MDARD Horticulture Fund Project Title: Efficiency of Bioreactor Nutrient Remediation in the Presence of the Organophosphate Chlorpyrifos

2018 2nd Year Report

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The overarching goal of this study was to identify the effects that pesticide residues may have on bioreactors for agricultural runoff water treatment. Over the course of two years, simulated runoff comprised of nitrate (NO_3^-) and phosphate (PO_4^{3-}) – the major nutrients responsible for eutrophication – were supplied to a two-stage treatment system. The first stage was a woodchip bioreactor for NO_3^- removal and the second stage was an expanded/calcined aggregate for PO_4^{3-} adsorption. All of the treatment replications received the same nitrate/phosphate influent, with half of the treatment replications receiving the same nutrient influent supplemented with pesticides in order to identify changes in response to pesticide exposure. The efficacy of these treatment systems depends upon adequate contact time between the nutrient/pesticide laden simulated runoff and the treatment substrate – known as the hydraulic retention time. For the first year of the study, a hydraulic retention time of 3 days was selected and nutrient concentration and water chemistry were measured weekly to monitor remediation potential and changes within the system between bioreactors receiving a nutrient only influent and a nutrient influent supplemented with the organophosphate insecticide chlorpyrifos. In year two, the hydraulic retention time was reduced to between19-23 minutes, depending on minor fluctuations in flow rate, and the pesticide supplemented influent treatment was comprised of chlorpyrifos, bifenthrin, and oxyfluorfen. In year two, nutrient concentration and water chemistry conditions were again measured, as well as the concentration of chlorpyrifos, bifenthrin, and oxyfluorfen in treatment system effluent. Microbial samples were

harvested from bioreactors on the day preceding pesticide incorporation, the day following incorporation, ten days after initial incorporation, and at the termination of the study (37 days after application).

Year 1 Nutrient Remediation:

The first year of this study initiated on January 27th, 2017 and concluded on June 9th, 2017. During the first 14 weeks of the study, simulated runoff comprised of 90 $mg \cdot L^{-1}$ NO_3^- and 2.5 $mg \cdot L^{-1}$ PO_4^{3-} were supplied to the 20 bioreactor system replicates during the incubation stage – where the bacterial community could proliferate prior to pesticide exposure. After the incubation stage, chlorpyrifos was added to the simulated runoff for 10 of the bioreactor system replicates at a rate of 1 $mg \cdot L^{-1}$. Throughout the 151 day duration of the study, woodchip bioreactors consistently remediated influent concentrations of 90 $mg \cdot L^{-1}$ NO_3^- to below detection limits (0.2 $mg \cdot L^{-1}$) throughout the incubation stage, as well as following exposure to chlorpyrifos (Figure 1). While the addition of chlorpyrifos resulted in changes in oxidation-reduction potential and pH, the nitrate remediation capacity was unaffected. Phosphate remediation occurred for one of the expanded/calcined aggregates– the expanded shale product haydite. The other aggregate tested, a calcined clay product, was unable to effectively reduce phosphate concentrations in effluent – with periods of increased PO_4^{3-} export attributed to the initially high amounts of sorbed PO_4^{3-} being desorbed and continued equilibration between initial PO_4^{3-} and additional PO_4^{3-} throughout the study. The sequestration/export characteristics of PO_4^{3-} in both aggregates was maintained in the presence of chlorpyrifos (Figure 2). Conclusions drawn from the first year of this study indicate that with a 3 day hydraulic retention time NO_3^- can be effectively remediated using woodchip bioreactors and that PO_4^{3-} can be sorbed using expanded shale, and both processes are conserved in the presence of chlorpyrifos.

Year 2 Nutrient Remediation:

In year two, a reduced hydraulic retention time was selected in order to establish the kinetics of nutrient remediation in a manner more reflective of the quantity of runoff generated in nursery production. The study initiated on March 1st, 2018, and concluded on May 11th, 2018, with the incubation stage ending on April 5th 2018. Woodchip bioreactors were again used for the first stage, while the expanded shale was used as the sole secondary treatment. The experimental design supplied 1.3 L/minute of simulated runoff to each of the 20 bioreactor system replicates for 8 hours per day – resulting in a hydraulic retention time between 19-23 minutes, depending on minor fluctuations in flow rate. Similar to year one, an incubation period of nutrient only simulated runoff was provided to all bioreactors for system establishment (12 $mg \cdot L^{-1}$ NO_3^-, 0.75 $mg \cdot L^{-1}$ PO_4^{3-}), followed by the incorporation of pesticides thereafter and an increase in nutrient influent concentration (20 $mg \cdot L^{-1}$ $NO_3^-$and 1.5 $mg \cdot L^{-1}$ PO_4^{3-}). Under the reduced hydraulic retention time, nitrate remediation was not achieved within these systems – where influent concentrations were unable to be reduced (Figure 3). Similarly, PO_4^{3-} concentrations were unable to be reduced using this reduced hydraulic retention time in expanded shale aggregate reservoirs (Figure 4).

Year 2 Pesticide Remediation:

The pesticide concentrations supplementing the nutrient influent were 2 $mg \cdot L^{-1}$ chlorpyrifos, 500 $\mu g \cdot L^{-1}$ bifenthrin, and 500 $\mu g \cdot L^{-1}$ oxyfluorfen, respectively. Chlorpyrifos was effectively reduced to 500 $\mu g \cdot L^{-1}$ throughout the course of the study in the woodchip bioreactors, reducing influent concentrations by appx. 75% (Figure 5). Bifenthrin remediation was inconsistent, with effluent concentrations from the woodchip bioreactors reducing influent

concentrations to 200-400 $\mu g \cdot L^{-1}$ before export levels of 600 $\mu g \cdot L^{-1}$ (exceeding influent levels) was observed on the final sample date (Figure 6). Oxyfluorfen concentrations exiting woodchip bioreactors were reduced to between 300-400 $\mu g \cdot L^{-1}$ over the first three sample dates before effluent concentrations rose to 500 $\mu g \cdot L^{-1}$ (influent concentration) over the last three sample dates (Figure 7). The expanded shale reservoir used after the woodchip bioreactor yielded effluent concentrations of chlorpyrifos similar to the post woodchip treatment. Bifenthrin concentrations in expanded shale reservoir effluent increased over time, with concentrations reflecting reductions following woodchip bioreactors across all sample dates. Oxyfluorfen concentrations were reduced to under 400 ppb over the first three sample dates following passage through expanded shale, with the concentration on the latter three sample dates rising over 400 $\mu g \cdot L^{-1}$and at times exceeding the influent concentration of 500 $\mu g \cdot L^{-1}$. Pesticide remediation within these treatment systems are hypothesized to result from a combination of sorption (both to woodchips and to expanded shale), as well as subsequent degradation via microbial communities.

Year 2: Microbial Community Analysis

Bacterial communities within woodchip bioreactors are responsible for nitrate remediation. This study was conducted in an effort to identify if, and to what extent, the microbial consortium within woodchip bioreactors would be affected by the addition of pesticides. As agricultural runoff treatment systems would be subject to both nutrient and pesticide laden influent, identifying population shifts and dominant species in response to the pesticide content in runoff may be important for proper management of these systems. Three bioreactors were randomly selected that received a nutrient only influent as well as three bioreactors that were to receive the nutrient and pesticide influent, with each of the bioreactors from each treatment sampled in triplicate on each sample date. Samples were collected at the

termination of the incubation stage – where all bioreactor reps had received the same nutrient-only influent to establish baseline community analysis, followed by sampling the day after initial pesticide application, 10 days after application, and the termination of the study (37 days after application). Microbial community analysis was conducted through extraction of woodchip bioreactor water, culturing and incubation on an LB medium plate, colony forming unit counts, and finally up to 3 colonies per plate selected for genetic sequencing. Bacterial communities found at the end of the incubation stage (and subsequently thereafter in nutrient only receiving bioreactors) reflected that the most commonly identified species was *Bacillus aryabhattai*. In the pesticide receiving bioreactors, population shifts were identified with predominant species 10 days after pesticide exposure largely being within the *Exiguobacterium* genus, and at the termination of the study a range of *Pseuodomonas* species were identified in more abundance, as well as continued presence of *Bacillus aryabhattai* in both treatments (Figure 8).

Year 2 Conclusions:

Nitrate and phosphate remediation can be achieved using woodchip bioreactors and expanded shale reservoirs when provided hydraulic retention times of 3 days; however, hydraulic retention times of approximately 20 minutes were insufficient for nutrient remediation. Pesticide remediation can be achieved using hydraulic retention times of approximately 20 minutes; however, the rate in which various pesticides are remediated requires further study. Pesticides sorption to woodchips represents one avenue in which these compounds may be removed from agricultural runoff, but the time needed to breakdown these compounds may limit the effectiveness of woodchips and/or require extended hydraulic retention time for degradation to occur to avoid desorption and export. Bacterial communities within bioreactors may be modified in the presence of pesticide contaminated influent; however, population shifts reflect species

capable of tolerating or exploit pesticides, as well as the capacity to adapt to changing conditions, highlighting the diversity of species which may survive in these systems. Continued research into hydraulic retention times is essential to determining best management practices for these treatment systems. Nutrients can be effectively remediated using extended (3 day) hydraulic retention times, while the capacity for these systems to remediate and adapt to pesticides is evidenced in sub-20 minute hydraulic retention times. We believe that the limited hydraulic retention time was not sufficient for nitrate removal as there wasn't time for the dissolved oxygen in the system to be consumed in order to switch to nitrate use as a terminal electron acceptor in respiration.

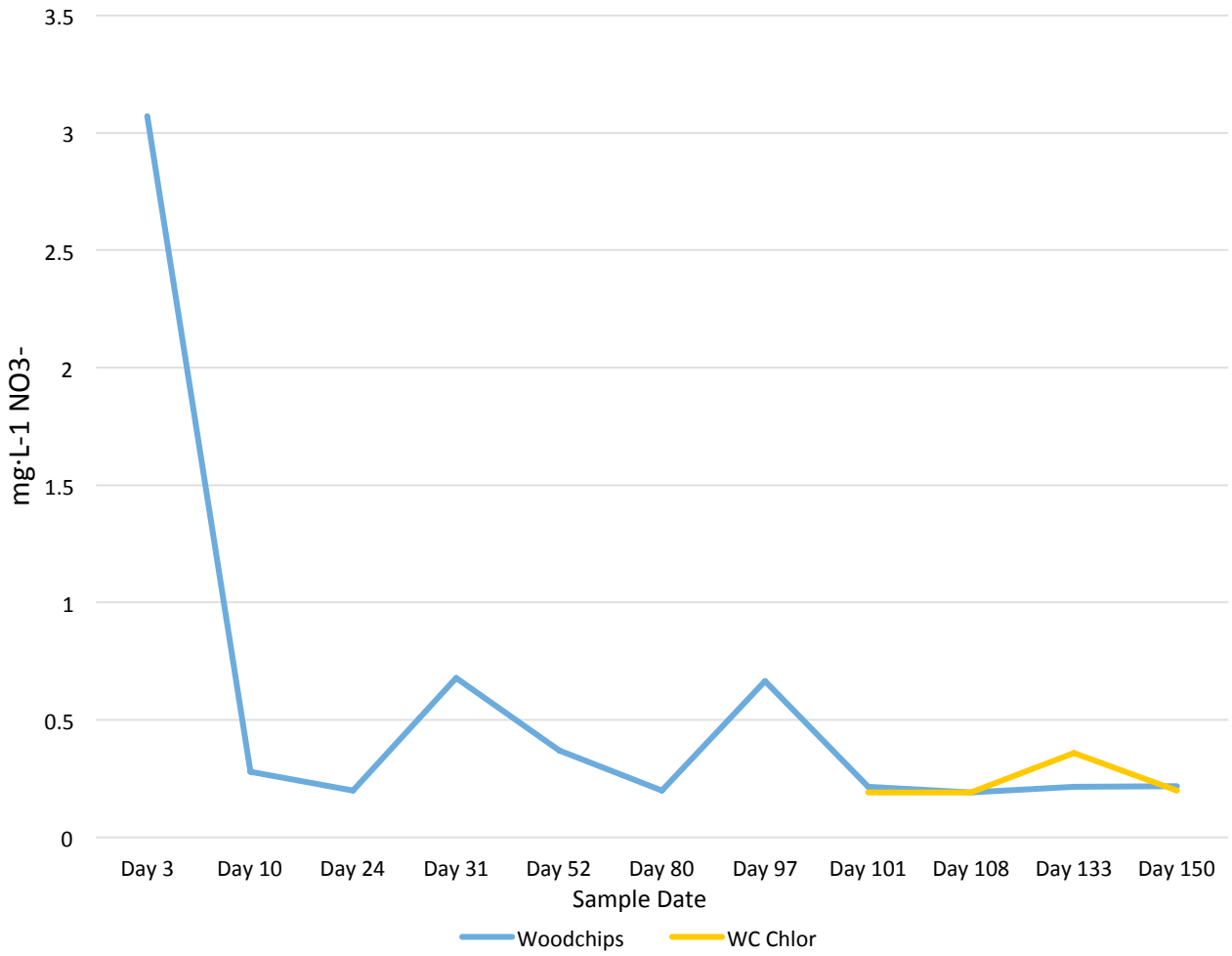

Figure 1: Under a three day hydraulic retention time, influent nitrate concentrations of 90 mg/L NO3- were reduced below detection limit 0.2 mg/L NO3- using woodchip bioreactors. The capacity to remediate nitrates was conserved in the presence of chlorpyrifos.

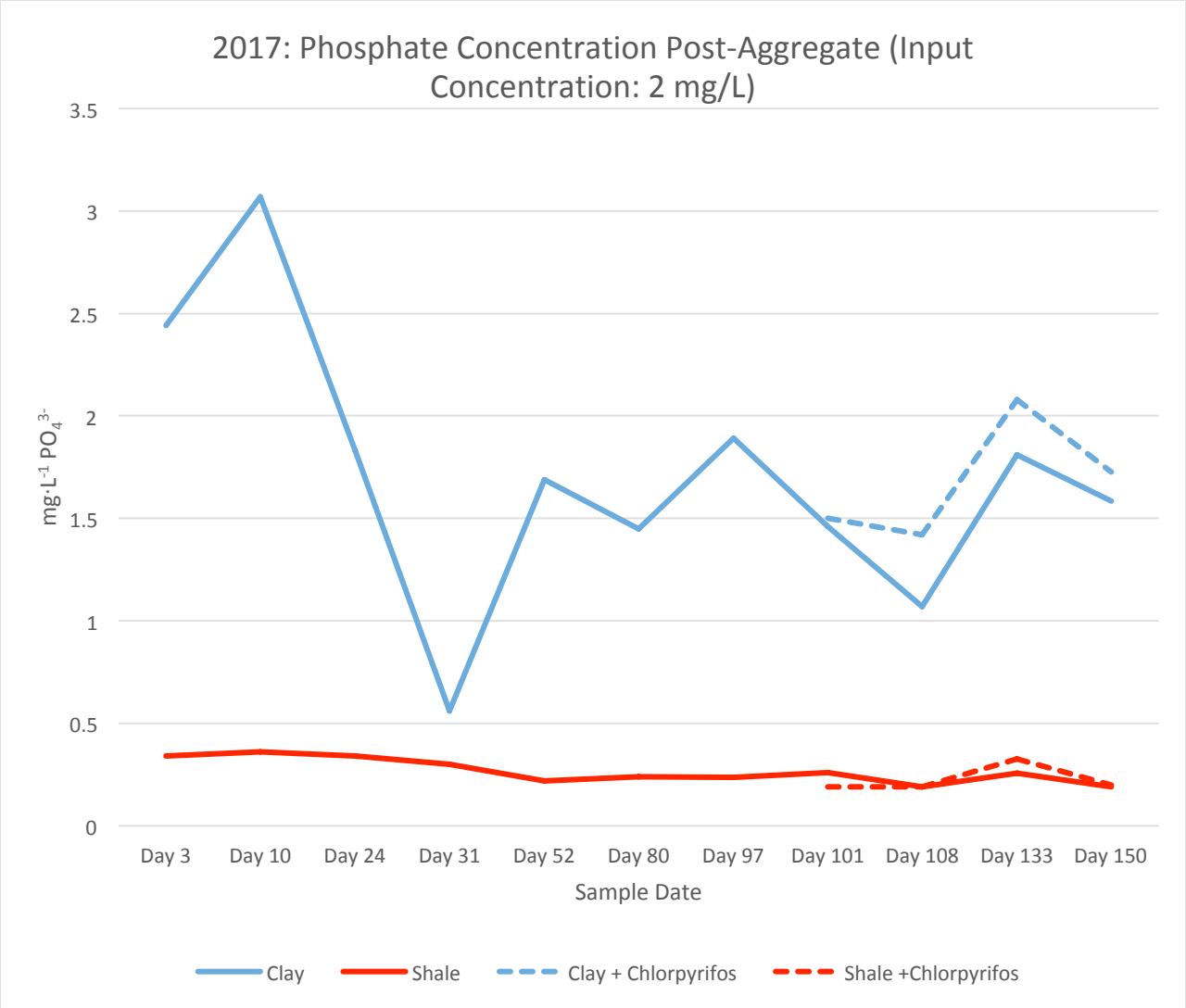

Figure 2: Under a three day hydraulic retention time, the expanded shale aggregate was capable of removing phosphate below detection (0.2 mg/L). The expanded clay desorbed initial phosphate, before subsequent adsorption and equilibration yielded limited phosphate removing potential. The addition of chlorpyrifos had no effect on phosphate sorption in either aggregate.

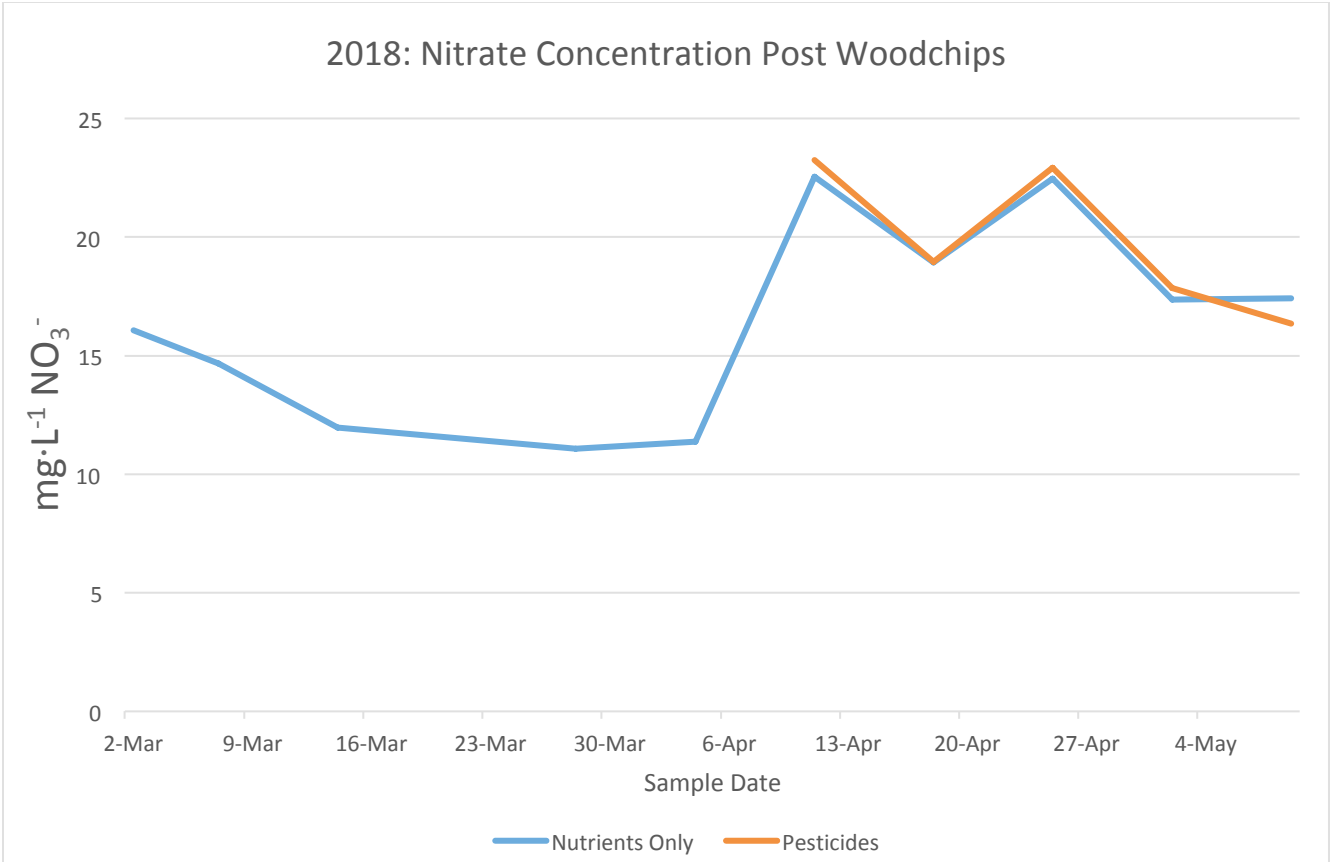

Figure 3: Influent nitrate concentrations of 12 mg/L during the incubation stage, and 20 mg/L post pesticide incorporation, were unable to be effectively remediated using aproximately 20 minute hydraulic retention time. The addition of chlorpyrifos, bifenthrin, and oxyfluorfen yielded no differences in nutrient remediation capacity of woodchip bipreactors under these conditions.

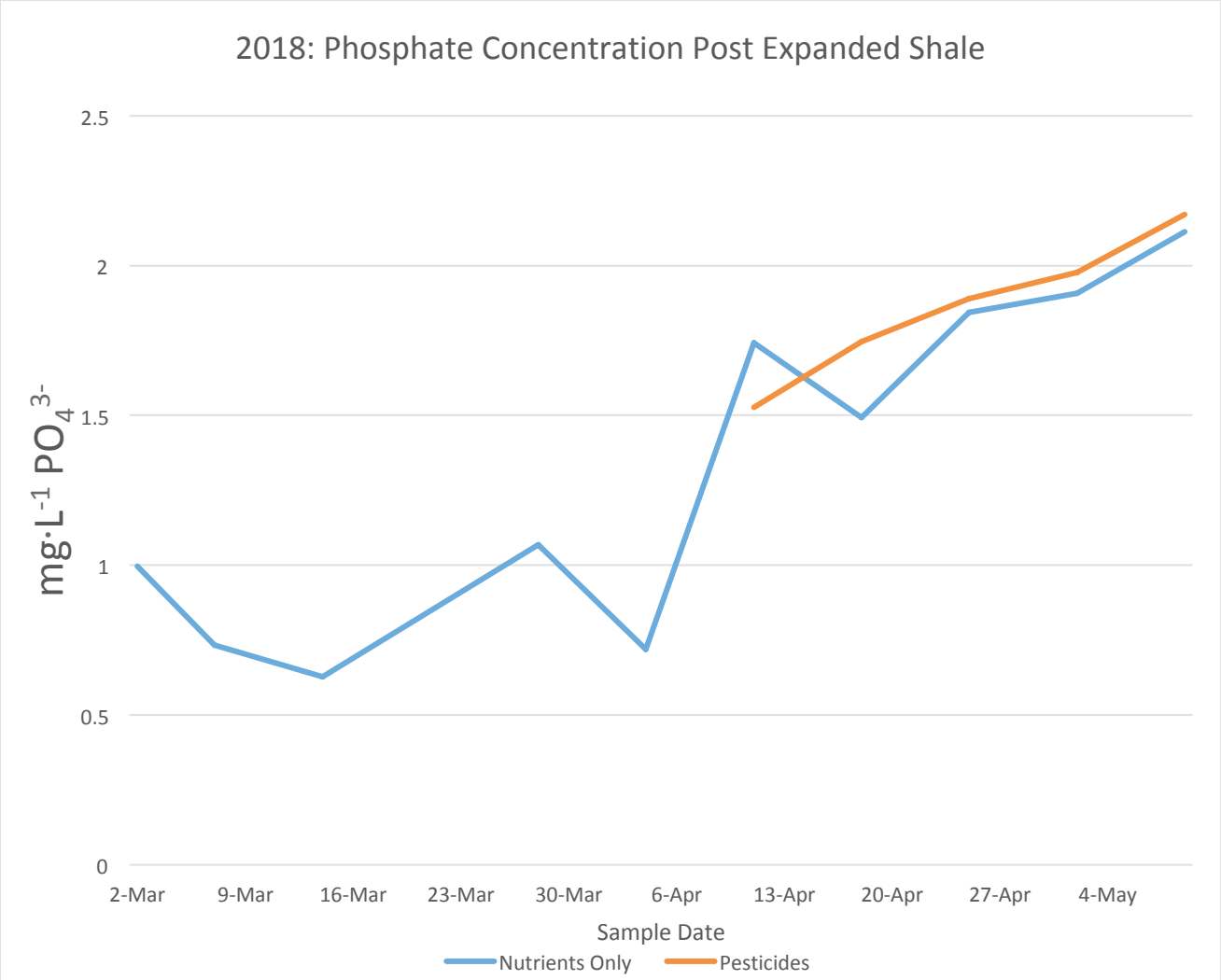

Figure 4: Influent phosphate concentrations of 0.75 mg/L during the incubation stage, and 1.5 mg/L post pesticide incorporation were not effectively remediated under aproximately 20 minute hydraulic retention time using expanded shale aggregate. The capability to adsorb phosphate was not affected by the presence of chlorpyrifos, bifenthrin, or oxyfluorfen.

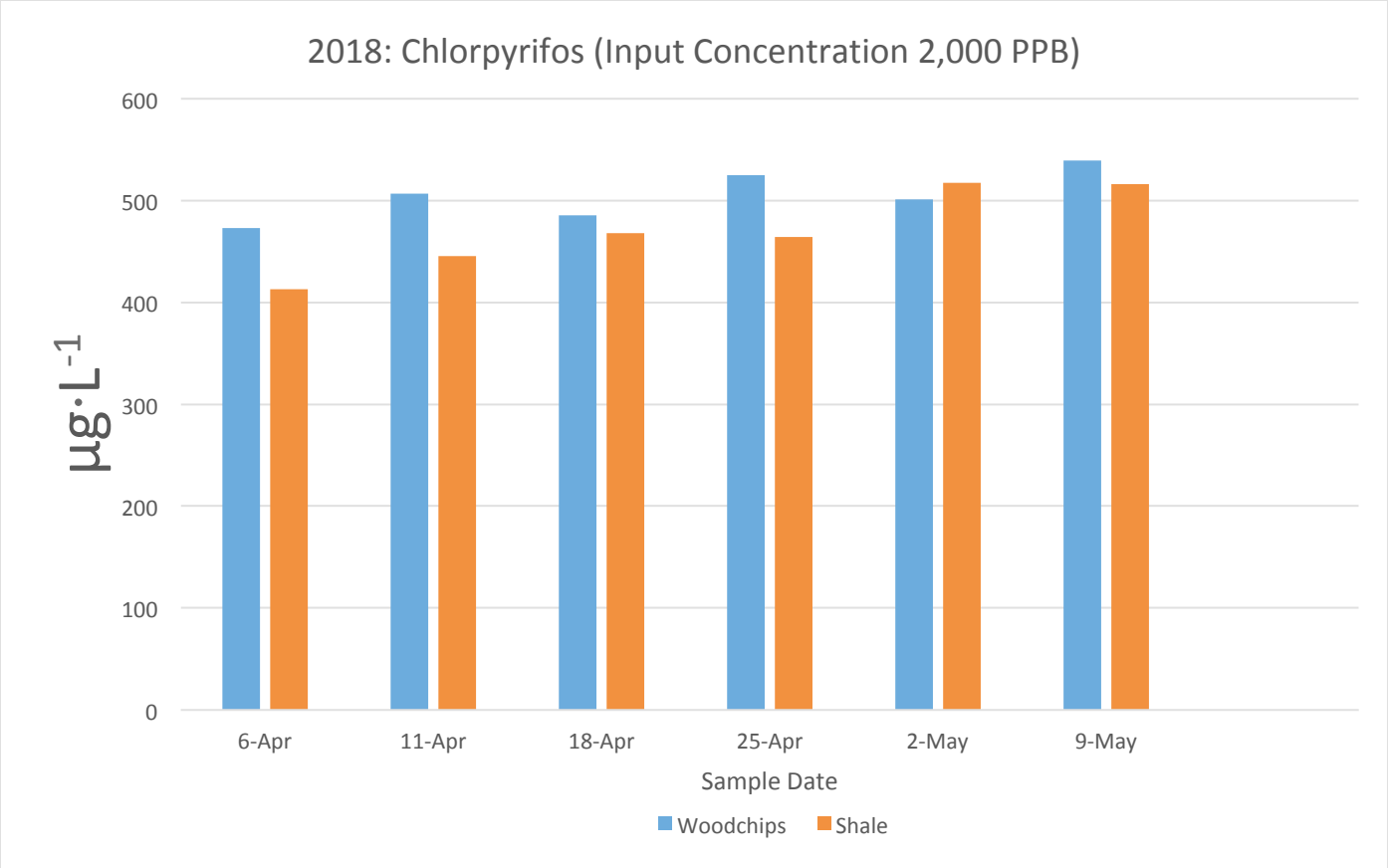

Figure 5: Chlorpyrifos concentration was consistently reduced over all sample dates by woodchip bioreactors, while the shale reservoirs at times removed chlorpyrifos.

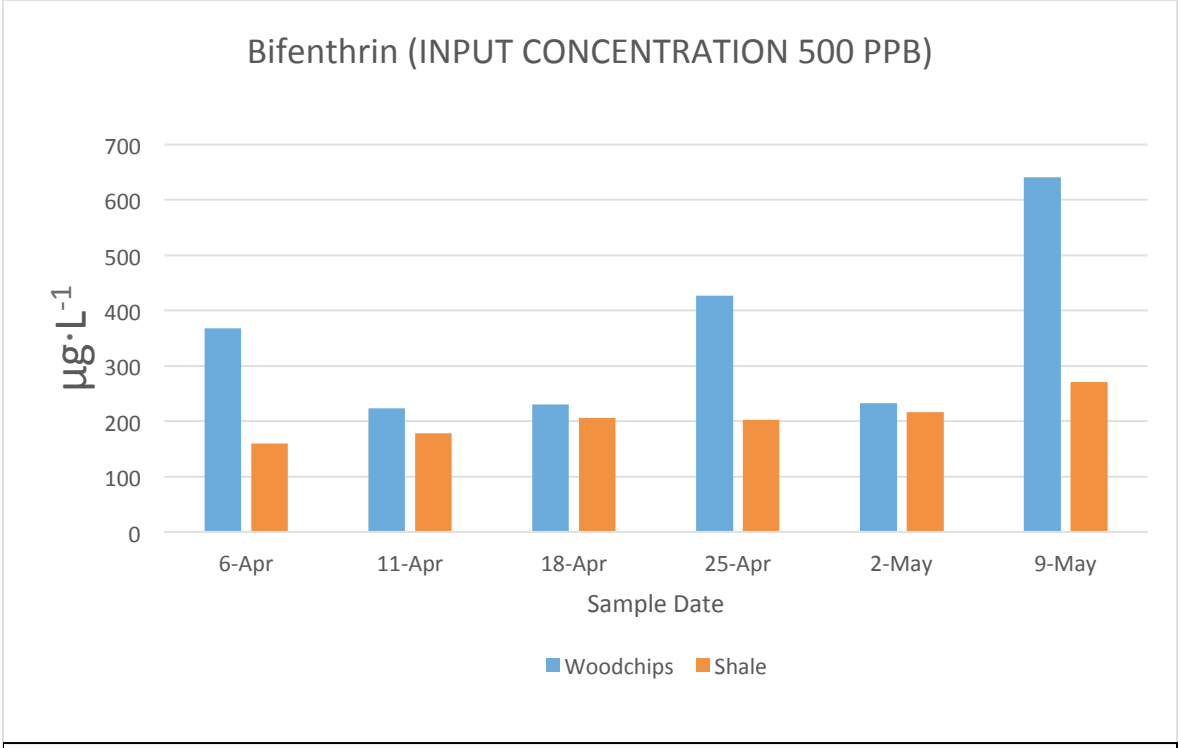

Figure 6: Bifenthrin concentration in effluent fluctuated post-woodchips, with net export on the final sample date. The capability of expanded shale to remove bifenthrin was stable across all sample dates, with steady increases alluding to increased saturation of binding sites.

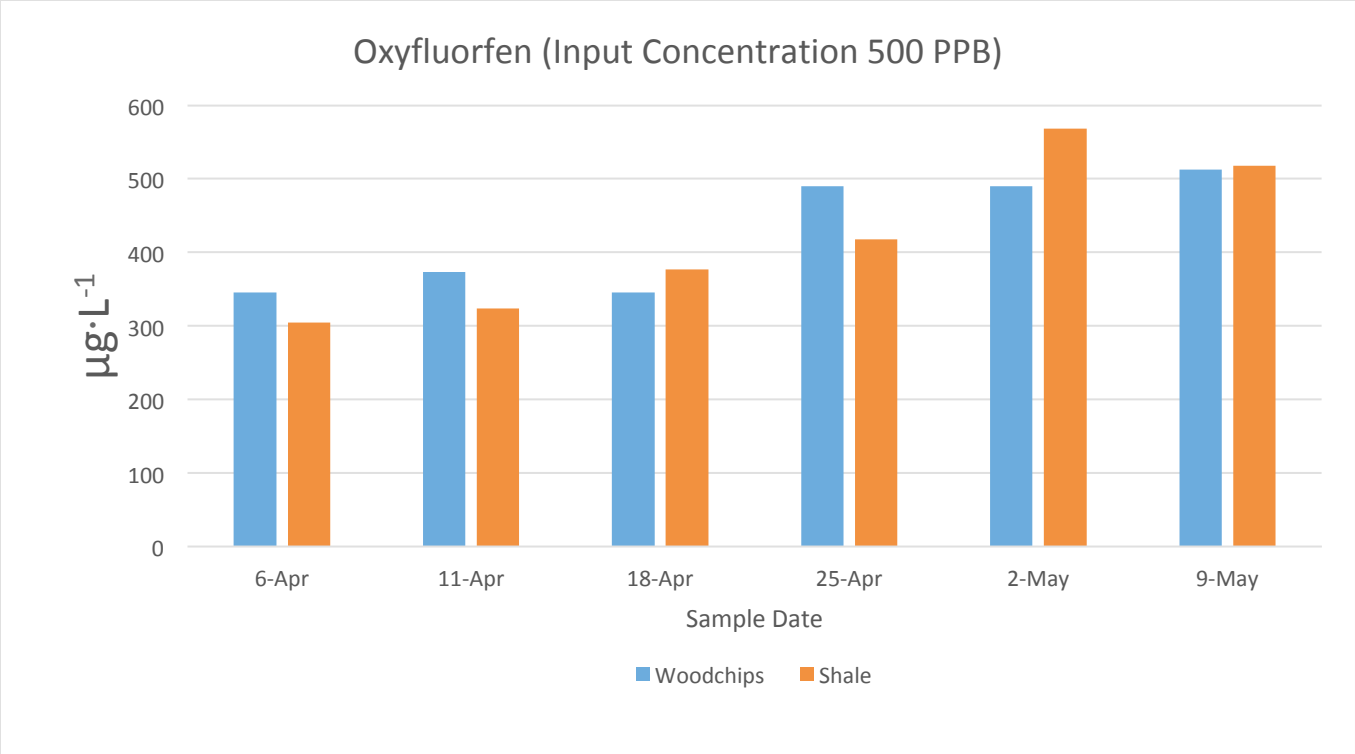

Figure 7: Oxyfluorfen concentration in effluent was reduced over the first three sample dates, while over the final three dates increased to influent levels.