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1 **The fate of fertiliser P in soil under pasture and uptake by subterranean clover – a field study**
2 **using ^{33}P -labelled single superphosphate**

3

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15

16 **Keywords:** fertilizer, improved grasslands, NSP, phosphorus cycling, *Trifolium subterraneum*

17

18 **Abstract**

19 *Background and aims* Single superphosphate (SSP) is a major source of phosphorus (P) used in grazing
20 systems to improve pasture production. The aim of this experiment was to determine the fate of
21 fertiliser P in clover pastures under field conditions.

22 *Methods* A procedure was developed to radiolabel SSP granules with a ^{33}P radiotracer, which was then
23 applied to the soil surface (equivalent to $\sim 12 \text{ kg P ha}^{-1}$) of a clover pasture. Recovery of fertiliser P
24 was determined in clover shoots, fertiliser granules and soil fractions (surface layer: 0 – 4 cm and sub-
25 surface layer: 4 – 8 cm).

26 *Results* The P diffusion patterns of the ^{33}P -labelled SSP granules were not significantly different to
27 those of commercial SSP granules ($P > 0.05$). Recovery of fertiliser P in clover shoots was 34 – 40 %.
28 A considerable proportion of the fertiliser P ($\sim 30 \%$) was recovered in the surface soil layer and was
29 largely inorganic P.

30 *Conclusions* Recovery of fertiliser P by clover plants was up to 40 % in the year of application. Much
31 of the fertiliser P in soil fractions was inorganic P, which suggests that the accumulation of organic P in
32 soils under clover pasture is not occurring on the single season timeframe at these sites.

33

34 **Abbreviations:** ANOVA – analysis of variance, ASPAC – Australasian Soil and Plant Analysis
35 Council, EC – electrical conductivity, EDTA – ethylenediaminetetraacetic acid, ICP-OES – inductively
36 coupled plasma optical emission spectroscopy, LSC – liquid scintillation counter, NIST – National
37 Institute of Standards and Technology, P – phosphorus, PBI – phosphorus buffering index, PUE –
38 phosphorus use efficiency, PVC – polyvinyl chloride cylinders, SSP – single superphosphate, TOC –
39 total organic carbon, TON – total organic nitrogen, WSP – water soluble phosphorus.

40

41 **Introduction**

42

43 Managed grazing systems occupy approximately 25 % of the world's land surface, but pasture growth
44 is often limited by phosphorus (P) (Asner et al. 2004). Single superphosphate (SSP) is one of the main
45 sources of P used across the world to overcome soil P deficiency and improve pasture (and crop)
46 production (Leikam and Achorn 2005). In Australia, considerable improvements in pasture production
47 have occurred since the introduction of subterranean clover and widespread use of SSP, which is
48 typically applied to the soil surface of a pasture at 9 – 12 kg P ha⁻¹ year⁻¹ (Donald and Williams 1954;
49 Russell 1960; Weaver and Wong 2011). This 'sub and super' philosophy continues to be an important
50 strategy for grazing systems in the high rainfall zone of south-eastern Australia, which accounts for
51 approximately 50 % of Australia's cattle and sheep production (Cayley et al. 1999; Mason and Kay
52 2000). However, the P balance efficiency of these grazing systems (i.e. the proportion of fertiliser P
53 that is removed in produce) is low and greater utilisation efficiency of fertiliser P is needed for both
54 economic and environmental reasons (McLaughlin et al. 2011; Simpson et al. 2011; Weaver and Wong
55 2011).

56 Historically, low P use efficiency (PUE) in fertilised pastures has been associated with the
57 accumulation of fertiliser P in the upper layers of soil profiles (McLaughlin et al. 2011; Simpson et al.
58 2011). This is largely based on soil P audits of long-term field sites that show much of the fertiliser P
59 can be accounted for as an increase in soil P between unfertilised and fertilised treatments (Haynes and

60 Williams 1992; Kohn et al. 1977; McCaskill and Cayley 2000; McLaren et al. 2014; Simpson et al.
61 1974; Watson 1969; Williams and Haynes 1992). McLaren et al. (2014) reported that approximately 90
62 % of the applied fertiliser P (across plots receiving between 15 and 24 kg P ha⁻¹ yr⁻¹ as triple
63 superphosphate) was recovered in the top 0 – 20 cm layer after 13 years of P fertilisation at a field site
64 under pasture located at the Ginninderra Experiment Station, near Hall, Australian Capital Territory,
65 Australia. Similarly, Watson (1969) reported that between 68 and 100 % of the applied fertiliser P
66 (across plots receiving between 4 and 35 kg P ha⁻¹ yr⁻¹ as single superphosphate) could be accounted
67 for in the top 0 – 10 cm layer after 10 years of P fertilisation at a field site under pasture located at the
68 Glen Lossie field station, near Kojonup, Western Australia, Australia. A limitation of these studies is
69 that they were not able to identify the pathways associated with the accumulation of fertiliser P in soils
70 under pasture. It is assumed that a low PUE corresponds directly with a low level of P availability due
71 to ‘fixation’ of fertiliser P by the soil after application. However, it is also possible that much of the
72 fertiliser P can be recovered by the plant but is subsequently returned to the soil surface via indirect
73 processes (e.g. plant decay or trampling, animal faeces and urine).

74 Direct labelling of fertiliser sources with P radiotracers (i.e. ³³P or ³²P) can be a powerful
75 technique for determining the fate of fertiliser P in agro-ecosystems (Frossard et al. 2011). Indeed,
76 many studies have successfully used P radiotracers to determine the recovery of fertiliser P by arable
77 crops (and in soil fractions) under field conditions (Dion et al. 1949; Mattingly and Widdowson 1958a;
78 b; McBeath et al. 2012; McLaughlin et al. 1988a; Mitchell et al. 1952; Nelson et al. 1948; Sharpley
79 1986; Spinks and Barber 1947). For example, McLaughlin et al. (1988a; 1988b; 1988c) investigated
80 the fate of ³²P-labelled monocalcium phosphate that was placed in a concentrated band 4 cm below the
81 soil surface and of ³³P-labelled medic residues that were mixed throughout the soil volume in wheat
82 crops grown in a loam soil near Mallala, South Australia, Australia. McLaughlin et al. (1988a) reported
83 that after 95 days of growth approximately 12 % of the added ³²P-labelled monocalcium phosphate and
84 5 % of the ³³P-labelled medic residues were recovered in wheat shoots. McLaughlin et al. (1988c) also
85 reported that in the 0 – 10 cm layer of the soil profile the majority of ³²P-labelled monocalcium
86 phosphate was recovered as inorganic P whereas much of the ³³P-labelled medic residues was
87 recovered as organic P, as determined using the ignition-H₂SO₄ extraction technique of Walker and
88 Adams (1958). Interestingly, between 22 and 28 % of the added ³³P-labelled medic residues, but less
89 than 5 % of the added ³²P-labelled monocalcium phosphate, could be recovered in the microbial

90 biomass of the 0 – 10 cm layer of the soil profile (McLaughlin et al. 1988b), as determined using the
91 hexanol method of McLaughlin et al. (1986). These studies provide detailed information on the
92 recovery of fertiliser P and leguminous pasture residue by arable crops under field conditions.
93 However, no previous published studies have used radiotracers to determine the recovery of fertiliser P
94 by clover plants under field conditions.

95 One of the challenges of the direct labelling technique is to ensure that the fertiliser source is
96 homogenously labelled with the radiotracer (Frossard et al. 2011). One way to overcome this is to
97 incorporate a radiotracer into the fertiliser source during the manufacturing process (Fardeau et al.
98 1995). For example, Bolan et al. (1987) made ^{32}P -labelled SSP by reacting rock phosphate with
99 sulfuric acid that contained a carrier-free $\text{H}_3^{32}\text{PO}_4$ radiotracer. This produced a mixture of ^{32}P -labelled
100 mono-calcium phosphate and gypsum (i.e. single superphosphate), which was then densed and made
101 into granules using a granulating drum. However, some of the issues with this procedure are: 1) it is
102 complex and time consuming (Hedley et al. 1988; Nunn and Dee 1954); 2) incomplete acidulation of
103 the rock phosphate can occur (Bolan et al. 1987; Nunn and Dee 1954); 3) as a by-product of the
104 acidulation process a cocktail of fluorosilicic and hydrofluoric acid vapour is emitted (Leikam and
105 Achorn 2005); 4) the source of phosphate rock can affect the chemical composition and solubility of
106 the SSP (Braithwaite et al. 1992; Hedley et al. 1988); and 5) there can be a large variation in granule
107 size and weight within each batch of fertiliser made (Braithwaite et al. 1992; Fogel 1960). The
108 relatively large amount of rock phosphate required for this procedure, and the low recovery of granules
109 with a consistent size and weight needed for experimentation purposes, can cause a considerable
110 dilution of radioactivity in the ‘ideal’ granules. It is likely that many of these issues have hindered
111 research on understanding the recovery of P from SSP in pastures under field conditions.

112 The first aim of this study was to develop a rapid and simple procedure to label SSP with a
113 ^{33}P radiotracer and to granulate using a ‘press and cut’ technique. The second aim was to compare the P
114 diffusion patterns of the labelled SSP granules with those of commercial SSP. The third aim of this
115 study was to determine the recovery of fertiliser P (i.e. ^{33}P -labelled SSP) in clover shoots and soil
116 fractions under field conditions in order to understand the processes associated with PUE in pastures.

117

118 **Materials and methods**

119

120 ³³P-labelled SSP granules

121

122 A rapid and simple procedure was used to label SSP with a ³³P radiotracer (half life 25.4 days), and
123 then ‘granulate’ (into cubes) using a cut and press technique. In summary, commercial SSP granules
124 were sourced and dried in a laboratory oven at 40 ° C overnight. The commercial SSP granules were
125 then ground to pass through a 150 µm sieve using a Siebtech© puck mill. Six × 7.0 g (± 0.1 g) amounts
126 of the SSP powder were then placed into separate 65 mm × 75 mm cylindrical plastic containers prior
127 to incubation with the ³³P radiotracer. A 2.0 mL aliquot of solution containing 60 MBq of ³³P was
128 added to each of these, resulting in a final moisture content of 22.2 %. The SSP slurry in each container
129 was stirred with a fine needle and then allowed to equilibrate overnight at 40 ° C in a laboratory oven.
130 Through this treatment, the moisture content of the ³³P-labelled slurry was reduced to 19.8 %, which
131 was ideal for pressing and cutting based on results of a pilot study. Each batch was worked into a
132 ‘bolus’ and placed into a 40 mm diameter ring, positioned between two plastic sheets and pressed into
133 a disc at a pressure of 1/3 ton (US) per 3.14 square inches (37.9 kPa) using a manual hydraulic press
134 (custom built by Templeton, Kenly & Co: Actuant Corporation, Menomonee Falls, WI). The discs
135 were then cut into 3 mm cubes using a knife and dried in a laboratory oven at 40 ° C overnight.
136 Granules were then weighed and grouped into batches prior to field application.

137

138 Visualisation of P diffusion pattern of SSP granules

139

140 The P diffusion pattern of the SSP granules made using the press and cut technique and that of the
141 commercial SSP granules was determined using the quantitative visualisation method of Degryse and
142 McLaughlin (2014). The Monarto soil as reported in Degryse and McLaughlin (2014) was used for the
143 test. In summary, eight Petri dishes (55 mm diameter × 10 mm deep) were filled with soil and wetted to
144 field capacity, then sealed with Parafilm and allowed to incubate overnight at 25 °C. A hole was made
145 in the soil at the centre of each Petri dish and a single granule was inserted. Each SSP granule weighed
146 55 – 60 mg and four replicates were used per SSP treatment. The granule was then covered over with
147 soil and the Petri dish re-sealed with Parafilm. Iron-oxide filter papers were prepared as set out by
148 Degryse and McLaughlin (2014). After 7 days incubation, iron-oxide filter papers were deployed for ~
149 10 mins on the soil surface of each Petri dish. The filter papers were then removed, stained with

150 malachite green solution, and the stained filter papers were scanned and analysed for the stained area
151 (i.e. area of P diffusion) using imaging software (GNU Image Manipulation Program, v. 2.8.10, Free
152 Software Foundation, Boston, MA). This involved the conversion of the scanned image to a binary
153 image (black-white) using a threshold value of 120 on a scale from 0 – 255, and the stained area
154 quantified (Degryse and McLaughlin 2014).

155

156 Field experimentation

157

158 *Site background and characterisation*

159

160 Two field sites were selected in the temperate region of south-eastern Australia that were under
161 permanent pasture. A site of high soil P fertility (i.e. predicted to not be fertiliser P responsive) located
162 at the Kybybolite Research Centre, near Naracoorte, South Australia (36°52' S, 140°55' E), and a site
163 of low soil P fertility (i.e. predicted to be fertiliser P responsive) located at the Ginninderra Experiment
164 Station, near Hall, the Australian Capital Territory (35°11' S, 149°3' E). Physico-chemical properties of
165 soils at the field sites used in this study are shown in Table 1.

166

167 [Suggested location of Table 1]

168

169 Chemical properties were determined on soil collected at pasture establishment and prior to
170 fertiliser application. Six soil samples were collected from four depth intervals (0 – 2.5 cm, 2.5 – 5 cm,
171 5 – 10 cm and 10 – 20 cm) and bulked within each depth and for each field site location. The soil was
172 then dried in a laboratory oven at 40 °C for one week and passed through a 2 mm sieve. Soil pH and
173 electrical conductivity (EC) were measured using a 1:5 soil to solution ratio in deionised water after
174 shaking for 1 hour. A measure of oxalate-extractable aluminium and iron was carried out as described
175 by McKeague and Day (1966). Total organic carbon (TOC) and nitrogen (TON) were determined on a
176 LECO TruSpec CN analyser (LECO Corporation, St Joseph, MI). An estimate of total organic P (and
177 inorganic P) was carried out using the ignition-H₂SO₄ method of Walker and Adams (1958). Available
178 soil P was determined by the bicarbonate extraction method of Colwell (1963), which is based on the
179 method of Olsen et al. (1954). In summary, 0.5 g (\pm 0.02) of soil was extracted with 0.5 M NaHCO₃

180 solution adjusted to pH 8.5 at a 1:100 soil to solution ratio and shaken for 16 hours. The Colwell
181 extracts were then centrifuged at $1610 \times g$ for 20 minutes, filtered through a Whatman no. 42 filter
182 paper, and concentrations of inorganic P in the filtrate were determined using the molybdenum blue
183 method of Murphy and Riley (1962). All values were blank corrected before standard volume and
184 weight conversions. A measure of P buffering capacity was determined using the single-point method
185 of Burkitt et al. (2008), referred to as the P buffering index (PBI).

186 Total soil P was determined using laboratory X-ray fluorescence carried out at Geoscience
187 Australia laboratories, Canberra, Australia. A Philips PW2404 4 kW sequential wavelength dispersive
188 spectrometer fitted with a rhodium X-ray tube was used to analyse fused beads made from 1.0 g
189 soil/6.0 g 12:22 flux (35 % lithium tetraborate/65 % lithium metaborate). Analytical recoveries were
190 calculated using reported values of the Canadian Certified Reference Materials Project (CCRMP) soil
191 standards (Till-1 and Till-4). On average, an analytical recovery of 99 % was obtained using laboratory
192 X-ray fluorescence.

193

194 *Experimental Design*

195

196 In May 2013, a 5 m \times 5 m fence was erected at each field site to prevent outside interference by
197 grazing animals (e.g. sheep, kangaroos and rabbits) and the pasture within the enclosure was sprayed
198 with knockdown herbicide to remove all vegetation. In June 2013, a subterranean clover sward was
199 established across the enclosure by broadcasting seed to ensure that there was at least 1 seed cm^{-2} and
200 then lightly scratching seed into the soil surface. After clover establishment, 18 'open-ended' cylinders
201 (polyvinyl chloride cylinders – PVC – cores) of 15 cm diameter and 18 cm in height were inserted 15
202 cm into the soil at each site, so that each core protruded 3 cm above the soil surface, as described by
203 McLaughlin et al. (1988a). Basal nutrients were then applied across the field sites in July 2013 to
204 include: 1) Ca as CaSO_4 to supply 6.4 kg Ca ha^{-1} ; 2) K as K_2SO_4 to supply 44.9 kg K ha^{-1} ; 3) Mg as
205 MgSO_4 to supply 12.1 kg Mg ha^{-1} ; 4) Mo as MoO_3 to supply 0.1 kg Mo ha^{-1} ; 5) B as H_3BO_3 to supply
206 0.3 kg B ha^{-1} ; 6) Cu as CuSO_4 to supply 0.7 kg Cu ha^{-1} ; 7) Zn as ZnSO_4 to supply 1.4 kg Zn ha^{-1} , and;
207 8) S as all previously mentioned sulphate salts to supply 40.5 kg S ha^{-1} .

208

209 Each treatment was replicated six times in a randomised block design. Treatments included a
control (no added P fertiliser), the addition of commercial SSP granules (8 % P) to supply ~ 12 kg P ha^{-1}

210 ¹, and the addition of ³³P-labelled SSP granules to supply ~12 kg P ha⁻¹. In August 2013, the clover
211 pasture sward was harvested to no less than 3 cm above the soil surface and discarded prior to fertiliser
212 placement. At the Ginninderra site, four granules were used to supply 250 mg (± 5 mg) of SSP core⁻¹
213 (average 62.5 mg of SSP granule⁻¹) to the soil surface of each core for the commercial SSP and ³³P-
214 labelled SSP treatments. At the Naracoorte site, four granules were used to supply 285 mg (± 5 mg) of
215 SSP core⁻¹ (average 71.3 mg of SSP granule⁻¹) to the soil surface of each core for the commercial SSP
216 and ³³P-labelled SSP treatments. The P rate of the commercial SSP treatment that was applied to the
217 Ginninderra and Naracoorte field sites was 11.4 and 12.9 kg P ha⁻¹, respectively. The P rate and
218 radioactivity of the ³³P-labelled SSP treatment that was applied to the Ginninderra and Naracoorte field
219 sites was 11.4 and 13.0 kg P ha⁻¹ and 4.2 and 4.8 MBq core⁻¹, respectively. In addition, the control
220 cores received a second application of nutrients to balance the S from the SSP treatments, which
221 included Ca and S as CaSO₄ to supply 15.6 and 12.5 kg (Ca + S) ha⁻¹ respectively. Each site was
222 irrigated to ensure the cumulative rainfall throughout the growing season was close to that of the long-
223 term average (Table 2). Small amounts of irrigation reported in Table 2 are mostly associated with
224 watering in applications of basal nutrients to ensure they were accessible to clover roots.

225

226 [Suggested location of Table 2]

227

228 *Field agronomy and harvest*

229

230 Four clover shoot cuts were collected at the Naracoorte field site and two clover shoot cuts were
231 collected at the Ginninderra site. The clover shoots were cut to 3 cm above the soil surface, except at
232 the last harvest where the clover shoots were removed to the soil surface. At the final harvest, the
233 residue of all fertiliser granules was collected from the soil surface, and the cores were removed and
234 sectioned into three layers; the surface layer (0 – 4 cm) and subsurface layer (4 – 8 cm), and a ‘buffer’
235 soil layer (8 – 15 cm) which was discarded. At the Ginninderra and Naracoorte sites, 92 % and 100 %
236 of the SSP granule residues was recovered from the soil surface, respectively.

237

238 *Plant digestion and total P analysis*

239

240 Clover shoots were dried in a laboratory oven at 60 °C for seven days. After drying, these samples
241 were weighed and then ground to pass through a 2 mm sieve using a rotor cross beater grinder (Retsch,
242 Haan, Germany) prior to chemical and isotopic analysis. Clover shoots were digested as set out by
243 Zarcinas et al. (1987) and subsequently analysed for P by inductively coupled plasma optical emission
244 spectroscopy (ICP-OES). Analytical recoveries of P by this method for the National Institute of
245 Standards and Technology (NIST) 1573a plant standard, and the Australasian Soil and Plant Analysis
246 Council (ASPAC) ASPAC-84 plant standard were 89 % and 95 % (average of nine replicates),
247 respectively.

248

249 *Granule digestion and total Ca, P and S analysis*

250

251 Concentrations of total P were determined on the commercial SSP granules and ³³P-labelled SSP
252 granules prior to field application, and on the granule residues collected from the soil surface after the
253 last harvest. The granules that were collected from the field were dried in a laboratory oven at 60 °C for
254 seven days prior to chemical analysis. All granules were digested as set out by Zarcinas et al. (1996)
255 and subsequently analysed for Ca, P and S by ICP-OES. Analytical recoveries of Ca, P and S by this
256 method for the Sigma-Aldrich BCR-032 rock phosphate standard were 97 %, 92 % and 86 % (average
257 of six replicates), respectively.

258

259 *Soil extraction and P analysis*

260

261 All soil fractions were extracted with sodium hydroxide–ethylenediaminetetraacetic acid (NaOH-
262 EDTA) at a 1:10 soil to solution ratio as described by Doolette et al. (2010). Concentrations of
263 inorganic and total P were determined on the filtrates using the molybdenum blue method of Murphy
264 and Riley (1962) and ICP-OES, respectively. Organic P in the extract was calculated as the difference
265 between total P and inorganic P.

266 The ignition-H₂SO₄ extraction technique of Saunders and Williams (1955) as modified by

267 Walker and Adams (1958) was carried out on all soil fractions. Concentrations of inorganic P in the
268 filtrates were determined using the molybdenum blue method of Murphy and Riley (1962).

269 Concentrations of inorganic P for the ignited and unignited extracts are referred to as ignition-H₂SO₄

270 extractable total and inorganic P, respectively. The difference between total and inorganic P
271 determined by ignition-H₂SO₄ extraction is referred to as organic P.

272

273 *Liquid scintillation counting for ³³P analysis*

274

275 The ³³P activity of all plant and granule digests, and all soil extracts was measured using a Rackbeta II
276 Wallac® liquid scintillation counter (LSC). A measure of ³³P activity in NaOH-EDTA and H₂SO₄
277 (ignited and non-ignited) soil extracts was carried out to determine the recovery of fertiliser P as
278 inorganic and total (organic by difference) forms of P in soil fractions. For total P, the ³³P activity was
279 determined on an aliquot of all plant and granule digests, and the filtrates of NaOH-EDTA and the
280 H₂SO₄ (ignited) soil extracts. For inorganic P fractions, the ³³P activity was determined on the NaOH-
281 EDTA extracts that had been acidified to flocculate organic P, and on the H₂SO₄ extract of the
282 unignited soil. This involved acidifying 4 mL of the NaOH-EDTA extract with 1 mL of 2.5 M H₂SO₄;
283 the resulting solution was then centrifuged at 1610 × g for 20 minutes and the supernatant analysed for
284 ³³P activity using LSC.

285 The solution colour of all digests and extracts were examined prior to ³³P analysis so that the
286 colour ranges of unknown samples were within that of the quench curve established for LSC analysis.
287 Consequently, the NaOH-EDTA extracts from the total P fraction samples were diluted using a 1:10
288 ratio of extract to water. The scintillant cocktail was made using 2 mL of sample and 10 mL of
289 scintillant (Perkin Elmer UltimaGold AB). All samples were analysed by LSC for 2 minutes in
290 duplicate, and the ³³P counts were corrected for sample volume, blanks, radioactive decay and dilution.
291 All ³³P counts were corrected to the same reference date (T₀), which allows for direct comparison of ³³P
292 radioactivity across all samples.

293 The specific activity of the granules (MBq mg⁻¹ water-soluble P – WSP) was calculated using
294 Equation 1 and corrected for WSP as the ³³P radiotracer would have only labelled the WSP fraction of
295 the total P in SSP, which was 99 % of total P. Briefly, 0.5 g of SSP was extracted with 50 mL of
296 deionised water and shaken for 16 hours (three replicates). The specific activity of the ³³P-labelled SSP
297 granules was 0.21 MBq mg⁻¹ WSP. The proportion of plant P derived from fertiliser P was calculated
298 using Equations 1 and 2, and the recovery of fertiliser P in various components of the pasture system

299 was calculated using Equation 3. In Equations 2 and 3, no correction was made for water-insoluble P in
 300 the SSP as this represented less than 1 % of the total P in the product.

301

302 Specific activity (SA) of samples (plant digests, initial and residue granule digests, and soil extracts)
 303 was calculated by:

304

$$305 \quad (1) \text{ Specific activity (MBq mg P}^{-1}\text{)} = \frac{\text{Total sample activity (MBq core}^{-1}\text{)}}{\text{Total sample P (mg P core}^{-1}\text{)}} \times 100$$

306

307 The proportion of plant P derived from the fertiliser was calculated by:

308

$$309 \quad (2) \text{ Plant P derived from fertiliser (\%)} = \frac{\text{SA of plant (MBq mg P}^{-1}\text{)}}{\text{SA of fertiliser (MBq mg WSP}^{-1}\text{)}} \times 100$$

310

311 The recovery of fertiliser P in samples (clover shoots, granule residues, soil extracts) was calculated
 312 by:

313

$$314 \quad (3) \text{ Fertiliser recovery (\%)} = \frac{\text{(Fertiliser P in sample (MBq core}^{-1}\text{))}}{\text{(Total fertiliser P added (MBq core}^{-1}\text{))}} \times 100$$

315

316 Statistical analyses

317

318 All statistical analyses were carried out using R 3.0.2 (R Core Team 2013). A one-way analysis of
 319 variance (ANOVA), orthogonal contrasts and the Tukey *post hoc* test of honest significance difference
 320 were used to compare treatment means at the 5 % ($P = 0.05$) level of significance. The blocking factor
 321 was not significant and was dropped from the ANOVA model. All regression models were checked for
 322 normality of residuals and constant variance using diagnostic plots, the Shapiro-Wilk test, and
 323 Levene's test (Levene 1960; Shapiro and Wilk 1965). Outliers were identified using Cook's distance
 324 plot (Cook and Weisberg 1982).

325

326 **Results**

327

328 Validation of isotopically labelled SSP granules

329

330 The visual appearance of the P diffusion patterns as determined using the method of Degryse and
331 McLaughlin (2014) was similar between the SSP granules made using the press and cut technique and
332 those of the commercial SSP granules (Figure 1). The binary images of these P diffusion patterns are
333 shown in Figure 2. The total area of P diffusion for the SSP granules made using the press and cut
334 technique were not significantly different ($P < 0.05$) to those of the commercial SSP granules after one
335 week incubation; average values were 10.4 cm^2 and 10.8 cm^2 , respectively.

336

337 [Suggested location of Figure 1]

338

339 The coefficient of variation (CV %) for the specific activity (MBq mg WSP^{-1}) of 10 granules
340 was very low (2.5 %). In addition, the elemental composition (i.e. Ca, P and S) of the commercial SSP
341 granules collected from the soil surface at the end of the field experiment was not significantly
342 different ($P < 0.05$) to those of the ^{33}P -labelled SSP granules (Table 3).

343

344 [Suggested location of Table 3]

345

346 Field Experimentation

347

348 *Site characteristics*

349

350 Soil pH ranged from acidic to slightly acidic for all surface and subsurface layers at the Naracoorte and
351 Ginninderra field sites (Table 1). Concentrations of total organic C, organic N and organic P were
352 higher at the Ginninderra site than at the Naracoorte site (Table 1). The concentration of Colwell-
353 extractable P was 12 mg P kg^{-1} when averaged across the 0 – 10 cm layer at the Ginninderra site; this
354 soil would be considered potentially responsive to fertiliser P (Colwell 1963; Reuter et al. 1995).
355 Conversely, the concentration of Colwell-extractable P was 57 mg P kg^{-1} (again averaged across the 0
356 – 10 cm layer) at the Naracoorte site; this soil would be predicted to be non-responsive to fertiliser P
357 (Colwell 1963; Reuter et al. 1995). The soil P sorption capacity at the Ginninderra site, as indicated by

358 PBI, oxalate-extractable aluminium and oxalate-extractable iron, was approximately double that of the
359 Naracoorte site (Table 1).

360 The in-season rainfall at the Ginninderra site was higher than at the Naracoorte field site
361 (Table 2). The annual rainfalls at the Ginninderra (622 mm) and Naracoorte (479 mm) field sites for
362 2013 were 90 and 98 % of the long-term averages, respectively (Table 2). The cumulative rainfall and
363 irrigation at the Ginninderra (708 mm) and Naracoorte (490 mm) field sites for 2013 were 103 and 100
364 % of the long-term averages, respectively.

365

366 *Dry matter, P uptake and proportion of plant P derived from fertiliser*

367

368 The cumulative biomass removal for the fertilised treatments was not significantly different ($P < 0.05$)
369 to that for the unfertilised control at both sites (Table 4). Concentrations of P in plant tissue of fertilised
370 treatments at the Ginninderra and Naracoorte field sites were generally higher than in non-fertilised
371 treatments (Figure 2). The cumulative P uptake for the fertilised treatments was significantly different
372 ($P < 0.05$) to that for the unfertilised control at the Ginninderra site but not at the Naracoorte site (Table
373 4). The cumulative P removal was approximately three times higher at the Naracoorte site than at the
374 Ginninderra site, despite similar amounts of biomass removal (Table 4).

375

376 [Suggested location of Figure 2]

377

378 The proportion of plant P that was derived from the fertiliser can be calculated by dividing the
379 specific activity of the plant by that of the fertiliser (see Equation 2). The proportion of plant P in
380 clover shoots that was derived from the fertiliser at the Ginninderra site (37 %) was approximately
381 double that at the Naracoorte field site (19 %) (Figure 3).

382

383 [Suggested location of Figure 3]

384

385 *Recovery of fertiliser P applied to clover pastures*

386

387 The proportion of fertiliser P recovered in clover shoots at the Ginninderra and Naracoorte field sites
388 was 34 % and 40 %, respectively (Table 5 and Figure 4).

389

390 [Suggested location of Table 5]

391

392 At both sites, 32 % of the applied fertiliser P was recovered by H₂SO₄ extraction of ignited
393 soil from the surface (0 – 4 cm) layer of the soil profile (Table 5 and Figure 4). Similarly, 24 and 31 %
394 of the applied fertiliser P was recovered by NaOH-EDTA extraction of the surface (0 – 4 cm) soil layer
395 at the Ginninderra and Naracoorte sites, respectively (Table 5 and Figure 4). Less than 5 % of the
396 applied fertiliser P was recovered from the subsurface layer (4 – 8 cm) at the Naracoorte site using the
397 ignition-H₂SO₄ extraction procedure (Table 5 and Figure 4). Concentrations of the ³³P radionuclide
398 were too low for detection at the Ginninderra site in ignition-H₂SO₄ extracts, and at both sites in
399 NaOH-EDTA extracts of the 4 – 8 cm layer (Table 5).

400 The majority of fertiliser P that was recovered from the surface soil layer at both sites using
401 the ignition-H₂SO₄ and NaOH-EDTA extraction techniques was identified as inorganic P, which
402 ranged from 20 to 30 % of the applied fertiliser P (Table 5). As a proportion of total extractable P,
403 between 89 to 95 % of the fertiliser P in soil extracts was identified as inorganic P using the ignition-
404 H₂SO₄ and NaOH-EDTA extraction techniques in all cases, except at the Ginninderra site using the
405 ignition-H₂SO₄ extraction technique, where 60 % of the total extractable P was identified as inorganic
406 P (Table 5).

407 Less than 5 % of the applied fertiliser P was recovered as organic P in the surface layer as
408 determined using the NaOH-EDTA extraction technique at the Ginninderra site, and also at the
409 Naracoorte site using both the ignition-H₂SO₄ and NaOH-EDTA extraction techniques (Table 5). The
410 exception to this was at the Ginninderra site using the ignition-H₂SO₄ extraction, where 13 % of the
411 fertiliser P was recovered as organic P in the surface layer (Table 5). As a proportion of total
412 extractable P, 5 to 11 % of the fertiliser P in soil extracts was identified as organic P using the ignition-
413 H₂SO₄ and NaOH-EDTA extraction techniques in all cases, except at the Ginninderra site using the
414 ignition-H₂SO₄ extraction technique, where 40 % of the total extractable P was identified as organic P
415 (Table 5).

416 Approximately 5 % of the applied ^{33}P was recovered in the fertiliser granule residues that
417 were collected from the soil surface at the Ginninderra and Naracoorte field sites (Table 5 and Figure
418 4). However, based on concentrations of total P in the granule residue, 13 and 14 % of the applied
419 fertiliser P (detected as ^{31}P) was recovered in the granule residues that were collected from the soil
420 surface at the Ginninderra and Naracoorte field sites, respectively.

421 The proportion of applied fertiliser P that was unaccounted for was determined as the
422 difference in radioactivity (MBq core^{-1}) added as ^{33}P -labelled SSP and that recovered in clover shoots,
423 fertiliser granule residues and extracted in soil fractions using the ignition- H_2SO_4 extraction. The
424 proportion of applied fertiliser P that was unaccounted for was 27 and 20 % of the applied fertiliser P at
425 the Ginninderra and Naracoorte field sites, respectively (Table 5 and Figure 4).

426

427 **Discussion**

428

429 Validation of isotopically labelled SSP granules

430

431 The labelling approach and subsequent granulation using the press and cut technique proved to be an
432 accurate method for radiolabelling SSP granules. Close agreement between the P release behaviour of
433 the ^{33}P -labelled SSP and commercial SSP granules is likely due to the matching chemical composition
434 of the fertiliser material (Braithwaite et al. 1992; Hedley et al. 1988), and because the majority of P
435 within SSP is water soluble (Degryse and McLaughlin 2014; Williams 1971b). Further evidence of this
436 is that both granule types had similar concentrations of elements in the granule residues collected from
437 the soil surface at the last harvest of the field experiment. The P diffusion pattern of both granule types
438 is consistent with previous studies that showed the majority of P movement from SSP granules occurs
439 within the first week of application to the soil, and is generally restricted to a few centimetres from the
440 granule (Lawton and Vomocil 1954; Williams 1971b). Indeed, the press and cut technique has
441 successfully been used before in non-isotopic studies for investigating the agronomic effectiveness of
442 various mixtures of fertiliser material and added impurities (Mullins et al. 1995; Prochnow et al. 2004).

443 An important consideration of the direct labelling approach is to ensure that the radiotracer is
444 uniformly distributed within the fertiliser source (Frossard et al. 2011). A low coefficient of variation
445 for the specific activity (MBq mg WSP^{-1}) of ^{33}P -labelled SSP granules indicates that the ^{33}P radiotracer

446 was homogenously labelled within the SSP. The homogeneous labelling can be attributed to the
447 incubation step where a fraction of the monocalcium phosphate is dissolved, mixed with ^{33}P , and then
448 re-formed on drying, thus incorporating the ^{33}P into the monocalcium phosphate matrix. Consequently,
449 the press and cut technique used to make ^{33}P -labelled SSP granules was successful in producing
450 granules that have equal P release properties to commercial SSP granules.

451

452 The fate of fertiliser P in pasture systems

453

454 A SSP rate of $\sim 12 \text{ kg P ha}^{-1}$ is commonly used in south-eastern Australia as a maintenance rate for soil
455 P fertility (Weaver and Wong 2011). A lack of clover response to the application of $\sim 12 \text{ kg P ha}^{-1}$ was
456 unsurprising at the Naracoorte site given that soil P fertility was well in excess required for optimum
457 pasture growth (Reuter et al. 1995). The application of $\sim 12 \text{ kg P ha}^{-1}$ at the Ginninderra site was likely
458 too small to result in a significant increase in cumulative dry matter. Simpson et al. (2010) have
459 previously shown that the application of $\sim 35 \text{ kg P ha}^{-1}$ is required to achieve maximum clover growth
460 with a single fertiliser application to low soil P at this site. However, there was a significant increase in
461 the cumulative P uptake between fertilised and non-fertilised treatments at the Ginninderra site,
462 concentrations of P in plant tissue also appeared higher in fertilised treatments than in non-fertilised
463 treatments at both field sites, which support an increased supply of P to clover plants in fertilised
464 treatments.

465 A lower initial soil P status at the Ginninderra site compared to that of the Naracoorte site is
466 likely the main reason for a higher proportion of clover P uptake that is derived from fertiliser sources
467 at the former than at the latter field site (McBeath et al. 2012; Morel and Fardeau 1990). It has been
468 shown that the relative importance of fertiliser P to plant P uptake is influenced by native soil P fertility
469 (Dean et al. 1948; Morel and Fardeau 1990). Morel and Fardeau (1990) reported a decrease in the
470 proportion of plant P derived from fertiliser P (^{32}P -labelled diammonium phosphate) when the quantity
471 of plant-available soil P increased for ryegrass pastures grown under glasshouse conditions.

472 The recovery of applied fertiliser P in clover shoots was 34 % and 40 % at the Ginninderra
473 and Naracoorte field sites respectively. No published studies have reported the recovery of fertiliser P
474 by clover pastures under field conditions, but several have done so for arable crops. Fertiliser
475 recoveries for P by arable crops under field conditions generally range from 5 % to 35 % (Dion et al.

476 1949; Mattingly and Widdowson 1958a; b; McBeath et al. 2012; McLaughlin et al. 1988a; Mitchell et
477 al. 1952; Nelson et al. 1948; Sharpley 1986; Spinks and Barber 1947). Relative to the recovery of
478 fertiliser P reported for arable crops, recoveries of 34 – 40 % suggest that clover plants at the
479 Ginninderra and Naracoorte field sites were at the upper end of fertiliser P recovery that is typically
480 found for arable crops. Some of the reasons for a relatively high recovery of fertiliser P in clover
481 pastures may include: 1) the root systems of clover pastures were established and concentrated in the
482 surface layer of the soil profile, which is near to where the fertiliser P is applied (Ozanne et al. 1961);
483 2) the majority of P within SSP is water soluble and would be readily available for plant uptake
484 (Williams 1971b); 3) the movement of fertiliser P is generally restricted to a few centimetres from the
485 granule and would remain in the root zone during the growing season (Lawton and Vomocil 1954); 4)
486 fertiliser P is unlikely to become rapidly unavailable to plants over the growing season in soils of low
487 to moderate sorption capacity (Dorahy et al. 2007; He et al. 2004); and 5) there were favourable
488 seasonal conditions at the Ginninderra and Naracoorte field sites. More research is needed to ascertain
489 if the results of the current study are typical for other clover-based pastures and whether the recovery
490 of fertiliser P by clover plants will vary under different agronomic management (e.g. fertiliser rate,
491 timing and placement).

492 Recently, McLaren et al. (2014) demonstrated that the ignition-H₂SO₄ and NaOH-EDTA
493 extraction techniques provide a reliable estimate of total, inorganic and organic P for soils collected
494 from a medium-term P fertiliser × grazing experiment at the Ginninderra site. McLaren et al. (2014)
495 suggested that it was likely that concentrations of organic P were overestimated using the ignition-
496 H₂SO₄ extraction technique and slightly underestimated using the NaOH-EDTA extraction technique.
497 However, they also found that concentrations of total extractable P using *aqua regia* digestion were
498 strongly correlated with the ignition-H₂SO₄ (100 % recovery of *aqua regia* digestion) and NaOH-
499 EDTA (63 % recovery of *aqua regia* digestion) extraction techniques. Consequently, the ignition-
500 H₂SO₄ and NaOH-EDTA extraction techniques were both used in the current study to identify the fate
501 of fertiliser P in soil fractions because they provide a reliable estimate of total, inorganic and organic P
502 (McLaren et al. 2014).

503 At both field sites, most of the fertiliser P in soil fractions was found in the surface layer (0 –
504 4 cm) of the soil profile in the year of application. Many studies have shown that concentrations of soil
505 P in surface layers of fertilised soils under pasture are enriched relative to those in subsurface layers

506 (Haynes and Williams 1992; McLaren et al. 2014; McLaughlin et al. 1990; Watson 1969). However,
507 the recovery of fertiliser P in soil fractions reported in the current study over a single growing season is
508 considerably lower than those reported in long-term studies using indirect techniques (soil P audits)
509 (McCaskill and Cayley 2000; Watson 1969). The estimated recovery of fertiliser P by pastures is
510 generally considered low in the year of application due to the accumulation of P in fertilised soil as
511 sparingly-available forms of inorganic and organic P (McLaughlin et al. 2011). Indeed, a large
512 proportion of the fertiliser P that is applied to pastures can be recovered in soil fractions of the upper
513 layers of the soil profile (McLaren et al. 2014; Watson 1969). Since a considerable proportion of the
514 fertiliser P was found to be accessible to pasture plants in the year of application, the low PUE of
515 pastures is unlikely due to the immediate 'fixation' of fertiliser P by soil constituents. Rather, fertiliser
516 P appears to be highly (re)cycled in pasture systems, whereby much of the fertiliser P can be taken up
517 by the plant but is subsequently returned to the soil surface via pasture decay, pasture trampling and/or
518 deposition of faeces and urine from grazing animals (Bircham and Hodgson 1983; Bromfield 1961).

519 The high recovery of fertiliser P in soil fractions associated with pools of inorganic P is
520 consistent with reports for arable crops grown under field conditions (McLaughlin et al. 1988c;
521 Sharpley 1986) and glasshouse conditions (Friesen and Blair 1988). However, the ignition-H₂SO₄
522 extraction technique suggests that a larger proportion of the fertiliser P was being recovered as organic
523 P than that indicated by the NaOH-EDTA extraction technique at the Ginninderra site. The reasons for
524 this are unclear but it is possible that the ignition-H₂SO₄ extraction technique has overestimated total
525 organic P in this soil. This can occur because the ignition step can increase the solubility of inorganic P
526 (McLaren et al. 2014; Oniani et al. 1973; Williams et al. 1970). Nevertheless, recovery of the majority
527 of fertiliser P as inorganic P in soil is consistent with previous studies for arable crops, which show that
528 there is little conversion of fertiliser P to organic forms in the year of application (Friesen and Blair
529 1988; McLaughlin et al. 1988c; Sharpley 1986). In contrast, it is well known from long-term studies
530 that concentrations of organic P in fertilised soils under pasture are a major sink of fertiliser P
531 (Condrón and Goh 1989; McLaren et al. 2014; Oniani et al. 1973; Simpson et al. 1974). McLaren et al.
532 (2014) determined the net accumulation of organic (and inorganic) forms of P after 13 years of
533 fertilisation in soils collected from a medium-term P fertiliser × grazing experiment at the Ginninderra
534 Experiment Station, near Hall, Australian Capital Territory, Australia. They reported that 28 % of the
535 fertiliser P had accumulated as organic forms in the topsoil layer (0 – 10 cm) when averaged across the

536 fertilised and grazing treatments, as determined by the summed concentrations of organic P using
537 sequential chemical fractionation.

538 A small proportion of the fertiliser P was recovered in the granule residues collected from the
539 soil surface at the last harvest, which is likely to consist of water insoluble phosphates (Gilkes and
540 Lim-Nunez 1980; Prochnow et al. 2001). The ~ 5 % of ^{33}P recovered in the granule residues relative to
541 that originally labelled with the ^{33}P radiotracer is likely to be from subsequently formed precipitates of
542 dicalcium phosphate or dicalcium phosphate dihydrate within the granule on the un-acidulated fraction
543 of the SSP (Lehr et al. 1959). Clearly, the majority of fertiliser P is released from the granule during a
544 single growing season upon application to pastures and is potentially available for plant uptake
545 (Williams 1971a).

546 The unrecovered fertiliser P is likely to be associated with three main components of the
547 pasture system. Possible fates of the unrecovered fertiliser P include: 1) incorporation into clover roots;
548 2) transport to below 8 cm from the soil surface; and 3) incorporation into pools not extracted by the
549 ignition- H_2SO_4 or NaOH-EDTA extraction techniques. We consider it unlikely that much fertiliser P
550 was transported below 8 cm from the soil surface because the recovery of fertiliser P in the subsurface
551 layer (4 – 8 cm) was low, and in many cases too low for detection (Table 5), and the movement of P
552 from SSP granules is generally restricted to a few centimetres from the granule (Lawton and Vomocil
553 1954; Williams 1971b), except in very sandy soils. We also consider it unlikely that much fertiliser P
554 has been incorporated into pools of soil P not extracted by the ignition- H_2SO_4 extraction and NaOH-
555 EDTA extraction techniques because: 1) the ignition- H_2SO_4 extraction technique provides a close
556 approximation of total soil P as measured using *aqua regia* digestion (McLaren et al. 2014), and; 2)
557 McLaren et al. (2014) found that pools of ‘residual P’ as determined using sequential chemical
558 fractionation did not significantly accumulate with longer-term addition of fertiliser P under pasture.
559 Consequently, we believe that much of the fertiliser P that is unaccounted for is likely to be in clover
560 roots.

561 It is well known that clover roots contain P and can be a quantitatively important fraction of
562 the total P in clover plants (Biddiscombe et al. 1969). In addition, subterranean clover is known to
563 translocate less P from their roots to shoots compared to other pasture species, particularly at low levels
564 of P fertility (Barrow 1975; Blair and Cordero 1978; Paynter 1990). Biddiscombe et al. (1969) reported
565 that the proportion of total plant P in the root fraction of subterranean clover after 92 days of growth at

566 low and high levels of P addition was 32 % and 22 %, respectively. Assuming similar partitioning
567 between roots and shoots occurred in our study, clover roots would account for 4.9 kg P ha⁻¹ at the
568 Ginninderra site (based on the partitioning value of 32 % in the low P soil of Biddiscombe et al. (1969))
569 and 7.9 kg P ha⁻¹ at the Naracoorte site (based on the partitioning value of 22 % in the high P soil of
570 Biddiscombe et al. (1969)). This would account for over half of the unrecovered fertiliser P at each site.

571 This study found that clover shoots were able to recover 34 % and 40 % of the P from SSP at
572 the Ginninderra and Naracoorte field sites, respectively, in the year of application. The recovery of
573 fertiliser P by clover plants might be even higher if fertiliser P uptake in roots is also measured, along
574 with the residual fertiliser P in soil from previous applications of fertiliser P. Indeed, several studies
575 have shown that fertiliser P from previous applications can be an important source of P for pasture
576 growth (Gallet et al. 2003; Morel and Fardeau 1989). Gallet et al. (2003) estimated that 14 to 62 % of P
577 in clover shoots was derived from previous applications of fertiliser P in three soils under glasshouse
578 conditions. However, it is also apparent that on a longer timeframe fertiliser P that has cycled through
579 the soil-plant system at least once is prone to being transformed into inorganic or organic forms of soil
580 P of low solubility (Condrón and Goh 1989; McLaren et al. 2014). Hence, regular inputs of fertiliser P
581 are still likely to be required in order to maintain the optimum agronomic level of soil P fertility.

582 The majority of the fertiliser P remaining in the soil surface layer at the end of the experiment
583 was inorganic, although it was unclear how available this P remained for clover growth. We propose
584 that much of this is still present in a readily available form for plant uptake, and is largely associated
585 with the sorption sites of aluminium and iron oxy-hydroxides (Dorahy et al. 2007; He et al. 2004). It is
586 likely that the addition of plant residues to the soil surface could also affect the distribution of P pools
587 in soil fractions and possibly the recovery of fertiliser P by clover plants (Friesen and Blair 1988;
588 McLaughlin et al. 1988c). However, it is unclear how much of the plant P in clover pastures is returned
589 to the soil surface via plant decay or trampling or when this occurs in its lifecycle.

590 In addition to chemical constraints, there also can be physical constraints to accessing the
591 fertiliser P in the surface layer (McBeath et al. 2012). Regular drying of surface layers in soils under
592 pasture may restrict the plant's ability to access P in this layer, and subsoil applications of fertiliser P
593 may be beneficial (Pinkerton and Simpson 1986; Scott 1973). In any case, the results of this study
594 indicate that the application of fertiliser P to the soil surface resulted in a relatively high recovery of
595 fertiliser P by clover plants in the year of application.

596

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605

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802 **List of figure captions**

803

804 **Fig. 1** A visual representation (left) and the binary image (right) of the diffusion patterns for
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832 **Table 1. Chemical properties of the soils used in this study. Analyses were carried out on soil samples collected prior to fertiliser addition.**

Location	Depth (cm)	pH _w (1:5)	EC _w ($\mu\text{S cm}^{-1}$; 1:5)	TOC (%)	TON (%)	Colwell P (mg kg^{-1})	PBI	Oxalate Al (mg kg^{-1})	Oxalate Fe (mg kg^{-1})	Total P ^a (mg kg^{-1})	Ignition-H ₂ SO ₄ (mg kg^{-1})		
											total	inorganic	organic
Ginninderra,	0 – 2.5	5.1	74	2.6	0.23	19	31	709	10100	362	213	50	163
ACT	2.5 – 5	4.6	70	1.6	0.15	11	35	773	11600	323	178	34	143
	5 – 10	4.7	64	1.1	0.10	8	33	726	10300	310	156	31	125
	10 – 20	4.7	35	0.6	0.05	5	46	873	11800	301	112	21	90
Naracoorte,	0 – 2.5	5.6	88	1.3	0.11	92	14	435	6260	358	254	159	95
SA	2.5 – 5	5.3	77	1.2	0.12	66	16	427	6740	358	207	123	84
	5 – 10	4.7	51	0.9	0.08	34	23	374	2770	201	140	75	66
	10 – 20	4.7	32	0.4	0.04	23	20	378	3630	148	81	40	41

833 ^a Determined by laboratory X-ray fluorescence.

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844 **Table 2. Monthly rainfall for the Ginninderra and Naracoorte field sites. Values in parentheses are added irrigation as rainfall equivalents.**

Location	Rainfall (mm)^a	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total annual
Ginninderra	2013	85	79	28	10	12 (52)	108 (1)	56	29 (3)	69	20 (30)	105	21	708
	Long-term ^b	58	55	56	46	47	46	56	60	67	69	69	59	688
Naracoorte	2013	1	6	15	15	40	60 (3)	90 (6)	123 (2)	39	59	17	14	490
	Long-term ^c	22	21	27	25	39	57	56	73	55	38	35	40	488

845 ^a Rainfall data was sourced from the nearest Bureau of Meteorology weather station.846 ^b Based on average monthly rainfall between 1962 to 2012 (complete records).847 ^c Based on average monthly rainfall between 1998 to 2012 (complete records).

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859 **Table 3. Concentrations of Ca, P and S (%) in commercial SSP and ³³P-labelled SSP granules**
 860 **collected at the end of the field experiment from the Ginninderra and Naracoorte field sites as**
 861 **determined by ICP-OES on acid digests (Zarcinas et al. 1987). Values in parentheses are**
 862 **standard errors.**

Field site	Element (%)	Commercial SSP	³³P-labelled SSP
Ginninderra, ACT	P	1.97 (0.09)	2.09 (0.07)
	Ca	20.12 (0.94)	20.55 (0.38)
	S	12.65 (0.70)	13.54 (0.40)
Naracoorte, SA	Ca/S ratio	1.60 (0.04)	1.52 (0.03)
	P	1.82 (0.05)	2.10 (0.02)
	Ca	22.40 (0.51)	22.73 (0.59)
	S	14.83 (0.49)	15.28 (0.44)
	Ca/S ratio	1.51 (0.03)	1.49 (0.02)

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884 **Table 4. Summary of the cumulative biomass removal (t DM ha⁻¹ equivalent) and P uptake (kg P**
 885 **ha⁻¹ equivalent) for the control, commercial SSP and ³³P-labelled SSP treatments at the**
 886 **Ginninderra and Naracoorte field sites. Values in parentheses are standard errors.**

Measurement	Field site	Control	Commercial SSP	³³P-labelled SSP
Cumulative biomass (t DM ha ⁻¹)	Ginninderra	6.7 (0.5)	7.5 (0.6)	7.3 (0.5)
	Naracoorte	8.0 (0.5)	7.7 (0.3)	6.9 (0.6)
Cumulative P uptake (kg P ha ⁻¹)	Ginninderra	7.8 (0.7)	10.8 (1.1)	10.5 (0.8)
	Naracoorte	28.4 (1.8)	29.9 (0.7)	28.1 (1.9)

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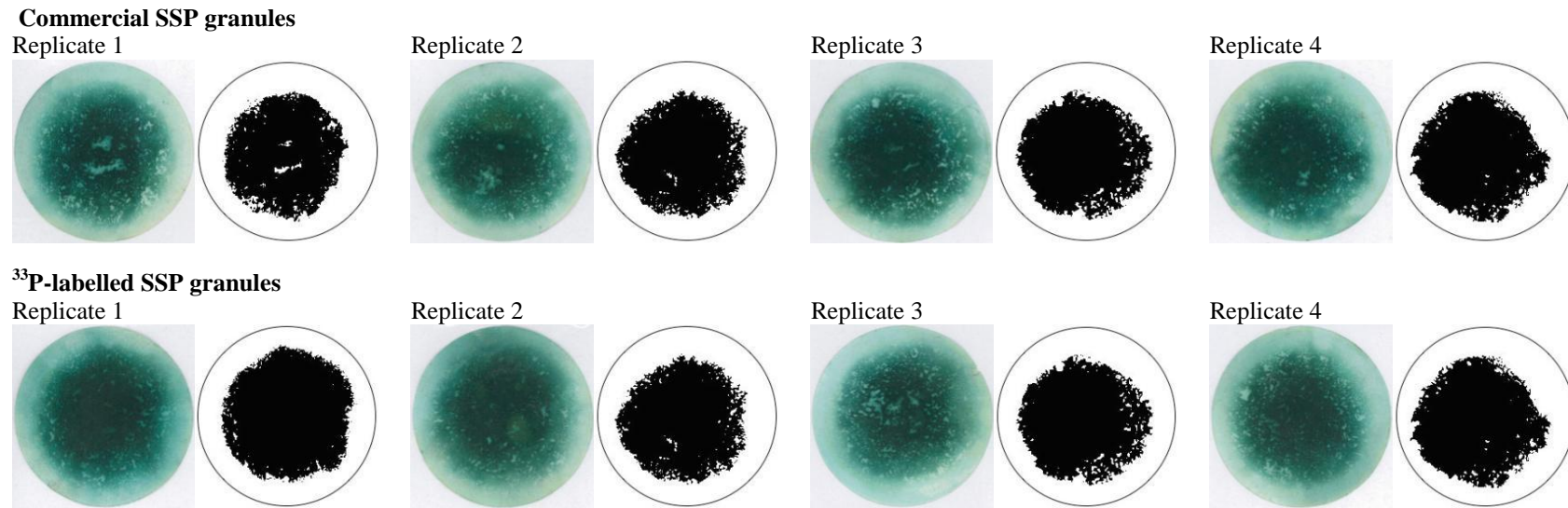
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910 **Table 5. Concentrations of ^{33}P radioactivity (MBq core $^{-1}$) from ^{33}P -labelled SSP detected in various components of the pasture system at two field sites. Values in**
 911 **parentheses are standard errors.**

Matrix/location	Radioactivity of ^{33}P in components of the pasture system (MBq core$^{-1}$)	Ginninderra^A	Naracoorte^A
Applied granule (soil surface)	Total applied as ^{33}P -labelled SSP	4.17	4.75
Clover (shoots)	Cumulative biomass removal	1.43 (0.09)	1.92 (0.10)
Recovered Granule (soil surface)	Total recovered as ^{33}P -labelled SSP	0.29 (0.04)	0.16 (0.01)
Soil (0 – 4 cm layer)	Ignition- H_2SO_4 extractable total P	1.32 (0.12)	1.53 (0.06)
	Ignition- H_2SO_4 extractable inorganic P	0.79 (0.10)	1.39 (0.07)
	Ignition- H_2SO_4 extractable organic P	0.53 (0.06)	0.17 (0.06)
	NaOH-EDTA extractable total P	1.02 (0.12)	1.49 (0.10)
	NaOH-EDTA extractable inorganic P	0.91 (0.11)	1.41 (0.09)
	NaOH-EDTA extractable organic P	0.11 (0.04)	0.08 (0.03)
Soil (4 – 8 cm layer)	Ignition- H_2SO_4 extractable total P	BD	0.21 (0.03)
	Ignition- H_2SO_4 extractable inorganic P	BD	0.20 (0.03)
	Ignition- H_2SO_4 extractable organic P	BD	0.02 (0.01)
	NaOH-EDTA extractable total P	BD	BD
	NaOH-EDTA extractable inorganic P	BD	BD
	NaOH-EDTA extractable organic P	BD	BD
Unknown	By difference	1.13 (0.09)	0.93 (0.11)

912 ^ABD = below detection. Refers to samples that contained ^{33}P activity that was too low for detection by liquid scintillation counting.

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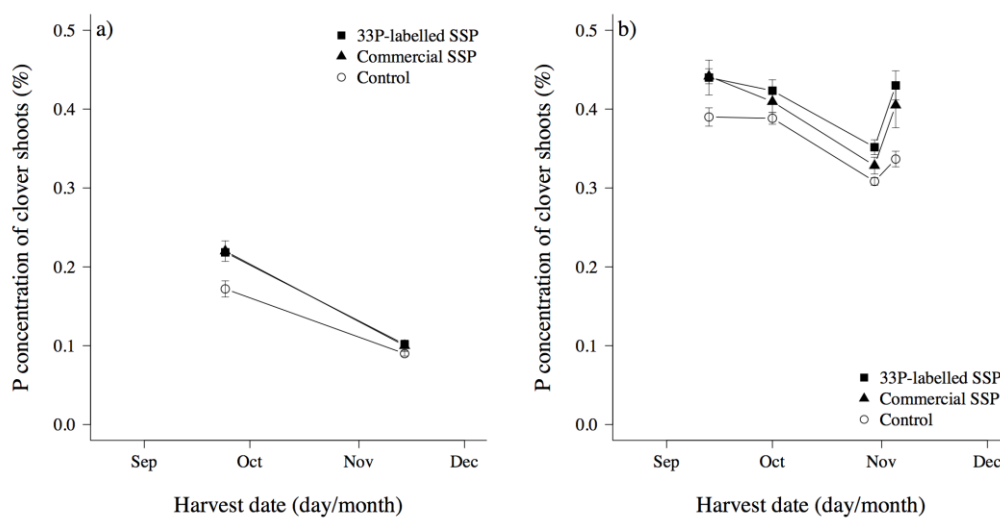
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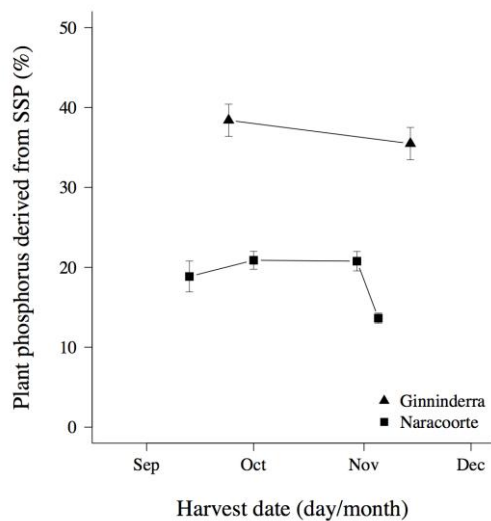
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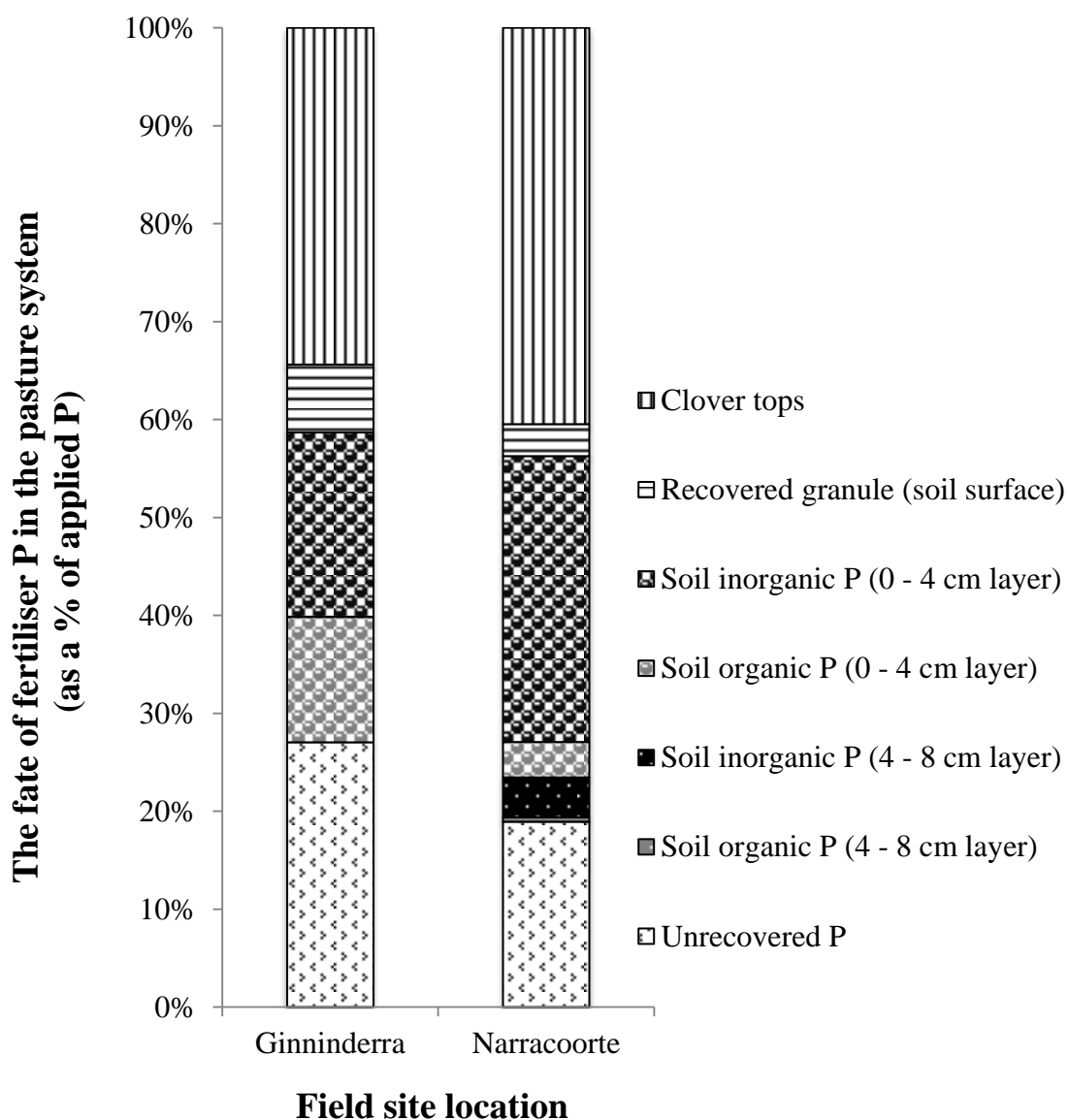
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