The Hidden Impacts of Phosphorus Pollution to Streams and Rivers

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Phosphorus (P) enrichment to streams, lakes, and estuaries is increasing throughout the United States. P loading is typically viewed from a harmful algal bloom perspective; if added P causes excess growths of phytoplankton or macroalgae, it may become targeted for control. However, P loading also contributes to two other non–algae-based aquatic problems. Field and experimental evidence shows that P loading directly stimulates growth of aquatic bacteria, which can increase to concentrations that exert a significant biochemical oxygen demand on water bodies, contributing to hypoxia, a widespread impairment. Experimental evidence also demonstrates that fecal bacterial growth can be significantly stimulated by P loading, increasing health risks through exposure or the consumption of contaminated shellfish and causing economic losses from beach and shellfish area closures. Resource managers need to look beyond algal bloom stimulation and should consider the broader roles that excess P loading can have on ecosystem function and microbiological safety for humans.

Keywords: phosphorus, bacteria, hypoxia, pathogens, nutrient management

Phosphorus is an essential macronutrient for biota, but it is also a common pollutant because, in many regions of the world, it is delivered in excess to receiving surface waters (Caraco 1993, Wetzel 2001). Phosphorus (P) is sourced naturally from rock via weathering but, to a far greater degree since the mid-1800s, has been mined for use, largely as fertilizer (fossil P; Forsberg et al. 2003). Natural weathering of P amounts to about 3 teragrams (Tg) per year (Falkowski et al. 2000), whereas recent estimates of human mining of P for fertilizer (figure 1a) and other uses put the amount at 25 Tg per year (Schlesinger and Bernhardt 2013), indicating that mining has increased P flux by approximately 800% over natural weathering. P naturally exists as a variety of oxidized forms that can be either dissolved or particle bound (adsorbed) to soils—that is, associated with soil particles through aluminum oxides, iron oxides, calcium compounds, or clays or bound to organic particles (Froelich 1988, Lebo 1991, Correll 1998). Dissolved and particle-bound P are both subject to rainfall-driven runoff from the landscape to water bodies (Sims et al. 1998), with approximately 75%–90% of the P entering receiving waters moving in association with the eroding soils (figures 1b, 1c; Sharpley et al. 1993). Once in the receiving waters, P becomes a pollutant, either as dissolved P or associated with suspended solids. Dissolved inorganic P is readily used by algae and microbes, but the amount of bioavailable particulate phosphate varies widely—from 10% to 90%, depending on source soils, water characteristics, and the algal–microbial consortia present (Sharpley et al. 1993, Wetzel 2001 and the references within). Dissolved P can also exist in organic forms (dissolved organic phosphorus, DOP), which may or may not be bioavailable; for example, a survey of 27 Midwestern aquatic systems showed the bioavailability of DOP to vary from 0% to 100%, with a median value of 78% (Thompson and Cotner 2018).

Phosphorus concentrations in US watercourses have been increasing over time (Stoddard et al. 2016), and such pollution is widespread (Paulsen et al. 2008), as was noted above. The majority of P that enters water bodies is contributed by stormwater runoff that contains unused commercial fertilizers, manure from livestock production, and untreated or incompletely treated human sewage (Correll 1998, Howarth et al. 2002, Withers and Jarvie 2008). With rapidly increasing human populations in many regions, an increase in P from greater wastewater processing (figure 1d) might be expected (NRC 1993) through normal discharge of effluents, as well as through releases from sewage leaks, spills, and storm or hurricane related accidents. In addition, mining of P for chemical fertilizers to support agriculture will increase with population rise (Peñuelas et al. 2012, Yang et al. 2016). There is a highly significant correlation ($r^2 = .37$, $p = .002$) between watershed human population density and phosphate yield to rivers in North America and Europe (Turner et al. 2003).

In addition to agricultural and urban runoff and wastewater effluents as sources of P, we note that major increases in industrial livestock manure loads have occurred, particularly in the Mid- and South Atlantic regions of the United States, because of swine and especially poultry production

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Figure 1. Common sources of phosphate pollution to the landscape and watercourses: (a) Phosphate mining industry worldwide has increased to 25 Tg per year (the mine shown is in Aurora, eastern North Carolina, Pamlico estuarine system in background). (b) Much of the P delivered to waterways in runoff is associated with suspended sediments. The photo shows turbid agricultural field runoff in eastern North Carolina. (c) Turbid urban stormwater runoff in Wilmington, North Carolina. (d) Wastewater treatment plants release P in effluents, also during leaks, spills, and outages from major storms. (e) Liquid swine waste being sprayed on adjoining fields in Duplin County, North Carolina. (f) Poultry manure prior to being spread as litter on adjoining fields, Duplin County, North Carolina. Photographs: Michael A. Mallin, except (a), courtesy of Sound Rivers, Inc.

(Burkholder et al. 1997, Sims et al. 1998, Kellogg 2000, Yang et al. 2016). For example, in five Atlantic coastal watersheds (the Cape Fear, Lumber, Neuse, Tar-Pamlico, and White Oak Rivers) the total number of poultry raised in confinement increased from approximately 762,224,000 in 1992 to 886,280,400 in 2014, an increase of more than 124,000,000 birds (Patt 2017). Such industrial animal production facilities are called *concentrated animal feeding operations* (CAFOs). Whereas both swine and poultry CAFOs are abundant in North Carolina, poultry CAFOs are also numerous in Delaware, Maryland, Virginia, Georgia, and South Carolina (USDA 2019). The massive amount of waste generated by swine CAFOs is pumped into outdoor cesspits called lagoons and periodically sprayed onto nearby fields (figure 1e), where it is subject to stormwater runoff. Poultry CAFO waste is spread as dry litter on surrounding fields (figure 1f), where it is also exposed to rainfall and subsequent runoff. These disposal practices, although they are legal, result in loads of concentrated nutrients, fecal microbes, and organic matter contaminating receiving streams and stimulating elevated biological oxygen demand (BOD; Hoorman et al. 2008, Mallin et al. 2015).

Subsurface movement of P to streams is usually low because P binds readily to soil particles (Correll 1998). Exceptions occur in regions where the soils are sandy, organic, or saturated by P from agricultural or urban sources, so that P is exported through the soil layer, particularly where the water table is high (Sims et al. 1998, Howarth et al. 2002). An example of P-saturated regions is areas where considerable amounts of P have been added to soil by land application of animal manures generated by CAFOs (figure 1e, 1f; Sims et al. 1998, Kellogg 2000, Cahoon and Ensign 2004, Yang et al. 2016). Over time, the number of US counties where manure nutrients exceed the potential plant uptake and removal has continued to increase (Howarth et al. 2002). P saturation of soils can also occur where septic systems are too highly concentrated or soils too wet to allow for proper immobilization in drain fields (Robertson et al. 1998, USEPA 2002, Cahoon et al. 2006) or even in select residential and recreational landscapes (Cahoon and Ensign 2004). On a broad scale, from preindustrial times to the present the accumulation of P in watershed soils has greatly increased (Bennett et al. 2001), and P concentrations in lakes and streams continue to increase (Stoddard et al. 2016), reflecting the switch from small-scale fertilization with recycled manure P to massive-scale use of mined fossil P as commercial fertilizer, as well as the disposal of CAFO wastes (figure 1).

Orthophosphate denotes ionized forms of H_3PO_4 (orthophosphoric acid) that are most readily available for plant and microbial uptake (Correll 1998). Once carried into receiving waters, orthophosphate concentrations are thought to remain within a general range because of adsorption equilibria, wherein dissolved P can be bound or leached from particles, depending on the dissolved P content in the water (Froelich 1988). However, the amount of particle-bound P can increase greatly according to inputs from anthropogenic sources. Passing down the river continuum to the estuary, P is usually desorbed from suspended sediments by increasing salinity and subsequent competition from other anions for sorption sites (Lebo 1991, Howarth and Marino 2006). In water, P is most frequently measured as orthophosphate P and as total P, consisting of inorganic plus organic P, both dissolved and particle bound. Total P is usually the P measurement considered most relevant in assessing water quality or trophic state for two reasons: First, algae tend to be luxury consumers of P, taking from the water more than they need, storing it, and accessing some of the stored P for later use (Wetzel 2001 and the references within). Second, dissolved inorganic P is mostly ephemeral in the water column—that is, low in concentration and taken up by algae and bacteria within seconds to minutes (Wetzel 2001 and the references within). Therefore, a dissolved inorganic P measurement provides only a snapshot of the P actually available to the biota (Withers and Jarvie 2008). Water-column total P concentrations exceeding the 70–84 micrograms (μg) of P per liter range are considered to be indicative of eutrophic fresh waters (Dodds et al. 1998, Wetzel 2001).

Stimulation of bacteria as well as algae

Aquatic ecologists generally view P as a nutrient that, if added in excess, directly stimulates only primary producers (algae and higher plants). P was historically recognized as the primary nutrient controlling nuisance algal growth in most freshwaters (Vollenweider 1968, Hecky and Kilham 1988, Howarth and Marino 2006), although nitrogen (N) plays that role in selected freshwater bodies (figure 2; Elser et al. 1990, Mallin et al. 2004a, Burkholder and Glibert 2013). As such, P inputs that drive algal blooms also indirectly lead to increased BOD in affected waterways, because decaying algal blooms are quite labile (NRC 2000). Excess P enrichment can also contribute to growth of toxigenic algae, including toxigenic cyanobacteria (blue-green algae) in fresh and tidal fresh systems (e.g., NAS 1969, Heisler et al. 2008, Burkholder and Glibert 2013). Although lakes and reservoirs are better studied, P enrichment can also stimulate benthic algal growth in freshwater streams and rivers (Correll 1998 and the references therein). Therefore, the P concentration is an integral part of many trophic state indices such as the well-known Carlson index (Carlson 1977) and is also the primary nutrient most often targeted for reduction in schemes to reduce lake eutrophication (Howarth and Marino 2006). Missing almost entirely from consideration, however, is that P pollution contributes significantly to ecosystem degradation through mechanisms unrelated to primary producers. Specifically, P enrichment causes increased bacterial production and subsequent BOD increases and causes the stimulation of fecal microbes (including potential pathogens) that threaten human health (figure 2).

P is an essential nutrient for natural bacterial growth, both structurally and physiologically. Bacteria contain P in membrane phospholipids and nucleic acids, with their small sizes

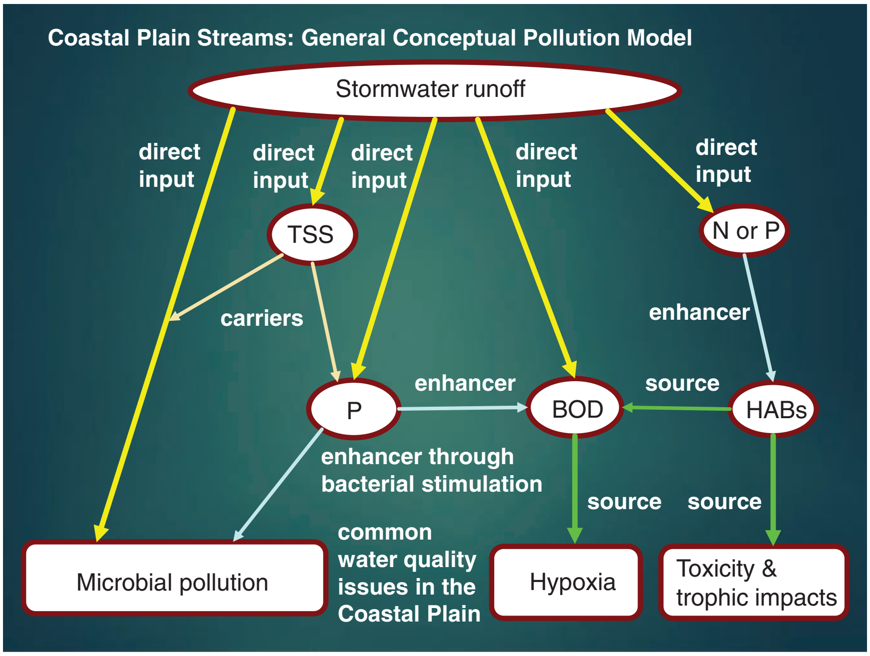

Figure 2. A conceptual model of stormwater-driven pollution parameter influences on Coastal Plain inland water bodies and estuarine tidal creeks. Stormwater is used in the present article to represent nonpoint pollution from sources such as cropland agriculture, urban runoff, and runoff or subsurface transport from swine, poultry, and cattle CAFOs. Phosphorus commonly drives harmful algal bloom formation in many freshwater locations, but N principally does in the Coastal Plain locations we have studied.

arguing for more P relative to carbon (C; Kirchman 1994). Although the C:P ratios in bacteria are variable, a number of studies have found them to be considerably lower than the C:P ratios in phytoplankton. For example, reported molar C:N:P ratios were 45:9:1 for marine bacteria (Goldman et al. 1987), 44:9:1 for lake bacteria (Chrzanowski et al. 1996), and 60:7:1 for soil bacteria (Cleveland and Liptzen 2007), compared with the Redfield ratio of 106:16:1 for pelagic marine phytoplankton (Redfield 1958). However, in a survey of 128 upper Midwestern US lakes, Cotner and colleagues (2010) found an average ratio of 102:12:1, close to the C:P Redfield ratio. The suggested reasons for variability in bacterial elemental composition include analytical methodology, trophic state, temperature, and C lability (Cotner et al. 2010). On a broad scale, Kirchman (1994) provided bacterial C:P estimates ranging from 7% to 72% of phytoplankton C:P ratios. Bacterial metabolism and growth rates also tend to be very fast relative to those of phytoplankton, creating a relatively rapid turnover of P-based ATP (adenosine triphosphate) and RNA (the growth rate hypothesis of Elser et al. 1996). Therefore, the bacterial requirement for P is considered to be greater than that for phytoplankton.

In many aquatic systems, P has stimulated or been positively correlated with bacterial growth, including lakes (Currie 1990, Toolan et al. 1991, Morris and Lewis 1992, Chrzanowski et al. 1995), streams and rivers (Elwood et al. 1981, Mallin et al. 2004a, Surbeck et al. 2010), coastal wetlands (Sundareshwar et al. 2003), estuaries (Cotner et al. 2000), and coastal marine waters (Bjorkman and Karl 1994, Thingstadt et al. 1998). Under P-limited conditions, bacteria in the field have been shown to be better competitors for P than phytoplankton (Currie 1990, Kirchman 1994, Cotner et al. 2000).

The need and uptake capabilities of bacteria for P lead to nonalgal water-quality impacts exacerbated by excessive P enrichment. In the present article, we focus on two of these impacts: hypoxia as a major ecosystem-level impact and microbial pathogen contamination, indicated by fecal bacteria, as an issue affecting human health (figure 2). Loading of P is a positive feedback mechanism that drives systems toward hypoxia and that can exacerbate fecal microbial pollution. As of 2016, microbial pathogen impairment was identified by the USEPA (2018) as the largest source of impairment to US streams and rivers, with 317,197 stream kilometers (km; 197,092 miles) threatened or impaired. Oxygen depletion was also described as a major source of impairment, affecting 157,119 stream km (97,629 miles). In the present article, we synthesize previous and present work from our laboratories to illustrate the important role of P enrichment in promoting hypoxia and fecal microbial contamination separate from effects of primary producers and therefore provide further impetus for managers to reduce P loading to water bodies.

Coastal Plain watercourses

The data used within this research was derived from Coastal Plain ecosystems. The Coastal Plain of the United States encompasses a vast area that stretches from southern New Jersey through north Florida and into the Gulf Coast region and that is bounded to the west by either Piedmont terrain or the Sandhills region in the south. The principal lotic water bodies characterizing the inland areas of the Coastal Plain are blackwater streams and rivers (Meyer 1990, Smock and Gilinsky 1992); such systems discharge either into larger rivers and riverine estuaries or into larger coastal sounds, and some blackwater systems are dammed for water supply or recreational use. They are darkly stained by dissolved organic material and drain flatlands with little slope (Hupp 2000, Smock and Gilinsky 1992). Another prominent type of lotic water body in the Coastal Plain is tidal creeks (MacMillin et al. 1992, Mallin et al. 2001a, 2004b, Sanger et al. 2015), which can be marine, mesohaline, or fresh to oligohaline, and they discharge into larger estuaries or the

Table 1a. Field correlations between turbidity and phosphorus in upper and lower Coastal Plain watercourses.

Water body	r	p	n
New River	.53	.0002	44
Burnt Mill Creek	.40	.035	29
Echo Farms stream	.79	.001	29
Silver Stream	.79	.001	29
Smith Creek	.47	.001	48
11 rural streams	.38	.001	66
5 tidal creeks	.19	.0001	650

Note: Presented as Pearson correlation coefficients (r). Source: from Mallin and colleagues (2001a, 2009).

Table 1b. Field correlations between turbidity and fecal bacteria in upper and lower Coastal Plain watercourses.

Water body	r	p	n
Cape Fear River	.86	.001	41
Prince Georges Creek	.44	.007	36
Smith Creek	.62	.001	48
11 rural streams	.77	.0001	66
5 tidal creeks	.36	.0001	650

Note: Presented as Pearson correlation coefficients (r). Source: from Mallin and colleagues (2001a, 2009).

lower reaches of rivers. Some tidal creeks, particularly fresh or oligohaline, are also blackwater streams. As with other areas of the United States, the Coastal Plain is experiencing increasing P levels in water bodies (Stoddard et al. 2016). Many of the large riverine estuaries and sounds are affected by algal blooms issues (Bricker et al. 2007), whereas many blackwater systems and tidal creeks are primarily affected by hypoxia and fecal microbial pollution (Lerberg et al. 2000, Mallin et al. 2001a, Mallin et al. 2006, Sanger et al. 2015).

Suspended solids as pollutant carriers

Direct anthropogenic inputs of P to receiving waters can originate from controllable point sources, such as human and industrial wastewater treatment plants (figure 1d), and from less easily controlled nonpoint sources, including septic systems, crop agriculture (figure 1b), urban runoff (figure 1c), and runoff and subsurface transport from swine, poultry, or cattle CAFOs. As was noted earlier, some nonpoint source P is dissolved, but particulate-bound P, in general, makes up 75%–90% of the erodible and runoff-transported P to water bodies (Sharpley et al. 1993). Once in the water, much P is bound to suspended sediments. In addition to the aforementioned source areas for erodible sediments and associated P, other source areas include urban and suburban development activities, silviculture, and road construction.

Suspended sediment pollution can be measured either by gravimetric methods (total suspended solids) or indirectly by methods using light transmission techniques (turbidity, Secchi depth). Suspended sediments in excess have well-known direct water quality impacts, including increasing light attenuation, interfering with fish behavior and prey detection, changing the bottom composition and affecting fish nesting, reducing benthic invertebrate diversity and abundance, and affecting benthic microalgal composition and abundance.

However, the function of runoff-driven suspended sediments as carriers of other pollutants is of principal interest for the purposes of the present discussion (figure 2). Bonding of pollutants to sediment particles occurs through both physical and chemical sorption (Olsen et al. 1982). The strong association between P and suspended solids is discussed above; additionally, a number of researchers have found an association of suspended particles with fecal bacteria (Pommepuy et al. 1992, Baudart et al. 2000, Jeng et al. 2005). This pollutant-transport function is suggested by statistical correlations between suspended matter (here represented as turbidity) and the above two parameters, P and fecal bacteria, from a wide variety of fresh and estuarine lotic systems on the Coastal Plain (table 1a, 1b). On occasion, positive correlations occurred between turbidity and N, but in far fewer systems than the positive association with P.

P enrichment promotes hypoxia through increased BOD: An ecosystem-level impact

A major ecosystem impact is critical reduction in water body dissolved oxygen, or hypoxia. An important measureable factor that influences dissolved oxygen is biochemical oxygen demand (BOD), which is the dissolved oxygen needed for heterotroph respiration to break down organic matter in water bodies. The BOD over 5 days (BOD5) is commonly measured in wastewaters as a means to assess labile (easily used) organic materials pollution (Clark et al. 1977, NRC 1993). The decomposition of algal blooms produces a labile source of BOD linked to hypoxia problems (NRC 2000, Mallin et al. 2006, Volkmar and Dahlgren 2006). The BOD over 20 days (BOD20) is measured to assess the more refractory organic material as a driver of ecosystem respiration. In broadscale statistical analyses of Southeastern, mostly blackwater systems, total P concentration was significantly correlated with BOD5 or BOD20 in a number of aquatic ecosystems (table 2a, 2b). Total N was correlated with BOD in several systems as well, although for a different reason than total P: The N stimulated increased chlorophyll *a* (highly labile organic matter), making N an indirect enhancer of BOD.

Phytoplankton production in blackwater lotic systems can be limited by light availability in the darkly stained waters (Smock and Gilinsky 1992, Phlips et al. 2000), although phytoplankton productivity in colored waters (especially lakes) can be very high at times (Nürnberg and Shaw 1999, Phlips et al. 2000). On the US Coastal Plain, however, where sufficient light is available, N inputs, rather than P, stimulated phytoplankton growth in blackwater systems (Mallin et al. 2001b, 2004a). In other large blackwater rivers, the N:P

Table 2a. Pearson correlations between total phosphorus and 5-day biological oxygen demand in a variety of upper and lower coastal plain blackwater rivers, streams, and impoundments (lakes).

Water body	r	p	n
NE Cape Fear River	.34	.008	58
Great Coharie Creek	.66	.0001	38
Hammond Creek	.42	.009	38
Stockinghead Creek	.63	.0001	35
Carolina Beach Lake	.63	.009	16
Greenfield Lake	.53	.0001	147

Source: From Mallin and colleagues (2006, 2015, 2016).

Table 2b. Pearson correlations between total phosphorus and 20-day biological oxygen demand in a variety of upper and lower coastal plain blackwater rivers, streams, and impoundments (lakes).

Water body	r	p	n
NE Cape Fear River	.34	.009	58
Black River	.33	.010	58
Brown's Creek	.40	.012	38
Colly Creek	.39	.015	38
Great Coharie Creek	.78	.0001	38
Hammond Creek	.69	.001	38
Six Runs Creek	.49	.002	38
Carolina Beach Lake	.61	.012	16

Source: From Mallin and colleagues (2006, 2015, 2016).

ratios are very low (Edwards and Meyer 1987, Meyer 1992), arguing for N limitation (again where light is available). Regarding estuaries, large systems on the US East Coast have been well studied, and phytoplankton tend toward N limitation mainly, with some spring P limitation especially in upper areas (Howarth and Marino 2006). Tidal creeks are a highly abundant estuarine feature of the Coastal Plain, and, as was noted previously, can be clear or blackwater. Nutrient addition experiments (Lewitus et al. 2004, Mallin et al. 2004b), as well as very low N:P ratios (MacMillin et al. 1992), have indicated that phytoplankton in these tidal creek systems are primarily N limited, with occasional P limitation in upper reaches.

Experimental evidence of BOD enhancement through P enrichment

We have conducted a series of nutrient addition experiments over several years to assess the influence of nutrient loading on BOD in water from various Coastal Plain blackwater systems, including two fifth-order blackwater rivers (the Black and Northeast Cape Fear Rivers), a pristine second-order blackwater stream (Colly Creek), a fourth-order blackwater stream affected by agriculture and livestock production (Great Coharie Creek), and an urban blackwater impoundment (Greenfield Lake). Response variables included phytoplankton biomass as chlorophyll *a*, bacterial abundance changes measured by acridine orange counts, biomass changes measured as ATP concentration, and 5- and 20-day BOD concentrations from assays conducted using light and dark cubitainers. Experimental details are presented in Mallin and colleagues (2001b, 2004a, 2016).

Eleven sets of nutrient addition bioassays were performed on water from the Black and Northeast Cape Fear Rivers (Mallin et al. 2001b). N additions (1.0 milligrams [mg] of N per liter) either as ammonium, urea, or an N and P combination often yielded significant increases in phytoplankton biomass as chlorophyll *a* production relative to controls without N additions, and sometimes significant ATP increases as well (table 3a–3c). Additions of P as orthophosphate or glycerophosphate (1.0 mg of P per liter) significantly stimulated chlorophyll *a* on only one occasion, but frequently stimulated significant ATP production without concurrent (significant) chlorophyll *a* production, indicating direct P stimulation of heterotrophic microbial growth by P stimulation (table 3a–3c).

The results from eight nutrient addition bioassays carried out on water from blackwater creeks revealed two pathways by which BOD—and, therefore, hypoxia—can be stimulated. First, additions of nitrate and a nitrate and urea combination at various concentrations yielded significant chlorophyll *a* production during 5-day experiments, and postexperimental analyses demonstrated significant subsequent increases in both BOD and bacterial counts. Although significant stimulation usually occurred at 0.20 mg of N per liter, more consistent chlorophyll *a* stimulation occurred at 0.50 mg of N per liter final concentrations (Mallin et al. 2004a). Therefore, hypoxia can be enhanced by N inputs via the traditionally accepted indirect pathway of labile phytoplankton to BOD to bacteria (equation 1):

$$\uparrow \text{nitrogen} \Rightarrow \uparrow \text{algal biomass} \Rightarrow \uparrow \text{BOD} \Rightarrow \downarrow \text{dissolved oxygen} \quad (1)$$

Note that, in many other types of water bodies, P would be the nutrient stimulating algal biomass (Howarth and Marino 2006) and enhancing BOD via the indirect pathway.

Bioassays demonstrated that chlorophyll *a* stimulation by P additions rarely occurred in the blackwater systems we tested. However, P additions significantly and directly stimulated bacterial abundance (figure 3a), which led to further significant BOD increases (figure 3b). The P concentration that generally stimulated bacterial and BOD stimulations was 0.50 mg of P per liter (Mallin et al. 2004a). This demonstrates the other (hidden) impact of P loading, a direct stimulation of microbial heterotrophy (equation 2):

$$\uparrow \text{phosphorus} \Rightarrow \uparrow \text{heterotroph biomass} \Rightarrow \uparrow \text{BOD} \Rightarrow \downarrow \text{dissolved oxygen} \quad (2)$$

Table 3a. Percentage of 5-day nutrient addition bioassays demonstrating significant ($p < .05$) stimulation by nitrogen as ammonium or urea on water from the Black and Northeast Cape Fear Rivers.

River	Response variable	Percentage showing stimulation
Black River	Chlorophyll *a*	45
Black River	ATP (adenosine triphosphate)	45
Northeast Cape Fear River	Chlorophyll *a*	64
Northeast Cape Fear River	ATP	18

Note: Out of 11 bioassays. Both rivers analyzed in each experiment.

Table 3b. Percentage of 5-day nutrient addition bioassays demonstrating significant ($p < .05$) stimulation by phosphorus as orthophosphate or glycerophosphate on water from the Black and Northeast Cape Fear Rivers.

River	Response variable	Percentage showing stimulation
Black River	Chlorophyll *a*	0
Black River	ATP (adenosine triphosphate)	82
Northeast Cape Fear River	Chlorophyll *a*	9
Northeast Cape Fear River	ATP	36

Note: Out of 11 bioassays. Both rivers analyzed in each experiment.

Table 3c. Percentage of 5-day nutrient addition bioassays demonstrating significant ($p < .05$) stimulation by nitrogen and phosphorus on water from the Black and Northeast Cape Fear Rivers.

River	Response variable	Percentage showing stimulation
Black River	Chlorophyll *a*	55
Black River	ATP (adenosine triphosphate)	64
Northeast Cape Fear River	Chlorophyll *a*	55
Northeast Cape Fear River	ATP	55

Note: Out of 11 bioassays. Both rivers analyzed in each experiment.

As an additional (nonbioassay) check of the impact of total P on ambient bacteria, we examined the available bacterial count data (from unamended controls), along with corresponding chlorophyll *a* data from Great Coharie Creek and Colly Creek ($n = 23$). Using literature conversion factors (Fukuda et al. 1998), we transformed the chlorophyll *a* and bacterial count data to biomass as µg of C per milliliter (mL), and plotted the ratio of algal biomass to bacterial biomass as a function of total P concentration (figure 4). The results were negative and significant ($r = -.589$, $p = .003$), indicating that, in this data set, algal/bacterial biomass ratios were lowest when total P concentrations were highest, again suggesting P is driving bacterial biomass production.

Phytoplankton limitation by N was also demonstrated in a Coastal Plain lentic system. A series of 15 nutrient addition bioassays were performed on water from Greenfield Lake, an urban blackwater impoundment (Mallin et al. 2016). The results demonstrated significant chlorophyll *a* stimulation over control by nitrate (100 µg of N per liter additions) in 11 out of 15 experiments. In contrast, P additions as orthophosphate (50 µg of P per liter additions) significantly stimulated chlorophyll *a* production on only 1 out of 15 occasions. Again, additional P loadings do not always stimulate algal growth in freshwater ecosystems.

To summarize, in 19 stream or river experiments, N additions (as either nitrate, ammonium, or urea) often significantly stimulated phytoplankton production in blackwater streams and rivers. This finding also occurred in bioassays carried out on water from a blackwater Coastal Plain impoundment (algal N limitation). BOD measurements performed following the incubations on blackwater streams showed that N additions yielded significant BOD increases via production of labile organic matter as phytoplankton, which, on decomposition, increases bacterial respiration (figure 2). This decomposition also significantly increased bacterial densities, whereas, bacterial production was not directly stimulated by N additions.

In contrast, P additions rarely increased chlorophyll *a* production in any of the Coastal Plain systems tested. However, P additions caused significantly greater (versus unamended controls) bacterial production measured as direct counts, and subsequently increased BOD in tested blackwater (bacterial P limitation). Also, in the Black and Northeast Cape Fear Rivers, additions of total P led to significant non–algae-based ATP increases. Inorganic P additions occasionally led to significantly higher ATP, bacterial counts, and BOD, but less frequently than total P additions (i.e., combined inorganic and organic P).

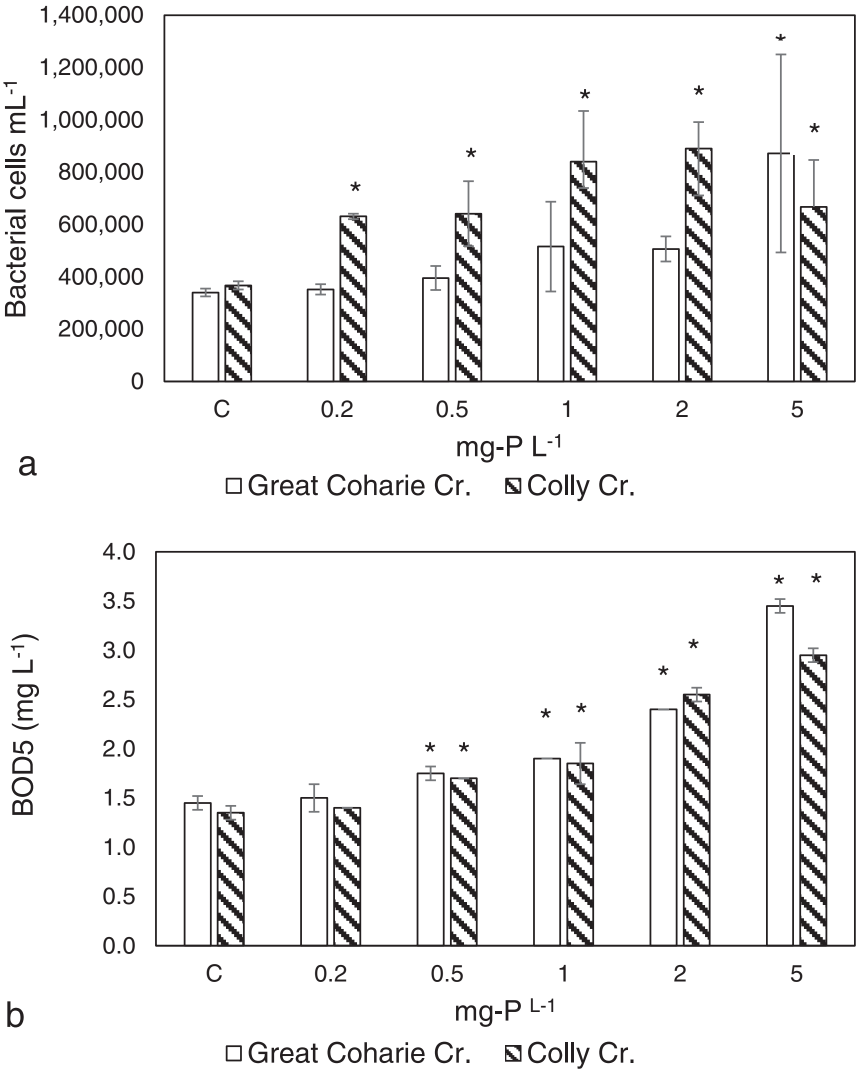

Figure 3. (a) Mean bacterial densities (the error bars represent the standard deviation; n *= 3) following phosphorus additions at varying concentrations in water from two Coastal Plain streams, November 1999 experiment. (b) Mean 5-day biological oxygen demand concentrations for those same 5-day experiments (see Mallin et al. 2004a for details). *p < .05.*

Reflecting similar findings while working in a South Carolina salt marsh environment, Sundareshwar and colleagues (2003) found simultaneous *Spartina alterniflora* stimulation by N additions and soil bacteria stimulation by P additions. Those researchers noted that the heterotrophs were primarily limited by P and secondarily limited by C. In fluvial blackwater systems, dissolved organic carbon (DOC) is abundant (Meyer 1990, Smock and Gilinsky 1992), so C would not often limit bacterial growth; however, we note that bioavailability of DOC can be quite variable (Thompson and Cotner 2018). Therefore, additions of P can directly lead to lower dissolved oxygen in Coastal Plain aquatic ecosystems by stimulating bacterial growth, increasing heterotrophy and BOD.

Phosphorus pollution: The human health connection

Fecal bacteria enter watercourses through human (Cahoon et al. 2016) or animal wastewater treatment system malfunctions (Burkholder et al. 1997) or stormwater runoff (Struck 1988, Jeng et al. 2005, Cahoon et al. 2006, Mallin et al. 2009). Domestic animals in particular can carry many pathogens (Hinton and Bale 1991), some of which are pathogenic to humans as well (Berger and Oshiro 2002). Elevated loads of pathogenic microbes in runoff carrying fecal material can lead to human health illness from exposure to contaminated recreational waters or consumption of contaminated shellfish (Alexander et al. 1992, Rippey 1994, Wittman and Flick 1995) and economic losses from illness and closures of beaches to recreation and shellfish beds to harvest (NRC 1993, Dwight et al. 2005). Microbial water safety is usually estimated (however imperfectly) from the indicator taxa fecal coliform bacteria, *Enterococcus* sp., or *Escherichia coli*). As with ambient bacteria in waterways, P loading increases the survival and in some cases reproduction of fecal-derived indicator bacteria in field situations outside of the host organism. This has been established by a number of enrichment and dilution experiments.

Experimental response of fecal bacteria to P

Some fecal bacteria associate with the sediments, which serve as reservoirs and incubators. In such an environment fecal bacteria are protected from solar irradiance and usually have sufficient carbon and other nutrients present for survival and even growth (Struck 1988, Jeng et al. 2005). In a series of experiments using sediment cores from oligohaline areas of tidal creeks, Toothman and colleagues (2009) investigated the influence of added labile organic carbon substrate and P on the fecal bacterium *Enterococcus*. Organic carbon inputs occasionally stimulated fecal bacteria growth. The results also showed that there was a significant positive impact of P inputs on fecal *Enterococcus* concentrations but only at sites with low initial sediment P concentrations (less than 31 μg of P per gram). In most sediments P is high enough not to be limiting to bacterial growth—except sandy coastal sediments.

Stimulation of fecal bacterial growth by P additions in surface waters was demonstrated by adding dissolved

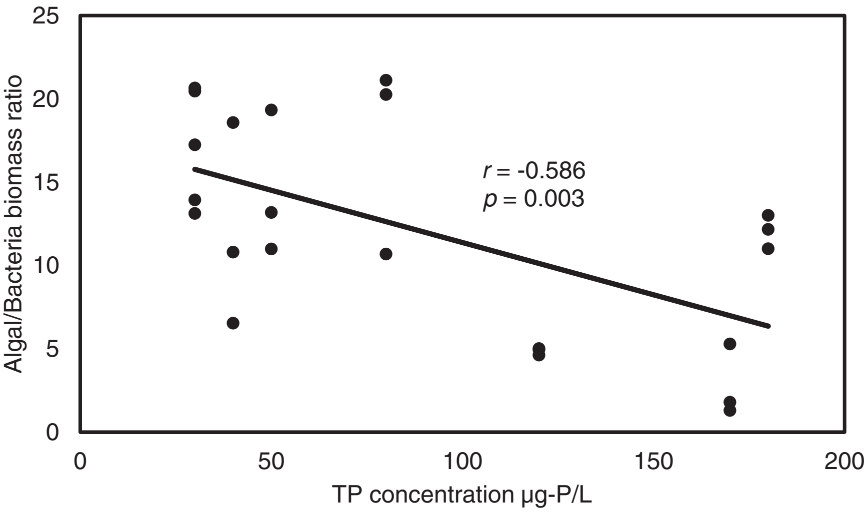

Figure 4. Correlation plot of algal/bacterial biomass ratio versus total phosphorus concentrations for Great Coharie Creek and Colly Creek demonstrating a significant negative relationship; that is, bacterial biomass increases along with increasing total phosphorus concentrations (**n** ***= 23).***

organic P (DOP) glycerophosphate to water from a constructed stormwater treatment wetland—in this case, fresh water that drained into a tidal creek (Chudoba et al. 2013). In a 21-day experiment (figure 5a), additions of inorganic or organic N alone provided no bacterial stimulation, additions of an N and P combination provided some stimulation, but additions of DOP alone provided significant and long-lasting stimulation of fecal bacteria growth. Testing DOP alone at varying concentrations (figure 5b) Chudoba and colleagues (2013) found significant stimulation by day 2 from all concentrations, but increased growth according to the amount of P added. Three of six experiments showed significant stimulation of fecal bacteria by P additions, whereas none showed stimulation by N additions. This is growth of fecal coliform bacteria that occurred outside of human or animal hosts in wetland water amended only with DOP.

The impacts of nutrient additions can be confounded by microzooplankton grazing; therefore, the effects of P on fecal coliform bacteria were further tested using another type of assay, called dilution assays. Originally developed to assess microzooplankton grazing on phytoplankton (Landry and Hassett 1982), such dilution assays were used by Chrzanowski and colleagues (1995) to test nutrient enrichment impacts on natural bacterioplankton in lake conditions.

We adapted this technique for several 24-hour experiments to determine whether microzooplankton grazing reduced fecal bacteria in a constructed stormwater treatment wetland, while at the same time determining whether nutrient additions stimulated fecal coliform bacterial growth (Chudoba et al. 2013). The concept behind such experiments is that dilution reduces predator–prey encounter rates, reducing grazing but not the growth rates of the prey. Fecal coliform growth rates for each treatment during the 24-hour period were plotted and regressed against the initial bacterial densities (as colony-forming units per 100 mL); the series of dilutions produced a line of best fit of measured specific growth rate versus prey concentration, and a negative and statistically significant slope (figure 6) implied that microzooplankton grazing was a significant factor affecting fecal coliform population growth. A significantly positive

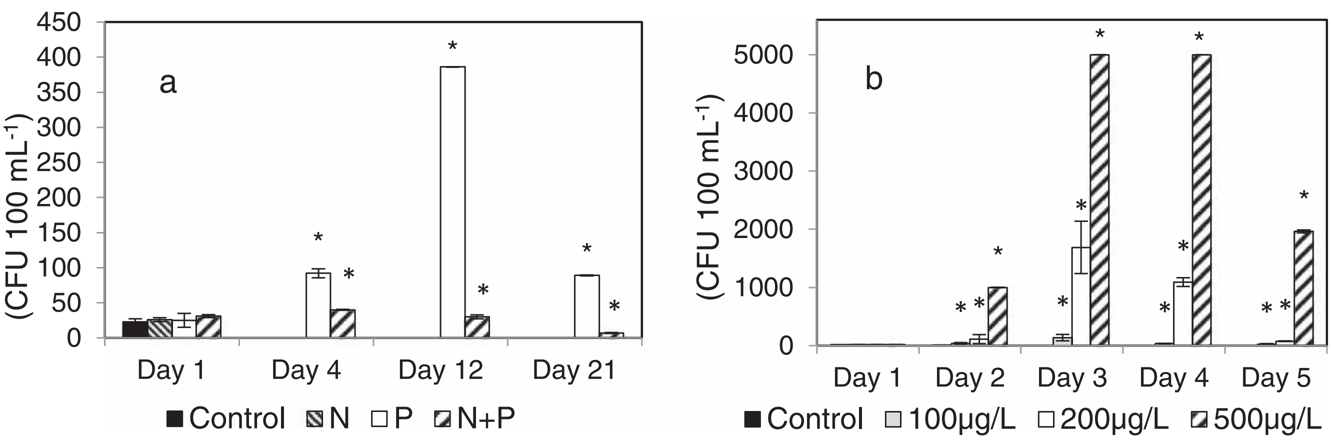

Figure 5. (a)Growth of fecal coliform bacteria following nutrient additions (1.5 mg per liter as N or P) for 21 day experiment, demonstrating significant impact of P addition; note rapid cell loss in control and N-amended treatment. (b) The growth of fecal coliform bacteria following organic P additions of various concentrations for 5-day experiment, demonstrating greater impact with increasing addition concentrations and bacteria concentrations as colony-forming units (CFU) per 100 milliliters (mL). Source: Reprinted from Chudoba and colleagues (2013) with permission.

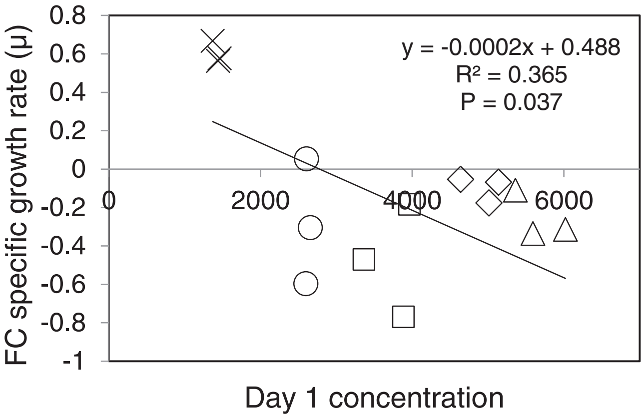

Figure 6. Example of an October 2011 24-hour dilution assay amended by 100 µg inorganic P per liter demonstrating significant negative slope, i.e., significant microzooplankton grazing of the fecal bacteria, positive* y*-intercept (i.e., a positive specific growth rate) in the theoretical absence of grazing, and significant (*p *= .03) P stimulation (over 24 hours) of fecal coliform bacterial growth over unamended control—that is., Xs versus diamonds. The diamonds represent unamended 100% unfiltered control water, the triangles represent 100% unfiltered amended water, the squares represent 75% unfiltered amended water, the circles represent 50% unfiltered amended water, and the Xs represent 25% unfiltered amended water. The* x*-axis represents fecal coliform counts (in colony-forming units per 100 milliliters) after 24 hours. Source: Reprinted from Steffy (2012) with permission.

y-intercept implied positive growth of the bacteria in the theoretical absence of grazing. Effects of added inorganic or organic P or N on bacterial growth rates were therefore compared by analyzing the *y*-intercept parameter, with the influence of grazing analyzed separately. Figure 6 also demonstrates a comparison of fecal bacteria densities between water amended with inorganic P versus unamended whole water (using analysis of variance, ANOVA). In the inorganic P addition experiment summarized by figure 6, the regression indicated a significant grazing effect ($p = .037$); as such, the ANOVA indicated that only the P amended 25% whole-water treatment (the least affected by grazing) produced significantly higher fecal bacterial growth rate versus the unamended control ($p = .008$).

Several such dilution experiments were performed on Coastal Plain wetland waters with amendments of either inorganic or organic P (Steffy 2012, Chudoba et al. 2013). Three of the six organic P treatments had a significantly higher growth rate than the inorganic P treatment (i.e., greater stimulation by organic P), whereas four of the six inorganic P treatments had a positive growth rate, but only two were higher than the organic P treatment, implying a somewhat better stimulation by organic P than by inorganic P (Steffy 2012, Chudoba et al. 2013). This is illustrated in a set of simultaneous experiments (figure 7a, 7b), in which organic P provided stimulation, but inorganic P did not, and microzooplankton grazing was not a factor.

To summarize, the dilution experiments demonstrated that microzooplankton grazing on bacteria occurs and at times could confound nutrient addition experiments. These experiments also demonstrated that, in the theoretical absence of grazing, on occasion, organic or inorganic P additions significantly stimulated fecal bacterial growth over control dilutions that lacked nutrient amendments. However, these experiments did not find significant stimulation of fecal bacterial growth by N amendments. The P addition concentration in these experiments was only 100 µg of P per liter, a level frequently exceeded by concentrations of total P in streams draining CAFO areas, as well as urbanized streams (Mallin et al. 2006, 2015). Using a combination of microassays and field studies in a sewage-influenced California stream, Surbeck and colleagues (2010) determined that dissolved P levels above 70 µg of P per liter were sufficient to prevent die-off of *E. coli* and enterococci. Therefore, P concentrations sufficient to enhance survival or reproduction of fecal bacteria are readily encountered in streams, rivers, and other water bodies influenced by urban runoff or by runoff of P from crop agriculture or animal production facilities.

Another unconventional impact of the P stimulation of excessive bacterial growth is food-web based. Burkholder and colleagues (2008) detailed how phagotrophy (feeding on particulate matter, including bacteria) is a mechanism used by many harmful algal species to supplement their nutrition. Taxa using bacterivory include dinoflagellates, haptophytes, and raphidiophytes, among others, mostly investigated from estuarine and coastal marine habitats (Burkholder et al. 2008). These impacts are ecosystem scale as blooms of many of the harmful algal species can lead to hypoxia or their toxins can cause massive die-offs of aquatic life (Burkholder et al. 2018 and the references therein). P stimulation of a bacteria-based aquatic food web can therefore cause harmful effects indirectly through increasing the food sources of mixotrophic harmful algae.

Where do we go from here?

Cumulative data from the USEPA (2018) indicate that oxygen depletion through organic enrichment is a pervasive source of impairment to assessed waters, among the top five causes of impairment for streams, rivers, wetlands, lakes, reservoirs, and coastal shoreline areas in the United States. Hypoxia has well documented deleterious effects on aquatic organism health and behavior (Diaz and Rosenberg 1995, Vaquer-Sunyer and Duarte 2008, Sturdivant et al. 2014). Severe hypoxia in bottom waters also creates conditions conducive to the release of bound P from aquatic sediments (Withers and Jarvie 2008), thus fueling algal and microbial growth in overlying waters (Correll 1998). Hypoxia can result from BOD loads generated by decomposing algal blooms (NRC 2000, Mallin et al. 2006, Volkmar and

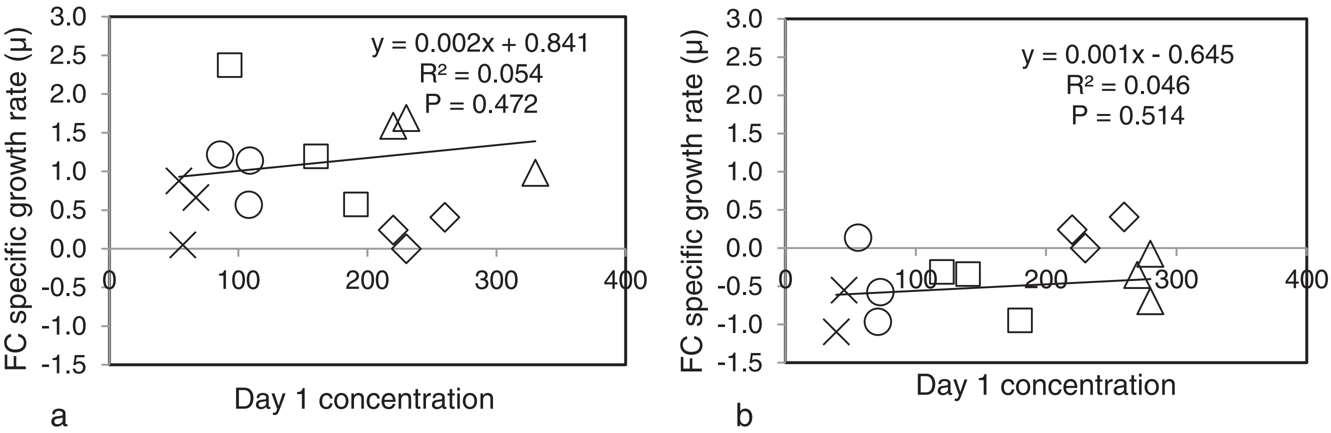

Figure 7. (a) September 2011 organic P addition 24-hour dilution assay in which no significant grazing effect occurred (p = .472), but growth rates of the 100% unfiltered (triangles) and 75% unfiltered (squares) organic P treatments were significantly (p < .001) higher than the unamended unfiltered control (diamonds). (b). Inorganic P addition experiment the same day in which no significant grazing effect occurred, and inorganic P additions did not provide stimulation over an unamended control. The bacteria concentrations are represented as colony-forming units (CFU) per 100 milliliters (mL). The circles represent 50% filtered treatment, and the Xs represent 25% unfiltered water. The x-axis represents fecal coliform counts (CFU per 100 mL) after 24 hours. Source: Reprinted from Steffy (2012) with permission.

Dahlgren 2006). However, it also occurs in numerous situations not involving harmful algal blooms, and our experimental data indicate that direct stimulation of bacterial growth through the increasing P loads to water bodies is a contributing factor.

Microbial pathogens are a major source of pollution to US waters as well, also among the top five causes of impairment in assessed streams, rivers, wetlands, and coastal shorelines (USEPA 2018). Increased P loads to water bodies increase the direct threat to human health in that P stimulation can cause increased survival and even growth of fecal bacteria in receiving water bodies outside of the host organism (human or animal). The increased environmental health threat can come from human contact in fresh and marine recreational waters or can come from contaminated shellfish consumption harvested from brackish waters. Economic losses occur from beach closures and when shellfish beds are closed to harvest because of high fecal bacterial counts.

All of the data and experiments described for this analysis were focused on Coastal Plain waters. Blackwater Coastal Plain ecosystems are known to be bacteria rich and tend toward heterotrophy (Meyer 1990, Smock and Gilinsky 1992, Nürnberg and Shaw 1999). Blackwater systems are also rich in DOC and not likely to be limited by the abundance of C substrate, although a high proportion of refractory DOC could limit bacterial use. From work in a California stream, Surbeck and colleagues (2010) experimentally determined that levels of DOC above 7 mg per liter released fecal bacteria from carbon limitation, suggesting significant C lability. This critical concentration probably varies geographically or geologically. In a 2011 5-month survey of seven Coastal Plain blackwater streams, DOC averaged 12.3 mg of C per liter (standard deviation [SD] = 7.4, range = 1.3–33.5). Moreover, DOC concentrations in the Cape Fear River were about 11.0 mg of C per liter (Avery et al. 2003) and ranged from about 6.0 to about 20.0 mg of C per liter in the Ogeechee River, Georgia (Leff and Meyer 1991). On a broader scale, in a study encompassing 27 Midwestern water bodies, DOC averaged 6.8 mg of C per liter (SD = 2.9, range = 2.0–11.9 mg of C per liter) and about 4%–54% was evaluated as bioavailable (Thompson and Cotner 2018). Both the concentration and bioavailability of DOC would likely also control the extent to which P alone stimulates bacterial growth in a given water body and whether organic P is more stimulatory (Bjorkman and Karl 1994, Avery et al. 2003, Thompson and Cotner 2018).

As was demonstrated earlier, grazing by microzooplankton may mask any stimulatory impact by P loading on bacterial population growth. The intensity of such grazing likely depends on location and climate. Burtchett and colleagues (2017) investigated such grazing on fecal coliform bacteria in a wet detention pond and a constructed stormwater wetland. Grazing rates in both situations were correlated with fecal bacterial abundance and water temperature. In turn, fecal bacterial densities were correlated with water temperature and rainfall. Interestingly, DOC in the wetland averaged 11.3 mg per liter and in the detention pond averaged only 5.4 mg per liter, and DOC concentration was only correlated with fecal bacterial counts in the pond, not in the DOC-rich wetland.

Research is needed to assess whether P stimulation through bacterial increases strongly influence BOD in geographic regions other than the Coastal Plain. Shaded streams in the Sandhills or Piedmont regions might be appropriate analogues; blackwater systems in more northerly regions should be assessed as well. As we have described, P stimulation of bacteria in general has been demonstrated in many types of water bodies, under various salinities and trophic states. Although it is widespread, the extent to which such stimulation of bacterial production causes significant ecosystem impacts will likely depend on factors such as hydrology, light attenuation, geography, and system trophic state.

Fungal growth during decomposition of organic matter is another aspect of aquatic heterotrophy that contributes to BOD and that was not specifically investigated in our work. Limited field investigations have demonstrated enhanced fungal growth in the field in nutrient-rich waters (Jenkins and Suberkropp 1995, Padgett et al. 2000). In controlled microcosm experiments, Suberkropp (1998) found that additions of N or P alone or of both together yielded enhanced conidium production, fungal biomass as ATP, and greater leaf material weight loss compared with controls. This suggests another pathway in which P (or N) inputs can contribute to BOD.

Another important question stemming from this research is how water body protection from these impacts can be strengthened. A major proportion of P loading, as well as significant fecal bacterial loading to water bodies, comes from non–point-source runoff in association with suspended sediments (figures 1 and 2, table 1a, 1b). Erosion, and stormwater runoff in general are issues for which proven technologies exist (generally, under the terms *best management practices* or *stormwater control measures*). Such technologies need broader distribution and stronger encouragement and enforcement in both urban and rural areas. We also note that tertiary P treatment of wastewater is not universal; rather, it gets mandated only in watersheds draining to receiving waters that are known to host algal blooms stimulated by P. As such, present efforts to protect water bodies from P loading are based solely on whether or not algal growth in a given system is limited by P. Scientists and managers need to move beyond this paradigm to account for the effects of P loading on other ecosystem and human environmental issues.

The hidden impacts of P loading to water bodies

Significant positive field correlations between total P and BOD in a variety of Coastal Plain lotic and lentic systems suggest an influence of P on hypoxia in these aquatic ecosystems. Targeted nutrient addition experiments using water from several different Coastal Plain watercourses demonstrated significant P stimulation of bacterial growth and associated increases in BOD and ATP concentrations as ecosystem-level effects. From a management perspective, the findings indicate that in systems affected by low dissolved oxygen, the direct contribution of P enrichment to hypoxia must be considered when devising total maximum daily loads required for remediation of impaired waters.

Both nutrient addition and dilution experiments showed a positive effect of P enrichment on fecal bacteria survival and reproduction, at concentrations typically found in human-affected water bodies. The significant P stimulation of fecal bacteria indicates that P enrichment can exacerbate the impacts of fecal microbial pollution on shellfish fishing and recreational waters as a human health concern. In systems where P does not directly stimulate noxious algae, it should nonetheless be included in management considerations, because it significantly contributes to other important water quality issues well beyond harmful algal blooms.

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