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Release of dissolved and colloidal phosphorus from riparian wetlands : a field and laboratory assessment of the mechanisms and controlling factors

Sen Gu

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Sen Gu

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CNRS 6118)

OSUR : Observatoire des Sciences de l'Univers de Rennes

**Release of dissolved
and colloidal
phosphorus from
riparian wetlands:
*a field and laboratory
assessment of the
mechanisms and
controlling factors***

**Thèse soutenue à Rennes
le 24 octobre 2017**

devant le jury composé de :

Erwin KLUMPP

Professeur/Forschungszentrum Jülich/Rapporteur

Per-Erik MELLANDER

Senior Research Officer/TEAGASC/Rapporteur

Jean-Marcel DORIOZ

Directeur de Recherche, INRA, UMR CARTELE/
Examineur

Chantal GASCUEL-ODOUX

Directrice de Recherche, INRA, UMR SAS/
Examineur

Gilles PINAY

Directeur de Recherche, CNRS, UMR Ecobio/
Examineur

Gérard GRUAU

Directeur de Recherche, CNRS, UMR Géosciences
Rennes/Directeur de thèse

Résumé

Le phosphore (P) est un nutriment essentiel dans le contrôle de l'eutrophisation des eaux de surface. En raison de la diminution des émissions urbaines et industrielles de P, la plupart du P causant cette eutrophisation dans les pays occidentaux est aujourd'hui du P issu des sols agricoles, expliquant pourquoi les recherches actuelles sur l'eutrophisation se focalisent sur la compréhension des mécanismes par lequel le P est relargué de ces sols. Dans cette thèse, nous avons étudié ces mécanismes en nous focalisant sur la fraction dissoute (DP) du P, fraction la plus menaçante du point de vue de l'eutrophisation. Une double approche a été utilisée, combinant le suivi de la composition d'eaux du sol et d'eaux de ruisseau dans un petit bassin versant (BV) agricole représentatif (BV de Kervidy-Naizin, France) et des simulations expérimentales au laboratoire.

Les suivis de terrain ont révélé que les zones humides ripariennes (RW) étaient les principales zones de relargage de DP dans le BV étudié, via deux mécanismes essentiellement déclenchés par les fluctuations des hauteurs de nappe, i) la réhumectation des sols (DRW) et ii) la dissolution réductrice des oxydes de fer du sol dans de périodes d'anoxie. Ces mêmes suivis ont révélé la présence de fortes variations spatiales de la nature chimique (inorganique et organique/colloïdale) du DP relargué, ces variations étant démontrées être en relation avec des différences dans les propriétés du sol et la topographie locale. Les variations saisonnières et interannuelles de l'hydroclimat, combinées aux variations locales de topographie ont été démontrées être les deux facteurs principaux contrôlant i) la fréquence des épisodes DRW, et ii) la durée des périodes anoxiques, entraînant au final de fortes variations saisonnières et interannuelles de la dynamique de relargage du DP. Comme indiqué dans un modèle conceptuel général, la topographie est sans doute le facteur clé de contrôle des variations observées, en raison de son rôle sur i) le transfert de P à partir des parcelles agricoles amont, ii) le taux de minéralisation du P organique du sol P et iii), le déclenchement des deux mécanismes de relargage précités.

Les expériences en laboratoire ont confirmé le rôle des événements DRW comme processus clé causant le relargage de DP dans les RWs. Les résultats ont démontré que le DP relargué consistait non seulement de "vrai" DP inorganique et organique, mais aussi de P colloïdal, le P colloïdal et le DP organique étant les plus réactifs aux événements DRW. Les données ont aussi révélées que ces différentes formes de P provenaient de différentes sources dans le sol (mésoporosité et macroporosité pour P colloïdal et le DP organique; microporosité pour DP inorganique), et que la quantité de P colloïdal relargué était positivement corrélée avec la teneur en matière organique et la taille de biomasse microbienne du sol. Ces mêmes expériences ont confirmé le rôle des conditions anoxiques comme conditions favorisant la libération de DP dans les RWs. La dissolution réductrice de sol Fe-oxyhydroxydes n'est cependant pas le seul processus impliqué, un autre processus étant la hausse du pH causée par des réactions de réduction. Les résultats obtenus démontrent que l'augmentation de pH contrôle la libération de DP dans les sols riches en matière organique, alors que ce relargage est contrôlé principalement par la réduction des oxydes de fer dans les sols pauvres en matière organique. Les données expérimentales démontrent également que l'apport de sédiments issus des sols agricoles amont accroît le relargage de DP dans les RW, probablement en raison de la dissolution des oxydes de fer de ces mêmes sédiments par les bactéries ferroséductrices des RWs.

Au final, cette thèse permet de mieux contraindre les mécanismes et facteurs responsables du relargage de DP dans les bassins versants agricoles. Une conséquence très pratique de ce travail est que la conception de stratégies pour limiter les fuites de DP dans ces bassins ne peut se faire sans une prise en compte des rôles de l'hydroclimat, de la topographie locale et des propriétés du sol sur ce relargage.

Mots clés: phosphore dissous et colloïdal, pollutions diffuses agricoles, bassin versant, zones humides, sols, processus d'oxydoréduction, mécanismes biogéochimiques, eaux souterraines

Abstract

Phosphorus (P) is a key nutrient in controlling surface water eutrophication. Because of the decrease of urban and industrial P emissions, most of the P nowadays causing surface water eutrophication in western countries consists of P transferred from agricultural soils, explaining why current eutrophication research focused on understanding the mechanisms by which P is released from soils. In this thesis, we studied these release mechanisms for dissolved P (DP) – i.e. the most bioavailable P component for algae - using an approach combining field monitoring of soil and stream water compositions in a small, headwater catchment typical of western countries agricultural catchments (the Kervidy-Naizn catchment, France), and laboratory experimental simulations.

Field monitoring data revealed that riparian wetlands (RW) are the main zones of DP release and DP production in the studied catchment, through essentially two mechanisms triggered by groundwater table fluctuations, namely i) rewetting of dry soils (DRW), and ii) reductive dissolution of soil Fe (hydr)oxides during anaerobic periods. Field monitoring data also revealed the presence of strong spatial variations in the chemical nature (inorganic *vs.* organic/colloidal) of the released DP, which was in relation to differences in soil properties and local topography. Seasonal and inter-annual hydroclimate variations, combined with variations in local topography were found to control the frequency of soil DRW events and duration of anaerobic periods, resulting in strong seasonal and inter-annual variations of DP release dynamics. As shown in a conceptual model, topography is likely to be the key driver of the observed spatial and temporal variations, because of its combined control on i) the transfer of P from upland fields to RW zones, ii) the mineralization rates of soil organic P and iii) the triggering of the above two release mechanisms.

Laboratory leaching experiments on the same soils confirmed the role of DRW events as a major process causing DP release pulses in RWs. The data demonstrated that the released DP consisted not only of true dissolved inorganic and organic P but also of colloidal P, the latter phase being the most reactive to DRW events. The data also revealed that the different P forms came from different P sources in the soil (soil macro/mesopores for colloidal P and organic DP; soil micropores for inorganic DP) and that the amount of released colloidal P correlated positively with the organic matter contents and soil microbial biomass size of the soil. Anaerobic incubation experiments, on their hand, confirmed the role of anoxic conditions as conditions favoring the release of DP in RW. Reductive dissolution of soil Fe-oxyhydroxide was, however, not the sole process involved in that release, another process being the rise in pH caused by reduction reactions. Experimental data showed that the pH rise controlled the DP release in organic-rich soils, this release being on the contrary mainly controlled by soil Fe-oxyhydroxides reductive dissolution in organic-poor soils. Experimental data also showed that the input of soil sediments from upland fields enhanced the release of DP in RW, most likely due to the enhanced dissolution of sediment Fe-oxyhydroxides by RW Fe-reducing bacteria.

Overall, this thesis allowed new constraints to be placed on the release mechanisms of DP in headwater agricultural catchments. One very practical output is that great care should be taken of hydroclimate variability, local topography, and soil property when designing and implementing management options to reduce DP release and transfer in agricultural catchments.

Keywords: dissolved phosphorus, groundwater, riparian wetland, biogeochemical processes, drying-rewetting, redox processes, hydroclimate, topography, soil properties, colloidal phosphorus

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Chapter 1- General Introduction

1.1 The global story of phosphorus

1.1.1 Why do we study phosphorus?

Phosphorus (P) is an essential element for all living organisms on earth. It is a major constituent of DNA/RNA (carriers of genetic information), ATP (intracellular energy molecules) and phospholipids (cell membrane formation), thus allowing the physiological functioning of all animals, plants, and microorganisms (Bundy et al., 2005). As an important component of our bone and teeth, “P minerals keep us upright and allow us to chew our food” (Oelkers and Valsami-Jones, 2008). Phosphorus also plays a key role in controlling or co-controlling the primary productivity of terrestrial and aquatic ecosystems (Carpenter et al., 1998; Correll, 1998).

In spite of its biological importance, P is only the 11th most abundant elements in the lithosphere, occurring only in small quantities (0.09% w/w) (Filippelli, 2008). Phosphorus is rarely found in its elemental form. Although P has a number of potential oxidation state (from -3 to +5), it occurs almost exclusively in its most oxidized form (PO_4^{3-}), whether P is dissolved or particulate, organic or inorganic (Bennett and Schipanski, 2012). Unlike other critical bio-nutrients, such as carbon and nitrogen, P has no stable atmospheric gas phases and its cycling is almost entirely dependent on its aqueous and solid transfer. As the P cycle on earth is one of the slowest biogeochemical cycles, i.e., on a geological time scale (millions of years), P is considered to be a non-renewable resource on a human time scale.

1.1.2 The global challenge of P resources

Humans started to mine P from P-rich rock (especially apatite which is a variety of tri-calcium phosphate minerals) since the late 18th century, and it has become the major source of P for human use ever since. Due to the lack of an atmospheric component in the P cycle, the availability of P in a terrestrial ecosystem depends entirely on the original soil P content and anthropogenic inputs (Delmas et al., 2015). Human has started to use P fertilizers to increase the agricultural yields since the 19th century, and

this use has then increased dramatically in the 20th century, due to the rapid population growth and the equally rapid food demand growth (**Figure 1.1**).

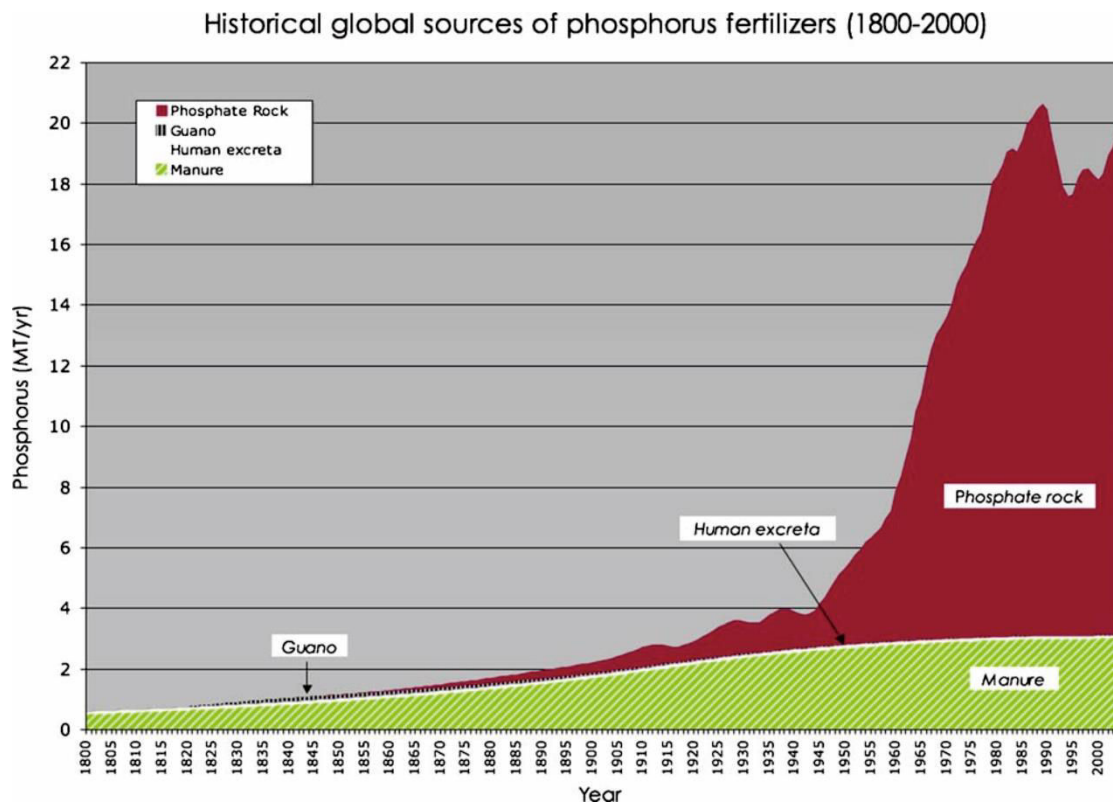


Figure 1.1. Historical sources of P used for soil fertilization, including manure, human excreta, guano and phosphate rock (1800–2000). From Cordell et al. (2009).

The mined P is used predominantly to produce P fertilizers, with the remaining being used in cattle-feed supplements, food preservatives, detergent additives and industrial cleaning agents (Elser and Bennett, 2011). Due to the increased demand for food and changing diets (more animal products), the demand for P fertilizers is expected to continue to increase in the next decades (Neset and Cordell, 2012). Cordell et al. (2009) predicted a “phosphorus peak” in approximately 2033, based on the P reserve estimates by the United States Geological Survey (USGS) in 2009 (**Figure 1.2a**).

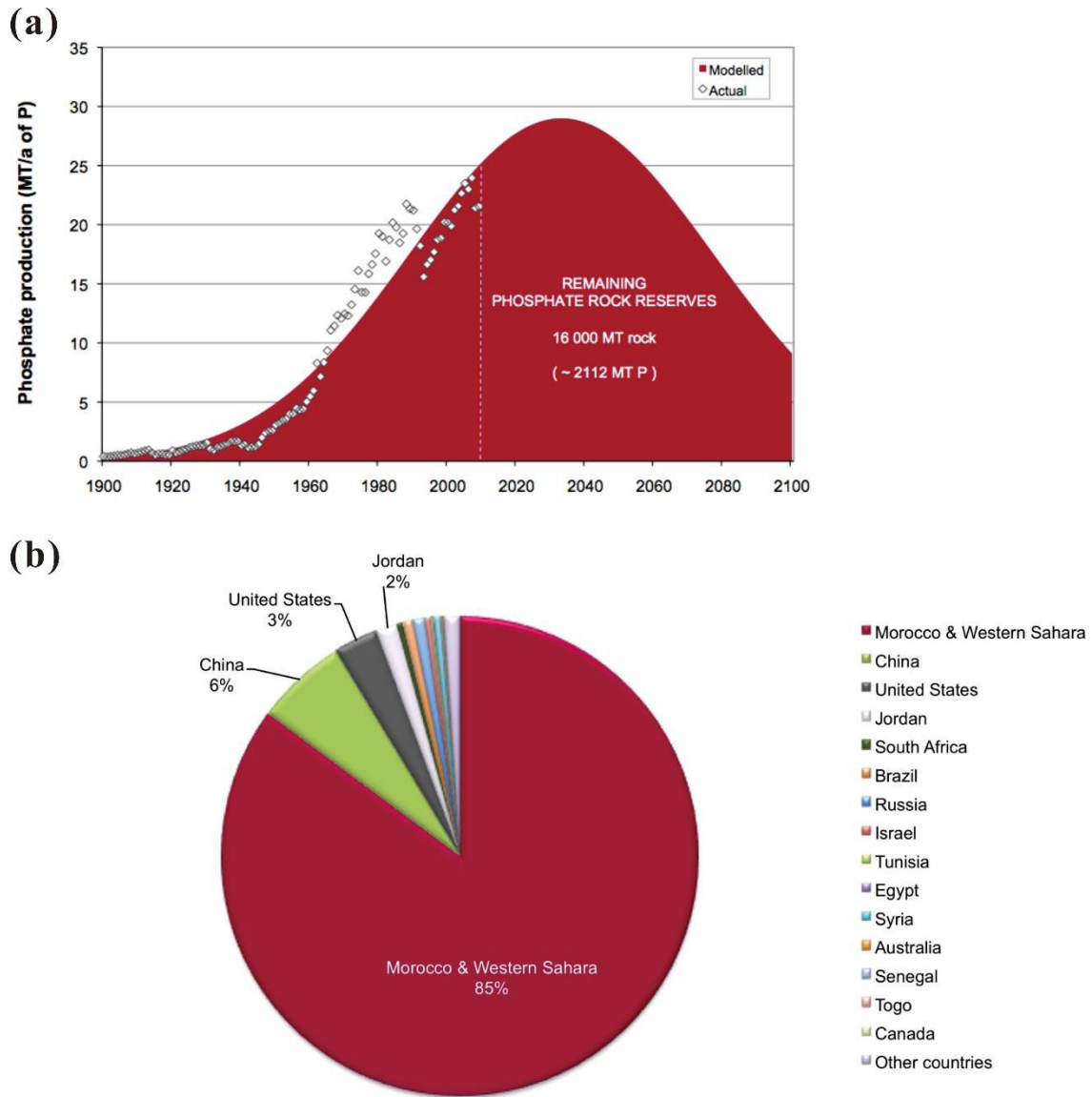


Figure 1.2. (a): Evolution of global P rock reserves, and predicted peak in production by 2033 as derived from US Geological Survey and industry data. From Cordell and White (2011). (b): Remaining global P rock reserves by countries of production as reported in 2010 by International Fertilizer Development Center (IFDC). After Cordell and White (2011)

The distribution of P rock reserves is not uniform on Earth, these reserves being mainly located in China, Morocco, Jordan, South Africa and the United States. These five countries control more than 95% of the known phosphate reserves (**Figure 1.2b**). Due to the rapid depletion of the known P reserves with easy accessibility and high grade (Cordell and White, 2011), the future P resources will certainly be more costly to extract and of lower quality. This will lead to geopolitical stresses and have significant implications for farmers and food security, such as the 800% price spike for phosphate rock and other fertilizer products in 2008 (Cordell and White, 2011).

1.1.3 The P dilemma: too much vs. too little

Despite the predictable oncoming of global P resources scarcity, the use efficiency of P fertilizers is still too low, with only 15-30% of the applied P fertilizers being taken up by harvested crop (FAO, 2006). Over the past two decades, the fertilizer industry, governmental institutes, and research organizations have actively supported more efficient fertilizer application practices, such as recycling of organic matter containing P, and using organic farming techniques to optimize soil conditions to increase soil phosphorus availability for plants (FAO, 2008). However, much of the world's cropland (70%) is still subjected to P surpluses, due to the historical over-application of P onto soils (MacDonald et al. 2011).

Thus, the application of P fertilizer meets the human's increasing food demand in western countries but meanwhile leads to the accumulation of high quantities of P in agricultural soils. This accumulation of P in soils generates a risk of P transfer from soils to surface waters through soil erosion and leaching (Bouraoui and Grizzetti, 2011). Once in streams, P will find its way to large rivers and estuaries and end up into oceans, it will be retransformed into rocks and eventually uplift to terrestrial layer over geological timescales, where it will form new mineable P rock reserves. Some parts of P in streams can also be deposited in wetlands, lakes, and reservoirs, which add time-lags to the delivery of P into oceans.

However, the excessive presence of bioavailable P in aquatic ecosystems due to human acceleration of the P cycle has damageable consequences for these ecosystems, because P together with nitrogen stimulates the growth of phytoplankton, algae, and aquatic plants, causing the process of water eutrophication (Smith and Schindler, 2009; see **Figure 1.3**). It is generally accepted that P is the limiting nutrient for eutrophication in freshwater ecosystems, while the coastal water eutrophication is limited by nitrogen (Schindler et al., 2008). Water eutrophication has many deleterious consequences for aquatic ecosystems and humans, including reduction of biological functions and biodiversity, decline of surface water quality for drinking water production and recreational purposes, development of toxin-producing algae like cyanobacteria, etc. In particular, the toxins produced by the proliferation of cyanobacteria in eutrophic freshwater can affect animal and human health through exposure during drinking or bathing (Serrano et al., 2015).

To limit P losses to waters is thus of critical importance, not only to avert the future

P crisis by keeping the P cycling in the terrestrial ecosystem but also to secure human health by preventing the eutrophication of water bodies.

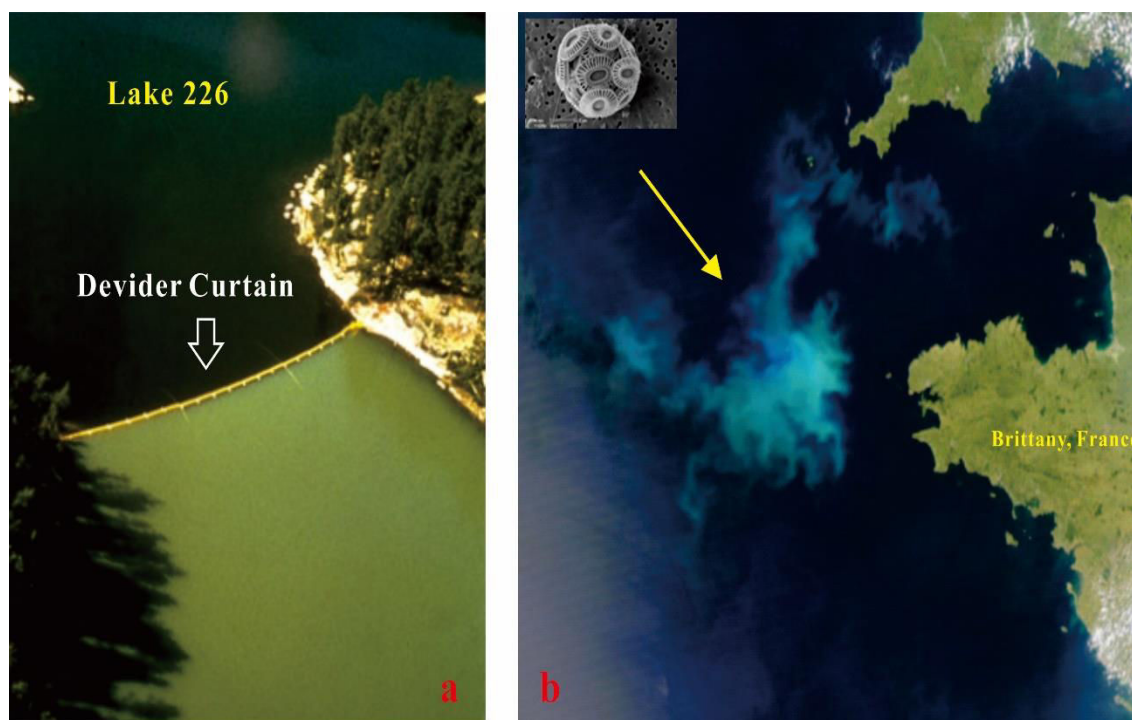


Figure 1.3. (a): Aerial photo of Lake 226 (Ontario, Canada) showing the result of lake fertilization experience with (south of the divider curtain) and without (north of the divider curtain) addition of P. The photo clearly shows the resulting algal blooms (cyanobacteria) in the lake side where P was included in the fertilization mixture. From Schindler (1977), see also Bennett and Schipanski, (2012). (b): Chalky, whitish blue bloom of marine algae (coccolithophoridae) in the Atlantic ocean in the west of Brittany, France, where is a world hotspot for nitrogen and P losses to coastal waters. Although coccoliths are small (micrometer scale), they often form large, concentrated blooms that are visible from space. Photo courtesy of NASA. From Filippelli (2008).

1.2 P losses to waters: point vs. diffuse sources

Phosphorus can be lost to waters from essentially two sources: i) point sources from urban and industrial wastewater treatment plants and ii) diffuse sources from agricultural landscapes, either through erosion of P-rich soil particles during overland flow, or P leaching during drainage and subsurface water flow (**Figure 1.4**). With the implementation of targeted measures such as banning P in detergents, precipitating P in sewage water treatment plants, or purifying industrial wastewater for P, P losses from point sources have generally been strongly reduced in many western countries (Collins et al., 2014; Schoumans et al., 2014). However, eutrophication is still occurring in many lakes and estuaries in these countries, pointing to the permanent

contribution of diffuse sources that transfer P from agricultural soils to surface waters (Billen et al., 2007; Grizetti et al., 2012; Jarvie et al., 2017; Scavia et al., 2014; Schoumann et al., 2014).



Figure 1.4. Examples of (a) plant water treatment system of urban sewage; (b) agricultural overland flow and channel drainage; (c) agricultural overland flow and soil erosion. Photos from Google (a and b) and Kervidy-Naizin catchment, Western France (c).

The diffuse transfer of P from soils to waters occurs mainly through soil erosion and overland flow, which are the most important P loss pathways in hilly and mountainous areas, and more importantly from soil leaching and artificial tile drainage in flatter areas (Chapman et al., 2005; Chardon and Schoumans, 2007; Heathwaite et al., 2005b; Nelson et al., 2005; Ulén et al., 2010). Managing agricultural diffuse P loss is difficult because of the large spatiotemporal variations in water pathways and soil erosion processes and the complexity of P forms in both soils and waters (i.e. dissolved organic and inorganic P, colloidal P, and particulate P). Combined with the continuously increasing food demand, the percentage of P loss to watercourses originating from diffuse agricultural sources has increased over the past two decades in Europe and North America. It was estimated that agricultural P loss contributed 50% of the total P

load to coastal waters in North America, Denmark, and Finland during 2000 (OECD, 2001). As shown in an assessment study at the scale of France, 46% of the total P input in watercourses came from diffuse agricultural sources between 2005 and 2009, versus 97% for nitrogen (Dupas et al., 2015a). Another study in Brittany, Western France, estimated that 70% of the phosphorus input in watercourses had an agricultural origin during the period 2007-2011 (Legeay et al., 2015). These proportions should be considered with caution due to the high uncertainty in flux data, but an average contribution of >50% of agricultural emissions to the total P input in watercourses is generally accepted in Western countries (Alexander et al., 2008; Dorioz et al., 2013).

Thus, research efforts and management options in many countries are currently focused on understanding and decreasing diffuse P losses from agricultural sources (Collins et al., 2014; Kleinman et al., 2007; Schoumans et al., 2014; Sharpley et al., 2013).

1.3 Knowledge and research trends on diffuse P loss processes

1.3.1 The need for an integrated catchment approach

It is essential to limit diffuse P losses at all spatial scales, including the plot, field, catchment, regional and global scales. There are generally two strategies to study P transfer processes and dynamics in agricultural landscapes namely reductionist-based and complexity based approaches (Coveney and Highfield, 1995; Haygarth et al., 2005). The reductionist-based approaches are usually conducted at small spatial scales (plot, lysimeter, mesocosm batch) by inductive mechanistic, replicated hypothesis testing. The advantage of this approach is that it allows the isolation of P release and transfer mechanisms with high degree of precision, whereas it has the disadvantage of being far away from real field conditions (catchment, river or lake) and there are no actual means to connect them to the larger scale transfer processes and dynamics. In contrast, complexity-based approaches are usually conducted on large scales (catchment, river or lake) by empirical observation approaches. The advantage of these approaches is that the research sites cross the management scale and integrate the small-scale processes into natural, wider patterns. The disadvantage of these approaches lies in the difficulty in explaining the observed patterns and the great contingency and uncertainty (Haygarth et al., 2005).

Many studies have shown that the P loss mechanisms identified at the plot scale could

not always cannot always produce an obvious signal when extrapolated to the scale of headwater catchments (Haygarth et al., 2012). In the past, most studies on agricultural P losses reduction have focused on batch experiments or rainfall simulations, which developed knowledge only on P release processes at the plot scale (millimeter to decameter), but not sufficient to study the catchment as a whole (1-100 km²). Thus, there is an essential need to integrate the two approaches for the development of the overall P transfer science.

Over the past two decades, there has been an increase in the number of catchment-based studies, especially at the small catchment scale (<10 km²) where the complexity and uncertainty regarding water pathways and dynamics are minimized (Aora et al., 2010; Dahlke et al., 2012; Dillon and Molloy, 1997; Haygarth et al., 2005; Heathwaite and Dils, 2000; Jordan-Meille and Dorioz, 2004; Mellander et al., 2012b, 2015, 2016; Moreau et al., 1998; Rodriguez-Blanco et al., 2013a, b; Siwek et al., 2013). The basic hypothesis of the catchment approach is that the P signal at the outlet of the catchment summarizes all the P mobilization and delivery processes that occur inside the catchment. As the one selected in this thesis, catchments in rural areas are preferred where point source P losses are minimized, with the purpose to focus on the diffuse P losses. As also shown in this thesis, the catchment approach does not exclude laboratory approaches at the small scale to fully assess the nature of P release mechanisms in agricultural soils.

1.3.2 Some key concepts about P mobilization processes in agricultural catchments

In agricultural landscape, it is generally accepted that not all catchment areas equally contribute to P losses. They occur predominantly from areas where high P concentrations and high transport capacities (water flow paths) coincide. These zones of coincidence are called critical source areas (Heathwaite et al., 2005b; McDowell et al., 2014; Shore et al., 2014). Although generally representing less than 20% of the total catchment area, these zones export most of the P to the catchment outlet (Pionke et al., 2000). Thus, in this critical-source-area concept, P losses may be managed by reducing the availability of P at the source level (e.g. decrease fertilizer P inputs) and/or by changing water flow pathways and trapping the exported P through the implementation of buffer zones at the interface between the hillslope and the stream.

Another concept to study the P transfer in agricultural landscapes is the “Phosphorus transfer continuum” which highlights the interdisciplinary and multi-scaled nature of the P transfer science (Haygarth et al., 2005). The P transfer continuum in agricultural landscapes assumes that the overall process can be decomposed into four steps, i.e. “sources”, “mobilization”, “deliver”, and “impact” (**Figure 1.5**).

The P “sources” can be of natural (indigenous soil-P mineralization and atmospheric deposition of P-rich micro-particles) or anthropogenic (fertilizers and manure applied to the soils) origins. Because the natural background concentrations of P in soils were generally insufficient to meet the increasing demand of farm yields, humans have applied fertilizers and imported animal feed to counterbalance the low natural P concentration in soils. This application and import are in many regions excessive, leading to the accumulation of P in soils, usually referred to as “legacy phosphorus” (Haygarth et al., 2014; Jarvie et al., 2013a; Sharpley et al., 2013). The “mobilization” describes the processes by which the P is separated from its sources in the soils, either by solubilization under the action of chemical/biochemical processes or by the physical detachment of P-rich particles. It has long been evidenced that the potential of P solubilization (indicated by the P concentrations in soil solutions) generally increases with the increasing concentration of extractable soil P (McDowell et al., 2001). Thus, agronomic P tests, which were originally designed to estimate the fertilization requirement of soils, are nowadays often used as environmental risk assessment test, as they have the capacity to evaluate the P solubilization potential in soils (Jordan-Meille et al., 2012; Wall et al., 2013). These soil P tests are even used as regulation tools in several European countries (Amery and Schoumans, 2014). The degree of P saturation (DPS) provides another tool to evaluate the risk of P solubilization in soils, which is currently used as a regulation and management tool in several countries (Khiari et al., 2000; Leinweber et al., 1999; McDowell et al., 2002; Schoumans and Chardon, 2015). The DPS can be calculated by adsorption isotherms or by chemical extraction of iron and aluminum oxides, which are considered as the two main P-sorbing phases in soils (Renneson et al., 2015). The physical detachment of soil P-rich particles is controlled by the capacity of soils to be eroded, either via surface runoff or through soil macropore water flows. The detachment capacity of soils can be estimated by structural stability

tests of soils (Le Bissonnais et al., 2002).

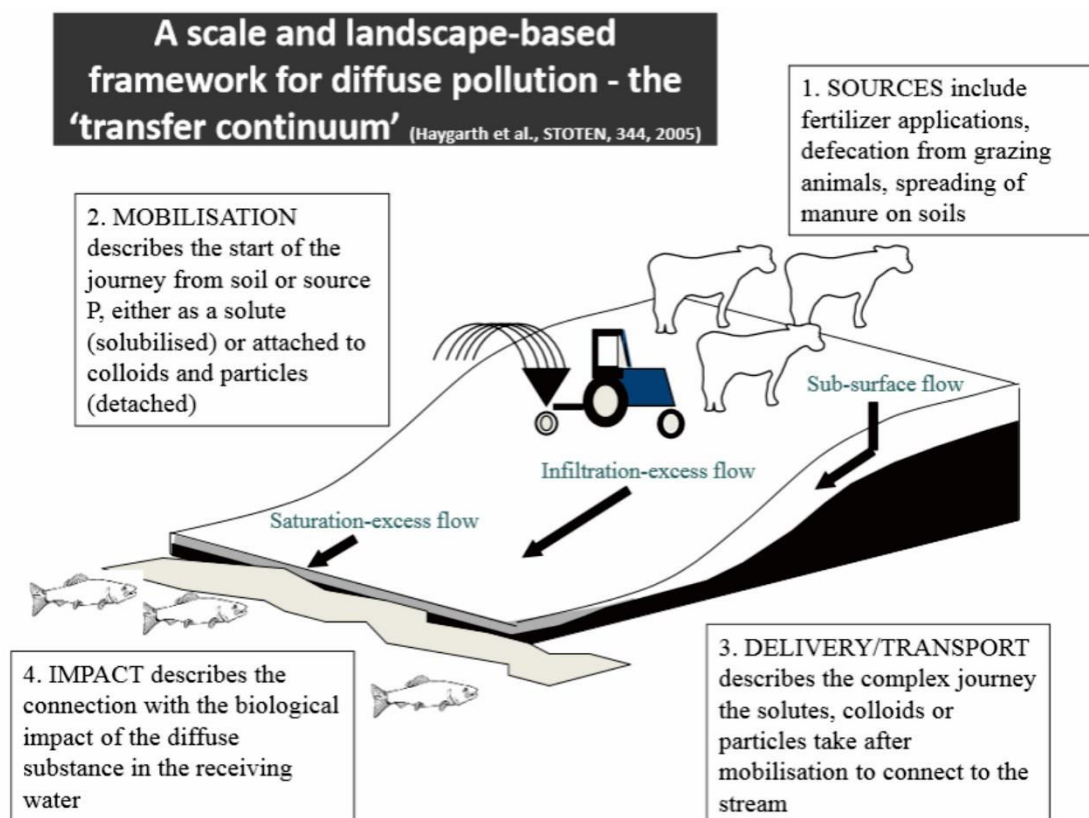


Figure 1.5. Phosphorus transfer continuum in agricultural landscapes. From Haygarth et al. (2005).

The “delivery” of the mobilized P to water bodies can only occur if there is hydrological connectivity between the P source in the catchment and the receiving water body. Overland flow caused by saturation excess, a situation generally confined to high-magnitude and high-intensity rainfall events, was for long considered as the dominant pathways of P transfer, mobilizing both particulate and dissolved P forms (Boardman, 1990; Fraser et al., 1999). More recently, however, contribution of subsurface transfer (natural or due to artificial drainage) of dissolved and colloidal P was acknowledged to be an important transfer pathway in agricultural catchments (Heathwaite and Dils, 2000; Jordan-Meille and Dorioz, 2004; Mellander et al., 2016; van der Salm et al., 2011). The soil properties, groundwater level, the presence of tile drains, and topography are all factors that control this subsurface transfer. These factors varied spatially (from point to plot, field, hillslope, and catchment) and temporally (due to temporal variations in water table levels), making the quantification and scaling-up of this P transfer pathway very complex (Haygarth et al., 2012). The complexity is even

enhanced due to the chemical nature of P, which can be adsorbed onto soil matrix and thus become retained during transport, a process which can strongly vary both in space and time (Haygarth et al., 2005).

Finally, the “impact” of P transfer refers to the perturbation that P inputs cause in a receiving water body. The present knowledge is able to identify some aspects of the P-associated deterioration of water quality (such as eutrophication). Moreover, more investigation is needed to quantify the exact linkage between source types and locations, transfer processes and pathways and impacts (McDowell et al., 2004). There is an increasing need to track P inputs backward, from delivery through mobilization to source locations of P in catchments, to fully link the processes of P release in farms and agricultural soils to the impacts of diffuse P losses in water bodies (Haygarth et al., 2005).

1.3.3 Are dissolved and colloidal P species major components of diffuse P losses in agricultural landscapes?

Originally, most of the P losses from agricultural landscapes were assumed to consist of particulate P, with the surface runoff and soil erosion as the main source and transport pathway. Recently, the situation has changed, with the recognitions that the mobilization of dissolved P (DP) is a larger contributor to P losses from agricultural lands than previously acknowledged (Hahn et al., 2014; Jordan-Meille and Dorioz, 2004; Kleinman et al., 2007, 2011). For example, Rodriguez-Blanco et al. (2013b) estimated that 54% of the P exported during base flow in a small Spanish agricultural catchment consisted of DP. In another study, Mellander et al. (2016) estimated that more than 50% of P lost via groundwater and consisted of DP. Even during storm flow, DP can be the dominant P fraction transferred. For example, a survey of 53 storm events in the Kervidy-Naizin catchment in Brittany, western France, revealed that the proportion of DP averaged ca. 30% of the total P fluxes with maximum fractions as high as 61% during some storms (**Figure 1.6**).

This change in paradigm poses very concrete issues regarding P management, demonstrating for example that reducing soil erosion could not be sufficient to protect surface waters from P inputs from agricultural soils (Kleinman et al., 2011). They also pose a series of very fundamental questions such as (i) where are DP source areas located in agricultural landscapes? ii) by which mechanisms is this DP component

generated and transferred from soils to waters, iii) what is the composition of the soil P reservoirs from which it is formed? and finally, iv) what is the exact chemical nature of the P released in solution.

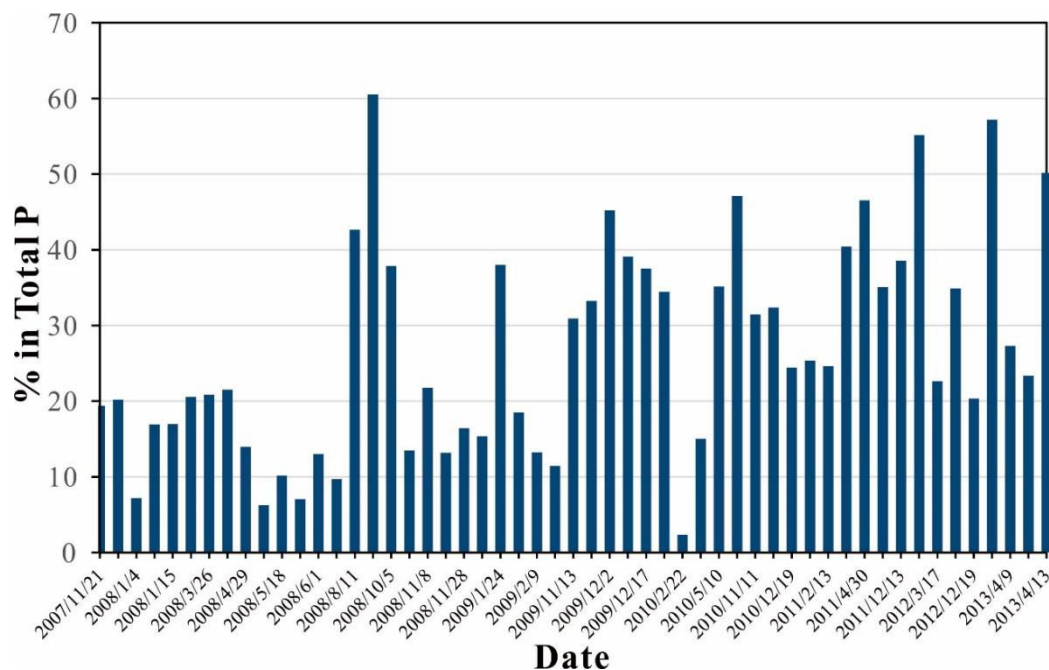


Figure 1.6. Proportion of the total P flux occurring as dissolved P (in weight %) during the 53 storm events monitored at Kervidy-Naizin (Western France) catchment outlet, from 2007 to 2013 (unpublished data).

DP is the target of many water quality programs for P. For example, the Water Framework Directive, which requires EU members to achieve at least ‘good’ ecological status in all waterbodies, sets a $50 \mu\text{g l}^{-1}$ DP limit for baseflow in low-alkalinity rivers in the UK (UK TAG, 2008). DP in soil solutions is operationally defined as P that can pass through $0.45 \mu\text{m}$ filters. The DP fraction which can directly react with the acid-molybdate solution is usually defined as the molybdate reactive (dissolved) P (MRP) fraction, the remaining fraction which does not react with the acid-molybdate solution is usually defined as the molybdate unreactive (dissolved) P (MUP) fraction. It is generally assumed that the MRP fraction consists mainly of ortho-phosphate, and is directly available for algal uptake, acting as a direct trigger of freshwater eutrophication. On the contrary, the MUP fraction is generally considered to consist of organic P, and to be less bioavailable than MRP.

There has been an increasing concern about this operationally defined P speciation. As a recent study showed, many of the P-bearing organic compounds could be in fact

more bioavailable than earlier thought (Li and Brett, 2013). Furthermore, there is actually no natural cutoff between true dissolved species and colloidal particles. Colloids, which have a size range from 1~1000nm, span the operational 0.45 μm limit, and could thus be accounted as DP. Several recent studies showed that colloids could be important carriers for P transfer from agricultural soils to aquatic ecosystems (Heathwaite et al., 2005a; Hens and Merckx, 2002; Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013; Siemens et al., 2008). Moreover, other studies suggest that the mobilized colloids could be transported across a long distance in soils because of their low reactivity with the soil matrix (Baalousha et al., 2005) and that some colloidal P forms could be bioavailable for algae (Van Moorlegem et al., 2013). These results point to the need to estimate the exact speciation of P when studying the transfer of DP from soils to surface waters.

1.3.4 Riparian buffer zones as potential sources of dissolved and colloidal P agricultural catchments

A widely recognized strategy to limit the agricultural P loss is to prevent the “delivery” of the mobilized P by the use of riparian buffer zones, which comprise non-cultivated land strips placed at the interface between agricultural fields and surface water bodies (Robert et al., 2012). These buffer zones can be components of the natural landscapes such as riparian wetlands, forests, swamps, etc., or be constructed zones including filter trenches, hedge rows, or grassed areas sown as vegetated buffers (Dorioz et al., 2006). The establishment of riparian buffer zones is very efficient at retaining particulate P by vegetation (**Figure 1.7**), with retention yield ranging from 41-95%, depending on the situations (Hoffmann et al., 2009).

However, riparian zones suffer from one severe drawback with respect to P retention, i.e. the possible development in these zones of two biogeochemical processes capable of transforming particulate P into dissolved (and possibly colloidal) P. The first of these biogeochemical processes is related to the sudden change in soil moisture condition, i.e. drying-rewetting events that these zones may experience at the end of the dry summer season or during rainfall events following dry periods. As long established by laboratory studies, dry/wet cycles in soils can result in DP release mechanisms in soils. (Blackwell et al., 2009, 2010, 2013; Turner et al., 2003). The DP released by this process may come from the lysis of microbial cells (Grierson et al., 1998; Turner et al.,

2003; Turner and Haygarth, 2001), from solutes emitted by the soil micro-organisms to protect themselves from desiccation (Halverson et al., 2000), or from organic matter previously protected in soil micro-aggregates and disrupted by the physical stresses causes by drying (Blackwell et al., 2010). The second biogeochemical process is due to the episodes of water saturation that affect these zones and the reducing conditions these episodes cause. During these reducing conditions, the soil Fe (hydr)oxides can be reductively dissolved (Jeanneau et al., 2014; Knorr, 2013; Lambert et al., 2013). Yet, Fe (hydr)oxides are important P-bearing phases in soils. The reductive dissolution of Fe (hydr)oxides induced by the soil waterlogging will thus concomitantly result in the solubilization of P previously adsorbed or co-precipitated onto/within these soil Fe (hydr)oxides (Carlyle and Hill, 2001; Hoffmann et al., 2009; Jeanneau et al., 2014; Knorr, 2013; Lambert et al., 2013; Surridge et al., 2007; Zak et al., 2004).

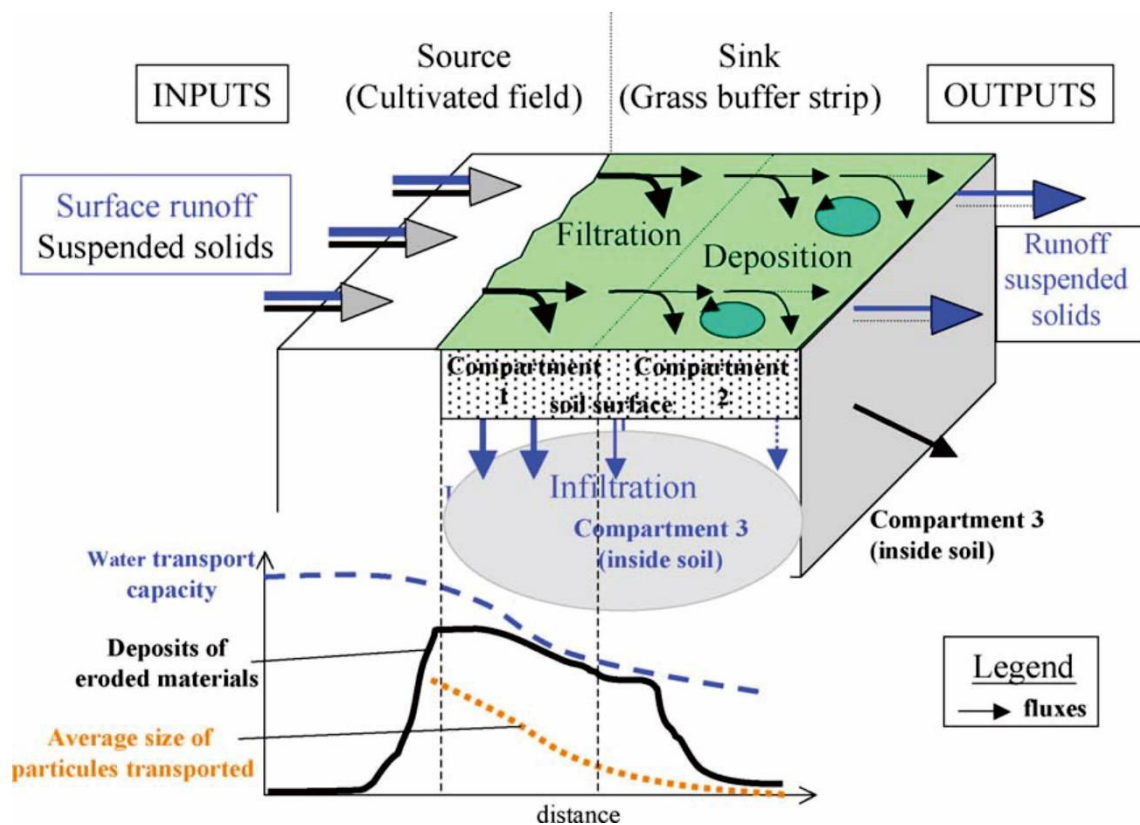


Figure 1.7 Schematic representation of the functioning of a vegetated buffer zone. (Dorioz et al., 2006)

Thus, there is a suspicion that riparian buffer zones could be the source zones of DP in catchments and possibly colloidal P components of diffuse P losses in agricultural landscapes. In fact, this possibility has been highlighted in several review studies, where riparian buffer zones have been hypothesized as temperate sinks of particulate P that could be transformed into DP via biogeochemical processes and thus increasing the delivery of DP into streams (Collins et al., 2009; Dorioz et al., 2006; Hoffmann et al., 2009; Robert et al., 2012; Stevens and Quinton, 2009). However, the exact links between water table fluctuation and redox dynamics in riparian buffer zones and DP release process at the catchment scale are still to be established, as well as the capacity of these zones to release colloidal P species. One fundamental reason for these lacks is that unraveling DP and colloidal P production mechanisms in riparian zones and quantifying their fluxes require the combination of high-frequency measurements of P concentrations and P forms both at the soil water-interface and at the catchment outlet, which requires time-consuming monitoring protocols. Another difficulty is the spatial variability of soil chemical properties and P status, as well as the temporal variations in hydroclimatic conditions (water table fluctuation) and water pathways.

1.4 The long-term Kervidy-Naizin Observatory: an ideal site for unraveling dissolved and colloidal phosphorus losses in agricultural catchments

A prerequisite for the implementation of mitigation strategies to reduce DP losses in the agricultural landscape is to identify sources within catchments, along with the mobilization mechanisms, transfer pathways, and their controlling factors. Excessive P (and nitrogen) in water bodies has been a regional challenge for water quality in Brittany, Western France, this region, representing a hotspot of intensive agriculture in France and Europe, and even in the world (**Figure 1.8**). Based on this situation, the Kervidy-Naizin catchment is monitored as a long-term field observatory (ORE AgrHys, https://www6.inra.fr/ore_agrhys) since 1993 to understand and model how the spatiotemporal variations in pedo-geology, hydroclimatology, landscape and agricultural activities control water and solute transport in this typical headwater catchment.

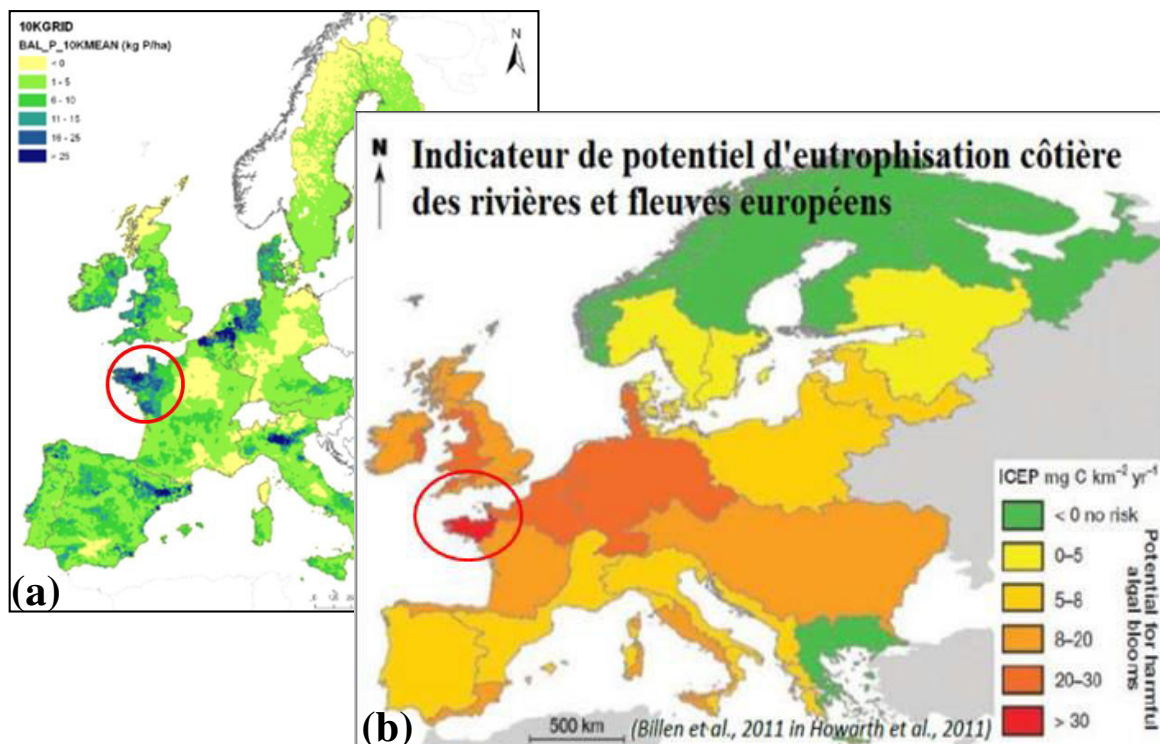


Figure 1.8. Maps of soil P surplus (a) and eutrophication risk (b) at the scale of Europe showing the unique, hotspot position of Brittany.

Because green algae blooms usually occur in coastal areas in Brittany, the research emphasis on the Kervidy-Naizin catchment was mainly nitrogen and dissolved organic carbon (DOC) until recently. However, 80% of the drinking water supply in Brittany comes from surface water reservoirs, which are frequently subjected to cyanobacteria blooms for which P is considered to be the limiting nutrient. The situation is similar for freshwater bathing sites in this region, which are regularly closed due to the proliferation of cyanobacteria in summer. For those reasons, the first systematic P study in this region was initiated in this catchment with the thesis of Dupas (2015) from 2013-2015, aiming at unraveling P transport processes and pathways at the catchment scale. Characterized by intensive agriculture (90% of Kervidy-Naizin land is dedicated to agriculture), very high animal densities (>35 000 pigs/5 km²), and high level of soil P content (up to 2.4 g/kg of total P) (Matos-Moreira et al., 2017), that study is rare even on the global scale. Before Dupas's work, a 6-years' of high-frequency monitoring of DP concentration at the outlet of the Kervidy-Naizin catchment indicated that DP is clearly a predominant part of P losses from this catchment during flood events (**Figure 1.6**). Other advantages of this catchment are that: i) it has been subject to ca. 20 years

of numerous pedological, hydrological and geochemical studies (Aubert et al., 2013; Crave and Gascuel-Oudou, 1997; Curmi et al., 1998; Dia et al., 2000; Dupas et al., 2015 a, b, c; Durand and Torres, 1996; Humbert et al., 2015; Lambert et al., 2011; 2013; Mérot et al., 1995; Molénat et al., 2002, 2008; Morel et al., 2009), so that soil characteristics, groundwater and stream water dynamics, soil biogeochemical processes, and nitrate and DOC transfer pathways are well constrained, providing a unique framework for implementation of the P transfer study, ii) this site benefits from numerous water sampling equipment thanks to other projects on nitrogen and DOC undergoing at the same time with the present thesis, such as zero tension lysimeters, gauging station, automatic samplers at the catchment outlet for continuous stream discharge and stream water composition monitoring (as detailed in Dupas 2015; see **Figure 1.9**). These preliminary studies make this catchment a unique site to conduct a combined field and laboratory assessment of the release mechanisms of DP and controlling factors.

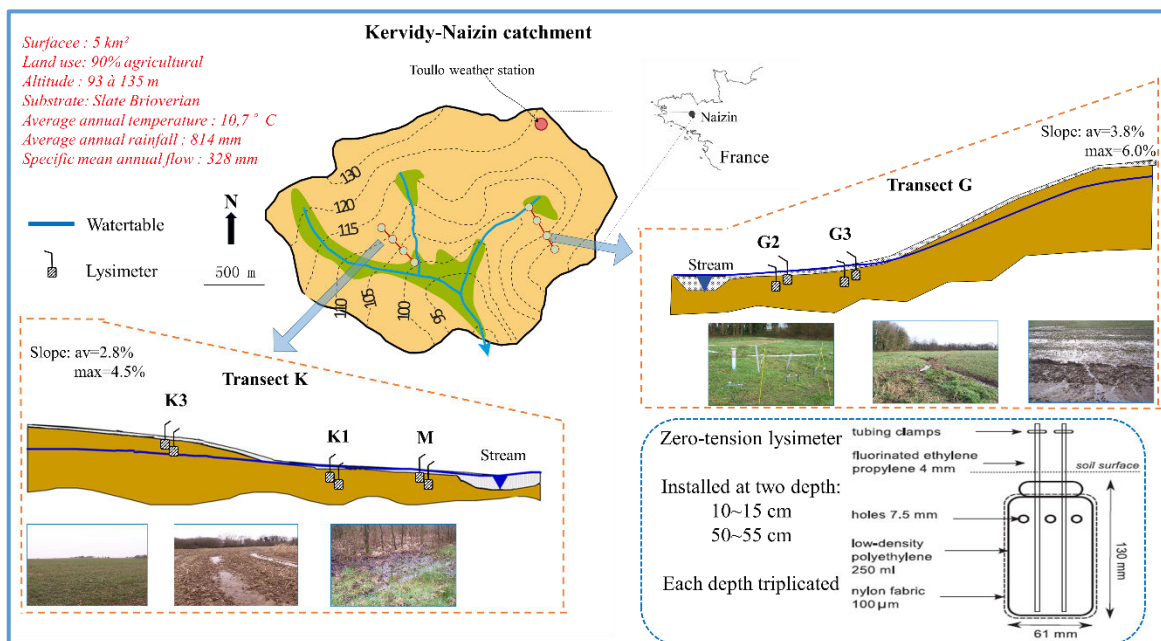


Figure 1.9. Sketch showing the main characteristics and equipment of the long-term observatory catchment of Kervidy-Naizin, Brittany, France.

1.5 General objectives and organization of the thesis

This thesis is based on the general context of understanding and reducing DP transfer in agricultural fields at the small catchment scale. This thesis estimated the DP release dynamics in soils in riparian wetlands (RWs) during baseflow periods, with the aim of identifying the mechanisms of DP (and colloidal P) release and their controlling factors. Field monitoring campaigns and laboratory simulation experiments are jointly conducted. The general objectives are fourfold:

1) What are the mechanisms that release dissolved P in RW soils under field condition? What is the role of water table fluctuations in controlling hydrological connectivity and DP solubilization mechanisms?

2) What are the factors controlling the spatial and temporal variability in DP release mechanisms in RWs? What is the role of hydroclimate variability and topography, and what are their influences on the water-table level and dynamic? Is the DP mobilized actually delivered to the stream? What is the chemical nature of the DP released (reactive or unreactive)? Do the soil properties play a role in this nature?

3) What is the speciation of the P released in soils during drying-rewetting cycles? What is the role of colloidal P? Do the different P forms react similarly to drying-rewetting cycles? How the soil properties and soil P status influence the speciation of the released P?

4) What are the mechanisms of the DP release in soils under reducing condition? What is the influence of soil properties? Does the addition of P-rich sediments from upland field runoff accelerate the DP release in RW soils?

Results related to these four objectives are presented and discussed in the following five chapters:

Chapter 2 tests whether RW zones are critical DP production zones in the Kervidy-Naizin catchments as hypothesized above, and explores the role of groundwater table dynamic as a key factor controlling the release dynamics and mechanisms of DP in RW soils;

Chapter 3 explores the interaction of hydroclimate variability, topography and soil properties in controlling the P release dynamics and the speciation of the released P;

Chapter 4 presents a laboratory simulation of the drying-rewetting cycles that naturally occur in RWs soils, to analyze the effects of these cycles on the various P species (truly dissolved P, colloidal-P), trying to evaluate the controlling factors of the

release of each P species;

Chapter 5 presents a laboratory simulation of the reducing conditions that naturally occur in RWs soils during winter water-saturation episodes, to analyze the role of soil Fe oxyhydroxide reductions on P release dynamics and P speciation in RW soils.

Finally, chapter 6 presents a general conclusion, summarizing the main results of the thesis, highlighting some implications of these results for managers, and finally summarizing possible future research directions.

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Chapter 2

Groundwater control of biogeochemical processes causing phosphorus release from riparian wetlands

This chapter corresponds to a paper published in the journal “Water Research”:
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Groundwater control of biogeochemical processes causing phosphorus release from riparian wetlands

Abstract:

Because of the high sorption affinity of phosphorus (P) for the soil solid phase, mitigation options to reduce diffuse P transfer usually focus on trapping particulate P delivered via surface flow paths. Therefore, placing riparian buffers between croplands and watercourses has been promoted worldwide, sometimes in wetland areas. To investigate the risk of P-accumulating riparian wetlands (RWs) releasing dissolved P into streams, we monitored molybdate-reactive P (MRP) in the soil pore water of two RWs in an agricultural watershed. Two main mechanisms released MRP under the control of groundwater dynamics. First, soil rewetting after the dry summer period was associated with the presence of a pool of mobile P, limited in size. Its mobilization started under water saturated conditions caused by a rise in groundwater. Second, anoxic conditions at the end of winter caused reductive dissolution of Fe (hydr)oxides along with a release of MRP. Comparison of sites revealed that the first MRP release occurred only in RWs with P-enriched soils, whereas the second was observed even in RWs with low soil P status. Seasonal variations in stream MRP concentrations were similar to concentrations in RW soils. Hence, RWs can act as a key component of the P transfer continuum in agricultural landscapes by converting particulate P from croplands into MRP transferred to streams.

2.1 Introduction

In agricultural landscapes, riparian wetlands (RWs) are highly reactive biogeochemical interfaces located between croplands and watercourses (Vidon et al., 2010).

Due to their topographic position in valley bottoms, fluxes of sediments, nutrients and pesticides converge in these zones. Therefore, establishment of vegetated buffers has been promoted in riparian areas worldwide to reduce pollutant transfer to streams. Riparian wetlands have proved effective in sediment retention (Ockenden et al., 2014), denitrification (Anderson et al., 2014; Oehler et al., 2007) and microbial degradation of pesticides (Maillard and Imfeld, 2014). Their role in the phosphorus transfer continuum

is less clear. Generally, RWs help decrease P delivery to watercourses by trapping particulate P and, to a lesser extent, sorbing dissolved P forms (Dorioz et al., 2006; Hoffmann et al., 2009).

However, RWs may also act as P sources for surface waters. This is due to the periodic water table fluctuations that affect these zones, which lead to a succession of dry periods and water-saturated periods (Obour et al., 2011; Song et al., 2007). Such unstable hydraulic conditions can increase P release from soil microbial biomass (Blackwell et al., 2010). Several laboratory studies have shown that soil rewetting after a dry period could lead to osmotic shock, causing microbial cell lysis and subsequent release of microbial P (e.g. Turner and Haygarth, 2001). According to Blackwell et al. (2009), up to ca. 70% of soil microbial biomass can be killed by osmotic shock caused by rewetting, with large variability in the amount of P released, depending on its recycling rate. Additionally, the periodic water table rises that affect RWs can modify the redox status of Fe (hydr)oxides, i.e. an important P sorbing compound in acidic soils (Li et al., 2012 ; Surridge et al., 2012). Anoxic conditions resulting from a high water table and low flow velocity can cause reductive dissolution of Fe (hydr)oxides in RW soils (Jeanneau et al., 2014 ; Knorr, 2013 ; Lambert et al., 2013 ; Li et al., 2012), which may cause release of previously adsorbed P (Carlyle and Hill, 2001 ; Hoffmann et al., 2009).

The presence of permanent vegetation in RWs can make biological P cycling in soils more intense than that in croplands as a result of more diversified plant and microbial communities (Roberts et al., 2012; Stutter et al., 2009). Permanent vegetation also results in high organic matter levels in RW soils, i.e. an important source of colloids (Haygarth et al., 2006), and provides favorable conditions for macropore formation. Hence, subsurface transfer of mobile P forms via preferential flow can be increased (Gachter et al., 1998; Haygarth et al., 1997). Although several laboratory experiments have highlighted mechanisms potentially involved in P solubilization and mobilization in RW soils (references above), a thorough literature search showed a lack of evidence regarding their contribution to diffuse P transfer in field conditions or the role of water table dynamics. In this study, we used zero-tension lysimeters to monitor molybdate-reactive P (MRP) concentrations in the free soil solution of two RWs in an intensively farmed watershed. The research questions addressed are: i) Does soil rewetting after a dry period and Fe (hydr)oxides reduction release MRP in RWs, and how are these

production mechanisms linked to water table dynamics? ; ii) Can we relate MRP concentrations in streams with P-release mechanisms in RW soils? ; and, iii) Which soil factors control the spatial variability of P-release mechanisms in RWs?

2.2 Materials and Methods

2.2.1 Study sites

The monitored RWs were located in Kervidy-Naizin, a 5 km² agricultural watershed belonging to the Agrhys environmental research observatory (http://www6.inra.fr/ore_agrhys_eng) in Brittany, France (Aubert et al., 2013; Aubert et al., 2014). The Kervidy-Naizin watershed is drained by a stream of second Strahler order. Climate is temperate oceanic, with a mean annual (2007 - 2013) temperature of 10.6°C and annual rainfall of 867 mm. Lithology consists of impervious Brioverian schists capped by up to 30 m of unconsolidated weathered materials, in which a shallow aquifer develops. The schist contains mainly quartz, muscovite and chlorite and, to a lesser extent, K-feldspar and plagioclase (Pauwels et al., 1998). The weathered schist is not likely to be a source of MRP in the watershed as mean MRP concentration measured below the soil depth (1m) was 7 µg l⁻¹ (unpublished results), which is lower than the long term mean baseflow concentration in the stream (18 µg l⁻¹, Dupas et al., 2015b). Soils are silty loams, classified as Luvisols. They are well-drained in the upland domain and hydromorphic in valley bottoms, where RWs develop. Agricultural activities are dominated by arable crops (cereals, maize) and animal production (pigs, dairy cows). We confined investigations to two RWs at the footslope of two transects equipped with piezometers (Molenat et al., 2008) (**Figure 2.1**). Transect A ranged in elevation from 110 to 120 m a.s.l. with a mean slope of 3.8% (max=6%). The RW in transect A was 51 m wide, and its vegetation consisted of unfertilized herbaceous species (*Dactylis glomerata* and *Agrostis canina*). Winter barley (*Hordeum vulgare*) was grown in the adjacent field during the study period (October 2013 - May 2014). Soil P content (0 - 15 cm) in this field was 315 mg P kg⁻¹ extractable P (Dyer method NF X 31-160) and 1283 mg P kg⁻¹ total P (NF X 31-147). Pig slurry was applied on this field on April 9, 2014 (61 kg P ha⁻¹). Transect B ranged in elevation from 104 to 109 m a.s.l. with a mean slope of 2.8% (max=4.5%). The RW in transect B was 64 m wide, and its vegetation consisted of shrubs and trees (*Populus negro*, *Salix caprea*, *Betula alba*). The adjacent field was left fallow during the study period, but maize (*Zea mays*) residues remained

on the soil surface. Soil P content (0 - 15 cm) in this field was 157 mg P kg⁻¹ extractable P (Dyer method NF X 31-160) and 1244 mg P kg⁻¹ total P (NF X 31-147). Pig slurry was applied on this field on May 2, 2014 (52 kg P ha⁻¹). Both RWs were managed as unfertilized riparian buffers for 20 years without exportation of biomass. Prior to conversion into a buffer-zone, RW A received up to 60 kg P ha⁻¹ yr⁻¹ as pig slurry and mineral fertilizer, whereas RW B received P input from grazing cattle (unquantified inputs).

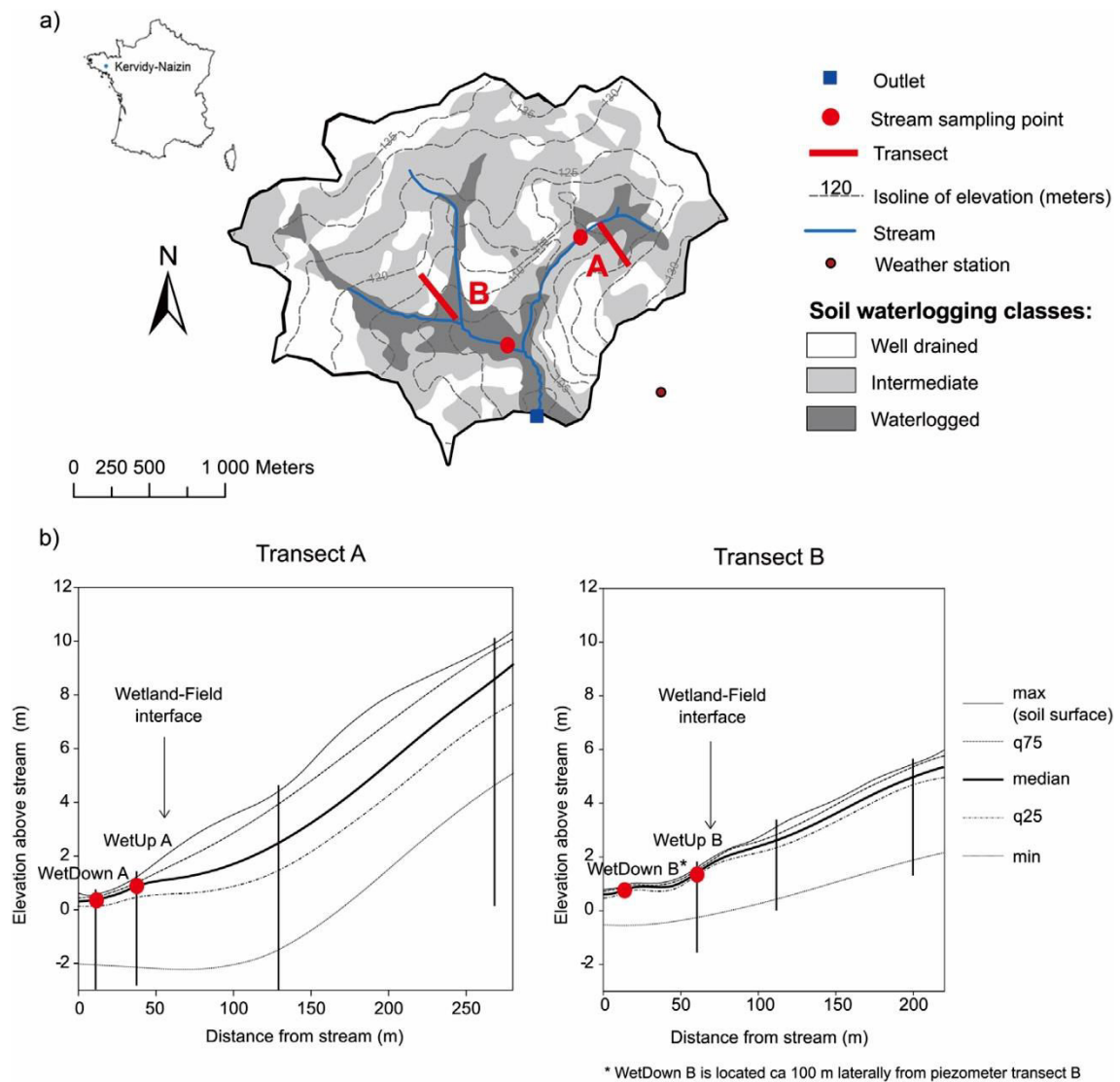


Figure 2.1. a) map of the Kervidy-Naizin catchment ; b) location of the sampling sites (red dots) and variability in water table level along the two transects. Vertical lines represent piezometers (depth 3 - 8 m; screening 1.5 - 4 m).

2.2.2 Soil and water sampling

Soil cores and soil pore water were sampled at two sites within each of the two RWs

(WetUp and WetDown). In transect A, WetUp A was 13 m downslope from the wetland-field interface and WetDown A was 40 m downslope from the interface. In transect B, distances from the wetland-field interface were 9 m and 52 m for WetUp B and WetDown B, respectively. The aim of this sampling design was to investigate the variability of soil P content and water table level, and their effect on soil MRP concentrations, along the flowpaths between the upslope and downslope side of the RWs (**Figure 2.1**).

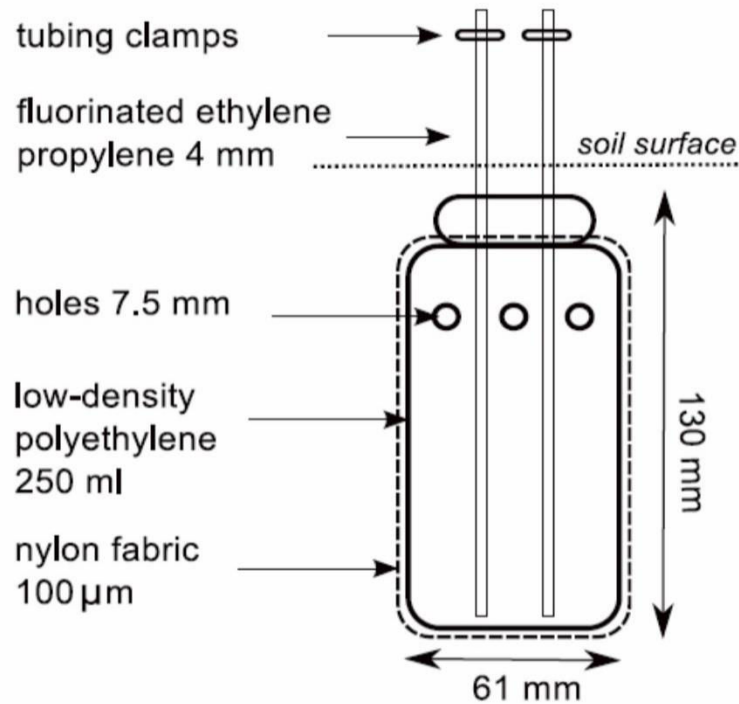


Figure 2.2 Zero-tension lysimeter (adapted from Bourrie et al., 1999).

Soil cores in the 0 - 15 cm and 25 - 40 cm horizons were collected with a 75 mm diameter sampler at each of the four sites in April 2014. WetDown B was only sampled in the surface due to water saturated soils below 15 cm. Soil pore water was collected with zero-tension lysimeters placed in triplicates (spaced ca. 1 m apart) at 10 - 15 cm and 50 - 55 cm depths, i.e. in the same soil horizons as the soil cores. The lysimeters were designed to collect free soil solution while maintaining in-situ anoxic conditions (**Figure 2.2**). After an equilibration period of three months, the 24 lysimeters were sampled weekly from October 2013 to January 2014 and every two weeks from February 2014 to May 2014. From June to October, soil moisture was too low to collect soil solution. Grab samples of stream water were collected at the same frequency downstream of the monitored RWs, and daily at the outlet of the 5 km² watershed.

Because we were interested in subsurface transfer, we focused analyses on baseflow concentrations by discarding grab samples collected during storms (surface runoff may contribute to storm flow). We considered storms as events with > 10% discharge rise and > 20 l s⁻¹ discharge (Dupas et al., 2015b). All samples were filtered (< 0.45 µm cellulose acetate filter) within 6 h after collection and kept refrigerated until analysis within 3 days.

2.2.3 Soil and water chemical analyses

Soil samples were air-dried, sieved to < 2 mm and analyzed for particle size fractions (NF X 31-107), organic matter/nitrogen/carbon contents (NF ISO 13878, NF ISO 10694), pH in water (1 :5 v :v water extraction NF ISO 10390) , extractable P (Dyer method, i.e. 1 :5 w/v extraction with citric acid 20 g l⁻¹ NF X 31-160), total P (ICP-AES after total solubilization with hydrofluoric and perchloric acid NF X 31-147), Al and Fe (ICPAES after extraction with ammonium oxalate and oxalic acid, according to Tamm 1922) (**Table 2.1**). Equilibrium P concentration (EPCo) and maximum sorption capacity (Qmax) were estimated from 6-point batch isotherms (0, 0.1, 0.5, 50, 100, 200 mg P l⁻¹; 1:25 w:v) in 0.01M CaCl₂ according to Graetz and Nair (2000). One drop of chloroform was added to inhibit microbial activity. After 24 h equilibration at 20±2°C, samples were centrifuged (3000 rpm; 10 min), filtered (< 0.45 µm) and analyzed for MRP. Qmax was determined by fitting a Langmuir equation (Van der Zee and Bolt, 2001) to the last three points (50, 100, 200 mg P l⁻¹):

$$Q = (c * K * Q_{max}) / (1 + K * c)$$

where c is the concentration of P in the equilibrium solution (mg l⁻¹), Q is the total amount of P sorbed (mg mg⁻¹) and K is an affinity parameter (l mg⁻¹). EPCo represents the solution P concentration at which no net sorption or desorption of P would occur between soil and solution (Stutter and Lumsdon, 2008). EPCo was determined by fitting a linear equation to the first three points (0, 0.1, 0.5 mg P l⁻¹). We consider EPCo as a reference MRP concentration in the soil solution, which we can compare to the actual MRP concentration of soil solution collected in-situ. Qmax served to calculate “Degree of P Saturation” (DPS), defined here as the ratio of Extractable P to Qmax. DPS is an index of P accumulation in the soil, either via direct application of fertilizers or enrichment via erosion (Schoumans and Chardon, 2015).

For each water sample collected in lysimeters or in the stream, MRP was determined colorimetrically by reaction with ammonium molybdate (ISO 15681). Because filtrates

< 0.45 μm can contain colloidal forms of molybdate reactive phosphorus, we chose to use the term MRP rather than soluble reactive phosphorus (Haygarth and Sharpley, 2000). Precision of MRP measurement was $\pm 4 \mu\text{g l}^{-1}$. Fe^{2+} was analyzed using the 1,10-phenanthroline colorimetric method, according to AFNOR NF T90-017, with a precision of 5%. Nitrate concentration was measured by ionic chromatography (DIONEX DX 100), with a precision of 2.5%.

Table 2.1. Soil properties in the riparian wetlands studied.

Site	WetUp A		WetDown A		WetUp B		WetDown B
Depth (cm)	0 - 15	25 - 40	0 - 15	25 - 40	0 - 15	25 - 40	0 - 15
Clay (< 2 mm) g kg ⁻¹	241	263	233	250	288	248	354
Silt (2-50 mm) g kg ⁻¹	615	584	589	574	599	583	603
Sand (50-2000 mm) g kg ⁻¹	205	223	268	271	169	265	57
Organic carbon g kg ⁻¹	40.9	9.5	37.1	10.7	60.2	14.8	89.1
Organic nitrogen g kg ⁻¹	3.7	1.2	3.3	1.4	4.7	1.4	6.4
C:N ratio	11.2	7.7	11.2	7.9	12.9	10.3	14.0
Organic matter g kg ⁻¹	70.7	16.5	64.2	18.5	104.0	25.6	154.0
pH	6.1	6.3	6.1	6.4	6.2	6.0	5.9
Dyer P mg kg ⁻¹	288.5	65.9	241.8	62.8	47.6	4.4	12.2
Al (Tamm method) mg kg ⁻¹	1690	1470	1410	1570	1710	1090	1560
Fe (Tamm method) mg kg ⁻¹	4250	3340	6000	7220	7710	1480	10200
Total P mg kg ⁻¹	1213.2	375.3	1051.7	384.5	680.8	198.6	458.2
DPS %	21	10	14	7	3	1	1
EPCo mg l ⁻¹	127.2	21.0	83.1	4.3	4.1	<4	<4
Soil solution MRP (mean ± standard deviation) mg l ⁻¹	220 ± 86	60 ± 73	126 ± 43	60 ± 81	16 ± 20	6 ± 8	6 ± 6

2.3 Results and discussion

2.3.1 Soil P content and water table depths in riparian wetlands

Soil total P content at WetUp A and B was 13% and 33% higher than that at WetDown A and B, respectively (**Figure 2.3**). A probable explanation for the higher soil P levels on the upslope side of the RWs is that P delivery from the adjacent fields has enriched RWs in P (Ockenden et al., 2014). Previous studies in the Kervidy-Naizin watershed have indeed evidenced that its loamy soils are vulnerable to erosion, which leads to spatial redistribution of soil and nutrients across the landscape (Le Bissonnais et al., 2002). Longterm monitoring in a larger number of sites would be necessary to quantify the importance of P accumulation in RWs at the watershed scale. Such a monitoring is however beyond the scope of this study, which focuses on the role of groundwater dynamics in P remobilization in RWs.

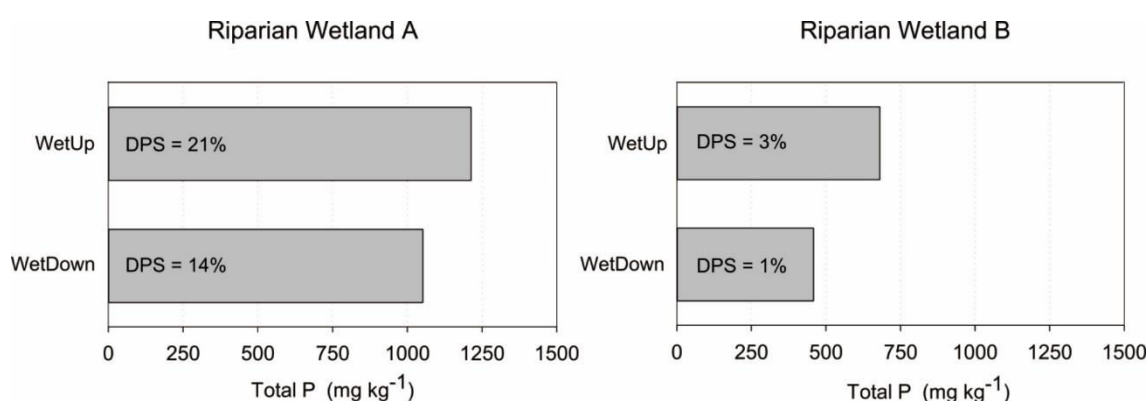


Figure 2.3. Total P content and Degree of P Saturation (DPS) in the 0-15 cm soil layer in four wetland sites.

Overall, data show contrasting hydrologic and soil conditions between the two RWs and within each RW. Mean water table level was shallower in RW B than in RW A (**Figure 2.1**), whereas soil P content and DPS were lower in RW B than in RW A (**Figure 2.3**). The legacy of past P inputs prior to conversion of these zones into unfertilized buffers, along with different rates of P enrichment from adjacent fields, are two possible explanations for the differences in soil P content between RW A and RW B (Dunne et al., 2011; Jarvie et al., 2013a; Sharpley et al., 2013). Within each RW, opposite gradients of mean water table and soil P content were observed, with higher soil P content on the upslope side close to the cropped field (WetUp) and a shallower

water table on the downslope side (WetDown).

2.3.2 Groundwater level controls P release in riparian wetlands

In both RW A and B, MRP concentrations in the soil solution appeared strongly linked to water table dynamics and/or stagnancy (**Figures 2.4 and 2.5**).

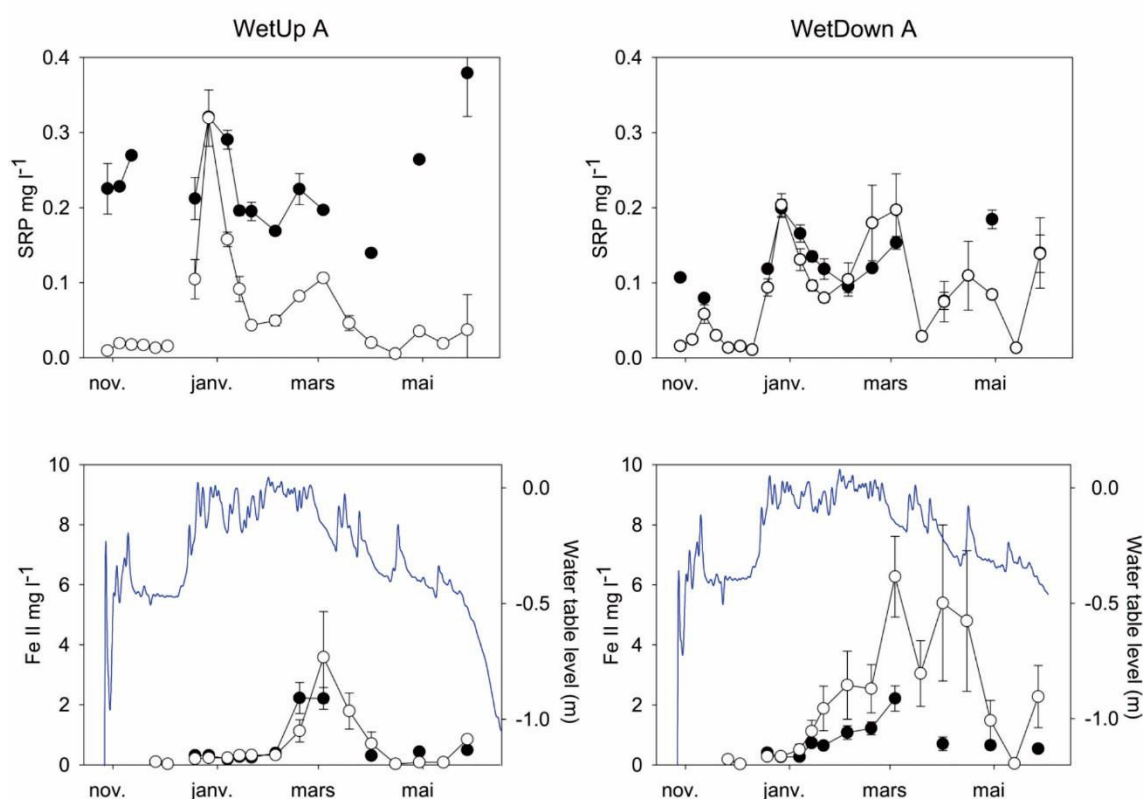


Figure 2.4. Water table level, molybdate-reactive P (MRP) and Fe²⁺ concentrations in soil solutions of riparian wetland A. Solid circles: 10-15 cm depth; empty circles: 50-55 cm depth. Error bars represent standard errors of triplicate samples.

At the beginning of the hydrological year (October - December), when the water table level was below -15 cm, soil MRP concentrations displayed a decreasing vertical gradient at all sites, reflecting the vertical gradient of soil extractable P (**Table 2.1**). Mean MRP concentrations from October 17th to December 10th were strongly correlated to the theoretical concentration EPCo ($r=0.98$), which suggests that the soil solution was at equilibrium with the soil solid phase at this time of the year, as is usually observed in well-drained soils (Vadas et al., 2007). Starting from December, MRP dynamics at the four sampling sites were dominated by the occurrence of two concentration peaks, whose amplitude depended on soil characteristics.

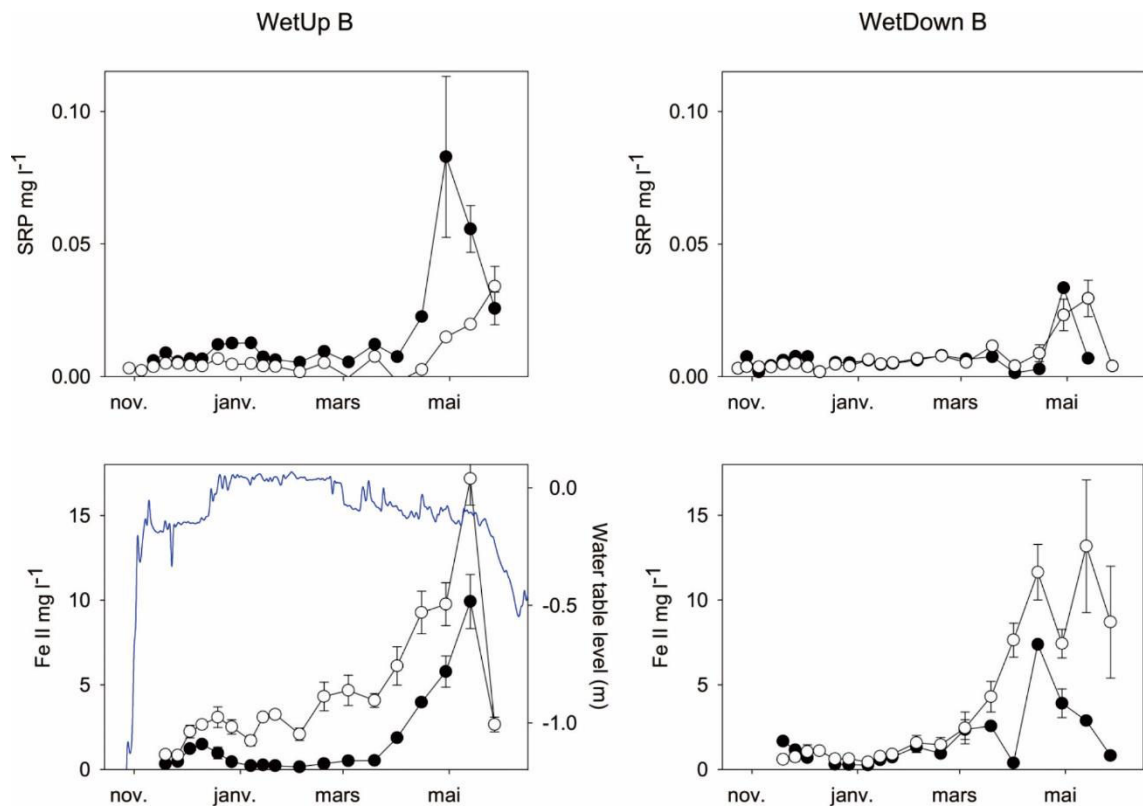


Figure 2.5. Water table level, molybdate-reactive P (MRP) and Fe^{2+} concentrations in soil solutions of riparian wetland B. Solid circles: 10-15 cm depth; empty circles: 50-55 cm depth. Error bars represent standard errors of triplicate samples.

A first MRP peak (December - January) was observed concomitantly with the sudden water table rise in the uppermost soil horizons of the wetlands. This peak was on average 31 times higher in RW A than RW B and, within each RW, it was higher in upslope sites than downslope sites (respectively 1.6 and 1.8 times in RW A and RW B). In RW A, MRP concentrations increased both at -15 cm and -50 cm, and the vertical concentration gradient disappeared. Concentrations at that time were also much higher than EPCo values, which suggest initiation of a P-release process. Most likely, the observed vertical homogenization of MRP concentrations was caused by the water table rising. Vadas et al. (2007) also observed that under conditions of water saturation, P mobilization into groundwater could result in MRP concentrations exceeding those in equilibrium with soils because of P leaching from the topsoil. In RW B, concentrations increased slightly and only at -15 cm at site WetUp, indicating limited P mobility compared to that in RW A; MRP concentrations did not vary in the other RW B lysimeters. Thus, it appears that this MRP peak was the highest at sites with high soil P content. This first MRP peak was followed by several weeks of gradually decreasing

MRP concentrations (January - February), although the water table remained near the soil surface. This decrease suggests exhaustion of a pool of mobile P present in the soil at the beginning of the hydrological year. Exhaustion of this P pool may be caused by its flushing or by its readsorption onto the soil matrix. Our observations are consistent with previous laboratory experiments that have shown that soil rewetting after a dry period could release organic and inorganic P as a result of microbial biomass being killed by osmotic shock (Blackwell et al., 2010; Turner and Haygarth, 2001). Previous monitoring campaigns in the same watershed, but focusing on the composition of dissolved organic matter using ultraviolet spectrometry, carbon isotopes and molecular biomarkers highlighted the release of microbial-derived organic compounds in the soil solution at the same time of the year (Jeanneau et al., 2014 ; Lambert et al., 2013). Hence, the most probable mechanism causing P release when the water table rose at the beginning of the hydrological season was mobilization of a P pool of microbial origin, limited in size, which can migrate under water saturated conditions (McGechan et al., 2005). Further work on P speciation (with a particular focus on organic forms) will help test the hypothesis that the P pool flushed at the beginning of the hydrological season is microbial-derived.

A second MRP peak (February - March in RW A, April - May in RW B) was observed concomitantly with an increase in Fe^{2+} concentrations in periods of low NO_3^- concentrations ($< 1 \text{ mg l}^{-1}$) in the soil solution (Supplementary material). This peak was again higher in RW A than RW B. In both RW A and B, MRP concentrations increased at 15 cm and -50 cm. At WetDown A, MRP concentrations at -50 cm exceeded that at -15 cm and that at -50 cm at WetUp A, similar to Fe^{2+} concentrations. In RW B, MRP concentrations increased the most at WetUp -15 cm, i.e. where soil P content was the highest. Hence, P release seemed more a function of the amount of Fe^{2+} being dissolved in RWA but more a function of soil P status in RW B. This indicates that reductive dissolution of Fe (hydr)oxides is the mechanism causing the P release observed under anoxic conditions (Carlyle and Hill, 2001). Anoxic conditions at this time of year were due to low rainfall in a period of high water table, which decreased water velocity and thus water oxygenation (Jeanneau et al., 2014). As a result, Fe (hydr)oxides became solubilized as Fe^{2+} (Bourri  et al., 1999), which released previously adsorbed P (Hoffmann et al., 2009 ; Vidon et al., 2010). The amount of P released by Fe (hydr)oxide reductive dissolution appeared to be controlled by both the amount of P sorbing sites

that were solubilized and the amount of P previously adsorbed on them. The timing of this second peak appeared to be influenced by NO_3^- dynamics, as reductive dissolution of Fe (hydr)oxides started after NO_3^- had been consumed arguably through denitrification.

Overall, seasonal variation in groundwater MRP concentration was controlled by water table fluctuation, and spatial variability among wetland sites depended on soil P status and propensity of Fe (hydr)oxides solubilization.

2.3.3 Linking MRP concentration in riparian wetlands and in the stream

In-situ observations of soil solution MRP concentrations can be directly linked to stream concentrations. We used stream MRP concentration measured downstream of the two RWs and at the watershed outlet. This combined soil and stream observation could provide a complete view of the solubilization-mobilization-transport continuum from RWs soil to surface water (Haygarth et al., 2005; Haygarth et al., 2012; Mellander et al., 2012a).

The water table rise in December - January was accompanied by a sudden increase in discharge (**Figure 2.6**). Stream MRP concentrations also increased downstream of both RW A and B and at the watershed outlet. They were higher downstream of RW A than RW B, similar to differences observed at the same time in the soil solution (**Figures 2.4 and 2.5**). Stream MRP concentrations then decreased, probably as a result of exhaustion of the previously mobilized P pool in wetland soils. They increased again in February - March downstream of RW A and in April - May downstream of RW B. Again, the timing of these MRP peaks and their relative amplitude between RW A and B was consistent with observations of P release caused by reductive dissolution of Fe (hydr)oxides in wetland soils. Hence, variation in stream MRP concentrations could be related to the two successive P-release mechanisms identified in RWs. Despite this synchronization of peaks between wetland soils and the stream, differences in the relative amplitude of the peaks suggest that retention processes occurred: for example, the second peak was not as large as the first one in the stream downstream of RW A, whereas the amplitude of P release within the soil was similar during the first and second peak. This suggests that some of the MRP released in wetland soils via Fe (hydr)oxides reductive dissolution was not transferred to the stream, probably because of Fe^{2+} re-oxidation on its way to the stream and re-adsorption of P on the newly formed Fe (hydr)oxides (Baken et al., 2015; van der Grift et al., 2014).

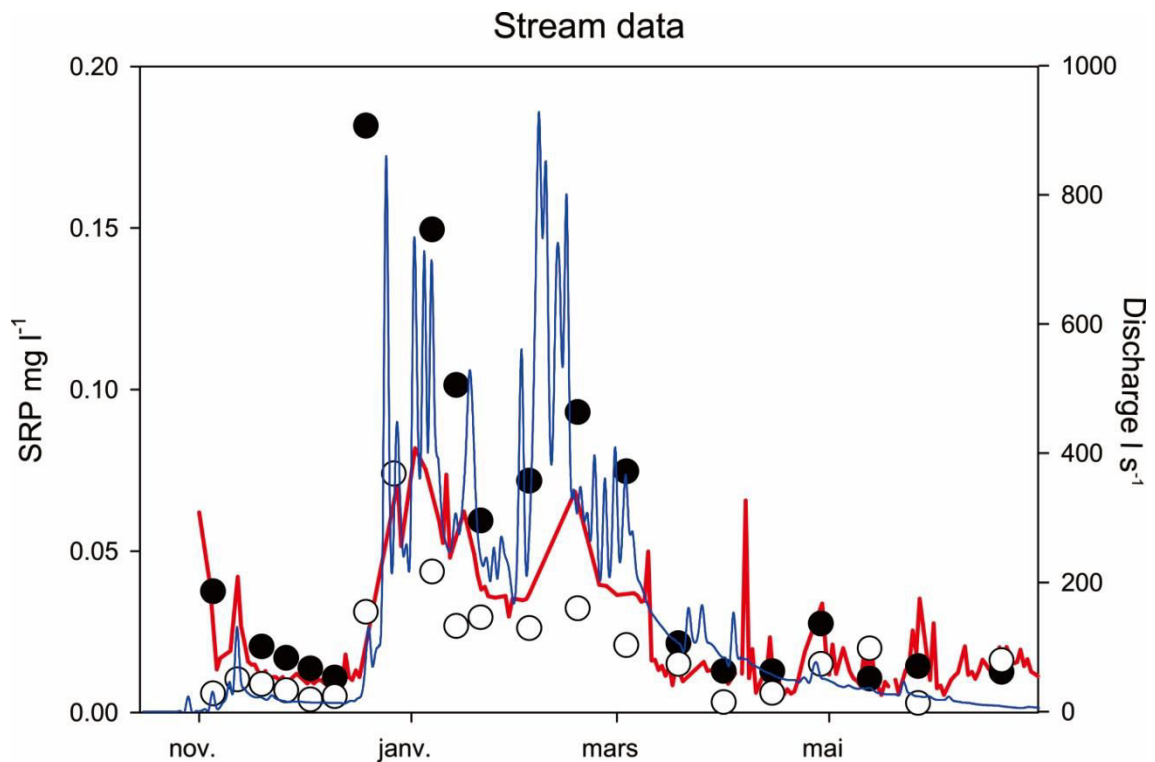


Figure 2.6. Discharge (blue line) and stream molybdate-reactive P (MRP) concentrations during baseflow periods near RW A (solid circles), RW B (empty circles) and at the watershed outlet (red line).

This connection between MRP concentrations in RWs and streams completely explains stream observations made earlier by (Dupas et al., 2015b) that periods of high MRP concentrations coincided with the first water table rise in RWs and periods of anoxic conditions in RW soils. This study also confirms that RWs can be major sources of MRP in agricultural watersheds through their enrichment in particulate P delivered from upland fields via erosion followed by the solubilization-mobilization of this allochthonous P in water saturated conditions (Roberts et al., 2012; Stutter et al., 2009).

2.4 Conclusion

Riparian wetlands are commonly promoted as buffer zones to reduce P transfer to streams. This study demonstrates that enriched and/or periodically anoxic RWs can actually be hot spots of MRP in agricultural watersheds. “Hot moments” of P release were related to water table dynamics, which activated two successive release mechanisms:

- Soil rewetting after the dry summer caused mobilization of a pool of mobile P, probably of microbial origin. Soil P status appeared to be the main controlling

factor of the spatial variability in autumn flush;

- Anoxic conditions at the end of winter caused reductive dissolution of Fe (hydr)oxides, which released MRP into soil pore water. Propensity of Fe (hydr)oxides solubilization (determined by soil wetness, Fe content of the soil and presence of NO_3^-) appeared to be the main controlling factor of the spatial variability in end of winter P release.

Phosphorus release mechanisms in wetlands produced a clearly discernable signal in the stream both downstream of the monitored RWs and at the outlet of the watershed. To further investigate the biogeochemical processes taking place in RW soils, future monitoring in the Kervidy-Naizin catchment will focus on P speciation in soil and soil pore water, with a particular focus on organic P and colloidal P forms.

Given the risk of P remobilization in P-accumulating RWs, management options should focus on:

- Preventing P mobilization in upslope fields to avoid further enrichment of RWs by erosion;
- Recovering legacy P from RWs by exporting biomass;
- In highly sensitive areas, emerging technologies involving the use of P-sorbing materials could be implemented to increase P immobilization in riparian zones.

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2.5 Supplementary materials

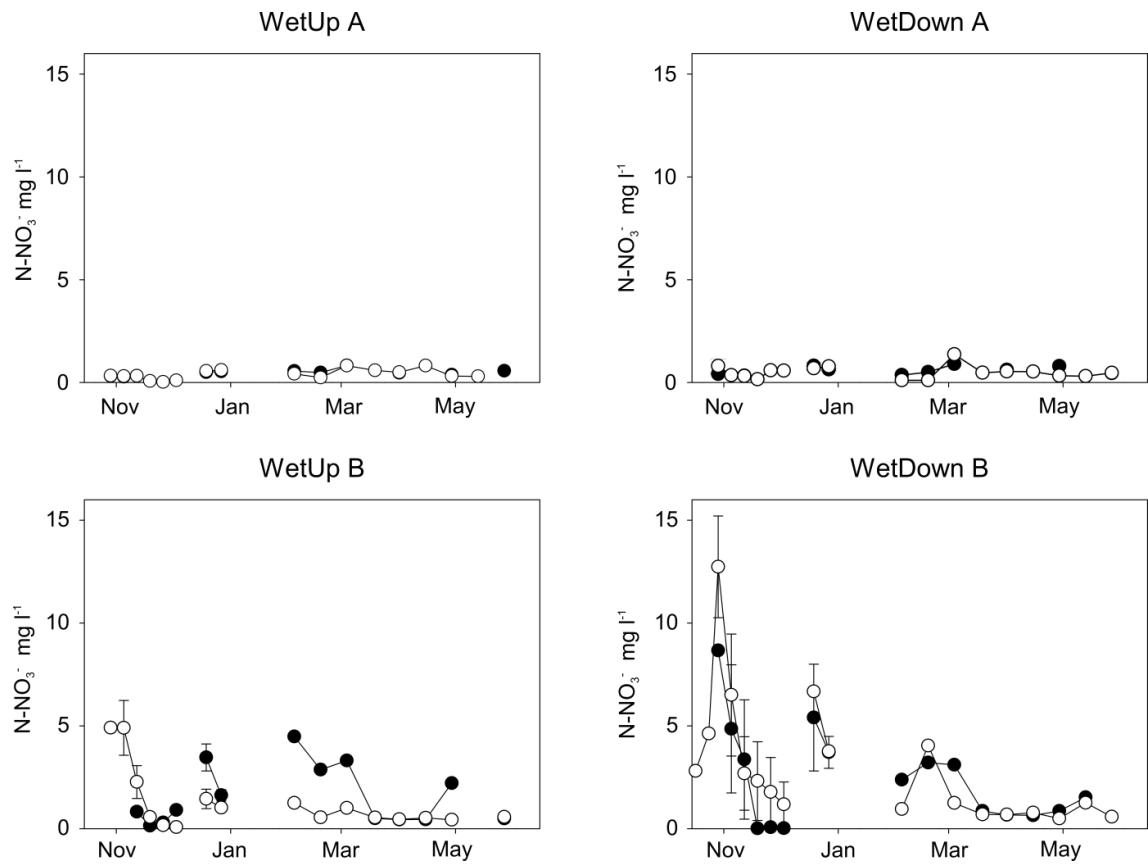


Figure S1. Nitrate concentrations in soil solutions of riparian wetland A and B. Solid circles: 10-15 cm depth; empty circles: 50-55 cm depth. Error bars represent standard errors of triplicate samples.

2.6 Conclusion of chapter 2 (one-year monitoring study)

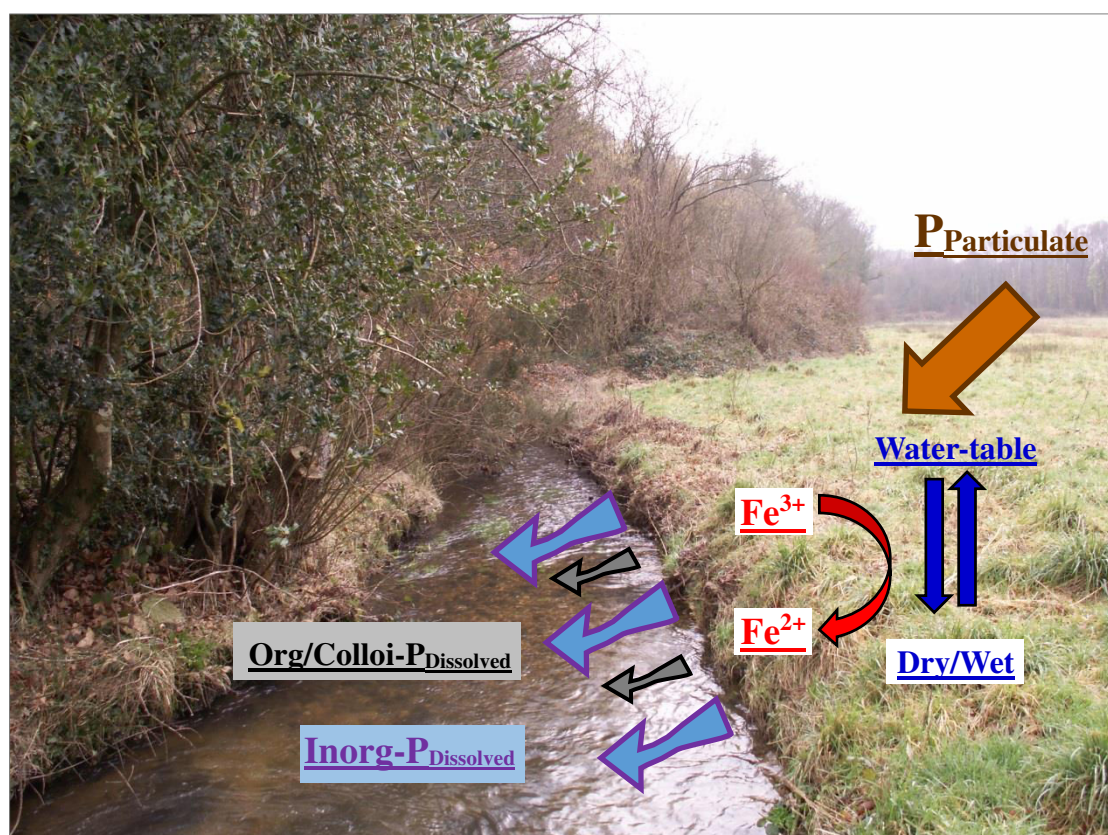
The results presented in this chapter have confirmed that riparian wetlands (RWs) are the main places of production of dissolved reactive P (MRP) in agricultural catchments, whereby confirming also the role of groundwater in the transfer of that component from the RWs to the stream. The in-situ monitoring of MRP concentrations in the natural soil solutions of the Kervidy-Naizin RWs during one entire hydrological year showed that the seasonal fluctuations in the water table had an effect not only on the transfer of MRP but also on the trigger of the biogeochemical processes at the origin of the MRP release pulses observed in these RWs. The bacterial cell lysis caused by rewetting of dried soils in autumn, and the reductive dissolution of Fe (hydr)oxides generated during the development of anoxic conditions in winter are the two identified mechanisms responsible for the release of MRP in soils of the two studied RWs. These two mechanisms produced a visible signal both in the soil solution and in the stream at the outlet of the catchment, suggesting a transfer (at least in part) of the mobilized P to watercourses.

To better assess the role of these two mechanisms in the overall MPR release and transfer processes, and also better constrain the way they react to water table dynamic and soil properties fluctuations, it would be useful to monitor the soil solutions during more than one single hydrological year (possibility of encompassing years with different hydroclimatic conditions and thus different water table dynamics). An attention should be paid also to the dissolved unreactive P (MUP), a dissolved P fraction which was not measured during the study presented in Chapter 2, though constituting possibly an important fraction of the dissolved P component released in RW soils. Thus, it was expected that the longer and coupled monitoring of both MRDP and MUDP would allow us to tackle more entirely and specifically how factors such as the hydroclimate variability and topography that jointly control the water table dynamics to trigger releases of DP in RWs. To present the results of this longer (three years in total) and more complete (measurements of both MRDP and MUDP) field monitoring survey is basically the objective of **Chapter 3** in this thesis.

Chapter 3

Release of dissolved phosphorus from riparian wetlands: Evidence for complex interactions among hydroclimate variability, topography and soil properties

This chapter corresponds to a paper published in the journal “Science of the Total Environment”: Release of dissolved phosphorus from riparian wetlands: Evidence for complex interactions among hydroclimate variability, topography and soil properties. Sen Gu, Gérard Gruau, Rémi Dupas, Cornélia Rumpel, Alexandra Crème, Ophélie Fovet, Chantal Gascuel-Odoux, Laurent Jeanneau, Guillaume Humbert, Patrice Petitjean. Science of the Total Environment. 2017. 598, 421-431.



Release of dissolved phosphorus from riparian wetlands: Evidence for complex interactions among hydroclimate variability, topography and soil properties

Abstract

In agricultural landscapes, establishment of vegetated buffer zones in riparian wetlands (RWs) is promoted to decrease phosphorus (P) emissions because RWs can trap particulate P from upslope fields. However, long-term accumulation of P risks the release of dissolved P, since the unstable hydrological conditions in these zones may mobilize accumulated particulate P by transforming it into a mobile dissolved P species. This study evaluates how hydroclimate variability, topography and soil properties interact and influence this mobilization, using a three-year dataset of molybdate-reactive dissolved P (MRDP) and total dissolved P (TDP) concentrations in soil water from two RWs located in an agricultural catchment in western France (Kervidy-Naizin), along with stream P concentrations. Two main drivers of seasonal dissolved P release were identified: i) soil rewetting during water-table rise after dry periods and ii) reductive dissolution of soil Fe (hydr)oxides during prolonged water saturation periods. These mechanisms were shown to vary greatly in space (according to topography) and time (according to intra- and interannual hydroclimate variability). The concentration and speciation of the released dissolved P also varied spatially depending on soil chemistry and local topography. Comparison of sites revealed a similar correlation between soil P speciation (percentage of organic P ranging from 35-70%) and the concentration and speciation of the released P (MRDP from <math><0.10</math> to 0.40 mg l⁻¹; percentage of MRDP in TDP from 25-70%). These differences propagated to stream water, suggesting that the two RWs investigated were the main sources of dissolved P to streams. RWs can be critical areas due to their ability to biogeochemically transform the accumulated P in these zones into highly mobile and highly bioavailable dissolved P forms. Hydroclimate variability, local topography and soil chemistry must be considered to decrease the risk of remobilizing legacy soil P when establishing riparian buffer zones in agricultural landscapes.

3.1 Introduction

Despite intensive efforts to decrease anthropogenic nutrient inputs to surface water, phosphorus (P) remains an important issue in many water-quality restoration programs worldwide (Jarvie et al., 2013b; Sharpley et al., 2013; Smith and Schindler, 2009). As a result of the general reduction in urban and industrial emissions, the percentage of P derived from diffuse agricultural sources has increased over the past two decades in Europe and North America. Agricultural P currently represents more than 50% of the total P load in many streams and rivers in Western countries (Dupas et al., 2015b; Kronvang, 2007). Therefore, research and management efforts in these countries currently focus on decreasing diffuse P losses from agricultural sources (Kleinman et al., 2007; Schoumans et al., 2014; Sharpley et al., 2013).

In agricultural landscapes, establishment of vegetated buffer zones in riparian wetlands (RWs) has long been promoted as an effective way to reduce P transfer to streams. P can be retained through two main mechanisms: i) using vegetation to trap particulate P and ii) retaining dissolved and colloidal P forms via soil adsorption or plant uptake (Dorioz et al., 2006; Haygarth et al., 2006; Vidon et al., 2010). RWs can also be sources of dissolved organic and inorganic P, which is more bioavailable than particulate P, for streams and rivers, in response to geochemical or biogeochemical mediated solubilization of particulate P, or P bound to RW soils (Gächter et al., 1998; Roberts et al., 2012; Stutter et al., 2009). In particular, the water-table fluctuations that seasonally influence these hydrologically unstable zones can induce a succession of drying-rewetting cycles, a situation that is known to trigger the release of dissolved P in soils (Blackwell et al., 2009, 2010, 2013; Turner and Haygarth, 2001). The dissolved P that is released can come from many sources, including lysed microbial cells (Grierson et al., 1998; Turner et al., 2003; Turner and Haygarth, 2001), solutes that soil micro-organisms produce to protect themselves from desiccation (Halverson et al., 2000) and organic matter previously protected in soil micro-aggregates and disrupted by physical stresses caused by drying (Blackwell et al., 2010). The hydrological instability of RWs has also been widely shown to influence redox conditions in soils. The high water table and low velocity flow episodes that usually develop in RWs during the wet season can create anoxic conditions, inducing reductive dissolution of Fe (hydr)oxides (Jeanneau et al., 2014; Knorr, 2013) and thus solubilization of the P previously adsorbed or co-precipitated onto/within these Fe (hydr)oxides (Carlyle and

Hill, 2001; Hoffmann et al., 2009; Surridge et al., 2007; Zak and Gelbrecht, 2007).

Thus, it is essential to understand how and under what conditions these biogeochemical processes develop in RWs because they can transform RWs into potential sources of dissolved P in streams. This is a complicated issue due to the diversity of the processes and factors involved, and because of the complex interactions between these processes and factors. In the same way that the speciation and content of P can vary by location in RW soils, both intra- and interannual variations in hydroclimatic conditions can influence groundwater dynamics and consequently the frequency and development of drying-wetting cycles and reducing conditions. Slope gradients and soil composition and structure in RWs may also influence dissolved P release by driving soil-groundwater contact time and soil P adsorption capacity (Darch et al., 2015). The hydroclimate and slope gradient together can influence hydrological connectivity between RWs and streams, ultimately regulating the risk of transfer of released P into streams (Wolf et al., 2013).

Most studies on the release of dissolved P in soils have been performed in the laboratory (e.g. Blackwell et al., 2010; Stutter et al., 2009; Turner and Haygarth, 2001), and little is known about interactions between the above factors and processes under field conditions. Recently, a field study by Dupas et al. (2015b) highlighted the complexity of these interactions by investigating release dynamics of molybdate-reactive (i.e. inorganic) dissolved P (MRDP) in two RWs in a small agricultural catchment (Kervidy-Naizin) in western France. Their results revealed i) a large difference in the ability of two RWs to release MRDP and ii) temporal succession of two P release mechanisms (soil rewetting in autumn and reductive dissolution of soil Fe (hydr)oxides in late winter), which are driven by seasonal fluctuations in water table.

The Dupas et al. (2015b) study, however, monitored dynamics for only one year; thus, it could not examine the influence of interannual hydroclimate variability on P release dynamics. Moreover, little is known about the molybdate-unreactive dissolved P (MUDP) forms in their study because only MRDP concentrations were measured. For the present study, we included a three-year monitoring of the two RWs investigated previously by Dupas et al. (2015b), measuring both MRDP and total dissolved P (TDP) in water from two RW soils, adjacent streams and the outlet stream of the catchment. Our objective was threefold: i) explore the interaction between soil properties and topography that influences the chemical nature (MRDP and MUDP) of the released P,

ii) explore interactions among intra- and interannual hydroclimate variability and topography that influence the variations in dissolved P release dynamics and iii) determine whether all of the dissolved P released is quantitatively transferred to the stream.

3.2 Materials and Methods

3.2.1 Research site

The Kervidy-Naizin catchment is a small (5 km²) headwater catchment located in central Brittany, western France (48° N, 3° W), that belongs to the AgrHys environmental research observatory (http://www6.inra.fr/ore_agrhys_eng/). The climate is temperate oceanic, with annual temperature and rainfall averaging 10.5°C and 875 mm, respectively, from 2006-2016. The catchment is drained by a second-order stream (Strahler classification) that usually dries up in summer. The substrate lithology consists of impervious Brioverian schists covered with 2-30 m of unconsolidated weathered materials in which a shallow aquifer develops. Soils are silty loamy Luvisols that are well-drained in upland domains and hydromorphic in valley bottoms, where RWs develop. Agricultural practices mainly involve arable crops (cereals, maize and winter barley) and livestock farming (dairy/beef cattle, pigs and poultry).

Many hydrological and biogeochemical research projects have been conducted in this catchment since the AgrHys environmental research observatory was established in 1993 (Aubert et al., 2013; Dupas et al., 2016; Lambert et al., 2013; Molénat et al., 2008), providing detailed knowledge of water chemistry and water pathways during base-flow and storm-flow periods. Water-table depths are recorded every 15 min using 10 piezometers (depth: 2-8 m, equipped with Orpheus OTT pressure probes) installed along two transects (A and B, **Figure 3.1**). Stream discharge is monitored at the outlet at 1-min intervals with an automatic gauge station; rainfall is recorded hourly at the Toullo weather station located to the east of the catchment outlet.

Three hydrological periods have been distinguished on an annual basis in this catchment (Humbert et al., 2015; Lambert et al., 2013; Molénat et al., 2008). Period A sees progressive rewetting of RW soils after the dry summer season. Period B sees the water-table rise in the upland domain, leading to a high hydraulic gradient between upland areas and RWs and strong lateral water flow in RW soils; this period (subdivided into B1 and B2, depending on the RW soil redox status (Lambert et al., 2013))

is associated with prolonged waterlogging of RW soils on an annual basis. Period C sees drawdown of the water table, which progressively dries catchment soils and decreases stream flow (Humbert et al., 2015; Lambert et al., 2013; Molénat et al., 2008).

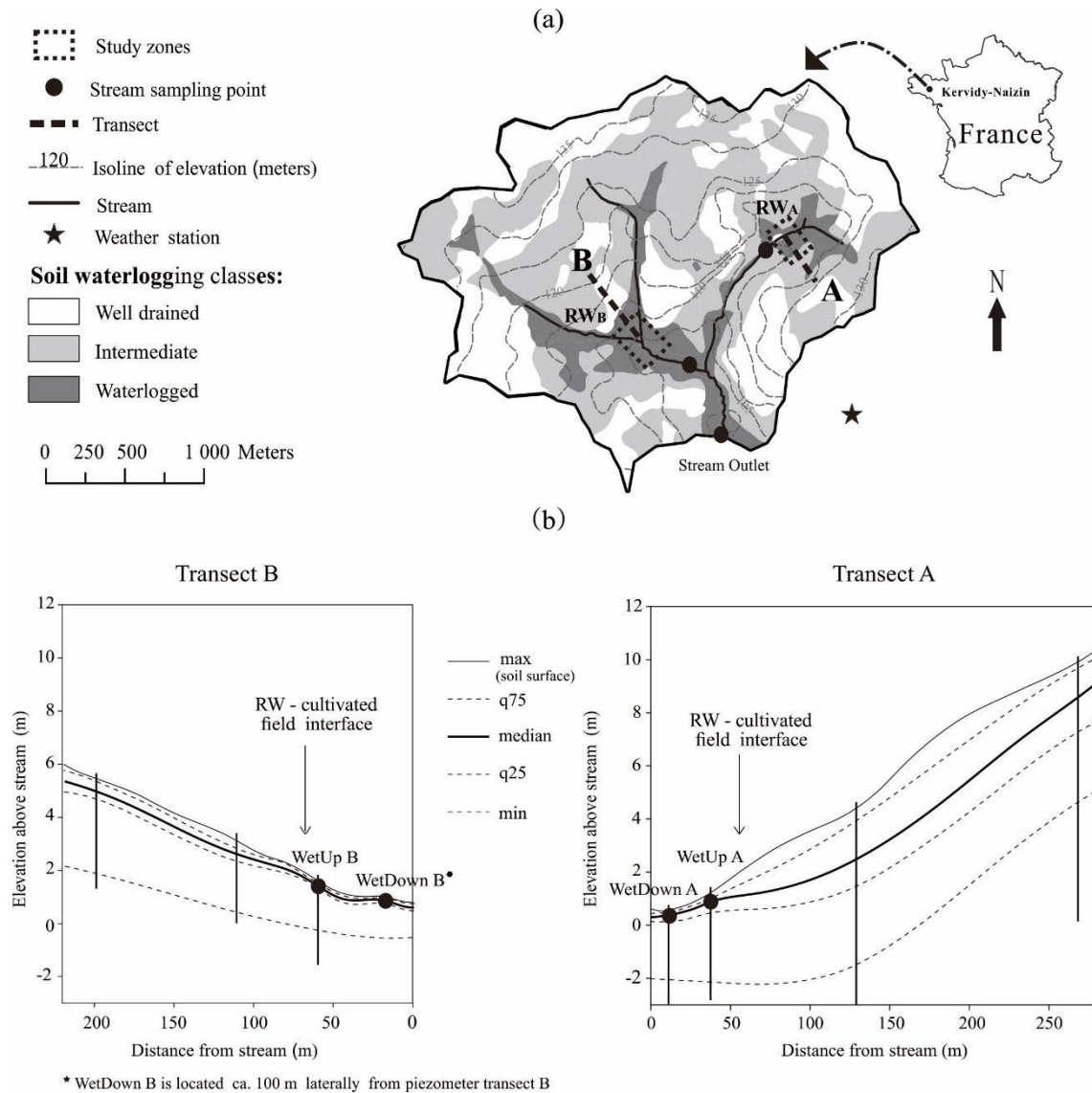


Figure 3.1. (a) Map of the Kervidy-Naizin catchment; (b) location of sampling sites (black dots) and variability of water-table level along the two transects, as established from analysis of 15 years of piezometric data. Vertical black lines represent piezometers.

RWs located at the foot-slope of transects A and B (RW_A and RW_B) were studied using zero-tension lysimeters installed by Dupas et al. (2015) designed to collect free soil water without disturbing *in-situ* redox conditions. Twenty-four lysimeters were set in triplicate at two depths (10-15 and 50-55 cm) and at two sites in each RW. The two sites in RW_A (hereafter called “WetUp-A” and “WetDown-A”) are located 13 m and 40 m downslope from the RW-Cultivated field interface, respectively, while those in

RW_B (hereafter called “WetUp-B” and “WetDown-B”) are located 9 m and 52 m downslope from the RW-Cultivated field interface, respectively (**Figure 3.1**). Transects A and B, and therefore RW_A and RW_B, differ morphologically; transect B and RW_B are flatter than transect A and RW_A (mean slope gradient of 2.8% and 3.8%, respectively). RW_A is 51 m wide, with vegetation consisting of unfertilized herbaceous species (*Dactylis glomerata* and *Agrostis canina*), while RW_B is 64 m wide, with vegetation consisting of a mixture of shrubs and trees (*Populus negro*, *Salix caprea* and *Betula alba*).

Both RWs were managed as unfertilized riparian buffer zones for ca. 20 years, without export of biomass. Before being managed as buffer zones, RW_A was fertilized with pig slurry and mineral fertilizer at amounts up to 60 kg P ha⁻¹ yr⁻¹, while RW_B was subjected to unquantified P inputs of cattle manure (assumed to be lower than those in RW_A). Dupas et al. (2015b) measured total P, extractable P (citric acid extractable P, Dyer method), clay, silt, sand, Al, Fe, organic carbon and organic nitrogen concentrations of soils in RW_A and RW_B. Their results indicated a three- to four-fold decrease in mean concentrations of total P and Dyer P with increasing depth (**Table S1**). They also showed higher total P and Dyer P concentrations in RW_A top-soils (1052-1213 and 242-289 mg/kg, respectively, depending on position in the RW) than in RW_B top-soils at equivalent positions and depths (458-681 and 12-48 mg/kg, respectively). The mean degree of P saturation calculated from oxalate-extractable Al and Fe contents was also higher in RW_A (7-21% depending on depth and position in the RW) than in RW_B (1-3%). Total P and Dyer P concentrations decreased from the top (i.e. near the cultivated fields) to the bottom (i.e. near the stream) of each RW.

3.2.2 Soil and water sampling

In April 2015, a 75-mm-diameter auger was used to collect soil samples in RW_A and RW_B at depths comparable to those of the lysimeters (i.e. 0-15 cm (“shallow”) and 30-40 cm (“deep”)) to determine P speciation in soils from these RWs. In RW_A, soil samples were taken at WetUp-A and WetDown-A; in RW_B, only the shallow soil (0-15 cm) at WetUp-B could be sampled due to the extreme moisture conditions in this RW at the time. Soil water samples were collected weekly to biweekly from October 2013 to June 2016. On each sampling date, samples from the triplicate lysimeters from each given depth and location were mixed to increase the amount of soil water available for chemical analysis.

Stream water samples were collected on the same dates as the soil water sampling in the stream reaches adjacent to the two RWs, and daily at the catchment outlet. Because this study focused on the release of P in RW soils and the subsequent transfer of this P to the stream through groundwater flow, samples collected during storms were removed from data analysis to avoid the potential contribution of overland flow to the stream's dissolved P budget (included when calculating annual TDP flux). Note that the storm periods are also important concerning P release from RWs, due to the high hydraulic gradient between RWs and streams which leads to strong exchange of water and solutes. Storm events were identified when discharge exceeded 20 l s^{-1} and when increases in discharge over a period of 10 min were greater than 10% (Dupas et al., 2015b). All water samples were passed through $<0.45 \mu\text{m}$ cellulose acetate filters within 6 h after sampling, then kept in a refrigerator in the dark at 4°C until analysis within 3 days.

3.2.3 Soil and water chemical analysis

Soil samples were transferred to the laboratory in plastic bags inside a cool box, then homogenized by wet sieving to $<5 \text{ mm}$. Soil P speciation was determined using the sequential fractionation method developed by Hedley (1982) and modified by Ivanoff et al. (1998). This method is based on sequential P extraction with different reagents (H_2O , 0.5 M NaHCO_3 at pH 8.5, 1 M HCl , and 0.1 M NaOH). P was extracted by shaking soil suspensions (1 g of dry weight soil in 30 ml solution) for 16-21 h. After centrifugation at 10 000 RPM for 10 min, the supernatant was removed and analyzed for TDP and MRDP. P extracted by H_2O and NaHCO_3 is considered labile P, while HCl - and NaOH - extracted P is defined as moderately labile P. Residual (i.e. non-labile) P, which may contain a mixture of organic and inorganic P forms, was determined as the total P measured after calcination at 550°C and acid digestion of the residue after the final extraction step. Extraction efficiency was checked by comparing the sum of P extracted using the sequential extraction method with the total P content measured after calcination and acid digestion of the bulk soil samples. All sequential experiments were performed in triplicate.

For all water samples, MRDP was determined colorimetrically via reaction with ammonium molybdate (Murphy and Riley, 1962) applied directly to the $<0.45 \mu\text{m}$ filtrates (natural water) or to the centrifuged solutions (soil extracts). The same method was used for TDP, but after digestion of the filtrates or centrifuged solution in acidic potassium persulfate. The precision of MRDP and TDP measurements was $\pm 4 \mu\text{g l}^{-1}$

and $\pm 13 \mu\text{g l}^{-1}$, respectively. The difference between TDP and MRDP was assumed to be organic P for soil extracts and MUDP for natural water samples. Development of reducing conditions in soil water was monitored by measuring Fe^{2+} concentrations using the 1.10 phenanthroline colorimetric method (AFNOR NF T90-017, 1997), with a precision of 5%. Fe^{2+} reactants were mixed with samples *in situ* to avoid the influence of oxidation on Fe^{2+} concentration during sample transport. The potential influence of Fe oxyhydroxide precipitation on MRDP concentrations during sample transport was checked by comparing MRDP concentrations in sample aliquots directly filtrated and acidified on-site to MRDP concentrations in sample aliquots filtrated in the laboratory. The results were found to be comparable with average differences below 12%, suggesting that potential Fe^{2+} oxidation had little influence on MRDP concentrations in the $<0.45 \mu\text{m}$ filtrates (**Figure S1**).

3.3 Results

3.3.1 Hedley P fractionation

The sums of TDP extracted by Hedley fractionation did not differ significantly (ANOVA, $p > 0.05$) from total P contents measured after digestion of the bulk soil samples, suggesting high efficiency of the selected P fractionation method (see supporting materials, **Table S2**). Consistent with results of the bulk soil analyses (**Table S1**), the amount of extracted P content decreased strongly with soil depth and was much higher in RW_A soils than in RW_B soils (**Figure 3.2**). However, the total P content from bulk soil analyses were 1.3-3.1 times as large as those obtained by Hedley fractionation for the same soils (**Tables S1-2**). Hedley fractionation may have underestimated total P content for two reasons: i) the difference in sample pretreatment (sieving wet soils at 5 mm for P fractionation vs. sieving air-dried soils at 2 mm for bulk soil analyses); ii) the difference in the acids used for digestion (calcination at 550°C followed by H_2SO_4 digestion for P fractionation vs. HF/HClO_4 digestion for bulk soil analyses).

According to Hedley fractionation, moderately labile P extracted was always the largest P fraction (up to 77%), while labile P and residual P were found in smaller percentages (12-18% and 9-33% of total extracted P, respectively). In the two main fractions (moderately labile and labile P), P speciation in shallow soils differed between RW_A and RW_B . Organic P represented ca. 70% of these two fractions in shallow RW_B soils but less than 35% of those in shallow RW_A soils. Interestingly, the percentage of

MRDP in these two fractions was linearly correlated with the percentage of Dyer P in the total P content from bulk soil analysis at the same sites ($r=0.9753$, **Figure S2**). This indicates that Dyer P content can be considered a proxy for the extracted MRDP (i.e. inorganic P content) in the soils studied; thus, the lower percentage of Dyer P in RW_B soils emphasized that organic P was the dominant P fraction in RW_B soils (**Table S1**).

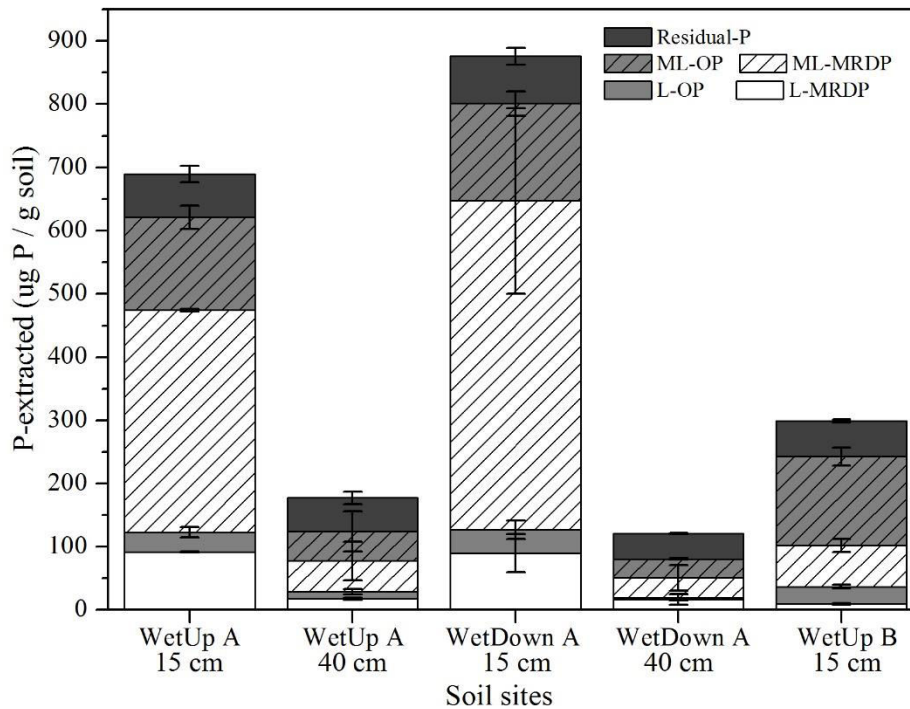


Figure 3.2. Amounts of organic and inorganic P in the studied soils as established from the Hedley fractionation results. “ML” and “L” refer to moderately labile and labile P, respectively. “OP” represents organic P, which is the difference between TDP and MRDP. Error bars represent the standard deviation of triplicate samples.

3.3.2 Rainfall, discharge and water-table variations

In this section, we highlight differences in hydroclimatic and water-table dynamics between the three P-monitored years to clarify their influence on processes that trigger release of different forms of dissolved P in RW soils. For simplification, the hydrological year monitored by Dupas et al. (2015b) (i.e. 2013-2014) is referred to as Y1, and the two hydrological years monitored in the current study (i.e. 2014-2015 and 2015-2016) are referred to as Y2 and Y3.

Cumulative values and distribution of discharge directly reflected rainfall (**Figure 3.3a**). Stream discharge at the catchment outlet in Y1 was nearly 2 times as high as that in Y2 and Y3, especially during the high-flow period B. For each of the three years, the

beginning of period B corresponded to an abrupt increase in stream discharge to $>100 \text{ l s}^{-1}$. In Y1 and Y3, stream discharge remained $>100 \text{ l s}^{-1}$ during most of period B, even though the magnitude was relatively lower in Y3. The magnitude of stream discharge in Y2 during period B was much lower, with <30 consecutive days with discharge $>100 \text{ l s}^{-1}$ (**Figure 3.3a**).

Consequently, water-table fluctuations and the number of days in which RW soils were saturated also varied during the three years (especially for high-flow period B) and between the two RWs (**Figure 3.3b**). The difference in transect topography resulted in a shallower mean water table in RWB than in RWA (**Figure 3.1b**). In RWB, even the shallow soil horizons remained saturated throughout period B, irrespective of interannual rainfall variability. In contrast, due to the steeper topography of RWA, interannual variability in rainfall amount and frequency resulted in strong spatio-temporal variability in the duration of saturation periods. Shallow soils remained saturated throughout most of period B at WetUp-A and WetDown-A locations in Y1. In contrast, the water table remained quite deep in “dry” Y2; only deep soils remained saturated during period B, while shallow soils experienced a frequent succession of dry/wet episodes. An intermediate situation was observed in Y3: shallow soils in WetDown-A remained saturated during most of period B, as in Y1, while those in WetUp-A experienced a frequent succession of dry/wet episodes, as in Y2 (**Figure S3**).

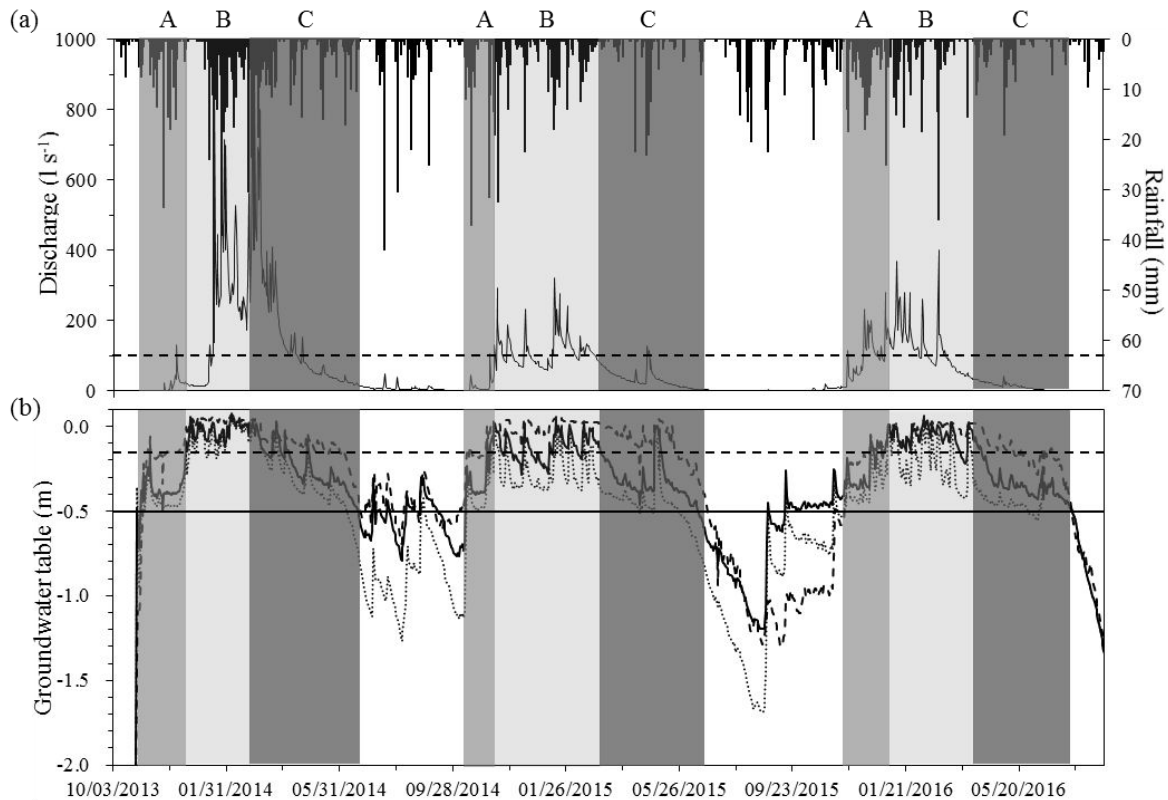


Figure 3.3. (a) Discharge (black line) and precipitation (black histogram) time-series data for the Kervidy-Naizin catchment during the three investigated years. Grey, light grey and dark grey areas represent the identified hydrologic periods A, B and C, respectively. Horizontal dashed line represents discharge value of 100 l s^{-1} . (b) Groundwater table of WetUp-A (dotted line), WetDown-A (solid line) and WetUp B (dashed line). Horizontal dashed and solid lines represent the depths of 15 cm and 50 cm lysimeters in RWA. Due to the flatness of RWB, the results for WetUp B and WetDown B are based on the same piezometric water-table data, with depths of lysimeters in RWB at 10 cm and 55 cm, respectively.

3.3.3 Soil water chemistry

Soil water chemistry data are reported as time-series plots that compare TDP, MRDP and Fe^{2+} concentrations measured at WetUp-A (**Figure 3.4.1**), WetDown-A (**Figure 3.4.2**), WetUp-B (**Figure 3.4.3**) and WetDown-B (**Figure 3.4.4**). During the three years, MRDP concentrations remained near the detection limit (ca. 0.005 mg l^{-1}) in RW_B lysimeters, except at WetUp-B, where MRDP concentrations in shallow lysimeters rose to 0.1 mg l^{-1} at the end of period B of each year (**Figure 3.4.3a**). These low MRDP concentrations in RW_B lysimeters contrasted with those in RW_A lysimeters, which frequently recorded MRDP concentrations $>0.1 \text{ mg l}^{-1}$. MRDP concentrations fluctuated in the same range from Y1 to Y3 in shallow lysimeters in RW_A ($0.15\text{-}0.40$ and $0.05\text{-}0.25 \text{ mg l}^{-1}$ at WetUp-A and WetDown-A lysimeters, respectively). The

positive concentration gradient observed between shallow WetDown-A ($0.1 \text{ mg l}^{-1} < \text{MRDP} < 0.2 \text{ mg l}^{-1}$) and WetUp-A ($0.2 \text{ mg l}^{-1} < \text{MRDP} < 0.4 \text{ mg l}^{-1}$) lysimeters in Y1 remained constant in Y2 and Y3. Mean TDP concentrations were consistently higher (70%, n=250) than mean MRDP concentrations in all lysimeters, suggesting that MUDP were always present (**Figures 3.4.1-4**). Interestingly, strong TDP concentration peaks (up to $0.3\text{-}0.4 \text{ mg l}^{-1}$) were observed in WetUp-B and WetDown-B lysimeters (**Figures 3.4.3-4 a-b**). These peaks indicate that the dissolved P released by RW_B soils had a different chemical nature (molybdate unreactive) compared to that released by RW_A soils (molybdate reactive).

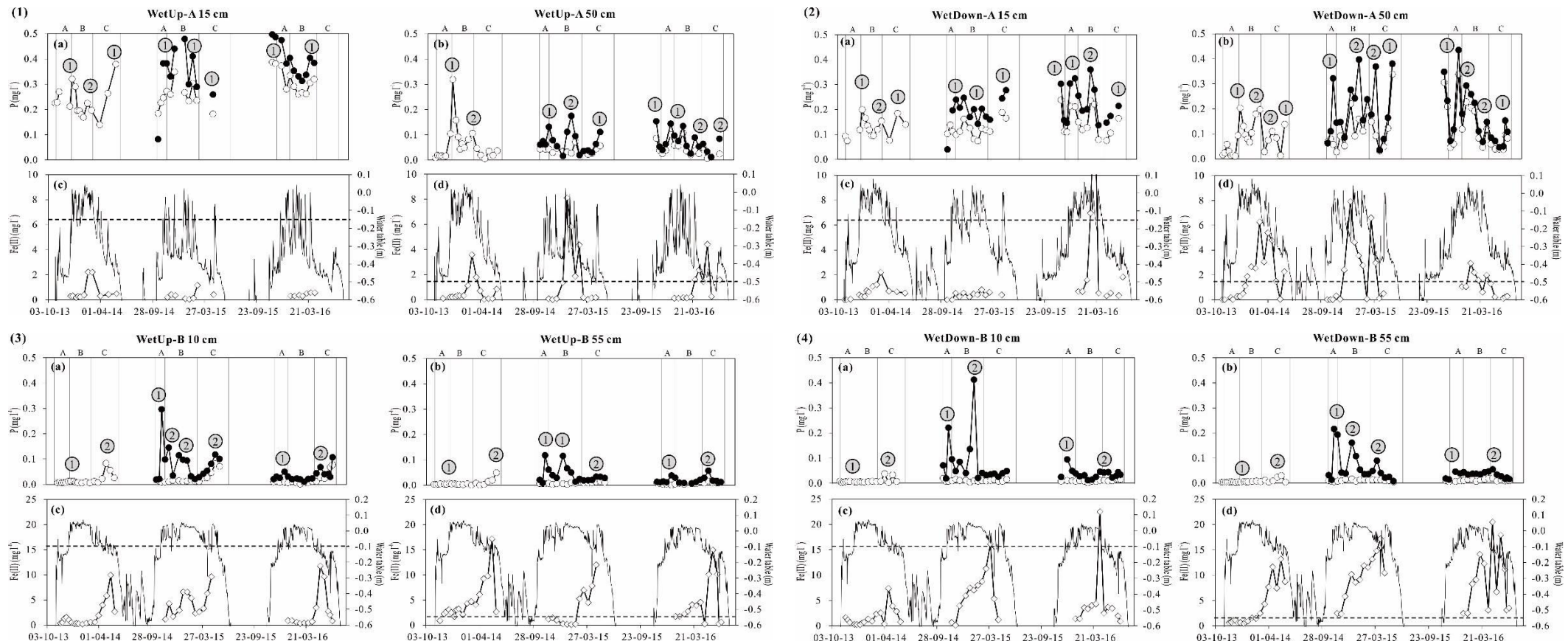


Figure 3.4. Time-series plots comparing the evolution of MRDP (empty circles) and TDP (solid circles) concentrations with Fe^{2+} concentrations and water-table depth fluctuations measured in soil solutions at (1) WetUp-A locations (15 cm and 50 cm depth), (2) WetDown-A locations (15 cm and 50 cm depth), (3) WetUp-B locations (10 cm and 55 cm depth), and (4) WetDown-D locations (10 cm and 55 cm depth). Circled numbers in grey (1 and 2) relate concentration peaks to P release mechanisms discussed in text. Horizontal black dashed lines represent the depths of lysimeters. Vertical black lines represent the limits between hydrological periods. Data for hydrological year 2013-2014 are from Dupas et al (2015b).

The Fe^{2+} concentration peaks varied in amplitude and timing among sampling sites and were in phase with variations in water-table dynamics and soil saturation periods. Deep lysimeters at WetUp-A and WetDown-A recorded Fe^{2+} concentration peaks (up to 17 mg l^{-1}) each year (**Figures 3.4.1-2d**). In shallow lysimeters, Fe^{2+} concentration peaks were only observed in Y1 and Y3 at WetDown-A (**Figures 3.4.2c**) and in “wet” Y1 at WetUp-A (**Figure 3.4.1c**). In contrast, all lysimeters in RW_B recorded Fe^{2+} peaks at the end of period B of each year (**Figures 3.4.3-4 c-d**). Thus, Fe^{2+} concentration peaks in RW_A and RW_B lysimeters depended entirely on water-table dynamics and the topographic position of the lysimeters.

By comparing water-table fluctuations to the temporal distribution of MRDP, TDP and Fe^{2+} concentration peaks, one can identify two situations that favored the occurrence of TDP and MRDP peaks in the two RWs. The first situation (“1” in **Figures 3.4.1-4**) corresponds to episodes of water-table rise after dry periods, especially the initial water-table rise event after the dry summer, i.e. at the beginning of period B. The second (“2” in **Figures 3.4.1-4**) corresponds to periods of Fe^{2+} release to soil water, which were particularly well identified in both shallow and deep RW_A lysimeters (**Figs 3.4.1-2**). Thus, our data support a combination of two types of dissolved P release peaks in the RWs studied, as highlighted by Dupas et al. (2015b).

3.3.4 Stream water chemistry

Stream water chemistry was similar to that of RW_A and RW_B soil water, in terms of differences in P speciation and temporal dynamics of TDP and MRDP concentrations (**Figure 3.5**). In particular, the stream branch that drains RW_A (stream A) had consistently higher MRDP concentrations than the stream branch that drains RW_B (stream B), as observed in the corresponding RW soil water. Concentration peaks observed in streams A and B were similar to those observed in RW soil water. However, stream waters differed from soil water in that MRDP concentrations measured in the stream in Y2 and Y3 were lower than those in Y1. This was particularly evident for stream A, whose mean MRDP concentration of 0.048 mg l^{-1} ($n=20$) in Y1 decreased to 0.016 ($n=15$) and 0.029 mg l^{-1} ($n=17$) in Y2 and Y3, respectively. This interannual decrease in MRDP concentration in stream A differed greatly from the consistently high level or increase in MRDP concentrations in RW_A soil water (**Figure 3.4a**). In contrast, MUDP produced in RW_B soil water in Y2 and Y3 was efficiently transferred to the stream in these years, which indicates that conditions or processes in Y2 and Y3

decreased the transfer of MRDP from soil waters to the stream.

3.4 Discussion

3.4.1 Influence of soil P content and soil P speciation

One major finding of the present study is the evidence that release of dissolved P following water-table rise in RWs is not restricted to MRDP (inorganic P). Other P species, which the ammonium molybdate method of Murphy and Riley (1962) cannot detect, are also released. These species include organic P carried by organic molecules such as phytates, sugar phosphates or phospholipids, or organic or inorganic P incorporated into or adsorbed onto colloidal particles (Haygarth and Sharpley, 2000). Release of organic P in RWs is potentially damaging due to the risk of eutrophication, since several P-bearing organic compounds are known to be highly bioavailable (Li and Brett, 2013). Many recent studies have indicated that Fe-rich colloids associated with organic matter are vectors for P transport in soils and watercourses (e.g. Baken et al., 2016b; Gottselig et al., 2014; Liu et al., 2014; Regelink et al., 2013; Rick and Arai, 2011) and that increased mobilization of these colloids in RWs can damage the quality of surface water because it could accelerate transfer of P from soils. Based only on our data, we cannot firmly identify the presence of these colloids in the two RWs studied. We can only assume that when they are present, they are likely to mobilize P more in RW_B soil water than in RW_A soil water, where most of the P released is MRDP.

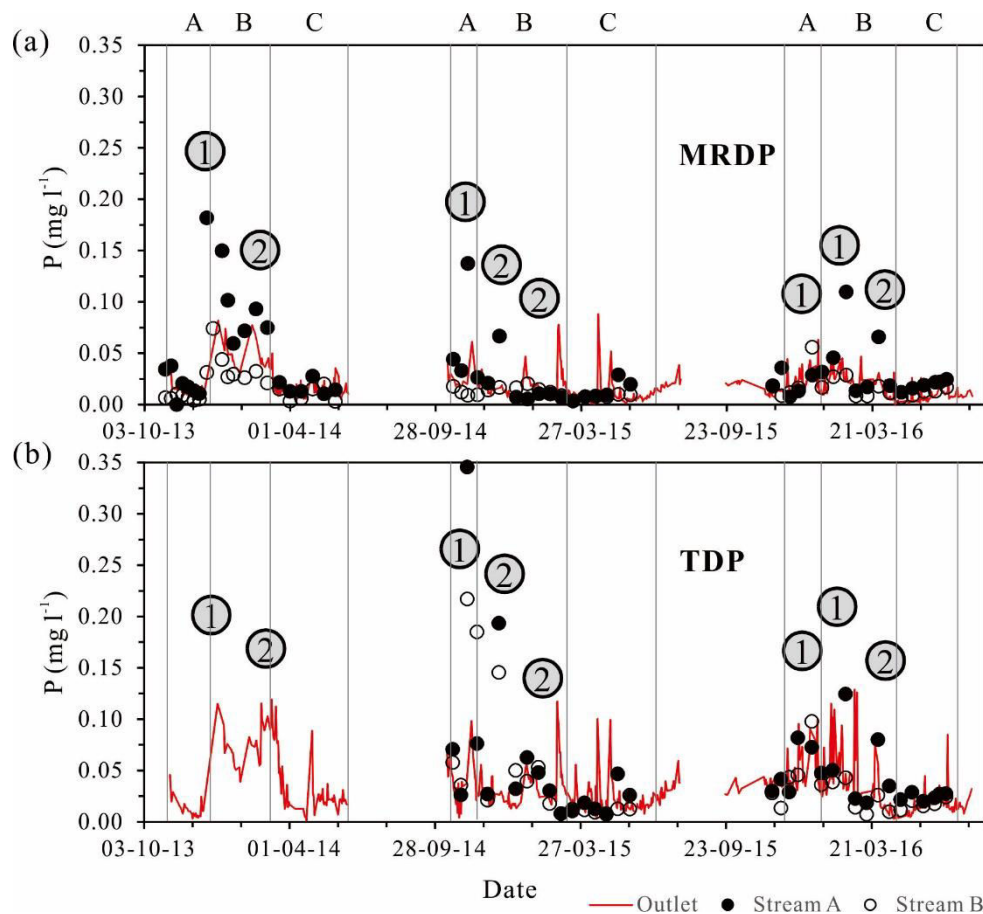


Figure 3.5. (a) MRDP and (b) TDP concentrations during baseflow periods (A, B and C) in streams near RW_A , RW_B and at the catchment outlet. Solid circles: MRDP or TDP in stream A waters; empty circles: MRDP or TDP in stream B waters; red line: MRDP or TDP in stream waters at the catchment outlet. Circled numbers in grey (1 and 2) relate concentration peaks to P release mechanisms discussed in text. Vertical grey lines represent the limits between hydrological periods.

We assume that the differences in P content and speciation observed between RW_A and RW_B soils are the main drivers of the observed spatial differences in dissolved P concentration and speciation. Bulk soil analyses and Hedley fractionation results showed that RW_B soils contained less total P and less easily exchangeable inorganic P than RW_A equivalents. The historical difference in P input ($RW_A > RW_B$) could explain the difference in total P content between RW_B and RW_A soils. There are several explanations for the difference in P speciation, including i) differences in past fertilizer inputs prior to conversion into vegetated buffer zones (pig slurry and mineral fertilizer in RW_A vs. cattle manure in RW_B), since fertilization history is known to play a key role in P speciation in soils (Eriksson et al., 2016; Khatiwada et al., 2014) and ii) differences in vegetation cover (mainly grass species in RW_A and shrubs and trees in RW_B), since vegetation is known to influence the inorganic-P:organic-P ratio in soils

(directly or indirectly) through its influence on soil pH (Osborne and Kovacic, 1993; Sugihara et al., 2015). The difference in topography between the two RWs could also explain the difference in P speciation. The interannual recurrence of prolonged saturation periods, as observed in RW_B soils, could have decreased the mineralization of soil organic matter, thereby favoring the release of a larger percentage of organic/colloidal P. Conversely, rapid oscillation of oxic/anoxic conditions induced by frequent water-table fluctuations in RW_A could have increased conversion of organic P into inorganic P forms in the soil, as suggested by recent experimental results (Scalenghe et al., 2012), which ultimately would explain why water leached from RW_A was rich in inorganic P and poorer in organic/colloidal P.

3.4.2 Key influence of interannual hydroclimatic variability on P release dynamics

Our data indicate that two hydrologically-driven processes influence release of dissolved P in the two RWs studied: i) rewetting of soils after dry periods and ii) reductive dissolution of soil Fe (hydr)oxides in response to soil saturation. The first process is consistent with laboratory experiments showing that soil rewetting after a dry period can release organic and inorganic P as a result of microbial biomass being killed by osmotic shock (Blackwell et al., 2010; Turner et al., 2003; Turner and Haygarth, 2001). This process was triggered each year in RW_A and RW_B during the water-table rise at the beginning of period B, and occasionally occurred in shallow RW_A soils in late period B (**Figures 3.4.1-4**). Concerning the second process, it is well known that reductive dissolution of Fe (hydr)oxides, as observed in saturated RW soils, can cause P release in soil water (Hofmann et al. 2009; Obour et al. 2011; Roberts et al., 2012; Scalenghe et al., 2012; Surridge et al., 2007; Young and Ross, 2001). Our results show, however, that triggering of this process can vary spatially and temporarily because it depends on the establishment of the anoxic conditions necessary to reduce soil Fe (hydr)oxides. These conditions were spatially and temporary stable in RW_B, where simultaneous reductive dissolution of soil Fe (hydr)oxides and dissolved P release occurred each year in both shallow and deep soils (**Figures 3.4.3-4**). It was unstable in shallow RW_A soils, occurring only in wet Y1 at WetUp-A, and only in Y1 and Y3 at WetDown-A (**Figures 3.4.1-2**). In addition to the amount and frequency of rainfall, topography appears to have a major influence on the temporal stability of reductive

dissolution of soil Fe (hydr)oxides. The flat topography of RW_B facilitated saturation of shallow and deep soils for long periods of time, irrespective of the annual weather conditions. In contrast, the steeper slope of RW_A meant that long-term soil saturation only occurred annually in deeper soils, and occurred in shallower soils only in relatively wet years (Y1 and Y3), when they were saturated long enough to allow reductive dissolution of Fe (hydr)oxides.

Using our data, we semi-quantitatively estimated the groundwater conditions required to induce reductive dissolution of Fe (hydr)oxides, i.e. the period of time the soil must remain saturated to generate the anoxic conditions necessary for the reductive dissolution to start. In shallow RW_B soils, 65 and 51 days were required at WetUp-B and WetDown-B, respectively. In shallow WetUp-A soils, Fe²⁺ release began after 26 consecutive days of saturation in Y1, but none was observed in Y2 and Y3 because they had no more than 5 and 8 consecutive days of saturation, respectively. In shallow WetDown-A soils, Fe²⁺ release began after 48 consecutive days of saturation in Y1 and Y3, but none was observed during Y2 because it had no more than 26 consecutive days of saturation. In deep soils, longer saturation was required to trigger reductive dissolution in RW_B soils (at least 95 days) than in RW_A soils (at least 66 days). Different mechanisms could explain these spatial variations, such as initial differences in the availability of other electron acceptors (mostly oxygen and nitrate) in the soil. Deep RW_B soils had higher nitrate concentrations than deep RW_A soils at the beginning of period B (data not shown), which could explain why reductive dissolution of Fe (hydr)oxides occurred later in RW_B soils than in RW_A soils.

Hydroclimate variability during the three years and topographic differences between RW_A and RW_B also induced spatio-temporal variations in the triggering of P mobilization through soil rewetting after drying. For example, in shallow WetUp-A soils, at least two release peaks occurred in Y2 and Y3 due to a succession of dry/wet episodes caused by water-table fluctuations, while only one peak occurred in Y1 (between periods A and B); the water table at this location remained near the soil surface throughout period B. This is in contrast to the flatter RW_B, in which P release upon soil rewetting was essentially confined to the transition between periods A and B of each year (**Figs 3.4.3-4**).

Overall, release of MRDP in RW_A soils was sustained by a combination of higher soil P content, predominant inorganic P forms and the less stable hydrological

conditions induced by a steeper topography. Although TDP dynamics observed in Y2 and Y3 suggest that RW_B soils can release dissolved P in organic/colloidal forms, the peak magnitudes and mean concentrations in these soil waters were much lower than those observed for MRDP in RW_A waters. MRDP concentrations in RW_A soil water were not only higher on average but also appeared to decrease less rapidly after a water-table rise. Although the MRDP pool in RW_A soils can become exhausted in wet years such as Y1, present results indicate the existence of effective interannual regeneration mechanisms of this pool (**Figures 3.4.1-2**). Irrespective of the nature of these mechanisms, the MRDP pool in RW_A soils appeared much larger and regenerated more rapidly than the pool of exchangeable organic/colloidal P in RW_B soils.

3.4.3 Topography as the potential ultimate driver of dissolved P release in RW soils

Using the Kervidy-Naizin catchment as an example, a conceptual model was constructed to describe how topography could ultimately drive processes that release P in RWs and the chemical nature and concentrations of the released P. Due to differences in topography, the mean water-table level is deeper in RWA than in RWB (**Figure 3.6**). RW_A soils experience more intra- and interannual water-table variations than RW_B soils, where the flatter topography causes the water table to remain in shallow soil horizons even in dry years. These topography-induced unstable groundwater dynamics explain the great spatial and temporal variations in the two main processes of dissolved P release, and why the P release linked to reductive dissolution of Fe (hydr)oxides can be deactivated in dry years in shallow soil horizons in RW_A. As discussed above, the effect of topography on soil moisture and organic matter mineralization rates could explain the higher inorganic-P:organic-P ratio in RW_A soils than in their RW_B equivalents. Overall, shallow RW_A soils have more inorganic P and a frequent, rapid cycle of dry/wet episodes. Since topography influences both of these features, this conceptual model provides a suitable explanation for why the steeper RW_A releases more inorganic P than the flatter RW_B.

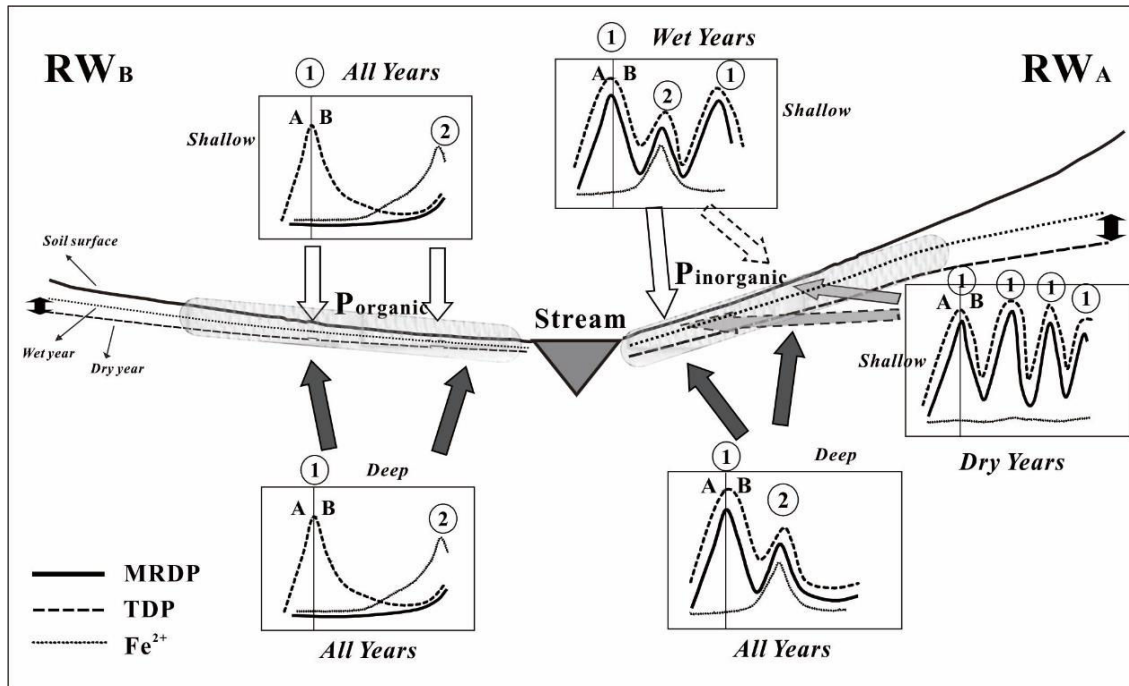


Figure 3.6. Sketch diagram summarizing the spatial and temporal variability of P release processes and species in the two studied RWs. The greater diversity of situations encountered in RWA soils is thought to be primarily due to the steeper slope compared to RWB, meaning that this RW is more susceptible to inter-annual hydroclimatic variations than RWB. The circled numbers “1” and “2” given opposite the cycles refer to the release processes discussed in the text. In each inset box, the solid line represents MRDP, the dashed line TDP and the dotted line the Fe^{2+} concentration, vertical line represents the transition between periods A and B. On each RW slope, the solid line represents the soil surface, the dotted line the mean water table in wet years and the dashed line the mean water table in dry years.

Steep topography could increase the ability of RW to transform particulate P into bioavailable MRDP at the catchment scale due to its influence on major soil biogeochemical processes involved in the production and release of MRDP. The potential negative influence on water quality could increase because steep topography also facilitates the transport of erosive soil particles from upland cultivated soils into the RWs, thereby providing the particulate P necessary to sustain chemical reactions. In the Kervidy-Naizin catchment, this downward transport of upland P-rich soil particles into the steeper RWA has been suggested as the main cause for the high P concentrations in RWA soils, especially at the WetUp-A location (Dupas et al., 2015c), which supports the hypothesis that steeper topography is a potential aggravating factor in RWA.

3.4.4 Delivery and retention of mobilized P

A strong connection appears to exist between dissolved P release in RW soil water and delivery of P to streams (Figures 3.4.1-4 and 3.5). We stress, however, that P

transfer is not quantitative and that P may be retained in certain years. This is suggested by the lower MRDP concentrations observed at point A in the stream in Y2 and Y3 than in Y1, despite the consistently high MRDP concentrations in RW_A soil water over the three years. At the sampling point in stream A, which is upstream in the catchment (**Figure 3.1**), the RW merges with the drainage area where stream A originates, and it is unlikely that this decrease in MRDP concentrations is related to a change in water pathways between years or to input into the stream of MRDP-poor water of a different origin during Y2 and Y3. Comparison of annual P and water fluxes at the catchment outlet also indicates the existence of retention processes. Using the high-frequency monitoring of water discharge and TDP concentrations at the catchment outlet, total annual TDP fluxes were estimated at 63-185 g·P ha⁻¹ y⁻¹, depending on the year (**Figure 3.7**), which is in the range of annual P fluxes exported from temperate catchments (e.g. Mellander et al., 2015, 2016; Pärn et al., 2012). Consistent with hydroclimate conditions, TDP and water fluxes were much higher in Y1 than in Y2 and Y3; however, the decrease in TDP fluxes between Y1 and Y2/Y3 was 1.5 times as large as the decrease in water fluxes (2 times as high).

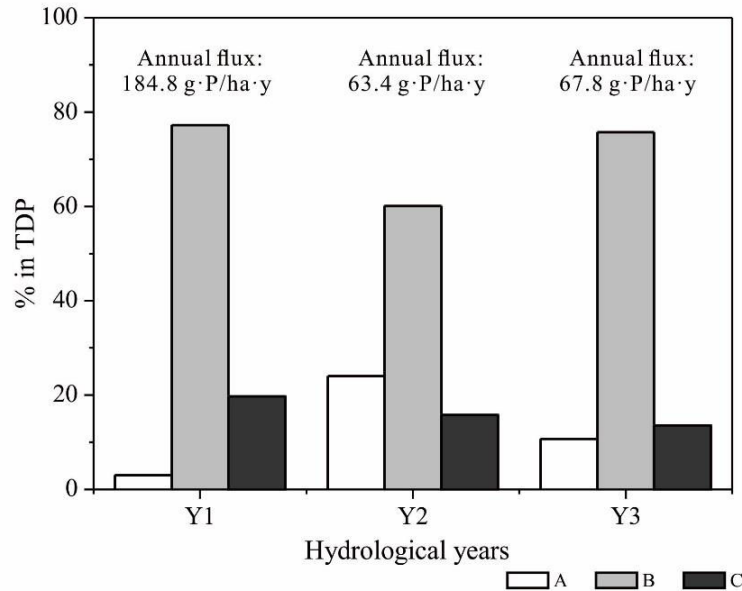


Figure 3.7. Relative contribution of each hydrological years and periods within each hydrological years to the total annual flux of TDP exported at the outlet of the Kervidy-Naizin catchment during the study period.

These two observations suggest that some of the TDP released from RW soils does not transfer to the stream and that this retention may have peaked in Y2 and Y3. Re-

oxidation of Fe^{2+} released from RW soils is a potential retention mechanism. Precipitation of Fe (hydr)oxides is a commonly observed phenomenon that traps P when reduced water flows over oxic interfaces (Baken et al., 2015; van der Grift et al., 2014). It most likely occurs on stream banks because they are oxic interfaces that RW water must cross before reaching the stream. This process may have been more active in Y2 and Y3, because the decreased groundwater flow rates caused by the lower hydraulic gradient could have increased Fe^{2+} oxidation reactions at the stream bank, thus causing re-adsorption of some of the TDP transported by the groundwater.

3.5 Conclusions

Establishment of vegetated buffer zones in RWs is a valuable strategy to reduce diffuse P transfer in agricultural areas (Schoumans et al., 2014) due to the ability of these zones to trap particulate P. However, biogeochemical processes can transform accumulated P in these zones into highly mobile and highly bioavailable dissolved P forms. Our study indicates that RW soils are hotspots of dissolved P release in an agricultural catchment in western France. We confirm the generalizability of the two mechanisms suggested to explain the release of dissolved P in RW soils: rewetting of soils after dry periods and reductive dissolution of soil Fe (hydr)oxides in response to soil saturation. This study provides three new major findings: i) differences in soil properties and topography can induce spatial variations in the chemical nature (inorganic vs. organic/colloidal) of the released P; ii) distinct intra- and interannual variations in dissolved P release dynamics can occur in RW soils as a function of the frequency of drying-rewetting cycles and the duration of soil saturation, both of which depend on the hydroclimate and local topography; and iii) the triggering of retention processes at the RW-stream interface can decrease P export from RW soils to streams.

Overall, the study reveals a complex interactions among soil chemistry, hydroclimate variability and topography in determining the location of hotspots of dissolved P release in RWs at the catchment scale. Topography is identified as the key driver due to its influence on the spatio-temporal variability of drying-rewetting cycles and subsequent redox oscillations. Steeper topography increases input of particulate P into zones able to efficiently transform particular P into bioavailable dissolved P. When developing management strategies for RWs with steep topography, the first consideration must be to decrease P mobilization due to erosion from upslope fields in order to prevent further

accumulation of particulate P in RWs.

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3.6 Supplementary materials

Table S1. Soil characteristics in the riparian wetlands studied. Data was from soil basic analyses in Dupas et al., 2015c.

Sampling Site	Clay (<2µm)	Silt (2-50µm)	Organic Carbon	Organic matter	pH	Dyer P	Al	Fe	Total P (HF)
	g kg ⁻¹								
WetUp A-S	241	615	40.9	70.7	6.1	288.5	1690	4250	1213.2
WetUp A-D	263	584	9.5	16.5	6.3	65.9	1470	3340	375.3
WetDown A-S	233	589	37.1	64.2	6.1	241.8	1410	6000	1051.7
WetDown A-D	250	574	10.7	18.5	6.4	62.8	1570	7220	384.5
WetUp B-S	288	599	60.2	104.0	6.2	47.6	1710	7710	680.8
WetUp B-D	248	583	14.8	25.6	6.0	4.4	1090	1480	198.6
WetDown B-S	354	603	89.1	154.0	5.9	12.2	1560	10200	458.2

Table S2. Hedley P fractionation of soils from studied sampling sites.

Soils	Labile P (H ₂ O + NaHCO ₃)			Moderately labile P (HCl + NaOH)			Residual -P	Ptotal (Sum)	Ptotal (H ₂ SO ₄)
	MRDP	TDP	Ratio Org-P	MRDP	TDP	Ratio Org-P	TDP	TDP	TDP
	ppb		%	ppb		%	ppb		
WetUp A-S	91.4 (0.7)	123.1 (9.2)	25.6 (5.0)	350.8 (2.2)	498.0 (21.2)	29.5 (2.6)	68.5 (13.1)	689.6 (43.5)	725.5 (9.3)
WetUp A-D	17.7 (1.6)	28.8 (5.8)	37.7 (6.9)	48.8 (30.5)	95.5 (62.4)	48.2 (1.9)	52.9 (9.8)	177.2 (77.9)	176.8 (46.2)
WetDown A-S	89.6 (30.3)	126.9 (15.6)	30.3 (15.3)	520.3 (146.9)	674.0 (166.3)	23.2 (2.8)	75.2 (13.2)	876.2 (168.7)	808.7 (202.3)
WetDown A-D	16.4 (8.7)	19.7 (13.5)	11.0 (16.8)	31.2 (20.1)	61.1 (21.3)	51.6 (16.1)	39.9 (0.5)	120.7 (34.3)	124.5 (45.1)
WetUp B-S	9.3 (1.2)	36.7 (3.5)	74.5 (3.0)	65.1 (10.1)	206.0 (23.4)	68.5 (2.1)	56.2 (2.8)	298.9 (26.4)	323.3 (20.7)

Note: “S” and “D” means the soils from surface (0~15 cm) and deep (30~40 cm) in studied sites, respectively. Numbers in brackets represent the relative standard deviation of triplicate samples. ‘Org-P’ means the organic P, which is the difference between TDP and MRDP.

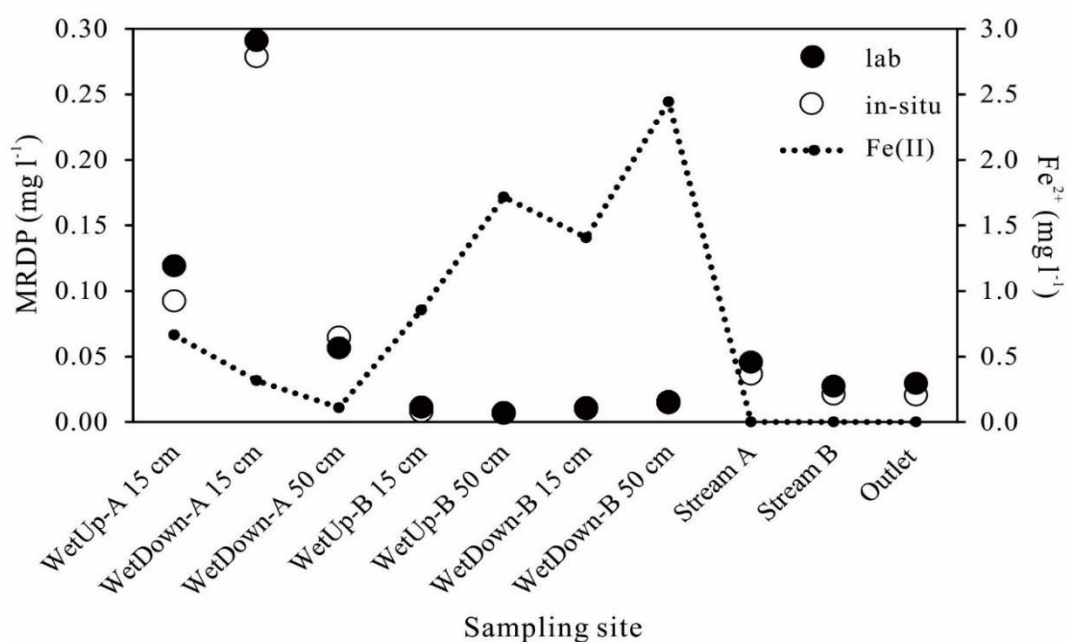


Figure S1. Concentration of MRDP in water samples filtrated in laboratory (solid cycles) and *in-situ* (empty cycles). Dashed line with solid cycles represents the Fe²⁺ concentration in corresponding water samples. The average difference between laboratory and *in-situ* was within 12%.

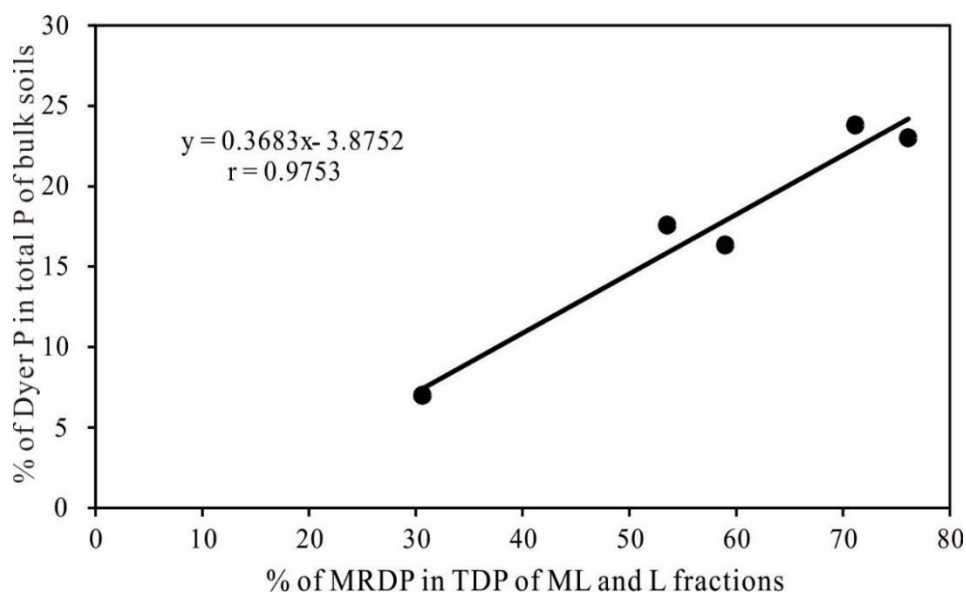


Figure S2. Correlation between the percentage of MRDP in TDP of moderate labile (ML) and labile P fractions and the percentage of Dyer P in total P content of bulk soil. Data was from Hedley P fractionation in Table S1 and soil basic analyses in Dupas et al., 2015c.

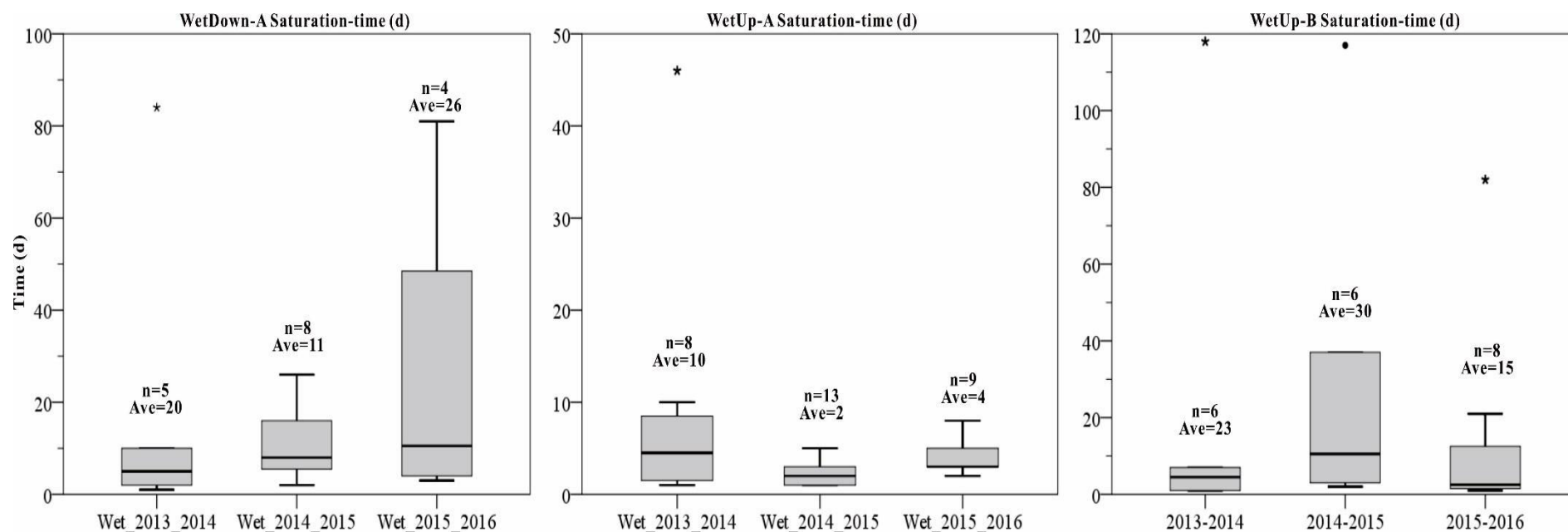


Figure S3. Distribution of consecutive soil saturation length of RW_A and RW_B surface lysimeters. The middle bar represents the median, the lower bar limits the 1st quartiles (q_{0.25}) and upper bar limits the 3rd quartile (q_{0.75}). The lower and upper lines are, respectively, the 1st quartile minus 1.5 times interquartile range and the 3rd quartile plus 1.5 times the interquartile range. ‘n’ represents the number of consecutive saturation periods, ‘Ave’ means the average length of saturation periods. Average saturation times at WetDown-A were similarly high during Y1 and Y3 (26 as against 20 days, respectively), being by contrast low during Y2 (11 days). At WetUp-A, the situation was different: average saturation times at this location were quite high during Y1 (10 days), being on the contrary very low during Y2 and Y3 (2 and 4 days, respectively). For RW_B, the consecutive saturation periods calculated for WetUp-B soils were >80 days for the three investigated years, further highlighted the difference in groundwater table dynamics between RW_A and RW_B. Note that, even though no piezometer was installed in WetDown-B, the flat topography makes it even wetter than WetUp-B.

3.7 Conclusion of chapter 3 (three years monitoring study)

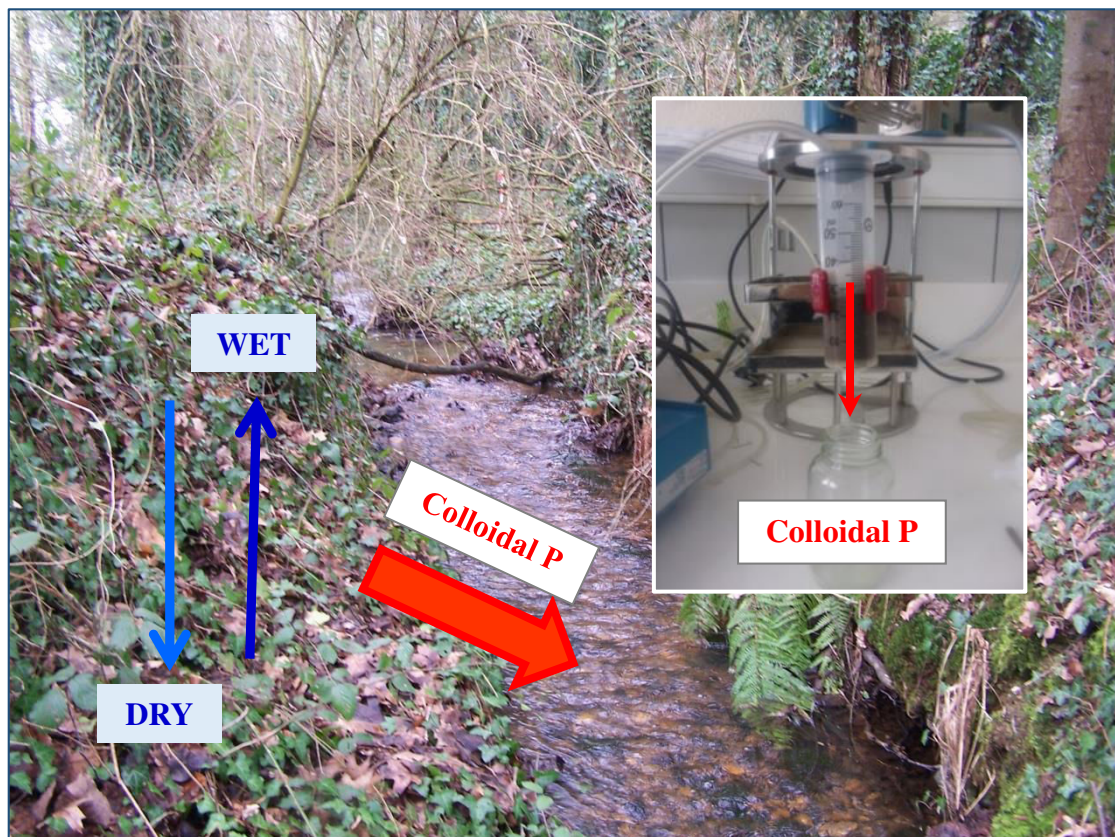
This chapter reveals that the intra- and interannual variations of dissolved P release dynamics in RW soils, and the concentrations and speciation of this P fraction are jointly controlled by the hydroclimate variability, topography, and soil properties. The topography was identified as the key ultimate driver because a steep topography can not only increase the input of particulate P into RWs, but also make RWs more efficient in transforming particulate P into more bioavailable DP by promoting the mineralization of soil organic P. The release DP in wetland soil solutions are not quantitatively transferred into streams, due to the trigger of retention processes such as the re-oxidation of Fe(II) when reduced water flows cross over oxic interfaces (e.g. soil-stream interface along stream banks). This retention process could be more active in relatively dry years due to the decreased groundwater flow rates. Results of the three-year monitoring study also showed that a dissolved unreactive P (MUP) fraction can also be released in RW soils, especially in soils that have higher organic matter and organic P content and are from a smooth topography which could decrease the mineralization rate of soil organic matter. The spatial differences in the concentration and speciation of DP in soil solutions are driven by the differences in P content and speciation in their corresponding soils, and also the topography of their corresponding RWs.

The results of the three-year monitoring study have confirmed the role of dry-wet cycles as one of the triggers of DP release in RWs, though revealing that the speciation of the released DP could be different from one RW to another. Drying/wetting cycles can be easily simulated in the laboratory, raising the possibility to set up experiments to understand more about why is the released P different in speciation, and where in the soil matrix are the different P components involved in the release P process located. The aim of **Chapter 4** is to present the results of column leaching experiments conducted to highlight these two points.

Chapter 4

Drying/rewetting cycles stimulate release of colloidal-bound phosphorus in riparian soils

This chapter corresponds to a paper submitted to the journal “Geoderma”: Drying/rewetting cycles stimulate release of colloidal-bound phosphorus in riparian soils. Sen Gu, Gérard Gruau, François Malique, Rémi Dupas, Patrice Petitjean, and Chantal Gascuel-Oudoux.



Abstract:

Drying and rewetting (DRW) events in soils cause the release of molybdate-unreactive dissolved phosphorus (MUP) into soil solutions; historically, it has been considered biologically-derived phosphorus (P) from microbial cell lysis. This unreactive P, however, could also represent P bound to soil colloids that are mobilized during DRW events, suggesting the involvement of more physically-based mechanisms, a point rarely reported in the literature. To explore the potential role of colloid-bound P (colloidal P) in the P released during DRW events, two riparian wetland soils (A and B) with contrasting soil P speciation were subjected to three successive DRW cycles in soil columns. Leachates were analyzed for molybdate-reactive P (MRP), total P (TP) and dissolved organic carbon (DOC). Colloidal P was determined by ultrafiltration at 30kDa and 5kDa and analyzed for MRP, TP, DOC and other elements. For both soils, distinct TP release peaks were observed upon each DRW event, while MRP and MUP concentrations remained relatively constant and decreased rapidly during leaching, respectively. Soil B showed larger TP release peaks than soil A and contained different P forms (more MUP and MRP, respectively). Colloidal P was a major fraction (up to 70%) of TP (<0.45 μm) in leachates immediately after rewetting and decreased at the end of each DRW cycle, with the mean colloidal P proportion much higher in soil B than in soil A (45% vs. 17%). Colloidal P contained a combination of Fe/Al oxides and DOC and was mainly colloidal MUP (mean=68%). The size of colloids in leachates was clearly larger at the beginning than at the end of DRW cycles. Thus, DRW events stimulated the release of both truly dissolved P and colloidal P into riparian soils. The amount of colloidal P released increased with soil organic matter content and porosity. Larger colloidal P and truly dissolved MUP had a greater response to DRW events and were exhausted more rapidly than truly dissolved MRP and smaller colloidal P, suggesting potential differences in their sources and production mechanisms. Colloidal P must be considered when trying to evaluate and decrease the loss of leached P from agricultural soils into surface waters.

4.1 Introduction

Drying and rewetting (DRW) events are one of the most common abiotic stresses that soils experience (Blackwell et al., 2010). Recent studies revealed that DRW events could cause the release of considerable amounts of dissolved phosphorus (P) into soil

solutions (Blackwell et al., 2009; Bünemann et al., 2013; Turner et al., 2003; Turner and Haygarth, 2001).

Originally, the dissolved P released during DRW events was attributed to physical processes such as disruption of dried soil aggregates upon rewetting followed by the subsequent mobilization of particles and associated P (Powlson and Jenkinson, 1976). Recent attention, however, has focused on the role of soil microbial biomass, with the hypothesis that the released P could derive from microbial cells that are lysed by osmotic shock upon rewetting (Blackwell et al., 2009, 2010, 2013; Bünemann et al., 2013; Turner et al., 2003; Turner and Haygarth, 2001). Turner et al. (2003) demonstrated that at least 88% of total dissolved P (TP) released during DRW events originated from lysed bacteria cells. Blackwell et al. (2009) later confirmed this and found that up to 70% of soil microbial biomass could be killed by the osmotic shock caused by DRW events. They attributed a portion of the P pulses observed during DRW events to microbial cell lysis. Another study, on grassland soils in Switzerland, attributed the pulse release of P during DRW events to microbial biomass, and at the same time emphasized the importance of physicochemical processes (Bünemann et al., 2013).

Most studies have shown that the dissolved P released during DRW events is mainly molybdate-unreactive, suggesting that it is organic (Blackwell et al., 2009; Turner et al., 2003). Although this molybdate-unreactive nature is consistent with the hypothesis of microbial cell lysis, it does not confirm that the dissolved P released is organic. In fact, dissolved P is operationally defined as P that can pass through 0.45 μm membranes, whose size lies within the range of colloids (1-1000 nm). The molybdate-unreactive P could also consist of inorganic P adsorbed onto, or incorporated into soil colloids, a possibility that has yet to be fully studied. This knowledge gap is potentially problematic for two reasons: i) an increasing number of studies show that colloids are important carriers of P in soil solution (Heathwaite et al., 2005a; Hens and Merckx, 2002; Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013; Siemens et al., 2008), and ii) drying is known to stimulate production of soil colloids, which are then released into soil solutions upon soil rewetting (Klitzke and Lang, 2007; Madjalani, 2008; Mohanty et al., 2015).

Thus, studying the influence of DRW events on the release of colloidal P into soil solutions is essential. Accordingly, we measured concentrations of colloidal P, truly

dissolved molybdate-reactive P (MRP) and molybdate-unreactive P (MUP) in laboratory rewetting experiments conducted on two air-dried soils with different organic matter (OM) content, soil microbial biomass, and soil P speciation. The two soils studied come from two riparian wetlands located in an agricultural watershed. We chose these soils because field-monitoring data indicated that both could release P upon rewetting after drying under natural field conditions and the released P was observed to enter the stream network (Dupas et al., 2015c; Gu et al., 2017). The same field monitoring data indicated that the two soils responded differently to DRW events in term of the speciation of the P released, with one releasing mainly MRP and the other releasing mainly MUP (Gu et al., 2017).

The objectives of the present study were to i) evaluate the role of colloids in the P release flushes observed during DRW cycles in soils, ii) determine the composition of the colloidal P fraction and compare it with truly dissolved MRP and MUP, iii) estimate the influence of soil properties and soil P speciation on the release of colloidal P and iv) verify the mechanisms that produce the various P species in soils during DRW events. More general objectives were to provide insight into the capacity of riparian zones, where DRW events naturally occur, to release colloidal P to surface water, and to assess potential consequences of this release on water eutrophication.

4.2 Materials and Methods

4.2.1 Soil properties and preparation

The studied soils come from two riparian wetlands in the Kervidy-Naizin watershed, a 5 km² agricultural headwater watershed located in Brittany, western France (48.012° N, 2.835° W). The two wetlands (wetlands A and B) are located in the same topographic position, at the interface between cultivated fields and the stream network. They differ in three main characteristics: i) mean slope (3.6% for A vs. 2.8% for B); ii) mean saturation time (<10 days for A vs. >25 days for B), and iii) vegetation cover (unfertilized herbaceous species for A vs. shrubs and trees for B).

Both wetlands were monitored for three years to understand the role of water table fluctuations on P release and P speciation in soil solutions (Dupas et al., 2015c; Gu et al., 2017). Results indicate pulses of dissolved P release in phase with the water-table rise and subsequent soil rewetting, particularly in autumn when rainfall resumes after the dry summer season. Stream data indicated that the dissolved P released was

transferred to the adjacent stream, making the two wetlands the main sources of dissolved P in the stream at the watershed scale. However, while the dissolved P released in wetland A consisted of mainly MRP (mean=72%), that in wetland B was mainly MUP (mean=63%). This difference in dissolved P speciation was related to differences in soil P speciation (more inorganic P in wetland A vs. more organic P in wetland B), which was caused by differences in topography and water saturation dynamics (Gu et al., 2017).

Soils from wetlands A and B (soils A and B, respectively) are silt loams, classified as Luvisols. Dupas et al. (2015) and Gu et al. (2017) described their chemical and mineral compositions; here, we provide only the information necessary to interpret our results (**Tables S1 and S2, Figure S1** in Supporting Information). Briefly, soils A and B have similar concentrations of clay, silt and sand (24%, 61% and 14% vs. 29%, 60% and 11%, respectively), N contents (3.7 vs. 4.7 g/kg), C:N ratios (11.2 vs. 12.9) and pH (6.1 vs. 6.2). In contrast, soil A differs from soil B in having lower OM content (70.7 vs. 104.0 g/kg), lower microbial biomass (437.5 vs. 791.7 mg/kg), and higher concentrations of TP (1.21 vs. 0.68 g/kg), Olsen-P (0.08 vs. 0.04 g/kg), and Dyer-P (0.288 vs. 0.048 g/kg). Soil A also has a degree of P saturation 7 times as high as that in soil B (21% vs. 3%) (**Table S1**). Most importantly, sequential extraction experiments (using Hedley fractionation) indicated that soil P speciation is predominantly inorganic (64% of TP) in soil A and predominantly organic (57% of TP) in soil B (**Table S2**). Soil B's higher OM content, microbial biomass, and proportion of organic P are related to wetland B's slower mineralization rate, caused by its flatter topography and longer water saturation time (Gu et al., 2017).

Approximately 1.5 kg of soil (fresh weight) from the surface horizon (0-15 cm) was sampled in the two wetlands with a 75-mm diameter auger in October 2015. Sampling sites lay within 1 m of the lysimeters used by Dupas et al. (2015) and Gu et al. (2017) to monitor P concentrations in soil solution (WetUp-A and WetUp-B sites, respectively, in their studies). The surface horizon was selected because it is more prone to DRW events and field monitoring indicated that it releases more P during DRW events than deeper soil horizons (Gu et al., 2017). After sampling, soils were transferred to the laboratory in plastic bags and air-dried in a clean room (20 days, at 25±2 °C). Soils were sieved to <2 mm after removing all visible non-soil materials. Soils were then stored in a refrigerator at 4 °C before use.

4.2.2 Experimental setup and conduct of DRW experiments

PVC syringes 11.5 cm long with a 3.1-cm inner diameter were used as leaching columns. For each soil, three columns were run in parallel, to provide triplicate measurements of P concentration dynamics (**Figure 4.1**). A solution of 0.01 M NaCl was used as the influent solution since its ion strength was similar to the natural solutions recovered in these soils. All column tops were sealed with rubber plugs with a hole pierced in the middle. Soft PVC tubes were used to inject the influent solution and drain leachates using a peristaltic pump. All junctions between rubber plugs, columns and soft tubes were tightly sealed with parafilm. Each column was filled with 20.0 g of dried soil. Glass fiber (0.5 g, 8 μm pore size, Sigma-Aldrich) was placed at the top and bottom of each column to avoid soil mass loss/disturbance during solution injection and leaching.

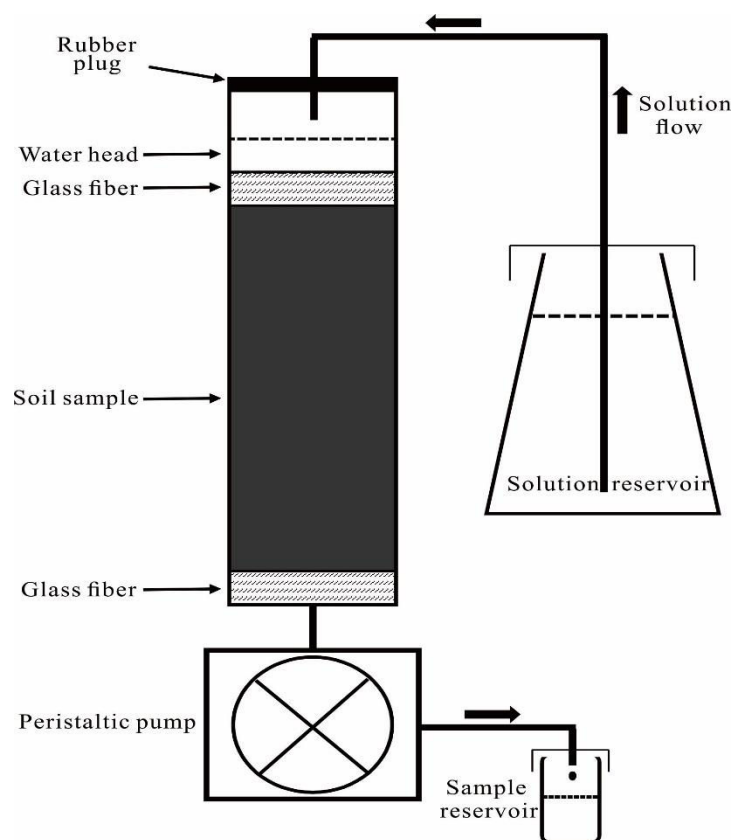


Figure 4.1. Simplified diagram of the column leaching experimental device.

In total, three consecutive DRW cycles were conducted for each soil (referred to as cycle-1, -2, and -3). Each leaching phase after drying began with a gentle rewetting

procedure to raise soil moisture to the soil's field capacity. About 15 ml of solution was required for this purpose, with no or only a few drops of leachate recovered at the bottom of each column. The columns were then sealed, and the leaching phases began. The pumping rate was 20 rpm, which corresponded to a leaching rate of 1.0-1.5 ml/min. Leaching generated a negative pressure in the column, which created a circulation gradient between the solution reservoir and the column. A water head of ca. 0.5 cm was maintained at the top of the column during the leaching phase.

After each leaching phase of ca. 30 h, the solution reservoir and peristaltic pump were disconnected, and the soils were progressively dried by flushing O₂ (90 L/h, at 25±2 °C) on the top of each column until a constant weight was reached. Soils were kept dry for 2 days before beginning the next leaching phase. A drying phase followed by a leaching phase in a soil column is hereafter considered a DRW cycle (the initial air-drying of soils served as the drying phase of the first DRW cycle). All experiments were performed in the laboratory at 25±2 °C.

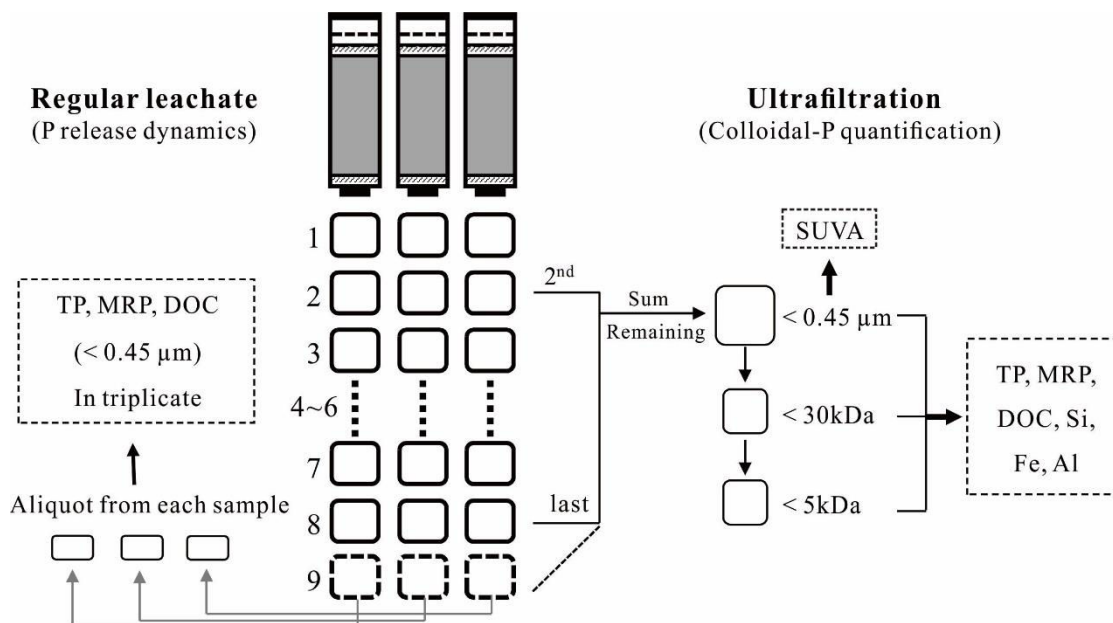


Figure 4.2. Diagram describing the sampling strategy and analyses performed according to the filtration/ultrafiltration procedures. MRP, TP, and DOC represent the molybdate-reactive phosphorus, total phosphorus, and dissolved organic carbon, respectively. SUVA represents the specific ultraviolet absorbance. Numbers “1-9” represent the numbers of sampling. “2nd” and “last” represent the second and last sampling, at which the remaining samples after aliquots taken for regular leachate analyses were summed together to ensure enough sample for ultrafiltration analyses.

4.2.3 Leachate treatments

Two types of samples were collected: regular-filtrated (RF) and ultrafiltrated (UF) (**Figure 4.2**). Both RF and UF samples were filtrated at 0.45 μm using cellulose acetate membranes, and the latter were then ultrafiltrated at smaller pore sizes (see below). Eight to nine RF samples were collected for each leaching phase to monitor TP, MRP and dissolved organic carbon (DOC) release dynamics. These samples were collected using a 60 ml PVC container every 50 min during the day and a 1 L glass beaker at night. Volumes were recorded as the mass of the solution and were then converted into relative volume (the millimeter of water passed through the column profile). Due to the cost and complexity of the methods, UF samples were restricted to leachates collected at the beginning (second RF sampling) and end (last RF sampling) of each leaching phase. These samples were used to determine the proportion of colloidal P in the P released and to identify how this proportion changed throughout each leaching phase. To ensure enough samples for chemical analysis, the UF samples for each leaching phase were prepared by combining the remaining RF leachates after analyzed TP, MRP, and DOC to form a single homogeneous sample, which was then sequentially ultrafiltrated (**Figure 4.2**).

Ultrafiltration was conducted using 15 ml centrifugal tubes (Vivaspin) equipped with permeable membranes of 30kDa and 5kDa. Aliquots of 0.45 μm -filtered samples were transferred into cells, then centrifuged at 3400 \times g for 15 and 25 min for 30 and 5kDa cells, respectively. Before use, cells were rinsed three times with deionized water, soaked twice in deionized water for 24 h, and then centrifuged with deionized water three times to avoid potential contamination.

4.2.4 Chemical analysis

MRP concentrations in RF and UF samples were determined colorimetrically by direct reaction with ammonium molybdate (Murphy and Riley, 1962). The same method was used for TP after digestion in acidic potassium persulfate. The precision of MRP and TP measurements was ± 4 and $\pm 13 \mu\text{g l}^{-1}$, respectively. MUP was calculated as TP minus MRP. DOC concentrations in both RF and UF samples were analyzed with a total organic analyzer (Shimadzu TOC-5050A), with a precision of $\pm 3\%$ (using potassium hydrogen phthalate as the standard solution). Specific Ultraviolet Absorbance (SUVA) values (expressed in $\text{mg l}^{-1} \text{m}^{-1}$, $\pm 5\%$ precision) were calculated

by dividing ultraviolet (UV) absorbance at 254 nm normalized by the DOC concentration. SUVA values were then converted into the aromaticity percentage of the OM using the equation of Weishaar et al. (2003) ($\text{aromaticity} = 6.52 \times \text{SUVA} + 3.63$). UV absorbance was measured with a Lambda 25 (PerkinElmer) spectrophotometer using deionized water as a blank.

The colloid composition was estimated by measuring Fe, Al, and Si concentrations in the UF samples (<0.45 μm , 30 and 5kDa) with an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700X). Calibration curves and accuracy controls were performed in accordance with Yeghicheyan et al. (2013), using river water reference material for trace elements with a wide compositional range (SLRS-5, National Research Council of Canada). De-ionized water purified with a Milli-Q (Millipore) system was used as a blank. Total relative uncertainties were $\pm 5\%$.

4.2.5 UF data treatment

P, DOC, Fe, Al, and Si were partitioned among the coarse colloidal (0.45 μm – 30kDa), fine colloidal (30-5kDa), and truly dissolved fractions (<5kDa) (**Figure 4.3**). The total amount of colloidal P (colloidal MUP and MRP) was considered as the sum of coarse and fine colloidal P fractions and was calculated as the difference in TP concentration between the <0.45 μm and <5kDa samples. MRP concentrations measured in the <5kDa samples were considered the truly dissolved MRP in leachates. The difference between TP and MRP concentrations in the <5kDa ultrafiltrates was considered the truly dissolved MUP and was considered to consist mostly of organic P-bearing molecules.

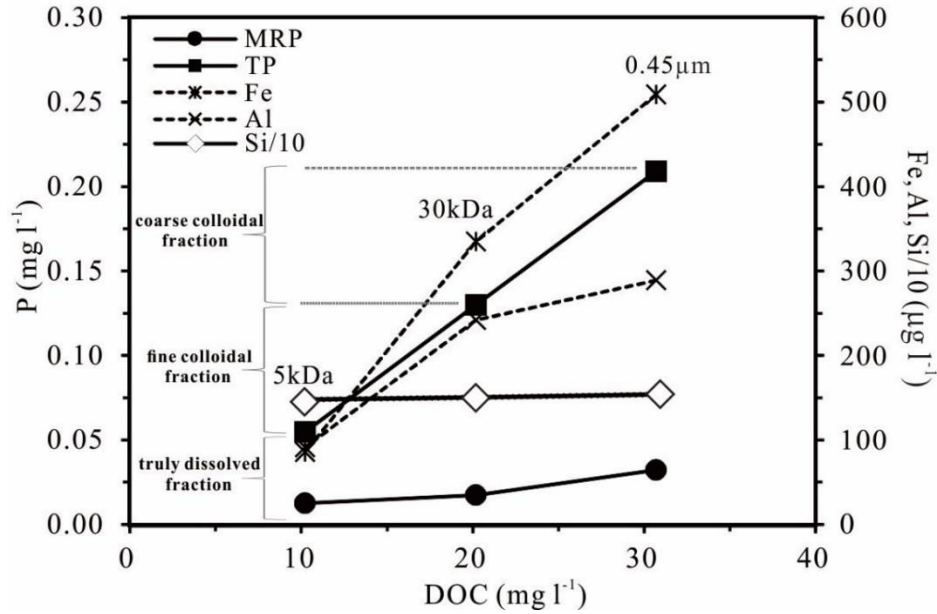


Figure 4.3. Diagram describing how ultrafiltration data were used to estimate molybdate-reactive phosphorus (MRP), total P (TP), dissolved organic carbon (DOC), Fe, Al and Si speciation in leachates. Labels “0.45 μm ”, “30kDa”, and “5kDa” indicate the pore sizes used for ultrafiltration. Using TP in soil B leachate at the beginning of cycle-2 as an example, the “coarse colloidal fraction” was calculated as the difference in TP concentration between 0.45 μm and 30kDa filtrated leachates, the “fine colloidal fraction” was calculated as the difference in TP concentration between 30kDa and 5kDa filtrated leachates, and the “truly dissolved fraction” was defined as TP concentration in <5kDa filtrated leachates.

4.2.6 Statistical analysis

The significance of the difference in maximum and minimum TP concentrations among the three successive DRW cycles for each soil was assessed using variance analysis tests (ANOVA) at a 5% significance level. Linear regression analyses were performed to estimate the co-variation of MUP and DOC concentrations. Pearson correlations were calculated among P, DOC, Fe, Al, and Si concentrations in UF samples to clarify the composition of the P-bearing colloids released. All statistical analyses were performed using the SPSS (IBM Statistics 22) software package.

4.3 Results

4.3.1 P and DOC concentrations in RF samples

Rewetting after air drying yielded distinct TP concentration peaks in leachates from soils A and B, followed by a progressive decrease to the end of each leaching cycle (Figure 4.4). TP concentrations at the beginning and end of each leaching cycle

remained relatively constant during three DRW cycles in soil A ($p=0.52$ and 0.10 , respectively). In soil B, however, both concentrations significantly decreased from cycle-1 to cycle-3 ($p<0.001$ and $p=0.02$, respectively). In addition, peak amplitude ratios (the ratio of concentration at the beginning of a leaching cycle to that at the end of the previous leaching cycle) were much higher in soil B leachates (4.3-4.8) than in soil A leachates (2.0-2.4) (**Table 4.1**). Thus, the RF data indicated a higher reactivity of TP release to DRW cycles in soil B than in soil A.

Table 4.1. Peak amplitude ratios^a of total phosphorus (TP), molybdate-reactive phosphorus (MRP), molybdate-unreactive phosphorus (MUP), and dissolved organic carbon (DOC) during the second (cycle-2) and third (cycle-3) drying-rewetting cycles in the soils studied.

	TP		MRP		MUP		DOC	
	cycle-2	cycle-3	cycle-2	cycle-3	cycle-2	cycle-3	cycle-2	cycle-3
Soil A	2.0	2.4	2.9	1.2	1.1	10.1	7.8	7.0
Soil B	4.3	4.8	3.8	1.9	4.7	7.7	8.9	23.1

^aCalculated as the ratio of concentration at the beginning of a leaching cycle to that at the end of the previous leaching cycle.

The nature of the TP released also differed between the two soils. In RF samples from soil A, TP concentrations ranged from 0.22-0.53 mg l⁻¹ (mean=0.34 mg l⁻¹). Most released TP consisted of MRP, with MRP concentrations ranging from 0.12-0.34 mg l⁻¹ (mean=0.26 mg l⁻¹) and corresponding to 76% of TP, on average (**Figure 4.4a**). Conversely, in RF samples from soil B, TP concentrations had a wider range: 0.07-0.91 mg l⁻¹ (mean=0.25 mg l⁻¹). MRP concentrations, however, remained low, ranging from 0.03-0.22 mg l⁻¹ (mean=0.08 mg l⁻¹), highlighting the much higher proportions (mean=63%) of MUP in the TP of soil B leachates (**Figure 4.4b**).

MUP was more reactive than MRP during rewetting and the subsequent leaching cycle. The difference in reactivity upon rewetting is illustrated by the peak amplitude ratios, which were much higher for MUP than for MRP, particularly in soil B (mean=5.9 for MUP vs. mean=2.5 for MRP) (**Table 4.1**). MUP was exhausted more rapidly than MRP during each leaching cycle. This difference was quantitatively illustrated by calculating the mean proportions of total leached MUP and MRP that was exhausted during the first 300 mm of each leaching cycle: 41-44% of MUP and 27-29% of MRP, in soils A and B, respectively. As expected, the faster exhaustion of MUP than MRP during leaching resulted in a progressive increase in the relative proportion of MRP in the TP released from the beginning to the end of each leaching cycle (55% to 84% and 22% to 46% on average in soils A and B leachates, respectively).

In both soils, TP release pulses upon rewetting were accompanied by clear DOC release pulses (**Figure 4.5**). However, DOC concentrations in the two soils' leachates differed during each release pulse, being much higher in soil B leachates (46.3-136.3 mg l⁻¹) than in soil A leachates (13.8-37.6 mg l⁻¹). This was consistent with the fact that soil B had higher total organic carbon concentrations than soil A (60.2 g/kg vs. 40.9 g/kg, **Table S1**). Besides, DOC was more sensitive to rewetting than P. In soil A, peak amplitude ratios of DOC were 7.8 and 7.0 for cycle-2 and cycle-3, respectively; in soil B, they were 8.9 and 23.1, respectively, all of which were higher than those of TP and MRP (**Table 4.1**). DOC and MUP releases followed the same release dynamics, particularly in soil B (**Figure 4.5**). The synchronized release of DOC and MUP in RF samples from soil B was confirmed by their strong linear correlation ($r=0.96$). In contrast, DOC and MUP concentrations in RF samples from soil A had a weaker linear correlation ($r=0.60$) (**Figure S2**).

The maximum degree of soil dryness between each rewetting cycle also differed between the two soils. Soil dryness remained higher (2.7-3.0% of the water left) in soil A, but progressively decreased from cycle-1 (4.5% left) to cycle-2 (9.7%) and cycle-3 (17.9%) in soil B (**Table S3**). This was likely due to the higher OM content of soil B (**Table S1**). Soil B also leached more leachate (mean=1700 mm) during each of the three DRW cycles than soil A (mean=1100 mm), most likely due to the former's higher content of plant debris, which increased soil porosity and decreased resistance to water irrigation.

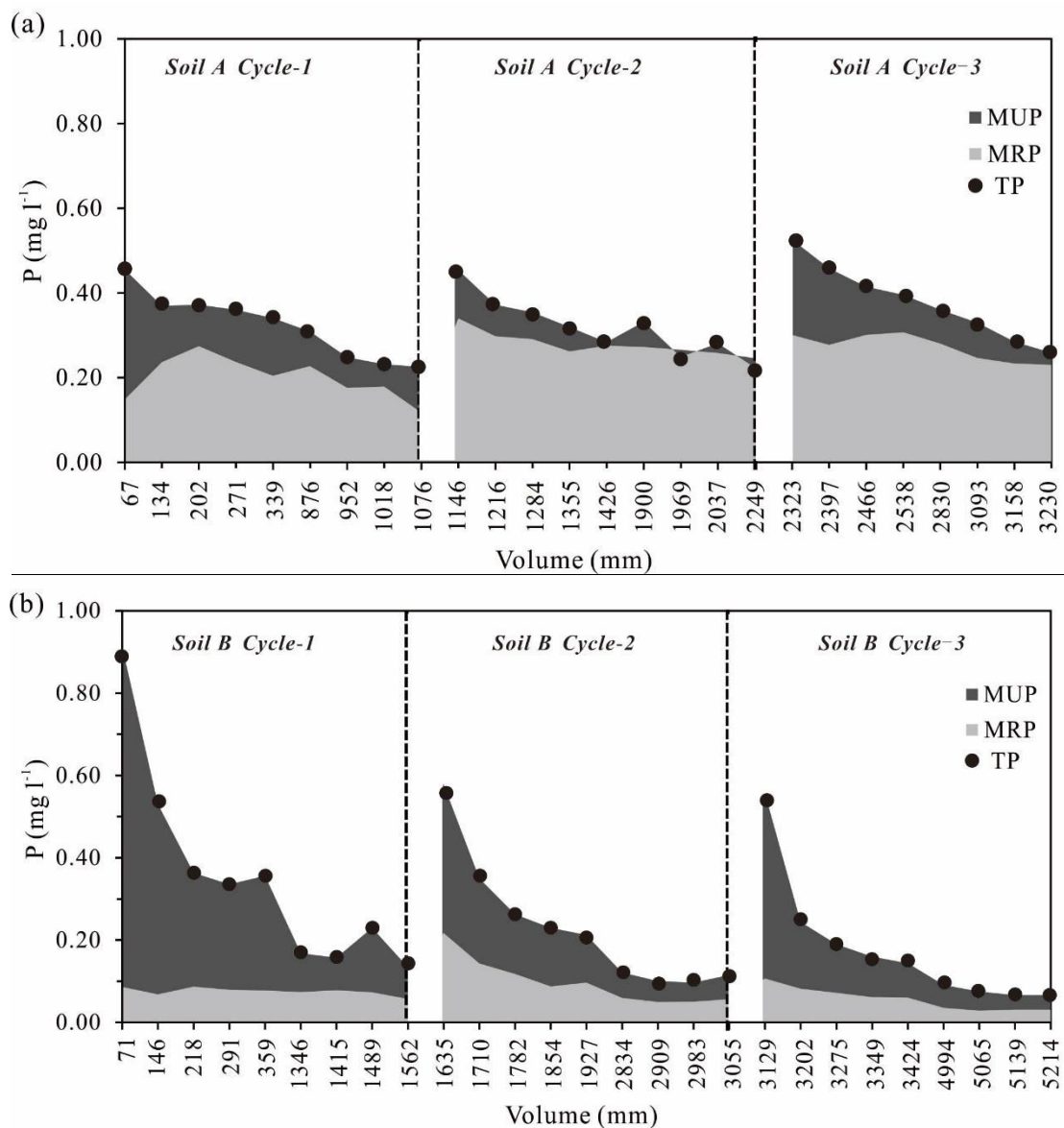


Figure 4.4. Phosphorus fractions (MUP: molybdate unreactive P; MRP: molybdate reactive P; TP: total P) in $<0.45 \mu\text{m}$ filtrated leachates from soils A and B. Vertical dashed lines represent the beginning of a drying-rewetting cycle (the drying of soil column with an O_2 flush). TP and MRP values are mean concentrations calculated from three leaching experiments conducted in parallel on the same soil. Mean standard deviations for TP and MRP were 16.0% and 14.0%, respectively, for soil A and 14.4% and 17.5%, respectively, for soil B. MUP concentrations were calculated as TP concentrations minus MRP concentrations.

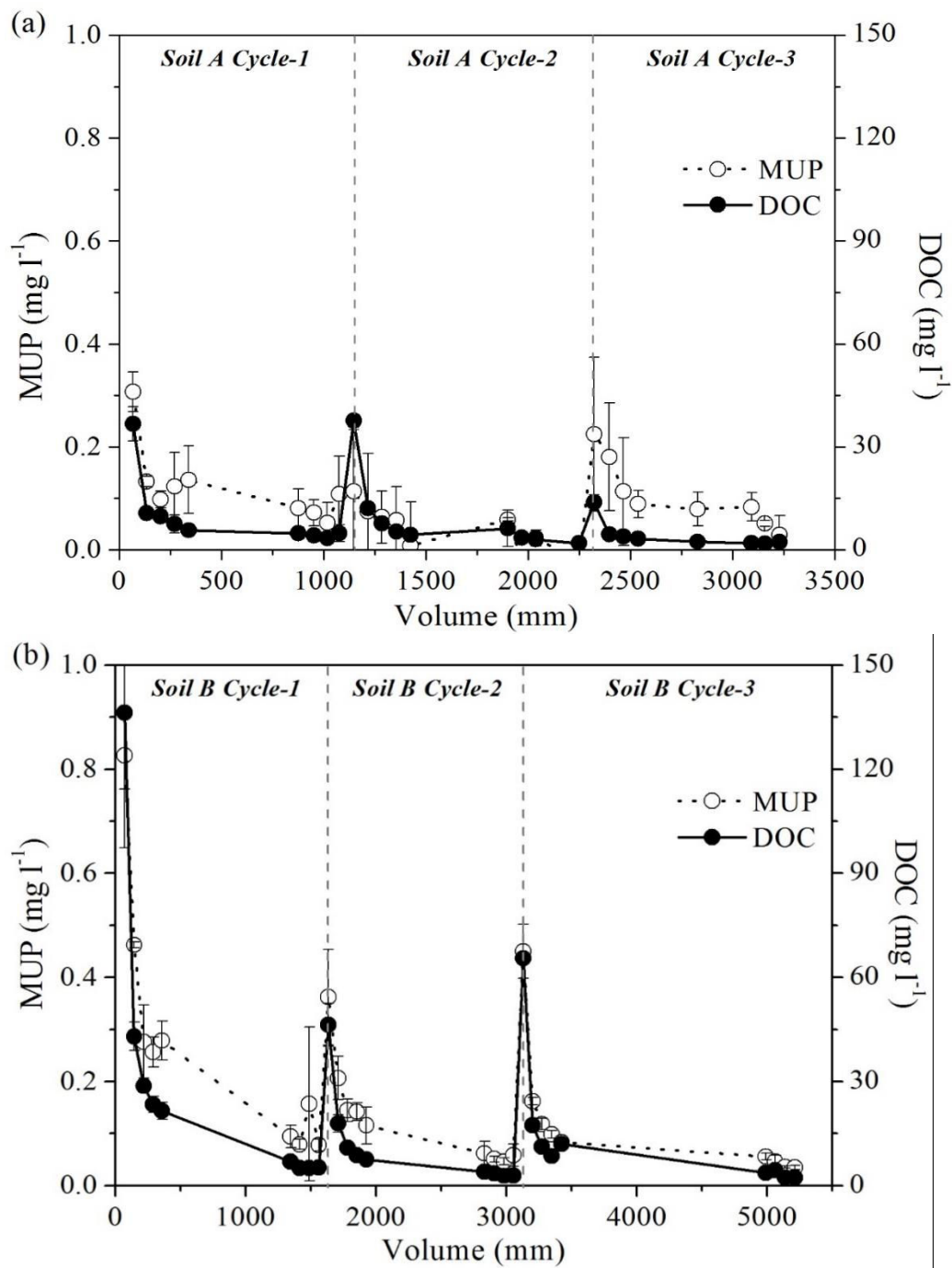


Figure 4.5. Dissolved organic carbon (DOC) concentrations in leachates from soils A and B. Molybdate-unreactive phosphorus (MUP) concentrations are shown to illustrate the synchronicity of DOC and MUP release dynamics. Error bars represent one standard deviation of triplicate samples. Vertical dashed lines represent the onset of rewetting after drying.

4.3.2 UF leachate results

In soil A, truly dissolved MRP was the dominant fraction in all UF samples (65-92%, mean=79%) of the released TP, which was higher than colloidal P (4-29%, mean=17%) or truly dissolved MUP (0-8%, mean=4%) (**Figure 6a**). In comparison, released TP in soil B contained higher proportion of colloidal P (21-74%, mean=45%), less truly dissolved MRP (2-75%, mean=35%) and more truly dissolved MUP (5-48%, mean=15%) (**Figure 4.6b**). Aromaticity of the <0.45 μm UF samples increased from the beginning (<18%) to the end (>20%) of cycle-1 in both soils but remained relatively constant in cycle-2 and cycle-3 with all values > 20%, suggesting a change in the composition of the DOC released in cycle-1.

UF results were also used to calculate the proportions of DOC, Fe and Al carried by colloids (**Figure 3S**). The colloidal fraction accounted for 52% and 46% of DOC, 76% and 65% of Fe, and 64% and 54% of Al in soils A and B, respectively, in the total amount found in the <0.45 μm samples. These results suggested the close association of DOC, Fe, and Al in the P-bearing colloids. Furthermore, the P:DOC, P:Fe, and P:Al ratios in these colloids varied little between fractions or soils in the beginning UF samples (**Table 4.2**), suggesting a consistency in the composition of these colloids.

Table 4.2. Molar ratios of phosphorus (P):dissolved organic carbon (DOC), P:Fe, and P:Al in the coarse (0.45 μm - 30kDa) and fine (30-5kDa) colloidal fractions in leachates of soils A and B at the beginning of drying-rewetting cycles.

Cycle	Colloidal fraction	Soil A			Soil B		
		P:DOC	P:Fe	P:Al	P:DOC	P:Fe	P:Al
1	Coarse	3.5	1.2	1.9	5.2	0.7	1.2
	Fine	3.0	0.3	0.4	3.4	0.5	0.6
2	Coarse	6.6	0.4	1.5	6.2	0.2	0.5
	Fine	6.6	0.3	0.4	7.5	0.3	0.5
3	Coarse	6.4	0.6	1.3	7.8	0.1	0.1
	Fine	6.5	0.3	0.5	7.7	0.6	0.9

Proportions of colloidal P and truly dissolved MUP decreased in UF samples from the beginning to the end of each leaching cycle, which increased the proportion of truly dissolved MRP (**Figure 4.6**). This evolution was consistent with the RF sample data that the relative MUP proportion decreased from the beginning to the end of each leaching cycle (**Figure 4.4**). The more pronounced decrease in these proportions in soil B was due to its larger proportion of colloidal P and truly dissolved MUP at the

beginning of each leaching cycle. Thus, the change in P speciation observed in both soils was likely due to the rapid exhaustion of reservoirs that supplied colloidal P and truly dissolved MUP to leachates. In contrast, the reservoir supplying truly dissolved MRP to leachates appeared infinite, particularly in soil A (**Figures 4.4 and 4.6**).

The size distribution of leached colloids also changed during leaching. This is deduced from the repartition of DOC, the major constituent of colloids, between the coarse (0.45 μm – 30kDa) and fine (30–5kDa) colloidal fractions (**Figure 4.7**). The proportion of coarse colloidal DOC was nearly equal to that of fine colloidal DOC at the beginning of the leaching cycle (ca. 50% of total colloidal DOC each) but was much lower than fine colloidal DOC at the end of each leaching cycle (3%-35%). This suggests that not only were the colloids rapidly exhausted from the soil column during leaching, but that this exhaustion resulted in the granulometric sorting of the colloids (larger colloids were exhausted faster than smaller colloids).

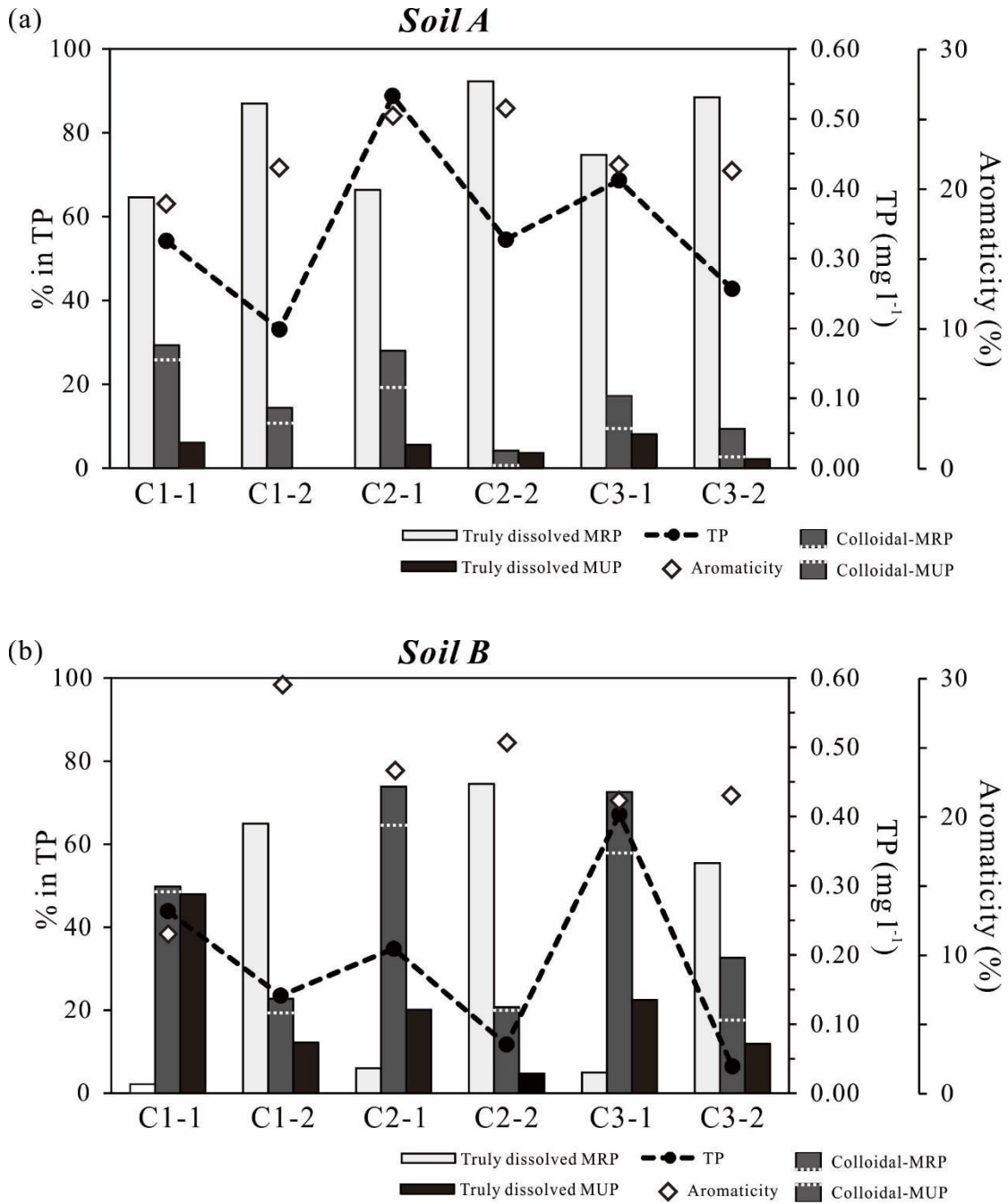


Figure 4.6. Histograms of proportions of colloidal phosphorus (P) and truly dissolved molybdate-reactive P (MRP) and molybdate-unreactive P (MUP) in leachates determined from ultrafiltration. C1-C3 represent drying-rewetting cycles (cycle-1, -2, and -3, respectively), while numbers after hyphen indicate whether the samples were collected (1) at the beginning or (2) end of each leaching cycle. Note that because the TP concentrations in the UF samples were analyzed in mixed samples of the remaining triplicate RF leachates with different remaining volumes, the TP concentrations were not exactly the same as concentrations in RF samples.

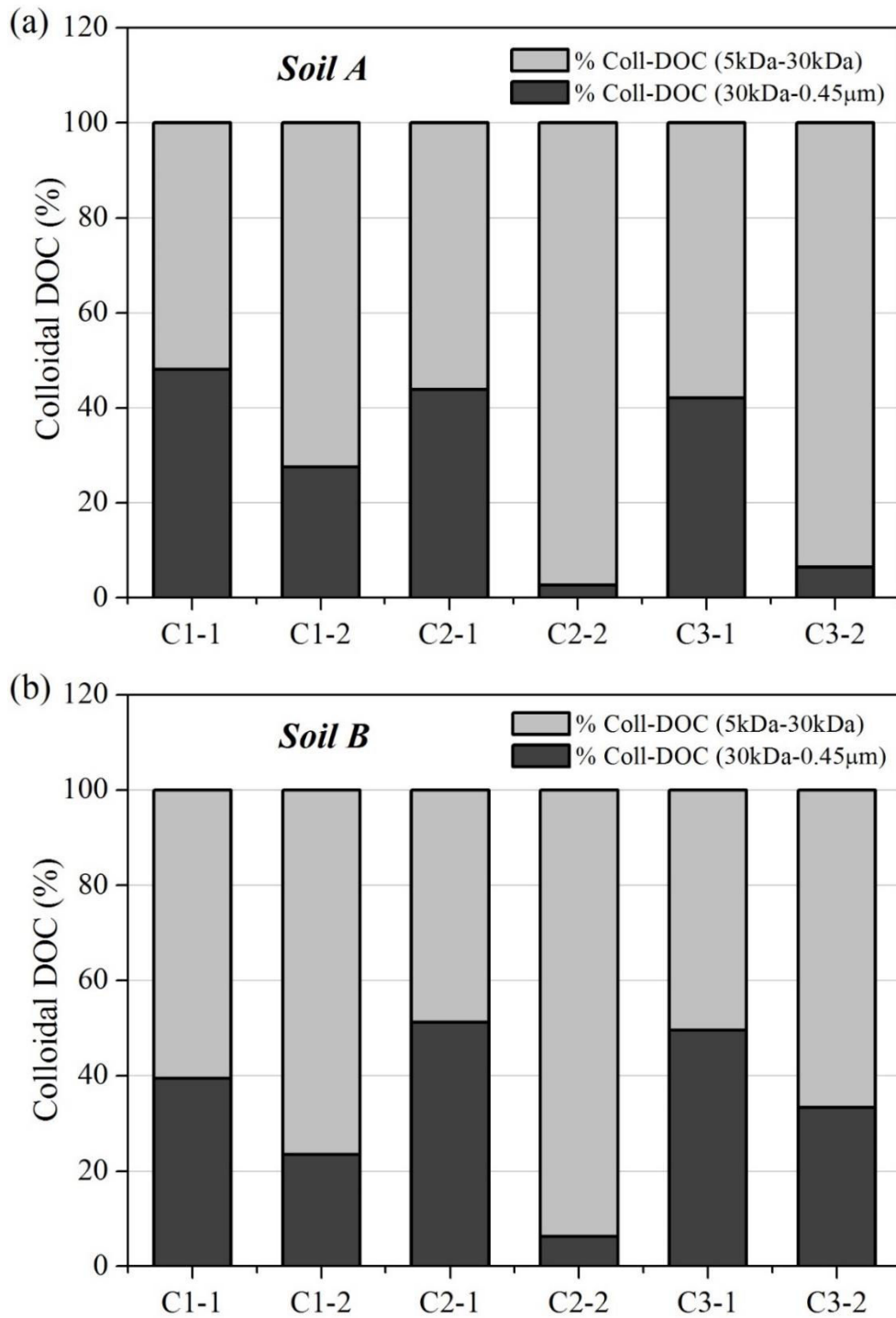


Figure 4.7. Distribution of dissolved organic carbon (DOC) between coarse and fine colloid fractions. C1-C3 represent drying-rewetting cycles (cycle-1, -2, and -3, respectively), while numbers after hyphen indicate whether the samples were collected (1) at the beginning or (2) end of each leaching cycle.

4.4 Discussion

4.4.1 *Soil rewetting stimulates release of colloidal P*

Based on the RF and UF data, it appears that not all P species responded similarly to DRW cycles in the two soils. Clearly, MUP species dominated the P speciation in the TP release immediately upon rewetting and the TP concentration decrease during each leaching cycle. This confirmed previous findings that DRW events strongly stimulate the release of MUP into the soil solution (Blackwell et al., 2009, 2013; Chen et al., 2016; Turner et al., 2003). The influence of DRW cycles on MRP was very limited as suggested in **figure 4.4**. A significant proportion of the MRP at the beginning of leaching cycles in RF samples was colloidal. It was measured as MRP because of the acidic nature of the ammonium molybdate solution used to quantify MRP (Hens and Mercks, 2002). Correcting this effect would result in an even lower sensitivity of truly dissolved MRP to DRW cycles.

The most striking finding is that up to 81% of the MUP released was bound to colloids. Although previous studies mentioned this possibility (e.g. Blackwell et al., 2009), the present study provides the first direct evidence that DRW cycles can stimulate the release of colloidal P in soils. The simultaneous removal of most DOC, Fe and Al along with colloidal P in the UF samples suggests that the colloidal P released could have consisted of P bound to organic-rich or Fe(Al)oxide-rich colloids, or a combination of both, with little or no role of clay-rich colloids. This was further confirmed by the Pearson correlation analysis between the amounts of colloidal TP removed and the amounts of DOC, Fe, and Al (but not Si) removed (**Table 4.3**). It is well established that colloidal P is ubiquitous in soils and streams, and is often strongly associated with P-rich colloidal phases such as OM and Fe(Al)oxyhydroxides (Baken et al., 2016a; Gottselig et al., 2014, 2017; Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013). However, uncertainty remains about the exact arrangement of the organic and mineral portions of P-rich colloids and the way P interacts with them. Jiang et al. (2015) isolated water-dispersible fine colloids in an arable soil in Germany and found that 65% of the colloidal P was bound to amorphous Fe-oxides, which functioned as the host phase of P and as a cement for smaller organic, P-bearing colloids. Gottselig et al. (2017), in their study on stream water, found that P was preferentially associated with Fe in the smallest fraction (i.e. 1-20 nm), but more with organic-rich colloids in

larger fractions (>60 nm). In the present study, however, the UF data indicated no fundamental difference in the composition of colloids in the coarse and fine fractions or between the two soils.

Table 4.3. Pearson correlation coefficients of chemical components in the colloidal fraction of leachates from soils A and B.

	MRP _{Coll} ^a	TP _{Coll} ^a	Al	Fe	DOC ^a	Si	MUP _{Coll} ^a
MRP _{Coll}	1.000						
TP _{Coll}	0.709	1.000					
Al	0.772	0.859	1.000				
Fe	0.784	0.962	0.943	1.000			
DOC	0.437	0.912	0.758	0.851	1.000		
Si	0.409	0.360	0.693	0.481	0.278	1.000	
MUP _{Coll}	0.563	0.982	0.800	0.917	0.952	0.313	1.000

^aMRP_{Coll}, MUP_{Coll}, TP_{Coll} and DOC represent the molybdate-reactive phosphorus, molybdate-unreactive phosphorus, total phosphorus and dissolved organic carbon in the colloidal fraction, respectively. MUP_{Coll} is the difference between TP_{Coll} and MRP_{Coll} concentrations.

Depending on the sample, up to 50% of MUP passed through the <5kDa filters. This MUP fraction may have been P-bearing organic molecules from microbial cells that lysed due to osmotic shock upon soil rewetting (Blackwell et al., 2010, 2013; Turner and Haygarth, 2001; Turner et al., 2003). It also may have consisted of P bound to nanoparticles (<5kDa) with the same composition and origin as larger organo-mineral colloids, or of small organic colloids, as highlighted by Jiang et al. (2015).

4.4.2 Co-existence of physically- and biologically-driven P release during rewetting

Results of the present study suggest that the physically- and biologically-driven processes (soil aggregates disruption and microbial cell lysis, respectively) are likely not exclusive, but rather co-exist in soils during DRW cycles.

It is well established that drying of the soil matrix causes soil colloids to mobilize, which is associated with the micro-fragmentation of macropore walls and soil aggregates due to increased shear forces and capillary stress (Klitzke and Lang, 2007; Madjalani et al., 2008; Mohanty et al., 2015). In this scenario, the amount of the leachable colloids physically generated from dry pore walls or soil aggregates ultimately depends on the duration of soil drying (Mohanty et al., 2015). During the three successive DRW cycles in the present study, the two soils experienced intense drying conditions similar to those in previous studies, suggesting that physical

processes played an important role in controlling colloidal P release upon rewetting of the two soils studied. Interestingly, X-ray diffraction analysis performed by Madjalani et al. (2008) showed that released colloids were enriched in Fe-bearing minerals, which is also true of colloids released from soils of the present study and many previous studies (Henderson et al., 2012; Ilg et al., 2008; Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013).

As described, soil microbial biomass decreases significantly when soils are dried to a certain extent. The osmotic shock caused by rewetting can cause remaining microbe cells to lyse, leading to the release of dissolved organic P in the soil solution (Blackwell et al., 2009; Butterly et al., 2009; van Gestel et al., 1993). In the present study, this process could account for the truly dissolved MUP found in the <5kDa fraction of the UF samples and assumed to be activated at least during the first DRW cycle. Microbial biomass P has been observed to recover slowly after its partial killing during rewetting, but no early than within 7-28 days, depending on the study (Chen et al., 2016; Grierson et al., 1998). In other studies, microbial biomass did not recover to its original level after 12 days of rewetting (Mondini et al., 2002), or it decreased after repeated DRW cycles (Butterly et al., 2009). In the present study, the duration of one complete DRW cycle was <4 days, and the maximum time for microbial biomass to recover was 30 h. It is assumed that contribution of the microbial cell lysis process was much smaller during cycle-2 and cycle-3 than during cycle-1 when microbial biomass was the maximum. The decrease in the relative importance of microbial-derived P from cycle-1 to cycle-3 was consistent with the change in aromaticity of the DOC released in the UF samples. Aromaticity was <18% in leachates at the beginning of cycle-1 in both soils, suggesting that the DOC released contained a large proportion of microbial metabolites, which generally have an aromaticity <15% (Pédrot et al., 2008). In contrast, DOC aromaticity was higher (21-30%) in leachates from cycle-2 and cycle-3, which indicates a higher proportion of dissolved humic substances in the DOC (aromaticity of ca. 35%; Weishaar et al., 2003).

Simultaneous release of colloidal P and truly dissolved MUP in each DRW cycle indicate the co-activation of physically- and biologically-driven P release. This is consistent with Bünemann et al. (2013), who investigated release dynamics of C and P during DRW of grassland soils in Switzerland. They concluded that both microbial biomass and physical perturbations were likely involved in the P release. Although both

P release processes can operate independently, they can also interact. For example, P-bearing metabolites released during microbial cell lysis can interact with P-poor Fe-oxyhydroxide colloids released by disaggregation of macropore walls to form P-bearing organo-mineral colloids. These colloids are nearly indistinguishable from P-bearing colloids, which physically detach from macropore walls or soil aggregates and have a mixed composition of Fe-oxyhydroxide/humic-substances.

4.4.3 Influence of soil properties

That soil B released more colloidal P and truly dissolved MUP, while soil A released more truly dissolved MRP, is likely a direct consequence of differences in P content and speciation between the soils.

For example, the release of more truly dissolved MRP from soil A appeared consistent with its higher extractable Dyer- and Olsen-P contents, which were 6-8 times as high as those in soil B. It is also consistent with the P status of soil A being composed of mainly (ca. 65%) inorganic species (**Tables S2**). Interestingly, the difference in released P forms in the two soils was similar to that observed in natural soil solutions from the same soils. Under field conditions, soil A released mainly MUP upon rewetting, while soil B released mainly MUP (Gu et al., 2017). Given the simplified experimental setup and the highly disturbed character of the soils placed in the columns, intrinsic soil properties exerted first-order control over the forms of P released upon rewetting, thus becoming much more important than factors such as physical properties or the geometry of macropore networks.

Soil B's release of more colloidal P and truly dissolved MUP than soil A was consistent with its higher OM content and microbial biomass, both of which can stimulate release of MUP from microbial cell lysis (Achat et al., 2012; Nguyen and Marschner, 2005) and produce more stable colloids (Klitzke and Lang, 2007). Another explanation is the legacy effect induced by the distinctly different soil moisture conditions that the two soils experienced. Soil A experienced frequent changes in soil moisture, with continuous saturation periods lasting <2 weeks, while soil B was saturated for >6 months each year (Gu et al., 2017). This long-term difference in moisture conditions could have influenced the contemporary response of microbes to DRW stress by increasing the persistence to abiotic changes (such as osmotic shock) or selecting a more resistant microbial community (such as fungi), as in soil A (Banerjee et al., 2016; Evans and Wallenstein, 2012), thus explaining the differences observed.

The longer saturation time and higher OM content of soil B also could explain its higher colloidal P concentration upon rewetting compared to soil A. In wetland soils, Fe-rich colloids form in response to water-table drawdown and the subsequent oxidation of previously released Fe(II). The size and crystallinity of these colloids depended greatly on the soil DOC:Fe ratio and the saturation time, with high DOC:Fe ratios and longer saturation (as in soil B) producing larger and more crystallized colloids (Pédrot et al., 2011). A difference in vegetation cover of the wetlands also may have influenced colloidal P concentrations. Forest soil B had more plant debris content, which increased soil column porosity, than grassland soil A, as indicated by soil B's larger leaching volume. The higher porosity provided more soil macropores and mesopores, in which more colloids could be mobilized than in micropores (Mohanty et al., 2015).

4.4.4 Linking sources and production mechanisms of P forms released during soil rewetting

Contrasted responses of MUP and MRP species (colloidal and truly dissolved) were identified during rewetting of the two studied soils. That colloidal P and truly dissolved MUP were rapidly exhausted from the soil columns suggests that their pools were finite and most likely produced within soil pores. Coarse colloids tend to leave columns faster than fine colloids, which can pass through soil micropores, thus increasing their mean transit time, as confirmed by the change in size distribution of colloids in the UF data. The dominant coarse colloid fraction probably came from soil macro/mesopores. During successive leaching experiments on arable soils, Sharma et al. (2015) observed initially high TP concentrations followed by a sharp decrease. They suggested that the peak P release observed at the onset of leaching was dominated by rapid macropore flow, while the more constant P release that followed was dominated by soil matrix flow. Soil macro/mesopores also mobilized more colloids than micropores in experiments that determined the influence of drying duration on colloid production rate and colloid flow path in soils (Mohanty et al., 2015). Thus, P-bearing coarse colloids and microbial-derived truly dissolved MUP originated mainly from soil macro/mesopores, which also served as the main conduit for their exhaustion from the soil columns via gravitational drainage.

The relatively constant truly dissolved MRP concentrations during leaching seemed

to come from an infinite pool that was less influenced by DRW, which suggests that its production mechanisms were not linked to those previously mentioned. We assumed that truly dissolved MRP was evenly distributed throughout the entire soil matrix, including soil micro-aggregates. DRW cycles may partially influence this P pool by driving the mineralization rate of soil OM, but the short duration of each DRW cycle (<4 days) was not long enough to cause biological mineralization to release MRP.

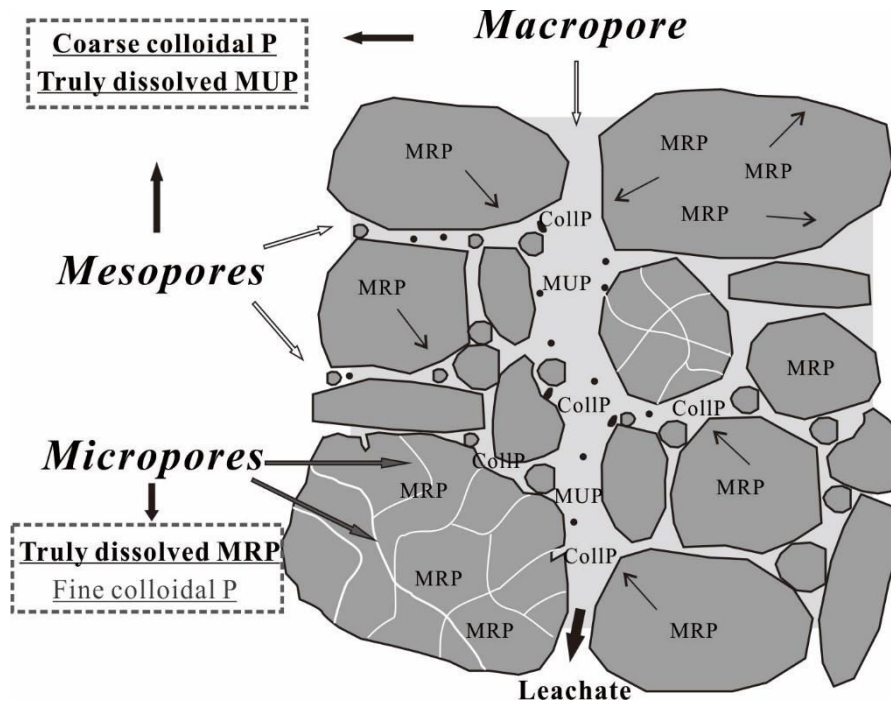


Figure 4.8. Diagram illustrating potential sources and transport pathways of forms of phosphorus (P) released during drying-rewetting events: truly dissolved molybdate-reactive P (MRP), truly dissolved molybdate-unreactive P (MUP), and colloidal P (ColIP). Although micropores exist in nearly all coarse soil aggregates, they are shown in only two aggregates for simplicity.

To summarize (**Figure 4.8**), upon rewetting, a P pool composed of larger colloidal P (both organic and inorganic) and truly dissolved MUP is activated by physical and biotic processes, respectively. Since these P pools are finite and come mainly from soil macro/mesopores, they are rapidly exhausted by gravitational water flow. Rewetting also mobilizes truly dissolved MRP, mainly in the soil matrix. Exhaustion of this P pool is slow and constant, suggesting a stationary equilibrium between its release from soil matrix to micropores and its diffusion from micropores to macro/mesopores and leachate.

4.4.5 Environmental and ecological implications

This study confirms that DRW events can trigger the release of dissolved and colloidal P into wetland soils, highlighting the important role of soil properties in influencing the nature and relative abundance of released P forms. This is significant given that the experimental results are highly consistent with field data for soil solutions from the two wetlands. This is particularly true for the difference in MUP and MRP concentrations in RF samples between the two soils, which was apparent in the field monitoring dataset published by Dupas et al. (2015) and Gu et al. (2017). The rapid exhaustion of colloidal and truly dissolved MUP during leaching from the two soils was also the usual response of the wetland soils when subjected to natural DRW cycles (Gu et al., 2017).

Besides confirming the role of riparian wetlands as potential sources of truly dissolved P species (MRP and MUP) for streams in the agricultural landscape, the present study also demonstrates that riparian wetlands are also potential sources of P-rich colloid production. Although potentially less bioavailable than MRP, colloidal P can contribute greatly to eutrophication of surface water. Colloids produced in riparian wetlands contain a combination of poorly crystallized, fine-grained Fe-oxyhydroxides and OM. These colloids are particularly sensitive to bioreduction in the presence of humic acids, which directly influence the size, shape, and density of Fe-oxyhydroxides or directly increase bioreduction of nanoparticles by electron shuttling and Fe complexation (Pédrot et al., 2011). Once transported into anoxic environments such as bottom sediments of rivers or lakes, these colloids can become a P source for aquatic organisms. A recent study of P-bearing colloids in streams also indicated that labile P contained in P-rich colloids could be released into holding water bodies within seven days (Baken et al., 2016a). Thus, subsurface leaching of colloidal P from riparian wetlands could increase potentially available P in surface water, perhaps as much as the direct transfer of truly dissolved P species.

4.5 Conclusions

DRW events, a common abiotic stress that soils experience, can release dissolved P in soils and thus increase the risk of surface water eutrophication. We conducted DRW experiments on two riparian wetland soils to assess their ability to accelerate release and transfer of P from wetland soils to surface waters, aiming to bring further

constraints on the nature of the released P forms and the mechanisms that release them. We confirmed that DRW events can trigger pulses of dissolved P release into soil leachates. More importantly, we found that the P released during DRW cycles consisted not only of truly dissolved P but a large proportion of colloidal P. We also found that DRW cycles activated the biological release of truly dissolved MUP via microbial cell lysis. The release of colloidal P was activated by physical mechanisms of colloid mobilization or, to a lesser extent, biologically colloid formation. The truly dissolved MRP pool was less influenced by DRW cycles. These activation mechanisms indicated different potential sources of these P forms in the soil matrix. Colloidal P and truly dissolved MUP were generated mainly in soil macro/mesopores, which also were conduits that rapidly exhausted these two P pools from soil columns via gravitational water flow. Truly dissolved MRP was generated mainly in micropores throughout the soil matrix, and its release was constant and slow.

Soil properties such as P content and speciation influenced P forms (MRP or MUP) and concentrations released during DRW cycles, while properties such as soil OM and microbial biomass, and factors such as historical moisture conditions and vegetation cover influenced the amount of the colloidal P released. These soil properties should be considered when trying to evaluate and decrease the loss of leached P, especially colloidal P, from agricultural soils into surface water.

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4.6 Supplementary materials

Table S1. Main properties of the soils studied.

Soil property	Soil A	Soil B
Clay (<2 μm) (g/kg)	241	288
Fine loam (2-50 μm) (g/kg)	441	440
Coarse loam (20-50 μm) (g/kg)	174	159
Fine silt (50-200 μm) (g/kg)	83	57
Coarse silt (200-2000 μm) (g/kg)	61	56
Organic carbon (g C/kg)	40.9	60.2
Total nitrogen (g N/kg)	3.65	4.66
C:N ratio	11.2	12.9
Organic matter (g/kg)	70.7	104.0
pH	6.08	6.18
Microbial biomass (mg /kg)	437.5	791.7
P-Oxalate (g P/kg)	0.238	0.030
P-Dyer (g P/kg)	0.288	0.048
P-Olsen (g P/kg)	0.075	0.014
Si-Oxalate (g Si/kg)	0.29	0.31
Al-Oxalate (g Al/kg)	1.69	1.71
Fe-Oxalate (g Fe/kg)	4.25	7.71
Total P (g P/kg)	1.21	0.68
P_{Oxal}/P_{tot} (%)	19.6	4.4
P_{Dyer}/P_{tot} (%)	23.8	7.0
P_{Olsen}/P_{tot} (%)	6.2	2.1

Table S2. Mean (\pm standard deviation) concentrations of phosphorus species in the soils studied (Hedley fractionation)^a

Soil	Biomass	Biomass	H ₂ O	H ₂ O	NaHCO ₃	NaHCO ₃	HCl	HCl	NaOH	NaOH	P _{residual}	Tot	Tot
	P _{inorg}	P _{org}	P _{inorg}	P _{org}	P _{inorg}	P _{org}	P _{inorg}	P _{org}	P _{inorg}	P _{org}		P _{inorg}	P _{org}
mg/kg												%	%
A	1.0 \pm 0.5 (0.2)	0,3 \pm 0.1 (0.0)	7.3 \pm 1.4 (1.1)	5.2 \pm 0.4 (0.8)	84.1 \pm 0.7 (12.2)	26.5 \pm 8.9 (3.8)	290.7 \pm 24.9 (42.1)	16.1 \pm 12.4 (2.3)	60.1 \pm 27.1 (8.7)	131.1 \pm 6.5 (19.0)	68.5 \pm 13.1 (9.9)	64.3	25.8
B	0.5 \pm 0.3 (0.2)	0.5 \pm 0.2 (0.2)	0.1 \pm 0.0 (0.0)	1.3 \pm 0.0 (0.4)	9.3 \pm 1.3 (3.1)	26.5 \pm 2.4 (8.9)	52.0 \pm 12.9 (17.4)	6.0 \pm 4.5 (2.0)	13.1 \pm 3.4 (4.4)	134.9 \pm 11.3 (45.1)	56.2 \pm 2.8 (18.3)	25.1	56.6

^aBased on duplicate and triplicate analyses of soils A and B, respectively. Values in % and numbers in parentheses are proportions of the P species in the total P extracted.

Table S3. Soil water content measured in triplicate soil columns at the end of the drying cycles^a

Soil	Cycle	Column 1	Column 2	Column 3	Mean
A	1	2.65 %	2.66 %	2.68 %	2.66 %
	2	2.98 %	3.02 %	2.84 %	2.95 %
	3	2.78 %	2.68 %	2.75 %	2.73 %
B	1	4.43 %	4.47 %	4.49 %	4.46 %
	2	10.45 %	6.07 %	12.42 %	9.65 %
	3	17.98 %	17.71 %	17.89 %	17.86 %

^aSoil water content at cycle-1 before rewetting was the initial water content of air-dried soils relative to the constant weight at 105 °C of the same soil; water content at cycle-2 and -3 (dried using an O₂-flush) were calculated relative to the same constant weight at 105 °C as cycle-1. Soil columns lost almost no weight during leaching due to the protection of glass fiber.

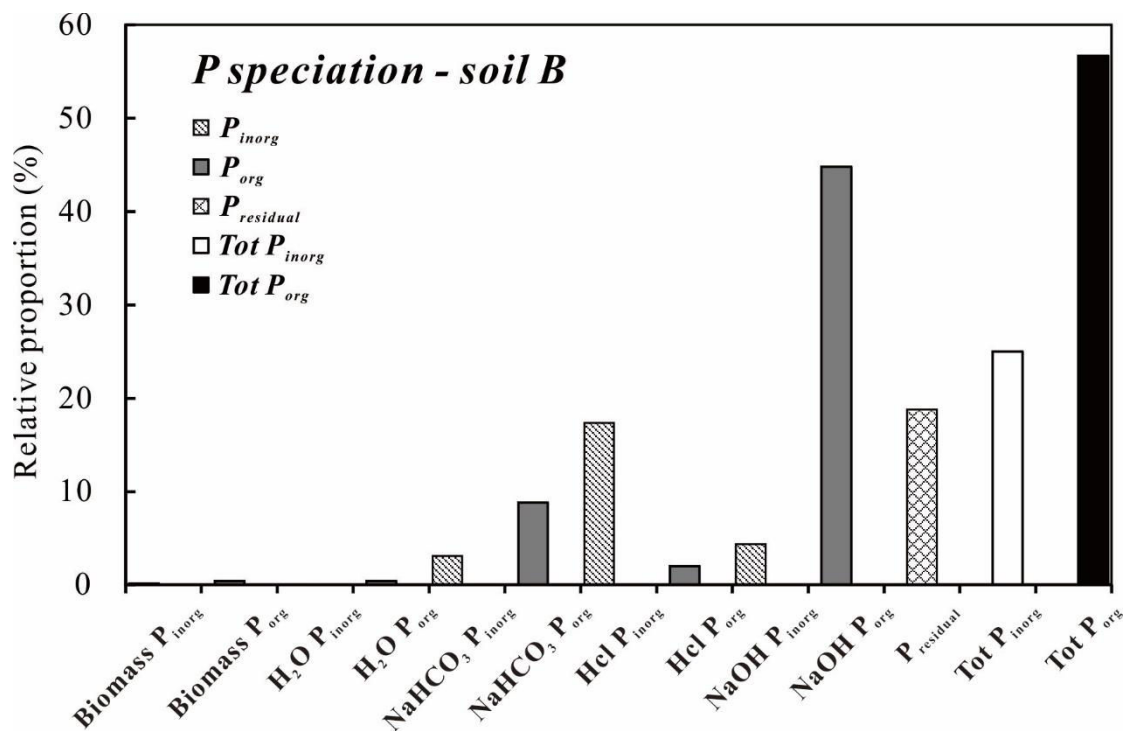
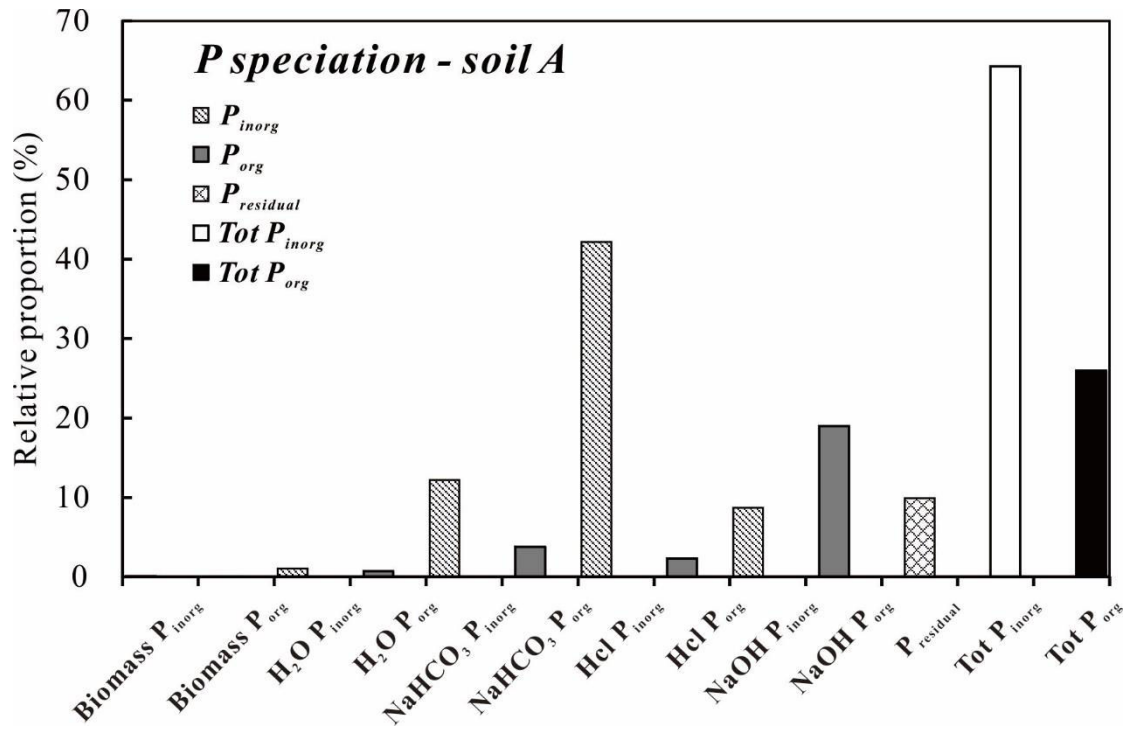


Figure S1. Phosphorus fractions in soils A and B determined by Hedley fractionation. In this fractionation method, P_{inorg} represents the molybdate-reactive P; Tot-P represents the total extracted P; P_{org} is the difference between Tot-P and P_{inorg} .

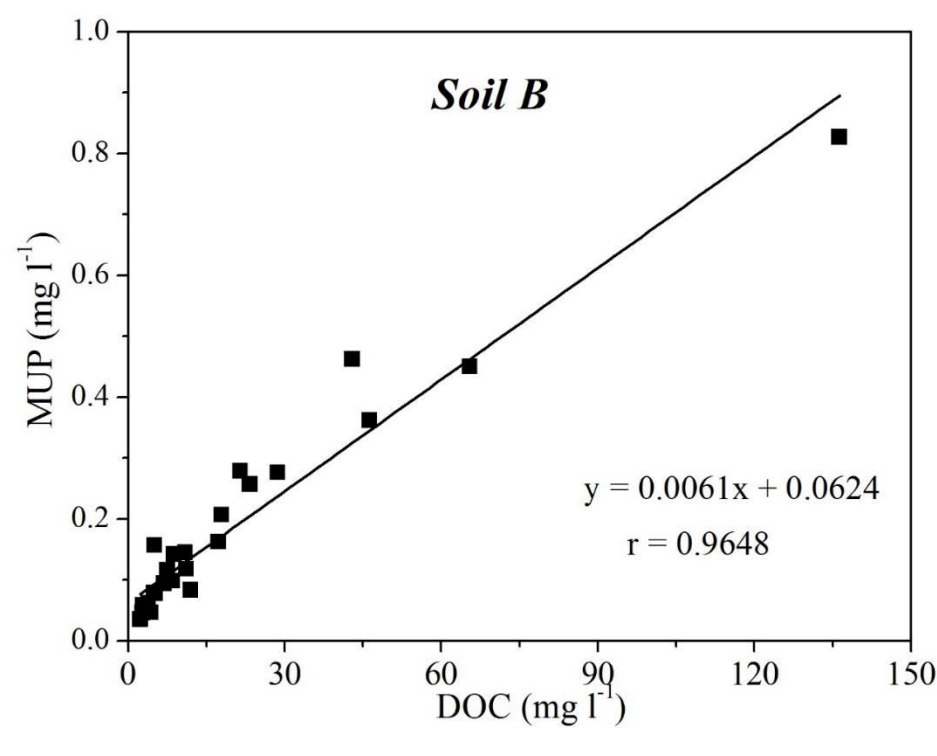
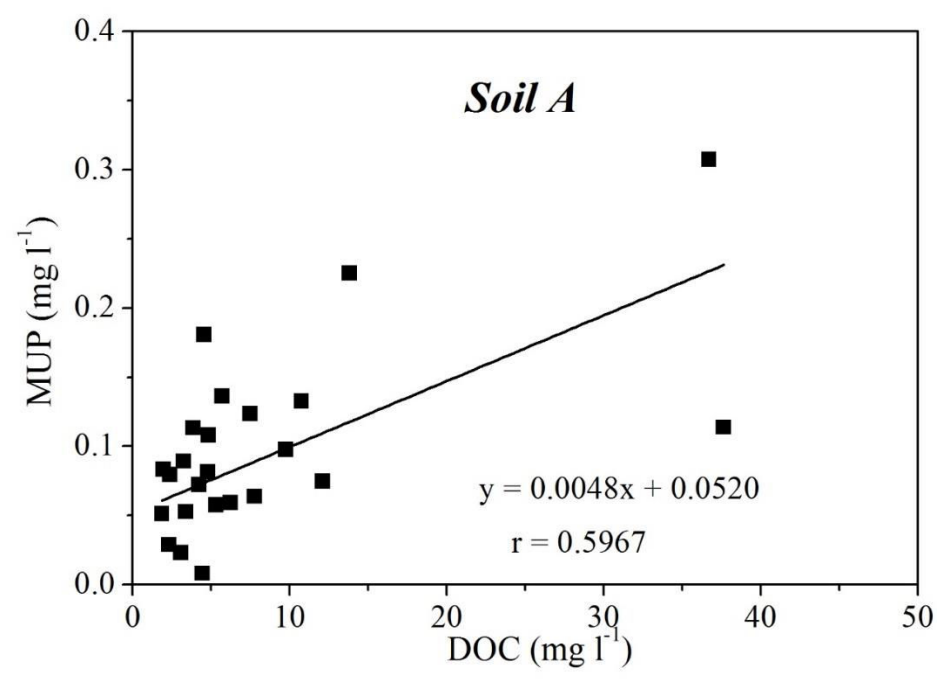


Figure S2. Linear correlation between MUP and DOC concentrations in soil A and B leachates.

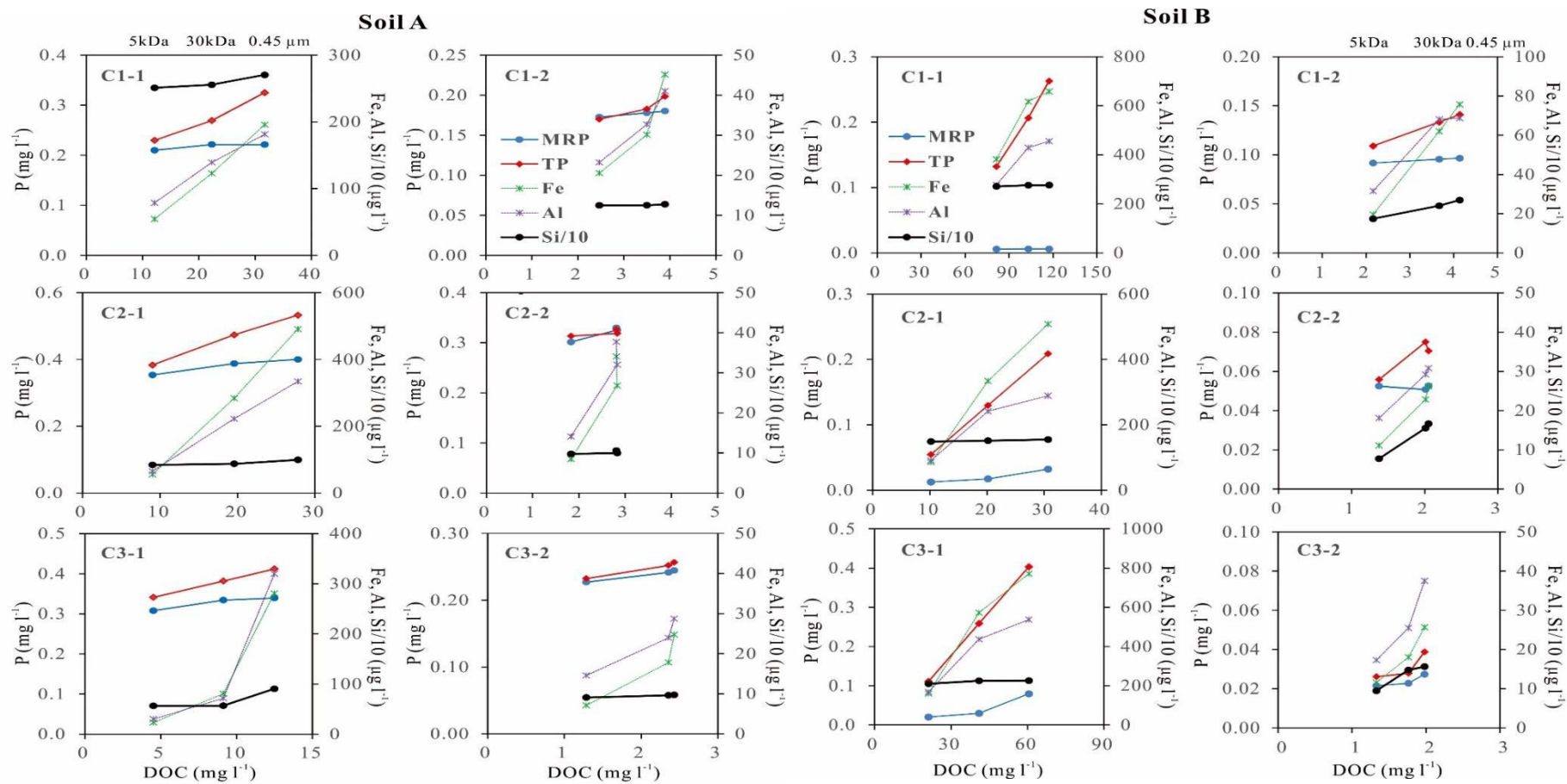


Figure S3. Covariation of phosphorus (P), dissolved organic carbon (DOC), Fe, Al and Si concentrations during ultrafiltration of soil A and B leachates. C1-C3 represent drying-rewetting cycles (cycle-1, -2, and -3, respectively), while numbers after hyphens indicate whether samples were collected (1) at the beginning or (2) end of each leaching cycle.

4.7 Conclusion of chapter 4 (laboratory simulation of drying-wetting cycle)

The drying-rewetting (DRW) column leaching experiments presented in this chapter confirmed the conclusions that have stemmed out of the field monitoring results reported in Chapters 2 and 3, showing that DRW cycle is one of the processes that trigger the release of DP in RWs. The results evidenced that the DP released during DRW cycles consisted not only truly dissolved P but also a large proportion of colloidal P, thereby confirming the emerging view that colloids play a great role in conveying P in soils. Most likely, the truly dissolved MUP pool released during those cycles consist of organic dissolved P coming from microbial cell lysis, the truly dissolved MRP comes from desorption and diffusion of inorganic P stored in the soil matrix. The production of colloidal P during DRW cycles is due to the mechanical erosion of macropores, a physical process known to efficiently produce colloids in soils, though it is not excluded that a smaller proportion of colloids could have a biological origin (e.g. cell debris). Thus, different P forms released upon rewetting of dried RW soils have different potential sources in the soil, and different exhaustion dynamics/pathways. The truly dissolved MUP and colloidal P are mainly from the soil macro/mesopores and are rapidly exhausted from the soil matrix via gravitational leaching flow. The truly dissolved MRP, on the other hand, is mainly from the micropores throughout the soil matrix and is constantly and slowly released. The results in this chapter also reveal that the colloidal and truly dissolved organic P fractions are more reactive to DRW cycles, with huge concentration peaks upon rewetting of dried soils. In fact, MRP fractions react much less to DRW cycles, suggesting that its release is produced by different and longer time-scale processes. Comparison of soil properties and colloidal P production during DRW cycles suggest that soils with high organic matter content and large microbial biomass size are likely the soils most reactive to DRW cycles, producing the largest amount of colloidal P during DRW cycles.

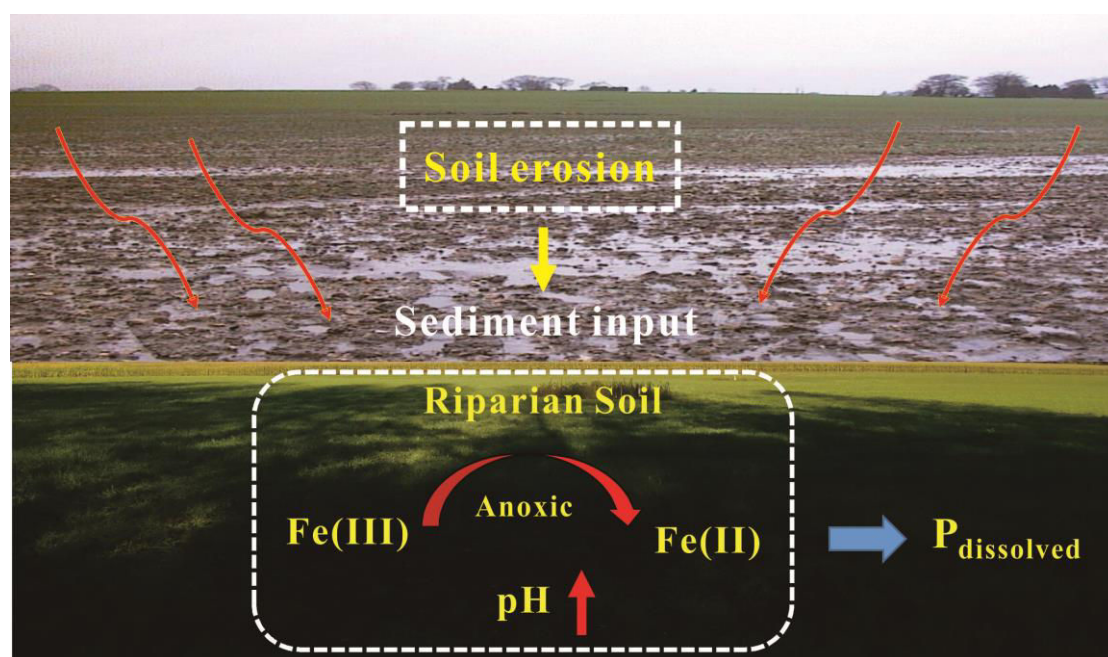
Besides the impact of DRW cycles, the field monitoring results in Chapters 2 and 3 have pointed out the reductive dissolution of soil Fe-oxyhydroxides during water saturation period as another process promoting the release of DP in RWs. As with DRW cycle, reduction processes can be simulated in the laboratory. The aim of **chapter 5** is

to present the results of laboratory simulations performed to further assess how exactly these reduction processes control the release of DP in RWs.

Chapter 5

Release of dissolved phosphorus upon reduction of wetland soils: a laboratory study of the respective roles of soil Fe-oxyhydroxides dissolution, pH changes, sediment inputs and soil phosphorus speciation

This chapter corresponds to a paper by Sen Gu, Gérard Gruau, Rémi Dupas, Patrice Petitjean, Qingman Li and Gilles Pinay, in preparation for submission to Geoderma or European Journal of Soil Science.



Release of dissolved phosphorus upon reduction of wetland soils: a laboratory study of the respective roles of soil Fe-oxyhydroxides dissolution, pH changes, sediment inputs and soil phosphorus speciation

Abstract

The development of anoxic conditions in riparian wetland (RW) soils has been widely recognized to release dissolved (<0.45µm) phosphorus (P), but the respective roles of soil P speciation, soil Fe oxyhydroxide reductive dissolution, pH changes during reduction reactions and sediment inputs on this release remain a controversial issue. In this study, we attempted to unravel and quantify these different roles by performing laboratory anaerobic/aerobic incubations on RW soils with and without sediment addition. The selected soils are two RW soils with contrasting soil P status and organic matter (OM) content, coming from a small agricultural catchment in western France. The added sediments come from the agricultural field immediately above the RW. Soil solutions from all incubations were analyzed for dissolved molybdate-reactive P (MRP), dissolved total P (TP), dissolved organic carbon (DOC), nitrate, sulfate, and Fe²⁺ concentrations. We found that the concentrations of released TP and MRP correlated positively with the soil total P and extractable P contents, but negatively with the OM contents of corresponding soils. The molar Fe:P ratios of anaerobic soil solutions were lower (<1.4) in soils with higher oxalate extractable P (P-Oxalate) and lower oxalate extractable Fe (Fe-Oxalate) than in soils (up to 12.0) with lower P-Oxalate and higher Fe-Oxalate. The dissolved P release was mainly controlled by the Fe-oxyhydroxides reductive dissolution mechanism (83%) in the soils with high extractable P and low OM contents, whereas that released in soils with low extractable P but high OM contents was mainly controlled by the pH change mechanism (88%). Moreover, the mixture of eroded soil sediments with RW soils enhanced the release of dissolved P, DOC and Fe(II) by 16, 4 and 18%, respectively, as compared to the reduction of the sediment and RW soils alone without mixing. The results of the present study suggest that soil properties controlled the concentration, speciation of dissolved P released under anoxic conditions and also their potential transfer risk. There was a

clear difference in the controlling mechanisms of dissolved P release between soils, implying that management options to reduce dissolved P transfer from RW soils should be also designed with reference to soil properties. Management efforts, such as preventing P mobilization in upslope fields to avoid further enrichment of RWs in P or recovering legacy P from RWs by exporting biomass, should focus in priority on RWs whose Fe:P ratios in soil pore water during reduction are the lowest, as these soils are at high risk with respect to the potential transfer of DP to watercourses.

5.1 Introduction

Riparian wetlands (RWs) – i.e. the non-cultivated buffer zones between agricultural fields and watercourses - have long been promoted to reduce diffuse losses of phosphorus (P) from agricultural sources. However, studies investigating the sources of dissolved P (DP) in streams draining agricultural catchments revealed that RWs could be source zones of DP release at the catchment scale. (Dupas et al., 2015c; Gu et al., 2017). Two mechanisms have been proposed to account for this release, both related to the periodic water table fluctuations that affect these zones. The first is due to the rewetting of soils after the dry summer period, which causes osmotic shock in soils and the subsequent release of P from the microbial cell (Turner and Haygarth, 2001). The second mechanism is due to the waterlogging of RW zones during wet seasons, which favors the development of anoxic conditions in the RW soils. Studies investigating the dynamics of DP release in RW soils during water-saturation periods both in the field and laboratory reported positive correlations between DP and Fe(II) concentrations (SurrIDGE et al., 2007; Knorr et al., 2013; Dupas et al., 2015c; Gu et al., 2017). This suggests that the establishment of anoxic conditions able to reduce Fe-oxyhydroxides during water-saturation periods could be a key factor in controlling DP release in RW soils.

At least two hypotheses can explain this concomitance between the process of Fe reduction and DP release in RW soils: i) reductive dissolution of Fe-oxyhydroxides by soil bacteria, leading to the solubilization of associated inorganic and organic P species and ii) pH rise during the Fe-oxyhydroxide reduction reactions resulting in desorption of inorganic and organic P species from soil particles (Kirk et al., 2004). The first “dissolution” hypothesis assumes that the released DP consists of P originally trapped into, or adsorbed onto soil Fe-oxyhydroxides. Indeed, because of their highly reactive

and strongly protonated surfaces, Fe-oxyhydroxides have strong ability to adsorb oxyanions like phosphate groups or P-bearing, negatively charged dissolved organic molecules/colloids (Borch and Fendorf, 2007; Wilson et al., 2004). When soils become water-saturated and flow velocity decreased, O₂ depletion is often observed. In reaction, the microorganisms change their terminal electron acceptors from O₂ to other acceptors such as iron. This leads to the reductive dissolution of Fe-oxyhydroxides (Stumm and Sulzberger, 1992; Zak et al., 2004), and thus the release of the associated substances, including the adsorbed phosphate groups and/or P-bearing, dissolved organic molecules/colloids (Knorr et al., 2013; Jeanneau et al., 2014; Surridge et al., 2012). In this case, the ultimate sources of the dissolved P species released in RWs under anoxic conditions are the soil Fe-oxyhydroxides. The “desorption” hypothesis assumes that the DP release process is associated with the pH rise caused by reduction reactions. Several studies have reported pH rises in soil solutions following the consumption of protons in reduction reactions of NO₃⁻, Fe or Mn (Stumm and Sulzberger, 1992; Ponnampereuma, 1972; McBride, 1994; Quantin et al., 2001). At pH below 7, protonated hydroxyl groups generate a positive charge on soil mineral surfaces, which induces adsorption of negatively charged species such as phosphate groups or P-bearing organic molecules/colloids on mineral surfaces through formation of surface complexes (Buffle et al., 1989; Zak et al., 2004). However, when the pH rises to neutral or slightly basic conditions during reduction reactions, soil particles become electronegative. Hence, mineral surfaces and phosphate groups or/and negatively charged P-bearing organic molecules/colloids repel each other, limiting the complexation of these P compounds by soil mineral surfaces. Therefore, DP, including dissolved organic/inorganic P or colloidal P species, are released into soil solutions (Vandervoort et al., 2013; Liang et al., 2010). In this case, the ultimate sources of the dissolved P species released in RWs under anoxic conditions are not limited solely to soil Fe-oxyhydroxides, but extend to all mineral surface areas of the soil.

Another important aspect of P-release processes in RW soils is that these soils receive P-rich soil particles derived from adjacent cultivated fields through erosion and surface transport (Ockenden et al., 2014). It is commonly accepted that this soil particle input to RWs occurs mainly through overland flow processes developed during high-magnitude rainfall events (Haygarth and Jarvis, 1999). In a study published in 2009, Stutter and coworkers evaluated the effects of sediment addition on dissolved P release

processes in vegetated buffer strips installed in riparian zones between cropland and watercourse. Using laboratory experiments, they showed that the addition of fine sediments into RW soils stimulated the release of inorganic DP in these soils, an effect they interpreted to be largely caused by biological processes (Stutter et al., 2009). However, these experiments were all carried out under aerobic conditions, which did not allow to evaluate whether the addition of P-rich sediments into RW soils also stimulated the release of DP during anoxic episodes undergone by these soils. This possibility is nevertheless worth considering given that RW soils (i) generally have greater microbial biomass and diversity than adjacent cultivated soils and (ii) contain bacteria used to reduce soil Fe-oxyhydroxides (Krutz et al., 2006, Pédrot et al, 2011; Dia et al., 2015). The encounter between bacteria accustomed to Fe-oxyhydroxides reduction and soil particles rich in P-bearing Fe-oxyhydroxides such as in RW soils might enhance the release of DP in these soils during installation of anoxic conditions.

Assessing the relative effects of Fe-oxyhydroxide dissolution, pH rise and sediment input on DP release during installation of anoxic conditions in RW soils is important for understanding further how RW zones control DP solubilization in agricultural landscapes. However, this assessment is difficult because these processes operate simultaneously as the soil becomes reduced. In this study, we conducted soil incubation experiments in order to separately study and quantify each of these processes. Two soils coming from two natural RW zones were selected for that purpose. These two soils differ each other in their organic matter (OM) content and also their inorganic P versus organic P ratio, thus allowing also investigating the effects of soil P speciation on DP release during soil reduction. The two RWs from which the incubated soil samples come from are located in a small agricultural catchment in western France (so-called Kervidy-Naizin catchment). Field monitoring data indicated that both zones released DP under anoxic conditions in natural field conditions, and the released DP was transferred to the stream network (Dupas et al., 2015c; Gu et al., 2017). The same field monitoring data also indicated that the P speciation of the released DP was different in the two soils. The soil with a high inorganic P/organic P ratio released mainly molybdate-reactive P (MRP), while the other soil with a low inorganic P/organic P ratio released mainly molybdate-unreactive P (MUP) (Gu et al., 2017). A sample of sediment eroded from the cultivated fields adjacent to these two RWs through overland flow was also collected and included in the experiments to evaluate the role of sediment inputs on DP

release in RW soil under anoxic conditions.

Thus, the objectives of this study are to use laboratory incubations to: i) estimate the relative effects of the pH rise and Fe-oxyhydroxide dissolution processes on DP release in RW soils under anoxic conditions; ii) assess the control of soil P speciation on the concentration and speciation of the DP released, and finally iii) evaluate in which respect the inputs of P-rich sediments from upland cultivated fields affect the overall DP release process in RW soils under anoxic conditions.

5.2 Materials and methods

5.2.1 Sampling sites and soil preparation

The soil samples come from two RWs (wetland A and B) of the Kervidy-Naizin catchment, a small-scale (5 km²) agricultural headwater catchment, located in Brittany, western France (48.012° N, 2.835° W). Belonging to the Agrhys environmental research observatory (http://www6.inra.fr/ore_agrhys_eng), this catchment has been intensively investigated since 1993 so that its hydrological, pedological and geochemical characteristics are well constrained (Mérot et al., 1995; Lambert et al., 2013; Aubert et al., 2014). The substrate lithology of this catchment consists of impervious Brioverian schists capped by 2-30m of unconsolidated weathered materials, in which a shallow aquifer develops. The upland domains are well-drained, whereas the valley bottoms are hydromorphic, leading to the development of RWs at the interface between cultivated fields and the stream network. Soils from wetlands A and B (soils A and B, respectively) are silt loam, classified as Luvisols.

Fresh soil cores (ca. 1.5 kg) from the surface layer (0-15 cm) were sampled in the two RWs with a 75-mm diameter auger in January 2014. Sampling sites were within 1 m² of the lysimeters investigated by Dupas et al. (2015) and Gu et al. (2017) for in-situ monitoring of P concentrations in the soil solution (WetDown-A and WetDown-B sites, respectively, in their studies). Meanwhile, eroded sediments (soil S, ca. 1.0 kg) re-deposited at the interface between wetland B and the upland cultivated field were collected manually with a PVC cup. Soils and sediments were transferred to the laboratory in plastic bags and air-dried in a clean room (20 days, at 25±2 °C). They were sieved to <2 mm after removing visible roots and stones, then stored in a refrigerator at 4 °C before use.

5.2.2 Experimental setup

The following experiments were carried out. Firstly, soil A and B were incubated anaerobically under a nitrogen stream, without pH buffer, to simulate natural anoxic conditions observed in these two soils. Secondly, these two soils were incubated aerobically, but in applying a pH rise equivalent to that recorded during their anaerobic incubations; these second experiments were used to quantify the effect of the pH rise (from 6 to 7.6) on the amount of DP released during the anaerobic incubation (and by difference the effect of reductive dissolution of Fe-oxyhydroxides alone). Finally, in a third series of experiments, sediment S was incubated anaerobically, first alone and secondly mixed in equal proportion (50% each in weight of dry soil/sediment) with soil B (referred hereafter as sample “M”); this third series of experiments was used to evaluate whether the sediment released more DP upon reduction when incorporated into RW soils, as would be expected by the accelerating role of wetland Fe-reduction bacteria on the reduction of soil Fe-oxyhydroxides.

The incubation system and procedures were essentially similar to those developed by Grybos et al. (2009), which investigated similar research questions but focused on dissolved organic carbon (DOC) and neglecting P. Suspensions were prepared at 1:20 soil dry weight/solution ratio, with a solution consisting of 0.48, 0.85 and 0.1 mol l⁻¹ of NO₃⁻, Cl⁻, and SO₄²⁻, respectively, to mimic the anion composition of the shallow groundwater in RWs during the water-saturation period. The suspensions (50 g dried soil or soil/sediment mixture in 1 L solution in this study) were placed in an air-tight, 1-litre batch Prelude reactor (Guerin, Biolafite) in a water bath with temperature controlled at 20±2 °C. The suspension was continuously stirred at a speed of 150 rpm, and simultaneously supplied with a continuous stream of N₂ gas via an automatic gas-injection system at 0.5 l min⁻¹ for the first 2 h and then at 0.15 l min⁻¹. For the aerobic incubations simulating the pH changes alone, the pH was adjusted by the addition of 1.0 M NaOH, with the rest of the incubation parameters (except for N₂ addition) being similar to those used during anaerobic incubations.

Aliquots (ca. 20 ml) of soil suspension were sampled with a sterile syringe into flasks prefilled with argon gas (for anaerobic incubations only) twice a day in the first 3 days of incubation, and then once a day during the remaining 10-days incubation. pH and Eh were immediately measured with combined electrodes (Malter Pt 4805 DXK-S8/225 and Malter HA 405-DPA-SC-S8/225, respectively). Flasks were then centrifuged for 10

min at 3340 rpm. The supernatant was collected using a sterile syringe equipped with a soft PVC tube at the mouth, allowing its transfer without contacting the air. The supernatant was then filtrated using pre-washed cellulose acetate filters of 0.45 μm and 0.2 μm pore size for P and for DOC, anions (nitrate, sulfate), and Fe(II) analyses, respectively. Aliquots for P, DOC, and Fe(II) analyses were immediately acidified with a diluted sulfuric acid, and the samples for anions analysis were directly placed in argon-filled analysis tubes. The quick sampling (few minutes), together the acidification of filtrated aliquots by sulfuric acid and the short storage time of subsamples prevented the redox sensitive parameter (Fe(II)) and precipitation of Fe-hydroxides prior to measurement.

5.2.3 Analyses

5.2.3.1 Soil and sediment analyses

Portions of the air-dried soils and sediment samples were analyzed at the INRA laboratory, Arras, France, for particle size fractions (NF X 31-107), OM/nitrogen/carbon contents (NF ISO 13878, NF ISO 10694), pH in water (1:5 v:v water extraction NF ISO 10390), extractable P (P-Oxalate, NF X 31-161; P-Dyer, NF X 31-160; P-Olsen, NF ISO 11263), Si/Al/Fe (ICP-AES after extraction with ammonium oxalate and oxalic acid, according to Tamm, 1922), and total P (ICP-AES after total solubilization with hydrofluoric and perchloric acid, NF X 31-147) contents.

5.2.3.2 Solution analyses

MRP concentrations of filtrates were determined using the standard colorimetric method of Murphy and Riley (1962). The same method was used for total P (TP), but after digestion of filtrates in acidic potassium persulfate. The precision of MRP and TP measurements was ± 4 and $\pm 13 \mu\text{g l}^{-1}$, respectively. MUP was calculated as TP minus MRP.

Nitrate and sulfate concentrations were analyzed by ionic chromatography (Dionex, DX120), with a precision of $\pm 4\%$. DOC concentrations were analyzed with a total organic analyzer (Shimadzu TOC-5050A), with a precision of $\pm 5\%$ (using potassium hydrogen phthalate as the standard solution). Specific Ultraviolet Absorbance (SUVA) values ($\text{mg l}^{-1} \text{m}^{-1}$, $\pm 5\%$ precision) were calculated by dividing ultraviolet (UV) absorbance at 254 nm normalized by the DOC concentration. SUVA values were then converted into the aromaticity percentage of the OM using the equation of Weishaar et

al. (2003) (aromaticity= $6.52 \times \text{SUVA} + 3.63$). UV absorbance measurements were performed on a Lambda 25 (PerkinElmer) spectrophotometer using deionized water as a blank. Fe(II) concentrations were determined using the 1,10 phenanthroline colorimetric method (AFNOR NF T90-017, 1997), with a precision of $\pm 5\%$. The same method was used for total Fe, but after reduction of the samples by the ascorbic acid solution.

5.3 Results

5.3.1 Soil/sediment composition

As shown in **Table 5.1**, soil A and sediment S have similar concentrations of clay, loam, and silt (24, 60 and 16% vs. 22, 66 and 12%, respectively), whereas soil B has comparatively higher clay (35%) and lower silt (4%) concentrations. Soil B also distinguishes from soil A and sediment S by having higher organic carbon, total nitrogen, and OM contents, as well as higher C:N ratio. pH values of the three soil/sediment samples are slightly acidic in the range 5.9 – 6.5. Soil A and sediment S held comparable total P content, with concentrations (0.93-1.07 g/kg) about two times higher than in soil B (0.46 g/kg). Extractable P concentrations, including P-oxalate, P-Dyer and P-Olsen, all decrease in the order of soil A > sediment S > soil B, so do their proportions relative to total P contents. Noticeably, all extractable P concentrations are by one order of magnitude higher in soil A than those measured in soil B. This suggests a major difference of the P speciation in these two soil samples, P in soil A comprising of more easily exchangeable P fraction than P in soil B. Otherwise, the oxalate extractable Si and Al contents are in the order of sediment S > soil B > soil A, while the oxalate extractable Fe contents are similar in sediment S and soil B that are both higher than in soil A.

Table 5.1. Main properties of the studied soil and sediment samples.

Soil property	Soil A	Soil B	Sediment S
Clay (<2 µm) (g/kg)	242	354	222
Fine loam (2-50 µm) (g/kg)	430	456	408
Coarse loam (20-50 µm) (g/kg)	166	147	249
Fine silt (50-200 µm) (g/kg)	87	29	72
Coarse silt (200-2000 µm) (g/kg)	75	14	49
Organic carbon (g C/kg)	37.6	89.1	34.0
Total nitrogen (g N/kg)	3.38	6.35	2.98
C:N ratio	11.1	14.0	11.4
Organic matter (g/kg)	65.0	154.0	58.8
pH	6.08	5.92	6.53
P-Oxalate (g P/kg)	0.20	0.01	0.06
P-Dyer (g P/kg)	0.72	0.03	0.25
P-Olsen (g P/kg)	0.32	0.01	0.11
Si-Oxalate (g Si/kg)	0.25	0.28	0.33
Al-Oxalate (g Al/kg)	1.16	1.56	1.89
Fe-Oxalate (g Fe/kg)	6.01	10.20	9.46
Total P (g P/kg)	1.07	0.46	0.93
P_{Oxal}/P_{tot} (%)	19.1	2.6	6.0
P_{Dyer}/P_{tot} (%)	29.5	2.7	11.5
P_{Olsen}/P_{tot} (%)	5.8	1.5	3.4

5.3.2 Anaerobic incubations of RW soils

During soil A and B anaerobic incubations, the pH values increased rapidly in the first ca. 100 h (75 h for soil A), and then increased more slowly to reach an equilibrium at values in between 7.5 and 7.6 at the end of the incubation (**Figures 5.1-2, a**). Eh values evolved similarly but the other way, decreasing first rapidly from approximately 300 mv to 0 during the first 70-80 h incubation, then more slowly to reach equilibrium values in between -150 and -200 mv at the end of the incubation.

In both soils, NO_3^- concentrations decreased rapidly from the initial concentration of 30 mg l⁻¹ to 0 after ca. 75h of incubation (**Figures 5.1-2, b**). SO_4^{2-} concentrations remained constant throughout the two incubations (**Figures 5.1-2, b**), unlike DOC concentrations which increased all along the incubations. The DOC release was

stronger in soil B than in soils A, as indicated by the much higher final DOC concentrations recovered in the corresponding incubations (149 and 78 mg l⁻¹ for soil B and A, respectively). The aromaticity of the released DOC was low at the beginning of the incubations then increasing (more slowly in soil A than in soil B) to reach comparable equilibrium values of 35.1±1.8 and 31.8±1.4% for soil A and B, respectively (**Figure 5.3**).

The release Fe(II) was negligible before the NO₃⁻ concentrations reached to 0, as indicated by the very low concentrations of Fe(II) recorded during that part of the incubations (**Figures 5.1-2, c**). Then, Fe(II) concentrations started to increase and continued increasing to the end of the incubations with no equilibrium concentrations reached. The amount of Fe(II) release was much stronger in soil B than in soil A, as indicated by the much higher final Fe(II) concentration recorded in the former soil compared to the latter (9.7 vs. 2.6 mg l⁻¹, respectively) (**Figures 5.1-2, c**). In both incubations, ca.100% of the Fe released in solution was in the form of Fe(II).

Similarly to Fe(II) concentrations, MRP and TP concentrations continuously increased during anaerobic incubations of soils A and B, without reaching any equilibrium value. Interestingly, the releases slightly accelerated after the first 50h when NO₃⁻ concentrations reached to 0 and Fe(II) concentration started to increase, evidencing a close linkage between the TP and Fe(II) release processes (**Figures 5.1-2, b-c; S1**). This is suggested further by the strong positive correlations existing between Fe(II), TP and MRP concentrations in soils A and B ($r^2 > 0.849$, **Figure S2**), except for MRP concentrations in soil B whose very low values resulted in a poor correlation with Fe(II) concentrations ($r^2 = 0.277$).

Contrary to DOC and Fe(II) concentrations, both MRP and TP concentrations were higher in soil A than in soil B. More importantly, the speciation of the TP released was very different in soils A and B incubations, the released TP being dominantly of MRP in soil A (mean = 75%) as against being dominantly of MUP in soil B (mean = 71%) (**Figures 5.1-2, c**).

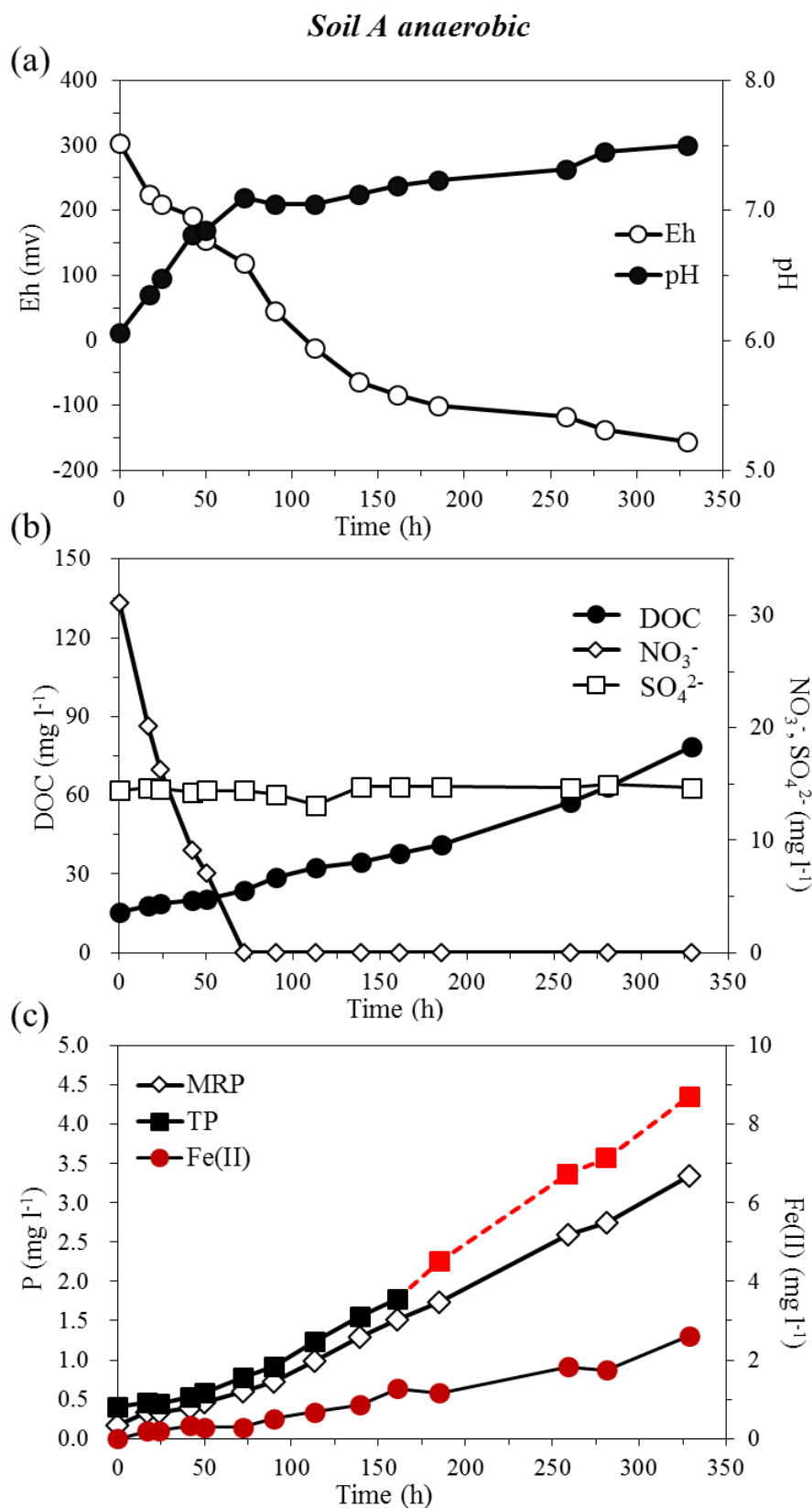


Figure 5.1. Time-variation of parameters during anaerobic incubation of soil A. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentrations. Note that TP concentrations for samples after 200h are not measured concentrations, but concentrations calculated using a constant TP/MRP ratio of 1.3.

Soil B anaerobic

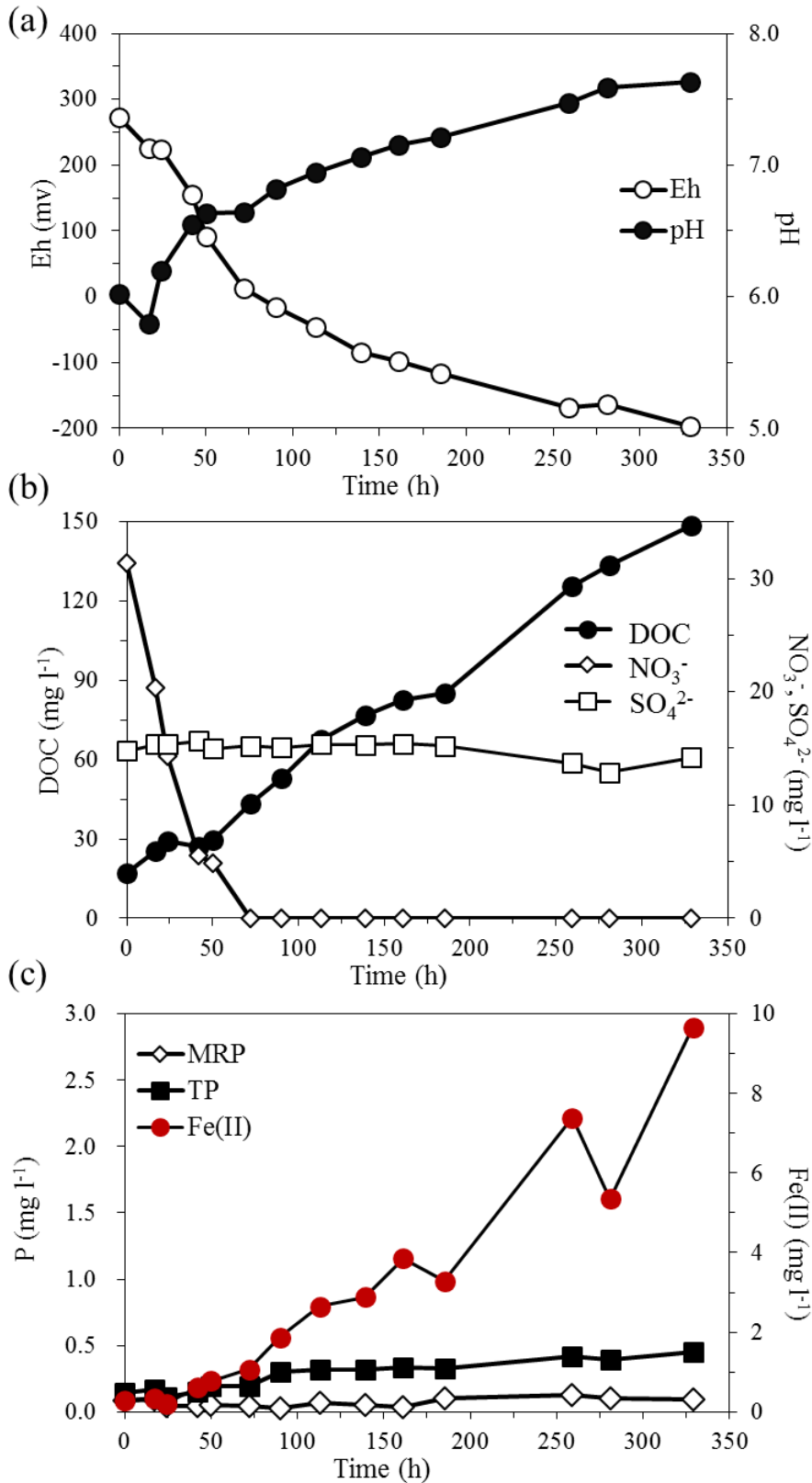


Figure 5.2. Time-variation of parameters during anaerobic incubation of soil B. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentration.

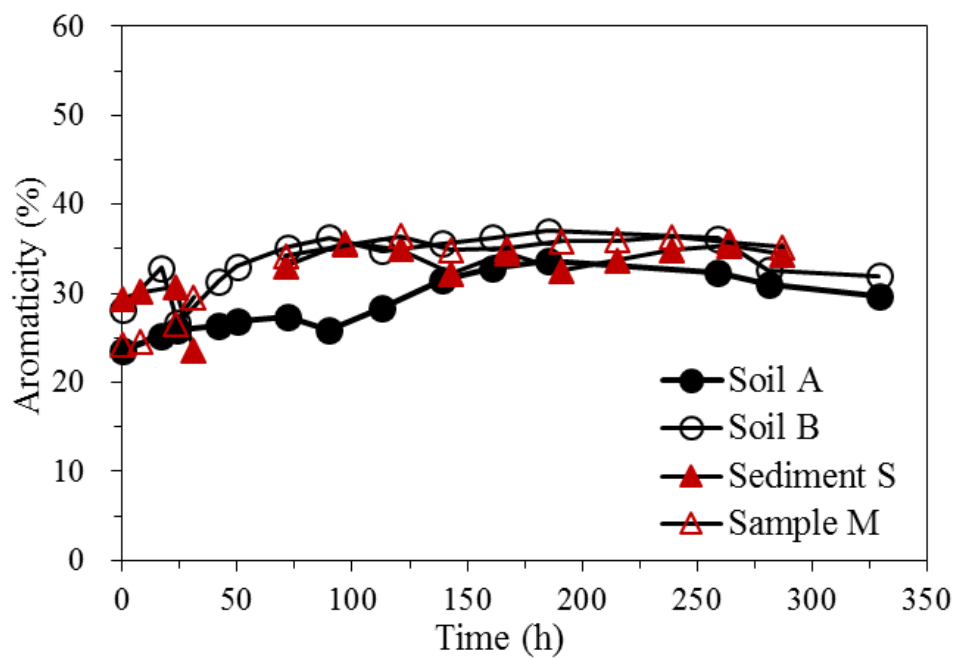


Figure 5.3. Time-variation of aromaticity of released dissolved organic matter during anaerobic incubations of studied soils and sediments.

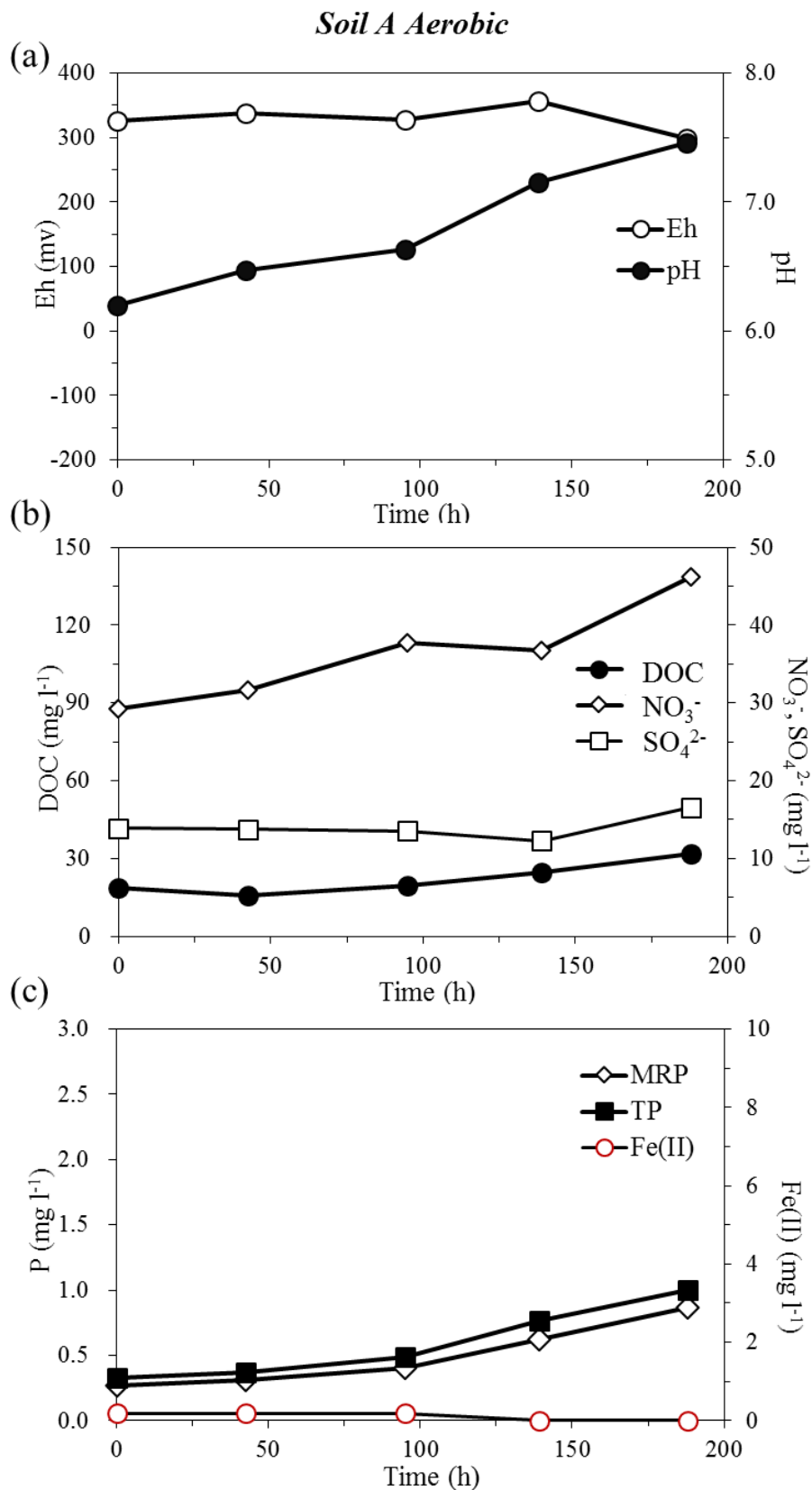


Figure 5.4. Time-variation of parameters during aerobic incubation of soil A. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentrations.

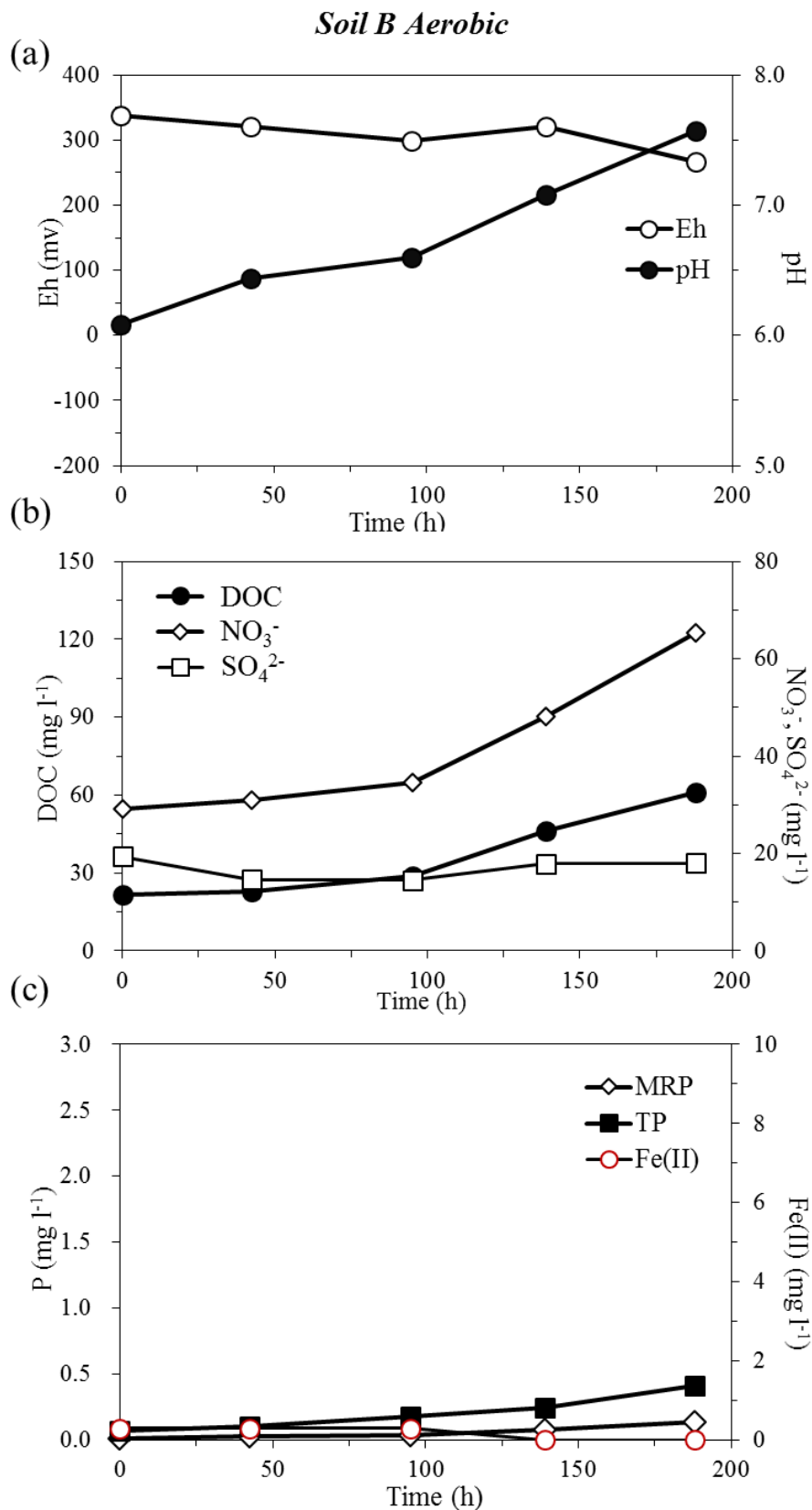


Figure 5.5. Time-variation of parameters during aerobic incubation of soil B. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentrations.

5.3.3 Aerobic incubations of RW soils

Eh values and SO_4^{2-} concentrations remained constant during the 200h of aerobic incubation of RW soils A and B at increasing pH (from 6.0 to 7.5-7.6) (**Figures 5.4-5, a-b**). In contrast with the corresponding anaerobic incubations, NO_3^- concentrations increased from the initial value of ca. 30 mg l^{-1} up to 46 and 65 mg l^{-1} for soils A and B, respectively, from the beginning to the end of the incubations (**Figures 5.4-5, b**). As in the anaerobic incubations, DOC concentrations turned out to increase during the aerobic incubations at variable pH (from ca. 20 mg l^{-1} to 32 and 61 mg l^{-1} in soil A and B, respectively), but the final concentrations were by a factor of ca. 2.5 lower than in the corresponding anaerobic incubations (**Figures 5.1-2, 5.4-5, b**).

Concentration in Fe(II) remained very low in the two aerobic incubations (below 0.29 mg l^{-1}) (**Figures 5.4-5, c**). On the contrary, both MRP and TP concentrations increased during these experiments, in line with the pH increase. The increases in TP and MRP were however much less pronounced than in the corresponding anaerobic incubations, particularly for soil A that the final TP concentration was by a factor of 4 lower than in the anaerobic incubation (**Figures 5.1c, 5.4c**). Similar with the corresponding anaerobic incubations, the TP speciation was very different between soils A and B, with the released TP being dominantly of MRP in soil A (mean = 83%), as against being dominantly of MUP in soil B (mean= 75%) (**Figures 5.4-5, c**). Note that DOC, NO_3^- , MRP and TP concentrations did not reach equilibrium values after the 200 h of aerobic incubations imposed in our experiments.

5.3.4 Anaerobic incubations of sediment with and without RW soil addition

Anaerobic incubation of sediment S alone and mixed with soil B (sample M) yielded Eh, pH, NO_3^- , SO_4^{2-} , DOC and Fe(II) release curves (**Figures 5.6-7**) much alike those observed during anaerobic incubations of soils A and B (see **Figures 5.1-2**). In those parameters, Fe(II) and DOC concentrations continued rising from the beginning to the end of the incubations (**Figures 5.6-7, b-c**). Quite clearly, sample M released more Fe(II) and DOC compared to the incubation of sediment S alone (**Figures 5.6, b-c**), which was not unexpected given the high DOC and Fe(II) release potential of soil B under anaerobic conditions (see **Figures 5.2, a-b**). Interestingly, the final Fe(II) and DOC concentrations recorded at the end of the incubation of sample M were close to half of

the values found at the end of the incubation of soil B alone (5.3 and 94 mg l⁻¹ respectively for sample M vs. 9.7 and 150 mg l⁻¹ respectively for soil B). Similar with those of soils A and B samples, the aromaticity of the released DOC was low at the beginning of incubations of sediment S and sample M, then increasing to reach equilibrium values (**Figure 5.3**). Final equilibrium values were 34.2±1.2% and 35.7±0.6%, for sediment S and sample M, respectively.

Comparable to results from the anaerobic incubations of RW soils A and B, the anaerobic incubations of sediment S and sample M resulted in a progressive released of TP and MRP (**Figures 5.6-7, c**), which turned out to be quite linearly correlated with the release of Fe(II) ($r^2 > 0.9116$, **Figure S2**). Final TP concentrations obtained during sediment S and sample M incubations were not very different and were quite comparable to the values found during incubation of soil B alone. None of the two incubations performed with sediment S yielded extreme TP and MRP concentrations as those of soil A. Regarding the speciation of the TP released, MRP was the dominant fraction in TP during the incubation of sediment S alone (mean=63%) (**Figure 5.6c**). During incubation of sample M, the proportion of MRP in the TP released was averaged 47%, which was almost exactly at mid distance of the values found during anaerobic incubations alone of sediment S (63%) and soil B (29%) (**Figure 5.7c**).

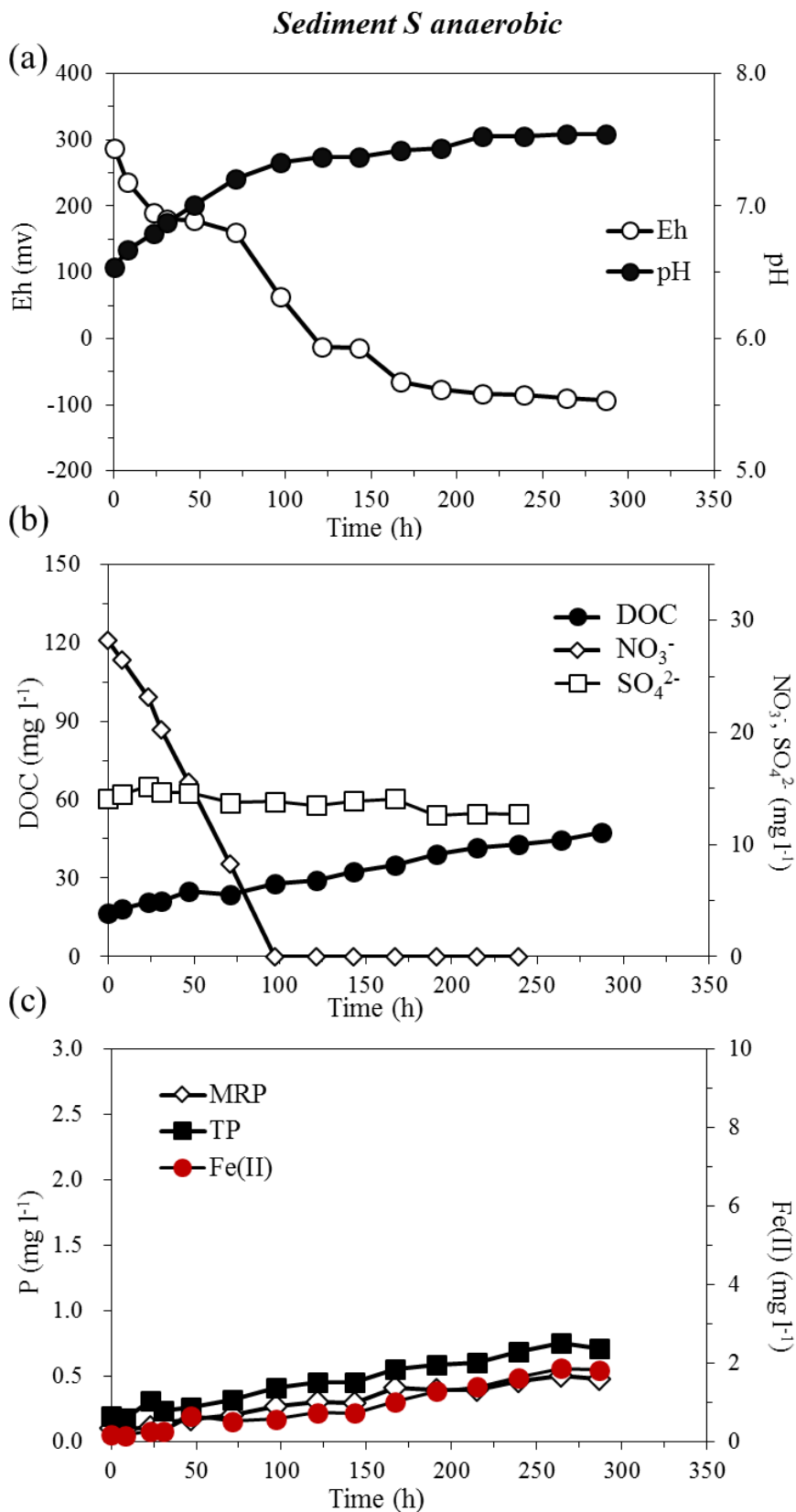


Figure 5.6. Time-variation of parameters during anaerobic incubation of sediment S. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentration.

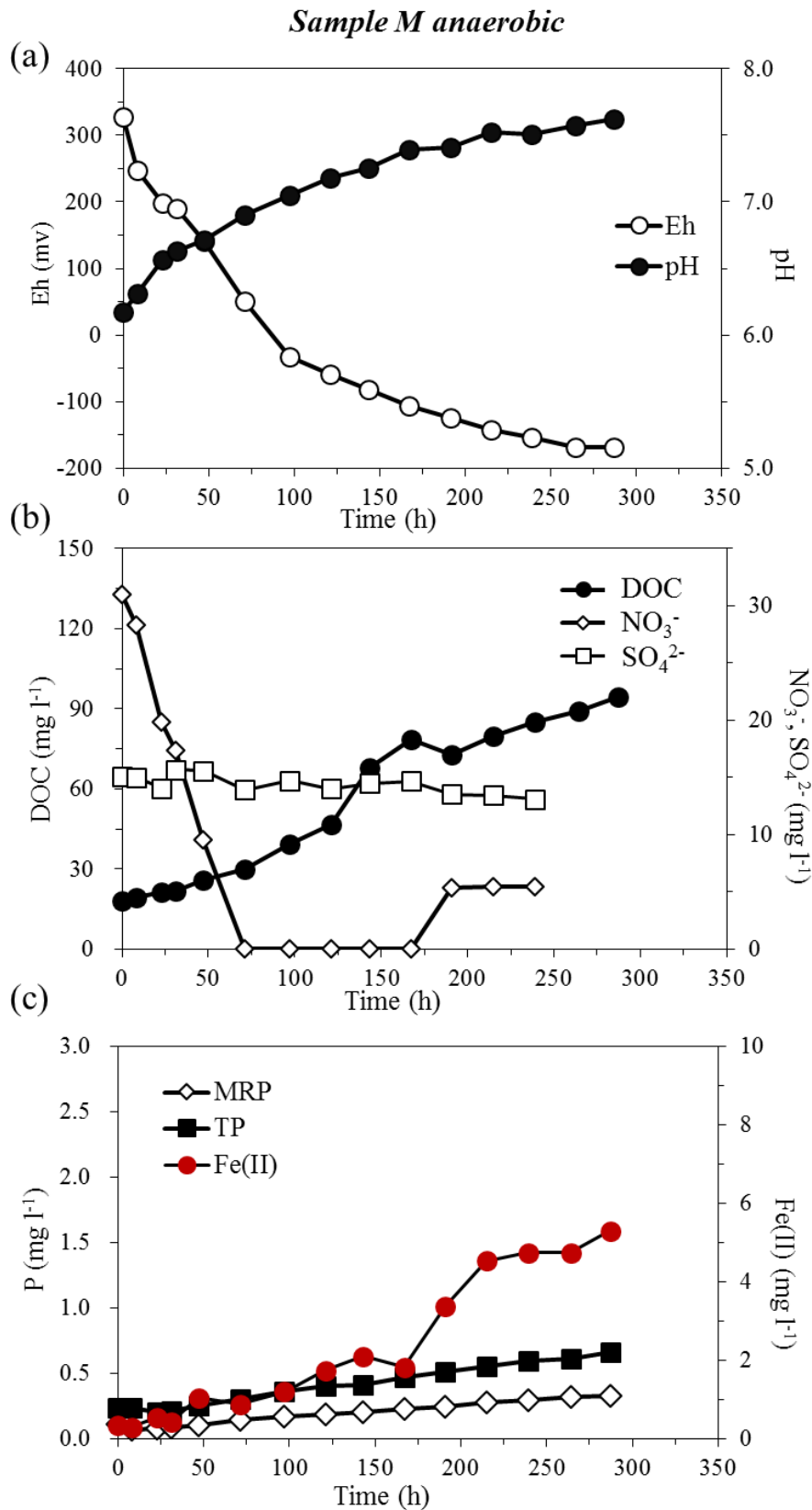


Figure 5.7. Time-variation of parameters during anaerobic incubation of soil/sediment mixed sample M. (a): Eh and pH; (b): dissolved organic carbon (DOC), NO₃⁻ and SO₄²⁻ concentrations; (c): MRP, TP, and Fe(II) concentration.

5.4 Discussion

The experimental approach used here demonstrates that DP can be mobilized under anoxic conditions in RW soils, as previously shown by several field studies (Dupas et al., 2015c; Gu et al., 2017; Knorr et al., 2013; SurrIDGE et al., 2012). In anaerobic incubations, DP release was concomitant with the release of Fe(II) in the experimental soil solutions, a fact already noted during surveys of these two soils under natural, field anoxic conditions (Dupas et al., 2015c; Gu et al., 2017). The release of Fe(II) indicate that a process of reductive dissolution of Fe-oxyhydroxides that started only after the complete nitrate reduction by soil bacteria (**Figures 5.1-2, 5.6-7, b-c**), which is consistent with the order of electron acceptors used by bacteria to oxidize OM in anaerobic soils (Christensen et al., 2000; Stumm and Sulzberger, 1992). Moreover, the Eh–pH values recorded during anaerobic incubations in this research (–100 mV; pH=7.5/7.6, after 325h) correspond to the critical Eh–pH for Fe reduction in soils (Gotoh and Patrick, 1974; Patrick and Henderson, 1981; Patrick and Jugsujinda, 1992). The observed DOC release and pH increase during reduction reactions were already observed during previous incubations carried out on the same soils for the study of DOM and trace metal release dynamics under anoxic conditions (Grybos et al., 2007, 2009).

5.4.1 Controls of soil properties on concentration and speciation of released DP

We found that the amount and speciation of DP released from RW soils under anoxic conditions can be of huge difference from one soil to another, which is likely under the control of the differences in P content and speciation between these soils. The release of more TP with higher proportion of MRP by soil A is indeed correlated with the higher total P and extractable inorganic P (Oxalate-P, Dyer-P, and Olsen-P) contents in this soil than in soil B. Previous studies have suggested that the differences in P content and speciation observed between soils A and B likely resulted from a combination of factors, including differences in P inputs (annual rate and fertilizer type) before converted into buffer zones, differences in vegetation covers, and differences in soil OM mineralization rates (Dupas et al., 2015c; Gu et al., 2017). Moreover, the fact that soil B released mainly MUP in TP appears consistent with the high soil OM and organic P contents of RW soils in this part of the Kervidy-Naizn catchment (Dupas et al., 2015c;

Gu et al., 2017). As suggested by Gu et al. (2017), the occurrence of prolonged saturation periods in Kervidy-Naizin wetland B could have decreased the mineralization rate of soil OM in this wetland, thereby promoting the accumulation of organic P in soil, and thus explaining why soil B releases essentially MUP.

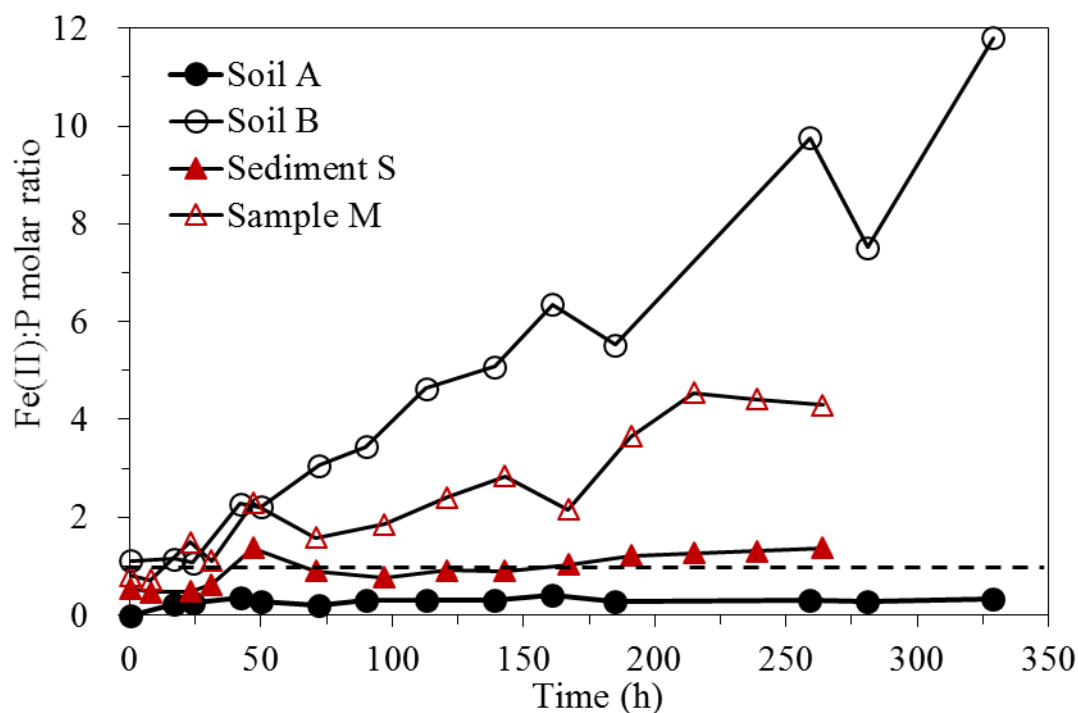


Figure 5.8. Time-variation of Fe:P molar ratios in anaerobic incubations of soils studied. “P” in the Fe(II):P ratio is the TP in the solution. Dash line refers to the Fe:P ratio of 1.0.

The two soils differed also in terms of the Fe:P molar ratios recovered in the soil solutions. The Fe:P ratios of reduced soil solutions is an important factor to evaluate the risk if the DP released could be transferred to watercourses or not, because it determines the capacity of Fe to immobilize DP once re-oxidized at the soil/surface water interface (Zak et al., 2004; Surridge et al., 2007, 2012). Fe:P ratios in soil A were low, being below or close to 1.0 during the entire anaerobic incubation, whereas ratios in soil B were higher, reaching to 11.8 at the end of the incubation (**Figure 5.8**). The higher Fe:P ratios recorded by soil B are clearly due in part to the lower amount of TP released by this soil. However, they could also be due to the presence of higher amount of reducible Fe in this soil than in soil A. Soil Fe-oxyhydroxides mobilized by the oxalate solutions are regarded to consist of amorphous and poorly crystalline Fe phases, which are known as more susceptible to bioreduction reactions under anoxic conditions (Lovley and

Phillips, 1986; Ehrlich 1990), resulting in the release of associated P (the P-Oxalate). Thus, the differences in Fe:P ratios between soils A and B solutions could be related to the higher proportion of poorly crystallize, easily reducible Fe oxides in the latter soil, as manifested by its higher Fe-Oxalate content (**Table 5.1**). In their field study of German peatlands, Zak et al. (2004) proposed that a Fe:P ratio of 3.0 could be used as a threshold to evaluate the risk that the MRP released in anaerobic pore water could be exported to stream and river water. According to their results, a Fe:P ratio above 3.0 would decrease MRP concentrations to below 1 mmol l⁻¹ when soil water enters aerobic conditions at the soil/surface water interface. On the contrary, a Fe:P ratio lower than 3.0 would result in the export of MRP to the adjacent surface waters, because the Fe re-oxidation process could not precipitate sufficient amount of Fe-oxyhydroxides to immobilize all the DP present in the anoxic soil water. Thus, Fe:P ratios in the present study suggested that soil A poses a greater threat than soil B with regards to the export of DP from RW soils to adjacent surface waters under anoxic conditions. Interestingly, field monitoring of Kervidy-Naizin soil solutions and stream water has established that most of the DP conveyed by the stream at the outlet of this catchment comes from wetland A with little contribution from wetland B (Gu et al., 2017).

5.4.2 Assessing the respective roles of reductive dissolution of Fe-oxyhydroxides and pH rise

The DP release under anoxic conditions was not only simultaneous of the reductive dissolution of Fe-oxyhydroxides but also concomitant with a marked rises of the soil solution pH. As pointed out earlier, an increase in pH during anaerobic incubation of Kervidy-Naizin RW soils has already been observed and widely discussed in previous studies (Grybos et al., 2007; 2009). The increase in pH values during reduction of such acidic soils is commonly interpreted as a by production of the reduction of NO₃⁻ and Fe(III) by bacteria, given that both these two reduction reactions consume protons (McBride, 1994; Quantin et al., 2001). The non-equilibrated Fe(II) concentrations could explain the continuous increase in pH and decrease in Eh during anaerobic incubations.

What are the proportions of the released DP that are respectively due to the processes of soil Fe-oxyhydroxide reduction and pH rise? This can be assessed by comparing the results of the aerobic (pH rise process alone) and anaerobic (combination of both processes) incubations of RW soils A and B. For that purpose, we calculated the TP

released during each incubation, avoiding the amount released immediately after the preparation of the soil suspension, this first DP release pulse being due to the rewetting of the dry soil (Turner and Haygarth, 2001; Blackwell et al., 2010). As shown in **Table 5.2**, the TP corresponded to concentrations of 3.95 and 0.34 mg l⁻¹, respectively, in the anaerobic incubations of soils A and B, and of 0.67 and 0.30 mg l⁻¹, respectively, in the aerobic incubations of corresponding soils with controlled pH rise. Thus, the mass balance calculation indicates that the proportion of DP release due to pH rise corresponded to 17 and 88% of the TP released during anaerobic incubations of soils A and B, respectively. This suggested that the DP release in soil A was mainly controlled by the reductive dissolution of Fe-oxyhydroxides, whereas in soil B being controlled mainly by the pH rise. The process of soil Fe-oxyhydroxide reduction dominates in soil A as a major DP donor for the soil solution, indicating that soil Fe-oxyhydroxides in this soil are a major host phase for P. On the contrary, soil Fe-oxyhydroxides, though more abundant in soil B than in soil A, seem to host only a small amount of P because their reductive dissolution released very little DP. We hypothesize that this difference in controlling mechanisms of DP release between the two studied soils originates from the difference in the P content of the soil Fe-oxyhydroxides: the Fe-oxyhydroxides in soil A being rich in P, but not those of soil B. This hypothesis strongly fits with the Fe- and P-oxalate data obtained for these two soils. Indeed, while soil A shows a Fe-oxalate:P-oxalate ratio of ca. 30, this ratio is >1000 in soil B. As already mentioned earlier, the soil Fe-oxyhydroxides quantified by the oxalate solution are assumed to be the most poorly crystallized of all soil Fe-oxyhydroxides, which are also the most easily reducible by bacteria. The Fe-oxalate:P-oxalate ratio data yields to the same conclusion that Fe-oxyhydroxides in soil A contain much richer P than those in soil B.

Table 5.2. Calculated amounts of total dissolved P (TP) and dissolved organic carbon (DOC) released in anaerobic and aerobic incubations of soils A and B.

Soils	Incubations	Concentration (mg l ⁻¹)			Released TP (mg l ⁻¹)	Released TP (%) ^a	Released DOC (%) ^a
		Fe(II)	Initial TP	Final TP			
A	Anaerobic	2.62	0.40	4.35	3.95	100	100
	Aerobic	0	0.33	1.00	0.67	17.0	36.6
B	Anaerobic	9.66	0.11	0.45	0.34	100	100
	Aerobic	0	0.11	0.41	0.30	88.2	37.8

^a Calculated with reference to the anaerobic incubation experiments (anaerobic incubation = 100% of released TP).

^b Calculated DOC release percentages with the same method as TP (anaerobic incubation = 100% of released DOC).

This difference in the capacity of soil Fe-oxyhydroxides to accommodate P between soil A and soil B could be also a consequence of the different P speciation exhibited by these two soils. As shown in a recent study, the soil P speciation in soil B is predominantly organic (57%) (Gu et al., 2017). Unlike inorganic P which can be easily accommodated inside soil Fe-oxyhydroxide crystal lattices, larger organic P species only form surface complexes, leading to the difference in P content in Fe-oxyhydroxides of soils A and B. Otherwise, OM is generally negatively charged in soils and its sorption on soil minerals is highly reactive to pH changes (Allison, 1973). Thus, the predominance of organic P in soil B and its much higher OM content could explain why the linkage between DP release and pH rise is so strong in this soil.

Interestingly, the same mass-balance calculation for DOC indicated that >60% of its release was due to the reductive dissolution of Fe-oxyhydroxides in both soils. These proportions are different of those published earlier by Grybos et al. (2009), in which the pH rise dominated the DOC release under anoxic conditions. The difference in experimental temperatures (30°C in their study vs. 20°C in this study) and pH control protocols of aerobic incubations (direct incubation at pH 7.4 in their study vs. gradual increase of the pH from 6 to 7.6 in this study) could be at the origin of this difference. More importantly, the results of the present study indicated a decoupling of the control factors regarding TP and DOC release under anoxic conditions in soil B, suggesting a potential control of soil properties on the TP and DOC behavior under anoxic conditions.

Note that the mineralization of soil organic P could have contributed to release DP in

the present incubations. Microbes mineralize organic P molecules using $\text{NO}_3^-/\text{Fe(III)}$ -oxides (anaerobic conditions) or O_2 (aerobic conditions) as electron acceptors. The experimental setup used in this study could not avoid the potential contribution of soil organic P mineralization on the observed DP release processes. The quantified DP release due to pH rise in this study included in fact also the microbial mineralized P. Further studies are needed to fully evaluate the potential role of this third mechanism.

5.4.3 Influence of sediment deposition in RWs on DP release under anoxic conditions

We found that the eroded soil sediment (sediment S) had higher TP and extractable P concentrations than soils in their adjacent RWs (soil B). Moreover, the Fe-oxalate:P-oxalate ratio (157) suggests that the Fe-oxyhydroxides present in this sample are significant P carrier. These findings emphasized the importance of evaluating the fate of the P carried by this sediment when mixed with RW soils. In this study, we use soil B as the RW soil end-member, given its high easily reducible Fe content (and thus presumably its high Fe-reduction bacterial biomass) and low inherent DP release activity upon reduction. The results of anaerobic incubations of samples B (reference RW soil), S (eroded sediment) and M (mixture of B and S) were used to calculate the sediment-soil mixture effect on DP release under anoxic conditions (**Table 5.3**). The possible positive effect of mixing soil B and sediment S on DP release under anoxic conditions was evaluated by comparing the amount of DP released during incubation of sample M (taking into account of a possible enhance effect of soil B on sediment S release), with the amount of DP that should be theoretically released by simple addition the amounts released during their separate incubations (no enhance effect of soil B on sediment S release). Results showed that the sediment-soil mixture released more TP, DOC, and Fe(II) compared to what expected from the simple addition of the two samples, as established separately, the increases being of 16, 4 and 18%, for TP, DOC and Fe(II), respectively. Thus, the soil erosion not only is able to physically transfer the P-rich sediments into RWs but also seems to stimulate the release of DP from the input sediment component. As judged from the 16 and 18% increase in the DP and Fe(II) release, and only 4% increase in DOC, this stimulation most likely occurs because of the enhancement of the reductive dissolution of the sediment Fe-oxyhydroxides by the RW Fe-reduction bacteria.

After their long-term function to limit sediments and nutrients flux from field to watercourses, RWs have been reported to act as source zones of DP in agricultural catchments (Dorioz et al., 2006; Stutter et al., 2009; Dupas et al., 2015c; Gu et al., 2017). This could partially explain the re-increase of dissolved reactive P concentrations in some large rivers since the mid-1990s (Jarvie et al., 2017; Michalak et al., 2014), which has caused harmful algal blooms in receiving water bodies (Smith et al., 2015). Several biogeochemical processes have been identified to cause the DP release in soil solutions, such as drying-rewetting of soils, reductive dissolution of P-rich Fe/Mn oxides, etc (Blackwell et al., 2009; Bünemann et al., 2013; Dupas et al., 2015c; Gu et al., 2017). The present study suggested that the deposition of eroded soil sediments into RWs has the potential to enhance the DP release during the undergoing of identified biogeochemical processes. More future experiments are needed to unravel the detailed mechanisms behind this enhance effect.

Table 5.3. Comparison of total dissolved P (TP), dissolved organic carbon (DOC) and Fe(II) concentrations measured during anaerobic incubation of sample M, and concentrations anticipated from separated anaerobic incubations of soil B and sediment S.

Parameters		^a Soil B (329h)	^b Soil B (normalized) (287h)	^a Soil S (287h)	^c Sample M (calculated) (287h)	^d Sample M (measured) (287h)	^e Increased release (%)
TP	mg l ⁻¹	0.45	0.43	0.71	0.57	0.66	15.71
DOC	mg l ⁻¹	148.82	134.12	47.40	90.76	94.26	3.86
Fe(II)	mg l ⁻¹	9.66	7.13	1.83	4.48	5.30	18.34

^aConcentration measured at corresponding time during separate anaerobic incubations of sediment S and soil B.

^bConcentrations calculated using the concentration vs time linear regression equations shown in **Figure S3**.

^cConcentrations calculated of Soil B (normalized) (287h) and Soil S (287h) by mass balance equations, assuming no effect of the mixing on the TP, DOC and Fe(II).

^dConcentrations measured during anaerobic incubation of samples M.

^eCalculated as the difference between measured and calculated concentrations. Soil M (measured) to the Soil M (calculated).

5.5 Conclusions

The present study firstly confirmed the solubilization of DP in RW soils under anoxic conditions. The concentration and speciation of the released DP were found to be controlled by the soil chemical properties, especially the soil OM content and the soil P status. The Fe:P ratios in soil solutions from anaerobic incubations were also

controlled by the soil properties, especially the oxalate extractable P and Fe, thus controlling the risk of the released DP to be transferred to surface watercourses. This study also confirmed the two release mechanisms of DP under anoxic conditions: i) reductive dissolution of Fe-oxyhydroxides and ii) rise in pH. More importantly, the present study provided an approach to unravel the nature of the mechanisms involved in the mobilization of DP under anoxic conditions in RW soils. We found a difference in the controlling factors between soils: the DP release in the soils with high extractable P and low OM contents was mainly controlled by the first mechanism, whereas that released in soils with low extractable P but high OM contents was mainly controlled by the second mechanism. Moreover, under anoxic conditions, the input of eroded sediments into RW soils enhanced the release of DP, DOC, and Fe(II) compared to the components incubated separately.

Thus, an important step to reduce the DP transfer from RWs to watercourses is to limit the soil erosion from upland fields to avoid the deposition of P-rich sediments into RW soils, such as by optimizing the timing and type of irrigation. Management should be also made according to the basic soil properties, notably the values of Fe:P molar ratios in soil solutions (e.g. soil A), which could be used as an estimate of the risk of potential transfer the released DP to watercourses, and thus of potential eutrophication of surface waters. Management efforts, such as preventing P mobilization in upslope fields to avoid further enrichment of RWs by erosion or recovering legacy P from RWs by exporting biomass, should focus preferentially on RW soils whose Fe:P ratios upon release during reduction are the lowest, because these soils are at high risk with respect to the potential transfer of DP to watercourses. Management options to limit P transfer from agricultural catchments to rivers should not only focus on installing buffer zones to limit delivery of P to waters but should also limit soil erosion in cultivated fields.

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5.6 Supplementary materials

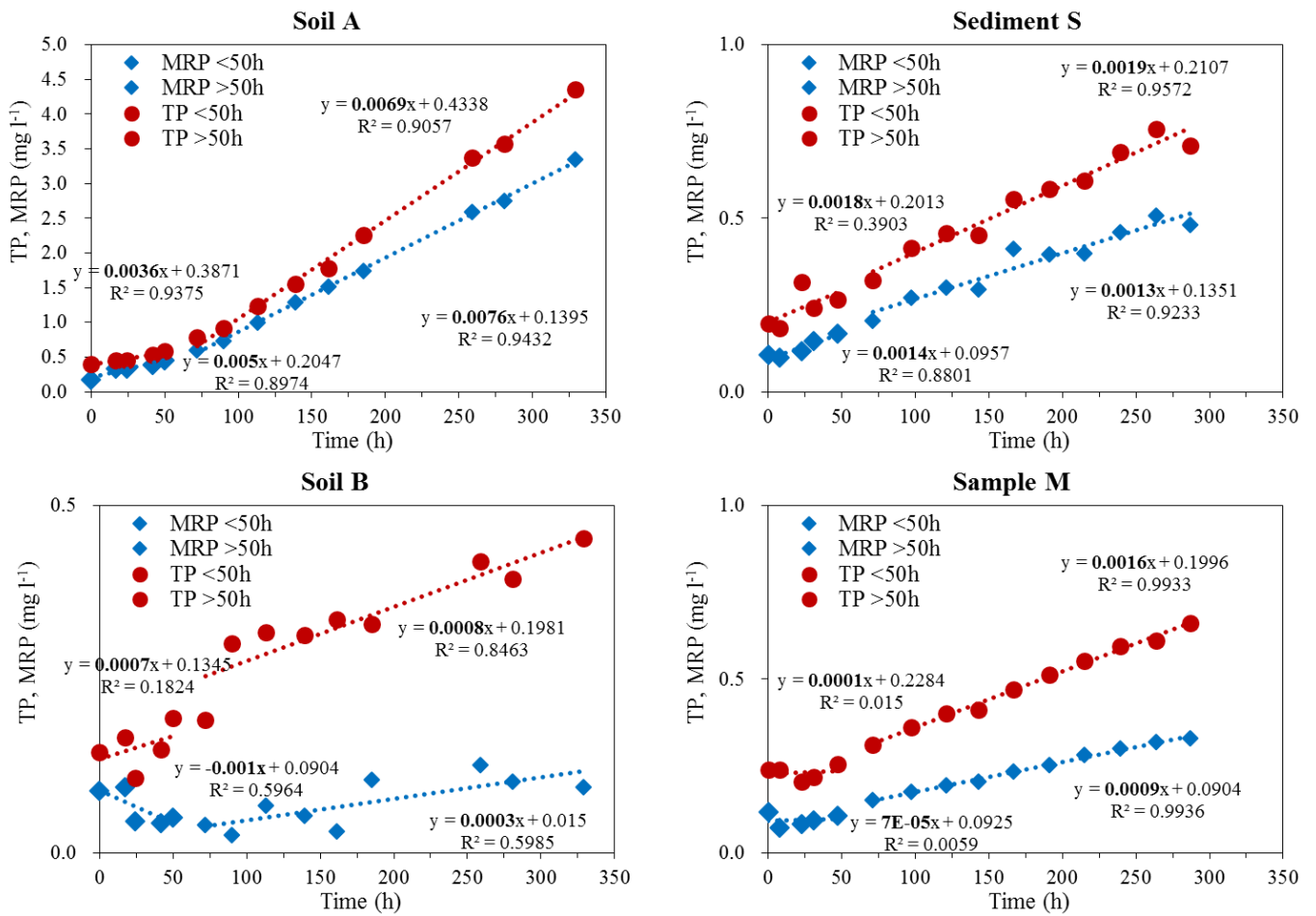


Figure S1. Comparison of release kinetics of MRP and TP during < 50h and > 50h anaerobic incubations of the four studied sample. All MRP and TP release speed were higher during > 50h incubation period, expect during anaerobic incubation of sediment S alone.

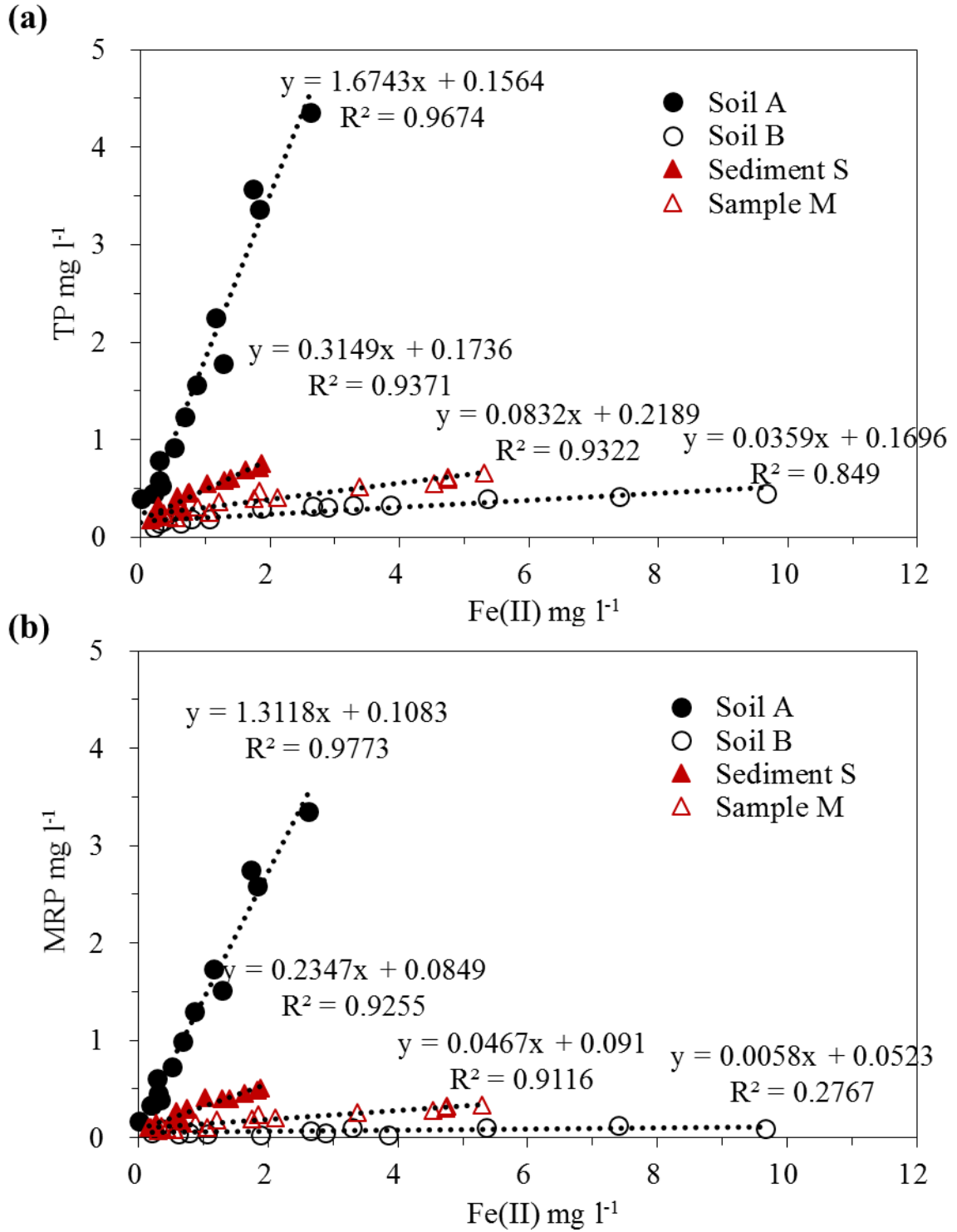


Figure S2. Linear correlations between (a) TP and Fe(II), (b) MRP and Fe(II) concentrations during anaerobic incubations of the four studied samples.

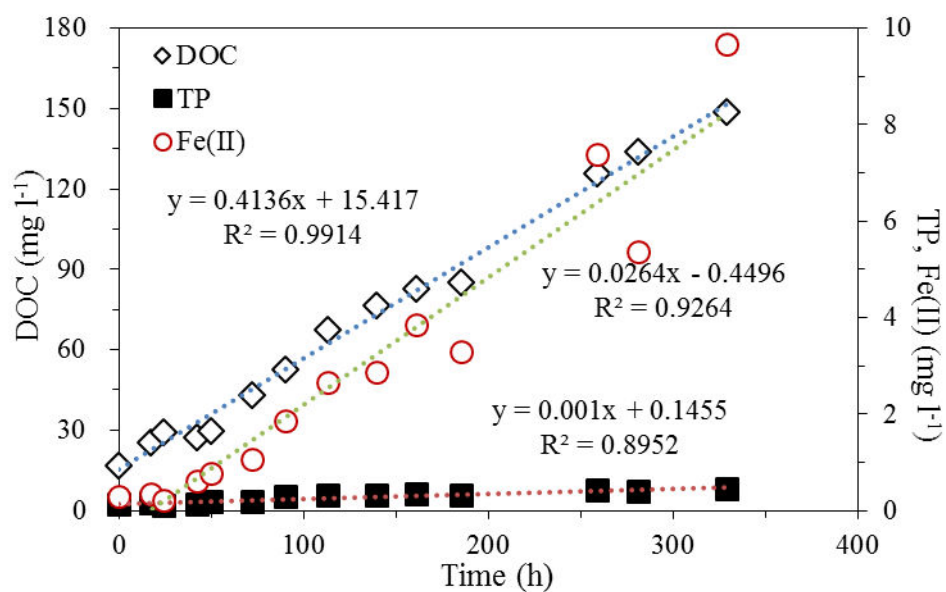


Figure S3. Time-series variation of dissolved organic carbon (DOC), total dissolved P (TP) and Fe(II) concentrations during anaerobic incubation of mixed soil-sediment sample M.

5.7 Conclusion of chapter 5 (laboratory simulation of reduction processes)

The anaerobic incubation results presented in this chapter confirmed the field monitoring data of Chapters 2 and 3 that there was a pulse release of dissolved P (DP) in riparian wetland (RW) soils under anoxic conditions. The reductive dissolution of Fe-oxyhydroxides upon installation of anoxic conditions, along with the concomitant rises in pH generated by reduction reactions are the two biologically driven mechanisms that cause this DP release. The comparison between the anaerobic and aerobic incubations of the same soil samples revealed that DP release is mainly controlled by the Fe-oxyhydroxides reductive dissolution mechanism in the soil with high extractable P and low organic matter content. In contrast, the DP release is mainly controlled by the pH rise mechanism in the soil with lower extractable P but higher organic matter content. This difference is likely due to the fact that Fe-oxyhydroxides in soil with low organic matter content and high extractable P are richer in P than Fe-oxyhydroxides in soil richer in organic matter. There appears also a decoupling of control factors with regards to DP and dissolved organic carbon (DOC) release under reducing conditions because the DOC release is mainly controlled by the Fe-oxyhydroxides reductive dissolution mechanism in both soil types. Finally, the release of DP, DOC, and Fe(II) by RW soils under anoxic conditions appears to be enhanced if sediments eroded from upland fields are added to the former. Thus the input of eroded soil sediments in RWs can not only enrich the RW soils in P but also make the RW soils more efficient to release P into soil solutions under reducing conditions. This chapter emphasized the importance to control the soil erosion in the cultivated field. It also suggested that a soil property test aimed at determining soil oxalate-extractable P and Fe concentrations could be useful in evaluating the transferring risk of the DP released under anoxic conditions to the stream.

Chapter 6

General conclusions

6.1 Recall of thesis objectives

This thesis is placed on the general perspective of limiting dissolved P (DP) transfer in agricultural landscapes, with the specific aims of i) testing further the hypothesis that riparian wetlands (RWs) are active DP release zones in these landscapes, and ii) unravelling the main mechanisms by which this DP (and colloidal P) release occurs, along with iii) identifying what are the main factors that ultimately trigger the release of DP (and colloidal) in RWs. In this thesis, DP release dynamics and mechanisms in RW soils were investigated by field monitoring campaigns conducted in a small, well-equipped agricultural catchment located in Brittany, Western France, during baseflow periods. Laboratory experiments with soils coming from the same RW zones with field monitoring were jointly conducted, to test and further constrain field-monitoring derived hypotheses. The four main series of research questions central to the thesis are recalled here:

1) What are the mechanisms that release dissolved P in RW soils under field condition? What is the role of water table fluctuations in controlling hydrological connectivity and DP solubilization mechanisms?

2) What are the factors controlling the spatial and temporal variability in DP release mechanisms in RWs? What is the role of hydroclimate variability and topography, and what are their influences on water-table level and dynamic? Is the DP mobilized actually delivered to the stream? What is the chemical nature of the DP released (reactive or unreactive)? Do the soil properties play a role in this nature?

3) What is the speciation of the P released in soils during drying-rewetting cycles? What is the role of colloidal P? Do the different P forms react similarly to drying-rewetting cycles? How the soil properties and soil P status influence the speciation of the released P?

4) What are the mechanisms of the DP release in soils under reducing condition? What is the influence of soil properties? Does the addition of P-rich sediments from

upland field runoff accelerate the DP release in RW soils?

6.2 Summary of conclusions

6.2.1 Constraints from field monitoring on the mechanisms and factors causing DP releases in riparian wetlands

Previous studies of the Kervidy-Naizin catchment suggested that riparian wetlands (RWs) in this catchment could be the main source zones of the DP conveyed by the stream. The DP released in RW soils was transferred to the stream by subsurface groundwater flow. Thus, the first year of field monitoring (2013-2014) aimed at i) verify hypothesis that RWs are DP sources and ii) identify the involved release mechanisms, with the question that whether the water table controlled only the transfer of DP by hydrologically connecting RW soil waters to stream waters, or it controlled also the type and intensity of the release mechanisms involved. As shown in Chapter 2, results of this first-year field monitoring confirmed the hypothesis that RWs in the Kervidy-Naizin catchment are zones of DP (dissolved molybdate-reactive P, the MRP) release in this catchment. They also suggested two biogeochemical processes controlling this release in RW soils: i) rewetting of soils after dry periods and ii) reductive dissolution of soil Fe (hydr)oxides in response to soil saturation, thereby evidencing a strong control of water table dynamics on the triggering and intensity of these two processes. The first process was related to the water table rise during the autumn. The release of MRP at that time was triggered by the re-saturation of dried soils and the MRP released was gradually exhausted with the groundwater flow in RW soils in winter. The soil P status appeared to be the main controlling factor of the spatial variability of this mobile MRP pool, which was very limited in the Kerolland RW (wetland B) with organic-rich soils. The prolonged stagnation of groundwater in RW soils during late winter/early spring caused the second release peak of MRP, due to the reductive dissolution of Fe (hydr)oxides. The propensity of Fe (hydr)oxides to be solubilized, which is determined by soil wetness, Fe content of the soil and presence of NO_3^- , appeared to be the main controlling factor of the spatial variability of MRP release in winter. The two release mechanisms of MRP create clear signals in the streams that downstream of the monitored RWs and at the outlet of the catchment, indicating the transfer (at least in part) of the released MRDP to the stream.

During the second, longer field monitoring survey (2013-2016), we estimated the

influence of the hydroclimate variability and topography on the triggering of the previously identified biogeochemical processes that release DP. We also added the measurement of dissolved molybdate-unreactive P (MUP) to fully investigate the speciation of the DP fractions released. The results, as shown in Chapter 3, revealed the existence of strong spatial differences in DP concentration and speciation between the two investigated wetlands (Guerinieac – RWA; Kerolland – RWB), which was likely driven by the differences in P content and speciation in their corresponding soils. The soils of RWA with higher total and exchangeable P contents released higher concentrations of total dissolved P (TP) and MRP into soil solutions. By contrast, soils of RWB with more organic P were found to release essentially molybdate-unreactive dissolved P (MUP, organic/colloidal). Topography was suggesting to be the key factor controlling the reactivity/speciation of the DP released in RWs, by its critical control on water table dynamic and soil organic mineralization. RWs with a smooth topography and thus prone to low mineralization rates would release more MUP than steeper RWs in which mineralization rates are higher (more MRP released).

The intra- and interannual variations in DP release dynamics were also distinct between RWA and RWB, reflecting the difference in the frequency of drying-rewetting cycles and duration of consecutive days of soil saturation between the two RWs. Both parameters were tightly related with the groundwater level dynamics, which are dependent on the hydroclimate and local topography. The interplay of hydroclimate and topography variations resulted in a spatial and temporal variation in the trigger of the two identified biogeochemical processes that release DP in soils.

The field monitoring data also evidenced that the DP released in RW soil solutions were not quantitatively transferred into the stream, which was likely due to the re-oxidation of Fe(II) and subsequent co-precipitation of DP when the reduced soil solutions flowed through the oxic RW-stream interface. This retention process could be more active in relatively dry years due to the decreased groundwater flow rates. Thus, the field monitoring results identified a complex interaction among soil properties, hydroclimate variability, and topography in controlling the location of hotspots of DP release in RWs. A conceptual model was built to illustrate how these various factors interact with each other to locate these hotspots in agricultural landscapes. Finally, the results pointed out that topography could be the main driver because of its critical influence on the spatial and temporal variability of drying-rewetting cycles and

associated redox oscillations in RWs.

6.2.2 Highlighting how drying-wetting cycles stimulate the release of colloidal P in wetland using column leaching experiments

As revealed by field monitoring data, rewetting of dry soils in autumn that caused by the water table rise activated a mobile P pool and resulted in pulses of DP release in RW soils. The repeated drying-rewetting (DRW) leaching experiments conducted during this thesis on RWA and RWB soils brought new constraints on the release mechanisms of DP during these rewetting processes and on the nature of the released P forms.

These laboratory experiments, as shown in Chapter 4, firstly confirmed that the DRW events can trigger pulses of DP release into soil leachates, thereby supporting the hypotheses put forwards from the field monitoring data. More noticeably, the experimental data revealed that the DP released during DRW cycles consisted not only truly dissolved P but also of a large proportion of colloidal P. Thus, the P forms in DP released during rewetting of dried RW soils could be separated into three parts: truly dissolved MUP, colloidal P and truly dissolved MRP. These different P forms reacted differently to DRW cycles, suggesting different activation/production mechanisms. The colloidal P fraction was the most reactive fraction and was mainly activated by the physical mechanisms of colloid mobilization or, to a lesser extent, biologically colloid formation. This discovery demonstrates further the important role of colloids in mobilizing P in soils. The truly dissolved MUP pool was also reactive to DRW cycles but to a lesser extent and was mainly comprised of organic P derived from the lysis of microbial cells upon rewetting. These two P forms (colloidal and organic P) were rapidly exhausted during the leaching experiments, suggesting that the reservoir they came from were limited in size and were located mostly in soil macropores. On the contrary, the truly dissolved MRP reacted little to DRW cycles and was not rapidly exhausted from soil columns. This suggested that this component in the two studied was i) likely produced by other processes operating over longer time scales than the selected DRW duration in this study, ii) likely came from the soil microporosity through a combination of desorption and diffusion processes.

Otherwise, the proportions and concentrations of the different P forms (Colloidal P, MRP, and MUP) released during DRW cycles were found to be strongly controlled by

the content and speciation of P in corresponding soils. In particular, the proportion of released colloidal P appeared to be positively correlated to the organic matter content and microbial biomass of soils, a result consistent with the factors known to favor the production of colloids upon rewetting of dried soils.

6.2.3 Constraints on the processes releasing P under anoxic conditions

The anaerobic incubations conducted in this thesis were aimed at unraveling the nature of the mechanisms involved in the release of DP during installation of anoxic conditions in RWs. The incubation results, as shown in Chapter 5, firstly confirmed the results stemming out from field monitoring that the installation of anoxic conditions resulted in a DP release process. Two mechanisms were identified that contributed to this release: i) reductive dissolution of Fe-oxyhydroxides which serve as a P sink in soils and ii) pH rise due to proton consumption by reduction reactions. More importantly, this thesis provided an approach to unravel the respective contribution of each mechanism to the DP release and the way RW soil properties control this contribution proportion. In RWA soil with high extractable P and low organic matter content, the DP release was mainly ($\approx 80\%$) controlled by the Fe-oxyhydroxide reductive dissolution mechanism. In RWB soil with low extractable P but high organic matter contents, the DP release was mainly ($>80\%$) controlled by the pH rise mechanism. Once again, the concentration and speciation of the released DP (MRP or MUP) appeared to be strongly controlled by soil chemical properties such as the soil organic matter content and soil P status. The Fe:P molar ratio in anaerobic soil solutions was also found to be controlled by soil chemical properties, specifically the soil oxalate extractable P and Fe contents. This ratio could be important in evaluating the risk for the released DP to be transferred to surface watercourses, by determining the proportion of DP that will be immobilized by re-oxidized Fe(II) at the oxic RW-stream interface and thus stored in streambank soils before reaching the stream network.

Finally, anaerobic incubations with the mixture of RWB soil with eroded sediments from upland cultivated fields revealed an enhanced DP release, compared to what expected from their separate incubations. Thus, the input of soil eroded from upland fields can not only raise the P concentration of RW soils but also stimulate the release of part of the P conveyed by these eroded soils, most likely because of the function of Fe-reduction bacteria in RW soils.

6.3 Possible implications for management

The objectives of the present thesis were to provide the scientific basis to constrain the mechanisms involved in the mobilization and delivery of DP in agricultural catchments with shallow water tables like the Kervidy-Naizin catchment. DP has been proved to be the predominant fraction of the P exported from this catchment. Results from this thesis show that the RWs in this catchment behave as hotspots of DP release in the landscape, emphasizing further the importance of controlling both the mobilization of the legacy P present in these zones due to past cultivation and the inputs of any additional P from upland cultivated fields.

As illustrated in the conceptual model developed in this thesis, the spatiotemporal variations of DP release in RW soils are controlled by the interplay of hydroclimate, topography and soil property variations. The topography is identified as the main ultimate driver because of its key control on P input from upland fields and organic P mineralization. Thus, a first practical implication stemming out of this thesis is that topography should be used as a guide to identify those RWs in the landscape at high risk regarding DP release. Managing strategies should be designed in priority to reduce the DP release from those high-risk RWs. Therefore, the management strategies for that purpose should focus on:

- i) Limit the input of P from upland cultivated fields (for example in implementing hedges or embankments at the RW-fields interface);
- ii) Export biomass to decrease the P content of RW soils;
- iii) Promote the immobilization of soil P by spreading adsorbent materials on RW soil surfaces or along drains or ditches, to form reactive, P-sorbing barriers between RW soils and streams.

Management strategies should also be considered in connection with the season of the year and the sensitivity of receiving downstream water to eutrophication. Indeed, the present results show that DP concentration and speciation will be different depending on whether the release mechanism is dominantly related to drying-wetting cycles (mainly activated in autumn/early winter) or soil Fe-oxyhydroxide reduction (mainly activated in late winter/early spring). Limiting the transfer of DP during drying-rewetting events in autumn/early winter would require reduction not only of the transfer of truly dissolved P (MRP and MUP) but more importantly the transfer of colloidal P, which was the predominant fraction of the P released at that time. Reducing the export

of truly dissolved P could be achieved by use of the adsorbent material addition method mentioned above. This strategy would be, however, of no use for limiting the export of colloidal P. An option for reducing this colloidal P export could be to decrease the soil pH to enhance the adsorption of colloids to soil matrix. However, this is hard to achieve and the potential negative ecological influence of such a pH decrease should be evaluated before it could be applied. Limiting the transfer of DP during late winter/early spring waterlogged periods could be achieved by avoiding the development of reducing conditions, or by trying to make the released P retained within RW soils. The reducing condition can be avoided by improving the drainage of the RWs, to shorten the number of consecutive days of water saturation. However, this solution should be taken with care because the ecosystem services provided by RWs such as denitrification and enhanced biodiversity could be altered or even lost by such a practice. To enhance the retention of released DP under reducing conditions, one option could be to reduce the groundwater flow rate, such as by increasing the width of RWs. The reduced groundwater flow rate would increase the chance for Fe(II) to be oxidized when crossing the oxic RW-stream interface and could also favor the denitrification rate of nitrate. However, this option could be hard to implement as it would almost certainly bring potential conflict with farmers.

Changing the Fe:P molar ratios of the soil solutions could be another management option to reduce the risk of DP transfer (mobilization and delivery) during waterlogged periods. Published data indeed show that when the Fe:P ratio in anaerobic soil solution is >3.0 , then the re-oxidation of Fe(II) has the potential to reduce DP concentrations to values as low as $< 1 \text{ mmol l}^{-1}$ (Zak et al., 2004). As suggested in this thesis, Fe:P ratios are tightly associated with the oxalate extractable P and Fe contents of the soil. It could thus be appropriate to use the oxalate extractable P and Fe contents of RW soils as a test to evaluate the risk of DP transfer, especially in RWs where anoxic conditions frequently take place. The addition of Fe(III)-minerals could be applied to increase the Fe:P ratio of soils naturally poor in Fe, to increase the Fe(II) concentrations of RW soil waters and favor the capture of P by re-oxidized Fe(II) at the RW-stream interface.

Because the re-oxidation of Fe(II) is expected to occur at the RW-stream redox interface, it comes that soils near the stream could be enriched in P in many RWs, the stored P being associated with amorphous or mineral Fe compounds. Thus, this thesis also suggests that any management designed to enhance the retention of released P in

RW should be applied with simultaneous consideration of preventing stream bank erosion, for example by planting riparian vegetation, restoring the natural watercourse morphology, or restricting livestock access to the stream.

To sum up, the new constraints placed by this thesis on the detailed nature of the DP release mechanisms operating in RW soils could serve as a basis to design and apply new specific management options to limit the transfer of DP from these zones. An important lesson is that whatever option is chosen, it will have to take a combined consideration of hydroclimate variations, local topography, and soil properties of RWs.

6.4 Perspectives

6.4.1 Nature, source, and significance of organic P fraction

The field monitoring data presented in this thesis showed that a significant fraction of the DP released by RWs was in the form of MUP, especially for those RWs where the soils have high organic matter and organic P content. Part of this MUP fraction is likely to consist of organic P, the significance and origin of which remain, however, uncertain. In fact, this fraction could be organic P carried by organic molecules such as phytates, sugar phosphates or/and phospholipids, or organic P incorporated into or adsorbed onto colloidal particles. Given that these fractions are all potentially bioavailable (Li and Brett, 2015; Montalvo et al., 2015), it would be interesting to know more about their composition, origin and overall significance. Solution ^{31}P NMR and enzyme addition assays in NaOH-EDTA extracts are the two current main methods for identifying and quantifying soil organic P compounds (Jarosch et al., 2015; Menun and Liu, 2014). However, because the amounts of samples required by these methods are quite large, most efforts to characterize soil organic P compounds have been focused so far on analysis of laboratory soil extracts prepared from air-dried and sieved soils. By using the benefit of the soil solution sampling devices implemented on the Kervidy-Naizin catchment, we believe that it should be possible to conduct this organic P analysis and characterization directly on natural soil solutions. Ultra-filtration and ultra-centrifugation techniques could be used to concentrate the soil solution to get the required amounts of organic P. These organic P compound analyses and characterizations would provide a better understanding of the meaning of this fraction with respect to the whole DP release process, and of the potential environmental threat it poses if transferred to streams and rivers.

6.4.2 Towards a better characterization of colloid composition and colloid properties regarding P transfer in soils

As revealed in this thesis, colloidal P is a major fraction of the DP released in response to DRW events and is generally also an important compartment of the operationally defined MUP fractions found in natural RW soil solutions. It has been widely recognized that colloids are important carriers for P in soil solutions (Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013; Siemens et al., 2008). Colloids have the potential to transport P across long distance through subsurface leaching (Ryan and Elimelech, 1996) and the colloidal P is also potentially bioavailable (Montalvo et al., 2015). Thus, it is necessary to make further investigation to know more about: i) the composition of the colloidal P carriers (Are they organic, mineral, mixtures? Do their composition change in space and time?), ii) the way these carriers are produced in RW soils (where and when?) as well as iii) the way they bond and transport P. So far, most published researches on colloidal P transport in soils have been conducted on soil extracts prepared in the lab using air-dried soil samples mixed with either deionized water or synthetic solutions of various ionic force and pH (Heathwaite et al., 2005a; Hens and Merckx, 2002). Although these researches are important for providing basic information on the role of colloids in soil P transport, they suffer from the drawback that the amount and composition of colloids may be different, from those occurring naturally in soils. Thus, one future research direction on colloidal P would be to gain more information on the colloidal P under natural field conditions. Once again, the soil solution sampling device installed on the Kervidy-Naizin catchment and the knowledge already acquired on this catchment about P release dynamics provide an ideal framework for such a future work. Ultra-filtration and asymmetric flow field flow fractionation coupled with ICP-MS and organic carbon detector are common methods for characterizing the colloidal P fraction in soil solution, and it would be interesting to make a comparison between methods with natural or laboratory extracted soil solutions. Another possibility is to enrich the colloids present in natural soil solution using ultra-centrifugation techniques, and then to characterize the colloids to get detailed information on their composition and the exact way P is bound to them. The X-ray adsorption near edge structure could be used for that purpose.

Another research direction on colloidal P is to study the spatial and temporal variation of the colloidal P fraction in natural soil solutions and streams during one or more

complete hydrological years. The sampling equipment along transects in the Kervidy-Naizin catchment makes this catchment again a perfect site for this research. The spatial variation of colloidal P between different transect could provide information on how soil properties control the composition and properties of the soil colloids involved in P transport. The sampling of colloids along water pathways, from near upland fields to RW zones and stream banks and finally to the stream could enable evaluation of the possible changes in the composition and properties of colloids during transport in soils and streams. The possible temporal variations of colloid composition and properties with respect to P could reveal possible seasonal variation in the importance of colloidal P transport in the agricultural catchment, possibly related to the temporal variations of DP release processes in RW mentioned above.

Finally, colloidal P in soil solutions and stream waters have been found to be strongly associated with P-rich colloidal phases such as organic matter (OM) and Fe(Al)oxyhydroxides (Baken et al., 2016a; Gottselig et al., 2014, 2017; Jiang et al., 2015; Liu et al., 2014; Regelink et al., 2013). However, no direct study has been performed so far to determine the detailed partitioning of OM, Fe(Al)oxyhydroxides and P in these P-bearing colloids, which is essential to characterize the mechanisms by which these P-bearing colloids in agricultural catchment soils are produced. It has been recently indirectly suggested by Gottselig et al. (2017), that P could be preferentially associated with Fe in the smallest fraction (i.e. 1-20 nm), but more with organic-rich colloids in larger fractions (>60 nm). However, besides being indirect, this characterization has been made on streams draining a forested catchment, not an agricultural one. Thus, another important direction for future researches would be to study the detailed composition of the P-bearing colloids, in streams draining agricultural landscapes. Along with analysis of natural colloids, laboratory experiments simulating the direct production of colloids under various Fe/OM/P ratios could be helpful for that purpose.

6.4.3 Test the generality of the conceptual model developed from the Kervidy-Naizin catchment

This thesis indicates a complex interaction among hydroclimate variability, local topography and soil properties in controlling the DP release from agricultural catchments. The conceptual model developed in this thesis provides a framework to

evaluate and predict the DP release dynamics in an agricultural catchment, intra- or inter-annually. The Kervidy-Naizin catchment belongs to the AgrHys environmental research observatory, the catchments in which are part of the national RBV watershed network (<http://rnbv.ipgp.fr/>) that are diverse in agro-climatic issues and contexts. There are also national networks from other European countries where the context is very similar to Brittany such as the “Demonstration Test Catchment” in the United Kingdom (<http://www.demonstratingcatchmentmanagement.net/>) or the "Agricultural Catchments Program" in Ireland (<http://www.teagasc.ie/agcatchments/>). It is worth trying to apply the schematic framework developed in this thesis to these catchments with similar or contrast context, for the better understanding and future control of the DP release from agricultural catchments to watercourses.

Chapter 7

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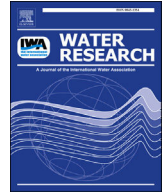
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Annexe A

An innovative approach for sequential extraction of phosphorus in anaerobic sediments



An innovative approach for sequential extraction of phosphorus in sediments: Ferrous iron P as an independent P fraction



Sen Gu ^{a, b, 1}, Yiguang Qian ^{a, 1}, Yang Jiao ^a, Qingman Li ^{a, *}, Gilles Pinay ^c, Gerard Gruau ^c

^a Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China

^b Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China

^c OSUR – CNRS, University of Rennes 1, Rennes, 35042, France

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ABSTRACT

Accurate identification of phosphorus (P) forms is crucially important for understanding the geochemical cycle of P; however, until now the role of ferrous iron P (Fe(II)-P) buried in sediments has been completely ignored in nearly all sequential extraction procedures developed. Using sediment cores sampled from Donghu Lake in Wuhan, China, this study explored a modified version of widely used sequential P extraction method (SEDEX; [Ruttenberg, 1992](#)) in which Fe(II)-P was identified as an independent fraction. Based on the high selectivity of the extractant (0.2% 2,2'-bipyridine+0.1 M KCl) and the dissolution equilibrium of P, procedures for extracting Fe(II)-P were optimized using a 1:100 solid:liquid ratio and extraction at 50 ± 1 °C for 24 h. The sedimentary P extracted was divided into five fractions: loosely-bound P, Fe(II)-P, CDB-P, Ca-P and O-P. Fe(II)-P was the predominant fraction in fresh sediments in Donghu Lake, accounting for 15.7–49.9% of TP, with a mean of 31.6%. The mean values of Ca-P, O-P, CDB-P and loosely-bound P were 28.4%, 22.7%, 17.1% and 4.3%, respectively. Combined with component analysis of extracts and recovery experiments of standard reference minerals (vivianite, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) in natural sediments, extraction of Fe(II)-P with 0.2% 2,2-bipyridine and 0.1 M KCl was robust, with a good recovery rate (88.7–100.6%) and little of the Ca-P dissolved. It is possible to use this innovative SEDEX not only to distinguish the contribution of different P matrices in fresh sediments, but also to investigate the transformation of sedimentary P under different redox conditions. Therefore, greater focus on Fe(II)-P is necessary, because it is a major sink for the geochemical process of sedimentary P.

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1. Introduction

Accurate identification of phosphorus (P) forms is crucially important for understanding the geochemical cycle of P. Sediments are commonly regarded as both a source and a sink for P, which plays a vital role in water eutrophication ([Søndergaard et al., 1996](#); [Jarvie et al., 2005](#); [Huang et al., 2015](#)). Most P originating from point sources (e.g. industrial and domestic sewage) and non-point sources (e.g. rainfall, runoff and soil leakage) that flows into water bodies is buried in sediments ([Withers and Jarvie, 2008](#); [März et al., 2014](#)). The P released from sediments acts as an internal source ([Spivakov et al., 1999](#); [Wang et al., 2009a, 2009b](#)). Even when

external P is controlled, P release from sediments sustains water eutrophication for several decades ([Søndergaard et al., 2003](#); [Schindler, 2006](#); [Lewis et al., 2007](#)). In some lakes, the quantity of internal P is several orders of magnitude higher than that of external sources ([Wang et al., 2013](#)). Thus, it is highly important to scientifically identify P forms in sediments to facilitate management of internal P.

P forms in sediments depend greatly on mineral composition and the depositional environment ([Søndergaard et al., 1996](#); [Aydin et al., 2009a](#)). The conventional sedimentary matrices interacting with P are iron/aluminum oxides and calcium carbonate, which occur either through adsorption or precipitation of minerals such as apatite, a Ca-phosphate ([Khare et al., 2007](#); [Filippelli, 2008](#)). The proportion of apatite in calcareous sediments is commonly higher than that in non-calcareous sediments ([Berg et al., 2004](#)), whereas P coupling with metal oxides predominates in non-calcareous sediments ([Berg et al., 2004](#); [Li et al., 2012](#)). At pH < 6.5, P is mainly deposited with metal oxides, and with calcium carbonate if

* Corresponding author. Institute of Hydrobiology, Chinese Academy of Sciences, No. 7 Donghu South Road, Wuchang District, Wuhan, 430072, China.

E-mail address: qmli@ihb.ac.cn (Q. Li).

¹ Sen Gu and Yiguang Qian contributed equally to this study and should be regarded as joint first authors.

pH > 6.5 (Wang et al., 2009b; Yin et al., 2013). Accordingly, it is necessary to consider sediment composition to develop methods to identify P forms in sediments.

Several methods have been developed to distinguish P forms in sediments, and sequential extraction procedures are frequently applied. One commonly applied extraction protocol, especially for marine sediments, is SEDEX (Ruttenberg, 1992), a sequential multi-step fractionation, and P in sediments is generally divided into loosely-bound P, P bound to metal oxides (CDB-P), calcium-carbonate-precipitated P (Ca-P) and organic P (O-P), depending upon their chemical characteristics (Tiyapongpattana et al., 2004; Aydin et al., 2009a). SEDEX has several advantages over other methods: samples need no special treatment, extraction is performed in batches, special equipment is not required, and costs are low (Ruttenberg, 1992; Ruban et al., 1999; Aydin et al., 2009a). The original SEDEX protocol has been modified multiple times for various reasons. Some modifications were designed to streamline the protocol (Anderson and Delaney, 2000; Ruttenberg et al., 2009), while others were designed to improve the selectivity of SEDEX. CDB-P, for example, can be further fractionated into readily reductive P (generally defined as P bound to both amorphous and partially crystalline Fe(hydr)-oxides), P bound to crystalline Fe(hydr)-oxides and P bound to amorphous and crystalline Al(hydr)-oxides (Kraal et al., 2009; Wang et al., 2009b). Ca-P can be artificially divided into metastable calcium phosphate, hydroxylapatite, fluorapatite and chlorapatite due to their stability (Aydin et al., 2009b). Some modifications added new extraction steps to the original SEDEX protocol to extract, for example, fish bone hydroxyapatite (Schenau et al., 2000) or biogenic opal-bound P (Latimer et al., 2006; März et al., 2014). Though the P forms in sediments that SEDEX identifies are contentious, reasonable scientific SEDEX procedures show reliable results (Ruttenberg et al., 2009). The credibility of SEDEX has been widely verified with the development of mineral analysis technology (Brandes et al., 2007; Kruse et al., 2015).

Several studies have indicated that the potential of anoxic sediments to bind P in freshwater, brackish and fully marine environments, is attributed to the occurrence of Fe(II) phases (Roden and Edmonds, 1997; Zak et al., 2006; Egger et al., 2015), which interact directly with P to form the Fe(II) phosphate phase (operationally defined as Fe(II)-P). Furthermore, the stability of Fe(II)-P in sediments is highly sensitive to environmental factors such as pH, redox potential, ion strength and natural ligands (Schultz and Grundl, 2004; Statham et al., 2012). It is expected that fractionating Fe(II)-P as an independent P fraction is extremely helpful for gaining more information about the geochemistry of P in sediments.

The SEDEX developed by Ruttenberg (1992) for marine sediments is widely used for P fractionation in sediments, with an initial intention to separate the authigenic carbonate fluorapatite (CFAP) from detrital apatite of igneous or metamorphic origin. When more sedimentary P was fractionated from fresh anoxic sediments, Fe(II)-P was generally considered as Fe-bound P because the extractants (2.5% Na-dithionite with 0.3 M Na₃-citrate and 1.0 M NaHCO₃ (pH 7.6)) could dissolve Fe(II) phases (Jensen et al., 1998). Our previous research found that Fe(II)-P was one of the main sedimentary P forms in Dianchi Lake, accounting for 23.4–39.8% of total phosphorous (TP) buried (Li et al., 2012). Therefore, it is imperative to develop a new extraction method for P fractionation that more accurately and precisely estimates P distribution patterns in sediment under anaerobic conditions.

Accordingly, this study developed an innovative SEDEX for anaerobic sediments with Fe(II)-P as an independent fraction. This new method is based on modifying Ruttenberg's procedures with Fe(II)-P extraction methods. The feasibility of this new SEDEX was

tested by comparing its results to those of Ruttenberg's method.

2. Materials and methods

2.1. Sampling site

Sediments were collected from three representative sampling sites (S1–S3) in two lake regions of Donghu Lake, Guozheng and Tanglin (30°32.173'–35.628' N, 114°20.899'–24.924' E) (Fig. S1). S1 was near the outlet of domestic and municipal sewage, one of the most heavily polluted locations in the area. S2 was near the Moshan scenic spot and may be heavily contaminated by tourist wastewater. S3 was in a less exploited region where anthropogenic pollution was relatively lower. Depths of these three sites were 3.3 m, 3.6 m and 3.7 m, respectively. The degree of contamination was assumed to decrease from S1 to S3.

2.2. Sediment sampling and characterization

Sediment cores (30 cm) were collected in March 2013 using a piston sediment corer equipped with polymethyl methacrylate (PMMA) tubes. Cores were immediately cut into three layers at 10 cm intervals and taken to the laboratory in sealed polyethylene bags in iceboxes, then stirred until homogenized in a glove box. All samples were divided into two batches. One sub-sample was stored anaerobically in brown bottles filled with high-purity N₂ at 4 °C, and the other sub-samples were naturally air-dried without illumination, then ground and sieved with a 0.145 mm sieve to obtain a uniform size, and were stored in a refrigerator at 4 °C. Physico-chemical characteristics of selected sediments are shown in Table 1.

Redox potential and pH of sediments were measured anaerobically using a potentiometer with a platinum and glass electrode, respectively. Fresh sediments were centrifuged at 4500 rpm for 30 min and filtered through a cellulose-nitrate membrane (0.45 μm) to obtain the pore water. Total nitrogen (TN) and TP of the pore water were measured by an auto-analyzer according to the former method (Johnes and Heathwaite, 1992). TN and TP of the sediment were measured as ammonium and orthophosphate by colorimetry after the acid digestion of several samples. Organic matter content was determined as loss on ignition (LOI) by combusting samples at 550 °C.

2.3. Optimizing the extraction procedures of Fe(II)-P

Fe(II)-P was extracted at pH 7.5 using a 2,2'-bipyridine (0.2%) + KCl (0.1 M) solution as the extractant. The procedure was as follows: sediment (ca. 0.5 g dry weight (DW)) from each batch was transferred quickly along with a 2,2'-bipyridine (0.2%) and KCl (0.1 M) solution into a brown bottle that was sealed with a rubber plug and then shaken on a reciprocal shaker. At certain intervals, 20 ml of suspension was sampled during shaking and filtered through a cellulose nitrate membrane (0.45 μm). The residual on the membrane was rinsed several times in 0.1 M KCl solution to obtain the final volume of 100 ml. The final solution was used to determine Fe(II) and Fe(II)-P. To improve extraction yield, the solid:liquid ratio (1:200, 1:500 and 1:1000), extraction temperature (25 °C, 40 °C and 50 °C) and shaking frequency (200 and 300 rpm) of extraction procedures were modified. All extraction experiments were conducted without anaerobic conditions, because Fe(II) oxidation can be inhibited when 2,2'-bipyridine is bound to the sediment's surface.

Table 1
Physicochemical characteristics of selected sediments from Donghu Lake.

Sample site	Depth cm	pH	Eh mv	LOI %	TN	TP	Fe		
							TFe	^r TFe ²⁺	^d TFe ²⁺
mg/g DW									
S ₁	0–10	7.28	–47	10.22 ± 0.23	3.33 ± 0.18	1.53 ± 0.01	25.13 ± 0.04	7.05 ± 0.09	6.03 ± 0.16
	10–20	7.19	–121	9.98 ± 0.02	2.40 ± 0.39	1.67 ± 0.09	26.48 ± 1.30	18.13 ± 0.15	7.28 ± 0.32
	20–30	7.16	–144	10.75 ± 0.03	2.75 ± 0.67	1.06 ± 0.01	29.51 ± 2.54	20.01 ± 0.06	7.66 ± 0.15
S ₂	0–10	7.13	30	10.58 ± 0.06	3.45 ± 0.26	1.29 ± 0.05	26.31 ± 1.87	5.83 ± 0.24	5.23 ± 0.10
	10–20	7.10	–58	10.98 ± 0.16	3.81 ± 0.08	1.22 ± 0.02	27.01 ± 2.17	10.97 ± 1.01	6.16 ± 0.28
	20–30	7.11	–115	6.26 ± 0.02	1.16 ± 0.20	0.82 ± 0.02	24.63 ± 0.81	17.01 ± 1.25	10.49 ± 1.15
S ₃	0–10	7.37	–64	7.71 ± 0.39	2.12 ± 0.02	1.00 ± 0.01	25.78 ± 0.08	7.16 ± 0.09	6.11 ± 0.21
	10–20	7.15	–76	7.43 ± 0.45	2.60 ± 0.02	0.75 ± 0.01	26.90 ± 2.07	14.83 ± 0.07	7.15 ± 0.31
	20–30	7.16	–105	7.77 ± 0.11	2.32 ± 0.14	0.61 ± 0.03	24.84 ± 1.05	17.24 ± 2.59	8.19 ± 0.13

Note: Subscripts indicate sites at which sediment samples were collected. Values of LOI, TN, TP, TFe and TFe²⁺ are mean ± standard deviation of three replicates analysis. LOI, TN, TP and TFe represent loss of ignition, total nitrogen, phosphorus and iron of sediments, respectively. ^rTFe²⁺ and ^dTFe²⁺ represent Fe²⁺ in fresh sediments and Fe²⁺ in air-dried sediments.

2.4. Evaluating the extraction characteristics of the new extracting agent

Extraction experiments of several Fe(II) and P bearing minerals were performed separately and in combination to explore the selectivity and effectiveness of this new extractant (0.2% 2,2'–bipyridine + 0.1 M KCl). First, reference Fe(II) and P bearing minerals, including pyrite (FeS₂), siderite (FeCO₃), vivianite (Fe₃(PO₄)₂·8H₂O), AlPO₄, FePO₄·2H₂O, and hydroxyapatite (Ca₅(PO₄)₃OH), as well as their combinations (1:1 in weight), such as vivianite + pyrite (V + P), vivianite + siderite (V + S), vivianite + AlPO₄ (V + A), and vivianite + FePO₄·2H₂O (V + F), were chosen for the single and combined mineral extraction experiments. Then, 40 mg of each single and combined minerals were added into 100 ml of four extractants (H₂O, 0.1 M KCl, 0.2% 2,2'–bipyridine, and 0.2% 2,2'–bipyridine + 0.1 M KCl), and all the extraction experiments proceeded according to the new extraction protocol (Section 2.3). Concentrations of Fe (II) and P in the extracting solution were determined and all the experiments were operated in triplicate. As before, all extraction experiments were performed without anaerobic conditions.

2.5. Establishing the innovative SEDEX of P in sediments

This innovative SEDEX is based on a modification of Ruttenberg's method (Ruttenberg, 1992); the extractants and corresponding P fractions are shown in Table 2. P in sediment was sequentially extracted in strict accordance with the original method, except that the duration of CDB-P extraction was extended to facilitate dissolution of poorly crystalline Fe/Al-oxyhydroxides. Our new SEDEX (Table 2) was performed by extracting Fe(II)-P after extracting loosely-bound P, which means starting by extracting CDB-P using the washed residual of the Fe(II)-P extraction. All extracts were filtered through 0.45 μm P-free membranes before the P and iron analysis. To evaluate the reliability of the developed SEDEX, the effect of P fraction after adding Fe(II)-P extraction was compared with those obtained by a slightly modified Ruttenberg's method, in which biogenic apatite + CaCO₃-P and detrital apatite are extracted with 1.0 M HCl for 16 h, and called Ca-P. In addition, the recovery rate of this new sequential extraction method was evaluated by adding a standard reference mineral (vivianite) in known quantities to the natural sediment.

2.6. Determining the total iron, Fe(II) and Fe(II)-P in sediments

Sediment extraction filtrates with 3.0 M HCl and filtrates of

Fe(II)-P extraction were analyzed for total iron and Fe(II) by colorimetry. Approximately 5 ml of filtrate was transferred into a 50 ml beaker. After being adjusted to approximately pH 5.0 with NaOH, 10 ml of 10% ascorbic acid and 1% 2,2'–bipyridine were added, and the red color was left to develop for at least 15 min. Next, absorbance was measured at 520 nm with a spectrophotometer (752 UV–visible, Shanghai Oppler Instrument Company), and total iron concentration was determined following the standard calibration. The ascorbic acid reduction step was omitted in the determination of Fe(II). To determine Fe(II)-P, analyses were conducted after removing the 2,2'–bipyridine by sorption with active carbon containing no P and adopting the following procedures: 5 ml of filtrate solution were transferred into 50 ml volumetric flasks containing 0.5 g non-P active carbon and 5 ml of 5.0 M KCl. Before being diluted to a constant volume, the flasks were shaken to remove the 2,2'–bipyridine rapidly. The suspensions were then filtered through 0.45 μm membranes, and P in the filtrate was measured with the molybdenum blue method. Separate standard calibrations and reagent blanks were generated for each kind of filtrate to eliminate the interference of the solution matrix and operational differences.

2.7. Statistical analyses

Differences among fresh, freeze-dried and air-dried sediment, as well as between the two P fractionation methods, were statistically analyzed by applying analysis of variance (ANOVA) at a 5% significance level to certify the reliability of the SEDEX developed. Linear regression was performed among the Fe(II)-P fraction, total extracted P and the sum of labile-P and CDB-P from the original SEDEX method. All analyses were performed using the SPSS (v. 13.0) software package.

3. Results

3.1. Optimization of procedures for Fe(II)-P extraction

In our previous study, Fe(II)-P was extracted using a 1:1000 solid:liquid ratio under static conditions and room temperature (Li et al., 2012). However, this approach is not only time-consuming but also unfit for batch analysis, given such a low solid:liquid ratio, which makes it unsuitable for P fractionation. Extraction effects were analyzed under different solid:liquid ratios (1:200, 1:500 and 1:1000), temperatures (25 °C, 40 °C and 50 °C) and shaking frequencies (200 and 300 rpm) (Fig. 1). At a low solid:liquid ratio, the addition of shaking greatly shortened extraction time (Fig. 1A). For

Table 2

Comparison between this innovative sequential extraction procedure (SEDEX) and the slightly modified SEDEX of Ruttenberg (1992).

P Fraction	Extractants	
	New SEDEX	SEDEX of Ruttenberg (1992)
Loosely-bound P: Loosely-sorbed P, pore water P	MgCl ₂ 1 M (pH 8.0, 2 h)	MgCl ₂ 1 M (pH 8.0, 2 h)
Fe(II)-P: P precipitated by Fe ²⁺ and less by Ca ²⁺	0.2% 2,2'-bipyridine + 0.1 M KCl at 50 °C (24 h)	Not included
CDB-P: Reductant-soluble P, Fe/Al-bound P	Citrate dithionite bicarbonate (CDB) 0.3/1/1 M (pH 7.6, 16 h)	Citrate dithionite bicarbonate (CDB) 0.3/1/1 M (pH 7.6, 16 h)
Ca-P: Detrital and metastable apatite	HCl 1 M (16 h)	HCl 1 M (16 h)
O-P: Organic P	Calcination (550 °C) + HCl 1 M (16 h)	Calcination (550 °C) + HCl 1 M (16 h)

instance, when a 1:1000 solid:liquid ratio was used, extraction time of 150 h without shaking was sharply reduced to 36 h with a shaking frequency of 200 rpm. However, the effect of shaking frequency on Fe(II)-P extraction at higher solid:liquid ratios (1:200 and 1:500) seemed insignificant. Even when a shaking frequency of 300 rpm was used, P release was equivalent to that at 200 rpm, and extraction equilibrium was not observed. These results indicated that shortening extraction time by increasing shaking frequency was substantially limited at high solid:liquid ratios. The temperature was changed to further shorten extraction time at a high solid:liquid ratio (1:100). Raising the temperature greatly shortened extraction time (Fig. 1B). It took about 24 h for the release of P to reach equilibrium at an extraction temperature of 50 °C, while no equilibrium was observed in the same amount of time at 25 °C or 40 °C, suggesting temperature strongly influenced the extraction efficiency of Fe(II)-P. Also, when release of P at 50 ± 1 °C reached equilibrium, the quantity of P extracted was nearly equivalent to that obtained with a 1:1000 solid:liquid ratio in 36 h. Moreover, the relative standard deviations (RSD) of all the data (Table S1 and S2) obtained from the Fe(II)-P extraction in triplicate lay in the 2.7–7.6% range, which indicates that this new Fe(II)-P extraction method can be reproduced reliably. Based on these results, new procedures for Fe(II)-P extraction were developed: a solid:liquid ratio of 1:100, extraction temperature of 50 ± 1 °C, shaking frequency of 200 rpm, and extraction time of 24 h.

To determine the recovery rate of the new Fe(II)-P extraction method, the standard reference mineral (vivianite, Fe₃(PO₄)₂·8H₂O) was added to natural fresh sediment subsamples (S1,0–10 cm) in known quantities and mixed thoroughly, then extraction efficiencies were measured. All results from the three replicates are presented in Table 3. The recovery rates were 88.7–100.6%, and RSD of the data were small (0.3–1.0%), which indicates that it is a scientific and available method for Fe(II)-P extraction.

3.2. Selectivity of the new extracting agent

The selectivity of this new extractant (0.2% 2,2'-bipyridine + 0.1 M KCl) was evaluated using several Fe(II) and P bearing minerals separately and in combination. Extraction efficiencies of 0.2% 2,2'-bipyridine + 0.1 M KCl, H₂O, 0.1 M KCl, and 0.2% 2,2'-bipyridine were compared. For single minerals (Fig. 2), except vivianite (Fe₃(PO₄)₂·8H₂O), P and Fe(II) concentrations in all extracting agents were extremely low and varied little, with 0–0.6 mg/L of P and 0.03–0.9 mg/L of Fe(II). For vivianite, P and Fe(II) concentrations were very low in H₂O (0.01 ± 0.0002 and 0.03 ± 0.0002 mg/L, respectively) and 0.1 M KCl (0.02 ± 0.0003 and 0.04 ± 0.0001 mg/L, respectively), but much higher in 0.2% 2,2'-bipyridine (18.2 ± 0.3 and 48.4 ± 1.2 mg/L, respectively) and 0.2% 2,2'-bipyridine + 0.1 M KCl (23.4 ± 0.6 and 62.2 ± 2.3 mg/L, respectively). The solubility of both Fe(II) bearing minerals (FeS₂ and FeCO₃) in H₂O and 0.1MKCl was lower than that in 0.2% 2,2'-bipyridine+0.1 M KCl; however, the solubility of Ca₅(PO₄)₃OH

in H₂O and 0.1MKCl was higher than that in 0.2% 2,2'-bipyridine+0.1 M KCl, suggesting 0.2% 2,2'-bipyridine could limit its dissolution. These results demonstrated that 0.2% 2,2'-bipyridine+0.1 M KCl exhibits high selectivity for Fe(II) extraction in minerals, especially vivianite.

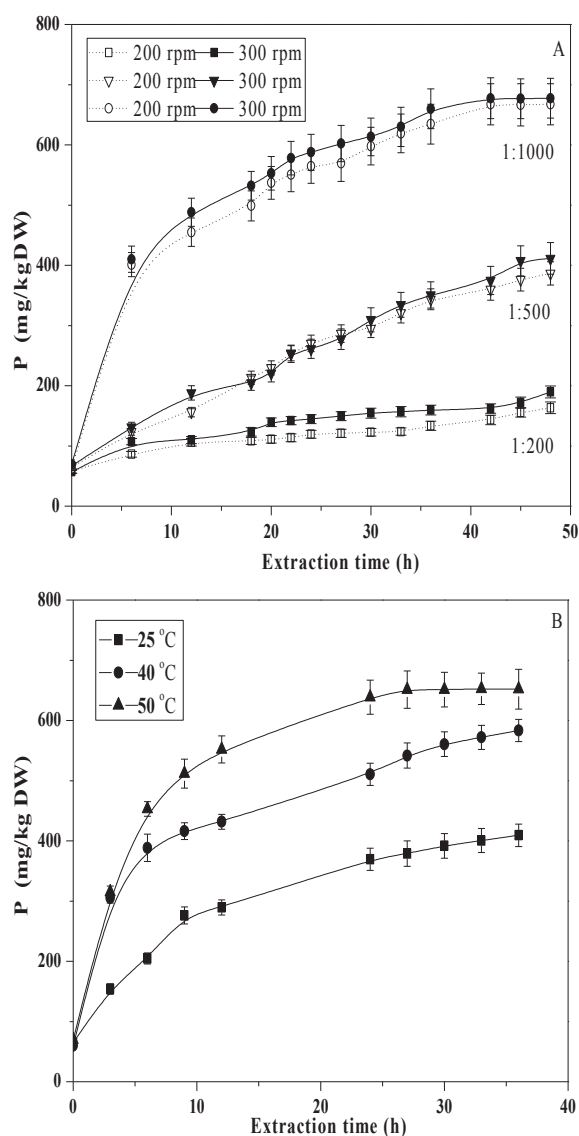


Fig. 1. Relationship between extraction efficiency of Fe(II)-P and extraction conditions for surface sediment at sampling point S3. (A) Dependence of Fe(II)-P extraction on the solid:liquid ratio and shaking frequency; temperature = 25 °C. (B) Dependence of Fe(II)-P on temperature; solid:liquid ratio = 1:100. Error bars represent standard deviations of three replicates analysis.

Extraction was performed on a combination of vivianite and other minerals to further testify the selectivity of 2,2'-bipyridine for Fe(II) and P dissolution (Fig. 3). As with single minerals, P and Fe(II) concentrations in 0.2% 2,2'-bipyridine and 0.2% 2,2'-bipyridine + 0.1 M KCl were several orders of magnitude higher than those in H₂O and 0.1 M KCl. In the mineral combinations, V + P, V + S, V + A and V + F, P and Fe(II) concentrations in 0.2% 2,2'-bipyridine and 0.2% 2,2'-bipyridine + 0.1 M KCl were almost the same, approximately 18.8 ± 0.4 and 23.7 ± 0.2 mg/L, respectively, for P, as well as 48.1 ± 0.8 and 65.4 ± 0.7 mg/L, respectively, for Fe(II), which is highly consistent with the results for extraction of vivianite alone using 0.2% 2,2'-bipyridine and 0.2% 2,2'-bipyridine + 0.1 M KCl. These results could validate that 0.2% 2,2'-bipyridine + 0.1 M KCl is an ideal extractant for extracting Fe(II)-P, with a very high selectivity.

3.3. Fractionation of P in sediments using the innovative SEDEX

Based on the main composition of sedimentary P and the optimized procedures for Fe(II)-P extraction, sedimentary P was sequentially divided into five fractions, i.e. loosely-bound P, Fe(II)-P, CDB-P, Ca-P and O-P. The loosely-bound P in Donghu Lake sediments ranged from 29.4 to 68.6 mg/kg DW (mean = 46.1), accounting for 3.9–4.9% of TP (Table 4). Fe(II)-P was 95.8–835.9 mg/kg DW (mean = 379.0), equivalent to 16.1–50.5% of TP. CDB-P, Ca-P and O-P were 74.2–271.9, 217.7–342.4, and 156.7–292.9 mg/kg DW, respectively, accounting for 12.5–22.6%, 16.5–45.0% and 17.7–26.4% of TP, respectively. According to the means, the distribution of P fractions was ranked in decreasing order as Fe(II)-P > Ca-P > O-P > CDB-P > loosely-bound P. The sum of P fractions was similar to the TP obtained using the digestion method, suggesting that the procedures developed can extract nearly all P in sediments.

Fe(II)-P in surface sediments (0–20 cm) was commonly higher than in bottom layers, and the heavier the P load, the higher the percentage of Fe(II)-P (Table 4), suggesting that external P flowing into Donghu Lake sediments predominantly appeared as Fe(II)-P. The correlation analysis between concentrations of TP and Fe(II)-P, Ca-P, O-P, and CDB-P respectively (Fig. 4), showed that Fe(II)-P was most significantly correlated with TP ($R^2 = 0.93$), while O-P and CDB-P were less significantly correlated with TP ($R^2 = 0.85$ and $R^2 = 0.71$, respectively). In contrast, Ca-P was not correlated with TP. This indicates that Fe(II)-P is an extremely important and independent P fraction in sediments, even though Fe(II)-P has been ignored in nearly all universal P fraction method until now. CDB-P is defined as P bound to ferric and aluminum oxides (Ruttenberg, 1992) and is generally considered the dominant matrix that binds P in Donghu Lake sediments (Zhang et al., 2015; Zhou et al., 2001). After Fe(II)-P is fractionated as an independent fraction, the percentage of CDB-P in sediments is even lower than that of O-P. Thus, when Fe(II)-P is fractionated as an independent P fraction, the

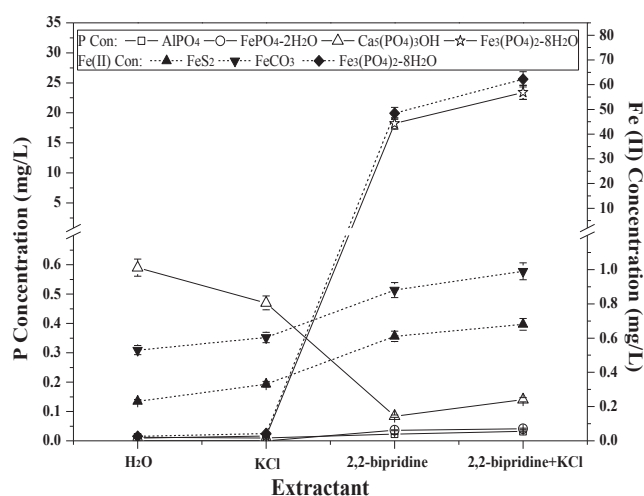


Fig. 2. Extraction characteristics of extracting agents (H₂O, KCl, 2,2-bipyridine and 2,2-bipyridine + KCl) for several individual Fe(II)- and P-bearing minerals under identical conditions (200 rpm, 50 °C). Error bars represent standard deviations of three replicates analysis.

contributions of ferric and aluminum oxides in sediments to P retention needs to be re-evaluated.

3.4. Evaluation of extracted Fe(II)-P in sediment

As shown above, Fe(II)-P extracted with our method dominated over sedimentary P. To clarify the extraction target of 2,2'-bipyridine + 0.1 M KCl, dynamics of Ca²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Al³⁺ and Fe³⁺ in extracts were monitored (Fig. 5). With increased extraction time, all Ca²⁺, Mn²⁺, Mg²⁺, and Fe²⁺ concentrations also increased to different degrees. Al³⁺ tended to decrease and Fe³⁺ was almost not detected by colorimetry (data not shown), which indicates that the contribution of Al³⁺ and Fe³⁺ to released P was negligible. For Mg²⁺ and Mn²⁺, their concentrations in extracts were several orders of magnitude lower than those of P; thus, their contribution to released P was extremely small.

The released P might have come from dissolution of either Fe²⁺- or Ca²⁺-bearing minerals. When extraction of P reached equilibrium, Fe(II) concentration increased by 4 orders of magnitude, while Ca²⁺ was nearly 100 times as high as that in the initial extraction. The correlation analysis (Fig. S2) between Fe²⁺ or Ca²⁺ and P in extracts of 2,2'-bipyridine + 0.1 M KCl indicated that the amount of P released depended greatly on dissolution of Fe²⁺ and Ca²⁺; however, results for Ca²⁺ in extracts of 0.1 M KCl suggested that a small amount of P was related to the dissolution of Ca²⁺-bearing minerals. According to the P released by KCl, the percentage of P related to Ca²⁺ in Fe(II)-P was less than 10%. Thus the Fe(II)-P

Table 3

Recovery of standard reference minerals (Fe₃(PO₄)₂·8H₂O) added to the natural sediment extracted by this innovative sequential extraction procedure.

Original Fe(II)-P (mg/kg)	Added Fe (II)-P (mg/kg)	Extracted Fe(II)-P (mg/kg)	Recovery rate	RSD*
202.4	160.6	332.1	91.5%	0.8%
202.4	160.6	335	92.3%	
202.4	160.6	329.6	90.8%	
211.2	200.7	409.4	99.4%	1.0%
211.2	200.7	406.5	98.7%	
211.2	200.7	414.4	100.6%	
213.6	240.8	405.8	89.3%	0.3%
213.6	240.8	403.1	88.7%	
213.6	240.8	404.9	89.1%	

Notes: RSD* is the relative standard deviation of all Fe(II)-P extracted in three replicates analysis, the used natural sediment sample is from S1, 0–10 cm.

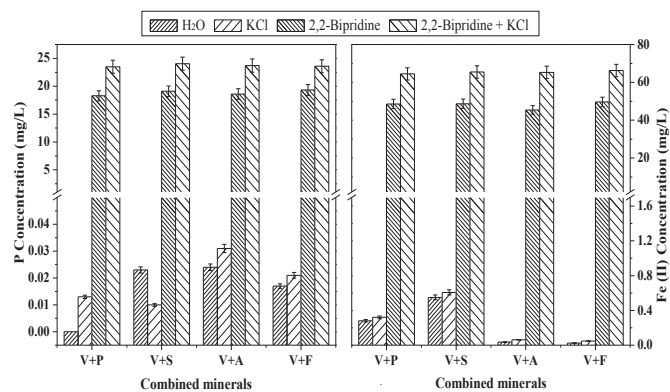


Fig. 3. Extraction characteristics of extracting agents (H₂O, KCl, 2,2-bipyridine and 2,2-bipyridine + KCl) for several Fe(II)- and P-bearing minerals in combination under identical conditions (200 rpm, 50 °C). “V” means vivianite (Fe₃(PO₄)₂·8H₂O), “P” means pyrite (FeS₂), “S” means siderite (FeCO₃), “A” means AlPO₄, and “F” means FePO₄·2H₂O respectively. Error bars represent standard deviations of three replicates analysis.

obtained in the new SEDEX came mainly from dissolution of Fe²⁺- and P-bearing minerals and then from labile Ca²⁺- and P-bearing minerals. To further test the above inference, sediment minerals were analyzed after Fe(II)-P was extracted (Fig. 6). Vivianite was present in sediments before Fe(II) extraction but not afterwards. Some pyrite was also dissolved by 2,2'-bipyridine. A high molar ratio of Fe(II):P suggested that the dissolved Fe(II) came not only from vivianite but from other Fe(II) minerals such as pyrite.

3.5. Application of the new SEDEX to oxidized sediments

Fe(II) in sediments rapidly oxidizes during drying, and the forms of sedimentary P change. To test the practicality of the new SEDEX, the P fractions of air-dried sediments from Donghu Lake were fractionated. Some of the Fe(II) oxidized during air drying (Table 1). For instance, mean total Fe(II) content in fresh anaerobic sediments was 17.14 mg/g DW but decreased to 8.19 mg/g DW after air drying. As a result, the percentage of Fe(II)-P greatly decreased. As mentioned, mean Fe(II)-P content in fresh anaerobic sediments (379.0 mg/kg DW) significantly decreased (to 279.3 mg/kg DW) when sediments were air-dried (Fig. 7). Conversely, mean CDB-P content significantly increased from 187.1 to 280.7 mg/kg DW. For other P fractions, O-P remained constant, Ca-P significantly decreased, and loosely-bound P significantly increased ($P < 0.05$, $n = 9$), which is consistent with previous results (Lukkari et al., 2007a,b; Kraal et al., 2009; Zhang et al., 2013, 2014). Kraal et al. (2009) concluded that P fractionation in anoxic, carbonate-poor

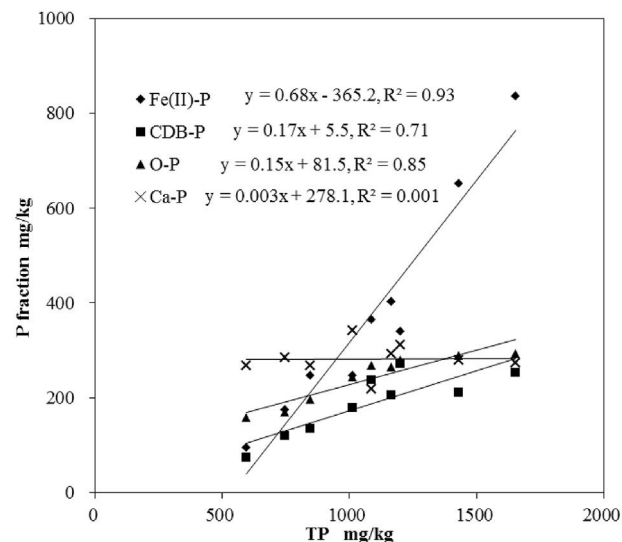


Fig. 4. Relationship between different P fractions and total P (TP) in sediments.

sediments is strongly affected by pyrite oxidation that occurs when these sediments are exposed to oxygen; pyrite oxidation produces sulfuric acid and iron oxyhydroxides. The acid dissolves apatite, and the released phosphate is subsequently bound in the freshly formed iron oxyhydroxides. Pyrite oxidation thus leads to a conversion of authigenic Ca-P to Fe-bound P. These results indicate that shielding sediments from atmospheric oxygen is vital to preserve the *in situ* P fractionation and to enable a valid reconstruction of P cycling based on investigating the transformation of sedimentary P records using the new SEDEX. Some pyrite was also dissolved by 2,2'-bipyridine. A high molar ratio of Fe(II):P suggested that the dissolved Fe(II) came not only from vivianite but from other Fe(II) minerals such as pyrite.

4. Discussion

4.1. Reliability of the innovative SEDEX

Sequential extraction of sedimentary P is based mainly on the chemical properties of sedimentary P. Extraction conditions may also affect the percentages of P fractions. In our method, the Fe(II)-P fraction was extracted with 0.2% 2,2'-bipyridine + 0.1 M KCl at 50 °C for 24 h, which was necessary to evaluate the effect of Fe(II)-P extraction on other P fractions. To evaluate the reliability of the SEDEX developed, the P fractions after adding Fe(II)-P extraction were compared to those obtained by the slightly modified

Table 4
P fractions of fresh sediments in Donghu Lake extracted by this innovative sequential extraction procedure.

Site	Depth cm	Loosely- P mg/kg DW	Fe(II)-P	CDB-P	Ca-P	O-P	TP*	TP**
S ₁	0–10	40.2 ± 0.1	246.2 ± 44.0	178.8 ± 20.4	342.4 ± 5.6	243.7 ± 2.9	1011.2 ± 21.9	896.9 ± 13.7
	10–20	36.7 ± 0.2	174.4 ± 11.8	118.9 ± 19.6	284.3 ± 13.6	168.8 ± 25.4	746.3 ± 31.8	751.2 ± 24.1
	20–30	29.4 ± 4.2	95.8 ± 6.9	74.2 ± 2.8	267.6 ± 12.0	156.7 ± 20.9	594.3 ± 49.0	611.0 ± 30.9
S ₂	0–10	48.6 ± 5.7	339.8 ± 11.0	271.9 ± 1.8	310.9 ± 15.7	278.5 ± 8.7	1201.1 ± 24.4	1291.8 ± 9.3
	10–20	47.8 ± 0.7	402.3 ± 7.8	204.8 ± 18.8	293.3 ± 7.5	263.9 ± 13.9	1164.2 ± 55.3	1221.4 ± 15.5
S ₃	20–30	34.2 ± 1.8	246.4 ± 19.4	135.2 ± 1.3	268.5 ± 3.1	194.9 ± 14.7	844.9 ± 7.1	815.9 ± 6.9
	0–10	68.6 ± 0.2	652.3 ± 23.9	210.1 ± 23.6	278.6 ± 5.9	288.2 ± 17.1	1429.2 ± 27.6	1533.6 ± 36.8
	10–20	63.9 ± 4.2	835.9 ± 56.2	252.1 ± 37.6	273.3 ± 14.9	292.9 ± 11.7	1654.2 ± 23.0	1674.1 ± 28.4
	20–30	45.0 ± 2.2	363.7 ± 10.7	238.2 ± 20.9	217.7 ± 16.6	267.7 ± 1.3	1087.2 ± 44.6	1061.6 ± 37.9
Overall mean		46.1 ± 2.1	379.0 ± 14.3	187.1 ± 35.9	281.8 ± 9.9	239.5 ± 12	1081.4 ± 29.1	1095.3 ± 21.1

Notes: TP* is the sum of all P fractions; TP** is obtained through the digestion method; data are mean ± standard deviation of three replicates analysis.

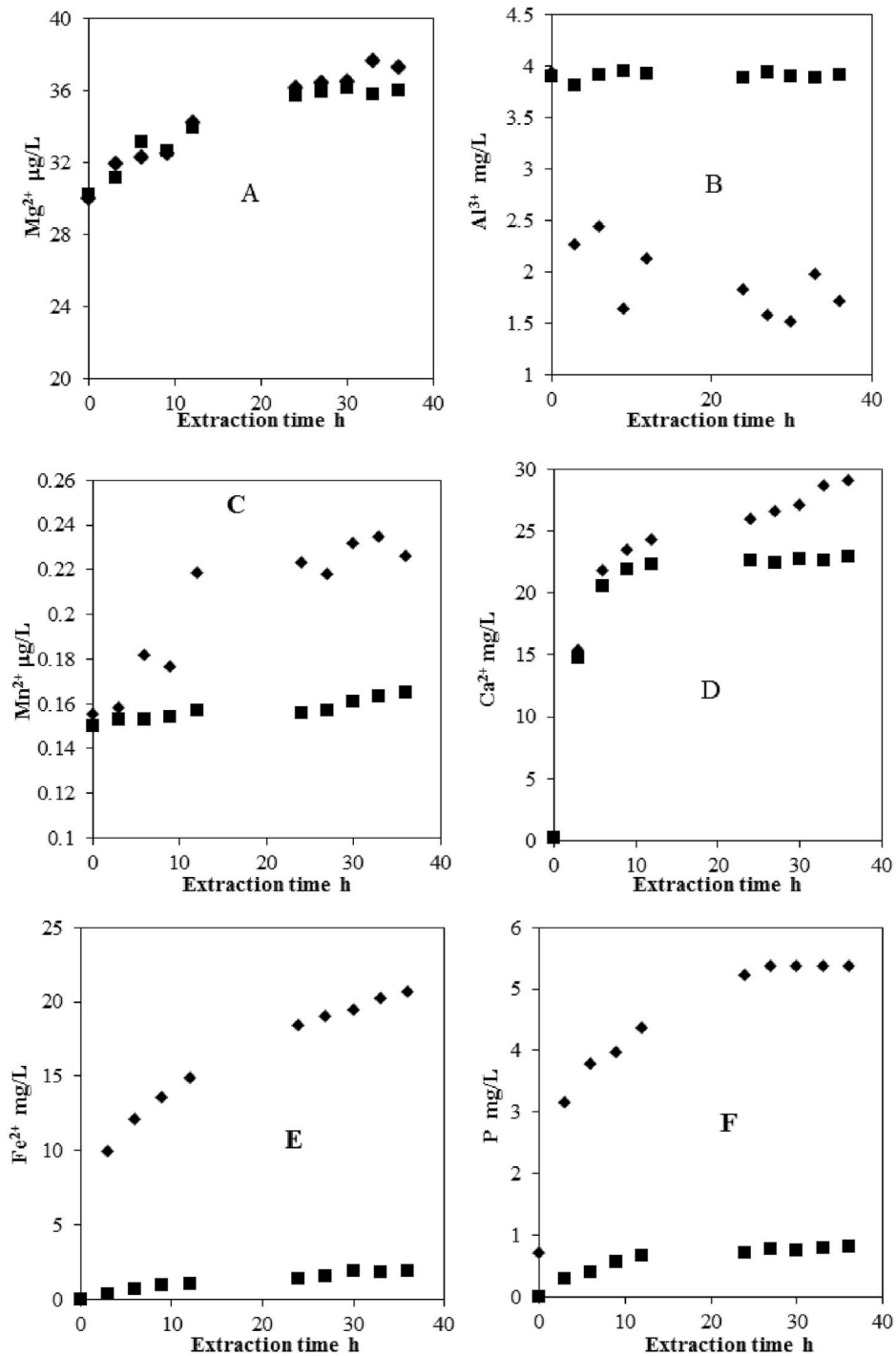


Fig. 5. Dynamics of cations and P in extracts at 50 °C of surface sediment at sampling point S3. Solid diamonds indicate the mixed extract of 2,2'-bipyridine and KCl, which was composed of 0.2% bipyridine and 0.1 M KCl. Solid squares indicate the simple extract of 0.1 M KCl, which was chosen as a control. The solid:liquid ratio was 1:100.

Ruttenberg's method. After Fe(II)-P was extracted, both CDB-P and Ca-P significantly decreased ($P < 0.05$) (Fig. 8). O-P obtained by both methods was similar ($P > 0.05$), suggesting that it is not affected by Fe(II)-P extraction. CDB-P in Ruttenberg's procedures is extracted by a citrate + dithionite + bicarbonate solution. Both citrate and dithionite are able to react with the Fe(II) produced from reduced Fe(III) as well as bulk Fe(II) in sediments. Since the bulk Fe(II) in vivianite was dissolved by both citrate and dithionite, the P in vivianite was extracted. Thus, the CDB-P obtained by Ruttenberg's method should include partial Fe(II)-P. When this portion of Fe(II)-P

is preferentially extracted with 2,2'-bipyridine, the amount of CDB-P inevitably decreases. The results indicate that using 2,2'-bipyridine as an extracting agent distinguishes Fe(II)-P from CDB-P. The presence of KCl is considered to indicate the effect of Fe(II)-P extraction on Ca-P. Among sedimentary P, a minor portion is labile calcium phosphate, such as $\text{Ca}_3(\text{PO}_4)_2$, whose solubility is related to the effect of salt. Solubility increases with neutral salt concentration (Inouye et al., 1998), which agrees with our results. Though P was released in the control (with KCl alone) (Fig. 5F), the amount of P released by KCl is less than 10% Fe(II)-P. According to

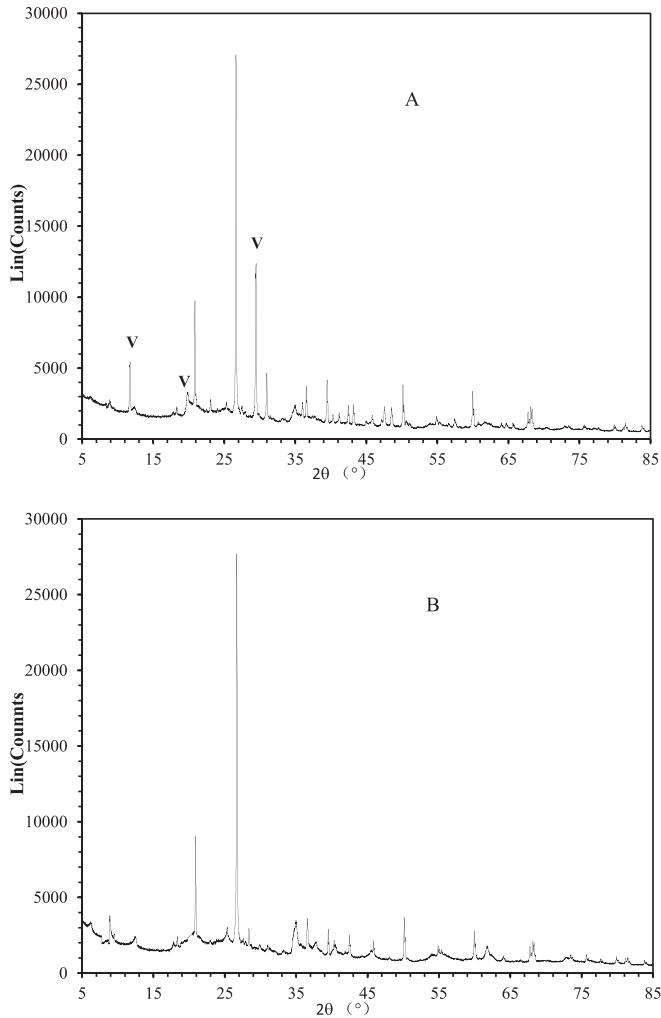
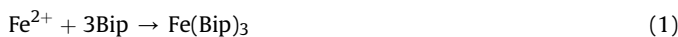


Fig. 6. Change in mineral components induced by 2,2′-bipyridine in surface sediment at sampling point S3. XRD pattern of sediment (A) before and (B) after extraction. “V” means vivianite.

both methods, the Ca-P in Fe(II)-P is estimated as less than 3.5% of TP. Thus, the effect of Fe(II)-P extraction on Ca-P is limited. O-P in our method is determined after all inorganic P is extracted by using ignition and acid dissolution. Because 2,2′-bipyridine is an organic compound that readily decomposes during ignition, its residue had nearly no impact on O-P.

4.2. Solubility of vivianite in the extracting agent

Fe(II)-P, extracted with 2,2′-bipyridine, is found at a relatively high percentage in sedimentary P, but the extraction efficiency of Fe(II) is only 21.4% (Li et al., 2012), suggesting that most Fe(II) in sediments is resistant to 2,2′-bipyridine. Furthermore, the molar ratio of Fe(II):P is higher than in the stoichiometry of vivianite. Thus, it is necessary to delineate the solubility of vivianite co-existing with other Fe(II)-bearing minerals. The mechanism for dissolution of Fe(II)-bearing minerals enhanced by 2,2′-bipyridine is described by the following reaction:



where Fe^{2+} is soluble Fe(II) equilibrated with respect to Fe(II)-bearing minerals such as vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$), pyrite (FeS ,

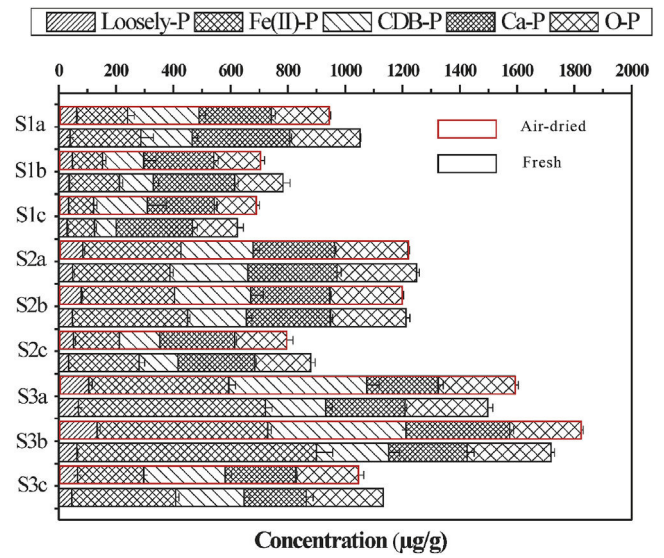


Fig. 7. Comparison of mean concentrations of sedimentary P forms in both fresh and air-dried sediments in three sediment layers at three sampling points. S1, S2 and S3 indicate sediment sampling sites, while “a”, “b” and “c” represent 0–10, 10–20 and 20–30 cm depths, respectively. Error bars represent standard deviations of three replicates analysis.

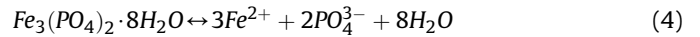
FeS_2), siderite (FeCO_3) and magnetite (Fe_3O_4); “Bip” represents 2,2′-bipyridine; and $\text{Fe}(\text{Bip})_3$ is the complex of Fe(II) with 2,2′-bipyridine. When dissolution of Fe(II)-bearing minerals reaches equilibrium, the relationship among soluble Fe(II), Bip and $\text{Fe}(\text{Bip})_3$ is constructed as follows:

$$K_{\text{Fe-bip}} = \frac{[\text{Fe}(\text{Bip})_3]}{[\text{Fe}^{2+}][\text{Bip}]^3} \quad (2)$$

$$[\text{Fe}^{2+}] = \frac{[\text{Fe}(\text{Bip})_3][\text{Bip}]^{-3}}{K_{\text{Fe-bip}}} \quad (3)$$

where $K_{\text{Fe-bip}}$ is the stability constant of $\text{Fe}(\text{Bip})_3$, and $K_{\text{Fe-bip}}$ is ca. 1.0×10^{15} at 50 °C, determined by our previous experiments.

The dissolution of vivianite is described as:



$$K_{\text{SP}_{\text{vivianite}}} = [\text{Fe}^{2+}]^3 [\text{PO}_4^{3-}]^2 \quad (5)$$

where $K_{\text{SP}_{\text{vivianite}}}$ (1.0×10^{-36} at 50 °C) is the solubility constant of vivianite (Alborno and Tomson, 1994).

Hydrolysis of PO_4^{3-} is given as:



Based on Equations (6–8), the relationship between the concentration of soluble PO_4^{3-} and TP is calculated at a given pH as:

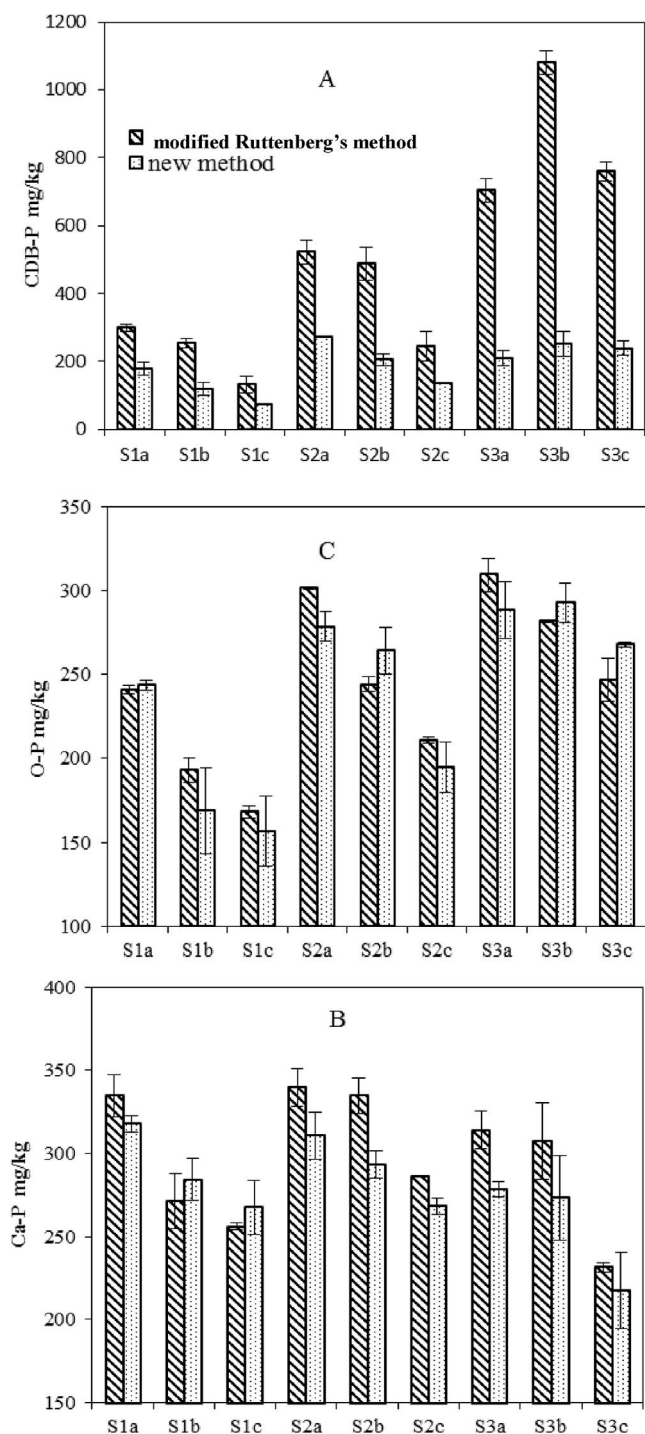


Fig. 8. Evaluation of effects of Fe(II)-P extraction on other P fractions. The t-values of CDB-P, Ca-P and O-P obtained by both methods are 3.66, 2.77 and 0.75, respectively, ($t_{8, 0.05} = 2.31$, $n = 9$). S1, S2 and S3 indicate sediment sampling sites, while "a", "b" and "c" represent 0–10, 10–20 and 20–30 cm depths, respectively. Error bars represent standard deviations of three replicates analysis.

$$P_{total} = \left(1 + \frac{10^{-pH}}{K_3} + \frac{10^{-2pH}}{K_2 K_3} + \frac{10^{-3pH}}{K_1 K_2 K_3} \right) [PO_4^{3-}] \quad (9)$$

where K_1 , K_2 and K_3 are dissociation constants of H_3PO_4 , $H_2PO_4^-$ and HPO_4^{2-} , respectively: $K_1 = 7.5 \times 10^{-3}$, $K_2 = 6.3 \times 10^{-8}$ and $K_3 = 4.4 \times 10^{-13}$ (Acquaviva et al., 2014). When the dissolution of

vivianite approaches equilibrium, the concentration of Fe^{2+} is calculated based on Equation (5) as follows:

$$[Fe^{2+}] = \sqrt[3]{K_{SP, vivianite} \cdot [PO_4^{3-}]^{-2}} \quad (10)$$

To combine Equation (3) with Equation (10), the relationship between extracted Fe^{2+} and PO_4^{3-} is obtained as:

$$[Fe(Bip)_3] [PO_4^{3-}]^{\frac{2}{3}} = \sqrt[3]{K_{SP, vivianite} \cdot K_{Fe-bip} \cdot [Bip]^3} \quad (11)$$

where [Bip] is the concentration of 2,2'-bipyridine as Fe(II) extraction approaches equilibrium. Using Equations (9) and (11) and assuming that the molar ratio of soluble $Fe(Bip)_3:TP$ is n when extraction reaches equilibrium, the solubility of vivianite co-existing with other Fe^{2+} -bearing minerals in 2,2'-bipyridine can be estimated (Fig. S3). For example, when the molar ratio of soluble $Fe(Bip)_3:TP$ is 1.5, P extracted from the dissolution of vivianite is 62.9 mg/100 mL; the percentage of P calculated in sediments equals 6.3%. With an increased $Fe(Bip)_3:TP$ molar ratio, the ability of bipyridine to extract P in vivianite decreases. Even so, when the $Fe(Bip)_3:P$ molar ratio is ca. 30, extracted P is 10.4 mg/100 mL; the percentage of P in sediment is as high as 1.0%. It was reported that P in vivianite accounts for 50% of TP in sediments. Evidently, 0.2% 2,2'-bipyridine is sufficient to extract all vivianite in the normal amount of P content in sediments. If vivianite in sediments is exceedingly high, it is necessary to increase the concentration of 2,2'-bipyridine.

5. Conclusions

Fe(II)-P is a crucial P form in sediments. New SEDEX were developed by optimizing Fe(II)-P extraction procedures. Sedimentary P is sequentially divided into five fractions, i.e. P loosely bound to the surface of sediment particles (loosely-bound P, extracted with 1.0 M $MgCl_2$), P precipitated by Fe(II) (Fe(II)-P, extracted with 0.2% 2,2'-bipyridine + 0.1 M KCl), reductant-soluble P and Fe/Al-bound P (CDB-P, extracted with citrate dithionite bicarbonate), apatite (Ca-P, extracted with 1.0 M HCl) and organic P (O-P, calcination at 550 °C followed by 1.0 M HCl extraction). The advantage of the new SEDEX is that it can distinguish the contribution of different matrices of P retention in fresh sediments and can also investigate the transformation of sedimentary P during sediment oxidation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2016.07.058>.

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Thèse de Sen Gu

Titre et résumé en français de la thèse

Titre de la thèse

Une analyse combinée de terrain et de laboratoire des mécanismes et facteurs responsables des relargages du phosphore dissous et colloïdal dans les zones humides ripariennes ;

Résumé de la thèse

Contexte et objectifs

Le Phosphore (P) est un nutriment clé responsable de l'eutrophisation croissante des eaux de surface. Les émissions diffuses de P en provenance des sols agricoles sont une des principales sources en cause de ce processus, constituant de ce fait une menace pour la santé des écosystèmes aquatiques. Dans l'optique de protéger les cours d'eau des émissions diffuses de P agricole, une stratégie largement utilisée de par le monde consiste à implanter des bandes enherbées à l'interface entre champs cultivés et réseau hydrographique avec l'idée de capter le P. De fait, ces bandes enherbées ont prouvé leur efficacité dans la réduction des émissions de P particulaire, par leur capacité à intercepter et stocker physiquement les particules de sol porteuses de P. Ceci étant, l'efficacité de ces bandes enherbées ripariennes du point de vue de la rétention du P dissous (P_{dis}) est beaucoup plus problématique. Dans certains cas, les bandes enherbées ripariennes ont même été prouvées être des sources de P_{dis} au cours d'eau. Comprendre les mécanismes de solubilisation et de mobilisation du P_{dis} dans ces zones est devenu un enjeu majeur des recherches visant à lutter contre l'eutrophisation des eaux de surface.

Des suivis préliminaires réalisés sur le bassin versant agricole de Kervidy-Naizin situé dans l'Ouest de la France ont mis en évidence que les sols des zones ripariennes de ce bassin étaient des zones sources de P_{dis} pour le cours d'eau (P_{dis} ici = P réactif au molybdate, noté aussi MRDP pour *molybdate reactive dissolved P*), faisant de ce bassin un site particulièrement bien adapté pour élucider les différents mécanismes impliqués dans le processus de libération de P_{dis} au sein des bandes enherbées ripariennes. En utilisant les données de composition des solutions de sol recueillies durant l'année hydrologique 2013-2014 le long de deux transects (G et K) internes à ce bassin, nous avons pu révéler que deux mécanismes biogéochimiques paraissaient impliqués dans ce processus de libération: 1) le ré-humectage du sol après l'été ; 2) la dissolution réductrice des oxyhydroxydes de Fer du sol pendant les périodes anoxiques survenant lors de la saturation hivernale en eau des sols. Les données ont montré que ces deux processus entraînaient des variations temporelles de la teneur en MRDP des solutions de sol, en phase avec les mouvements de la nappe. Des variations spatiales de la teneur en MRDP des solutions de sol ont également été identifiées au cours de cette première année de suivi, que nous avons supposé être en lien avec des différences dans la propension des oxydes de Fer des sols des zones ripariennes étudiées à être réduits, et/ou à des différences de spéciation du P au niveau de la phase solide du sol. Enfin, cette première année de suivi a montré que le MRDP libéré dans les solutions de sol était transféré par écoulement de sub-surface vers le cours d'eau, confirmant le rôle de zones source

de P_{dis} pour les rivières des sols des bandes enherbées ripariennes.

Partant de ces résultats préliminaires, plusieurs questions ont émergé qui ont constitué le cœur de cette thèse: 1) l'année 2013-2014 ayant été une des années les plus humides des dix dernières années, et les deux mécanismes présumés de relargage de P_{dis} dépendant très fortement de la dynamique de la nappe, la question s'est posée de savoir si ces relargages reflétaient un fonctionnement normal ou atypique des zones ripariennes étudiées ; autrement dit, nous avons voulu savoir comment ces deux mécanismes répondaient dans le cadre de conditions hydroclimatiques plus sèches, et quelles étaient les variations temporelles de concentrations en P_{dis} , tant dans les sols que dans la rivière, que des alternances d'années sèches et humides étaient capables d'engendrer ; 2) sur le plan spatial, la question s'est posée de savoir quelles étaient les causes exactes des variations de réponses observées entre les sols des transect G (fort relargage de MRDP) et K (faible relargage de MRDP) ; ces variations spatiales reflètent-elles de véritables différences dans la capacité nette de relargage de P_{dis} par les sols de ces deux transects, ou reflètent-elles des variations dans la nature chimique du P_{dis} libéré? Autrement dit, l'absence de MRDP libéré dans les sols du transect K dénote-t-elle une absence totale de libération de P_{dis} , ou bien est-elle due à la libération de formes de P_{dis} non réactives au molybdate, comme du P colloïdal ou du P organique, par exemple, et si oui, quelles sont les causes de ces variations de composition du P_{dis} relargué entre transects? 3) Du point de vue des mécanismes, la question s'est posée de savoir si les mécanismes de libération du P_{dis} observés sur le terrain étaient reproductibles au laboratoire, et si oui, de savoir ce que les expériences de laboratoire permettaient de dire quant à leur nature exacte ? Enfin 4) concernant le devenir du P_{dis} libéré dans les sols, la question a été soulevée de savoir si la totalité de ce P était transférée au cours d'eau quelles que soient les conditions hydroclimatiques, ou bien si une partie du P_{dis} pouvait être retenue le long des berges, du fait de la réoxydation et de la re-précipitation des oxydes de Fer au niveau de ces zones d'interfaces oxiques entre sols et cours d'eau ?

Stratégie de recherche

La stratégie de recherche a consisté à combiner des approches de terrain et de laboratoire. Sur le terrain, nous avons répété les suivis réalisés en 2013-2014, en 2014-2015 et 2015-2016, aboutissant ainsi à la collecte de données de composition des eaux du sol et des eaux de rivière au cours de trois années hydrologiques successives. Contrairement à l'année 2013-2014 où seule les teneurs en MRDP ont été mesurées, les années 2014-2015 et 2015-2016 ont vu également la mesure des teneurs en P colloïdal (P_{coll}) et P_{dis} organique ($P_{dis-org}$). Au laboratoire, des expériences simulant la réduction des oxydes de Fer du sol et les alternances de conditions sèches-humides subies par les sols des bandes enherbées ripariennes ont été réalisées. L'objectif ultime visé en réalisant ce travail combiné de terrain et de laboratoire était de clarifier les mécanismes de production, la spéciation et le transfert, sous toutes ses formes chimiques, du P_{dis} de manière à contribuer à l'élaboration de stratégie de réduction des émissions de P dans les bassins versants agricoles.

Résultats et interprétations

Données de terrain: Les données hydroclimatiques ont montré que les précipitations totales et le débit du cours d'eau à l'exutoire en 2014-2015 (année 2) et 2015-2016 (année 3) étaient deux fois plus faibles qu'en 2013-2014 (année 1). Après la remontée de la nappe à l'automne, les sols des zones ripariennes ont connu des alternances de périodes sèches et humides lors des années 2 et 3, contrairement à l'année 1 durant laquelle les sols étaient restés continuellement saturés en eau durant la période hivernale. Ces variations temporelles des conditions hydriques du sol ont été particulièrement

marquées dans le transect G, les sols du transect K restant, eux, saturés en eau quelle que soit l'année. Cette différence de comportement a été reliée à une différence de topographie, le transect K étant plus plat que le transect G. Au niveau du transect G, les variations interannuelles de la dynamique de nappe engendrées par les variations climatiques ont eu des répercussions sur les concentrations en MRDP des eaux du sol, les années 2 et 3 se caractérisant par un pic principal au moment de la remontée de nappe, contrairement à l'année 1 où un 2^{ème} pic a été observé en phase avec la réduction des oxydes de Fer du sol. L'absence de ce deuxième pic lors des années 2 et 3 a été mise en lien avec l'absence de périodes prolongées de saturation en eau du sol lors de ces années sèches, absence empêchant le déclenchement du processus de réduction des oxydes de Fer du sol. Du point de vue des variations spatiales de concentrations des teneurs en MRDP des eaux du sol, les années 2 et 3 ont confirmé l'opposition des eaux des transects K et G. Toutefois, les données des années 2 et 3 ont montré que l'absence de MRDP dans les eaux des sols du transect K ne signifiait pas une absence de relargage de P_{dis} par ces sols. De fait, il est apparu que les sols de ce transect relarguaient du P_{dis} non réactif au molybdate, P_{dis} que les analyses ont montré être de nature colloïdale (>50% du P_{dis}) ou organique. Du P_{coll} a également été observé dans les eaux des sols du transect de Guériniec, mais en moindre proportion (<30%). Cette présence de P_{coll} dans les eaux des sols étudiés confirme les travaux récents publiés dans la littérature suggérant un rôle important des vecteurs colloïdaux dans le transport du P dans les sols. Des analyses physico-chimiques ont démontré que ces vecteurs étaient dans le cas du bassin étudié principalement des nano-oxydes de Fer associés à de la matière organique, et que leur plus grande abondance dans les eaux des sols du transect K était liée à une spéciation solide du P dans les sols de ce transect dominée par des formes organiques du P. Du point de vue du transfert à la rivière, le flux de P_{dis} transféré lors des années 2 et 3 est apparu beaucoup plus faible que lors de l'année 1. La réduction plus importante du flux de P_{dis} par rapport au flux d'eau a été mise en lien avec l'existence de processus de rétention au niveau des berges lors des années 2 et 3, stimulées par le caractère plus sec de ces deux années et la prépondérance de conditions oxydantes au niveau des berges lors de ces deux années.

Incubations au laboratoire en conditions anoxiques: Les expériences réalisées ont confirmé le rôle des conditions anoxiques comme conditions favorisant la libération de P_{dis} dans les sols des zones ripariennes. La dissolution réductrice des oxyhydroxydes de Fer du sol n'est cependant pas le seul processus impliqué, un autre processus étant la désorption du P liée à la hausse du pH causée par les réactions de réduction. Les résultats obtenus démontrent que l'augmentation de pH contrôle la libération de P_{dis} dans les sols riches en matière organique, alors que ce relargage est contrôlé principalement par la réduction des oxydes de Fer dans les sols pauvres en matière organique. Les données expérimentales démontrent également que l'apport de sédiments issus des sols agricoles amont accroît le relargage de DP dans les sols des bandes enherbées ripariennes, probablement en raison de la dissolution des oxydes de Fer de ces mêmes sédiments par les bactéries feroréductrices présentes dans ces mêmes sols.

Simulation au laboratoire des alternances sec-humide: Les expériences en laboratoire ont confirmé le rôle des processus de réhumectation comme processus clé causant le relargage de P_{dis} dans les sols des zones ripariennes. Les résultats ont démontré que le P_{dis} relargué consistait non seulement de "vrai" DP inorganique et organique, mais aussi de P colloïdal, le P colloïdal et le $P_{dis-org}$ étant les plus réactifs aux alternances sec-humide. Les données ont aussi révélées que ces différentes formes de P provenaient de différentes sources dans le sol (mésoporosité et macroporosité pour P_{coll} et le P_{dis} organique; microporosité pour DP inorganique), et que la quantité de P_{coll} relargué était positivement corrélée avec la teneur en matière organique et la taille de biomasse microbienne du sol. Ces mêmes expériences ont confirmé le rôle des alternances sec-humide comme facteur favorisant la libération de P_{dis} dans les sols des zones

ripariennes.

Conclusions et perspectives

Les suivis de terrain et les expériences de laboratoire réalisés dans le cadre de cette thèse ont permis de jeter des éclairages nouveaux sur les interactions complexes entre hydroclimat, topographie, composition des sols et processus biogéochimiques à l'origine des relargages de P_{dis} observés dans les sols des zones ripariennes des bassins versants agricoles. Les résultats acquis ont montré que la complexité de ces interactions conduisait à de fortes variabilités spatio-temporelles du point de vue de la capacité de ces sols à relarguer du P_{dis} , ainsi que du point de vue de la capacité du P_{dis} relargué à être effectivement transféré au cours d'eau. Les expériences de laboratoire ont fourni d'importantes clés, que ce soit du point de vue de la nature des mécanismes à l'origine de ces relargages, ou des liens existants entre le statut du P présent dans les sols et la nature chimique du P_{dis} relargué. Prise dans son ensemble, cette thèse fournit de nouveaux éléments de compréhension sur les mécanismes et facteurs à l'origine des émissions agricoles de P aux cours d'eau, émissions que l'on sait être une des causes principales de l'eutrophisation croissante des eaux de surface.

Production scientifique

Articles dans des revues à comité de lecture

Directement lié à la thèse:

- R. Dupas, G. Gruau, **S. Gu**, G. Humbert, A. Jaffrézic, C. Gascuel-Oudou (2015). Groundwater control of biogeochemical processes causing phosphorus release from riparian wetlands. *Water Research*, 84, 307-314
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