Is resorption efficiency linked to nutrient concentrations in old-growth tropical forests?

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1 Abstract

Although it has been the subject of numerous studies, the mechanisms driving the paradox of having nutrient rich old-growth tropical forests growing on nutrient depleted soils, remains a mystery. Various studies have suggested efficient nutrient cycling to be the key, postulating a negative relationship between soil nutrient concentrations and resorption efficiencies. Empirical evidence supporting this hypothesis is, however, scarce and often contradictory. This study sheds light on the issue by coupling measurements and analyses of bulk soil elemental concentrations, canopy (fresh leaves) elemental concentrations, litter elemental concentrations and resorption efficiencies in both nutrient-rich and nutrient-poor old-growth tropical forests. Using the TropSOC database we tracked nutrient concentrations from soil, to canopy, to litter, in order to assess how this pathway differs between nutrient rich and nutrient poor environments. Using ICP-OES data, we were able to show that the two analysed regions did indeed differ in nutrient richness and that there may well be a relationship between soil P and K concentrations and the respective resorption efficiencies. Overall regression of K showed that bulk soil K concentrations had a negatively significant relationship with K resorption efficiencies (p-value = 0.00151, R2 = 0.6913, DF = 9). Additionally, the individual regression of P in the nutrient-poor region showed a significant negative relationship between P resorption rates and P bulk soil concentrations (p-value = 0.00151, R2 = 0.6913, DF = 9). Although not steadfast proof, these results indicate that a relationship between nutrient concentrations and resorption efficiencies may well be present in old-growth tropical forests.

2 Introduction

Despite often growing on extremely weathered soils, tropical forests store large amounts of above-ground nutrients [Vitoussek and Sanford, 2003]. It is said that these forests have a rich nutrient economy above a poor nutrient substrate [Vitoussek and Sanford, 2003]. Especially rock derived nutrients such as calcium (Ca), magnesium (Mg), phosphorous (P), and potassium (K), which are vital for plant growth, are strongly depleted in tropical soils [Bauters et al., 2022]. This paradox and the mechanisms thereof have been the focus of many studies throughout the years. One mechanism that is postulated to contribute to this phenomenon, is efficient nutrient cycling. Hereby nutrient losses are minimized via nutrient retention and internal cycling of essential nutrients [Brant and Chen, 2015, Vitoussek and Sanford, 2003]. This is most commonly referred to as nutrient resorption and is a key process for nutrient availability, conservation and cycling [Brant and Chen, 2015, Vergutz et al., 2012]. Understanding the mechanisms that drive this resorption is therefore of global relevance and is essential for the establishment and accuracy of ecosystem and biogeochemical models [Brant and Chen, 2015]. Despite this, such understanding remains limited and exact relationships remain unclear [Brant and Chen, 2015].

It is often assumed that species living in nutrient poor environments have higher resorption efficiencies than species inhabiting nutrient-rich environments [Aerts, 1996]. However, despite decades of research, results on relationships between nutrient resorption and nutrient concentrations are contradictory [Brant and Chen, 2015, Killingbeck, 2004, Yuan and Chen, 2015]. While several studies along fertility gradients and in fertilisation experiments have found no relationship between the two, other studies, including meta-analyses based on global databases, suggest a coupling between nutrient status and nutrient resorption efficiency [Brant and Chen, 2015, Killingbeck, 2004, Yuan and Chen, 2015].

This study aims to shed light on this issue by providing measurements and analyses of bulk soil elemental concentrations, canopy (fresh leaves) elemental concentrations, litter elemental concentrations and resorption efficiencies across various old-growth tropical forests in the Eastern part of the Congo Basin. This data was taken from the data gathered within the TropSOC database and all subsidiary information is taken from this database and the attached publication[Doetterl et al., 2021]. The chosen locations can be differentiated into regions characterised by different parent material and geological age. Due to these differences they also differ in weathering status and nutrient concentrations.

The wide variety of nutrient concentrations and measurements (ICP-OES, ECEC, plant available phosphorous) as well as the structured approach (soil-canopy-litter), allows for a comprehensive overview of the relationship between nutrient concentrations and nutrient resorption in senescing leaves. We hypothesise that, despite controversial reports, such a relationship exists and that it is a key driver of nutrient cycling in old-growth tropical forests and can offer some explanation of the discrepancy between below-ground and above-ground resource distribution. This report will focus for the most part on the ICP-OES analysis.

3 Material and Methods

The "TropSOC project" gathered data throughout the Eastern Congo and East African Rift Valley System, gathering not only soil and leaf data but data on soil, vegetation, environmental properties and land management of 136 tropical forest and cropland plots as well [Doetterl et al., 2021]. The overall objective of this project was to develop a mechanistic understanding of soil organic carbon dynamics, including plant responses to soil properties in the African tropics. As such, this report falls within these objectives and provides a minor part of the research output achieved. The data used for this report was collected between 2017 and 2020 and exact locations and in-depth descriptions of the study sites, the sampling structure, the material and methods and the overall project can once again be found within the TropSOC database and the attached publication [Doetterl et al., 2021]. The study sites were located between the Congo and the Nile basin and encompassed sites across South and North Kivu, Western Rwanda and Southwestern Uganda. These different regions are characterized by three different and geochemically distinct parent materials and throughout the report will therefore be referred to as "mafic", "felsic" and "sedimentary", describing the underlying parent material. Although the TropSOC database contains both forest and cropland sites, this report only looked at resorption rates and nutrient concentrations in the forest sites, as these represent near pristine and untouched habitats and are therefore more relevant for deducing natural relationships. The individual plots were sampled using a strict catena approach in order to investigate differences between geochemistry and soil and plant parameters. Twelve 40 m x 40 m forest plots were established for each geochemically distinct region and were subdivided into 20 m x 20 m subplots. 4 soil cores were extracted from each subplot using a cylindrical soil corer and provided the material upon which the bulk soil measurements were taken.

3.1 Bulk Soil Measurements

These bulk soil measurements included the measurements of plant available phosphorous concentrations, effective cation exchange capacity and total elemental composition as determined by inductively coupled plasma optical emission spectrometry (ICP-OES). These steps did not constitute a part of the research project and therefore the following only offers a brief overview of the applied methods.

3.1.1 Plant Available Phosphorous

Plant available phosphorous was measured according to the Bray 2 method, after passing bulk soil through a 2 mm sieve. In this method 50 ml of a 0.03 NH4 and 0.1 M HCl solution are added to 2.5 g of bulk soil. Following 5 min of shaking, the extract can be filtered and analyzed on a spectrophotometer. The amount of phosphorous content in solution is determined colorimetrically, measuring the intensity of signal turbation at 880 nm, following treatment with a molybdate-ascorbic acid reagent.

3.1.2 Plant Available Potassium

Potential cation exchange capacity (CEC) and effective cation exchange capacity (ECEC) were similarly analysed on 2 mm sieved bulk soil. Potential cation exchange capacity was measured via percolation with BaCl2 at a pH of 8.1. Effective cation exchange capacity on the other hand was analyzed by percolation with NH4Cl at soil pH. The resulting percolate was analyzed for exchangeable bases using flame photometry and atomic absorption spectrophotometry. Base saturation, defined as the relative availability of all base cations (Na, K, Mg, Ca) for CEC, was calculated in percent of ECEC. Of these elements, the following analysis only made use of the potassium measurements, henceforth referred to as plant available K [Doetterl et al., 2021].

3.1.3 Total Element Composition via ICP-OES analysis

Total element composition was measured using inductively coupled plasma optical emission spectrometry (ICP-OES) (5100 ICP-OES Agilent Technologies, USA). This analysis was performed on both bulk soil and physically separated mineral fractions of soil. For these measurements, 1g of powdered sample

material was placed in a digestion tube and boiled in aqua regia for 90 minutes at 120Â°C, using a DigiPREP digestion system (DigiPREP MS SCP Science, Canada). All extracts and standards were then filtered through a 41 grade Whatman filter and diluted at a ratio of 1:2 for Ca, Mg, Na, K, P and 1:1000 for Al, Fe and Mn, using a diluting system (Hamilton 100, USA). This was followed by ICP-OES measurement [Doetterl et al., 2021].

3.2 Fresh Leaf Measurements

Fresh leaf samples were obtained via specially trained tree climbers. These samples were comprised of leaves taken from sun-exposed shoots of the outer canopy of selected tree species, constituting 80% of the standing basal area per plot. When sampling of the outer leaves was not possible, partially shaded leaves situated below the uppermost canopy were sampled instead. Between 5 and 17 trees per plot underwent this sampling scheme. All leaves were oven-dried at 70°C, dry-weighed and milled for later chemical analysis. This analysis entailed the measurement of total elemental composition based on ICP-OES. For brevity's sake, this method will be further discussed in the section "Litterfall Measurements" as, following sampling, both fresh and litterfall plant material received the same treatment. Following analysis, values were aggregated according to relative abundance within a plot, to create one weighted average per plot. Similar to the bulk soil measurements, fresh leaf measurements were not part of the research project and therefore detailed accounts of the methods and sampling structure can once again be taken from the TropSOC database [Doetterl et al., 2021].

3.3 Litterfall Measurements

Litterfall on the other hand was sampled using 10 litter traps, installed and distributed evenly within each plot, following a standardized protocol for measuring tropical forest carbon allocation and cycling [Marthews, 2015]. These litter traps had a diameter of 60 cm and were installed at a height of 1.0 m above the ground. They were emptied once every 2 weeks between August 2018 and February 2020 and the collected material included all organic residues, save those from larger animal bodies and woody material > 2 cm. These samples were pooled as composite samples, representative of their respective plots, were oven-dried and were subsequently weighed. Additionally, the samples were aggregated into seasons, categorized based on the average precipitation for each period, with each sample consisting of 10 g of each constituent month. As sampling had not occurred during all months in all plots, some of these aggregated samples consisted merely of samples taken during 1 or 2 months. The aforementioned categories included a "weak dry season" (December to February), a "strong rain season" (March to May), a "strong dry season" (June to August) and a "weak rain season" (September to November). The final set of samples that underwent further processing was comprised of 118 samples. Following sampling and aggregation the samples were ground in a blender at the lowest setting for 1.5 minutes plus an additional half minute per month sampled in the aggregate. The ground samples were then transferred into the receptacles of a ball mill. A ceramic ball was added, and each sample was milled for 2.5 min until the sample had been fully pulverized

3.3.1 Total Elemental Concentrations via ICP-OES Analysis

200 mg +- 10 mg of the ground and milled samples were weighed into Teflon tubes and were covered with a plastic cap to avoid contamination. Exact weights were noted, as well as the placement within the extraction rack. Additionally, two reference samples and two blanks were created per rack using the standards IPE 2012-1-4-157 and IPE 2012-2-1-124. The 118 samples were extracted in 3 batches entailing the extraction of acid extractable cations such as K, Ca, Mg, Al, Fe, Mn and P and further analysis with inductively coupled plasma-optical emission spectroscopy (ICP-OES). Following the weighing in, nitric acid (HNO3) was added to each sample, followed by extraction at 120°C for 150 min. In a subsequent extraction hydrogen peroxide was added to the samples and once again extraction was performed at 120°C for 150 min. Following the extractions, the solution was transferred to 50ml centrifuge tubes and nanopure H2O was used to rinse the Teflon vials three times each. The volume was adjusted to the next 5ml mark using nanopore water; in all but two samples this was 40 ml. Following

the mixing of the samples via repeated shaking, the samples were diluted by a factor of 4 with nanopure H2O and were processed via ICP-OES Analysis.

3.4   Data Preparation and Statistical Analysis

Following the measurements, all values were converted to mg per g of dry weight and further analysis was performed in Rstudio [R Core Team, 2022]. The individual plots were averaged in order to produce a region-specific average. Nutrient resorption is often estimated as resorption efficiency, which is defined as the percentage of nutrient reduction between green and senesced leaves[Killingbeck, 2004]. This is also true for our study and rates of resorption between canopy and litter were calculated as the inverse ratio of the litter concentrations divided by the fresh leaf concentrations. They can therefore be seen as the percentage of the element that was lost in transition from fresh leaf to litter.

Our analysis mainly involved data visualization with boxplots, calculation of resorption efficiencies and regression analysis of the relationship between nutrient concentrations and resorption efficiency. The regression analyses were performed for both Potassium and Phosphorous and included an overall regression as well as individual regressions for each region, in order to avoid spatial autocorrelation. Due to large differences in values, for the comparison of fresh leaves and litter, calcium was used as a tracer as it is assumed that calcium is not resorbed and therefore concentrations remain constant in senescing leaves. In these comparisons all values were adapted according to the change in calcium concentrations. For simplification, the mafic regions were excluded from further analysis, as the sedimentary and felsic regions showed the largest differences in terms of nutrient concentrations and therefore had the most relevance for this study. Additionally, as they were not further analysed in this report, the 4 categories of litterfall samples were averaged for better data visualization, creating a total of 23 comparable data points. As the plant available phosphorous and potassium measurements had numerous negative values, these were excluded from the analysis. All bulk soil measurements therefore refer to the total elemental concentrations as measured via ICP-OES analysis.

4 Results and Discussion

Due to time constraints results and discussion will only be briefly visited. A full analysis and discussion of the results can be found in Dr. Bukombe's forthcoming publication.In general, the produced data showed a high degree of variability and uncertainty. This was to be expected as different sampling strategies were used, especially between canopy (fresh leaves) and litter samples, and the regions showed a high degree of heterogeneity. Additionally, due to the relatively few number of data points, only clear and strong relationships were going to be able to be observed. Nevertheless, some interesting patterns emerged.

Sedimentary regions showed slightly higher concentrations of metals such as aluminium and iron. Soils from this region are regarded as highly leached and therefore lack most nutrients essential for plant growth. As metals are generally not leached from soils, their concentrations are relatively high. These relatively high concentrations are reflected in both the bulk soil and the plant litter (Fig. 1 and Fig. 2).
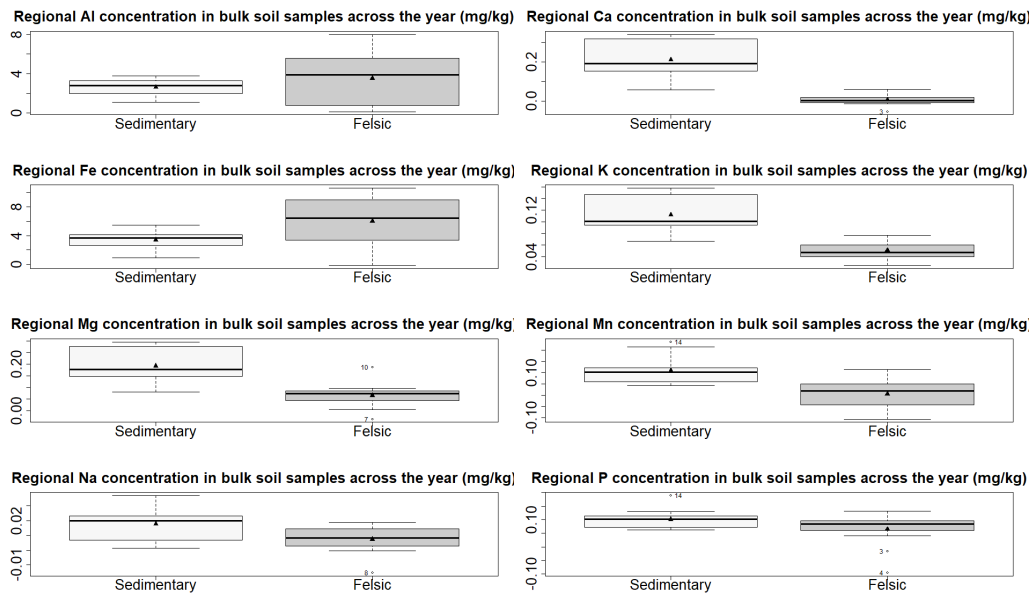

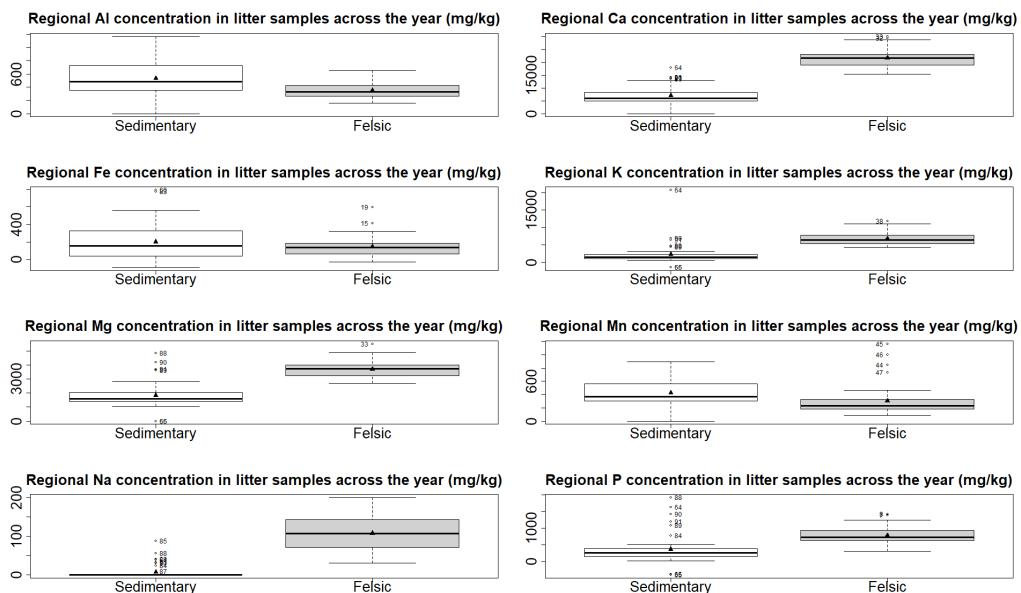Figure 1: Bulk soil nutrient concentrations per region

Figure 2: Litterfall nutrient concentrations per region

On the other hand, as to be expected, most other elemental concentrations, including those necessary for plant growth, seem to be substantially higher in the felsic region (Fig. 1 and Fig. 2). This may be in part due to the fact that this region has received volcanic ash input and therefore has relatively fertile topsoil. For our further analysis we therefore considered the sedimentary region to be poor in nutrients and the felsic region to be rich in nutrients.

Our results also show a high degree of similarity between the patterns of elemental concentrations in the bulk soil and in the plant litter, suggesting a high degree of dependency between the two (Fig. 1 and Fig. 2). This indicates that the elemental concentrations in the litter samples are most likely primarily dependent on the concentrations in the bulk soil. This pattern was not replicated in the manganese concentrations. Although this was not explored further, the large number of outliers may suggest that this is an artifact of the data rather than a systematic relationship.
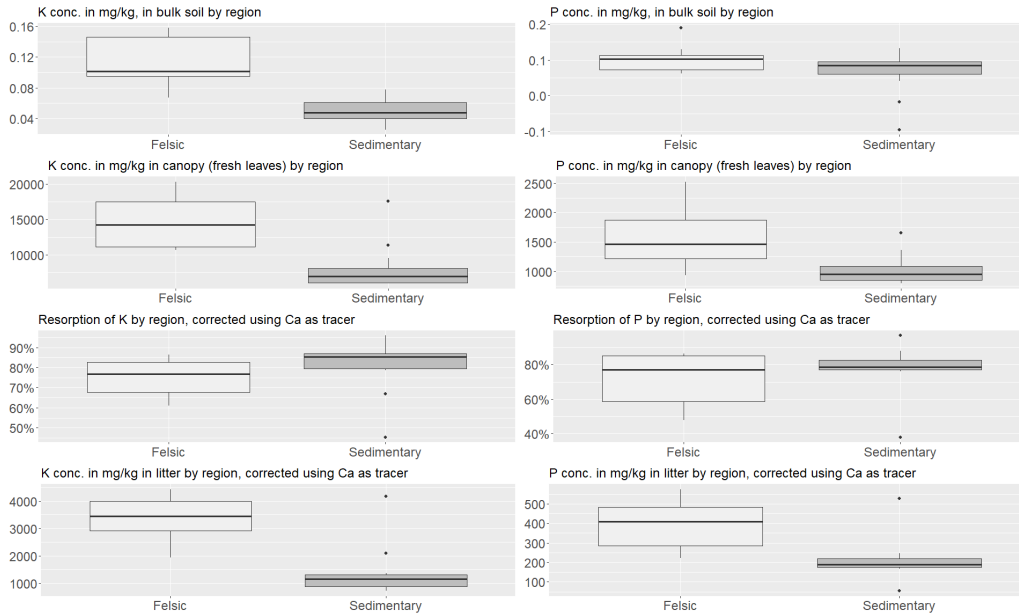

Figure 3: Elemental concentrations and resorption efficiencies across multiple stages

Although all measurements were looked at whilst studying differences in nutrient concentrations, further analysis was only performed using Potassium (K) and Phosphorous (P). These are amongst the most important nutrients for plant growth and therefore, of the measured elements, should be the most affected by differing resorption patterns [Aerts and Chapin, 1999]. Looking at K and P measurements across bulk soil, canopy and litter, we can see the same distinction between nutrient rich felsic sites and nutrient poor sedimentary sites as described before (Fig. 3). K resorption rates seem to mostly lie between 65 % and 90 %. P resorption rates on the other hand seem to lie between 55 % and 85 %. As hypothesized, the resorption rates seem to be indicating that the nutrient poor sedimentary sites have more efficient resorption, than their nutrient rich counterparts (Fig. 3). This is especially true for the potassium (K) measurements (Fig. 3).

The P regression analysis showed that the overall linear model did not detect a significant relationship between the P resorption efficiencies and the P bulk soil concentrations (p-value = 0.182, R2 = 0.08326, DF = 21) (Fig. 4). This is not surprising as the boxplots already showed a high degree of variability and only slight differences between the resorption efficiencies of nutrient rich and nutrient poor sites. Individual regression of the felsic region also showed a non-significant relationship between the P resorption efficiencies and P bulk soil concentrations (p-value = 0.274, R2 = 0.1182, DF = 10) (Fig. 4). However, regression of the sedimentary region showed a significant negative relationship between P resorption efficiencies and P soil concentrations explaining a large amount of the variation in the data (p-value = 0.00151, R2 = 0.6913, DF = 9) (Fig. 4). The detection of this relationship in the sedimentary region could be explained by the fact that this region showed the lowest nutrient concentrations and therefore, according to our hypothesis, the relationship between resorption efficiencies and nutrient concentrations should be strongest (Fig. 4).
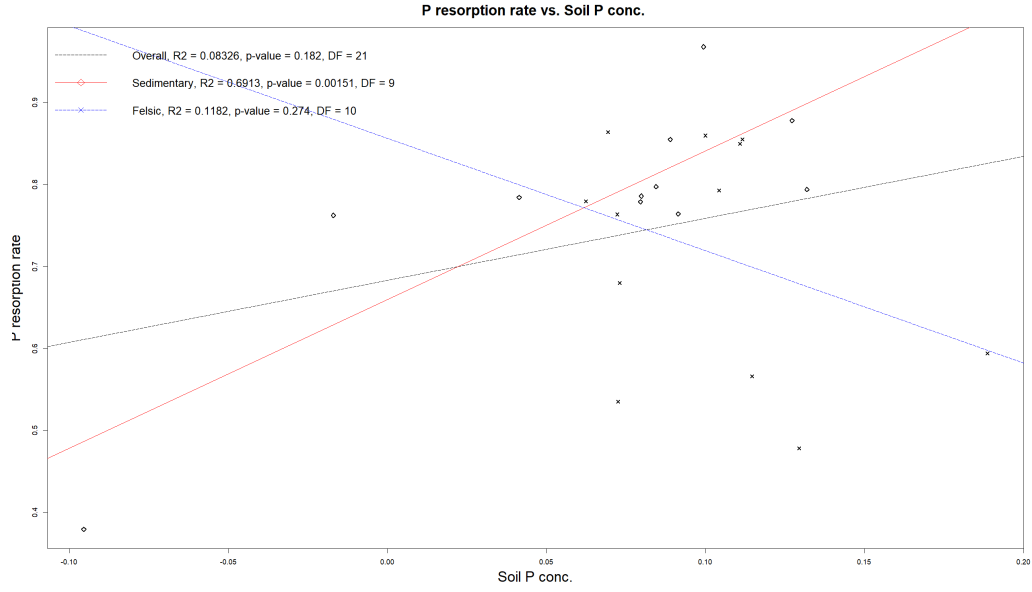
Figure 4: Regression analysis of resorption efficiencies vs. P concentration (mg/g) in bulk soil; dashed lines indicate non-significant results
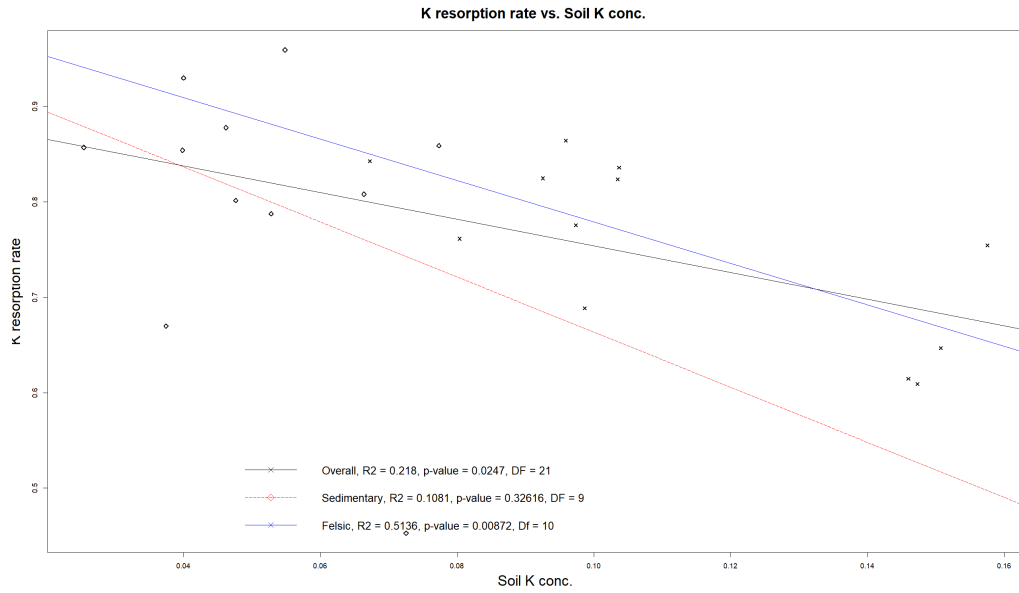

Figure 5: Regression analysis of resorption efficiencies vs. K concentration (mg/g) in bulk soil; dashed lines indicate non-significant results

The overall K regression analysis on the other hand showed a significant negative relationship between the K resorption efficiencies and the K bulk soil concentrations (p-value = 0.0247, R^2 = 0.218, DF =21), supporting the hypothesis that resorption efficiencies increase in nutrient limited environments. As the boxplots already showed relatively strong differences between the K resorption efficiencies of nutrient rich and nutrient poor regions, this was to be expected. Surprisingly, however, the sedimentary region did not show the same relationship (p-value = 0.32616, R^2 = 0.1081, DF = 9). The felsic region on the other hand also showed a significant negative relationship between bulk soil K concentrations and K resorption rates (p-value = 0.00872, R^2 = 0.5136, DF = 10). We have no explanation as to why this relationship would be present in the felsic region but not in the sedimentary region.

4.1 Conclusion

Despite the high degree of variability in our data and the limited number of observations, the observed relationships indicate nutrient concentrations may indeed be negatively correlated with resorption efficiency. Although true for both phosphorous and potassium, we found these relationships to be stronger and more evident in potassium. Although some significant relationships were found many other factors explaining nutrient resorption efficiencies might still be at play. Other studies have suggested that resorption efficiencies differed among plant growth types and were highly dependent on phenology, as well as water availability, as this causes premature abscission of leaves and therefore lower nutrient resorption [Brant and Chen, 2015, Killingbeck, 2004]. In order to conclusively say if a relationship between nutrient concentration and nutrient resorption efficiency exists, more data and a more thorough analysis is required, taking the aforementioned factors into account. Additionally, an analysis using plant available phosphorous and potassium measurements may reveal a tighter coupling of resorption efficiencies and nutrient concentrations and therefore more conclusive statements. Lastly, some studies have suggested that foliar nutrient concentrations also play an important role in nutrient resorption and that resorption only decreases with foliar nutrient status[Vergutz et al., 2012]. As such, a regression analysis of foliar nutrient concentrations and resorption efficiencies may also prove to be insightful. Such analyses will be provided in Dr. Bukombe's forthcoming publication.

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