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

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Abstract

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

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

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

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

Keywords

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

Introduction

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

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

In this study, we investigated the feasibility of using a density fractionation method to determine the distribution of OP in soils, so as to separate the heavy mineral-rich material from the 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

Methods

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

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

Extraction of OLF was carried out using sonication (Sonics Vibracell CV18 probe). To avoid aggregate breakdown of the HF, a pilot test for each of the soil types, based on bulk soil texture (Toberman et al. 2016; Table S1), was carried out to find the optimal sonication energy input, following the procedure of Schrumpf et al. (2013). For sandy and silty soils (there were no clay rich soils), target energy inputs of 100 and 300 J mL^{-1} respectively were used. The samples were periodically checked for complete aggregate disruption using a 0.1 mL subsample observed under a microscope at 100x magnification. Complete disruption was assumed when no further OLF material could be seen 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 CO_2 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.

Results and discussion

Performance of the fractionation method

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

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

Concentrations of P forms in the LF and HF

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

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

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

Element recoveries in the fractionation process

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

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

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

Assessment of the methodology for phosphorus

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

The analysis procedures for soil P differ from those for OC and TN in two respects. Firstly, the concentrations of the P forms are relatively low, being about an order of magnitude less than that of TN, and two to three orders less than that of OC. Secondly, the combined analytical method for OC and TN is simple and reproducible, comprising full combustion and gas analysis (CO_2 and NO_2 in the method that we used here). In contrast, our analytical method for TP involved combustion in a muffle furnace, extraction of inorganic P into H_2SO_4, and then determination of the resulting SRP, while determination of IP omits the combustion step. Therefore there is more scope for errors to arise. As shown by the results in Fig. 2 we obtained quite good reproducibility in the P measurements on individual soils, which may suggest that the problems arise from variability in the extraction steps, i.e. the combustion of OM and conversion of OP to IP, or the extraction of the so-formed organic P into H_2SO_4. However, to account for the low recoveries and variabilities, difficulties with the combustion and extraction would have to apply only to fractionated soil, since full recovery of TP in bulk soils was achieved (see Methods) and we cannot see an obvious reason why that should occur.

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

Element relationships in LF and HF

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

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

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

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

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

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

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

Conclusions

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

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465

Tables

Table 1. Abbreviations

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

Table 2. Summary of element recoveries.

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

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

Figure captions

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

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

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

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

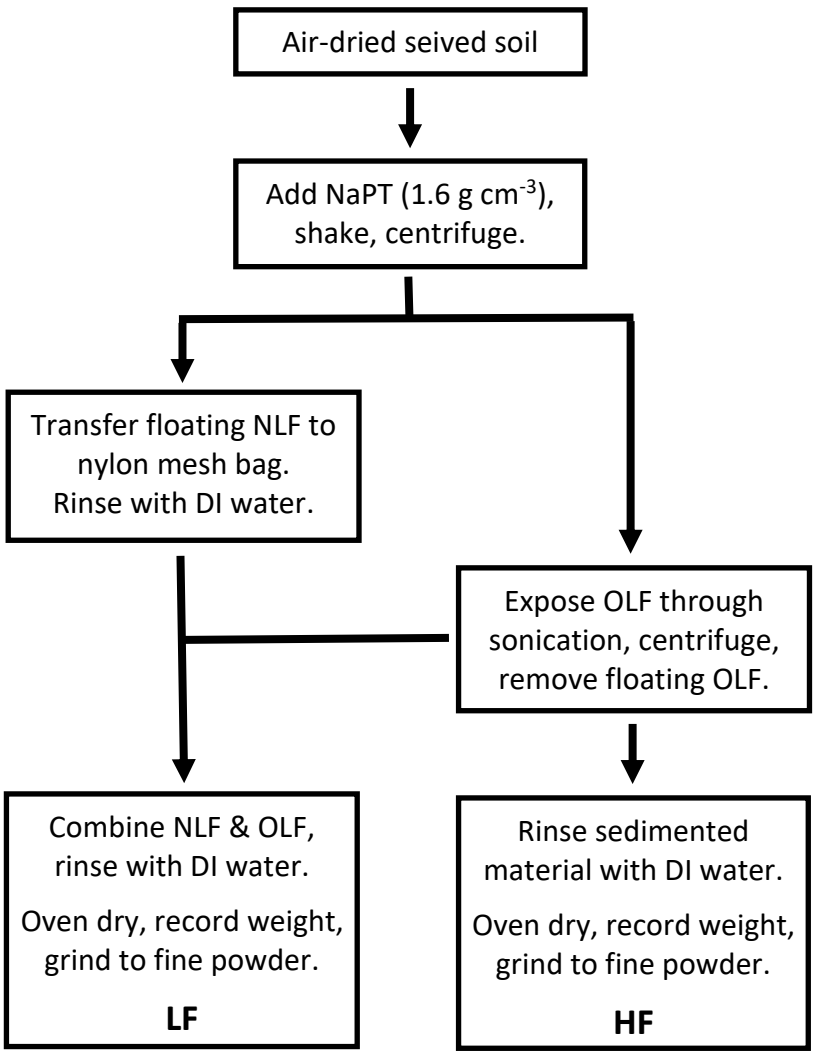
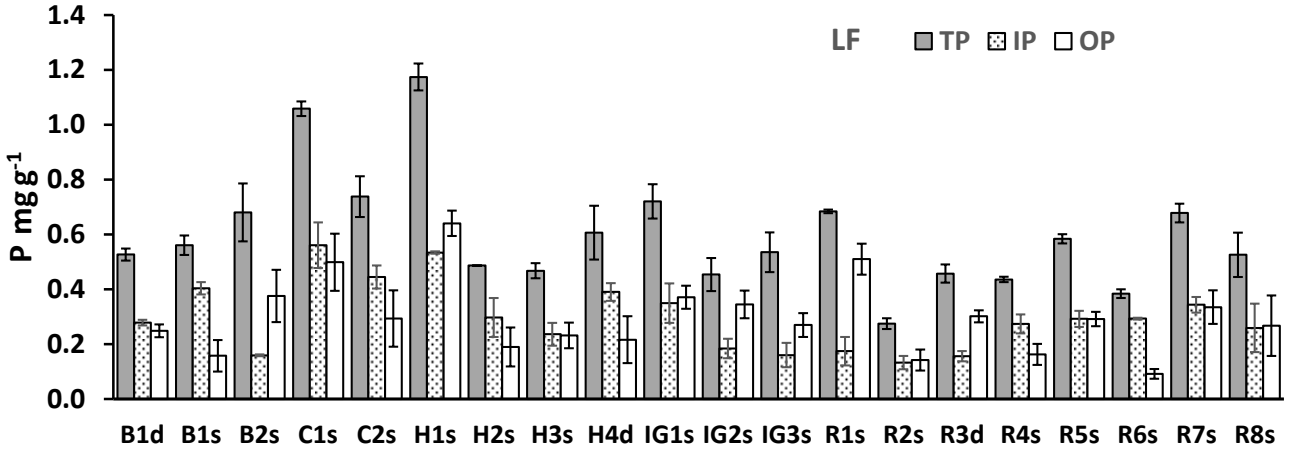
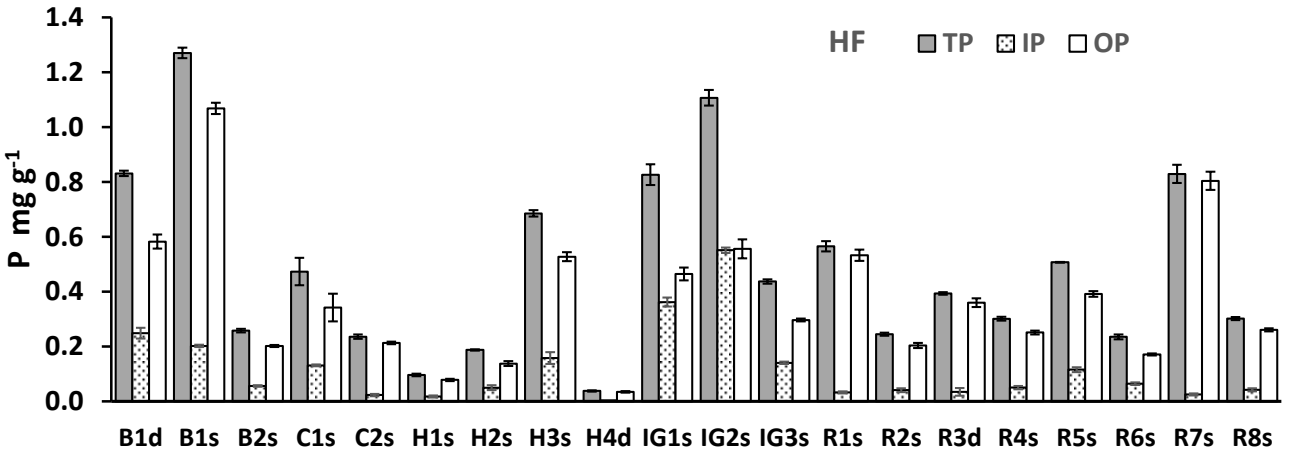

Air-dried seived soil
Add NaPT (1.6 g cm-3), shake, centrifuge.
Transfer floating NLF to nylon mesh bag. Rinse with DI water.
Expose OLF through sonication, centrifuge, remove floating OLF.
Combine NLF & OLF, rinse with DI water.
Oven dry, record weight, grind to fine powder.
LF
Rinse sedimented material with DI water.
Oven dry, record weight, grind to fine powder.
HF

Fig. 1





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518 Fig. 2

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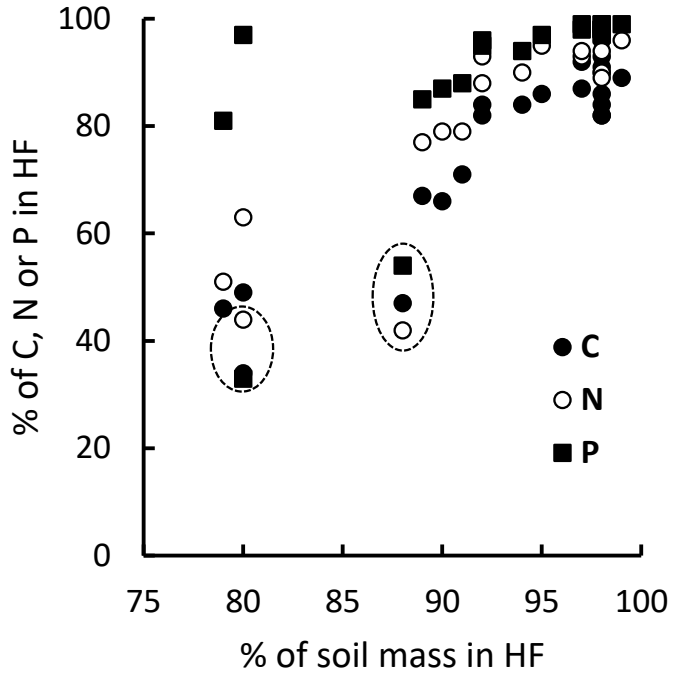

522

523 Fig 3.

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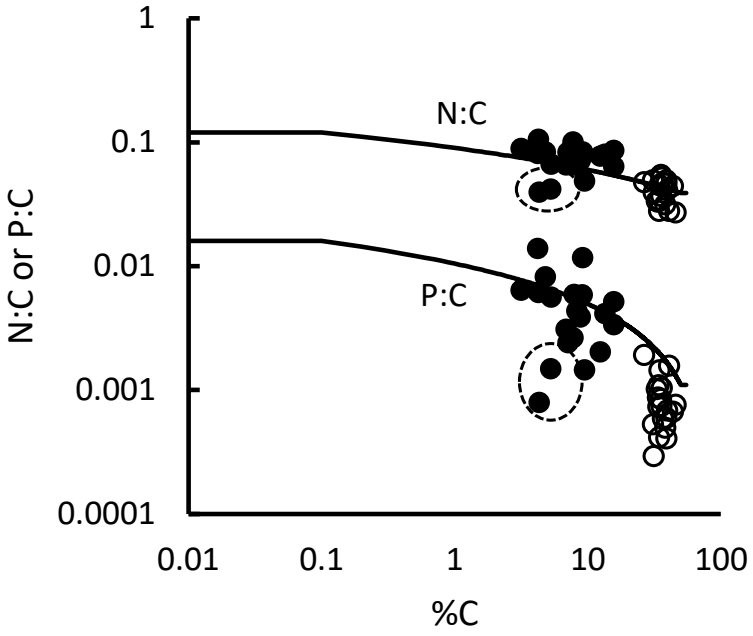

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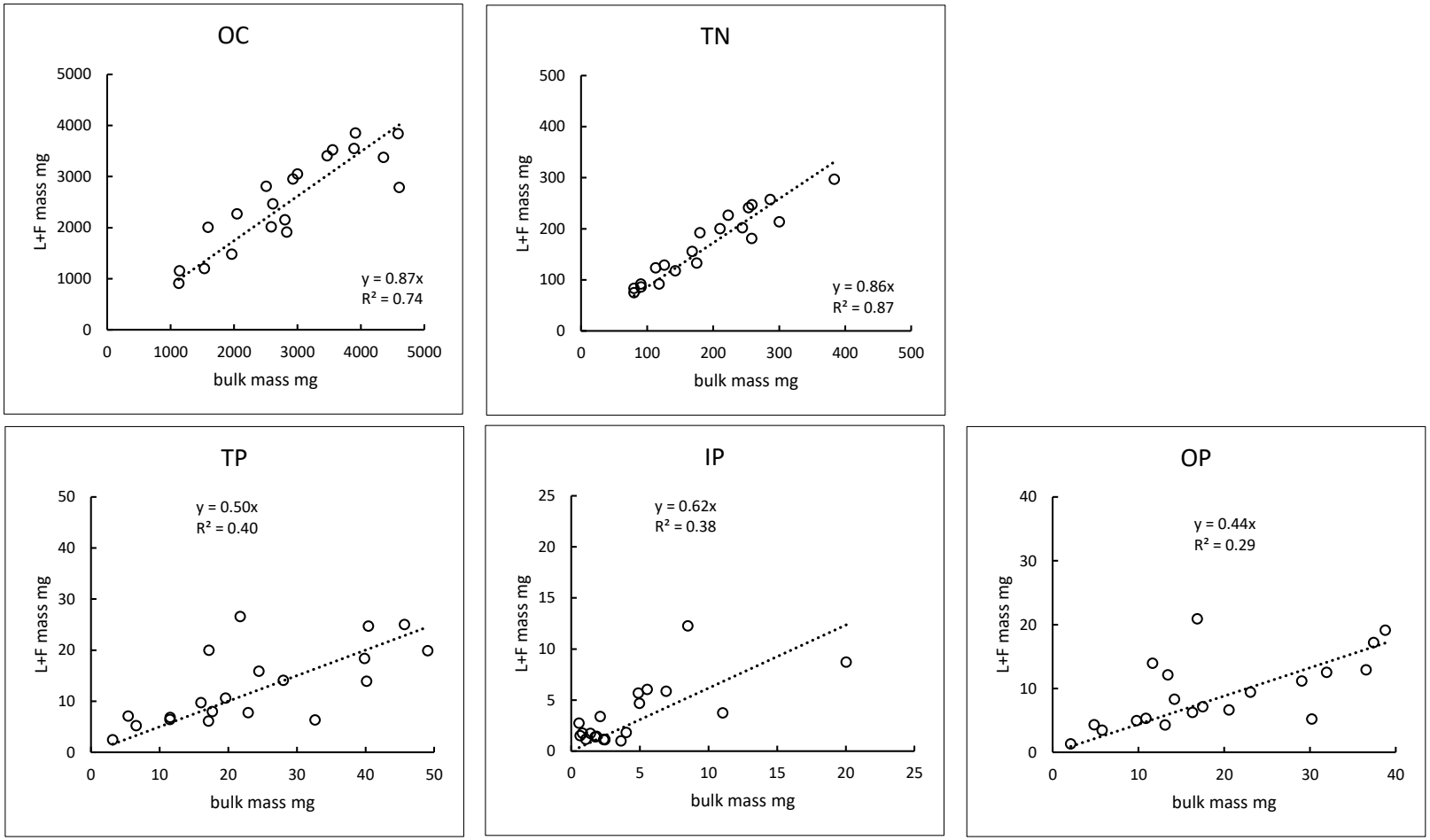
527 Fig. 4



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	long	MAP	MAT	land use	soil type	upper depth	lower depth	n[1]	clay	silt	sand	pH[2]	BD_{FE}[3]	OC
			deg	deg	mm	°C			cm	cm		%	%	%		g cm^{-3}	%
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 H_2O

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