doi.org/10.1016/j.scitotenv.2021.147622

Initiating pedogenesis of magnetite tailings using *Lupinus angustifolius* (narrow-leaf lupin) as an ecological engineer to promote native plant establishment

Hongtao Zhong [1,2]*, Hans Lambers [1,2], Wei-San Wong [1,2], Kingsley W. Dixon [2], Jason C. Stevens [2,3], Adam T. Cross [4,5]*

[1] School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia

[2] Centre for Mine Site Restoration, School of Molecular and Life Sciences, Curtin University, Kent Street, Bentley, WA 6102, Australia

[3] Kings Park Science, Department of Biodiversity, Conservation and Attractions, 2 Kattidj Close, Kings Park, WA 6005, Australia

[4] EcoHealth Network, 1330 Beacon St, Suite 355a, Brookline, MA 02446, United States. https://ecohealthglobal.org.

[5] School of Molecular and Life Sciences, Curtin University, GPO Box U1987, Bentley, WA 6102, Australia.

*Corresponding authors: Hongtao Zhong, hongtao.zhong@uwa.edu.au, School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia; Adam Cross, adam.cross@curtin.edu.au, School of Molecular and Life Sciences, Curtin University, Kent Street, Bentley, WA 6102, Australia

Keywords: Magnetite tailings; Eco-engineering; *Lupinus angustifolius*; Pedogenesis; Phosphorus; Ecological restoration

Acknowledgement

This research was supported by the Australian Government through the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (Project Number ICI150100041) and Australian Research Council Linkage Project (LP160100598). ATC was supported by the Research Fellowship in Restoration Ecology jointly funded by the EcoHealth Network, Gelganyem Limited, and Curtin University. The authors thank Michael Blair for help in the use of glasshouse facilities.

1 ABSTRACT

Mine tailings pose physical and chemical challenges for plant establishment. Our aim was to learn from natural processes in long-term soil and ecosystem development to use tailings as novel parent materials and pioneer ecological-engineering plant species to ameliorate extreme conditions of tailings, and facilitate the establishment of subsequent native plants. A glasshouse trial was conducted using magnetite tailings containing various amendments, investigating the potential of the nitrogen (N)-fixing, non-native pioneer species *Lupinus angustifolius* (Fabaceae), narrow-leaf lupin, as a potential eco-engineer to promote soil formation processes, and whether amendment type or the presence of pioneer vegetation improved the subsequent establishment and growth of 40 species of native plants. We found that *L. angustifolius* eco-engineered the mine tailings, by enhancing the N status of tailings and mobilising primary mineral P into organic P via a carboxylate-exudation strategy, thereby enabling subsequent growth of native species. The substantial increases of the soil organic P (from ca. 10 to 150 mg kg^{-1}) pool and organo-bound Al minerals (from 0 to 2 mg kg^{-1}) were particularly evident, indicating the initiation of pedogenesis in mine tailings. Our findings suggest that the annual legume *L. angustifolius* has eco-engineering potential on mine tailings through N-fixation and P-mobilisation, promoting the subsequent growth of native plants. We proposed *Daviesia* (Fabaceae) species as native species alternatives for the non-native *L. angustifolius* in the Western Australian context. Our findings are important for restoration practitioners tasked with mine site restoration in terms of screening pioneer eco-engineering plant species, where native plants are required to restore after mine operations.

2 Introduction

Globally, large quantities of fine-particulate mined materials, generally referred to as mine tailings, continue to be produced as a result of increasing demands for mineral resources worldwide (Aznar-Sánchez et al., 2018). Australia is one of the largest global producers and exporter of Fe ores, accounting for 36% of world production in 2019 (Senior et al., 2020). However, Fe-ore mining activities in Western Australia occur in areas harbouring high plant diversity with high rates of localised endemism (Gibson et al., 2012). It is generally a legislated requirement in regions such as Western Australia that post-mining landscapes must be restored as part of mine closure by reinstating locally-native vegetation communities (Stevens and Dixon, 2017).

Tailings are among the most hostile and challenging mined substrates for plant re-colonisation, both physically and chemically (Cross et al., 2017). Various strategies have been explored to facilitate the revegetation of tailings *in situ*. These strategies include collection and broadcasting of native seeds, seed-based enablement technologies (Erickson et al., 2017; Madsen et al., 2016), precision stripping, storage and return of natural topsoil (Merino-Martín et al., 2017), fertilising amendments and engineering such as slope stabilisation and contouring (Koch, 2007), and amendment with organic waste material or application of plant growth-promoting microbes (Ottenhof et al., 2007; Wang et al., 2017). However, no single rehabilitation or ecological restoration technique thus far has successfully achieved the full recovery of a reference ecosystem on a tailings landform often due to hostile substrate conditions (Cross and Lambers, 2017).

To establish vegetation on tailings landforms, it is often necessary to alleviate or ameliorate extreme edaphic conditions. Mine tailings have potential to be a novel soil-forming parent material (Santini and Banning, 2016; Wu et al., 2019c) if capable of being ‘re-engineered’ to be effective growth substrates. Bradshaw (1997) suggested that facilitating the return of natural processes to post-mining landscapes (i.e. ecological engineering) might represent the most effective way to restore these areas and establish self-sustaining ecosystems in the long-term. Monitoring soil development must therefore be considered in any effort to undertake restoration of mined lands, because soil and ecosystems co-evolve over long periods in response to environmental and edaphic factors (Bradshaw and Chadwick, 1980; Hotchkiss et al., 2000). Soil phosphorus (P) dynamics have long been used as a key indicator of soil and ecosystem development along natural soil chronosequences in different climatic zones (Eger et al., 2011; Turner and Laliberté, 2015; Zhou et al., 2013). However, changes in soil P dynamics, such as inorganic and organic P fractions, have only recently been used as a tool to evaluate soil development in the rehabilitation and restoration of mine tailings (Wang et al., 2018; Zhong et al., 2020).

At the early stage of soil development, soil P dynamics are highly influenced by pioneer plants that have the potential to 'kick-start' pedogenic processes (Lambers et al., 2012). Some pioneer plants that symbiotically fix nitrogen (N), such as legume species (Fabaceae), may have potential as eco-engineers and facilitate ecological succession in young ecosystems (Cross and Lambers, 2017). Some Fabaceae are capable of mobilising P, for example *Lupinus* species with their carboxylate-exudation capacity (with or without cluster roots), nitrogen fixation and fast growth rates are potential eco-engineering pioneer species (Pearse et al., 2007). If changes in soil N and P dynamics can be accelerated in unweathered mine tailings, it may facilitate the establishment of other native plants. In the present study we investigated the potential of *Lupinus angustifolius* (narrow-leaf lupin) as an ecosystem engineer on magnetite tailings by conducting a 20-month glasshouse experiment. We assessed whether *L. angustifolius* can eco-engineer the magnetite tailings by improving the growth medium because of its specific traits and development (N fixation and P mobilisation) and potentially enhance the growth of subsequent native plants.

3 Material and Methods

3.1 Study area

Growth substrates and native plant species were sourced from a magnetite mining operation in the Mid-west region of Western Australia, located 400 km northeast of Perth. This area experiences a semi-arid Mediterranean climate with mild wet winters (mean monthly maximum 19 °C) and hot dry summers (mean monthly maximum 37 °C). The study area receives 311 mm annual rainfall, about 65% of which falls between May and September (winter; Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/, Station 10195).

The mining area is located in the Yalgoo Bioregion according to the Interim Biogeographic Regionalisation for Australia (version 7; http://www.environment.gov.au/land/nrs/science/ibra), and the local plant communities are typical Eremaean sclerophyll shrublands (Beard, 1990). Plant communities are generally low to open woodlands, predominantly comprising shrubs or trees of *Acacia* spp., *Eucalyptus* spp., *Melaleuca leiocarpa*, *Allocasuarina acutivalvis*, *Callitris columellaris*, and *Hakea recurva* subsp. *recurva*, with an understorey of shrubs, grasses and herbaceous annuals (Markey and Dillon, 2008).

Dry-stacked magnetite tailings (hereafter referred to as tailings) are generated as fine-textured (processed by high-pressure grinding to <4 mm) waste materials at the end of magnetite-ore processing. Three substrate amendments were sourced from the mine site, including crushed overburden rock (a heterogeneous mix of rocks and stones, hereafter referred to as waste rock), stockpiled (ca. 4–5 years) natural topsoil (hereafter referred to as topsoil), and leaf litter collected from beneath native vegetation including *Acacia ramulosa*, *Eucalyptus loxophleba* and *Allocasuarina acutivalvis* (pooled to provide one composite litter).

Natural topsoils at the study site comprise highly-weathered red earth, from stony red loamy sand to loamy clay soil (Leptic Rudosol, Orthic Tenosol or Red Kandosol according to Australian Soil Classification; Ferrasols or Fluvisols according to FAO Soil Units; Aridisols, Entisols or Fluvisols according to Soil Survey Staff 2014). These soils usually contain abundant fragments (usually 2–20 mm) of ironstone gravel (Payne et al., 1998). All rocky material (>40 mm) and all large woody debris (branches and twigs) were removed prior to experimental use. Selected physical and chemical profiles for tailings, topsoil and waste rock are presented in Cross et al. (2018), with mineralogy presented in Wu et al. (2019b).

3.2 Suitability of tailings as a growth medium for pioneer and native plants

Experiments were established in rectangular polycarbonate boxes (400 mm in depth × 400 mm in width × 1000 mm in length) filled with tailings to a depth of 350 mm and the tailings overlain by an additional 50 mm of growth media treatment mimicking standard industry practice at the study site. Growth media treatments included (1) tailings only, resulting in 400 mm depth of tailings (TAL), (2) tailings mixed with 10% (w/w) native vegetation litter (VEG), (3) tailings mixed with 10% (w/w) waste rock (ROC), and (4) tailings mixed with 10% (w/w) topsoil (TOP). Three replicate boxes were established for each treatment, with each box divided transversely into two sub-treatments by insertion of a central impermeable plastic plate yielding 24 sub-treatments in total.

The potential of *Lupinus angustifolius* (narrow-leaf lupin) as an ecological engineer to promote the accumulation of organic matter and to enhance N cycling in the tailings was tested by sowing 20 seeds of this species into each of half of the sub-treatments, leaving the other half unplanted (yielding a +/- *L. angustifolius* sub-treatment for each growth media treatment). An unplanted and un-amended tailings treatment was used as control. This was undertaken to address the lack of organic matter and available N as major constraints for plant growth in magnetite tailings in previous studies (Cross et al., 2019). Seeds were inoculated with commercial rhizobium (EasyRhiz Soluble Legume Inoculant 'Serradella Lupin', Alosca Technologies, Australia) to promote nodulation and N_2 fixation. Seeds of *L. angustifolius* were sown in late March 2016, with plants harvested in September 2016 to determine aboveground biomass. Following weighing, harvested *L. angustifolius* were returned to respective sub-treatments and left to decompose on the substrate surface prior to seeding with native species. A small amount of *L. angustifolius* leaves (ca. 10 g) was sampled from each sub-treatment for nutrient analysis.

The suitability of tailings as a growth medium for native plants was investigated by broadcasting seeds of 40 local native species into all sub-treatments (Appendix S1). Selected species provided a representative sample of growth forms, life histories and nutrient-acquisition strategies desired in restoration based on the local native reference communities (Cross et al., 2019; Cross and Lambers, 2020). Seeds of all native species were collected during 2014 and 2015, cleaned to pure seed, and stored in a controlled-environment room prior to experimental use. Filled seeds of all species were broadcast onto the soil surface of all boxes in March 2017 (seeding rate ranging from 12.5–75 seeds m^{-2} for each species; Appendix S1), after the harvested *L. angustifolius* had been left to decompose. Physical seed dormancy in species of *Acacia* and *Senna* was broken prior to seeding by immersion in boiling water for one minute (Erickson et al., 2016; Kildisheva et al., 2020). All aboveground parts of established native seedlings in each treatment were harvested and weighed in November 2017. We attempted to harvest roots for each individual, but compaction of the tailings rendered this unsuccessful.

The trial ran from March 2016 to November 2017. Descriptions of activities at the four sampling times are provided in Figure 1. The experimental setup is shown in Figure S1. The experiment was conducted in a temperature-controlled glasshouse (20–25 ℃) at the Shenton Park Field Station, University of Western Australia. All boxes were watered weekly during the annual growing season (April to September), and remained un-watered from October to March each year to simulate the semi-arid climate of the study site. The pH of tap water in Perth region is about 7.8 (Drinking water quality, www.watercorporation.com.au).

3.3 Soil analyses

Soil samples were collected at four times over the course of the experiment: immediately after growth media treatments were established (prior to the sowing of *L. angustifolius* seeds) in March 2016, immediately prior to the harvesting of *L. angustifolius* plants in September 2016, immediately prior to broadcasting the seeds of native species in March 2017, and finally in November 2017 immediately prior to the harvesting of native plants.

Soil samples were collected using an auger (35 mm diameter, 150 mm depth), with approximately 200 g of soil collected from each sub-treatment at each sampling point. All samples were sieved to remove large organic debris, roots and small gravel (<2 mm stainless steel sieve) and stored in zip-lock polyethylene bags at 4 °C for up to one week prior to analysis, with 150 g subsamples air-dried (35 °C for up to one week) and stored in zip-lock polyethylene bags at 27 °C until analysis.

Soil microbial biomass carbon (MBC) was measured by $CHCl_3$ fumigation and 0.5 M K_2SO_4 extraction of fresh soil as described in Vance et al. (1987). Soluble organic carbon (C) in extracts was analysed using an Aurora O.I 1030W wet oxidation Total Organic Carbon analyser (College Station, TX, USA). A conversion factor (K*c*) of 0.45 was applied to the MBC results as described in Wu et al. (1990). Soil microbial biomass P (MBP) was measured by $CHCl_3$ fumigation and 0.5 M $NaHCO_3$ extraction of fresh soil, as described in Brookes et al. (1982). Phosphorus concentrations were determined spectrophoto-metrically using a UV160A spectrophotometer at 880 nm (Shimadzu, Kyoto, Japan) after reaction with molybdate blue (Blakemore et al., 1987; Murphy and Riley, 1962). A conversion factor (K*p*) of 0.4 was applied to the MBP results as described in Brookes et al. (1982). Soil mineral N (NH_4-N and NO_3-N) was extracted with 2 M KCl from fresh soil, and determined by LACHAT FIA QuikChem 8500 Series 2 (Loveland, CO, USA).

Soil pH (both in deionised water and in 0.01 M $CaCl_2$) and electrical conductivity (EC) (deionised water) were measured for each sample using soil pH and EC probes calibrated with pH 4 and 7 buffer solutions (Orion 720a, Beverly, MA, USA). Soil total organic matter content was determined by the loss on ignition (LOI) method. Soil organic C concentration was estimated by dividing the total organic matter (measured as LOI) with a conversion factor of 1.72 (Blakemore et al., 1987). Total N was measured by the combustion method via a Leco analyser (FP628, St. Joseph, MI, USA).

Exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ (2 h, 1:30 soil-to-solution ratio), with detection by an ICP–OES (Thermo iCAP 6000 series ICP-OES, Freemont, CA, USA) following the methods of Blakemore et al. (1987). Effective cation exchange capacity (ECEC) was calculated as the sum of [Al], [Ca], [K], [Mg], and [Na]. Colwell-P and -potassium (K) were extracted with 0.5 M $NaHCO_3$ (adjusted to pH = 8.5) from air-dried soil (16 h, 1:40 soil-to-solution ratio). Phosphorus and K concentrations in the extracts were determined colorimetrically (Rayment and Lyons, 2010), using a UV160A spectrophotometer at 880 nm (Shimadzu, Kyoto, Japan) and atomic absorption spectroscopy (Varian SpectrAA55AAAS, Palo Alto, CA, USA), respectively.

Total P concentration was determined by ignition (550 ℃, 1 h) and extraction in 1 M HCl (16 h, 1:50 soil to solution ratio) modified after Saunders and Williams (1955). Organic [P] was calculated by subtracting 1 M HCl-extracted P without ignition from 1 M HCl-extracted P after ignition. While this procedure may underestimate true total P in strongly weathered soil compared with the NaOH fusion method, and overestimate organic P due to potential increase in acid extractability of P in ignited soil (Condron et al., 1990), this ignition procedure was appropriate in this instance, because the tailings used in this study were not heavily weathered (Wu et al., 2019b). Soil extracts were filtered through Whatman No. 42 filter paper, and filtrates were stored at 4 ℃ until analysis. Phosphorus concentrations in all extracts were determined by an ICP-OES Model 5300DV (Perkin Elmer, Shelton, CT, USA). Results are expressed on a dry soil mass basis.

Soil metal-organic Fe and Al complexes (Fe_p and Al_p) were extracted with 0.1 M sodium pyrophosphate (Blakemore et al., 1987). Soil extracts were filtered through Whatman No. 42 filter paper and filtrates were stored at -20 ℃ until analysis. Fe and Al concentrations in all extracts were determined using an ICP-OES Model 5300DV (Perkin Elmer, Shelton, CT, USA).

3.4 Leaf analyses

Leaf samples of *L. angustifolius* were oven-dried at 70 ℃ for two days, followed by ball-mill grinding (Geno/Grinder 2010, SpexSamplePrep, NJ, USA) using zirconium beads. Ground leaf samples were digested with hot concentrated HNO_3:$HClO_4$ (3:1). Leaf elemental concentrations (i.e. P) were determined by an ICP-OES Model 5300DV (Perkin Elmer, Shelton, CT, USA). Leaf N concentration was determined by an Elementar Vario-Max CN Elementary Analyser (Elementar, Langenselbold, Germany).

3.5 Statistical analyses

Data and statistical analyses were performed using JMP® 15 (SAS Institute Inc.). The fit model (standard least squares function) was used to analyse the interactions between *L. angustifolius* (with or without), treatments (TAL, VEG, ROC, and TOP) and time (T0: Mar-2016, T1: Sep-2016, T2: Mar-2017, and T3: Nov-2017), with *L. angustifolius*, treatments and time as fixed effects and Pot ID as random factor, on selected soil chemical properties. Model results are presented in Supporting Information (Table S2). Selected soil chemical properties, MBC, MBP, NH_4-N and NO_3-N concentrations, at T3: Nov 2017 were analysed to determine the differences amongst experimental conditions using a Kruskal-Wallis test with a Games-Howell post-hoc test. Differences between native plant total biomass and leaf area amongst treatment groups were determined by Welch's Test followed by Games-Howell post-hoc testing. All data were square-root or log transformed to normalise distribution before analysis.

4 Results

4.1 Growth of *Lupinus angustifolius* and native plants

ROC and TOP showed the greatest *L. angustifolius* aboveground biomass at the September 2016 harvest (Table 1), followed by TAL and VEG treatments. There was no significant difference in leaf [N] among substrates, while VEG and TAL showed the greatest leaf [P].

In the TAL (-) treatment the greatest number of native species survived at the end of the trial. Aboveground biomasses of native plants were greatest in TAL (both with and without prior *L. angustifolius*) and TOP (+), which were about twice that in VEG (+) and 10 times that in VEG (-), respectively (Table 2).

4.2 Substrate pH, organic C, total N, organic P and metal-organic complexes

Substrate pH decreased gradually in all treatments over the two years of the glasshouse trial (Figure 2). The main effect of sampling time on substrate pH was highly significant (*p*=0.0004, F=13.77), but there was no difference between treatments with and without *L. angustifolius* (*p*=0.2879, F=1.15) and among substrate treatments (*p*=0.2147, F=1.59). However, soil organic C concentration did not increase significantly (mainly about 2 mg C g^{-1}) over the course of the trial in any treatment, except in TOP (Figure 3). The main effect of different substrates on organic C was significant (*p*=0.0104, F=4.5093), but not between with and without *L. angustifolius* (*p*=0.7071, F=0.1428) and sampling time (*p*=0.5230, F= 0.4123). There were interactive effects between sampling time and substrate types on soil organic C concentration (*p*=0.0319, F=3.1196).

Total N concentrations did not increase significantly in TAL and VEG (stabilised around 0.3 mg N g^{-1}), while total N concentration reached 0.4 mg N g^{-1} soil in both ROC and TOP at the end of the trial (Figure 4). The main effects of sampling time (*p*=0.0019, F=10.4298) and different substrates (*p*=0.015, F=4.01) on total N concentrations were significant, but there was no difference between with and without *L. angustifolius* (*p*=0.8097, F=0.0587).

Organic P concentration increased almost 10-fold over the first 12 month of *L. angustifolius* growth and decomposition in three substrate types, and then decreased towards the end of the experiment (to about 50 mg P kg^{-1}), with the exception of ROC (Figure 5). The magnitude of the increasing trend (from below 10 mg kg^{-1} in March 2016 to almost 150 mg kg^{-1} in March 2017) and differences between with and without *L. angustifolius* were most pronounced in March 2017. The main effect of sampling time on organic P concentration was significant (*p*=0.0029, F=9.5917), but there was no effect of *L. angustifolius* (*p*=0.7061, F=0.1439) and substrate (*p*=0.4202, F=0.9692).

The concentrations of pyrophosphate-extractable Al (Al_p), mainly Al-organic complexes, increased significantly in all four types of substrates towards the end of the experiment (Figure 6). This pattern was not observed for Fe_p concentrations (Figure S2). Notably, organic-bound [Al] showed the greatest increase during the growth of native plants (from March 2017 to November 2017) in TAL (both with and without *L. angustifolius*). The main effects of sampling time (p=0.0001, F=17.0262) and different substrates (p<0.0001, F=23.8289) on Al_p were highly significant, but there was no effect of *L. angustifolius* (p=0.1130; F=2.6058).

Overall, the interactions among substrates, with and without *L. angustifolius*, and sampling times had no significant effect on selected soil properties, with the exception of soil organic P concentration (Table S2, S3).

5 Discussion

5.1 Effectiveness of *Lupinus angustifolius* as an ecological engineer

This study found that use of the legume, *L. angustifolius,* significantly enhanced the subsequent growth of native species (more than 6 months) in magnetite tailings with a clear improvement compared to previous studies of the same tailings material (Cross et al., 2019). We used a similar assemblage of native plant species as Cross et al. (2019), who observed high mortality rates in seedlings of nine out of 40 tested species two months after germination without prior growth of *L. angustifolius*. This suggests the potential of *L. angustifolius* as an ecosystem engineering species to promote the subsequent growth and survival of native species (Table 2).

The eco-engineering effect of *L. angustifolius* may, in part, be attributed to the accumulation of N as a result of biological N fixation during the growth of *L. angustifolius*, because N is a major limiting nutrient for plant growth in tailings used in this study as it is in young substrates along chronosequences (Turner & Condron, 2013). However, the increase of the N concentration in the tailings was relatively small (Figure 4), probably due to the inhibition of biological N fixation by the high pH (Tang et al., 1995). Furthermore, the sharp increase of substrate total N concentration in the ROC treatment suggests that the decomposition of *L. angustifolius* biomass (high in N) facilitated the growth of native plants. This agrees with Nyenda et al. (2020) that an N-fixing species (*Senegalia polyacantha*) produced fertility islands and facilitated the establishment of understorey vegetation on nickel-mine tailings. However, there are also studies that raised concerns of N-fixing plants becoming 'weedy' and hampering the return of other plant species during rehabilitation of post-mining landscapes (e.g., Frouz et al., 2015; Woodbury et al., 2020).

The role of *Lupinus lepidus* in ecosystem engineering and facilitating the primary succession at Mount St Helens (Washington, USA) has been investigated thoroughly following an eruption in 1980 (del Moral et al., 1995), but mainly focused on the contribution of N building up. However, the contribution of soil P mobilization in the young pumice-based soils by *L. lepidus* through carboxylate exudation was initially overlooked (Bishop et al., 2010). Like pumice material, magnetite tailings used in the present study had a high [total P] (about 1200 mg kg^{-1}), but only a minor fraction was available for plant growth (about 10 mg kg^{-1} of Colwell P) (Cross et al., 2018), suggesting a possible P limitation for early plant establishment and growth of plants lacking a P-acquisition a mechanism based on carboxylate release. Our results indicate that *L. angustifolius* may have functioned as an ecosystem engineer, mobilising P for subsequent plants during succession, which was evident in high leaf [P] in TAL (Table 1).

Lupinus angustifolius releases large quantities of carboxylates into the rhizosphere mobilising P, although it does not form specialised cluster roots, as does other species such as *L. albus* (Hocking and Jeffery, 2004; Pearse et al., 2006). The substrate organic P concentration first increased during the growing phase of *L. angustifolius*, as a result of *L. angustifolius* mobilising the primary mineral P in the tailings, followed by incorporation of inorganic P into organic P in biomass, which then ended up in the litter. The organic P pool subsequently declined during the growth of native plants in the tailings, because the organic P would have been hydrolysed by plant and microbial phosphatases, converting organic P into inorganic P available for uptake. These observations suggest that *L. angustifolius* roots mobilised the primary form of P from the

P-rich tailings, and made this available to the subsequently grown native plants, either by directly releasing inorganic P from the tailings, or indirectly from decomposing biomass following hydrolysis of organic P (Lambers et al., 2012). At the very early stages of soil development, monoester P, a relatively labile P form, tends to be the most important fraction of soil organic P (Zhou et al., 2019). Some native Western Australia plants are able to utilise relatively labile forms of soil organic P through release of root phosphatases (Png et al., 2017; Zhong et al., 2021). Therefore, we suggest that *L. angustifolius* promoted the growth of subsequent native plants through eco-engineering the P availability in the magnetite tailings.

5.2 Initial pedogenesis in magnetite tailings

The build-up of soil organic C and alleviation of extreme acidity or alkalinity are often used as important indicators for initial soil formation in mined tailing materials (Arocena et al., 2010; You et al., 2018). The organic C concentration in tailings remained relatively unchanged over the 20-month duration of the present glasshouse trial, probably due to the overestimation of organic C concentration in the tailings (2 mg g^{-1}) by use of the 500 ℃ dry-combustion method for the Mar-2016 harvest (You et al., 2015). However, soil microbial biomass C (approx. 160 mg kg^{-1}) in the tailings at the end of the present trial showed a major increase (Figure S4), compared with a 2.5-year field trial of wood-chip-amended and planted Cu-Pb-Zn tailings (about 80 mg kg^{-1}) under subtropical and semi-arid climatic conditions (You et al., 2018).

The decrease of the soil pH was most evident during the decomposition of *L. angustifolius* biomass, although pH was still much higher than that of natural topsoil in the study site, which generally ranged from 4–6 (Cross et al., 2018). Amending tailings with organic material (i.e. sugarcane mulch, C to N ratio about 80) has been proposed as a method to achieve more significant pH neutralisation in tailings (from >9 to about 7.5) (Wu et al., 2019a). This emphasises the importance of using organic amendments with a low C to N ratio that are more accessible to soil decomposers and promote a reduction in soil pH; the sclerophyllous plant material utilised in the VEG treatment in the present study (mainly *Eucalyptus loxophleba* and *Allocasuarina acutivalvis* leaf litter) had a much higher C to N ratio of >150 (unpublished data,

H. Zhong). We observed a slight pH increase during the growth of native plants (Figure 2), similar to that reported in tailings colonised by *Maireana brevifolia* (Wu et al., 2019a). The plants in low-N tailings likely acquired more anions (i.e. NO_3^-) than cations, balancing this by net H^+ uptake (or release of HCO_3^- or OH^-) from their roots to achieve charge balance (De Wit et al., 1963; Dijkshoorn et al., 1968). This is partially supported by the low nitrate concentrations in the tailings at the end of the glasshouse trial (Figure S5).

In addition, soil P dynamics plays an important role during pedogenesis (Turner and Condron, 2013; Walker and Syers, 1976; Zhou et al., 2020). The accumulation of soil organic P is due to the transformation of weathered primary mineral inorganic P into organic forms in microbial or plant biomass which then becomes accessible for soil microbes and plants (Turner et al., 2007). Soil P dynamics are rarely used as an indicator to assess soil formation in the context of mine tailings rehabilitation. However, the increase of organic P concentration indicated the commencement of pedogenesis by *L. angustifolius* P-mobilisation with subsequent biomass decomposition enabling growth of native plant plants. Similar eco-engineering and promotion of primary mineral weathering by a carboxylate-exuding P-mobilising strategy has been reported for *Hippophae rhamnoides* (Elaeagnaceae) in very early stages of soil and ecosystem development in a glacial retreat soil chronosequence, with organic P progressively accumulating in the surface soil (Zhou et al., 2013).

Pyrophosphate-extractable Al and Fe fractions are good estimates of soil organo-bound Fe and Al minerals, respectively (Parfitt and Childs, 1988). In the present study, these fractions provided a good indication of soil-forming processes in terms of weathered primary Al or Fe minerals, mainly magnetite and biotite-like mica (Wu et al., 2019b), being bound by organic matter. However, Wu et al. (2019b) did not include pyrophosphate-extractable Al and Fe fractions in their study. In the fresh tailings, there were no organo-bound Al minerals. This soil-forming process was particularly evident by the sharp increase of the Al_p concentrations during the native plant growth phase but did not increase during *L. angustifolius* growth (Figure 6). This was contrary to the changes in soil organic P concentration in this trial. The underlying mechanisms of such differences are not clear and worthy of further study. Organo-mineral associations are important, because they contribute to the formation of soil aggregates, which are abundant in natural soils near mine sites (Wu et al., 2019c). Therefore, our study confirmed that soil formation processes were initiated in the magnetite tailings and that the restoration of some subsequent native species was facilitated through eco-engineering effects of *L. angustifolius*. This was achieved mainly via carboxylate-releasing mobilisation of primary mineral P and transforming it into organic P, while the formation of organo-bound Al mineral was most evident during the growth of tested native plants.

6 Concluding remarks

This study demonstrated soil formation in magnetite tailings as a result of using a fast-growing, annual legume *L. angustifolius* as an ecological engineer that promoted growth of native plants in a 20-month glasshouse trial. Apart from relatively small changes in soil organic C and pH, transformation of P (from primary mineral P to organic P) was evidently promoted by the carboxylate-releasing *L. angustifolius*. The formation of organo-bound Al minerals was largely promoted by growth of native plants. We suggest that the substrate P dynamics is a useful indicator of soil formation investigating restoration capacity of substrates when mine tailings are used as soil-forming parent material. We recommend the use of plant species with N_2-fixing and P-mobilising traits as eco-engineering plants to initiate soil formation and N cycling, as well as improving pioneer plant community establishment by facilitating the return of other plants, in ecological restoration of mined land. Finally, rather than using exotic pioneer plants such as species of *Lupinus*, future studies should examine whether perennial native legumes such as the cluster-rooting species in the genus *Daviesia* would provide more ecologically appropriate natural analogues in a Western Australian context (Nge et al., 2020).

References

Arocena J. M., van Mourik J. M., Schilder M. L. M., Faz Cano A. 2010. Initial soil development under pioneer plant species in metal mine waste deposits. Restor. Ecol. 18: 244-252. https://doi.org/10.1111/j.1526-100X.2009.00582.x

Aznar-Sánchez J. A., García-Gómez J. J., Velasco-Muñoz J. F., Carretero-Gómez A. 2018. Mining waste and its sustainable management: advances in worldwide research. Minerals. 8: 284. https://doi.org/10.3390/min8070284

Beard J. S. 1990. Plant life of Western Australia. Kenthurst [N.S.W]: Kangaroo Press.

Bishop J. G., O'Hara N. B., Titus J. H., Apple J. L., Gill R. A., Wynn L. 2010. N-P co-limitation of primary production and response of arthropods to N and P in early primary succession on Mount St. Helens volcano. PLOS ONE. 5: e13598. https://doi.org/10.1371/journal.pone.0013598

Blakemore L. C., Searle P. L., Daly B. K. 1987. Methods for chemical analysis of soils (Rev. ed.). Lower Hutt, N.Z.: NZ Soil Bureau, Department of Scientific and Industrial Research.

Bradshaw A. 1997. Restoration of mined lands—using natural processes. Ecol. Eng. 8: 255-269. https://doi.org/10.1016/S0925-8574(97)00022-0

Bradshaw A. D., Chadwick M. J. 1980. The restoration of land: the ecology and reclamation of derelict and degraded land. Berkeley, Calif.: Univ of California Press.

Brookes P. C., Powlson D. S., Jenkinson D. S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Bio. Biochem. 14: 319-329. https://doi.org/10.1016/0038-0717(82)90001-3

Condron L. M., Moir J. O., Tiessen H., Stewart J. W. B. 1990. Critical evaluation of methods for determining total organic phosphorus in tropical soils. Soil Sci. Soc. Am. J. 54: 1261-1266. https://doi.org/10.2136/sssaj1990.03615995005400050010x

Cross A. T., Ivanov D., Stevens J. C., Sadler R., Zhong H., Lambers H., et al. 2019. Nitrogen limitation and calcifuge plant strategies constrain the establishment of native vegetation on magnetite mine tailings. Plant Soil. https://doi.org/10.1007/s11104-019-04021-0

Cross A. T., Lambers H. 2017. Young calcareous soil chronosequences as a model for ecological restoration on alkaline mine tailings. Sci. Total Environ. 607-608: 168-175. https://doi.org/10.1016/j.scitotenv.2017.07.005

Cross A. T., Lambers H. 2020. Calcicole-calcifuge plant strategies limit restoration potential in a regional semi-arid flora. Ecol. Evo.

Cross A. T., Stevens J. C., Dixon K. W. 2017. One giant leap for mankind: can ecopoiesis avert mine tailings disasters? Plant Soil. 421: 1-5. https://doi.org/10.1007/s11104-017-3410-y

Cross A. T., Stevens J. C., Sadler R., Moreira-Grez B., Ivanov D., Zhong H., et al. 2018. Compromised root development constrains the establishment potential of native plants in unamended alkaline post-mining substrates. Plant Soil. https://doi.org/10.1007/s11104-018-3876-2

De Wit C. T., Dijkshoorn W., Noggle J. C. 1963. Ionic balance and growth of plants. Mededeling / Instituut voor biologisch en scheikundig onderzoek van landbouwgewassen, Wageningen, pp. 72.

del Moral R., Titus J. H., Cook A. M. 1995. Early primary succession on Mount St. Helens, Washington, USA. J. Veg. Sci. 6: 107-120. https://doi.org/10.2307/3236262

Dijkshoorn W., Lathwell D. J., De Wit C. T. 1968. Temporal changes in carboxylate content of ryegrass with stepwise change in nutrition. Plant Soil. 29: 369-390.

Eger A., Almond P. C., Condron L. M. 2011. Pedogenesis, soil mass balance, phosphorus dynamics and vegetation communities across a Holocene soil chronosequence in a super-humid climate, South Westland, New Zealand. Geoderma. 163: 185-196. https://doi.org/10.1016/j.geoderma.2011.04.007

Erickson T., Barrett R., Merritt D., Dixon K. 2016. Pilbara Seed Atlas and Field Guide: Plant Restoration in Australia's Arid Northwest. Collingwood, AU: CSIRO PUBLISHING.

Erickson T. E., Muñoz-Rojas M., Kildisheva O. A., Stokes B. A., White S. A., Heyes J. L., et al. 2017. Benefits of adopting seed-based technologies for rehabilitation in the mining sector: a Pilbara perspective. Aust. J. Bot. 65: 646-660. https://doi.org/10.1071/BT17154

Frouz J., Vobořilová V., Janoušová I., Kadochová Š., Matějíček L. 2015. Spontaneous establishment of late successional tree species English oak (*Quercus robur*) and European beech (*Fagus sylvatica*) at reclaimed alder plantation and unreclaimed post mining sites. Ecol. Eng. 77: 1-8. https://doi.org/10.1016/j.ecoleng.2015.01.001

Gibson N., Meissner R., Markey A. S., Thompson W. A. 2012. Patterns of plant diversity in ironstone ranges in arid south western Australia. J. Arid Environ. 77: 25-31. https://doi.org/10.1016/j.jaridenv.2011.08.021

Hocking P. J., Jeffery S. 2004. Cluster-root production and organic anion exudation in a group of old-world lupins and a new-world lupin. Plant Soil. 258: 135-150. https://doi.org/10.1023/B:PLSO.0000016544.18563.86

Hotchkiss S., Vitousek P. M., Chadwick O. A., Price J. 2000. Climate cycles, geomorphological change, and the interpretation of soil and ecosystem development. Ecosystems. 3: 522-533. https://doi.org/10.1007/s100210000046

Kildisheva O.A., Dixon K.W., Silveira F.A.O., Chapman T., Di Sacco A., Mondoni A., Turner S.R., Cross A.T. 2020. Dormancy and germination: making every seed count in restoration. Rest. Ecol. 28: S256–S265. https://doi.org/10.1111/rec.13140

Koch J. M. 2007. Alcoa's Mining and Restoration Process in South Western Australia. Restor. Ecol. 15: S11-S16. https://doi.org/10.1111/j.1526-100X.2007.00288.x

Lambers H., Bishop J. G., Hopper S. D., Laliberté E., Zúñiga-Feest A. 2012. Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. Ann. Bot. 110: 329-348. https://doi.org/10.1093/aob/mcs130

Madsen M. D., Davies K. W., Boyd C. S., Kerby J. D., Svejcar T. J. 2016. Emerging seed enhancement technologies for overcoming barriers to restoration. Restor. Ecol. 24: S77-S84. https://doi.org/10.1111/rec.12332

Markey A. S., Dillon D. J. 2008. Flora and vegetation ofthe banded iron formations of the Yilgarn Craton: central Tallering Land System. Conservation Science Western Australia 7: 121-149.

Merino-Martín L., Commander L., Mao Z., Stevens J. C., Miller B. P., Golos P. J., et al. 2017. Overcoming topsoil deficits in restoration of semiarid lands: Designing hydrologically favourable soil covers for seedling emergence. Ecol. Eng. 105: 102-117. https://doi.org/10.1016/j.ecoleng.2017.04.033

Murphy J., Riley J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27: 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nge F. J., Cambridge M. L., Ellsworth D. S., Zhong H., Lambers H. 2020. Cluster roots are common in *Daviesia* and allies (Mirbelioids; Fabaceae). Journal of The Royal Society of Western Australia. 103: 111-118.

Nyenda T., Gwenzi W., Gwata C., Jacobs S. M. 2020. Leguminous tree species create islands of fertility and influence the understory vegetation on nickel-mine tailings of different ages. Ecol. Eng. 155: 105902. https://doi.org/10.1016/j.ecoleng.2020.105902

Ottenhof C. J. M., Faz Cano Á., Arocena J. M., Nierop K. G. J., Verstraten J. M., van Mourik J. M. 2007. Soil organic matter from pioneer species and its implications to phytostabilization of mined sites in the Sierra de Cartagena (Spain). Chemosphere. 69: 1341-1350. https://doi.org/10.1016/j.chemosphere.2007.05.032

Parfitt R., Childs C. 1988. Estimation of forms of Fe and Al - a review, and analysis of contrasting soils by dissolution and Mossbauer methods. Soil Res. 26: 121-144. https://doi.org/10.1071/SR9880121

Payne A. L., van Vreeswyk A. M. E., Leighton K. A., Pringle H. J., Hennig P. 1998. An inventory and condition survey of the Sandstone-Yalgoo-Paynes Find area, Western

Australia. Technical Bulletin 90, Department of Agriculture and Food, Western Australia, pp. 372.

Pearse S. J., Veneklaas E. J., Cawthray G., Bolland M. D. A., Lambers H. 2007. Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. New Phytol. 173: 181-190. https://doi.org/10.1111/j.1469-8137.2006.01897.x

Pearse S. J., Veneklaas E. J., Cawthray G. R., Bolland M. D. A., Lambers H. 2006. Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. Plant Soil. 288: 127-139. https://doi.org/10.1007/s11104-006-9099-y

Png G. K., Turner B. L., Albornoz F. E., Hayes P. E., Lambers H., Laliberté E. 2017. Greater root phosphatase activity in nitrogen-fixing rhizobial but not actinorhizal plants with declining phosphorus availability. J. Ecol. 105: 1246-1255. https://doi.org/10.1111/1365-2745.12758

Rayment G. E., Lyons D. J. 2010. Soil Chemical Methods - Australasia. Collingwood, Vic.: CSIRO Publishing.

Santini T. C., Banning N. C. 2016. Alkaline tailings as novel soil forming substrates: Reframing perspectives on mining and refining wastes. Hydrometallurgy. 164: 38-47. https://doi.org/10.1016/j.hydromet.2016.04.011

Saunders W. M. H., Williams E. G. 1955. Observatioms on the determination of total organic phosphorus in soils. J. Soil Sci. 6: 254-267. https://doi.org/10.1111/j.1365-2389.1955.tb00849.x

Senior A., Britt A. F., Summerfield D., Hitchman A., Champion D., Huston D., et al. 2020. Australia's Identified Mineral Resources 2019. Geoscience Australia, Canberra, AU.

Stevens J., Dixon K. 2017. Is a science-policy nexus void leading to restoration failure in global mining? Environ. Sci. Policy. 72: 52-54. https://doi.org/10.1016/j.envsci.2017.01.006

Tang C., Robson A., Adams H. 1995. High Ca is not the primary factor in poor growth of *Lupinus angustifolius* L. in high pH soil. Australian Journal of Agricultural Research. 46: 1051-1062.

Turner B. L., Condron L. M. 2013. Pedogenesis, nutrient dynamics, and ecosystem development: the legacy of T.W. Walker and J.K. Syers. Plant Soil. 367: 1-10. https://doi.org/10.1007/s11104-013-1750-9

Turner B. L., Condron L. M., Richardson S. J., Peltzer D. A., Allison V. J. 2007. Soil organic phosphorus transformations during pedogenesis. Ecosystems. 10: 1166-1181. https://doi.org/10.1007/s10021-007-9086-z

Turner B. L., Laliberté E. 2015. Soil development and nutrient availability along a 2 million-year coastal dune chronosequence under species-rich Mediterranean shrubland in southwestern Australia. Ecosystems. 18: 287-309. https://doi.org/10.1007/s10021-014-9830-0

Vance E. D., Brookes P. C., Jenkinson D. S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-707. https://doi.org/10.1016/0038-0717(87)90052-6

Walker T. W., Syers J. K. 1976. The fate of phosphorus during pedogenesis. Geoderma. 15: 1-19. https://doi.org/10.1016/0016-7061(76)90066-5

Wang L., Ji B., Hu Y., Liu R., Sun W. 2017. A review on in situ phytoremediation of mine tailings. Chemosphere. 184: 594-600. https://doi.org/10.1016/j.chemosphere.2017.06.025

Wang Y., Shen Z., Zhang Z. 2018. Phosphorus speciation and nutrient stoichiometry in the soil-plant system during primary ecological restoration of copper mine tailings. Pedosphere. 28: 530-541. https://doi.org/10.1016/S1002-0160(18)60031-1

Woodbury D. J., Yassir I., Arbainsyah, Doroski D. A., Queenborough S. A., Ashton M. S. 2020. Filling a void: Analysis of early tropical soil and vegetative recovery under leguminous, post-coal mine reforestation plantations in East Kalimantan, Indonesia. Land Degrad. Dev. 31: 473-487. https://doi.org/10.1002/ldr.3464

Wu J., Joergensen R. G., Pommerening B., Chaussod R., Brookes P. C. 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biol. Biochem. 22: 1167-1169. https://doi.org/10.1016/0038-0717(90)90046-3

Wu S., Liu Y., Bougoure J. J., Southam G., Chan T.-S., Lu Y.-R., et al. 2019a. Organic matter amendment and plant colonization drive mineral weathering, organic carbon sequestration, and water-stable aggregation in magnetite Fe ore tailings. Environ. Sci. Tech. 53: 13720 - 13731. https://doi.org/10.1021/acs.est.9b04526

Wu S., Liu Y., Southam G., Robertson L., Chiu T. H., Cross A. T., et al. 2019b. Geochemical and mineralogical constraints in iron ore tailings limit soil formation for direct phytostabilization. Sci. Total Environ. 651: 192-202. https://doi.org/10.1016/j.scitotenv.2018.09.171

Wu S., Nguyen T. A. H., Liu Y., Southam G., Wang S., Chan T.-S., et al. 2019c. Deficiencies of secondary Fe (oxy)hydroxides associated with phyllosilicates and organic carbon limit the formation of water-stable aggregates in Fe-ore tailings. Chem. Geo. 523: 73-87. https://doi.org/10.1016/j.chemgeo.2019.06.002

You F., Dalal R., Huang L. 2018. Initiation of soil formation in weathered sulfidic Cu-Pb-Zn tailings under subtropical and semi-arid climatic conditions. Chemosphere. 204: 318-326. https://doi.org/10.1016/j.chemosphere.2018.04.037

You F., Dalal R., Mulligan D., Huang L. 2015. Quantitative measurement of organic carbon in mine wastes: methods comparison for inorganic carbon removal and organic carbon recovery. Commun. Soil Sci. Plant Anal. 46: 375-389. https://doi.org/10.1080/00103624.2014.989113

Zhong H., Smith C., Robinson B., Kim Y.-N., Dickinson N. 2020. Soil phosphorus dynamics along a short-term ecological restoration trajectory of a coastal sandplain forest in New Zealand. Land Degrad. Dev. 1-12. https://doi.org/10.1002/ldr.3782

Zhong H., Zhou J., Azmi A., Arruda A. J., Doolette A. L., Smernik R. J., et al. 2021. *Xylomelum occidentale* (Proteaceae) accesses relatively mobile soil organic phosphorus without releasing carboxylates. J. Ecol. 109: 246-259. https://doi.org/10.1111/1365-2745.13468

Zhou J., Wu Y., Prietzel J., Bing H., Yu D., Sun S., et al. 2013. Changes of soil phosphorus speciation along a 120-year soil chronosequence in the Hailuogou Glacier retreat area (Gongga Mountain, SW China). Geoderma. 195-196: 251-259. https://doi.org/10.1016/j.geoderma.2012.12.010

Zhou J., Wu Y., Turner B. L., Sun H., Wang J., Bing H., et al. 2019. Transformation of soil organic phosphorus along the Hailuogou post-glacial chronosequence, southeastern edge of the Tibetan Plateau. Geoderma. 352: 414-421. https://doi.org/10.1016/j.geoderma.2019.05.038

Zhou J., Zúñiga-Feest A., Lambers H. 2020. In the beginning, there was only bare regolith-then some plants arrived and changed the regolith. J. Plant Ecol. 13: 511-516. https://doi.org/10.1093/jpe/rtaa030

Table 1

Aboveground biomass and selected foliar nutrient concentrations of *Lupinus angustifolius* harvested in Sep 2016. Data in columns are means (n=3) with standard errors in parenthesis. The same letters indicate no significant difference (p<0.05). TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

Substrate	Fresh aboveground biomass (g)	Total N ($mg\ g^{-1}$)	Total P ($mg\ g^{-1}$)
TAL	3.3 (0.4)^{ab}	23.7 (2.8)^{a}	1.1 (0.1)^{ab}
VEG	2.9 (0.1)^{b}	23.1 (0.9)^{a}	1.2 (0.1)^{a}
ROC	4.3 (0.1)^{a}	27.4 (0.5)^{a}	0.9 (< 0.1)^{b}
TOP	4.3 (0.2)^{a}	28.0 (2.2)^{a}	0.9 (0.1)^{b}

Table 2

Total aboveground biomass and leaf area of native plants harvested in Nov 2017. Data in columns are means (n=3) with standard errors in parenthesis. The same letters indicate no significant difference ($p<0.05$). Symbols of ‘+’ and ‘-‘ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

Substrate	*L. angustifolius*	Number of native species survived	Fresh aboveground biomass (g)
TAL	+	20 (2)^{ab}	11.8 (0.8)^{a}
	-	22 (2)^{a}	10.2 (1.6)^{a}
VEG	+	16 (3)^{ab}	5.0 (1.8)^{ab}
	-	13 (2)^{b}	1.7 (0.2)^{b}
ROC	+	20 (3)^{ab}	9.0 (2.5)^{ab}
	-	18 (1)^{ab}	6.9 (2.3)^{ab}
TOP	+	17 (4)^{ab}	5.4 (2.5)^{ab}
	-	20 (3)^{ab}	12.8 (6.1)^{ab}

Figure 1. Schematic illustration of times of soil samplings and plant biomass harvestings during a 21-months glasshouse trial from March 2016 to November 2017.

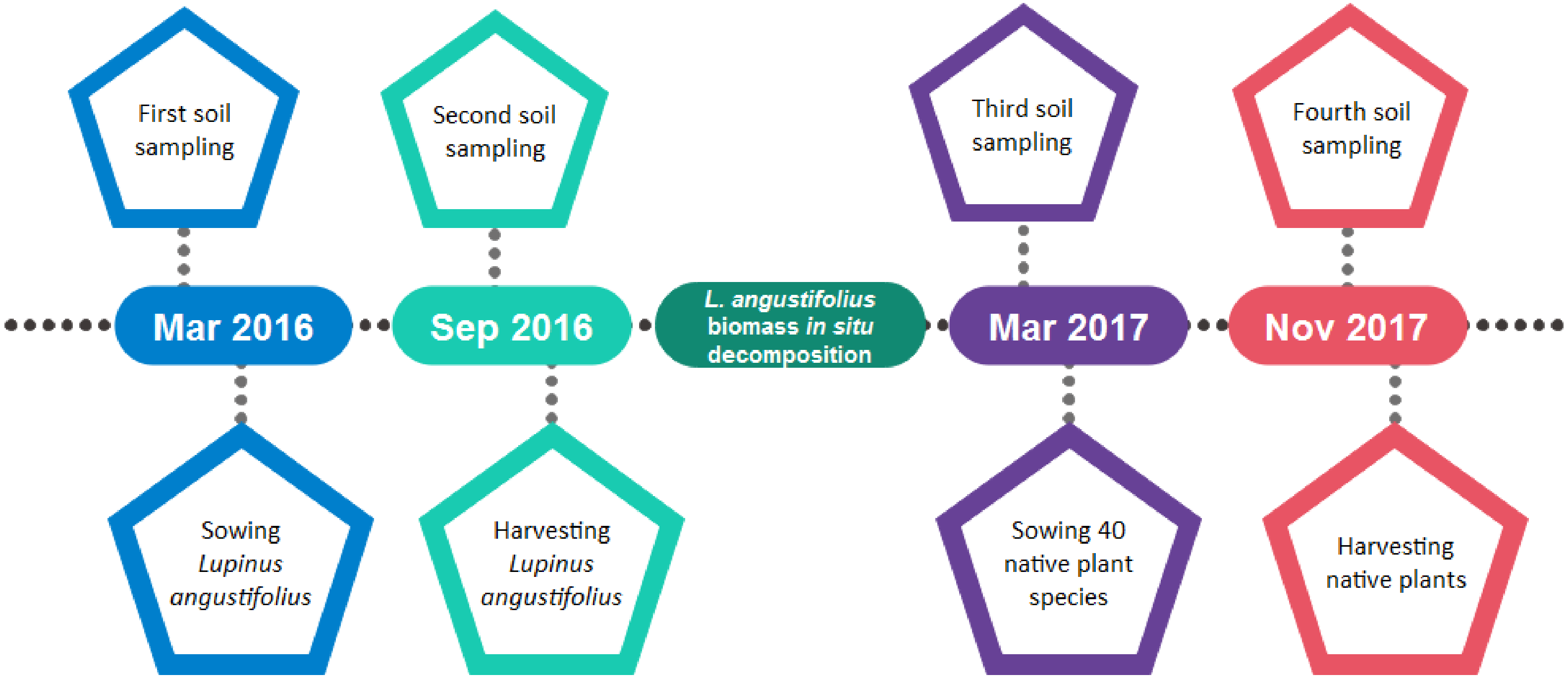

Figure 2. Changes of soil pH during a 21-months glasshouse trial using different substrates. Values are means ± standard errors (n=3). Symbols of 🟩 and ▲ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

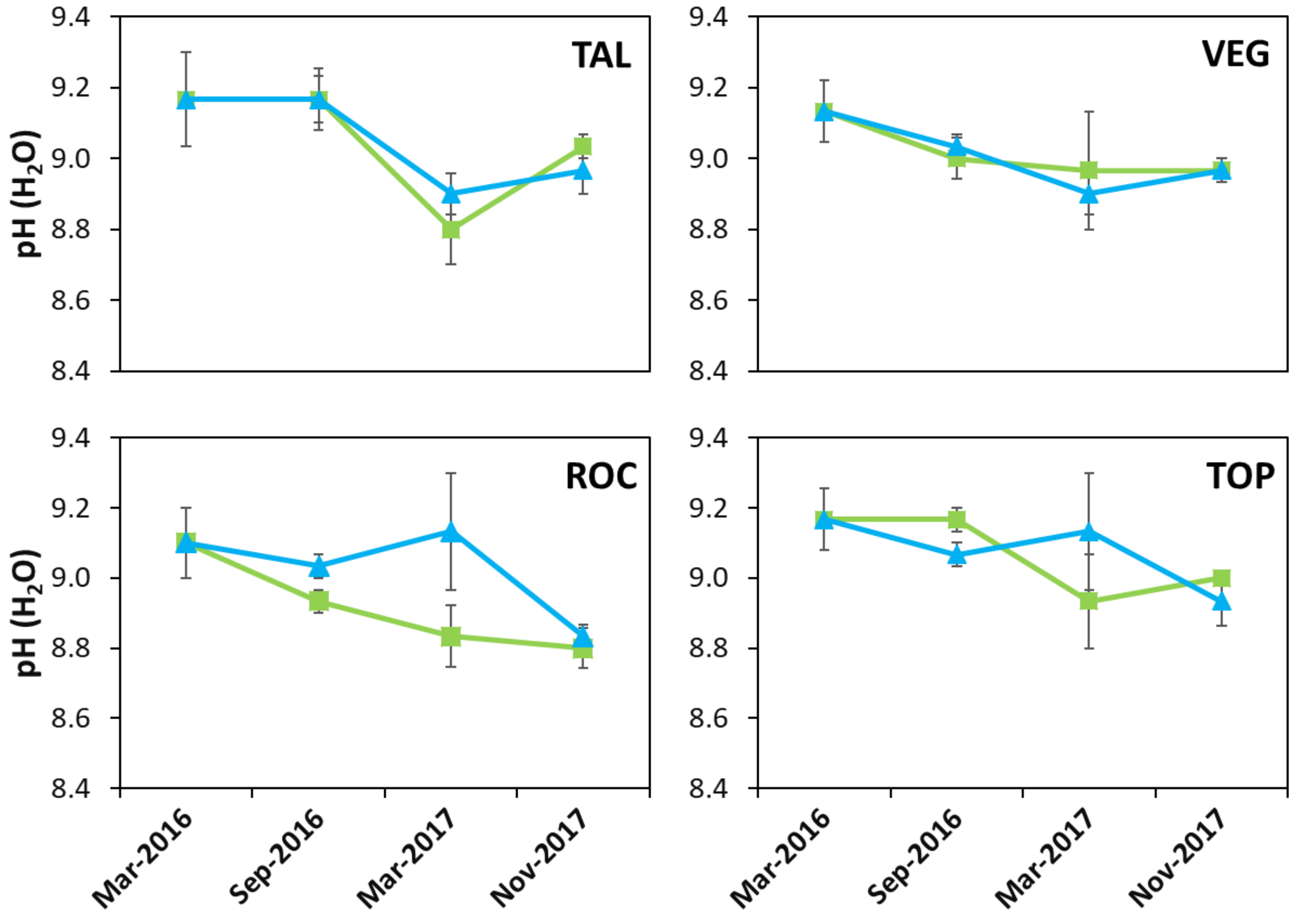

Figure 3. Changes of soil organic carbon (C) during a 21-months glasshouse trial using different substrates. Values are means ± standard errors (n=3). Symbols of 🟩 and ▲ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

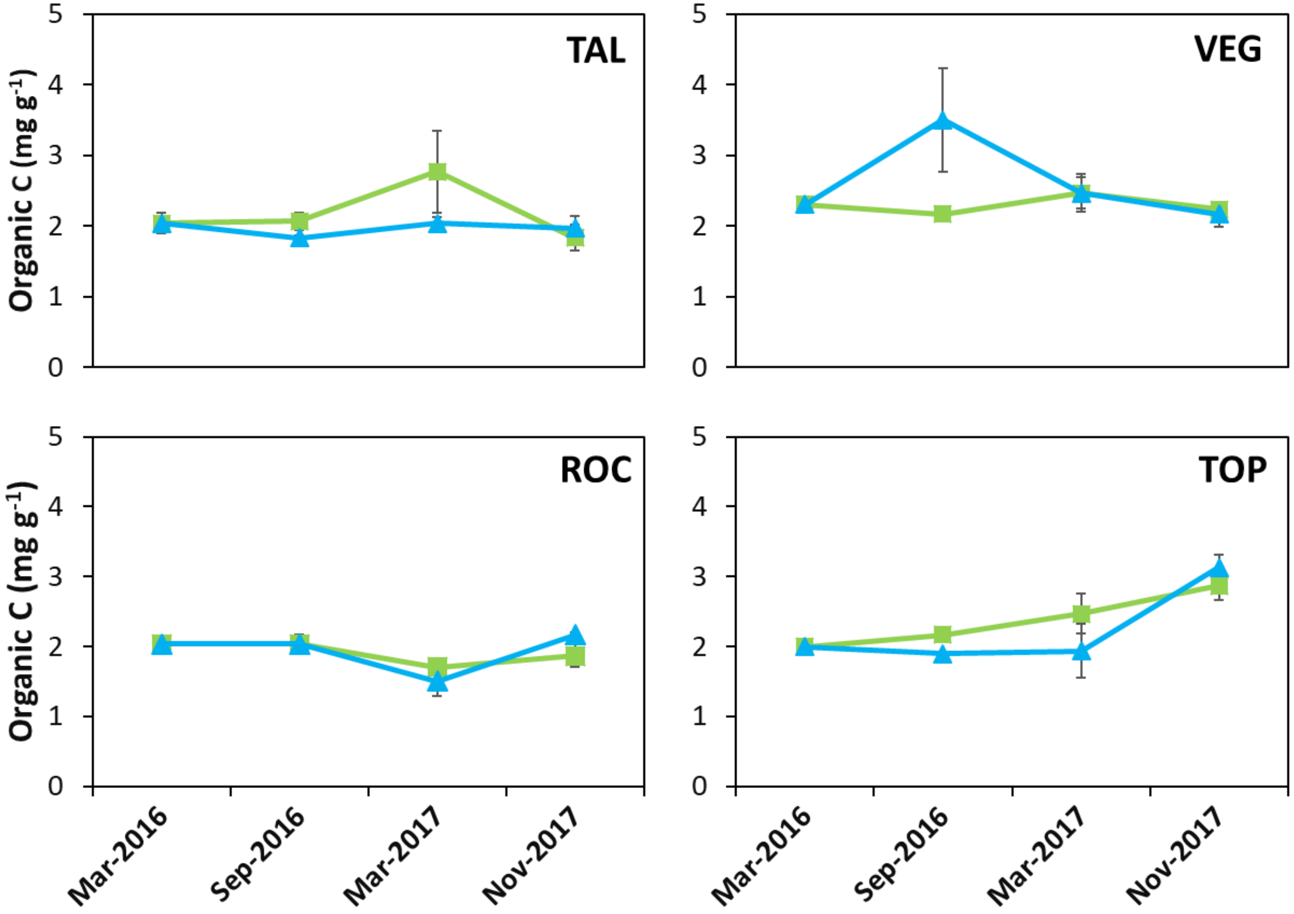

Figure 4. Changes of soil total nitrogen (N) during a 21-months glasshouse trial using different substrates. Values are means ± standard errors (n=3). Symbols of 🟩 and ▲ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

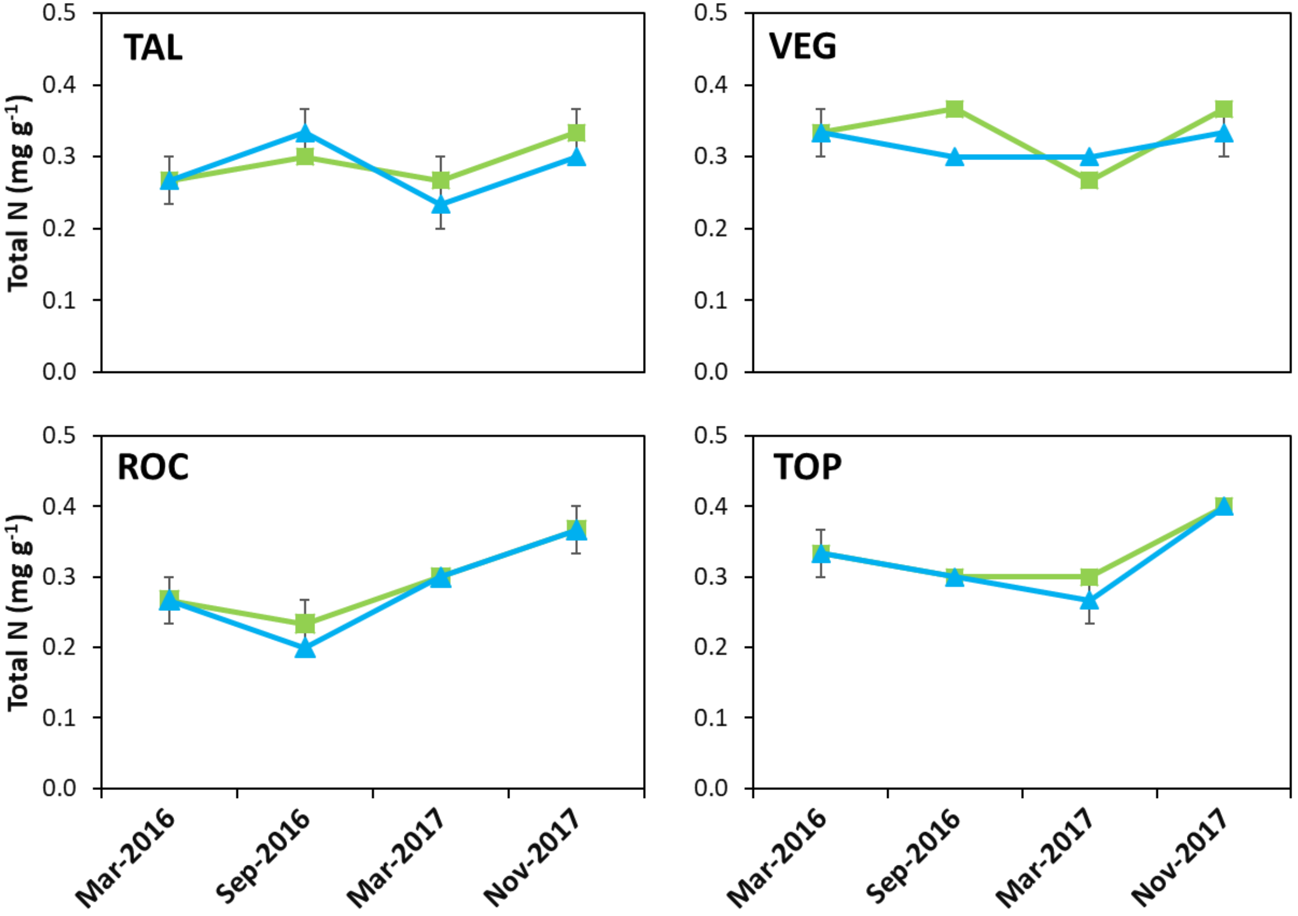

Figure 5. Change of soil organic phosphorus (P) during a 21-months glasshouse trial using different substrates. Values are means ± standard errors (n=3). Symbols of 🟩 and ▲ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.

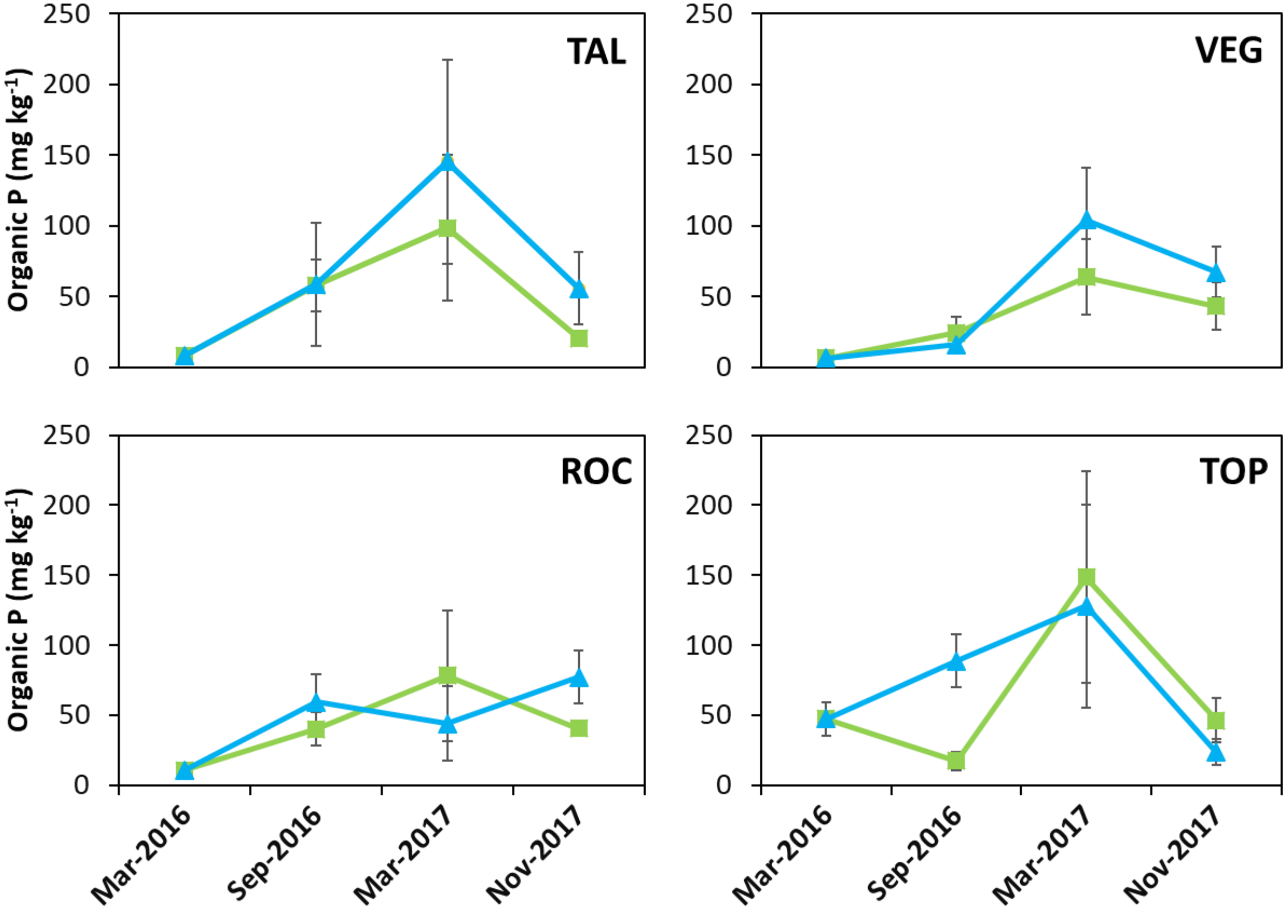

Figure 6. Change of soil pyrophosphate-extractable Al concentrations (Al_p) during a 21-months glasshouse trial using different substrates. Values are means ± standard errors (n=3). Symbols of 🟩 and ▲ indicate with and without *Lupinus angustifolius*, respectively; TAL: tailings, VEG: tailings mixed with 10% (w/w) native plant litter, ROC: tailings mixed with 10% (w/w) waste rock; and TOP: tailings mixed with 10% (w/w) stockpiled soil.