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Linking soil erosion to instream dissolved phosphorus cycling and periphyton growth

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Abstract

Phosphorus (P) is a limiting nutrient in freshwater systems and when present in runoff from agricultural lands or urban centers may contribute to excessive periphyton growth. In this study, we examined the link between soil erosion and delivery of eroded soil to streams during flow events, and the impact of that freshly-deposited soil on dissolved reactive P (DRP) concentrations and periphyton growth under baseflow conditions when the risk of stream eutrophication is greatest. A microcosm experiment was designed to simulate the release of P from soil which had been amended with different amounts of P fertilizer to overlying water during base flow conditions. Unglazed tiles, which were inoculated for 5 days in a second order stream, were incubated for 7 days in microcosms containing soil with eight levels of soil Mehlich-3' plant available phosphorus (M3P) ranging from 20 to 679 mg/kg M3P. Microcosm DRP was monitored. Following incubation tiles were scraped and the periphyton analyzed for chlorophyll *a*. Microcosm DRP concentrations increased with increasing soil M3P and equilibrium phosphorus concentration (EPC_0). Relationships between M3P, EPC_0 and DRP were non-linear and increases in soil M3P and/or DRP had a greater impact on biomass accumulation when these parameters were above threshold values of 30 mg/kg M3P and 0.125 mg/L DRP. Significantly, this ecological threshold corresponds to the agronomic thresholds above which increased soil M3P does not increase plant response.

Key terms: Fluvial-sediment; phosphorus; ecology; freshwater; agriculture; diffuse pollution; chlorophyll *a*.

Introduction

Phosphorus (P) is a key limiting nutrient of primary production in many freshwater systems whose biological availability degrades water quality and limits commercial and recreational water use (Schindler et al., 2008). Remedial efforts to address this have focused on the control of point-sources, particularly nutrient loading from urban wastewater treatment plants (Jarvie et al., 2006b; Bowes et al., 2010; Neal et al., 2010) and nutrient and sediment losses from agricultural lands (Jarvie et al., 2013b; USDA NRCS, 2012a,b). However, the measurement of water quality improvements resulting from changes in agricultural management is complicated by "legacy effects", which may delay any response to improvements in management practices (Schulte et al. 2010, Jarvie et al. 2013a,b). Streambed sediment and freshly-deposited soil in a streambed may act as a P sink or source depending on stream dissolved reactive phosphorus (DRP), sediment P sorption capacity, and degree of P saturation of the soil (Dodds 2003, Jarvie et al., 2005, 2006a). "Legacy P" is the term given to a portion of P that accumulates at various points throughout the transport pathways within the terrestrial-freshwater continuum (Sharpley et al. 2013). Accumulation of legacy P in soils, or along hydrologic flow paths, may take years and, in many instances, decades to return to equilibrium levels (Jarvie et al. 2013a, Haygarth et al. 2014, Powers et al., 2016).

Periphyton are assemblages of algal species, typically diatoms, filamentous green algae, and cyanobacteria growing on, or attached to stream and river substrates, such as sediment, woody debris and rocks (Stevenson et al. 1996; Larned 2010). Periphyton comprise a species-rich group of microalgae, which are considered to be important primary producers at the sediment-water

interface within riverine systems (Giller and Malmquist 1998, Scott and Marcarelli 2012). Periphyton produce oxygen at the sediment surface. Thereby they reduce P transport from deposited sediment as a result of anaerobic P release (Palmer-Felgate et al., 2010), and serve as a major source of food for invertebrates (Adey et al. 1993, Giller and Malmquist 1998, Brönmark and Hansson 2005). Phosphorus is often a limiting element for periphyton (Scott et al. 2009) and periphyton can play an important role in P cycling through assimilating P from the water column (Jarvie et al. 2002). Periphyton also influence the exchange of P across the sediment/water interface (Drake et al. 2011). Periphyton can also intercept P released from benthic sediments which increases P deposition through altering biochemical conditions within the river system (Dodds 2003, Withers and Jarvie 2008). In addition periphyton can trap particulate material from the water column (Adey et al. 1993).

However, excess inputs of P from anthropogenic sources (Dodds et al. 1997, Shilling 2007), together with high water temperatures during low flow periods, may result in excessive periphyton growth in riverine systems (Hilton et al. 2006, Bowes et al., 2007, 2012). Excessive periphyton growth can negatively impact streams and rivers through changes in particulate and dissolved organic carbon (C) budgets, nutrient cycling, biological and chemical oxygen demand, pH (Shilling 2007), and loss of macrophyte and invertebrate communities (Flynn et al., 2002; Hilton et al. 2006). Periphyton communities vary compositionally with changing nutrient levels, responding rapidly to changes in environmental conditions. Consequently, they may act as ecological indicators of increasing nutrient concentrations, particularly those caused by anthropogenic disturbances (Shilling 2007, Stone et al. 2012). The rate of periphyton accumulation such as cell volume, number of cells, and biomass of periphyton per unit area

106 (Bowes et al, 2007; McCall et al., 2014; Hilton et al. 2006, van der Valk 2012), are commonly
107 used metrics to estimate the degree of eutrophication within an aquatic ecosystem.
108
109 Understanding the P release characteristics of deposited soil particles provides the key link
110 between non-point P sources delivered especially during the spring/summer storm events (times
111 of greatest stream eutrophication risk) (Stamm et al, 2014). This study makes the crucial link
112 between soil erosion and delivery of eroded soil to streams during flow events, and the impact of
113 that freshly-deposited soil on dissolved P concentrations and periphyton growth under low flows.
114 The direction and extent of exchange of P between sediment and stream water can be estimated
115 from the relationship between DRP concentration within the stream and equilibrium P
116 concentration (EPC_0) of suspended and deposited sediment (Jarvie et al. 2005). Sediment EPC_0
117 is defined as the aqueous phase P concentration at which no net P adsorption or desorption by
118 sediment occurs (Haggard et al. 1999, Taylor and Kunishi 1971). The combination of EPC_0 and
119 soil P status, which is measured using Mehlich-3 P (M3P) extraction, accounted for over half of
120 the variability in DRP concentrations in 22 Ozark streams, USA (Haggard et al. 2007),
121 suggesting that M3P might be a suitable predictor of P uptake or release from soil. Soil M3P
122 tests are routinely used in laboratories throughout the world for soil P management decisions. It
123 can be used for all soils and has been used by researchers as a surrogate test for sediment P
124 availability to represent legacy sources of P that can become available with time (i.e., 1 to 2
125 years) (Haggard et al., 2007). Several studies have examined this relationship for stream
126 sediments (McDowell and Sharpley 2001, McDowell and Sharpley 2003, Ekka et al. 2006).
127 Although these studies were generally limited by the M3P range of the selected stream sediments
128 in which they observed increased DRP concentrations with increasing M3P and EPC_0. Typically

129 stream sediment M3P concentrations range from 2.7 to 39 mg/kg M3P, while soil M3P
130 concentrations can range from 0.01 to in excess of 900 mg/kg (Table 1).
131
132 Stream-bed sediments typically reflect an unknown depositional history and rapidly reach
133 equilibrium with the overlying river water (Haggard and Stoner, 2009). Freshly deposited
134 agricultural soils may pose a greater risk to water quality than stream sediments as such soils
135 typically have higher soil M3P and EPC_0 than stream sediments (Sharpley et al., 1996). During
136 erosion and transport to the stream channel, soils undergo particle sorting (Sharpley, 1985), with
137 changes in particle size distributions, having a potential impact on P-sorption properties of the
138 deposited soils. In the current study, a fine silt loam soil with relatively low M3P (20 mg/kg),
139 enriched with P to achieve a range of M3P values from 20 to 679 mg/kg M3P, was used to
140 determine the impact of freshly-deposited agricultural soil on periphyton biomass accumulation.
141
142 This study was undertaken in order to test the hypothesis that sediment-bound P stimulates
143 periphyton growth through sorption/desorption processes within the aqueous solution, and
144 examined: (1) the impact of soil M3P and EPC_0 on P release from soil to overlying-water and (2)
145 the effect of release P on periphyton biomass and nutrient stoichiometry.
146
147 **Materials and Methods**
148
149 *Study Approach*
150
151 The conceptual framework shown in Fig. 1 was developed to define the study hypothesis based
152 on typical chemograph data (Richards et al., 2001; Jordan et al., 2007; Stamm et al., 2014).

153 Specifically we aimed to determine if P bound to eroded soil deposited during storm flow events
154 stimulated periphyton growth through P sorption/desorption processes during baseflow
155 conditions (i.e. sustained low flows during spring and summer). During storm flow suspended
156 sediment (SS) and DRP increase with increasing flow rate (Q). As Q decreases, SS and TP
157 concentrations which comprise of particulate P (PP) and DRP decrease as sediment is deposited
158 on stream bottoms. This study addresses a key research gap in understanding the links between P
159 bound in soils deposited on stream beds, P release from deposited soil to stream water and the
160 impacts of P release on periphyton biomass and nutrient uptake.
161
162 A microcosm experiment was designed to simulate the release of P from soil deposited in a
163 stream to overlying water during base flow conditions. Unglazed mosaic tiles were inoculated for
164 5 days in Mud Creek Tributary which is a low nutrient second order stream that has been
165 extensively characterized by Rogers et al. (2011). Following this, soil enriched with different soil
166 M3P concentrations were added to microcosms and allowed to equilibrate before inoculated tiles
167 were incubated in the microcosm. A Pembroke silt loam soil was chosen because it represents
168 the main soil type under agricultural use in the Mud Creek watershed, and thus, dominates soil-
169 related processes occurring in this watershed. Such microcosms are commonly used in nutrient
170 cycling studies (Drake et al., 2012; Scott et al., 2013) and while they do not replicate exact
171 stream conditions they have the advantage of allowing for a wide range of treatments to be
172 examined in controlled conditions.
173
174 *Soil Preparation*
175

A Pembroke silt loam soil was collected from the Research Farm, University of Arkansas, Fayetteville, Arkansas (36°5′50″N, 94°10′44″W). The upper 10-20 cm depth of soil was collected and air dried before being sieved to pass through a 4-mm sieve. The upper 10 cm was discarded to minimize the inclusion of grass roots. Following this the loam soil with native M3P concentration 20 mg/kg (labeled M3P20 hereafter) was spiked with different concentrations of superphosphate (9 g/kg total phosphorus (TP)) fertilizer. Fertilizer was added to increase M3P concentrations to 23, 30, 44, 62, 97, 187, 428, and 679 mg/kg (after McDowell et al. 2011). It was then incubated for 168 days with periodic soil wetting to maintain an approximate soil moisture content of 30% by weight approximately equivalent to saturated field moisture. Sub-samples of soil from each of the nine M3P levels were air dried and sieved (<2 mm) and plant available soil P was determined by M3P extractant (Mehlich 1984). Soil EPC_0 was determined using the procedure described by Haggard et al. (2007). Two grams of air dried soil were added to 50 mL of deionized water, spiked with potassium dihydrogen orthophosphate (KH_2PO_4) to give DRP concentrations of 0, 0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/L before shaking in a reciprocating shaker for 1 h. The soil and water suspension was then allowed to settle for approximately 30 min before filtering supernatant through 0.45 μm membrane filter. Following this DRP was determined colorimetrically after Murphy and Reily (1962). The amount of P absorbed per dry weight soil (mg P/kg soil) was plotted against initial P concentration of the standard solutions (mg/L) and sediment EPC_0 was estimated as the x-intercept of the linear portion of this plot (after Haggard et al. 2007)**.**

Periphyton inoculation and analysis

Two 300 x 300 mm mosaic tiles, each containing thirty-six 50 mm square unglazed tiles, held together with flexible unreactive bonding material, were placed on the sediment surface in Mud Creek Tributary (N 36° 06' 45", W 94° 07' 24", Fayetteville, Arkansas, USA) on November 28th, 2012 as shown in Fig. 2a. Water within Mud Creek Tributary had low DRP (0.001 mg/L) and TP (0.024 mg/L) concentrations, with oligotrophic levels of chlorophyll-*a* (0.08 µg/cm) measured on the inoculated tiles, typical for a mid-order stream with low nutrient inputs. Periphyton were allowed to accumulate on the tiles until a film of periphyton was visible (five days), after which they were transported to the laboratory for a benchtop experiment. The bonding material holding the tiles together in the mosaic was cut using a razor blade, and tiles with grazers (macroinvertebrates) or outliers (approximately 12 tiles that were visibly different, or damaged) were excluded from the study.

Periphyton were scraped from ten arbitrarily selected tiles (50 mm x 50 mm) using a stiff-bristled brush and then rinsed with aerated tap-water to form a periphyton slurry. The composite slurry total volume was recorded and the slurry was divided into four subsamples for periphyton chlorophyll *a* estimation, and determination of total periphyton P, C, and nitrogen (N) (each conducted in duplicate). Chlorophyll *a* was measured as a proxy for biomass accumulation. It was determined in duplicate by filtering the chlorophyll *a* subsample onto non-muffled Whatman GF/F filters and freezing, before chlorophyll *a* concentration was determined using a Turner Fluorometer (APHA 2007). Periphyton P content was determined by adding peroxodisulfate, boric acid and sodium hydroxide to samples before autoclaving at 550°C. Phosphorus content of digestate was determined colorimetrically using the ascorbic acid method (APHA 2007). The remaining subsample was filtered onto pre-muffled Whatman GF/F filters (500°C for 4 h to

desiccate carbon on filter) and frozen. The frozen filter discs were dried for 24 to 48 h (50°C) and analyzed for C and N content with a Thermo Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Delft, The Netherlands). The ratios of C:P, C:N and C:Chlorophyll *a* (an adapted form of the autrophic index used by Drake et al., 2011) were calculated for analysis of periphyton stoichiometry. Nutrient limitation status was inferred from stoichiometric ratios based on the following: C:P > 180 and N:P > 22 indicating P limiting conditions; C:P > 10 and N:P < 13 indicating N limiting conditions (Hillebrand and Kahlert 2001).

Stream water grab samples were taken less than 1 m upstream of the tiles three mornings during the five-day inoculation period (days 1, 2 and 5) to record water quality conditions in Mud Creek during inoculation. Upon collection, samples were transported to the laboratory and stored at 4 °C until water quality analysis was completed (within 24 h). Samples were filtered through 0.45 µm filter paper and analyzed colorimetrically for DRP, NO_3-N, chloride (Cl), sulphate (SO_4) and NH_4-N. Dissolved reactive P and TP (following persulphate digestion of unfiltered sample) were determined colorimetrically after Murphy and Reily (1962). Nitrate-N, Cl, SO_4 and NH_4-N concentrations were determined using ion chromatography (Dionex ICS-1600) and turbidity was determined using a turbidimeter (WTW Turbo 550). Following combustion, TN was determined using ion chromatography and TOC and DOC were analysed following the EPA-600/4-79-020 procedure (EPA 1979). All samples were analyzed in accordance with Standard Methods (APHA 2007).

Microcosm Experiment

245 A microcosm experiment was designed to simulate the release of P from soil deposited in a
246 stream to overlying water during base flow conditions. The nine levels of soil M3P were
247 examined in triplicate. For each microcosm, twenty grams of air-dried soil were added to a 1 L
248 laboratory beaker (27 beakers), before adding 700 mL of aerated tap water with pH of 8.3, DRP
249 of 0.001 mg/L and NO_3-N of 0.86 mg/L. The soil and water were allowed to equilibrate for 72 h
250 (Fig. 2b), before being amended with NO_3-N (as KNO_3) to achieve a concentration of 2.5 mg
251 NO_3-N/L in the overlying water (to ensure that NO_3-N did not limit periphyton accumulation
252 even at high P concentrations). One unglazed tile inoculated with periphyton from Mud Creek
253 Tributary was then placed in each microcosm (t = 0 d). The tiles were suspended 25 mm above
254 the soil surface using non-reactive supports, and care was taken to minimize suspension of soil
255 particles into the overlying water. The microcosms were placed in a temperature-controlled
256 laboratory (20°C) and artificial lighting (> 500 $\mu E/m^2/S$) with 12 h day / 12 h day night cycle for
257 168 h (Fig. 2c). This temperature was chosen as it is representative of Ozark streams during
258 spring/fall, where temperatures generally vary between 17 and 25°C. Aerated tap water was
259 added daily by hand to replenish evaporative losses and 30 mL samples were collected from
260 mid-depth of the water overlying the tile at 0, 1, 2, 3, 5, and 7 days after the start of incubation.
261
262 All samples were filtered immediately using 0.45-µm filters and analyzed within 24 h for DRP.
263 The DRP mass in overlying water was calculated taking into account the dilution effect caused
264 by addition of water to replenish samples removed as the experiment progressed. Nitrate
265 concentrations in the overlying water of selected microcosms (M3P20, 23, 62, 427 and 679
266 treatments) were measured by sampling overlying water throughout the experiment to ensure that
267 NO_3-N was not limiting. The experiment was terminated after 168 h, and the tiles (Fig. 2d) were

268 removed from the microcosms. Periphyton biomass was calculated by quantifying the amount of
269 chlorophyll *a,* total carbon, N and P on each tile. The ratios of C:P, C:N and C:Chlorophyll *a*
270 were calculated to determine the effect of treatment on periphyton stoichiometry.
271
272 Microcosm experiments allow controlled experiments, with full replication and have been used
273 to examine nutrient cycling in streams (Drake et al., 2012; Scott et al., 2013; Rodriguez Castro et
274 al., 2015). This study is unique in that these microcosm experiments were used to simulate the
275 effects of freshly deposited agricultural eroded soils, whereas most microcosm incubations use
276 stream/wetland sediments which have already undergone a period of equilibration in the
277 stream/wetland environment (Reddy et al., 1999; Wang et al., 2013; Li et al., 2013; Lin et al.,
278 2015). This design allows for examination of a wide range of soil M3P quickly and ensures that
279 all other factors including are constant.
280
281 *Statistical analysis*
282
283 Linear regression analysis was conducted on chlorophyll *a*, M3P, EPC_0, DRP (at start of
284 incubation (t=0)), periphyton total carbon, periphyton total nitrogen and periphyton total
285 phosphorus. For the relationship between EPC_0 and M3P, the linear model was fit using
286 log(EPC_0) and log(M3P) and the results were back-transformed for presentation. The
287 relationships between DRP and M3P as well as between DRP and EPC_0 were also fit on the log-
288 log scale and then back-transformed for presentation. Significant relationships were plotted and
289 equations presented in results section. Logarithmic transformations were required for DRP, M3P,
290 EPC_0 and periphyton total phosphorus data, which were not normally distributed. Quantile plots

for the studentized residuals were used as a graphical check for normality. Least square difference analysis was used to allow comparisons between treatments. Piecewise regression was used to determine breakpoints in the relationships between DRP and M3P and chlorophyll-a. All statistical analyses were performed using SAS 9.1 (SAS 2004).

Results and Discussion

Phosphorus release from soil

Dissolved reactive P concentrations in overlying water were positively related to P levels in deposited soil, with DRP concentrations of 0.009 mg/L for the lowest treatment (M3P20) and 1.61 mg/L for the highest treatment (M3P679) after the 72-h equilibrating period (i.e. at the start of the incubation) (Table 2). In addition to the linear plots between DRP and soil M3P/EPC_0 these relationships were plotted logarithmically (Fig. 3a and Fig. 4a, respectively) to demonstrate the nature of the relationship between soil EPC_0 and M3P concentrations and overlying water DRP at low concentrations. Logarithmic plots magnify the response of DRP to a relatively small increase in soil EPC_0 /M3P which was of particular interest since threshold responses have been reported when correlating soil M3P and runoff DRP in rainfall simulation (Vadas et al., 2005) and laboratory P release studies (Mulqueen et al., 2004). Soil M3P and EPC_0 were positively correlated, with a gradual increase in soil EPC_0 per unit change in soil M3P. The best fit model (p<0.001) was:

$$EPC_0 = 0.00047(M3P)^{1.64} \quad [1]$$

315 Microcosm DRP and soil M3P were positively correlated (Fig.3b) and the best fit model
316 (p<0.001) was:
317
318 $DRP=6.5x10^{-5}(M3P)^{1.59}$ [2]
319
320 DRP and EPC_0 were positively correlated (Fig. 4b) and the best fit model (p<0.001) was:
321
322 $DRP=0.112(EPC_0)^{0.96}$ [3]
323
324 Relationships between M3P, EPC_0 and DRP were non-linear and DRP release from soil increased
325 exponentially with soil M3P values. These findings were similar to those reported by Rogers et al.
326 (2011). Rogers et al. (2011) examined the relationship between M3P and DRP for five streams in
327 the Upper Illinois River Watershed (slope: 0.0016, R^2=0.75). Haggard et al. (2007) reported a
328 slope of 0.020 between M3P of benthic sediments and stream water DRP. The EPC_0 of the M3P679
329 treatment was 19 mg/L which was an order of magnitude greater than the sediment EPC_0 in similar
330 streams in Arkansas (Ekka et al., 2006; Haggard et al., 2007). Sediment EPC_0 has been reported
331 to vary from -0.62 mg/L (Smith et al., 2009) to a max of 6.99 mg/L reported downstream of a
332 wastewater treatment plant discharge point (Ekka et al., 2006). These results were also in
333 agreement with findings of field runoff studies (Vadas et al., 2006). In a meta-analysis of runoff
334 studies (rainfall simulation, field, etc.), Vadas et al. (2005) observed a similar break-point
335 relationship between soil P sorption capacity and surface runoff DRP concentrations in a field-
336 scale runoff study. Sims et al. (2002) demonstrated that soil P sorption capacity was strongly
337 correlated with soil M3P (R^2=0.72) in rainfall simulations studies. This was consistent with similar

findings in column leaching studies (Maguire and Sims, 2002) and rainfall simulation studies (Torbert et al., 2002). Recently eroded sediments which possess higher soil M3P levels than stream streams, may pose a risk to water quality during storm events if they are located in a critical source area, a zone of frequent runoff generation that readily connects high P sources in soils to streams (Thompson et al., 2012).

Impact of DRP released from soils on periphyton accrual

Introduction of inoculated periphyton tiles to the microcosms resulted in a general decrease in overlying water DRP concentrations during the 168-hr incubation (Table 2). This was not significant for M3P20, 23, 30, 44, 97, and 187 treatments, while DRP concentrations in M3P428 and 679 treatments were significantly lower at the end of the study (compared to t=0) (Table 2). Overall, trends showed a sharp decrease in DRP during the first 24 h of incubation (ranging from <0.001 mg DRP/h (M3P20 treatment) to 0.057 mg DRP/h (M3P679 treatment), followed by a slower decrease in DRP over the remaining 144 hours (<0.001 mg DRP/h). Overlying water DRP concentrations decreased for all microcosms with the exception of M3P97 (72 h sample), M3P428, and M3P679 (120 h samples). In these microcosms, DRP was observed to increase between the 72 h and 120 h sampling events and decrease for the remainder of the experiment. This may have been a result of an observed die-off of periphyton between 72 h and 120 h, followed by a recovery of periphyton (i.e. some of the initial periphyton observed to change colour and new periphyton developed on tile). However, DRP concentrations at 0 h observed in microcosms receiving M3P97 (0.078 mg/L), 187 (0.242 mg/L), 428 (0.637 mg/L) and 679 (1.61 mg/L) treatments were significantly higher than those observed in the low M3P treatments with differences between these treatments also statistically significant (p<0.01). Standard deviations

were significantly higher in the case of the higher treatments which was perhaps indicative of the level of variability in soil M3P. The average NO_3-N concentration of overlying water in the microcosms was approximately 1.68±0.24 mg/L after 24 h, before gradually decreasing to 0.219±0.283 mg/L at 168 h indicating that periphyton assimilated NO_3-N during the study.

Periphyton biomass was greater in the microcosms with higher DRP concentrations (Fig.5) and soil M3P (Fig. 6). Periphyton biomass followed a log function, with large increases in chlorophyll *a* concentrations occurring in response to small increases in DRP, followed by potential P-saturation of the periphyton and little change in chlorophyll *a* concentrations even with large increases in DRP. These findings were in agreement with previous work that used nutrient diffusion substrates to directly link nutrient availability to periphyton biomass in streams (Lang et al. 2012). There was a three-fold increase in chlorophyll *a* biomass when overlying water DRP at the start of the study increased from 0.009 to 1.61 mg/L These results are similar to flume experiments conducted by Bowes et al. (2012), who reported chlorophyll *a* levels between 8 to 12 µg/cm^2 (DRP concentrations between 0.03 and 0.373 mg/L) in river studies, which was higher than that observed in the current study (0.2 to 1.5 µg/cm^2). This was likely due to the fact that P immobilized by periphyton was not replaced by an incoming P flux, as would occur in a running stream.

The soil M3P was positively correlated with chlorophyll *a* and total periphyton C, N, and P (Table 3). There was a sharp increase in chlorophyll *a* per unit area of tile in response to increase and overlying water DRP (Fig. 6) followed by a plateau level of chlorophyll *a* (approximately 0.9 µg/cm^2), above which there was no increase - even when DRP increased significantly. Total

384 periphyton P was strongly correlated with chlorophyll *a* (R^2=0.72), with both having a similar
385 relationship with DRP, where an initial steep slope was followed by a plateau. Total periphyton C,
386 and N generally increased with increases in overlying water DRP; however, concentrations were
387 not strongly correlated with overlying water DRP or chlorophyll *a*. Nitrate-N concentrations were
388 not limiting during the study, with the exception of the possibility of NO_3-N limitation for the high
389 P treatments between the 120 and 168 h samplings.
390
391 *Threshold soil M3P and water DRP values*
392
393 A key finding of this study is the threshold response of chlorophyll-*a* to soil M3P and DRP
394 concentrations. While piecewise regression did not allow determination of a threshold DRP value
395 using the data shown in Fig. 5, it was possible to determine a threshold M3P of 30 mg/kg using
396 data shown in Fig. 6. The difficulty obtaining a breakpoint for Fig. 5 data using piecewise
397 regression was likely a result of the relatively large number of similar DRP concentrations
398 observed for the lower M3P treatment. Using Equation 2, a threshold value of 0.125 mg/L DRP
399 was determined. The threshold values observed in this study are specific to a Pembroke soil in an
400 artificial environment (i.e. microcosm). Following from this the level of response and threshold
401 value will vary between soils with a range of possible threshold values. The DRP threshold of
402 0.125 mg/L is higher than the 0.075 mg/L TP mesotrophic-eutrophic boundary suggested by Dodds
403 et al. (1998) and the upper threshold reported by Evans-White et al. (2013) in a review of stream
404 nutrient criteria development in the US, which presented P threshold values of between 0.006 and
405 0.074 mg/L. This value was also greater than the biological breakpoint with median concentrations
406 of TP (0.033 mg/L) observed by Crain and Caskey (2010). Bowes et al 2007 reported a threshold

of 0.090 mg P/L on the River Frome in the UK. In recent unpublished work on the Hampshire Avon a threshold of ~0.11 mg-P/L was observed (Bowes, *per com*.). This indicated that the threshold observed in the current study is reasonable.

The results are in agreement with P runoff studies and show that soils with an M3P greater than approximately 30 mg/kg pose a risk to water quality both directly (when deposited in stream) and indirectly (when P mobilized in subsurface and overland flow; Sharpley et al. 1996). The existence of threshold or breakpoint relationships between soil M3P and water DRP is long established (Sharpley et al. 1995) and this study has now demonstrated threshold responses of chlorophyll-*a* to soil M3P and DRP concentrations for the soil examined in this study. This ecological threshold corresponds to the agronomic thresholds above which increased soil M3P does not increase plant response, typically between 30 and 70 mg/kg M3P (Sharpley et al., 1996). Future work must examine these relationships across a wide range of soils, sediments and climatic conditions. This current research emphasises the need to address P loss from critical source areas (areas with high connectivity and high soil M3P) within the landscape to mitigate both dissolved and particulate P losses to streams.

Concluding remarks/wider implications

These results have implications for catchment managers dealing with 'legacy P' within streams (Sharpley et al. 2013, Haygarth et al. 2014). The greatest risk of periphyton proliferation is under sustained low flows during spring and summer (Shilling 2007). Thus, if deposited soils release P to overlying water during this period which favours periphyton biomass accumulation, it could have a greater impact than P released during high flow periods (Withers and Jarvie 2008, Jarvie

et al. 2012). The findings demonstrates that the conceptual framework outlined in this paper accurately describes the release of DRP following deposition of soil in a stream following a storm flow event and the subsequent release and uptake by periphyton. These data suggest that increased soil M3P content within the watershed has the potential to increase available P in the sediment and overlying water, which is further supported by the observation of increased water quality degradation with increased human development (agricultural and urban land use; Giovaaneti et al. 2013).

While microcosms do not accurately replicate in-stream conditions as overlying water is stagnant, not reproducing flowing water environment, with implications for periphyton growth rates, waste accumulation, dissolved oxygen, redox, no replenishment of nutrients from upstream sources, or periphyton innocula from upstream (Jungmann et al., 2001). This allows development of robust relationships between M3P and DRP/chlorophyll *a*. This could not be readily achieved using in-stream studies, where controlling other variables affecting P dynamics is considerably more challenging. Future work must examine these processes in dynamic systems that allow water to flow over the periphyton and across a wider range of soils types.

This study highlights the risk of P release from eroded soil which is deposited in a stream bed and demonstrates that soil eroded from agricultural landscapes can lead to increased periphyton biomass. It may be beneficial for catchment managers to focus on reducing erosion of high P soils to prevent nuisance periphyton growth in streams. Relationships between M3P, EPC_0 and DRP were non-linear and DRP release from soil increased exponentially with soil M3P values. Small increases in M3P and/or DRP have a greater impact on biomass accumulation when these

parameters are below key threshold 0.125 mg/L DRP and 30 mg/kg M3P found in this study. Periphyton biomass followed a log function, with large increases in chlorophyll *a* concentrations occurring in response to small increases in DRP, followed by potential P-saturation of periphyton and little change in chlorophyll a concentrations even with large increases in DRP.

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695 **Table 1** Summary of Mehlich-3 phosphorus (M3P), equilibrium phosphorus concentration
696 (EPC_0) and dissolved reactive phosphorus (DRP) from previous studies.
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Reference	Sediment type	M3P (mg/kg)	EPC_0 (mg/L)	DRP (mg/L)	Comments
Current study	Soil	20 - 679	<0.001 -19	<0.001 - 1.98	Microcosm after 72 h equilibrium phase (no mixing)
McDowell and Sharpley, 2003	Stream	6.8 - 38.6	0.01 - 0.04	0.05 - 0.16	Laboratory re-circulating fluvarium after 24 h uptake phase
Haggard et al., 2007	Stream	2.7 - 19.4	<0.001 - 0.329	0.003 - 0.072	Catchment scale study examining 22 streams
Rogers et al., 2011	Stream	13 - 39		0.03 - 0.07	Field study
Ekka et al., 2006	Stream		< 0.01 - 6.99	<0.001 - 7.03	Catchment scale study examining sediment P downstream of WWTP's
McDowell and Sharpley, 2001	Stream	14	0.02		Field study
	Stream	22	0.043		
Sallade and Sims, 1997	Stream	3-62	0.02-0.28	0.04-0.74	Sediments from 17 ditches classified in Deleware
Sims et al, 2007	Soil	0.01-14.7		0-0.4	Runoff experiment
		0.1-75.6		6-10	Column experiment
Smith 1999	Stream	5.7-126	-0.616-0.2	0.001-0.177	Catchment scale study
Zhuan et al., 2009	Stream		0.031-0.052	0.02-0.25	Batch experiment
Palmer-Felgate et al., 2009	Stream		0.003-0.044	0.001-1.3	Stream-bed sediments from three catchments
Range		2.7 - 679	<0.001 -19	<0.001 - 10	Min - Max

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708 **Table 2** Mean dissolved reactive phosphorus (mg/L) in overlying water during the microcosm
709 experiment for each soil M3P level of soil added to the microcosm.

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Treatment	Time (hours)					
	0	24	48	72	120	168
M3P 20	0.009 (0.009)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.001 (0.001)	0.002 (0.002)
M3P 23	0.009 (0.001)	0.001 (0.001)	0.001 (0.001)	0.002 (0.002)	0.001 (0.001)	0.002 (0.002)
M3P 30	0.008 (0.001)	0.002 (0.002)	0.002 (0.000)	0.002 (0.001)	0.001 (0.001)	0.001 (0.001)
M3P 44	0.016 (0.006)	0.012 (0.006)	0.010 (0.005)	0.003 (0.001)	0.002 (0.004)	0.100 (0.001)
M3P 62	0.049 (0.004)	0.034 (0.016)	0.015 (0.006)	0.010 (0.005)	0.002 (0.002)	0.004 (0.008)
M3P 97	0.078 (0.068)	0.041 (0.022)	0.036 (0.023)	0.059 (0.042)	0.028 (0.024)	0.024 (0.029)
M3P 187	0.242 (0.110)	0.114 (0.058)	0.181 (0.163)	0.201 (0.119)	0.143 (0.102)	0.277 (0.281)
M3P 428	0.637 (0.306)	0.345 (0.184)	0.379 (0.207)	0.242 (0.010)	0.489 (0.256)	0.103 (0.091)
M3P 679	1.609 (0.611)	0.367 (0.322)	0.507 (0.012)	0.394 (0.120)	1.352 (0.608)	0.262 (0.093)

LSD to compare means at same M3P level = 0.010; LSD to compare means at different M3P level = 0.0109
Mean (standard deviations in parentheses); Minimum detection limit for dissolved reactive P analyses was ± 0.002 mg/L.

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Table 3 Characterization of periphyton following a 168 hour incubation period for each soil M3P level of soil added to the microcosm.

Treatment	Chlorophyll *a* content	Carbon total	Nitrogen total	Phosphorus total	C:P by moles	C:N by moles	N:P by moles	[1]AI (C:Chla)
	µg/cm^2	mg/cm^2	mg/cm^2	mg/cm^2				
M3P20	0.39 (0.08)	0.12 (0.03)	0.016 (0.004)	0.001 (0.001)	275 (15)	9 (12)	32 (6)	0.33
M3P23	0.52 (0.06)	0.16 (0.05)	0.023 (0.005)	0.002 (0.001)	246 (46)	8 (9)	30 (4)	0.32
M3P30	0.79 (0.06)	0.19 (0.03)	0.028 (0.005)	0.002 (0.001)	259 (30)	8 (8)	33 (7)	0.24
M3P44	0.79 (0.17)	0.15 (0.02)	0.022 (0.006)	0.003 (0.002)	166 (61)	8 (8)	21 (7)	0.20
M3P62	0.82 (0.60)	0.22 (0.06)	0.028 (0.005)	0.005 (0.001)	116 (58)	9 (9)	12 (1)	0.39
M3P97	0.76 (0.04)	0.17 (0.04)	0.024 (0.006)	0.005 (0.003)	138 (105)	8 (8)	17 (14)	0.23
M3P187	1.07 (0.19)	0.23 (0.04)	0.031 (0.006)	0.003 (0.001)	203 (84)	9 (8)	24 (11)	0.22
M3P428	0.90 (0.40)	0.19 (0.03)	0.028 (0.005)	0.006 (0.002)	100 (37)	8 (8)	12 (4)	0.24
M3P679	1.20 (0.32)	0.18 (0.04)	0.029 (0.007)	0.009 (0.002)	56 (3)	7 (7)	8 (1)	0.16

[1]Autotrophic Index (C:Chlorophyll *a*) (standard deviations in parentheses)

List of Figures

Fig. 1 Chemograph showing conceptual framework of changes in water quality parameters following a storm event (shaded area indicates the transition between storm and base flow conditions).

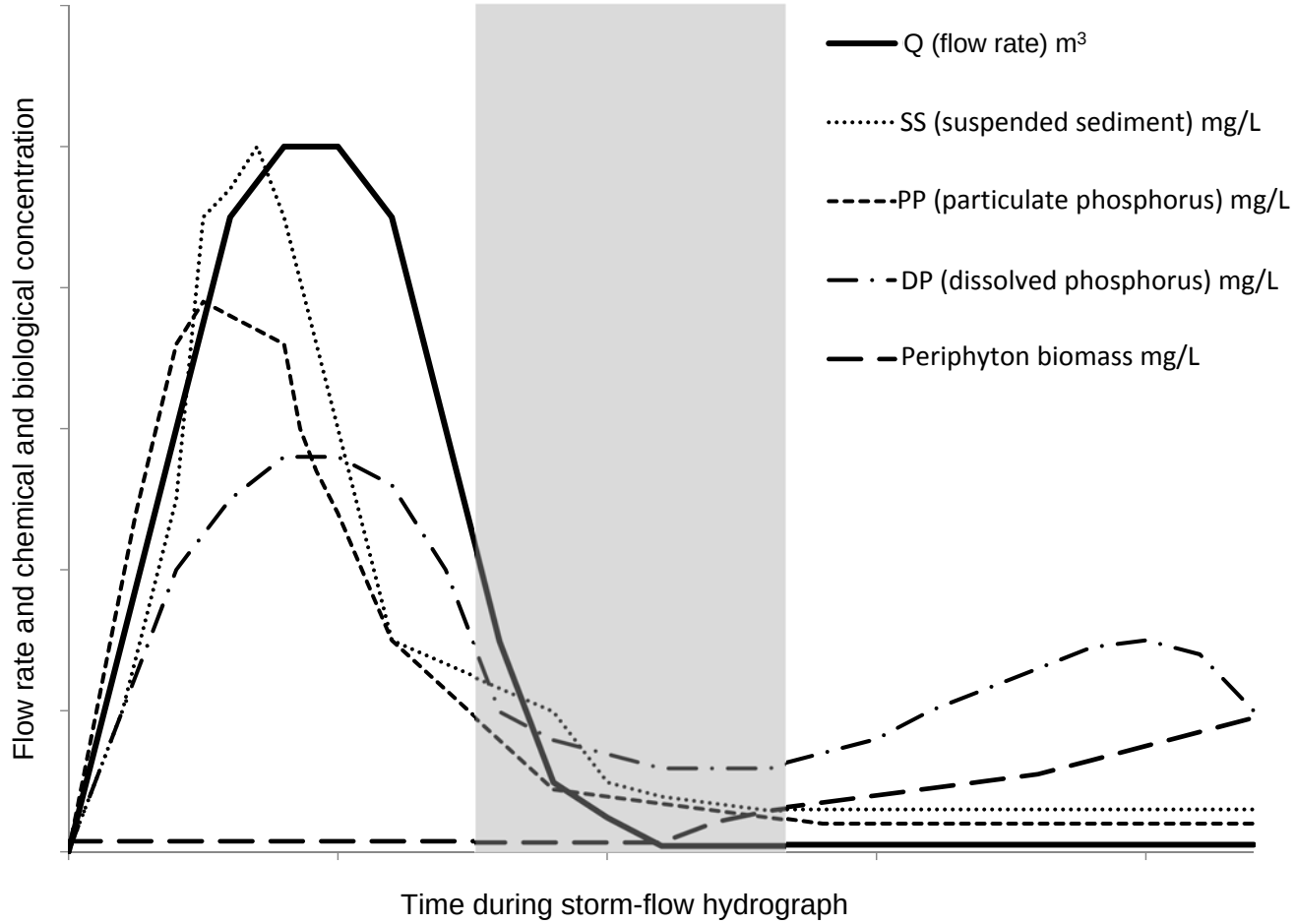
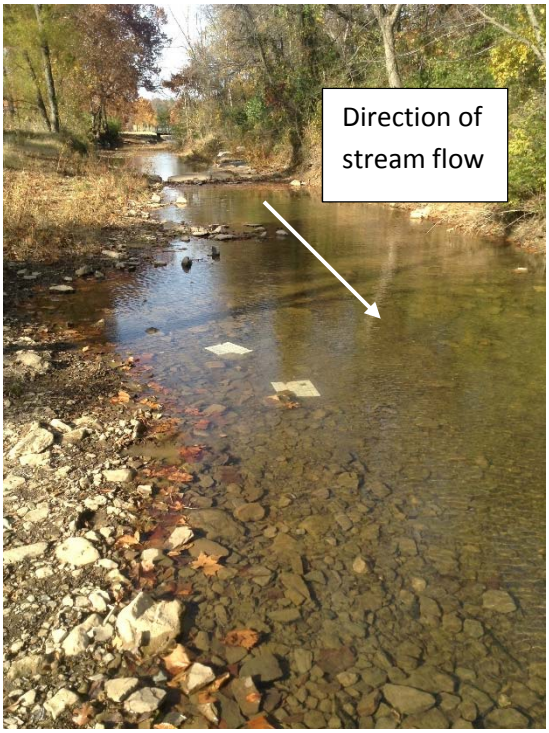


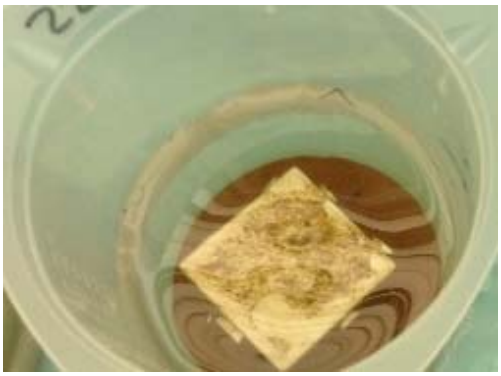
Fig. 2 Photographs showing (a) mosaic tile inoculation, (b) soil equilibration, (c) microcosm setup and (d) tiles at the end of the incubation period.



a. Photo of mosaic tiles inoculated for 120 hrs in Mud Creek in run in approx. 150 mm depth of stream water.

c. Mosaic tiles placed on soil and incubated for 168 hrs at approx. 20 °C and artificial lighting (>500 µE/m^2/S).

b. Soil placed in beaker, water added and mixture allowed to equilibrate for 72 hrs.



d. Following 168 hrs of incubation mosaic sols removed from microcosm and periphyton destructively sampled.

Fig. 3 Relationship between dissolved phosphorus (DRP) levels in microcosm water and soil Mehlich-3 extractable phosphorus (M3P) (a) logarithmic plot and (b) linear plot superimposed inside the logarithmic plot (same units for each graph).

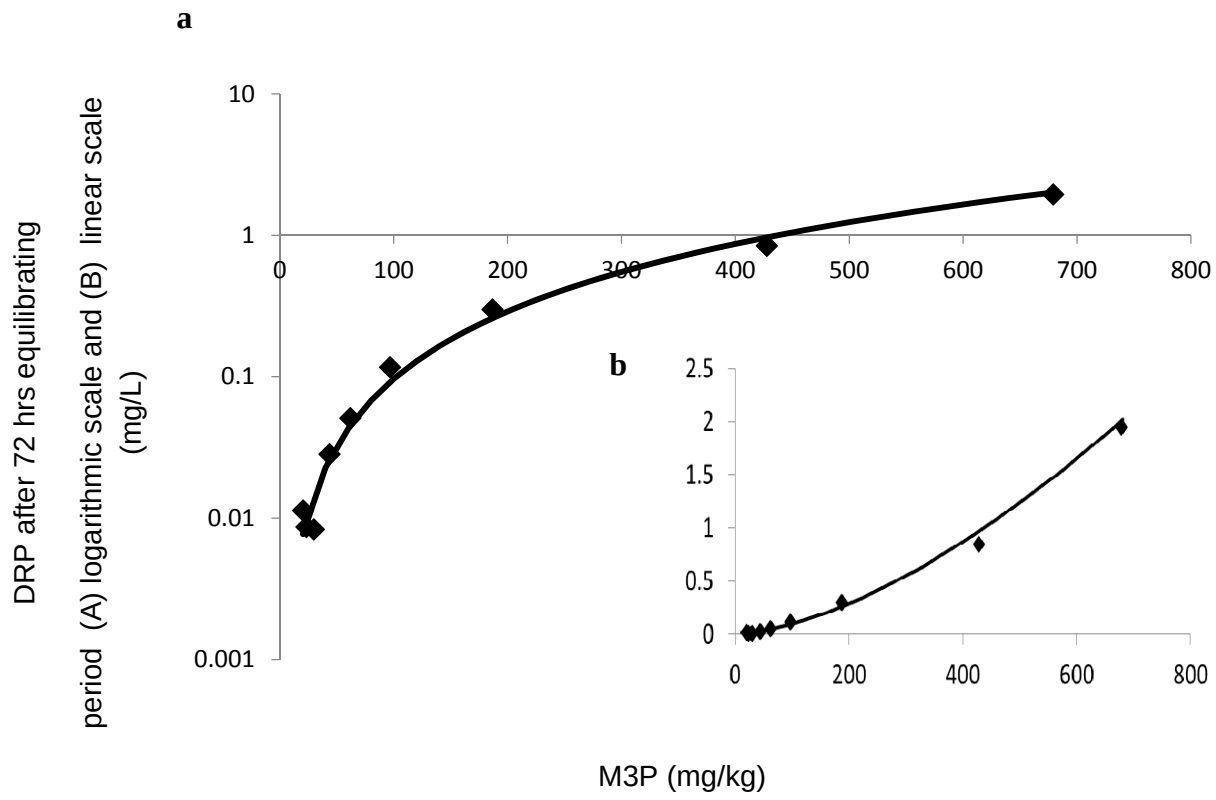


Fig. 4 Relationship between dissolved phosphorus (DRP) levels in microcosm water and equilibrium phosphorus concentration (EPC0) (a) logarithmic plot and (b) linear plot superimposed inside the logarithmic plot (same units for each graph).

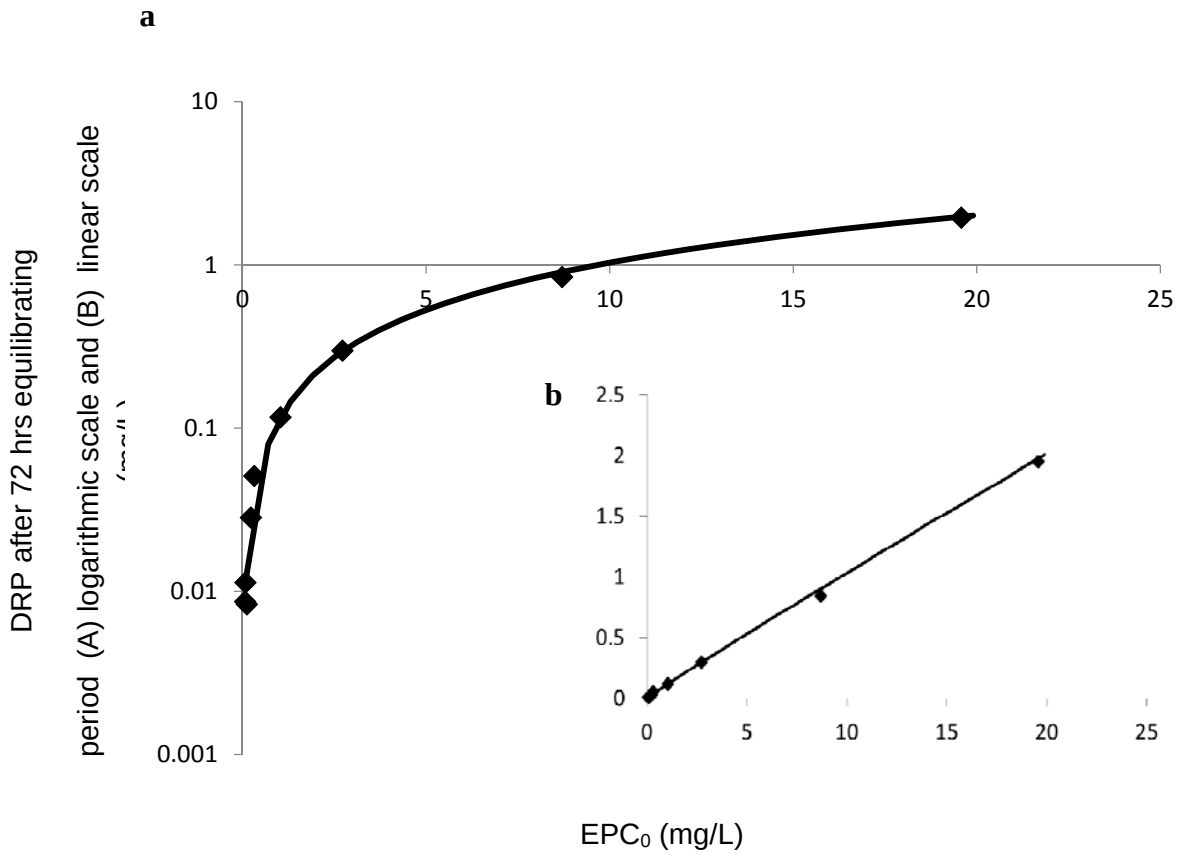


Fig. 5 Relationship between chlorophyll-a and dissolved reactive phosphorus (DRP) during the microcosm experiment with best fit moel.

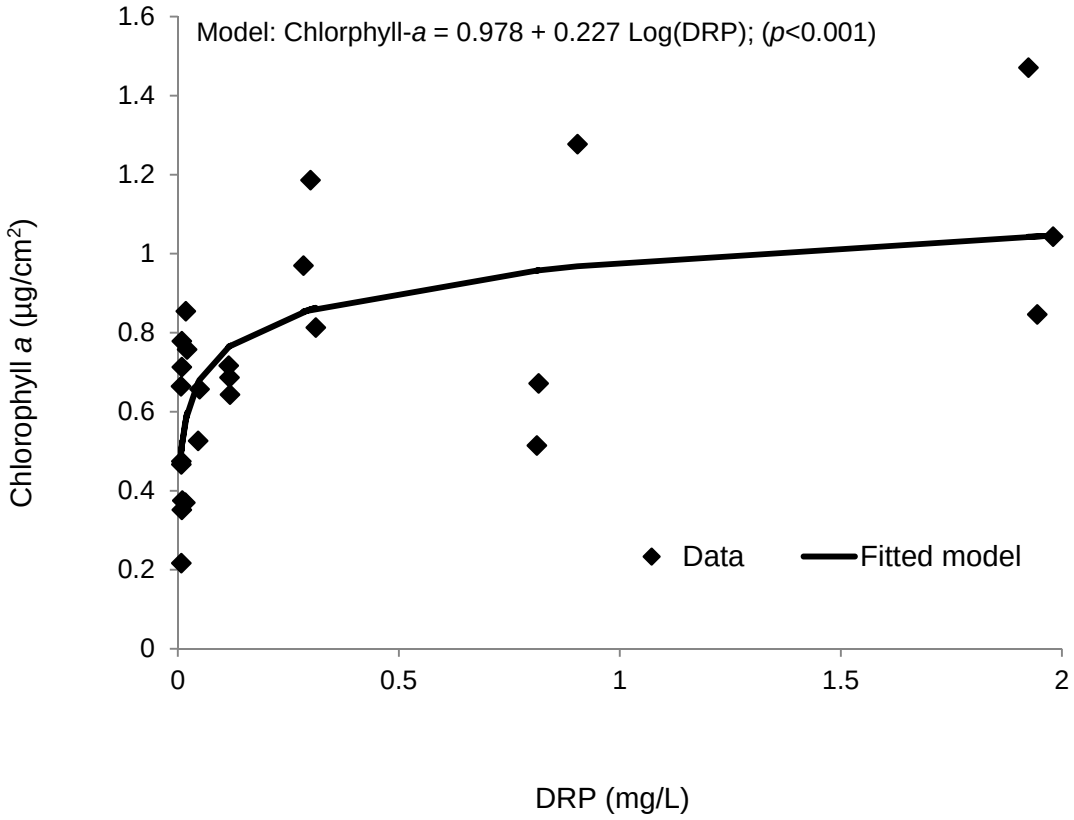

Model: Chlorphyll-a = 0.978 + 0.227 Log(DRP); (p<0.001)
Chlorophyll a (µg/cm2)
DRP (mg/L)
Data
Fitted model
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
0.5
1
1.5
2

Fig. 6 Relationship between chlorophyll-a and underlying soil Mehlich 3 (M3P) during the microcosm experiment with best fit model.