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Stable soil organic matter: a comparison of C:N:P:S ratios in Australian and other world soils

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Abstract

Sequestering soil carbon (C) relies upon the availability of stabilising elements, nitrogen (N), phosphorus (P) and sulphur (S) which are known to be essential components of the stable organic C pool (Himes 1998; Lal 2008). The C:N:P:S ratios were investigated for a series of soils to test the hypothesis that the stable portion of the soil organic material (humus) has constant ratios of C:N:P:S. Constant ratios, if established, would provide an excellent tool to evaluate the feasibility, cost and strategies to sequester soil C in terrestrial ecosystems. Freshly-collected Australian soils cited in the literature were analysed for total C, N, P, organic P (OP) and S, and the ratios were compared with values for soils from numerous locations around the world, hereafter known as the International soils.

Total N and S were highly correlated with C for the International and Australian soils and the relationships were similar for both sets. The correlation of C with P for Australian soils was not as strong as the correlations with N and S, however, a stronger relationship was found for OP than P with C.

The correlation of OP with C for the International soils was not as strong as for the Australian soils probably due, in part, to the different methodologies used to analyse soil for OP in the International soils compared with the single method used for the Australian soils. The weaker relationship between OP and C for both sets of soils, compared with the relationship between N, S and C was probably also due, in part, to the wide variety of compounds in the soil OP pool which vary in their relationship with humus and the wide C:P ratio found in the soil microbial biomass.

Overall, the C:N:OP:S ratios were constant for the stable portion of the soil organic material and these were consistent across a wide range of global soils and should provide a reliable basis with which to determine the level to which the availability of N, P and S may limit humus-C sequestration in terrestrial ecosystems although further research is needed to more accurately determine the amount of OP in humus.

Key words: soil organic matter, humus, C:N:P:S ratios, carbon sequestration.

Introduction

The non living component of soil organic matter (SOM) is primarily the remains of dead organisms. Also, the organisms on this planet can be referred to as “CHNOPS” organisms because of the very large proportion of the mass of all organisms from varying classes that is contributed by these six elements (Morowitz, 1968). Thus, if we assume that hydrogen and oxygen are not limiting then C, N, P and S are the elements that are most likely to limit organism growth and the eventual formation of SOM. It has been suggested that the amount of total SOM

is essentially linearly related to the amount of residues returned to the soil (e.g. Christopher and Lal 2007 and references therein) however there are many studies that have shown surprisingly little response of SOM to differences in residue input. Soon (1998) compared complete residue removal and residue incorporated over a ten year period. Soil-C levels decreased in all treatments and there was no significant difference between treatments at the end of the study. Campbell et al (1991) compared different rotations with varying fertiliser applications over thirty one years. Despite some of the treatments varying by up to 50% in the amount of C returned to the soil there was no significant difference in soil-C at the end of the study. Rumpel (2008) compared the effects of long term residue burning with residue incorporation on both soil-C stocks and soil-C composition. Even after thirty one years soil-C stocks and chemical composition of SOM remained unchanged.

Thus, while a lack of nutrients can limit plant biomass production or, conversely, that fertilisation can increase plant biomass production and thus increase the amount of crop residues available to be returned to the soil, we hypothesise that nutrient availability can affect SOM levels in ways unrelated to increased biomass production. While it is generally recognised that only a small proportion of crop residue-C will ever be converted to humus understanding what influences the humification efficiency (% plant-C converted into humus-C) is vital to understanding soil-C dynamics.

While Williams and Donald (1957) and Walker and Adams (1958) suggested that SOM, presumably as a whole, has constant proportions of C:N:P:S, it was Himes (1998) who suggested it is only the humus that has constant proportions of these elements, and suggested the same ratios as used by Lal (2008), namely humus C:N:P:S is equal to 10,000:833:200:143. This opens up the possibility that the availability of N, P and S may limit the formation of humus not just by limiting primary production but by limiting humification efficiency as well.

It is therefore important to resolve which of the various fractions which make up the total soil organic material, if any, conform to these ratios and whether they can be readily separated for analysis and this is the primary focus of this paper. Two forthcoming papers will report on the effect of nutrient availability on humification efficiency.

This paper reports on an investigation of the stoichiometry of the SOM of freshly-collected Australian soils. The soils were analysed for total C, N, P, OP and S, and the ratios were compared with values for soils from numerous locations around the world, hereafter known as the International soils. Cleveland and Liptzin (2007) also investigated soil stoichiometry but did not consider S, correlated C with P not OP and dealt mainly with forest and untilled soils where plant residues generally make up a significant portion of the total soil organic

fraction. Thus there are some fundamental difference between the research reported on by Cleveland and Liptzin (2007) and the research reported on here.

Materials and Methods

Definition of SOM and humus

Baldock and Skjemstad (1999) have one of the widest definitions of SOM - "all organic materials found in soils irrespective of origin or state of decomposition". This definition though, is not universally accepted. Prior to analysis some researchers routinely discard any organic material described as "large pieces of stubble or root" (Cambardella and Elliot 1992, Chowdhury et al 1999), others discard organic material retained on top of a 2 mm sieve (e.g. Walker and Adams 1958; Gregorich and Ellert 1993; Boone, 1994) while Spycher et al (1983) discarded all live or dead root material not passing through a 0.85 mm sieve. The glossary of soil science terms (SSSA 2008) defines SOM as the organic fraction of the soil exclusive of un-decayed plant and animal residues and is considered synonymous with humus. It is this definition that we accept and imply throughout this paper. For the freshly-collected Australian soils used in this study a method, based on dry sieving and winnowing, similar to Thedorou (1990), was developed to separate obviously identifiable light pieces of plant residues from the heavier mostly mineral fraction. This material is hereafter referred to as the light fraction (LF) while the remaining, mostly mineral fraction, will be referred to as the heavy fraction (HF). The organic matter associated with the HF is considered to be analogous to SOM or humus as defined in the glossary of soil science terms (SSSA 2008). No washing or separation by floatation was involved as significant quantities of C and N can be lost with any such procedures (Crow et al 2007, Kaiser et al 2009).

International soils

Table 1 presents a list of the references and a summary of the data for the C, N, OP and S values for the International soils. They included cropped, pasture, virgin, surface and sub-surface soils. Some researchers removed visible plant remains prior to analyses while all passed the soils through a 2mm sieve and presumably discarded any plant remains that did not pass through the sieve. Although similar analytical techniques were used in many of the papers to analyse for soil C, N, OP and S there were some variations. For example the most common methods for C and N analysis were dichromate oxidation and Kjeldahl digestion respectively but estimation using dry combustion in a multi element analyser was also used. Similarly total S was estimated by dry combustion in a multi element analyser, X-ray fluorescence spectroscopy and inductively coupled plasma

spectroscopy following acid digestion. Two main types of methods were used to analyse for OP. In one the OP is measured as an increase in inorganic P extracted by a suitable acid solution after the soil organic material has been destroyed by ignition or by treatment with an oxidising agent such as hydrogen peroxide. The other main type utilise strong acids to break the bonds which bind OP to metal cations in soils and subsequent extraction of the released OP by an alkali. These will be referred to as the ignition or extraction methods for OP analysis respectively throughout this paper.

(Place Table 1 near here)

Australian soils

Four of the Australian soils, from different agro-ecological regions, were collected as part of a wider series of experiments investigating the effect of nutrient availability on SOM formation and humification efficiency. They were chosen as being representative of agricultural soils in four widely-separated Australian areas. Approximately 50 kg of surface soil (0-15 cm) was collected from five positions at each site. The soils from each site were combined giving one composite sample from each of the four sites. Table 2 gives the geographic position of each site, the mean annual rainfall, the land use preceding sampling and some basic properties of these soils. To get a wider variety of soils for comparison with these four Australian soils and the International soils farmers in the region near each experimental site were invited to submit soils for evaluation with no restriction on the type of soil to be submitted. A wide selection of soils was submitted including cropped, pasture and virgin soils. The cropped soils were generally regularly fertilised while the pastures soils ranged from irregularly to rarely fertilised. Fifty five soils were submitted, making fifty-nine Australian soils in total. Farmers were asked to collect approximately 250 g soil as a composite sample collected from 0-15 cm from several positions within each paddock or virgin area. There was no replication of any of the soils submitted by the farmers.

(Place Table 2 near here)

Australian soil preparation - Heavy/light material fractionation

The main soils were fractionated as shown in Figure 1. The soils were air dried and gently crushed to separate soil, plant material and gravel. They were then passed through a 5 mm sieve and any organic material retained on top of the sieve was discarded. Material passing through the 5 mm sieve was then passed through a 2 mm sieve and gravel retained on top of the sieve was subsequently discarded. Organic matter retained on top of the 2 mm sieve was designated LF material. The material passing through the 2 mm sieve was then fractionated to produce

a LF and a HF. The LF material was separated from the heavy, mostly mineral fraction, material using a dry sieving/winnowing procedure in the following manner.

(Place Figure 1 near here)

The 2 mm sieved samples were re-sieved using a 0.4 mm sieve and any material passing through the sieve was designated HF material. Organic material greater than 0.4 mm has been shown to be largely labile and easily decomposed and thus forms part of the LF (Magid and Kjaergaard 2001). The mixture of LF and HF material retained on top of the 0.4 mm sieve was then placed on a large metal tray forming a layer a few millimetres thick. The tray was subsequently gently shaken back and forth a few times causing the light fraction of mostly-identifiable plant material to float to the top, while the heavy, mostly-mineral fraction, sank to the bottom. A gentle stream of compressed air was directed across the top of this material and the LF could be gently blown away from the HF. The two separated fractions, the largely-plant LF material and largely-mineral HF material, were put through this procedure several times to ensure no heavy mineral fraction remained in the LF material and that no easily-visible plant-like LF material remained in the largely mineral HF. The LF material 0.4–2 mm in size was combined with the LF material 2-5 mm in size that had previously been separated from the sample as described above. The fractionation procedure thus produced two fractions, a largely-mineral HF (<2mm in size) and a largely-plant LF (0.4-5 mm in size).

A 50 g sub-sample of six of the soils were used to compare fractionation procedures (see below) and these were treated differently as shown in Figure 2. The soils were air dried and gently crushed to separate soil, plant material and gravel. They were then passed through a 5 mm sieve and any organic material retained on top of the sieve was discarded. Material passing through the 5 mm sieve was then passed through a 2 mm sieve and gravel retained on top of the sieve was subsequently discarded. All material passing through the 2 mm sieve and the organic matter retained on top of the 2 mm sieve was recombined to form an un-fractionated sample.

(Place Figure 2 near here)

The dry sieving/winnowing fractionation process described above was tested against two commonly used floatation procedures; floating off the light fraction with 30% sodium chloride solution, density 1.2 g cm^{-3}, and floating off the light fraction with a sodium iodide solution, density 1.5 g cm^{-3} using six of the Australian soils. Soils for this test were sampled and analysed in triplicate. The light fraction material, 0.4–10 mm in size, removed by the dry sieving/winnowing method (Figure 1) from the six soils and used in the comparison of fractionation procedures was analysed for C, N, P and S and their ratios calculated.

Chemical analyses

Prior to chemical analysis each of the HF samples was thoroughly mixed and approximately half of each sample was milled to pass through a 100 mesh sieve (150μm opening) and the milled material was again thoroughly mixed to ensure that sub-samples subsequently analysed were representative of the whole sample. The soils were checked for the presence of carbonates using dilute hydrochloric acid (~ 1M) and this was detected and removed in only one soil by treating the soil with dilute hydrochloric acid and stirring until no further effervescence was observed. All of the LF material analysed was milled to pass through a 100 mesh sieve and then thoroughly mixed, again to ensure that sub-samples subsequently used for chemical analysis were representative of the whole light fraction sample.

Total C and N were determined using a dry combustion analyser (Europa Scientific Model 20-20, Crewe, UK). Total acid extractable P and S (hereafter known as acid P or acid S respectively) were determined by inductively-coupled plasma optical-emission spectroscopy (ICP-OES, Varian Vista-Pro (axial) Melbourne, Australia) following microwave-assisted acid digestion using reverse-aqua regia according to method 3051A of the USEPA (1998).

Total and organic P was also determined by the ignition-extraction procedure (Olsen and Sommers 1982), hereafter known as ignition P or ignition OP respectively. Briefly P was determined colorimetrically on an 0.5M sulphuric acid extract of an oven dried soil sample (105^{o}C overnight – hereafter known as the unignited sample) after reaction with malachite green (Irving and McLaughin 1990). A second soil sample was placed in covered borosilicate glass test tubes and put in a muffle furnace at 450^{o}C for 4 hours (hereafter known as the ignited sample). The P in the ignited sample was again extracted with 0.5M sulphuric acid and the P in the extract determined after reaction with malachite green. Total P was taken as the P measured in the ignited sample and OP was taken as the difference between the ignited and unignited samples.

Results

Fractionation procedure used on Australian soils

Removal of the LF material by any of the methods generally produced soils with similar C, N, P and S levels (Table 3). The means of the C, N, P and S levels of the fractionated soils were significantly reduced (P=0.05) compared to the un-fractionated soils (Table 3). With the exception of the C:N for the Harden long term pasture and the C:S for the Buntine soil, removal of the LF from soils with more than 50 g LF per kilogram un-

fractionated soil (5% LF) significantly reduced the C:N, C:P and C:S ratios compared to the un-fractionated soils (Table 3, P=0.05).

(Place Table 3 near here)

In our study the composition of the LF was remarkably consistent despite its origin from soils separated by large distances and contrasting management (Table 4). The low C content of the LF, mean 15% compared to 46% for fresh wheat residue, contributed to the low C:N, C:P and C:S ratios of the LF (Table 4). Overall these data suggest that across different soils the C:N:P:S ratios are more consistent in the organic material associated with the HF than the LF (Tables 3 and 4), which, morphologically appears to consist largely of undecomposed or partly decomposed easily identifiable plant material (Figure 3).

(Place Table 4 and Figure 3 near here)

In soils with more than 5% LF the LF-C contributed approximately 40% or more of the total soil C compared to less than 10% in soils with less than 5% LF (Table 5). Similarly, in soils with more than 5% LF the LF-N contributed 35% or more of the total N compared to approximately 6% in soils with less than 5% LF; 20% or more of the total P, compared to less than 5%; and approximately 30% or more of the total S compared to less than 6% (Table 5).

(Place Table 5 near here)

Analyses of International soils

Despite the different procedures used in preparing soils for subsequent analysis, particularly with regards to retaining all plant residues or removing larger pieces, Figure 4 and Table 6 confirms there is a significant correlation (coefficient of determination, R^2~0.8 and P<0.001) between total soil N and S with C in the International soils while the relationship between OP and C is weaker (R^2=0.44).

(Place Figure 4 and Table 6 near here)

Strictly speaking organic C, N and S should be used to calculate these ratios but generally the easier to obtain total soil C, N and S values can be used instead. Total soil C essentially equates to soil organic C, unless there are appreciable quantities of carbonate, and organic forms of N and S generally constitute 95% or more of the total N and S in most soils (Duxbury et al., 1989; Zech et al., 1997) and thus these total elemental values can be used. However this is not the case with soil P because the amounts of OP generally constitute a smaller and much more variable proportion of the total soil P, 20-75% (Duxbury et al., 1989; Zech et al., 1997), and OP values must be used. Even then, however, the correlation between OP and C is still quite low for this composite collection of data (Fig 4 and Table 6).

If the data from each of the papers used to obtain the C and OP is analysed individually a confusing picture emerges. The regression of C with OP from only nine of the nineteen data sets produced an $R^2 > 0.5$ and $P<0.001$, indicating a reasonable correlation of C with OP (Table 7). However, even with the data sets that produced R^2 values greater than 0.5 the slopes of the regression lines, indicating the amount of C associated with each unit of OP, varied widely from a low of 12 to a high of 176 (Table 7). Figure 6 shows an example of three of the C and OP International data sets, along with the Australian C with OP. All the R^2 values of these regressions lines are greater than 0.6, $P<0.001$ (Tables 6 and 7) but it illustrates the wide variability in the relationship of C with OP. Possible reasons for this variability will be discussed later.

(Place Table 7 near here)

Analyses of Australian soils

The relationship of total soil N and S with C for the Australian soils is even stronger than for the International soils, (Figure 4, Table 6). The relationship between C and OP for the Australian soils is less defined, as was the case for the International soils (Figure 4, Table 6). While the relationships between soil C and acid P or ignition P (total P) for the Australian soils is stronger than C with OP for the International soils (Figs 4 and 5) they are still not particularly strong. In contrast the relationship between C and OP for the Australian soils is strong (Fig 4B, Table 6). The relationship between acid P and ignition P is also strong, with a slope close to one (Figure 5C), suggesting that suitable total P determinations could be obtained by either method.

(Place Figure 5 near here)

Comparison of C:N, C:OP and C:S ratios for Australian and International soils

There was no significant difference in relationship between N and S with C for both the Australian and International soils, P=0.01, (Table 6). The data indicates that essentially identical amounts of C are associated with each unit of N or S for both sets of soils (Table 6).

There was a significant difference in the relationship between OP with C for the Australian and International soils, P=0.05, (Table 6). Each unit of OP for the International soils was associated with significantly less C than for the Australian soils. It should be noted however that although the P value of the slope of the regression line for OP with C for the International soils was significant ($P<0.001$) it had a low R^2 value, 0.44, and the combined data, gathered from different papers (Table 1), failed the normality test. All linear regressions require a source population to be normally distributed about the regression line and failure of the normality test can indicate the presence of outlying influential points or an incorrect regression model (Bailey 1972).

Discussion

Does methodology matter?

Removal of the LF material by any of the three methods we used produced soils with similar C, N, P and S levels and so similar C:N, C:P and C:S ratios (Table 3) suggesting any of these methods would be suitable. We say this with some reservation as we believe the dry sieving/winnowing method consistently produced slightly cleaner samples, more complete removal of LF material, as suggested by the consistently lower C:N, C:P and C:S ratios of the soils fractionated by this method (Table 3). The key factor we believe is to remove the LF as there were significant differences between fractionated and un-fractionated soils (Table 3) and we believe that constant C:N, C:S and possibly C:OP ratios (C:OP once others issues have be resolved) will only be found in the HF or humus.

Methodological issues with measuring soil OP do matter. The reviews of Turner et al (2003a) and Agbenin et al (1999) highlight methodological problems very clearly. Both researchers analysed common sets of soils by different methods and obtained different, sometimes very different, values for total P and OP. For example, soil 12 from Agbenin et al (1999) had a total P of 40 mg kg^{-1} as analysed by his BOW-1 method and 233 mg kg^{-1} as analysed by his BOW-2 method. Similarly soil 12 had an OP value of 18 mg kg^{-1} as analysed by his BOW-1 method and 156 mg kg^{-1} as analysed by his BOW-2 method. This resulted in a C:OP ratio of 574 or 65 for the same soil analysed in the same laboratory. Clearly issues' surrounding the analysis of soil for OP needs resolving before one tries to do stoichiometric scaling between C and OP.

Dealing with the LF

Comparison of the three Harden farmer soils (Table 3), that came from the same property quite close to each other indicate the substantial contribution that the LF can make to the C, N, P and S levels of the un-fractionated virgin and pasture soils compared with cropped soil. The un-fractionated virgin and pasture soils had ten times the amount of LF material compared to the cropped soil (Table 5). Once this material was removed from the virgin and pasture soils the C, N, P and S levels declined by an average 39%, 34%, 17% and 33% respectively compared to 12%, 11%, 6% and 12% respectively when the LF was removed from the cropped soil (Table 3). It is possible that the rapid decomposition of this LF material following cropping and cultivation is largely responsible for the large reductions in C, N, P and S levels observed when virgin or pasture soils are brought into cropping. This needs further investigation but supports the suggestion of Cambardella and Elliott (1992) that the loss of total soil organic matter during the first years of cultivation is primarily the result of LF decomposition.

If these observations hold more widely, then future investigations of changes in soil C related to changes in land management should fractionate soil samples before C analysis, and analyse both light and heavy fractions to determine if C loss occurs primarily from the light fraction, or from the whole soil C pool.

The relatively low amount of LF material in the Hamilton pasture soil, 2.3%, compared to the Harden virgin or pasture soils, approximately 9%, (Table 4) is probably due to its past management. This pasture had been heavily grazed but unfertilised for more than ten years and this probably resulted in a significant proportion of the LF being mineralised and not replaced during this time. Comparison with soils with a similar management history would need to be done to verify this suggestion. The relatively high amount of LF material in the Buntine and Leeton cropping soils is probably a result of differences in soil type, climate and cropping history. Buntine is in an area with a much lower rainfall than Harden and is also much sandier, Table 2. Taken together these two facts suggest that LF decomposition would be much slower at Buntine compared to Harden resulting in a higher base load of LF material. The Leeton soil was sampled immediately after a rice crop and the soil had a large amount of rice root material remaining in the soil. Farmers in this area routinely follow a rice crop with 3-4 years of wheat during which time the rice root material decomposes reducing the LF amount significantly. Sampling immediately after the rice crop has given a skewed impression of the amount of LF material commonly found in these soils.

The mean C:N ratio of the LF isolated by our sieving/winnowing method (>0.4 mm) is very similar to the C:N ratio of particulate organic matter fraction (>53 μm) from a mixture of cultivated and non cultivated Australian soils obtained by dispersion/washing procedures by Chan (2001), 18.2 compared to 17.5 respectively, and suggests our procedure is isolating a similar LF without the complication that nutrients might be lost in the dispersion/washing procedure as suggested by Crow et al (2007) and Kaiser et al (2009).

The low C:N, C:P and C:S, ratios of the LF compared to fresh wheat residue (Table 4) would normally suggest rapid decomposition under favourable conditions and yet it remains in these soils, unprotected (not coated in clay) and in large amounts, particularly in the virgin and pasture soils. Why such large amounts of this unprotected, nutrient rich LF material remains in undisturbed soils, but appears to decompose rapidly following cultivation, requires further investigation.

The ratios

C:N

The correlation of total soil N and S with C for the Australian soils is even stronger than for the International soils (Figure 4, Table 6) presumably because the Australian soils had the LF removed before analysis, while the International soils had varying amounts of the LF remaining. The stronger correlation of soil N and S with C for the fractionated Australian soils supports the hypothesis that the constancy in C:N:S stoichiometry is probably only in the organic material associated with the HF and not in the LF organic material.

The question as to the specific value of humus C:N and C:S ratios and what is the most significant immediate precursor of humus requires some discussion. Until quite recently it has generally been accepted that humus-C is predominantly plant derived (Kogel-Knaber 2002). However Kindler et al (2009) point out that plant derived C can be integrated directly into humus as plant biomolecules or indirectly by first being incorporated into microbial biomass and there is now ample evidence that microbial biomass detritus is a significant source for humus formation (e.g. Poirer et al 2005; Kindler et al 2006; Six et al 2006; Miltner et al 2009). While the stoichiometry of soil organisms has been quite extensively studied (e.g. Cromack et al 1975; Stark 1972; Cleveland and Liptzin 2007) Boberg (2009) points out that the C:N ratio of an organism reflects how much N in relation to C the organism requires for biomass production. Since C is used both as an energy source, and thereby lost as carbon dioxide, and to produce biomass, the C:N ratio of the substrate must be higher than that of the organism in order to balance the need, and the C:N ratio of the new organism will be lower than the substrate. As stated earlier, the C:N ratio of plant residues is approximately 100 while the C:N ratio of fungi and bacteria is in the range 8-25 and 5-10 respectively (Pinck and Allison 1944; Chapin et al 2002) and while a C:N ratio for the general soil microbial biomass will vary with the fungal:bacterial ratio, Griffin (1972) has suggested that a range of 10:1 to 12:1 is reasonable, which is in the same general range that we found for humus. It is also quite likely that only some components of the microbial biomass detritus will contribute to humus formation. For example it is generally accepted that microbial cell wall material is much more stable in soil than the intracellular components (Hurst and Wagner, 1969; Webley and Jones, 1971; Brookes 2001). Pinck and Allison (1944) demonstrated the synthesis of lignin-like complexes by twelve cultures of filamentous fungi on various media that used simple sucrose as a carbon source and various nitrogen sources. The materials were found to resemble lignin, including resisting digestion with 72% sulphuric acid, but had much lower methoxyl contents than commonly found in plant lignin. The C:N ratio and other properties were also similar to soil humus. It is suggested that while microbial cell walls and these lignin-like complexes, at least, may contribute to humus formation some intracellular components probably do not.

C:S

Information on C:S ratios for a range of microbes from various habitats is very scarce but Heldal et al (1996) suggest a bacterial mass C:S ratio range of 31-94 while Olsson et al (2008) suggest a fungal mass C:S ratio range of 54-192 for young hyphae of *Glomus intraradices*. This covers the values for baker's yeast of 82 given by Waites et al (2001) and a value of 156 for *Penicillium atrovenetum* given by Tashpulatov et al (2000). Overall this is similar to the C:S range of the mean values for the International soils of 54-132 (Table 1). We would again suggest that these tight ratios support the hypothesis that humus is largely microbial in origin and the tight ratio seen in soils is as a result of this microbial origin. More data from a range of bacteria and fungi from various habitats are needed to test this hypothesis.

C:OP

Soil P is fundamentally different to soil C, N or S. P is a fundamental building block of some soil minerals, e.g. the apaptite group, and does not have a gaseous form. In natural situations gaseous C and N are primarily brought into the soil system by photosynthesis, nitrogen fixation and rainfall deposition. Although S is a natural component of volcanic soils it primarily enters other soils through rainfall deposition. Because P is a fundamental building block of some soil minerals we believe, as already stated, that there cannot be a constant ratio of soil C with total soil P. However, because P is also a fundamental building block of soil microbes, which we hypothesise make a significant contribution to humus formation, there could be a constant ratio of soil OP with soil C. The weak correlation actually found between OP and C for the International soils (Figure 4, Table 6) could be due to several reasons. First, combining results obtained by a variety of methods has produced a data set that suggests the relationship of OP with C is highly variable (Figure 4). However, examination of the data from the individual papers (Table 8) shows that when the researchers used only one method they generally obtained a much stronger relationship, although not always. Second, the relationship between OP and C sometimes varied widely even when researchers used similar methods (Figure 6, Table 7) and these results highlight the problems associated with analysing soils for OP as discussed in the reviews of Agbenin et al (1999) and Turner et al (2003a) as mentioned earlier. Third, P is found in many organic compounds and the proportions vary in different soils (e.g. Turner et al 2003a; 2003b). The major organic P compounds are the inositol phosphates (up to 50% of total OP), which contain neither N nor S but are generally considered to be associated with the soil heavy fraction component (Borie et al.,1989; Dalal, 1977). Hong and Yamane (1980) found that 60% of the OP in extracted fulvic acid, generally considered a heavy fraction component, was inositol hexakisphosphate and 40%

was other forms of OP. Paing et al (1999) found humic-bound P accounted for 80% of OP but whether it was an integral part of the humus materials or only complexed with them is largely unknown. Williams and Steinbergs (1958) suggested that OP could be divided into two parts, the P intimately bound to the C, N and S of the humus, or heavy fraction, and a varying proportion of more or less independent OP compounds. Forth, soil microbial C:P ratios are highly variable. In the review paper by Manzoni et al (2010) they reported mass C:P ratios for soil microbial biomass ranging from 23 to 333. Cross et al (2005) reported an even wider C:P ratio for fungi and bacteria in a freshwater benthic system. The molar C:P ratio range for bacteria and fungi was 5-370 and 300-1190 respectively. The bacterial and fungal C:N ratio range in the same system was only 2.9-7.6 and 6.5-9.0 respectively. Thus, if humus has a largely microbial component then these highly variable humus C:OP ratios (Figure 4, Table 6) may simply be an expression of the highly variable microbial C:P ratios and may in fact reflect differences in the composition of the soil microbial population. Fifth, most microbial P resides within the cell membrane and cytoplasm while C is an important constituent of the microbial cell wall that contains little P. As a result microbial P has a much shorter turnover time than microbial C (Brookes, 2001; Kouno et al 2002a) and this may also contribute to the highly variable C:OP ratio.

(Place Figure 6 near here)

While the correlations between total soil C and acid P or ignition P for the Australian soils are actually stronger than the correlation between total soil C and soil OP for the International, and may be used to suggest a relationship between soil C and total P, they are still not particularly strong. The relatively weak correlations between total soil C and acid P or ignition P (Figure 5) probably results not only from variations associated with measuring OP, as mentioned above, but also from variations in the inorganic P fraction itself. Some of the Australian soils were cropping or pasture soils which had received varying fertiliser applications and depending on soil mineralogy and P fixation potential, would have retained different amounts of inorganic P. In contrast, correlating total soil C with soil OP for the fractionated Australian soils produces quite a strong correlation (Figure 4B, Table 6). This again suggests there is a constancy in the C:OP ratio but only for the organic material associated with the HF.

While we have focussed on methodological issues, the complex nature of soil OP compounds and the reported highly variable microbial C:P ratios in regards to possible explanations for the weaker relationship between soil C and OP, especially for the International soils, it is quite likely that, considering the sampling was from many different geographic areas (Tables 1 and 2), differences in site geology, biogeochemistry and differences in how

microbes enzymatically attack OP versus N or S could also play a substantial role but we consider a detailed discussion on these issues beyond the scope of this paper.

It is clear that not all the microbial biomass detritus will contribute to humus formation and thus the humus C:N:P:S ratios will not be exactly the same as for the microbial biomass as a whole, but the similarity in the C:N:P:S ratios for the soil microbial biomass and humus supports other extensive evidence that microbial biomass detritus is a significant source for humus formation. While plant material is the ultimate precursor for humus formation it may not be the most significant immediate precursor but the penultimate precursor. Given that there is a large body of evidence that the availability of nutrients, especially N, P and S, may limit microbial-C assimilation efficiency (e.g. Lukito et al 1997; Kouno et al 2002b; Fontaine et al 2004) more research is needed to provide reliable estimates of the proportion of plant-C and microbial-C present in humus as microbial-C assimilation efficiency, which is affected by N, P and S availability, may directly limit the humification efficiency of fresh organic matter inputs.

Implications for sequestering carbon in soil

The similarity in the relationships between N and S with C for both the Australian and International soils (Figure 4, Table 6), suggest that an approximately equal C:N and C:S ratio is a fundamental property of the organic material associated with the HF which we call SOM or humus. The wide difference in the relationship between OP and C for the Australian and International soils (Figure 4, Table 6) is reflected in the wide variety also seen when the individual papers are examined (Table 7).

There appears to be strong evidence that there is an approximate constancy in the relationships of N and S with C in SOM and may imply that SOM-C levels could be limited by the supply of N and S and not just by C input. Because the constancy only appears to be in SOM, and not in the light fraction material, internationally accepted soil fractionation procedures need to be used to test this across a broad range of localities and land uses. There is probably also constancy in the relationship between SOM-C and a fraction of the soil OP but techniques are needed to elucidate the relationship of the different OP fractions with SOM. Similar soil fractionation procedures need to be used and techniques need to be developed that facilitate the extraction, separation and detection of organic phosphates in soils, especially those associated with SOM. Only then will we be able to give a reliable value for the amount of P required per unit of SOM-C.

(Place Table 8 near here)

Consistent stoichiometric ratios for these nutrients in the stable SOM pool across soils globally, could mean that provision of all these nutrients will be necessary to stabilise higher C content in soils. This does not mean that all nutrients would have to be supplied externally in all cases. Soils of volcanic origin may have sufficient S to support higher C levels but low N or P. Similarly soils naturally high in P may be deficient in S or N. Opportunities also exist through increased inputs of inorganic or organic fertilisers, capturing more N through symbiotic (Herridge et al 2008) or non-symbiotic N-fixation (Wakelin et al 2007), stimulation of otherwise unavailable nutrients by various solubilising microorganisms or symbionts (Shehana and Abraham 2001), or better capture of S and N deposition (Fowler et al 2007). Given that these nutrients have to be "locked up" for as long as the carbon is stored the question arises about the economic value (or lost opportunity cost) of these required nutrients, however it occurs. The simplest assumption is to assume that their value equals or is similar to, the value of replacing them with fertiliser.

Using the C:N:P:S ratios suggested by both Himes (1998) and Lal (2008) each tonne of humus-C will "lock up" 80kg N, 20kg P and 14kg S and their potential value can be estimated by comparing the cost of the same quantity of nutrients provided by fertiliser. Table 8 shows that the potential cost of the N, P and S associated with each tonne of humus-C if all these nutrients were supplied via common fertlisers to be $248. The current trading prices for carbon dioxide (CO_2) in existing markets range from $5-10 per tonne (Chicago Climate Exchange) to $40 (EU/Kyoto compliance protocols). Based on these trading prices for CO_2 a tonne of humus-C (equivalent to 3.7 t CO_2) would currently be worth $20-$150, considerably less than the estimated value of nutrients "locked up", as shown in Table 8. We are not suggesting that if the nutrients were supplied in this way there would be a 100% conversion of these fertiliser nutrients to humus nutrients as there would undoubtedly be losses which would vary with climate, soil type etc. Thus, given the complexity of agricultural systems and nutrient cycles this estimated value could easily be out by a factor of two. Also, producing fertiliser by the Haber-Bosch process is costly in terms of carbon dioxide emissions and this would also have to be factored in. These "hidden costs" need to be accounted for when considering any future carbon trading scheme. While fertilising strictly for enhancing soil-C stocks is probably not currently amenable to current carbon pricing, small incremental improvements in productivity and other factors associated with improved soil condition in systems which maintain a positive nutrient balance (e.g. Kirkby et al 2006), might alter the decision as to whether it makes economic and environmental sense. Increasing the absolute plant biomass available for sequestration may not be feasible in many instances, and so understanding if the humification efficiency can be increased for a given crop residue input, will be important in understanding whether the carbon sequestration can be increased in

many systems. As already mentioned, the effect of N, P and S availability and SOM stoichiometry on humification efficiency will be reported in two forthcoming papers.

Our study provides strong evidence for a constant stoichiometric ratio of C:N:OP:S for the stable portion of the soil organic material across a wide range of global soils. Such a ratio should provide a reliable basis with which to determine the extent to which the availability of both C and the stabilising nutrients may limit humus-C sequestration, and to consider implications for management strategies to optimise retention of stable organic matter in soils.

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Table 1: References used and C, N, P, S data summary for International soils represented in Figure 4

Reference	Number soils	Analyses as well as C			mean			Soil types or sites sampled	Soils from
		N	OP	S	C:N	C:OP	C:S		
Aceves et al (1999)	21	+			13.0			5	Spain
Acquaye and Kang (1987)	48	+	+		10.7	116		> 10	Ghana
Agbenin et al (1999)	45		+			287		6	Nigeria
Aguilera et al (2002)	8	+		+	11.9		71	4	Chile
Bailey (1985)	51	+		+	12.2		90	> 10	Canada
Chapman (1987)	9	+		+	16.5		89	4	Scotland
Chen et al (2000)	8	+	+		10.2	68		8	China
Chen and He (2004)	11	+			11.0			11	China
Chen et al (2004)	15	+	+		12.6	117		7	New Zealand
Chowdhury et al (1999)	19	+		+	11.1		72	3	Japan
Gharmakher et al (2009)	22	+		+	9.9		77	> 10	France
Goller et al (2006)	10	+		+	10.3		68	5	Ecuador
Groenendijk et al (2002)	30	+		+	13.2		96	4	New Zealand
Han et al (2005)	12	+	+		10.5	58		12	China
Lilienfein et al (2000)	10	+		+	13.6		54	2	Brazil
Lilienfein et al (2003)	15	+		+	14.7		69	3	Brazil
Makarov et al (1997) *	12	+	+		10.2	87		6	Russia
Makarov et al (2004)	21		+			85		2	Russia
Makarov & Malysheva (2006)	21		+			77		> 10	Russia
Mansfeldt and Blume (2002)	14	+		+	12.8		56	> 10	Germany
Matula (2004)	48	+		+	9.8		95	> 10	Czech Republic
McLaren and Swift (1977)	14			+			67	7	Scotland
Neptune et al (1975)	12	+	+	+	15.2	156	114	> 10	Brazil & USA
Nguyen and Goh (1992)	12	+		+	12.0		82	4	New Zealand
Nishiyama (2001)	10	+			13.0			5	Japan
Saikh et al (1998)	36	+	+		10.4	82		> 10	India
Singh and Singh (1995)	7	+			13.9			5	India
Stanko-Golden & Fitzgerald (1991)	9			+			59	9	Puerto Rico
Sturgis (1932)	10	+			10.8			1	USA
Tabatabai & Bremner (1972)	68	+		+	10.8		68	> 10	USA
Tsuji et al (2005)	26			+				> 10	Zambia
Turner et al (2001)	29	+			9.8			> 10	UK
Turner et al (2003a)	36		+			100		> 10	USA
Turner et al (2003c)	29	+	+		9.8	126		> 10	UK
Turner et al (2003d)	29	+	+		9.8	74		> 10	UK
Uriyo and Kesseba (1975)	43		+			200			Tanzania
Walker and Adams (1958)	66	+	+	+	11.0	44	89	> 10	New Zealand
Walker and Adams (1959)	45	+	+	+	17.5	164	132	> 10	New Zealand
Wang et al (2001)	12	+		+	12.7		100	4	Mongolia
Wang et al (2006)	33	+		+	10.6		57	> 10	Canada & USA

* 3 outliers (C>30%) not used in analyses

Table 2: Geographic position, rainfall, land use and selected properties of soils from the main Australian sites

Site	Geographic Position	Mean Annual Rainfall (mm)	Land Use
Hamilton, Vic	142.02E 37.65S	686	rain-fed very degraded unimproved pasture
Harden, NSW	148.37E 34.56S	610	rain-fed cereal cropping
Leeton, NSW	146.41E 34.57S	432	irrigated rice/wheat, sampled after rice crop
Buntine, W.A.	116.57E 29.99S	357	rain-fed cereal cropping

Site	USDA texture	% clay	% silt	% sand	pH†	% C	% N	% P*	% OP	%S
Hamilton	sandy clay loam	25	19	56	5.15	3.006	0.261	0.0326	0.0224	0.0508
Harden	sandy loam	15	10	75	5.29	0.994	0.091	0.0274	0.0129	0.0118
Leeton	clay loam	60	12	28	5.84	1.180	0.125	0.0318	0.0119	0.0160
Buntine	sand	8	3	89	4.80	0.725	0.060	0.0116	0.0050	0.0078

†$CaCl_2$ * P = acid extractable P

Table 3: Percent total C, N, acid extractable P and S of 6 Australian soils with light fraction included or light fraction removed by one of three procedures *

Soil	Fractionation method	C	N	P	S	C:N	C:P	C:S
Hamilton soil; long term unimproved pasture	0	**3.163**	**0.269**	**0.033**	**0.042**	**11.8**	**96**	**75**
std error		0.0055	0.0014	0.0003	0.0001	0.05	0.68	0.25
	1	3.084	0.264	0.031	0.035	11.7	98	88
	2	3.147	0.247	0.031	0.033	12.8	97	95
	3	2.930	0.229	0.030	0.031	12.8	97	95
mean of three fractionated soils	1, 2 and 3	**3.057**	**0.247**	**0.031**	**0.033**	**12.4**	**97**	**93**
pooled std error		0.0014	0.0003	0.00005	0.0001	0.01	0.15	0.32
Mean % loss from un-fractionated soil		***3***	***8***	***6***	***21***			
Harden soil; virgin soil	0	**3.594**	**0.241**	**0.025**	**0.023**	**14.9**	**146**	**160**
std error		0.0129	0.0007	0.0002	0.0001	0.02	1.51	0.97
	1	2.049	0.165	0.021	0.016	12.4	97	132
	2	2.100	0.160	0.020	0.015	13.1	106	140
	3	2.120	0.156	0.020	0.015	13.6	104	143
mean and pooled std error of three fractionated soils	1, 2and 3	**2.090**	**0.161**	**0.020**	**0.015**	**13.0**	**102**	**139**
		0.0128	0.0001	0.00003	0.0001	0.08	0.66	0.88
Mean % loss from un-fractionated soil		***42***	***33***	***20***	***65***			
Harden soil; long term pasture	0	**4.047**	**0.364**	**0.039**	**0.032**	**11.1**	**104**	**128**
std error		0.0121	0.0007	0.00002	0.0001	0.02	0.37	0.76
	1	2.743	0.253	0.036	0.025	10.9	77	112
	2	2.616	0.233	0.033	0.022	11.2	80	121
	3	2.541	0.225	0.032	0.020	11.3	79	126
mean of three fractionated soils	1, 2 and 3	**2.633**	**0.237**	**0.034**	**0.022**	**11.1**	**79**	**120**
pooled std error		0.0009	0.0001	0.00003	0.0001	0.01	0.07	0.15
Mean % loss from un-fractionated soil		***35***	***35***	***13***	***31***			
Harden soil; long term cropping	0	**2.179**	**0.185**	**0.035**	**0.017**	**11.8**	**62**	**125**
std error		0.0104	0.0011	0.00008	0.0002	0.05	0.44	1.99
	1	1.957	0.169	0.034	0.016	11.6	58	123
	2	1.980	0.167	0.033	0.016	11.9	60	127
	3	1.825	0.157	0.033	0.014	11.7	56	128
mean of three fractionated soils	1, 2 and 3	**1.921**	**0.164**	**0.033**	**0.015**	**11.7**	**58**	**126**
pooled std error		0.0006	0.0001	0.00006	0.0001	0.01	0.09	0.33
Mean % loss from un-fractionated soil		***12***	***11***	***6***	***12***			
Buntine soil; long term cropping	0	**1.845**	**0.115**	**0.010**	**0.018**	**16.0**	**182**	**104**
std error		0.0007	0.0001	0.0001	0.0005	0.01	2.94	3.11
	1	1.005	0.070	0.007	0.012	14.3	141	85
	2	1.183	0.077	0.010	0.010	15.3	123	114
	3	0.959	0.061	0.007	0.009	15.8	142	110
mean of three fractionated soils	1, 2 and 3	**1.049**	**0.069**	**0.008**	**0.010**	**15.1**	**135**	**103**
pooled std error		0.0017	0.0002	0.00002	0.0001	0.04	0.47	0.47
Mean % loss from un-fractionated soil		***45***	***40***	***20***	***44***			
Leeton soil; long term cropping	0	**2.383**	**0.150**	**0.023**	**0.015**	**15.9**	**104**	**155**
std error		0.0303	0.0028	0.0004	0.0002	0.12	2.31	2.87
	1	1.081	0.087	0.020	0.011	12.4	55	100
	2	1.245	0.092	0.020	0.011	13.6	61	111
	3	1.203	0.092	0.019	0.010	13.1	62	116
mean three fractionated soils	1, 2 and 3	**1.176**	**0.090**	**0.020**	**0.011**	**13.0**	**59**	**109**
pooled std error		0.0028	0.0004	0.0001	0.0001	0.03	0.22	0.41
Mean % loss from un-fractionated soil		***51***	***40***	***13***	***27***			

* **0:** Light fraction not removed
1: Light fraction removed by dry sieving/winnowing method
2: Light fraction removed by floatation using 30% NaCl solution (density 1.2 g cm^{-3})
3: Light fraction removed by floatation using NaI solution (density 1.5 g cm^{-3})

Table 4: Mass of light fraction material, % C, N, acid extractable P and S of light fraction material and C:N, C:P and C:S ratios of light fraction removed by dry sieving/winnowing method

Soil	Mass light fraction (g kg^{-1} un-fractionated soil)		C	N	P	S	C:N	C:P	C:S
Hamilton unimproved pasture	23		12.780	0.715	0.056	0.118	17.9	228	109
Harden virgin soil	95		19.150	0.910	0.050	0.080	21.0	383	240
Harden long term pasture	94		16.730	1.320	0.080	0.100	12.7	198	163
Harden long term cropping	9		16.150	0.898	0.066	0.102	18.0	245	159
Buntine long term cropping	51		15.790	0.807	0.055	0.081	19.6	287	194
Leeton long term cropping	137		9.968	0.502	0.043	0.044	19.9	230	228
		means	**15.095**	**0.859**	**0.058**	**0.088**	**18.2**	**262**	**182**
fresh wheat residue (analysed in this laboratory)			**46**	**0.71**	**0.07**	**0.07**	**65**	**657**	**657**

Table 5: Proportion of C, N, acid extractable P and S in light fraction material as a percentage of total element in un-fractionated soil

Soil	Mass light fraction (g kg^{-1} un-fractionated soil)		C	N	P	S
Hamilton unimproved pasture	23		9.3	6.5	4.4	7.0
Harden virgin soil	95		49.4	36.7	20.0	35.9
Harden long term pasture	94		38.6	34.9	19.2	32.1
Harden long term cropping	9		6.7	4.5	1.7	5.6
Buntine long term cropping	51		45.5	39.4	29.8	28.5
Leeton long term cropping	137		59.6	48.7	26.5	38.9
		means	**34.9**	**28.4**	**16.9**	**24.7**

Table 6: Selected parameters associated with the linear regressions between soil C, N, OP and S shown in Figure 4

Reference	N	R^2	Y intercept	Slope (SE)	*P* value of slope	C fixed per unit N, OP or S
Figure 3A (C:N)						
International soils	761	0.86	0.09	11.1 (0.2)	<0.001	11.2
Australian soils	59	0.99	0.11	11.7 (0.1)	<0.001	11.8
Figure 3B (C:OP)						
International soils*	478	0.44	1.10	52 (3)	<0.001	53
Australian soils	59	0.74	-1.03	189 (15)	<0.001	187
Figure 3C (C:S)						
International soils	531	0.76	0.28	70 (2)	<0.001	70
Australian soils	59	0.97	0.73	68 (2)	<0.001	68

* data failed normality test N = number soil samples SE = std error

Table 7: Selected parameters associated with linear regression between soil C and OP for the International soils

Reference	OP estimated by†	N	R^2	Y intercept	Slope (SE)	P value of slope	C fixed per unit OP
Acquaye & Kang (1987)	1	48	0.58	0.363	56(7)	<0.001	57
Agbenin et al (1999)	1	15	0.60	1.201	130 (29)	<0.001	131
	2	15	0.60	0.794	175 (40)	<0.001	176
	2	15	0.24	0.917	105 (52)	0.065	105
Chen et al (2000)	1	8	0.09	1.166	15(20)	0.473	16
Chen et al (2004)	1	15	0.30	2.471	59 (25)	0.033	61
Han et al (2005)	2	12	0.14	0.655	39 (31)	0.230	40
Makarov et al (1997)	2	15	0.20	0.192	12 (7)	0.098	12
Makarov (2004)	1	21	0.54	1.468	56 (12)	<0.001	58
Makarov & Malysheva (2006)	2	21	0.74	0.148	75 (10)	<0.001	75
Neptune et al (1975)	2	12	0.28	1.082	72 (36)	0.075	73
Saikh et al (1998)	2	36	0.09	1.116	47 (25)	0.068	49
Turner et al (2003a)	1	18	0.65	-0.204	89 (16)	<0.001	89
	2	18	0.82	-1.225	157 (18)	<0.001	157
Turner et al (2003c)	2	29	0.25	3.117	40 (13)	0.0053	43
Turner et al (2003d)	2	29	0.44	2.214	38(8)	<0.001	40
Uriyo & Kesseba (1975)*	1	43	0.66	0.404	105 (12)	<0.001	105
Walker & Adams (1958)	1	66	0.63	0.556	31 (3)	<0.001	32
Walker & Adams (1959)*	1	45	0.34	0.818	92 (20)	<0.001	93

† 1 = ignition method 2 = extraction method * data failed normality test N = number soil samples SE = std error

Table 8: Estimated potential value of N, P and S locked up with each tonne of humus-C

nutrient	Amount (kg)	Approx price/kg nutrient	Approx Cost ($)
N	80	1.50	120
P	20	5.00	100
S	14	2.00	28
			$248

Prices are in Australian dollars and calculated from 2009 fertiliser costs

Figure Captions

Figure 1: Flow diagram for separation of soil light and heavy fractions

Figure 2: Flow diagram for preparation of un-fractionated soil

Figure 3: Representative selection of light fraction material removed from Australian soils

(This figure does not have to be reproduced in colour for the print edition)

Fig 4. (A) Total C:N for 598 ; (B) C:organic P for 408 and (C) C:S ratios for 527 International soils (open circles) and 59 Australian soils (closed circles) respectively (see Tables 7 and 8 for regressions).

Fig 5. (A) Total C vs Acid P, (B) Total C vs Ignition P and (C) Acid P vs Ignition P for the 59 Australian soils

Fig 6. Total soil C vs Organic P for the 59 Australian and selected International soils