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The where, when and what of phosphorus fertilisation for seedling establishment in a biodiverse jarrah forest restoration after bauxite mining in Western Australia

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Key words: fertiliser, legume, nitrogen, rehabilitation,

Abstract

Fertiliser application to restore nutrients lost in the mining process and facilitate early plant establishment and growth is a key step in the restoration of sites disturbed by mining. However, few studies have investigated the effects of different fertiliser types and application methods on mine restoration outcomes, especially in highly biodiverse ecosystems such as the jarrah forest. This forest is a unique, floristically diverse landscape with species adapted to growth on highly weathered phosphorus impoverished Ferralsol. In this study we investigated the effect of fertiliser type (rock phosphate, single superphosphate, and an NPK fertiliser), application method (top-dressed versus incorporated), and the timing of application (winter vs. summer) on the trajectory of jarrah (*Eucalyptus marginata*) forest restoration following bauxite mining compared to an unfertilised control. All fertilised soil had elevated Colwell-P concentrations (bar rock phosphate) and had considerably less N than found in the native forest, even after N fertilisation. Fertiliser incorporation resulted in a more even distribution of P down the soil profile and increased overall plant growth (as assessed by percentage cover) compared with either top-dressed fertiliser application and no fertiliser, potentially offering better erosion control. In contrast, native species richness was highest in the zero fertiliser and NPK treatments and lowest in the phosphorus incorporation treatments. On average, unfertilised plots had 10 more native species per plot than those fertilised with P only. Fertiliser application also reduced the abundance and cover of *Bossiaea ornata* and *Lomandra* spp., both of which are small slow-growing understorey taxa. In contrast, the legume *Acacia celastrifolia* exhibited a vigorous growth response to fertiliser, with growth being greatest when P (either rock phosphate or SSP) was incorporated. These data suggest that P fertiliser incorporation is a potential strategy to both maximise early plant growth and cover and increase the efficiency of P application. However, if the goal of restoration is to maximise diversity then moderation in P application and using fertilisers that also contain N and K may be appropriate.

Keywords: *Acacia*, biodiversity, fertiliser, legume, nitrogen, rehabilitation

1. Introduction

Vegetation removal and the processes of soil removal, stockpiling and mixing when soil is stripped and respread result in significant losses and redistribution of soil nutrients during the mining process. Consequently, applying fertiliser to replace lost nutrients is generally viewed as a key step in the restoration of sites disturbed by mining (Tibbett, 2010). Fertiliser addition is also regarded as beneficial by increasing plant growth and thereby reducing the risk of erosion in newly restored (bare) sites (Ward et al., 1990). However, the effects of different fertiliser types and application methods in post mining restoration have received relatively little attention.

Both neutral and positive effects of fertiliser application on plant growth have been observed in restored mine sites (e.g. Malakondaiah et al., 1981; Wali, 1999; Rokich and Dixon, 2007; Williamson et al., 2011; Soliveres et al., 2012), which suggests species-specific responses to fertiliser that may, in turn, affect competitive interactions among species. For example, fertiliser addition reduced seedling survival of some woody species in quarry restoration in Spain due to increased competition with herbaceous species (Soliveres et al., 2012) and increased mortality of proteoid shrubs in fynbos restoration in South Africa (Holmes, 2001). In addition, fertiliser application can increase the growth and establishment of weed and native annuals with negative impacts on native species richness and slower growing species (Daws et al., 2015, 2019a; Nussbaumer et al., 2016). Consequently, there is a need to better understand fertiliser impacts on both species responses and community composition in mine site restoration.

The jarrah (*Eucalyptus marginata*) forest in Western Australia has highly weathered, nutrient deficient soils. Post mining restoration in this, and other environments with nutrient deficit soils can result in soil phosphorus concentrations remaining elevated for 20 or more years after a single, initial fertiliser application (e.g. Banning et al., 2008; Spain et al., 2018;

Daws et al., 2019b). In contrast, when fertiliser containing inorganic N is applied, N is often rapidly lost (Daws and Richardson, 2015; Sloan et al., 2016). For example, in newly restored jarrah forest, a single application of 40 kg N ha^{-1} is undetectable after just 4.5 months (Daws and Richardson 2015). As a result, it is common practice for eucalypt forest restoration after bauxite mining to receive fertiliser only containing P (e.g. Standish et al., 2010; Spain et al., 2018), based on the assumption that N_2-fixing legumes, which are likely to increase N_2-fixation in response to P-application (Hingston et al., 1982), will increase soil-N (Grant et al., 2007). However, any potential impacts on the restored plant community of applying solely P based fertiliser, rather than fertiliser also containing N, have not been assessed.

In post mining restoration, inorganic fertilisers are typically applied as a top-dressing (e.g. Koch, 2007; Nussbaumer et al., 2016; Sloan et al., 2016). For example, in jarrah forest restoration following mining for bauxite, newly restored sites are top-dressed with fertiliser the first winter/spring after the completion of restoration and seeding in the preceding summer (Koch, 2007; Standish *et al*., 2015). However, P in these fertilisers is likely to remain concentrated at the soil surface. For example, in agricultural systems vertical stratification of P can occur when fertiliser is either top-dressed or shallow buried adjacent to seeds (Eckert, 1985; Mackay et al., 1987; Morrison and Chichester, 1994; Ryan et al., 2017). Furthermore, the availability of shallow/surface applied fertilisers to plants is likely to be restricted in restored mine sites where rapid drying of surface soils may occur. This will particularly be the case for P, as diffusion of phosphate ions to plants is limited in dry soil (Nye and Tinker, 1977). Indeed, surface application limits P uptake in a range of agricultural systems (Piper and de Vries, 1964; Scott, 1973; Jarvis and Bolland, 1991), with incorporation of P fertiliser increasing crop yields relative to surface applications in a number of studies (Nable and Webb, 1993; Sander and Eghbell, 1999; Teutsch et al. 2000; Singh et al. 2005).

Consequently, the benefits to plant growth of fertiliser application in restored mine sites may be greater when the fertiliser is incorporated into the soil rather than applied as a top-dressing. However, this remains to be tested.

In jarrah forest restoration, fertiliser is typically applied the first winter after the completion of earthworks in the preceding summer (Koch, 2007): establishing seedlings may be several months old before fertiliser is applied. However, responses to fertiliser addition may be expected to be greater if the applied fertiliser is available to establishing seedlings from the onset of germination in autumn. While this remains to be tested, anecdotal evidence shows various trends for some key native jarrah forest species: spring fertiliser application produced optimal growth for some keystone jarrah forest species (Humphrys, 1987), while Lockley and Koch (1996) found summer application (at the time of seeding) produced a higher density of jarrah seedlings.

In this study, we investigated the effects of a range of fertiliser treatments on establishment of one-year-old jarrah forest in the process of being restored after bauxite mining in Western Australia. Specifically, we investigated whether fertiliser incorporation versus a top-dressed application impacts on the distribution of available (Colwell) P in the soil profile and tested the hypothesis that incorporation will result in greater plant growth. Secondly, we assessed the effect of fertiliser application relative to an unfertilised control to test the hypothesis that fertiliser application will increase overall plant growth, but increase weed abundance and reduce native plant species richness. Thirdly, we tested the effect of fertilisers containing only P (including slow release rock phosphate and highly soluble single superphosphate) compared with an NPK-based fertiliser on plant responses to test the hypothesis that applied-N will have limited impact on vegetation responses due to only short-term availability after application. Finally, we tested the effect of the timing of fertiliser application (summer versus winter) on plant responses, to test the hypothesis that a greater response will be evident when fertiliser is present from the onset of germination / seedling emergence (i.e. when applied in summer).

2. METHODOLOGY

2.1. Description of study location

The experiment was established in the northern jarrah forest of Western Australia located approximately 130 km south-east of the state capital Perth (32° 48' S 116° 28' E). The region experiences a Mediterranean climate with hot, dry summers and mild, wet winters. Mean January and July temperatures are 32.1 and 15.8°C, respectively and total rainfall is approx. 720 mm yr^{-1} and strongly seasonal, most falling during the winter months of June to August (Australian Bureau of Meteorology, 2021).

The forest vegetation comprises of the dominant overstorey species *Eucalyptus marginata* (jarrah), which constitutes around 80 % of stems in both restored and unmined forest (Daws et al., 2015). The remaining stems are mostly comprised of the subdominant species *Corymbia calophylla* (marri). In addition, there is a mid-storey layer of *Banksia grandis*, *Allocasuarina fraseriana* and *Xanthorrhoea preisii* with large woody shrubs of *Bossiaea aquifolium*, various *Acacia* species and a diverse understorey (Gardner and Bell, 2007). Jarrah forest soils developed on ca. 2.6-billion-year-old granite-gneiss metamorphic batholith of the Yilgarn craton (Nemchin & Pidgeon, 1997), within the bauxitic province of the Darling Range (McArthur, 1991). Here, the deep weathering of regolith is among the oldest in the world, with weathering events as early as the Cretaceous Period. This has led to subsoil accumulation of bauxite ores and also a depletion of nutrients, particularly phosphorus. The resulting soils are gravelly with low concentrations of available N, P and K (Table 1 and see Hingston et al., 1989) with high rates of phosphorus fixation on the remaining 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 in April 2004 at two previously surfaced-mined sites with the objective of studying the effects of ground-based phosphorus fertiliser application (type of fertiliser, placement and time of application) on restoration. The area was cleared of native jarrah forest vegetation in 2002, two years prior to the commencement of this experiment. During the two years following vegetation clearing, topsoil and gravel overburden were removed to expose the bauxite ore which was blasted and mined. Subsequently the area was re-shaped to blend in with the surrounding landscape and the entire area deep-ripped to relieve mining-related compaction. The overburden was then replaced followed by fresh topsoil sourced from an adjacent area that had just been cleared for mining. Due to the processes of being stripped, transported to and then re-spread across the area being restored, the topsoil spread across the trial sites was relatively homogenous.

Following topsoil replacement, but prior to a final contour ripping stage, an incomplete randomised block design was established at both sites. Six treatments that were operationally feasible, including the current prescription of applying fertiliser as a broadcast treatment in the winter following seeding (Table 2), were established. The design was incomplete as impractical treatments such as fertiliser incorporation in winter were excluded. For example, this treatment would result in both the burial of emerging seedlings and soil compaction due to wet soil conditions. Treatments were replicated either 8 times (the 3 single super phosphate [SSP] treatments) or 4 times (the control, NPK or rock phosphate) (Table 2). Treatment plots were 25 × 25 m in size. In the incorporation treatments, fertiliser was applied prior to the final contour ripping with the ripping step used to incorporate the fertiliser down the soil profile.

Tines incorporated material to a depth of approximately 1 m. Following contour ripping, a seed mix of 162 species representing forest sub-types of northern Jarrah forest (comprising understorey and tree species) was broadcast at the rate of 88 g plot^{-1}. *Acacia celastrifolia* was not included in the seed mix as it was well represented in the soil seed bank. In the broadcast fertiliser treatments, fertiliser was applied by hand once contour ripping had taken place, either immediately (summer) or in the following winter.

The chemical composition of the applied fertilisers was: 1) Single superphosphate at 450 kg ha^{-1} 9.1% total P (equivalent by weight to 40.9 kg ha^{-1} P), 10.1% sulphur, 9.0% calcium, 0.6% copper, 0.3% zinc and 0.06% molybdenum; 2) NPK (commercial name K-Till) at 340 kg ha^{-1} (8.6% N, 12.0% P, 9.8% K, 6.7% S, 3.8% Ca, 0.1% Cu and 0.2% Zn) containing 40.8 kg P ha^{-1}, and 3) Rock phosphate at 1,200 kg ha^{-1} (~15% total P content with very low solubility).

2.3. Soil sampling and analysis

Soil samples were collected in May 2005 from four 1 m × 1 m quadrats located 5 m inside the treatment plot boundary (Fig. 1A). For consistency, soil samples were taken from each of two furrows and two ridges, formed by ripping. Soil was sampled at 0-5, 5-10, 10-20 and 20-30 cm depth-intervals to investigate treatments effects on fertiliser distribution down the soil profile. Soil samples were stored in plastic zip lock bags, sealed for transport and re-opened within 24 hours. Samples were air-dried (in a drying room maintained at a constant temperature of 40°C) and sieved to 2 mm prior to further analysis. For comparative purposes, soils were also sampled from three reference jarrah forest sites and in restored sites prior to the addition of fertiliser. Samples were analysed at a commercial laboratory (CSBP Soil and Plant Laboratories, Bibra Lake, Perth, Australia). Soils were hand textured and phosphate retention index was assessed using the method of Allen and Jeffey (1990). Soil pH was determined using a 1:5 ratio of soil: either distilled water or 0.01 M calcium chloride solution and Colwell (available) phosphorus (Colwell, 1963), NO_3-N and NH_4-N were also analysed.

2.4. Floristic survey and analysis

In May 2005, a 20 m × 20 m plot was established within the centre of each 25 m × 25 m plot. Each 20 m × 20 m plot was further divided into twenty 2 m × 2 m quadrats, with a total of 80 m^2 sampled per plot. For each species, species identity, density and percentage cover were recorded separately for each 2 m × 2 m quadrat. Density and cover were then summed for the entire plot.

2.5. Statistical Analysis

One-way ANOVA implemented in Minitab 17 (Minitab Inc., State College, PA, US), followed by Tukey´s *post hoc* test was used to test for an effect of fertiliser treatment on soil P concentration and vegetation responses (species richness, total density, total cover and non-native weed species richness). In addition, for four relatively abundant taxa (*Acacia celastrifolia*, *Banksia grandis*, *Bossiaea ornata* and *Lomandra* spp.) one-way ANOVA was used to test for fertiliser effects on density and cover. Data was tested for normality and did not require transforming. For soil P, the ANOVA was followed by Fisher's Least Significance Difference test.

Multivariate data analysis was undertaken using PRIMER™ (Plymouth Routines in Multivariate Ecological Research, U.K). Floristic trends were analysed using a nonmetric multi-dimensional scaling – nMDS procedure (using Primer-E Ver 6.0 software, www.primer-e.com) to explore patterns of variation in community composition related to fertiliser treatment. nMDS was selected over other multivariate data analysis methods as it can better explain the spatial configuration of the data with minimal distortion to the structure. The raw floristic data were initially subjected to a fourth-square root transformation followed by calculation of the Bray-Curtis similarity of the distance between points. Subsequently, ANOSIM was used to test the significance of effects of fertiliser treatment on community composition.

3. RESULTS

3.1. Effects of fertiliser treatment on soil N and P

With the exception of rock phosphate, P fertilised soils had elevated Colwell-P concentrations compared to the native forest soils, pre-treatment values (Table 1) and the unfertilised control (Fig 1). Across the top-dressed fertilised treatments, Colwell-P was consistently higher in furrows (Fig 1A) than in the ridges formed following ripping (Fig 1B). Furthermore, in the top-dressed treatments elevated Colwell-P was largely restricted to the 0-5 cm depth. In the incorporated SSP treatment, Colwell-P concentrations were similar in the furrows and ridges at 0-5 cm depth and there was a more uniform distribution of P down the soil profile compared with top-dressing (Fig. 1).

Fertiliser application, including NPK, had no effect on soil NO_3^- at a depth of 0-5 cm in furrows (One-way ANOVA, $P > 0.05$; Table 3). This pattern was similar for ridges (data not shown). Soil NH_4^+ differed with treatment (One-way ANOVA, $P < 0.05$) and was significantly higher in the undisturbed reference forest soils than in restored soils, except for the two SSP treatments where fertiliser was applied in summer (Table 3).

3.2. Fertiliser effects on plant species richness, density and cover

The SSP and rock phosphate treatments had significantly reduced native plant species richness compared with the control and NPK treatment (One-way ANOVA, $P < 0.05$; Fig.

2A). On average, across the SSP and rock phosphate treatments there were 10.7 fewer native species per plot compared with the control (58.2 species per plot). Stem density in the control and NPK treatments was similar, and both were significantly higher than in the rock phosphate treatment (Fig. 2B).

The number of non-native weed species was highest in the two fertiliser incorporation treatments (rock phosphate and SSP) and all fertiliser treatments had significantly higher weed species richness than the control (One-way ANOVA, $P < 0.05$; Fig. 2C). Total plant cover also responded significantly to fertiliser treatment (One-way ANOVA, $P < 0.05$; Fig. 2D) and was highest in the two fertiliser incorporation treatments (rock phosphate and SSP) and lowest in the control. Fertiliser addition resulted in cover being up to six times higher (SSP incorporated down the profile) than the control.

3.3. Fertiliser effects on taxa level responses

For all four taxa for which responses were individually investigated, fertiliser treatment had significant effects on both stem density and total percentage cover (One-way ANOVA, $P < 0.05$; Fig. 3). For *A. celastrifolia*, both stem density and total cover were highest in the two fertiliser incorporation treatments and lowest in the control. For example, cover in the two incorporation treatments ranged from 26-30 % compared with ca. 3 % cover in the control treatment (Fig. 3B).

Stem density of *Banksia grandis* was significantly lower in the rock phosphate treatment compared with the other five treatments (One-way ANOVA, $P < 0.05$; Fig. 3C). Total cover of *B. grandis* was significantly lower in the rock phosphate, SSP incorporation and top-dressed SSP in summer treatments compared with the control treatment.

For *Bossiaea ornata*, stem density was similar across the five fertiliser addition treatments but was nearly 2.5 times higher in the control (One-way ANOVA, $P < 0.05$; Fig.

3E). Percentage cover of *B. ornata* was also significantly affected by treatment with cover being highest in the control followed by the NPK treatment. For example, cover of *B. ornata* in the control was more than four times higher than in the SSP incorporation treatment.

All of the fertiliser treatments resulted in a significant reduction in the stem density of *Lomandra* species compared with the control (One-way ANOVA, $P < 0.05$; Fig. 3G). Cover of the *Lomandra* species was also significantly reduced in all the fertiliser addition treatments: cover was approximately three times higher in the control than the fertiliser treatments.

3.4. Community level responses to fertiliser treatments

In the MDS ordination, there was significant overlap in vegetation composition among the five fertiliser treatments. The control plots appeared to cluster as a separate group. (Fig. 4). This was supported by the ANOSIM which indicated a significant effect of fertiliser treatment on community composition (global r = 0.141, $P < 0.05$; Table 4). Pair-wise comparisons among the six treatments indicated that all five of the fertiliser treatments had a significant impact on community composition relative to the control ($P < 0.05$). Based on the magnitude of the r-statistic the community composition in the NPK treatment was most similar to the control (Table 4).

4. Discussion

Fertiliser application is generally a routine step in mine restoration, with fertiliser typically top-dressed either concurrent with, or following, seeding (e.g. Spain et al., 2015; Koch, 2007). However, our current data indicate that when fertiliser was applied as a top-dressing, available-P remains concentrated within the top 5 cm of soil, predominately within the furrows caused by ripping. In contrast, when the fertiliser was incorporated, the distribution of P down the soil profile was more even and, in agreement with our first hypothesis, resulted in increased plant growth. Phosphorus is generally relatively immobile in soil and is rapidly sorbed as iron and aluminium hydroxides in jarrah forest soils (Lambers et al., 2008); available-P can remain elevated, close to the soil surface, for 20 or more years in both the jarrah forest and elsewhere following a single top-dressed application (Banning et al., 2008; Spain et al., 2018; Daws et al., 2019b). Consequently, there may be long-term impacts on the distribution of P down the soil profile depending on the method of fertiliser application.

In restored mine sites where rapid drying of surface soils may occur, especially during summer in Mediterranean climates such as in the jarrah forest, the availability of shallow/surface applied fertilisers may be further restricted compared with fertiliser incorporated throughout the soil profile. This will particularly be the case for P as diffusion of phosphate ions to plants is limited in dry soil (Nye and Tinker, 1977). Indeed, surface application can limit uptake of applied P and crop yields in a range of agricultural systems (Piper and de Vries, 1964; Scott, 1973; Jarvis and Bolland, 1991; Nable and Webb, 1993; Sander and Eghbell, 1999; Teutsch et al., 2000; Singh et al., 2005). In a mine restoration context these results suggest that the same growth benefit resulting from a top-dressed application may be achievable at lower application rates if the fertiliser is incorporated.

In newly restored sites, the positive effect of fertiliser incorporation on plant growth / cover may be advantageous through an increase in site stabilisation and reduction in erosion risk. Vegetation cover has a significant effect on controlling run-off and soil erosion when at least 30–40% of the soil surface is covered (Thornes, 1988; Thornes, 1990). While cover was less than 10 % in the control, both of the fertiliser incorporation treatments resulted in total cover in excess of 30 % within the first twelve months after seeding, demonstrating the potential of fertiliser addition, and especially fertiliser incorporation, for reducing erosion.

The P-supply in soils is typically heterogeneous and consequently most plant roots grow preferentially in regions that contain high concentrations (Drew, 1975; Fransen et al., 1999; Hodge, 2004). For example, in agricultural systems, when fertiliser is applied as a band beneath or adjacent to seeds, root proliferation is encouraged in the region of the band (Anghinoni and Barber, 1980; Yao and Barber, 1986, Sander et al., 1990). Consequently, a top-dressed application may encourage root proliferation in surface soils with a potential negative impact on seedling survival during summer drought. While we did not investigate root distribution down the soil profile in our current study, P placement at the surface altered root distributions in two Australian native herbs (Denton et al., 2006). Incorporating fertiliser down the soil profile would militate against this risk. Further studies of the impacts of fertiliser incorporation on root responses in restored systems, and potential impacts on seedling survival during summer drought would be of value.

In support of hypothesis 2, fertiliser application increased overall plant growth, and generally resulted in fewer native species and more weed species than the control. Fertiliser addition has been demonstrated to increase weed growth in a range of restoration studies (Whisenant, 1999; Prober and Wiehl, 2012) and weed proliferation may impact negatively on establishing native species (Nussbaumer et al., 2016). Further, other recent studies in restored Jarrah forests have shown that unfertilised treatments are more similar in composition to native Jarrah forest communities than fertilised treatments (Daws et al., 2013, 2015, 2019a). On an individual species / taxa level, there were also mixed responses to fertiliser application.

The understorey legume *Acacia celastrifolia* responded vigorously to all fertiliser treatments, but especially incorporation. N_2-fixing legumes, such as *A. celastrifolia*, are generally P- rather than N-limited and many respond vigorously to applied-P in mine restoration (Grant et al., 2007; Daws et al., 2015, 2019b). Indeed, higher soil NH_4^+ concentrations in the single superphosphate incorporation treatment compared with the control (12 versus 2.2 mg kg^{-1}, respectively) likely reflect greater growth and N_2-fixation by legume species such as *A. celastrifolia* (Hingston et al., 1982; Koutika et al. 2014). In contrast, *B. ornata* and *Lomandra* spp. responded negatively to all the fertiliser treatments. *B. ornata* is a small, slow growing shrub and *Lomandra* spp. are small, grass-like understorey plants. While the negative effect of applied fertiliser on abundance / growth of these taxa may be mediated by direct negative effects of P (e.g. Lambers et al., 2002; Williams et al., 2019), it is also likely that these slow-growing species are susceptible to competition from highly P responsive species such as *A. celastrifolia*. Indeed, negative competitive effects of vigorous legume growth, in response to applied P, on slow-growing understorey species have been reported elsewhere (e.g. Boyes et al., 2011; Le Stradic et al., 2014; Daws et al., 2015, 2019ab), and may be a key mechanism altering species competitive dynamics and consequent ecological trajectories.

Banksia grandis (proteaceae) is a mid-storey tree that produces cluster roots to facilitate P uptake in P-deficient soils (Lambers et al., 2002). Many proteaceae including *B. grandis* are sensitive to high levels of applied-P (Shane et al. 2004; Handreck 1991; Lambers et al., 2002; de Campos et al., 2013) as they have limited ability to regulate P uptake when external concentrations are high (Shane et al., 2004). Whilst relatively unresponsive to top-dressed fertiliser application, abundance and cover were reduced when P (including rock phosphate) was incorporated further reinforcing the suggestion that P-availability to plants may be greater when fertiliser is incorporated. It seems likely that the potentially toxic effects of P on these P-sensitive species are minimised when P is applied as a top-dressing, presumably due to roots being placed away from surface soils where P is concentrated.

Fertiliser type had a significant and contrasting effect on available P and N and on plant communities. The rock phosphate treatment had the seemingly incongruous effect of affecting plant responses while having no effect on available (Colwell)-soil P. In terms of use as a fertiliser in agriculture, rock phosphates have been concluded to be ineffective because they do not dissolve rapidly in Western Australian soil (Boland and Gilkes, 1990). However, the gradual release of phosphate ions from the mineral may be appropriate for the restoration of native forest as applied rock phosphate is known to leave a considerable residue of undissolved rock phosphate in the soil for several years after application. The lack of detectable differences in soil test P is likely due to the sparing solubility of rock phosphate in the bicarbonate (buffered at pH 8.5) used as an extractant in Colwell P: studies have concluded that bicarbonate solution poorly predicts potential P release from the residual rock phosphate in soil (Rajan et al., 1996; Saggar et al., 1999). Despite rock phosphate having low solubility, the impact on plant responses may also have resulted from the rapid release of P from easily dissolvable mineral surfaces. Indeed, a two-phase release of P from rock phosphate has been reported previously with an initial rapid release of P from the surface followed by a much lower release of P as the bulk mineral dissolves (Rafael et al., 2018). In addition, many jarrah forest species, including *B. grandis*, produce large quantities of carboxylates to release P from strongly sorbed forms (Lambers et al., 2002). Consequently, an alternative explanation for the plant responses to rock phosphate, including the negative effect on growth and abundance of *B. grandis* is carboxylate mediated P dissolution.

Despite having the same P application rate as the P-only treatments, the species richness in the NPK treatment was significantly higher than all but one P-only treatment and was similar to the unfertilised control (Figure 2A). Further, the NPK treatment had a smaller negative effect on abundance and / or cover of *B. ornata* and *Lomandra* species. Consistent with the univariate results, our multivariate ANOSIM indicated that the community composition in the NPK treatment was the least different to the unfertilised plots, providing little support for our third hypothesis, and suggesting some interaction with N fertilisation that our simple soil analysis may not be detecting. ANOSIM also revealed a significant effect of all fertilisers on plant community composition, where all five of the fertiliser treatments caused a significant shift in relative floristic composition compared to unfertilised plots. This demonstrates clearly that fertilisers have an unbalanced effect on early forest development, that is quite different to simply encouraging greater uniform plant growth. In fact, the effects we show are highly selective on a species by species basis. While being far from conclusive, we can postulate that lower rate fertiliser regimes may be more suitable for this forest, and that the effect of co-applied N needs to be better understood in terms of plant community response.

While the mechanism(s) behind the response to N is unclear, applying N containing fertilisers may, at least initially, maintain a more natural N:P ratio in the soil. It is also possible that applying N limits the establishment / reduces the competitiveness of N_2 fixing species. Indeed, nitrate and ammonium addition can depress nodule prediction in seedlings of *Acacia* species (e.g. *A. auriculiformis*; Goi et al., 1992). Notably, despite containing the same quantity of P as the single superphosphate treatments, the NPK fertiliser treatment resulted in soil ammonium concentrations (3.1 mg kg^{-1}) that were nearly as low as in the control, (3.12.2 mg kg^{-1}; Table 3), suggesting a lower rate of atmospheric N_2-fixation by legumes (Koutika et al., 2014).

Soil ammonium concentrations were also considerably higher in the native (unmined) forest soils compared to all experimental plots, including those fertilised with nitrogen. After P fertilisation in particular, our data suggest that this may signify a shift in ecosystem stoichiometry, changed from a natural state of P limitation to an N-limited system, at least in the initial stages after restoration. Studies on the biogeochemistry of these restored forest systems is required to confirm this supposition.

Finally, our data indicated that there were no effects of the timing of fertiliser application on species richness, individual species or plant community composition providing no support for our fourth hypothesis that having fertiliser present from the start of restoration will be beneficial. Consequently, this current study provides no support to change from a winter to summer top-dressing. However, since these developing plant communities were only 12 months old when sampled, longer-term studies of the effect of fertiliser treatment on community development would be of value.

5. Conclusion

Ultimately, restoration practitioners aim to achieve a rate of fertiliser application that both compensates for nutrient losses and reflects the aims of restoration. Our current study suggests that if the aims of restoration are to maximise ground cover (to minimise erosion or maximise productivity), then higher rates of fertiliser application and fertiliser incorporation might be needed, while to maximise floristic diversity little or no fertilisation may be appropriate. To optimise the use of fertiliser in mine site restoration to address these competing outcomes will require a better understanding of the effects of applied fertiliser, including longer term impacts.

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Table 1: Soil parameters in unmined forest and in restored sites prior to the addition of fertiliser.

Parameter	Unit	Unmined forest	Pre-treatment
Gravel	%	51.5 ± 14.7	70.0 ± 2.0
Texture	Class	Loam	Loam
NO_3^-	mg kg^{-1}	< 1	1.1 ± 0.1
NH_4^+	mg kg^{-1}	4.3 2.0	7.0 ± 1.8
Total P	mg kg^{-1}	131.6 ± 11.7	ND
Colwell P	mg kg^{-1}	3.7 ± 0.9	3.5 ± 1.2
P-retention index	ratio	90.5 ± 14.3	ND
pH (water)	mg kg^{-1}	6.0 ± 0.1	5.9 ± 0.1
pH ($CaCl_2$)	mg kg^{-1}	5.1 ± 0.1	5.3 ± 0.1
Organic C	%	5.7 ± 0.7	4.6 ± 0.5

Table 2: Details of the six fertiliser treatments used in the current study.

Treatment number	Fertiliser type	Fertiliser placement	Timing of fertiliser application	P-application rate (kg ha^{-1})
1*	Single super phosphate	Top-dressed	Winter	40.8
2	Single super phosphate	Top-dressed	Summer	40.8
3	Single super phosphate	Incorporated	Summer	40.8
4	NPK	Top-dressed	Winter	40.9
5	Rock phosphate	Incorporated	Summer	180
6	Control - No fertiliser application	N/A	N/A	0

*Current practice at Boddington Bauxite Mine (George et al., 2006)

Table 3: the effect of fertiliser type and application method on NO_3^- and NH_4^+ measured at 0-5 cm depth in the furrows caused by ripping in one-year old restored jarrah forest. For comparison, values for unmined forest were also determined. Treatment numbers relate to the numbers used in Table 1. Data are ±1 standard error of the mean.

Fertiliser type	**Application type**	**Soil NO_3 (mg kg^{-1})**	**Soil NH_4 (mg kg^{-1})**
Single super phosphate	Top-dressed in Winter	1.8 ± 1.3^a	6.3 ± 2.7^b
Single super phosphate	Top-dressed in Summer	2.1 ± 0.5^a	13.6 ± 5.1^{ab}
Single super phosphate	Incorporated in Summer	1.7 ± 0.6^a	12.0 ± 3.4^{ab}
NPK fertiliser	Top-dressed in Winter	3.0 ± 1.0^a	3.1 ± 2.3^b
Rock phosphate	Incorporated in Summer	≤ 1	5.2 ± 2.1^b
Control – no fertiliser	-	≤ 1	2.2 ± 1.3^b
Unmined forest	-	2.0 ± 0.5^a	26.3 ± 4.3^a

Values with the same letters within each column were not significantly different at $P < 0.05$ (One-Way ANOVA with Tukey´s pairwise comparisons).

Table 4: Pair-wise comparisons of the R-statistic for effects of the six fertiliser treatments on plant community composition in the ANOSIM. Treatment numbers relate to the numbers used in Table 2.

Treatment	SSP top dressed in Winter (1)	SSP top-dressed in Summer (2)	SSP incorporated in Summer (3)	NPK top-dressed in Winter (4)	Rock phosphate incorporated in Summer (5)	Control (6)
SSP top-dressed in Winter (1)	-					
SSP top-dressed in Summer (2)	-0.011	-				
SSP incorporated in Summer (3)	-0.039	-0.102	-			
NPK top-dressed in Winter (4)	0.111	0.228	0.148	-		
Rock phosphate incorporated in Summer (5)	-0.235	-0.228	-0.099	0.259	-	
Control (6)	0.593*	0.667*	0.647*	0.298*	0.722*	-

Test statistic 0.141, 3.9 percent; * $P < 0.05$

List of Figures

Figure 1: The effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate), placement (incorporated (INC) and top-dressed (TD)) and timing of application on soil available (Colwell-)phosphorus in (A) furrows and (B) ridges (formed following deep ripping) (*** $P < 0.01$ for pooled Fisher's Least Significant Difference) in one-year-old jarrah forest restored after mining. Vertical bar charts represent 0-5 cm soil samples and horizontal bar charts represent depth increments. Errors bar are ± 1 standard error of the mean.

Figure 2: The effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate [RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of application (S: summer, or W: winter) on vegetation responses (A: native plant species richness, B: total stem density, C: species richness of non-native weed species, and D: total plant cover) in one-year-old jarrah forest restored after mining. Errors bar are ± 1 standard error of the mean.

Figure 3: The effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate [RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of application (S: summer, or W: winter) on stem density (A, C, E, G) and percentage cover (B, D, F, H) for four taxa (*Acacia celastrifolia*, *Bossiaea ornata*, *Banksia grandis* and *Lomandra* spp.) in one-year-old jarrah forest restored after mining. Errors bar are ± 1 standard error of the mean.

Figure 4: NM-MDS of the effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate [RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of application (S: summer, or W: winter) on vegetation composition in one-year old restored jarrah forest.

Figure 1

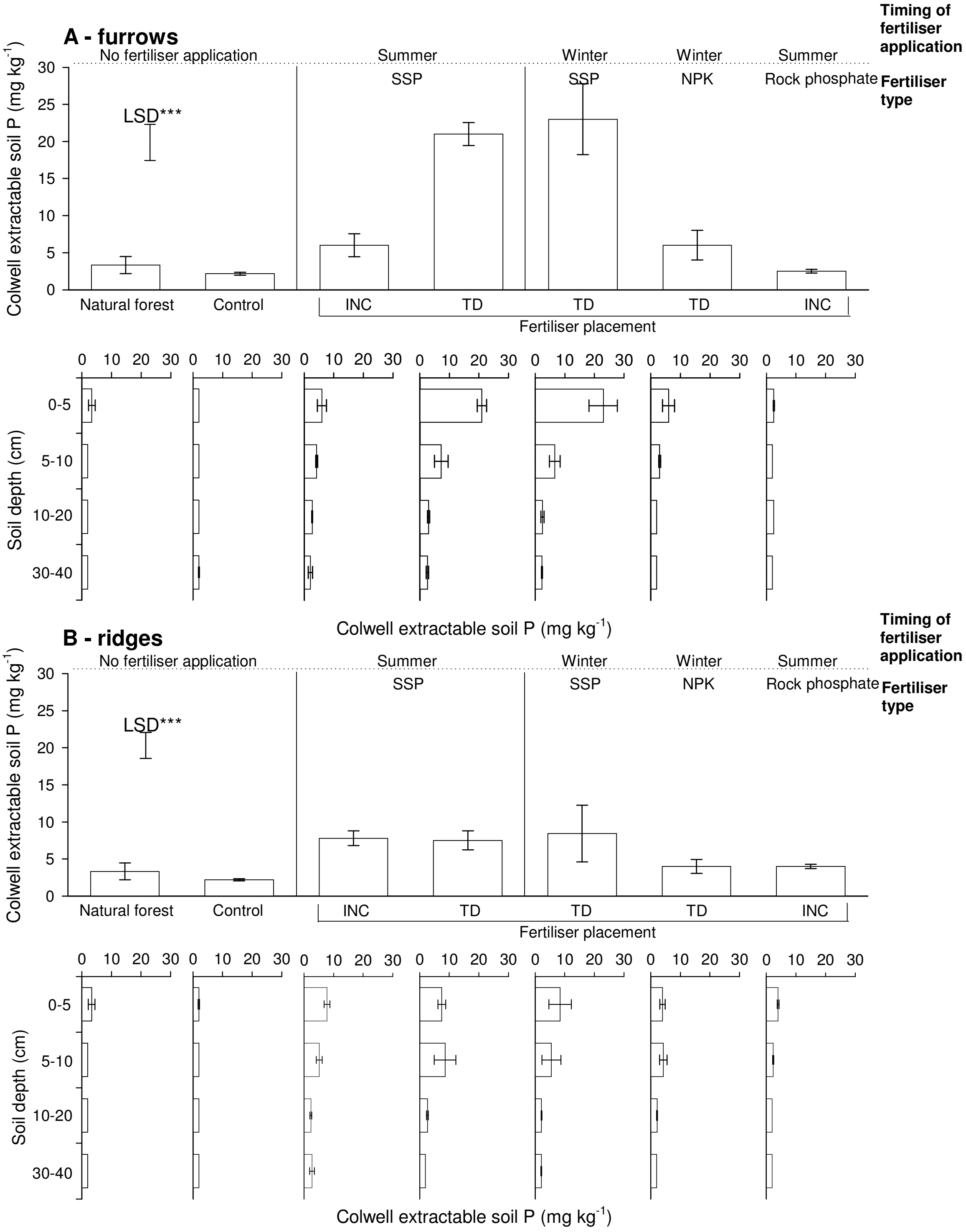
A - furrows
B - ridges
Timing of fertiliser application
Fertiliser type
No fertiliser application
Summer
Winter
Winter
Summer
SSP
SSP
NPK
Rock phosphate
LSD***
Colwell extractable soil P (mg kg^{-1})
0
5
10
15
20
25
30
Natural forest
Control
INC
TD
TD
TD
INC
Fertiliser placement
Soil depth (cm)
0-5
5-10
10-20
30-40
0 10 20 30

Figure 2

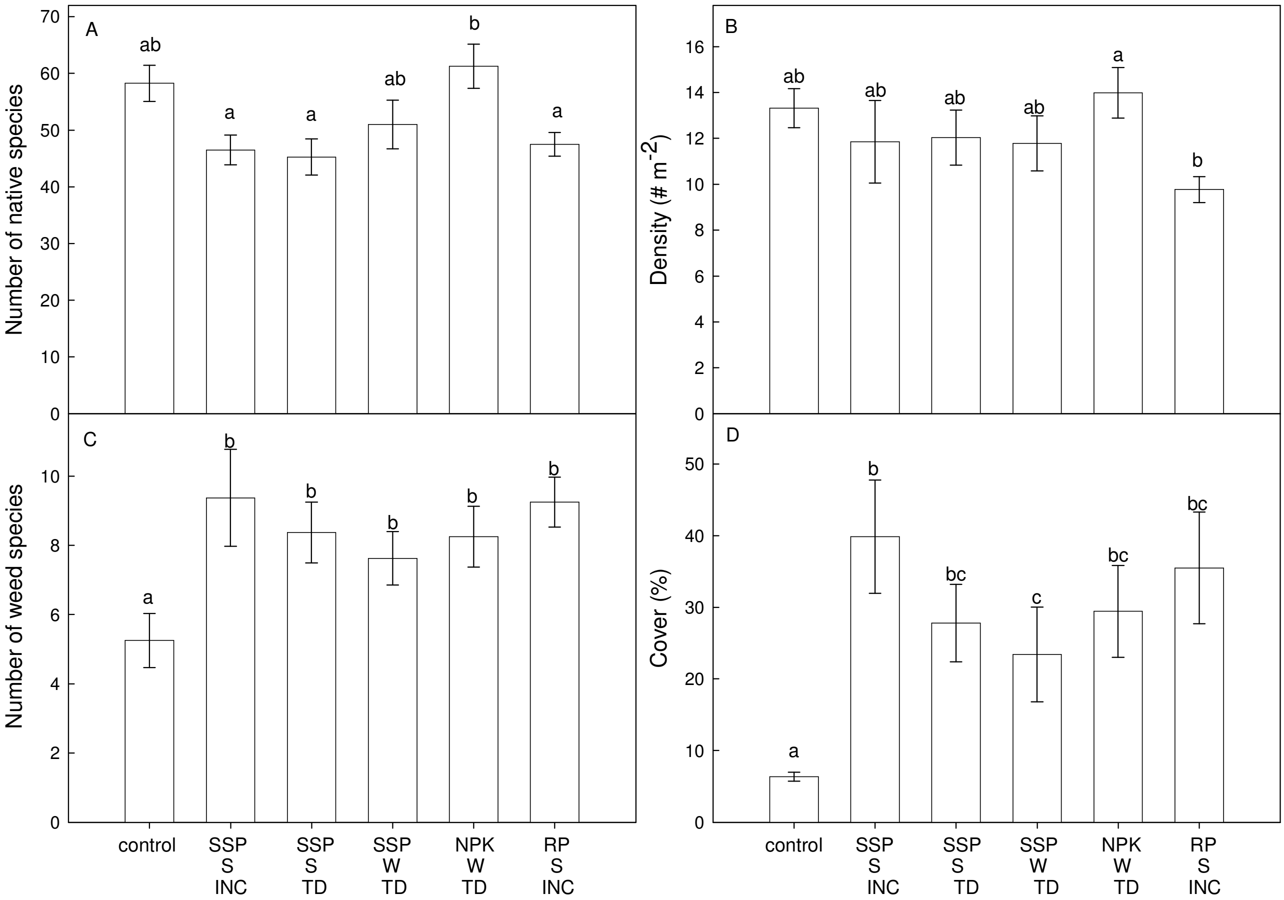
A
Number of native species
0
10
20
30
40
50
60
70
ab
a
a
ab
b
a
B
Density (# m^{-2})
0
2
4
6
8
10
12
14
16
ab
ab
ab
ab
a
b
C
Number of weed species
0
2
4
6
8
10
a
b
b
b
b
b
D
Cover (%)
0
10
20
30
40
50
a
b
bc
c
bc
bc
control
SSP S INC
SSP S TD
SSP W TD
NPK W TD
RP S INC

Figure 3

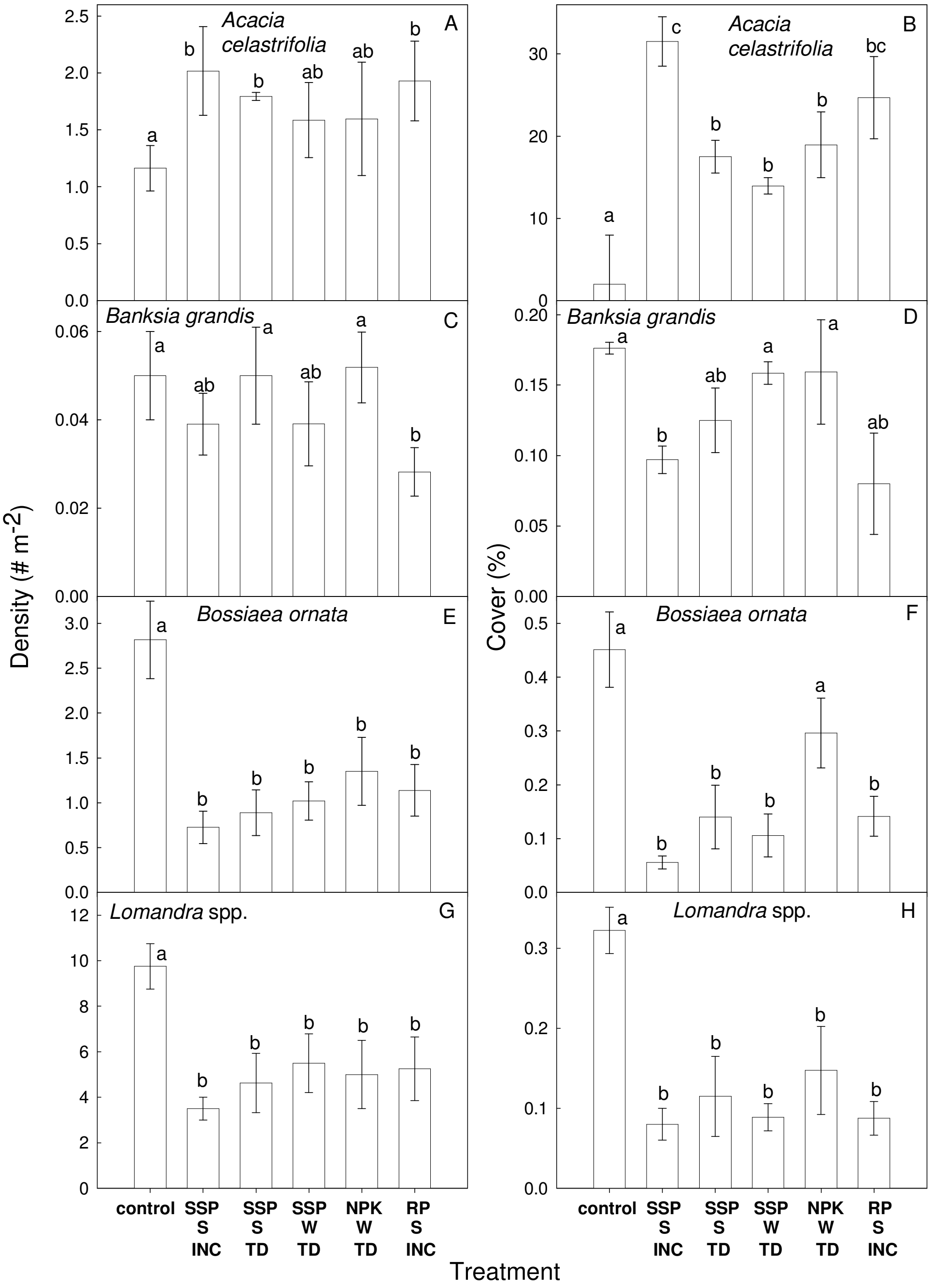
Acacia celastrifolia
A
B
Banksia grandis
C
D
Bossiaea ornata
E
F
Lomandra spp.
G
H
Density (# m-2)
Cover (%)
a
b
ab
bc
c
control
SSP S INC
SSP S TD
SSP W TD
NPK W TD
RP S INC
Treatment
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.00
0.02
0.04
0.06
0.05
0.10
0.15
0.20
0.1
0.2
0.3
0.4
0.5
0
2
4
6
8
10
12
20
30

Figure 4