

1 Revision submitted to Plant and Soil August 2017

2

3 **An investigation of the distribution of phosphorus between free and mineral associated soil organic**
4 **matter, using density fractionation**

5

6 Jessica L Adams · Edward Tipping · Sarah A Thacker · John N Quinton

7

8 Jessica L Adams · Edward Tipping · Sarah A Thacker

9 Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP, UK

10 e-mail jesams@ceh.ac.uk

11 tel +44 (0)1524 595 811

12

13 John N Quinton

14 Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

15

16

17 **Acknowledgements**

18 This work received National Capability funding from the UK Natural Environment Research Council
19 (CEH project number NEC04841). We are grateful to Alan Lawlor, Patrick Keenan, Manisha Patel and
20 Binoti Tanna for help with analysis, and to Aidan Keith (CEH) and two anonymous referees for their
21 constructive comments.

22

23

24

25

26

27

28

29

30

31

32

33

34

35 **Abstract**

36

37 *Aims* We investigated whether density fractionation can be used to determine the distribution of
38 organic phosphorus (OP) between free and mineral-associated soil organic matter (SOM).

39 *Methods* We performed density fractionations using sodium polytungstate solution (specific gravity
40 1.6 g cm^{-3}) on 20 soils from UK semi-natural and pasture ecosystems, to obtain a light fraction (LF) and
41 a heavy fraction (HF) for each soil. The fractions were quantified by weight, and analysed for organic
42 carbon (OC), total N (TN), total P (TP), inorganic P (IP), and OP (by difference).

43 *Results* Good recoveries of soil mass (96%), OC and TN (both $\sim 90\%$) were obtained, but recovery of
44 OP only averaged 56%. The average P:C ratio of HF SOM exceeded that of LF SOM by a factor of six,
45 greater than the factor of two obtained for TN:OC. For the soils studied, the elements of SOM were
46 predominantly in the HF, with averages of 75% for C, 82% for N, and 90% for P.

47 *Conclusions* The incomplete recovery of OP demands further work. Nonetheless, the results show that
48 HF SOM is much richer in P than LF SOM.

49

50

51

52 **Keywords**

53 Carbon · Density fractionation · Nitrogen · Phosphorus · Soil organic matter · Stoichiometry

54 Introduction

55

56 The N (nitrogen) and P (phosphorus) contents of bulk SOM (soil organic matter) vary appreciably; C:N
57 ratios can be as low as 8 and higher than 30, while C:P ratios range from c. 20 to 1000 (Tipping et al.
58 2016). Such variation arises from differences in the element stoichiometry of input litter, the
59 processing of elements during decomposition, stabilisation processes, and within-soil transport.
60 Accounting for the variation is necessary in order to understand and quantify the interlinked
61 biogeochemical cycles of the elements. From analysis of data obtained with the Hedley fractionation
62 procedure (Hedley et al, 1982), Yang and Post (2011) found that C and N in SOM were closely linked,
63 but that P was correlated to neither C nor N, and they concluded that OP is decoupled from OC and
64 ON. However, from an analysis of data for c. 2000 soils, including topsoils and subsoils under both
65 natural and agricultural vegetation, with %OC ranging from 0.1 to 50%, Tipping et al. (2016) found a
66 strong positive relationship between the N:C and P:C ratios of SOM. This was attributed to the
67 preferential adsorption by mineral matter (i.e. accumulation at mineral surfaces owing to physical and
68 chemical interactions) of N-rich and P-rich organic compounds. Therefore, further insight might be
69 gained by fractionating soils according to density, then analysing the fractions for organic phosphorus.

70 The physicochemical fractionation of SOM involves the separation of SOM through flotation,
71 sedimentation and aggregate disturbance. During fractionation, the organic debris including plant and
72 animal material, referred to here as the light fraction (LF), but sometimes referred to as particulate
73 organic matter (Zimmermann et al. 2007), is separated from organic material bound to mineral
74 matter, referred to as the heavy fraction (HF), using a dense solution. A range of fractionation
75 methods are available (Sohi et al. 2001; Kirkby et al. 2011; Zimmermann et al. 2007), and there is no
76 standardised procedure, therefore research into methodological aspects continues (Cerli et al, 2012).
77 For well-drained soils, the HF is generally regarded as the stable SOM pool, on the basis of stable
78 isotope ($\delta^{13}\text{C}$) and radiocarbon (^{14}C) analyses (Trumbore 1993; Swanston et al. 2005; Tan et al. 2007;
79 Kögel-Knabner et al. 2008). Almost all studies report a low C:N ratio within the HF whereas the LF
80 mostly has a high C:N ratio. We conducted a thorough literature search for reports of measurements
81 of the distribution of OP between the HF and LF, and found none, although several studies have
82 reported the OP content of light material (Rodkey et al. 1995; O'Hara et al. 2006; Wick & Tiessen
83 2008).

84 In this study, we investigated the feasibility of using a density fractionation method to
85 determine the distribution of OP in soils, so as to separate the heavy mineral-rich material from the
86 lighter free organic matter, and analysing the fractions for organic carbon (OC), total nitrogen (TN),

87 total phosphorus (TP) and inorganic phosphorus (IP), and obtaining organic phosphorus (OP) by
88 difference.

89 Abbreviations are listed in Table 1.

90

91 **Methods**

92

93 Soil samples had been collected in a survey of the catchments of the Rivers Avon (Hampshire),
94 Conwy (N Wales), Dee (NE Scotland), and Ribble (NW England) carried out between 2013 and 2015
95 (Toberman et al. 2016). Samples had been bulked from 6 or 10 separate cores, and included both
96 topsoils and subsoils. We chose 20 soils to provide a range of SOM contents, and with sufficient light
97 material to analyse. Most soils were under seminatural vegetation, three were from improved
98 grassland. Arable soils were not analysed owing to their low contents of light material.

99 We applied a physicochemical density fractionation method based on the procedure of
100 Schrumpf et al. (2013), which in turn was derived from those of Golchin et al. (1994) and Sohi et al.
101 (2001). Fig 1. is a schematic of the fractionation method. We distinguished non-occluded and
102 occluded light fractions (NLF, OLF), which were combined to make the light fraction (LF), and the heavy
103 fraction (HF). One fractionation was performed for each soil. Twenty-five g subsamples of sieved soil
104 were placed in 400 mL centrifuge bottles, with 250 mL sodium polytungstate (NaPT; Sometu, Belgium)
105 at a density of 1.6 g cm⁻³ (Cerli et al. 2012). The bottles were gently shaken by hand, then centrifuged
106 at 5500 rpm for 30 minutes. If the quantity of floating material (NLF) was low, it was removed using a
107 wide-tipped pipette and placed into 60 µm nylon mesh bags. For samples of heathland and forest soil
108 with higher quantities of NLF, material was removed using a spatula and placed in 60 µm nylon mesh
109 bags. The remaining suspension was brought back to its initial volume with fresh NaPT (this required
110 c. 20 mL) re-centrifuged, and then residual light fraction was removed, this procedure being repeated
111 (no more than twice) until all NLF was accounted for. The material in the mesh bags was rinsed with
112 deionised water, and the leachate repeatedly measured for conductivity using a Jenway 4510 probe;
113 complete removal of excess NaPT was assumed when the conductivity fell below 50 µs cm⁻¹, except
114 that for calcareous soils conductivities < 200 µs cm⁻¹ were considered acceptable, because of
115 dissolution of carbonates (Schrumpf et al. 2013). The rinsed samples were weighed, oven dried at 40
116 °C, weighed again, and once completely dried they were stored in a desiccator until further analysis.

117 Extraction of OLF was carried out using sonication (Sonics Vibracell CV18 probe). To avoid
118 aggregate breakdown of the HF, a pilot test for each of the soil types, based on bulk soil texture
119 (Toberman et al. 2016; Table S1), was carried out to find the optimal sonication energy input, following
120 the procedure of Schrumpf et al. (2013). For sandy and silty soils (there were no clay rich soils), target
121 energy inputs of 100 and 300 J mL⁻¹ respectively were used. The samples were periodically checked
122 for complete aggregate disruption using a 0.1 mL subsample observed under a microscope at 100x
123 magnification. Complete disruption was assumed when no further OLF material could be seen
124 attached to minerals under the microscope. During sonication, the bottle was submerged in an ice

125 bath and the temperature of the sample was measured and maintained at < 40 °C (Schrumpf et al.
126 2013). Once fully sonicated, samples were left to stand for 1 hour and then centrifuged again at 5500
127 rpm for 30 minutes and the OLF extracted by pipette; if necessary, further centrifugation was
128 performed (once or twice) to maximise the capture of OLF material. The OLF was added to the NLF in
129 the 60 µm mesh bags, the resulting LF was rinsed again until conductivity was < 50 or < 200 µs⁻¹, dried
130 at 40 °C, weighed and ground to a fine powder using a Retsch MM400 mixer mill.

131 The centrifuge bottles containing the remaining material (HF) were refilled with ultra-pure
132 deionised water and centrifuged at 5500 rpm for 10 minutes. After each centrifugation, the
133 supernatant was decanted into plastic beakers and measured for conductivity. This process was
134 repeated until the waste water had a conductivity of < 50 or < 200 µs⁻¹. The samples were then
135 transferred into aluminium trays, oven dried at 40 °C and weighed. The dried HFs were ground to a
136 fine powder using a ceramic pestle and mortar.

137 We tested for displacement of P forms from soil by NaPT by suspending 25 g subsamples of
138 four of the sieved soils in 250 mL NaPT at a density of 1.6 g cm⁻³ in 400 mL centrifuge bottles, as in the
139 density fractionations. After sonication and centrifugation, the clear supernatant solution beneath
140 the suspended light fraction was removed with a pipette and filtered (Whatman GFF). The solution
141 was analysed for soluble reactive P (SRP) and total dissolved P (TDP) as described below.

142 Soil OC and TN were determined by the procedures given by Emmett et al. (2008). Before analysis
143 for C and N, any samples that might have contained inorganic carbonate (bulk soil pH > 5.5) were
144 treated with 0.1 M HCl and observed under microscope until all CO₂ release had occurred. These
145 samples were then re-dried at 40 °C. Single determinations of total organic carbon (TOC) and total
146 nitrogen (TN) in milled subsamples were made with a Vario EL elemental analyser. Repeated
147 determinations by this method on three representative UK soils over the period of this study gave
148 relative standard deviations of between 2.1 and 3.6 % for TOC and between 1.7 and 3.1% for TN.

149 Total P (TP) was determined by the ignition-extraction method as described in Olsen and
150 Sommers (1982). First, 0.5 g subsamples were ignited in a Pyrotherm muffle furnace at 550 °C for 1-2
151 hours, placed in 50 mL centrifuge bottles with 25 mL 0.5 M sulphuric acid and shaken for 16 hours.
152 These were then centrifuged at 10000 rpm for 30 minutes, filtered using Whatman 1573 1/2 (12-25
153 µm) filter papers and refrigerated at 4 °C until further analysis. The extracts were analysed for soluble
154 reactive phosphorus using the molybdate method (Olsen and Sommers, 1982). Measurements on a
155 reference sample (ISE sample 921 from Wageningen University, Netherlands) gave an average TP
156 value that was 96.9% (sd 1.1%, n = 4) of the expected value. Inorganic P (IP) was determined by
157 extracting 0.5 g of soil with 25 mL of 0.5 M sulphuric acid, then analysing the extract with molybdate.
158 Organic P was obtained as the difference between TP and IP. These analyses were replicated four-

159 fold. The molybdate method was used to measure SRP in the supernatants of soil/NaPT suspensions
160 (see above), and concentrations of TDP were also determined with molybdate after digestion with
161 acid persulphate (Rowland and Haygarth 1997). The supernatants were diluted 100 times with
162 deionised water before making the measurements, and at the resulting concentrations of NaPT, no
163 interference with the molybdate method was found for SRP. However, acid persulphate digestion of
164 the dilute NaPT solutions reduced the sensitivity of the molybdate assay, and this was taken into
165 account in estimating TDP concentrations.

166 Bulk analyses of the soils were reported by Toberman et al. (2016), using the same methods
167 for C, N and IP, but with a different method for TP, involving treatment of the samples with aqua regia
168 and microwave digestion. Resource limitations meant that we were unable to determine soil TP by
169 the same method for both bulk and fractionated soils. However tests on six bulk soil samples showed
170 that results from the two TP methods were in agreement; the ratio of TP values from the ignition-
171 extraction method to those from the aqua regia-microwave method ranged from 0.94 to 1.18, with a
172 mean of 1.02 (not significantly different from 1.00, $p > 0.05$).

173 Statistical analyses (t-tests and linear regressions) were performed with Microsoft Excel.
174 Before conducting linear regression analyses, data were tested for normality using quantile–quantile
175 plotting. For t-testing the D’Agostino-Pearson test was used to check for normality. Non-normal data
176 were transformed using log transformations where necessary.

177 **Results and discussion**

178

179 Performance of the fractionation method

180

181 Good recoveries of soil mass from the density fractionation procedure were achieved for all the
182 samples, with an average of 96% and a range over the 20 soils of 90 – 105% (Table S2). Regression
183 analyses indicated that recovery depended upon neither the amount of material in the heavy fraction,
184 nor the carbon content of the bulk soil (data not shown). The average recovery fell between the
185 averages of 100% obtained by Swanston et al. (2002) for 7 soils, and 83% obtained by Schrumpf et al.
186 (2013) for 48 samples; we used essentially the same method as these previous studies. The loss of
187 some material in these types of methods is probably from some soluble compounds dissolving into
188 the NaPT solution and some solid material was probably lost during rinsing and collection of the
189 separate fractions (Cerli et al. 2012). We found no measurable SRP in the supernatants of four soils
190 that had been suspended in NaPT and the suspensions sonicated, but small amounts of TDP were
191 detected, corresponding to between 3 and 8% (average 4.6%) of the soil TP. The fraction of soil mass
192 in HF ranged from 78.6 to 98.5 % (Table S2).

193 Light fraction element concentrations of OC showed only modest variation (relative standard
194 deviation, RSD, 12%), with a range of 26.5 to 45.5% and a mean of 36.1% (Table S3). This indicates
195 that the LF was predominantly but not entirely SOM (%C ~ 55%), i.e. some mineral matter was present.
196 Crow et al. (2007) reported values of 27 and 29% OC in two soils, and Swanston et al. (2002) obtained
197 a mean of 25% OC from 7 soils. Cerli et al. (2012) observed decreasing OC content in the light fraction
198 with increasing sonication time and intensity, suggesting a higher content of mineral matter through
199 aggregate breakdown. Thus the fractionation procedure certainly concentrates SOM in the LF, but
200 some mineral matter is retained.

201

202 Concentrations of P forms in the LF and HF

203

204 Concentration data for TP, IP and OP (by difference) are presented in Fig. 2 and Table S4. Based on
205 relative standard errors, the average reproducibility was $\pm 14\%$ for the LF forms of P, and $\pm 6\%$ for the
206 HF forms, which can be considered satisfactory, bearing in mind the several steps that are involved in
207 the analytical procedure.

208 For the majority of HF samples, most of the P is organic (range 50 to 97%, average 79%),
209 whereas in the LF OP and IP are similar (the OP range is 24 to 77%, average 50%). The IP content of
210 the LF is surprising, given that this material is thought to consist mainly of plant residues (Six et al.

211 2002). One possible explanation is that the strong acid reagent used to extract IP caused hydrolysis
212 of some of the LF SOM, releasing IP; however, Turner et al. (2005) considered this to apply to only a
213 small fraction of OP. To explore this further, we compared the results for the LF with data for “natural
214 LF”, i.e. the organic horizons of Swiss forest soils (Walthert et al. 2004; Blaser et al. 2005, Zimmermann
215 et al. 2006) for which IP was analysed by the same method that we used here. We took data for 16 F
216 (Oe) and 16 H (Oa) horizons, each dominated by SOM. For the F horizons the mean IP was 8% of the
217 total (range 0 - 30%), for the H horizons it was 16% (range 0 - 53%). Therefore the LF material isolated
218 by density fractionation in the present study appears to possess a higher fraction of its P in the
219 inorganic form than high-SOM bulk soils. One possible explanation is that IP owes its presence in the
220 LF to the coordination of inorganic phosphate with Al and Fe complexed by the SOM, which occurs to
221 different extents in the Swiss forest soils and the soils studied here. Another possibility is that IP is
222 associated with mineral matter, present at a higher concentration in the LF compared to the F and H
223 horizon soil samples.

224

225 Element recoveries in the fractionation process

226

227 Recoveries were calculated by combining the mass data with measured element concentrations in
228 bulk soil and in the two density-separated fractions. The results are summarized in Table 2 and
229 detailed in Tables S5 and S6. Average recoveries of OC and TN were each 91% (Table 2). The results
230 for OC fall within the range of published values, 72-101%, which come from data reported for two
231 soils by Crow et al. (2007), one soil by Cerli et al. (2012), and 48 soils by Schrumpf et al. (2013), all
232 fractionated by a similar method to that used here; the overall average recovery for all 51 soils was
233 94%. Our average recovery of 91% for TN exceeds those of 85% reported for one soil by Cerli et al.
234 (2012), and 74% reported for two soils by Crow et al. (2007). Therefore our processing of the soils
235 with respect to mass, OC and TN achieved similar levels of recovery to those of previous studies.

236 Average recoveries of TP, IP and OP for individual soils were 62%, 117% and 56% respectively
237 (Table 2). In each case the variability in the recoveries is appreciably greater than for OC and TN
238 (Tables S5 and S6). However, regressions of the sums of the recovered forms of P in LF and HF against
239 the starting (bulk) values (Fig. S1) gave highly significant slopes, suggesting some consistency in the
240 behaviours of the P forms during the fractionation and analytical procedure. It appears that on
241 average not much IP was lost, whereas definite losses of TP and OP occurred. The absolute losses of
242 TP and OP were similar (OP loss was equal to 90% of TP loss on average), and highly correlated ($r^2 =$
243 0.90, $p < 0.001$), indicating that most of the loss of TP was due to loss of OP; this follows because OP

244 was obtained as the difference between TP and IP, because IP was a minor part of TP in HF (see above),
245 and because overall IP losses were minor.

246

247 Assessment of the methodology for phosphorus

248

249 Two aspects of the results obtained give cause for concern about the methodology, the loss of
250 appreciable amounts of OP from some soils in the fractionation procedure (Table S6), and the high
251 variability in percentage recoveries (Tables 2 and S6, Fig. S1). Since these problems were not found
252 for OC and TN, they are specific to phosphorus forms, and are presumably due either to the behaviour
253 of OP during the density fractionation procedure or to errors in the analysis of TP and/or IP (OP is
254 derived by difference). Experimental tests for the solubilisation, and therefore loss, of P forms during
255 extraction revealed only small losses of TP (see above), not at all sufficient to explain the low
256 recoveries of TP and OP. This rules out any major displacement of sorbed IP or OP by NaPT, which
257 seemed plausible in view of the fact that monotonungstate can displace inorganic phosphate from
258 ferrihydrite (Gustafsson 2003). Therefore it seems unlikely that the fractionation procedure is at fault.

259 The analysis procedures for soil P differ from those for OC and TN in two respects. Firstly, the
260 concentrations of the P forms are relatively low, being about an order of magnitude less than that of
261 TN, and two to three orders less than that of OC. Secondly, the combined analytical method for OC
262 and TN is simple and reproducible, comprising full combustion and gas analysis (CO₂ and NO₂ in the
263 method that we used here). In contrast, our analytical method for TP involved combustion in a muffle
264 furnace, extraction of inorganic P into H₂SO₄, and then determination of the resulting SRP, while
265 determination of IP omits the combustion step. Therefore there is more scope for errors to arise. As
266 shown by the results in Fig. 2 we obtained quite good reproducibility in the P measurements on
267 individual soils, which may suggest that the problems arise from variability in the extraction steps, i.e.
268 the combustion of OM and conversion of OP to IP, or the extraction of the so-formed organic P into
269 H₂SO₄. However, to account for the low recoveries and variabilities, difficulties with the combustion
270 and extraction would have to apply only to fractionated soil, since full recovery of TP in bulk soils was
271 achieved (see Methods) and we cannot see an obvious reason why that should occur.

272 Further work is clearly needed to improve the yields from the density fractionation procedure.
273 The incomplete recoveries must be borne in mind when interpreting our results, in particular the
274 consequences of different relative losses of OP from HF and LF.

275

276 Element relationships in LF and HF

277

278 To explore element relationships in SOM, we assume TN (Table S3) is all organic. According to
279 Stevenson (1986), inorganic N comprises 10% of TN on average, while Schulten and Schnitzer (1998)
280 estimated only 5%. The inorganic contribution is highest in deeper soils and soils poor in SOM,
281 opposite circumstances to our relatively SOM-rich topsoils. In this section, for simplicity and clarity
282 we use N:C, P:C, C:N and C:P to refer to ratios of organic forms of the elements when discussing SOM
283 compositions.

284 Table 3 shows averaged OC concentrations and element ratios (g g^{-1}) for LF and HF. The
285 average N:C ratio of the HF is significantly ($p < 0.001$) higher than that of the LF, the HF N:C ratio
286 exceeding the LF ratio for 19 of the 20 soils. Such a difference also applies for P:C ($p < 0.001$), again
287 with 19 of the 20 soils fitting the pattern. However, the difference is considerably greater for P:C,
288 since there is nearly six times as much P per unit C in the HF compared to the LF, whereas the factor
289 for N:C is only 1.9. Higher N:C ratios (lower C:N ratios) in the HF have been reported before, by Sollins
290 et al. (2006), Crow et al. (2007), Cerli et al. (2012) and Schrumpf et al. (2013), but we could not find
291 published information for P:C ratios in density-fractionated soil. Therefore this appears to be the first
292 time that the difference between HF and LF has been demonstrated for P:C.

293 A comparable study is that of Kirkby et al. (2011), who used a dry sieving and winnowing
294 method to separate light fractions from six Australian soils, two natural or semi-natural and four
295 agricultural. They obtained an average OC content of 15.1% and average TN:OC and TP:OC ratios of
296 0.057 and 0.0039 respectively. The higher element ratios, compared to the values for LF in Table 3,
297 can be explained in terms of the lower OC concentration, as discussed below. The remaining soils had
298 an average OC content of about 3%, and TN:OC and OP:OC ratios of 0.085 and 0.0053 respectively,
299 similar to the values in Table 3. Another relevant study is by O'Hara et al. (2006), who used
300 fractionation with water to obtain LFs from several native eucalypt forest soils in Australia, and
301 determined their OC and total P concentrations. The average OC concentration was 45.5% (SD 0.9%),
302 and the average TP:OC ratio 0.0011 (SD 0.0003) g g^{-1} . Although the TP:OC ratio must be considered a
303 maximum estimate of the SOM P:C (since some of the TP could have been IP), the key point is that the
304 P:C ratio of SOM in these LFs was low, similar to the values in Table 3. Also relevant are data for the
305 "natural LF" of Swiss forest soils, considered above in relation to IP contents. Data for the Oe and Oa
306 horizons gave an average OC concentration of 36.4 (SD 6.3) % and an average TN:OC ratio of 0.045
307 (SD 0.007), both very similar to our values for LF shown in Table 3. For OP:OC, the Swiss forest soils
308 average was 0.0019 (SD 0.0009), which is about double our LF value (Table 3), but still substantially
309 lower than the average of 0.0049 that we find for the HF. The similarities between these literature
310 data and our values for density-fractionated LF strongly suggest that although losses of OP from LF
311 material may have contributed to the overall losses of OP in the fractionations, these losses were not

312 disproportionate, i.e. did not bias the estimated distributions of OP between LF and HF. In view of the
313 relatively low levels of OP in the LF, this suggests that most of the OP losses were from the HF.

314 Fig. 3 shows how the organic forms of the elements are partitioned into the HF, in relation to
315 the partitioning of soil mass. In 18 of the 20 soils C, N and P are predominantly in the heavy fraction,
316 and the HF percentages are in the order $P > N > C$. The preferential occurrences of N and P in the HF
317 result from both the HF:LF partitioning of organic matter per se, and also the enrichments of the two
318 elements in HF SOM (see above). This leads to the especially strong partitioning of OP into the HF.

319 Stoichiometric relationships among the elements can also be seen in log-log plots of N:C and
320 P:C against %C (Fig. 4), following the approach of Tipping et al. (2016) in their analysis of bulk C-N-P-S
321 data for c. 2000 soils. Tipping et al. (2016) formulated a model of SOM stoichiometry in which the
322 SOM of a soil is considered to be a mixture of two end-members, nutrient-poor SOM (NPSOM) which
323 has low N:C and P:C ratios (0.039 and 0.0011 g g^{-1} respectively), and nutrient-rich SOM (NRSOM) which
324 has high ratios (0.12, 0.016 g g^{-1}). All NRSOM is considered to be adsorbed to mineral matter, while
325 NPSOM may or may not be adsorbed. All unadsorbed SOM is NPSOM. Because mineral matter is the
326 obverse of the measured quantity OC%, the fraction of NPSOM increases linearly with \log_{10} %C,
327 between limits of 0.1% C and 50% C, and as a result the \log_{10} N:C and \log_{10} P:C values are predicted to
328 fall with %C as shown by the lines in Fig. 4. If it is assumed that the adsorption processes responsible
329 for SOM accumulation on mineral matter are unaffected by the physical fractionation of the soil then
330 the model should also hold for the results reported here, and the HF and LF N:C and P:C ratios should
331 follow the predicted relationships, but be separated according to the OC concentrations. As shown
332 by the plots in Fig. 4, the expected trends are indeed approximately followed. Apart from two outliers
333 (heathland soils), the N:C values fall close to the model line, and this is also true of the P:C values for
334 HF, while for LF the ratios are somewhat lower than expected. The heathland outlier HF results may
335 reflect the sandy nature of the soils, which may limit adsorption. Overall, we can conclude that the
336 present results support the Tipping et al. (2016) model.

337 The key result of this work is that the N:C ratios, and especially the P:C ratios, of the HF are
338 significantly and substantially higher than those of the LF. This is consistent with the preferential
339 adsorption by mineral matter of N- and P-rich compounds, proposed by Tipping et al. (2016) as a
340 principal mechanism by which NRSOM is formed. The C-N-P stoichiometry of NRSOM does not reveal
341 much about its molecular constituents, which could include recognisable biochemicals from plants
342 and microbes, and their breakdown products. The material may also comprise larger molecules
343 produced by humification, perhaps by reactions occurring at the mineral surface (Collins et al. 1995;
344 Johnson et al. 2016). Interestingly, McLaren et al. (2015a) presented evidence that about two-thirds
345 of the OP in five differing topsoils occurred in high molecular weight material. At least some of the

346 mineral-associated SOM has accumulated over hundreds to thousands of years, and therefore must
347 reflect the long-term supply of competing adsorbates, as well as post-adsorption modifications.

348 Our findings demonstrate that density fractionation is a promising approach to investigate the
349 interactions governing soil OP and its relationships to OC and ON. However, as already discussed, in
350 view of the incomplete recoveries that we obtained, there is a need to improve the basic
351 methodology. This might extend to the use of different analytical techniques for the determination
352 of different chemical forms, in view of recent evidence that the ashing-extraction technique used here
353 may underestimate TP (McLaren et al. 2015b). It is also important to recognise the different ways that
354 organic P might be measured (Olsen and Summers 1982; Turner et al 2005). Interesting possibilities
355 to obtain additional information are the coupling of density fractionation with subsequent chemical
356 fractionation, e.g. by the Hedley scheme (Hedley et al. 1982), and/or size fraction (Makarov et al.
357 2004).

358

359 **Conclusions**

360

- 361 1. The density fractionation method yielded good recoveries of soil mass, OC and TN for 20 semi-
362 natural and pasture soils with OC concentrations ranging from 4.5 to 18%.
- 363 2. Average recoveries of TP and OP were relatively low, 62% and 56% respectively (c. 50%), and
364 further work is need to improve them.
- 365 3. Organic matter of the heavy fraction was richer in N and P than that of the light fraction, on
366 average by a factor of two in N, and by a factor of six in P.
- 367 4. The elements of organic matter were predominantly in the heavy fractions of the soils, with
368 averages of 75% for C, 82% for N and 90% for P.
- 369 5. The variations with soil %C of stoichiometric ratios (P:C, N:C) in HF and LF agree approximately
370 with the predictions of the two end-member mixing model of SOM advanced by Tipping et al.
371 (2016), in which organic molecules rich in P and N preferentially accumulate on mineral matter
372 surfaces through strong adsorption.

373

374 **References**

- 375 Blaser P, Zimmermann S, Luster J, Walthert L, Lüscher P (2005) Waldböden der Schweiz Vol 2, Hep
376 Verlag, Bern, 920 pp
- 377 Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and heavy organic matter
378 fractions in soil - Testing for proper density cut-off and dispersion level. *Geoderma* 170: 403–
379 416
- 380 Collins MJ, Bishop AN, Farrimond P (1995) Sorption by mineral surfaces - rebirth of the classical
381 condensation pathway for kerogen formation. *Geochim Cosmochim Acta* 59: 2387–2391
- 382 Crow SE, Swanston CW, Lajtha K, Brooks JR, Keirstead H (2007) Stabilization and destabilization of soil
383 organic matter: a new focus. *Biogeochem* 85: 69-90
- 384 Emmett BA, Frogbrook ZL, Chamberlain PM, Griffiths R, Pickup R, Poskitt J, Reynolds B, Rowe E,
385 Rowland P, Spurgeon D, Wilson J. & Wood CM (2008) Countryside Survey Soils Manual.
386 Countryside Survey Technical Report No. 04/07, Centre for Ecology & Hydrology, Wallingford.
- 387 Golchin A, Oades JM, Skjemstad JO, Clarke P (1994) Study of free and occluded particulate organic
388 matter in soils by solid state ¹³C CP/MAS NMR spectroscopy and scanning electron microscopy.
389 *Aust J Soil Res* 32: 285-309
- 390 Gustafsson JP (2003) Modelling molybdate and tungstate adsorption to ferrihydrite. *Chem Geol* 200:
391 105-115
- 392 Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in the inorganic and organic soil phosphorus
393 fractions induced by cultivation practices and by laboratory incubations. *Soil Sci Soc Am J* 46:
394 970–976
- 395 Johnson K, Purvis G, Lopez-Capel E, Peacock C, Gray N, Wagner T, März C, Bowen L, Ojeda J, Finlay N,
396 Robertson S, Worrall F, Greenwell C (2016) Towards a mechanistic understanding of carbon
397 stabilization in manganese oxides. *Nature Comm* 6:7628, DOI: 10.1038/ncomms8628
- 398 Kirkby CA, Kirkegaard JA, Richardson AE, Wade LJ, Blanchard C, Batten G (2011) Stable soil organic
399 matter: A comparison of C:N:P:S ratios in Australian and other world soils. *Geoderma* 163:
400 197–208
- 401 Kögel-Knabner I, Guggenberger G, Kleber M, Leinweber P (2008) Organo-mineral associations in
402 temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *J Plant Nutr*
403 *Soil Sci* 171: 61–82
- 404 Makarov MI, Haumaier L, Zech W, Malysheva TI (2004) Organic phosphorus compounds in particle-
405 size fractions of mountain soils in the northwestern Caucasus. *Geoderma* 118: 101–114

406 McLaren TI, Smernik RJ, McLaughlin MJ, McBeath TM, Kirby JK, Simpson RJ, Guppy CN, Doolette AL,
407 Richardson AE (2015a) Complex forms of soil organic phosphorus—a major component of soil
408 phosphorus. *Environ Sci Technol* 49: 13238–13245

409 McLaren TI, Simpson RJ, McLaughlin MJ, Smernik RJ, McBeath TM, Guppy CN, Richardson AE (2015b)
410 An assessment of various measures of soil phosphorus and the net accumulation of
411 phosphorus in fertilized soils under pasture. *J Plant Nutr Soil Sci* 178: 543–554

412 O’Hara CP, Bauhus J, Smethurst PJ (2006) Role of light fraction soil organic matter in the phosphorus
413 nutrition of *Eucalyptus globulus* seedlings. *Plant Soil* 280: 127–134

414 Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil*
415 *analysis: Part 2. Chemical and microbiological properties. Agron. Mongr. 9. 2nd ed. ASA and*
416 *SSSA, Madison, WI, pp 403-430*

417 Rodkey KS, Kaczmarek DJ, Pope PE (1995) The distribution of nitrogen and phosphorus in forest floor
418 layers of oak-hickory forests of varying productivity. *Proc 10th Central Hardwood Forest*
419 *Conference, Gen Tech: 94–108*

420 Rowland AP, Haygarth PM (1997) Determination of total dissolved phosphorus in soil solutions. *J*
421 *Environ Qual* 26: 410–415

422 Schrumpf M, Kaiser K, Guggenberger G, Persson T, Kögel-Knabner I, Schulze E-D (2013) Storage and
423 stability of organic carbon in soils as related to depth, occlusion within aggregates, and
424 attachment to minerals. *Biogeosci* 10: 1675–1691

425 Schulten H-R, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. *Biol Fertil Soil* 26:1–
426 15

427 Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter:
428 Implications for C-saturation of soils. *Plant Soil* 241: 155–176

429 Sohi SP, Mahieu N, Arah JRM, Powlson DS, Madari B, Gaunt JL (2001) A Procedure for isolating soil
430 organic matter fractions suitable for modeling. *Soil Sci Soc Am J* 65: 1121-1128

431 Sollins P, Swanston C, Kleber M, Filley T, Kramer M, Crow S, Caldwell BA, Lajtha K, Bowden R (2006)
432 Organic C and N stabilization in a forest soil: evidence from sequential density fractionation.
433 *Soil Biol Biochem* 38: 3313–3324

434 Stevenson FJ (1986) *Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients.* Wiley, New
435 York

436 Swanston CW, Caldwell BA, Homann PS, Ganio L, Sollins P (2002) Carbon dynamics during long-term
437 incubation of separate and recombined density fractions from seven forest soils. *Soil Biol*
438 *Biochem* 34: 1121–1130

439 Swanston CW, Torn MS, Hanson PJ, Southon JR, Garten CT, Hanlon EM, Ganio L (2005) Initial
440 characterization of processes of soil carbon stabilization using forest stand-level radiocarbon
441 enrichment. *Geoderma* 128: 52–62

442 Tan Z, Lal R, Owens L, Izaurralde RC (2007) Distribution of light and heavy fractions of soil organic
443 carbon as related to land use and tillage practice. *Soil Tillage Res* 92: 53–59

444 Tipping E, Somerville CJ, Luster J (2016) The C:N:P:S stoichiometry of soil organic matter. *Biogeochem*
445 130: 117-131

446 Toberman H, Adams J, Tipping E, Schillereff D, Somerville C, Coull M, Helliwell R, Carter H, Guyatt H,
447 Keenan P, Lawlor A, Pereira MG, Patel M, Tanna B, Thacker S, Thomson N, Owens J, Gibbs S,
448 Smith D, Bryant C, Elliot F, Gulliver P (2016). Soil survey in England, Scotland and Wales carried
449 out during 2013 and 2014 [LTLS]. NERC Environmental Information Data Centre.
450 <https://doi.org/10.5285/17bebd7e-d342-49fd-b631-841ff148ecb0>

451 Trumbore SE (1993) Comparison of carbon dynamics in tropical and temperate soils using radiocarbon
452 measurements. *Global Biogeochem Cycles* 7: 275–290

453 Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus.
454 *Talanta* 66: 294–306

455 Walthert L, Zimmermann S, Blaser P, Luster J, Lüscher P (2004) *Waldböden der Schweiz Vol 1*, Hep
456 Verlag, Bern, 768 pp

457 Wick B, Tiessen H (2008) Organic matter turnover in light fraction and whole soil under silvopastoral
458 land use in semiarid northeast Brazil. *Rangeland Ecol Management* 61: 275–283

459 Yang X, Post WM (2011) Phosphorus transformations as a function of pedogenesis: A synthesis of soil
460 phosphorus data using Hedley fractionation method. *Biogeosci* 8: 2907–2916

461 Zimmermann S, Luster J, Blaser P, Walthert L, Lüscher P (2006) *Waldböden der Schweiz Vol 3*, Hep
462 Verlag, Bern, 848 pp

463 Zimmermann, M. Leifeld J, Schmidt MWI, Fuhrer J (2007) Measured soil organic matter fractions can
464 be related to pools in the RothC model. *Eur J Soil Sci* 58: 658–667

465

466 **Tables**

467

468 Table 1. Abbreviations

469

Abbreviation	Full title
C	Carbon
HF	heavy fraction
IP	inorganic phosphorus
LF	light fraction
N	Nitrogen
NaPT	sodium polytungstate
NLF	non-occluded light fraction
NPSOM	nutrient-poor soil organic matter
NRSOM	nutrient-rich soil organic matter
OC	organic carbon
OLF	occluded light fraction
ON	organic nitrogen
OP	organic phosphorus
P	Phosphorus
RSD	relative standard deviation
SD	standard deviation
SOM	soil organic matter
SRP	soluble reactive phosphorus
TDP	total dissolved phosphorus
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus

470

471

472

473

474 Table 2. Summary of element recoveries.

475

Element	Range %	Mean %	SD %	Range %
IP	28-486	117	105	28-486
TP	19-121	62	30	19-121
OP	17-124	56	29	17-124
OC	61-123	91	18	61-123
TN	70-110	91	12	70-110

476

477

478

479

480 Table 3. Concentrations of OC and ratios of N and P to C (g g^{-1}) in SOM of the LF and HF, for the 20 soil
 481 samples. The LF and HF ratios are significantly different in all cases (t-test; $p < 0.001$). C:N and C:P
 482 ratios are also shown.

483

	LF			HF		
	range	mean	SD	range	mean	SD
%OC	26.5-45.5	36.1	4.4	3.2-15.7	8.1	3.7
N:C	0.027-0.054	0.040	0.009	0.039-0.106	0.074	0.017
P:C	0.00029-0.0019	0.00084	0.00042	0.00080-0.0139	0.0049	0.0033
C:N	18.4-37.0	26.1	6.1	9.4-25.4	14.7	4.2
C:P	1260-3430	1490	730	72-1260	337	284

484

485 **Figure captions**

486

487 Fig. 1 Schematic of the fractionation procedure. Key: NLF non-occluded light fraction, OLF occluded
488 light fraction, LF light fraction, HF heavy fraction.

489

490 Fig. 2 Concentrations of total, inorganic and organic phosphorus (TP, IP, OP) in the light and heavy
491 fractions (LF and HF) of the 20 soil samples. The error bars indicate standard errors. Key: B broadleaf
492 woodland, C conifer plantation, H heathland, IG improved grassland, R rough grassland; d subsoil, s
493 topsoil.

494

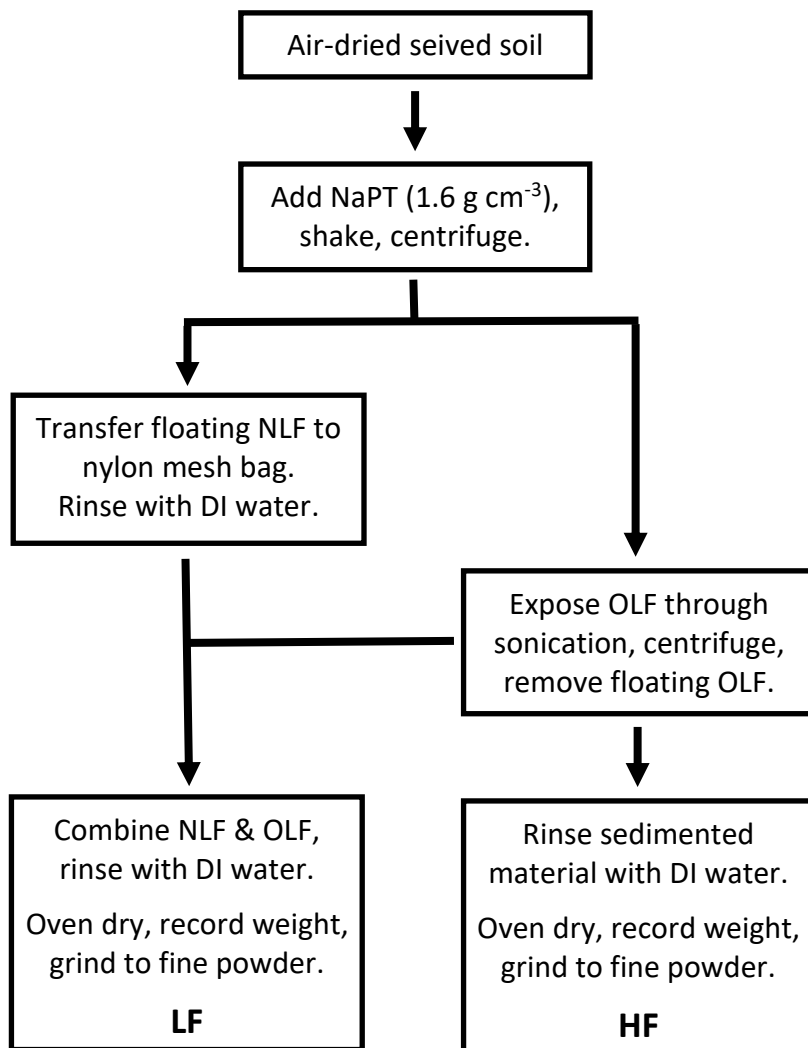
495 Fig. 3 Percentage of OC, TN and OP in the heavy fraction (HF) vs percentage of soil mass in the HF.
496 Data for two “outliers” (H1s, H4d) that do not fit the general pattern are indicated by dashed outlines.

497

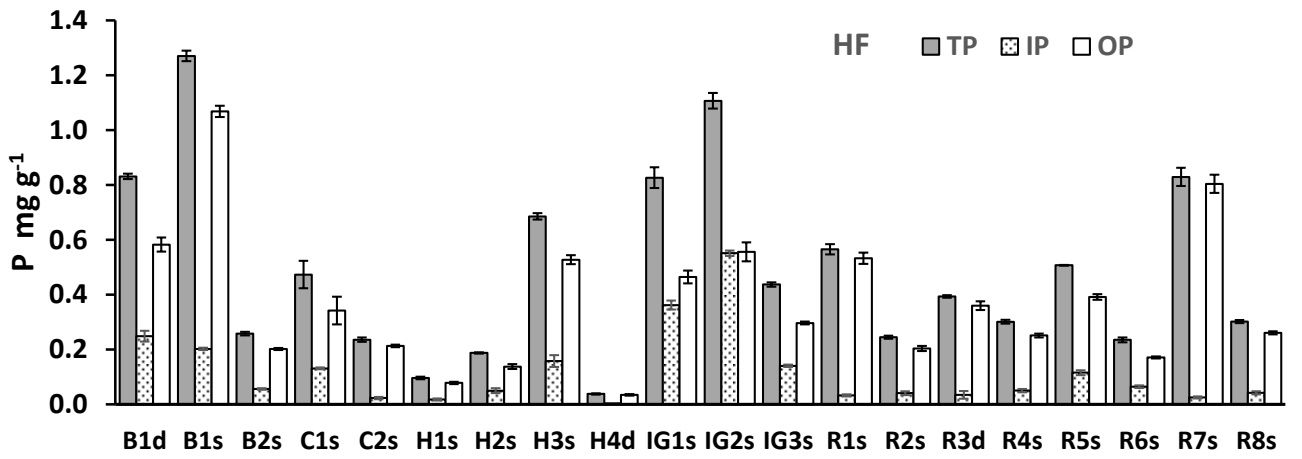
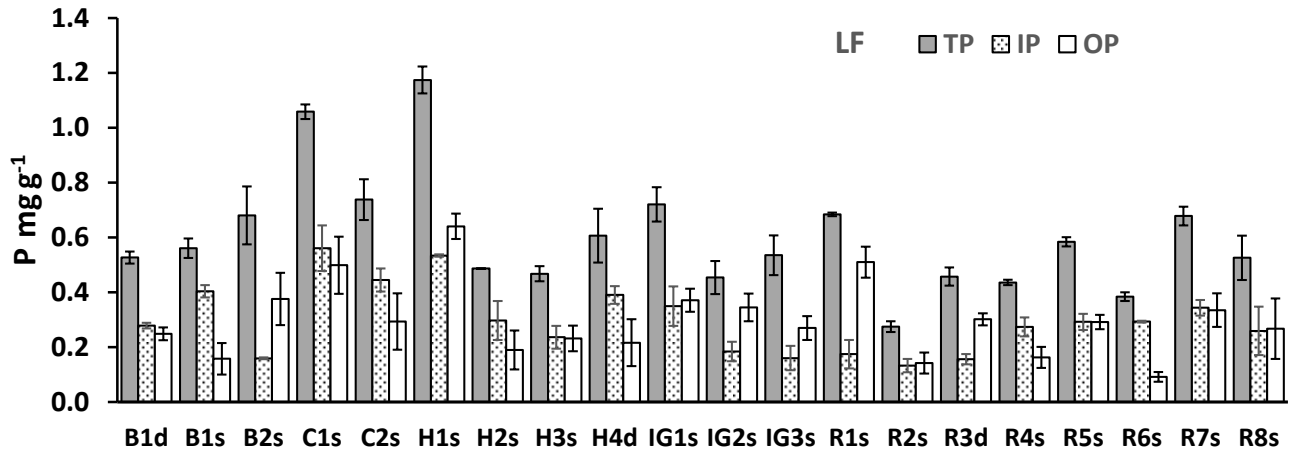
498 Fig. 4 Variations of N:C and P:C in SOM with %C for the light fraction (LF, open circles) and the heavy
499 fraction (HF, filled circles); ON is assumed equal to TN. The lines are predictions from the two end-
500 member mixing model of Tipping et al. (2016). The full range of %C is plotted to show end-member
501 ratios at ≤ 0.1 %C and ≥ 50 % C. Data for two “outliers” (H1s, H4d) that do not fit the general pattern
502 for HF are indicated by dashed outlines.

503

504
505
506
507
508
509
510
511
512
513
514
515



516 Fig. 1



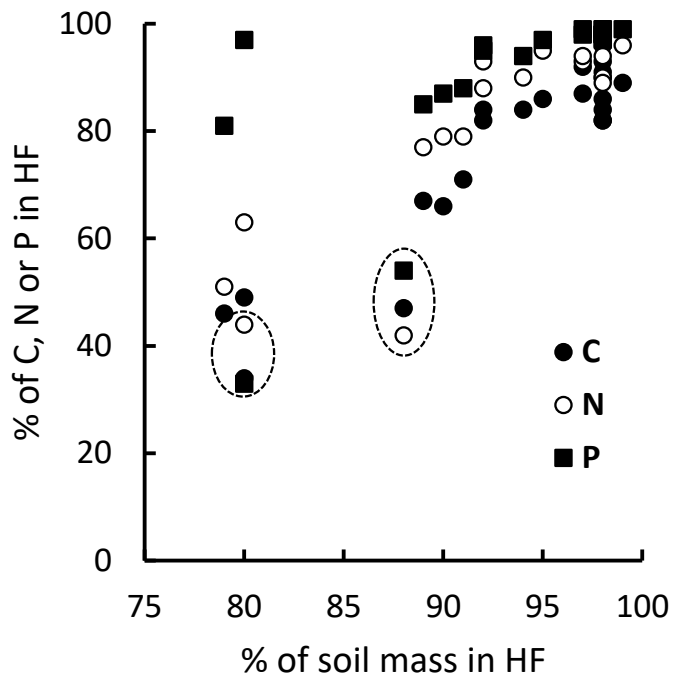
517

518 Fig. 2

519

520

521

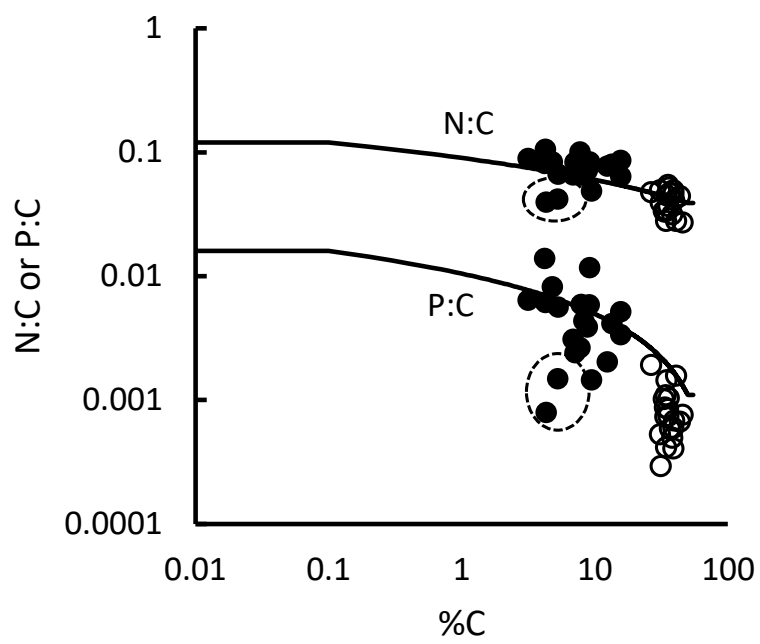


522

523 Fig 3.

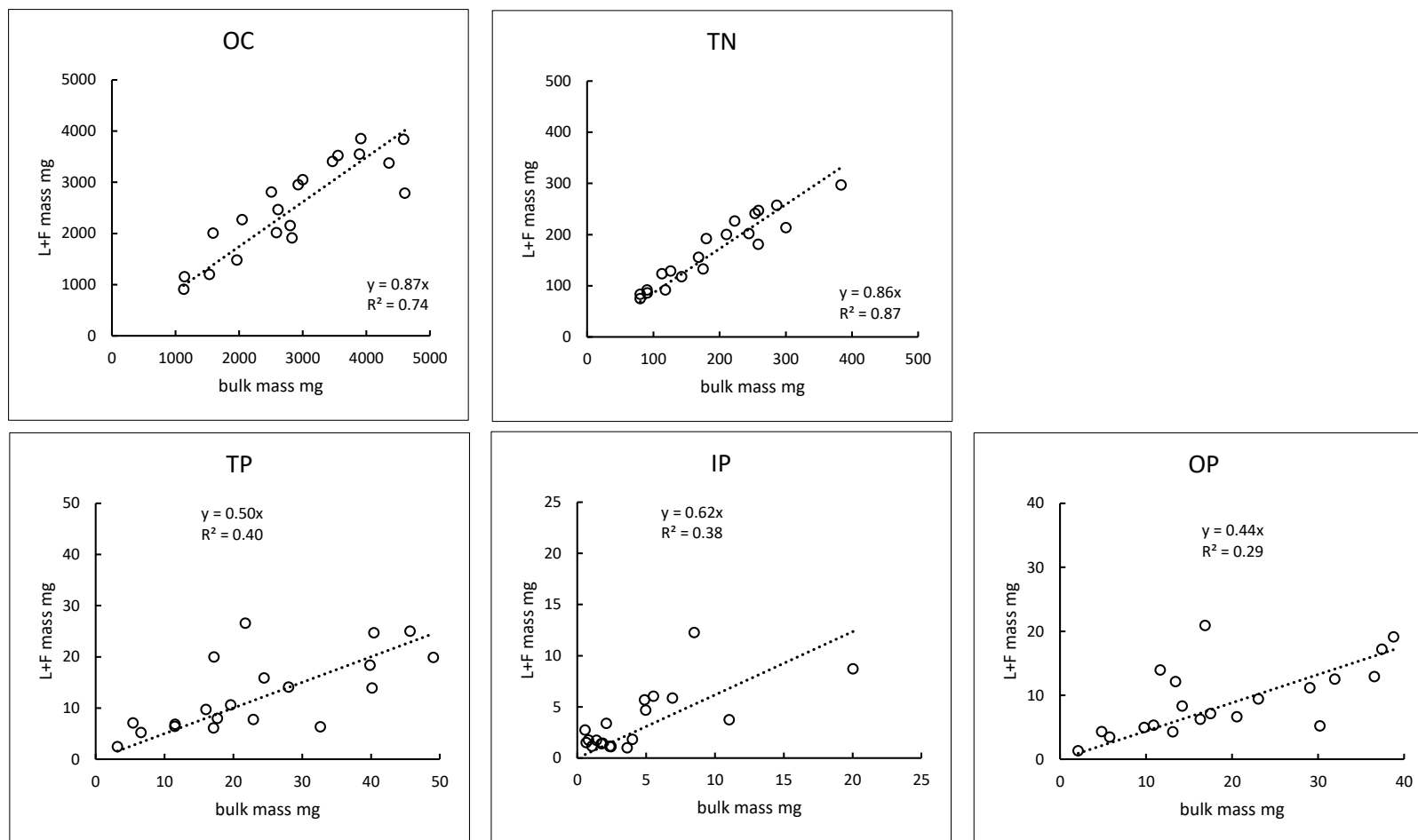
524

525



526

527 Fig. 4



528

529 Fig. S1 Linear regressions of recovery data, forced through zero. Only for TN was the Y-intercept significant ($p = 0.048$), and it was small (2.6 mg).

Table S1. Information about the soil samples

sample ID	database ID	date	lat deg	long deg	MAP mm	MAT °C	land use	soil type	upper depth cm	lower depth cm	n ¹	clay %	silt %	sand %	pH ²	BD _{FE} ³ g cm ⁻³	OC %
B1d	CW1d	05/08/13	53.19	-3.85	1391	9.9	broadleaf woodland	podzol	15	33	10	10.2	50.3	39.5	5.64	0.28	4.52
B1s	CW1s	05/08/13	53.19	-3.85	1391	9.9	broadleaf woodland	podzol	0	15	10	11.9	63.7	24.4	5.31	0.22	14.20
B2s	RW1s	25/04/13	54.00	-2.39	1614	7.9	broadleaf woodland	surface water gley	0	20	10	9.9	49.6	40.5	6.09	0.57	4.51
C1s	CC1s	06/08/13	53.03	-3.85	2309	7.3	conifer plantation	podzol	0	15	10	9.3	64.9	25.8	4.02	0.25	12.00
C2s	RC1s	01/05/13	54.01	-2.40	1614	7.9	conifer plantation	surface water gley	0	20	10	9.7	46.2	44.0	4.03	0.45	8.16
H1s	AH1s	23/07/13	50.83	-1.90	863	10.1	heathland	groundwater gley	0	15	10	nd ⁴	nd	nd	4.15	0.61	10.00
H2s	CH1s	06/08/13	53.26	-3.90	862	10.4	heathland	ranker	0	15	10	nd	nd	nd	4.32	0.50	11.60
H3s	CM1s	29/10/13	53.15	-4.00	2707	5.3	heathland	surface water gley	15	26	10	4.3	39.0	56.7	4.40	0.27	15.60
H4d	RH1d	01/05/13	54.18	-2.29	1827	5.9	heathland	ranker ⁶	20	38	10	8.3	61.3	30.4	3.68	0.55	11.30
IG1s	AIG4s	24/07/13	51.13	-1.95	865	9.5	improved grassland ⁵	groundwater gley ⁷	0	15	6	11.7	70.0	18.3	7.51	0.67	6.34
IG2s	RIG4s	12/04/13	54.25	-2.32	2053	5.7	improved grassland ⁵	groundwater gley	0	20	10	10.1	52.5	37.3	5.42	0.40	13.80
IG3s	RIG5s	30/04/13	54.00	-2.70	1220	8.4	improved grassland ⁵	groundwater gley	0	20	10	8.0	53.3	38.7	5.92	0.62	7.82
R1s	ACG1s	22/07/13	51.13	-2.00	865	9.5	rough grassland	rendzina	0	15	10	nd	nd	nd	7.24	0.56	10.40
R2s	ACG2s	23/07/13	51.38	-1.85	868	9.2	rough grassland	brown earth	0	15	10	24.6	68.6	6.7	6.51	0.58	10.90
R3d	CAG1d	06/08/13	53.08	-3.97	3105	6.1	rough grassland	surface water gley	20	44	10	nd	nd	nd	4.37	0.41	17.40
R4s	CAG3s	29/10/13	53.14	-4.00	3105	6.1	rough grassland	ranker	0	15	10	9.9	70.3	19.7	4.95	0.26	15.50
R5s	RAG1s	01/05/13	54.25	-2.32	2053	5.7	rough grassland	groundwater gley	0	20	10	nd	nd	nd	5.60	0.38	9.20
R6s	RAG2s	01/05/13	54.19	-2.35	1943	6.8	rough grassland	groundwater gley	0	20	10	11.9	65.1	23.0	5.59	0.33	10.30
R7s	RCG1s	22/02/13	54.14	-2.32	1491	6.8	rough grassland	ranker	0	15	10	10.7	56.0	33.4	7.46	0.24	18.30
R8s	RCG2s	25/04/13	54.20	-2.38	1943	6.8	rough grassland	ranker	0	20	10	9.7	55.7	34.5	5.40	0.46	5.80

1 number of cores (48 mm diameter) that were combined to make the sample

2 pH in H₂O

3 bulk density of the fine earth (<2mm)

4 not determined

5 periodically treated with mineral fertiliser

6 mapped as raw peat, reassigned

7 mapped as earthy peat, reassigned