

Master Thesis

Marc Pérez

Hier kommt das Abstract

Preface

Abstract

(Knuth 1984)

Introduction

Complexity of Phosphorous

Phosphorous displays a wide range of behaviours in soils, in places where organic, mineral and aqueous phases interface. In phases that contain oxygen Phosphorous is almost exclusively present as several derivatives of Orthophosphate PO_4^{3-} . It can be found as organic molecules as anhydric- and ester-groups, being needed by all known species as a constituent of DNA and energy transfer-processes. It can be present as anorganic Phosphate either as mono-orthophosphate PO_4^{3-} or poly-orthophosphate $HO - (PO_2)_n - OH$, where it can strongly interact with water, forming, depending on pH HPO_4^{2-} or $H_2PO_4^-$. The dissolved species of phosphate are subject to adsorption to clay- and oxide-surfaces of the solid soil-phase, they also form fallout-products such as Apatite, Vivianite etc. With the present metal-cations in the solution. While the solubility constant of most phosphate-salts are comparably low (Wert eingeben), meaning that the fallout and formation of minerals happens at low chemical activities of phosphate, phosphate often is leached from soil-surface-layers, heavily reducing the efficacy of P-fertilization and presenting a disturbance to P-limited ecosystems. Those phenomena, many of them being physicochemically controlled, are influenced by parameters such as pH, ionic-strength, clay-content, specific-surface of the solid phase, amorphous $Fe(OH)_3$ -content, amorphous $Al(OH)_3$ -content, in short the phenomena depend heavily on the composition, distribution and geometry of the soil. Those properties are considered to be stable

respectively long-term properties of a soil, when looked at it with the interest of modelling the transport processes of Phosphate in soils. Factors such as water-content, temperature, vegetation and precipitation are factors that temporally can vary fast and to a certain degree unpredictably. Organic forms of phosphates, prominently DNA or oligonucleotides and phytate are also subject to physicochemical reactions, mainly decomposition, but are foremost controlled in their presence by enzymatic processes, where i.e. plants form phytates in seeds to provide the embryo a compact and specific reserve of phosphate, but many bacteria possess via Phytases the ability to hydrolyse phytate and use it for their own means. To assess and cover those phenomena, models, dynamically describing the motion of Phosphorous in soils, differentiate several pools of Phosphorous, most prominently the organic-P, dissolved-P, adsorbed-P, mineral-P, where the difference in temporal behaviour, such as the mean-reside-time invite a differentiation between labile-P, semi-labile-P and so on.

Principle Mechanism of P-transport to the Plant

Plants that live in ground, interface with their roots the soil-matrix, soil-solution and air-pockets. It is the interface between root and soil-solution, where the exchange, particularly the uptake of water and nutrients happens. High-affinity phosphate transporters are mainly responsible for the transport of phosphate from the soil-solution into the cytosol. The momentary concentration of orthophosphate [$H_nPO_4^{n-3}$] in the soil-solution is called the intensity, meaning the Pi that is immediately available to the plant. It is influenced by sinks such as biological uptake, precipitation, adsorption and leaching, sources are dissolution of phosphate-minerals, mineralization resp. enzymatic hydrolyzation of organic Phosphate-esters and -anhydrides. Since it is difficult and sometimes up to now impossible to accurately assess all these factors, it has been shown beneficial to use a more agnostic approach and regard the diffusion rates, as a black-box model. For the sake of simplicity and feasibility, all biological-uptake can be removed, such that only the interaction between soil-matrix and soil-solution can be observed. Such a model would consist of the soil-matrix, containing the adsorption-surfaces and the interfacing soil-solution that exchanges phosphates until an equilibrium is reached. There have been different approaches to capture the diffusion of Phosphate. The IEK method employs ^{31}P and assesses the amount of P, that is exchangeable between both phases and the temporal development of that amount. Flossmann & Richter assessed the net-diffusion rate and equilibrium-concentration, modelling the diffusion-process as a first-order-kinetic. Whilst IEK observes the diffusion in a steady-state, the approach of Flossmann & Richter did start with a system of dried fine soil and deionized water, and subsequently sampling at different time-points, observing the desorption-reaction-speed from start to equilibrium.

Prediction of biological sinks

The prediction of biological sinks, particularly plant uptake is of great practical and academic interest. To adequately estimate the ability of a soil to provide sufficient Pi by assessing the

status of both soil and soil-solution is the basis for economically and ecologically sustainable fertilization practice. Several approaches and models exist and aim to connect estimations of the P_i -availability and the expected requirements of crops. The predominant group of models in use try to measure the extractable P_i via different extractants, and depending on aggressivity of the extractant to identify different pools of P_i . The extraction of P_i with H_2O or weakly extracting aqueous solutions are generally identified as the immediately available P_i or the Intensity. Extractants with strong acids or chelators, able to dissolve and desorb strongly bound P_i are identified as the P_i -Pool that will eventually become available to plants and is often called the Capacity. Such models continue to calculate the required amount of P_i by starting with the expected uptake of the planned crop.

Current models in the DACH-region

P-models deployed by the VDLUFA as well as GRUD share basic concepts and mathematical form; they will serve as an example how estimations of P-availability from soil tests and fertilization recommendations can be connected. The explained goal of those models is to reach an ecologically adequate level of P-availability such that only the plant-uptake has to be provided via fertilizer and said level can be maintained. Soil tests with nationally different extractants are therefore performed to assess the current P-availability as mentioned above, obtaining different measures of soil-test-phosphorus (STP). Depending on the STP value and the covariates clay-content (sensory measurement) and C_{org} -content the tested soil is placed in a P-supply-class ranging from A, depleted to E, eutrophicated. The estimated P-export, meaning the P-uptake of the harvesting organs, obtained by long-term-trials, most often the national mean-value is then adjusted by the affinity of the root-system for P-uptake, notably this is done for only a few major crops, resulting in the uncorrected fertilizer norm. This uncorrected fertilizer norm is subsequently multiplied with the supply-class parameter. This parameter is 1.5 for class A, in order to increase the amount of P_i available, while it is 0 for class E, such that the soil can be depleted to reach class C, designating adequate P-availability. Switzerland currently uses three concurrent STP-methods, with increasing order of extraction strength:

1. H2010-method: P is extracted using deionized H_2O for 10 minutes
2. CO2-method: P is extracted using deionized H_2O with 1M CO_2 for HOW MANY?? minutes
3. AAE10-method: P is extracted using edionized H_2O with ???M NH_4CH_3COO and ??? Na_4EDTA for 10 minutes

The three STP-methods shown above notably differed in their amount of isotopically recoverable P_i , where P-H2010 and P-CO2 displayed 97% of the P_i extracted being isotopically exchangeably, while AAE10 had below 25% and so did not represent P_i that eventually would become available ((demaria2005?))

A new approach from the VDLUFA published in 2018 () uses the coefficients obtained from

following the approach of Flossmann & Richter and using them as additional information to improve the supply-classification and modifying the correction term to the expected P-export.

Requirements to model parameters

Upon close inspection of the models in use, with particular focus on the established and known mode of P-transport, certain requirements have to be met, such that the models can work reliably across different types of soils, crops and weather scenarios. The assessed covariates in particular have to display certain correlations.

- Since the solubility of P_i in most soil-solutions, interfacing real soils is limited by the presence of metallic cations and adsorptive surfaces typically below 20 ppm per kg soil, plants usually deplete the soil-solution, surrounding the rhizosphere of P_i . The intensity of a nutrient must not be changed much or at all, it is to serve as a predictor for a reaction progress and equilibrium. Most of the P_i uptaken by a plant was delivered by a diffusive mass-transport.
- The covariates used to adjust the fertilization amount, aiming to reach a steady state, where fertilization equals export need to correlate (moderately strongly) to P-export, P-balance and in cases of low P-levels to the yield of a crop.
- At least one of the members of the parameter set used must correlate positively with amount of P fertilized, such that it “responds” to the treatment.
- Given the known mode of transportation of P, being dependend on diffusive mass-transport, the temporal reactivity of the soil-solution to changes in P-concentration, so the P-desorption-kinetic must in some form be present.

In this study soils from the STYCS-trial, under swiss management were used, a modified version of the Flossman & Richter P-desorption-kinetic was used to test, whether the STP-methods P-CO₂ and P-AAE10, and the new kinetic model parameters satisfy those requirements, and compare their performance:

Research Questions

- I: Is the **method** presented by Flossmann and Richter (1982) with the double extraction **replicable** with the soils from the **STYCS-trial**?
- II: How do GRUD-measurements of **STP correlate** to the soil properties C_{org} -**content**, **clay-content**, **silt-content** and **pH**?
- III: Are the kinetic coefficients k and PS **correlated to soil properties**?
- IV: How well can current GRUD methods of **STP** ($P - CO_2$ & $P - AAE10$) **predict** the **Yield-parameters**, **P-Export** and **P-Balance**?
- V: How well can the **kinetic parameters** k & PS **predict** **Yield-parameters**, **P-Export** and **P-Balance**?

Materials and Methods

The Long-Term Phosphorus Fertilization Experiment

The soil samples for this thesis originate from a set of six long-term field trials in Switzerland, established by Agroscope between 1989 and 1992. The primary objective of these experiments was to validate and re-evaluate Swiss phosphorus (P) fertilization guidelines by assessing long-term crop yield responses to varying P inputs across different pedoclimatic conditions. A detailed description of the experimental design and site characteristics can be found in Hirte et al. (2021).

The experiment was set up as a **completely randomized block design** with four field replications at each site. The core of the experiment consists of six fixed-plot treatments representing different P fertilization levels, which were applied annually as superphosphate before tillage and sowing. These levels were based on percentages of the officially recommended P inputs: 0% (Zero), 33% (Deficit), 67% (Reduced), 100% (Norm), 133% (Elevated), and 167% (Surplus).

Experimental Sites

The six experimental sites are located in the main crop-growing regions of Switzerland: **Rümlang-Altwi (ALT)**, **Cadenazzo (CAD)**, **Ellighausen (ELL)**, **Grabs (GRA)**, **Oensingen (OEN)**, and **Zurich-Reckenholz (REC)**. The key soil properties are summarized below.

Table 1: Soil characteristics of the six long-term experimental sites. Data adapted from Hirte et al. (2021).

Site	Soil Type (WRB)	Clay (%)	Sand (%)	Organic C (g/kg)	pH (H2O)
ALT	Calcaric Cambisol	22	48	21	7.9
CAD	Eutric Fluvisol	8	40	14	6.3
ELL	Eutric Cambisol	33	31	23	6.6
GRA	Calcaric Fluvisol	17	34	16	8.3
OEN	Gleyic-calc. Cambisol	37	32	24	7.1
REC	Eutric Gleysol	39	25	27	7.4

Source: [Article Notebook](#)

Soil samples for this thesis were collected on [Your Sampling Date] from the topsoil layer (0-20 cm). [Add any further specific details about your sampling strategy here].

Phosphorus Desorption Kinetics

The analysis of phosphorus (P) desorption kinetics was based on the principles of sequential extraction established by Flossmann and Richter (1982). The original method is described below, followed by the specific protocol adapted for this study.

Original Method of Flossmann and Richter (1982)

The foundational method aims to characterize the P replenishment capacity of the soil. The procedure is as follows:

1. **Removal of Soluble P:** 17.5 g of air-dried soil is shaken with 350 ml of deionized water for one hour at $25 \pm 1^\circ\text{C}$. The suspension is centrifuged and the supernatant is decanted to remove the readily soluble P fraction. This first extract is referred to as:
 - **Solution A:** Contains easily soluble P, which is discarded.
2. **Kinetic Extraction:** The remaining soil pellet is resuspended with another 350 ml of deionized water. Subsamples of the suspension are taken at specific time intervals, yielding the following extracts for kinetic analysis:
 - **Solution B:** Subsample taken after **10 minutes**.
 - **Solution C:** Subsample taken after **30 minutes**.
 - **Solution D:** Subsample taken after **120 minutes**.
3. **Analysis:** The P concentration in Solutions B, C, and D is determined colorimetrically using the molybdenum blue method according to Murphy and Riley (1962).

Adapted Kinetic Protocol for This Study

For this thesis, the original method was modified to capture the desorption process with a higher temporal resolution and using a different soil-to-solution ratio.

1. **Soil Suspension:** 10 g of air-dried soil was suspended in 200 ml of deionized water. Unlike the original protocol, a pre-washing step to remove soluble P was not performed, meaning the measured desorption includes both the release of readily soluble P and the subsequent replenishment from the solid phase.
2. **Kinetic Extraction:** The suspension was shaken continuously, and subsamples were taken at eight time points to generate a detailed kinetic curve. The resulting extracts were:
 - **Extract 1:** Subsample taken after **2 minutes**.
 - **Extract 2:** Subsample taken after **4 minutes**.

- **Extract 3:** Subsample taken after **10 minutes**.
- **Extract 4:** Subsample taken after **15 minutes**.
- **Extract 5:** Subsample taken after **20 minutes**.
- **Extract 6:** Subsample taken after **30 minutes**.
- **Extract 7:** Subsample taken after **45 minutes**.
- **Extract 8:** Subsample taken after **60 minutes**.

3. **Analysis:** Each subsample was immediately filtered. The concentration of orthophosphate in the filtered extracts was determined colorimetrically using the **malachite green method**.

Statistical Analysis

The statistical analysis aimed to derive the kinetic parameters of phosphorus (P) desorption for each soil sample. A two-stage approach was employed, starting with a linearized model and progressing to a more robust non-linear mixed-effects model.

Initial Approach: Linearized Model

[cite_start]Following the conceptual framework of Flossmann and Richter (1982), a first attempt was made to linearize the first-order kinetic model[cite: 2, 3]. The differential equation describing P release was solved and rearranged to yield a linear relationship:

$$\log \left(1 - \frac{P(t)}{P^S} \right) = -kt$$

In this formulation, $P(t)$ is the concentration of desorbed P at time t , k is the rate constant, and P^S is the maximum desorbable P, acting as the asymptote. [cite_start]For this initial model, the P^S parameter was not estimated from the kinetic data itself but was calculated *a priori* for each sample as the difference between the phosphorus concentration measured by the Olsen method (P_{Olsen}) and the initially water-soluble P concentration (P_{H_2O})[cite: 3, 7].

While this approach allowed for the use of simple linear regression to estimate k , the reliance on an externally calculated asymptote led to poor model fits and physically unrealistic results for several samples. Consequently, this method was discarded in favor of a more direct modeling approach.

Final Approach: Non-Linear Mixed-Effects Model

[cite_start]To overcome the limitations of the linearized model, a non-linear mixed-effects model (**nlme**) was implemented to estimate both the rate constant k and the asymptote P^S simultaneously and directly from the time-series data[cite: 29].

The model was fitted to the exact solution of the first-order rate equation:

$$P(t) = P^S \times (1 - e^{-k \times t'})$$

Here, t' represents the adjusted time. [cite_start]Based on preliminary analysis, it was observed that some P dissolves rapidly before the first measurement point[cite: 25]. [cite_start]To account for this, the measured time was adjusted by adding a 3-minute offset ($t' = t_{\min} + 3$)[cite: 7, 25].

[cite_start]The model was implemented in R using the **nlme** package[cite: 29]. To account for the hierarchical structure of the data, where multiple time points are nested within each unique soil sample, the model was specified with both fixed and random effects. The overall population means for P^S and k were estimated as **fixed effects**. [cite_start]To capture the unique desorption characteristics of each individual soil sample (defined by its unique ID, **uid**), subject-specific deviations from the fixed effects for both P^S and k were modeled as **random effects**[cite: 29].

This approach allowed for the robust estimation of individual kinetic parameters for each soil sample, providing the k and P^S coefficients used for all subsequent analyses in this thesis.

[The subsequent part of your analysis, where these coefficients are merged with the long-term trial data, would be described next.]

Results

Discussion

Conclusion

Acknowledgments

Legal Disclosure

References

Knuth, Donald E. 1984. “Literate Programming.” *Comput. J.* 27 (2): 97–111. <https://doi.org/10.1093/comjnl/27.2.97>.

Appendix

Supplements