al., 1992
GAM42[a]	GCCTTCCCACATCGTTT	35	Gammaproteobacteria	Manz et al., 1992
RHC175	TGCTCACAGAATATGCGG	30	*Rhodocyclus*/Accumulibacter	Hesselmann et al., 1999
RHC439	CNATTTCTTCCCCGCCGA	30	Most *Rhodocyclaceae*	Hesselmann et al., 1999
Rc988	AGGATTCCTGACATGTCAAGGG	ND	*Rhodocyclus* group	Crocetti et al., 2000
PAO462[c]	CCGTCATCTACWCAGGGTATTAAC	35	Most Accumulibacter	Crocetti et al., 2000
PAO651[c]	CCCTCTGCCAAACTCCAG	35	Most Accumulibacter	Crocetti et al., 2000
PAO846[c]	GTTAGCTACGGCACTAAAAGG	35	Most Accumulibacter	Crocetti et al., 2000
Acc-I-444	CCCAAGCAATTTCTTCCCC	35	PAO clade IA and other Type I clades	Flowers et al., 2009
Acc-II-444	CCCGTGCAATTTCTTCCCC	35	PAO clade IIA, IIC, and IID	Flowers et al., 2009
Actino-1011	TTGCGGGGCACCCATCTCT	30	*Tetrasphaera*-relatives	Liu et al., 2001
Actino-221[a]	CGCAGGTCCATCCCAGAC	30	*Tetrasphaera*-relatives	Kong et al., 2005
Actino-658[a]	TCCGGTCTCCCCTACCAT	40	*Tetrasphaera*-relatives	Kong et al., 2005
Tet1-266	CCCGTCGTCGCCTGTAGC	25	*Tetrasphaera*-relatives	Nguyen et al., 2011
Tet2-892	TAGTTAGCCTTGCGGCCG	5	*Tetrasphaera*-relatives	Nguyen et al., 2011
Tet2-174	GCTCCGTCTCGTATCCGG	20	*Tetrasphaera*-relatives	Nguyen et al., 2011
Tet3-654	GGTCTCCCCTACCATACT	35	*Tetrasphaera*-relatives	Nguyen et al., 2011
Tet3-19	CAGCGTTCGTCCTACACA	0	*Tetrasphaera*-relatives	Nguyen et al., 2011
BET135	ACGTTATCCCCCACTCAATGG	45	*Dechloromonas*-relatives	Kong et al., 2007
MIC179	GAGCAAGCTCTTCTGAAACCG	10	*Microlunatus phosphovorus*	Kawaharasaki et al., 1998
G123T	CCTTCCGATCTCTATGCA	40	*Thiothrix*-relatives	Kanagawa et al., 2000; Rubio-Rincón et al., 2017b
EUB338	GCTGCCTCCCGTAGGAGT	60	Most Bacteria[b]	Amann et al., 1990
EUB338-II	GCAGCCACCCGTAGGTGT	60	Most Bacteria[b]	Daims et al., 1999
EUB338-III	GCTGCCACCCGTAGGTGT	60	Most Bacteria[b]	Daims et al., 1999

[a] Competitor probes required.

[b] Use EUB338, EUB338-II, and EUB338-III together to obtain an estimate of total bacteria.

[c] Use PAO462, PAO651, PAO846 together to obtain an estimate of total CAP PAOs.

Table S9. A summary of polyphosphate accumulating organism (PAO) polymerase chain reaction (PCR) primer sequences.

Gene Target	Primer Pair	Sequence (5’-3’)	Reference
Most *ppk1*	NLDE-0199F	CGTATGAATTTTCTTGGTATTTATTGTACTAATCTngaygarttyt	McMahon et al., 2002; 2007
	TGNY-1435R	GTCGAGCAGTTTTTGCATGAwarttnccngt	
CAP *ppk1*	ACCppk1-254F	TCACCACCGACGGCAAGAC	McMahon et al., 2002; 2007; Kunin et al., 2008
	ACCppk1-1376R	ACGATCATCAGCATCTTGGC	
CAP *ppk1*	ppk274f	ACCGACGGCAAGACSG	Kunin et al., 2008
	ppk1156r	CGGTAGACGGTCATCTTGAT	
CAP *ppk1*	ppk734f	CTCGGCTGCTACCAGTTCCG	Kunin et al., 2008
	ppk1601r	GATSCCGGCGACGACGTT	
CAP Clade 1A *ppk1*	Acc-ppk1-763f	GACGAAGAAGCGGTCAAG	He et al., 2007; He and McMahon, 2011b
	Acc-ppk1-1170r	AACGGTCATCTTGATGGC	
CAP Clade 1A *ppk1*	Acc-ppk1-974f	TGATGCGCGACAATCTCAAATTCAA	Zhang et al., 2016
	Acc-ppk1-1113r	AATGATCGGATTGAAGCTCTGGTAG	
CAP Clade 1B *ppk1*	Acc-ppk1-372f	TGAAGGCATTCGCTTCCT	Zhang et al., 2016
	Acc-ppk1-653r	AAGCAGTATTCGCTGTC	
CAP Clade 1C *ppk1*	Acc-ppk1-362f	AGCTGGCGAGTGAAGGCATTCG	Zhang et al., 2016
	Acc-ppk1-758r	AACAGGTTGCTGTTGCGCGTGA	
CAP Clade 1D *ppk1*	Acc-ppk1-634f	TGCGACAGCGAATACAG	Zhang et al., 2016
	Acc-ppk1-848r	ACTTCGAGGCGGACG	
CAP Clade 2A *ppk1*	Acc-ppk1-893f	AGTTCAATCTCACCGACAGC	He et al., 2007; He and McMahon, 2011b
	Acc-ppk1-997r	GGAACTTCAGGTCGTTGC	
CAP Clade 2B *ppk1*	Acc-ppk1-870f	GATGACCCAGTTCCTGCTCG	He et al., 2007
	Acc-ppk1-1002r	CGGCACGAACTTCAGATCG	
CAP Clade 2C *ppk1*	Acc-ppk1-254f	TCACCACCGACGGCAAGAC	He et al., 2007
	Acc-ppk1-460r	CCGGCATGACTTCGCGGAAG	
CAP Clade 2D *ppk1*	Acc-ppk1-375f	GGGTATCCGTTTCCTCAAGCG	He et al., 2007
	Acc-ppk1-522r	GAGGCTCTTGTTGAGTACACGC	
CAP Clade 2E *ppk1*	Acc-ppk1-757f	TTCGTGGACGAGGAAGA	Zhang et al., 2016
	Acc-ppk1-1129r	ATTGTTCGAGCAACTCGATG	
CAP Clade 2G *ppk1*	Acc-ppk1-410f	CCGAGCAACGCGAATGG	Zhang et al., 2016
	Acc-ppk1-514r	TGTTGAGTACGCGCGGGA	
CAP Clade 2H *ppk1*	Acc-ppk1-701f	ACTCCTTCGTATTCCTCTCT	Zhang et al., 2016
	Acc-ppk1-928r	TCATCGCTTCGGAGCA	
CAP Clade 2I *ppk1*	Acc-ppk1-688f	AGTGATTATGCTTTCGTCTTTC	Zhang et al., 2016
	Acc-ppk1-946r	TGAACTGTCCGAGCAGGA	
CAP 16S	CAP438f	GGTTAATACCCTGWGTAGAT	Zhang et al., 2016
	CAP846r	GTTAGCTACGGCACTAAAAGG	
CAP 16S	PAO-518f	CCAGCAGCCGCGGTAAT	He et al., 2007; He and McMahon, 2011b
	PAO-846r	GTTAGCTACGGCACTAAAAGG	
CAP Clade 1A 16S	16S-Acc-1Af	TTGCTTGGGTTAATACCCTGA	He et al., 2010
	16S-Acc-1Ar	CTGCCAAACTCCAGTCTTGC	
CAP Clade 2A 16S	16S-Acc-2Af	TTGCACGGGTTAATACCCTGT	He et al., 2010
	16S-Acc-2Ar	CTCTGCCAAACTCCAGCCTG	
Halomona-related 16S	Pse136f	TAGTAGTGGGGGATAACGTC	Lane, 1991; Nguyen et al., 2012
	1492R	GCYTACCTTGT TACGACTT	

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