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 plant research has enhanced our knowledge of microbial mechanisms influencing P cycling, especially related to 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 identifying articles in Web of Science on polyP and its use by PAOs. We grouped our meta-analysis by discipline—wastewater treatment, soils and sediments (terrestrial), freshwater, marine, and agriculture—and discussed whether PAO behavior in wastewater treatment plants can be extended to natural habitats, including agricultural settings like soils, undergoing alternating anaerobic/aerobic conditions. We also synthesized knowledge gaps and research opportunities. Terrestrial, freshwater, and marine disciplines had fewer polyP and PAO articles compared to wastewater treatment and there was limited article overlap between disciplines. 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 and tile drains 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 changes in environmental conditions 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 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, federal support of agricultural water quality management programs can cost taxpayers millions of dollars (Gregory et al., 2007).

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 meta-analysis 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 evidence of their activity outside wastewater treatment plants (Section 6). The objectives of this critical review are to: (1) synthesize 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) and determine whether results support PAO-mediated P cycling that has been 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.

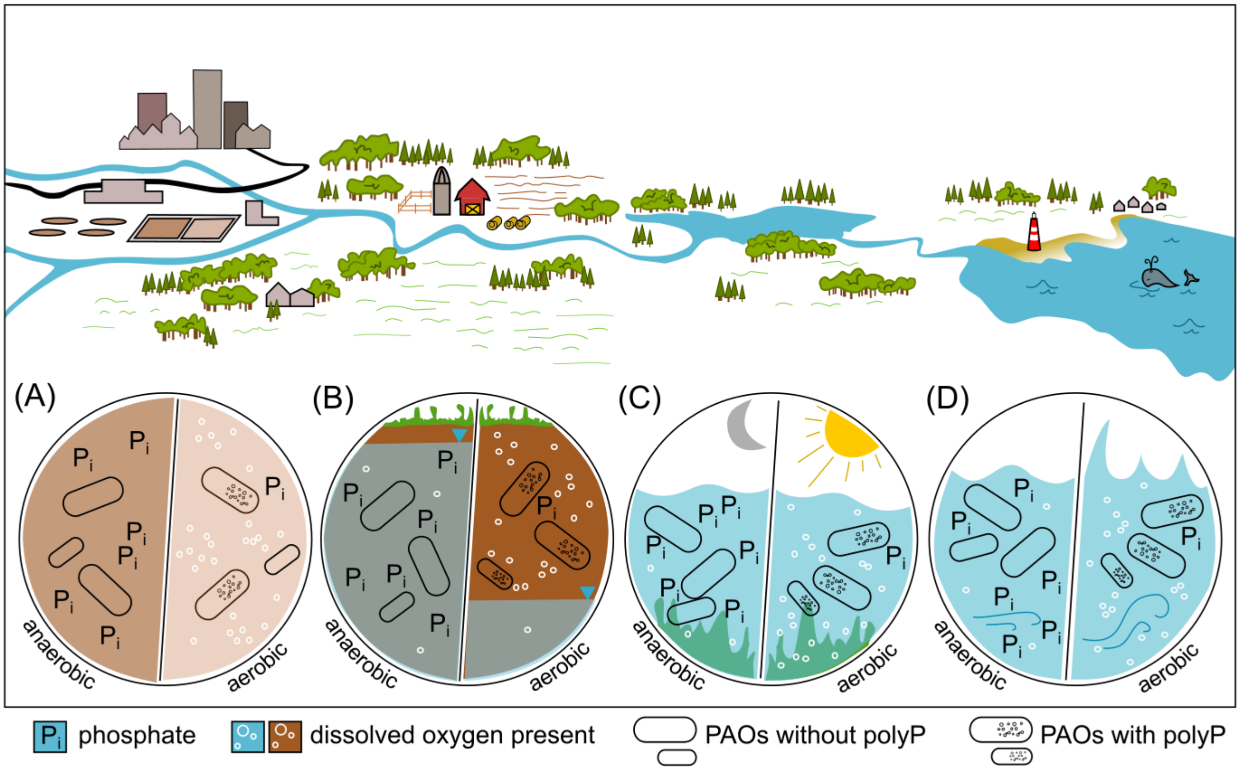

Figure 1. Schematic depicting (A) known microbially-mediated phosphate (P_i) cycling in wastewater treatment plants under alternating anaerobic/aerobic conditions. Hypothesized (this study) microbially-mediated P cycling in (B) soils (including agricultural and uncultivated soils) and sediments, (C) freshwater (streams and lakes), and (D) marine water (estuaries and oceans). Blue triangles in (B) denote water table depth. Abbreviations: polyphosphate accumulating organism (PAO) and polyphosphate (polyP).

To address our objectives, we conducted a meta-analysis of articles in Web of Science (https://webofknowledge.com) and synthesized the 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: wastewater treatment, terrestrial, freshwater, marine, and agriculture (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 as well as text from the article title and abstract. 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 synthesized research articles that did not directly address polyP and PAOs but provided support for hypothesized microbial P mechanisms outlined in Figures 1B-D (Section 6). We also relied on results from the Web of Science meta-analysis and research article synthesis when summarizing knowledge gaps related to microbial P forms and cycling mechanisms in agricultural water quality management research. We will not synthesize 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. STATE OF THE (AGRICULTURAL) FIELD

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 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 synthesizing 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

Agricultural water quality management research should consider microbial P forms such as polyphosphate (polyP), in addition to integrating multiple P forms and cycling mechanisms. 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, but is commonly included alongside organic P analyses because of its intracellular nature (e.g., Cade-Menun, 2017) and can be chemically liberated using common organic extraction procedures (e.g., Eixler et al., 2005). There are several reasons why polyP may be critical component to better 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, it 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., from journals like *Polymer Degradation and Stability* and *Journal of Biological Chemistry*). 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 found only one PAO article, which was published in 2016 (Figures 2C, 2D, S2B, Table S2). We discuss agricultural discipline results in further detail 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 (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.

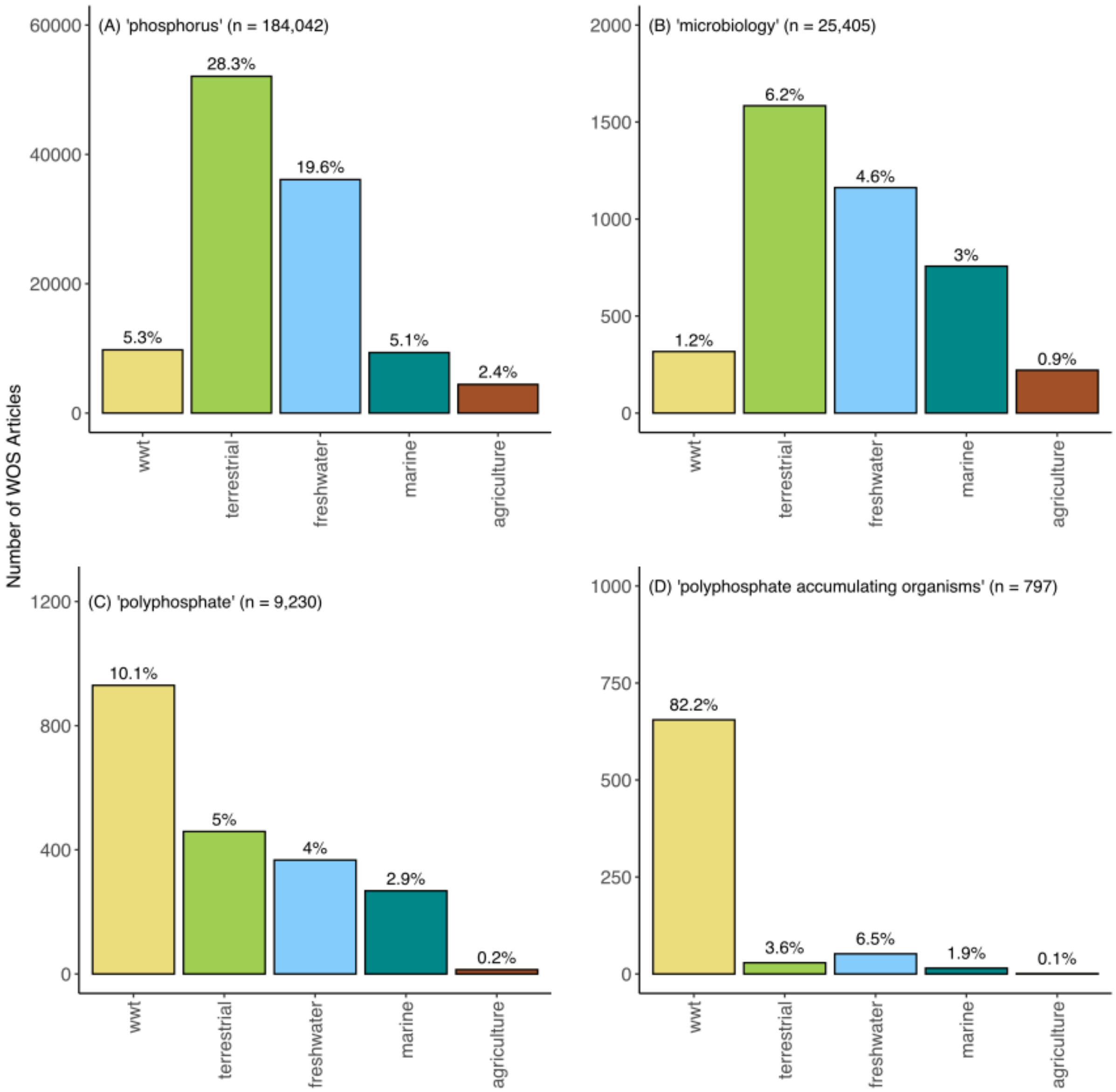

Figure 2. Number and percentage of articles returned from Web of Science (WOS) by category for (A) ‘phosphorus’, (B) ‘microbiology’, (C) ‘polyphosphate’, and (D) ‘polyphosphate accumulating organisms’ keyword searches. Percentages on top of each bar are relative to the number of articles in the full figure subset search (i.e., n = 184,042 for ‘phosphorus’); they may not sum to 100% when search results 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.

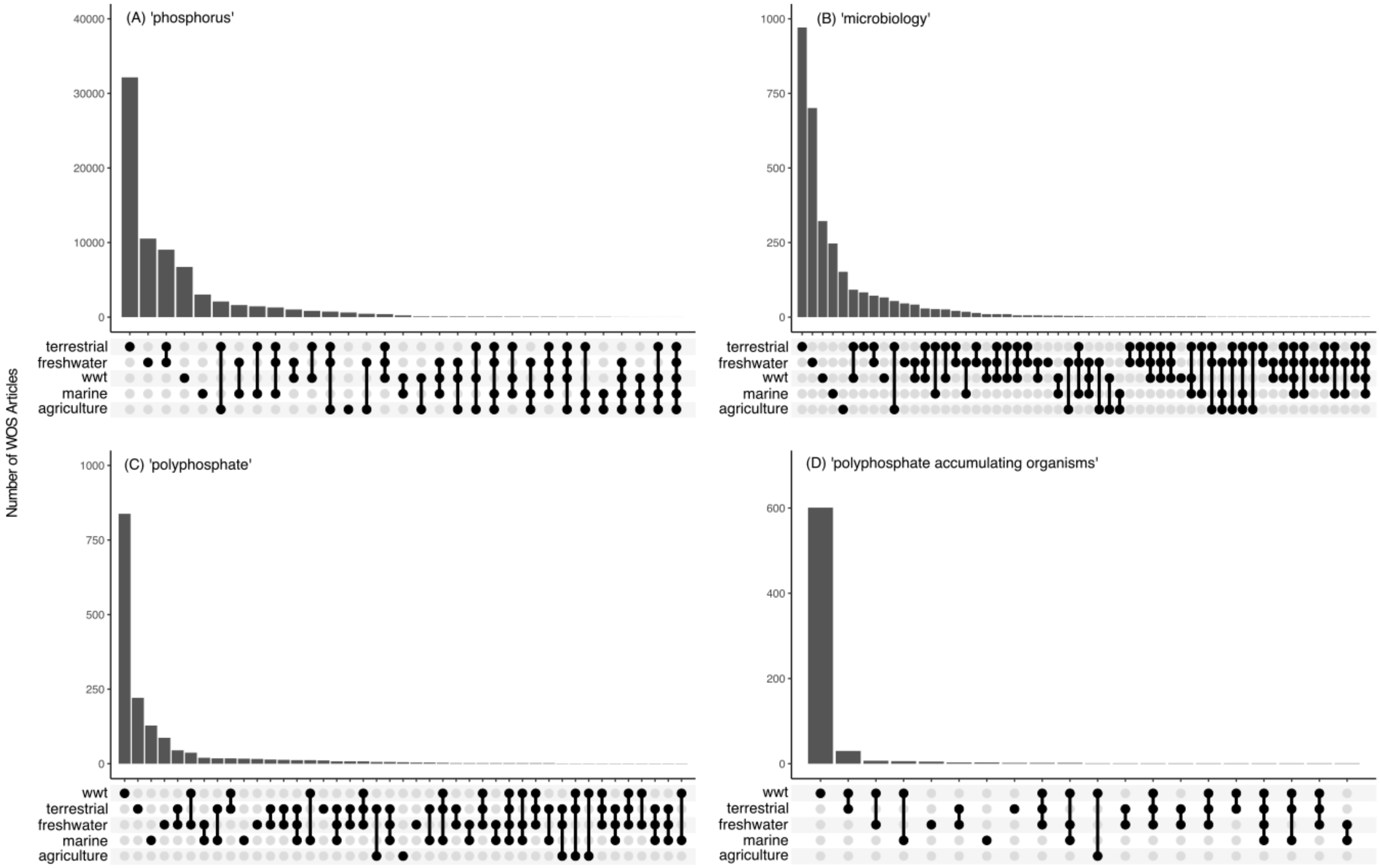

Figure 3. Number of overlapping Web of Science (WOS) articles by category for (A) ‘phosphorus’, (B) ‘microbiology’, (C) ‘polyphosphate’, and (D) ‘polyphosphate accumulating organisms’ keyword searches. Set diagrams under each bar plot show connections between categories; a closed circle represents articles 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 TREATEMENT & 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; Seviour et al., 2003). EBPR wastewater treatment plant designs include three main operating components: (1) an anaerobic reactor with an organic (carbon-containing) energy source (e.g., 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; 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 other non-PAO EBPR heterotrophs with less flexible metabolismc 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; Quang et al., 2019; Rubio-Rincón et al., 2017; Seviour et al., 2003; Seviour & McIlroy, 2008; Skennerton et al., 2014). 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.

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 papers 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 S4) 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 is 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 has 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 microorganism 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 synthesize 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 plant. 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 synthesis, 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 phylogenically 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 S4). However, none of these studies directly addressed the role of 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 29 articles that included the keyword “polyphosphate accumulating organisms”; this is in contrast to 52,076 and 1,584 articles in the terrestrial category that included the keywords “phosphorus” and “microbiology”, respectfully (Figures 2, S4, Table S2). Upon closer inspection of results within the terrestrial category, most polyP and PAO papers in the terrestrial category addressed soil environments (Figures S4C, S4D). For example, 3.1% of all polyP papers 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). Another polyP article, demonstrated polyP accumulation and release in sediment bacteria under aerobic and anaerobic laboratory conditions, respectively (Khoshmanesh et al. 2002). This study did not identify the specific organisms involved but this behavior supports PAO-mediated P cycling in sediments. 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 1 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 S4). 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 synthesis 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 S4). 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), 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 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 over four mid-Atlantic ecoregions 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). Overall, proportional relationships between polyP storage and

P availability support/do not support the idea that polyP is 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); however, many of these 30 articles focused on EBPR PAO research. There were 45 polyP and PAO articles that overlapped with one another and were both in the freshwater category. For example, one of these studied the distribution of *Candidatus* Accumulibacter phosphatis P functional genes in lake water (Peterson et al., 2008). Again, meta-analysis results support the need for interdisciplinary research addressing the role of PAOs in water quality management between wastewater treatment, terrestrial, freshwater, and marine disciplines.

6.3 Marine Habitats (Estuaries & 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 synthesis 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 S4). 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 S5) 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 estuarian waters and sediments. One noted the widespread distribution of PAOs and PAO-related P functional genes (i.e., *ppk*; Text S2, Table S5) in estuarian 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 number of P functional genes (i.e., *ppk* and *ppx*; Text S2, Table S5) increased when annual P concentrations were lower (Temperton et al., 2011). In contrast to these findings, one study along an urban to estuary gradient (i.e., from P-abundant inland to P-depleted open ocean) demonstrated a decrease in the number 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). Most PAO articles in the marine category overlapped with articles in the wastewater treatment category (n = 6, Figure 3D). As mentioned previously, several discussed the impact of salinity on EBPR processes and provide examples of how interdisciplinary research could benefit both 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 was not commensurate with 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 synthesis, 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* Accumulbacter phosphatis PAOs. For example, studies used established molecular biology tools to assess the presence and/or quantity of *Candidatus* Accumulbacter 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* Accumulbacter phosphatis and non-*Candidatus* Accumulbacter phosphatis PAOs (Table S4). 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. Since it is likely that *Candidatus* Accumulbacter 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 S5) 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 S5) 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 S5). 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 synthesized 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. 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 S6 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. Molecular biology tools such as quantitative real-time polymerase chain reaction (qPCR) 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 was used to quantify P functional genes (Temperton et al., 2011). 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 which 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 changing environmental conditions. 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.

Identify and Quantify PAOs and P Functional Genes – Studies identifying and quantifying known PAOs (e.g., *Candidatus* Accumulibacter phosphatis), new PAOs (i.e., microbes exhibiting PAO metabolism where P release and uptake corresponds with anaerobic and aerobic conditions, respectively), and 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.

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 augment the effective physical and chemical P retention mechanisms of existing agricultural management strategies with biological P retention. 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. 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.

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).

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REFERENCES

Acevedo, B., Camiña, C., Corona, J. E., Borrás, L., & Barat, R. (2015). The metabolic versatility of PAOs as an opportunity to obtain a highly P-enriched stream for further P-recovery. *Chemical Engineering Journal*, *270*, 459–467. https://doi.org/10.1016/j.cej.2015.02.063

Albertsen, M., Hansen, L. B. S., Saunders, A. M., Nielsen, P. H., & Nielsen, K. L. (2012). A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. *The ISME Journal*, *6*(6), 1094–1106. https://doi.org/10.1038/ismej.2011.176

Alexander, R. B., Smith, R. A., Schwarz, G. E., Boyer, E. W., Nolan, J. V., & Brakebill, J. W. (2008). Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environmental Science and Technology*, *42*(3), 822–830. https://doi.org/10.1021/es0716103

Amirbahman, A., Lake, B. A., & Norton, S. A. (2013). Seasonal phosphorus dynamics in the surficial sediment of two shallow temperate lakes: a solid-phase and pore-water study. *Hydrobiologia*, *701*(1), 65–77. https://doi.org/10.1007/s10750-012-1257-z

Archibald, J. (2010). *Dissolved phosphorus dynamics in shallow groundwater*. Cornell University.

Aschar-Sobbi, R., Abramov, A. Y., Diao, C., Kargacin, M. E., Kargacin, G. J., French, R. J., & Pavlov, E. (2008). High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. *Journal of Fluorescence*, *18*(5), 859–866. https://doi.org/10.1007/s10895-008-0315-4

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*, 505–511. https://doi.org/10.1038/nature13855

Barnard, J. L. (1976). A review of biological phosphorus removal in the activated sludge process. *Water SA*, *2*(3), 136–144.

Barnard, J. L., Dunlap, P., & Steichen, M. (2017). Rethinking the Mechanisms of Biological Phosphorus Removal. *Water Environment Research*, *89*(11), 2043–2054. https://doi.org/10.2175/106143017X15051465919010

Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., & Packmann, A. I. (2016). The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, *14*(4), 251–263. https://doi.org/10.1038/nrmicro.2016.15

Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impactd on erodable phosphorus and eutrophication: a global perspective. *BioScience*, *51*(3), 227–234. https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Bernhardt, E. S., Blaszczak, J. R., Ficken, C. D., Fork, M. L., Kaiser, K. E., & Seybold, E. C. (2017). Control points in ecosystems: moving beyond the hot spot hot moment concept. *Ecosystems*, *20*(2), 1–18. https://doi.org/10.1007/s10021-016-0103-y

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., Nemergut, D. R., Osborne, B. B., Ruiz-Gonz??lez, C., Schimel, J. P., Waldrop, M. P., & Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS Microbiology Ecology*, *91*(10), 1–11. https://doi.org/10.1093/femsec/fiv113

Bieroza, M., Bergström, L., Ulén, B., Djodjic, F., Tonderski, K., Heeb, A., Svensson, J., & Malgeryd, J. (2019). Hydrologic Extremes and Legacy Sources Can Override Efforts to Mitigate Nutrient and Sediment Losses at the Catchment Scale. *Journal of Environmental Quality*, *48*, 1314–1324. https://doi.org/10.2134/jeq2019.02.0063

Blackwell, M. S. A., Brookes, P. C., de la Fuente-Martinez, N., Gordon, H., Murray, P. J., Snars, K. E., Williams, J. K., Bol, R., & Haygarth, P. M. (2010). Phosphorus solubilization and potential transfer to surface waters from the soil microbial biomass following drying-rewetting and freezing-thawing. In *Advances in Agronomy* (1st ed., Vol. 106, Issue 10). Elsevier Inc. https://doi.org/10.1016/S0065-2113(10)06001-3

Bock, E., Smith, N., Rogers, M., Coleman, B., Reiter, M., Benham, B., & Easton, Z. M. (2015). Enhanced nitrate and phosphate removal in a denitrifying bioreacdtor with biochar. *Journal of Environmental Quality*, *44*(2), 605–613. https://doi.org/10.2134/jeq2014.03.0111

Bourke, D., Kurz, I., Dowding, P., O'Reilly, C., Tunney, H., Doody, D. G., O'Brien, J. E., & Jeffrey, D. W. (2009). Characterisation of organic phosphorus in overland flow from grassland plots using 31P nuclear magnetic resonance spectroscopy. *Soil Use and Management*, *25*(3), 234–242. https://doi.org/10.1111/j.1475-2743.2009.00229.x

Brady, N. C., & Weil, R. R. (2008). *The Nature and Properties of Soils* (14th ed.).

Brown, M. R. W., & Kornberg, A. (2004). Inorganic polyphosphate in the origin and survival of species. *Proceedings of the National Academy of Sciences*, *101*(46), 16085–16087. https://doi.org/10.1073/pnas.0406909101

Brown, M. R. W., & Kornberg, A. (2008). The long and short of it - polyphosphate, PPK and bacterial survival. *Trends in Biochemical Sciences*, *33*(6), 284–290.

https://doi.org/10.1016/j.tibs.2008.04.005

Bünemann, E. K., Smernik, R. J., Marschner, P., & McNeill, A. M. (2008). aMicrobial synthesis of organic and condensed forms of phosphorus in acid and calcareous soils. *Soil Biology and Biochemistry*, *40*(4), 932–946. https://doi.org/10.1016/j.soilbio.2007.11.012

Bünemann, E., Oberson, A., & Frossard, E. (Eds.). (2011). *Phosphorus in Action – Biological Processes in Soil Phosphorus Cycling*. Springer-Verlag.

Burgin, A. J., & Groffman, P. M. (2012). Soil O2 controls denitrification rates and N2O yield in a riparian wetland. *Journal of Geophysical Research*, *117*(1), 1–10. https://doi.org/10.1029/2011JG001799

Buttigieg, P. L., & Ramette, A. (2014). A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. *FEMS Microbiology Ecology*, *90*(3), 543–550. https://doi.org/10.1111/1574-6941.12437

Cade-Menun, B. J. (2017). Characterizing phosphorus forms in cropland soils with solution 31P-NMR: past studies and future research needs. *Chemical and Biological Technologies in Agriculture*, *4*(1), 1–13. https://doi.org/10.1186/s40538-017-0098-4

Cade-Menun, B. J., & Paytan, A. (2010). Nutrient temperature and light stress alter phosphorus and carbon forms in culture-grown algae. *Marine Chemistry*, *121*(1–4), 27–36. https://doi.org/10.1016/j.marchem.2010.03.002

Cade-Menun, B., & Liu, C. W. (2013). Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy of Soils from 2005 to 2013: A Review of Sample Preparation and Experimental Parameters. *Soil Science Society of America Journal*, *78*(1), 19–37. https://doi.org/10.2136/sssaj2013.05.0187dgs

Carini, P., Marsden, P. J., Leff, J. W., Morgan, E. E., Strickland, M. S., & Fierer, N. (2016).

Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology*, *2*, 16242. https://doi.org/10.1101/043372

Carlton, R. G., & Wetzel, R. G. (1988). Phosphorus flux from lake sediments: effect of epipelic algal oxygen production. *Limnology and Oceanography*, *33*(4), 562–570. https://doi.org/10.4319/lo.1988.33.4.0562

Carpenter, S. R. (2005). Eutrophication of aquatic ecosystems: bistability and soil phosphorus. *Proceedings of the National Academy of Sciences*, *102*(29), 10002–10005. https://doi.org/10.1073/pnas.0503959102

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. N. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, *8*(3), 559–568.

Castle, D., & Kirchman, D. L. (2004). Composition of estuarine bacterial communities assessed by denaturing gradient gel electrophoresis and fluorescence in situ hybridization. *Limnology and Oceanography: Methods*, *2*, 303–314. https://doi.org/10.4319/lom.2004.2.303

Chacon, N., Flores, S., & Gonzalez, A. (2006). Implications of iron solubilization on soil phosphorus release in seasonally flooded forests of the lower Orinoco River, Venezuela. *Soil Biology and Biochemistry*, *38*(6), 1494–1499. https://doi.org/10.1016/j.soilbio.2005.10.018

Chapra, S. C. and D. M. Dolan. 2012. Great Lakes total phosphorus revisited: 2. Mass balance modeling. Journal of Great Lakes Research. 38:741-754.

Choudoir, M. J., Panke-Buisse, K., Andam, C. P., & Buckley, D. H. (2017). Genome Surfing As Driver of Microbial Genomic Diversity. *Trends in Microbiology*, *25*, 624–636.

https://doi.org/10.1016/j.tim.2017.02.006

Christianson, L. E., Harmel, R. D., Smith, D., Williams, M. R., & King, K. (2016). Assessment and synthesis of 50 years of published drainage phosphorus losses. *Journal of Environmental Quality*, *45*(5), 1467–1477. https://doi.org/10.2134/jeq2015.12.0593

Cock, P. J. A., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B., & De Hoon, M. J. L. (2009). Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics*, *25*(11), 1422–1423. https://doi.org/10.1093/bioinformatics/btp163

Cohen, M. J., Kurz, M. J., Heffernan, J. B., Martin, J. B., Douglass, R. L., Foster, C. R., & Thomas, R. G. (2013). Diel phosphorus variation and the stoichiometry of ecosystem metabolism in a large spring-fed river. *Ecological Monographs*, *83*(2), 155–176. https://doi.org/10.1890/12-1497.1

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, et al. 2014. Controlling Eutrophication: Nitrogen and Phosphorus. Science. 323:1014-15

Crocetti, G. R., Hugenholtz, P., Bond, P. L., Schuler, A., Keller, J., Jenkins, D., Linda, L., Keller, R. G., & Blackall, L. L. (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Applied and Environmental Microbiology*, *66*(3), 1175–1182. https://doi.org/10.1128/AEM.66.3.1175-1182.2000.Updated

Cross, A. F., & Schlesinger, W. H. (1995). A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma*, *64*(3–4), 197–214. https://doi.org/10.1016/0016-7061(94)00023-4 da Veiga Leprovost, F., Barbosa, V. C., Francisco, E. L., Perez-Riverol, Y., & Carvalho, P. C. (2014). On best practices in the development of bioinformatics software. *Frontiers in Genetics*, *5*, 199.

Darch, T., Blackwell, M. S. A., Chadwick, D., Haygarth, P. M., Hawkins, J. M. B., & Turner, B. L. (2016). Assessment of bioavailable organic phosphorus in tropical forest soils by organic acid extraction and phosphatase hydrolysis. *Geoderma*, *284*, 93–102. https://doi.org/10.1016/j.geoderma.2016.08.018

Davelaar, D. (1993). Ecological significance of bacterial polyphosphate metabolism in sediments. *Hydrobiologia*, *253*(1), 179–192. https://doi.org/10.1007/BF00050737

DeAngelis, K. M., Silver, W. L., Thompson, A. W., & Firestone, M. K. (2010). Microbial communities acclimate to recurring changes in soil redox potential status. *Environmental Microbiology*, *12*(12), 3137–3149. https://doi.org/10.1111/j.1462-2920.2010.02286.x

DebRoy, S., Mukherjee, P., Roy, S., Thakur, A. R., & Raychaudhuri, S. (2013). Draft genome sequence of a phosphate-accumulating Bacillus sp .,. *Genome Announcements*, *1*(1), e00251-12. https://doi.org/10.1128/genomeA.00251-12.Copyright

Diaz, J. M., Ingall, E. D., Snow, S. D., Benitez-Nelson, C. R., Taillefert, M., & Brandes, J. A. (2012). Potential role of inorganic polyphosphate in the cycling of phosphorus within the hypoxic water column of Effingham Inlet, British Columbia. *Global Biogeochemical Cycles*, *26*(2), 1–14. https://doi.org/10.1029/2011GB004226

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science*, *321*(5891), 926–929. https://doi.org/10.1126/science.1156401

Dinh, M.-V., Schramm, T., Spohn, M., & Matzner, E. (2016). Drying-rewetting cycles release phosphorus from forest soils. *Journal of Plant Nutrition and Soil Science*, *179*(5), 670–

678. https://doi.org/10.1002/jpln.201500577

Dolan, D. M. and S. C. Chapra. 2012. Great Lakes total phosphorus revisited: 1. Loading analysis and update (1994–2008). Journal of Great Lakes Research. 38:730-740.

Dodd, R. J., & Sharpley, A. N. (2015). Recognizing the role of soil organic phosphorus in soil fertility and water quality. *Resources, Conservation and Recycling*, *105*, 282–293. https://doi.org/10.1016/j.resconrec.2015.10.001

Dodds, W. K., & Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. *Inland Waters*, *6*(2), 155–164. https://doi.org/10.5268/IW-6.2.909

Dodds, W.K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser, J. T., & Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters: analysis of potential economic damages. *Environmental Science and Technology*, *43*(1), 12–19. https://doi.org/10.1021/es801217q

Dodds, Walter K. (2003). The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. *Journal of Phycology*, *39*(5), 840–849. https://doi.org/10.1046/j.1529-8817.2003.02081.x

Dubrovsky, N. M., Burow, K. R., Clark, G. M., Gronberg, J. A. M., Hamilton, P. A., Hitt, K. J., Mueller, D. K., Munn, M. D., Nolan, B. T., Puckett, L. J., Rupert, M. G., Short, T. M., Spahr, N. E., Sprague, L. A., & Wilber, W. G. (2010). The quality of our Nation's waters-nutrients in the Nation 's streams and groundwater, 1992 – 2004. In *U.S. Geological Survey Circular 1350*. http://water.usgs.gov/nawqa/nutrients/pubs/circ1350

Dunbar, J., Barns, S. M., Ticknor, L. O., & Kuske, C. R. (2002). Empirical and Theoretical Bacterial Diversity in Four Arizona Soils Empirical and Theoretical Bacterial Diversity in Four Arizona Soils. *Applied and Environmental Microbiology*, *68*(6), 3035–3045.

https://doi.org/10.1128/AEM.68.6.3035

Dupas, R., Gruau, G., Gu, S., Humbert, G., Jaffrézic, A., & Gascuel-Odoux, C. (2015). Groundwater control of biogeochemical processes causing phosphorus release from riparian wetlands. *Water Research*, *84*, 307–314. https://doi.org/10.1016/j.watres.2015.07.048

Eixler, S., Selig, U., & Karsten, U. (2005). Extraction and detection methods for polyphosphate storage in autotrophic planktonic organisms. *Hydrobiologia*, *533*(1), 135–143. https://doi.org/10.1007/s10750-004-2406-9

Ekblom, R., & Wolf, J. B. W. (2014). A field guide to whole-genome sequencing, assembly and annotation. *Evolutionary Applications*, *7*(9), 1026–1042. https://doi.org/10.1111/eva.12178

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, *103*(3), 626–631. https://doi.org/10.1073/pnas.0507535103

Findlay, S., & Fischer, D. (2013). Ecosystem attributes related to tidal wetland effects on water quality. *Ecology*, *94*(1), 117–125. https://doi.org/10.1890/12-0464.1

Fleischer, S. (1978). Evidence for the anaerobic release of phosphorus from lake sediments as a biological process. *Naturwissenschaften*, *434*, 109–110.

Ford, W. I., King, K., & Williams, M. R. (2018). Upland and in-stream controls on baseflow nutrient dynamics in tile-drained agroecosystem watersheds. *Journal of Hydrology*, *556*(3), 800–812. https://doi.org/10.1016/j.jhydrol.2017.12.009

Franklin, D. H., Steiner, J. L., Duke, S. E., Moriasi, D. N., & Starks, P. J. (2013). Spatial considerations in wet and dry periods for phosphorus in streams of the fort cobb watershed, United States. *Journal of the American Water Resources Association*, *49*(4), 908–922. https://doi.org/10.1111/jawr.12048

Gächter, R., & Meyer, J. S. (1993). The role of microorganisms in mobilization and fixation of phosphorus in sediments. *Hydrobiologia*, *253*, 103–121. https://doi.org/10.1007/BF00050731

Gächter, R., Meyer, J. S., & Mares, A. (1988). Contribution of bacteria to release and fixation of phosphorus in lake sediments. *Limnology and Oceanography*, *33*(6 part 2), 1542–1558. https://doi.org/10.4319/lo.1988.33.6_part_2.1542

Gans, J., Wolinsky, M., & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science*, *309*(5739), 1387–1390. https://doi.org/10.1126/science.1126853

Garcia, A. M., Alexander, R. B., Arnold, J. G., Norfleet, L., White, M. J., Robertson, D. M., & Schwarz, G. (2016). Regional effects of agricultural conservation practdices on nutrient transport in the Upper Mississippi River Basin. *Environmental Science and Technology*, *50*(13), 6991–7000. https://doi.org/10.1021/acs.est.5b03543

Gebremariam, S. Y., Beutel, M. W., Christian, D., & Hess, T. F. (2011). Research Advances and Challenges in the Microbiology of Enhanced Biological Phosphorus Removal—A Critical Review. *Water Environment Research*, *83*(3), 195–219. https://doi.org/10.2175/106143010X12780288628534

George, T. S., Giles, C. D., Menezes-Blackburn, D., Condron, L. M., Gama-Rodrigues, A. C., Jaisi, D., Lang, F., Neal, A. L., Stutter, M. I., Almeida, D. S., Bol, R., Cabugao, K. G., Celi, L., Cotner, J. B., Feng, G., Goll, D. S., Hallama, M., Krueger, J., Plassard, C., … Haygarth, P. M. (2018). Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. *Plant and Soil*, *427*(Issue 1-2), 181–208. https://doi.org/10.1007/s11104-017-3391-x

Gerke, J. (2010). Humic (organic matter)-Al(Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. *Soil Science*, *175*(9), 417–425. https://doi.org/10.1097/SS.0b013e3181f1b4dd

Goyette, J. O., Bennett, E. M., & Maranger, R. (2018). Low buffering capacity and slow recovery of anthropogenic phosphorus pollution in watersheds. *Nature Geoscience*, *11*, 921–925. https://doi.org/10.1038/s41561-018-0238-x

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J. C., Glanville, H. C., Jones, D. L., Angel, R., Salminen, J., Newton, R. J., B??rgmann, H., Ingram, L. J., … Nemergut, D. R. (2016). Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Frontiers in Microbiology*, *7*, 214. https://doi.org/10.3389/fmicb.2016.00214

Graham, E. B., Wieder, W. R., Leff, J. W., Weintraub, S. R., Townsend, A. R., Cleveland, C. C., Philippot, L., & Nemergut, D. R. (2014). Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil Biology and Biochemistry*, *68*, 279–282. https://doi.org/10.1016/j.soilbio.2013.08.023

Gregory, S., Allen, A. W., Baker, M., Boyer, K., Dillaha, T., & Elliot, J. (2007). Realistic expectations of timing between conservation and restoration actions and ecological responses. In *Managing agricultural landscapes for environmental quality: strengthening the science base* (pp. 111–142). Soil and Water Conservation Society.

Grierson, P. F., Comerford, N. B., & Jokela, E. J. (1998). Phosphorus mineralization kinetics and response of microbial phosphorus to drying and rewetting in a Florida Spodosol. *Soil Biology and Biochemistry*, *30*(10–11), 1323–1331. https://doi.org/10.1016/S0038-0717(98)00002-9

Hall, E. K., Bernhardt, E. S., Bier, R. L., Bradford, M. A., Boot, C. M., Cotner, J. B., del Giorgio, P. A., Evans, S. E., Graham, E. B., Jones, S. E., Lennon, J. T., Locey, K. J., Nemergut, D., Osborne, B. B., Rocca, J. D., Schimel, J. P., Waldrop, M. P., & Wallenstein, M. D. (2018). Understanding how microbiomes influence the systems they inhabit. *Nature Microbiology*, *3*(9), 977–982. https://doi.org/10.1038/s41564-018-0201-z

Hamlin, Q. F., Kendall, A. D., Martin, S. L., Whitenack, H. D., Roush, J. A., Hannah, B. A., & Hyndman, D. W. (2020). Quantifying Landscape Nutrient Inputs With Spatially Explicit Nutrient Source Estimate Maps. *Journal of Geophysical Research: Biogeosciences*, *125*(2), 1–24. https://doi.org/10.1029/2019JG005134

Harold, F. (1964). Enzymic and genetic control of polyphosphate accumulation in Aerobacter aerogenes. *Journal of General Microbiology*, *35*(1964), 81–90. https://doi.org/10.1099/00221287-35-1-81

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, *60*(4), 579–598. https://doi.org/10.1007/s13213-010-0117-1

Haygarth, P. M., Harrison, A. F., & Turner, B. L. (2018). aOn the history and future of soil organic phosphorus research: a critique across three generations. *European Journal of Soil Science*, *69*(1), 86–94. https://doi.org/10.1111/ejss.12517

Haygarth, Philip M, Jarvie, H. P., Powers, S. M., Sharpley, A. N., Elser, J. J., Shen, J., Peterson,

H. M., Chan, N., Howden, N. J. K., Burt, T., Worrall, F., Zhang, F., & Liu, X. (2014). Sustainable phoshorus management and the need for a long-term perspective: the legacy hypothesis. *Environmental Science and Technology*, *48*(15), 8417–8419.

Helm, K. P., Bindoff, N. L., & Church, J. A. (2011). Observed decreases in oxygen content of the global ocean. *Geophysical Research Letters*, *38*(23), 1–6. https://doi.org/10.1029/2011GL049513

Hesselmann, R. P., Werlen, C., Hahn, D., van der Meer, J. R., & Zehnder, a J. (1999). Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. *Systematic and Applied Microbiology*, *22*(3), 454–465. https://doi.org/10.1016/S0723-2020(99)80055-1

Howe, A. C., & Chain, P. S. G. (2015). Challenges and opportunities in understanding microbial communities with metagenome assembly (accompanied by IPython Notebook tutorial). *Frontiers in Microbiology*, *6*, 678. https://doi.org/10.3389/fmicb.2015.00678

Howe, A. C., Jansson, J. K., Malfatti, S. A., Tinge, S. G., Tiedje, J. M., & Brown, C. T. (2014). Tackling soil diversity with the assembly of large, complex metagenomes. *Proceedings of the National Academy of Sciences*, *111*(13), 4904–4909. https://doi.org/10.1073/pnas.1405263111

Hug, L. A., Baker, B. J., Anantharaman, K., Brown, C. T., Probst, A. J., Castelle, C. J., Butterfield, C. N., Hernsdorf, A. W., Amano, Y., Ise, K., Suzuki, Y., Dudek, N., Relman, D. A., Finstad, K. M., Amundson, R., Thomas, B. C., & Banfield, J. F. (2016). A new view of the tree of life. *Nature Microbiology*, *1*(5), 1–6. https://doi.org/10.1038/nmicrobiol.2016.48

Hupfer, M, Gloess, S., & Grossart, H. (2007). Polyphosphate-accumulating microorganisms in aquatic sediments. *Aquatic Microbial Ecology*, *47*(3), 299–311. https://doi.org/10.3354/ame047299

Hupfer, Michael, & Lewandowski, J. (2008). Oxygen controls the phosphorus release from lake sediments - A long-lasting paradigm in limnology. *International Review of Hydrobiology*, *93*(4–5), 415–432. https://doi.org/10.1002/iroh.200711054

Hupfer, Michael, Rube, B., & Schmieder, P. (2004). Origin and diagenesis of polyphosphate in lake sediments: a 31P-NMR study. *Limnology and Oceanography*, *49*(1), 1–10. https://doi.org/doi:10.4319/lo.2004.49.1.0001

Iho, A., Ahlvik, L., Ekholm, P., Lehtoranta, J., & Kortelainen, P. (2017). Optimal Phosphorus Abatement Redefined: Insights From Coupled Element Cycles. *Ecological Economics*, *137*, 13–19. https://doi.org/10.1016/j.ecolecon.2017.02.023

International Panel on Climate Change (IPCC). 2014. Climate Change 2014: Synthesis Report. Contribution ofWorking Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (eds.) Core Writing Team, RK Pachauri, LA Meyer. IPCC, Geneva, Switzerland. 151p.

Jarvie, H. P., Johnson, L. T., Sharpley, A. N., Smith, D. R., Baker, D. B., Bruulsema, T. W., & Confesor, R. (2017). Increased soluble phosphorus loads to Lake Erie: unintended consequences of conservation practices? *Journal of Environmental Quality*, *46*(1), 123–132. https://doi.org/10.2134/jeq2016.07.0248

Jarvie, H. P., Sharpley, A. N., Flaten, D., Kleinman, P. J. a, Jenkins, A., & Simmons, T. (2015). The pivotal role of phosphorus in a resilient water–energy–food security nexus. *Journal of Environmental Quality*, *44*(4), 1049–1062. https://doi.org/10.2134/jeq2015.01.0030

Jarvie, H. P., Sharpley, A. N., Withers, P. J. a, Scott, J. T., Haggard, B. E., & Neal, C. (2013).

Phosphorus mitigation to control river eutrophication: murky waters, inconvenient truths, and “postnormal” science. *Journal of Environmental Quality*, *42*(2), 295–304. https://doi.org/10.2134/jeq2012.0085

Javot, H., Pumplin, N., & Harrison, M. J. (2007). Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant, Cell, and Environment*, *30*(3), 310–322. https://doi.org/10.1111/j.1365-3040.2006.01617.x

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., & Barea, J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils*, *37*(1), 1–16. https://doi.org/10.1007/s00374-002-0546-5

Jeffries, T. C., Schmitz Fontes, M. L., Harrison, D. P., Van-Dongen-Vogels, V., Eyre, B. D., Ralph, P. J., & Seymour, J. R. (2016). Bacterioplankton dynamics within a large anthropogenically impacted urban estuary. *Frontiers in Microbiology*, *6*, 1–17. https://doi.org/10.3389/fmicb.2015.01438

Jones, S. E., & Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Science*, *107*(13), 5881–5886. https://doi.org/10.1073/pnas.0912765107

Kaushal, S. S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., Newcomer, T. A., Duan, S., Welty, C., Belt, K. T., Sujay, S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., New-, T. A., Duan, S., Welty, C., Belt, K. T., Use, L., & Amplify, C. V. (2014). Land Use and Climate Variability Amplify Carbon, Nutrient, and Contaminant Pulses: A Review with Management Implications. *Journal of the American Water Resources Association*, *50*, 585–614. https://doi.org/10.1111/jawr.12204

Kawakoshi, A., Nakazawa, H., Fukada, J., Sasagawa, M., Katano, Y., Nakamura, S., Hosoyama, A., Sasaki, H., Ichikawa, N., Hanada, S., Kamagata, Y., Nakamura, K., Yamazaki, S., & Fujita, N. (2012). Deciphering the genome of polyphosphate accumulating Actinobacterium Microlunatus phosphovorus. *DNA Research*, *19*(5), 383–394. https://doi.org/10.1093/dnares/dss020

Kellogg, L. E., & Bridgham, S. D. (2003). Phosphorus retention and movement across an. *Biogeochemistry*, *63*(3), 299–315.

Kenney, W. F., A. D. Chapman, C. L. Schelske. 2015. Comment on ‘‘The Chemical Nature of Phosphorus in Subtropical Lake Sediments’’. Aquatic Geochemistry. 21:1-6.

Khoshmanesh, A., Hart, B. T., Duncan, A., & Beckett, R. (1999). Biotic Uptake and Release of Phosphorus by a Wetland Sediment. *Environmental Technology*, *20*, 85–91.

Khoshmanesh, A., Hart, B. T., Duncan, A., & Beckett, R. (2002). Luxury uptake of phosphorus by sediment bacteria. *Water Research*, *36*, 774–778. https://doi.org/10.1016/S0043-1354(01)00272-X

King, K. W., Williams, M. R., Macrae, M. L., Fausey, N. R., Frankenberger, J., Smith, D. R., Kleinman, P. J. A., & Brown, L. C. (2015). Phosphorus transport in agricultural subsurface drainage: a review. *Journal of Environmental Quality*, *44*(2), 467–485. https://doi.org/10.2134/jeq2014.04.0163

Kleinman, P. J. A., Sharpley, A. N., Buda, A. R., Mcdowell, R. W., & Allen, A. L. (2011). aSoil controls of phosphorus in runoff: management barriers and opportunities. *Canadaian Journal of Soil Science*, *91*(3), 329–338. https://doi.org/10.4141/CJSS09106

Kleinman, P. J. A., Sharpley, A. N., McDowell, R. W., Flaten, D. N., Buda, A. R., Tao, L., Bergstrom, L., & Zhu, Q. (2011). bManaging agricultural phosphorus for water quality protection: principles for progress. *Plant and Soil*, *349*(1–2), 169–182.

https://doi.org/10.1007/s11104-011-0832-9

Kleinman, P. J. A., Sharpley, A. N., Saporito, L. S., Buda, A. R., & Bryant, R. B. (2009). Application of manure to no-till soils: phosphorus losses by sub-surface and surface pathways. *Nutrient Cycling in Agroecosystems*, *84*(3), 215–227. https://doi.org/10.1007/s10705-008-9238-3

Kleinman, P. J. A., Smith, D. R., Bolster, C. H., & Easton, Z. M. (2015). Phosphorus fate, management, and modeling in artificially drained systems. *Journal of Environmental Quality*, *44*(2), 460–466. https://doi.org/10.2134/jeq2015.02.0090

Kornberg, A. (1995). Inorganic polyphosphate: toward making a forgotten polymer unforgettable. *Journal of Bacteriology*, *177*(3), 491–496. https://doi.org/10.1128/jb.177.3.491-496.1995

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120. https://doi.org/10.1128/AEM.01043-13

Krishnaswamy, U., Muthuchamy, M., & Perumalsamy, L. (2011). Biological removal of phosphate from synthetic wastewater using bacterial consortium. *Iranian Journal of Biotechnology*, *9*, 37–49.

Kristiansen, R., Thi, H., Nguyen, T., Saunders, A. M., Lund Nielsen, J., Wimmer, R., Le, V. Q., Mcilroy, S. J., Petrovski, S., Seviour, R. J., Calteau, A., Lehmann Nielsen, K., Nielsen, P. H. H., Nguyen, H. T. T., Saunders, A. M., Nielsen, J. L., Wimmer, R., Le, V. Q., Mcilroy, S. J., … Nielsen, P. H. H. (2013). A metabolic model for members of the genus

Tetrasphaera involved in enhanced biological phosphorus removal. *The ISME Journal*, *7*(3), 543–554. https://doi.org/10.1038/ismej.2012.136

Kunin, V., He, S., Warnecke, F., Peterson, S. B., Garcia Martin, H., Haynes, M., Ivanova, N., Blackall, L. L., Breitbart, M., Rohwer, F., McMahon, K. D., & Hugenholtz, P. (2008). A bacterial metapopulation adapts locally to phage predation despite global dispersal. *Genome Research*, *18*(2), 293–297. https://doi.org/10.1101/gr.6835308

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, *75*(15), 5111–5120. https://doi.org/10.1128/AEM.00335-09

Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, *9*(2), 119–130. https://doi.org/10.1038/nrmicro2504

Li, X., Yuan, H., Yang, J., & Li, B. (2013). Genome sequence of the polyphosphate-accumulating organism Arthrobacter sp. strain PAO19 isolated from maize rhizosphere soil. *Genome Announcements*, *1*(4), 11039–11040. https://doi.org/10.1186/1471-2164-13-534.9.

Lin, Y., Bhattacharyya, A., Campbell, A. N., Nico, P. S., Pett-Ridge, J., & Silver, W. L. (2018). Phosphorus Fractionation Responds to Dynamic Redox Conditions in a Humid Tropical Forest Soil. *Journal of Geophysical Research: Biogeosciences*, *123*, 3016–3027. https://doi.org/10.1029/2018JG004420

Lisboa, M. S., Schneider, R. L., Sullivan, P. J., & Walter, M. T. (2020). Drought and post-drought rain effect on stream phosphorus and other nutrient losses in the Northeastern

USA. *Journal of Hydrology: Regional Studies*, *28*, 100672. https://doi.org/10.1016/j.ejrh.2020.100672

Liu, J., Macrae, M. L., Elliott, J. A., Baulch, H. M., Wilson, H. F., & Kleinman, P. J. A. (2019). Impacts of Cover Crops and Crop Residues on Phosphorus Losses in Cold Climates: A Review. *Journal of Environmental Quality*, 850–868. https://doi.org/10.2134/jeq2019.03.0119

Loman, N., & Watson, M. (2013). So you want to be a computational biologist? *Nature Biotechnology*, *31*(11), 996–998. https://doi.org/10.1038/nbt.2740

Lyons, J. B., Gorres, J. H., & Amador, J. A. (1998). Spatial and temporal variability of phosphorus retention in a riparian forest soil. *Journal of Environmental Quality*, *27*(4), 895–903.

Maccoux, M. J., A. Dove, S.M. Backus, D.M. Dolan. 2016. Total and soluble reactive phosphorus loadings to Lake Erie: a detailed accounting by year, basin, country and tributary. Journal of Great Lakes Research. 42:1151-1165.

MacDonald, G. K., Jarvie, H. P., Withers, P. J. A., Doody, D. G., Keeler, B. L., Haygarth, P. M., Johnson, L. T., McDowell, R. W., Miyittah, M. K., Powers, S. M., Sharpley, A. N., Shen, J., Smith, D. R., Weintraub, M. N., & Zhang, T. (2016). Guiding phosphorus stewardship for multiple ecosystem services. *Ecosystem Health and Sustainability*, *2*(12), 1–12. https://doi.org/10.1002/ehs2.1251

Manzoni, S., Schaeffer, S. M., Katul, G., Porporato, A., & Schimel, J. P. (2014). A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. *Soil Biology and Biochemistry*, *73*, 69–83. https://doi.org/10.1016/j.soilbio.2014.02.008

Mao, Y., Graham, D. W., Tamaki, H., & Zhang, T. (2015). Dominant and novel clades of Candidatus Accumulibacter phosphatis in 18 globally distributed full-scale wastewater treatment plants. *Scientific Reports*, *5*, 11857. https://doi.org/10.1038/srep11857

Mao, Y., Wang, Z., Li, L., Jiang, X., Zhang, X., Ren, H., & Zhang, T. (2016). Exploring the shift in structure and function of microbial communities performing biological phosphorus removal. *PLoS ONE*, *11*(8), 1–16. https://doi.org/10.1371/journal.pone.0161506

Mao, Y., Yu, K., Xia, Y., Chao, Y., & Zhang, T. (2014). Genome reconstruction and gene expression of "Candidatus Accumulibacter phosphatis" Clade IB performing biological phosphorus removal. *Environmental Science and Technology*, *48*(17), 10363–10371. https://doi.org/10.1021/es502642b

Margenot, A. J., Kitt, D., Gramig, B. M., Berkshire, T. B., Chatterjee, N., Hertzberger, A. J., Aguiar, S., Furneaux, A., Sharma, N., & Cusick, R. D. (2019). Toward a Regional Phosphorus (Re)cycle in the US Midwest. *Journal of Environmental Quality*, *48*(5), 1397–1413. https://doi.org/10.2134/jeq2019.02.0068

Markelov, I., Couture, R. M., Fischer, R., Haande, S., & Van Cappellen, P. (2019). Coupling Water Column and Sediment Biogeochemical Dynamics: Modeling Internal Phosphorus Loading, Climate Change Responses, and Mitigation Measures in Lake Vansjø, Norway. *Journal of Geophysical Research: Biogeosciences*, *124*, 3847–3866. https://doi.org/10.1029/2019JG005254

Martin, P., Dyhrman, S. T., Lomas, M. W., Poulton, N. J., & Van Mooy, B. A. S. (2014). Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus. *Proceedings of the National Academy of Sciences*, *111*(22), 8089–8094. https://doi.org/10.1073/pnas.1321719111

Martin, P., & Van Mooy, B. A. S. (2013). Fluorometric quantification of polyphosphate in environmental plankton samples: extraction protocols, matrix effects, and nucleic acid interference. *Applied and Environmental Microbiology*, *79*(1), 273–281. https://doi.org/10.1128/AEM.02592-12

Martins, G., Terada, A., Ribeiro, D. C., Corral, A. M., Brito, A. G., Smets, B. F., & Nogueira, R. (2011). Structure and activity of lacustrine sediment bacteria involved in nutrient and iron cycles. *FEMS Microbiology Ecology*, *77*(3), 666–679. https://doi.org/10.1111/j.1574-6941.2011.01145.x

Martiny, J. B. H., Jones, S. E., Lennon, J. T., & Martiny, A. C. (2015). Microbiomes in light of traits: a phylogenetic perspective. *Science*, *350*(6261), 649–656. https://doi.org/10.1126/science.aac9323

McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., Hart, S. C., Harvey, J. W., Johnston, C. a., Mayorga, E., McDowell, W. H., & Pinay, G. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecoystems. *Ecosystems*, *6*(4), 301–312. https://doi.org/10.1007/s10021-003-0161-9

McLaren, T. I., Smernik, R. J., McLaughlin, M. J., McBeath, T. M., Kirby, J. K., Simpson, R. J., Guppy, C. N., Doolette, A. L., & Richardson, A. E. (2015). Complex Forms of Soil Organic Phosphorus-A Major Component of Soil Phosphorus. *Environmental Science and Technology*, *49*(22), 13238–13245. https://doi.org/10.1021/acs.est.5b02948

McMahon, K. D., & Read, E. K. (2013). Microbial contributions to phosphorus cycling in eutrophic lakes and wastewater. *Annual Review of Microbiology*, *67*, 199–219. https://doi.org/10.1146/annurev-micro-092412-155713

McParland, E., Benitez-Nelson, C. R., Taylor, G. T., Thunell, R., Rollings, A., & Lorenzoni, L. (2015). Cycling of suspended particulate phosphorus in the redoxcline of the Cariaco Basin. *Marine Chemistry*, *176*, 64–74. https://doi.org/10.1016/j.marchem.2015.07.008

Mekonnen, M. M., & Hoekstra, A. Y. (2018). Global Anthropogenic Phosphorus Loads to Freshwater and Associated Grey Water Footprints and Water Pollution Levels: A High-Resolution Global Study. *Water Resources Research*, *54*(1), 345–358. https://doi.org/10.1002/2017WR020448

Metson, G. S., & Bennett, E. M. (2015). Phosphorus cycling in Montreal's food and urban agriculture systems. *PLoS ONE*, *10*(3), 1–18. https://doi.org/10.1371/journal.pone.0120726

Mino, T., Van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*, *32*(11), 3193–3207. https://doi.org/10.1016/S0043-1354(98)00129-8

Mukherjee, C., Chowdhury, R., & Ray, K. (2015). Phosphorus recycling from an unexplored source by polyphosphate accumulating microalgae and cyanobacteria-a step to phosphorus security in agriculture. *Frontiers in Microbiology*, *6*, 1421. https://doi.org/10.3389/fmicb.2015.01421

Narancic, T., Djokic, L., Kenny, S. T., O'Connor, K. E., Radulovic, V., Nikodinovic-Runic, J., & Vasiljevic, B. (2012). Metabolic versatility of Gram-positive microbial isolates from contaminated river sediments. *Journal of Hazardous Materials*, *215–216*, 243–251. https://doi.org/10.1016/j.jhazmat.2012.02.059

National Academies of Sciences, Engineering, and Medicine (NASEM). 2018. *Science Breakthroughs to Advance Food and Agricultural Research by 2030*. Washington, DC:

The National Academies Press. doi: https://doi.org/10.17226/25059.

Nemergut, D., Shade, A., & Violle, C. (2014). When, where and how does microbial community composition matter? *Frontiers in Microbiology*, *5*, 2012–2014. https://doi.org/10.3389/fmicb.2014.00497

Noe, G. B., Scinto, L. J., Taylor, J., Childers, D. L., & Jones, R. D. (2003). Phosphorus cycling and partitioning in an oligotrophic everglades wetlands ecosytem: a radioisotope tracing study. *Freshwater Biology*, *48*(2003), 1993–2008. https://doi.org/10.1046/j.1365-2427.2003.01143.x

Oehmen, A., Lemos, P. C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L. L., & Reis, M. A. M. (2007). Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research*, *41*(11), 2271–2300. https://doi.org/10.1016/j.watres.2007.02.030

Oliverio, A. M., Bradford, M. A., & Fierer, N. (2016). Identifying the microbial taxa which consistently respond to soil warming across time and space. *Global Change Biology*, *23*, 2117–2129. https://doi.org/10.1111/gcb.13557

Ota, S., Yoshihara, M., Yamazaki, T., Takeshita, T., & Hirata, A. (2016). Deciphering the relationship among phosphate dynamics , electron-dense body and lipid accumulation in the green alga Parachlorella kessleri. *Scientific Reports*, *6*, 25731. https://doi.org/10.1038/srep25731

Paerl, H. W., & Otten, T. G. (2013). Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, *65*, 995–1010. https://doi.org/10.1007/s00248-012-0159-y

Pallen, M. J. (2016). Microbial bioinformatics 2020. *Microbial Biotechnology*, *9*(5), 681–686. https://doi.org/10.1111/1751-7915.12389

Perez-Riverol, Y., Gatto, L., Wang, R., Sachsenberg, T., Uszkoreit, J., Leprevost, F. da V., Fufezan, C., Ternent, T., Eglen, S. J., Katz, D. S., Pollard, T. J., Konovalov, A., Flight, R. M., Blin, K., & Vizca??no, J. A. (2016). Ten simple rules for taking advantage of Git and GitHub. *PLoS Computational Biology*, *12*(7), 1–11. https://doi.org/10.1371/journal.pcbi.1004947

Peterson, S. B., Warnecke, F., Madejska, J., Mcmahon, K. D., & Hugenholtz, P. (2008). Environmental distribution and population biology of Candidatus Accumulibacter, a primary agent of biological phosphorus removal. *Environmental Microbiology*, *10*(10), 2692–2703. https://doi.org/10.1111/j.1462-2920.2008.01690.x

Pett-Ridge, J., & Firestone, M. K. (2005). Redox fluctuation structures microbial communities in a wet tropical soil redox fluctuation structures microbial communities in a wet tropical soil. *Applied and Environmental Microbiology*, *71*(11), 6998–7007. https://doi.org/10.1128/AEM.71.11.6998

Powell, J. R., Welsh, A., Hallin, S., & Allison, S. D. (2015). Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. *Ecology*, *96*(7), 1985–1993. https://doi.org/10.1890/14-1127.1

Powers, S. M., Bruulsema, T. W., Burt, T. P., Chan, N. I., Elser, J. J., Haygarth, P. M., Howden, N. J. K., Jarvie, H. P., Lyu, Y., Peterson, H. M., Sharpley, A. N., Shen, J., Worrall, F., & Zhang, F. (2016). Long-term accumulation and transport of anthropogenic phosphorus in three river basins. *Nature Geoscience*, *9*(5), 353–356. https://doi.org/10.1038/ngeo2693

Price, K. J., & Carrick, H. (2011). Meta-analytical approach to explain variation in microbial phosphorus uptake rates in aquatic ecosystems. *Aquatic Microbial Ecology*, *65*(1), 89–102. https://doi.org/10.3354/ame01537

Price, K. J., & Carrick, H. J. (2016). Effects of experimental nutrient loading on phosphorus uptake by biofilms: evidence for nutrient saturation in mid-Atlantic streams. *Freshwater Science*, *35*(2), 503–517. https://doi.org/10.1086/686269

Prosser, J. I. (2013). Think before you sequence. *Nature*, *494*, 41. https://doi.org/10.1038/494040a

Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P., Green, J. L., Green, L. E., Killham, K., Lennon, J. J., Osborn, A. M., Solan, M., Van, der G., & Young, J. P. W. (2007). The role of ecological theory in microbial ecology. *Nature Reviews Microbiology*, *5*, 384–392. http://dx.doi.org/10.1038/nrmicro1643

Quang, M. N., Rogers, T., Hofman, J., & Lanham, A. B. (2019). Global Sensitivity Analysis of Metabolic Models for Phosphorus Accumulating Organisms in Enhanced Biological Phosphorus Removal. *Frontiers in Bioengineering and Biotechnology*, *7*, 234. https://doi.org/10.3389/fbioe.2019.00234

R Core Team. 2017. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available online: https://www.R-project.org/.

Rabbani, G., Rahman, A., & Mainuddin, K. (2013). Salinity-induced loss and damage to farming households in coastal Bangladesh. *International Journal of Global Warming*, *5*, 400–415. https://doi.org/10.1504/IJGW.2013.057284

Rao, N. N., Gómez-García, M. R., & Kornberg, A. (2009). Inorganic polyphosphate: essential for growth and survival. *Annual Review of Biochemistry*, *78*, 605–647. https://doi.org/10.1146/annurev.biochem.77.083007.093039

Read, E. K., Ivancic, M., Hanson, P., Cade-Menun, B. J., & McMahon, K. D. (2014). Phosphorus speciation in a eutrophic lake by 31P NMR spectroscopy. *Water Research*, *62*, 229–240. https://doi.org/10.1016/j.watres.2014.06.005

Reddy, K. R., Kadlec, R. H., Flaig, E., & Gale, P. M. (1999). Phosphorus retention in streams and wetlands: a review. *Critical Reviews in Environmental Science and Technology*, *29*(1), 83–146. https://doi.org/10.1080/10643389991259182

Reed, D. C., Algar, C. K., Huber, J. A., & Dick, G. J. (2014). Gene-centric approach to integrating environmental genomics and biogeochemical models. *Proceedings of the National Academy of Sciences*, *111*(5), 1879–1884. https://doi.org/10.1073/pnas.1313713111

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., Harvey, P. R., Ryan, M. H., Veneklaas, E. J., Lambers, H., Oberson, A., Culvenor, R. A., & Simpson, R. J. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, *349*, 121–156. https://doi.org/10.1007/s11104-011-0950-4

Richardson, A. E., & Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability. *Plant Physiology*, *156*, 989–996. https://doi.org/10.1104/pp.111.175448

Richardson, C. J. (1985). Mechanisms Controlling Phosphorus Retention. *Science*, *228*, 1424–1427.

Rier, S. T., Kinek, K. C., Hay, S. E., & Francoeur, S. N. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. *Freshwater Science*, *35*(2), 490–502. https://doi.org/10.1086/685859.

Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. *Annual Reviews of Genetics*, *38*, 525–552. https://doi.org/10.1146/annurev.genet.38.072902.091216

River, M., & Richardson, C. J. (2018). Stream transport of iron and phosphorus by authigenic nanoparticles in the Southern Piedmont of the U.S. *Water Research*, *130*, 312–321. https://doi.org/10.1016/j.watres.2017.12.004

Rocca, J. D., Hall, E. K., Lennon, J. T., Evans, S. E., Waldrop, M. P., Cotner, J. B., Nemergut, D. R., Graham, E. B., & Wallenstein, M. D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *ISME Journal*, *9*(8), 1693–1699. https://doi.org/10.1038/ismej.2014.252

Rodríguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, *17*(4–5), 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rousk, J., Brookes, P. C., Glanville, H. C., & Jones, D. L. (2011). Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. *Applied and Environmental Microbiology*, *77*(8), 2791–2795. https://doi.org/10.1128/AEM.02870-10

Rubio-Rincón, F. J., Welles, L., Lopez-Vazquez, C. M., Nierychlo, M., Abbas, B., Geleijnse, M., Nielsen, P. H., van Loosdrecht, M. C. M., & Brdjanovic, D. (2017). Long-term effects of sulphide on the enhanced biological removal of phosphorus: The symbiotic role of Thiothrix caldifontis. *Water Research*, *116*, 53–64. https://doi.org/10.1016/j.watres.2017.03.017

Safarzadeh-Amiri, A., Walton, J., Mahmoud, I., & Sharifi, N. (2017). Iron(III)-polyphosphates as catalysts for the liquid redox sulfur recovery process. *Applied Catalysis B: Environmental*, *207*, 424–428. https://doi.org/10.1016/j.apcatb.2017.02.022

Saia, S. M. M., Sullivan, P. J. J., Regan, J. M. M., Carrick, H. J. J., Buda, A. R. R., Locke, N. A.

A., & Walter, M. T. T. (2017). Evidence for polyphosphate accumulating organism (PAO)-mediated phosphorus cycling in stream biofilms under alternating aerobic/anaerobic conditions. *Freshwater Science*, *36*(2), 284–296. https://doi.org/10.1086/691439

Scavia, D., J. D., Allan, K. K. Arend, S. Bartell, D. Beletsky et al. 2014. Assessing and addressing the re-eutrophication of Lake Erie: central basin hypoxia. Journal of Great Lakes Research. 40:226–246.

Schachtman, D. P., Reid, R. J., Ayling, S. M., S, D. B. D. P., & A, S. S. S. M. (1998). Update on Phosphorus Uptake Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiology*, *116*, 447–453.

Schimel, J., & Gulledge, J. (1998). Microbial community structure and global trace gases. *Global Change Biology*, *4*(7), 745–758. https://doi.org/10.1046/j.1365-2486.1998.00195.x

Schimel, J. P., Gulledge, J. M., Clein-Curley, J. S., Lindstrom, J. E., & Braddock, J. F. (1999). Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. *Soil Biology and Biochemistry*, *31*(6), 831–838. https://doi.org/10.1016/S0038-0717(98)00182-5

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science*, *195*(4275), 260–262.

Schindler, David W. (2012). The Dilemma of Controlling Cultural Eutrophication of Lakes. *Proceedings of the Royal Society B*, *279*(1746), 4322–4333. https://doi.org/10.1098/rspb.2012.1032

Schloss, P. D. (2017). Preprinting microbiology. *MBio*, *8*(3), 1–11. https://doi.org/10.1128/mBio.00438-17

Schloss, P. D. (2020). Reintroducing mothur: 10 years later. *Applied and Environmental Microbiology*, *86*, 02343–19. https://doi.org/10.1128/AEM.02343-19

Schloss, P. D., & Handelsman, J. (2006). Toward a census of bacteria in soil. *PLoS Computational Biology*, *2*(7), 786–793. https://doi.org/10.1371/journal.pcbi.0020092

Schulz, H. N., & Schulz, H. D. (2005). Large sulfur bacteria and the formation of phosphorite. *Science*, *307*, 416–418. https://doi.org/10.1126/science.1103096

Scinto, L. J., & Reddy, K. R. (2003). Biotic and abiotic uptake of phosphorus by periphyton in a subtropical freshwater wetland. *Aquatic Botany*, *77*(3), 203–222. https://doi.org/10.1016/S0304-3770(03)00106-2

Sekaluvu, L., Zhang, L., & Gitau, M. (2018). Evaluation of constraints to water quality improvements in the Western Lake Erie Basin. *Journal of Environmental Management*, *205*, 85–98. https://doi.org/10.1016/j.jenvman.2017.09.063

Seviour, R. J., & McIlroy, S. (2008). The microbiology of phosphorus removal in activated sludge processes-the current state of play. *Journal of Microbiology*, *46*(2), 115–124. https://doi.org/10.1007/s12275-008-0051-0

Seviour, R. J., Mino, T., & Onuki, M. (2003). The Microbiology of Biological Phosphorus Removal in Activated Sludge Systems. *FEMS Microbiology Reviews*, *27*(1), 99–127. https://doi.org/10.1016/S0168-6445(03)00021-4

Seviour, R. J., & Nielsen, P. H. (2010). *Microbial Ecology of Activated Sludge* (R. J. Seviour & P. H. Nielsen (Eds.)). IWA Publishing.

Shade, A., & Teal, T. K. (2015). Computing workflows for biologists: a roadmap. *PLOS Biology*, *13*(11), 1–10. https://doi.org/10.1371/journal.pbio.1002303

Shan, B., Li, J., Zhang, W., Di, Z., & Jin, X. (2016). Characteristics of phosphorus components in the sediments of main rivers into the Bohai Sea. *Ecological Engineering*, *97*, 426–433. https://doi.org/10.1016/j.ecoleng.2016.10.042

Sharpley, A., Jarvie, H. P., Buda, A., May, L., Spears, B., & Kleinman, P. (2013). Phosphorus legacy: overcome the effects of past management practices to mitigate future water quality impairment. *Journal of Environmental Quality*, *42*(5), 1308–1326. https://doi.org/10.2134/jeq2013.03.0098

Sharpley, A. N., & Menzel, R. G. (1987). The impact of soil and fertilizer phosphorus on the environment. *Advances in Agronomy*, *41*, 297–324.

Sharrer, K. L., Christianson, L. E., Lepine, C., & Summerfelt, S. T. (2016). Modeling and mitigation of denitrification “woodchip” bioreactor phosphorus releases during treatment of aquaculture wastewater. *Ecological Engineering*, *93*, 135–143. https://doi.org/10.1016/j.ecoleng.2016.05.019

Sherson, L. R., Van Horn, D. J., Gomez-Velez, J. D., Crossey, L. J., & Dahm, C. N. (2015). Nutrient dynamics in an alpine headwater stream: use of continuous water quality sensors to examine responses to wildfire and precipitation events. *Hydrological Processes*, *29*(14), 3193–3207. https://doi.org/10.1002/hyp.10426

Sicko-Goad, L., & Lazinsky, D. (1986). Quantitative ultrastructural changes associated with lead-coupled luxury phosphate uptake and polyphosphate utilization. *Archives of Environmental Contamination and Toxicology*, *15*(6), 617–627. https://doi.org/10.1007/BF01054908

Siebers, N., Hofmann, D., Schiedung, H., Landsrath, A., Ackermann, B., Gao, L., Mojzeš, P., Jablonowski, N. D., Nedbal, L., & Amelung, W. (2019). Towards phosphorus recycling for agriculture by algae: Soil incubation and rhizotron studies using 33P-labeled microalgal biomass. *Algal Research*, *43*, 101634. https://doi.org/10.1016/j.algal.2019.101634

Silver, W. L., Lugo, A. E., & Keller, M. (1999). Soil oxygen availability and biogeochemical cycling along elevation and topographic gradients in Puerto Rico. *Biogeochemistry*, *44*, 301–328.

Skennerton, C. T., Barr, J. J., Slater, F. R., Bond, P. L., & Tyson, G. W. (2014). Expanding our view of genomic diversity in Candidatus Accumulibacter clades. *Environmental Microbiology*, *17*(5), 1574–1585. https://doi.org/10.1111/1462-2920.12582

Smith, D. R., King, K. W., Johnson, L., Francesconi, W., Richards, P., Baker, D., & Sharpley, A. N. (2015). aSurface runoff and tile drainage transport of phosphorus in the midwestern United States. *Journal of Environmental Quality*, *44*(2), 495–502. https://doi.org/10.2134/jeq2014.04.0176

Smith, D. R., King, K. W., & Williams, M. R. (2015). bWhat is causing the harmful algal blooms in Lake Erie? *Journal of Soil and Water Conservation*, *70*(2), 27A-29A. https://doi.org/10.2489/jswc.70.2.27A

Smith, M. S., & Tiedje, J. M. (1979). Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry*, *11*(3), 261–267. https://doi.org/10.1016/0038-0717(79)90071-3

Stevenson, J. R., & Stoermer, E. F. (1982). Luxury Consumption of Phosphorus by Five Cladophora Epiphytes in Lake Huron. *Transactions of the American Microscopical Society*, *101*(2), 151–161.

Sulu-Gambari, F., Seitaj, D., Meysman, F. J. R., Schauer, R., Polerecky, L., & Slomp, C. P. (2016). Cable bacteria control iron-phosphorus dynamics in sediments of a coastal hypoxic basin. *Environmental Science and Technology*, *50*(3), 1227–1233. https://doi.org/10.1021/acs.est.5b04369

Tapia-Torres, Y., Rodríguez-Torres, M. D., Elser, J. J., Islas, A., Souza, V., García-Oliva, F., & Olmedo-Álvarez, G. (2016). How to live with phosphorus scarcity in soil and sediment: lessons from bacteria. *Applied and Environmental Microbiology*, *82*(15), 4652–4662. https://doi.org/10.1128/AEM.00160-16

Taylor, S. (2016). *Polyphosphate accumulation in stream biofilms evaluated among sites of various land use using an in-situ enrichment experiement in Pennsylvania, USA*. Central Michigan University.

Temperton, B., Gilbert, J. a, Quinn, J. P., & McGrath, J. W. (2011). Novel analysis of oceanic surface water metagenomes suggests importance of polyphosphate metabolism in oligotrophic environments. *PLOS One*, *6*(1), 1–14. https://doi.org/10.1371/journal.pone.0016499

Todd-Brown, K. E. O., Hopkins, F. M., Kivlin, S. N., Talbot, J. M., & Allison, S. D. (2012). A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry*, *109*(1–3), 19–33. https://doi.org/10.1007/s10533-011-9635-6

Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., Detter, J. C., Bork, P., Hugenholtz, P., & Rubin, E. M. (2005). Comparative Metagenomics of Freshwater Microbial Communities. *Science*, *308*(5721), 554–557. https://doi.org/10.1126/science.1107851

Turner, B. L., & Haygarth, P. M. (2001). Phosphorus solubilization in rewetted soils. *Nature*, *411*, 258.

Uhlmann, D., & Bauer, H.-D. (1988). A remark on microorganisms in lake sediments with emphasis on polyphosphate-accumulating bacteria. *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie*, *73*(6), 703–708.

United States Global Change Research Porgram (USGCRP). 2018. *Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Volume II.* [Reidmiller, D.R., C.W. Avery, D.R. Easterling, K.E. Kunkel, K.L.M. Lewis, T.K. Maycock, and B.C. Stewart (eds.)]. U.S. Global Change Research Program, Washington, DC, USA, 1515 pp. doi: 10.7930/NCA4.2018

Valdivia, M. V. S. (2009). *The role of microbial processes in soil phosphorus dynamics* (Issue May). Cornell University.

Vidon, P., Allan, C., Burns, D., Duval, T. P., Gurwick, N., Inamdar, S., Lowrance, R., Okay, J., Scott, D., & Sebestyen, S. (2010). Hot spots and hot moments in riparian zones: potential for improved water quality management. *Journal of the American Water Resources Association*, *46*(2), 278–298. https://doi.org/10.1111/j.1752-1688.2010.00420.x

Wallenstein, M. D., & Hall, E. K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry*, *109*(1–3), 35–47. https://doi.org/10.1007/s10533-011-9641-8

Wan, B., Huang, R., Diaz, J. M., & Tang, Y. (2019). Polyphosphate Adsorption and Hydrolysis on Aluminum Oxides. *Environmental Science and Technology*, *53*, 9542–9552. https://doi.org/10.1021/acs.est.9b01876

Wang, Z., Dunne, A., van Loosdrecht, M. C. M., & Saikaly, P. E. (2018). Effect of salt on the metabolism of “Candidatus Accumulibacter” clade I and II. *Frontiers in Microbiology*, *9*, 479. https://doi.org/10.3389/fmicb.2018.00479

Watson, S. J., Needoba, J. A., & Peterson, T. D. (2019). Widespread detection of Candidatus

Accumulibacter phosphatis, a polyphosphate-accumulating organism, in sediments of the Columbia River estuary. *Environmental Microbiology*, *21*, 1369–1382. https://doi.org/10.1111/1462-2920.14576

Watts, D. B., & Torbert, H. A. (2009). Impact of gypsum applied to grass buffer strips on reducing soluble P in surface water runoff. *Journal of Environmental Quality*, *38*(2000), 1511–1517. https://doi.org/10.2134/jeq2008.0378

Weinke, A. D., & Biddanda, B. A. (2018). From Bacteria to Fish: Ecological Consequences of Seasonal Hypoxia in a Great Lakes Estuary. *Ecosystems*, *21*(3), 426–442. https://doi.org/10.1007/s10021-017-0160-x

Wieder, W. R., Bonan, G. B., & Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change*, *3*(10), 909–912. https://doi.org/10.1038/nclimate1951

Wilfert, P., Kumar, P. S., Korving, L., Witkamp, G. J., & Van Loosdrecht, M. C. M. (2015). The Relevance of Phosphorus and Iron Chemistry to the Recovery of Phosphorus from Wastewater: A Review. *Environmental Science and Technology*, *49*, 9400–9414. https://doi.org/10.1021/acs.est.5b00150

Williams, M. R., & King, K. W. (2020). Changing Rainfall Patterns Over the Western Lake Erie Basin (1975–2017): Effects on Tributary Discharge and Phosphorus Load. *Water Resources Research*, *56*, 1–17. https://doi.org/10.1029/2019WR025985

Williams, M. R., King, K. W., Ford, W., Buda, A. R., & Kennedy, C. D. (2016). Effects of tillage on macropore flow and phosphorus transport to tile drains. *Water Resources Research*, *52*, 2868–2882. https://doi.org/10.1002/2015WR017650.Received

Willis, A. (2016). Extrapolating abundance curves has no predictive power for estimating microbial biodiversity. *Proceedings of the National Academy of Sciences*, *113*(35), E5096–E5096. https://doi.org/10.1073/pnas.1608281113

Willis, A., Bunge, J., & Whitman, T. (2017). Improved detection of changes in species richness in high diversity microbial communities. *Journal of the Royal Statistical Society. Series C: Applied Statistics*, *66*(5), 963–977. https://doi.org/10.1111/rssc.12206

Wilson, G., Bryan, J., Cranston, K., Kitzes, J., Nederbragt, L., & Teal, T. K. (2016). Good Enough Practices in Scientific Computing. *PLoS Computational Biology*, *13*, 1–20. https://doi.org/10.1371/journal.pcbi.1005510

Withers, P. J. A., Sylvester-Bradley, R., Jones, D. L., Healey, J. R., & Talboys, P. J. (2014). Feed the crop not the soil: Rethinking phosphorus management in the food chain. *Environmental Science and Technology*, *48*(12), 6523–6530. https://doi.org/10.1021/es501670j

Wu, S., Vymazal, J., & Brix, H. (2019). Critical review: biogeochemical networking of iron in constructed wetlands for wastewater treatment [Review-article]. *Environmental Science and Technology*, *53*, 7930–7944. https://doi.org/10.1021/acs.est.9b00958

Yall, L., Boughton, B. H., Roinestad, S. A., & Sinclair, N. A. (1972). Logical removal of phosphates. *Logical Removal of Phosphates. Applications of New Concepts of Physical Chemical Waste Water Treatment*.

Ye, Y., Gan, J., & Hu, B. (2015). Screening of Phosphorus-Accumulating Fungi and Their Potential for Phosphorus Removal from Waste Streams. *Applied Biochemistry and Biotechnology*, *177*, 1127–1136. https://doi.org/10.1007/s12010-015-1801-1

Yevdokimov, I., Larionova, A., & Blagodatskaya, E. (2016). Microbial immobilisation of phosphorus in soils exposed to drying-rewetting and freeze-thawing cycles. *Biology and*

Fertility of Soils, *52*(5), 685–696. https://doi.org/10.1007/s00374-016-1112-x

Young, E. O., & Briggs, R. D. (2008). Phosphorus concentrations in soil and subsurface water: a field study among cropland and riparian buffers. *Journal of Environmental Quality*, *37*(1), 69–78. https://doi.org/10.2134/jeq2006.0422

Young, E. O., & Ross, D. S. (2016). Total and labile phosphorus concentrations as influenced by riparian buffer soil properties. *Journal of Environmental Quality*, *45*(1), 294–304. https://doi.org/10.2134/jeq2015.07.0345

Yuan, Z., Pratt, S., & Batstone, D. J. (2012). Phosphorus recovery from wastewater through microbial processes. *Current Opinion in Biotechnology*, *23*(6), 878–883. https://doi.org/10.1016/j.copbio.2012.08.001

Zhang, A. N., Mao, Y., Zhang, T., Oehmen, A., Kong, Y., Nielsen, J. L., Nielsen, P. H., Mielczarek, A. T., Nguyen, H. T., Nielsen, J. L., Nielsen, P. H., Hesselmann, R. P. X., Werlen, C., Hahn, D., Meer, J. R. van der, Zehnder, A. J. B., McMahon, K. D., He, S., Gall, D. L., … Lee, C. (2016). Development of quantitative real-time PCR assays for different clades of "Candidatus Accumulibacter." *Scientific Reports*, *6*, 23993. https://doi.org/10.1038/srep23993

Zhang, F., Blasiak, L. C., Karolin, J. O., Powell, R. J., Geddes, C. D., & Hill, R. T. (2015). Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. *Proceedings of the National Academy of Sciences*, *112*(14), 4381–4386. https://doi.org/10.1073/pnas.1423768112

Zhang, Haiyu, Ishige, K., & Kornberg, A. (2002). A polyphosphate kinase (PPK2) widely conserved in bacteria. *Proceedings of the National Academy of Sciences*, *99*(26), 16678–16683. https://doi.org/10.1073/pnas.262655199

Zhang, Hui, Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y., & Nakamura, K. (2003). Gemmatimonas aurantiaca gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum Gemmatimonadetes phyl. nov. *International Journal of Systematic and Evolutionary Microbiology*, *53*(4), 1155–1163. https://doi.org/10.1099/ijs.0.02520-0

Zhu, R., Wu, M., Zhu, H., Wang, Y., & Yang, J. (2011). Enhanced phosphorus removal by a humus soil cooperated sequencing batch reactor using acetate as carbon source. *Chemical Engineering Journal*, *166*, 687–692. https://doi.org/10.1016/j.cej.2010.11.048

Zimmerman, N., Izard, J., Klatt, C., Zhou, J., & Aronson, E. (2014). The unseen world: environmental microbial sequencing and identification methods for ecologists. *Frontiers in Ecology and the Environment*, *12*(4), 224–231. https://doi.org/10.1890/130055

Supporting Information for

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

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File Contents

This file contains Text S1-S2, Figures S1-S4, Tables S1-S8, References for Supporting Information Text, Tables, and Figures. *Number of Pages:* 34.

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. *Candidatus* Accumulibacter phosphatis Metabolism

The metabolism of model polyphosphate accumulating organisms (PAO), *Candidatus* Accumulibacter phosphatis, in operational EBPR wastewater treatment plants is described as follows. During anaerobic 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 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).

Text S2. Phosphorus Cycling Functional Genes

There are several known functional genes associated with PAO-mediated P uptake and release in EBPR (Table S5). 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 S5). 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 S5). 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 S5). 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.

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 S5. As we mentioned previously for *ppk* and *ppx*, more work needs to be done to characterize the abundance, diversity, and role of these genes in P cycling with respect to engineered systems. While we only focus on P-related genes here, little is known about functional genes regulating other important PAO polymers: PHA and glycogen (Seviour et al., 2003).

FIGURES

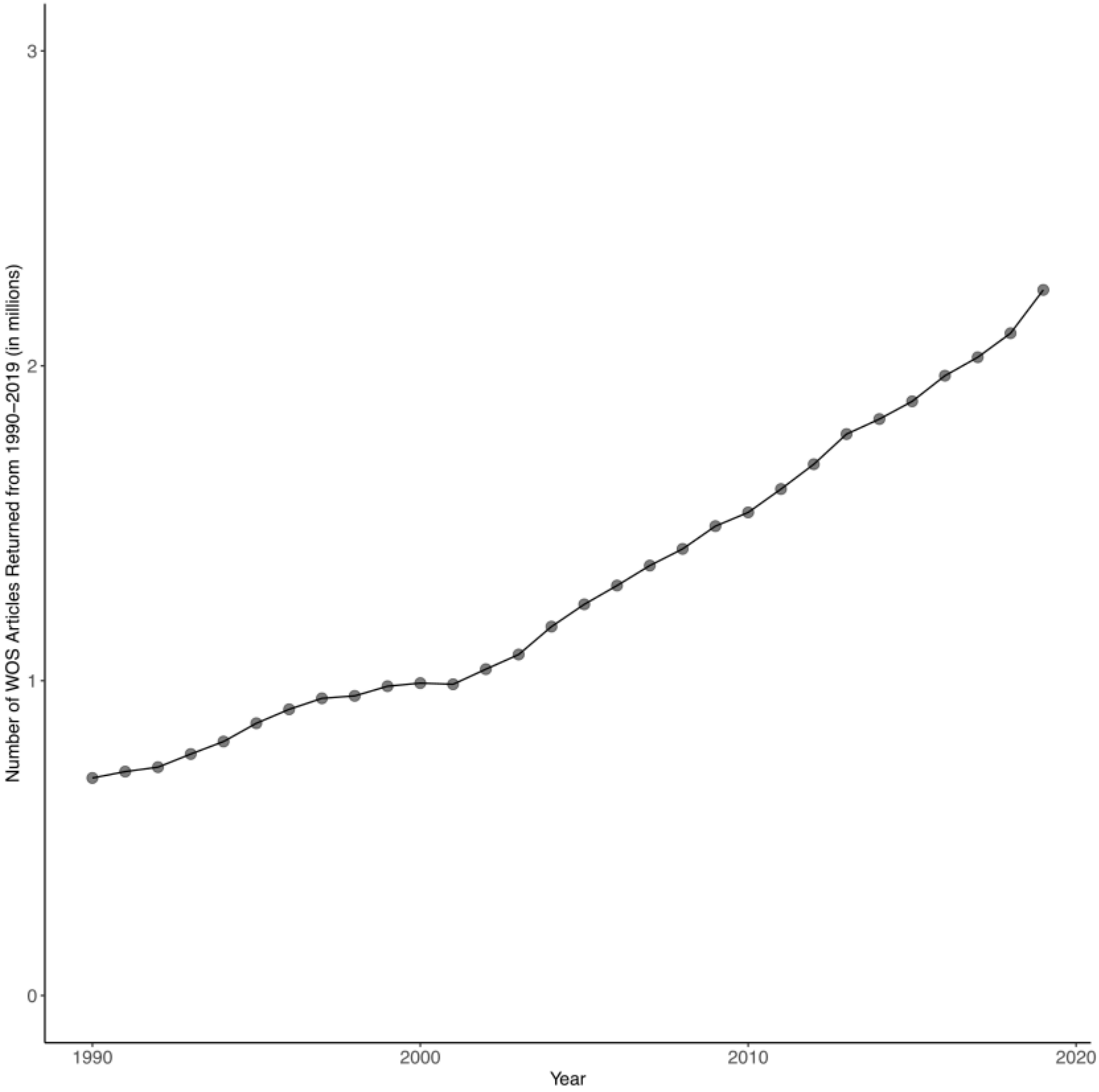

Figure S1. Number of the total number of Web of Science (WOS) articles published per year from 1990 to 2019.

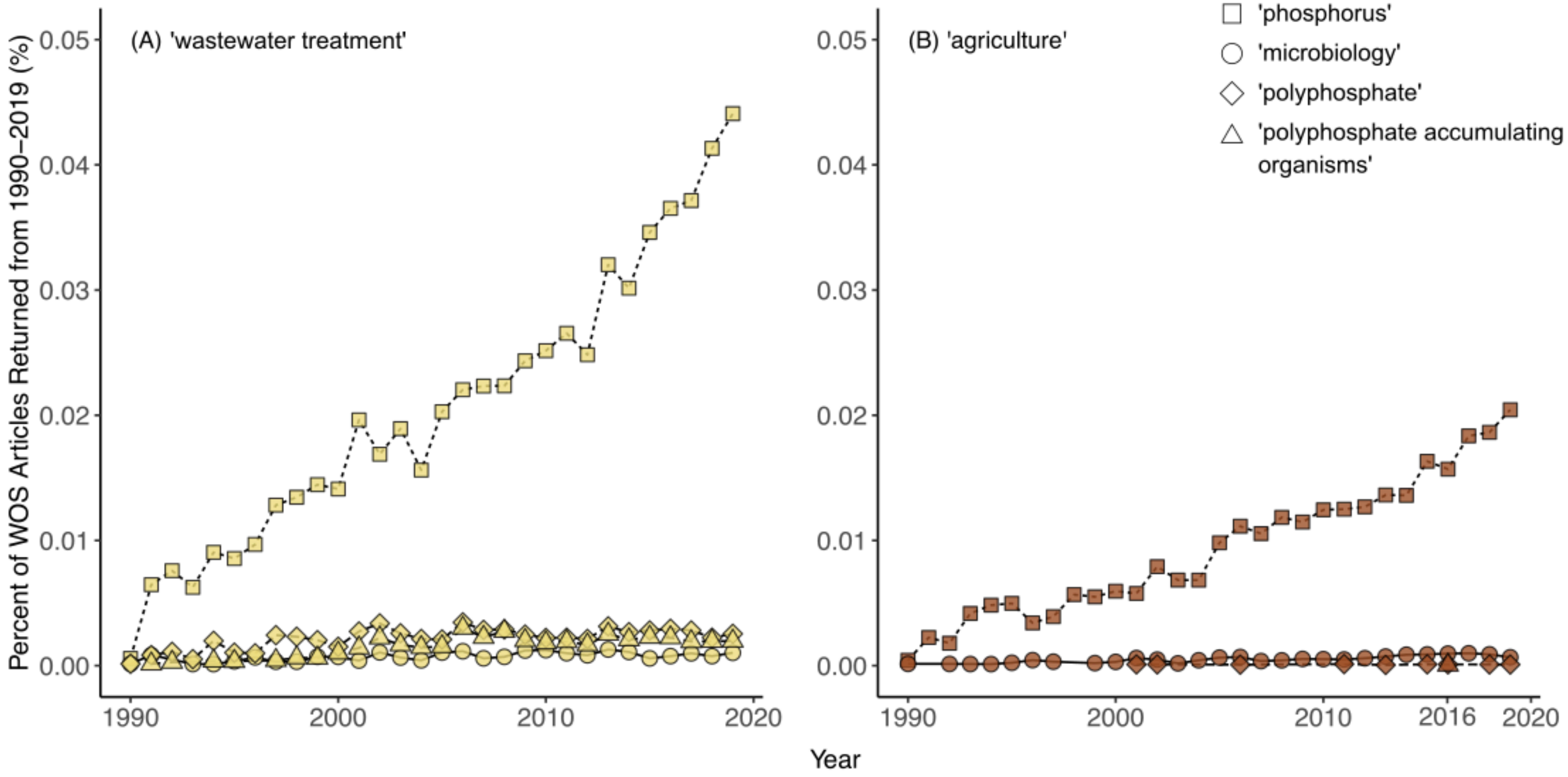

Figure S2. Percent of Web of Science (WOS) articles returned from 1990-2019 for various (A) ‘wastewater treatment’ and (B) ‘agriculture’ keyword searches. For example, the open box line represents the WOS search “ ’wastewater treatment’ AND ‘phosphorus’ ”. Note: There is only one “ ‘agriculture’ AND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

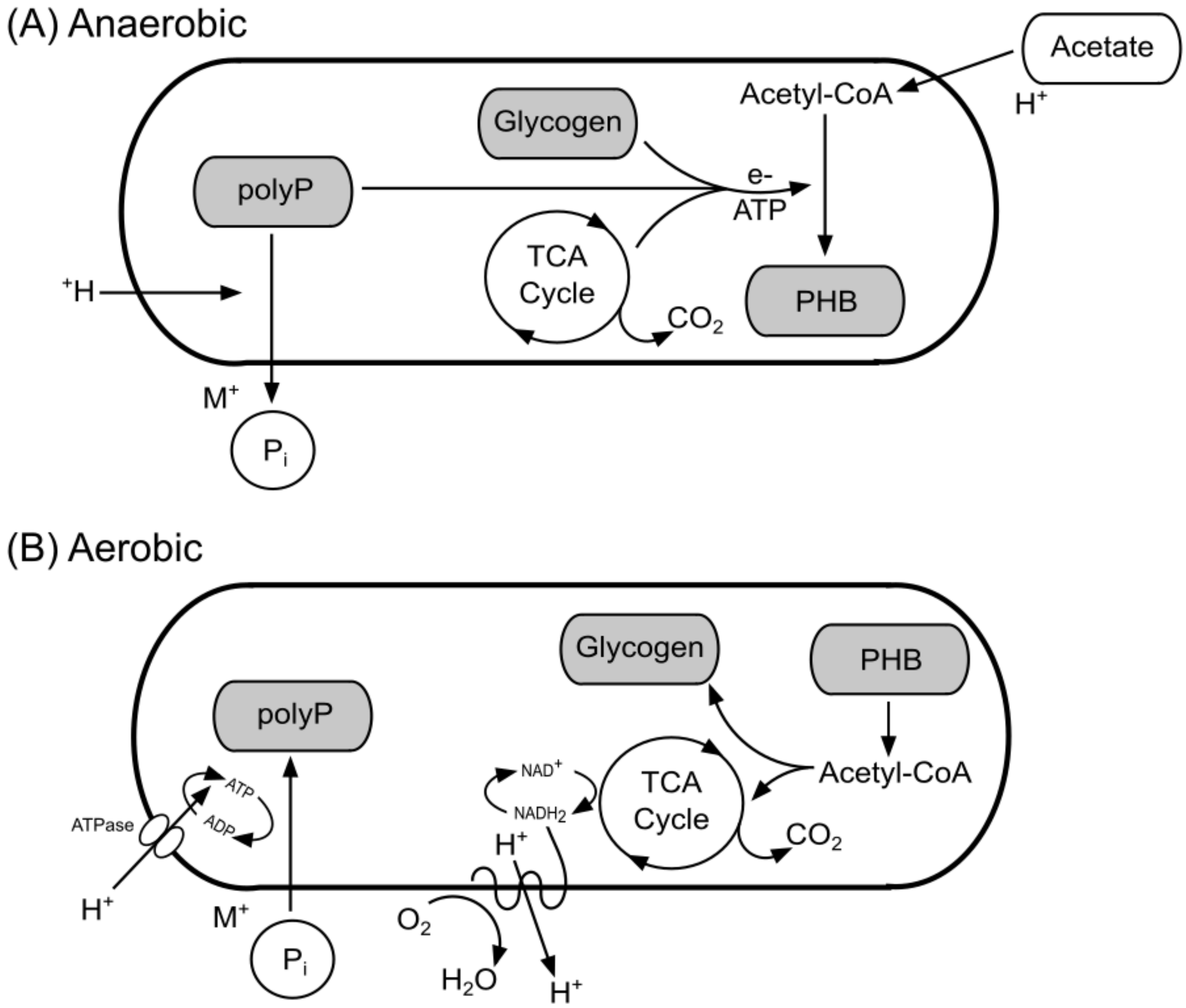

Figure S3. (A) Anaerobic and (B) aerobic metabolism of model polyphosphate accumulating organism (PAO), *Candidatus* Accumulibacter phosphatis. The poly-β-hydroxyalkanoate (PHA) known as poly-β-hydroxybutyrate (PHB) is specific to CAP. Abbreviations: metal cations (M^+), phosphate (P_i). Adapted from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

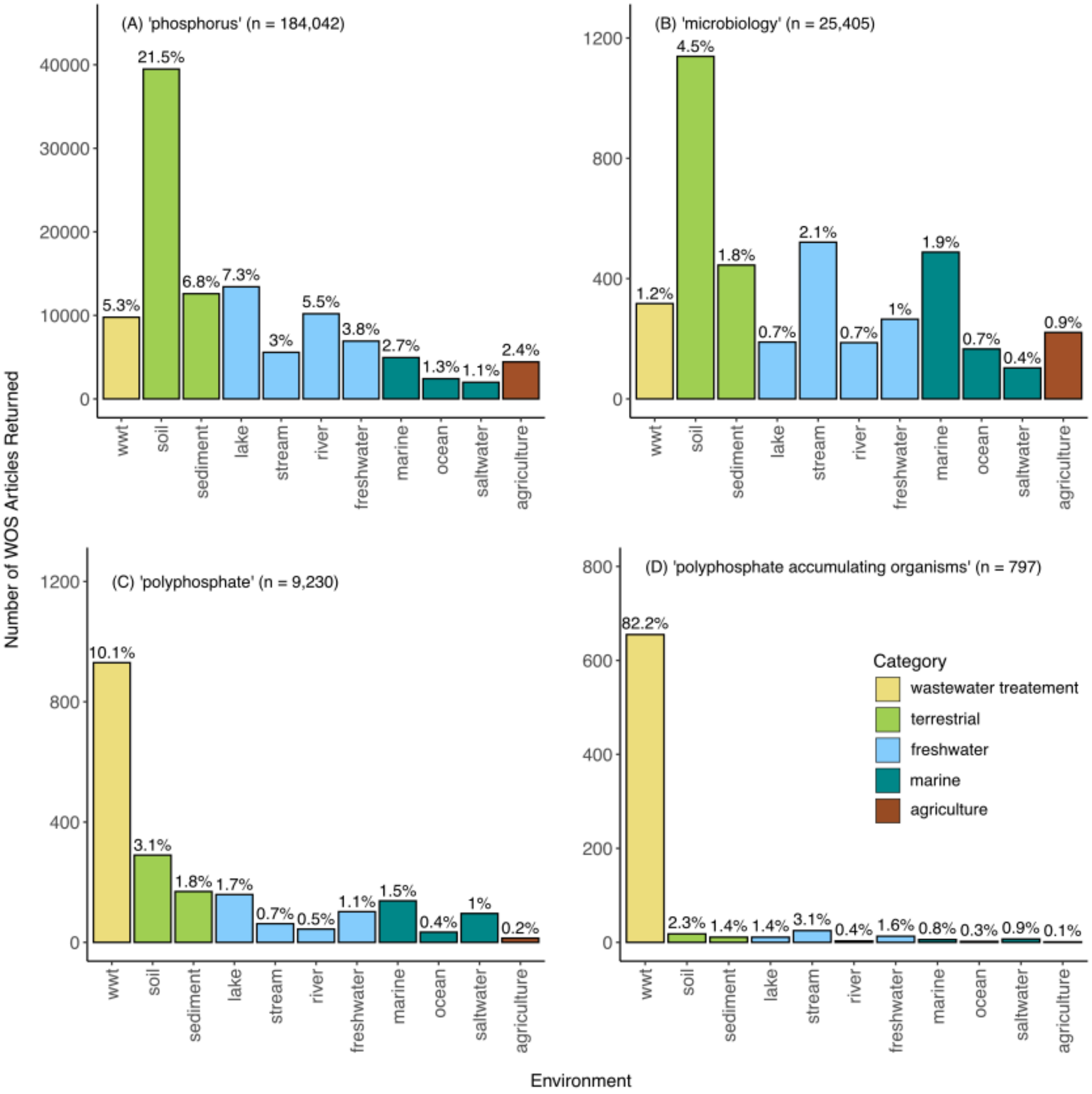

Figure S4. Number and percentage of articles returned from Web of Science (WOS) by environment for (A) ‘phosphorus’, (B) ‘microbiology’, (C) ‘polyphosphate’, and (D) ‘polyphosphate accumulating organisms’ keyword searches. Environments are grouped into five categories: wastewater treatment (wwt), terrestrial, 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.

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. 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., 2017
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 S5. Functional genes and associated proteins related to phosphorus (P) cycling. Abbreviations: polyphosphate (polyP).

Gene	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 S6. 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 S7.	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
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-species (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 S7. 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	

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., 2017
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.

SUPPORTING INFORMATION REFERENCES

Ahn, K., & Kornberg, A. (1990). Polyphosphate kinase from Escherichia coli. Biochemistry, 265(20), 11734–11739.

Akiyama, M., Crooke, E., & Kornberg, A. (1993). An exopolyphosphatase of Escherichia coli. The Journal of Biological Chemistry, 268(1), 633–639.

Akiyama, M., Crookes, E., & Kornberg, A. (1992). The polyphosphate kinase gene of Escherichia coli. The Journal of Biological Chemistry, 267(31), 22556–22561.

Albertsen, M., Hansen, L. B. S., Saunders, A. M., Nielsen, P. H., & Nielsen, K. L. (2012). A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. The ISME Journal, 6(6), 1094–1106. https://doi.org/10.1038/ismej.2011.176

Alcántara, C., Blasco, A., Zúñiga, M., & Monedero, V. (2014). Accumulation of polyphosphate in Lactobacillus spp. and its involvement in stress resistance. Applied and Environmental Microbiology, 80(5), 1650–1659. https://doi.org/10.1128/AEM.03997-13

Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., & Stahl, D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology, 56(6), 1919–1925. https://doi.org/10.1111/j.1469-8137.2004.01066.x

Amann, R. I., Fuchs, B. M., & Behrens, S. (2001). The identification of microorganisms by fluorescence in situ hybridisation. Current Opinion in Biotechnology, 12(3), 231–236.

Archibald, J. (2010). Dissolved phosphorus dynamics in shallow groundwater. Cornell University.

Aschar-Sobbi, R., Abramov, A. Y., Diao, C., Kargacin, M. E., Kargacin, G. J., French, R. J., & Pavlov, E. (2008). High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. Journal of Fluorescence, 18(5), 859–866. https://doi.org/10.1007/s10895-008-0315-4

Auling, G., Pilz, F., Busse, H. J., Karrasch, S., Streichan, M., & Schon, G. (1991). Analysis of the polyphosphate-accumulating microflora in phosphorus- eliminating, anaerobic-aerobic activated sludge systems by using diaminopropane as a biomarker for rapid estimation of Acinetobacter spp. Applied and Environmental Microbiology, 57(12), 3585–3592.

Bark, K., Kampfer, P., Sponner, A., & Dott, W. (1993). Polyphosphate-dependent enzymes in some coryneform bacteria isolated from sewage-sludge. FEMS Microbiology Letters, 107(2–3), 133–138.

Baumhardt, R. L., Stewart, B. A., & Sainju, U. M. (2015). North American Soil Degradation: Processes, Practices, and Mitigating Strategies. Sustainability, 7, 2936–2960. https://doi.org/10.3390/su7032936

Beer, M., Stratton, H. M., Griffiths, P. C., & Seviour, R. J. (2006). Which are the polyphosphate accumulating organisms in full-scale activated sludge enhanced biological phosphate removal systems in Australia? Journal of Applied Microbiology, 100(2), 233–243. https://doi.org/10.1111/j.1365-2672.2005.02784.x

Benitez-Nelson, C. R. (2000). The biogeochemical cycling of phosphorus in marine systems. Earth Science Reviews, 51(1), 109–135. https://doi.org/10.1016/S0012-8252(00)00018-0

Bergström, L., Kirchmann, H., Djodjic, F., Kyllmar, K., Ulén, B., Liu, J., Andersson, H., Aronsson, H., Börjesson, G., Kynkäänniemi, P., Svanbäck, A., & Villa, A. (2015).

Turnover and losses of phosphorus in Swedish agricultural soils: long-term changes, leaching trends, and mitigation measures. Journal of Environmental Quality, 44(2), 512–523. https://doi.org/10.2134/jeq2014.04.0165

Bock, E., Smith, N., Rogers, M., Coleman, B., Reiter, M., Benham, B., & Easton, Z. M. (2015). Enhanced nitrate and phosphate removal in a denitrifying bioreacdtor with biochar. Journal of Environmental Quality, 44(2), 605–613. https://doi.org/10.2134/jeq2014.03.0111

Bourke, D., Kurz, I., Dowding, P., O'Reilly, C., Tunney, H., Doody, D. G., O'Brien, J. E., & Jeffrey, D. W. (2009). Characterisation of organic phosphorus in overland flow from grassland plots using 31P nuclear magnetic resonance spectroscopy. Soil Use and Management, 25(3), 234–242. https://doi.org/10.1111/j.1475-2743.2009.00229.x

Brock, J., & Schulz-Vogt, H. N. (2011). Sulfide induces phosphate release from polyphosphate in cultures of a marine Beggiatoa strain. International Society for Microbial Ecology Journal, 5(3), 497–506. https://doi.org/10.1038/ismej.2010.135

Buda, A. R., Koopmans, G. F., Bryant, R. B., & Chardon, W. J. (2012). Emerging technologies for removing nonpoint phosphorus from surface water and groundwater: introduction. Journal of Environmental Quality, 41(3), 621–627. https://doi.org/10.2134/jeq2012.0080

Cade-Menun, B. J. (2015). Improved peak identification in 31P-NMR spectra of environmental samples with a standardized method and peak library. Geoderma, 257–258, 102–114. https://doi.org/10.1016/j.geoderma.2014.12.016

Cade-Menun, B. J. (2017). Characterizing phosphorus forms in cropland soils with solution 31P-NMR: past studies and future research needs. Chemical and Biological Technologies in Agriculture, 4(1), 1–13. https://doi.org/10.1186/s40538-017-0098-4

Cade-Menun, B. J., Benitez-Nelson, C. R., Pellechia, P., & Paytan, A. (2005). Refining 31P nuclear magnetic resonance spectroscopy for marine particulate samples: storage conditions and extraction recovery. Marine Chemistry, 97(3), 293–306. https://doi.org/10.1016/j.marchem.2005.05.005

Callahan, M. P., Kleinman, P. J. A., Sharpley, A. N., & Stout, W. L. (2002). Assessing the efficacy of alternative phosphorus sorbing soil amendments. Soil Science, 167(8), 539–547. https://doi.org/DOI 10.1097/01.ss.0000026971.27546.b0

Castle, D. and D. L. Kirchman. 2004. Composition of estuarine bacterial communities assessed by denaturing gradient gel electrophoresis and fluorescence in situ hybridization. Limnology and Oceanography: Methods. 2:303-314.

Choi, J., Yang, F., Stepanauskas, R., Cardenas, E., Garoutte, A., Williams, R., Flater, J., Tiedje, J. M., Hofmockel, K. S., Gelder, B., & Howe, A. (2016). Strategies to improve reference databases for soil microbiomes. The ISME Journal, 11, 829–834. https://doi.org/10.1038/ismej.2016.168

Christianson, L. E., Harmel, R. D., Smith, D., Williams, M. R., & King, K. (2016). Assessment and synthesis of 50 years of published drainage phosphorus losses. Journal of Environmental Quality, 45(5), 1467–1477. https://doi.org/10.2134/jeq2015.12.0593

Cober, J. R., Macrae, M. L., & Van Eerd, L. L. (2019). Winter Phosphorus Release from Cover Crops and Linkages with Runoff Chemistry. Journal of Environmental Quality. https://doi.org/10.2134/jeq2018.08.0307

Cohen, M. J., Kurz, M. J., Heffernan, J. B., Martin, J. B., Douglass, R. L., Foster, C. R., & Thomas, R. G. (2013). Diel phosphorus variation and the stoichiometry of ecosystem metabolism in a large spring-fed river. Ecological Monographs, 83(2), 155–176. https://doi.org/10.1890/12-1497.1

Crocetti, G. R., Hugenholtz, P., Bond, P. L., Schuler, A., Keller, J., Jenkins, D., Linda, L., Keller, R. G., & Blackall, L. L. (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. Applied and Environmental Microbiology, 66(3), 1175–1182. https://doi.org/10.1128/AEM.66.3.1175-1182.2000.Updated

Daims, H., Brühl, A., Amann, R., Schleifer, K. H., & Wagner, M. (1999). The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. Systematic and Applied Microbiology, 22(3), 434–444. https://doi.org/10.1016/S0723-2020(99)80053-8

DebRoy, S., Mukherjee, P., Roy, S., Thakur, A. R., & Raychaudhuri, S. (2013). Draft genome sequence of a phosphate-accumulating Bacillus sp .,. Genome Announcements, 1(1), e00251-12. https://doi.org/10.1128/genomeA.00251-12.Copyright

Diaz, J. M., & Ingall, E. D. (2010). Fluorometric quantification of natural inorganic polyphosphate. Environmental Science and Technology, 44(12), 4665–4671. https://doi.org/10.1021/es100191h

Fares, A., Awal, R., & Bayabil, H. (2016). Soil water content sensor response to organic matter content under laboratory conditions. Sensors, 16(8), 1239. https://doi.org/10.3390/s16081239

Flowers, J. J., He, S., Yilmaz, S., Noguera, D. R., & McMahon, K. D. (2009). Denitrification capabilities of two biological phosphorus removal sludges dominated by different “Candidatus Accumulibacter” clades. Environmental Microbiology Reports, 1(6), 583–588. https://doi.org/10.1111/j.1758-2229.2009.00090.x

Gao, D., Huang, X., & Tao, Y. (2016). A critical review of NanoSIMS in analysis of microbial metabolic activities at single-cell level. Critical Reviews in Biotechnology, 36(5), 884–890. https://doi.org/10.3109/07388551.2015.1057550

García Martín, H., Ivanova, N., Kunin, V., Warnecke, F., Barry, K. W., McHardy, A. C., Yeates, C., He, S., Salamov, A. a, Szeto, E., Dalin, E., Putnam, N. H., Shapiro, H. J., Pangilinan, J. L., Rigoutsos, I., Kyrpides, N. C., Blackall, L. L., McMahon, K. D., & Hugenholtz, P. (2006). Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nature Biotechnology, 24(10), 1263–1269. https://doi.org/10.1038/nbt1247

Gassman, P. W., Reyes, M. R., Green, C. H., & Arnold, J. G. (2007). The Soil and Water Assessment Tool: Historical Development, Applications, and Future Research Directions. Transactions of the ASAE, 50(4), 1211–1250. https://doi.org/10.1.1.88.6554

Ge, H., Batstone, D. J., & Keller, J. (2015). Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO clade Comamonadaceae. Water Research, 69, 173–182. https://doi.org/10.1016/j.watres.2014.11.026

George, T. S., Giles, C. D., Menezes-Blackburn, D., Condron, L. M., Gama-Rodrigues, A. C., Jaisi, D., Lang, F., Neal, A. L., Stutter, M. I., Almeida, D. S., Bol, R., Cabugao, K. G., Celi, L., Cotner, J. B., Feng, G., Goll, D. S., Hallama, M., Krueger, J., Plassard, C., … Haygarth, P. M. (2018). Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant and Soil, 427(Issue 1-2), 181–208. https://doi.org/10.1007/s11104-017-3391-x

Good, A. G., & Beatty, P. H. (2011). Fertilizing nature: A tragedy of excess in the commons. PLOS Biology, 9(8), 1–9. https://doi.org/10.1371/journal.pbio.1001124

Gregory, S., Allen, A. W., Baker, M., Boyer, K., Dillaha, T., & Elliot, J. (2007). Realistic expectations of timing between conservation and restoration actions and ecological responses. In Managing agricultural landscapes for environmental quality: strengthening the science base (pp. 111–142). Soil and Water Conservation Society.

Günther, S., Trutnau, M., Kleinsteuber, S., Hause, G., Bley, T., Röske, I., Harms, H., & Müller, S. (2009). Dynamics of polyphosphate-accumulating bacteria in wastewater treatment plant microbial communities detected via DAPI (4’,6’-diamidino-2- phenylindole) and tetracycline labeling. Applied and Environmental Microbiology, 75, 2111–2121. https://doi.org/10.1128/AEM.01540-08

Harold, F. (1964). Enzymic and genetic control of polyphosphate accumulation in Aerobacter aerogenes. Journal of General Microbiology, 35(1964), 81–90. https://doi.org/10.1099/00221287-35-1-81

He, S., & McMahon, K. D. (2011a). bMicrobiology of “Candidatus Accumulibacter” in activated sludge. Microbial Biotechnology, 4(5), 603–619. https://doi.org/10.1111/j.1751-7915.2011.00248.x

He, S., & McMahon, K. D. (2011b). a’Candidatus Accumulibacter’ gene expression in response to dynamic EBPR conditions. The ISME Journal, 5(2), 329–340. https://doi.org/10.1038/ismej.2010.127

He, Shaomei, Bishop, F. I., & McMahon, K. D. (2010). Bacterial community and “Candidatus Accumulibacter” population dynamics in laboratory-scale enhanced biological phosphorus removal reactors. Applied and Environmental Microbiology, 76(16), 5479–5487. https://doi.org/10.1128/AEM.00370-10

He, Shaomei, Gall, D. L., & McMahon, K. D. (2007). “Candidatus Accumulibacter” population structure in enhanced biological phosphorus removal sludges as revealed by polyphosphate kinase genes. Applied and Environmental Microbiology, 73(18), 5865–5874. https://doi.org/10.1128/AEM.01207-07

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Science Society of America Journal, 46(5), 970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Herrmann, A. M., Ritz, K., Nunan, N., Clode, P. L., Pett-Ridge, J., Kilburn, M. R., Murphy, D. V., O’Donnell, A. G., & Stockdale, E. A. (2007). Nano-scale secondary ion mass spectrometry - a new analytical tool in biogeochemistry and soil ecology: a review article. Soil Biology and Biochemistry, 39(8), 1835–1850. https://doi.org/10.1016/j.soilbio.2007.03.011

Hesselmann, R. P., Werlen, C., Hahn, D., van der Meer, J. R., & Zehnder, a J. (1999). Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. Systematic and Applied Microbiology, 22(3), 454–465. https://doi.org/10.1016/S0723-2020(99)80055-1

Hill, C. R., & Robinson, J. S. (2012). Phosphorus flux from wetland ditch sediments. Science of the Total Environment, 437, 315–322. https://doi.org/10.1016/j.scitotenv.2012.06.109

Hoffmann, C. C., Kjaergaard, C., Uusi-Kämppä, J., Hansen, H. C. B., & Kronvang, B. (2009). Phosphorus Retention in Riparian Buffers: Review of Their Efficiency. Journal of Environmental Quality, 38(5), 1942–1955. https://doi.org/10.2134/jeq2008.0087

Hoppe, P., Cohen, S., & Meibom, A. (2013). NanoSIMS: technical aspects and applications in cosmochemistry and biological geochemistry. Geostandards and Geoanalytical Research, 37(2), 111–154. https://doi.org/10.1111/j.1751-908X.2013.00239.x

Howe, A. C., & Chain, P. S. G. (2015). Challenges and opportunities in understanding microbial communities with metagenome assembly (accompanied by IPython Notebook tutorial). Frontiers in Microbiology, 6, 678. https://doi.org/10.3389/fmicb.2015.00678

Howe, A. C., Jansson, J. K., Malfatti, S. A., Tinge, S. G., Tiedje, J. M., & Brown, C. T. (2014). Tackling soil diversity with the assembly of large, complex metagenomes. Proceedings of the National Academy of Sciences, 111(13), 4904–4909. https://doi.org/10.1073/pnas.1405263111

Hung, C., Peccia, J., Zilles, J. L., & Noguera, D. R. (2002). Physical Enrichment Accumulating Activated Polyphosphate in Organisms Sludge of. Water Environment Research, 74(4), 354–361.

Ishige, K., Kameda, A., Noguchi, T., & Shiba, T. (1998). The polyphosphate kinase gene of Pseudomonas aeruginosa. DNA Research, 5(3), 157–162.

Johnson, K. N., Kleinman, P. J. A., Beegle, D. B., Elliott, H. A., & Saporito, L. S. (2011). Effect of dairy manure slurry application in a no-till system on phosphorus runoff. Nutrient Cycling in Agroecosystems, 90(2), 201–212. https://doi.org/10.1007/s10705-011-9422-8

Kanagawa, T., Kamagata, Y., Aruga, S., Kohno, T., Horn, M., & Wagner, M. (2000). Phylogenetic Analysis of and Oligonucleotide Probe Development for Eikelboom Type 021N Filamentous Bacteria Isolated from Bulking Activated Sludge. Applied and Environmental Microbiology, 66(11), 5043–5052. https://doi.org/10.1128/AEM.66.11.5043-5052.2000.Updated

Kaushal, S. S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., Newcomer, T. A., Duan, S., Welty, C., Belt, K. T., Sujay, S., Mayer, P. M., Vidon, P. G., Smith, R. M., Pennino, M. J., New-, T. A., Duan, S., Welty, C., Belt, K. T., Use, L., & Amplify, C. V. (2014). Land Use and Climate Variability Amplify Carbon, Nutrient, and Contaminant Pulses: A Review with Management Implications. Journal of the American Water Resources Association, 50, 585–614. https://doi.org/10.1111/jawr.12204

Kawaharasaki, M., Kanagawa, T., Tanaka, H., & Nakamura, K. (1998). Development and application of 16S rRNA targeted oligonulceotide probe for the detection of the phosphorus-accumulating bacterium Microlunatus phosphovorus in an enhanced biological phosphorus removal process. Water Science and Technology, 37(4–5), 481–484.

Kawakoshi, A., Nakazawa, H., Fukada, J., Sasagawa, M., Katano, Y., Nakamura, S., Hosoyama, A., Sasaki, H., Ichikawa, N., Hanada, S., Kamagata, Y., Nakamura, K., Yamazaki, S., & Fujita, N. (2012). Deciphering the genome of polyphosphate accumulating Actinobacterium Microlunatus phosphovorus. DNA Research, 19(5), 383–394. https://doi.org/10.1093/dnares/dss020

Keasling, J. D., Bertsch, L., & Kornberg, A. (1993). Guanosine pentaphosphate phosphohydrolase of Escherichia coli is a long-chain exopolyphosphatase. Proceedings of the National Academy of Sciences, 90(15), 7029–7033. https://doi.org/10.1073/pnas.90.15.7029

Kim, J. M., Lee, H. J., Kim, S. Y., Song, J. J., Park, W., & Jeon, C. O. (2010). Analysis of the fine-scale population structure of “Candidatus Accumulibacter phosphatis” in enhanced biological phosphorus removal sludge, using fluorescence in situ hybridization and flow cytometric sorting. Applied and Environmental Microbiology, 76(12), 3825–3835. https://doi.org/10.1128/AEM.00260-10

King, K. W., Williams, M. R., Macrae, M. L., Fausey, N. R., Frankenberger, J., Smith, D. R., Kleinman, P. J. A., & Brown, L. C. (2015). Phosphorus transport in agricultural subsurface drainage: a review. Journal of Environmental Quality, 44(2), 467–485. https://doi.org/10.2134/jeq2014.04.0163

Kleinman, P. J. a, Salon, P., Sharpley, a N., & Saporito, L. S. (2005). Effect of cover crops established at time of corn planting on phosphorus runoff from soils before and after dairy manure application. Journal of Soil and Water Conservation, 60(6), 311–322.

Kong, Y., Nielsen, J. L., & Nielsen, P. H. (2004). Microautoradiographic study of Rhodocyclus - related polyphosphate-accumulating bacteria in full-scale enhanced biological phosphorus removal plants. Applied and Environmental Microbiology, 70(9), 5383–5390. https://doi.org/10.1128/AEM.70.9.5383

Kong, Y., Nielsen, J. L., & Nielsen, P. H. (2005). Identity and ecophysiology of uncultured actinobacterial polyphosphate-accumulating organisms in full-scale enhanced biological phosphorus removal plants. Applied and Environmental Microbiology, 71(7), 4076–4085. https://doi.org/10.1128/AEM.71.7.4076-4085.2005

Kong, Y., Xia, Y., Nielsen, J. L., & Nielsen, P. H. (2007). Structure and function of the microbial community in a full-scale enhanced biological phosphorus removal plant. Microbiology, 153(12), 4061–4073. https://doi.org/10.1099/mic.0.2007/007245-0

Kornberg, A. (1995). Inorganic polyphosphate: toward making a forgotten polymer unforgettable. Journal of Bacteriology, 177(3), 491–496. https://doi.org/10.1128/jb.177.3.491-496.1995

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology, 79(17), 5112–5120. https://doi.org/10.1128/AEM.01043-13

Kristiansen, R., Thi, H., Nguyen, T., Saunders, A. M., Lund Nielsen, J., Wimmer, R., Le, V. Q., Mcilroy, S. J., Petrovski, S., Seviour, R. J., Calteau, A., Lehmann Nielsen, K., Nielsen, P. H. H., Nguyen, H. T. T., Saunders, A. M., Nielsen, J. L., Wimmer, R., Le, V. Q., Mcilroy, S. J., … Nielsen, P. H. H. (2013). A metabolic model for members of the genus Tetrasphaera involved in enhanced biological phosphorus removal. The ISME Journal, 7(3), 543–554. https://doi.org/10.1038/ismej.2012.136

Kruse, J., Abraham, M., Amelung, W., Baum, C., Bol, R., Kühn, O., Lewandowski, H., Niederberger, J., Oelmann, Y., Rüger, C., Santner, J., Siebers, M., Siebers, N., Spohn, M., Vestergren, J., Vogts, A., & Leinweber, P. (2015). Innovative methods in soil phosphorus research: a review. Journal of Plant Nutrition and Soil Science, 178(1), 43–88. https://doi.org/10.1002/jpln.201400327

Kumble, K. D., & Kornberg, A. (1996). Endopolyphosphatases for long chain inorganic polyphosphate in yeast and mammals. Journal of Biological Chemistry, 271(43), 27146–27151. https://doi.org/10.1074/jbc.271.43.27146

Kunin, V., He, S., Warnecke, F., Peterson, S. B., Garcia Martin, H., Haynes, M., Ivanova, N., Blackall, L. L., Breitbart, M., Rohwer, F., McMahon, K. D., & Hugenholtz, P. (2008). A bacterial metapopulation adapts locally to phage predation despite global dispersal. Genome Research, 18(2), 293–297. https://doi.org/10.1101/gr.6835308

Lanham, A. B., Oehmen, A., Saunders, A. M., Carvalho, G., Nielsen, P. H., & Reis, M. A. M. (2013). Metabolic versatility in full-scale wastewater treatment plants performing enhanced biological phosphorus removal. Water Research, 47(19), 7032–7041. https://doi.org/10.1016/j.watres.2013.08.042

Li, X., Yuan, H., Yang, J., & Li, B. (2013). Genome sequence of the polyphosphate-accumulating organism Arthrobacter sp. strain PAO19 isolated from maize rhizosphere soil. Genome Announcements, 1(4), 11039–11040. https://doi.org/10.1186/1471-2164-13-534.9.

Lichko, L. P., Kulakovskaya, T. V., & Kulaev, I. S. (2006). Inorganic polyphosphate and exopolyphosphatase in the nuclei of Saccharomyces cerevisiae: dependence on the growth phase and inactivation of the PPX1 and PPN1 genes. Yeast, 23, 735–740. https://doi.org/10.1002/yea

Liu, J., Macrae, M. L., Elliott, J. A., Baulch, H. M., Wilson, H. F., & Kleinman, P. J. A. (2019). Impacts of Cover Crops and Crop Residues on Phosphorus Losses in Cold Climates: A Review. Journal of Environmental Quality, 850–868. https://doi.org/10.2134/jeq2019.03.0119

Liu, W. T., Nielsen, A. T., Wu, J. H., Tsai, C. S., Matsuo, Y., & Molin, S. (2001). In situ identification of polyphosphate- and polyhydroxyalkanoate-accumulating traits for microbial populations in a biological phosphorus removal process. Environmental Microbiology, 3(2), 110–122. https://doi.org/10.1046/j.1462-2920.2001.00164.x

Liu, Y., Engel, B. A., Flanagan, D. C., Gitau, M. W., McMillan, S. K., & Chaubey, I. (2017). A review on effectiveness of best management practices in improving hydrology and water quality: Needs and opportunities. Science of the Total Environment, 601–602, 580–593. https://doi.org/10.1016/j.scitotenv.2017.05.212

Locke, N. (2015). Classification of polyphosphate-accumulating bacteria in benthic biofilms. Pennsylvania State University, State College, Pennsylvania.

Manz, W., Amann, R., Ludwig, W., Wagner, M., & Schleifer, K.-H. (1992). Phylogenetic Oligodeoxynucleotide Probes for the Major Subclasses of Proteobacteria: Problems and Solutions. Systematic and Applied Microbiology, 15(4), 593–600. https://doi.org/http://dx.doi.org/10.1016/S0723-2020(11)80121-9

Mao, Y., Graham, D. W., Tamaki, H., & Zhang, T. (2015). Dominant and novel clades of Candidatus Accumulibacter phosphatis in 18 globally distributed full-scale wastewater treatment plants. Scientific Reports, 5, 11857. https://doi.org/10.1038/srep11857

Mao, Y., Yu, K., Xia, Y., Chao, Y., & Zhang, T. (2014). Genome reconstruction and gene expression of "Candidatus Accumulibacter phosphatis" Clade IB performing biological phosphorus removal. Environmental Science and Technology, 48(17), 10363–10371. https://doi.org/10.1021/es502642b

Margenot, A. J., Nakayama, Y., & Parikh, S. J. (2018). Methodological recommendations for optimizing assays of enzyme activities in soil samples. Soil Biology and Biochemistry, 125, 350–360. https://doi.org/10.1016/j.soilbio.2017.11.006

Martin, P., & Van Mooy, B. A. S. (2013). Fluorometric quantification of polyphosphate in environmental plankton samples: extraction protocols, matrix effects, and nucleic acid interference. Applied and Environmental Microbiology, 79(1), 273–281. https://doi.org/10.1128/AEM.02592-12

Maszenan, A. M., Seviour, R. J., Patel, B. K. C., Schumann, P., Burghardt, J., Tokiwa, Y., & Stratton, H. M. (2000). Three isolates of novel polyphosphate-accumulating Gram-positive cocci, obtained from activated sludge, belong to a new genus, Tetrasphaera gen. nov., and description of two new species, Tetrasphaera japonica sp. nov. and Tetrasphaera australiensis sp. no. International Journal of Systematic and Evolutionary Microbiology, 50(2), 593–603. https://doi.org/10.1099/00207713-50-2-593

McDowell, R. W., Dils, R. M., Collins, A. L., Flahive, K. A., Sharpley, A. N., & Quinn, J. (2016). A review of the policies and implementation of practices to decrease water quality impairment by phosphorus in New Zealand, the UK, and the US. Nutrient Cycling in Agroecosystems, 104, 289–305. https://doi.org/10.1007/s10705-015-9727-0

McDowell, R. W., & Hill, S. J. (2015). Speciation and distribution of organic phosphorus in river sediments: a national survey. Journal of Soils and Sediments, 15(12), 2369–2379. https://doi.org/10.1007/s11368-015-1125-3

Mcdowell, R W, Moreau, P., Salmon-monviola, J., & Durand, P. (2014). bContrasting the spatial management of nitrogen and phosphorus for improved water quality: Modelling studies in New Zealand and France. European Journal of Agronomy, 57, 52–61. https://doi.org/10.1016/j.eja.2013.09.011

Mcdowell, Richard W, & Nash, D. (2012). A Review of the Cost-Effectiveness and Suitability of Mitigation Strategies to Prevent Phosphorus Loss from Dairy Farms in New Zealand and Australia. Journal of Environmental Quality, 41, 680–693. https://doi.org/10.2134/jeq2011.0041

McMahon, K. D., Dojka, M. A., Pace, N. R., Jenkins, D., & Keasling, J. D. (2002). Polyphosphate kinase from activated sludge performing enhanced biological phosphorus removal. Applied and Environmental Microbiology, 68(10), 4971–4978. https://doi.org/10.1128/AEM.68.10.49710

McMahon, K. D., Yilmaz, S., He, S., Gall, D. L., Jenkins, D., & Keasling, J. D. (2007). Polyphosphate kinase genes from full-scale activated sludge plants. Applied Microbiology and Biotechnology, 77(1), 167–173. https://doi.org/10.1007/s00253-007-1122-6

Menzel, P., & Krogh, A. (2016). Kaiju: Fast and sensitive taxonomic classification for metagenomics. Nature Communications, 7, 11257. https://doi.org/10.1101/031229

Morrison, E., Newman, S., Bae, H. S., He, Z., Zhou, J., Reddy, K. R., & Ogram, A. (2016). Microbial genetic and enzymatic responses to an anthropogenic phosphorus gradient within a subtropical peatland. Geoderma, 268, 119–127. https://doi.org/10.1016/j.geoderma.2016.01.008

Mueller, C. W., Weber, P. K., Kilburn, M. R., Hoeschen, C., Kleber, M., & Pett-Ridge, J. (2013). Chapter 1 - Advances in the analysis of biogeochemical interfaces: NanoSIMS to investigate soil microenvironments. Advances in Agronomy, 121, 1–46. https://doi.org/10.1016/B978-0-12-407685-3.00001-3

Nakamura, K., Hiraishi, A., Yoshimi, Y., Kawaharasaki, M., Masuda, K., & Kamagata, Y. (1995). aMicrolunatus phosphovorus gen. nov., sp. nov., a new Gram-positive polyphosphate-accumulating bacterium isolated from activated sludge. International Journal of Systematic Bacteriology, 45(1), 17–22. https://doi.org/10.1099/00207713-45-1-17

Nakamura, K., Ishikawa, S., & Kawaharasaki, M. (1995). bPhosphate uptake and release activity in immobilized polyphosphate-accumulating bacterium Microlunatus phosphovorus strain NM-1. Journal of Fermentation and Bioengineering, 80(4), 377–382. https://doi.org/10.1016/0922-338X(95)94207-8

Nakamura, K., Masuda, K., & Mikami, E. (1991). Isolation of a new type of polyphosphate accumulating bacterium and its phosphate removal characteristics. Journal of Fermentation and Bioengineering, 71(4), 258–263. https://doi.org/10.1016/0922-338X(91)90278-O

Nguyen, H. T. T., Le, V. Q., Hansen, A. A., Nielsen, J. L., & Nielsen, P. H. (2011). High diversity and abundance of putative polyphosphate-accumulating Tetrasphaera-related bacteria in activated sludge systems. FEMS Microbiology Ecology, 76(2), 256–267. https://doi.org/10.1111/j.1574-6941.2011.01049.x

Nguyen, H. T. T., Nielsen, J. L., & Nielsen, P. H. (2012). “Candidatus Halomonas phosphatis”, a novel polyphosphate-accumulating organism in full-scale enhanced biological phosphorus removal plants. Environmental Microbiology, 14(10), 2826–2837. https://doi.org/10.1111/j.1462-2920.2012.02826.x

Nobu, M. K., Tamaki, H., Kubota, K., & Liu, W. T. (2014). Metagenomic characterization of “Candidatus Defluviicoccus tetraformis strain TFO71”, a tetrad-forming organism, predominant in an anaerobic-aerobic membrane bioreactor with deteriorated biological phosphorus removal. Environmental Microbiology, 16(9), 2739–2751. https://doi.org/10.1111/1462-2920.12383

Nocek, B., Kochinyan, S., Proudfoot, M., Brown, G., Evdokimova, E., Osipiuk, J., Edwards, A. M., Savchenko, A., Joachimiak, A., & Yakunin, A. F. (2008). Polyphosphate-dependent synthesis of ATP and ADP by the family-2 polyphosphate kinases in bacteria. Proceedings of the National Academy of Sciences, 105(46), 17730–17735. https://doi.org/10.1073/pnas.0807563105

Ohtake, H., Kuroda, A., Chandrasekaran, M., Wu, H., & Tanaka, S. (2001). Molecular genetics of bacterial polyphosphate accumulation to better understand the mechanism underlying biological phosphorus removal. In M. Healy, D. L. Wise, & M. Moo-Young (Eds.), Environmental Monitoring and Biodiagnostics of Hazardous Contaminants (pp. 181–196). Kluvwer Academic Publishers.

Ota, S., Yoshihara, M., Yamazaki, T., Takeshita, T., & Hirata, A. (2016). Deciphering the relationship among phosphate dynamics , electron-dense body and lipid accumulation in the green alga Parachlorella kessleri. Scientific Reports, 6, 25731. https://doi.org/10.1038/srep25731

Oyserman, B. O., Martirano, J. M., Wipperfurth, S., Owen, B. R., Noguera, D. R., & McMahon, K. D. (2017). Community Assembly and Ecology of Activated Sludge under Photosynthetic Feast–Famine Conditions. Environmental Science & Technology, 51(6), 3165–3175. https://doi.org/10.1021/acs.est.6b03976

Pellerin, B. A., Stauffer, B. A., Young, D. A., Sullivan, D. J., Bricker, S. B., Walbridge, M. R., Clyde, G. A., & Shaw, D. M. (2016). Emerging tools for continuous nutrient monitoring networks: sensors advancing science and water resources protection. Journal of the American Water Resources Association, 52(4), 993–1008. https://doi.org/10.1111/1752-1688.12386

Peng, X., Chen, L., Lv, Z., Hao, G., & Fang, H. (2010). Communications in Soil Science and Plant Analysis Characterization of Phosphorus in Sewage Sludge from Different Sources by Phosphorus Nuclear Magnetic Resonance Spectroscopy Characterization of Phosphorus in Sewage Sludge from Different Sources by 31 Pho. Communications in Soil Science and Plant Analysis, 3624, 1237–1244. https://doi.org/10.1080/00103621003721429

Peterson, S. B., Warnecke, F., Madejska, J., Mcmahon, K. D., & Hugenholtz, P. (2008). Environmental distribution and population biology of Candidatus Accumulibacter, a primary agent of biological phosphorus removal. Environmental Microbiology, 10(10), 2692–2703. https://doi.org/10.1111/j.1462-2920.2008.01690.x

Ragot, S. A., Kertesz, M. A., & Bünemann, E. K. (2015). phoD alkaline phosphatase gene diversity in soil. Applied and Environmental Microbiology, 81(20), 7281–7289. https://doi.org/10.1128/AEM.01823-15

Ragot, S. A., Kertesz, M. A., Mészáros, É., Frossard, E., & Bünemann, E. K. (2016). Soil phoD and phoX alkaline phosphatase gene diversity responds to multiple environmental factors. FEMS Microbiology Ecology, 93(1), 1–15. https://doi.org/10.1093/femsec/fiw212

Rangarajan, E. S., Nadeau, G., Li, Y., Wagner, J., Hung, M. N., Schrag, J. D., Cygler, M., & Matte, A. (2006). The Structure of the exopolyphosphatase (PPX) from Escherichia coli O157:H7 suggests a binding mode for long polyphosphate chains. Journal of Molecular Biology, 359(5), 1249–1260. https://doi.org/10.1016/j.jmb.2006.04.031

Rao, N. N., Gómez-García, M. R., & Kornberg, A. (2009). Inorganic polyphosphate: essential for growth and survival. Annual Review of Biochemistry, 78, 605–647. https://doi.org/10.1146/annurev.biochem.77.083007.093039

Read, E. K., Ivancic, M., Hanson, P., Cade-Menun, B. J., & McMahon, K. D. (2014). Phosphorus speciation in a eutrophic lake by 31P NMR spectroscopy. Water Research, 62, 229–240. https://doi.org/10.1016/j.watres.2014.06.005

Reizer, J., Reizer, A., & Saier, M. H. (1993). Exopolyphosphatase and guanine pentaphosphate phosphatase belong to the sugar kinase/actin/hsp70 superfamily. Trends in Biochemical Sciences, 18, 247–248.

Rier, S. T., Kinek, K. C., Hay, S. E., & Francoeur, S. N. (2016). Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. Freshwater Science, 35(2), 490–502. https://doi.org/10.1086/685859.

Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: genomic analysis of microbial communities. Annual Reviews of Genetics, 38, 525–552. https://doi.org/10.1146/annurev.genet.38.072902.091216

Rittenburg, R. A., Squires, A. L., Boll, J., Brooks, E. S., Easton, Z. M., & Steenhuis, T. S. (2015). Agricultural BMP effectiveness and dominant hydrological flow paths: concepts and a review. Journal of the American Water Resources Association, 51(2), 305–329. https://doi.org/10.1111/1752-1688.12293

Rode, M., Wade, A. J., Cohen, M. J., Hensley, R. T., Bowes, M. J., Kirchner, J. W., Arhonditsis, G. B., Jordan, P., Kronvang, B., Halliday, S. J., Skeffington, R. A., Rozemeijer, J. C., Aubert, A. H., Rinke, K., & Jomaa, S. (2016). Sensors in the stream: the high-frequency wave of the present. Environmental Science and Technology, 50(19), 10297–10307. https://doi.org/10.1021/acs.est.6b02155

Rubio-Rincón, F. J., Welles, L., Lopez-Vazquez, C. M., Nierychlo, M., Abbas, B., Geleijnse, M., Nielsen, P. H., van Loosdrecht, M. C. M., & Brdjanovic, D. (2017). Long-term effects of sulphide on the enhanced biological removal of phosphorus: The symbiotic role of Thiothrix caldifontis. Water Research, 116, 53–64. https://doi.org/10.1016/j.watres.2017.03.017

Saif, S. R. (1981). The Influenc of Soil Aeration on the Efficiency of Vasicular-arbuscular Mycorrhizae. The New Phytologist, 88, 649–659.

Schachtman, D. P., Reid, R. J., Ayling, S. M., S, D. B. D. P., & A, S. S. S. M. (1998). Update on Phosphorus Uptake Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiology, 116, 447–453.

Schulz, H. N., & Schulz, H. D. (2005). Large sulfur bacteria and the formation of phosphorite. Science, 307, 416–418. https://doi.org/10.1126/science.1103096

Seviour, R. J., Mino, T., & Onuki, M. (2003). The Microbiology of Biological Phosphorus Removal in Activated Sludge Systems. FEMS Microbiology Reviews, 27(1), 99–127. https://doi.org/10.1016/S0168-6445(03)00021-4

Seviour, R. J., & Nielsen, P. H. (2010). Microbial Ecology of Activated Sludge (R. J. Seviour & P. H. Nielsen (eds.)). IWA Publishing.

Sharpley, A.N., W.W. Troeger, and S.J. Smith, 1991. The Measure- ment of Bioavailable Phosphorus in Agricultural Runoff. Jour- nal of Environmental Quality 20:235-238.

Sharpley, A. N., Kleinman, P. J. A., Flaten, D. N., & Buda, A. R. (2011). Critical source area management of agricultural phosphorus: experiences, challenges and opportunities. Water Science and Technology, 64(4), 945–952. https://doi.org/10.2166/wst.2011.712

Sharrer, K. L., Christianson, L. E., Lepine, C., & Summerfelt, S. T. (2016). Modeling and mitigation of denitrification “woodchip” bioreactor phosphorus releases during treatment of aquaculture wastewater. Ecological Engineering, 93, 135–143. https://doi.org/10.1016/j.ecoleng.2016.05.019

Shi, X., & Kornberg, A. (2005). Endopolyphosphatase in Saccharomyces cerevisiae undergoes post-translational activations to produce short-chain polyphosphates. FEBS Letters, 579(9), 2014–2018. https://doi.org/10.1016/j.febslet.2005.02.032

Sicko-Goad, L., & Lazinsky, D. (1986). Quantitative ultrastructural changes associated with lead-coupled luxury phosphate uptake and polyphosphate utilization. Archives of Environmental Contamination and Toxicology, 15(6), 617–627. https://doi.org/10.1007/BF01054908

Skennerton, C. T., Barr, J. J., Slater, F. R., Bond, P. L., & Tyson, G. W. (2014). Expanding our view of genomic diversity in Candidatus Accumulibacter clades. Environmental Microbiology, 17(5), 1574–1585. https://doi.org/10.1111/1462-2920.12582

Soo, R. M., Skennerton, C. T., Sekiguchi, Y., Imelfort, M., Paech, S. J., Dennis, P. G., Steen, J. A., Parks, D. H., Tyson, G. W., & Hugenholtz, P. (2014). An expanded genomic representation of the phylum Cyanobacteria. Genome Biology and Evolution, 6(5), 1031–1045. https://doi.org/10.1093/gbe/evu073

Stante, L., Cellamare, C. M., Malaspina, F., Bortone, G., & Tilche, A. (1997). Biological phosphorus removal by pure culture of Lampropedia spp. Water Research, 31(6), 1317–1324. https://doi.org/10.1016/S0043-1354(96)00351-X

Stevenson, J. R., & Stoermer, E. F. (1982). Luxury Consumption of Phosphorus by Five Cladophora Epiphytes in Lake Huron. Transactions of the American Microscopical Society, 101(2), 151–161.

Streichan, M., Golicki, J. R., & Schön, G. (1990). Polyphosphate-accumulating bacteria from sewage plants with different processes for biological phosphorus removal. FEMS Microbiology Ecology, 73, 113–124.

Sulu-Gambari, F., Seitaj, D., Meysman, F. J. R., Schauer, R., Polerecky, L., & Slomp, C. P. (2016). Cable bacteria control iron-phosphorus dynamics in sediments of a coastal hypoxic basin. Environmental Science and Technology, 50(3), 1227–1233. https://doi.org/10.1021/acs.est.5b04369

Temperton, B., Gilbert, J. a, Quinn, J. P., & McGrath, J. W. (2011). Novel analysis of oceanic surface water metagenomes suggests importance of polyphosphate metabolism in oligotrophic environments. PLOS One, 6(1), 1–14. https://doi.org/10.1371/journal.pone.0016499

Tomer, M. D., & Locke, M. A. (2011). The challenge of documenting water quality benefits of conservation practices: a review of USDA-ARS's conservation effects assessment project watershed studies. Water Science & Technology, 64(1), 300–310. https://doi.org/10.2166/wst.2011.555

Trelstad, P. L., Purdhani, P., Geißdörfer, W., Hillen, W., & Keasling, J. D. (1999). Polyphosphate kinase of Acinetobacter sp. strain ADP1: Purification and characterization of the enzyme and its role during changes in extracellular phosphate levels. Applied and Environmental Microbiology, 65(9), 3780–3786.

Uhlmann, D., & Bauer, H.-D. (1988). A remark on microorganisms in lake sediments with emphasis on polyphosphate-accumulating bacteria. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie, 73(6), 703–708.

USEPA (US Environmental Protection Agency). 1978. Method 365.3. Phosphorous, all forms (colorimetric, ascorbic acid, two reagent). US Environmental Protection Agency, Washington, DC.

Valdivia, M. V. S. (2009). The role of microbial processes in soil phosphorus dynamics (Issue May). Cornell University.

Von Sperber, C., Kries, H., Tamburini, F., Bernasconi, S. M., & Frossard, E. (2014). The effect of phosphomonoesterases on the oxygen isotope composition of phosphate. Geochimica et Cosmochimica Acta, 125, 519–527. https://doi.org/10.1016/j.gca.2013.10.010

Wagner, M., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wedi, D., & Schleifer, K. H. (1994). Development of an rRNA-targeted oligonucleotide probe specific for the genus Acinetobacter and its application for in situ monitoring in activated sludge. Applied and Environmental Microbiology, 60(3), 792–800.

Wang, Q., Fish, J. A., Gilman, M., Sun, Y., Brown, C. T., Tiedje, J. M., & Cole, J. R. (2015). Xander: employing a novel method for efficient gene-targeted metagenomic assembly. Microbiome, 3, 32. https://doi.org/10.1186/s40168-015-0093-6

Watson, S. J., Needoba, J. A., & Peterson, T. D. (2019). Widespread detection of Candidatus Accumulibacter phosphatis, a polyphosphate-accumulating organism, in sediments of the Columbia River estuary. Environmental Microbiology, 21, 1369–1382. https://doi.org/10.1111/1462-2920.14576

Williams, M. R., King, K. W., Ford, W., Buda, A. R., & Kennedy, C. D. (2016). Effects of tillage on macropore flow and phosphorus transport to tile drains. Water Resources Research, 52, 2868–2882. https://doi.org/10.1002/2015WR017650.Received

Wurst, H., & Kornberg, A. (1994). A soluble exopolyphosphatase of Saccharomyces cerevisiae. Journal of Biological Chemistry, 269(15), 10996–11001.

Zago, A., Chugani, S., & Chakrabarty, A. M. (1999). Cloning and characterization of polyphosphate kinase and exopolyphosphatase genes from Pseudomonas aeruginosa 8830. Applied and Environmental Microbiology, 65(5), 2065–2071.

Zeckoski, R. W., Smolen, M. D., Moriasi, D. N., Frankenberger, J. R., & Feyereisen, G. W. (2015). Hydrologic and Water Quality Terminology as Applied to Modeling. Transactions of the ASABE, 58(6), 1619–1635. https://doi.org/10.13031/trans.58.10713

Zhang, A. N., Mao, Y., Zhang, T., Oehmen, A., Kong, Y., Nielsen, J. L., Nielsen, P. H., Mielczarek, A. T., Nguyen, H. T., Nielsen, J. L., Nielsen, P. H., Hesselmann, R. P. X., Werlen, C., Hahn, D., Meer, J. R. van der, Zehnder, A. J. B., McMahon, K. D., He, S., Gall, D. L., … Lee, C. (2016). Development of quantitative real-time PCR assays for different clades of "Candidatus Accumulibacter." Scientific Reports, 6, 23993. https://doi.org/10.1038/srep23993

Zhang, F., Blasiak, L. C., Karolin, J. O., Powell, R. J., Geddes, C. D., & Hill, R. T. (2015). Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. Proceedings of the National Academy of Sciences, 112(14), 4381–4386. https://doi.org/10.1073/pnas.1423768112

Zhang, Haiyu, Ishige, K., & Kornberg, A. (2002). A polyphosphate kinase (PPK2) widely conserved in bacteria. Proceedings of the National Academy of Sciences, 99(26), 16678–16683. https://doi.org/10.1073/pnas.262655199

Zhang, Hui, Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y., & Nakamura, K. (2003). Gemmatimonas aurantiaca gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum Gemmatimonadetes phyl. nov. International Journal of Systematic and Evolutionary Microbiology, 53(4), 1155–1163. https://doi.org/10.1099/ijs.0.02520-0

Zhu, Y., Huang, W., Lee, S. S. K., & Xu, W. (2005). Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis. EMBO Reports, 6(7), 681–687. https://doi.org/10.1038/sj.embor.7400448

Zilles, J. L., Hung, C. H., & Noguera, D. R. (2002). aPresence of Rhodocyclus in a full-scale wastewater treatment plant and their participation in enhanced biological phosphorus removal. Water Science and Technology, 46(1–2), 123–128. http://www.ncbi.nlm.nih.gov/pubmed/12216613

Zilles, J. L., Peccia, J., Kim, M., Hung, C., & Noguera, D. R. (2002). bInvolvement of Rhodocyclus -related organisms in phosphorus removal in full-scale wastewater treatment plants. Applied and Environmental Microbiology, 68(6), 2763–2769. https://doi.org/10.1128/AEM.68.6.2763

Zimmerman, N., Izard, J., Klatt, C., Zhou, J., & Aronson, E. (2014). The unseen world: environmental microbial sequencing and identification methods for ecologists. Frontiers in Ecology and the Environment, 12(4), 224–231. https://doi.org/10.1890/130055