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**Phosphorus availability and fractionation vary among forest site types in
reconstructed oil sands soils**

Mihiri C.W. Manimel Wadu, Min Duan, Scott X. Chang*

442 Earth Sciences Building, Department of Renewable Resources, University of Alberta,
Edmonton, Alberta, Canada T6G 2E3

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*Corresponding author. Tel.: +1 780 492-6375; Fax: +1 780 492-1767

E-mail address: manimelw@ualberta.ca / mihiri.wadu@gmail.com (M. Manimel Wadu),
mduan@snut.edu.cn (M. Duan), Scott.chang@ualberta.ca (S. X. Chang).

Abstract: We investigated relationships between phosphorus (P) availability/fractionation and growth of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) in the Athabasca oil sands region. The study sites had peat-mineral soil mix (PMM) as cover soil over a tailings sand substrate planted to pine or an overburden substrate planted to spruce. Available P was determined using a modified Kelowna extraction and sequentially extracted to obtain exchangeable, Fe- and Al-bound, Ca- and Mg-bound, organic, and residual P. The organic P content in PMM explained 83 and 65% of the variations in available P in the pine and spruce sites, respectively. Phosphorus bound to Fe and Al in PMM was also positively related to available P on pine sites ($p < 0.001$) but not on spruce sites. Available P in PMM was correlated with aboveground biomass increment of spruce ($p < 0.05$) and explained 78% of the variation in foliar P. Foliar P in both pine and spruce was lower than the adequate level for tree growth. Low foliar P and available P in PMM affect the successful establishment of pine and spruce trees on the reclaimed sites and proper P nutrition management is important to overcome P limitation in reclaimed soils.

Keywords: phosphorus availability, reconstructed soils, tree growth, forest productivity, phosphorus fractionation

1. Introduction

The Athabasca oil sands region (AOSR) in Alberta, Canada is the world's second largest reserve of recoverable oil (Humphries 2008). Reclamation of open-pit mines is a land rebuilding process aimed at creating ecosystems with land capability equivalent to or better than that existed pre-disturbance. Surface mining in the AOSR removes vegetation, surface and subsurface materials and therefore, the initial step in land reclamation is reconstructing the soil. Reconstructed soils usually have a capping material (cover soil) consisting of organic-rich soil that is placed over substrates (i.e., subsoil) such as tailings sand (TS) or overburden (OB) materials (Rowland et al. 2009). In past reclamation activities, peat-mineral soil mix (PMM) has commonly been used as the capping material. The PMM is a mixture of salvaged peat and surface mineral soils, while the TS is a by-product of the process that extracts bitumen from the oil sand, and all material that overlies the economically mineable oil sands is referred to as OB.

Re-establishment of nutrient cycling, soil development, and vegetation growth play a critical role in restoring ecosystem functions on reconstructed soils (Macdonald et al. 2012), but properties of different substrates affect nutrient availability and root development in reconstructed soils in the AOSR (Jung et al. 2014; Jamro et al. 2015). Research has revealed that growth-limiting factors vary among different reclamation prescriptions and tree species growing on those sites; the growth of lodgepole pine (*Pinus contorta* var. *latifolia*) on PMM over TS was mainly affected by water availability while nitrogen (N) availability, salinity and soil compaction affected the growth of white spruce (*Picea glauca* (Moench) Voss) on PMM over OB in reconstructed soils (Duan et al. 2015; Duan and Chang 2015). Moreover, changes in water and N availabilities cause variations in fine root biomass in TS profiles, while salinity and soil compaction affect fine root biomass in OB profiles (Jung et al. 2014; Jamro et al. 2015).

Increased soil pH (Howat 2000) and high concentration of residual bitumen (Fung and Macyk 2000) can also negatively affect the growth of trees on reconstructed soils.

Phosphorus (P) as an essential macronutrient required by plants can be a limiting nutrient in addition to N (Portsmuth et al. 2005; Nordin et al. 2001); however, few have studied the implication of P availability for the establishment of vegetation on reclaimed sites. Oil sands sites reclaimed with a peat-mineral capping soil require application of fertilizer P to provide an early boost to ecosystem development (Rowland et al. 2009). Moreover, studies on germination and initial growth of trembling aspen (*Populus tremuloides*) on contrasting oil sands reclamation soils showed a positive relationship between P fertilization and tree growth (Pinno et al. 2012). Available soil P varied with time in reclaimed plots with a peak at year 5 and then declined to year 20 and available soil P in mature natural stands was approximately three times greater than that in reclaimed stands at year 20 (Pinno and Hawkes 2015). In reclaimed soils with TS comprising half or more of the soil profile to 1 m, available P was significantly less than that in the coarse natural analogues in the AOSR (Lanoue 2003). Low available P in reclaimed soils suggests the necessity for conducting further research to identify factors controlling P availability in these soils since low available P in soils can pose a long-term risk on ecosystem development.

Phosphorus occurs in soil in a wide range of organic and inorganic compounds which differ in their biological availability in the soil (Moazed et al. 2010) and the distribution of P within chemically defined pools will ultimately determine the bioavailability, mobility and mineralization of P in the soil (Dick and Tabatabai 1978; Liu et al. 2014). For instance, P fractions in soil may be characterized as the loosely adsorbed P, metal oxide bound P (i.e., mainly of Fe and Al), Ca bound P, and organic P that can be released through mineralization

processes (Hedley et al. 1982). Most of the P in peat is bound to organic matter and as occluded Fe, Al, and Ca phosphate (e.g., Nieminen and Jarva 1996; Nieminen and Penttilä 2004). Both Fe-bound inorganic and organic P were also correlated with the stand volume growth on some drained peat sites (Nieminen and Penttilä 2004). As such, soil P availability is extremely complex and needs to be systematically evaluated due to the dynamic transformations of P between different pools; a comprehensive understanding of tree nutrition is not possible without a better characterization of P in soils.

Considerable attention has been given to investigate P availability and fractionation in reclaimed mine sites in different parts of the world. Phosphorus was a limiting factor in restoring soil fertility on a newly reclaimed land after coal mining in China and dicalcium phosphate was identified as the main source of variation in the capacity of soil to supply P (Chen et al. 1998). Some post-mining soils in northeast Germany were identified as P-deficient and less P saturation in soils was coupled with rather small contents of oxalate extractable iron and aluminum (Slazak and Freese 2015). In some reclaimed mine sites in the Appalachian region, plant-available P (i.e., P measured using bicarbonate extraction) was 32% lower than in a managed forest (Simmons and Currie 2005). These studies indicate that P limitation in reclaimed soils is related to many different factors. However, P fractionation in reconstructed soils in the AOSR has not been studied to the best of our knowledge. Therefore, there is a need to determine the differences in P forms and P availability in soils constructed with different reclamation prescriptions in the AOSR.

Changes in soil physical and chemical properties after reclamation would influence the transformation of P and the distribution of P forms in the cover soil and substrate layers. Organically bound P dominates the soil P pool in the humus layer and mineralization of P in the

organic form is thus a major pathway for the release of plant-available P (Wood et al. 1984; Cade-Menun et al. 2000). Phosphorus fixation is generally restricted to the mineral horizon due to the accumulation of Al and Fe in this horizon (Wood et al. 1984; Li et al. 1999) and P fixation in soil is also dependent on soil pH (Sample et al. 1980). The ratio of peat to mineral soil in the PMM cover soil ranges from pure peat to 60:40 depending on the site being reclaimed in the AOSR (Pinno and Hawkes 2015). Thus, the proportions of organic and inorganic P in PMM may vary depending on the composition of PMM. Salinity and sodicity can also affect P forms and P dynamics in reclaimed saline marsh soils and short-term P sorption by these soils was related to poorly crystalline Fe oxides, silicate clays, and Ca activity in the soil solution (Domínguez et al. 2001). Also, varying concentrations of Ca, Mg and Al in reconstructed soils (Jung et al. 2014) may determine the extent of P precipitation in PMM and substrate layers depending on the pH of different materials.

The goal of this study was to improve our understanding of P availability and forms in reconstructed soils and their relationship with foliar P concentration and tree growth of lodgepole pine and white spruce planted on PMM over TS and PMM over OB, respectively. We hypothesized that P availability varies among different reclamation prescriptions due to varying soil properties associated with PMM, TS and OB, and that P fractionation would provide a comprehensive understanding of P availability in different reclamation materials. We further hypothesized that P availability in reconstructed soils is related to foliar P and ratios between foliar P and other nutrients to the extent that the growth of pine and spruce is affected. This study will provide information related to P fertility in reconstructed soils that can help improve the re-establishment of healthy ecosystems on reclaimed lands in the AOSR.

2. Materials and Methods

2.1. Site description

Study sites were located 22 km north of Fort McMurray (56°59 'N and 111°32 'W) in northeastern Alberta, Canada. The study area was reclaimed from open-pit mining to upland forests and was characterized by a continental boreal climate having short, cool summers and long, cold winters. The mean annual temperature from 1971 to 2000 was 0.7 °C with mean daily temperatures ranging from -18.8 °C (January) to 16.8 °C (July). The mean annual precipitation for the same period was 456 mm with 342 mm occurring during the growing season (Environment Canada 2013).

The open-pit mine sites were re-constructed using PMM as the cover soil above reclamation substrates including TS and OB materials. Pine and spruce were planted on TS and OB sites, respectively. Details on site characteristics and understory plant communities are given elsewhere (Jung et al. 2014; Duan et al. 2015). Pine in different stands was planted in during 1991-1996, while spruce was planted during 1982-1992.

2.2. Experimental design and soil sampling

A total of eighteen sites, with nine pine sites and nine spruce sites were set up and soil samples were collected from PMM and the substrate in each site in July 2012. The top 20 cm of PMM and the upper 20 cm of substrates were collected using an auger in five randomly selected locations within each plot to obtain a composite PMM or substrate sample. The thickness of the PMM layer in pine and spruce sites ranged from 12 to 30 cm and 11 to 48 cm, respectively (Duan et al. 2015). When the thickness of the PMM was less than 20 cm, the entire

PMM layer was sampled. Each soil sample was placed in a plastic bag immediately after sampling, stored in a cooler and taken back to the laboratory. Upon returning to the laboratory, a subsample of each soil sample was air-dried for chemical and physical analyses.

2.3. Measurements of basic soil properties

The gravimetric water content of each soil sample was measured after oven-drying a subsample at 105 °C for 24 h. Soil texture was determined using the hydrometer method (Gee and Or 2002). Soil pH was measured in deionized water using a digital DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) with a soil to water ratio of 1:2 (w:v). Mehlich III extraction (Mehlich 1984) of soil samples was conducted as a measure of extractable cations namely, Ca^{2+} , Mg^{2+} , Fe^{3+} and Al^{3+} , which will be denoted as Ca_M , Mg_M , Fe_M , and Al_M hereafter. A 2.5 g of soil was placed in a centrifuged tube and 25 mL of Mehlich III solution (0.2 mol L⁻¹ CH₃COOH, 0.25 mol L⁻¹ NH₄NO₃, 0.015 mol L⁻¹ NH₄F, 0.013 mol L⁻¹ HNO₃, 0.001 mol L⁻¹ EDTA) was added. The mixture was then shaken for 5 min on a mechanical shaker at 120 strokes per min and the extract was filtered using a Whatman No. 40 filter paper. Cation concentrations were measured using PerkinElmer Optima 3000 DV inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Shelton, CT). Total C and N concentrations of air-dried soil samples were measured with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy) at the Lethbridge Research Centre of Agriculture and Agri-Food Canada (Duan et al. 2015).

2.4. Soil P extraction and fractionation

Plant available P in soil samples was determined by the modified Kelowna method (Qian et al. 1994), which is the most appropriate extractant for the determination of available P in natural and reclaimed soils in the AOSR (Lanoue 2003). The extraction was done by shaking 2.5 g of soil with 25 mL of the Kelowna reagent ($0.015 \text{ mol L}^{-1} \text{ NH}_4\text{F}$, $0.25 \text{ mol L}^{-1} \text{ CH}_3\text{COONH}_4$, and $0.25 \text{ mol L}^{-1} \text{ CH}_3\text{COOH}$) for 15 min on a mechanical shaker. The suspension was filtered and P in solution was determined colorimetrically by the molybdate-ascorbic acid method (Murphy and Riley 1962) using a scanning spectrophotometer (GENESYSTM 10 Series) at a wavelength of 882 nm. As an estimation of the P adsorption maxima (P_{150}), a single point adsorption experiment was conducted at 150 mg P L^{-1} concentration (Ige et al. 2005). Two grams of air-dried soil were weighed into a 50 mL centrifuge tube and 20 mL of P solution in $0.01 \text{ mol L}^{-1} \text{ KCl}$ was added. Two drops of toluene were added to inhibit microbial activity and the suspension was shaken for 24 hours on a mechanical shaker at room temperature and then centrifuged at $3000 \times g$ for 10 min and filtered through a $0.45 \mu\text{m}$ syringe filter. Phosphorus concentrations in the solution were determined colorimetrically using the scanning spectrophotometer at a wavelength of 882 nm.

Sequential extraction of P was conducted according to the modified method of Nair et al. (1995) to distinguish five forms of P, with the extractants listed in parentheses: exchangeable ($1 \text{ mol L}^{-1} \text{ NH}_4\text{Cl}$), Fe- and Al-bound ($0.1 \text{ mol L}^{-1} \text{ NaOH}$), Ca- and Mg-bound ($0.5 \text{ mol L}^{-1} \text{ HCl}$), organic (see below for definition), and residual P. Phosphorus fractions extracted by different extractants will be abbreviated as follows: Ex-P, exchangeable or easily removable P; Fe/Al-P, P associated with Fe and Al; Org-P, organically bound P; Ca/Mg-P, Ca and Mg associated P. Inorganic P in various soil extracts and digests was determined colorimetrically using the molybdate- ascorbic acid method. The difference between the total amount of P in the digested

NaOH extract and the amount of inorganic P detected in undigested NaOH extract was taken as the amount of organic P present. Total P in the soil was determined after Kjeldahl digestion (HClO_3 and HNO_3 acid digestion) of soil samples (Sparks et al. 1996) and P was measured colorimetrically using the molybdate-ascorbic acid method. The sum of Ex-P, Fe/Al-P, Org-P and Ca/Mg-P will be referred to as the total extractable P fraction. Residual P was calculated as the difference between total P and the total extractable P determined by the fractionation method.

2.5. Measurement of tree growth parameters and foliar P analysis

On each site, a 10 x 10 m plot was set up for soil and plant sampling and measurement of tree growth. Tree size was measured in September 2011 and 2012 for twelve sites and the rest in May and September 2012 assuming that there was minimal growth during the winter season. Tree height and diameter at breast height (1.3 m above the ground) of all trees within each plot were measured using a height pole and a diameter tape, respectively. Aboveground biomass of trees of each site was calculated using the diameter at breast height and height-based allometric equations (Lambert et al. 2005; Ung et al. 2008). The difference of aboveground biomass of trees between two measurement intervals was calculated as the current annual increment of aboveground biomass (ABI). In addition, annual aboveground biomass increment based on tree age and the growth measured over one year (current year) were significantly correlated ($R^2 = 0.55$; $p < 0.05$), Supplementary Figure S1). But only the ABI based on the current year measurements was considered as the most accurate measure of current stand growth in young stands. In October 2012, five trees were randomly selected in each plot, and one branch from the upper one-third of the live crown of each tree was collected to form a composite branch

sample for each plot. Branch samples were placed in paper bags and taken back to the laboratory. The foliage from each branch was separated into current-year and 1-year-old needles. After washing twice with distilled water, needles were oven-dried at 65 °C for 24 h or until constant weight. Three hundred needles were randomly selected and weighed to determine the unit needle weight. The sample was subsequently ground to pass through a 0.15 mm sieve in preparation for nutrient analyses. The total P and micronutrients (Fe, Mn, Zn, Cu and B) in needles were analyzed by ICP-MS after digestion using concentrated HNO₃ and 30% H₂O₂ on a digestion block at 125 °C for 4 h (Campbell and Plank 1998). Concentration of total N in needles was determined with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy).

2.6. Statistical analysis

All statistical analyses of soil and plant measurements were performed by tree species since the two species were planted on different substrates and the term ‘site type’ will be used to indicate the specific tree species and substrate combinations hereafter. As such, the purpose of the following statistical analyses was to identify relationships within a particular site type but not to compare between site types. Pearson correlation analyses between foliar P concentration, soil properties, and P fractions in PMM were performed. The absolute concentrations of soil P fractions (mg kg⁻¹) were included in the correlation analysis. Linear regression analysis was conducted between available soil P, soil P fractions and foliar P for both pine and spruce. An α value of 0.05 was chosen to indicate statistical significance in all analyses except for the correlation analysis between ABI and soil and foliar parameters for spruce sites where an α value of 0.10 was considered. The large α value of 0.1 was selected to identify any possible

relationship between ABI and soil and foliar nutrient parameters in spruce sites. Tree establishment at different times has caused a large variability in ABI data and detecting significant correlations between ABI and other parameters were not possible at a lower α value. All statistical analyses were conducted using the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

3. Results

3.1. Soil properties, available P and P fractionation in pine sites

Large differences in soil properties were found between PMM and TS with regards to pH, organic matter content, clay content and extractable cations (Table 1). The highest available P was found in the PMM layer (1.9 mg kg^{-1}) in the pine sites which was more than 4-fold greater than that in the TS (Table 1). Around 30 and 50% of total P was extracted during the sequential P fractionation in PMM and TS, respectively (Fig. 1a). High concentrations of Org-P and Fe/Al-P fractions were found in both PMM and TS (Fig. 1b). Although the largest Org-P fraction (%) among all samples was found in TS (Fig. 1a), the Org-P concentration was about 4 and 16 mg kg^{-1} in TS and PMM, respectively (Fig. 1b).

In PMM, Ex-P, Fe/Al-P, and Org-P were all positively correlated with available P (Table 2). A significant correlation was found only between Fe/Al-P and Org-P among all the P fractions measured in PMM in pine sites. Both Mehlich extractable Ca and Fe were related to P sorption in PMM of pine sites as shown by the positive correlations between P_{150} and Ca_M and Fe_M . Mehlich extractable Al was significantly correlated with Org-P and available P, while Fe_M was negatively correlated with available P. Both Org-P and Fe/Al-P were negatively correlated

with pH in PMM (Table 2). Moreover, Org-P and Fe/Al-P explained 83 and 86%, respectively, of the variation in available P in PMM (Fig. 2).

3.2 Soil properties, available P and P fractionation in spruce sites

Among soil parameters only bulk density, EC, organic matter content and Al_M were different between PMM and OB (Table 1). Both available P and total P concentrations were not significantly different between PMM and OB. Total extractable P in OB was twice as much as that in PMM (Fig. 1a). A characteristic feature is the highest Ca/Mg-P concentration in OB as compared to PMM and the greater Org-P concentration in PMM as compared to OB (Fig. 1b).

Among different P fractions, only Ex-P and Fe/Al-P in PMM were positively correlated (Table 2). Available P and Org-P, Mg_M , Al_M , Fe_M , P_{150} and organic matter content in PMM were significantly correlated. Both Mg_M and Fe_M were positively correlated with Org-P but not with Al_M in PMM. In contrast to the pine sites, a positive correlation between available P and P_{150} in PMM was noted for spruce sites. In PMM, Org-P explained 65% of the variation in available P although Fe/Al-P did not have a significant relationship with available P in PMM (Fig. 2).

3.3. Relationships between available P, foliar P and tree growth in pine sites

Foliar P concentration in pine varied in a narrow range from 1.1 to 1.3 mg kg⁻¹ with a foliar N:P ratio of 12 ± 0.7 (Table 1). Foliar P concentrations in current and one-year-old needles were not significantly different (Fig. 3a). There was no significant relationship between ABI and available P in either PMM or TS in the pine sites (Supplementary Table S3). However,

foliar P in current-year needles was related to soil available P in PMM in the pine sites (Fig. 3b), with foliar P concentration showed a tendency to decrease when available P was greater than $\sim 3.5 \text{ mg kg}^{-1}$ in PMM. Among different P fractions, only Ca/Mg-P and ABI were strongly correlated in the pine sites ($r = -0.67$, $p < 0.05$).

3.4. Relationships between available P, foliar P and tree growth in spruce sites

Foliar P concentration in the current-year needles of spruce varied from 0.9 to 1.9 mg kg^{-1} , and the foliar N:P ratio was 9 ± 2.2 (Table 1). The available P in OB was not related with either foliar P or ABI (Supplementary Table S4). Available P in PMM in spruce sites explained 78% of the variation in current-year foliar P concentration (Fig. 3b). In contrast with the pine sites, there was a significant correlation between ABI and foliar P concentration in current-year needles (Table 3). Also, the ratios between P and Fe, Mn and Zn in spruce needles were positively and significantly correlated with ABI (Table 3), but none of the P fractions was correlated with ABI.

4. Discussion

4.1. P availability and fractionation varied in pine and spruce sites

The detailed analysis of different soil P forms provides insights into P availability in reconstructed soils. The difference in P fractions between the PMM and substrates in this study supports the hypothesis that varying soil properties caused differences in P fractionation and availability among PMM, TS, and OB. Since Org-P and Fe/Al-P were related to P availability in the studied soils (Fig. 2), the pathways for supplying P into the available P pool can be Org-P

mineralization (Adams and Pate 1992), dissolution of inorganic Fe/Al-P, and dissolution of amorphous Al- and Fe-organic matter coatings that contain occluded P (Fox and Comerford 1990). Relationships between available P and Org-P in PMM (Fig. 2) in both site types were different despite having similar Org-P concentrations (Fig 1a) due to the varying degrees of P retention by the Org-P (Table 2).

The weak relationship between available P and Fe/Al-P in the spruce compared to the pine sites is not surprising since different Fe/Al oxides (i.e., amorphous and crystalline Fe/Al oxides) (Mahieu et al. 2000) exhibit varying P adsorption capacities (Arai and Livi 2005; Arai et al. 2013). Negatively charged P compounds attach themselves to hydrous Fe or Al oxides (Turner et al. 2005) and thereby could turn a large proportion of soil P into unavailable forms (Arai and Livi 2013; Solomon and Lehmann 2000). On the other hand, the presence of organic acids can result in the desorption of surface complexed P (Fox 1995; Jones and Darrah 1994), enhancing the availability of P in Fe/Al-P fraction as in the pine sites (Fig. 2). Formation of sparingly soluble Ca/Mg-P reduces P availability when the Ca concentration and pH is high in soils (Sample et al. 1980), similar to that in most pine and spruce sites (Table 1). Surprisingly, a direct relationship between Ca/Mg-P and available P was not found in this study.

The Org-P mineralization may be important phenomena in pine and spruce sites due to the marked influence of Org-P on available P. Although the total P in PMM is high (Table 1), mineralization into the available form was small as evident by the large differences between Org-P and available P in PMM (Table 1, Fig. 2). The mineralization of Org-P can be influenced by the different C:P and C:N ratios in PMM in the two site types (Table 1). A greater immobilization of P results in lower available P in sites with a high C:P ratio, which ranges from 112 to 501 (Stevenson 1986; Enwezor 1976). The ratio between total N and P in PMM

was about four- or five-fold higher than that in tree foliage in some spruce sites (Supplementary Figure S2) and when N and P are released by microbial mineralization (Alexander 1967), it often results in a shortage of available P relative to the available N leading to P limitation for tree growth.

4.2. Low foliar P in pine and spruce and the need for P fertilization in reclaimed sites

Foliar P concentration was lower than the adequate level needed for lodgepole pine (adequate when foliar P $>1.5 \text{ mg g}^{-1}$; Carter 1992) but none of the pine sites had a foliar P level (Fig. 3a) that indicates severe P deficiency ($<0.9 \text{ mg g}^{-1}$; Carter 1992) when compared with the thresholds that establish P deficiency. The foliar N:P ratio of pine sites was greater than the threshold value of 11 indicating slight to moderate P deficiency (Brockley 2001) where foliar N concentration ($>13.5 \text{ mg kg}^{-1}$) was greater than the deficiency level for pine (Brockley 2001). Therefore, the foliar P and N:P ratio corroborate with each other to indicate moderate P deficiency in the pine sites. Pine is less sensitive to soil nutrient deficiencies than spruce (Périé and Munson 2000; Bothwell et al. 2001) and often succeed in nutrient-poor soils (Weetman et al. 1985). Therefore, the absence of a relationship between foliar P and ABI in pine is possible even with low foliar P and the imbalance between foliar P and N concentrations (Supplementary Table S3).

Foliar P in pine needles did not increase with increasing available P in PMM (Fig. 3b), suggesting that other factors control foliar P in pine sites. Nutrient supply for trees is not only determined by the concentrations of available nutrients in the soil but also by the nutrient uptake kinetics of their (mycorrhizal) roots (Bassirirad et al. 1999). Phosphorus concentrations decreased in above- and belowground tissues in trees under low water availability in the soil

(Peuke and Rennenberg 2004). Therefore, low water availability in pine sites (Duan et al. 2015) may be the reason for low foliar P concentration when the concentration of soil available P was high (Fig. 3b). Further research is needed to investigate underlying mechanisms for low foliar P in pine regardless the high available P in the soil. Although very low P concentration in TS can reduce the capacity of the soil to supply P for pine growth (Fig. 1), neither available P nor P fractions in TS were significantly correlated with ABI (Supplementary Table S3).

The foliar P concentration in spruce was also lower than the adequate P concentration (adequate when foliar P $>1.6 \text{ mg g}^{-1}$; Carter 1992) for tree growth. Most of the sites (Fig. 3a) showed some level of P deficiency when compared with the thresholds established in the literature (Carter 1992) and the growth of spruce was significantly related to available P in PMM (Table 3). The foliar N:P ratio of spruce was below the critical value of 11 reported for trees in natural upland ecosystems (Tessier and Raynal 2003). The low foliar N concentration (i.e., $<14.5 \text{ mg g}^{-1}$, Carter 1992) in spruce indicates that both N and P limitations had caused the low foliar N:P ratio. In addition, foliar P:Fe, P:Mn and P:Zn ratios were significantly and positively related with ABI (Table 3). Since foliar Fe, Mn and Zn concentrations (Table 1) in spruce were present at adequate levels (Carter 1992), the low nutrient ratios was caused by low foliar P concentrations causing adverse effects on ABI. Similar to our result, strong relationships between available P and site productivity (based on site index) of white spruce have been reported in other studies (Harding 1985; Wang 1995).

Increasing foliar P is beneficial for improving the balance between P and micronutrients in spruce trees and white spruce is generally a more nutrient-demanding coniferous species that requires a higher concentration of P than other boreal coniferous species (Wilde 1966), including pine species (Ingstad 1979). Improving P fertility would be essential for enhancing

the long-term productivity of spruce stands and nutrient content in current-year needles of spruce was sensitive to fertilization (Munson et al. 1994). Thus fertilizer trials to determine P application rate to ensure adequate supply of P and other nutrients in spruce sites with OB substrate are essential to achieve sustainable land reclamation. Attention should also be paid to the need for long-term P fertility management in spruce sites by monitoring available P in soil and foliar P levels after fertilizer application to determine the effectiveness of P application. Additional research is clearly needed to further elucidate the mechanisms controlling P uptake in pine sites in light of the low foliar P measured in those sites with high available P in PMM.

5. Conclusions

Both the lodgepole pine and white spruce stands we studied had below optimum levels of foliar P concentrations, with foliar N and P ratios imbalanced in pine trees. Our results demonstrated that there was no relationship between foliar P and tree growth rate in the pine sites and the main limitation was with other growth factors such as water availability while P was a limitation for the growth of trees on the spruce sites. Phosphorus availability varied between the two site types owing to the variations in P fractionations. Low P availability is a risk for long-term ecosystem development on some reconstructed sites in the AOSR and could especially be a concern with planting nutrient demanding species such as white spruce on such reclaimed soils. Phosphorus fertilization would be required to achieve better growth performance in newly established ecosystems. Also, regular monitoring of soil P availability in soil and foliar P levels is essential due to the potential for some reclamation materials to lower the availability of P after it has been applied to reclaimed sites. While a direct comparison between the two site types is not possible due to the confounding of tree species and substrate

type effects, further research is needed to separate the tree species vs. soil substrate effect on soil P availability and tree growth to improve the management of reconstructed soils in the oil sands.

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- 586

Table 1. Tree growth, current-year foliar nutrient concentrations and soil properties (means with SD in parentheses) of lodgepole pine and white spruce sites selected for this study in the Athabasca oil sands region, Alberta.

Parameter	Pine site		Spruce site	
Above ground biomass increment ($\text{Mg ha}^{-1} \text{ yr}^{-1}$)	4.0 (1.1)		2.8 (1.0)	
Foliar P (mg g^{-1})	1.2 (0.1)		1.2 (0.2)	
Foliar N (mg g^{-1})	14.2 (0.9)		11.1 (2.6)	
Foliar N:P ratio	12.0 (0.7)		9.0 (2.2)	
Foliar Fe (mg g^{-1})	0.12 (0.04)		0.11 (0.001)	
Foliar Mn (mg g^{-1})	0.07 (0.002)		0.05 (0.04)	
Foliar Zn (mg g^{-1})	0.06 (0.002)		0.07 (0.01)	
	Pine site		Spruce site	
	Peat-mineral soil mix	Tailings sand	Peat-mineral soil mix	Overburden
pH (1:2 w:v)	7.6 (0.4)	6.7 (0.8)	7.8 (0.4)	7.6 (0.2)
Bulk density (Mg m^{-3})	0.9 (0.3)	1.4 (0.2)	0.9 (0.3)b	1.4 (0.2)a
Organic matter (%)	10.7 (3.5)a ^a	0.4 (0.1)b	11.9 (6.5)a	6.8 (0.9)b
Clay content (g kg^{-1})	230 (41)	< DL ^b	300 (18)	262 (43)
Electrical conductivity (dS m^{-1})	1.0 (0.2)	0.2 (0.01)	3.9 (3.5)a	0.5 (0.2)b
Available P (mg kg^{-1})	1.9 (0.7)a	0.4 (0.1)b	0.8 (0.4)	0.5 (0.2)
Total P (g kg^{-1})	0.2 (0.6)a	0.02 (0.004)b	0.18 (0.1)	0.17 (0.05)
Total N (g kg^{-1})	2.5 (1.2)a	0.02 (0.01)b	3.9 (2.1)a	0.5 (0.2)b
Total C (g kg^{-1})	70.3 (39.1)a	1.8 (0.7)b	87.7 (67.7)	39.4 (5.4)
N:P ratio	10.5 (3.1)a	1.4 (0.9)b	26.2 (21.1)	3.29 (1.5)
C:P ratio	302.7 (132.3)a	113.3 (38.1)b	586.1 (302.1)	255.7 (65.5)
C:N ratio	28.1 (8.1)b	100.4 (77.3)a	31.1 (24.2)	87.2 (27.3)
Ca _M ^c (mg kg^{-1})	253.1 (20.8)a	11.6 (4.0)b	519.6 (237.8)	218.1 (25.1)
Mg _M ^c (mg kg^{-1})	48.5 (7.0)a	2.7 (0.7)b	58.6 (15.5)	47.4 (13.5)
Al _M ^c (mg kg^{-1})	3.9 (3.7)a	1.1 (0.1)b	0.7 (0.4)b	3.1 (2.0)a
Fe _M ^c (mg kg^{-1})	26.3 (3.8)a	6.4 (1.3)b	22.5 (17.5)	20.0 (4.7)

a

Different letters indicate significant differences in soil properties between PMM and substrate within each site type at $p < 0.05$ ($n = 6$)

^b DL stands for detection limit.

^c Ca_M, Mg_M, Al_M and Fe_M indicate Mehlich-3 extractable Ca, Mg, Al and Fe, respectively.

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Table 2. Pearson correlation coefficients and significance levels for correlations among foliar P concentration, P fractions and soil properties of peat-mineral soil mix in lodgepole pine and white spruce sites.

Variable	Available P	Foliar P ^a	Ex-P ^b	Fe/Al-P ^b	Org-P ^b	Ca/Mg-P ^b	P ₁₅₀ ^c	OM ^d	Mg _M ^e	Al _M	Ca _M	Fe _M
	(mg kg ⁻¹)	(mg g ⁻¹)	(mg kg ⁻¹)					%	(μg g ⁻¹)			
Lodgepole pine												
Foliar P	0.16											
Ex-P	0.73^{*/f}	0.46										
Fe/Al-P	0.93^{***}	-0.14	0.52									
Org-P	0.91^{***}	0.13	0.53	0.87^{**}								
Ca/Mg-P	-0.39	-0.09	-0.22	-0.32	-0.26							
P ₁₅₀	-0.41	0.24	-0.28	-0.39	-0.37	-0.19						
OM	0.24	0.83^{**}	0.31	0.02	0.16	0.05	0.43					
Mg _M	-0.04	0.90^{**}	0.36	-0.35	-0.02	-0.21	0.43	0.63				
Al _M	0.90^{**}	-0.15	0.48	0.98^{***}	0.85^{**}	-0.28	-0.62	0.01	-0.32			
Ca _M	-0.38	0.70[*]	0.04	-0.68[*]	-0.40	-0.03	0.83^{**}	0.51	0.83^{**}	-0.70[*]		
Fe _M	-0.68[*]	-0.12	-0.78[*]	-0.42	-0.35	-0.09	0.73^{**}	-0.14	0.01	-0.65[*]	0.36	
pH	-0.88^{**}	0.05	-0.41	-0.95^{***}	-0.92^{***}	0.33	0.54	-0.08	0.26	-0.96^{***}	0.66	0.58
White spruce												
Foliar P	0.88^{**}											
Ex-P	0.21	0.48										
Fe/Al-P	0.52	0.74[*]	0.84[*]									
Org-P	0.81^{**}	0.13	0.41	0.54								
Ca/Mg-P	-0.25	-0.14	0.37	0.28	0.10							
P ₁₅₀	0.69[*]	0.55	0.41	0.32	0.68[*]	-0.34						
OM	0.93^{**}	-0.10	-0.09	0.21	0.72[*]	-0.40	0.55					
Mg _M	0.94^{**}	0.32	0.13	0.32	0.72[*]	-0.43	0.81^{**}	0.85^{**}				
Al _M	0.83^{**}	0.03	-0.01	0.39	0.57	-0.09	0.32	0.75[*]	0.78^{**}			
Ca _M	-0.09	0.32	0.10	0.18	-0.31	-0.58	0.37	-0.13	0.10	-0.38		
Fe _M	0.71[*]	-0.10	0.01	0.29	0.67[*]	0.03	0.47	0.68[*]	0.72[*]	0.79[*]	-0.46	
pH	-0.82[*]	0.18	0.12	-0.06	-0.62	0.46	-0.44	0.89^{**}	-0.70[*]	-0.54	0.11	-0.47

^a Foliar P concentration in current-year needles.

^b Ex-P, Fe/Al-P, Org-P and Ca/Mg-P represent exchangeable P, P associated with Fe and Al, organically bound P and P associated with Ca and Mg, respectively.

^c P₁₅₀ represents the adsorption maxima determined using a one-point P sorption isotherm conducted at 150 mg P L⁻¹.

^d OM stands for organic matter content.

^e Cations with subscript M denote Mehlich III extractable cations.^f *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

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Table 3. Pearson correlation coefficients between current above ground biomass increment and soil and current-year foliar P data of white spruce sites. (*p* values are given in parentheses).

	Available P (mg kg ⁻¹)	Soil C:P	Foliar P (mg g ⁻¹)	Foliar P:Fe	Foliar P:Mn	Foliar P:Zn
Aboveground biomass increment (Mg ha ⁻¹ yr ⁻¹)	0.71 (0.03)	0.65 (0.05)	0.59 (0.06)	0.77 (0.01)	0.84 (0.01)	0.78 (0.01)

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Fig. 1. Distribution of various forms of P (a) expressed as a percent of the total P and (b) absolute concentration of P forms in peat-mineral soil mix and substrate in lodgepole pine and white spruce sites. Ex-P, exchangeable P; Fe/Al-P, P associated with Fe and Al oxides; Org-P, organically bound P; Ca/Mg-P, Ca and Mg-associated P. Vertical error bars in (b) indicate standard deviations and different letters indicate significant differences among the concentration of P forms in each soil layer at $p < 0.05$.

Fig. 2. Relationships between available P and organic or Fe and Al bound P fractions in peat-mineral soil mix in lodgepole pine and white spruce sites. The asterisks indicate the significance of the regression lines, with ** and *** indicating $p < 0.01$ and $p < 0.001$, respectively.

Fig. 3. Relationship between (a) foliar N and P concentrations in current-year and one-year-old needles in lodgepole pine and white spruce, and (b) available P concentration in PMM and foliar P concentration in current-year needles in lodgepole pine and white spruce sites. The asterisks indicate the significance of the regression lines at $p < 0.01$

Phosphorus availability and fractionation vary among forest site types in reconstructed oil sands soils

Mihiri C.W. Manimel Wadu, Min Duan, Scott X. Chang

Supplementary Materials

S1. Relationship between increment of aboveground biomass based on one year growth measurement and mean annual increment based on total biomass and tree age for lodgepole pine and white spruce.

S2. Relationship between foliar and soil N:P ratios of lodgepole pine and white spruce sites.

S3. Pearson correlation coefficients among aboveground biomass increment, foliar P concentration (current-year needle), available P and P fractions in tailings sand in lodgepole pine sites. Bold values are p values.

Tailings sand							
	Foliar P	ABI ^a	Ex-P ^b	Al/Fe-P ^b	Org-P ^b	Ca/Mg-P ^b	Total P
Available P	0.03	-0.28	-0.03	0.31	-0.13	0.4	0.12
	0.93	0.46	0.95	0.41	0.74	0.28	0.75
Foliar P		0.34	-0.02	-0.22	0.56	0.23	0.03
		0.37	0.96	0.56	0.12	0.54	0.93
ABI ^a			0.11	0.05	0.54	-0.3	0.13
			0.77	0.91	0.13	0.43	0.74
Peat-mineral soil mix							
	Foliar P	Available P	Ex-P ^b	Al/Fe-P ^b	Org-P ^b	Ca/Mg-P ^b	Total P
ABI ^a	0.05	0.34	0.23	-0.14	-0.20	-0.67	0.11
	0.09	0.37	0.55	0.71	0.67	0.04	0.79

^a Aboveground biomass increment.

^b Ex-P, Fe/Al-P, Org-P and Ca/Mg-P represent exchangeable P, P associated with Fe and Al, organically bound P, and P associated with Ca and Mg, respectively.

S4. Pearson correlation coefficients among aboveground biomass increment, foliar P concentration (current-year needle), available P and P fractions in overburden in white spruce sites. Bold numbers are *p* values.

	Foliar P	ABI ^a	Ex-P ^b	Al/Fe-P ^b	Org-P ^b	Ca/Mg-P ^c	bTotal P
Available P	0.21 0.58	-0.39 0.30	0.34 0.38	0.69 0.04	0.86 <0.01	0.22 0.58	0.53 0.14
Foliar P		0.60 0.09	0.14 0.71	0.51 0.16	0.40 0.29	0.59 0.10	0.28 0.47
ABI			-0.07 0.86	-0.24 0.54	-0.29 0.46	0.19 0.62	0.00 1.00

^a Aboveground biomass increment.

^b Ex-P, Fe/Al-P, Org-P and Ca/Mg-P represent exchangeable P, P associated with Fe and Al, organically bound P, and P associated with Ca and Mg, respectively.