

## **Influence of stormflow and baseflow phosphorus pressures on stream ecology in agricultural catchments**

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## **Abstract**

Stormflow and baseflow phosphorus (P) concentrations and loads in rivers may exert different ecological pressures during different seasons. These pressures and subsequent impacts are important to disentangle in order to target and monitor the effectiveness of mitigation measures. This study investigated the influence of stormflow and baseflow P pressures on stream ecology in six contrasting agricultural catchments. A five-year sub-hourly dataset was used consisting of stream discharge, P chemistry, macroinvertebrate and diatom ecology, supported with microbial source tracking and turbidity data.

Total reactive P (TRP) loads delivered during baseflows were low (1-7% of annual loads), but TRP concentrations frequently exceeded the environmental quality standard (EQS) of  $0.035 \text{ mg L}^{-1}$  during these flows (32-100% of the time in five catchments). A pilot microbial source tracking exercise in one catchment indicated that both human and ruminant faecal effluents were contributing to these baseflow P pressures but were diluted at higher flows. Seasonally, TRP concentrations tended to be highest during summer due to these baseflow P pressures and corresponded well with declines in diatom quality during this time ( $R^2 = 0.79$ ). Diatoms tended to recover by late spring when storm P pressures were most prevalent and there was a poor relationship between antecedent TRP concentrations and diatom quality in spring ( $R^2 = 0.23$ ). Seasonal variations were less apparent in the macroinvertebrate indices; however, there was a good relationship between antecedent TRP concentrations and macroinvertebrate quality during spring only ( $R^2 = 0.51$ ) and summer only ( $R^2 = 0.52$ ).

Reducing summer point source discharges may be the quickest way to improve ecological river quality, particularly diatom quality in these and similar catchments. Aligning estimates of P sources with ecological impacts and identifying ecological signals which can be attributed to storm P pressures are important next steps for successful management of agricultural catchments at these scales.

## 1. Introduction

Eutrophication of rivers is a continuing international concern and phosphorus (P) can sometimes be a key limiting nutrient in many of these water-bodies (McDowell et al., 2009; O'Neil et al., 2012; Dodds and Smith, 2016). Management of eutrophication and other water resource issues in the European Union (EU) is addressed by the Water Framework Directive (WFD) (OJEC, 2000) which is reviewed in six year cycles. This legislative framework requires all EU member states to achieve at least 'good' and non-deteriorating status in all water-bodies. For rivers this includes good ecological and good chemical status but greater emphasis is placed on the former (Borja, 2007). The WFD requires that River Basin Management Plans and Programmes of Measures (PoM) are implemented at catchment scales, which offer a natural unit for integrated, ecosystem-based water management.

The major sources of P to rivers include those from human population centres (waste water effluent) and intensive agriculture (organic and inorganic nutrients in runoff), with the former considered to be primarily a point source issue and the latter a more diffuse phenomenon (Bowes et al., 2005). For agriculture, mitigation measures are generally targeted at a combination of residual (e.g. soil P stores where P is also included in the territorial regulations), incidental (e.g. recently applied fertilisers) and point (farmyards and facilities) sources of nutrients (DEFRA 2004; Swedish Board of Agriculture, 2009; SI 31 2014). These PoMs under the WFD are expected to contribute to the achievement of good ecological status. However, the results of the first round of River Basin Management Plans show that more than half of Europe's surface water-bodies are in less than good ecological status (European Environment Agency, 2012). Furthermore, national environmental quality standards (EQS) for riverine P concentrations (e.g. 0.035 mg L<sup>-1</sup> unfiltered molybdate reactive P in Ireland) are still exceeded in many parts of Europe (European Environment

Agency, 2010). With the second round of River Basin Management Plans under development, the lack of clear improvements may prompt the introduction of additional mitigation measures. However, the risk of increasing economic burdens on farming communities (or increasing support) will be an important consideration to ensure that existing measures and expectations of improvement (and any further measures) are based on robust supporting science.

Knowledge of the links between agricultural P transfers and ecological quality is improving and an important emerging issue in the scientific literature is the discontinuity between the timing of greatest P transfers from land and the timing of greatest eutrophication risk (Jarvie et al., 2013; Stamm et al., 2013; Withers et al., 2014b). The majority of P losses from agriculture generally occur during winter storms, whereas the ecological quality of rivers can be linked to P concentration pressures during periods of ecological sensitivity (spring and summer low river flows; Mainstone and Parr, 2002; Hilton et al., 2006). However, there is high uncertainty regarding the source, magnitude and ecological impacts of baseflow P concentrations in agricultural catchments during the summer season. In groundwater-fed agricultural catchments diffuse P sources can contribute to elevated P concentrations in baseflows owing to slow-flow subsurface pathways linking groundwater to surface water during very long recession periods following storm events (Mellander et al., 2016). Bed sediments may also provide a direct source of P to the water-column during baseflows. However, studies have shown that the significance of the pool of sediment-associated P for soluble P release and nuisance algal proliferation is low (Jarvie et al., 2005) and these sediments may primarily act as chemical sinks for water-column P (Shore et al., 2016). Recent studies have shown that rural point sources can maintain rivers in a eutrophic state in

the long durations between storms and especially during dry periods (see Withers et al., 2014b).

The literature suggests these point sources of P (and other pollutants) are mostly related to human and animal faecal effluents from municipal waste water outfalls, domestic septic systems and farmyards (Jarvie et al., 2006; Withers et al., 2014b; Old et al., 2012). The use of specific tools to detect faecal pollution in rivers and other water bodies is an emerging science (Fenech et al., 2012). Enumerating *Escherichia coli* (*E. coli*) is a standard method for the indirect detection of faecal matter but this bacteria can also be present as part of normal soil flora (Winfield and Groisman, 2003). Microbial source tracking (MST) encompasses several techniques to detect bacteria in water (Field and Samadpour, 2007; Scott *et al.*, 2002; Stapleton *et al.*, 2007). The most common MST method incorporates molecular techniques to detect host-associated bacteria such as the faecal non-coliform group, *Bacteroidales* (Ahmed et al., 2008; Bernhard and Field, 2000; Kildare *et al.*, 2007). As these strict anaerobes display a high level of association with specific hosts they can be used to approximately distinguish, between human and, for example, agricultural ruminant and other animal faecal pollution (Bernhard and Field, 2000; Harwood *et al.*, 2013a Kildare *et al.*, 2007; Schriewer *et al.*, 2010).

While smaller catchment rivers (<10km<sup>2</sup>) are not fully considered in WFD monitoring, and some rural point source influences may dissipate at larger scales (Gill and Mockler, 2016), there is growing recognition of their ecological importance as nurseries and refugia (Biggs et al., 2016). Accordingly, there needs to be more robust scientific evidence of the ecological impacts of the different P sources delivered from agricultural catchments. The aim of this study was to contribute to this evidence base by combining high resolution nutrient

monitoring and microbial source tracking with concurrent ecological quality data in rivers over a five year period from 2010-2015. This information is critical for assessing if and how agricultural measures can contribute to the achievement of good ecological status in rivers as partly required by the WFD.

The objectives were to:

1. Characterise the magnitude of storm and baseflow P pressures in agricultural catchments (in terms of their effect on stream P concentrations).
2. Investigate the effects of these pressures on P concentrations seasonally.
3. Investigate concurrent impacts on stream ecology.
4. In a pilot study in one catchment, use microbial source tracking (MST) methods, including host-associated molecular markers, to associate the origin of certain P sources with different sources of faecal pollution.

It was hypothesised that ecological quality declined over the summer period due in part to elevated baseflow P concentrations, which could be linked to point source pressures. The study focused on characterising the relative and combined influence of stormflow and baseflow P pressures, based on the assumption that stormflows are mostly dominated by diffuse P pressures and baseflows are mostly dominated by point source P pressures (Bowes et al., 2005).

### 1.1 Study areas

Data from six meso-scale (3.5 km<sup>2</sup> to 30 km<sup>2</sup>) agricultural catchments in Ireland were used in this study (Figs. 1, 2). Catchment characteristics including land-use and geological settings are described in Table 1. Four are dominated by grassland land use ranging from relatively

extensive beef to intensive dairying (termed Grassland A, B, C, D) and two have high proportions of spring barley or winter wheat cropping (termed Arable A, B). Based on their soil and geological characteristics and the natural soil drainage typologies of Schulte et al (2005), Grassland A, D and Arable A are characterised as free-draining, Arable B as moderately drained and Grassland B and Grassland C as poorly drained. The streams ranged from 1<sup>st</sup> to 3<sup>rd</sup> Strahler order and were mostly in the size range included in national intensive monitoring programmes (*ca.* 10km<sup>2</sup>) (Wall et al., 2011; Owen et al., 2012; Kyllmar et al., 2006). The outlet of Grassland D, a karst zone of contribution, was an emergent spring and subsequent 1<sup>st</sup> order stream (Mellander et al., 2012). These catchments have been subject to both P and N management regulations since 2006 (S.I 31 2014).

There is a single, central waste water treatment plant in the Arable A catchment based on a batch reactor facility for up to 75 people with the remaining population (approximately 208 people) on septic tank systems. In all other catchments waste water is generally treated by single housing septic tank systems. While there are no published data relating to P load inputs to the rivers from these systems, Table 1 shows the approximate inputs into combined septic and waste water treatment facilities based on P production of 0.7 kg human<sup>-1</sup> yr<sup>-1</sup> and Central Statistics Office data on average household populations in each catchment area. The human P loading is based on EU averages of 1.6 g P person<sup>-1</sup> day<sup>-1</sup> in faeces and urine and 0.3 g P person day<sup>-1</sup> in dishwasher and laundry detergents (Schmid Neset et al., 2008; de Madariaga, 2007). Ruminant input P loads are based on organic P produced per animal from statutory information relating to Good Agricultural Practice guideines (SI 31, 2014) and animal type and numbers in each catchment, obtained from the Irish Department of Agriculture Food and the Marine.

## 2. Methods

### 2.1 Stream flow and phosphorus chemistry data

Stream discharge, total P (TP, i.e. unfiltered and digested) and total reactive (TRP, i.e. unfiltered and undigested) concentrations have continuously been measured on a sub-hourly basis at each catchments' outlet since *ca.* 2009/ 2010, depending on the catchment. Discharge is calculated from measuring water level (OTT Orpheus-mini), rated at a Corbett flat-V non-standard weir (Mellander et al., 2012). Phosphorus is measured using a Phosphax Sigma (Hach) bankside analyser. The instrument alternates TP and TRP measurements with approximately three of each taken per hour over the range 0.010-5.000 mg L<sup>-1</sup>. A cleaning cycle (using heated and concentrated H<sub>2</sub>SO<sub>4</sub> acid) and calibration cycle (2 mg L<sup>-1</sup>) is carried out automatically every 24 hours. At the same locations, turbidity was measured continuously using a Solitax (Hach) turbidity meter (Sherriff et al. 2016). For the purposes of this study P data were analysed for the period from 1<sup>st</sup> Feb 2010 – 28<sup>th</sup> Feb 2015 where available<sup>1</sup>, which includes five of each of the four seasons (for comparison with seasonal ecology data) and four full hydrological years (for comparative assessments of P pressures across catchments). All data were managed in the WISKI 7 (Kisters, 2010) database system with aggregation to average hourly and daily time-series, where necessary, following quality control. Unless shown, most of the P-ecology investigations were undertaken with the TRP fraction as this is operationally equivalent (unfiltered, undigested) to molybdate reactive P (MRP), often referred to as orthophosphate as P, in national statutory datasets (Jarvie et al., 2002).

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<sup>1</sup> Data-sets were shorter in Grassland C (Stream phosphorus and discharge data available from 01/10/2010 – 28/02/2015) and Grassland D (Stream phosphorus available from 26/02/2010 - 16/09/2014, stream discharge available from 10/05/2010 – 09/09/2014).



## 2.2 Hydrological dependence and seasonality of P concentrations

For objectives 1 and 2, stormflows and baseflows were identified by separating daily stream discharges into five categories based on flow percentiles for each catchment. The upper 10<sup>th</sup> percentiles of flows were classed as ‘very high’ flow and the upper 10<sup>th</sup> - 30<sup>th</sup> as ‘high’ flow. The values of these percentiles are shown in Supplementary Information (Table S1). Collectively, these were referred to as stormflows and were regarded as the times when stream P concentrations mainly reflected diffuse P sources. The lower 10<sup>th</sup> percentile of flows were classed as ‘very low’ flow and the lower 10<sup>th</sup>-30<sup>th</sup> as ‘low’ flow. Collectively, these were referred to as baseflows and were regarded as the times when stream P status was mainly vulnerable to point P sources. The remaining range of flows, from the upper 30<sup>th</sup> to the lower 30<sup>th</sup> percentiles, were termed ‘intermediate’ flows and were regarded as the times when stream P concentrations could not be primarily attributed to either diffuse or point P sources. In order to characterise the magnitude of ‘diffuse’ (i.e. referring to stormflows) and ‘point’ source (i.e. referring to baseflows) P pressures in these catchments in terms of their effect on stream P concentrations, the duration of the time the Irish riverine environmental TRP (i.e. MRP) quality standard (EQS) of 0.035 mg L<sup>-1</sup> was exceeded during these five flow categories was compared over the four hydrological years (October 2010-October 2014) in each catchment. Average annual TRP loads were also computed for these hydrological years and separated into those that occurred during stormflows and those that occurred during baseflows. The effects of ‘diffuse’ and ‘point’ source P pressures on seasonal P concentrations was investigated by (i) quantifying the duration that each flow category occurred seasonally and (ii) comparing the resultant seasonal TRP concentrations.

### 2.3 Ecological impacts of diffuse and point source P pressures

The subsequent impacts of ‘diffuse’ and ‘point’ source P pressures on stream ecology were investigated for objective 3 by comparing seasonal P concentrations with concomitant ecological quality metrics that were derived from surveys taken at the catchment outlets each May and September between 2009 and 2014. Median TRP concentrations (of continuous hourly data) during summer (i.e. June, July and August) were compared with macroinvertebrate and diatom quality indices for September. Similarly, median TRP concentrations during ‘spring’ (here referring to the months February, March and April, i.e. the three months prior to the ecology survey) were compared with macroinvertebrate and diatom quality indices for May. Whilst two ecological surveys per year are not expected to fully capture the temporal community dynamics in response to sudden stressors (e.g. Feeley et al., 2012), they nevertheless provide seasonal insights which are not afforded by national statutory data-sets (one survey every three years) and which capture time-integrated diffuse and point source P pressures (over seasonal time-scales).

The ecological surveys were conducted independently (Aquatic Services Unit; University College Cork) and methods are described by Murphy et al. (2015). In summary, benthic macroinvertebrate communities were collected using standard 2-minute, travelling, kick-samples in the riffles using a long-handled pond net (250 mm width, mesh size 1 mm) according to ISO Standards (ISO 7828; 1985) and national protocols (McGarrigle et al., 1992). Macroinvertebrates were identified in the laboratory to the highest taxonomic level possible and Q-values were calculated (an inter-calibrated ecological quality metric - McGarrigle et al, 1992). Diatoms were collected from natural substrates near to macroinvertebrate sampling sites and were assessed in accordance to the Diatoms for Assessing River Ecological Status (DARES) protocol including (i) identification of diatom

community assemblage; and, (ii) calculation of Trophic Diatom Index (TDI) as a measure of the effect of nutrients on diatom assemblages (Kelly et al., 2008). Macroinvertebrate Q-values and diatom TDIs were expressed as Ecological Quality Ratios (EQRs) by comparing the observed TDI or Q-value with values expected if the site was at reference condition.

In order to attribute seasonal variations in ecological metrics to seasonal P pressures it was considered important to account for natural seasonal life cycle histories. The diatom EQR was adjusted for natural seasonal variation using the model of Kelly et al. (2007). However, there is currently no published methodology for adjusting macroinvertebrate EQR for natural seasonal variation so expert judgement ([www.limnos-consultancy.com](http://www.limnos-consultancy.com)) on species life histories was applied and both seasonally un-adjusted and seasonally adjusted EQRs are presented. The former (un-adjusted) provides a good representation of the range of species present in each catchment.

Aquatic vegetation cover values were recorded at each catchment outlet in order to aid interpretation of the macroinvertebrates and diatom indices. Vegetation cover values were estimated from within a representative section in a stretch approximately four times the stream width. The estimates included the broad groupings of macroalgae (filamentous algae), macrophytes, bryophytes and higher plants. These were identified to the highest possible taxonomic level.

#### 2.4 Pilot microbial source tracking

The Arable B catchment had previously been identified as a catchment with high P concentration patterns at both baseflow and stormflow (Jordan et al., 2012; Melland et al., 2012). To augment the work undertaken in objectives 1, 2 and 3, a pilot study in objective 4

sought to investigate the sources associated with organic pollution, specifically faecal pollution.

An autosampler (ISCO 6712 Portable Sampler) was deployed at the outlet of Arable B to take a 1 L sample every 2 h over 36-96 h time periods during specific hydrological phases. Samples were analysed for *E. coli* occurrence by the IDEXX Colisure® Quanti Tray®/2000 system. Colisure reagent (IDEXX laboratories, ME, USA) was aseptically added to 100 ml of water sample and mixed to dissolve. The solution was poured into a 97-well quanti-tray, sealed and incubated at 37°C for 24 hours. Red/magenta wells that fluoresce under UV light indicated detection of *E.coli*. Ambiguously coloured wells (pink/orange) were further incubated for up to 48 h and re-tested. Faecal Indicator Organism colony forming units (CFU)/100 ml were calculated using the most probable number (MPN) table provided by IDEXX.

Quantitative Polymerase Chain Reaction (qPCR) was employed for rapid and highly specific detection of the *Bacteroidales* group from faecal and water samples (Bernhard and Field, 2000; Kildare *et al.*, 2007). Animal and human faecal samples were used to build a DNA archive of potential faecal pollution sources and also to evaluate qPCR assay specificity. Animal faecal samples included cow, sheep, horse, pig and goat. Collected water samples were filtered through 0.2 µm cellulose nitrate membrane filters to concentrate bacterial cells, which then underwent chemical extraction of total nucleic acids. These DNA extracts were then tested using the following TaqMan *Bacteroidales* assays; a universal faecal marker (BacUni-UCD), a human associated faecal marker (Bac-Hum-UCD) and a bovine associated faecal marker (BacCow-UCD). For all TaqMan assays, 10 µl of DNA extract, diluted 1:3, 1:9, 1:27, 1:81 were assayed in duplicate in a final reaction volume of 25 µl. Each 25 µl

reaction contained a final concentration of 1x TaqMan<sup>®</sup> Environmental Master Mix 2.0 (Applied Biosystems<sup>®</sup>, Carlsbad, CA, USA) with 400 nM each of forward and reverse primer, and 80 nM probe. The samples were amplified in an ABI StepOnePlus<sup>™</sup> Real-Time PCR System (Applied Biosystems<sup>®</sup>). Standard amplification conditions were used: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Detailed methods used in this pilot study are described in Supplementary Information.

## Results

### 3.1 Phosphorus – EQS and flow dependence (objective 1)

The emergent spring in Grassland D was the only surface water without any observed significant P pressures (i.e. TRP concentrations never exceeded the EQS, Fig. 3, Table 2). No clear point sources have been noted in this catchment. Phosphorus pressures were present during baseflows, to varying degrees, in the other five catchments and were particularly high in Arable B (Fig. 3, Table 2). In three catchments, Grassland C, Arable A and Arable B, P pressures (i.e. in terms of TRP concentrations) were greater during baseflows than during stormflows. However, TRP loads were much lower during baseflows (i.e. excluding intermediate and storm flows) accounting for just 3-7% of total annual loads (Table 2). Patterns of increases in TRP concentrations with decreases in flow from low to very low were observed in four of the catchments (Fig. 3)

Phosphorus pressures (i.e. in terms of TRP concentrations) were present during stormflows in four of the catchments (all except Arable A and Grassland D, Fig. 3, Table 2). Grassland B was the only catchment where P pressures were greater during stormflows than during baseflows and is likely an artefact of the heavy soils in this catchment (Table 1) which facilitate rapid runoff and delivery of P in overland flow (Mellander et al., 2015). Nevertheless, regardless of the concentration patterns, TRP loads were much higher during stormflows (i.e. excluding intermediate and baseflows) in all catchments accounting for 60 – 90% of total annual loads (Table 2).

### 3.2 Seasonality of P concentrations (objective 2)

The summer of 2012 was exceptionally wet and discharges in all catchments except Grassland D did not fall to baseflow levels (i.e. the lower 30<sup>th</sup> percentile of discharges) during this time (Fig. 4, Table S2). Instead, stormflows occurred on average 38 – 63% of this season across catchments. During the other summer seasons, storm flows were less frequent (<7% of the season, Fig. 4, Table S2) and were dominated by baseflows, which occurred on average 74 – 99% of the summer season across catchments. Winters were generally dominated by storm flows, occurring on average 55 – 68% of the winter season across catchments (Fig. 4, Table S2). Flows rarely dropped to baseflow levels during the winter seasons. The spring and autumn seasons tended to be dominated by intermediate flows, with both baseflows and stormflows occurring for shorter periods of time (Fig. 4, Table S2).

In general, stream TRP concentrations tended to peak during the summer and decline throughout the following seasons in all catchments, reaching their lowest levels in winter or spring (Fig. 5). In 2010, 2011 2013 and 2014, elevated TRP concentrations during the summer reflected the high TRP concentrations and long duration of baseflows during this time and generally resulted in median TRP concentration in excess of the EQS of 0.035 mg L<sup>-1</sup> in all five catchments. The seasonal trend of high TRP concentrations in summer compared to winter persisted in Grassland B despite this catchment having higher P concentrations in stormflow than baseflow (Fig. 5, Table 2) and reflects the relatively long duration of baseflows in summer (84% when 2012 is removed) compared to the duration of stormflows in winter (52% when 2012 is removed) in this catchment. The seasonal trends were weakest in Grassland A, reflecting the similar magnitudes of TRP pressures during both stormflows and baseflows in this catchment (Fig. 3) and the similar durations of these pressures in summer and winter (Table S2).

In 2012, elevated TRP concentrations during the summer reflected the high TRP concentrations and long durations of stormflows during this time. Stormflows were equally, if not more, prevalent during the following winter (2012/2013, Table S2) but had lower (than summer) stream TRP concentrations. Despite the prevalence of stormflows (and associated P pressures) during the summer of 2012, TRP concentrations were generally lower during this summer than during the other drier and more typical summers in three of the catchments (Arable B, Arable A, Grassland C) - with median TRP concentrations falling below the EQS in the latter two catchments in summer 2012.

### 3.3 Ecological impacts of P pressures (objective 3)

The trends in diatom quality across catchments and years broadly reflected the trends in median TRP concentrations with no ecological impacts (i.e. achieving at least 'Good' WFD status) in Grassland D and greatest ecological impacts (i.e. furthest from 'Good' WFD status) in Arable B (Fig. 6a). There was a general positive response in diatom EQR to TRP concentrations  $< 0.035 \text{ mg L}^{-1}$  and a negative response to TRP concentrations above this threshold. Overall, there was a moderate ( $R^2 = 0.47$ ), significant ( $P < 0.05$ ) logarithmic relationship between diatom EQR and stream TRP concentrations. Diatom quality exhibited marked seasonal variations in the four most impacted catchments with a summer decline followed by a winter/spring recovery. Diatom seasonal trends often resulted in reductions from 'Good' (EQR = 0.78) to 'Moderate' (EQR = 0.52) ecological status, except for relatively weak seasonal trends in 2012 (due to the wet summer). The seasonal variations were larger than 'natural' variation indicating the presence of seasonal pressures during this time and reflected the seasonality in the stream TRP concentrations in these catchments. The relationship between stream TRP concentrations and diatom ecological quality became much stronger ( $R^2 = 0.70$ , logarithmic relationship,  $P < 0.05$ ) for the summer data only ( $R^2 = 0.70$ ,



logarithmic relationship,  $P < 0.05$ ), particularly when the wet summer of 2012 was removed ( $R^2 = 0.79$ , logarithmic relationship,  $P < 0.05$ ) and was weaker for the spring data only ( $R^2 = 0.23$ ,  $P < 0.05$ ). The diatom species traits reflected a combination of eutrophic and non-eutrophic (e.g. saprobity, siltation) pollution at all sites, the effects of which often increased between seasons (see Supplementary Information, Tables. S3a-h).

The trends in seasonally unadjusted macroinvertebrate quality across catchments and years broadly reflected the trends in median TRP concentrations (Fig. 6b) with the least chemical and ecological impacts in Grassland C and greatest impacts in Arable B. Overall, there was a moderate ( $R^2 = 0.49$ ), significant ( $P < 0.05$ ), linear relationship between seasonally unadjusted macroinvertebrate EQR and stream TRP concentrations (Fig. 6b). Seasonally unadjusted macroinvertebrate quality exhibited marked seasonal variations in the four most impacted catchments, with a summer decline followed by a recovery the following spring, concurrent with seasonal changes in the stream TRP concentrations. These seasonal variations persisted in 2012 despite the stormy conditions during the summer period. These seasonal trends were stronger than any inter-annual trends. However, when natural life cycle variations were taken into account, much of this seasonal variation was removed (Fig. 6c) and there was a weaker relationship between macroinvertebrate EQR and stream TRP concentration ( $R^2 = 0.35$ ). This relationship was stronger for the summer data only ( $R^2 = 0.42$ ), particularly when the summer data of 2012 were removed ( $R^2 = 0.52$ ), and was also stronger for the spring data only ( $R^2 = 0.51$ ). Species abundance data are presented in Supplementary Information (Table S4a-e) and provide further insights into the pollution impacts at each site. Of note was the regularly high abundance of the collector/filterer feeding faunal groups *Simuliidae* and *Chironomidae* in Arable B and the excessive abundance of these groups in September 2012 in Grassland A.

Year to year, Grassland A and Arable A periodically experienced prolific instream algal growth, usually most abundant during the September sampling. Grassland A regularly had high levels of *Cladophora spp.* and *Vaucheria spp.*, with a combined coverage of up to 75% in September 2013. Arable A periodically had a high abundance of *Ranunculus spp.* (up to 80% coverage in May 2013). Arable B, Grassland B and Grassland C rarely had instream algal growth apart from low levels of ephemeral filamentous green algae.

### 3.4 Microbial Source Tracking (objective 4)

The *Bacteroidales* assays used in this pilot study in the Arable B catchment were a universal marker associated with faecal matter (BacUni-UCD), a marker associated with human faecal matter (BacHum-UCD) and a marker associated with bovine faecal matter (BacCow-UCD). BacUni-UCD was assayed against cow, sheep, goat, human, horse, and pig faecal samples. It detected *Bacteroidales* in all faecal samples (Table S5). Both of the host-associated *Bacteroidales* qPCR assays were tested against various faecal samples in order to establish assay specificity (Table 3). The BacHum-UCD assay detected all human faecal samples and did not detect sheep, cow, pig, goat or horse faecal samples. The BacCow-UCD assay detected cow but it also detected sheep samples at similar Cycle threshold (Ct) values of 14, and goat (1 in 10 dilution) was detected shortly after at a Ct value of 20 (Fig. S1). As a consequence of this high level of cross-reactivity between cow, sheep, and goat, the BacCow-UCD assay was determined to be more suitable for detecting agricultural ruminant faecal pollution in this pilot study and catchment rather than just bovine faecal pollution. BacCow-UCD did not detect human, horse or pig faecal *Bacteroidales*.

Six events were captured across the very low to very high flow range in Arable B between 2010 and 2012 (Figs. S2a-f). Total P, TRP and turbidity were extracted from the continuous

datasets from the time of sample collection and compared with discharge. Following normalisation by log-transformation, the P relationships could be described overall by a ‘U-shaped’ curve (Figs 7a and 7b - in this case a second order polynomial), as is specifically expected when catchments have both point source and diffuse source influences due to loss of dilution and increase in concentration, respectively (Bowes et al., 2010). The stronger relationship ( $R^2 = 0.77$ ) between flow and TP was influenced by the stronger turbidity (and hence sediment) signal at higher flows (Fig. 7c).

*E. coli* was determined in the collected samples and counts across the sampled events could also be described by a (weaker) polynomial curve (Fig. 8a) indicating, as with P, some independence and dependence for transport at both low and high flows, respectively. In terms of the host-associated faecal markers also tested in the samples, the universal marker indicated presence across the flow range but with a particularly increased presence during the November 2010 high flow event (Fig. 8b) – but which didn’t increase to the same extent during the higher October 2011 event. This was less apparent in the human and ruminant associated markers with a tendency for these markers to be diluted to less than detection in the majority of high flow samples, particularly for the BacHum (UCD) human marker (Figs. 8c and 8d).

## 4 Discussion

### 4.1 Phosphorus pressures and source tracking

The low magnitude of the baseflow P loads found in all catchments means that these are unlikely to have a significant influence on the water-quality of downstream standing water-bodies such as lakes and estuaries (e.g. Vollenweider, 1968). However, the integration of these low loads into low and extreme low flows provides the conditions for the catchment river P concentrations to remain above the EQS for long durations during critical summer periods of growth. Phosphorus transfer via groundwater likely has a part to play in elevated baseflow P concentrations, particularly in the well-drained catchments (such as Grassland A and Arable A), which have typically long hydrological recession periods and can account for up to 45% of total annual TRP loss (Holman et al., 2008; Mellander et al. 2015). However, groundwater P concentrations would not account for the observed pattern of increases in P with decreases in flow as found here and in other studies (e.g. Neal et al., 2010). Furthermore, previous detailed physico-chemical analyses of ditch bed sediments in Arable A and Grassland B showed that they were unlikely to release soluble P and had capacity for further soluble P retention (Shore et. al., 2016). Li et al., (2013) also showed that Irish river sediments tend to have high P sorption capacities. Therefore, other low-flow P pressures are likely acting on these catchments, either in tandem with, or independent of groundwater.

In the Arable B pilot study, human faecal matter was shown to be present at low and very low flows in the spring and summer periods and appeared to be effectively diluted at higher flows. The presence of *E. coli* at both low and high flow in Arable B indicates a decoupling of this marker from human faecal pollution at high flows, likely because *E. coli* is present in soils in the natural microbial flora and can be transferred as part of high flow runoff processes over soils (Muirhead et al., 2006) – which is a known process in the Arable B catchment

(Sherriff et al., 2016). The ruminant organic P inputs in each catchment (Table 1) may also be a significant contributor to the observed TRP losses in baseflow (Tables 1 and 2) and, in comparison to human sewage effluent, less is known about the preponderance of agricultural point source influences on baseflows. Flynn et al. (2016) found signals of bovine faecal matter in summer but not winter baseflows, similar in part to the findings of the pilot study in Arable B where ruminant (BacCow) faecal matter was shown to be present at low and very low flows in the spring and summer periods. The maintenance of high TRP above the EQS at low and very low flows in this catchment, even as stream water decreases in turbidity, and the presence of both human and ruminant faecal matter at low and extreme low flows, is supportive of low level point source influences which most likely persist in colloidal form for long durations.

Whilst MST work is needed to identify the sources contributing to elevated baseflow P concentrations elsewhere, the similar catchment characteristics (Tables 1 and 2) and large ranges in TRP concentrations during low-flows in Arable B, Grassland A and Grassland B suggests these point source influences may also be of an intermittent nature in these catchments.

There is no doubt that the overall P loads are dominated by high magnitude, short duration winter storms but the main findings here are consistent with Stamm et al. (2013) who question the ecological relevance of P loads for flowing waterbodies. The main sources contributing to elevated TRP concentrations during stormflows are likely residual losses from soil P stores and incidental losses from recently applied fertilisers, of which the former is likely to dominate (Shore et al., 2016). The inconsistent pattern of the universal faecal marker in two very high flow events (Fig. 7b) and the decoupling of the *E. coli* marker from the

universal faecal marker, support this theory. The reduction in stream TRP concentrations between the summer and winter of 2012/2013 likely partly reflects a loss of the more labile P during the wetter summer of 2012 and earlier flushing of proximal P sources (Sherriff et al. 2016).

#### 4.2. Ecology

Despite the introduction of a range of P management measures across these study catchments in 2006, the present study showed no clear evidence of ecological improvement trends in five impacted catchments over a five year period from 2010-2015. Diatom quality tended to be poorer than macroinvertebrate quality in all catchments, thus diatom quality limits the overall ecological quality status, as assessed under the WFD (using the one-out-all-out principle), in these catchments.

The strong relationship ( $R^2 = 0.80$ ) between stream TRP concentrations and diatom quality during the 'typical' summer periods (i.e. excluding 2012) indicate that summer baseflow P pressures, which are indicative of point source influences as described in section 4.1, may be having trophic impacts in these streams. The general decline in the nutrient sensitive diatom species *Achnanthes minutissimum* in the four most impacted catchments over the summer periods further supports this notion. Whilst organic pollution impacts were also apparent (presence of diatom species indicative of organic pollution, Tables S3a-h) in these catchments and often increased during the sensitive summer time (e.g. *Cocconeis spp.*), in the 'typical' summers of 2010, 2011, 2013 and 2014, such organic pollution impacts are likely more associated with point sources than diffuse (the landscape is least hydrologically connected during this time) and therefore implications for management would be similar. Cases of good TRP quality (i.e. low concentrations) and poor diatom quality occurred, mostly in Arable A, suggesting that other pollutants may also be limiting the diatom quality in this

catchment. For example, motile diatom species, siltation and high covers of the rooted macrophyte *Ranunculus spp.* were frequently recorded at the Arable A site suggesting that sediment may be the limiting factor for the diatom quality in this catchment. Furthermore, nitrate-N, or other N fractions, either co-limiting or as the main limiting nutrient, is a possible next stage in this research and some studies have shown the increasing importance of N limitation in rivers even at smaller scales (Dodds et al., 2011; Elshohi et al., 2011).

When macroinvertebrate life cycles were taken into account, seasonal patterns of summer declines followed by winter recovery were less apparent for these biota than for the diatoms. This is supported by the findings of Johnson and Hering (2009), who showed that in their lowland study streams, benthic diatom assemblages indicated a stronger and earlier response to elevated nutrient concentrations than macroinvertebrates. Furthermore, overall, variation in the benthic diatom assemblage was more strongly correlated to the nutrient gradient than for macroinvertebrates. Hering et al. (2006) further suggested that benthic macroinvertebrates are more directly affected by stream oxygen conditions, whilst diatoms are more directly affected by nutrient conditions. Nevertheless, strong empirical relationships between stream P (MRP) concentrations and macroinvertebrate EQR have been demonstrated using river monitoring data in Ireland (EPA, pers. comm.) and the broad relationship between stream P concentrations and macroinvertebrate EQR ( $R^2 = 0.49$ ) found in the present study across catchments and years, supports this. However, the precise mechanisms by which P affects macroinvertebrates in eutrophic and polluted rivers are not well understood or documented. The high levels of in-stream vegetation growth in Arable A and Grassland A indicate the mechanism may be trophic in these catchments. However, in-stream vegetation growth was minimal in Arable B where stream P concentrations were highest and macroinvertebrate quality was most impacted. Excessive abundance of the collector/filterer feeding faunal

groups *Simulidae* and *Chironomidae* in this catchment point to the possibility of high in-stream levels of fine particulate organic matter (FPOM) at the site which is often associated with point discharges from agricultural or domestic/municipal sewage waste. For baseflow P pressures, FPOM is likely to be similarly often associated with point sources and, therefore, management of this pressure is likely to be similar to that for point sources. Many cases of high TRP concentrations ( $>$  EQS) and good macroinvertebrate quality occurred (23%) supporting the notion that other factors such as the availability of FPOM and in-stream biological oxygen demand are also important determinants of benthic macroinvertebrate quality.

Whilst episodic storm-driven P transfers were present to some degree during most of the summer periods (particularly in 2012), the additional impact on diatom and macroinvertebrate ecology of the occasional summer storm (occurred  $<$  7% of the summer time across all catchments) which resulted in periodic TRP concentration spikes, versus the persistence of elevated summer baseflow TRP conditions, could not be disentangled. Further research is needed in this area. The findings of the present study, showing improved relationships between higher summer TRP concentrations and poorer September diatom and macroinvertebrate quality when the 2012 data (wet summer) were removed, support the theory that persistent low and baseflow P concentrations, likely from point sources, have a significant impact on instream ecological quality. Furthermore, in the presence of both low-flow point P sources and stormflow-driven diffuse P sources, the point sources are likely to be more manageable, whereas reducing episodic storm-driven P sources would require further agricultural measures such as the identification and management of hydrologically-sensitive areas (Thomas et al., 2016). Therefore whilst both types of pressure need to be managed, at small catchment scales such as those included in this study, river-quality



improvements may be more expediently realised by firstly focusing on the the persistent point sources.

## **Conclusion**

This study demonstrated that in six agricultural catchments, P pressures (in terms of magnitude and duration of concentration) were generally greater during baseflow than during stormflow. The baseflow P pressures appeared to have an impact on stream ecological quality, particularly diatom ecology. A pilot MST exercise in one catchment indicated that both human and ruminant faecal effluents were contributing to these baseflow P pressures and were less consistent during high flows. Therefore, mitigating ecological impacts in these streams will likely at least require a reduction in point source discharges. Whilst summer stormflow P pressures were present to some degree in most catchments, the prevalence and persistence of elevated summer low- and baseflow P concentrations was a dominant feature.

The implications are for:

- i. Development of source-specific measures to target both point and diffuse pollution and an understanding of how the mitigating benefits of these measures are likely to be best measured. While diffuse P mitigation measures are likely to reduce the downstream load (and hence the burden to downstream standing water bodies), these reductions in the continuing presence of baseflow P concentrations may not improve riverine ecology. Such a development could better align the objectives of agricultural measures to improve water quality with those of the WFD at the river scale.

- ii. Development and use of empirical source apportionment tools, which would include validation by source markers, particularly in small rural catchments, to provide a more accurate representation of the causes of ecological impairment.

Further work is warranted beyond this study at larger catchment scales, using MST or other source associated markers to refine risk assessments and especially when seasonal EQS are exceeded for long durations (i.e. during baseflows).

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