

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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Page 1

1	The where, when and what of phosphorus fertilisation for seedling establishment in a
2	biodiverse jarrah forest restoration after bauxite mining in Western Australia
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15	Key words: fertiliser, legume, nitrogen, rehabilitation,

Page 2

17 Abstract

18 Fertiliser application to restore nutrients lost in the mining process and facilitate early plant 19 establishment and growth is a key step in the restoration of sites disturbed by mining. 20 However, few studies have investigated the effects of different fertiliser types and application 21 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 22 23 to growth on highly weathered phosphorus impoverished Ferralsol. In this study we 24 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 25 26 (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 27 28 Colwell-P concentrations (bar rock phosphate) and had considerably less N than found in the 29 native forest, even after N fertilisation. Fertiliser incorporation resulted in a more even 30 distribution of P down the soil profile and increased overall plant growth (as assessed by 31 percentage cover) compared with either top-dressed fertiliser application and no fertiliser, 32 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. 33 34 On average, unfertilised plots had 10 more native species per plot than those fertilised with P 35 only. Fertiliser application also reduced the abundance and cover of Bossiaea ornata and 36 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 37 38 being greatest when P (either rock phosphate or SSP) was incorporated. These data suggest 39 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 40

- 41 maximise diversity then moderation in P application and using fertilisers that also contain N
- 42 and K may be appropriate.

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

Page 4

45 **1. Introduction**

46 Vegetation removal and the processes of soil removal, stockpiling and mixing when soil is 47 stripped and respread result in significant losses and redistribution of soil nutrients during the 48 mining process. Consequently, applying fertiliser to replace lost nutrients is generally viewed 49 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 50 51 erosion in newly restored (bare) sites (Ward et al., 1990). However, the effects of different 52 fertiliser types and application methods in post mining restoration have received relatively 53 little attention.

54 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 55 56 Dixon, 2007; Williamson et al., 2011; Soliveres et al., 2012), which suggests species-specific 57 responses to fertiliser that may, in turn, affect competitive interactions among species. For 58 example, fertiliser addition reduced seedling survival of some woody species in quarry 59 restoration in Spain due to increased competition with herbaceous species (Soliveres et al., 60 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 61 62 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 63 64 a need to better understand fertiliser impacts on both species responses and community composition in mine site restoration. 65

66 The jarrah (*Eucalyptus marginata*) forest in Western Australia has highly weathered, 67 nutrient deficient soils. Post mining restoration in this, and other environments with nutrient 68 deficit soils can result in soil phosphorus concentrations remaining elevated for 20 or more 69 years after a single, initial fertiliser application (e.g. Banning et al., 2008; Spain et al., 2018;

70	Daws et al., 2019b). In contrast, when fertiliser containing inorganic N is applied, N is often
71	rapidly lost (Daws and Richardson, 2015; Sloan et al., 2016). For example, in newly restored
72	jarrah forest, a single application of 40 kg N ha ⁻¹ is undetectable after just 4.5 months (Daws
73	and Richardson 2015). As a result, it is common practice for eucalypt forest restoration after
74	bauxite mining to receive fertiliser only containing P (e.g. Standish et al., 2010; Spain et al.,
75	2018), based on the assumption that N_2 -fixing legumes, which are likely to increase N_2 -
76	fixation in response to P-application (Hingston et al., 1982), will increase soil-N (Grant et al.,
77	2007). However, any potential impacts on the restored plant community of applying solely P
78	based fertiliser, rather than fertiliser also containing N, have not been assessed.
79	In post mining restoration, inorganic fertilisers are typically applied as a top-dressing
80	(e.g. Koch, 2007; Nussbaumer et al., 2016; Sloan et al., 2016). For example, in jarrah forest
81	restoration following mining for bauxite, newly restored sites are top-dressed with fertiliser
82	the first winter/spring after the completion of restoration and seeding in the preceding
83	summer (Koch, 2007; Standish et al., 2015). However, P in these fertilisers is likely to remain
84	concentrated at the soil surface. For example, in agricultural systems vertical stratification of
85	P can occur when fertiliser is either top-dressed or shallow buried adjacent to seeds (Eckert,
86	1985; Mackay et al., 1987; Morrison and Chichester, 1994; Ryan et al., 2017). Furthermore,
87	the availability of shallow/surface applied fertilisers to plants is likely to be restricted in
88	restored mine sites where rapid drying of surface soils may occur. This will particularly be
89	the case for P, as diffusion of phosphate ions to plants is limited in dry soil (Nye and Tinker,
90	1977). Indeed, surface application limits P uptake in a range of agricultural systems (Piper
91	and de Vries, 1964; Scott, 1973; Jarvis and Bolland, 1991), with incorporation of P fertiliser
92	increasing crop yields relative to surface applications in a number of studies (Nable and
93	Webb, 1993; Sander and Eghbell, 1999; Teutsch et al. 2000; Singh et al. 2005).
94	Consequently, the benefits to plant growth of fertiliser application in restored mine sites may

Page 6

95 be greater when the fertiliser is incorporated into the soil rather than applied as a top-96 dressing. However, this remains to be tested.

97 In jarrah forest restoration, fertiliser is typically applied the first winter after the 98 completion of earthworks in the preceding summer (Koch, 2007): establishing seedlings may 99 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 100 from the onset of germination in autumn. While this remains to be tested, anecdotal evidence 101 102 shows various trends for some key native jarrah forest species: spring fertiliser application 103 produced optimal growth for some keystone jarrah forest species (Humphrys, 1987), while 104 Lockley and Koch (1996) found summer application (at the time of seeding) produced a 105 higher density of jarrah seedlings.

In this study, we investigated the effects of a range of fertiliser treatments on 106 107 establishment of one-year-old jarrah forest in the process of being restored after bauxite 108 mining in Western Australia. Specifically, we investigated whether fertiliser incorporation 109 versus a top-dressed application impacts on the distribution of available (Colwell) P in the 110 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 111 112 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 113 114 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 115 116 hypothesis that applied-N will have limited impact on vegetation responses due to only short-117 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 118

- response will be evident when fertiliser is present from the onset of germination / seedlingemergence (i.e. when applied in summer).
- 121

122 2. METHODOLOGY

123 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).

130 The forest vegetation comprises of the dominant overstorey species *Eucalyptus*

131 marginata (jarrah), which constitutes around 80 % of stems in both restored and unmined 132 forest (Daws et al., 2015). The remaining stems are mostly comprised of the subdominant 133 species Corymbia calophylla (marri). In addition, there is a mid-storey layer of Banksia 134 grandis, Allocasuarina fraseriana and Xanthorrhoea preisii with large woody shrubs of Bossiaea aquifolium, various Acacia species and a diverse understorey (Gardner and Bell, 135 136 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 137 138 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 139 140 subsoil accumulation of bauxite ores and also a depletion of nutrients, particularly 141 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 142

Page 8

remaining amorphous iron and aluminium oxides. Generically these soils are classified as
lateritic oxisols (USDA, 1999) or ferralsols (FAO, 2012).

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146 2.2. Experimental design

147 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 148 149 application (type of fertiliser, placement and time of application) on restoration. The area was 150 cleared of native jarrah forest vegetation in 2002, two years prior to the commencement of this 151 experiment. During the two years following vegetation clearing, topsoil and gravel overburden 152 were removed to expose the bauxite ore which was blasted and mined. Subsequently the area 153 was re-shaped to blend in with the surrounding landscape and the entire area deep-ripped to 154 relieve mining-related compaction. The overburden was then replaced followed by fresh 155 topsoil sourced from an adjacent area that had just been cleared for mining. Due to the 156 processes of being stripped, transported to and then re-spread across the area being restored, 157 the topsoil spread across the trial sites was relatively homogenous.

158 Following topsoil replacement, but prior to a final contour ripping stage, an incomplete 159 randomised block design was established at both sites. Six treatments that were operationally 160 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 161 162 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 163 164 wet soil conditions. Treatments were replicated either 8 times (the 3 single super phosphate 165 [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 166 167 contour ripping with the ripping step used to incorporate the fertiliser down the soil profile.

Page 9

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.

174 The chemical composition of the applied fertilisers was: 1) Single superphosphate at

175 450 kg ha⁻¹ 9.1% total P (equivalent by weight to 40.9 kg ha⁻¹ P), 10.1% sulphur, 9.0%

176 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).

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181 2.3. Soil sampling and analysis

182 Soil samples were collected in May 2005 from four $1 \text{ m} \times 1 \text{ m}$ quadrats located 5 m inside the treatment plot boundary (Fig. 1A). For consistency, soil samples were taken from each of 183 two furrows and two ridges, formed by ripping. Soil was sampled at 0-5, 5-10, 10-20 and 20-184 185 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 186 187 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, 188 soils were also sampled from three reference jarrah forest sites and in restored sites prior to 189 190 the addition of fertiliser. Samples were analysed at a commercial laboratory (CSBP Soil and 191 Plant Laboratories, Bibra Lake, Perth, Australia). Soils were hand textured and phosphate 192 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), NO₃-N and NH₄-N were also analysed.
- 196 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.

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203 2.5. Statistical Analysis

204 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 205 206 concentration and vegetation responses (species richness, total density, total cover and non-207 native weed species richness). In addition, for four relatively abundant taxa (Acacia 208 celastrifolia, Banksia grandis, Bossiaea ornata and Lomandra spp.) one-way ANOVA was 209 used to test for fertiliser effects on density and cover. Data was tested for normality and did not 210 require transforming. For soil P, the ANOVA was followed by Fisher's Least Significance 211 Difference test.

Multivariate data analysis was undertaken using PRIMERTM (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, <u>www.primer-</u>
<u>e.com</u>) 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

Page 11

218 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, 219 220 ANOSIM was used to test the significance of effects of fertiliser treatment on community 221 composition. 222 223 224 3. RESULTS 225 3.1. Effects of fertiliser treatment on soil N and P 226 With the exception of rock phosphate, P fertilised soils had elevated Colwell-P 227 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 228 consistently higher in furrows (Fig 1A) than in the ridges formed following ripping (Fig 1B). 229 230 Furthermore, in the top-dressed treatments elevated Colwell-P was largely restricted to the 0-231 5 cm depth. In the incorporated SSP treatment, Colwell-P concentrations were similar in the 232 furrows and ridges at 0-5 cm depth and there was a more uniform distribution of P down the 233 soil profile compared with top-dressing (Fig. 1). Fertiliser application, including NPK, had no effect on soil NO₃⁻ at a depth of 0-5 cm 234 235 in furrows (One-way ANOVA, P > 0.05; Table 3). This pattern was similar for ridges (data not shown). Soil NH₄⁺ differed with treatment (One-way ANOVA, P < 0.05) and was 236 significantly higher in the undisturbed reference forest soils than in restored soils, except for 237 the two SSP treatments where fertiliser was applied in summer (Table 3). 238 239 240 3.2. Fertiliser effects on plant species richness, density and cover The SSP and rock phosphate treatments had significantly reduced native plant species 241 richness compared with the control and NPK treatment (One-way ANOVA, P < 0.05; Fig. 242

243	2A). On average, across the SSP and rock phosphate treatments there were 10.7 fewer native
244	species per plot compared with the control (58.2 species per plot). Stem density in the control
245	and NPK treatments was similar, and both were significantly higher than in the rock
246	phosphate treatment (Fig. 2B).
247	The number of non-native weed species was highest in the two fertiliser incorporation
248	treatments (rock phosphate and SSP) and all fertiliser treatments had significantly higher
249	weed species richness than the control (One-way ANOVA, $P < 0.05$; Fig. 2C). Total plant
250	cover also responded significantly to fertiliser treatment (One-way ANOVA, $P < 0.05$; Fig.
251	2D) and was highest in the two fertiliser incorporation treatments (rock phosphate and SSP)
252	and lowest in the control. Fertiliser addition resulted in cover being up to six times higher
253	(SSP incorporated down the profile) than the control.
254	

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255 3.3. Fertiliser effects on taxa level responses

For all four taxa for which responses were individually investigated, fertiliser treatment had 256 257 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 258 259 fertiliser incorporation treatments and lowest in the control. For example, cover in the two 260 incorporation treatments ranged from 26-30 % compared with ca. 3 % cover in the control 261 treatment (Fig. 3B).

Stem density of *Banksia grandis* was significantly lower in the rock phosphate 262

263 treatment compared with the other five treatments (One-way ANOVA, P < 0.05; Fig. 3C).

264 Total cover of *B. grandis* was significantly lower in the rock phosphate, SSP incorporation

265 and top-dressed SSP in summer treatments compared with the control treatment.

For Bossiaea ornata, stem density was similar across the five fertiliser addition 266 267 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 268 269 being highest in the control followed by the NPK treatment. For example, cover of B. ornata 270 in the control was more than four times higher than in the SSP incorporation treatment. 271 All of the fertiliser treatments resulted in a significant reduction in the stem density 272 of *Lomandra* species compared with the control (One-way ANOVA, P < 0.05; Fig. 3G). 273 Cover of the Lomandra species was also significantly reduced in all the fertiliser addition 274 treatments: cover was approximately three times higher in the control than the fertiliser 275 treatments. 276 277 3.4. Community level responses to fertiliser treatments 278 In the MDS ordination, there was significant overlap in vegetation composition among the 279 five fertiliser treatments. The control plots appeared to cluster as a separate group. (Fig. 4). 280 This was supported by the ANOSIM which indicated a significant effect of fertiliser 281 treatment on community composition (global r = 0.141, P < 0.05; Table 4). Pair-wise 282 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

284 magnitude of the r-statistic the community composition in the NPK treatment was most

similar to the control (Table 4).

Page 14

4. Discussion

288 Fertiliser application is generally a routine step in mine restoration, with fertiliser typically 289 top-dressed either concurrent with, or following, seeding (e.g. Spain et al., 2015; Koch, 290 2007). However, our current data indicate that when fertiliser was applied as a top-dressing, 291 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 292 of P down the soil profile was more even and, in agreement with our first hypothesis, resulted 293 294 in increased plant growth. Phosphorus is generally relatively immobile in soil and is rapidly 295 sorbed as iron and aluminium hydroxides in jarrah forest soils (Lambers et al., 2008); 296 available-P can remain elevated, close to the soil surface, for 20 or more years in both the 297 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 298 299 distribution of P down the soil profile depending on the method of fertiliser application. 300 In restored mine sites where rapid drying of surface soils may occur, especially during 301 summer in Mediterranean climates such as in the jarrah forest, the availability of 302 shallow/surface applied fertilisers may be further restricted compared with fertiliser 303 incorporated throughout the soil profile. This will particularly be the case for P as diffusion of 304 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 305 (Piper and de Vries, 1964; Scott, 1973; Jarvis and Bolland, 1991; Nable and Webb, 1993; 306 307 Sander and Eghbell, 1999; Teutsch et al., 2000; Singh et al., 2005). In a mine restoration 308 context these results suggest that the same growth benefit resulting from a top-dressed 309 application may be achievable at lower application rates if the fertiliser is incorporated. 310 In newly restored sites, the positive effect of fertiliser incorporation on plant growth / 311 cover may be advantageous through an increase in site stabilisation and reduction in erosion

Page 15

312 risk. Vegetation cover has a significant effect on controlling run-off and soil erosion when at 313 least 30–40% of the soil surface is covered (Thornes, 1988; Thornes, 1990). While cover was 314 less than 10 % in the control, both of the fertiliser incorporation treatments resulted in total 315 cover in excess of 30 % within the first twelve months after seeding, demonstrating the 316 potential of fertiliser addition, and especially fertiliser incorporation, for reducing erosion. The P-supply in soils is typically heterogeneous and consequently most plant roots 317 grow preferentially in regions that contain high concentrations (Drew, 1975; Fransen et al., 318 319 1999; Hodge, 2004). For example, in agricultural systems, when fertiliser is applied as a band 320 beneath or adjacent to seeds, root proliferation is encouraged in the region of the band 321 (Anghinoni and Barber, 1980; Yao and Barber, 1986, Sander et al., 1990). Consequently, a 322 top-dressed application may encourage root proliferation in surface soils with a potential 323 negative impact on seedling survival during summer drought. While we did not investigate 324 root distribution down the soil profile in our current study, P placement at the surface altered 325 root distributions in two Australian native herbs (Denton et al., 2006). Incorporating fertiliser 326 down the soil profile would militate against this risk. Further studies of the impacts of 327 fertiliser incorporation on root responses in restored systems, and potential impacts on seedling survival during summer drought would be of value. 328

329 In support of hypothesis 2, fertiliser application increased overall plant growth, and 330 generally resulted in fewer native species and more weed species than the control. Fertiliser 331 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 332 333 establishing native species (Nussbaumer et al., 2016). Further, other recent studies in restored 334 Jarrah forests have shown that unfertilised treatments are more similar in composition to 335 native Jarrah forest communities than fertilised treatments (Daws et al., 2013, 2015, 2019a). 336 On an individual species / taxa level, there were also mixed responses to fertiliser application.

337	The understorey legume Acacia celastrifolia responded vigorously to all fertiliser treatments,
338	but especially incorporation. N ₂ -fixing legumes, such as A. celastrifolia, are generally P-
339	rather than N-limited and many respond vigorously to applied-P in mine restoration (Grant et
340	al., 2007; Daws et al., 2015, 2019b). Indeed, higher soil NH_4^+ concentrations in the single
341	superphosphate incorporation treatment compared with the control (12 versus 2.2 mg kg ⁻¹ ,
342	respectively) likely reflect greater growth and N ₂ -fixation by legume species such as A.
343	celastrifolia (Hingston et al., 1982; Koutika et al. 2014). In contrast, B. ornata and Lomandra
344	spp. responded negatively to all the fertiliser treatments. B. ornata is a small, slow growing
345	shrub and Lomandra spp. are small, grass-like understorey plants. While the negative effect
346	of applied fertiliser on abundance / growth of these taxa may be mediated by direct negative
347	effects of P (e.g. Lambers et al., 2002; Williams et al., 2019), it is also likely that these slow-
348	growing species are susceptible to competition from highly P responsive species such as A.
349	celastrifolia. Indeed, negative competitive effects of vigorous legume growth, in response to
350	applied P, on slow-growing understorey species have been reported elsewhere (e.g. Boyes et
351	al., 2011; Le Stradic et al., 2014; Daws et al., 2015, 2019ab), and may be a key mechanism
352	altering species competitive dynamics and consequent ecological trajectories.
353	Banksia grandis (proteaceae) is a mid-storey tree that produces cluster roots to
354	facilitate P uptake in P-deficient soils (Lambers et al., 2002). Many proteaceae including B.

355 *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

357 external concentrations are high (Shane et al., 2004). Whilst relatively unresponsive to top-

358 dressed fertiliser application, abundance and cover were reduced when P (including rock

- 359 phosphate) was incorporated further reinforcing the suggestion that P-availability to plants
- 360 may be greater when fertiliser is incorporated. It seems likely that the potentially toxic effects

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Page 17

362 presumably due to roots being placed away from surface soils where P is concentrated. 363 Fertiliser type had a significant and contrasting effect on available P and N and on 364 plant communities. The rock phosphate treatment had the seemingly incongruous effect of 365 affecting plant responses while having no effect on available (Colwell)-soil P. In terms of use 366 as a fertiliser in agriculture, rock phosphates have been concluded to be ineffective because 367 they do not dissolve rapidly in Western Australian soil (Boland and Gilkes, 1990). However, 368 the gradual release of phosphate ions from the mineral may be appropriate for the restoration 369 of native forest as applied rock phosphate is known to leave a considerable residue of 370 undissolved rock phosphate in the soil for several years after application. The lack of 371 detectable differences in soil test P is likely due to the sparing solubility of rock phosphate in 372 the bicarbonate (buffered at pH 8.5) used as an extractant in Colwell P: studies have 373 concluded that bicarbonate solution poorly predicts potential P release from the residual rock 374 phosphate in soil (Rajan et al., 1996; Saggar et al., 1999). Despite rock phosphate having low 375 solubility, the impact on plant responses may also have resulted from the rapid release of P 376 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 377 378 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 379 380 carboxylates to release P from strongly sorbed forms (Lambers et al., 2002). Consequently, 381 an alternative explanation for the plant responses to rock phosphate, including the negative 382 effect on growth and abundance of *B. grandis* is carboxylate mediated P dissolution. 383 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 384 385 was similar to the unfertilised control (Figure 2A). Further, the NPK treatment had a smaller

of P on these P-sensitive species are minimised when P is applied as a top-dressing,

Page 18

386 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 387 388 composition in the NPK treatment was the least different to the unfertilised plots, providing 389 little support for our third hypothesis, and suggesting some interaction with N fertilisation 390 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 391 392 caused a significant shift in relative floristic composition compared to unfertilised plots. This 393 demonstrates clearly that fertilisers have an unbalanced effect on early forest development, 394 that is quite different to simply encouraging greater uniform plant growth. In fact, the effects 395 we show are highly selective on a species by species basis. While being far from conclusive, 396 we can postulate that lower rate fertiliser regimes may be more suitable for this forest, and 397 that the effect of co-applied N needs to be better understood in terms of plant community 398 response.

399 While the mechanism(s) behind the response to N is unclear, applying N containing 400 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 N₂ fixing 401 402 species. Indeed, nitrate and ammonium addition can depress nodule prediction in seedlings of 403 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 404 soil ammonium concentrations (3.1 mg kg^{-1}) that were nearly as low as in the control, (3.12.2)405 mg kg⁻¹; Table 3), suggesting a lower rate of atmospheric N₂-fixation by legumes (Koutika et 406 407 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

Page 19

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.

421

422 5. Conclusion

423 Ultimately, restoration practitioners aim to achieve a rate of fertiliser application that both 424 compensates for nutrient losses and reflects the aims of restoration. Our current study 425 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 426 might be needed, while to maximise floristic diversity little or no fertilisation may be 427 428 appropriate. To optimise the use of fertiliser in mine site restoration to address these 429 competing outcomes will require a better understanding of the effects of applied fertiliser, 430 including longer term impacts.

431

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439

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- 634 P uptake and growth of wheat. Commun. Soil Sci. Plant Anal. 17, 819–827.

- 635 Table 1: Soil parameters in unmined forest and in restored sites prior to the addition of
- 636 fertiliser.
- 637

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
$\mathrm{NH_4^+}$	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

Treatment	Fertiliser type	Fertiliser	Timing of	P-application
number		placement	fertiliser	rate (kg ha
			application	
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	N/A	N/A	0
	application			
*Current	t practice at Boddington B	auxite Mine (Geo	rge et al., 2006)	

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

- 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
- 645 comparison, values for unmined forest were also determined. Treatment numbers relate to the
- 646 numbers used in Table 1. Data are ± 1 standard error of the mean.

Fertiliser type	Application type	Soil NO ₃	Soil NH4
		(mg kg ⁻¹)	(m g 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	-	$2.0 \pm 0.5^{\mathrm{a}}$	26.3 ± 4.3^{a}

647 Values with the same letters within each column were not significantly different at P < 0.05648 (One-Way ANOVA with Tukey's pairwise comparisons).

Page 33

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

Treatment	SSP top	SSP top-	SSP	NPK top-	Rock phosphate	Control
	dressed in	dressed in	incorporated in	dressed in	incorporated in	(6)
	Winter (1)	Summer (2)	Summer (3)	Winter (4)	Summer (5)	
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*	-

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

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List of Figures

Page 34

2	Figure 1: The effect of fertiliser type (single super phosphate [SSP], NPK and rock
3	phosphate), placement (incorporated (INC) and top-dressed (TD)) and timing of application
4	on soil available (Colwell-)phosphorus in (A) furrows and (B) ridges (formed following
5	deep ripping) (*** $P < 0.01$ for pooled Fisher's Least Significant Difference) in one-year-
6	old jarrah forest restored after mining. Vertical bar charts represent 0-5 cm soil samples and
7	horizontal bar charts represent depth increments. Errors bar are ± 1 standard error of the
8	mean.
9	Figure 2: The effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate
10	[RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of application (S:
11	summer, or W: winter) on vegetation responses (A: native plant species richness, B: total
12	stem density, C: species richness of non-native weed species, and D: total plant cover) in
13	one-year-old jarrah forest restored after mining. Errors bar are ± 1 standard error of the
14	mean.
15	Figure 3: The effect of fertiliser type (single super phosphate [SSP], NPK and rock phosphate
16	[RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of application (S:
17	summer, or W: winter) on stem density (A, C, E, G) and percentage cover (B, D, F, H) for
18	four taxa (Acacia celastrifolia, Bossiaea ornata, Banksia grandis and Lomandra spp.) in
19	one-year-old jarrah forest restored after mining. Errors bar are ± 1 standard error of the
20	mean.
21	Figure 4: NM-MDS of the effect of fertiliser type (single super phosphate [SSP], NPK and
22	rock phosphate [RP]), placement (incorporated (INC) and top-dressed (TD)) and timing of
23	application (S: summer, or W: winter) on vegetation composition in one-year old restored
24	jarrah forest.

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1 Figure 1



1 Figure 2

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1 Figure 3

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1 Figure 4

