Training report

4th year

Restoration of wetlands and impact on the phosphorus cycle

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I. Presentation of the host organization

I worked in the Ecoscience department in Aarhus University for 12 weeks (01/05/23 – 21/07/23) where approximately 275 employees work there. The department's missions are, education, research and advise government agencies on solving societal problem through research-based knowledge. This department is conducting world class research on different areas including (*AU Ecoscience - Themes and Projects*, s. d.):

- The Artic (the department works to advise Greenlandic authorities on mineral resource activities and the environmental problems that it creates. They also work on the effects of climate change on Artic ecosystems).

- Marine ecology (understanding the function and structure of different marine ecosystems, from coastal zone to open seas but also from the Artic to tropical systems. The department examine closely at separating changes that occur because of human activity and changes that occur from natural variations).

- Terrestrial ecosystems (the department works to develop strategies for a more sustainable agriculture and forestry. They are the data center for 'Dry nature and species' for the national monitoring program NOVANA).

- Fresh water ecology (the department study and measure biological and chemical processes in fresh ecosystems. Their research includes lake and river restoration but also nutrient dynamics, greenhouse gases and interaction between catchment and freshwater. They take a special look at nitrogen and phosphorus release from fields and transported to streams).

The department collaborates on research themes or approaches that are frequently shared or commonly found within the four academic strengths (Figure 1).

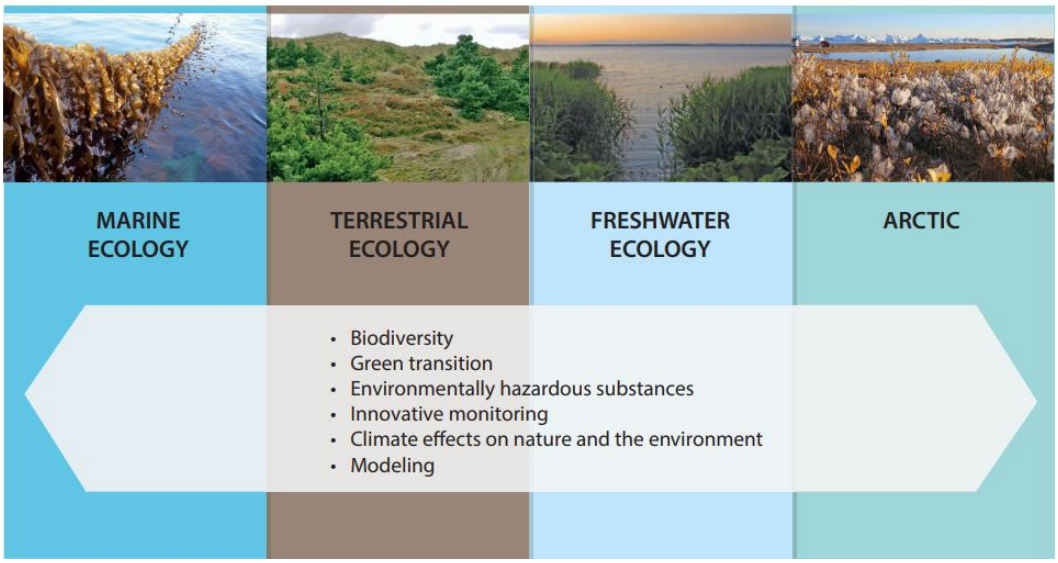


Figure 1 - Ecoscience department's research themes within the four academic strengths (Department of Ecoscience, 2021)

II. Introduction

A. Context of wetland restoration

In Europe, many wetlands were drained to promote agricultural activities but also for urban development and water reclamation. Areas of wetlands, water holes and lakes significantly decreased because of this drainage within the last centuries (Hoffmann & Baattrup-Pedersen, 2007). Streams have been modified to insure a more efficient runoff of drained water from agricultural lands (Hoffmann & Baattrup-Pedersen, 2007). All these modifications have severe consequences on aquatic ecosystems, like eutrophication due to loss of fertilizers from agriculture. Indeed, intensive agriculture is an important source of nutrient in streams, lakes, and groundwater (Grizetti et al., 2011). To reduce nutrients losses, the European Union adopted a Frame Water Directive in the year 2000 to prevent and reduce water pollution but also to improve the state of aquatic ecosystems, to promote a more sustainable use and limit the effects of floods and droughts. This directive states that good ecological quality is expected by 2015 in water bodies and if it is not achieved by then it is expected by 2027.

Denmark started early with wetland restoration since a national program to reduce nitrogen losses was implemented in 1998. This Danish Action Plan for the Aquatic Environment II (DAPAE-II) included the restoration of 16 000 ha of wetlands (Audet et al., 2020). Wetlands are important because they are significant carbon and nitrogen sinks. They also are called “kidneys” of landscapes (Mitsch and Gosselink 1986) because they purify water with for example denitrification, which is a process where nitrate (NO_3^-) or nitrite are reduced and ultimately produces molecular nitrogen (N_2). Wetlands therefore act as nutrient buffers between land and streams (Walton et al., 2020). Moreover, they offer habitats for many endangered species within the group of birds, plants, insects but also amphibians. Therefore, they are also denoted as hotspots of biodiversity (Kaden et al., 2023).

B. The issue of phosphorus release after wetland restoration

To have more agricultural land available, Denmark conducted wetland drainage by using different methods: ditches, channelization of the watercourse, tile drains and/or pumping. In response to the drainage of wetlands and intensive agriculture soils accumulated nutrient including nitrogen (N) and phosphorus (P) that can be in mobile mineral forms. As the soil has been drained, the water table has dropped and therefore the oxygen (O_2) has been able to enter deeper into the soil. The contribution of O_2 in the soil leads to the degradation of the organic part due to promoted microbial respiration and thus organic matter becomes mineralized, leading to an accumulation of inorganic nitrogen and phosphorus forms. This is an issue, because when a wetland is rewetted, water slows down the diffusion of oxygen in the soil (because its diffusion is much weaker in this environment), and it leads to anoxic conditions. Phosphorus is bound to several compounds, including iron oxides (Prasad & Chakraborty, 2019). If the area is rewetted, the oxygen in the soil will be completely consumed by reduction processes and the change in redox conditions from oxic to anoxic may result in the reduction of iron-oxides. The ferric iron (Fe^{3+}), which is in its solid form will act as an electron acceptor and become ferrous iron (Fe^{2+}), soluble in water. Therefore, the P that was bound to Fe^{3+} will be released (Aldous et al. 2007).

Rewetting a drained wetland can therefore cause a release of P that has been accumulated in the soil, even decades after the restoration, especially when the area in question was formerly agricultural used (Audet et al., 2020). Hence, restored wetlands may act as sources of phosphorus due to different mechanisms like desorption of dissolved phosphate (Zak & Gelbrecht, 2007) while others

can act as sinks of phosphorus (Audet et al., 2020). Therefore, it is important to understand phosphorus cycle and binding forms depending on environmental conditions like, nutrient status, pH, and redox conditions, in order to assess better the amount of mobilizable phosphorus and estimate P release and retention before rewetting a wetland**.** It is also important to have a long-term monitoring surveillance program to characterize phosphorus storage or release in restored wetlands.

Aarhus University and more precisely the department of Ecoscience is focusing on water quality and therefore on monitoring nitrogen and phosphorus before and after wetland restoration. This research is part of the NIFA project network, which is about peatland restoration and the environmental effects on hydrology, water quality, carbon sequestration and biodiversity and is financed by the Environmental Ministry of Denmark. The department's mission is through research-based knowledge advise government agencies on solving societal problems (*AU Ecoscience - Themes et projects*, s. d.).

For now, three restoration methods are used to try to stop or at least mitigate phosphorus release. It is possible to do a "top-soil removal" (Zak & McInnes, 2022), which consist in removing the upper part rich in nutrients (used then for the agricultural fertilization) and to give again a hydraulic and ecological dynamics more natural to the peat (zone where water circulates well and where there are few nutrients). The second option is to harvest the vegetation. When plants grow, they capture nutrients present in the soil, including nitrogen and phosphorus and when they die, they release those in the soil. Harvesting the vegetation prevent this turn back of nutrients in the soil. Another idea to limit the release of phosphorus is the "slow rewetting". This consists in slowly rewetting a former wetland which would allow the release of small quantities of phosphorus but over a longer time in order to not disturb the downstream systems too much (Zak & McInnes, 2022).

C. Phosphorus in the soil

As mentioned above, rewetting a former wetland may result in a high release of phosphorus from degraded soil layers. Thus, depending on the area's history (drainage, characteristics of the soil, land use…), the restoration can have different impacts like water quality downstream of the wetland's restored area. Phosphorus accumulated in the soil, because of drainage and intensive agriculture, can be found under different forms and can be potentially mobilized by different mechanisms.

In the soil, phosphorus can be found under different forms:

- Dissolved P (which can be inorganic (orthophosphate) or organic)
- Particulate P (which can be inorganic or organic)

The inorganic particulate P can be fixed or adsorbed on the surface of other elements such as iron (Fe) and aluminum (Al) oxides and hydroxides or it can also be bound to other elements such as calcium (Ca), iron, aluminum, or manganese (Mn) (Figure 2). Soils with higher concentrations of iron and aluminum oxides possess a greater capacity to adsorb phosphorus compared to soils that have relatively lower amounts of iron and aluminum oxides (Prasad & Chakraborty, 2019).

The different mechanisms in which P can be released (the major part of phosphorus released with those mechanisms is phosphate, but it can also be dissolved organic P) after rewetting are:

- Phosphorus loosely bounds to the surface of some elements such as iron (Fe) and aluminum (Al) hydroxides can be released when it is in contact with water due to equilibrium reactions including anion exchange.

- “Redox-sensitive P”. In anoxic condition, redox-sensitive P (P bound to redox sensitive elements, mostly Fe^{3+} and Mn) can be released.

- “pH-sensitive P”. When pH is lower or higher than 7, the phosphorus bound to elements that are sensitive to pH will be released because of a chemical dissolution.

- The organic phosphorus can be released by bacteria because they release exoenzymes which induce cleavage of organic bound P.

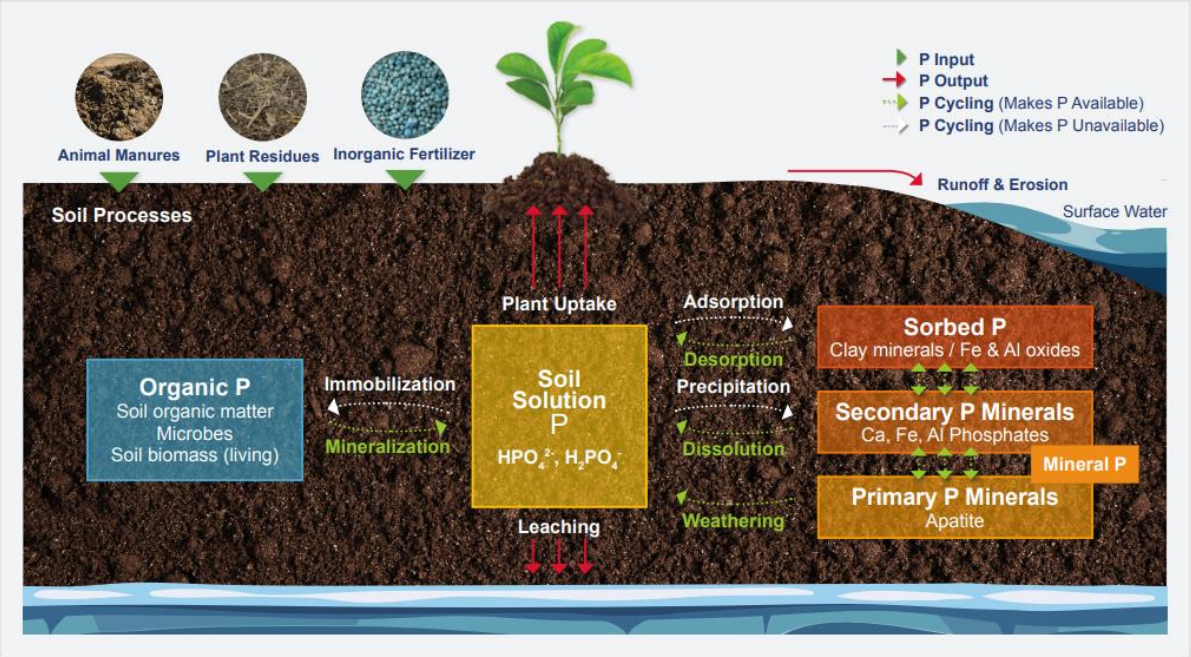


Figure 2 - Phosphorus cycling in the soil (Prasad & Chakraborty, 2019)

In their study, Zak et al. (2008) revealed a strong association between increased concentrations and mobilization rates of Fe and P in rewetted fens. They observed a notable correlation between the quantity of 'reductant soluble P' and the rates of P mobilization. A conservative calculation emphasized the significant role of 'reductant soluble P' as a key source of P mobilization in rewetted fens. This pool is projected to remain a potent P source for approximately 40-60 years in highly decomposed peat layers. Regarding P bound to pH-sensitive elements, it can be taken into account when low-lime fens have undergone acidification (Lamers et al., 1998; Van Dijk et al., 2004 in Zak et al., 2008).

Hoffmann et al. (2011) demonstrated with their results that wetlands capacities to act as buffers for P were varying. Such different results for phosphorus retention in different areas shows that it is hard to establish a phosphorus balance for restored riparian wetlands (Hoffmann et al., 2009 in in Hoffmann et al., 2011). The significant variation observed in P retention across the four riparian wetlands in this study underscores the importance of conducting pre- and post-restoration monitoring for wetland restoration projects in order to demonstrate the effects of rewetting on P retention. Furthermore, it is recommended to conduct extended post-restoration monitoring to account for the potential impact of phosphorus erosion and release processes, which can act as a new source of phosphorus for varying durations and obscure the long-term effectiveness of wetland restorations in terms of phosphorus retention (Hoffmann et al., 2011).

D. Purpose of the internship

The major objective of the internship was the investigation of P binding forms in agricultural altered soils. This work will focus on the investigation of different phosphorus fractions by established chemical extraction method.

These methods allow to characterize phosphorus cycling in soils depending on environmental conditions like nutrient status, pH, and redox conditions, allowing to draw conclusion on the risk of P release upon rewetting. Soil samples will be obtained from different drained peatlands and from a mineral site used for crop production. The drained peatlands will be rewetted in the nearest future and after rewetting a high P mobilization can be expected due to soil mineralization and fertilization during drainage and land use. On the mineral site experimental plots are established where manure applications are tested with different sulfuric acid concentrations. The investigation sites are part of three different projects supported by the environmental ministry of Denmark:

- 1) Rewetting of organic lowland soils in Denmark (NIFA),
- 2) "Environmental effects of acidified slurry",
- 3) "Rewetting of the lake Gammelgård sø".

This report is focusing more on the chemical extraction method and experiments than on the results. The chemical extraction results to study the P binding forms from the samples that will be taken are arriving after my internship. Therefore, a little data will be available but not the full results from the experiments. Moreover, those projects just started, hence it will take a few years to have the complete results of the projects.

E. Projects description

Three different projects are treated in this report. The purpose of the first one is to know the environmental effects of acidified slurry and here especially regarding phosphorus. The other two are aimed at determining the amount of phosphorus in the soil of areas that are going to be rewetted in the nearest future.

1. Environmental effects of acidified slurry

The aim of this study is to unravel the effects of acidified slurry environmental factors on the field scale. For this, in 2023, 4 replicates of a randomized complete blocks design (RCBD) in grass-clover on sandy soil at the coordinates, 55.442152, 9.102387 (WGS84). In compliance with current Danish regulations, cattle manure undergoes controlled acidification, which involves the use of higher quantities of sulfuric acid. This process ensures the stability of the acidified manure reducing both the loss of ammonia and methane. Additionally, the manure is spread four times throughout the growing season. Soils samples were taken and will be taken at five different dates. First, before the manure application, then, after the second and after the fourth mowing, after the growing season and at last, about one year after the first manure application (D. Zak, comm.pers.).

Various parameters will be examined in the soil samples in this study, including dry mass, loss on ignition, pH, as well as the levels of water extractable phosphate, total dissolved phosphorus,

nitrate, ammonium, nitrite, iron, aluminum, calcium, cadmium, and arsenic. Additionally, the concentration and composition of dissolved organic matter in the water extracts will be analyzed for 12 randomly selected plots to quantify the presence of humic-like substances, which could potentially serve as complexing agents for phosphorus and metals (D. Zak, comm.pers.). What I did for this project, was to extract P, Fe, Al, Mn soluble in the oxalate solution in order to know the degree of P saturation (DPS) and P sorption capacity (PSC).

For this project it was not required, for me, to go out on the field and withdraw soil samples because it was already done. Therefore, it was only necessary to do the phosphorus extraction in the lab with the soil taken before the manure application.

2. Rewetting of organic lowland soils in Denmark (NIFA)

The NIFA project aims to restore peatlands and analyze their impact on hydrology, water quality, carbon sequestration, and biodiversity. At Aarhus University, the wetland group is dedicated to six specific subprojects or research areas, which are (D. Zak, comm.pers.):

- Removing nitrogen and phosphorus
- Studying water sources and fluxes
- Assessing the risk of nutrient swapping through modeling
- Investigating the effects of plant harvesting and filter materials as phosphorus traps
- Examining the impact of topsoil removal on phosphorus loss, greenhouse emissions, and biodiversity
- Developing a toolbox to assess the environmental effects of rewetting.

Financed by the Environmental Ministry of Denmark, the project network is monitoring ten different peatlands (Figure 3).

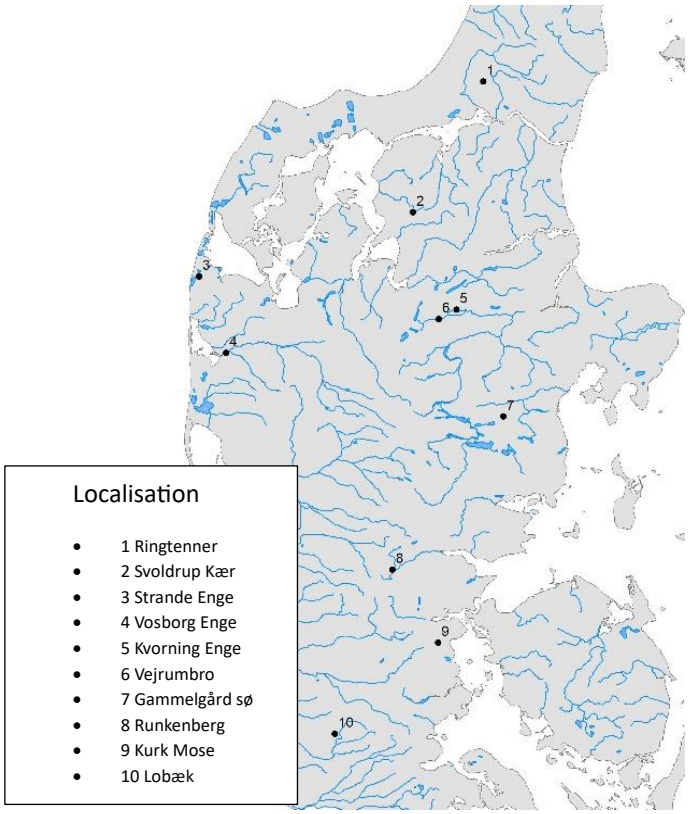


Figure 3 - The different peatlands being monitored in Denmark (Department of Ecoscience)

The sites I am studying for this project are Vosborg, Kvorning, and Vejrumbro respectively number 4, 5 and 6 on figure 3. Those three sites were drained and used for agricultural practices for centuries due to the extremely fertile soils. The first two, Kvorning and Vosborg have not been rewetted yet but the last one, Vejrumbro, has already been rewetted.

3. Rewetting of the lake Gammelgård sø

In summer 2023, the Danish Nature Agency Søhøjlandet will successfully complete a restoration project in the Gammelgård Sø area, located in Skanderborg municipality. This initiative is part of the broader climate action plan targeting peat-rich lowland soils. Not only will it help sequester greenhouse gases, but it will also play a vital role in expanding and connecting precious natural habitats. As an additional benefit, the project will serve as a pilot study to evaluate the efficacy of biomass harvesting in mitigating phosphorus leaching (*Gammelgård Sø lavbundsprojekt*, n.d.).

The captivating landscape of Gammelgård Lake, nestled amid the hilly glacial terrain between Søballe and Galten, consists of a drained lake area. Historically, the water was controlled through a pump system and an intricate network of drainage channels. However, with the completion of the project, the current pump operation will be decommissioned, and efforts will be made to cover old ditches, reorganize drains, and establish distribution channels. These crucial measures will lead to the resurrection of a significant portion of the original Gammelgård Lake. Moreover, the entire 44-hectare area will be thoughtfully restored to its natural state, transitioning from agricultural use to a harmonious blend of shallow lake, meadow, and marshland (*Gammelgård Sø lavbundsprojekt*, n.d.).

Throughout the process, utmost care will be taken to ensure the successful recreation of Gammelgård Lake. By embracing sustainable practices and safeguarding biodiversity, the project seeks to create a haven for local flora and fauna, further enhancing the ecological significance of the region (*Gammelgård Sø lavbundsprojekt*, n.d.).

III. Materials and method

A. Phosphorus extraction with an oxalate solution

For the assessment of environmental effects of acidified slurry, the oxalate protocol was applied (Baumann et al., 2020). The purpose of this protocol is to characterize P sorption capacities of mineral soils. Several soil samples from different plots were taken on the field experiment before slurry application in February 2023. For each plot, three layers of different depths were taken above the water table. The first layer corresponds to a depth of 0 to 5cm, the second corresponds to a depth of 5 to 30cm and finally the last corresponds to a depth of 75 to 100cm. Those depths represent the leaching zone in all the plots. It is important to note that each plot is attributed a lab number and each depth layer correspond to a letter (layer 0-5cm: a; layer 5-30cm: b; layer 75-100cm: c). The soil texture can be characterized, for all three layers, as sandy soil. For this protocol, not all 12 plots were used, only 4 plots were sampled, which mean that for each plot there is three samples, one for each layer. This makes 12 samples to analyze but each layer of each plot is sampled twice, which makes a total of 24 samples to analyze.

Step 1: Preparation of the oxalate solution

To extract P, Al, Mn, Ca, Fe from soil samples, an oxalate buffer solution was prepared. This solution contains di-ammonium oxalate monohydrate and oxalic acid dihydrate dissolved in a 1L flask with ultra-pure water. This buffer solution is used to extract phosphorus and metals acting as P binding partners from the soil. The pH of this solution must be 3 because when it's less than 3.5, the initial step involves the protonation of oxide surfaces, followed by the subsequent adsorption of oxalate and the release of complexed Al3+ and Fe3+ ions. Moreover, the extraction of organically bound Fe, Al and Mn also occurs (Schwertmann, 1964).

Step 2: Soil sampling stage

The first step of the extraction is to put 2 grammes of each layer of each plot in a centrifuge tube with the help of a spoon, a beaker and a scale (Figure 4).

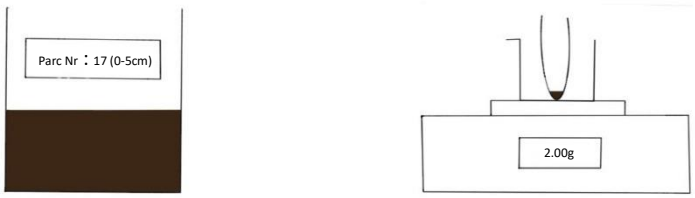


Figure 4 - Weighing stage

Step 3: The shaking stage

In each tube, 30mL of the oxalate solution must be added. Then the tubes must be shaken for 2 hours without interruption (Figure 5).

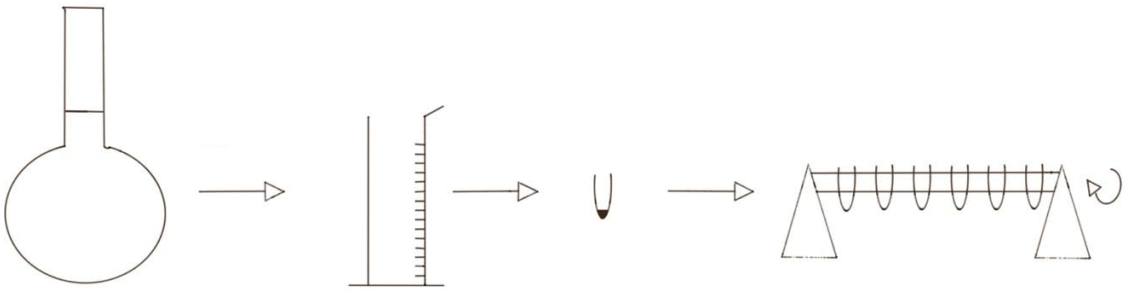


Figure 5 - Shaking stage

Step 4: The centrifuge stage

Once all the samples have been stirred for 2 hours without interruption, they can go into the centrifuge to separate the liquid and the solid phase.

Step 5: Filtration stage

After 5 minutes tops in the centrifuge at 4500 rpm, the samples are ready to be filtrated with a filtration unit, cellulose-acetate filters (0.45 µm pore size), and pure water (Figure 6). The first thing to do before filtering the samples is to clean everything with pure water. Then, the filter needs to be placed and washed with pure water. To filtrate the pressure gauge must be open. After this, the filter is washed with a few millimeters of the sample and then the whole sample is filtrated. The filter is changed after each sample and again the new will be washed as described before.

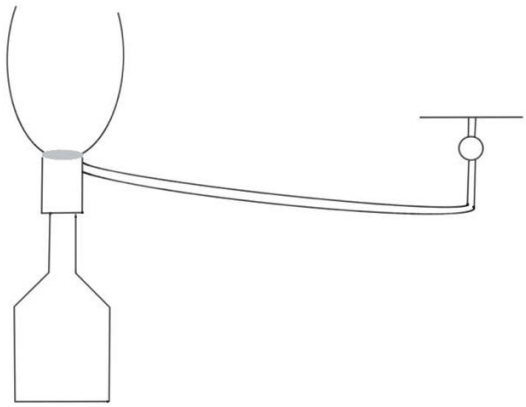


Figure 6 - Filtration stage

Step 6: Samples analysis and calculations

Once all samples have been filtered, they went into the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) analyzer. This analyzer is employed for the identification and quantification of chemical elements within a liquid sample. The sample is subjected to heat, which leads to the excitation of atoms and ions, causing them to emit light. The intensity of this emitted light is typically proportional to the concentration of a specific element in the sample. Through accurate measurement of the light intensity, it becomes possible to ascertain the presence of different elements in the sample and their respective proportions (SpectroAI, 2019). After the ICP-OES analysis, calculations to find to the concentrations of P, Fe, Al, and Mn were made in order to know at the end, the DPS (Degree of P saturation) and the PSC (P Sorption Capacity) (Annex 1).

To find the concentration of each element in g/kg, the following formula was applied (example for Fe):

$$Fe\left(\frac{g}{kg}\right) = \frac{C - C_0 * V * f}{m * 1000},$$

Equation 1 - Determination of elements' concentration

Where C = concentration in the test sample (mg/L); C_0 = blank value concentration in the measuring solution (mg/L); V = Extract volume (mL); f = Dilution factor (extract volume/measuring volume) and m = weight/dry weight (g).

Since, the extract volume is almost the same as the measuring volume, we consider the f as equal to 1, which simplifies the calculations. The same equation was applied to find P, Al, and Mn.

B. Litterbags experiment and determination of phosphorus fractions

1. Litterbags experiment

One of the purposes of the NIFA project is to investigate the efficiency of plant harvesting on removing surplus P from soils. The purpose of the litterbag experiment is to estimate the impact of harvesting on the plant-available phosphorus pools in peat upper soil layers (0-30cm). In this context, an experimental set-up to measure the changes in these pools in three different wetland sites was built. Two different, adjacent plots per site named "control" and "treatment" respectively, based on their use were determined to implant the bags that contain the soil that is going to be analyzed after. Both areas will receive litterbags but, the difference between the two is that the treatment area is going to be harvested and not the control area. For the "litterbags" nylon socks were used. The role of the litterbag sock is to expose the initial soil sample under the two plots surfaces at different incubation times. Part of the initial soil sample was analyzed for the plant-available phosphorus forms and harvesting is scheduled to take place in the "treatment" plot in three different growing seasons per year for three consecutive years so that at the end of the experiment it is possible to assess if the vegetation harvesting can be used as a supplementary mitigation measure for the reduction of mobilized phosphorus in rewetted wetlands.

The advantage of the litterbags is to put the same soil in all of the bags (soil that were taken from the field), and then put them in the soil to later investigate the efficiency of plant harvesting on P retention. The soil in the bags from the treatment and the control area is going to be analyzed and compare to see how harvesting alter P fractions from the soil and thus mitigate P losses to adjacent streams and lakes.

The three sites determined to do the litterbags experiment were, Kvorning, Vosborg and Vejrumbro. The experiments were done respectively on the 30th of May, 6th of June and 8th of June 2023.

Figure 7 - Litterbags being shaken

As said before, litterbags were used to put the soil in for all three sites where this experiment was made. Before using them on the field, the bags were first washed to control if any phosphorus was already present in them, in order to be more precise with the calculations when they will be taken out of the soil.

The litterbags needed for the three experimentations were put in a tray that contained pure water. After this leaching, they were put on a shaker for 22 hours (Figure 7) and then dried to be ready for the experiment on the field. Two wash steps were made, one before the first experimentation, on the 25th of May with 42 socks and another one for the other two on the 1st of June with 84 socks.

The water that results from the two washing were filtered on the 8th of June and P analyses have shown that no P was leached from the nylon socks.

For the experiment, always two subareas are chosen on the field, a control area, and a treatment area (Figure 8 and 9). They both have the same shape and size (6m*7m for Kvorning site). At Kvorning (Figure 8), which is peat mixed with sandy soil, the distance between the two areas is about 2 meters, and a piezometer station (KE-175P) is located between the two. One area is going to be the control area and the other one, the treatment area. After the experimentation, a map with the localization of the litterbags is made (Figure 10).

Figure 8 - Kvorning site, control and treatment area

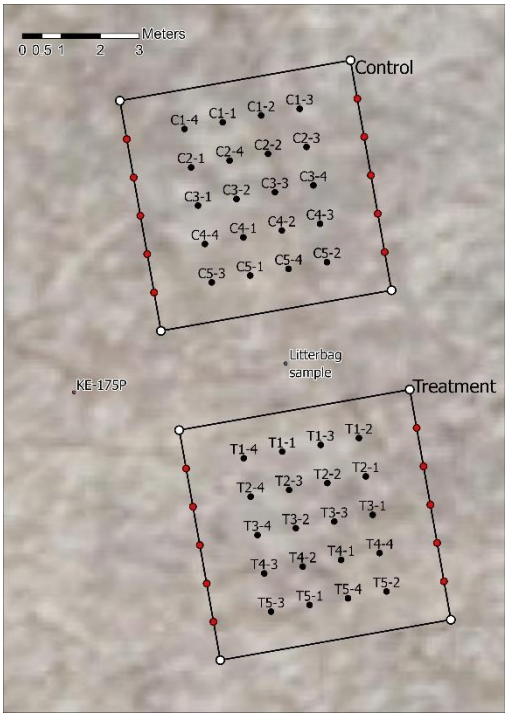


Figure 9 - Localization of the litterbags in Kvorning after the experimentation (Department of Ecoscience)

A portion of the peat was excavated (around 5kg) between the control and treatment areas. This portion corresponded to the upper layer of peat, from a 10cm depth to 30cm. The soil was put in a bucket and mixed thoroughly with a spoon. After that, the bags are filled with the soil from the bucket (about 100g, using the spoon and a scale (Figure 10). Once the sock is filled, it is knotted and turned upside down to be knotted again with a nylon clamp to protect the sample and simplify the recovering after exposure of 0.5, 1 and 2 years.

Figure 10 - Litterbag weighed and buried

Wooden sticks are used to set the marks where the litterbags are going to be buried in the soil, based. The litterbags are separated by 1m each and are separated from the wooden sticks that are represented by red dots on figure 8. The holes in which the litterbags are put into are about 7cm of diameter and 25cm depth. Once the litterbags are in the hole, the nylon clamp that is jutting out from the soil is cut (Annex 2). The soil that was taken out to dig the hole is used to cover the bags. Finally, the GPS data of each corner of both control and treatment area is taken. The same procedure is applied in the control area and the treatment area. Once all the litterbags are in the soil, the peat that is still in the bucket is put in a plastic bag and brought back to the laboratory for analysis for essential bulk parameters like total contents of carbon, nitrogen and phosphorus as well as different mobile P fractions (see below).

The procedure is the same for all three sites, a control and a treatment area is chosen for the other two sites and then represented on a sketch after the experimentation (Figure 11). Small details change, like the distance between the control and the treatment area (2,20m for Vosborg and 2,90m for Vejrumbro), the size and shape of both areas (squares with 6m faces for both Vosborg and Vejrumbro), and the weight of the soil sample that is took from the field to be analyzed. These are:

- 1900g of soil for Kvorning
- 1125g for Vosborg
- 892g for Vejrumbro

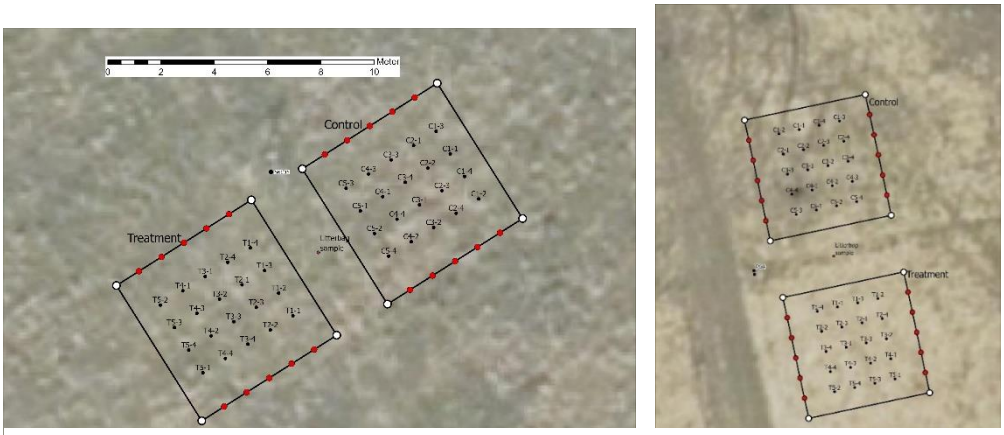


Figure 11 - Localization of the litterbags in Vosborg (left) and in Vejrumbro (right) (Department of Ecoscience)

The soil's properties and characteristics differ from one site to another (Table 1). Those differences can easily be observed on the field and are very important for the results. Since the soil is very different from site to site, it does not have the same characteristics. Hence, it is possible to expect a P release that differs according to the sites because of those differences.

Table 1 - Soil description of the three study sites

Sites	***Soil description***
Kvorning	Highly decomposed peat soil mixed with sand
Vosborg	Mineral soil with high organic carbon contents and iron and manganese intrusions
Vejrumbro	Moderately to highly decomposed peat

2. Phosphorus fractionation

We applied a modified protocol for P fractionation (Zak et al., 2008). This protocol allows us to determine the fraction of P bound to soluble elements or P loosely bound to the surface, the P bound to redox-sensitive compounds and the P bound to pH-sensitive compounds. Moreover, at the end, when these mobile P fractions are analyzed, it is possible to determine the 'rest P', which is almost all less mobile organic phosphorus.

It is important to note that the technical extract solutions in particular the bicarbonate-dithionite solution is prepared at the day of usage (Annex 3).

This protocol is applied to the soil samples that were taken during the litterbag experiment in Kvorning (Kvo), Vosborg (Vos), and Vejrumbro (Vej), as explained above. For this protocol, centrifuge tubes of 50ml are needed. Since there are three sites and since there is five replicates for each site, 15 centrifuge tubes must be labelled before the experiment. The labels are:

- Kvo – 1; Kvo – 2; Kvo – 3; Kvo – 4; Kvo – 5
- Vos – 1; Vos – 2; Vos – 3; Vos – 4; Vos – 5
- Vej – 1, Vej – 2; Vej – 3; Vej – 4; Vej – 5

Another set of 15 centrifuges tubes of 50ml is going to be needed in order to separate the liquid phase from the solid phase after centrifugation, but also to serve for the wash step that will be described later. There are labelled with a W at the end to signify that there are the ones for the wash step like the following:

- Kvo – 1 - W; Kvo – 2 - W; Kvo – 3 - W; Kvo – 4 - W; Kvo – 5 - W
- Vos – 1 - W; Vos – 2 - W; Vos – 3 - W; Vos – 4 - W; Vos – 5 - W
- Vej – 1 - W, Vej – 2 - W; Vej – 3 - W; Vej – 4 - W; Vej – 5 – W

In addition to the P fractionation protocol, an iron extraction protocol was carried out to determine the ratio of oxidised and reduced iron in the soil samples. As soil samples were aerated due to water tables lower than the sampling depth, we expected only minor amount or absence of reduced iron. For this extraction, the same amount of centrifuge tubes is necessary, 15 where the soil is going

to be, and 15 more that are going to be needed to separate the liquid phase from the solid phase after centrifugation. The labelling is globally the same, only with Fe before the name of the site.

Step 1: Weighing of soil

This step is the same as the one that was made for the oxalate protocol (figure 3). The difference is that in each centrifuge tubes, not 2 but 5g of soil must be weighed. The soil is put in the tubes labelled (Kvo – 1; Kvo – 2; …). All 15 tubes need to be filled. To do so, the centrifuge tube is placed in the beaker which is on the scale. Then, it is important to remember to press tare to weigh only the soil that is added with the help of a spoon into the centrifuge tube. A 5% marge of error weigh difference was accepted.

Step 2: The sequential P extraction and iron extraction

- ***1. KCl step***

In each centrifuge tube, 25ml of a 1 molar KCl solution is added. Then the tubes are placed into the overhead shaker so that they can be shaken for 30 minutes without interruption (figure 4).

Once the samples are done being shaken, they are centrifuged for 5 minutes at 4500 rpm. After that, the liquid phase and the solid phase are separated, and the liquid phase is transferred into the wash step centrifuge. Before filtering the KCl, the second step, which is the BD step, is prepared. The tubes containing the soil are filled with 25ml of a BD solution (0.115 M bicarbonate-dithionite). Then, the tubes are placed into the shaker so that they can be shaken for 1 hour without interruption. While those are being shaken, the filtration of the KCl samples was conducted.

The first thing to do before filtering the samples is to clean everything with pure water. Then, the filter needs to be placed and washed with pure water. To filtrate the pressure gauge must be open. After this, the filter is washed with a few millimeters of the sample and then the whole sample is filtrated. The filter is changed and washed after each sample. Once the filtration is done, the samples are placed in small centrifuge tubes of 15ml. On those ones is written, the name of the solution that was added to the soil, the name of the site, and the number of the replicate (for example KCl -KVO – 1; KCl – VOS – 1; KCl – VEJ – 1…). In each sample two drops of a 2M HCl solution is added to preserve the samples until their analysis.

The KCl step is used to determine the total dissolved P with the ICP-OES analyzer. The total dissolved P is the inorganic dissolved P (orthophosphates) and the dissolved organic P. The purpose of this extraction agent is to eliminate dissolved and loosely bound substances, such as ferrous iron and Ca, that may disrupt the following extraction procedures (Zak et al., 2008).

- ***2. BD step***

After the filtration of the KCl, the samples that were being shaken are put in the centrifuge for 5 minutes at 4500 rpm. Once this is done, like for the KCl, the transfer is made. The liquid phase goes into the centrifuge tubes for the wash step. After that, in the centrifuge tube that contains the soil, 25ml of pure water is added to wash the soil from the BD solution that may still be in before putting in the last chemical. The tubes are place for 5 minutes in the shaker and then again for 5 minutes in the centrifuge. When it is done, the liquid phase is added to the centrifuge tube that already contains the BD solution.

Before filtering the BD, the third step, which is the HCl step, is prepared. The tubes containing the soil are filled with 25ml of an HCl solution (0.5 M HCl). Then, the tubes are placed into the shaker so that they can be shaken for 16 hours without interruption. After that, the filtration of the BD samples is made.

For the filtration of the BD solution plus the wash step solution, the same procedure as for the KCl was applied. During the filtration process, one site, the last one Vejrumbro was a little difficult to filter. To alleviate this issue, a dilution was made for all five replicates of this site. Contrary to what is written in the protocol (Annex 3), only 4ml of the solution was taken and 36ml of pure water was added to have a factor 10 dilution. To make the dilution, two micropipettes are used. One of 4ml for the solution and another one of 9ml for the pure water. After the filtration, the result of the filtration is put in sample vessels of 60ml (BD – Kvo – 1; BD – Vos – 1; BD – Vej – 1; …).

This BD step is used to extract the redox-sensitive P, which means the P bound to Fe, Mn and other redox-sensitive compounds.

- ***Preparation for the iron extraction***

For this extraction, the weighing of the soil needs to be done once more. The procedure is the same as the one for the P fractionation. After this step, 25ml of a sulfuric acid solution is added in each centrifuge tubes that contains soil. Then the samples are placed in the shaker alongside the HCl ones. Those ones also need to be shaken for 16 hours.

- ***3. HCl step***

The samples that were being shaken are put in the centrifuge for 5 minutes. Once this is done, the transfer like for the KCl and the BD is made. After that, a wash step like for the BD is also made. Once the transfer from the was step is done, the filtration can begin. The solution that results from the filtration is put in small centrifuge tubes of 15ml. On those ones is written, the name of the solution that was added to the soil, the name of the site, and the number of the replicate (HCl -KVO – 1; HCl – VOS – 1; HCl – VEJ – 1…).

The HCl is used to extract the pH-sensitive P, which means the P bound to elements like Ca or Al that might be released in acid conditions.

- ***4. Iron extraction step***

After the filtration of the HCl, the extra samples used only for this extraction, that were being shaken are placed in the centrifuge for 5 minutes at 4500 rpm. Once this is done, the transfer like for the KCl and the BD is made. In this case, a wash step like for the BD and the HCl is also made. Once the transfer from the was step is done, the filtration can begin (Figure 12). The same procedure as before is applied. This time, for all samples a dilution step is made (Figure 12). For this dilution, 2ml of the solution is taken out with a micropipette and put in a sample vessel. Then 9ml of pure water is taken two times and put in the sample vessel in order to have a factor 10 dilution.

Figure 12 - Filtration of the iron samples (left) and dilution of the iron samples (right)

The iron step is used to distinguished Fe^{2+} and Fe^{3+}. In the following picture (Figure 13) it is possible to see the step of iron species determination.

Figure 13 - Iron species determination

After the experiment, all samples are stored at 5 °C until their analysis. The steps for determining the P fractions area summarized in Figure 14.

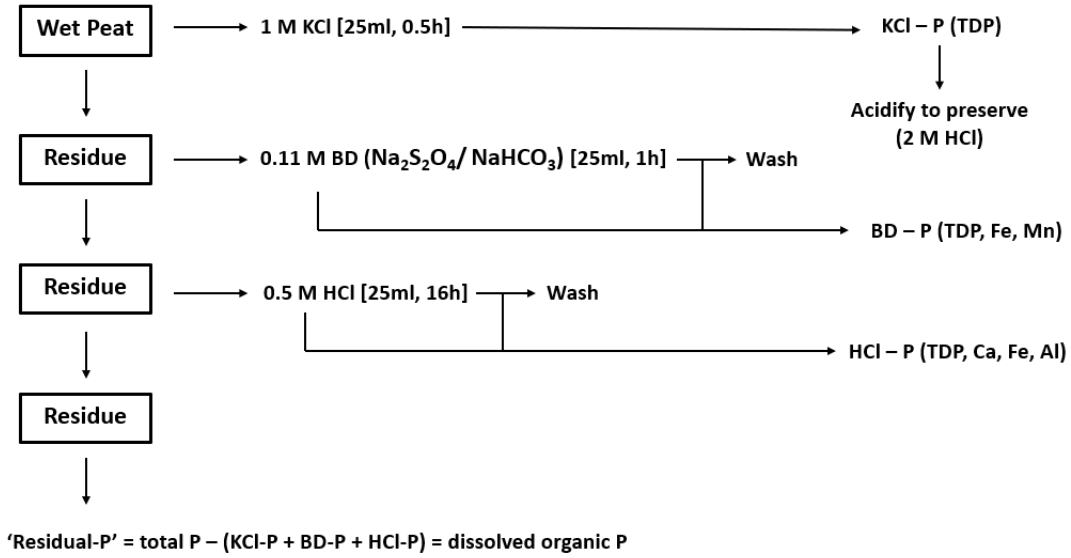


Figure 14 - The extraction steps of the sequential chemical extraction to determine P fractions (modified from Zak et al., 2008)

C. Rewetting of the lake Gammelgård sø

1. Soil samples took on the field

At Gammelgård sø, three plots were determined to take out soil samples. The plots were chosen because some samples had already been taken out there and the GPS coordinates were known. On each plot, three soil samples of 1 meter were taken in plastic tubes. After that, back at the university, the plastic tubes were cut, and the soil was cut in different layers (0-5cm; 5-10cm; 10-15cm; 15-20cm; 20-25cm; 25-40cm; 40-60cm; 60-100cm). Two of the three soil samples for a plot were cut and placed into plastic bags according to the layer. It is the soil from those plastic bags that is going to be analyzed after the chemical extraction.

2. P fractionation

For the samples took at Gammelgård sø, the P fractionation was also realized but in a shortened way (Figure 15).

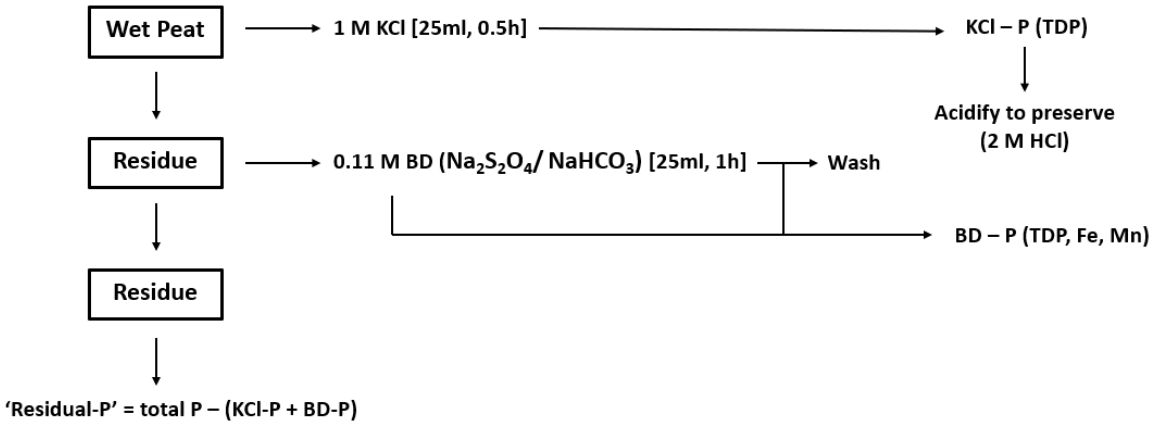


Figure 15 - P fractionation with samples took at Gammelgård sø (modified from Zak et al., 2008)

IV. Results

As said before, the results that are presented here are only the beginning of the two projects that just started. To be able to draw conclusion on the effect of acidified slurry on P leaching and, on the efficiency of plant harvesting on P retention, further investigations must be conducted. In this case, no conclusions can be drawn in this report regarding those projects. Some of the early results of these projects can be shown but the discussion will be limited accordingly.

A. Environmental effects of acidified slurry

The content of P ranged between 3 and 18 mmol/kg, with the highest concentration in the first layer (a: 0-5cm) (Annex 1). The content of Fe ranged between 11 and 65 mmol/kg with again, the highest concentration found in depth a of all soil profiles. The Al was the one that had the highest concentration in all depths and samples, varying from 47 to 83 mmol/kg and, with the highest concentration found in the second layer (b: 5-30cm). As for Mn, it is the one that had the lowest concentration in almost all depths and samples, varying from 1 to 4 mmol/kg. The highest Mn concentration was found in last layer (c: 75-100cm).

The PSC varied between 33 and 73 mmol/kg or between 1 and 3 g/kg and was highest at 5-30 cm depth. The DPS varied between 9 and 30% with high proportion in depth a and b with an average of respectively 27% and 20%.

On the graph below (Figure 16) a correlation can be observed between the depth and the P sorption capacity of the soil. The highest values of P sorption capacity are observed at lower depths. The same can be observed for the depth and the degree of P saturation in the soil. The highest values for P saturation are also observed at the soil surface.

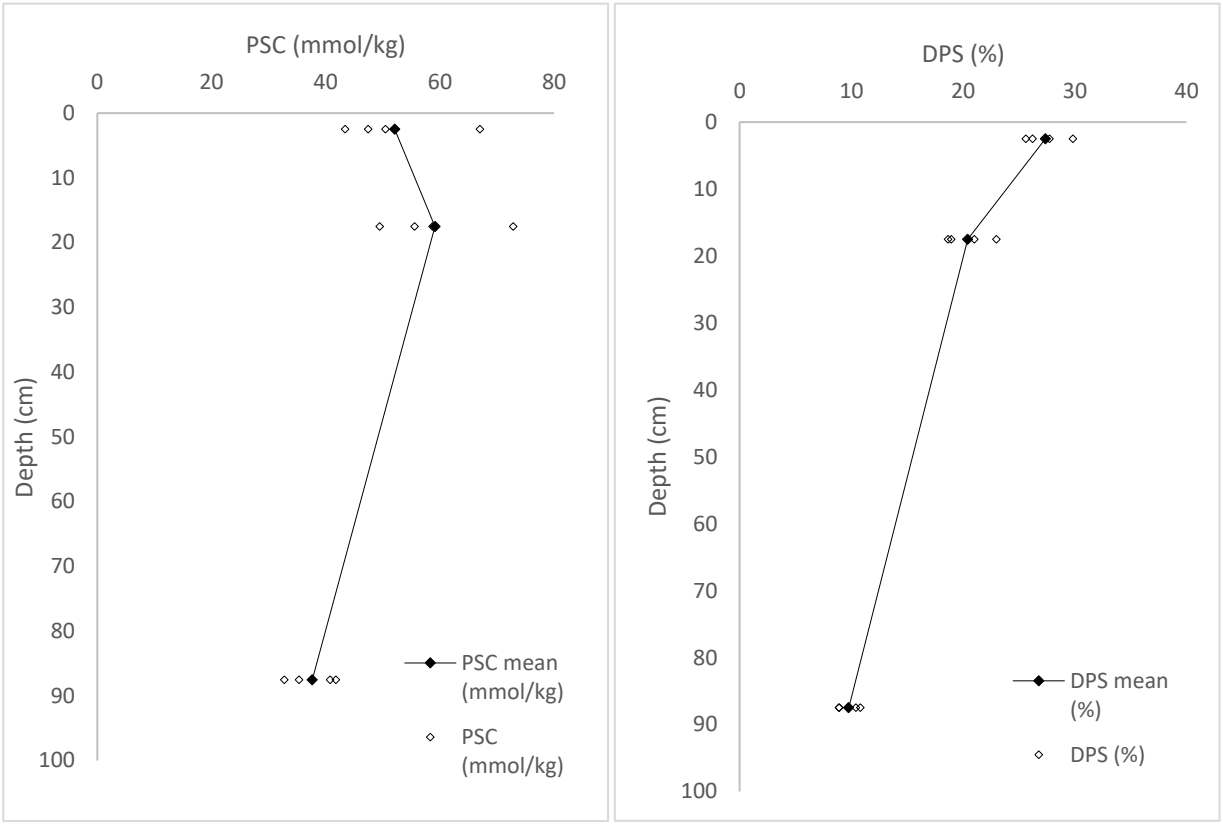


Figure 16 - P sorption capacity and degree of P saturation

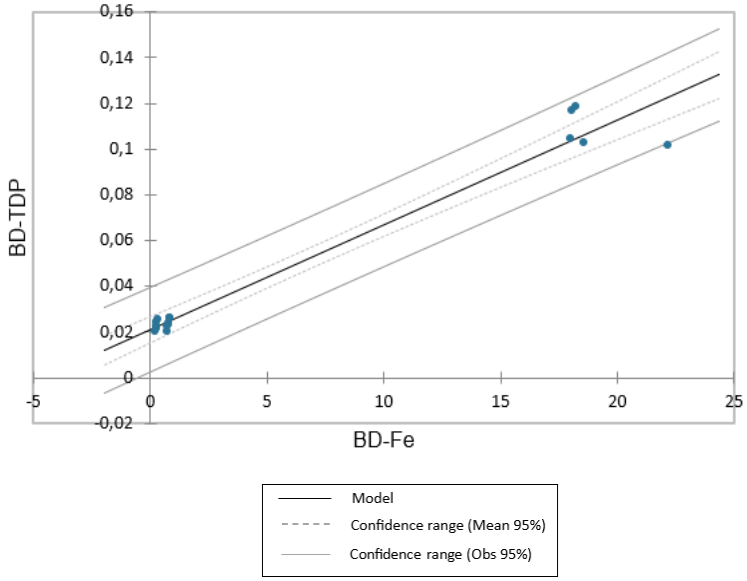
The dependance of the variable DPS on depth was proven using the ANOVA statistical test using RStudio and XLSTAT software. The p-value is inferior to 0.001 (RStudio Team, 2022), which mean that it is a high statistical significance. Given the R^2, 96% variability of the dependent variable DPS is explained by the explanatory variable which is the depth (Lumivero, 2023). The dependance of the variable PSC on depth was also proven using the ANOVA statistical test using RStudio and XLSTAT software. The p-value for this test is 0.0208 (RStudio Team, 2022). This test states that given the R^2, 58% of the variability of the dependent variable PSC is explained by the explanatory variable which is the depth (Lumivero, 2023). There was no significant relation (P = 0.108) between the DPS and the PSC.

B. Rewetting of organic lowland soils in Denmark (NIFA)

The P fractionation that was applied on the three different sites was to determine the total dissolved P, the redox-sensitive P and the pH-sensitive P using respectively a KCl solution, a BD solution and a HCl solution. For now, only the results for the redox-sensitive P were available (Table 2).

Table 2 - Results of the analysis after P fractionation

Sample	Weighing freshmass [g]	DM of freshmass [%]	P-content realted to DM			Metall content related to DM					
			KCl TDP [mg/g]	BD TDP [mg/g]	HCl TDP [mg/g]	KCl Ca [mg/g]	BD Fe [mg/g]	BD Mn [mg/g]	HCl Fe [mg/g]	HCl Ca [mg/g]	HCl Al [mg/g]
KVO 1	4,97	47,32121021		0,02056			0,7335	0,0098			
KVO 2	4,87	47,32121021		0,0263			0,844	0,01			
KVO 3	4,81	47,32121021		0,02388			0,7974	0,0108			
KVO 4	4,96	47,32121021		0,02284			0,7499	0,0104			
KVO 5	4,9	47,32121021		0,02312			0,7892	0,0106			
VOS 1	5	33,90383493		0,10152			22,18	2,2918			
VOS 2	5,01	33,90383493		0,11869			18,251	1,9163			
VOS 3	5,02	33,90383493		0,11669			18,038	1,6569			
VOS 4	5,04	33,90383493		0,10306			18,581	1,7674			
VOS 5	5,05	33,90383493		0,10461			17,989	1,9741			
VEJ 1	5,05	87,42457746		0,02412			0,265	0,0017			
VEJ 2	4,98	87,42457746		0,02561			0,3342	0,0017			
VEJ 3	4,98	87,42457746		0,02044			0,2308	0,0017			
VEJ 4	5,08	87,42457746		0,02173			0,295	0,0017			
VEJ 5	5,02	87,42457746		0,0229			0,2632	0,0017			



A statistical test was made to see if there were a correlation between the BD-P content and the Fe content. The test reveals a high correlation between the BD-TDP and the BD-Fe (R^2 = 0.965, P<0.0001). According to the statistical test, given the R^2, 97% of the variability of the dependent variable BD-TDP is explained by the explanatory variable BD-Fe (Lumivero, 2023).

Figure 17 - Calculations for DBD and BD-P pool in Gammelgård sø

Knowing the BD-TDP and the BD-Fe, it possible to determine the Iron/Phosphorus ratio in molar. For that the BD-Fe need to be divided by the BD-TDP and multiply by the molar mass of phosphorus divided by the molar mass of iron. This result (Table 3) allows us to make an estimation on the potential P release that can happen regarding the P bound to redox-sensitive Fe.

Table 3 - Iron/Phosphorus ratio

Probe	BD-Fe:BD-TDP	Mean BD-Fe:BD-TDP
KVO 1	19,8	18,7
KVO 2	17,8	
KVO 3	18,6	
KVO 4	18,2	
KVO 5	19,0	
VOS 1	121,4	97,7
VOS 2	85,4	
VOS 3	85,9	
VOS 4	100,2	
VOS 5	95,5	
VEJ 1	6,1	6,7
VEJ 2	7,2	
VEJ 3	6,3	
VEJ 4	7,5	
VEJ 5	6,4	

The variability in P release rates can be attributed to the properties of the peat, including the quantity of P attached to Fe3+ compounds that are sensitive to redox conditions as well as the molar ratios of Fe:P or Al:P (Zak et al., 2010). These characteristics help explain the wide range of P release rates observed. The net rates of P release in peat cores containing extensively decomposed peat were found to have a significant correlation with the quantity of P attached to redox-sensitive compounds, as well as the molar ratios of Fe:P and Al:P in the peat (Zak et al., 2010).

In Table 3, it is possible to observed that the Fe:P molar ratio is quite different in the three sites. In Vosborg it is close to a 100 while it is low for Kvorning (18.7) and even lower for Vejrumbro (6.7). Those differences can come from the different characteristics of the soil (Table 1). Indeed, as it was said before, the three sites have completely different soils meaning that the P release can be very different from one site to another.

C. Rewetting of the lake Gammelgård sø

Concerning the results of the samples of Gammelgård sø, only the BD-TDP content was available right now. Therefore, it was not possible to determine the Fe:P molar ratio like for the NIFA project. However, it was possible to determine the DBD (the mass of soil solids per unit volume of soil), while it was not possible for the NIFA project because some data were missing. To calculate the DBD, it is necessary to have the fresh weight of the samples, the dry mass, the dry weight, and the volume of the samples (Table 4).

Table 4 - Calculations for DBD and BD-P pool in Gammelgård sø

Site	Number	Freshweight(g)	Length (dm)	Diameter (dm)	Volume (dm3)	Drymass %	Dry weight (g)	DBD (g/dm3)	Mass of Soil in m2 (0.3 m depth) (kg)	BD-P (g/kg)	BD-P pool (g)	Mean from 0-40cm Mean BD-P pool (g) (40cm)
G17 0-5	1	92	0,5	0,47	0,087	26,164	24,071	277,484	83,245	0,041	3,395	4,753
G17 5-10	2	104	0,5	0,47	0,087	25,141	26,147	301,412	90,424	0,076	6,889	
G17 10-15	3	122	0,5	0,47	0,087	29,547	36,047	415,544	124,663	0,052	6,533	
G17 15-20	4	127	0,5	0,47	0,087	24,491	31,104	358,561	107,568	0,037	4,020	
G17 20-25	5	140	0,5	0,47	0,087	25,562	35,787	412,539	123,762	0,040	4,968	
G17 25-40	6	413	1,5	0,47	0,260	19,970	82,475	316,915	95,075	0,029	2,713	
G17 40-60	7	574	2,0	0,47	0,347	18,693	107,300	309,231	92,769	0,012	1,096	
G17 60-100	8	596	4,0	0,47	0,694	21,932	130,716	188,358	56,507	0,022	1,268	
G4 0-5	9	90	0,5	0,5	0,098	23,445	21,101	214,931	64,479	0,100	6,419	6,433
G4 5-10	10	98	0,5	0,5	0,098	37,361	36,614	372,945	111,884	0,050	5,562	
G4 10-15	11	95	0,5	0,5	0,098	38,551	36,624	373,044	111,913	0,099	11,134	
G4 15-20	12	87	0,5	0,5	0,098	43,318	37,686	383,871	115,161	0,049	5,622	
G4 20-25	13	98	0,5	0,5	0,098	41,818	40,982	417,439	125,232	0,035	4,438	
G4 25-40	14	494	1,5	0,47	0,260	40,223	198,701	763,525	229,057	0,024	5,426	
G4 40-60	15	610	2,0	0,47	0,347	30,370	185,258	533,901	160,170	0,041	6,514	
G4 60-100	16	633	4,0	0,47	0,694	15,835	100,233	144,433	43,330	0,051	2,230	
G11 0-5	17	173	0,5	0,47	0,087	75,250	130,182	1500,703	450,211	0,262	117,863	99,374
G11 5-10	18	212	0,5	0,47	0,087	73,171	155,124	1788,224	536,467	0,188	100,882	
G11 10-15	19	207	0,5	0,47	0,087	72,256	149,570	1724,209	517,263	0,268	138,421	
G11 15-20	20	197	0,5	0,47	0,087	64,232	126,538	1458,692	437,608	0,219	95,963	
G11 20-25	21	199	0,5	0,47	0,087	70,926	141,143	1627,060	488,118	0,207	100,846	
G11 25-40	22	583	1,5	0,47	0,260	73,300	427,338	1642,080	492,624	0,086	42,271	
G11 40-60	23	707	2,0	0,47	0,347	33,851	239,328	689,727	206,918	0,083	17,264	
G11 60-100	24	836	4,0	0,47	0,694	50,387	421,237	606,989	182,097	0,015	2,653	

With the DBD information, it is possible to estimate how many years it would take for the plants to empty the BD-P pool (Table 5). No studies were made to exactly know which plants is present in the three different plots. Since the site of Gammelgård sø was a former lake, we make the hypothesis that the plants present there are the ones from a lake system. Therefore, to make the calculations on plant uptake Reed becks are chosen. Based on Walton et al., 2020 study, the P stock that these can take is from 0.4 to 4.7 $g.m^{-2}$. Only the first 30cm were taken to make the calculations because the rooting zone of the plants is in the first layer of the soil (0-30cm). Therefore, the P that can be removed from the soil by the plants is the one is this first 40cm.

Table 5 - Estimation of the time Reed becks would take to empty the BD-P pool

Site	Mean BD-P pool (g) (40cm)	Plant uptake 0,4g/m² (years)	Plant uptake 4,7g/m² (years)
G17 0-40cm	4,753	11,9	1,0
G4 0-40cm	6,433	16,1	1,4
G11 0-40cm	99,374	248,4	21,1

In Table 4 and 5 it is possible to see that the last site in Gammelgård sø has a lot more BD-P content than the other two. Therefore, it is not surprising to see that the time it would take for the plants to remove the BD-P pool is way higher than the other. It is also possible to note that the impact on the BD-P pool depletion time is influenced by the changes in the plants' ability to uptake P. Indeed, it would take only 1 year for the plants (if harvested) to empty the BD-P pool when they can uptake 4.7 g of P per m² but it would take them almost 12 years if they only take up 0.4 g of P per m².

V. Discussion

A. Environmental effects of acidified slurry

The results from the oxalate protocol show that, the PSC and the DPS was higher in the first depth layer (Figure 16), meaning that the soil has a better P sorption capacity near the soil surface, but it also has a higher degree of P saturation of 27% and 20% for the first two layers. This indicates a risk of P leaching in those layers. Since the soil samples show a risk of P leaching and it is the ones before the first manure application, it may indicate that after the manure application, the risk for P leaching might be higher, which could lead to P release to groundwater and subsequently into streams and lakes.

The results that were obtained with the oxalate protocol were compared to the ones in another study, with similar conditions. Indeed, in Baumann et al., 2020, the soil texture is rather similar between all depth and profiles and can be described as sandy loam. Since the soil texture was approximately the same between the two studies, the results were compared in order to see if the soil from the 'Environmental effects of acidifies slurry' project had a good capacity to filter and retain phosphorus compared to other soils. In comparison with the study from Baumann et al., 2020, the results here, show a better capacity for the soil to retain P. The PSC here varied between 33 and 73 mmol/kg whereas in Baumann et al., 2020, it varied between 13 and 32 mmol/kg. The DPS was almost the same, it ranged between 9 and 30% here compared to variation from 12 to 36% in Baumann et al., 2020. Some similarities in the two studies can be mentioned. In both studies, the PSC was higher in the first to depths than in the third one and the DPS was higher in the first depth layer.

Regarding the statistical tests, it would have been preferable to carry out the calculations on a larger number of plots than the four we did in order to have more data to compare and more data to carry out the statistical tests.

Even though the soil here has a better capacity to retain P (between 33 and 73 mmol/kg or between 1 and 3g/kg per year) as the one in the Baumann et al., 2020 study, it is very low compared to the different filters that exist to remove P. The most commonly utilized are natural materials, industrials byproducts and man-made products. Industrial byproducts such as furnace slags, demonstrated the highest capacity for phosphorus removal, reaching up to 420 grams of phosphorus per kilogram. Natural materials, like heated opoka, followed with a maximum P removal capacity of 40 grams per kilogram, while man-made filter media, such as Filtralite, achieved a maximum of 12 grams per kilogram (Vohla et al., 2011). Most of the filter media examined in Vohla et al., 2011, exhibited a pH level greater than 7 and contained significant amounts of calcium (CaO). The analysis revealed a strong positive correlation, as determined by the Spearman Rank Order Correlation, between the retention of phosphorus and the calcium oxide (CaO) and calcium content of the filter materials (with R2 values of 0.51 and 0.43, respectively). However, the relationship between phosphorus retention and pH level was comparatively weaker, yet still statistically significant.

B. Rewetting of organic lowland soils in Denmark (NIFA)

As shown by the results, the molar Fe/P ratio for Vosborg is almost a hundred (Table 3), which signifies that to release 1 mol of P, 97.7 mol of Fe needs to be released. In that case, the BD-P pool in Vosborg concerning Fe, is not a risk of P leaching. Indeed, a significant export of P to nearby lakes or

rivers can only be expected when the molar Fe:P ratios in highly decomposed peat are below 10 (Zak et al., 2010). Contrary to Vosborg, in the Vejrumbro site a higher export of P can be expected. The fact that the molar ratio is under 10 signifies that it is possible to assume that there is a potential risk of P being released in this site regarding the P bound to redox-sensitive Fe.

The redox process in the mobilization of P in rewetted fens is an important process to consider. The statistical test made to prove the correlation between redox sensitive Fe and P bound to redox-sensitive compounds, is in agreement with previous studies, that have showed that the rates of P release in highly decomposed peat were greatly influenced by the quantity of mobilizable P under anoxic circumstances (BD-P) (Zak et al., 2010).

However, it is important to note with the results available here, it is only a rough estimate of P release because there are other things to consider for a good estimation, like, the Al:P ratio, the sorption capacity of elements like Al and Mn, but also the pH. Moreover, these results (once they will be complete) mark the initial phase of the project on plant harvesting, offering valuable insights into the soil characteristics of the three distinct locations before harvesting. These results will contribute to quantify the efficiency of plant harvesting on P retention once they will be compared to the ones after harvesting.

C. Rewetting of the lake Gammelgård sø

According to the results, it can take decades and even centuries to empty the P pools in the soil without human management depending on plants capacity to retain P. Since the Gammelgård sø site is going to be rewetted, a release of P in the newly formed lake cannot be excluded. Moreover, since only the BD-P was taking into account here, a higher risk may occur when every factor that influenced P release is considered.

Previous studies have shown the importance of plant uptake to remove P from soils in a temporary basis, since a significant portion of the plant P reservoir is liberated through leaching and mineralization following die-back (Zak et al., 2014). By taking this in consideration, and by looking at the results that are available for now, it is possible to make a conjecture that the Gammelgård sø site, will maybe need human management (like harvesting or P filters implementation) to mitigate P losses into streams and lakes. If not, a high P release may occur, especially because rewetted fens that have been subjected to decades of drainage and agricultural activities are likely to exhibit a considerable potential for P mobilization (Zak et al., 2008). Such fens often have upper soil layers containing highly decomposed peat (which is the case especially for the plot G11 and 17), which results in notably higher levels of 'reductant-soluble P' and 'acid-soluble P' compared to the underlying layers with less decomposed peat or, the upper soil layers of undisturbed fens with slightly decomposed peat (Zak et al., 2008). The substantial rise in the provision of oxidizing substances [Fe^{3+}-hydroxides and sulfate], along with the greater availability of organic matter (OM) serving as electron donors in highly decomposed peat, is accountable for the pronounced mobilization of P after the rewetting of the fen (Zak & Gelbrecht, 2007). However, the results presented here represent only a very small fraction of the analysis that followed the chemical extraction. Therefore, it is necessary to wait for further results from this analysis in order to be able to estimate better the potential P leaching that may happen.

The results presented here are only preliminary and have some shortcomings, as no study has been carried out to determine which species or communities of plants are present on the site, in order to calculate how long it would take them to empty the BD-P pool. Further investigations are needed to improve the P risk assessment. That way, the estimation of how many years it would take for the plants to empty the P pools would be more precise and would better reflect the reality. It is also

important to integrate, if possible, the hydrology of the site and the soil characteristics to make better conclusions on P cycle and estimate the potential P release.

VI. Conclusion

As wetlands act as carbon and nitrogen sinks and contribute to reduce water pollution by acting as buffers between land and streams, their restoration appears like a good solution to fight global warming and reduce water pollution. However, with only the rewetting of wetlands, it is not likely that they will return to a low nutrient ecosystem. Therefore, human management to restore natural conditions of wetlands appear essential. Studying the potential P release is crucial when implementing wetland restoration strategies, in order to prevent harmful impacts on P-limited ecosystems.

The three projects presented here all aim at reducing P release from soils. Though the results presented in this report are not thoroughly evaluating the potential for P release yet, they point out the risk of P release in all sites that were studied. The Rewetting of organic lowland soils in Denmark project shall have interesting results on how to manage nutrient removal and retention from soil before wetland restoration. Conducting extended monitoring for this project will allow us to have more insight on P retention or release in restored wetlands or from mineral soils. The environmental effects of acidified slurry should also bring interesting results concerning the use of acidified slurry on environmental factor such as P. This may lead to a better control of the use of acidified slurry in order to reduce its impact on environmental factors and ecosystems.

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VII. Reflective feedback on experience

This internship has provided me with valuable insights into wetland ecosystems and the complexities involved in their restoration. Studying the potential P release and its impacts on ecosystems relay shown me the challenges of restoring wetlands. I learned that the necessity of human management to restore natural wetland conditions highlights the importance of human intervention in conservation efforts. This understanding is crucial in developing effective strategies for wetland restoration, as well as monitoring. Monitoring a wetland after restoration gives great insight on the nutrient cycle, therefore it is an important part of restoration.

Throughout my internship, I was able to work in different fields which was really rewarding. I was able to help on the field and do things by myself for the projects I worked on during those three months. Being on the field really help understand the experiment. I also was able to work in the laboratory and do the chemical extractions alongside my sub-supervisor. As with the fieldwork, carrying out the protocol yourself really helps understanding all the stages of the protocol. Working on the field, in the laboratory and in the office made me realized what it was like to be a researcher in Denmark.

Even though the duration of my internship did not allow me to have access to the results of the different projects I worked on, since it was only the beginning of those, it was very interesting to be part of this for three months.

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IX. Appendix

Annex 1: Calculations table to determine the PSC and DPS

					ICP and P and metals (mg/L)				P and metal content (g/kg)				P and metal conten (mmol/kg)						
Lab Nbr	Plot Nbr	Fresh weight (g)	Dry weight (%)	Dry weight (g)	P	Fe	Al	Mn	P	Fe	Al	Mn	P	Fe	Al	Mn	PSC (mmol/kg)	PSC (g/kg)	DPS (%)
43	17a	2,00	81,9	1,64	28,57	188,1	101,1	5,8	0,52	3,44	1,85	0,11	16,86	61,49	68,54	1,93	66	3	26
43*	17a	2,02	81,9	1,65	31,36	199,6	103,7	5,8	0,57	3,62	1,88	0,11	18,33	64,62	69,63	1,91	68	3	27
44	17b	2,04	83,9	1,71	27,17	205,8	127,8	6,3	0,48	3,60	2,24	0,11	15,34	64,36	82,92	2,01	75	3	21
44*	17b	2,03	83,9	1,70	26,9	198,1	119,2	6,3	0,47	3,49	2,10	0,11	15,26	62,25	77,73	2,03	71	3	21
45	17c	2,03	92,0	1,87	6,41	60,1	93,5	7,8	0,10	0,96	1,50	0,13	3,31	17,20	55,60	2,28	38	1	9
45*	17c	2,03	92,0	1,87	7,68	78,5	105,8	8,7	0,12	1,26	1,70	0,14	3,96	22,49	62,89	2,53	44	2	9
31	13a	2,00	85,0	1,70	23,6	118,6	74,6	5,9	0,42	2,09	1,32	0,10	13,42	37,34	48,75	1,88	44	2	31
31*	13a	2,01	85,0	1,71	22,07	115,4	72,0	8,1	0,39	2,02	1,26	0,14	12,48	36,14	46,82	2,58	43	2	29
32	13b	2,01	86,3	1,73	20,17	122,5	95,2	6,7	0,35	2,12	1,65	0,12	11,23	37,78	60,96	2,11	50	2	22
32*	13b	2,00	86,3	1,73	20,54	118,0	89,9	8,2	0,36	2,05	1,56	0,14	11,50	36,59	57,82	2,58	48	2	24
33	13c	2,02	93,5	1,89	5,97	40,7	85,5	12,6	0,09	0,64	1,36	0,20	3,04	11,51	50,29	3,64	33	1	9
33*	13c	2,03	93,5	1,90	5,56	40,2	86,5	13,1	0,09	0,63	1,37	0,21	2,82	11,30	50,59	3,76	33	1	9
1	1a	2,02	80,6	1,63	21,7	150,6	72,9	3,7	0,40	2,77	1,34	0,07	12,88	49,54	49,70	1,24	50	2	26
1*	1a	2,03	80,6	1,64	21,96	152,2	73,7	4,2	0,40	2,79	1,35	0,08	12,97	49,80	50,06	1,41	51	2	26
2	1b	2,03	83,4	1,69	21	170,2	97,2	3,9	0,37	3,01	1,72	0,07	11,99	53,83	63,72	1,25	59	2	20
2*	1b	2,00	83,4	1,67	17,79	149,6	100,5	3,4	0,32	2,69	1,81	0,06	10,30	48,00	66,90	1,11	58	2	18
3	1c	2,01	92,0	1,85	8,39	62,9	104,8	7,9	0,14	1,02	1,70	0,13	4,38	18,19	62,94	2,33	42	1	10
3*	1c	2,00	92,0	1,84	8,27	61,3	105,5	7,1	0,13	1,00	1,72	0,12	4,33	17,81	63,70	2,11	42	1	10
13	5a	2,02	84,4	1,71	22,91	136,1	76,6	5,8	0,40	2,39	1,35	0,10	12,98	42,71	49,84	1,84	47	2	28
13*	5a	2,01	84,4	1,70	23,42	135,7	77,4	5,7	0,41	2,40	1,37	0,10	13,34	42,78	50,65	1,81	48	2	28
14	5b	2,01	87,0	1,75	18,06	140,0	102,9	5,2	0,31	2,40	1,76	0,09	9,98	42,84	65,36	1,60	55	2	18
14*	5b	2,00	87,0	1,74	19,38	148,8	101,8	5,1	0,33	2,56	1,75	0,09	10,76	45,78	64,99	1,60	56	2	19
15	5c	2,00	92,4	1,85	7,15	55,7	79,1	9,9	0,12	0,90	1,28	0,16	3,73	16,13	47,54	2,91	33	1	11
15*	5c	2,00	92,4	1,85	7,46	69,0	88,0	6,1	0,12	1,12	1,43	0,10	3,89	19,97	52,91	1,80	37	1	10
ZAK-K					0,03	0,1	0,0	0,0											

Annex 2: litterbag being buried in Kvorning

Annex 3: Protocol for determination of phosphorus fractions (litterbag experiment)

Steps		Practical work		Analytics	Who
1.Weighing of soils	1. Weighing for extraction	5 g soil (remove thicker roots (> 1mm)) in 50 mL centrifuge tubes			Clemence, Karolina, Dominik
	2. Determination dry mass and loss on ignition (LOI)	ca.100 g soil in aluminum vessels: a) Dry mass at 105 °C fort wo days b) LOI at 450 °C 4h		Dry mass, LOI	Karina
	3. Elemental composition	Extra dried soil sample ca. 50g at 105 °C grounded with a disk mill. Total phosphorus (TP) content: „sulfuric acid digestion “ Metal content: „aqua regia		TP, Fe, Al, Mn, Ca, C, N (in Berlin)	Dominik
2.Sequential P- Extraction	1. Step	25 mL 1 M KCl-solution (1h N_2 degassed to remove oxygen)	0,5 h shaking, centrifugation (5 minutes at 4500 rpm), decant, and filtrate	total dissolved P (ICP-OES)	Clemence, Karolina, Dominik (analytics)
	2. Step	25 mL 0.115 M (bicarbonate-dithionite solution)	1 h shaking, centrifugation (5 minutes at 4500 rpm) and decant.	total dissolved P, Fe, Mn, (Al) (TP-analytics and AAS for metals), samples will be pre-diluted by factor 10 (5 ml BD+45 ml pure water)	Clemence, Karolina, Dominik (analytics)
	Wash step	25 mL deionized water	5 min shaking, centrifugation (5 minutes at 4500 rpm), combine with “BD decant”, and filtrate afterwards		
	3. Step	25 ml 0.5 M HCl	16 h shaking (overnight), centrifugation (5 minutes at 4500 rpm) and decant.	total dissolved P, Ca, Fe, Al (ICP-OES)	Clemence, Karolina, Dominik (analytics)
	Wash step	25 mL deionized water	5 min shaking, centrifugation (5 minutes at 4500 rpm), combine with “BD decant”, and filtrate afterwards		
	4. Step	„Rest-P“(organic P)	Rest pellet discard!	Rest P = total P minus (KCl-P + BD-P + HCl-P)	
3. Iron species extraction	1. Step	25 ml 5M H_2SO_4	16 h shaking (overnight), centrifugation (5 minutes at 4500 rpm) and decant.	total dissolved iron and total dissolved ferrous iron)	Clemence, Karolina, Emma (analytics)
	Wash step	25 mL deionized water	5 min shaking, centrifugation (5 minutes at 4500 rpm), combine with “BD decant”, and filtrate afterwards		
1 M KCl	18,5 g fill up to 500 ml with pure water				Marlene
0.11 M BD	10 g $Na_2S_2O_4$ (natrium dithionite) + 4.6 $NaHCO_3$ fill up to 500 ml with pure water				
0.5 M HCl	21 mL 36 % HCl fill up to 500 ml with pure water (Først vandet, så syren, ellers sker der noget uhyrligt.)				
5M H_2SO_4	500 ml				
2 M HCl	50 mL for conserving the KCl-samples				
Further Materials	• „gas diffuser “, overhead shaker, filter unit, centrifuge, scale, spoon, 25 mL volumetric flask, 100 mL beaker (1piece) • cellulose-acetate filters (0.45 µm pore size) (45 filters) • 30 x 50 mL centrifuge tubes (one set for chemical extraction and on set for later combining decants) (label example Kvo-1, 2, 3, 4, 5; Kvo-1-W, … (by hand with permanent marker) • 45 x 50 mL sample vessels (label example: KCl-Kvo-1, 2, 3, 4, 5; KCl-Vos-1, ….. (“label-machine”) • 30 x 50 mL centrifuge tubes (one set for chemical extraction and on set for later combining decants) (label example Kvo-1-Fe, 2, 3, 4, 5; Kvo-1-Fe-W, … (by hand with permanent marker) • 15 x 50 mL sample vessels (label example: Fe-Kvo-1, 2, 3, 4, 5; Fe-Vos-1, ….. (“label-machine”)				Clemence, Karolina

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Restoration of wetlands and impact on the phosphorus cycle

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Abstract :

As wetlands act as carbon and nitrogen sinks and contribute to reduce water pollution by acting as buffers between land and streams, their restoration appears like a good solution to fight global warming and reduce water pollution. Aarhus University and more precisely the department of Ecoscience is focusing on water quality and therefore on monitoring nitrogen and phosphorus before and after wetland restoration. The purpose of this internship was to focus on the potential P release after the rewetting of former wetlands. For the three projects this internship was focusing on, a risk of P release can be expected. Though the results presented in this report are not thoroughly evaluating the potential for P release yet, they point out the risk of P release in all sites that were studied. The future results of those projects could make a significant contribution in terms of wetland management before and after restoration. They will also be of interest in terms of the application of acidified manure to limit impacts and improve management.

Keywords :

Wetlands, restoration, rewetting, phosphorus (P)

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