Nutrient enrichment diminishes plant diversity and density, and alters long-term ecological trajectories, in a biodiverse forest restoration

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ABSTRACT

Nutrient enrichment can negatively affect natural plant communities and result in the loss of species diversity and productivity. Despite this, fertiliser (especially phosphorus) is typically applied to restore highly biodiverse communities. Long-term effects of nutrient addition to restored plant communities, particularly those adapted to inherently low nutrient soils, have received little attention. We report results of a large-scale 20-year field experiment established in West Australian jarrah forest restored after bauxite mining Three P-application rates were applied (0, 80 and 120 kg ha^{-1}) once at the beginning of the experiment, and plant communities monitored after 1, 6, 13 and 20 years. One year after the onset of restoration, native plant species richness and plant density was highest at 80 and 120 kg P ha^{-1}. Subsequently, native species richness, plant density, and the richness and density of seeder and slow-growing resprouter species were highest without fertilisation, establishing the negative impact of P enrichment on plant community and ecosystem development in P impoverished soils. Total plant cover was similar for all P treatments across the chronosequence which, when combined with higher stem densities at zero P, suggests zero P favoured smaller, slower growing species. Applied-P initially favoured weeds and ephemerals and, while these species declined over time, other species were lost from these plots. The similarity of the restored communities to unmined reference jarrah forest increased over time and was consistently highest at in the absence of P fertiliser. Jarrah forest restoration is assumed to follow the initial floristic model of plant succession. However, we question this assumption and instead suggest that successional outcomes are contingent on P fertilisation rather than initial floristics per se., Applied P retarded recruitment of resprouter species that were present at zero P, debunking the assumption under IFM that these species do not disperse to restored areas. Consequently, based on the most comprehensive long-term study of P-fertilisation in the context of restoration of P-impoverished ecosystems yet reported, we propose that P limitation is important for the recreation of species diversity in inherently P impoverished forests. These results highlight the necessity of long-term experiments for understanding forest successional dynamics and implications for restoration practices.

Key words: competition, initial floristics model, legume, nitrogen, rehabilitation

1. Introduction:

Studies in a range of native ecosystems have shown long-term negative effects of nutrient enrichment on plant community composition, species richness and ecosystem productivity (Wheeler and Shaw, 1991; Wassen et al., 2005; Isbell et al., 2013). Understanding this relationship is key to restoring historic plant communities because soil nutrients are often modified by human activity, either enriched in the case of old-fields (e.g., Standish et al., 2006; Tibbett et al., 2019a) or depleted in the case of mine sites (e.g. Prematuri et al., 2020). Consequently, adding fertiliser to restore nutrients lost during mining is generally viewed as a key step in restoring native plant communities (e.g., EPA, 1996; Bell, 2001; Tibbett, 2010; DFAT, 2016). Fertiliser addition can also increase under- and over-storey plant growth thereby having a range of potential benefits such as a reduced risk of soil erosion (Ward et al., 1990) and increased carbon sequestration and timber production (Brancalion et al., 2019). However, longer term effects of fertiliser addition on community composition in restored mine sites have received little attention. Long-term data are needed to establish this relationship, particularly for projects with goals focused on restoring community composition and species richness similar to undisturbed reference communities.

There is increasing evidence in post-mining restoration that fertiliser application may be detrimental, at least in the short-term, to re-establishing diverse native vegetation communities. For example, in three-year-old restored sites in the Cape Floristic Region, South Africa, NPK fertiliser increased weed growth, but decreased survival of proteaceous shrubs (Holmes, 2001) that are sensitive to high P (Stock and Allsopp, 1992). Similarly, up to five years following restoration, fertiliser-P addition increased the growth of weeds and native ephemerals in restored jarrah forest in Western Australia, while decreasing the abundance of long-lived resprouter species (Daws et al., 2013; Daws et al., 2015; Tibbett et al. 2020). Resprouters are a diverse and dominant component of undisturbed jarrah forest

(Norman et al., 2006a; Koch, 2007). Nitrogen fertiliser did not have the same detrimental short-term effects (Daws et al., 2013) perhaps because excess N-fertiliser can be rapidly lost from soils and instead, nitrogen is provided by early-successional legumes (Grant et al., 2007). In contrast, P-fertiliser can persist in restored soils for at least 20 years (Banning et al., 2008; Standish et al., 2008; Spain et al., 2018; Daws et al., 2019a) and therefore potentially affects vegetation dynamics in both the short and longer terms.

The jarrah forest occurs within one of the world's biodiversity hotspots (Hopper and Gioia, 2004) with 300–400 understorey plant species in areas where mining occurs (Koch, 2007). Jarrah forest soils are highly weathered and naturally P-impoverished (Hopper, 2009). Consequently, many understorey species have specialised adaptations for P-acquisition including cluster roots, mycorrhizal symbioses and exudation of carboxylases (Lambers et al., 2008). These species can exhibit toxicity to applied-P at elevated external concentrations due to a limited ability to regulate P uptake (e.g., de Campos et al., 2013). Many Western Australian resprouter species also have low growth rates (Pate et al., 1990; Bowen, 1991; Bowen and Pate, 1993). Thus, there are two possible, non-exclusive hypotheses for negative effects of applied-P on restored jarrah forest communities. Firstly, effects on composition may result from species-specific direct negative effects of elevated P on plant survival (e.g., Holmes, 2001). Secondly, since the growth of many resprouter species to applied-P is conservative compared with that of weeds, ephemerals and N_2-fixing legumes (Daws et al., 2015; Standish et al., 2008). they may be simply out-competed in high P soils.

Jarrah forest restoration is assumed to follow the initial floristics model of plant succession (Norman et al., 2006a; Koch, 2007) whereby the composition of the developing vegetation reflects the initial community that establishes (Egler, 1954). To date, the assumption has been that this model holds because late-successional species do not arrive or arrive but fail to establish. Empirical support for the model comes from restored jarrah-forest trajectories up to 14 years of age that received P-fertiliser at the onset (Norman et al., 2006a; Koch, 2007). Plant available soil-P can remain elevated for at least 20 years in restored jarrah forest (Banning et al., 2008; Daws et al. 2019a) following a single initial P-fertiliser application. Consequently, legacy effects of P-fertiliser could help explain why late arriving species do not establish: they may be outcompeted by species that respond to, and continue to benefit from, increased soil P. These possibilities have not been explored as long-term effects of P-fertiliser on plant community composition in restored jarrah forest sites have received little attention.

In this study, we report results from a long-term (20-year) experiment of the effects of a single initial application of P-fertiliser, at rates of 0, 80 or 120 kg P ha^{-1}, on vegetation responses in restored jarrah forest. Plots were monitored 1, 6, 13 and 20 years after initial restoration. Specifically, we explore the effects of fertility (applied-P) on diversity and development of the understorey community to assess whether fertility affects the diversity and trajectory of the developing community.

2. Materials and methods

2.1. Study site

The experiment was established within the Alcoa of Australia Ltd. bauxite mining lease in the northern jarrah forest, located 60 to 100 km south-east of Perth, Western Australia (Standish et al., 2015). The area has a Mediterranean-type climate with cool, wet winters and hot, dry summers. Annual rainfall is ~1,200 mm with average summer maximum temperatures of 29 °C and average winter minimum temperatures of 6 °C (Australian Bureau of Meteorology, 2015).

The forest overstorey vegetation comprises the dominant species *Eucalyptus marginata* (jarrah) and *Corymbia calophylla* (marri). There is a mid-storey layer dominated by *Banksia grandis*, *Allocasuarina fraseriana* and *Xanthorrhoea preisii* with woody shrubs of *Bossiaea aquifolium*, various *Acacia* species, and a diverse understorey (Koch, 2007). Jarrah forest soils are gravelly with low concentrations of available N, P and K (Hingston et al., 1989; Tibbett et al., 2020) with high rates of phosphorus fixation due to the presence of amorphous iron and aluminium oxides. Generically these soils are classified as lateritic oxisols (USDA, 1999) or ferralsols (FAO, 2012).

2.2. Experimental design

A large-scale field experiment was established on recently mined pits. Restoration activities comprise pit landscaping, deep ripping, topsoil return, contour ripping and native flora seeding (Standish et al., 2015). Treatment plots of 25 m × 25 m were established in four replicate mine pits using a randomised complete block design. Plots were seeded during summer 1994 (January to April) with 1.44 kg ha^{-1} jarrah seed, 0.60 kg ha^{-1} marri seed and an understorey seed mix containing 0.72kg ha^{-1} of legume species and 0.20 kg ha^{-1} of non-legume species. Following seeding, fertiliser was applied at 0, 80 or 120 kg ha^{-1} for P (as double superphosphate) and 80 kg ha^{-1} for N (as ammonium sulphate) (Lockley and Koch, 1996).

2.3. Vegetation monitoring

In September 1995, one year after the establishment of the experiment, a 20 m × 20 m plot was established within the centre of each 25 m × 25 m plot and subdivided into twenty 2 m × 2 m quadrats with a total of 80 m^2 sampled per plot. Species identity and density were recorded for all shrub and understorey species in each quadrat with density summed for the entire plot. Plots were re-monitored in spring 2000, 2007 and 2014, i.e. at 6, 13 and 20 years of age, except that percentage cover (a visual estimate that included overhang) was also recorded for each species. Cover estimates accounted for vertical structuring of vegetation, such that the sum of all cover estimates could exceed 100%.

2.4. Soil sampling

In April 2014 six soil samples were collected from random locations, within each 20 m × 20 m plot. Samples were collected at 0–10 cm depth from the furrows of the rip-lines and mixed. 0–10 cm depth was chosen because jarrah forest soils have high rates of P fixation on amorphous iron and aluminium oxides. As a result, limited downward movement of P occurs through the soil profile (e.g. Tibbett et al., 2020). Soil was air-dried at 26 °C, gravel removed using a 2 mm sieve (Rayment and Higginson, 1992) and samples analysed at a commercial laboratory (CSBP Soil and Plant Laboratories, Bibra Lake, Perth, Australia) assessing ammonium (NH_4^+), nitrate (NO_3^-), Colwell(available)-P and pH (in water).

2.5. Statistical analysis

Each plant species was assigned to one of four categories reflecting hypothesised responses to P. We predicted that weeds and native ephemerals would respond positively to fertiliser application (Prober and Wiehl, 2012), resprouter species to respond negatively (Lambers et al., 2008) and that seeders would be mixed in their responses. Seeders must re-establish through germination and seedling establishment, whereas resprouters can re-establish by sprouting from surviving underground structures (Bell, 2001; Clarke et al. 2015). In Bell's categorisation, used for fire response, ephemerals and weeds are also seeders; whereas the seeder category used here consisted only of longer-lived species. Species were classified based on published literature (Bellairs and Bell, 1990; Bell et al., 1993; Ward et al., 1997; Smith et al., 2000; Norman et al., 2006b; Burrows et al., 2008; Western Australian Herbarium, 2012).

Analyses for the effects of P-application rate and restoration age on (1) species richness, (2) density, and (3) cover for all species combined, and the four growth forms were undertaken using linear mixed effects models in the lme4 package using the *lmer* function (Bates et al., 2011) for R (R Development Core Team 2009). P-application rate and site age were treated as fixed effects and site (pit) and subject (individual plot) as random effects.

To assess which species were associated with unfertilised plots after 20 years, species occurring more than four times in zero P plots and fewer than four times in 80 and 120 kg P ha^{-1} plots were determined. For species associated with fertilised plots, the species that occurred more than four times in 80 and 120 kg P ha^{-1} plots and fewer than four times in the zero P plots were determined (Standish et al., 2008).

A dissimilarity matrix using Sorensens dissimilarity was created based on species abundance data for the 18 reference forest plots using PC-ORD v. 6.0 (MjM Software, Gleneden Beach, OR, US), resulting in an overall forest plot: forest plot similarity value (1 – dissimilarity). Subsequently, for each experimental plot, pair-wise similarity values were determined by comparison with each and every forest reference plot resulting in 18 pair-wise similarity values per experimental plot; the average of these values was then expressed as a percentage of the overall forest plot: forest plot similarity value.

3. Results:

3.1. Soil analysis

Twenty years after P-application, soil (Colwell) P concentrations were significantly higher as P-application rates increased (Table 1, P = 0.001). P-application had no effect on soil NH_4^+ concentrations (P = 0.496), and NO_3^- concentrations were lower than the detection limits of the analysis (≤ 1 mg kg^{-1}). Soil pH was significantly lower at 80 and 120 kg P ha^{-1} compared with zero P (P < 0.01, Table 1).

3.2. Effects of applied-P and time since restoration on plant species richness

After one-year, native plant species richness was lower at zero P than 80 or 120 kg P ha^{-1}. Subsequently, species richness increased at zero P, whilst generally declining in the 80 and 120 kg P ha^{-1} treatments. By 6 years of age, richness was highest at zero P. This was maintained up to 20 years of age (Fig. 2A): after 20 years, there were 33.5 species at zero P compared with 20.4 and 24.5 at 80 and 120 kg P ha^{-1}, respectively. The effects of applied-P, time since restoration and the P × Time interaction on species numbers were significant (Table 2).

After one year there were, on average, an additional 5.5 weed species at 80 and 120 kg P ha^{-1} compared with zero P (Fig. 3A).For the remainder of the experiment, the number of weed species at 80 and 120 kg P ha^{-1} declined to be similar to those observed at zero P (Fig 3A), although even after 20 years three of the nine species associated with fertilised plots were weeds (Table 3). The effects of applied-P, time since restoration and the Time × P interaction on the number of weed species were significant (Table 2). Ephemeral species exhibited a similar trend to weeds (Fig 3B). Time since restoration and the Time × P interaction had a significant effect on the number of ephemeral species (Table 2): the effect of P was not significant (Table 2).

The number of seeder species was initially similar at all three P-application rates (Fig. 3C). From six years onwards, the number of seeders declined, although this decline was least pronounced at zero P. As a result, species richness of this group at the 6, 13 and 20-year monitoring intervals was highest at zero P (Fig. 3C). The effects of time and P-application rate on species richness of seeders were significant (Table 2).

Species richness of resprouters was initially similar at all three P-application rates. Subsequently, the number of resprouters increased, particularly at zero P where the number of species more than doubled over the duration of the experiment (Fig. 3D). After 20 years, 15 of the 19 species associated with the unfertilised plots were resprouters (Table 3). The effects of time since restoration, applied P and the Time × P interaction on the number of resprouter species were significant (Table 2).

3.3. Effects of applied-P and time since restoration on stem density and cover

The number of individual native plants was initially (after one year) highest at 80 and 120 kg P ha^{-1} (Figure 2B). Subsequently stem density increased at zero P while declining in the P addition treatments: after 20-years, stem density was nearly twice as high at zero P compared with either 80 or 120 kg P ha^{-1}. This cross-over in density related to P-application rate was reflected in a significant Time × P interaction (Table 2). The effect of time since restoration on the density of native species was also significant (Table 2).

Weed density exhibited little change over time (Fig 4A): neither the effects of applied-P nor time were significant (Table 2). After one year, applied-P resulted in ~2 additional ephemeral plants m^{-2} compared with zero P (Fig. 4B). Subsequently, the density of ephemerals increased slightly at zero P and declined in the two P-addition treatments. This result was supported by a significant effect of time and a significant Time × P interaction (Table 2).

At 80 and 120 kg P ha^{-1}, the density of seeder species exhibited little change over the 20-year experiment (Fig. 4C). However, at zero P, seeder density increased significantly between six and 13 years, thereafter declining (Fig. 4C). The effects of both time and applied-P on seeder density were significant (Table 2). At all three P-application rates, stem density of resprouter species increased with time: the effect of time was highly significant (Table 2). However, the relative increase was greatest at zero P, where the resprouter density was initially lowest: density increased more than three-fold in this treatment (Fig 4D) and after 20 years resprouter density was highest at zero P. Both the main effect of applied-P and the Time × P interaction were significant (Table 2).

Between 6 and 13 years, total plant cover approximately doubled in all three P treatments, before declining at 20 years (Figure 2C). After six years cover was lowest at zero P but after 20 years this treatment resulted in the highest cover: the Time × P interaction was significant (Table 2), although the main effect of P was not (Table 2). The change in percentage cover over time was also highly significant (Table 2).

Percentage cover of weeds and ephemerals was low throughout the experiment, neither time nor applied-P had a significant effect on percentage cover (Table 2; Fig 5AB). The cover of seeder species changed significantly over time (Table 2; Figure 5C), peaking at 13 years before declining by 20 years. There was no effect of P-treatment on cover associated with seeders (Table 2).

Percentage cover of resprouters changed significantly over time (Table 2). Cover increased between 6 and 13 years and then declined slightly (Fig 5D). Cover was initially (after 6 years) higher at zero P than either 80 or 120 kg P ha^{-1} and this difference increased over time: after 20 years, cover at zero P was more than twice that in the P-addition treatments (Fig. 5D). This was supported by a significant main effect of P-treatment and a significant Time × P interaction (Table 2).

3.4. Changes in community composition with time since restoration

Applied-P had a significant effect on the similarity of the restored communities to reference unmined forest plots: similarity was consistently highest at zero P (General Linear Mixed Model, $P < 0.05$; Fig. 6). In addition, the similarity of the restored communities to the forest sites increased significantly over time (General Linear Mixed Model, $P < 0.001$).

4. Discussion:

Fertiliser application is broadly advocated as best practice for re-establishing native plant communities in post-mining restoration (e.g., EPA, 1996; Minerals Council of South Africa, 2007; Tibbett, 2010; DFAT, 2016). However, we found that P-fertiliser had significant, long-term effects both above- and below-ground on restored jarrah forest. In particular, we report negative impacts of P-fertilisation on long-term soil-P concentrations, species richness and community composition. Negative impacts likely result from direct effects of elevated P (i.e. toxicity; Lambers et al., 2008; Williams et al., 2019) and facilitation of competitive plant species (e.g., legumes and overstorey trees; Daws et al., 2015). Our findings have significant implications for restoring nutrient-limited systems world-wide.

Following a single application of P-fertiliser at the outset of restoration, available soil P remained elevated after 20 years compared with the zero P treatment (this study), and concentrations measured in undisturbed jarrah forest (Ward, 2000; Standish et al. 2008; Tibbett et al., 2020). In Brazilian Atlantic rainforest restored after bauxite mining Bizuti et al. (2020) found that, soil P pools remained lower than unmined forest, even with fertiliser inputs. However, previous studies in jarrah forest and other restored *Eucalyptus* forests reported that a single P-fertiliser application elevated soil-P concentrations for at least 20 years (Spain et al., 2018; Banning et al., 2008). These differences may result from the jarrah forest soils having soil nutrient levels that are low by global standards and, at least for the jarrah forest, suggests P application may have long-term impacts on plant responses.

P-fertiliser addition initially benefitted weeds and native ephemerals. This effect of P-addition has been reported previously in both restored jarrah forest and other restored vegetation communities (e.g., Prober and Wiehl 2002; Holmes, 2001; Daws et al., 2013). However, as the establishing vegetation developed, these species declined in abundance as also reported by Norman et al. (2006a). However, given their decline and the low percentage cover associated with these two groups, they are unlikely to be responsible for driving the negative long-term responses to P observed for other understorey groups (e.g. resprouters).

Species richness and density of seeder species was generally highest in one-year old restored sites, declining thereafter. Conversely, percentage cover of this group of species reached a maximum after 13 years before declining. Many seeders are N_2-fixing legumes that exhibit significant initial growth to applied-P (e.g., Daws et al., 2013, 2015) before declining over time as individuals senesce (Grant et al., 2007). Apart from year one, both the number of species and stem density of seeders was highest at zero P suggesting this group generally benefitted from a less competitive environment. Similarly, Daws et al. (2015) reported that among P-responsive legumes, while total cover responded positively to applied P, species richness was reduced at either 20 or 80 kg P ha^{-1} compared with zero P.

For resprouter species, including many cluster-root forming species, richness, stem density and cover were highest at zero P throughout the 20-year duration of this experiment. One explanation for this response is that reduced competition at zero P benefitted establishment of these slow-growing species. Competition was likely to be more intense in the applied-P treatments due to increased growth of reseeders (see above) as well as potentially increased competition from the establishing overstorey layer. In newly established jarrah stands, tree growth responds positively to P-application, although these benefits of fertiliser application largely disappear by 15 years of age (Daws et al., 2019b). Furthermore, studies in a range of forested systems have shown negative effects of intense overstorey competition on understorey species richness (e.g. Chan et al., 2006). Alternatively, there may have been direct negative effects of applied-P on growth and survival in the 80 and 120 kg P ha^{-1} treatments. Since P-toxicity has been observed for various Western Australian species (de Campos 2013; Williams et al., 2019), further experiments are required to disentangle relative effects of competition and P-toxicity on the (re-)establishment of resprouter species in jarrah forest restoration.

Resprouter species are under-represented in restored jarrah forest compared with reference forest (Norman et al., 2006a; Koch, 2007). Our data suggest that their low abundance may partially reflect the routine application of P-fertiliser (80 kg ha^{-1} prior to 2004 and 40 kg P ha^{-1} from 2004 onwards; Standish et al., 2015). Since many species associated with zero P plots were resprouters, this finding has significant practical implications: Alcoa propagates and plants seven resprouter species (listed exclusively as zero P species in Table 3) into restored sites to increase their abundance (A.H. Grigg pers. comm.). A low P-fertiliser regime may potentially increase the success of planting these species.

Applied P reduced the overall similarity of the composition of restored forest to reference forest sites. Similarly, P-application rates as low as 20 kg P ha^{-1} reduced the similarity of 2.5-year-old restored sites compared with reference forest sites (Daws et al. 2013). In addition, the composition of all restored sites became more similar over time to the reference sites, although this was most pronounced at zero P. Increasing similarity was likely driven by, (1) fewer weeds and ephemerals at zero P, (2) a higher number of resprouter species at zero P, with this number increasing over time, and (3) a decrease in abundance of seeders over time in all three treatments.

Consistent with previous studies (e.g., Norman et al., 2006a), we found some support for the Initial Floristics Model (IFM) in plots that received 80 or 120 kg P ha^{-1}. Specifically, the number of resprouter species was relatively constant over time for plots receiving 80 and 120 kg P ha^{-1} and overall species richness declined, reflecting species losses rather than gains. In contrast, there was no support for the IFM in the successional trajectory of unfertilised plots. Support for the model was lacking in key parameters including the increase in similarity of the restored communities to reference forest plots, and the more than doubling in species richness of resprouter species at zero P over the 20-year experiment. Indeed, the data suggest a relay floristics model could be appropriate whereby early colonists such as weeds and ephemerals, and ultimately Acacias and other legumes, are replaced by resprouter species. Ongoing recruitment of resprouter species may result from extended seed dormancy in the soil seed bank, or seed dispersal into plots (or both). Rather than resprouter species failing to arrive at restored sites over time, which has been the assumption under the IFM (Koch, 2007), our data suggest elevated P may retard species recruitment and hence disrupt, succession. The mechanisms for these effects could be: (1) P-responsive competitive plants limiting establishment of these typically slow-growing species, (2) an accumulation of fine woody debris and leaf litter, which can occur at high P-application rates (Grant et al., 2007), inhibiting seedling emergence, or (3) direct toxicity from elevated P. Consequently, we propose that jarrah forest succession is contingent on P fertilisation rather than initial floristics per se.

One year after restoration, applied-P resulted in higher species richness of native species and a higher plant density. Combined with applied-P increasing plant cover in newly restored sites (e.g., Daws et al., 2013), this suggests an early assessment of restoration success would come to the *incorrect* conclusion that applying P was beneficial. However, at 20-years, 80 or 120 kg P ha^{-1} resulted in fewer native species, fewer individual plants, a similar level of understorey cover and lower similarity to target vegetation than zero applied-P, demonstrating the value of long-term monitoring for determining restoration success.

5. Conclusion

We propose that P limitation and moderation of fertiliser inputs may be important for re-establishing long-term species diversity in naturally P-impoverished forests, such as the jarrah forest. Since there is considerable overlap between areas of high plant species richness, nutrient deficient soils and current and prospective mining areas (Tibbett et al., 2019b), these findings have potential broad applicability in post mining restoration.

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References

Banning, N.C., Grant, C.D., Jones, D.L., & Murphy, D.V. (2008) Recovery of soil organic matter, organic matter turnover and nitrogen cycling in a post-mining forest rehabilitation chronosequence. Soil Biology and Biochemistry, 40, 2021–2031.

Bates, D., Maechler, M., & Bolker, B. (2011) Lme4: linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.Rproject.org/package=lme4. Accessed March 2013.

Bell, D.T. (2001) Ecological response syndromes in the flora of southwestern Western Australia: fire resprouters versus reseeders. Botanical Review, 67, 417–440.

Bell, I.C. (2001) Establishment of native ecosystems after mining - Australian experience across diverse biogeographic zones. Ecological Engineering, 17, 179–186.

Bell, D.T., Plummer, J.A., & Taylor, S.K. (1993) Seed germination ecology in southwestern Western Australia. Botanical Review, 59, 24–73.

Bellairs, S.M., & Bell, D.T. (1990) Canopy-borne seed store in three Western Australian plant communities. Australian Journal of Ecology, 15, 299–305.

Bizuti, D.T.G., Soares, T.M., Duarte, M.M., Casagrande, J.C., Moreno, V.S., Peinado F.J.M., de Medeiros, S.D.S., van Melis, J., Schweizer D., & Brancalion, P.H.S. (2020) Recovery of soil phosphorus on former bauxite mines through tropical forest restoration. Restoration Ecology 28, 1237-1246.

Bowen, B.J. (1991) Fire response within the family Proteaceae: A comparison of plants displaying the seeder and resprouter mode of recovery. PhD thesis, University of Western Australia, Perth, WA, Australia.

Bowen, B.J., & Pate, J.S. (1993) The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae). Annals of Botany, 72, 7–16.

Brancalion, P.H.S., Campoe, O., Mendes, J.C.T., Noel, C., Moreira, G.G., van Melis, J., Stape, J.L. & Guillemot, J. (2019) Intensive silviculture enhances biomass accumulation and tree diversity recovery in tropical forest restoration. Ecological Applications 29, e01847.

Burrows, N.D., Wardell-Johnson, G., & Ward, B. (2008) Post-fire juvenile period of plants in south-west Australia forests and implications for fire management. Journal of the Royal Society of Western Australia, 91, 163–174, Fire response database available at http://naturemap.dec.wa.gov.au

Chan, S.S., Larson, D.J., Maas-Hebner, K.G., Emmingham, W.H., Johnston, S.R. & Mikowski, D.A. (2006) Overstory and understory development in thinned and underplanted Oregon Coast Range Douglas-fir stands. Canadian Journal of Forest Research 36, 2696-2711.

Clarke, K.R., & Warwick, R.M. (1994) Change in marine communities: an approach to statistical analysis and interpretation. Natural Environment Research Council, Swindon, UK.

Clarke, P.J., Lawes, M.J., Murphy, B.P., Russell-Smith, J., Nano, C.E.M., Bradstock, R., Enright, N.J., Fontaine, J.B., Gosper, C.R., Radford, I., Midgley, J.J., Gunton, R.M. (2015) A synthesis of postfire recovery traits of woody plants in Australian ecosystems. Science of the Total Environment 534, 31-42.

Daws, M.I., Standish, R.J., Koch, J.M., & Morald, T.K. (2013) Nitrogen and phosphorus fertiliser regime affect jarrah forest restoration after bauxite mining in Western Australia. Applied Vegetation Science, 16, 610–618.

Daws, M.I., Standish, R.J., Koch, J.M., Morald, T.K., Tibbett, M., & Hobbs, R.J. (2015) Phosphorus fertilisation and large legume species affect jarrah forest restoration after bauxite mining. Forest Ecology and Management, 354, 10–17.

Daws, M.I., Grigg, A.H., Standish, R.J., & Tibbett, M. (2019a) Applied phosphorus has long-term impacts on vegetation responses in restored jarrah forest, In: A.B. Fourie and M. Tibbett (eds.) Proceedings of the 13th International Conference on Mine Closure, Australian Centre for Geomechanics, Perth, pp. 693-704.

Daws, M.I., Grigg, A.H., Tibbett, M., & Standish, R.J. (2019b) Enduring effects of large legumes and phosphorus fertiliser on jarrah forest restoration 15 years after bauxite mining. Forest Ecology and Management, 438, 204–214.

de Campos, M.C.R., Pearse, S.J., Oliveira, R.S. & Lambers, H. (2013) Downregulation of net phosphorus-uptake capacity is inversely related to leaf phosphorus-resorption proficiency 464 in four species from a phosphorus-impoverished environment. Annals of Botany 111, 445–454.

DFAT (2016) Mine Rehabilitation: Leading Practice Sustainable Development Program for the Mining Industry. Department of Foreign Affairs and Trade, Commonwealth Government of Australia, Canberra, Australia.

Egler, F.E. (1954) Vegetation science concepts. I. Initial floristic composition, a factor in oldfield vegetation development. Vegetatio, 4, 412–417.

(EPA) Environment Protection Agency. 1995. Rehabilitation and revegetation. Best Practice Environmental Management in Mining, Commonwealth of Australia, Canberra, Australia

Grant, C.D., Ward, S.C., & Morley, S.C. (2007) Return of ecosystem function to restored bauxite mines in Western Australia. Restoration Ecology, 15, S94–S103.

Grigg A.H., & Grant C.D. (2009) Overstorey growth response to thinning, burning and fertiliser in 10–13-year-old rehabilitated jarrah (*Eucalyptus marginata*) forest after bauxite mining in south-western Australia. Australian Forestry 72, 80–86.

Holmes, P.M. (2001) Shrubland restoration following woody alien invasion and mining: effects of topsoil depth, seed source, and fertilizer addition. Restoration Ecology, 9, 71–84.

Hopper, S.D. (2009) OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. Plant and Soil 322, 49-86.

Hopper, S.D., & Gioia, P. (2004) The Southwest Australian Floristic region: evolution and conservation of a global hot spot of biodiversity. Annual Review of Ecology, Evolution and Systematics 35, 623-650.

Isbell, F., Reich, P.B., Tilman, D., Hobbie, S.E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. Proceedings of the National Academy of Sciences, 110, 11911-11916.

Koch, J.M. (2007) Restoring a Jarrah forest understorey vegetation after bauxite mining in Western Australia. Restoration Ecology, 15, S26–S39.

Lambers, H., Raven, J.A., Shaver, G.R. & Smith, S.E. (2008) Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution, 23, 95–103.

Lockley, I.R., & Koch, J.M. (1996) Response of two eucalypt species to fertilizer application on rehabilitated bauxite mines in Western Australia. Alcoa of Australia Ltd., Research Bulletin No.27.

Minerals Council of South Africa, (2007) Guidelines for the rehabilitation of mine land. Chamber of Mines of South Africa / Coaltech.

Norman, M.A., Koch, J.M., Grant, C.D., Morald, T.K., & Ward, S.C. (2006a) Vegetation succession after bauxite mining in Western Australia. Restoration Ecology, 14, 278–288.

Norman, M.A., Plummer, J.A., Koch, J.M., & Mullins, G.R. (2006b) Optimising smoke treatments for jarrah (*Eucalyptus marginata*) forest rehabilitation. Australian Journal of Botany, 54, 571–581.

Pate, J.S., Froend, R.H., Bowen, B.J., Hansen, A., & Kuo, J. (1990) Seedling growth and storage characteristics of seeder and resprouter species of Mediterranean-type ecosystems of S.W. Australia. Annals of Botany, 65, 585–601.

Prematuri, R., Turjaman, M., Sato, T., & Tawaraya, K. (2020) Post bauxite mining land soil characteristics and its effects on the growth of *Falcataria moluccana* (Miq.) Barneby & J. W. Grimes and *Albizia saman* (Jacq.) Merr. Applied and Environmental Soil Science article ID 6764380.

Prober, S.M., & Wiehl, G. (2012) Relationships among soil fertility, native plant diversity and exotic plant abundance inform restoration of forb-rich eucalypt woodlands. Diversity and Distributions, 18, 795–807.

R Development Core Team (2009) R 2.9.0. The R Foundation for Statistical Computing, Vienna.

Rayment, G.E., & Higginson, F.R. (1992) Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne.

Smith, M.A., Grant, C.D., Loneragan, W.A., & Koch, J.M. (2004) Fire management implications of fuel loads and vegetation structure in jarrah forest restoration on bauxite mines in Western Australia. Forest Ecology and Management, 187, 247–266.

Smith, M.A., Loneragan, W.A., Grant, C.D., & Koch, J.M. (2000) Effect of fire on the topsoil seed banks of rehabilitated bauxite mine sites in the jarrah forest of Western Australia. Ecological Management and Restoration, 1, 50–58.

Spain, A.V., Tibbett, M., Ridd, M., & McLaren, T.I. (2018) Phosphorus dynamics in a tropical forest soil restored after strip mining. Plant and Soil, 427, 105–123.

Standish, R.J., Cramer, V.A., Hobbs, R.J., & Kobryn, H.T. (2006) Legacy of land-use evident in soils of Western Australia's wheatbelt. Plant and Soil, 280, 189–207.

Standish, R.J., Morald, T.K., Koch, J.M., Hobbs, R.J., & Tibbett, M. (2008) Restoring jarrah forest after bauxite mining in Western Australia: the effect of fertiliser on floristic diversity and composition. In: Fourie, A.B., Tibbett, M., Weiersbye, I.M., Dye, P.J. (Eds.), Mine Closure 2008. Australian Centre for Geomechanics, Perth, Western Australia, pp. 717–725.

Standish, R.J., Daws, M.I., Gove, A.D., Didham, R.K., Grigg, A.H., Koch, J.M., & Hobbs, R.J. (2015) Long-term data suggest jarrah-forest establishment at restored mine sites is resistant to climate variability. Journal of Ecology, 103, 78–89.

Stock, W.D., & Allsopp, N. (1992) Functional perspective of ecosystems. Pages 241–259 in R. M. Cowling, editor. The ecology of fynbos: nutrients, fire and diversity. Oxford University Press, Cape Town.

Tibbett, M. (2010) Large-scale Mine Site Restoration of Australian Eucalypt Forests After Bauxite Mining: Soil Management and Ecosystem Development. In: Ecology of Industrial Pollution, L.C. Batty & K. Hallberg, (Eds), pp. 309-326. Cambridge University Press, UK.

Tibbett, M., Gil-Martínez, M., Fraser, T., Green, I.D., Duddigan, S., De Oliveira V., … Diaz, A. (2019a) Long-term acidification of pH neutral grasslands affects soil biodiversity, fertility and function in a heathland restoration. Catena, 180, 401–415.

Tibbett, M., O'Connor, R & Daws, M.I. (2019b) Too much of a good thing: phosphorus over-fertilisation in rehabilitated landscapes of high biodiversity value. In: A.B. Fourie and M. Tibbett (eds.) Proceedings of the 13th International Conference on Mine Closure, Australian Centre for Geomechanics, Perth, pp. 651-665.

Tibbett, M., Daws, M.I., George, S.J., & Ryan, M.H. (2020) The where, when and what of phosphorus fertilisation for seedling establishment in a biodiverse jarrah forest restoration after bauxite mining in Western Australia. Ecological Engineering, 153, 105907.

Ward, S.C. (2000) Soil development on rehabilitated bauxite mines in south-west Australia. Australian Journal of Soil Research, 38, 453–464.

Ward, S.C., Koch, J.M., & Grant, C.D. (1997) Ecological aspects of soil seed-banks in relation to bauxite mining. I. Unmined jarrah forest. Australian Journal of Ecology, 22, 169–176.

Ward, S.C., Koch, J.M., & Nichols, O.G. (1990) Bauxite mine rehabilitation in the Darling Range, Western Australia. Proceedings of the Ecology Society of Australia, 16, 557–565.

Wassen, M.J., Venterink, H.O., Lapshina, E.D., & Tanneberger, F. (2005) Endangered plants persist under phosphorus limitation. Nature, 437, 547–550.

Western Australian Herbarium (2012) FloraBase – the Western Australian Flora. Department of Environment and Conservation. <http://florabase.dec.wa.gov.au/>.

Wheeler, B.D., & Shaw, S.C. (1991) Aboveground crop mass and species richness of the principal types of herbaceous rich-fen vegetation of lowland England and Wales. Journal of Ecology, 79, 285–301.

Williams, A., George, S., Birt, H.W.G., Daws, M.I., & Tibbett, M. (2019) Sensitivity of seedling growth to phosphorus supply in six tree species of the Australian Great Western Woodlands. Australian Journal of Botany, 67, 390-396.

Table 1: Mean (±1SE) soil NH_4^+, NO_3^- Colwell(available)-P and soil pH, at 0-10 cm depth, across the phosphorus fertiliser treatments in 20-year-old restored jarrah forest.

Fertiliser treatment	Application rate (kg ha^{-1})	Colwell P (mg kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	Soil pH
	0	1.22 ± 0.11[a]	4.73 ± 0.56[a]	< 1	4.97 ± 0.06[a]
Phosphorus	80	4.58 ± 0.51[b]	4.72 ± 0.49[a]	< 1	4.70 ± 0.07[b]
	120	6.98 ± 0.81[c]	4.77 ± 0.36[a]	< 1	4.63 ± 0.06[b]

Superscripts indicate a significant difference (One-way ANOVA, $P < 0.05$) between values within each column.

Table 2: Summary of results from the general linear model analyses of the effects of P-application rate and time since initiation of restoration on responses of the species groupings.

Factors	All native species	Weeds	Ephemerals	Seeder species	Resprouter species
Species richness					
P-application rate	*	*	n.sig.	*	*
Time	***	***	***	***	***
P × time	***	*	*	n.sig.	*
Stem density					
P-application rate	n.sig.	n.sig.	n.sig.	*	*
Time	**	n.sig.	**	***	***
P × time	*	n.sig.	*	n.sig.	*
Plant cover					
P-application rate	n.sig.	n.sig.	n.sig.	n.sig.	*
Time	***	n.sig.	n.sig.	***	***
P × time	**	n.sig.	n.sig.	n.sig.	*

n.sig. $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 3: Species associated with unfertilised and fertilised plots in restored sites at 20 years. Species denoted with an asterisk are exotic, those in bold are resprouters.

0P plots		**80P and 120P plots**	
Anthericaeae	***Thysanotus fastigiatus***	Asteraceae	*Hypochaeris glabra**
Apiaceae	***Pentapeltis peltigera***	Colchicaceae	***Burchardia congesta***
	Xanthosia candida	Euphorbiaceae	*Phyllanthus calycinus*
Campanulaceae	*Wahlenbergia preisii*	Fabaceae	*Acacia extensa*
Cyperaceae	***Tetraria capillaris***†		*Paraserianthes lophantha*
Dasypogonaceae	***Lomandra caespitosa***†	Orchidaceae	***Caladenia latifolia***
	L. hermaphrodita†	Pittosporaceae	*Billardiera heterophylla*
	L. sonderi†	Poaceae	*Aira caryophyllea**
Epacridaceae	*Andersonia lehmanniana*		*Pentaschistis airoides**
Dilleniaceae	***Hibbertia acerosa***		
	H. amplexicaulis†		
Fabaceae	*Acacia drummondii*		
Goodeniaceae †	***Lechenaultia biloba***		
	Scaevola calliptera†		
Haemodoraceae	***Conostylis setosa***		
Orchidaceae	***Microtis media***		
Proteaceae	***Hakea undulata***		
Rutaceae	***Boronia fastigiatus***		
Tremandraceae	***Tetratheca hirsuta***		

†Currently propagated by Alcoa and planted into newly restored sites.

Figure legends:

Figure 1. Photograph of one of the 20-year-old experimental plots.

Figure 2. Effects of P-fertiliser application rate on: (A) species richness, (B) stem density, and (C) total native species cover, over time. Error bars ±1SE of the mean.

Figure 3. Effects of P-fertiliser application rate on the species richness of, (A) weeds, (B) ephemerals, (C) seeders, and (D) resprouters, over time. Error bars ±1SE of the mean.

Figure 4. Effects of P-fertiliser application rate on density of, (A) weeds, (B) ephemerals, (C) seeders, and (D) resprouters, over time. Error bars ±1SE of the mean although, for clarity, error bars for the weeds are +1SE of the mean. High weed density at 120 kg P ha^{-1} at 13 years was driven by an extremely high density of *Aira caryophyllea* in a single plot.

Figure 5. Effects of P-fertiliser application rate on the percentage cover of, (A) weeds, (B) ephemerals, (C) seeders, and (D) resprouters, over time. Error bars ±1SE of the mean. High weed cover at 120 kg P ha^{-1} at 13 years was driven by an extremely high abundance of *Aira caryophyllea* in a single plot.

Figure 6. Effects of P-fertiliser application rate on the similarity of species composition between the fertiliser treatments and adjacent reference forest plots over time. Similarity values for the treatment plots are expressed as a percentage of the average value of forest plot: forest plot similarity. Error bars ±1SE of the mean.

Figure 1:

Figure 2:

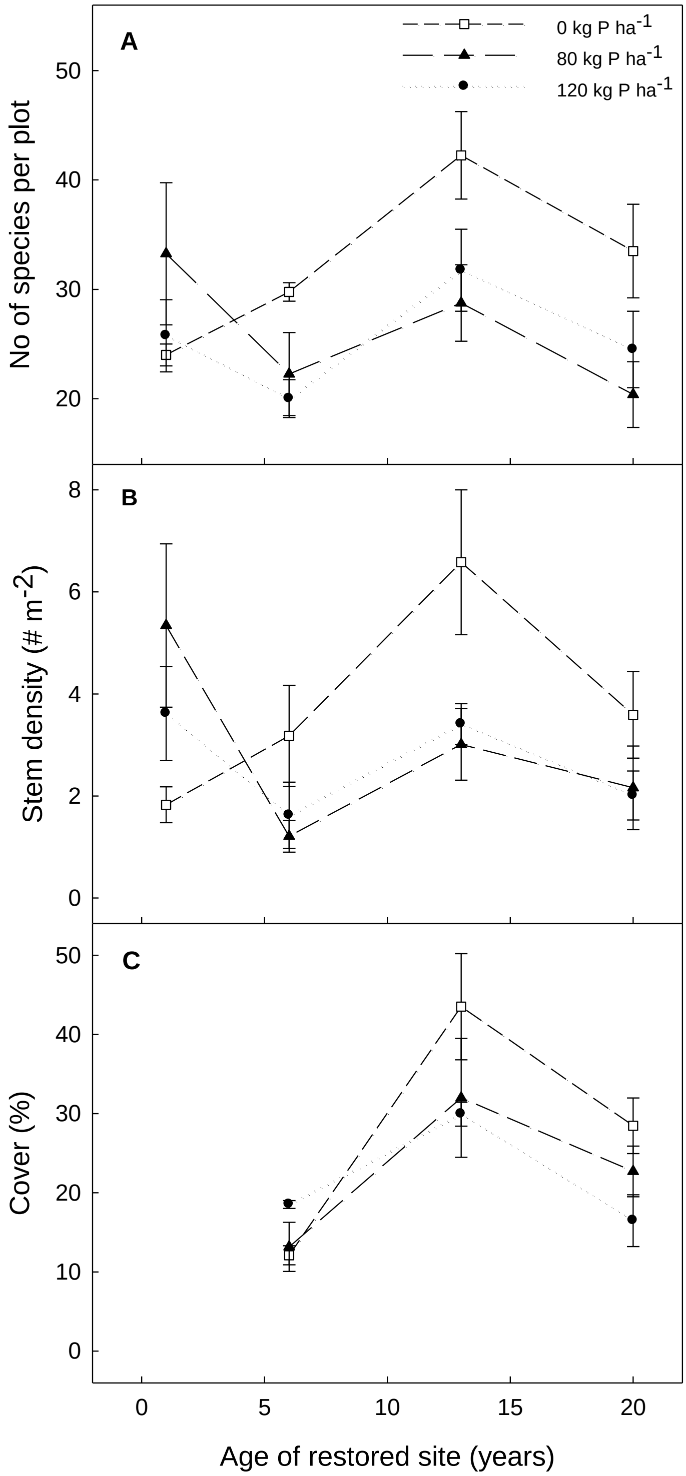
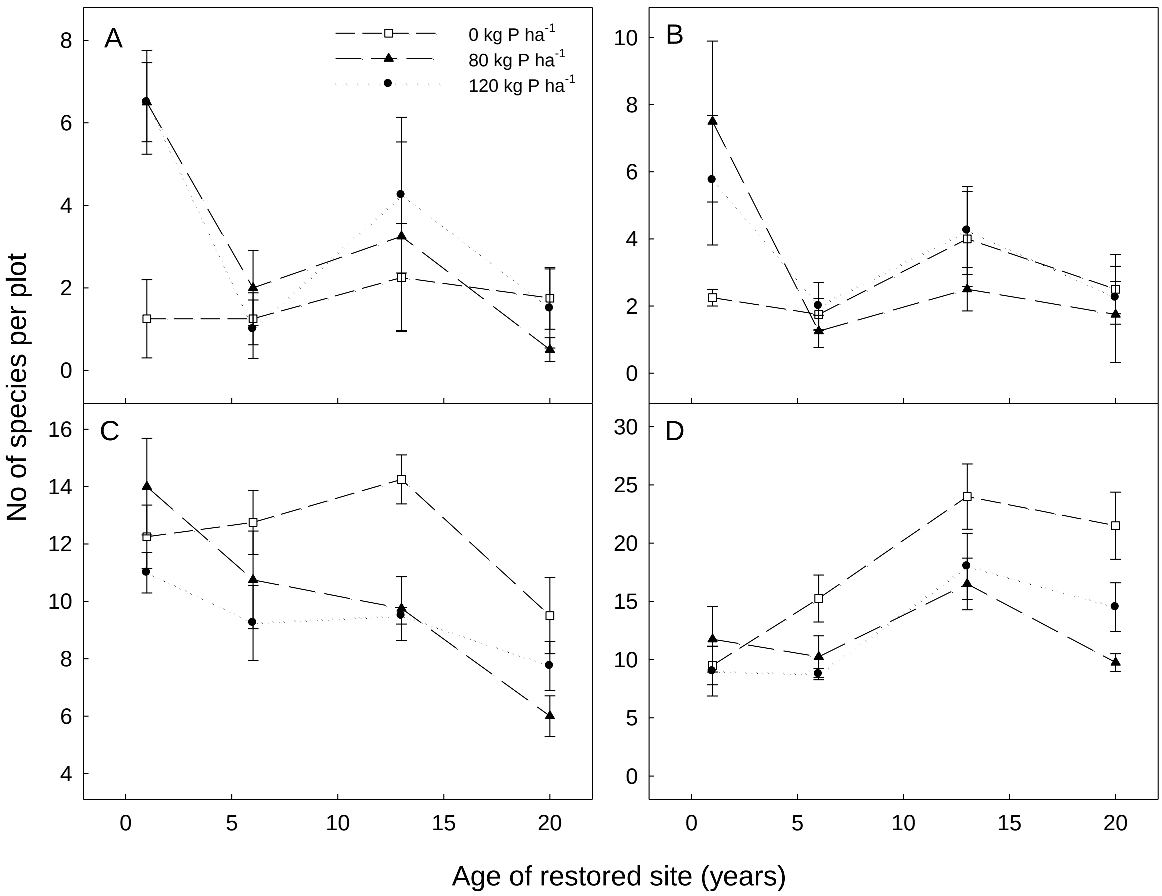
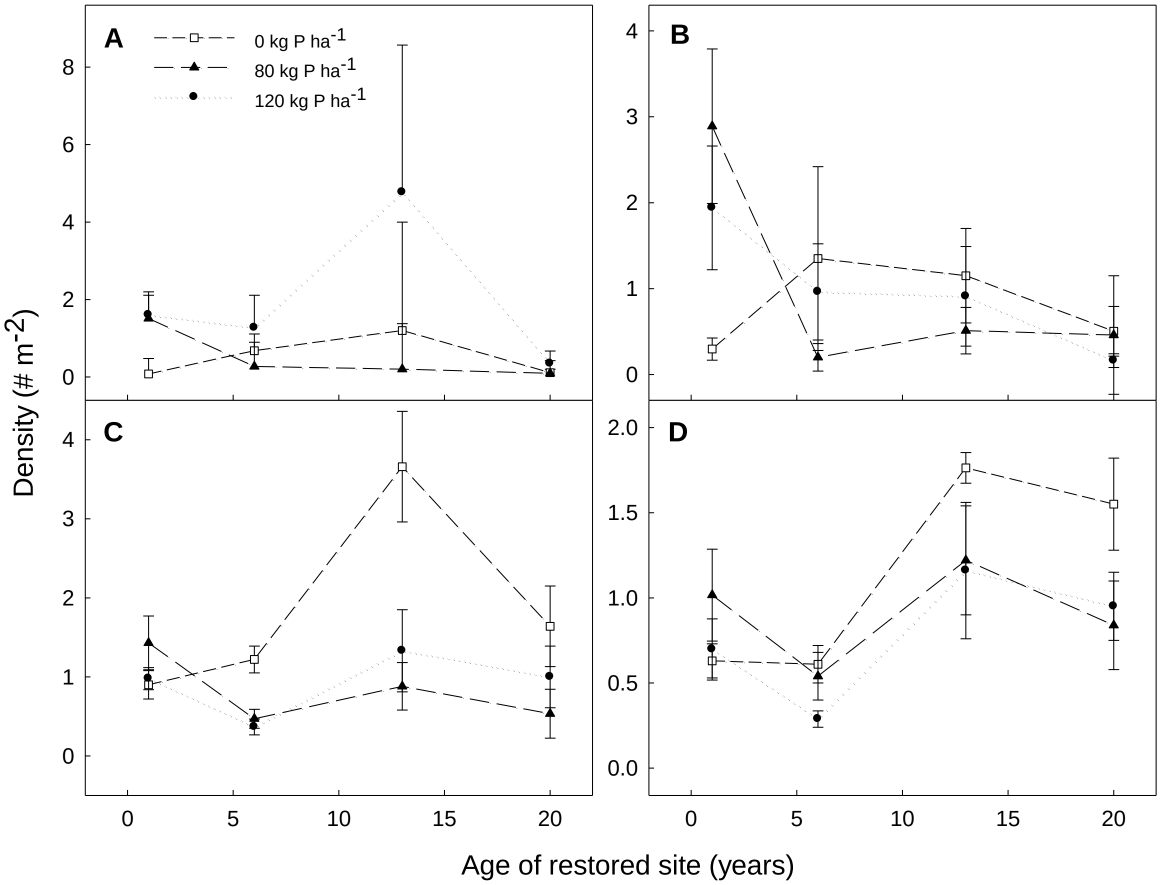

A
B
C
0 kg P ha-1
80 kg P ha-1
120 kg P ha-1
No of species per plot
Stem density (# m-2)
Cover (%)
Age of restored site (years)
0
5
10
15
20
30
40
50
2
4
6
8

Figure 3:



A
B
C
D
0 kg P ha-1
80 kg P ha-1
120 kg P ha-1
No of species per plot
Age of restored site (years)
0
5
10
15
20

596 Figure 4:



597

Figure 5:

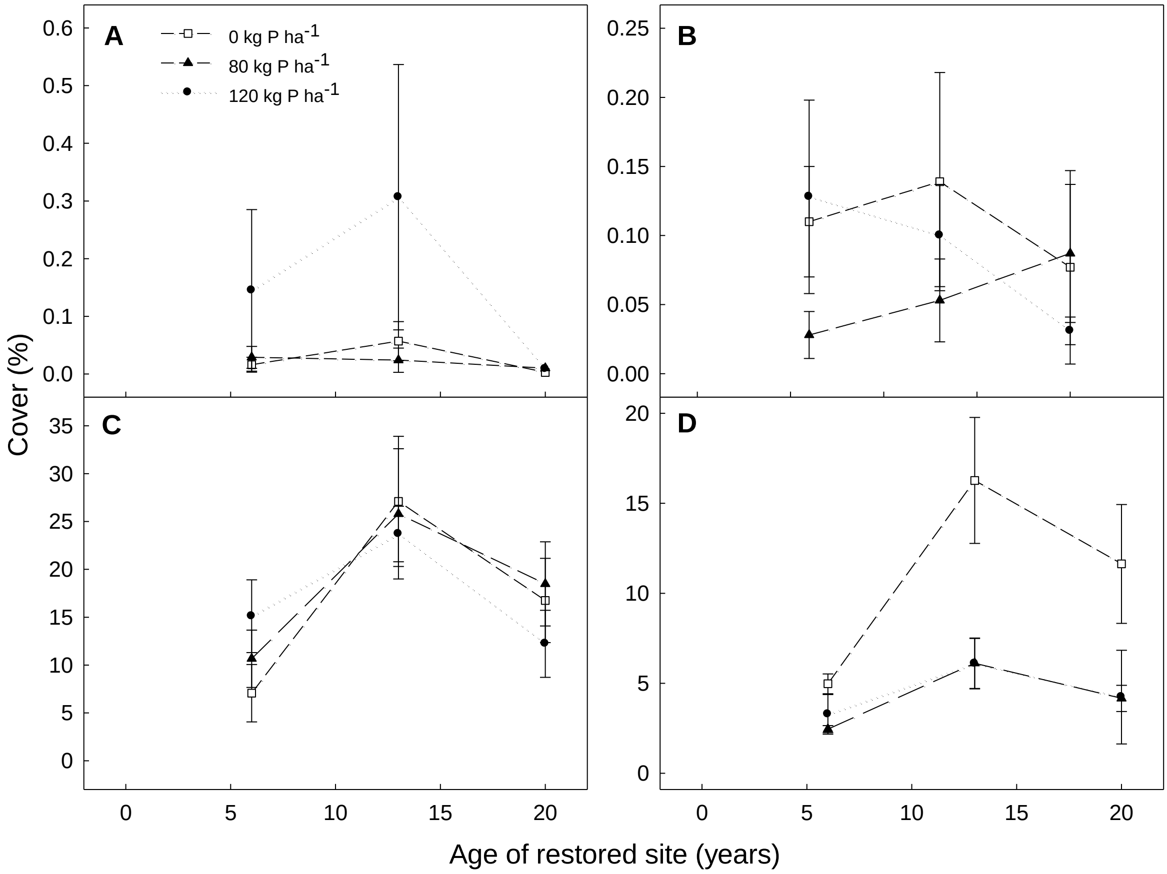
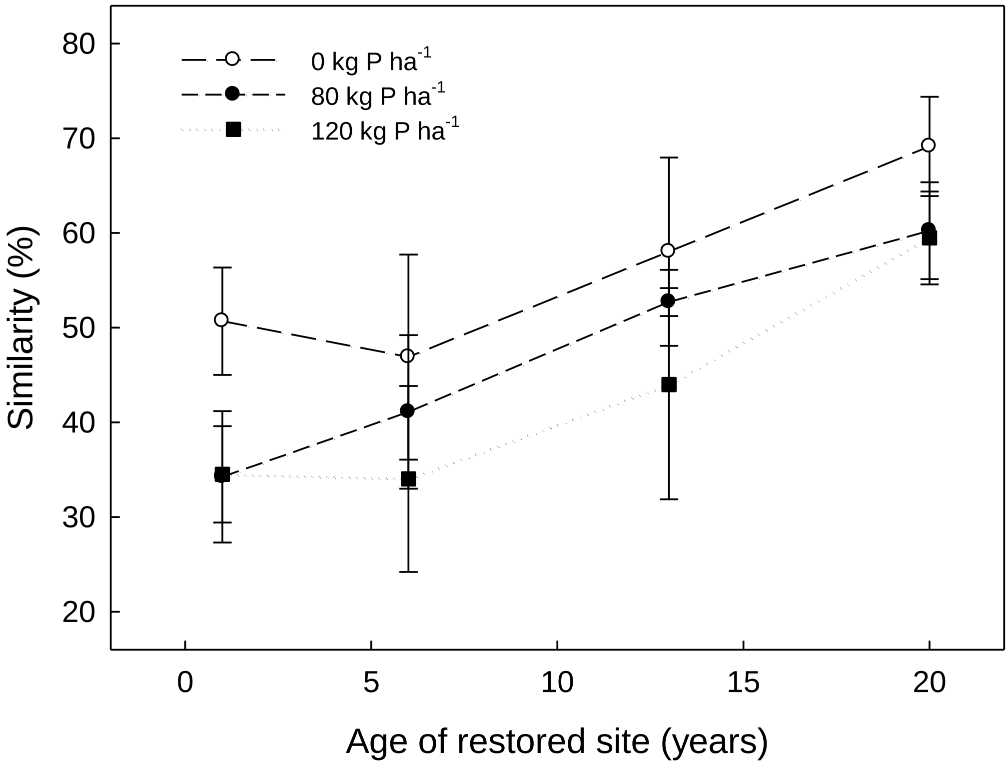

A
B
C
D
0 kg P ha-1
80 kg P ha-1
120 kg P ha-1
Cover (%)
Age of restored site (years)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.00
0.05
0.10
0.15
0.20
0.25
0
5
10
15
20
25
30
35
0
5
10
15
20

Figure 6:


0 kg P ha-1
80 kg P ha-1
120 kg P ha-1
Similarity (%)
80
70
60
50
40
30
20
0
5
10
15
20
Age of restored site (years)