Mendel University in Brno

Faculty of Forestry and Wood Technology

Department of Geology and Pedology

The Effect of Drought on Forest Tree Species' Nourishment: The Chosen Path of Phosphorus Cycling

DIPLOMA THESIS

2015 Theodore Danso Marfo

Declaration

I declare that I carried out this thesis: **"The effect of drought on forest tree species' nourishment: the chosen path of phosphorus cycling"** independently except for references to the work of other authors which were accordingly cited.

I agree that my work will be published in accordance with Section 47b of Act No. 111/1998 Coll. on Higher Education as amended thereafter and in accordance with the Guidelines on Publishing University Student Theses.

I understand that my work relates to the rights and obligations under the Act No. 121/2000 Coll., the Copyright Act, as amended, in particular the fact that Mendel University in Brno has the right to conclude a license agreement on the use of this work as a school work pursuant to Section 60 paragraph 1 of the Copyright Act.

Before closing a license agreement on the use of my thesis with another person (subject), I undertake to request for a written statement of the university that the license agreement in question is not in conflict with the legitimate interests of the university, and undertake to pay any contribution, if eligible, to the costs associated with the creation of the thesis, up to their actual amount.

In Brno, 04/2015

…….

Theodore Danso Marfo

Acknowledgements

I give all thanks and glory to God for the gift of life, strength and sense of reasoning to be able to reach this height in my knowledge seeking quest.

My deepest gratitude goes to Prof. Ing. Klement Rejsek, CSc (my thesis supervisor) for accepting my request for his supervision. His patience and down to earth suggestions kept me on my toes.

Secondly, I thank Doc. RNDr. Pavel Formanek, PhD (my thesis consultant) for his technical guidance and advice.

Thirdly, I thank all the staff and PhD students at the Department of Geology and Pedology especially doc. Ing. Valerie Vranová, PhD; Ing. Karel Marosz; Ing. Jindřich Figala; Ing. Rahul Datta and Ing. Soňa Kočvarová for the diverse ways I was aided to conduct this research.

Dedication

I dedicate this piece of work to the memory of my late parents, **Joseph Danso Marfo** and **Sophia Pat-Williams**. Their guidance and advice has made me the person I am today.

Abstract

This research sought to evaluate the effect of drought on forest tree species with emphasis on bio-available phosphorus obtained via phosphorus cycling. Soil samples were collected at root zone depth from areas of varied altitudes and tree species thus H-horizon soils from the mountain Spruce forest at Bily Kriz, A-horizon soils from both the young Spruce monoculture at Rajec and Beech forest at Stitna. Acid phosphatase activity of the various soil samples was measured in optimal conditions of the enzyme in the laboratory using the already in use protocol developed by Tabatabai and Bremner (1969) and some modifications made by Rejsek (1991). Two other protocols were developed by this research by replacing the buffer component of the first with water to mimic the unstable pH situation which occurs in nature and simulating a drought situation in the laboratory by incubating the soil sample with powdered form of the substrate used in the original protocol and adding water after the hour-long incubation. The laboratory analyses proved that global climate changes affects conservation of forest soil fertility via decreasing soil biological activity evaluated through phosphorus bioavailability. Soil phosphorus is indispensable for forest tree species' breeding hence important for European Forestry.

Key words: Drought, acid phosphatase, phosphorus cycle, climate change, forest tree species

Abstrakt

Cílem tohoto výzkumu je zhodnotit vliv sucha na druhy lesních stromů. Důraz byl kladen na biopřístupný fosfor, který je získáván z koloběhu. Vzorky půdy byly sbírány v kořenové hloubce v oblastech s odlišnou nadmořskou výškou a s odlišnými druhy. Půdy horizontu H byly odebrány v horském smrkovém lese na Bílém Kříži, půdy horizontu A v mladé smrkové monokultuře v Rájci a v bukovém lese ve Štítné. Aktivita kyselé fosfatázy ve vzorcích byla měřena za optimálních laboratorních podmínek za použití protokolu vyvinutého v Tabatabai a Bremmer (1969) s mírnou modifikací od Rejška (1991). V rámci tohoto výzkumu byly vyvinuty další dva protokoly, první nahrazuje pufr vodou pro napodobení nestálého pH, které se vyskytuje v přírodě. Sucho bylo simulováno inkubací vzorku půdy s práškovou formou substrátu, který byl použit v původním protokolu, a následným přidáním vody po hodině inkubace. Provedené laboratorní analýzy dosvědčili vliv globální klimatické změny na úrodnost testovaných lesních půd prostřednictvím snížení biologické aktivity půdy, zde měřené v přímé vazbě na přístupnost základního faktoru plodnosti rostlin, fosforu. Tento poznatek má všeobecnou platnost pro lesnictví Evropy.

Klíčová slova: sucho, kyselá fosfatáza, koloběh fosforu, klimatické změny, druhy lesních stromů

Table of contents

List of tables

List of figures

CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of problem

Future forests have to provide sufficient amounts of high quality timber due to the increasing demand. However, reduction in water availability as a result of the global climate change is expected to greatly reduce forest stability and productivity (Churkina and Running 1998, Chapin III, Callaghan et al. 2004, Malhi, Roberts et al. 2008). Prompt response is needed to curb the negative effects of climate change on forests given the long life span and thus the long rotation periods of trees.

1.2 Water availability in a future climate

Global climate change has markedly increased rainfall levels in the winter and spring in the North of Europe (Bardossy and Caspary 1990, Beck, Jacobeit et al. 2007). Climate models reveals that, this sequence will be deepened in the future as higher precipitation levels in the winter and spring is to be expected in these regions, resulting in a higher risk of flooding periods (Kramer, Vreugdenhil et al. 2008, Krysanova, Buiteveld et al. 2008). On the other hand, rainfall levels in the Central and North of Europe is expected to experience marked reduction in future summers (Gregory, Mitchell et al. 1997, Beniston, Stephenson et al. 2007). Current studies predicts warmer summers warming which will lead frequent summer drought periods (Fink, Brücher et al. 2004, Schär and Jendritzky 2004, Schär, Vidale et al. 2004, Fischer, Seneviratne et al. 2007, Founda and Giannakopoulos 2009, Kreuzwieser and Gessler 2010). There is a prediction of great variability in the weather, which is expected to hasten the occurrence of extreme weather periods such as prolonged drought periods. Though rainfall levels are expected to reduce in Europe during summer, heavy rainfall periods is also expected to occasionally occur during this same period. This will lead to flooding even during

vegetative periods (Christensen and Christensen 2003, Meehl, Arblaster et al. 2005), especially in areas with low water permeability like areas with high clay content.

1.3 Tree nutritional aspects

Inorganic nutrient uptake by the roots of trees is not just dependent on nutrient availability but also on how efficient the uptake systems of especially the mycorrhizal roots of trees (Geßler, Jung et al. 2005, Selle, Willmann et al. 2005). The root surface area is very important for nutrient uptake. Organic matter turn-over by microbes positively influences nutrient availability in the ecosystem.

On a smaller level, a function of the spatial spread of nutrients in the vertical manner within the soil profile and in the horizontal manner is influenced by the heterogeneity of the soil and the gaps in the forest canopy. The three-dimensional spread of roots in the soil which relates to the varying nutrient concentration gradients is critical. The ability of roots to take up soil area in search of nutrients thus the soil's foraging capacity is the yardstick to measure the soil's resource acquisition efficiency. Plants ability to acquire available nutrients in the rhizosphere can be explained by the kinetic nature the transporters of the nutrients thus the transporter affinity and maximum uptake capacity (von Wirén, Gazzarrini et al. 1997, Bassirirad 2000). In the case of plant species in forest ecosystems, biotic interactions that cause competitive hindrance or facilitation become crucial nutrient availability and its uptake efficiency. These interactions occur among plants and between plants and microbes. The nature of these interactions might cause a rise or fall in nutrient availability for roots and/or uptake efficiency of roots (Ortíz-Castro, Contreras-Cornejo et al. 2009). Both flooding and drought affect the above and below-ground physiology of trees as well as properties of the soil. Aside, the drawbacks water deficit has on the water and carbon balance of trees and forests (Ciais, Reichstein et al. 2005), mineral nutrition is a crucial factor which will also be affected by extreme water conditions in the future.

Current reviews evaluating the effects of the forecasted global climate change mainly focuses on the effects of temperature on tree nutrition (Bassirirad 2000). Reviews that takes into consideration flood or drought situations is mostly focused on the activity of enzymes (Kreuzwieser and Gessler 2010) or on specific tree species (Rennenberg, Loreto et al. 2006).

This thesis will attempt to also evaluate how drought affect forest tree nutrition with emphasis on bio-available phosphorus obtained via phosphorus cycling. Forest tree nutrition in drought conditions has become a topic of great importance because of the decreasing water availability resulting from the global climate change. All experiments will be conducted in a laboratory setting with drought simulation and will be preceded by the following hypothesis generated from literature and researches made by other scientists;

- Water stress or drought has negative effect on microbial activities and in effect enzymatic activities hence limits bioavailability of phosphorus.
- H-horizon soil has the highest soil enzymatic activities due to high microbe content as a result high organic matter content.
- Unstable soil pH negatively affects soil enzymatic activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Drought and its effect on nutrient availability to plants/trees

Although organic forms of nitrogen in soil and the rhizosphere play a crucial role in nitrogen supply to forest trees in boreal and temperate ecosystems (Näsholm, Huss-Danell et al. 2000, Persson, Högberg et al. 2003), available forms of inorganic nitrogen such as nitrates and ammonium, is still deemed crucial (Lucash, Eissenstat et al. 2007, Rennenberg, Dannenmann et al. 2009). Inorganic sulphate represents the most important sulphur source taken up by roots of plants (Buchner, Takahashi et al. 2004, Kopriva 2006). Phosphorus and a lot of nutrient elements are mainly taken up in the inorganic mineral forms (Schachtman, Reid et al. 1998, Marschner and Marschner 2012). This suggests that, any negative effect of drought on microbe mineralization actions that affects the amount of available nutrient is detrimental to tree nutrition.

Less available soil water hinders microbe action in the soil. Depending on the level of the water stress, the microbe metabolism can be completely hindered (Muhr and Borken 2009). In another scenario, the diffusion of the organic substrates to enhance microbial mineralisation becomes restricted (Lawrence, Neff et al. 2009). Again, the movement of microbes and other co-enzymes such as proteases decreases with increasing drought. Drought periods cause a reduction in bacterial activity which comes with dehydration and as the drought persists it leads to dieback of soil microbes (Schimel, Balser et al. 2007).

Clearly, the decrease in microbe action has something to do with the length and persistence of the drought period and the manner in which microorganisms will adapt to it. Generally, the specific effects of drought on mineralization are not spot on due to the inconsistency of results in existing literature. In terms of nitrate and ammonium concentrations in soil solution (Melillo, Butler et al. 2011), Johnson et al. (2002) observed no effects of drought in a deciduous forest for nitrogen availability. Higher ammonification rates was noted in the summer on a drought beech area in comparison to a cool and moist area (Geßler, Jung et al. 2005). Carbon and nitrogen mineralization were noted to be influenced variedly by drought

(Beier, Emmett et al. 2009). In the case of organic carbon, decomposition was mainly hinged on temperature but ammonification though also depends on temperature but reduced soil water availability is a great hindrance to this process. The amount of organic nitrogen in the soil increases during drought events, probably due to dieback of microbes (Luyssaert, Ciais et al. 2010). The benefits of this potential nitrogen during the drought periods are not known. Therefore the extent to which drought affects nutrition availability in terms of microbial action can still not be evaluated.

2.2 Phosphorus presence in the soil

The majority of phosphorus in the soil exists in the form of organic phosphorus, calcium bound inorganic phosphorus and iron or aluminium bound inorganic phosphorus (Witkowski and Mitchell 1987). The average amounts of organic and inorganic phosphorus differs markedly from soil to soil. The soil solution contains both organic and inorganic phosphorus, but a lot of the phosphorus in these groups is insolubility hence unavailable to plants. Phosphorus content in soil solution is usually around 0,001 mg/ l in very infertile soils to about 1 mg/ l in fertile as well as heavily fertilized soils (Pierzynski, Logan et al. 1990). A number of organic phosphorus compounds can be found in the soil, but it is difficult to know their identity and measure their amounts. Currently, Inositol phosphates are regarded as the most abundant organic phosphorus compound in the soil (Turner, Papházy et al. 2002). Phosphorus mineralization and immobilization are governed by a lot of the same processes that govern decomposition of soil organic matter thus temperature, moisture and nitrogen availability (FOG 1988). The conversion of organic phosphorus to mineral phosphorus in soils of temperate regions generates between 5 and 20 kg P/ ha/yr (Stevenson and Cole 1999). The mineralised phosphorus enters the soil solution where it is re-immobilised into organic matter and taken up by plants. As a general rule of thumb, available phosphorus for plant uptake is within soil pH of 6 and 7 (Jackson, Manwaring et al. 1990). Most of the compounds with which phosphorus reacts are in the finer soil particles. If soils with similar pH values and mineralogy are compared, phosphorus fixation will to be more evident in soils with higher clay contents (Parton, Stewart et al. 1988).

2.3 Mineralization and immobilization of Phosphorus

Mineralization and immobilization of organically held phosphorus is by the same general processes that release nitrogen from organic matter in the soil. Floors of forests represent sinks and sources of phosphorus (Bohlen, Scheu et al. 2004). When organic debris with low phosphorus but high carbon and other nutrients are incorporated to the soil, the microbes will immobilize the phosphorus in the biomass. According to Brady & Weil (1999), net immobilization of soluble phosphorus occur if the residues added to the soil have a carbon to phosphorus ratio more than about 300:1 (Ström, Owen et al. 2005), while net mineralization occur if the ratio is smaller than 200:1. However, Moore et al. (2006) presented markedly different ratios for Canadian temperate to subarctic forests (Manzoni, Trofymow et al. 2010). They found a strong relationship between the initial phosphorus content in litter and the pattern of phosphorus release. Litters with high initial phosphorus contents lost phosphorus and those with low contents retained phosphorus, with a critical carbon to phosphorus quotient for release of 700-900. They suggested that net phosphorus loss probably occurred at carbon to phosphorus quotients between 800 and 1,200. Osono & Takeda (2004) investigated the influence of lignin to phosphorus ratios as an indicator of phosphorus dynamics in litter in a cool temperate forest in Japan. The lignin to phosphorus ratio from which mobilization began was 500-620 (Osono and Takeda 2004). The critical values of the lignin to phosphorus ratios showed convergent trends among litter types as compared to their initial values, and reached those of the underlying humus layer (Osono and Takeda 2004). According to Brady & Weil (1999), mineralization of organic phosphorus in soils of temperate regions typically release between 5 and 20 kg P/ha/yr. These values can be juxtaposed with the yearly uptake of phosphorus by crops, trees and grasses, which generally range from 5 to 30 kg P/ha/yr. In spite of the fact that phosphorus may hinder productivity of forest ecosystems, much less attention has been given to phosphorus mineralization as compared to nitrogen mineralization. According to Brady & Weil (1999), mineralization of organic phosphorus is subject to many of the same influences that control the general decomposition of soil organic matter thus temperature, moisture and availability of nitrogen. Because of the lack of information available with regard to the mineralization of phosphorus, a description of the major factors that controls decomposition in general follows. A number of studies have also showed markedly increased rates of organic phosphorus mineralization in response to soil phosphatase activity.

2.4 Phosphorus cycling

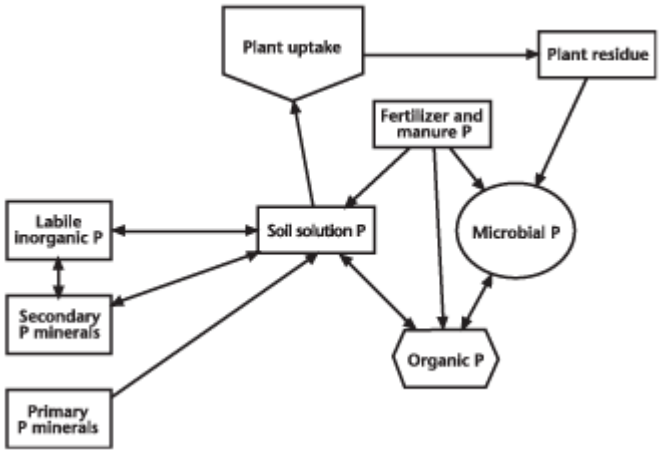

Figure 1: Phosphorus cycling diagram

Source: Canola Encyclopedia, "Crop Nutrition: Canola Council of Canada (online)", Accessed February, 28, 2015.

Phosphorus cycling is governed by varied processes hence can be more complication compared to nitrogen cycling. In the nitrogen cycle, it is possible to remove nitrogen through export of dissolved nitrogen to streams, during plant uptake, and gas emissions. While the conversion to nitrogen gas is a permanent nitrogen removal process, the phosphorus cycle does not have such a mechanism thus almost no gas phosphorus by-products are released. Phosphorus removal is through export in solution, during plant uptake, precipitation and adsorption onto mineral surfaces (Kadlec, 2005). Plant uptake of Phosphorus is not lasting since microbe decomposition of dead plant remains eventually converts organic Phosphorus into mineral Phosphorus. However, there can be slow decomposition as a result of sediment deposition and burial of plant material. This increases the potential to permanently store Phosphorus (Kadlec, 2005). Adsorption is the only long-term phosphorus removal process due to the generally low solubility of some phosphate minerals (Dunne and Reddy, 2006).

Phosphorus in the soil can exist as both organic and inorganic. Total phosphorus can be subdivided into Particulate phosphorus, Dissolved organic phosphorus and Soluble reactive phosphorus with the latter being the bio-available form of phosphorus.

Biological transformation is required to convert the particulate and organic forms of phosphorus to bio-available forms (Dunne and Reddy, 2006). At the heart of this transformation is phosphatase, a system of enzyme which breaks down the particulate and organic forms of phosphorus into mineral phosphorus.

In a study by Freeman et al. (1996), phosphatase enzyme activities in peat soils markedly rose over an 18 week water table drawdown experiment compared to an unchanged wetland control. Since microbial respiration was not enhanced by water table drawdown, the authors were not able to link these outcomes to increased microbial action. Rather, Freeman et al., (1996) noted that increased enzyme action during drawdown was caused by mobilization of enzymes attached to mineral surfaces and organic matter.

By using a slightly varied hydrologic treatment, Corstanje and Reddy (2004) examined the effect of water table drawdown followed by re-flooding on phosphatase enzyme production and microbial action. It was realised that, the phosphatase action in the beginning increased during the 30 day re-flooding part of the experiment, which reflected the anaerobic respiration rates. These two studies suggest that phosphatase enzyme action can be improved by both water table drawdown and re-flooding in marshy soils.

Calcium contents in basic marshy soils, determines the availability of inorganic phosphorus but in acidic soils, Iron and Aluminum are the determinants (Dunne and Reddy, 2006). Phosphate has a negative charge hence attracted to cations with positive charges (Ca^{2+}, Fe^{3+} and Al^{3+}). This results in phosphorus precipitation.

The solubility of iron bearing minerals is also affected by redox potential and in effect the availability of Phosphorus in soils. Iron exists as ferric iron in oxidizing conditions but in more reducing conditions it is reduced to ferrous iron. For this reason, phosphorus adsorbed onto iron-containing minerals may become mobile when ferric iron is reduced to ferrous iron. Since phosphorus retention depends on the hydrologic conditions in soils, many publications have sought to describe the impact of drying and flooding soils and its influence on nutrient retention in both laboratory and field researches. Aldous et al. (2005) did a laboratory experiment using restored and natural wetland soils to measure phosphorus release when soils were maintained under varied hydrologic conditions which included flooding, moist and dry followed by re-flooding. Results revealed that soils from restored wetlands released phosphorus in all hydrologic conditions, with the largest release from flooded and dried soils (Aldous et al., 2005). Phosphorus release after flooding was explained by the presence of high

aluminum and iron bound phosphorus parts in both natural and restored wetland areas. This was pinned on phosphorus release due to changes in redox potential.

Under flooded conditions thus lower redox potential, ferric iron was reduced to ferrous iron hence phosphorus originally attached to iron minerals was released into the water column. It was again speculated that mineralization of organic phosphorus during the dry treatment could be another factor that aided phosphorus release upon flooding (Aldous et al., 2005).

Another study revealed that the rate at which water is added to dry or moist soils can also affect phosphorus mobilization in a laboratory setting. Blackwell et al (2009) collected soils from the United Kingdom to find out how Phosphorus contents changed after the addition of water at different rates to moist and dried soils. Dried soils released marked amounts of dissolved Phosphorus in all forms during the first rewetting period as compared to the field moist soils. These results established that rewetting dry soils can improve phosphorus release as compared to moist soils. A faster rate of adding water also increased dissolved phosphorus release during the beginning of the rewetting period for dried soils, reflecting that dry antecedent soil conditions have the potential to release phosphorus during heavy rainfall periods (Blackwell et al., 2009).

2.5 Drought impact on the nutrient status of trees in the European context

Regardless of whether changes in the availability of soil nutrients or in the normal nature of nutrient transporters in mycorrhizal tree roots are affected, drought generally causes a reduction of the nutrient content and concentrations in trees (Minoletti and Boerner 1994, Sardans, Peñuelas et al. 2008). However, not all nutrients get affected in the same way. A 6 year long drought which caused about a third of the total phosphorus content in a stand biomass of Holm oak to reduce was observed (Sardans and Peñuelas 2007). In contrast, there was no effect of water deficit for contents of potassium. Peuke and Rennenberg (2004) observed that phosphorus and phosphate contents reduced in above and below ground tissues in varied European beech stands after 3 weeks of drought treatment to simulate a typical summer drought period (Peuke and Rennenberg 2004). However, concentrations of nitrogen and sulphur were not clearly affected in these experiments. Apart from the nutrient-specific variations, varied tree species show varied stoichiometric plasticity in response to water stress

conditions. Penuelas et al.(2008) compared the variations in element concentrations in response to water stress conditions for varied woody Mediterranean species in order to define biogeochemical niches for varied species in a multi-dimensional nutrient niche area. It was observed that, nutrient/element stoichiometry is highly different in the midst of co-existing species and there is a species-specific plasticity concerning the total and relative nutrient content in response to water stress conditions. It was also observed that water stress conditions reduces nutrient content in above ground biomass especially for a lot of drought-sensitive species, predominantly via the reduction in growth and transpiration (Sardans, Peñuelas et al. 2008)

Studies directed towards drought effects on nitrogen nutrition of trees showed that concentrations and compound detail of the soluble non-protein nitrogen compounds can be a crucial indicator of short-term physiological responses, highlighting variations in the state of their internal nitrogen more detail than total nitrogen concentrations, which are constant within a wide range of climatic and nutritional conditions (Rennenberg, Dannenmann et al. 2009). Moderately lower water availability resulted in reduced content of soluble nitrogen in various tissues of European beech and was taken as an indication of lower nitrogen uptake in summer and changes in nitrogen re-mobilization in spring and storage patterns in autumn (Nahm, Radoglou et al. 2006). However, an increase in the concentration of amino acids has also been noticed in various tissues under water stress (Fotelli, Rennenberg et al. 2002). This was as a result of the decomposition of proteins under severe drought indicated by the simultaneous reduction in protein nitrogen content. There is no straightforward picture of how drought influences tree nutrition but in general terms, reduction of nutrient contents is observed but that might not be true for all nutrients hence we require in-depth information on the cumulative effects of drought on the availability nutrient elements and the plant's capacity to take them. We also require experiments evaluating how variations in these factors influence tree nutrition and growth. There is also the lack of experiments focusing on the changing drought conditions and evaluation of nutrient balance during repeated drought and recovery periods. Such experiments will be very helpful because the global climate changes in Europe with respect to drought is predicted to be followed by intermittent heavy downpour of rain which will most likely to result in floods. Although it is a proven fact that water stress limits the bioavailabilty of a lot of soil nutrients, long drought periods followed by flood as a global climate change prediction for Europe could push European Forestry to depend more on natural cycles such as phosphorus cycle for its nutrition.

2.6 Measurement of soil phosphatase activities

Before the use of the simple, accurate and rapid enzyme assay based on the use of p-nitrophenyl phosphate (pNPP) by Tabatabai and Bremner (1969) (Eivazi and Tabatabai 1977) and modifications by Rejsek (1991) (Dundek, Holík et al. 2014), phosphatase assays used natural substrates such as b-glycerolphosphate and nucleic acids (Malcolm 1983).

The use of artificial substrates began with phenyl phosphate (Marchesi and Barrnett 1963), phenolphthalein phosphate (Malcolm 1983), pNPP with Bertrand and de Wolf 1968 (Nannipieri, Giagnoni et al. 2011), a-naphthyl phosphate with Hochstein 1962 (Nannipieri, Giagnoni et al. 2011) and b-naphthyl phosphate (Ramirez-Martinez and McLaren 1966). The choice of artificial substrates eliminated the determination of released phosphate. The success of the pNPP assay can also pinned on the fact that hydrolysis of pNPP is more rapid than that of natural substrates such as nucleic acids. The pNPP is hydrolysed to p-nitrophenol (pNP), which is usually analysed with the spectrophotometer at 410 nm under alkaline conditions.

Although, this current use of pNPP assay has obvious drawbacks, which have been noted and well discussed by several authors, it is almost always not considered when interpreting soil enzyme activities. It should be clearly noted that this assay only measures potential enzyme activities because the conditions created are different from the real in situ conditions where temperature and soil moisture keep changing as well as having a rarely optimum soil pH and substrate nature for enzymatic activities.

2.7 How the Spectrophotometer works

The spectrophotometer is designed to record the amount of light absorbed by a solution (Sakthivel, Janczarek et al. 2004). This sensitive instrument is a very important tool for biologists. It can be used to find out a molecule's absorption spectrum, the wavelengths of light that the specific molecule absorbs (for example, DNA is able to best absorb UV light with a wavelength of 260 nm and 410 nm for the measurement of acid phosphatase)

(Sreekanth, Krishnamurthy et al. 2011). A more concentrated solution will take up more light than a less concentrated one.

Using the spectrophotometer, we can quantitatively measure absorbance. This information can be used to find out the concentration of the absorbing molecule. The spectrophotometer can be used to quantify what is going on in all kinds of biological processes. For example, if an enzyme reaction generates or destroys a coloured molecule, the spectrophotometer can be used to measure how much of that molecule is present hence can quantity the activity of the enzyme.

Technically, the spectrophotometer does not measure the amount of light absorbed, but the amount transmitted. However, the percentage transmitted could be easily converted to absorbance. Most spectrophotometers do this conversion for automatically. There are no units for absorbance, but the wavelength should always be mentioned when absorbance data is reported.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Site and Stand description

This laboratory research was done with soils from three areas namely the Young Spruce monoculture in the mid-altitude of the Ecosystem station at Rajec-Nemcice, Mountain Spruce of the Bily Kriz Experimental Ecological Research Station and Beech forest of Ecosystem station at Stitna.

3.1.1 Brief description of the Ecosystem station at Rajec-Nemcice

The Institute of Forest Ecology of the Mendel University, Brno in 1968 created this study area. This station has some connection with The United Nations Educational, Scientific and Cultural Organization (UNESCO) in the International Biological Programme as well as 'Man and the Biosphere' programme. The Spruce stand in this area was artificially established by reforestation of a clear-cut area. This was done by felling the mature spruce stand of the first generation in 1978 (Pokorný, Rajsnerová et al. 2013). The Geographical coordinates of the area are 49º 29´ N, 16º 43´ E with a mid-altitude which ranges from 610 – 625 m above sea level. The main soil type in this area is Modal Oligotrophic Cambisol (KAmd´) with very acidic nature thus very low pH.

Climatic condition at The Ecosystem station at Rajec-Nemcice: Annual precipitation during growing season and duration of growing season are 430 mm and 140 – 160 days respectively.

Figure 2: Spruce stand at Ecosystem station at Rajec-Nemcice

3.1.2 Brief description of the Experimental Ecological Research Station at Bily Kriz

The Spruce stand in this area was established on a clear-cut area. This was done by felling the mature spruce stand of the first generation and planting 4-year old seedlings in 1981 (Pokorný and Tomášková 2007). The Geographical coordinates of the area are 49º 30´ N, 18º 30´ E with a high-altitude which ranges from 860 – 910 m above sea level. The main soil type in this area is Humo-ferric podzol with very acidic nature thus very low pH.

Climatic condition at The Experimental Ecological Research Station at Bily Kriz: Annual precipitation during growing season and duration of growing season are 650 mm and 120 – 140 days respectively.

Figure 3: Spruce forest stand at The Experimental Ecological Research Station at Bily Kriz

Source: Zajímavá místa v okolí Gruně, "Horska Chata v Beskydech (online)", Accessed Feb 28, 2015

3.1.3 Brief description of the Ecosystem station at Stitna

The Geographical coordinates of the area are 49° 2´ 52.142" North latitude 18° 0´ 28.180" East longitude with an above sea level elevation of 540 m. This station is located at the Bile Karpaty and the ecosystem has a Beech forest aged 107 years. This Ecosystem has been under the watch of the CzechGlobe, who are constantly making some physiological investigations (e.g. transpiration, photosynthesis etc) to better understand the Ecosystem and predict how it will respond to the inevitable future changes in climate.

Climate condition at the Ecosystem station at Stitna: Annual precipitation and temperature from 2009 to 2012 are 770.9 mm and 8.37 degree Celsius respectively.

.

Figure 4: Beech forest stand at the Ecosystem station at Stitna

Source: CzechGlobe, "Ekosystémová stanice Štítná nad Vláří (online)", Accessed Feb 28, 2015.

3.2 Soil sampling

The main guide was to sample soil at root zone because it is at this depth that trees can derive nutrition. For this reason, soils were sampled from the A-Horizon from the Beech forest of Stitna and the Young spruce monoculture of Rajec. The enzymatic activities at this horizon are predicted to be low but it is very important because it will give a vivid tree nutrition status in terms of natural nutrient cycling. At Bily Kriz, because the root zone is mainly in the organic layer due to the shallow rooting system of the Mountain Spruce, H-Horizon soils were sampled. The sampling which was done using Kopecky's soil sample rings of 100 cubic centimetre capacity. It was randomly done and not dependent on season or plot differentiation.

Figure 5: Organic layer (H-horizon) soil sampling

Figure 6: Mineral layer (A-horizon) soil sampling

3.3 Laboratory procedures

The main laboratory analysis performed was the measurement of the activity of acid phosphatase using p-nitrophenyl phosphate. This laboratory procedure mimics how extracellular acid phosphatase converts organic phosphorus to inorganic phosphorus for the bioavailability of phosphorus for plant uptake.

All the three separate soil samples were sieved in a 2 mm sieve and mixed or homogenised to get a representative sample. The representative samples of the three areas were duly stored in the refrigerator at about 5 degrees Celsius with their original moisture content.

Three protocols were employed to measure acid phosphatase activity. The first protocol is the one already in use developed by Tabatabai and Bremner (1969) and some modifications made

by Rejsek (1991). The other two protocols were attempts to mimic real in-situ situations in terms of real soil pH and most importantly, drought conditions.

Protocol 1

Chemicals used:

Succinate – Borate buffer of pH 4.8

p – nitrophenylphosphate (p-NPP) solution prepared by dissolving 1 tablet in 100 ml of Succinate – Borate buffer (p-NPP represents the artificial substrate)

1 M KOH

Standard p – nitrophenol solution (p-NP)

Procedure followed:

12 ml of S-B buffer was added to a gram of soil sample and put in an incubator at 37 degree Celsius for an hour. After incubation, filtrate was alkalised with 8 ml of 1 M KOH and later diluted in 1:2 thus a portion of filtrate to 2 portions of S-B buffer. The resultant solution was analysed for acid phosphatase activity using the spectrophotometer at a wavelength of 410 nm. The outcome was then recalculated to µg p-NP/g dry soil /hour.

Protocol 2

This protocol followed the same procedure as Protocol 1 except using water instead of S-B buffer. This was to get close to the natural pH of the soil as a laboratory improvised strategy to mimic natural situation.

Protocol 3

This protocol represents the drought simulation approach done in a laboratory setting. This protocol was a combination of Protocols 1 and 2 in the sense that, water was used instead of S-B buffer and the artificial substrate (p-NPP) was added in powder form to the soil samples and put in an incubator without water or S-B buffer. After the hour long incubation, water instead of S-B buffer was added. All parameters apart from the changes made remained the same at in protocol 1. This in real natural situation represents a period of drought.

3.4 Calibration curve for data evaluation

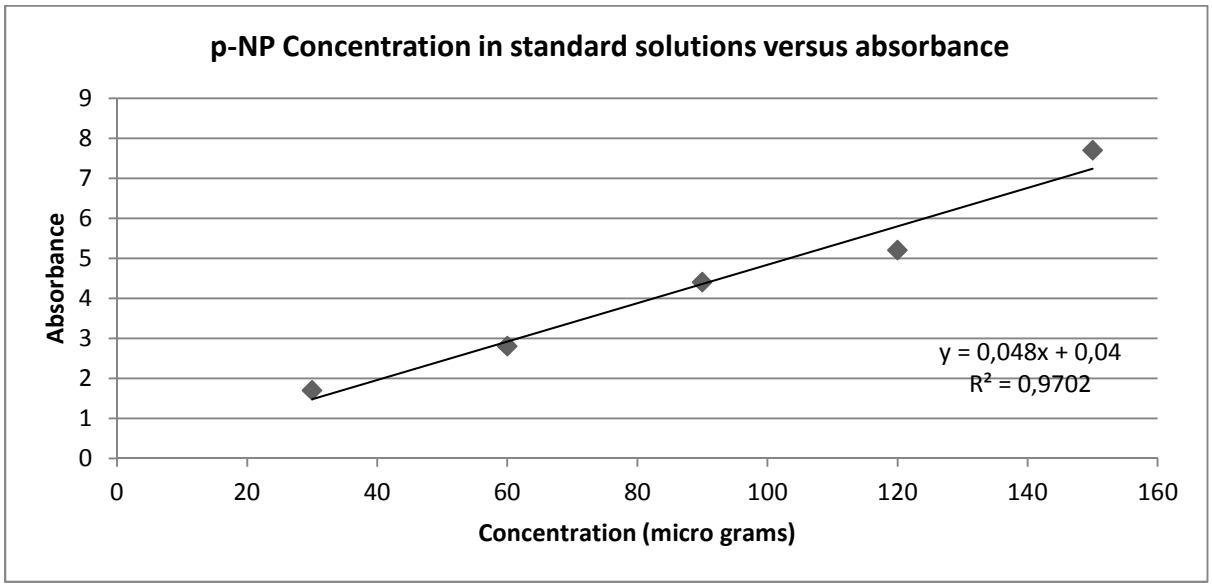

Figure 7: Standard curve used to evaluate data

3.5 Statistical data analysis

Analysis was done using A Single Factor Anova with an alpha value of 0.05 thus a confidence interval of 95%.

CHAPTER FOUR

4.0 RESULTS

4.1 Acid phosphatise activity measured using the various Protocols employed

Acid phosphatase activity measured using Protocol 1

	Absorbance values				
Area of sampling	Trial 1	Trial 2	Trial 3	Average	Acid phosphatase activity (µg p-NP)
Rajec (A-horizon)	0.7	0.9	0.8	0.8	15.8
Bily Kriz (H-horizon)	2.6	2.1	2.1	2.3	47.1
Stitna (A-horizon)	1.8	1.8	1.1	1.6	32.5

Table 1: Acid phosphatase activity measured using Protocol 1

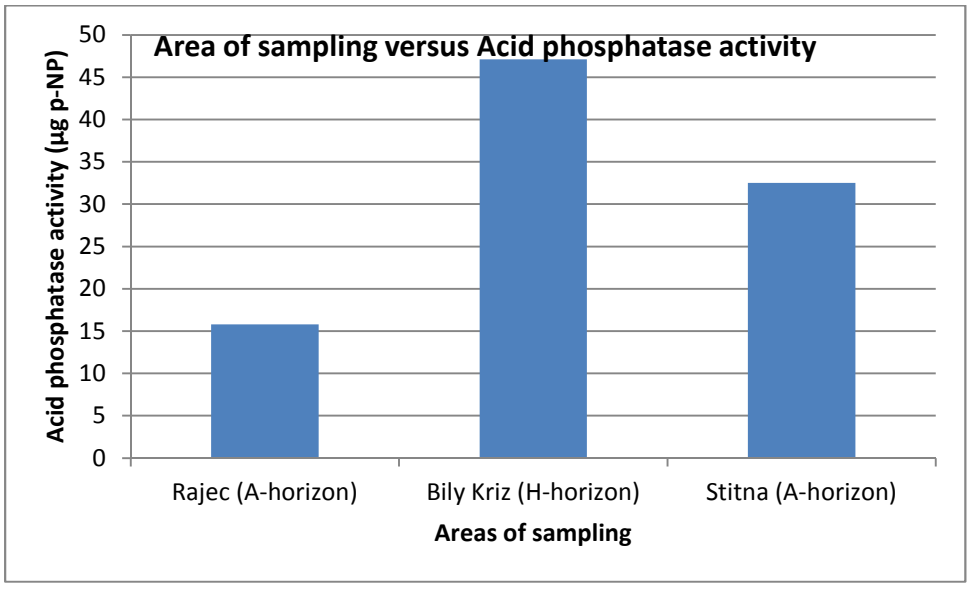

Figure 8: A graph of Acid phosphatase activity measured using Protocol 1

Acid phosphatase activity measured using Protocol 2

	Absorbance values				
Area of sampling	**Trial 1**	**Trial 2**	**Trial 3**	**Average**	**Acid phosphatase activity (µg p-NP)**
Rajec (A-horizon)	0.52	0.54	0.51	0.5	9.6
Bily Kriz (H-horizon)	1.37	1.39	1.36	1.4	28.3
Stitna (A-horizon)	0.73	0.74	0.71	0.7	13.8

Table 2: Acid phosphatase activity measured using Protocol 2

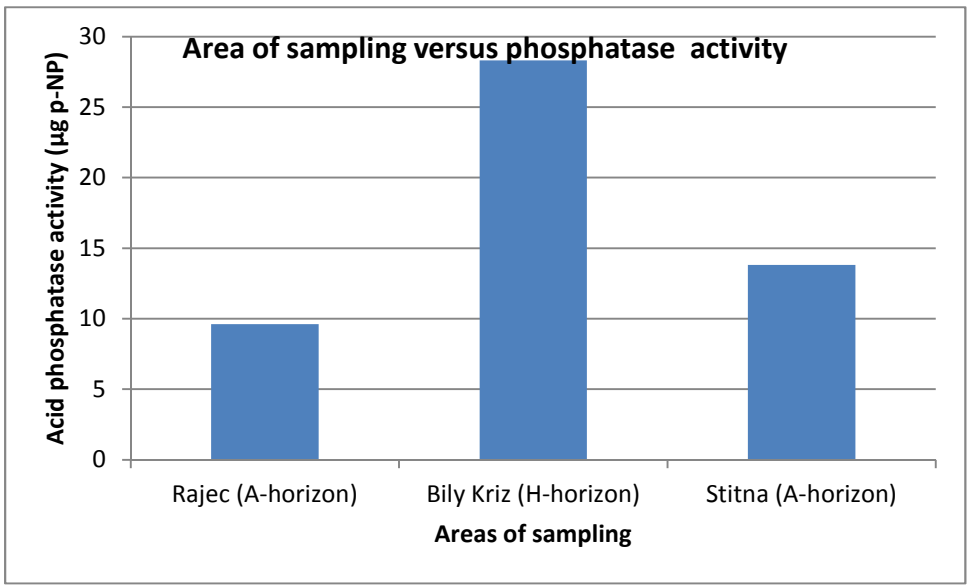

Figure 9: A graph of Acid phosphatase activity measured using Protocol 2

Acid phosphatase activity measured using Protocol 3

	Absorbance values				
Area of sampling	Trial 1	Trial 2	Trial 3	Average	Acid phosphatase activity (µg p-NP)
Rajec (A-horizon)	0.29	0.33	0.34	0.32	5.8
Bily Kriz (H-horizon)	0.81	0.81	0.86	0.83	16.5
Stitna (A-horizon)	0.61	0.63	0.63	0.62	12.1

Table 3: Acid phosphatase activity measured using Protocol 3

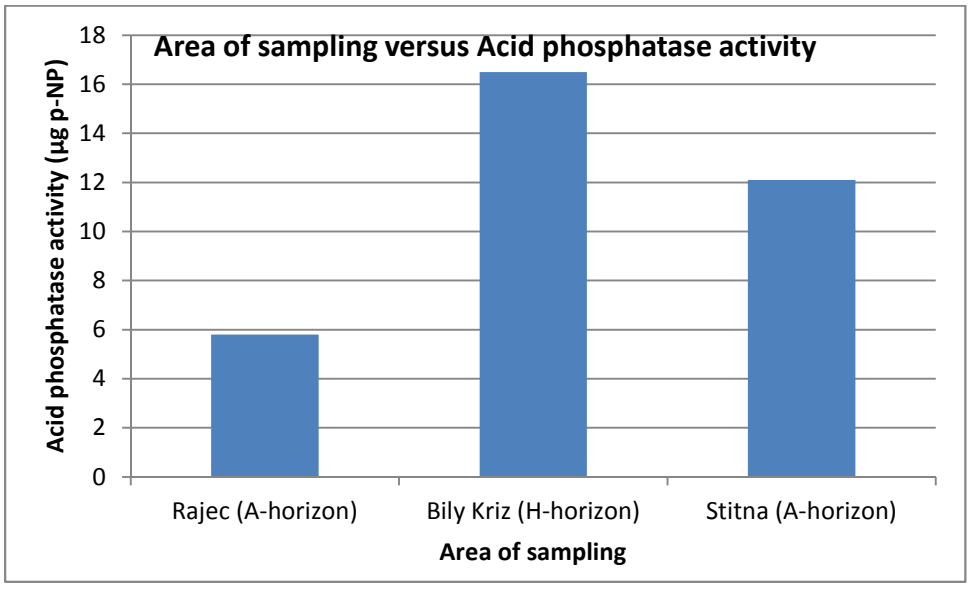

Figure 10: A graph of Acid phosphatase activity measured using Protocol 3

4.2 Comparison of Acid Phosphatase activity measured in each area sampled using the various Protocols 1, 2 & 3.

A-horizon soil from the young spruce monoculture of Rajec

Protocol	Acid phosphatase activity (µg p-NP)
Protocol 1	15.8
Protocol 2	9.6
Protocol 3	5.8

Table 4: Acid phosphatase activity measured for all three protocols for Rajec sample

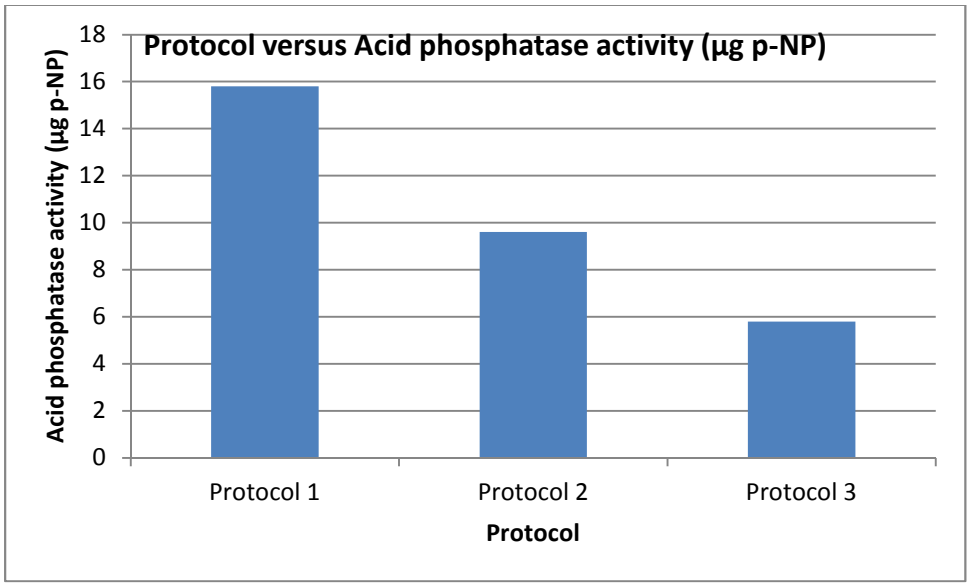

Figure 11: A graph of Acid phosphatase activity measured from all three protocols for Rajec sample

H-horizon soil from the Spruce stand at Bily Kriz

Protocol	Acid phosphatase activity (µg p-NP)
Protocol 1	47.1
Protocol 2	28.3
dProtocol 3	16.5

Table 5: Acid phosphatase activity measured from all three protocols for Bily Kriz sample

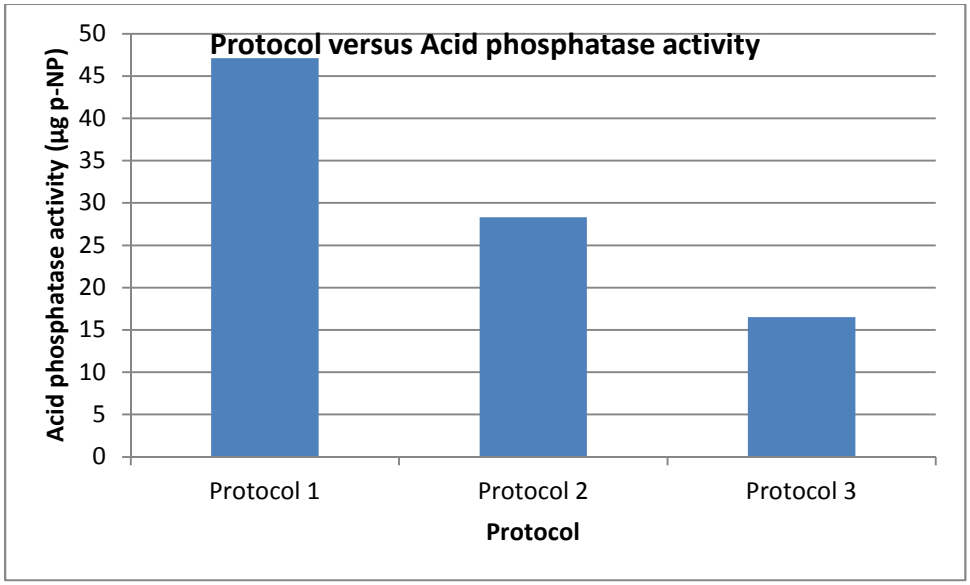

Figure 12: A graph of Acid phosphatase activity measured from all three protocols for Bily Kriz sample

A-horizon soil from the Beech forest of Stitna

Protocol	Acid phosphatase activity (µg p-NP)
Protocol 1	32.5
Protocol 2	13.8
Protocol 3	12.1

Table 6: Acid phosphatase activity measured from all three protocols for Stitna sample

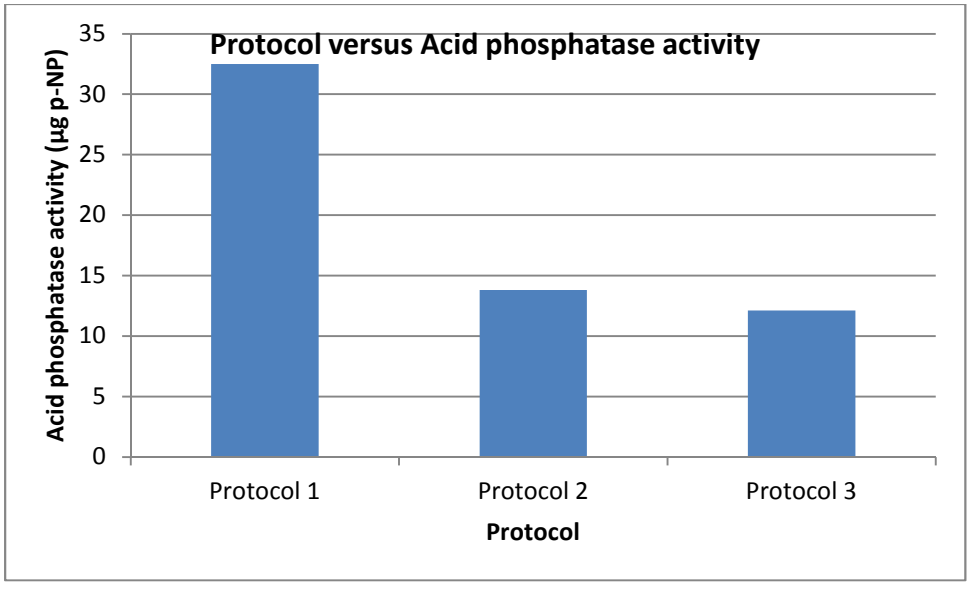

Figure 13: A graph of Acid phosphatase activity measured from all three protocols for Stitna sample

4.3 Statistical analysis of data using single factor ANOVA

NB: *Values in the columns represents Acid phosphatase activity (µg) measured using Protocols 1, 2 & 3*

Rajec (A-horizon)	Bily Kriz (H-horizon)	Stitna (A-horizon)
15.8	47.1	32.5
9.6	28.3	13.8
5.8	16.5	12.1

Table 7: Acid phosphatase activity measure for all samples using all three protocols

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Rajec (A-horizon)	3	31.2	10.4	25.48
Bily Kriz (H-horizon)	3	91.9	30.63333	238.1733
Stitna (A-horizon)	3	58.4	19.46667	128.1233

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	616.2866667	2	308.1433	**2.359584**	**0.175376**	**5.143253**
Within Groups	**783.5533333**	6	130.5922			
Total	1399.84	8				

Table 8: Anova for Acid phosphatase activity measure for all samples using all three protocols

CHAPTER FIVE

5.0 DISCUSSION

5.1 Overview of results obtained from the statistical analysis data (ANOVA)

From the statistical analysis data as shown in Tables 7 & 8, there were marked variations in the measured enzymatic action firstly between soil samples and secondly between the different Protocols employed.

From Table 8, it can be noticed that the p-value was far more than the alpha value of 0.05 used to make this single factor ANOVA. This shows the significant variations in the groups of values. The sources of variations which were stated in the ANOVA as "Within groups and Between groups" represents the variations in the measurement of enzymatic action due changes in Protocol used and differences in soil samples. The former had the greatest effect in pushing the p-value far above the alpha value used to calculate the single factor ANOVA. This is evidence that the simulated drought and soil pH situation had great impact on the phosphorus mineralisation. It should also be noted that the soil samples were not dried hence had some natural moisture content and this could be the reason for this not so strong drought effect as compare to soil pH effect. The details of the factors that contributed to significant variations of the measured enzymatic actions are discussed below:

5.1.1 The effect of soil pH on acid phosphatase activity and in effect bioavailability of phosphorus

The pH of soil solution exerts a strong control on enzyme activity, because it influences the conformation of the enzyme, its adsorption on solid surfaces, and the ionization and solubility of substrates.

Protocol 1was conducted at the optimum pH and temperature of 4.8 and 37 degrees Celsius respectively for maximum potential enzyme action hence the measured enzymatic activity

was markedly greater than that of Protocols 2 and 3 as shown in Table 1 and Figure 8 in the results. The values measured represent the maximum enzymatic activity level since all the factors for example the soil pH needed for enzymatic action were in optimal levels and also stable which seldom occurs in nature.

This was evident in Protocol 2 where water was used in place of buffer. The buffer serves as a control for pH fluctuations. This was an attempt to get to the real soil pH which though averagely hovers around the 4.8 in the sampled areas used in Protocol 1. The soil pH in real natural situations, is hardly optimum for the action of enzymes and not always stable as it was made to be by the used of buffer in Protocol 1. The unstable and uncertain soil pH affected the enzymatic activity in Protocol 2 hence the values measured was almost half of that measure in the optimal enzymatic factors of Protocol. This outcome is a strong indication of the significance of soil pH in the bioavailability of phosphorus to tree species.

5.1.2 The effect the sampling depth on acid phosphatase activity and in effect bioavailability of phosphorus

The samples for this research were taken from the root zone because at that depth, bio-available nutrients and in this case phosphorus can be taken up by trees. At Rajec and Stitna, because the Spruce stands were deep rooted, A-horizon soils were sampled and H-horizon soils sampled in the case of the Beech forest stand at Bily Kriz due to its shallow rooting system. The enzymatic activity recorded for all the three Protocols employed for the A-horizon soil sample from Bily Kriz, was significantly higher than the other two samples. This is a confirmation of the positive impact organic matter has on enzyme activities and in effect bioavailability of phosphorus.

5.1.3 The effect of soil texture and age of stand on acid phosphatase activity and in effect bioavailability of phosphorus

Parent material affects the mineralisation rate of phosphorus. Finer textured soils favours higher mineralization rates as compared to coarse textured soils. Again, older forest stands has more microbial action in the soil hence higher mineralisation is realised. This was evident in this research particularly when the enzymatic activities of soil samples collected from Rajec and Stitna are compared. In all Protocols employed, the soil sample from Stitna recorded higher enzymatic action compared to soil sample from Rajec. This sequence is attributed to the finer soil particles, almost clayey of soil sample from Stitna and the older age of the Spruce stand from which the soil samples were taken.

5.1.4 The effect of drought on acid phosphatase activity and in effect bioavailability of phosphorus and the likely implications for European Forestry

This final part of the discussion is the highlight to the entire research. The results supported the already proven phenomenon of the negative effect drought has of enzymatic action in the soil. Water stress puts stress on microbes hence impedes their rate of action in the soil which in effect affects enzymatic action and consequently the bioavailability of nutrients to tree species which in this case is phosphorus. The measured enzymatic activity of Protocol 1 which had all factors that aids enzymatic action in optimal state for all soil samples was markedly higher than for Protocol 2 and 3. This clearly supports literature and numerous researches made by scientists in that respect.

The activity and diverse nature of soil microbes is very crucial in sustainability as they control very important soil health functions which involve carbon and nutrient cycling. Soil microbial activity is an important soil health indicator. It is now more than ever crucial for the nourishment of European forests due to the droughts and intermittent heavy downpours Europe has began facing in the summers and will continue to face intensively as global climate changes takes full effect.

CHAPTER SIX

6.0 CONCLUSION

Forestry in Europe is presently under the influence of global climate changes. This situation has directly and indirectly affected atmospheric temperatures and precipitation. Because this MSc-thesis dealt with drought, the reinforcing interrelationship between the scientific work for this MSc-thesis and European forestry is needed.

Considering the results of this MSc-thesis, a mitigation of available phosphorus content due to true soil pH degree, can be directly linked to the proven fact that a water deficit reduces the activity of enzyme studied. It is obvious that true pH of soil was not sufficient for the conversion of phosphorus sources into its bio-available form. Therefore, drought caused by an impact of global climate change can negatively affect European forestry through soil biochemistry as well. The results obtained from this MSc-thesis appear to be important from forest management point of view. In addition, the result punctuates the importance of forest soil biochemistry for a modern forestry at the European scale and can add value to forestry science in general.

Finally, in view of the prediction of dry summers with intermittent heavy downpours or precipitations and the supporting researches by some scientists that reveals that more bioavailable phosphorus is released by enzymes when flood follows a long drought period (Aldous et al., 2005), phosphorus cycling will be crucial for forest tree species in the uncertain future of climate changes.

The value of this piece of research can be, thus, seen in the other evidences for European forestry about the role of global climate changes and forest soil biological activity.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

Finally, the under-listed approaches are duly suggested to guide future researches geared towards investigating the actual rate of acid phosphatase activity in the mineralisation of phosphorus into bio-available form to tree nutrition;

- If an experiment of this nature will be done in a laboratory setting, the temperature should be altered to suit close to real temperature found in soils at the areas under investigation. This will be time consuming since at the averagely low temperatures of most European forests, the mineralisation of phosphorus will be at slow pace in the laboratory but it will be worth it because it will bring to bear what is likely to happen in nature.
- Secondly, this research investigation should be frequently done on the field to get a clearer understanding of enzymatic activities to guide forest management planning process in terms of forest tree nutrition.
- Thirdly, a flooding dimension should be added to future experiment so that a clearer case can be made for the importance of phosphorus cycling in the future of European forestry.

8.0 Summary

Future forests have to meet the ever increasing demand for high quality timber. However, reduction in water availability due to global climate change is expected to greatly reduce the stability and productivity of forests. Phosphorus is often lacking in tropical and subtropical forests. Global climate changes has led to changes in precipitation patterns in the forests of temperate regions in the recent years, which has inevitably affected phosphorus cycling and in effect tree nutrition. It is very important to keep the forest alive and well-nourished as it plays an irreplaceable role in mitigating global climate change through carbon capture but at the same time it must adapt to climate change. This research sought to evaluate the effect of drought on forest tree species with emphasis on bio-available phosphorus obtained via phosphorus cycling. Soil samples were collected at root zone depth from areas of varied altitudes and tree species thus H-horizon soils from the mountain Spruce forest at Bily Kriz, A-horizon soils from both the young Spruce monoculture at Rajec and Beech forest at Stitna. Acid phosphatase activity of the various soil samples was measured in optimal conditions of the enzyme in the laboratory using the already in use protocol developed by Tabatabai and Bremner (1969) and some modifications made by Rejsek (1991). Two other protocols were developed by this research by replacing the buffer component of the first with water to mimic the unstable pH situation which occurs in nature and simulating a drought situation in the laboratory by incubating the soil sample with powdered form of the substrate used in the original protocol and adding water after the hour-long incubation. The results supported the earlier assumptions held before start of the research which were;

1. Water stress or drought has negative effect on microbial activities and in effect enzymatic activities hence limits bioavailability of phosphorus.
2. H-horizon soil has the highest soil enzymatic activities due to high microbe content as a result high organic matter content.
3. Unstable soil pH negatively affects soil enzymatic activities.

Again soil sample from Stitna though had the same sampling depth as the Rajec soil sample, had higher acid phosphatase activity due to its clayey texture. The laboratory analyses proved that global climate changes affects conservation of forest soil fertility via decreasing soil biological activity evaluated through phosphorus bioavailability. Soil phosphorus is indispensable for forest tree species' breeding hence important for European Forestry.

8.0 Souhrn

Spotřeba dřeva vysoké kvality neustále narůstá a je třeba, aby lesy budoucnosti tuto potřebu pokryly. Ovšem snížení dostupnosti vody, které je způsobené klimatickými změnami, snižuje také stabilitu a produktivitu lesa. V tropických a subtropických lesích je nedostatek fosforu. Globální klimatické změny vedly ke změnám v počtu srážek v lesích mírného pásma, což má za následek změny v koloběhu fosforu a z nich vyplývající změny ve výživě stromů. Je důležité udržovat zdravé a dobře živené lesy, protože hrají klíčovou roli při zmírňování dopadů klimatických změn skrze lapání uhlíku. Zároveň se však lesy musí změnám přizpůsobovat. Cílem tohoto výzkumu je zhodnotit vliv sucha na druhy lesních stromů. Důraz byl kladen na biopřístupný fosfor, který je získáván z koloběhu. Vzorky půdy byly sbírány v kořenové hloubce v oblastech s odlišnou nadmořskou výškou a s odlišnými druhy. Půdy horizontu H byly odebrány v horském smrkovém lese na Bílém Kříži, půdy horizontu A v mladé smrkové monokultuře v Rájci a v bukovém lese ve Štítné. Aktivita kyselé fosfatázy ve vzorcích byla měřena za optimálních laboratorních podmínek za použití protokolu vyvinutého v Tabatabai a Bremmer (1969) s mírnou modifikací od Rejška (1991). V rámci tohoto výzkumu byly vyvinuty další dva protokoly, první nahrazuje pufr vodou pro napodobení nestálého pH, které se vyskytuje v přírodě. Sucho bylo simulováno inkubací vzorku půdy s práškovou formou substrátu, který byl použit v původním protokolu, a následným přidáním vody po hodině inkubace. Výsledky potvrdily dřívější předpoklady, které byly následující:

1. Velké množství vody nebo sucho negativně ovlivňují aktivitu mikrobů a tím také aktivity enzymů, čímž je omezována biopřístupnost fosforu.

2. Půda horizontu H má nejvyšší míru enzymatické aktivity díky přítomnosti velkého množství mikrobů, které je důsledkem vysokého obsahu organické hmoty.

3. Nestálé pH negativně ovlivňuje aktivity enzymů.

Vzorek ze Štítné, který byl ze stejné hloubky jako vzorec z Rajce, vykazuje vyšší aktivitu kysele fosfatázy díky svojí jílovité struktuře. Provedené laboratorní analýzy dosvědčili vliv globální klimatické změny na úrodnost testovaných lesních půd prostřednictvím snížení biologické aktivity půdy, zde měřené v přímé vazbě na přístupnost základního faktoru plodnosti rostlin, fosforu. Tento poznatek má všeobecnou platnost pro lesnictví Evropy.

REFERENCES

9.0 Citation of literature resources used:

ALDOUS, A., P. McCormick, C. Ferguson, S. Graham, and C. CRAFT. (2005). *'Hydrologic regime controls soil phosphorus fluxes in restoration and undisturbed wetlands.'* Rest. Ecology. 13:341-347.

BARDOSSY, A. and H. CASPARY (1990). *"Detection of climate change in Europe by analyzing European atmospheric circulation patterns from 1881 to 1989."* Theoretical and Applied Climatology 42(3): 155-167.

BASSIRIRAD, H. (2000). *"Kinetics of nutrient uptake by roots: responses to global change."* New Phytologist 147(1): 155-169.

BECK, C., et al. (2007). *"Frequency and within-type variations of large-scale circulation types and their effects on low-frequency climate variability in central europe since 1780."* International Journal of Climatology 27(4): 473-491.

BEIER, C., et al. (2009). *"Carbon and nitrogen balances for six shrublands across Europe."* Global Biogeochemical Cycles 23(4).

BENISTON, M., et al. (2007). *"Future extreme events in European climate: an exploration of regional climate model projections."* Climatic Change 81(1): 71-95.

BLACKWELL, M.S.A., P.C. Brookes, N. de la Fuente-Martinez, P.J. Murray, K.E. Snars, J.K. Williams, and P.M. HAYGARTH.(2009). *'Effects of soil drying and rate of re-wetting on concentrations and forms of phosphorus in leachate'*. Bio. Fert. Soils 45:635-643.

BOHLEN, P. J., et al. (2004). *"Non-native invasive earthworms as agents of change in northern temperate forests."* Frontiers in Ecology and the Environment 2(8): 427-435.

BUCHNER, P., et al. (2004). *"Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport."* Journal of Experimental Botany 55(404): 1765-1773.

CANOLA ENCYCLOPEDIA, "*Crop Nutrition: Canola Council of Canada (online)*", Accessed February, 28, 2015, http://www.canolacouncil.org/canola-encyclopedia/crop-nutrition/crop-nutrition/

CHAPIN III, F. S., et al. (2004). *"Global change and the boreal forest: thresholds, shifting states or gradual change?"* AMBIO: A Journal of the Human Environment 33(6): 361-365.

CHRISTENSEN, J. H. and O. B. CHRISTENSEN (2003). *"Climate modelling: severe summertime flooding in Europe."* Nature 421(6925): 805-806.

CHURKINA, G. and S. W. RUNNING (1998). *"Contrasting climatic controls on the estimated productivity of global terrestrial biomes."* Ecosystems 1(2): 206-215.

CIAIS, P., et al. (2005). *"Europe-wide reduction in primary productivity caused by the heat and drought in 2003."* Nature 437(7058): 529-533.

CORSTANJE R. and K.R REDDY.(2004). *"Response of biogeochemical indicators to a drawdown and subsequent re-flood."* J. Environ. Qual. 33:2357-2366.

CZECHGLOBE, *" Ekosystémová stanice Štítná nad Vláří (online)"*, Accessed Feb 28, 2015. http://www.czechglobe.cz/cs/ekosystemova-stanice-stitna-nad-vlari/

ZAJÍMAVÁ MÍSTA v okolí Gruně *"Horska Chata v Beskydech (online)"*, Accessed Feb 28, 2015, http://www.dsvj.cz/aktivity-mista/mista.html

DUNDEK, P., et al. (2014). *"The effect of amino acid enantiomers on activity of selected enzymes in soil."* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 59(3): 29-34.

DUNNE, E.J., K.R. Reddy, and M.W. CLARK. (2006). *'Phosphorus release and retention by soils of natural isolated wetlands.'* Int. J. Environ. Pollut. 28:496-516.

EIVAZI, F. and M. TABATABAI (1977). *"Phosphatases in soils." Soil Biology and Biochemistry 9(3): 167-172.*

FINK, A. H., et al. (2004). *"The 2003 European summer heatwaves and drought–synoptic diagnosis and impacts."* Weather 59(8): 209-216.

FISCHER, E., et al. (2007). *"Contribution of land-atmosphere coupling to recent European summer heat waves."* Geophysical Research Letters 34(6).

FOG, K. (1988). *"The effect of added nitrogen on the rate of decomposition of organic matter."* Biological Reviews 63(3): 433-462.

FOTELLI, M., et al. (2002). *"Effects of drought on the competitive interference of an early successional species (Rubus fruticosus) on Fagus sylvatica L. seedlings: 15N uptake and partitioning, responses of amino acids and other N compounds."* Plant Biology 4(3): 311-320.

Founda, D. and C. Giannakopoulos (2009). "The exceptionally hot summer of 2007 in Athens, Greece—A typical summer in the future climate?" Global and planetary change 67(3): 227-236.

FREEMAN, C., G. Liska, N.J. Ostle, M.A. Lock, B. Reynolds, and J. HUDSON. (1996). *'Microbial activity and enzymatic decomposition processes following peat-land water table drawdown.'* Plant Soil 180:121-127.

GEßLER, A., et al. (2005). *"Climate and forest management influence nitrogen balance of European beech forests: microbial N transformations and inorganic N net uptake capacity of mycorrhizal roots."* European Journal of Forest Research 124(2): 95-111.

GREGORY, J., et al. (1997). *"Summer drought in northern midlatitudes in a time-dependent CO2 climate experiment."* Journal of Climate 10(4): 662-686.

JACKSON, R., et al. (1990). *"Rapid physiological adjustment of roots to localized soil enrichment."* Nature 344(6261): 58-60.

KADLEC, R.H. (2005). *'Phosphorus removal in emergent free surface wetlands.'* J. Environ. Sci. Health. 40:1293-1306

KOPRIVA, S. (2006). *"Regulation of sulfate assimilation in Arabidopsis and beyond."* Annals of Botany 97(4): 479-495.

KRAMER, K., et al. (2008). *"Effects of flooding on the recruitment, damage and mortality of riparian tree species: A field and simulation study on the Rhine floodplain."* Forest Ecology and Management 255(11): 3893-3903.

KREUZWIESER, J. and A. GESSLER (2010). *"Global climate change and tree nutrition: influence of water availability."* Tree physiology 30(9): 1221-1234.

KRYSANOVA, V., et al. (2008). *"Practices and lessons learned in coping with climatic hazards at the river-basin scale: floods and droughts."* Ecology and Society 13(2): 32.

LAWRENCE, C. R., et al. (2009). *"Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment."* Soil Biology and Biochemistry 41(9): 1923-1934.

LUCASH, M. S., et al. (2007). *"Estimating nutrient uptake by mature tree roots under field conditions: challenges and opportunities."* Trees 21(6): 593-603.

LUYSSAERT, S., et al. (2010). *"The European carbon balance. Part 3: forests."* Global Change Biology 16(5): 1429-1450.

MALCOLM, R. (1983). *"Assessment of phosphatase activity in soils."* Soil Biology and Biochemistry 15(4): 403-408.

MALHI, Y., et al. (2008). *"Climate change, deforestation, and the fate of the Amazon."* science 319(5860): 169-172.

MANZONI, S., et al. (2010). *"Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter."* Ecological Monographs 80(1): 89-106.

MARCHESI, V. and R. BARRNETT (1963). *"The demonstration of enzymatic activity in pinocytic vesicles of blood capillaries with the electron microscope."* The Journal of cell biology 17(3): 547-556.

MARSCHNER, H. and P. MARSCHNER (2012). *"Marschner's mineral nutrition of higher plants"*, Academic press.

MEEHL, G. A., et al. (2005). *"Understanding future patterns of increased precipitation intensity in climate model simulations."* Geophysical Research Letters 32(18).

MELILLO, J. M., et al. (2011). *"Soil warming, carbon–nitrogen interactions, and forest carbon budgets."* Proceedings of the National Academy of Sciences 108(23): 9508-9512.

MINOLETTI, M. L. and R. E. BOERNER (1994). *"Drought and site fertility effects on foliar nitrogen and phosphorus dynamics and nutrient resorption by the forest understory shrub Viburnum acerifolium L."* American Midland Naturalist: 109-119.

MUHR, J. and W. BORKEN (2009). *"Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil."* Journal of Geophysical Research: Biogeosciences (2005–2012) 114(G4).

NAHM, M., et al. (2006). *"Physiological performance of beech (Fagus sylvatica L.) at its southeastern distribution limit in Europe: seasonal changes in nitrogen, carbon and water balance."* Plant Biology 8(1): 52-63.

NANNIPIERI, P., et al. (2011). *"Role of phosphatase enzymes in soil. Phosphorus in action"*, Springer: 215-243.

NÄSHOLM, T., et al. (2000). *"Uptake of organic nitrogen in the field by four agriculturally important plant species."* Ecology 81(4): 1155-1161.

ORTÍZ-Castro, R., et al. (2009). *"The role of microbial signals in plant growth and development."* Plant signaling & behavior 4(8): 701-712.

OSONO, T. and H. TAKEDA (2004). *"Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species."* Ecological Research 19(6): 593-602.

PARTON, W. J., et al. (1988). *"Dynamics of C, N, P and S in grassland soils: a model."* Biogeochemistry 5(1): 109-131.

PERSSON, J., et al. (2003). *"Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field."* Oecologia 137(2): 252-257.

PEUKE, A. and H. RENNENBERG (2004). *"Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (Fagus sylvatica L.): phosphorus most affected by drought."* Trees 18(6): 639-648.

PIERZYNSKI, G., et al. (1990). *"Phosphorus chemistry and mineralogy in excessively fertilized soils: Quantitative analysis of phosphorus-rich particles."* Soil Science Society of America Journal 54(6): 1576-1583.

POKORNÝ, R., et al. (2013). *"Effect of early to late wood proportion on Norway spruce biomass."* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 60(6): 287-292.

POKORNÝ, R. and I. TOMÁŠKOVÁ (2007). *"Allometric relationships for surface area and dry mass of young Norway spruce aboveground organs."* J. For. Sci 53(12): 548-554.

RAMIREZ-Martinez, J. and A. MCLAREN (1966). "*Some factors influencing the determination of phosphatase activity in native soils and in soils sterilized by irradiation*", Univ. of California, Berkeley.

RENNENBERG, H., et al. (2009). *"Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses."* Plant Biology 11(s1): 4-23.

RENNENBERG, H., et al. (2006). *"Physiological responses of forest trees to heat and drought."* Plant Biology 8(5): 556-571.

SAKTHIVEL, S., et al. (2004). *"Visible light activity and photoelectrochemical properties of nitrogen-doped TiO2."* The Journal of Physical Chemistry B 108(50): 19384-19387.

SARDANS, J. and J. PEÑUELAS (2007). *"Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest."* Functional Ecology 21(2): 191-201.

SARDANS, J., et al. (2008*). "Drought and warming induced changes in P and K concentration and accumulation in plant biomass and soil in a Mediterranean shrubland."* Plant and Soil 306(1-2): 261-271.

SCHACHTMAN, D. P., et al. (1998). *"Phosphorus uptake by plants: from soil to cell."* Plant physiology 116(2): 447-453.

SCHÄR, C. and G. JENDRITZKY (2004). *"Climate change: hot news from summer 2003."* Nature 432(7017): 559-560.

SCHÄR, C., et al. (2004). *"The role of increasing temperature variability in European summer heatwaves."* Nature 427(6972): 332-336.

SCHIMEL, J., et al. (2007). *"Microbial stress-response physiology and its implications for ecosystem function."* Ecology 88(6): 1386-1394.

SELLE, A., et al. (2005). *"The high-affinity poplar ammonium importer PttAMT1. 2 and its role in ectomycorrhizal symbiosis."* New Phytologist 168(3): 697-706.

SREEKANTH, B., et al. (2011). *"Synthesis, DNA binding, and oxidative cleavage studies of Fe (II) and Co (III) complexes containing bioactive ligands."* Nucleosides, Nucleotides and Nucleic Acids 30(2): 83-96.

STEVENSON, F. J. and M. A. COLE (1999). *"Cycles of soils"*: carbon, nitrogen, phosphorus, sulfur, micronutrients, John Wiley & Sons.

STRÖM, L., et al. (2005). *"Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling."* Soil Biology and Biochemistry 37(11): 2046-2054.

TURNER, B. L., et al. (2002). *"Inositol phosphates in the environment."* Philosophical Transactions of the Royal Society B: Biological Sciences 357(1420): 449-469.

VON WIRÉN, N., et al. (1997). *"Regulation of mineral nitrogen uptake in plants."* Plant and Soil 196(2): 191-199.

WITKOWSKI, E. and D. MITCHELL (1987). *"Variations in soil phosphorus in the fynbos biome, South Africa."* The Journal of Ecology: 1159-1171.