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PHOSPHORUS AVAILABILITY FOLLOWING REVEGETATION WITH NOOTKA LUPINE AT TWO CONTRASTING SITES IN ICELAND

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ABSTRACT

Phosphorus scarcity is one of the major global environmental challenges. Understanding how phosphorus can be recovered is important for a sustainable future. The objectives of this study were to (i) assess if Nootka lupine, a nitrogen fixing legume, mobilizes phosphorus in Icelandic soils, and (ii) to provide insights into methods that are used to recover and reuse phosphorus. This was done to effectively test the hypothesis suggested in this study; that if Nootka lupine mobilizes soil phosphorus, there would be more phosphorus availability at sites colonised by Nootka lupine as compared to control sites. Soil samples and vegetation biomass data were collected at randomly selected sites colonized by Nootka lupine and control sites at Gunnarsholt and Holasandur, Iceland. Soil samples were analysed for plant-available phosphorus using the Bray method. Other measurements included total phosphorus, total iron, total aluminium and soil physical properties. Biomass was determined by oven dried vegetation samples. Data were analysed using ANOVA and Pearson correlation. The results showed that Nootka lupine sites had significantly ($p < 0.05$) higher plant-available phosphorus compared to the control sites, which implies that Nootka lupines mobilize phosphorus in Icelandic Andosols and therefore increase plant-available phosphorus. This study thus concluded that Nootka lupine mobilizes soil phosphorus; a potential cost effective method for mobilizing phosphorus for other plants in the ecosystem.

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1. INTRODUCTION

The world is facing a new and unprecedented era of global environmental change (Cordell et al. 2009). Our responses to such changes are largely dependent on our understanding of various natural and anthropogenic factors that shape them. One such factor is phosphorus (P) scarcity. Phosphorus is an essential element to life, and a key ingredient for plant growth (Schachtman et al. 1998; Raven et al. 2005; Taiz & Zeiger 2006). Phosphorus is required by plants for photosynthesis, nitrogen fixation, flowering, fruiting and maturation (Brady & Weil 2003), making up approximately 0.2% of a plant's dry weight (Schachtman et al. 1998). Thus, the net primary production (NPP) of terrestrial ecosystems is greatly dependent on P availability (Okin et al. 2004; Smit et al. 2009).

Phosphorus has long been used to enhance agricultural production as humans strive to meet the food demand for a growing world population (Cordell et al. 2011; Schröder et al. 2011). Phosphorus scarcity is a major constraint for agricultural productivity around the world (Smit et al. 2009). According to Brady and Weil (2003), lack of access to fertilizers such as P is often cited as one of the factors causing soil degradation as farmers clear more land and abandon unproductive land in attempts to survive. The scarcity of P is hence considered as one of the major global environmental challenges of the 21st century (Vance 2001; Cordell et al. 2011).

It is important to identify ways to recover and reuse P in order to ensure a sustainable future. Phosphorus is an essential element with no substitute (Ashley et al. 2011), and the source of P, phosphate rock, is a non-renewable resource which is increasingly becoming an expensive commodity on the world market (Cordell et al. 2011). The world population growth and the consequent increasing demand for food production has significantly increased P demand, putting pressure on available reserves (Cooper et al. 2011). Moreover, there is an alarming environmental concern of eutrophication (Correll 1998; Smil 2000; Bechmann et al. 2005) and the leakage of P from terrestrial ecosystems; a result of inefficient handling of P resources as well as the lack of recovery and reuse efforts (Cordell et al. 2012). Recovery and reuse methods could thus be used to recover P for use in sectors such as agriculture and land reclamation, lessening the dependence on commercial P fertilizers.

In agriculture and land reclamation, there has been a growing interest in using P efficient plants. Phosphorus efficient plants are able to acquire P not available to other plants, or make it accessible to other plants in the rhizosphere. This phenomenon has been demonstrated in white lupines (*Lupinus albus*) (Neumann et al. 2000). Phosphorus efficient plants could provide a cost effective opportunity for restoring degraded land as they require little or no fertilizer. Except for some legumes, few such plants are known to date.

In Iceland, the Nootka lupine (*Lupinus nootkatensis*), a legume species introduced from Alaska, has been used for land reclamation purposes to stop soil erosion on degraded land. A small amount of research has been conducted on this plant, demonstrating various aspects such as nitrogen fixation in Icelandic soils (Pálmason et al. 2004), good potential for revegetating degraded land (Arnalds et al. 2004), and the potential for greater biomass yield (Björnsson et al. 2004). Other studies have addressed issues of vegetation succession under Nootka lupine (Magnússon et al. 2004) as well as coexistence of Nootka lupine with native vegetation in Iceland (Aradóttir et al. 2004). Since its introduction in Iceland, this nitrogen-fixing perennial plant has been successful in growing on degraded land which is relatively poor in nutrients (Ágústsdóttir 2004). Due to the successful growth of lupine in poor soils, it is likely that it is acquiring both nitrogen and P from the soil. Although the mechanism of nitrogen fixation in Nootka lupine has been demonstrated (Pálmason et al. 2004), it is not well

understood how this plant acquires P from Icelandic Andosol soils which are characterized by a very high P retention capacity, a major limiting factor for P availability in Iceland (Arnalds et al. 1995). It is therefore interesting to know if Nootka lupine mobilizes such P despite the high P retention capacity of the Andosols.

Therefore, the objectives of this study were (i) to assess if Nootka lupine mobilizes P in Icelandic soils, as well as (ii) to provide a review of methods used to recover and reuse P. The study hypothesis suggests that if Nootka lupine mobilizes soil P, there would be more P availability at sites colonised by Nootka lupine as compared to control sites.

2. LITERATURE REVIEW

In order to ensure P security, it is important to understand the roles humans play in the entire P geochemical cycle, as well as opportunity for sustainable use. This section presents an overview of the P cycle, and a handful of opportunities for recovery and reuse.

2.1 Phosphorus cycle

The P cycle is a system under which P moves between terrestrial ecosystems and ocean sediments (Cross & Schlesinger 1995). This cycle is largely dependent on geochemical and biological processes whereby P is brought to terrestrial ecosystems through P rock weathering, whereas surface runoff and erosion wash P into rivers and oceans (House 2003). The P cycle shows a source and sink of P, as much P is removed from terrestrial ecosystems which are buried under ocean sediments (Paytan & McLaughlin 2007). This is partly a result of current anthropogenic systems that carry P away from terrestrial ecosystems, thereby modifying the natural P cycle flow. For example, mined P is used in agriculture or as detergents in households, of which its P-rich wastes are discharged into rivers and oceans (Fig. 1).

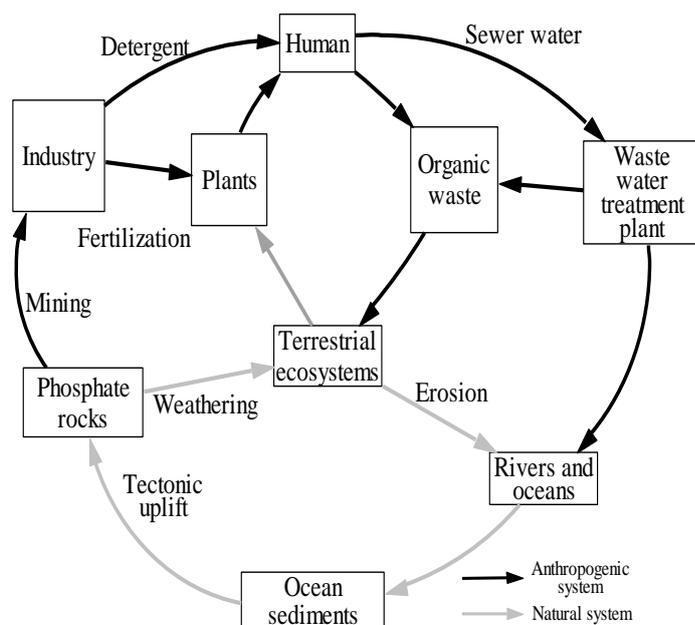


Fig. 1. P-cycle in the natural and anthropogenic system model: re-drawn and modified from Cornel and Schaum (2009).

Environmental problems such as deforestation, increased surface run-off and poor agricultural practice are the major factors attributed to P loss from terrestrial ecosystems (Smil 2000; Fullen & Catt 2004; Lawrence et al. 2007). For example, changes in vegetation cover, biomass production, soil organic matter (SOM) and nutrients in the ecosystem affect the amount of P and other nutrients being recycled to the ecosystems (Herbeck et al.; Bonsdorff et al. 1997; Glibert et al. 2002; Solomon et al. 2002; Brady & Weil 2003; Fullen & Catt 2004; Domagalski et al. 2007; Anderson et al. 2008; Cordell et al. 2009; Mihelcic et al. 2011). Such environmental problems have led to a significant amount of P and other nutrients being deposited in water streams leading to problems such as eutrophication of fresh water (Lowe & Keenan 1997; Correll 1998; Tilman 1999; Coelho et al. 2004; Bechmann et al. 2005; Garnier et al. 2005; Jarvie et al. 2006; Domagalski et al. 2007). Such biogeochemical changes vary in space and time, owing to the management of soil resources. Therefore, the maintenance of terrestrial ecosystems plays a crucial role for the management of soil resources such as P. Reduction in deforestation and increased efficiency in agricultural production could reduce the amount of P lost from soil.

2.2 Phosphorus scarcity

Phosphorus is essential for agricultural production. With global food production estimated to increase up to 70% in 2050 (Tomlinson 2011), P demand is expected to increase, leading to a rapid depletion of available reserves. For example, at the current extraction rate, the USA and China are expected to have depleted their reserves by 2060-70 (Fig. 2), resulting in a significant production deficit after the 2070s. The increasing demand for food products such as meat, milk and egg consumption in the world is estimated to create a demand for larger feed volume (Smit et al. 2009), which in return will increase the demand for fertilizer needed to grow hay or animal feed supplements. In fact, scientists have already warned that at the current extraction rate, P will probably be depleted by half at the turn of the century (Van Vuuren et al. 2010).

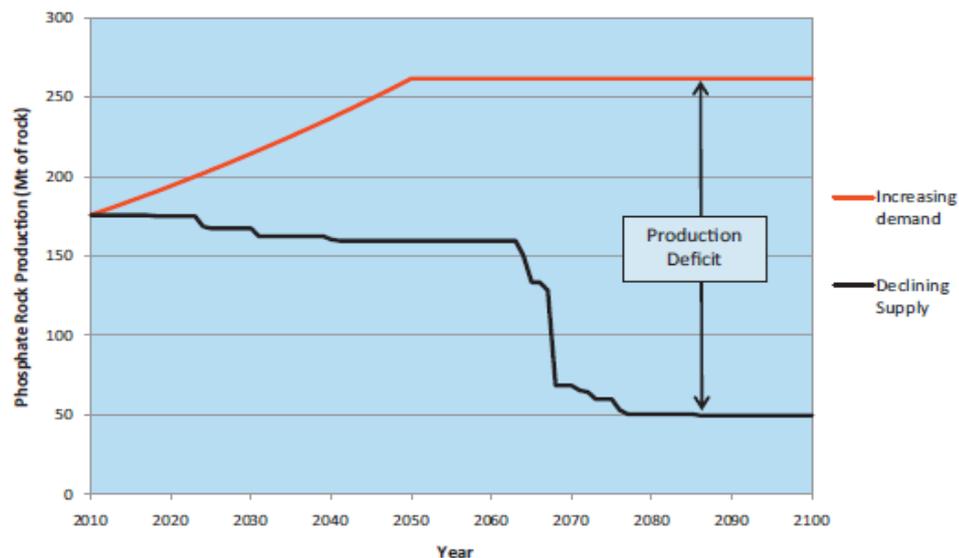


Fig. 2. The projected global P production deficit between 2010-2100. (Source: Cooper et al. 2011).

At the global scale, the existing P reserves are mainly found in countries such as Morocco, Western Sahara, China and the USA (Cordell et al. 2009). Such resources are hardly accessible to farmers, more especially in Sub-Saharan Africa (Cooper et al., 2011). For example, Africa is the largest exporter of P (Morocco and Western Sahara), yet the continent suffers the largest food shortage in the world (Cordell et al. 2009). Statistics from FAO (2008) indicate that Africa accounts for only approximately 3% of world P consumption.

In order to avoid this crisis, Cordell et al. (2009) proposed that there is a need to introduce an integrated approach to the management of the P cycle, an approach that will address the future P scarcity and explore options to recover and reuse P. Further, Cordell et al. (2009) stated that the important period for P scarcity and need for recovery is not when all the reserves are completely depleted, but when the high quality and most accessible reserves are depleted.

In addition, there has been reported inefficiency in the mining and utilization of P resources, thus an improvement in handling of the resource will potentially help to reduce the avoidable losses (Fig. 3), as well as reduce P demand by changing diets and increase efficiency in agriculture (Fig. 4). Cordell et al. (2011) found that only a fifth of P mined actually reaches the food industry. This would imply that much P is lost in the system between the mining sites and application to agricultural land. An efficient handling of such a resource would thus be expected to create good opportunities for recovery. Research and policy options may help increase the efficiency in handling, recovery and reuse of P to increase food production and maintain soil quality.

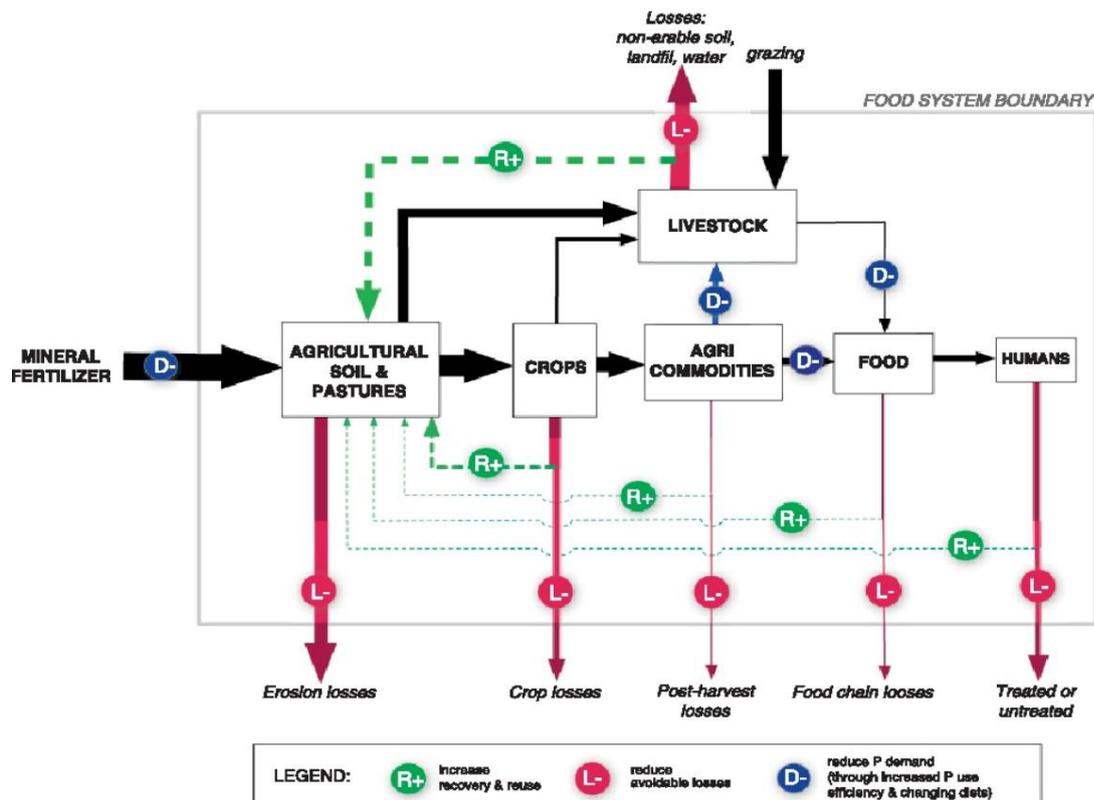


Fig. 3. Conceptual model illustrating how increased efficiency in agriculture through recovery and reuse may reduce P loss (Source: Cordell et al. 2012).

Swedish authorities for example, are already recycling P from sources such as wastewater, with a target for recycling up to approximately 70% of its P by 2015 (Levlin & Hultman 2004; Stark 2004). Such P is channelled to agricultural land.

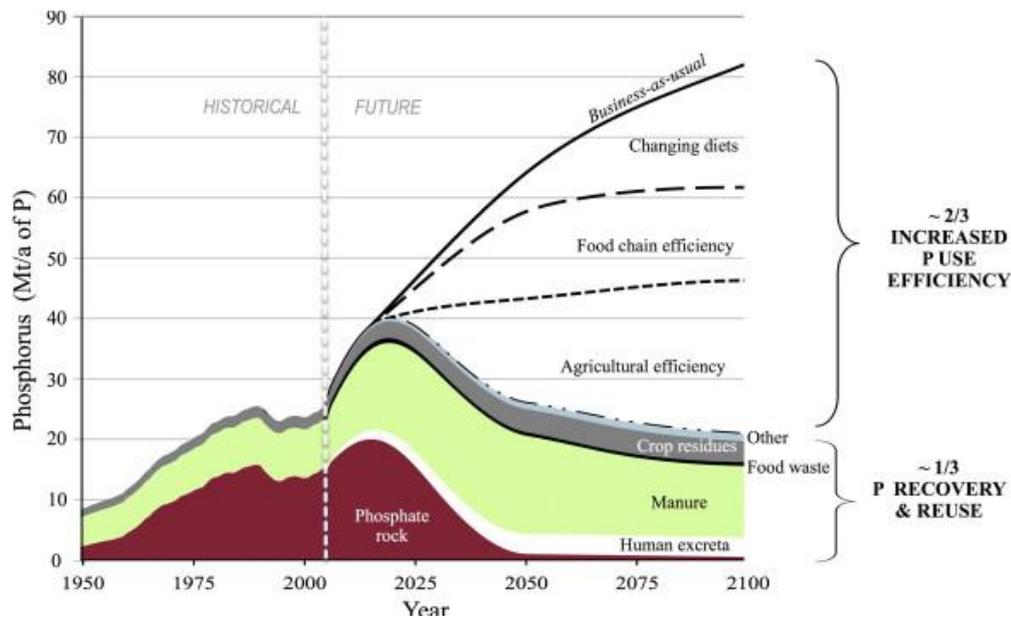


Fig. 4. Phosphorus resource demand under certain management scenarios. (Source: Cordell et al. 2011).

2.3 The uses of P efficient plants

In soils, P occurs in several chemical forms depending on the soil pH (Ashman & Puri 2009). Not all such forms are available to plants. For example, most acidic soils found in warm, humid and sub-humid regions around the world are characterized by low P availability, owing to dominance of other elements such as iron (Fe) and aluminium (Al) which precipitate P in soil, making it hardly available to plants (Brady & Weil 2003). Aluminium in particular is known for creating stunted roots in plants which diminish the plant root's ability to take up water and nutrients, particularly P (Rowell 1994; Ashman & Puri 2009).

Plants take up P in soluble form such as orthophosphates (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) (Worsfold et al. 2005). Soluble forms of P range from 0.001 mg l^{-1} in very infertile soils, to about 1 mg l^{-1} in most fertile soils (Brady & Weil 2003). Efforts have been made to increase the amount of P available for plant uptake. For example genetic modification (GM) of soybean (*Glycine max*) has been found to increase its ability to access P not available to other plants (Wang et al. 2010). This in return mobilizes P for other crops such as maize in an intercropping system. However, there is a growing scepticism towards the use of genetically modified crops (Gregorowius et al. 2011). This is because of uncertainty about future consequent environmental and health risks (Kvakkestad & Vatn 2011).

As an alternative, several non-GM plant species have been found to be efficient in acquiring P from soil. In New Zealand for example, land use change from grassland to forestland using *Pinus radiata* was found to increase P availability in soil (Chen et al. 2003; Chen et al. 2004). In Northern China, *Pinus tabulaeformis* has been found to increase soil P availability following afforestation in the plantation (Zhao et al. 2008).

Furthermore, research has demonstrated that white lupine (*Lupinus albus*) is very efficient in accessing P that is not available to other plants (Abdolzadeh et al. 2010). When presented with P limitation, White lupine secretes citric acid that releases P from the soils by developing short densely clustered roots (Dinkelaker et al. 1989; Peek et al. 2003; Shen et al. 2003; Watt

& Evans 2003). Citric acid is known for its role in mobilizing P in soil (Wang et al. 2008; Wei et al. 2010).

It is therefore possible that other legume plants could also potentially improve soil P availability, apart from nitrogen fixation. Identifying such plants could be used to source P for other plants in intercropping agriculture systems, or be used in land reclamation where soil has been severely degraded or in soils with high P retention capacity such as the Icelandic Andosols.

In the absence of P efficient plants, there are other methods that can be used to recover P to be used for maintaining soil quality, particularly in agriculture and land reclamation. Such methods can be used in regions such as Sub-Saharan Africa where research on P efficient plants is lagging behind. Those methods involve recycling organic wastes that are rich in P.

2.4 Phosphorus recovery from agricultural crop residues and livestock manure

It is estimated that 90% of all P mined from P reserves in the world is used as fertilizers in agriculture (Cooper et al. 2011). Therefore, the agricultural sector presents an uncontested opportunity for P recovery and reuse. A variety of organic residues from agricultural land has been used as a source of P. For example, Lupwayi et al. (2007) found that crop residue plays an important role in maintaining soil P, thus recycling such materials is important for P nutrient cycling. Residues that can be used include straw, husk and stalks (Cordell et al. 2011).

Various hypotheses have been used to link crop residues to soil P. For example, Iyamuremye and Dick (1996) found that decomposed crop residues produce organic acid which is effective in decreasing P adsorption into soils, consequently making it available to plants. This phenomenon was also demonstrated in wheat and soybean crop residues in the Vitrisol soils in India (Reddy et al. 2001). Meanwhile, Lupwayi et al. (2003) found a high soil P concentration after decomposing crop residues into soil. Also, Lupwayi et al. (2007) found that green manure is the most important source of soil nutrients, and can release approximately 5–6 kg ha⁻¹ of P.

Therefore, using organic agricultural waste such as crop residues is an important step in recovering nutrients lost during agricultural production. Such techniques can be used by farmers with relative ease, in the absence of heavy machinery. This can supplement the current process whereby crop residues are burned (Lupwayi et al. 2006), a practice found in most developing countries such as Namibia. However, crop residues are also used as animal fodder in mixed farming systems, perhaps making it difficult to use it for a composite purpose.

2.5 Phosphorus recovery from domestic wastewater and sewage sludge

Since the uses of P in agriculture and other anthropogenic sectors, there has been major leakage of nutrients such as P. Such losses are theoretically avoidable, owing to a modification of the anthropogenic P system (Fig. 3). On average, humans require approximately 1.2 g person⁻¹ day⁻¹, which translates into about approximately 3 million tons⁻¹ P⁻¹ year⁻¹ for the global population (Cordell et al. 2012). Prior to the introduction of modern sewage systems, such P often found its way into the environment, replacing the amount of P removed with food products (Smit et al. 2009). With more influx of people living in urban areas, towns and cities could become a potential source for the recovery and reuse of P. In realising the importance of such recovery opportunities, recent research has thus focused on

recovering P from human excreta in wastewater and sewage sludge (Levlin & Hultman 2004; Stark 2005; Bhuiyan et al. 2008; Cornel & Schaum 2009; Cordell et al. 2011).

Modern sanitary systems aim to limit contact between human and harmful pathogens in water from both domestic and industrial uses (Mihelcic et al. 2011). This is done by discharging wastewater into sewage systems. However, a study by Ronteltap (2009) showed that such wastewater often is rich in nutrients such as nitrogen, P and potassium. Such nutrients are often discharged into water streams and oceans. While this study values the adoption of such sanitary systems due to their role in maintaining environmental health, it is equally important that such systems allow nutrients to be returned to the environment. Smit et al. (2009) and Cordell et al. (2011) thus recommended that there is a need for new technologies that will ensure that such nutrients are returned to agricultural land. Furthermore, most wastewater treatment processes produce approximately 90% of the P-load in the raw sewage, which is removed from the wastewater and transferred into the sludge (Petzet et al. 2012). It has thus been suggested that sewage sludge can potentially provide a good opportunity to recover P (Biswas et al. 2009). Meanwhile, according to Lougheed (2011) recycling of such nutrients will not only reduce eutrophication but will also reduce the amount of fertilizers being imported as well as contributing to sustainable urban systems. According to the European Commission (2001) approximately 25% of P is recovered from municipal water and sludge in Europe. This practice is mainly found in countries such as Sweden and the Netherlands (Levlin & Hultman 2004; Stark 2004).

Recovering nutrients from wastewater and sewage sludge did not come unchallenged. Firstly, it has been found to be difficult to get a consensus on the uses of sludge in agricultural production due to low social acceptance (Stark 2004), as well as the concern for contaminants and heavy metals that could be passed through the food chain (Levlin & Hultman 2004). Secondly, recovering P in such a manner will require a major change in the physical infrastructure (Cordell et al. 2011). For example, new infrastructure would be required to channel nutrients to agricultural areas. Lastly, extracting P from wastewater and sludge requires technical expertise in chemical analysis, which is not always readily available, unlike crop residues and organic manure which are applied directly into soil.

3. METHODS AND MATERIALS

3.1 Description of the study area

This study was conducted at Gunnarsholt, southern Iceland and at Hólasandur, northern Iceland (Fig. 5). Iceland is an active volcanic island located along the mid-Atlantic ocean, extending between 63° 23'–66° 32'N and 13° 30'–24° 32'W. Iceland is characterised by a maritime climate due to its proximity to the Atlantic Ocean (Einarsson 1984).

Prior to settlement some 1100 years ago, the Icelandic landscape was approximately 60% covered with vegetation, of which birch woodland (*Betula pubescens*), the only native woodland species, occupied some 20–30% (Church et al. 2007). However, after settlement, Iceland has suffered major land degradation, an ecological catastrophe well documented in Icelandic literature. Various factors such as unsustainable land use practice (overgrazing, cutting of firewood, etc.) (Gísladóttir et al. 2011), extreme climate conditions (Ólafsdóttir & Guðmundsson 2002), as well as volcanic activities experienced over the years are often cited to that effect (Greipsson 2012). As a result, current vegetation cover amounts to approximately 25%, with the birch woodland occupying as little as 1% (Ministry for the Environment 2001). Clearing of woody vegetation led to severe soil erosion of the Andosols,

a volcanic soil type which is highly susceptible to erosion by both wind and water (Arnalds & Barkarson 2003). Other factors which modify the soils are frequent tephra fall from volcanic eruptions and freeze-thaw cycles (Arnalds et al. 1995).

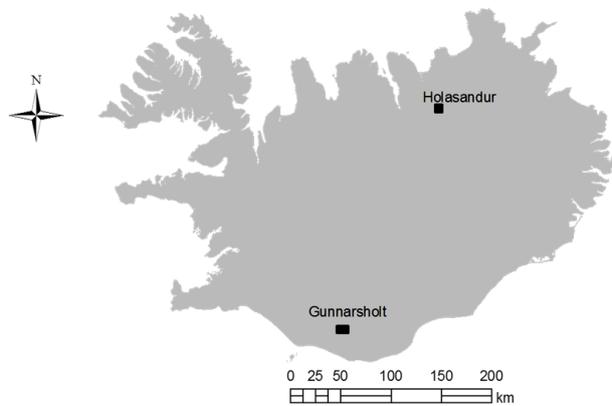


Fig. 5. Location of the sampling sites (Gunnarsholt) in south Iceland and (Hólasandur) in the north.

In order to combat land degradation and repair damaged land in Iceland, restoration of degraded land was promoted as a necessity. Early restoration work dated back to the 17th century with the construction of stone walls against drifting sand drift by Rev. Björn Halldorsson in south-west Iceland (Croft 2011). Since then, Iceland has initiated various approaches to combat land degradation (Magnússon 1997). For example, seed for native grass species such as *Leymus arenarius*, *D. caespitosa* and *Festuca richardsonii*, have been seeded on eroded land, and the non-native *Deschampsia beringensis* was introduced later. Other recent approaches include the introduction of non-native tree species such as the Siberian Larch (*Larix sibirica*), *Picea abies*, *P. sitchensis*, *P. sylvestris* and *P. contorta* (Eysteinnsson 2009). It was under this framework that Nootka lupine was introduced to Iceland.

Located at an altitude of 300 m (Greipsson et al. 2002), Hólasandur soils are classified as Sandy Vitrisol that form from aeolian deposition (Arnalds 2004). At an altitude 50 m (Gretarsdottir et al. 2004), the Gunnarsholt control sites' soils are classified as Combic Vitrisols, while those at the lupine sites are Brown Andosols, according to Arnalds (2004). These soils are largely influenced by the volcanic activities of Mount Hekla (Strachan et al. 1998). The control sites exhibit the typical characteristics of eroded landscapes in Iceland, with less than 5% vegetation cover, while the lupine sites were reclaimed.

Data from the SCSi (2012) indicate that the lupine fields used for sampling at Hólasandur were reclaimed between 1996 and 2002, while those at Gunnarsholt were reclaimed with lupine in 1999. Since then, grazing has been excluded from these sites. Data from the Icelandic Meteorological Office (2012) for 2001 to 2004 report an annual average temperature at Hella, near Gunnarsholt, of 4.9°C, and precipitation of 1344 mm year⁻¹. At Reykjahlíð, near Hólasandur, the annual average temperature is 3.01°C and annual precipitation is 447 mm year⁻¹.

3.2 Sampling and data collection

Sampling was carried out at 6 sites at Gunnarsholt; 3 of the sites were revegetated with Nootka lupine, while 3 sites were controls. At each site, three sampling plots (10 x 10 m) were randomly selected. In each sampling plot, five 0.5 x 0.5 m frames were randomly selected for sampling. From each 0.5 x 0.5 m frame, vegetation and soil samples were collected. Vegetation was clipped to the ground surface. Soil samples were collected at 0-

5 cm depth using a 5 cm diameter handheld auger. Prior to preparations samples were kept at room temperature.

In order to determine total biomass content, vegetation samples were milled in a Retsch SM 200 mill (Retsch GmbH, Germany) to ≤ 2 mm grains. Vegetation samples were then dried in a thermo-scientific Heraeus oven at 105 °C for 24 hours. The resultant dry matter was weighed and recorded. These data were used to calculate total biomass per hectare (kg ha^{-1}).

For soils, samples from each site were mixed as pooled samples and sieved through a 2 mm sieve. The samples retained on the 2 mm sieve were classified as either belowground biomass or gravels. Gravels were discarded. The soil samples were processed on the vial rotator (SampleTek, USA) for 16 hours. This was done to break down aggregates in the samples as well creating finer soil fractions to enable detection of P.

For Holasandur, both vegetation biomass and soil sample data were retrieved from the Soil Conservation Service of Iceland's (SCSI) soil archive. The Holasandur samples used in this study were collected in 2009 and biomass data as well as C and N data were available for these samples. These samples have been handled the same way as those at Gunnarsholt.

3.3 Soil chemical properties analysis

3.3.1 Total P

Sulphuric acid (25 ml, 0.5M) was added to soil samples (0.5 g) and shaken for 16 hours (200 rpm) using a reciprocal shaker (Brunswick Scientific, UK). Three replicate samples of each pooled soil sample were prepared. Samples were then filtered (Schleicher and Schull Rundfilter filter paper, Germany). Total phosphorus was analysed using inductively coupled plasma atomic emission spectrometry (ICP-OES; Spectro Ciros Vision).

3.3.2 Bray and Kurtz P

Plant-available P was determined using the Bray and Kurtz P-1 method as described in Kovar and Pierzynski (2000). This is the method mostly recommended for acidic to neutral soils such as the Icelandic Andosols. Soil samples (3.00 g) were accurately weighed and transferred into a 50 ml plastic tube. Then 30 ml of extraction solution (0.025 M HCl in 0.003 M NH_4F) was added to each flask and shaken at 200 rpm for 5 minutes using the reciprocal shaker. Three replicate samples of each pooled soil sample were prepared. Thereafter, samples were filtered. In cases when the first extract was not clear, samples were re-filtered. In order to avoid P contamination at the laboratory, all glassware used was cleaned with a phosphorus free detergent.

3.3.3 Olsen P

Plant-available P was also determined using the Olsen P method for Holasandur samples, using 1.00 g of soil in 20 ml of Olsen P extracting solution (0.5M NaHCO_3 , pH 8.5). Three replicate samples of each pooled soil sample were prepared. Samples were shaken at 200 rpm and left to settle for 30 minutes. Then samples were filtered (Schleicher and Schull Rundfilter filter papers, Germany).

3.3.4 Colorimetric methods

A colorimetric method was used to analyse P in the samples. Firstly, a colour reagent was prepared by mixing 25 ml of sulphuric acid (5N, H_2SO_4 , prepared by dissolving 70 ml of

H₂SO₄ in 500 ml of distilled water), 2.5 ml potassium antimonyl nitrate solution, potassium antimonyl tartrate solution (K(SbO)C₄H₄O₆ · 1/2H₂O, prepared by dissolving 1.37 g K(SbO)C₄H₄O₆ · 1/2H₂O in 400 mL of distilled water), ammonium molybdate solution ((NH₄)₆M₀₇O₂₄ · 4H₂O, prepared by dissolving 20 g (NH₄)₆M₀₇O₂₄ in 500 ml of distilled water), as well as 0.01M ascorbic acid prepared by dissolving 1.76 g of ascorbic acid in 100 ml of distilled water (Kovar & Pierzynski 2000).

Then 5 ml of the extracted sample were pipetted into a test tube and thoroughly mixed with 0.8 ml of the colour reagent and the absorbance of the resultant colour was measured at 880 nm using a UV-visible spectrophotometer (Cecil Instruments Ltd, England). A total of six standard solutions with different P concentrations ranging from 0.01 to 2.0 mg l⁻¹ were also prepared to generate a standard calibration curve for computing P concentration in the soil samples based on absorbance. This method was adapted from Kovar & Pierzynski (2000).

3.4 Soil physical properties analysis

Selected soil physical properties were also analysed: soil pH, soil organic matter (SOM) based on soil loss on ignition (%LOI), soil moisture content, and bulk density (BD).

3.4.1 Soil pH measured in water (H₂O)

Soil pH was measured in water. A 5 g soil sample was placed into a 50 ml plastic tube and mixed with 25 ml of distilled water to achieve a ratio of 1:5 (weight : volume). The samples were shaken for 2 hours using a reciprocal shaker. The samples were then left to settle for 30 minutes before pH was measured (pH meter Orion, USA). Prior to taking the pH measurement, the pH meter was calibrated using two buffers with known pH. This method was adapted from Blakemore et al. (1987).

3.4.2 Soil organic matter and soil moisture content

Soil organic matter and soil moisture content were measured using the oven-drying method described in Heiri et al. (2001). For each of the 12 sites, samples were measured in triplicate using 15 g of air-dried soil. The samples, in crucibles, were heated in the oven at 105°C for 24 hours. Thereafter, the same soil samples were ignited in the high temperature oven at 550°C for 16 hours and allowed to cool down. At each point, soil weight was recorded at 105°C as the weight of the crucible changes at varying temperatures. The SOM was calculated as follows:

$$\text{SOM} = ((\text{DW}105 - \text{DW}550) / \text{DW}105) * 100$$

where DW105 is the weight of the soil samples heated at 105°C and DW550 is the weight of the soil after being ignited at 550 °C. Meanwhile, soil moisture content was computed as:

$$\text{Soil moisture content (\%)} = ((\text{WS}105 - \text{DS}105) / \text{DW}105) * 100$$

where WS105 is the weight of the sample before drying at 105°C, whereas DS105 is the weight of the sample after drying at 105°C.

3.4.3 Soil bulk density

For soil BD of the total soil that was sampled from each sampling plot, a fraction of ≤ 2 mm referred to as active soil volume (ASV) was used to calculate the soil bulk; the BD. This

approach excludes the volume of other matters such as gravels and belowground biomass. This is based on air-dried soil at room temperature. Soil BD was calculated as:

$$\text{Soil BD} = (\text{Soil weight (g)} / \text{Soil volume (cm}^{-3}\text{)})$$

where soil weight (g) is the weight of the soil after air-drying and soil volume (cm⁻³) is the volume of the soil handheld auger used for sampling at 0-5 cm depth.

3.5 Statistical analysis

Data were analysed with two way analysis of variance (ANOVA) in R 2.15 (R Development Core Team 2012). Firstly the data were subjected to testing of normality as a prerequisite for ANOVA. Soil BD, loss on ignition, soil pH, biomass, total P and Bray P were log transformed to achieve normality. Study sites (Gunnarsholt and Holasandur) as well as site type (lupine and control) were used as predictors for all soil properties and vegetation biomass data presented. The Pearson correlation test was also used to test correlation among variables.

4. RESULTS

4.1 Soil physical properties

Results for soil BD, soil pH, soil moisture content and SOM are presented in Table 1. These results are further summarised in Fig. 6. Results from ANOVA on the effect of study site and site type on soil BD, soil pH, soil moisture content and SOM are presented in Table 2. Soil BD was not significantly different, statistically, between the control and lupine sites ($p > 0.05$). Soil BD was significantly different between the Gunnarsholt and Holasandur sites ($p < 0.05$). Soils at Holasandur had a higher BD than those of Gunnarsholt. The control site at Holasandur had a larger range in BD than all other sites (Fig. 6). Soil pH was significantly higher ($p < 0.05$) at the control sites than at the lupine sites for both Gunnarsholt and Holasandur (Fig. 6). Soil pH was also significantly higher ($p < 0.05$) at Gunnarsholt than at Holasandur (Fig. 6). At both sites, soils were slightly acidic to neutral.

Table 1. Soil physical properties (Mean \pm SD) at Gunnarsholt (n=6) and Holasandur (n=6)

Site type	BD (g cm ⁻³)	Soil pH	Soil moisture content (%)	SOM
Gunnarsholt				
Control	1.04 \pm 0.01	6.69 \pm 0.02	4.50 \pm 1.06	2.43 \pm 0.27
Lupine	0.90 \pm 0.03	6.65 \pm 0.02	3.11 \pm 0.36	3.30 \pm 0.24
Holasandur				
Control	1.21 \pm 0.41	6.61 \pm 0.12	1.37 \pm 0.20	1.27 \pm 0.28
Lupine	1.30 \pm 0.16	6.34 \pm 0.16	1.70 \pm 0.56	1.76 \pm 0.53

Soil moisture content (%) was not significantly different between the control and lupine sites ($p > 0.05$). There was a significant difference ($p < 0.05$) in soil moisture content between Gunnarsholt and Holasandur (Table 2). At Gunnarsholt soil moisture was higher, ranging between 3-5%, while at Holasandur soil moisture was less than 2%. The SOM was significantly different ($p < 0.05$) between the lupine and the control sites (Table 2), as well as between the Gunnarsholt and Holasandur soils ($p < 0.001$). Gunnarsholt soil had a greater SOM than soils at Holasandur (Table 1).

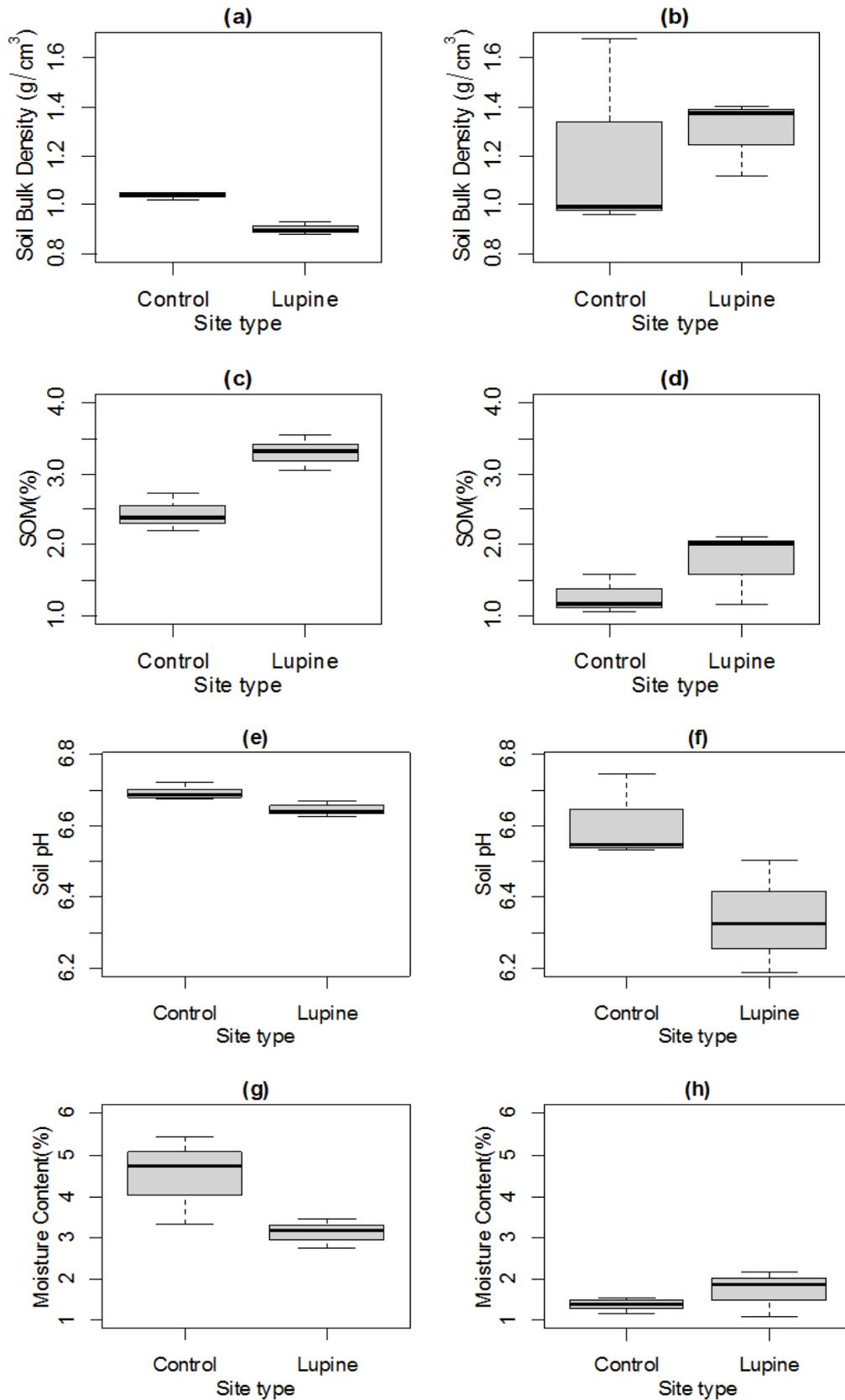


Fig. 6. Box and Whisker plots showing the minimum, lower quartile, median, upper quartile and maximum value for each soil property measured at control and lupine sites; (a),(c),(e) and (g) are for Gunnarsholt site (n=6), while (b), (d), (f) and (h) are for Holasandur (n=6).

Table 2. ANOVA results for the effects of study site and site type on soil BD (g cm^{-3}), soil pH, soil moisture content (%) and SOM. Significance codes: *** = 0.001; ** = 0.01; * = 0.05; NS = not significant (n=12).

Variables	Predictors	Df	SS	MS	F	P
BD (g cm^{-3})	Site	1	0.17	0.17	6	*
	Site type	1	0.00	0.00	0.04	NS
	Site x Site type	1	0.04	0.04	1.45	NS
Soil pH	Site	1	0.11	0.11	11.26	*
	Site type	1	0.07	0.07	7.30	*
	Site x Site type	1	0.03	0.03	3.52	NS
Soil Moisture Content (%)	Site	1	15.47	15.47	38.19	***
	Site type	1	0.81	0.82	2.02	NS
	Site x Site type	1	2.22	2.22	5.48	*
SOM (%)	Site	1	1.31	1.31	30.09	***
	Site type	1	0.28	0.28	6.47	*
	Site x Site type	1	0.00	0.00	0.00	NS

Soil carbon (C) and nitrogen (N) were significantly ($p < 0.05$) higher at the lupine sites than at the control (Fig. 7). The average C:N ratio was 8:1 at the lupine sites and 7:1 at the control site. The SOM was significantly correlated to soil C ($r = 0.85$, $p < 0.05$) and N ($r = 0.85$, $p < 0.05$). Soil BD and SOM were largely correlated, although the correlation was non-significant ($p > 0.05$). Soil moisture content was largely correlated to both soil pH and SOM. The correlation between soil moisture content and SOM was significant ($p < 0.05$).

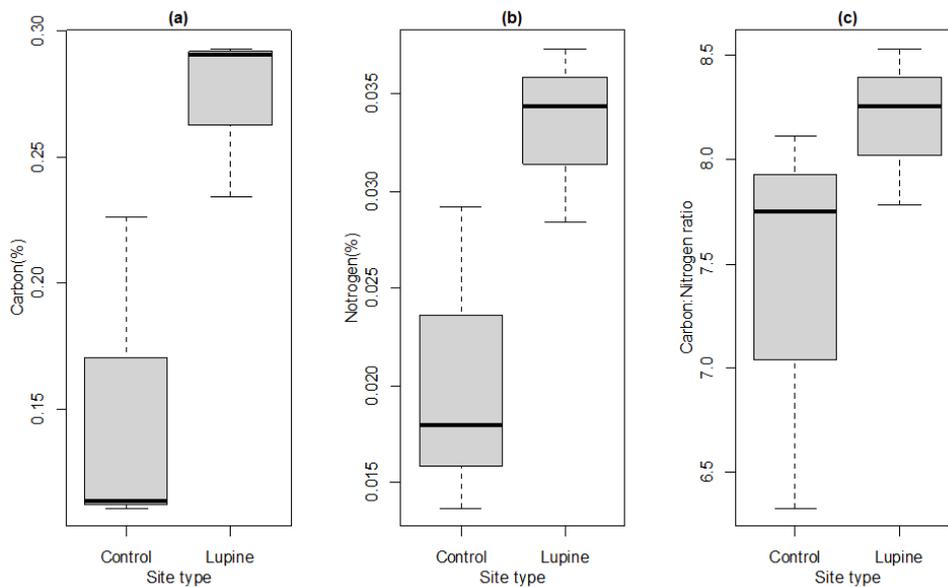


Fig.7. Box and Whisker plots showing the minimum, lower quartile, median, upper quartile and maximum value for Carbon (a) and Nitrogen (b) and Carbon : Nitrogen ratio at the Holasandur site (n=6).

4.2 Total P and plant-available P based on the Bray and Olsen methods

Results for total P, Bray P, total iron (Fe), total aluminium (Al), total calcium (Ca) and Olsen P are summarized in Table 3, while ANOVA results on the effects of site and site type on total P, Bray P, total iron (Fe), total aluminium (Al), and total calcium (Ca) are presented in Table 4. There was no significant difference ($p > 0.05$) in total P between the lupine and control sites (Table 4). Total P was significantly higher ($p < 0.001$) at Gunnarsholt than in the Holasandur soils (Table 4). At Gunnarsholt, total P was approximately double the concentration at the Holasandur sites (Table 3). Similarly, soil total Fe, total Al and total Ca were significantly higher at Gunnarsholt than at the Holasandur sites ($p < 0.05$).

Table 3. Results for total P, Bray P, total Fe, total Al, total Ca and Olsen P at Holasandur and Gunnarsholt sites (n=12).

Sites	Tot P (g kg ⁻¹)	Bray P (µg kg ⁻¹)	Tot Fe (g kg ⁻¹)	Tot Al (g kg ⁻¹)	Tot Ca (g kg ⁻¹)	Olsen P (mg/kg)
Gunnarsholt						
Control	0.31 ± 0.01	17.33 ± 0.72	23.98 ± 1.10	13.84 ± 0.50	8.80 ± 0.41	-
Lupine	0.37 ± 0.04	18.53 ± 0.40	26.13 ± 3.24	16.14 ± 1.77	10.54 ± 0.94	-
Holasandur						
Control	0.15 ± 0.01	18.43 ± 0.95	16.44 ± 1.12	10.10 ± 1.06	6.84 ± 0.88	0.56 ± 0.25
Lupine	0.16 ± 0.03	19.30 ± 0.44	16.45 ± 3.40	10.58 ± 2.29	6.46 ± 0.71	0.38 ± 0.23

Table 4. Results of the ANOVA on the effects of study site (Gunnarsholt versus Holasandur) and site type (Control versus Lupine) on soil total P, Bray P, total Ca, total Al and total Fe. Significance codes:***= 0.00 **= 0.0 * 0.05= NS not significant (n=12).

Variables	Predictors	Df	SS	MS	F	P
Total P (g kg⁻¹)	Site	1	1.97	1.97	230.91	***
	Site type	1	0.03	0.03	4.32	NS
	Site x Site type	1	0	0.00	0.96	NS
Bray P (mg kg⁻¹)	Site	1	2.44 x 10 ⁻⁶	2.43 x 10 ⁻⁶	5.62	*
	Site type	1	3.26 x 10 ⁻⁶	3.25 x 10 ⁻⁶	7.50	*
	Site x Site type	1	9.20 x 10 ⁻⁸	9.20 x 10 ⁻⁸	0.21	NS
Total Ca (g kg⁻¹)	Site	1	27.33	27.33	46.90	***
	Site type	1	1.36	1.36	2.34	NS
	Site x Site type	1	3.36	3.36	5.76	*
Total AL (g kg⁻¹)	Site	1	64.73	64.73	26.55	***
	Site type	1	5.81	5.81	2.38	NS
	Site x Site type	1	2.48	2.48	1.01	NS
Total Fe (g kg⁻¹)	Site	1	222.22	222.22	36.16	***
	Site type	1	3.52	3.52	0.57	NS
	Site x Site type	1	3.46	3.46	0.56	NS

Plant-available P (Bray P) ranged between a maximum average of 19 µg kg⁻¹ to a minimum average of 17 µg kg⁻¹, whereas Olsen P results were exceptionally higher than Bray P, ranging from 380 ± 230 µg kg⁻¹ at lupine sites and 560 ± 250 µg kg⁻¹ for control sites at the Holasandur site. There were significant differences in Bray P between the lupine and control sites ($p < 0.05$). A higher concentration of available P was found at lupine sites than at the

control. The plant-available P was also significantly higher at Holasandur than at Gunnarsholt ($p < 0.05$). No interaction effect was found between the sites, which suggests that the concentration follows the same pattern at both sites.

The available-P based on the Bray method and the Olsen method was relatively low in relation to the total soil P (Fig. 8). The Olsen P was higher than the Bray P at both sites. The available-P (%) was higher at Holasandur than at Gunnarsholt (Table 5). There was no significant difference in Bray available-P (%) between the control and lupine sites ($p > 0.05$). The Bray available-P (%) was significantly higher at Holasandur than at Gunnarsholt ($p < 0.05$). The Gunnarsholt site had very low available-P (%) compared to the Holasandur site, although available-P concentration ranged within $18 \mu\text{g kg}^{-1}$ at both sites. This difference was not tested in the Olsen P results owing to the small sample size.

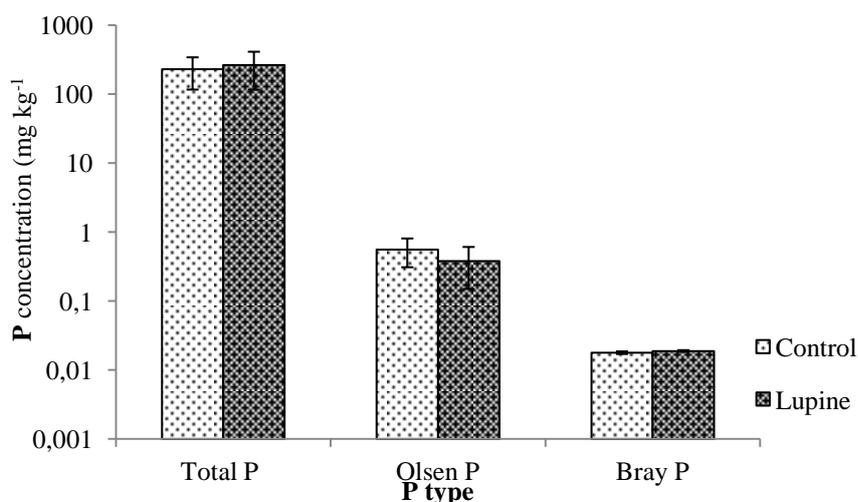


Fig. 8. Concentration (Mean \pm SD) of total P, Olsen P for Holasandur samples ($n=6$), and Bray P at the control and lupine sites ($n=12$).

Table 5. The proportion (%) of available phosphorus to total soil phosphorus ($n=12$), Olsen P ($n=12$) ($n=6$).

Sites	Site type	Tot P(g kg ⁻¹)	Bray P (%)	Olsen P (%)
Gunnarsholt	Control	0.31 ± 0.01	0.006	-
	Lupine	0.37 ± 0.04	0.005	-
Holasandur	Control	0.15 ± 0.01	0.013	0.18
	Lupine	0.16 ± 0.03	0.013	0.202

The plant-available (Bray) P was significantly correlated to soil pH and soil moisture content as well as C and N in the soils ($p < 0.05$). Plant available P showed a small to medium correlation to total P, total Fe, total Al and total Ca (Table 6). Total Ca, total Fe, total Al were significantly correlated to total P (Table 6). Both, total Fe, total Al, and total Ca were not significantly ($p > 0.05$) different between the control and lupine sites

Table 6. Pearson correlation coefficient (*r*) between soil physical properties, biomass yield and the chemical properties.
Significance codes: *** =0.001 ** =0.01 * = 0.05 NS =not significant (n=12).

Environmental Variables	Tot (g kg ⁻¹)	Bray P mg (kg ⁻¹)	Tot C (g kg ⁻¹)	Tot Al (g kg ⁻¹)	Tot Fe (g kg ⁻¹)	Tot C (g kg ⁻¹)	BD (g cm ⁻³)	Soil pH	SOM (%)	C (%)	N (%)	Soil Moisture content (%)	Above ground (kg ha ⁻¹)	Below ground Biomass (kg ha ⁻¹)
Tot P (g kg ⁻¹)	1:00													
Bray P mg (kg ⁻¹)	-0.35	1:00												
Total Al (g kg ⁻¹)	0.94**	-0.19	0.94**	1:00										
Total Fe (g kg ⁻¹)	0.95**	-0.39	0.91**	0.97**	1:00									
Total Ca (g kg ⁻¹)	0.94**	-0.18	1:00	0.94**	.91**	1:00								
BD (g cm ⁻³)	-0.61*	0.08	-0.61*	-0.49	-0.48	-0.61*	1:00							
Soil pH	0.56	-0.62*	0.58*	0.54	0.62*	0.58*	-0.12	1:00						
SOM (%)	0.89**	0.72**	0.87**	0.90**	0.87**	0.87**	-0.51	0.42	1:00					
C (%)	0.63	.90**	0.28	0.55	0.34	0.28	-0.03	-0.6	0.84*	1:00				
N (%)	0.67	.88**	0.32	0.58	0.37	0.32	-0.14	-0.57	0.85*	0.98	1.00			
Soil moisture content (%)	0.73**	-.57*	0.63*	0.66*	0.75**	0.63*	-0.38	0.55	0.67*	0.68		1:00		
Aboveground (kg ha ⁻¹)	0.49	0.22	0.54	0.54	0.45	0.54	-0.32	-0.12	0.43	0.60	0.55	-0.06	1:00	
Belowground (kg ha ⁻¹)	0.66*	0.14	0.69*	0.65*	0.57	0.69*	-0.49	0.13	0.76**	0.50	0.48	0.16	0.69*	1:00

4.3 Biomass yield at Gunnarsholt and Holasandur

Above-ground biomass and below-ground biomass results are presented as kg ha^{-1} in Fig. 9 and Fig. 10, respectively. Results showed that there was a significant statistical difference in aboveground biomass yield between Holasandur and Gunnarsholt ($F_{1,8} = 7.91, p < 0.01$).

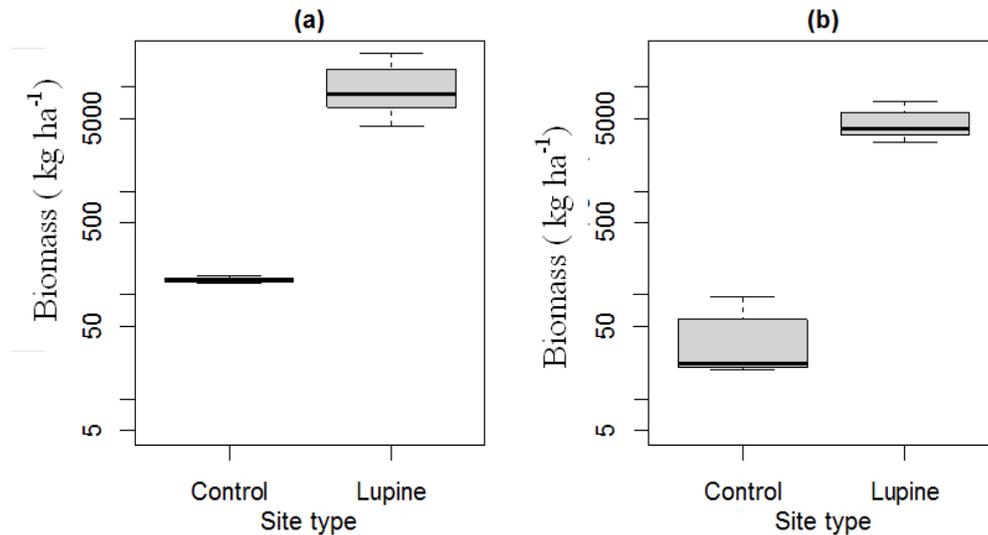


Fig.9. Box and Whisker plots showing the minimum, lower quartile, median, upper quartile and maximum value for aboveground biomass yield (kg ha^{-1}) at Gunnarsholt (a) and Holasandur (b) sites: log scale on the y axis ($n=12$).

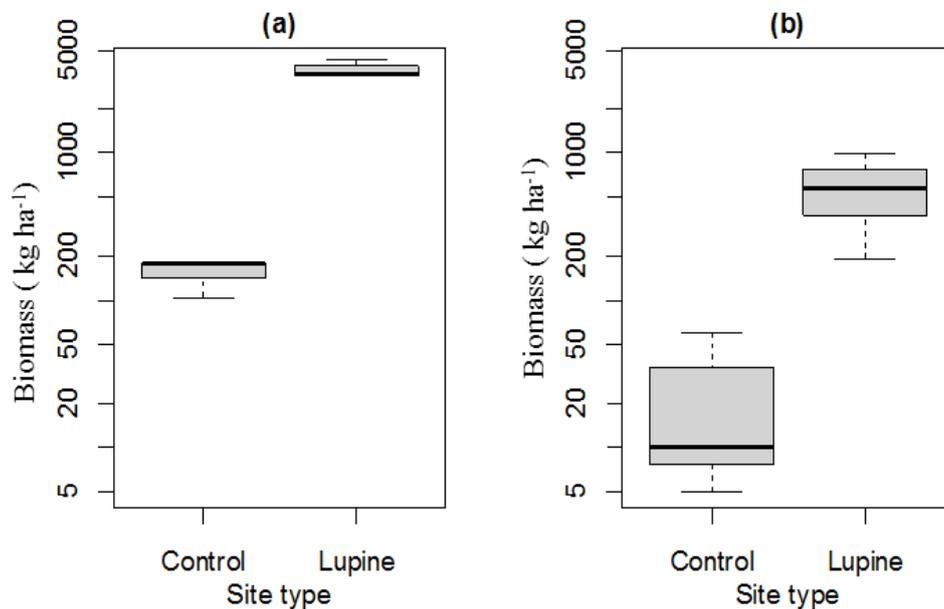


Fig.10. Box and Whisker plots showing the minimum, lower quartile, median, upper quartile and maximum value for below-ground biomass (0-5 cm depth) at Gunnarsholt (a) and Holasandur (b) site: log scale on the y axis ($n=12$).

This difference was also found between the lupine and control sites ($F_{1,8} = 151.20$, $p < 0.001$). Gunnarsholt had a greater biomass yield compared to the Holasandur site. Similarly, belowground biomass was significantly different between Holasandur and Gunnarsholt ($F_{1,8} = 23.44$, $p < 0.001$) as well as between the control and lupine sites ($F_{1,8} = 74.71$, $p < 0.001$). Belowground biomass and aboveground biomass were significantly correlated ($p < 0.01$; Table 6). No statistically significant relationship was found between aboveground biomass and soil properties such as total P, Bray P, total Ca, total Fe, total Al and soil pH. On the other hand, belowground biomass was found to be positively correlated to total Ca, total Al and total Fe (Table 6).

5. DISCUSSION

5.1 Total P and plant-available P

This study found that Nootka lupine mobilizes P by increasing the concentration of available-P. Although this was a relatively small experiment, results showed that plant-available P based on the Bray extraction method was significantly higher at the lupine site than at the control site at both the Gunnarsholt and Holasandur sites. The trend from these results supports those of Óskarsson et al. (2004) which were based on the Olsen method in a similar environment. The Olsen P results were similar to Óskarsson et al. (2004), though following a contrary trend whereby the control sites had more Olsen P than the lupine sites. This study used both the Bray extraction method recommended for acidic soils, as well as the Olsen method which is mainly used for alkaline soils (Kovar & Pierzynski 2000). The Bray results could be more reliable as none of the soil sample pH was alkaline.

Although the concentration of the available Bray P is relatively low ($19 \mu\text{g kg}^{-1}$), with time, it might increase in the ecosystem, which could benefit the Nootka lupine itself as well as other plants in the ecosystem. This perhaps in part explains the evident spread of Nootka lupine and the undergrowth of other herbaceous plants around it. However, as a result of the high P retention capacity of the Andosols ($> 95\%$) (Arnalds et al. 1995), it is also likely that much of such mobilized P would become re-bound to the soils in a non-soluble form which other plants cannot access.

This study also found that total soil P at Gunnarsholt was approximately double the amount of the concentration in soils at Holasandur. The non-significant difference in soil total P between the controls and lupine implies that revegetation with Nootka lupine had no vital impact on the total P concentration. This was not unexpected given the fact that the source of P is in the soil. These findings agree with those of Ritter (2007) who found that vegetation did not change soil total P in Icelandic Andosols. Although this study could find a statistically significant difference in available P concentration, the proportion of available Bray P (%) to the total P was not significantly different between the control and lupine sites, unlike between the Holasandur and Gunnarsholt. However, Total P was not strongly correlated to available P, which therefore means that available-P is not dependent on the total P, but perhaps on other factors such as soil pH.

Total P is influenced by the concentration of other soil properties such as total Al and total Fe which impact on P retention by forming allophane, which immobilizes P in Andosols (Dahlgren et al. 2004). For example, soils at Gunnarsholt had higher total Al, total Fe and total Ca than those at Holasandur, which significantly correlate to the total soil P at the two sites. The total Al and total Fe results from this study were consistent with those of Arnalds

and Kimble (2001). It is therefore not surprising that available P at both study sites was very low compared to other Andosols in places such as Japan and Hawaii (Dahlgren et al. 2004).

5.2 Soil physical properties

Results from this study showed that the soils at the Gunnarsholt and Hólasandur sites differ both in physical and chemical properties such as soil pH, soil moisture, and SOM. For example, soil BD was higher at Hólasandur compared to Gunnarsholt. This was expected given that the Hólasandur area is characterised by Vitrisol soils while the Gunnarsholt area is mainly characterised by Andosol soils, of which BD has been reported to be in a range of 0.3 – 0.8 g cm⁻³, while that of Vitrisol is often in a range of 0.8 – 1.2 g cm⁻³ (Arnalds 2004).

The results from this current experiment are therefore consistent with the BD at Hólasandur, being one of the deserts in Iceland, as well as those of Gunnarsholt, as stipulated in Arnalds (2004). The differences in soil BD between the two sites are probably a result of factors such as soil carbon, as SOM negatively correlated to soil BD ($r = -0.51$). Soils at Gunnarsholt had a higher SOM than those of Hólasandur, which therefore explains the low soil BD at Gunnarsholt as compared to Hólasandur.

The Icelandic Vitrisols, the soils found at Hólasandur as classified in Arnalds (2008), are characterised by low soil carbon as compared to Andosols. It was expected that soil BD would be different between the control and lupine sites, given the difference in accumulation of SOM between the two sites, but this study did not uncover evidence to support this. Other factors such as soil texture and compaction could probably explain this pattern. These were beyond the scope of this study and thus are not presented here. Although not significant, soil SOM had a greater influence on soil BD, which in turn affects other properties such as moisture content. For example, at Hólasandur where soil BD was higher, there was little soil moisture content. According to Hernanz et al. (2000), as soil BD increases, soil moisture holding capacity tends to decrease, owing to reduced pore space in soils. Arnalds (2008) also found a low water holding capacity in the Vitrisol soils; the soils found along the Hólasandur desert.

Soil pH at Gunnarsholt was slightly above the pH range measured earlier by Strachan et al. (1998) around Gunnarsholt, but within the range for Brown Andosols reported by Arnalds (2004). Hólasandur soil pH was slightly below pH 7, a common average soil pH for Vitrisols as reported in Arnalds (2004). While the difference in soil pH between Gunnarsholt and Hólasandur could be a result of differences in soil types between the two sites, control sites had a high soil pH compared to the lupine sites. It appears though that these results support the findings by Arnalds and Kimble (2001) that in Andosols, soil pH in vegetated soils tend to be lower compared to non-vegetated soils. This is because an increase in soil organic carbon in soils decreases soil pH, a pattern which was also demonstrated by Arnalds (2004). According to Brady and Weil (2003), when decomposed, SOM matter releases an organic acid which acidifies the soils. It is therefore not surprising that soil pH was low at lupine sites compared to control sites.

While lupine sites were found to have higher P availability, the overall differences in the aforementioned soil chemical properties between Gunnarsholt and Hólasandur are likely to be the results of differences in climatic factors and soil type between the two sites. It is also evident from this study that Nootka lupines at Gunnarsholt had a significantly greater biomass yield as compared to those at Hólasandur sites, given a higher mean temperature and precipitation at Gunnarsholt as opposed to Hólasandur.

This study has indicated that Nootka lupine may be able to increase soil P availability in Icelandic soils. With the global P scarcity, P efficient plants such as Nootka lupine could thus provide a sustainable and cost effective method of improving soil phosphorus for other plants, more especially in land reclamation projects. This is accompanied by advantages such as acquiring natural available P, reduced import of P as well as reduced environmental problems associated with the use of P fertilizers.

6. CONCLUSIONS

Nootka lupine used in Iceland for land reclamation was investigated in this study. The main objective was to assess whether Nootka lupine mobilizes P in Icelandic soils. Evidence from this study suggested that there was more plant-available P at sites colonized by Nootka lupines. Thus, Nootka lupine could provide a cost effective method of acquiring P from soil, compared to the conventional methods of using imported P. It is thus important for further studies to assess the ecological significance of this P to other plants in the ecosystem.

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