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Patricia Poblete-Grant, Paula Cartes, Sofía Pontigo, Philippe Biron, María de La Luz Mora, et al.. Phosphorus fertiliser source determines the allocation of root-derived organic carbon to soil organic matter fractions. *Soil Biology and Biochemistry*, 2022, 167, pp.108614. 10.1016/j.soilbio.2022.108614 . hal-03852481

HAL Id: hal-03852481

<https://hal-cnrs.archives-ouvertes.fr/hal-03852481>

Submitted on 25 Nov 2022

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Phosphorus fertiliser form determined the allocation of root-derived organic carbon to soil organic matter fractions

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Phosphorus fertiliser form **determined the allocation** of root-derived **organic carbon to** soil organic matter fractions

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Abstract

The efficiency of soil organic carbon (SOC) sequestration as a suitable negative emission technology depends mainly on plant-derived organic carbon input **and its allocation to stabilised SOC pools**. **These processes may be affected by fertiliser use and soil type**. The purpose of this study was to investigate the **effect of the organic fertiliser poultry manure compost, the mineral fertiliser rock phosphate and their mixture on** organic carbon (OC) transfer from plant to soil. We studied also its allocation to protected SOC pools of a Luvisol and a Neoluvisol. We carried out a growth chamber experiment with ¹³C-enriched atmosphere, where ryegrass plants were grown for 7 weeks on the **two different soil types in the presence of the three different fertilisers**. We quantified root-derived OC input in three SOC density fractions and soil microbial biomass at the end of the experiment. **Our results indicated that addition of poultry manure compost and its mixture with rock phosphate led to more root biomass and more root-derived OC transfer to active pools compared to rock phosphate alone**. **The amendments with poultry manure compost also led to higher microbial biomass contents compared to mineral fertilisation due to higher available organic phosphorus**. We also noticed variations in the dynamics of the stabilised OC pools amongst soils, which could be attributed to the different impacts of phosphorus fertiliser types on SOC stabilisation processes. We concluded that organic and mineral phosphorus fertilisers may have a contrasting impact on OC flow from plant to soil and in particular on the allocation of root-derived OC to labile or stable SOC fractions. The magnitude of these effects was more influenced by soil type than their pattern, which was similar for the two soil types.

Keywords: Poultry manure; Rock phosphate; C isotopes; SOM density fractionation

1 1. Introduction

2 To enhance soil organic carbon (SOC) sequestration in agricultural soils it is necessary to increase plant-
3 derived organic carbon inputs (Chenu et al., 2019), requiring nitrogen (N) and phosphorus (P) fertiliser
4 addition. This is problematic especially for P, since P fertilisers are made from rock phosphate (RP) and
5 may have limited availability in the future due to their global resource distribution and depletion of global P
6 reserves (Reijnders, 2014). In the context of a circular economy, alternative P fertilisers may include
7 poultry manure, a waste material that is available in large quantity due to a growing broiler industry (FAO,
8 2018). This material may be recycled into soil amendments by composting to prevent environmental
9 concerns. Indeed, poultry manure compost (PM) is a suitable fertiliser to improve yield and enhance soil
10 physical properties and SOC sequestration (Li et al., 2011; Malik et al., 2013; Mierzwa-Hersztek et al.,
11 2018; Mubeen et al., 2021; Poblete-Grant et al., 2019, 2020). To optimise resource use and to prevent
12 adverse effects (e.g., contamination with heavy metals), combined application of PM and RP has been
13 suggested (Mubeen et al., 2021; Poblete-Grant et al., 2019).

14
15 The use of organic or inorganic fertilisers may affect the functioning of plant and soil microbial
16 communities differently because of contrasting nutrient availability and input of exogenous organic matter.
17 Indeed, organic fertilisers were shown to increase root growth (Zhang et al., 2020) and labile SOC (Datta
18 et al., 2010). Root-derived compounds together with available nutrients may be taken up and incorporated
19 into the soil microbial biomass. The latter process was found to be a pre-requisite for SOC stabilisation
20 (Cotrufo et al., 2013). A recent study showed that different organic fertilisers alter root carbon input and its
21 use by the soil microbial biomass (Vidal et al., 2020) and therefore most probably SOC sequestration.
22 Accordingly, organic fertilisation strategies should be investigated in order to improve OC transfer from
23 plant to soil. To the best of our knowledge, the effect of contrasting P fertilisers on these processes has
24 never been assessed.

25

26 Therefore, in this study, we examined the influence of rock phosphate (RP), poultry manure compost (PM)
27 and their mixture (PMRP) on OC transfer from plant to soil and allocation of plant-derived OC (root-
28 derived OC) into particular SOC pools. To investigate the influence of edaphic properties on the general
29 nature of the response pattern and its magnitude, we used two soil types differentiated by their P status
30 and soil reaction. Our conceptual approach included continuous ^{13}C labelling of ryegrass plants grown on
31 a Luvisol and a Neoluvisol in a growth chamber under controlled conditions, followed by destructive
32 sampling and soil physical fractionation. We separated the unprotected OC fraction (FLf) from the one
33 occluded in aggregates (OLf) and OC in organo-mineral associations (Hf). We quantified plant-derived
34 OC, in the three different SOC pools with contrasting stability by using the isotope mass balance
35 approach. We made two hypotheses: (1) in treatments with organic P fertilisers we will observe greater
36 microbial biomass and more available nutrients than in those with mineral P fertilisers because of greater
37 plant-derived OC input and (2) among the three P fertiliser treatments, we will observe contrasting plant-
38 derived OC allocation to SOM fractions because of their specific effects on OC flow from plant to soil.

39

40 2. Materials and Methods

41 2.1 Materials

42

43 We used two soils differing in pH, available P (Olsen-P) and bulk density. Both soils belong to the French
44 observatory SOERE PRO (<https://www6.inra.fr/valor-pro/SOERE-PRO-les-sites>) and were collected at 0-
45 30 cm depth at Colmar (Eastern France) and Le Rheu (northwestern of France). They were classified as
46 Neoluvisol (NEO) (pH 6.1) in Colmar, and Luvisol (LUV) (pH 8.5) in Le Rheu (Baize and Girard, 2008).

47

48 The NEO soil was characterised by a bulk density of 1.54 g cm^{-3} and an available (Olsen's) P
49 concentration of 60 mg kg^{-1} . Total SOC concentration was 10 mg g^{-1} , and total N concentration was 1 mg
50 g^{-1} . The soil had a silty texture with a clay content of 14.6 %. The LUV soil was characterised by a bulk
51 density of 1.26 g cm^{-3} and an available (Olsen's) P concentration of 11 mg kg^{-1} . Its SOC concentration

52 was 12 mg g⁻¹, and its N concentration was 1 mg g⁻¹. Additionally this soil contained 128 mg g⁻¹ CaCO₃.

53 The soil had a silty texture with a clay content of 20.7%.

54

55 Poultry manure (PM) had a P concentration of 13.2 mg g⁻¹, and a humidity of 22%. It showed a carbon

56 concentration of, 288 mg g⁻¹ OC, a nitrogen concentration of 45 mg g⁻¹ total N, and a C:N ratio of 6.4. The

57 mineral fertiliser phosphate (RP) showed a P concentration of 300 mg g⁻¹ and a Ca content of 500 mg g⁻¹

58 as CaO. It had a residual C content of 20 mg g⁻¹, 0.5 mg g⁻¹ total N, and a C:N ratio of 45.9.

59

60 2.2 Growth chamber experiment

61

62 Perennial ryegrass (*Lolium perenne*) was grown in pots with 500 g of soil (unaltered or amended with

63 organic and/or inorganic P fertilisers). The species was chosen because of its high yields and ease of

64 establishment, making it a popular choice for permanent grasslands (Rivero et al., 2019). A total of 97

65 ryegrass seeds were sown in the pots containing the two different soil types. Organic and/or inorganic

66 fertilization was applied in form of dry powder to match a P input of 100 mg P kg⁻¹. We had the following

67 treatments: composted poultry manure (PM), rock phosphate (RP), a mixture of composted poultry

68 manure and rock phosphate (PMRP), unfertilized soil (control), and. unplanted soil (bare soil). To account

69 for the nutrient input from PM, N and potassium (K) in the control and treatments with RP were amended

70 with NH₄NO₃ and KCl, respectively (262 mg N and 221 mg K per kg soil) to reach similar concentrations

71 as in the PM treatment. PM addition was added based on its P content and corresponded to an

72 application of 14- and 9.8-ton ha⁻¹ in PM and PMRP treatments.. In addition, the OC inputs were 443 and

73 310 mg C g⁻¹ from PM and PMRP respectively. The fertilization rate of RP was equivalent to 0.8 and 0.25 t

74 ha⁻¹ in RP and PMRP treatments.

75

76 We used a completely randomized factorial experimental design with four replicates per treatment and set

77 up the experiment in a growth chamber (SERVATHIN Rubic V, iEES Paris, Thiverval-Grignon).

78 Temperatures were 24°C and 17°C during the day and night, respectively. They were selected as optimal

79 growth temperatures of temperate perennial ryegrass species, which ranges between 15 and 25°C
80 (Perera et al., 2020). For the first 13 days, the light intensity was $650 \text{ mol m}^{-2} \text{ s}^{-1}$, with a day length of 8 h
81 and 11 h thereafter until the end of the experiment. Plant development took place over the course of three
82 weeks in controlled soil moisture conditions. Moisture was monitored through the use of ten humidity
83 probes (Decagon Echo 5) randomly distributed in the pots. It was maintained on average at 40% of the
84 field capacity by regular watering. Twenty days after seeding (3 weeks), the growth chamber was
85 artificially labelled during the following 4 weeks using continuous $^{13}\text{C-CO}_2$ enrichment of the atmosphere
86 (approximately 145 g of 10 atom-% $^{13}\text{C-CO}_2$), leading to a mean ^{13}C ratio of 868‰ (standard error = 83).
87 The pots were harvested 7 weeks after seeding when plants were at tillering stage. Shoots and roots were
88 removed from the soil, washed with distilled water, and their fresh weight was determined. After oven
89 drying at 65°C for 48 h the plant material was ground to pass a 0.84 mm sieve. The remaining soil was
90 classified as rhizosphere soil and was oven-dried at 40°C and sieved at 2 mm. An aliquot of all samples
91 was ground for further analyses.

92

93 *2.3 Microbial biomass carbon*

94

95 We used the chloroform fumigation-extraction method (Vance et al., 1987) to determine microbial biomass
96 C (MBC) in 5 g of fresh soil. Briefly, fumigated and unfumigated soils were extracted with of K_2SO_4^- and
97 the TOC concentration of the extracts measured with a TOC analyser (TOC-VCSH by Shimadzu with a
98 sampler ASI-V by Shimadzu). The microbial biomass was determined using the difference between
99 fumigated and unfumigated samples.

100

101 *2.4 Soil organic matter density fractionation*

102

103 Soil organic matter (SOM) was divided into three fractions by density fractionation using 20 g of soil and
104 25 ml polytungstate following a method proposed by Adams et al., (2018). We isolated the free light
105 fraction (FLf), occluded light fraction (OLf), and the heavy fraction (Hf). The FLf and OLf fractions were

106 overdried at 40°C, and the Hf fraction was freeze-dried. Thereafter, an aliquot was ground and analysed
107 for elemental and isotopic composition.

108

109 2.5 Elemental and isotopic analyses

110

111 We determined the OC and N content of the bulk soil and physical fractions with an elemental analyser
112 Variopyrocube (Elementar). Before the analyses, decarbonation of the LUV soil was carried out by HCl-
113 fumigation (Harris et al., 2001). We used an Isotope Ratio Mass Spectrometer (Micromass Isoprime) to
114 determine the stable carbon isotope composition of soil and plant material relative to the Vienna Pee Dee
115 Belemnite (VPDB) international standard. It was calculated as follows:

$$116 \quad \delta^{13}\text{C} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) \times 10^3 \quad (1)$$

117 where R = $^{13}\text{C}/^{12}\text{C}$ (molar ratio).

118

119

120 2.6 Calculations of the contribution of the two different carbon types (plants and poultry manure compost)

121

122 Plant-derived OC in soil, which we hypothesised to be of root origin (RDC), was computed for bulk
123 samples and density fractions from the natural $\delta^{13}\text{C}$ abundance by using the following equation:

$$124 \quad RDC (\%) = \frac{(\delta^{13}\text{C OC treatment} - \delta^{13}\text{C OC bare soil})}{(\delta^{13}\text{C enriched root} - \delta^{13}\text{C OC bare soil})} \times 100 \quad (2)$$

125 where “ $\delta^{13}\text{C}$ treatment” refers to all treatments sampled at the end of the experiment, “ $\delta^{13}\text{C}$ bare soil” is
126 the soil without plants, and “ $\delta^{13}\text{C}$ enriched roots” is the mean $\delta^{13}\text{C}$ value of ryegrass roots of each
127 treatment.

128 For the PM and PMRP treatment, poultry manure-derived OC (PMC) was estimated as the difference in
129 total SOC following the treatments and the sum of the bare soil OC and root-derived OC (RDC) (Zhang et
130 al., 2015). Additionally, RDC (mg) and PMC (mg) allocated in each SOM fraction was calculated similarly
131 by using the mass balance.

132

133 2.7 Data analysis

134

135 The data sets were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test).
136 Statistical differences of means (95% significance level) were analysed using analyses of variance
137 (ANOVA). We tested the factors: soil type (S), treatment (T) and their interaction (S x T). Post hoc tests
138 with the function Tukey-test were made for the explanatory variables independently when the ANOVAs
139 detected significant differences. The relationships between soil physicochemical parameters (Poblete-
140 Grant et al., 2019) were tested by Pearson correlation analyses. Furthermore, a multiple linear regression
141 model was performed by selecting manually predictors from the full model to generate a simplified model.
142 Models were checked for normality and homogeneity using “nortest” and “lmtest” packages. Furthermore,
143 collinearity was tested using the “vif” function from the “car” package. Models were selected based on the
144 their R value the Akaike criterion (AIC). Finally, we performed a principal component analysis to identify
145 the response variables that best explained the treatment and soil type effect using the “facto-extra”,
146 “devtools”, and “ade4” packages. The statistical software R Foundation for Statistical Computing Version
147 3.6.3 was used to conduct the tests (R Development Core Team 2009-2018 RStudio, Inc). Effects were
148 declared significant at $p \leq 0.05$.

149

150 3. Results

151 3.1 Elemental content and stable isotope ratios of plant tissues and soils amended with different fertiliser 152 types

153 Plant OC concentration ranged from 334 to 410 mg g⁻¹ for shoots and from 153 to 355 mg g⁻¹ for roots
154 (Table 1). Shoot and root OC were lower for plants grown on LUV soil compared to those grown on NEO
155 soil (Table 1). In addition, root OC content significantly increased after PM and PMRP amendment of both
156 soils. Plant N concentrations ranged from 16.2 to 58.7 mg g⁻¹ in shoots and from 5.52 to 18.4 mg g⁻¹ in
157 roots (Table 1). The nitrogen concentrations of shoots of plants grown on the LUV soil were significantly

158 increased by RP followed by PMRP. However, root N concentrations were lowest in the RP treatment
159 (Table 1). When grown on the NEO soil, shoot and root N concentrations were most increased by RP
160 followed by PM and PMRP. The C:N ratios ranged from 5.80 to 24.8 and 13.9 to 28.1 for shoots and roots,
161 respectively. Soil type influenced C:N ratios of shoots and roots, being higher when plants were grown on
162 the NEO soil as compared to the LUV soil. The PM and PMRP amendments increased the C:N of roots for
163 the LUV soil, while it was decreased for the NEO soil (Table 1). When RP was added to both soils, shoot
164 C:N ratio decreased. The stable C isotope ratio ($\delta^{13}\text{C}$) ranged from 940 to 1,078 for shoots and from 611
165 to 1,047 for roots (Table 1). It was similar for controls, PM, and PMRP treatments of both soils. The $\delta^{13}\text{C}$
166 of shoots was highest for plants grown on the LUV soil amended with PM and PMRP, whereas for plants
167 grown on the NEO soil, RP and PMRP treatments showed the highest $\delta^{13}\text{C}$ followed by PM and control.
168 **The root $\delta^{13}\text{C}$ was increased only in the RP treatment of the NEO soil (Table 1).**

169
170 Insert Table 1, please

171
172 **The total SOC concentrations ranged between 10.5 and 16.5 mg g⁻¹ (Table 1). PM amendment led to**
173 **significantly increased SOC values in both soils, whereas PMRP addition increased SOC only in the NEO**
174 **soil (Table 1). Soil N concentrations ranged between 1.22 to 2.01 mg g⁻¹ and were significantly increased**
175 **only in the NEO soil by using PM. On the other hand, soil C:N ratios ranged from 7.7 to 8.9 and were**
176 **lowest for the NEO soil amended with RP (Table 1).** Soils differed in $\delta^{13}\text{C}$, being higher in the NEO soil as
177 compared to the LUV soil (Table 1). The $\delta^{13}\text{C}$ increased in the LUV soil by using PM and PMRP.
178 However, for the NEO soil, RP and PMRP treatments showed the greatest $\delta^{13}\text{C}$. The RP treatment
179 showed lower $\delta^{13}\text{C}$ for the LUV soil as compared to the NEO soil.

180
181 **In addition, soil type and treatments significantly affected root-derived OC (RDC) input ($p \leq 0.001$; Table 1).**
182 **It ranged from 0.11 and 6.67% corresponding to And ... mg in all treatments (Table 1). RDC input was**

183 higher in the NEO than the LUV soil. The PM and PMRP treatments increased RDC in both soils as
184 compared to the control and RP treatments.

185

186 *3.2 Microbial biomass, and elemental content of SOM pools as influenced by fertiliser types*

187

188 Microbial biomass carbon (MBC) ranged from 102 to 484 mg kg⁻¹. It showed significant differences for soil
189 type, treatment, and their interaction ($p \leq 0.001$; Fig. 1; Table S2). We found that the application of PM
190 significantly increased MBC in the LUV and NEO soils, with regards to the control. The RP treatment had
191 a negative effect on MBC leading to lower values (14–38%) for both soils as compared with the
192 unfertilised control. However, combination of RP with PM induced the greatest MBC increases (2-fold) for
193 the NEO soil. In the LUV soil amended with PMRP, similar MBC concentrations as in the control were
194 recorded.

195

196 Insert Figure 1, please

197

198 *3.3 SOC concentrations and distribution in physical fractions*

199 **The SOC** concentrations of the FLf fraction ranged between 147 to 237 mg g⁻¹ (Table 2). They were lower
200 than the SOC concentrations of the OLf fraction and higher than those of the Hf fraction, ranging between
201 206 to 328 mg g⁻¹ and 7.61 to 12.2 mg g⁻¹, respectively. Treatments showed significant differences for the
202 SOC concentration of the FLf, OLf, and Hf fractions ($p \leq 0.01$; Table 2), while only the SOC concentration
203 of the OLf fraction showed differences according to soil type ($p \leq 0.001$; Table 2). The interaction between
204 soil type and treatment was significant for the SOC concentration of the FLf fraction ($p \leq 0.001$; Table 2).

205

206 **Total N concentrations** ranged from 8.60 to 20.1 mg g⁻¹ in the FLf and from 11.6 to 26.2 mg g⁻¹ in the OLf
207 **fractions. They were strongly increased by PM and PMRP addition in both soils (Table 2). In the Hf**

208 fraction total N concentrations ranged from 1.01 to 1.33 mg g⁻¹ and showed no differences between
209 treatments.

210
211 The C:N ratio ranged between 12.4 to 19.3 in the FLf and between 11.8 to 16.2 in the OLf (Table 2). The
212 FLf and OLf of the PM and PMRP treatments showed the lowest C:N ratios. On the other hand, the C:N
213 ratio of the Hf ranged between 7.61 to 12.2 with no differences among the treatments.

214
215 The allocation of SOC to FLf was increased from 9% to 20 and 14% in the LUV soil amended with PM and
216 PMRP, respectively (Fig. 2A). Interestingly, RP reduced the SOC allocation to the FLf of the LUV soil. On
217 the other hand, the allocation of SOC to the OLf of the LUV soil was slightly increased by PM and PMRP
218 as compared to RP and control,. In the NEO soil, the allocation of SOC to the FLf was increased from
219 15% to 31 and 27% by the PM and PMRP, respectively.

220
221 *3.3 The allocation of plant and poultry manure compost-derived OC to SOM fractions is depending on the*
222 *fertiliser type*

223
224 PM and PMRP treatments increased RDC contribution to both soils, compared to the control soil, while
225 RP treatments showed no change. In the FLf, RDC contribution ranged from 3.66% to 17.6%, and was
226 highest in PM and PMRP treatments (Table 2). In RP treatments, values were similar as those of the
227 control. RDC allocated to the OLf, was lower, varying between 1.18 and 6.56% (Table 2). The PM
228 treatment showed the highest RDC in the OLf of the LUV soil, while PMRP showed the greatest RDC in
229 the OLf of the NEO soil. In the Hf fraction, RDC accounted for 2.09 to 5.82% (Table 2). It was higher than
230 the control in the PM treatment and lower than the control in the RP treatment for the NEO soil, while in
231 the LUV soil only PM treatment showed significantly higher values than the control.

232

233 Insert Figure 2, please

234

235 The total quantity of poultry manure-derived OC (PMC) was higher in the PM treatment (3.03 and 7.82
236 mg) as compared to PMRP (1.43 and 5.04 mg) in both soils (Fig. 3A). In PM and PMRP treatments of the
237 NEO soil, highest amounts of PMC were found in the FLf (47.7 and 26.9 mg) followed by the Hf (24.4 and
238 19.3 mg), and finally in the OLf (4.31 and 2.11 mg) (Fig. 3B-D). In contrast, the LUV soil showed
239 differences of PMC allocation in these two treatments. The PMC in the LUV soil amended with PM was
240 mainly accumulated in the FLf (18.8 mg) and in the Hf (16.0 mg), and the less PMC was found in the OLf
241 (1.50 mg). However, PMRP treatment showed the highest accumulation of PMC (43.7 mg) in the Hf as
242 compared to the PM treatment (16.0 mg) (Fig. 3D). Moreover, in the LUV soil amended with PMRP, the
243 accumulation of PMC was highest in Hf followed by FLf (8.02 mg), and finally in the OLf (1.10 mg) (Fig.
244 3D). Total PMC and PMC allocated in the FLf were significantly affected by soil type, treatment, and their
245 interaction ($p \leq 0.01$; Table S2). We also observed significant differences for the PMC allocated to the OLf
246 and Hf due to the treatment ($p \leq 0.001$; Table S2).

247

248 Insert Figure 3, please

249

250 3.4 Relationships between RDC allocation to physical fractions and biological and physicochemical
251 parameters

252 The multiple linear regression models showed that readily available organic P was the explanatory
253 variable with the greatest positive effect on the contribution of RDC in all SOM fractions of both soils
254 (Table 3). MBC and MBP also contributed to the development of these models. In the LUV soil, the readily
255 available organic P and MBP explained the 89% of the variability of RDC in the FLf, while in the NEO soil
256 both variables explained the 76% and 69% of the variability of RDC in the OLf and Hf. The readily
257 available organic P and MBC predictors, on the other hand, explained 66% and 94% of the variability of
258 RDC in the OLf and Hf of the LUV soil, respectively, as well as 96 of the variability of RDC in the FLf of the
259 NEO soil (Table 3). For the OC-FLf, the variables RDC-FLf and PMC-FLf explained the 72% and 74% of
260 its variability. In the NEO soil, readily available organic P explained 54% and 64% of the variability of OC-

261 FLf and OC-OLf, respectively. However, MBC and RDC-OLf were the variables, which were most
262 important and explained (78%) the variability of OC-OLf (Table 3).

263

264 Insert Table 3, please

265

266

267 **4. Discussion**

268 ***4.2 Soil microbial biomass is enhanced by organic P fertilisers through substrate provision and stimulation*** 269 ***of plant-derived organic matter input***

270

271 Our findings showed that poultry manure compost fertilisation increased MBC in both amended soils (Fig.
272 1), which is consistent with the findings of Malik et al. (2013), who investigated two alkaline soils (pH 8.3)
273 and Mierzwa-Hersztek et al. (2018), who studied a moderately acid soil (pH 6.4) amended with PM. On
274 the other hand, we found that RP amendment of both soils decreased the MBC compared to the
275 unfertilised control (Table 2). Similar negative fertiliser effects on soil microorganisms have previously
276 been reported (Lupwayi et al., 2005). According to He et al. (1997), the primary mechanism responsible
277 for the decreased MBC following RP amendment may be a rise in soil pH caused by liming materials
278 containing CaO such as RP.

279

280 Stimulation of MBC by organic fertilisers may be related to high plant-derived OC input resulting from
281 increased root biomass following the enhancement of the availability of both inorganic and organic P by
282 PM amendment (Poblete-Grant et al., 2019). Thus, MBC increase might be explained by either, i) the
283 enhanced availability of P and other nutrients, which allowed for higher root carbon input in the active
284 SOM pool (FLf) or ii) a direct input of PM (Fig. 3), which could provide a readily available OC-source and
285 thus stimulate MBC (Li et al., 2018; Lupwayi et al., 2019). It may also increase the available
286 macronutrients inputs, which may be benefitting MBC (Mierzwa-Hersztek et al., 2018; Spohn et al., 2013).
287 Soil microorganisms and SOM account for more than 10% of the plant-derived OC input belowground.

288 Microorganisms in the rhizosphere, whether alive or dead, may serve as a significant sink for root carbon
289 and promote the development of soil microstructures (Vidal et al., 2018). Plants rely on soil
290 microorganisms like bacteria and fungi to depolymerize and mineralize organic nutrient forms for their
291 nutrient uptake (Jacoby et al., 2017). Due to a lack of readily available substrates, a large number of soil
292 microorganisms may not be active. The readily accessible OC produced by roots most likely drives
293 microbial activity and growth in the rhizosphere, resulting in the production of extracellular enzymes and,
294 as a result, improved SOM decomposition and nutrient mineralisation (Kuzyakov and Xu, 2013). In these
295 conditions, microbial biomass can compete for nutrients with plants. Microbial P and N uptake can prevent
296 P fixation by soil colloids or N leaching. When the readily available OC is depleted, microbial biomass N
297 and P become available to plants through microbial turnover. Microorganisms can thus be conceptualised
298 as slow-release fertilisers that store P and other nutrients when their concentrations are high, and then
299 release them when nutrient concentrations are low (Malik et al., 2013).

300
301 In addition, we found that MBC was greatly affected by soil type. For example, the LUV soil amended with
302 PM showed the highest increase in MBC as compared to the control (Fig. 1), whereas in the NEO soil, the
303 MBC for the PM treatment was lower than in the combined treatment PMRP. Malik et al. (2013) also found
304 differences of MBC associated to soil type responses to PM amendment. These findings were attributed to
305 contrasting soil texture and organic matter content. However, in our experiment, the two soils had
306 comparable organic matter content and soil texture, but they differed in pH and initial available P status.
307 Therefore, soil reaction and nutrient availability may have affected microbial growth response to the
308 amendments. Furthermore, it was previously observed that RP was inefficient to promote belowground
309 growth (Poblete-Grant et al., 2019), which might diminish the release of easily available OC from roots
310 needed to increase the abundance of soil microorganisms.

311

312 **4.3 Organic fertilisers improve carbon transfer from plant to soil organic matter fractions**

313

314 Our study shows differences of root-derived organic carbon (RDC) contribution among soils and
315 treatments (Table 1 and 2). Our results indicated that organic fertilisers, such as poultry manure alone
316 (PM) or combined with rock phosphate (PMRP) were able to improve RDC similarly in both soil types. This
317 is in contrast to mineral fertilisers in form of rock phosphate (RP) . Our findings are consistent with the
318 results of Qiao et al. (2017) and Dou et al. (2016a, b), who found that pig manure can stimulate
319 rhizodeposition by increasing nutrient availability in several soil types. According to their findings higher
320 nutrient availability increased CO₂ fixation via photosynthesis, root biomass, and nutrient status in plants.,.
321 This agrees with the contrasting contribution of RDC reported for the NEO and LUV soils, which might be
322 related to their contrasting initial P availability. These results are in agreement with previously reported
323 differences in ryegrass-derived OC as influenced by soil type (Domanski et al., 2001; Kaštovská and
324 Šantrůčková, 2007; Kuzyakov et al., 2001). Indeed edaphic factors such as soil available P, N and pH
325 may influence SOC dynamics and allocation to stabilised pools (Di et al., 2018).

326
327 Our results showed a higher allocation of RDC to the FLf and OLf fractions of soils amended with PM and
328 PMRP compared to those amended with RP (Table 2 and Fig. 3B). The FLf is known as the active SOM
329 pool, while the Hf is generally regarded as the mineral-associated and thus stabilised SOM pool (Von
330 Lützwow et al., 2007). Moreover, both soil types amended with PM showed an increased RDC contribution
331 to OLf (Table 2). This fraction consists of partly decomposed organic matter occluded in soil aggregates
332 (Adams et al., 2018; Wagai et al., 2009) providing physical protection and thus limiting OC availability for
333 microbial decomposition (Rasse et al., 2005). Accordingly, previous data stated that long-term application
334 of PM may increase SOC accumulation by its positive effect on soil aggregate formation (Poblete-Grant et
335 al., 2020). In the Hf pool we found that the content of RDC was more increased in LUV and NEO soil
336 amended with PMRP as compared with RP amendment and the unfertilised soil (Table 2). In general,
337 RDC allocation in the three SOM pools was strongly influenced by the treatments containing PM;
338 however, the two soil types showed differences betweenamong FLf and either OLf or Hf.

339

340 The positive effect on RDC allocation to the SOM fractions could be explained by the enhancement of soil
341 organic P belonging to the readily fraction (extracted with H₂O and NaHCO₃). Multiple linear regression
342 models supported our findings, which showed that readily available organic P coupled with MBC or MBP
343 explained from 66% to 96% of the variability of RDC from all SOM fractions in both soils (Table 3).
344 Therefore, we suggest that short-term organic fertiliser application may improve SOC storage in all SOM
345 fractions due to their organic P input. This is in line with a recent literature review by Spohn (2020), who
346 showed the importance of organic P for SOC storage, indicating that large amounts of P are required to
347 achieve high levels of SOC accumulation promoted by initiatives such as 4 per mille (Soussana et al.,
348 2017).

349
350 In contrast, RP did not promote the transfer of RDC to SOM fractions even though in our previous study
351 showed an increase of root biomass in the NEO soil (Poblete-Grant et al., 2019). We found that RP
352 addition decreased RDC in the Hf of the LUV soil, and in all fractions from the NEO soil as compared to
353 their unfertilised control (Table 2). Thus, application of organic fertilisers may support both crop yield as
354 well as SOC sequestration through direct OC input and the stimulation of plant growth by nutrient and OC
355 input (Zhao et al., 2020).

356
357 Moreover, soil structure through aggregate stability induced by PM could play an important role in SOC
358 dynamics (Poblete-Grant et al., 2020). Accordingly, the increased accumulation of poultry manure-derived
359 OC (PMC) in the Olf fraction could be associated to its physical effect on enhancing soil
360 macroaggregates. Differences in PMC content between soils, on the other hand, may be attributed to
361 differences in soil mineralogy (Fig. 3). Accordingly, the high pH and concentration of CaCO₃ in the LUV
362 soil could explain the high allocation of PMC in the Hf of the PMRP treatment highlighting the role of Ca
363 from carbonates in the mineral bonds and interactions between minerals and SOM mediated by cation
364 bridges (Zhang et al., 2016).

365

366 In general, P fertiliser types greatly influenced the contribution of plant-derived carbon and its allocation to
367 SOM pools. Our study highlighted the important role of the soil biomass in the soil transfer of plant derived
368 OC. Moreover, our study indicated that the availability of organic P might promote the release of root
369 derived OC improving its incorporation to soil. We conclude that depending on soil type, the short-term
370 application of poultry manure or its combination with rock phosphate as P fertiliser could represent an
371 interesting alternative to stimulate plant- and poultry manure compost-derived carbon transfer to stabilised
372 SOC fractions. The general pattern of these effects was less influenced by soil type than their magnitude
373 Organic fertilisers may have beneficial effects on (1) the soil microbial biomass, crucial to ensure OC
374 transfer from plant to soil and (2) biomass production. . Field trials are required to determine the effects of
375 poultry manure alone and its combination with rock phosphate under real-world conditions in order to
376 assess the long-term importance of these mechanisms.

377
378 **Acknowledgments:** We are grateful with the financial support of the Fondo Nacional de Desarrollo
379 Científico y Tecnológico (FONDECYT) in Chile (projects n° 3210228, 1181050, 1201257, and 3200901),
380 as well as to the Chilean ANID scholarship n° 21150715, and ECOS C13U02. In addition, authors
381 acknowledge the support towards of the IEES laboratory in France. The SOERE-PRO and KOMECO
382 companies for supplying the soils and poultry manure compost used in this study. Finally, authors are
383 greatly grateful with the reviewers of Soil Biology and Biochemistry journal for their contribution in the
384 improvement of the manuscript.

385
386 **Author's contributions** CR, MLM, PB and PPG designed the experiment. PPG and PB carried out the
387 experiment. PPG, CR, MLM analysed the data and wrote the manuscript. CR, MLM, PC, and SP reviewed
388 the manuscript.

389
390 **Declarations**

391 **Conflict of interest** The authors mentioned that they do not have any financial or non-financial conflict of
392 interest, and that this article does not contain any studies with human participants or animals.

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