

# Effect of tree species and substrate properties on organic phosphorus forms in afforested technosols

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## Abstract

Planted vegetation is considered one of the most important soil-forming factors in the reclamation of degraded post-mining lands for forestry. The objective of this study was to compare the effect of N-fixing tree species and non-N-fixing species on the organic phosphorus ( $P_{org}$ ) forms in technosols developing from various substrates. Samples were taken from the uppermost layer (depth 0 – 5 cm) of technosols afforested with black locust ( *Robinia pseudoaccacia* ), black alder ( *Alnus glutinosa* ), silver birch ( *Betula pendula* ) and Scots pine ( *Pinus sylvestris* ). Samples of the tree foliage and the O horizons were taken as well. The studied technosols developed from Quaternary sands (Sands), fly ashes after lignite combustion (Ashes) and Miocene clays (Clays). The soil samples were measured for the contents of labile ( $P_{Olab}$ ) and moderately labile organic phosphorus ( $P_{Omod}$ ), phosphorus contained in fulvic and humic acids ( $P_{fulv}$  and  $P_{hum}$ , respectively) and residual organic phosphorus ( $P_{Ores}$ ). The foliage and O horizon samples were measured for the concentrations of C, N and P. The N-fixing trees had higher P concentration in the foliage than non-N-fixing species. However, in the O horizon the highest P concentration was determined under birch and not under N-fixers. The effect of tree species on the organic P ( $P_{org}$ ) concentrations in the mineral soil was limited with significantly lower  $P_{org}$  concentrations under pine. Soil under this species contained less  $P_{fulv}$ ,  $P_{hum}$  and  $P_{Ores}$ . However, the percentages of  $P_{Olab}$ ,  $P_{Omod}$ ,  $P_{fulv}$ ,  $P_{hum}$  and  $P_{Ores}$  in soil  $P_{org}$  were nearly the same under all tree species. The largest effect on the  $P_{org}$  concentration and on the contents of particular  $P_{org}$  fractions was from the substrate type. Sands contained much less  $P_{org}$  than the Clays and Ashes but their percentage of  $P_{Olab}$  in  $P_{org}$  was much higher than in two other substrates. We conclude that N-fixing trees do not affect the concentration of labile organic P and the major factor controlling this  $P_{org}$  fraction is the soil substrate quality.

## INTRODUCTION

Post-industrial wastelands are often reclaimed for forestry (Hüttl and Weber, 2001; Sheoran et al., 2010; Macdonald et al., 2015; Pietrzykowski, 2019). In this way of reclamation planted trees are to transform the uppermost layer of barrens into functional soils. However, a common feature of the substrates forming the uppermost layer of post-industrial barrens is low concentration of essential nutrients, in particular nitrogen and phosphorus (Zipper et al., 2011; Krzaklewski et al., 2012; Cross et al., 2019; Manimel Wadu et al., 2017). Insufficient nutrient contents in the dumped or excavated materials may hinder growth of the introduced vegetation (Sheoran et al., 2010; Pietrzykowski, 2019). In order to alleviate N deficiency in developing technosols planted are tree species capable of forming a symbiosis with N-fixing bacteria (hereafter referred to as ‘N-fixers’). Owing to the symbiosis with N-fixing bacteria these species may enrich the developing soils in nitrogen (Zipper et al., 2011; Vlachodimos et al., 2013; Bissonette et al., 2014; Pietrzykowski et al., 2015). However, the role of N-fixers in alleviating P deficiency has been less recognized although it is known that certain N-fixing plants can acquire P from compounds that are unavailable for other plant species (Hinsinger, 2001; Hinsinger et al., 2011) and in this way intensify P cycling in the forming soils.

Vegetation is one of the major factors affecting transformations and cycling of soil P (Hinsinger, 2015). Plants

take up phosphorus in mineral forms and deposit P-containing organic residues that become substrates for a variety of soil organic P forms (Hinsinger, 2015). In the uppermost soil horizons the organic P may constitute more than 50% of total P (Sulieman and Mühling, 2021). Organic P is not directly plant available, however there are several mechanisms that enable plants accessing organic P (Richardson et al., 2009). Chodak et al. (2021) reported that N-fixing tree species (black locust and black alder) improved capacity of technosols to release P by increasing the activity of phosphatases and enhancing microbial uptake of P. Organic P in soils occurs in a large variety of forms that differ in chemical composition, reactivity and plant availability (Turner et al., 2005). Recent studies suggest that organic P may be an important source of P for plants (Sulieman and Mühling, 2021). However, the effect of N-fixing plant species on the forms of organic phosphorus in soil is unclear and the published results are inconsistent. Increased contents of available P were reported for some N-fixing tree species and but not for others (Wang et al., 2010; Orczewska et al., 2012). Discrepant results might have been due to differences in the soil properties that obscured the effect of trees.

The objective in this study was to compare the effect of N-fixing tree species (black locust (*Robinia pseudoacacia*) and black alder (*Alnus glutinosa*)) and non-N-fixing species (silver birch (*Betula pendula*) and Scots pine (*Pinus sylvestris*)) on the forms of organic P in the uppermost horizons of reclaimed technosols developing from various parent materials.

## MATERIALS AND METHODS

### Study sites

The study was carried out in forest stands growing on technosols developing from three different types of substrates referred to as Clays, Sands and Ashes. Samples of Clays were taken at the external spoil heaps of the open-pit lignite mine Turów (number of sampling sites,  $n = 12$ ) and the open-pit sulphur mine near Piaseczno ( $n = 12$ ). Clays sampled at Turów and Piaseczno represented acid and alkaline materials, respectively. Sands were sampled in the reclaimed areas of the sand quarry Szczakowa ( $n = 17$ ) and on the external spoil heap of the open-pit lignite mine Bełchatów ( $n = 7$ ) while Ashes were taken at the combustion waste disposal site Lubień ( $n = 24$ ). Geographic localization and basic data on climate, parent material and initial fertilization of the sampling sites are given in Table 1.

### Soil and vegetation sampling

Samples representing the organic horizon (O) and the uppermost mineral soil (0 – 5 cm) were taken in late August and early September 2019 from sampling plots (10×10 m) covered by pure stands of black locust (*Robinia pseudoacacia*), black alder (*Alnus glutinosa*), Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*). The age of forest stands was 18 – 20 years for those growing on Ashes, 30 – 35 years for the stands growing on Sands and 38 – 44 years for the stands growing on Clays. At each sampling site one composite sample was taken from the O horizon and from the mineral soil. Each composite sample consisted of five subsamples (area of each subsample = 1.0 m<sup>2</sup> for the O horizon and 0.16 m<sup>2</sup> for the mineral soil) located at the corners and in the middle of the sampling plot. The samples were sieved (10 mm mesh for the O horizon and 2 mm mesh for the mineral soil). The mineral soil samples were divided into two parts. One part was air-dried and used for physical, physicochemical, and chemical analyses, and the other one was stored field-moist at 4°C and used for organic P fractionation. The samples of O horizon were air-dried and used for chemical analyses.

Leaves and needles were taken from trees growing within the sampling sites in direct vicinity of the O horizon and mineral soil sampling plots following Rautio and Fürst (2013) methodology. Within each sampling site the foliage material was taken from five trees. Leaves and needles were collected from the upper third of the crown with SW exposition. In case of Scots pine current year needles were collected. The collected leaves and needles from each sampling site were combined to produce a composite sample representative for the site. The foliage samples were dried to constant weight at 40 °C, homogenized and analyzed for C and nutrient contents.

Chemical analyses of mineral soils, O horizons and tree foliage

The mineral soil, O horizon and foliage samples were analyzed for total carbon and total nitrogen concentration using LECO TruMac<sup>®</sup> CNS analyzer. The inorganic C content in mineral soil samples was calculated based on the carbonate content measured with the gas-volumetric Scheibler method. The organic C ( $C_{org}$ ) content was then calculated as a difference between the total C and the inorganic C. Total phosphorus ( $P_t$ ) was determined colorimetrically by the molybdate blue method from *aqua regia* extracts by Varian CARY 300 Conc UV-Visible Spectrophotometer.

The texture of mineral soil samples was determined by hydrometer using Bouyoucos method. The pH of the samples was measured in  $H_2O$  ( $pH_{H_2O}$ ) and 1 M KCl solution ( $pH_{KCl}$ ) (soil/liquid ratio 1:5, w/v) at 20°C using a digital pH meter (CPC-411, ELMETRON). Data on the texture and pH of the studied soils are presented in supplementary material (Table S1).

#### Fractionation of organic phosphorus

Organic P fractionation was carried out according to Bowman and Cole (1978) method modified by Ivanoff et al. (1998), and described in Zhang and Kovar (2009) wherein determination of microbial P was excluded from the fractionation scheme. Briefly, 1 g of soil was sequentially extracted with 0.5 M  $NaHCO_3$  at pH = 8.5, 1.0 M HCl, and 0.5 M NaOH. The 0.5 M  $NaHCO_3$  extract contained labile P fraction ( $P_{Olab}$ ) and the 1.0 M HCl extract moderately labile P ( $P_{Omod}$ ). The 0.5 M NaOH extract was acidified to pH = 1.0 to distinguish P contained in fulvic ( $P_{fulv}$ ) and humic acids ( $P_{hum}$ ). The sample residue after sequential extraction was ashed at 550 °C and extracted with concentrated 1M  $H_2SO_4$  to determine residual, non-labile organic P fraction ( $P_{Ores}$ ). The extracts were centrifuged at 4000 rpm for 10 min, filtered and measured for inorganic P (IP) and total P (TP) by the molybdate colorimetric method. Total P (TP) in the extracts was determined after digestion with 2.5 M  $H_2SO_4$  and potassium persulfate. Organic P in the extracts was calculated as the difference between TP and IP.

#### Statistical analysis

Two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test ( $p < 0.05$ ) was used to test for the effect of tree species, and substrate type on the concentrations of organic P forms in the studied technosols. Prior to ANOVA the dependent variables were tested for distribution normality (Shapiro-Wilk test,  $p < 0.05$ ) and homoscedasticity (Levene's test,  $p < 0.05$ ) and transformed (logarithmic, square root or power transformation) if necessary.

All analyses were performed with Statgraphics Centurion XVII software (Statistical Graphics Corporation).

## Results

### Chemical properties of foliage and O horizons

The highest concentration of N was measured in the leaves of black locust (33.7 mg  $g^{-1}$ ), followed by black alder (28.1 mg  $g^{-1}$ ), silver birch (18.6 mg  $g^{-1}$ ) and Scots pine (12.1 mg  $g^{-1}$ ) (Table 2). The leaves of black alder had the highest P concentration (1492  $\mu g g^{-1}$ ) significantly higher than in the leaves of birch (1276  $\mu g g^{-1}$ ) and the Scots pine needles (1009  $\mu g g^{-1}$ ). The P concentration in the foliage of black alder (1401  $\mu g g^{-1}$ ) was significantly higher than in Scots pine needles but not than in the silver birch leaves (Table 2). The leaves of N-fixing species were characterized by significantly lower C-to-N and C-to-P ratios but significantly higher N-to-P ratio than the foliage of non-N-fixing trees. The largest C-to-N and C-to-P ratios and the smallest N-to-P ratio were determined in the Scots pine needles.

The O horizons under both N-fixing species contained more N than the O horizons under both non-N-fixing trees (Table 3). However, this was not the case for P. The highest concentration of P in the O horizons was determined under silver birch (851  $\mu g g^{-1}$ ) followed by black locust (808  $\mu g g^{-1}$ ). The P concentration under alder was significantly lower (747  $\mu g g^{-1}$ ) compared with birch but higher than under pine (649  $\mu g g^{-1}$ ). The C-to-N ratio was significantly lower and the N-to-P ratio significantly higher under N-fixing trees than under both non-N-fixing species. However, the C-to-P ratio was significantly higher under Scots pine and black alder than under birch and black locust.

## C<sub>org</sub>, N<sub>t</sub>, P<sub>t</sub> and P<sub>org</sub> in mineral soil

There were no significant differences in P<sub>t</sub> contents in mineral soil under the studied tree species (Table 4). However, the soil under Scots pine had lower concentration of C<sub>org</sub> (37.7 mg g<sup>-1</sup>); N<sub>t</sub> (1.38 mg g<sup>-1</sup>) and P<sub>org</sub> (149.7 µg g<sup>-1</sup>) than the soils under all remaining tree species (46.1 mg g<sup>-1</sup> – 49.7 mg g<sup>-1</sup> for C<sub>org</sub>, 2.00 mg g<sup>-1</sup> – 2.66 mg g<sup>-1</sup> for N<sub>t</sub> and 206.9 µg g<sup>-1</sup> – 211.9 µg g<sup>-1</sup> for P<sub>org</sub>) (Table 4). Tree species did not affect soil C<sub>org</sub>-to-P<sub>org</sub> ratio. The N<sub>t</sub>-to-P<sub>org</sub> ratio was the largest under black locust (12.7) but the difference was significant only compared with Scots pine (8.5).

The contents of C<sub>org</sub>, N<sub>t</sub>, P<sub>t</sub>, P<sub>org</sub> as well as C<sub>org</sub>-to-P<sub>org</sub> ratio depended on substrate type. The lowest contents of these elements were measured in Sands and the highest in Clays (although for P<sub>t</sub> the difference between Clays and Ashes was not significant). The C<sub>org</sub>-to-P<sub>org</sub> ratio was the lowest in Sands and the largest in Clays (Table 4). However, there was no effect of substrate type on N<sub>t</sub>-to-P<sub>org</sub> ratio.

## Organic P fractions in mineral soil

Tree species did not affect the contents of P<sub>Olab</sub> and P<sub>Omod</sub> in the uppermost layers of the studied soils (Figure 1). However, the tree species effect was observed for the remaining organic P fractions. The lowest contents of P<sub>fulv</sub>, P<sub>hum</sub> and P<sub>Ores</sub> were measured in soils under Scots pine (31.4 µg g<sup>-1</sup>, 19.8 µg g<sup>-1</sup> and 57.4 µg g<sup>-1</sup>, respectively) (Figure 1). The soils under the remaining tree species did not differ in terms of P<sub>fulv</sub> (39.5 µg g<sup>-1</sup> – 40.5 µg g<sup>-1</sup>), P<sub>hum</sub> (30.7 µg g<sup>-1</sup> – 41.5 µg g<sup>-1</sup>) and P<sub>Ores</sub> (76.9 µg g<sup>-1</sup> – 81.6 µg g<sup>-1</sup>) contents. When the contents of particular organic P fractions were expressed as percentage of P<sub>org</sub> no significant differences between the tree species were observed (Figure S1).

Concentrations of all organic P fractions (except P<sub>Olab</sub>) depended on the substrate type (Figure 1). The highest contents of P<sub>Omod</sub> were measured in Ashes (38.7 µg g<sup>-1</sup>), followed by Sands (20.7 µg g<sup>-1</sup>) and Clays (13.6 µg g<sup>-1</sup>). Sands were characterized by significantly lower P<sub>org</sub> concentrations (106.5 µg g<sup>-1</sup>; Table 4) and significantly lower contents of P<sub>fulv</sub>, P<sub>hum</sub> and P<sub>Ores</sub> fractions (20.8 µg g<sup>-1</sup>, 19.1 µg g<sup>-1</sup> and 21.5 µg g<sup>-1</sup>, respectively) than two other substrate types (Figure 1). Clays had the highest P<sub>org</sub> concentration (272.1 µg g<sup>-1</sup>; Table 4) resulting from the highest concentration of P<sub>Ores</sub> (143.1 µg g<sup>-1</sup>). Ashes contained significantly less of this fraction (56.8 µg g<sup>-1</sup>) than Clays but the contents of P<sub>fulv</sub> and P<sub>hum</sub> were similar for both substrate types (46.0 µg g<sup>-1</sup> – 46.6 µg g<sup>-1</sup> for P<sub>fulv</sub> and 37.4 µg g<sup>-1</sup> – 40.0 µg g<sup>-1</sup> P<sub>hum</sub>) (Figure 1). The C<sub>org</sub>-to-P<sub>org</sub> ratio depended on substrate type with Clays exhibiting the highest (271), Ashes intermediate (225) and Sands the lowest (166) value (Table 4). The effect of substrate type was evident also when the contents of particular organic P fractions were expressed as percentage of P<sub>org</sub>. Sands had the largest share of P<sub>Olab</sub>, Ashes of P<sub>fulv</sub> while Clays of P<sub>Ores</sub> (Figure S1).

## Discussion

### P and N concentrations in foliage and the O horizon

As expected the foliage of N-fixing tree species contained significantly more N than the foliage of Scots pine and silver birch (Table 2). The measured N concentrations in leaves of black alder and black locust represented average or relatively high values compared to data reported for these species growing at the natural and the reclaimed mine sites (Rodríguez-Barrueco et al., 1984; Côte et al., 1989; Kuznetsova et al., 2011; Deng et al., 2019; González et al., 2020; Woś et al., 2020). The N-fixing trees contained also significantly more P in the foliage than non-N-fixing species. However, the concentrations of P in the foliage of all the studied species were relatively low compared with literature data. For instance, P concentrations in leaves of black locust growing in a plantation on Loess Plateau, China varied from 1720 µg g<sup>-1</sup> to 2170 µg g<sup>-1</sup> (Deng et al., 2019) and of black locust growing in the areas affected by the industrial emissions from 1680 µg g<sup>-1</sup> to 2530 µg g<sup>-1</sup> (Tzvetkova and Petkova, 2015). Even higher P concentration (3410 µg g<sup>-1</sup>) was reported in the leaves of black locust growing in the reclaimed Green Valley coal mine, Indiana, USA (Jensen et al., 2010). The P concentration in black alder leaves in our study was close to the lower range of values reported by Kuznetsova et al. (2011) for the leaves of 2 – 7 years old black alders (1500 µg g<sup>-1</sup> – 1900 µg g<sup>-1</sup>) and by Rodríguez-Barrueco et al. (1984) for the leaves of 40-50 years old alders sampled in summer (1600

$\mu\text{g g}^{-1}$ ). However, it was distinctly lower than values reported by Temperton et al. (2003) for the leaves of 4 years old black alders growing on the sites after oil shale mining reclaimed for forestry ( $2200 \mu\text{g g}^{-1} - 4100 \mu\text{g g}^{-1}$ ). Concentration of P in the foliage of Scots pine represented the very low end of the range of values observed in needles of this species at various sites in Europe (Oleksyn et al., 2003; Fäth et al., 2019) and was well below the threshold value ( $1300 \mu\text{g g}^{-1}$ ; Göttlein, 2015). Similarly, the concentration of P in the leaves of silver birch was distinctly lower than values measured in afforested arable fields in Estonia ( $3890 \mu\text{g g}^{-1}$ ; Uri et al., 2007) and Finland ( $2650 \mu\text{g g}^{-1}$ ; Saramäki and Hytönen, 2004). Assessment of nutrient supply for trees includes not only the concentrations of particular elements in the foliage but also their ratio as the elements are required in appropriate proportions (Ingestad 1987; Knecht and Göransson, 2004; Marschner, 2012). The N-to-P ratio of 14 has been used as a general indicator of either P ( $>14$ ) or N ( $<14$ ) limitation for plant's growth (Knecht and Göransson, 2004). The N-to-P ratios observed in our study for all the species except Scots pine were higher than 14. Low concentrations of P in the foliage and high N-to-P ratios indicate insufficient P supply for all the studied tree species at all of the studied substrate types. Considering relatively high N concentrations and high N-to-P ratios in the foliage of the studied species (except for Scots pine) it seems that phosphorus and not nitrogen is the most limiting element in the studied technosols. Similarly, Manimel Wadu et al. (2017) identified insufficient P availability as a major long-term risk for ecosystem development at reclaimed sites after oil sands extraction in Canada.

Litterfall is the major source of organic matter accumulated in the O horizon. However, P concentrations in this horizon under the studied tree species did not correspond to P concentrations in the foliage. The highest P concentrations were found not under black alder but under silver birch followed by black locust. We presume that lower P concentration in O horizon under alder resulted from larger resorption of P from leaves prior to abscission. Côte and Dawson (1991) reported that black alder was able to resorb large amount of P from senesced leaves and that P resorption efficiency was particularly high at sites with low P availability. The P resorption efficiency of black alder was larger than efficiency of black locust and several non-N-fixing species growing on the swamp forest sites (Sürmen et al., 2014). Differences in the N and P resorption efficiency between the studied species may explain also the observed pattern in the N-to-P ratios in the O horizon. Nitrogen-fixing plants are less efficient at resorbing nitrogen than non-nitrogen-fixing plants but the opposite is true for P resorption (Stewart et al., 2008). Hence, the O horizons under N-fixers had higher N-to-P ratios than non-N-fixing species. Furthermore, black locust has been described as more efficient in N resorption from leaves than various alder species (Stewart et al., 2008). This together with higher P resorption efficiency of black alder compared with black locust (Sürmen et al., 2014) may explain significantly larger N-to-P ratio in the soil O horizon under black alder.

Low P concentration in the O horizon under Scots pine resulted probably from the low P concentration in green needles and significant P resorption from the senesced foliage. Blanco et al. (2007) reported high P resorption efficiency (49.6% – 54.0%) in Scots pines growing at two sites with different fertility in Pyrenees, Spain but noticed that the P resorption efficiency did not depend on soil P concentration. The highest C-to-N and C-to-P ratios of the O horizon under Scots pine resulted probably from chemical properties of pine needles. Similarly, Spohn and Stendahl (2022) reported higher C-to-N and C-to-P ratios in organic horizons under Scots pine stands compared to other tree species growing in Swedish forests and attributed this to elemental ratios of leaf/needle litter.

#### Concentrations of $C_{\text{org}}$ , $N_t$ and $P_{\text{org}}$ in mineral soil

Concentrations of  $C_{\text{org}}$ ,  $N_t$  and  $P_{\text{org}}$  in the mineral soil were significantly higher under deciduous trees compared with Scots pine (except for  $C_{\text{org}}$  which did not differ significantly between pine and birch) but there was no difference in  $P_t$  concentration between the studied species. The ultimate source of P in soil is the parent material as airborne inputs are usually negligible (Tipping et al., 2014). Similar  $P_t$  concentrations under the studied tree species indicate that within every substrate type particular tree species grew on soils developed from materials with similar phosphorus abundance. Hence, we can exclude a bias in the concentrations of organic P fraction resulting from planting any of the species on sites less abundant in this element.

The  $P_{org}$  concentration in the mineral soil under Scots pine was lower than under other tree species. Organic P in soil is mainly of plant origin (Spohn and Stendahl, 2022). Therefore, we presume that low  $P_{org}$  concentrations in the mineral soil under Scots pine were due to low input of organic P as indicated by low concentration of P in pine needles and in the O horizon. Lower  $P_{org}$  concentrations under Scots pine were mainly due to lower concentrations of relatively stable and recalcitrant fractions of organic phosphorus ( $P_{fulv}$ ,  $P_{hum}$  and  $P_{Ores}$ ). However, the concentrations of labile and moderately labile organic P fractions were lower under Scots pine than under other species as well (although the difference was not statistically significant). When the concentrations of particular P fractions were expressed as the percentage of  $P_{org}$  (Figure S1) there were nearly no significant differences between the studied species. This suggests that the tree species did not affect the quality of organic P in the mineral soil and that differences observed in the concentration of  $P_{org}$  and its fractions between Scots pine and other tree species resulted from lower organic P input under this species. This conclusion is supported also by lower  $C_{org}$  and  $N_t$  concentrations in the mineral soil under Scots pine compared with other species. Tree species are known to differently affect organic matter distribution in the soil profile (Gruba and Socha, 2019) and coniferous trees have been described to contain less organic C in the mineral soil than deciduous trees (Peng et al., 2020).

The remaining tree species did not differ in terms of  $P_{org}$  concentration in the mineral soil despite significant differences in P concentrations observed in the foliage and in the O horizon. They had also similar concentrations of  $P_{Olab}$ ,  $P_{Omod}$ ,  $P_{fulv}$  and  $P_{Ores}$  expressed either in absolute values or as percentages of  $P_{org}$ . This indicates that deciduous tree species - both N-fixing and non-N-fixing - have similar effect on the concentration of  $P_{org}$  and its fractions in the mineral soil. However, we cannot rule out that large variability in the properties of parent materials included in our study (e.g. pH, texture) obscured the tree species effect in the mineral soil. Spohn and Stendahl (2022) reported no substantial difference in the C-to- $P_{org}$  ratios in the mineral soil under different tree species and concluded that  $P_{org}$  concentrations were strongly affected by soil texture. On the contrary, Redel et al. (2008) found significant differences in soil P fractions under evergreen and deciduous forests growing on the same soil type in Chile.

Substrate type proved to be more important factor affecting the concentrations of  $P_{org}$  and  $P_t$  as well as  $C_{org}$  and  $N_t$  than tree species. Sands contained significantly less of all these elements and had significantly lower  $C_{org}$ -to- $P_{org}$  than Clays and Ashes. Fine textured materials with large specific surface area and high charge density are capable of sorbing more organic compounds than coarse textured ones as they have better ability to build-up of organo-mineral complexes resistant to microbial degradation (Franzluebbers et al., 1996; Hassink, 1997; Müller and Höper, 2004). In line with our results Spohn and Stendahl (2022) found significantly lower organic P concentrations and higher  $C_{org}$ -to- $P_{org}$  ratios in coarse textured forest soils compared to fine textured ones. Similarly, Chodak and Niklińska (2010) reported that coarse textured sands contained significantly less  $C_{org}$  and  $N_t$  than loamy sands. Next to the texture some other properties of parent materials such as initial concentrations of elements or pH also could have played a role in the observed differences in  $C_{org}$ ,  $N_t$  and  $P_{org}$  concentrations between various substrate types. Ashes in our study contained less clay particles than Sands, yet they contained significantly more  $C_{org}$ ,  $N_t$  and  $P_{org}$ . However, Ashes had higher percentage of silt particles that also contribute to stabilization of organic compounds in soil (Hassink, 1997), contained more P-rich minerals as indicated by their larger  $P_t$  concentrations and more  $C_{org}$  originating from unburnt lignite residues (Świątek et al., 2019). Significant difference in pH of Sands and Ashes also could affect the ability of these two substrate types to stabilize organic matter (Garrido and Matus, 2012; Rasmussen et al., 2018).

Classification of organic P availability based on chemical solubility has been criticized because various fractions haven't been correlated to P availability for plants and it was proven that plants can acquire P from various organic P fractions including those regarded as stable (Turner et al., 2005; Fox et al., 2011). Nevertheless the existing fractionation schemes remain useful in studying factors affecting development and transformations of soil P pools over time (DeBruler et al., 2019). In our study concentrations of particular fractions of organic P depended on the substrate type. When concentrations of  $P_{Olab}$ ,  $P_{Omod}$ ,  $P_{fulv}$ ,  $P_{hum}$  and  $P_{Ores}$  were expressed in absolute values ( $\mu\text{g g}^{-1}$ ) the observed differences followed the trend observed for  $P_{org}$ , with Sands having the lowest concentrations of all fractions except for  $P_{Olab}$ . However, very different

pattern was revealed when the organic P fractions were expressed as percentage of  $P_{org}$ . Sands had the highest relative content of  $P_{Olab}$  and the lowest of  $P_{Ores}$  among the studied substrate types, Ashes had the highest share of  $P_{fulv}$  while in Clays the organic P was dominated by  $P_{Ores}$  which constituted more than a half of organic P pool. The observed differences in relative contents of particular organic P fractions resulted apparently from parent material properties. Clays being rich in minerals with large specific surface area and high number of exchange sites retained considerable part of P-containing organic molecules in recalcitrant fraction ( $P_{Ores}$ ). On the contrary, Sands provided much less exchange sites and large part of organic P entering the mineral soil remained in labile fraction. High share of  $P_{Olab}$  in the  $P_{org}$  and low  $C_{org}$ -to- $P_{org}$  ratio of Sands could be important factors contributing to better P supply of trees growing on this substrate type indicated by higher concentration of P and lower N-to-P ratio in the foliage. It is known that soil microbial biomass plays a central role in cycling and transformations of soil organic P (Bünemann, 2015). However, soil microorganisms are usually C-limited and even in the P-poor soils the mineralization of organic P is mainly driven by microbial C demand (Heuck et al., 2015). Sands contained less  $C_{org}$  than Clays and Ashes but their organic matter was richer in organic P (as indicated by  $C_{org}$ -to- $P_{org}$  ratios) and contained relatively large pool of labile organic matter (extractable with  $NaHCO_3$ ) rich in phosphorus. Rapid microbial mineralization of this fraction might have resulted in high P release to soil solution while low content of clay minerals in Sands prevented adsorption of the released mineral P (Gérard, 2016).

## Conclusions

Black locust and black alder contained significantly more P in the foliage than silver birch and Scots pine indicating higher P uptake by the N-fixing tree species. However, the P concentrations in the O horizon followed a different trend with the highest concentration measured under silver birch and not under the N-fixers. We presume that this was due to more efficient P resorption from leaves by the N-fixing species (in particular by black alder). The effect of tree species on  $P_{org}$  concentrations in the uppermost mineral soil was limited. Only Scots pine exhibited significantly lower  $P_{org}$  concentrations in the mineral soil probably due to lower concentration of P in pine needles and the O horizon and slower decomposition rate of pine litter. The other tree species studied did not differ in the  $P_{org}$  concentrations in the mineral soil. Tree species did not affect the concentrations of organic P fractions with different lability as well. The concentrations of fulvic, humic and residual P were lower in the mineral soil under Scots pine only because there was generally less organic P under this tree. The percentages of labile, moderately labile, fulvic, humic and residual fractions in soil  $P_{org}$  were nearly the same under all tree species. The largest effect on the  $P_{org}$  concentration in the mineral soil as well as on the contents of its particular fractions was from the substrate type. Sands contained much less  $P_{org}$  than Clays and Ashes. However, the percentage labile P in  $P_{org}$  was much higher there resulting in better P supply for plants. We conclude that N-fixing trees do not affect the concentration of labile organic P as the major factor controlling this  $P_{org}$  fraction is the soil substrate quality.

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## Figure captions

Figure 1. Results of two-way ANOVA for the concentrations of labile organic P ( $P_{Olab}$ ), moderately labile organic P ( $P_{Omod}$ ), organic P contained in fulvic acids ( $P_{fulv}$ ) and humic acids ( $P_{hum}$ ) and residual organic P ( $P_{Ores}$ ) in mineral soils (0 – 5 cm) of technosols under different tree species. Studied factors include tree species (black locust, black alder, Scots pine, silver birch) and substrate class (Clays, Ashes, Sands). Whiskers indicate standard errors. Bars sharing the same letter do not differ significantly ( $p < 0.05$ , Student-Newman-Keuls test).

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