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Carbon-Nutrient stoichiometry to increase soil carbon sequestration

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Abstract

The more stable fine fraction pool of soil organic matter (FF-SOM; <0.4 mm) has more nitrogen, phosphorus and sulphur (NPS) per unit of carbon (C) than the plant material from which it originates and has near constant ratios of C:N:P:S. Consequently, we hypothesised that the sequestration of C-rich crop residue material into the FF-SOM pool could be improved by adding supplementary nutrients to the residues based on these ratios. Here we report on the effect of N, P and S availability on the net humification efficiency (NHE), the change in the size of the FF-SOM pool (as estimated by FF-C), following incubation of soil with wheaten straw. Four diverse soils were subjected to seven consecutive incubations cycles, with wheaten straw (10 t ha⁻¹ equivalent) added at the beginning of each cycle, with and without inorganic N, P, S addition (5 g N, 2 g P and 1.3 g S per tonne of straw). This nutrient addition doubled the mean NHE in all soils (from 7% to 15%) and when applied at twice the rate increased NHE further (up to 29%) for the two soils that received this treatment. The FF-N, -P and -S levels increased in concert with FF-C levels in all soils in close agreement with published stoichiometric ratios (C:N:P:S = 10,000:833:200:143). Microbial biomass-C (MB-C) levels were estimated during one incubation cycle and found to increase in parallel with FF-C from 448 µg MB-C g⁻¹ soil (no nutrient addition) to 727 µg MB-C g⁻¹ soil (plus nutrients) and 947 µg MB-C g⁻¹ soil (plus 2x nutrients). There was a significant relationship between MB-C and the change in FF-C during that incubation cycle, providing evidence of a close relationship between the microbial biomass and FF-SOM formation. The two to four-fold increases in NHE achieved with nutrient addition demonstrated that inorganic nutrient availability is critical to sequester C into the more stable FF-SOM pool irrespective of soil type and C input. This has important implications for strategies to build soil fertility or mitigate climate change via increased soil organic C, as the availability and value of these nutrients must be considered.

Keywords: Climate change, soil carbon, net humification efficiency.

1. Introduction

Soils are the largest sink of the global terrestrial C cycle holding approximately 1,500 Gigatonnes, three times the amount of C in the terrestrial biomass and twice as much as the C in the atmosphere (Post et al., 1982; Schlesinger, 1995; Eswaran et al., 2000). SOM, and the C it contains, is of fundamental importance to soil quality as it affects many soil chemical, physical and biological properties. Approximately half of all soil organic carbon (SOC) in managed ecosystems has been lost to the atmosphere during the last two centuries and it is generally accepted that this loss has been a major factor leading to soil degradation and declining soil quality (Schlesinger, 1984; Lal, 2004, 2006). Degraded soils can be repaired,

primarily by adopting management practices that lead to an increase in the size of the SOC pool. Such practices should not only improve soil quality but may help mitigate climate change by removing atmospheric carbon dioxide (CO₂) and transferring it to a more stable SOC pool.

Throughout this paper we accept the definition of SOM as the organic fraction of the soil exclusive of the coarse fraction material composed of un-decayed plant and animal residues (SSSA 2008). Magid and Kjaegaard (2001) showed that coarse fraction material (CF >0.4 mm) was synonymous with light fraction organic matter (LF <1.4 g cm⁻³) with regards to the amounts of C and N as well as physical appearance. This coarse fraction is often recognised as highly labile material with fast turnover rates in soil (Golchin et al. 1994; Wander 2004; Crow et al. 2007). On the other hand, FF organic material (<0.4 mm; analogous to heavy organic matter >1.4 g cm⁻³) is considered to be a more stabilised and slowly decomposing pool of SOM and is commonly referred to as soil humus (Golchin et al. 1994; Magid and Kjaegaard 2001). In this study we use FF-C to estimate this SOM pool. After removal of the CF (>0.4 mm), Kirkby et al. (2011) showed that the remaining FF-SOM matter had near constant C:N:P:S ratios. We propose that nutrient stoichiometry has important implications for SOM dynamics as it is central to microbial growth and turnover in soil the same way that Redfield (1958) demonstrated the importance of nutrient stoichiometry for phytoplankton growth, C-turnover and the biogeochemistry of the oceans.

Humification has been defined as the conversion of fresh organic matter inputs into more stabilized SOM (Tate, 1987). Consequently determination of the net change in the stabilised SOM pool after fresh OM additions (net humification efficiency - NHE) requires removal of CF material to avoid over-estimation. A wide range of values for the fraction of crop residues that is converted into more stable SOM have been reported from as low as 0.053 up to 0.43 (Ladd et al. 1981, 1985; Wilts et al. 2004; Cai and Qin 2006).

Many factors have been suggested to affect humification including the type of input material, soil type, climatic factors and soil nutrient status (Lin and Wen 1987; Galantini et al., 1992; Andren and Katterer, 1997). In particular, some models suggest low nutrient availability (especially N) will preclude increased soil-C and have shown that C storage increases as a result of N fertilisation. These models have focussed on the role that N fertilisation plays by increasing net primary plant production (NPPP) and increased organic matter inputs to the soil, rather than on a possible role for N (or P and S) on the humification process unrelated to NPPP (Rastetter et al. 1997; Hungate et al. 2003; van Groenigen et al. 2006).

Given that the more stabilised SOM has a near constant C:N:P:S ratios and is more nutrient (NPS) rich per unit of C than that of fresh plant material inputs due to microbial reprocessing (Himes, 1998; Kramer et al. 2003; Bol et al. 2009), we hypothesised that N, P and S addition with fresh C-rich plant residue could increase the size of the more stabilised SOM pool. We report soil incubation experiments on four contrasting soils to investigate (i) the impact of supplementary inorganic N, P and S on the change in the size of the more stabilised SOM pool, as estimated by the change in size of the FF-C pool (ii) the effects of these supplementary nutrients on the size of the microbial biomass and (iii) the relationship between the size of the microbial biomass and the FF-C pool. Our aim was to investigate the contribution of inorganic nutrients to the transformation of wheaten-straw-C into stabilized SOM.

2. Materials and Methods

2.1 Soils and crop residues

2.1.1 Collection and preparation of soils.

Four soils, from different agro-ecological regions in Australia and of varying texture and initial FF-C content, were chosen for the experiment (Table 1). Approximately 50 kg of surface soil (0-15 cm) was collected from five positions at each site and bulked to be used for a series of experiments investigating the role of inorganic nutrient availability on FF-SOM dynamics. All CF-SOM was removed from the soils using a dry sieving/winnowing procedure, described in detail in Kirkby et al. (2011), and the air dried soils were stored in covered plastic bins. Briefly, the air dried soils were gently crushed, using a steel rolling pin to break down aggregates and were then passed through a 2 mm sieve and gravel and any plant material retained on top of the sieve was subsequently discarded. The 2 mm sieved samples were re-sieved using a 0.4 mm sieve. 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 causing the LF, mostly-identifiable plant material to float to the top, while the heavy, mostly-mineral and mineral associated material sank to the bottom. A gentle stream of compressed air was used to separate the two fractions. This procedure was repeated several times on both fractions to ensure no heavy mineral fraction remained in the LF material and that no visible plant-like LF material remained in the largely mineral HF. This procedure effectively removed existing coarse (> 0.4 mm) LF organic material so impacts of added wheaten straw and nutrients on FF-SOM dynamics could be investigated. Other flotation and filtering methodologies for removal of endogenous LF material can substantially alter the microbiological response of the resulting soil (Crow et al. 2007), or potentially mobilise dissolved organic matter from the slowly decomposing SOM pool (Haider and Guggenberger 2005). The dry sieving/winnowing procedure adopted here resulted in a more complete removal of the LF material, with the remaining FF (or HF) material consistently having a lower C:N, C:P and C:S ratio than for the same soils fractionated by floatation methods (Kirkby et al. 2011). Nonetheless, with either method some of the fine (<0.4 mm) aggregates may also contain some potentially labile LF material. For this experiment 15 kg of each soil was removed from storage, thoroughly mixed, and a sub-sample removed and analysed for total C, N, P, S and FF-C, -N, -P and -S as described below.

2.1.2 Collection of wheaten straw

Standing wheaten straw (*Triticum aestivum*) was collected from a field site located at Harden (Table 2), oven dried (70°C) and stored. Stem internode material was separated and used to ensure uniformity of the material, as internode, node and leaf material may vary in the ratio of cellulose:hemicellulose and non-structural carbohydrates (Antongiovanni and Sargentini, 1991; McGrath et al., 1997). The straw was collected with the leaf sheath intact and this was removed prior to preparation to ensure the material was as free from surface soil contamination as possible. The straw was cut into pieces approximately five mm long prior to mixing with soil. A sub-sample of the straw was analysed for total C, N, P and S.

2.1.3 Addition of nutrient solution

For the purposes of calculating the amount of supplementary N, P and S needed, we assumed that the stoichiometry of the more stabilised SOM was as suggested by Himes (1998) with a C:N:P:S ratio of 10,000:833:200:143 (Table 2). Furthermore, and based on some of the highest reported values for the fraction of plant residue-C transformed into SOM-C reported in the literature (e.g. Jacinthe et al. 2002; Cai and Qin 2006), we assumed that a maximum of 30% of the straw-C could be transformed into stabilised SOM-C. Analysis of the straw

showed that it was nutrient poor compared to the FF-SOM (i.e. wheat straw has approximately one tenth the amounts of N, P and S per unit of C compared with the FF-SOM, Table 2) and additional nutrients were thus added (Table 2) to achieve a potential humification of 30%. This was done by adding 1 ml kg⁻¹ soil of a nutrient solution containing 64, 26 and 17 g per L of N, P and S, respectively, using ammonium nitrate, potassium dihydrogen phosphate and ammonium sulphate. The pH of the nutrient solution was adjusted to pH 7 using a 10M sodium hydroxide solution. In this instance, the availability of inorganic nutrients already in the soil were disregarded as they were expected to an order of magnitude less than the addition.

2.2 Experimental design

All soils were incubated with added wheaten straw (at 10 t ha⁻¹ equivalent) both with and without supplementary nutrient (NPS) treatments. There were three nutrient treatments; (i) soils with zero nutrient addition (0-Nu), (ii) soils receiving one dose (1x) of nutrient addition at the beginning of each incubation cycle (1-Nu) and (iii) soils receiving a double dose (2x) of nutrient addition at the beginning of each incubation cycle (2-Nu; Harden and Hamilton soils only). Seven consecutive incubation cycles, each of 84 days duration, were conducted with each soil and treatment combination. An 84-day incubation period was based on the results of a preliminary experiment that showed that after this time CO₂ efflux of the soils (incubated with or without added straw or supplementary nutrients) became low and approached an asymptote (e.g. Fig 1), suggesting that all potentially labile material had been decomposed or assimilated.

2.2.1 Treatments

0-Nu (control): soil + wheaten straw (no nutrient addition): Three replicates for each soil type consisting of 3500 g soil (equivalent oven dry weight) were prepared and brought up to 70% field capacity (18.0, 13.4, 5.0 and 23.3 ml water kg⁻¹ OD soil for the Hamilton, Harden, Buntine and Leeton soils respectively) using distilled water. Wheaten straw was added to the soil at a rate of 12 g kg⁻¹ dry soil and the soil and straw were thoroughly mixed. This addition of straw was equivalent to 10 t ha⁻¹ (to 7.5 cm soil depth) and is realistic of maximum rates of residue incorporation under field conditions in southern Australia.

1-Nu: soil + wheaten straw + (1x nutrient addition): This treatment was identical to the control except that a nutrient solution was added to the soil immediately prior to the addition of the straw. 1 ml of nutrient solution was added per kg dry whereby the soil was spread out on a plastic tray and the nutrient solution uniformly added to the soil surface. The soils were then brought up to 70% field capacity using distilled water and thoroughly mixed. 12 g straw was added per kg dry soil and the sample was again thoroughly mixed.

2-Nu: soil + straw + (2x nutrient addition): Identical to Treatment 1-Nu except that 2 ml of nutrient solution was added per kg dry soil, effectively doubling the amount of nutrients added to the soil. Only the Harden and Hamilton soils received this treatment.

2.2.2 Incubation conditions

Each replicate of the soil/straw mix was placed in a 4.5 L plastic mesocosm measuring 160 mm diameter and 150 mm high. The mesocosms had 8 rectangular drainage holes measuring 10 mm by 25 mm equally spaced around the base with nylon shade cloth in the bottom to prevent spilling but to allow gas exchange. The mesocosms were placed in large plastic boxes

with close fitting lids. Distilled water was placed in the bottom of the box (10 mm) to reduce soil drying during the incubation. The mesocosms were supported above the water by a 3 mm thick rigid perforated (12.5 mm diameter holes) plastic rack. Four mesocosms were placed in each box in a semi-random manner with no two replicates of any treatment in the same box. The boxes were incubated in a dark constant temperature room at 30°C for 84 days, after which time a sub-sample of soil was removed from each replicate and new straw, plus nutrients (according to treatment) were added to the remaining soil as described above.

During each 84 day incubation the mesocosms were managed as follows: After 7, 21, 35, 49, 63 and 77 days all mesocosms were weighed and distilled water added to bring the moisture content back to that corresponding to the initial 70% field capacity. After 14, 28, 42, 56 and 70 days the soil from each mesocosm was tipped onto a plastic tray and thoroughly mixed, brought back to the initial moisture content, and then returned to the mesocosm. The soils were taken out of the mesocosm and remixed to enhance straw decomposition and improve homogeneity by: (i) re-aerating the soil as it is generally acknowledged that oxygen is a requirement for the dominant heterotrophic soil organisms responsible for decomposition (Franzluebbers, 2005) (ii) simulating soil disturbance that occurs with cultivation and seeding operations, which preferentially increases the decomposition rate of coarse LF organic material (Cambardella and Elliott 1992) and (iii) redistributing the soil-straw-nutrient mixture to avoid 'nutrient poor' zones that may arise around straw pieces during decomposition.

After 84 days the soil from each mesocosm was tipped onto a plastic tray, thoroughly mixed and a sub-sample of 150 g soil, equivalent oven dry weight, was removed from each replicate. These sub-samples were dried at 40-45°C for several days. Evidence suggested that sulphur may be lost from soil if dried at a higher temperature (data not shown). This incubation cycle was repeated consecutively seven times during the course of the experiment.

2.3 Sample analysis

2.3.1 Soil fractionation and washing following incubation

After drying, the 150 g sub-samples had any CF organic material (partially decomposed wheat straw fragments >0.4 mm) removed by the dry sieving/winnowing procedure (Kirkby et al. 2011). Each sub-sample was also examined under a low-power stereo microscope to ensure no straw fragments remained in the sample. A 50 g sub-sample was pulverised (Labtechnics pulverising mill, model LM1, Adelaide) to pass through a 100 mesh sieve (150µm opening). Two 5 g sub-samples from the pulverised soil were washed twice with 0.1M HCl to remove residual inorganic N, P and S (Midwood and Boutton, 1998; Anderson and Schoenau, 2008). After drying, the two washed sub-samples were combined and pulverised again to give one composite 10 g washed sample. Thus, two pulverised samples (unwashed and washed) were produced from each replicate to be used for chemical analysis.

2.3.2 Analysis of soil for total C, N, P and S following incubation

Both washed and unwashed fractionated samples were analysed for total C, N, P and S. Total C and N were determined using a dry combustion analyser (Europa Scientific Model 20-20, Crewe, UK). Total acid extractable P and S 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, 0.5 g soil, 9 ml concentrated nitric acid and 3 ml concentrated hydrochloric acid, according to method 3051A of the USEPA (1998).

2.3.3 Analysis for FF-C, -N, -P and -S following incubation

Once the CF organic material was removed, the total-C of the fractionated and unwashed soils was analogous to the FF-C (Table 3; Kirkby et al., 2011). This was not the case for N, S and P as the unwashed samples contain some residual inorganic nutrients along with the FF-N, -P and -S (Kirkby 2011). After washing with 0.1M HCl, the N and S remaining in the soils is indicative of FF-N and FF-S and were used to derive FF C:N and C:S ratios. However this potentially underestimates the maximum FF-N and -S levels in unwashed samples because some FF-C (1-5%), and associated FF-N, -P and -S, was lost by washing (Kirkby 2011). Therefore FF-C, FF-N and FF-S lost by washing was estimated for all samples as follows. The FF-C value was calculated after removal of the CF organic material (Table 3). The FF-C:N and FF-C:S ratios were measured on samples that had the CF organic material removed followed by washing twice with 0.1M HCl (Table 3). The maximum FF-N and FF-S values for fractionated but unwashed soils were then estimated by dividing the FF-C value (measured on fractionated but unwashed soils) by the FF-C:N and FF-C:S ratios (measured on fractionated and washed soils, Table 3). A FF-P value was similarly estimated using the same procedure (Table 3), although it may overestimate FF-P as P in soil is a component of many common minerals that are not completely removed by washing (Kirkby 2011). We assume that the N, P, and S estimated in this way are indicative of the levels of N, P and S directly associated with FF-C notwithstanding the problems with FF-P as indicated above.

Straw-C, -N, -P and -S were determined in a similar manner to total C, N, P and S for soil. Straw-C and -N were determined using a dry combustion analyser and straw-P and -S by ICP-OES following microwave-assisted acid digestion using reverse-aqua regia according to method 3051A of the USEPA (1998).

2.3.4 Net humification efficiency

We define the net humification efficiency (NHE) as the change in the FF-C level over a given period per 100 units of fresh residue-C added. This definition implies any remaining CF organic material (>0.4 mm and largely undecomposed plant residues) must be removed from the soil before the soil is analysed for C so that the NHE is not over-estimated.

2.3.5 Microbial biomass

The microbial biomass was estimated as chloroform-labile C on treatments 0-Nu, 1-Nu and 2-Nu seven days after the start of the seventh incubation cycle using the fumigation-extraction method (Vance et al., 1987). A 7-day fumigation period was used, however, as it has been reported that the MB-C extracted from heavy clay soil (such as Leeton) only stabilises after such time (Amato and Ladd, 1988). Two subsamples were taken from each replicate, making a total of six samples for each treatment analysed for microbial biomass.

2.4 Statistical Analysis

The results were analysed using the SigmaStat statistical software program (Systat Software Inc. USA). The data were analysed using a one-, two-way or repeated measures ANOVA, as appropriate. If the ANOVA detected a statistical difference an all pair-wise multiple comparison was conducted using the Holm-Sidak method to identify which treatments were significantly different.

3. Results

3.1 CO₂ efflux

The initial (day 7, Fig 1) CO₂ efflux from all four fractionated soils without any wheaten straw added (all endogenous CF material >0.4 mm had been removed prior to the start of the experiment) was similar to the efflux measured for the latter periods (days 56 and 84, Fig 1).

In addition, the cumulative CO₂ emission from these soils was small and equivalent to the decomposition of between 1.9 - 6.8% (mean 4.4%) of the initial FF-C. Taken together this indicates there was little, if any, labile-C in these soils once the CF material had been removed. By contrast, high CO₂ efflux rates were observed over days 7 to 21 for all soils with added straw (Fig 1), indicating the presence of a significant amount of labile C. This is supported by the cumulative CO₂ efflux from the soils with added straw where the cumulative efflux was more than double (mean 9.2%, range 4.6 – 14.2) the initial total soil-C (initial FF-C + straw-C) compared to soils without added straw. Further, given that the soils were incubated under ‘ideal’ conditions and that they had been remixed after each measurement to encourage decomposition, we suggest most of the labile material in the straw had either been decomposed or assimilated by ~30 days as the CO₂ efflux rates for the last three measurement periods were very similar to the efflux from soils that had no straw added.

3.2 Changes in FF-C values after seven incubation cycles

The initial measured FF-C, FF-N, -P and -S values and the FF-C:N, C:P and C:S ratios of the four unwashed soils used are shown in Table 1. The maximum FF-N, -P and -S values were calculated as described in section 2.3.3. Although the P values have been reported along with the associated C:P ratio, it is acknowledged that they may over-estimate that associated with FF-SOM (Kirkby et al., 2011) as indicated by the wide FF-C:P ratios across the soils as compared with much closer FF-C:N and FF-C:S ratios.

After seven incubation cycles there was a significant increase in FF-C levels of all 0-Nu soils ($P < 0.01$), but no difference between soils ($P > 0.05$) with a mean overall increase in FF-C of 2280 mg kg⁻¹ soil (Figure 2). Nutrient addition (1-Nu) increased FF-C levels in all soils compared with the respective 0-Nu treatment ($P < 0.01$ for all soils) with a significant interaction ($P < 0.01$) between soil type and nutrient treatment. Adding two doses of nutrients to the Hamilton and Harden soils (2-Nu) increased the FF-C levels further for both soils ($P < 0.001$), with a mean increase of 4930 mg kg⁻¹ soil above the 1-Nu soils (Figure 2).

In addition to measuring an absolute change in FF-C (mg kg⁻¹ soil), the change in FF-C levels was also determined as a percentage of the straw-C that was added (Figure 2), defined as the NHE. The NHE was similar (4.5% to 7.5%) for all four soils not receiving supplementary nutrients. By contrast the NHE increased two to eight fold (from 6.7 to 12.2% and 5.4 to 42.6% for the Hamilton and Leeton soils respectively, Figure 2) with one dose of supplementary nutrients and for the two soils that received two doses (Hamilton and Harden) it increased again (from 12.2 to 27.2% and 18.1 to 29.1% for the Hamilton and Harden soils respectively).

3.3 Incremental change in FF-C, -N, -P and -S:

The FF-C levels in the 0-Nu soils showed little change over the first three or four incubation cycles but tended to increase in the later incubation cycles. Nutrient addition (1-Nu and 2-Nu soils) generated a more rapid increase in FF-C levels during early incubations and they continued to increase over the entire incubation period. As this was similar for all four soils only results for two of the soils (Hamilton and Buntine) are shown (Figures 3A-H). The FF-N, FF-P and FF-S levels generally moved in concert with the changes in FF-C (Figures 3B, C and D and 3F, G and H respectively) and over the course of the experiment correlations between FF-C and FF-N, -P and -S were generally significant for all treatments but were strongest where nutrients were applied (Table 4).

3.4 Changes in microbial biomass

Despite differences in soil texture and initial FF-C values, the microbial biomass was similar for three of the 0-Nu soils (soils with straw alone added), but greater in the Harden soil (Figure 4). Addition of one dose of nutrients with the straw (1-Nu) increased the microbial biomass of all four soils, compared with straw alone. There was also a significant interaction between soil type and nutrient addition for the 0-Nu and 1-Nu soils ($P < 0.001$), where a greater increase in the microbial biomass in response to nutrient addition was observed in the Buntine and Leeton soils ($P < 0.001$). The mean increase in microbial biomass of the Buntine and Leeton 1-Nu soils over the control soils was 156% compared to 62% for the Hamilton and Harden soils. Adding two doses of nutrients (2-Nu soils) to the Hamilton and Harden soils further increased the microbial biomass (mean increase of 27%) over the 1-Nu treatments ($P = 0.003$ for Hamilton, $P < 0.001$ for Harden), with no interaction between soil type and nutrient treatment ($P = 0.17$, Figure 4).

3.5 Relationship between supplementary nutrient addition, microbial biomass and humus-C
There was a significant correlation between the change in FF-C over the seventh incubation cycle and the microbial biomass estimated during the cycle (Pearson correlation coefficient = 0.710, $P < 0.001$, Figure 5). The overall pattern of change in the FF-C levels to straw and supplementary nutrient addition after seven incubations cycles closely matched the pattern of change seen in the microbial biomass measured on the soils during the seventh incubation cycle (Fig 5).

Discussion

Nutrient addition at least doubled FF-C sequestration in all soils irrespective of the initial FF-C or clay content. This increase was evident over seven consecutive incubation cycles. The concomitant incremental increases of FF-N and -S and the correlation of FF-C with FF-N and -S are consistent with previous observations that FF-SOM in surface soils has near constant proportions of C, N and S (Kirkby et al., 2011). Although the correlation of FF-C with FF-P was not as strong as with FF-N and -S (Table 4) these three nutrients are required, in relatively predictable amounts, for the transformation of C rich residue into N, P and S enriched FF-SOM (Christopher and Lal, 2007; Kirkby et al., 2011). The gradual increase in FF-C levels in soils receiving straw alone was probably due to an increase in inorganic N, P and S availability following the mineralisation of the straw added in earlier incubations.

We hypothesised that under favourable conditions for microbial activity the soil microbial biomass utilises incorporated crop residues with maximum microbial biomass increases achieved when nutrients (NPS) are not limiting. While it is well established that nutrients are required for microbial growth (Morowitz, 1968; Scheu, 1993; Christensen et al. 1996; Duah-Yentumi et al. 1998; Kouno et al. 2001) it is normally assumed that C and energy substrates are the main limiting factor for soil microorganisms (Morita, 1988; Egli, 1995; Harms and Bosma, 1997). When soil microorganisms utilise crop residues, portions are assimilated and transformed into microbial biomass (i.e. for C, termed the microbial growth efficiency, Herron et al. 2009). After the death of the organisms some microbial components are more resistant to decomposition than the original plant material and contribute to the more stabilised SOM pool (e.g. Sollins et al. 2006; Liang and Balsler 2008; Bol et al. 2009). Our results confirm that the microbial biomass increased in all four soils following the addition of labile-C inputs and these increases were greatest in the presence of added nutrients. However, the microbial biomass responded differently to nutrient addition across the four soils (i.e., Hamilton and Harden soils compared with the Buntine and Leeton soils) and this response was dose-dependent in the Harden and Hamilton soils. Whilst the bases of such differences were not investigated in the present experiments, they may be associated with differences in

the levels of initial inorganic nutrients in the soils (see table 1), soil texture, mineralogy and/or the composition of the microbial population (e.g. fungal:bacterial ratios). The significant correlations between the levels of FF-C and microbial biomass-C following straw and nutrient addition support the growing body of evidence that microbial transformations of plant residues contribute significantly to the formation of a more stabilised, slowly decomposing pool of SOM (e.g., Dijkstra et al., 2006; Kindler et al., 2006, 2009; Miltner et al., 2009;). Although evidence for this from the current experiments is indirect, further analyses of the soils might reveal such association. For example, analysing the soils for amino sugars known to be of microbial origin (e.g. Appuhn et al., 2004; Engelking et al., 2007) or soil analysis, using CPMAS-NMR, rRNA characterisation or metagenomics (e.g. Guggenberger et al., 1995; Kramer et al., 2003) would seem warranted.

Changes in FF-C levels expressed as a percentage of the residue-C added to the soil is a measure of NHE, provided that partially decomposed plant remains are removed before analysing for FF-C. Reporting changes in FF-C levels in this way is, in effect, a measure of the efficiency of a system to form and retain FF-C. Our results indicate that the addition of supplementary nutrients increased the NHE in all four soils used and this suggests that it may equally be possible to sequester FF-C in soils with varying textures and variable initial FF-C levels as long as inorganic nutrients are not limiting SOM formation.

The experimental systems used here were “closed” system soil mesocosms and any inorganic nutrients released could not be leached from the system or taken up by growing plants as might occur under field situations. This raises issues as to whether adding nutrients to stubble in the field might pose unacceptable environmental risks in some situations (e.g. lighter textured soils) where nutrients could leach into the groundwater or nearby waterways. Research is needed to investigate this issue which might include: types of nutrients used (e.g. slow release fertilisers), placement of the nutrients (e.g. spray soluble nutrients directly onto the stubble immediately prior to incorporation rather than broadcast granular fertiliser onto the soil surface prior to incorporation) or timing of nutrient addition (broadcast granular fertiliser on the soil surface after incorporation so that any leached nutrients have to pass through an active residue/soil/microbe layer).

These findings have several important implications. Firstly, they provide a possible explanation, namely an inadequate supply of N, P, and S, for the low levels of C-sequestration often observed in long-term experiments in conservation agriculture (e.g. Machado et al. 2003; Baker et al. 2007), where crop residues are retained *in situ* rather than burnt, incorporated or removed. If new residue-C is to be sequestered into the more stabilised SOM pool then new N, P and S will need to be sequestered with it. This is especially relevant in modern farming systems which aim to use nutrients more efficiently by supplying only the amounts needed for optimum economic return for crop production and a low risk of environmental damage when “surplus” nutrients are lost from the system. This conservative use of fertilisers, including the precise targeting of nutrient supply to a crop’s requirement, may inadvertently limit the supply of nutrients for the formation of more stabilised SOM.

Secondly, some models suggest that low nutrient availability, particularly N, may limit soil C storage through mechanisms that are not well understood (Rastetter et al. 1997; Hungate et al. 2003). Rastetter et al. (1997) further suggests that C sequestration can only be achieved in combination with changes in ecosystem properties that may include (i) increased C:N ratios of ecosystem components and/or (ii) an increase in total ecosystem N. Our results suggest the same rationale could be applied to P and S and C:P and C:S ratios. The meta-analysis by

Kirkby et al. (2011) showed that the C:N:P:S ratios of FF-SOM was relatively constant over a wide SOM-C range (~0.5 to 15% C). Increasing the C:N (and C:P and C:S) ratio of FF-SOM is therefore unlikely and we suggest soil C sequestration must generally be accompanied by an increased retention of soil N, P and S. This supports the hypothesis that C is only one of several elements needed to synthesise the more stabilised pool of SOM; NPS being the other major nutrient elements required (Jenkinson, 1988; Himes, 1998; Saroa and Lal 2004). While our results support this general hypothesis a limitation of one or more of the essential nutrients (NPS) may place a ceiling on the absolute quantity of SOM that can be synthesised (Stevenson 1994). Our application of a nutrient combination precludes such conclusions but further research is warranted to test this expanded hypothesis.

Finally, while our results show that adding supplementary nutrients can greatly increase FF-SOM levels in ways unrelated to increases in NPPP, and to a large extent is independent of the level of plant residues returned to the soil, they also imply that the nutrients will have to remain sequestered with the C in the FF-SOM for as long as it exists. Thus the potential value of these nutrients is a hidden cost generally overlooked when considering the processes involved in C sequestration in soil. This is an important consideration for strategies to build soil fertility and for C-trading systems currently being developed globally that target increased soil-C sequestration

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Figure legends

Figure 1

Effect of straw and nutrient treatment (addition or no addition) on CO₂-C efflux from four soils (A – Hamilton; B – Harden; C – Buntine and D – Leeton) over 84 days. Treatments with no straw addition (-) n = 3; treatments with straw addition (+) n = 5. Data are means and SEM.

Figure 2

Effect of three nutrient treatments, (i) no addition (open bars) (ii) 1X nutrient addition (shaded bars) or (iii) 2X nutrient addition (hatched bars) on the change FF-C levels, of four soils with added straw, after seven incubation cycles. LH axis = FF-C increase expressed as mg kg⁻¹ soil. RH axis = FF-C increase expressed as % of straw-C added. Data are means and SEM, n=3.

Significant differences between the soils and treatments are marked by different letters (P<0.05). Due to the orthogonal nature of the experiment the data for the two soils receiving all three nutrient treatments were analysed as one block and differences are marked by lower case letters. Data for the soils receiving two nutrient treatments (all four soils) were analysed as a separate block and differences are marked by upper case letters

Figure 3

Effect of nutrient treatment on the fine fraction-C, -N, -P and -S levels for one of the soils receiving three nutrient treatments (Harden: A-D) and one soil receiving two nutrient treatments (Buntine: E-H).

A and E: fine fraction-C; B and F: fine fraction-N; C and G: fine fraction-P; D and H fine fraction-S values over seven incubation cycles. Any remaining coarse fraction organic matter (>0.4 mm) removed before soils analysed. Data are means and SEM, n=3.

Figure 4

Effect of nutrient treatment (addition or no addition) on microbial biomass-C of four soils with added wheat straw seven days after the start of the 7th incubation cycle. Open bars = no nutrient addition; shaded bars = 1X nutrient addition; hatched bars = 2X nutrient addition. Data are means and SEM, n=6.

Significant differences between the soils and treatments are marked by different letters (P<0.05). Due to the orthogonal nature of the experiment the data for the two soils receiving all three nutrient treatments were analysed as one block and differences are marked by lower case letters. Data for the soils receiving two nutrient treatments (all four soils) were analysed as a separate block and differences are marked by upper case letters

Figure 5

Relationship between change in FF-C and microbial biomass-C of four soils with added wheat straw with 1x nutrient addition, 2x nutrient addition or no nutrient addition.

Figure 1

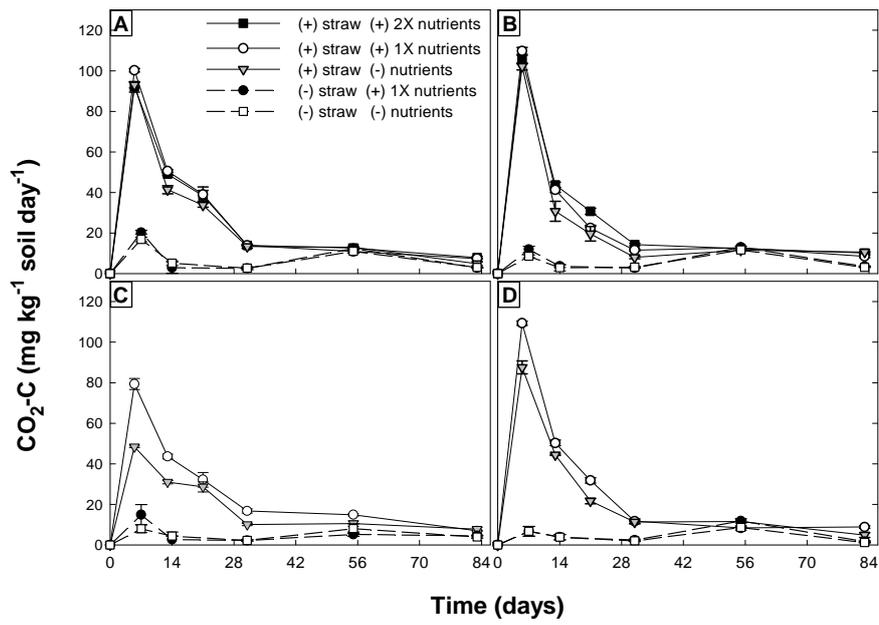


Figure 2

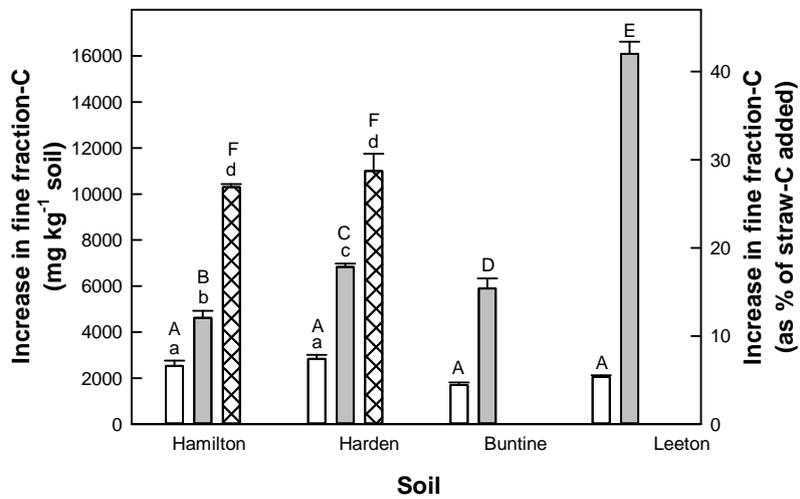


Figure 3

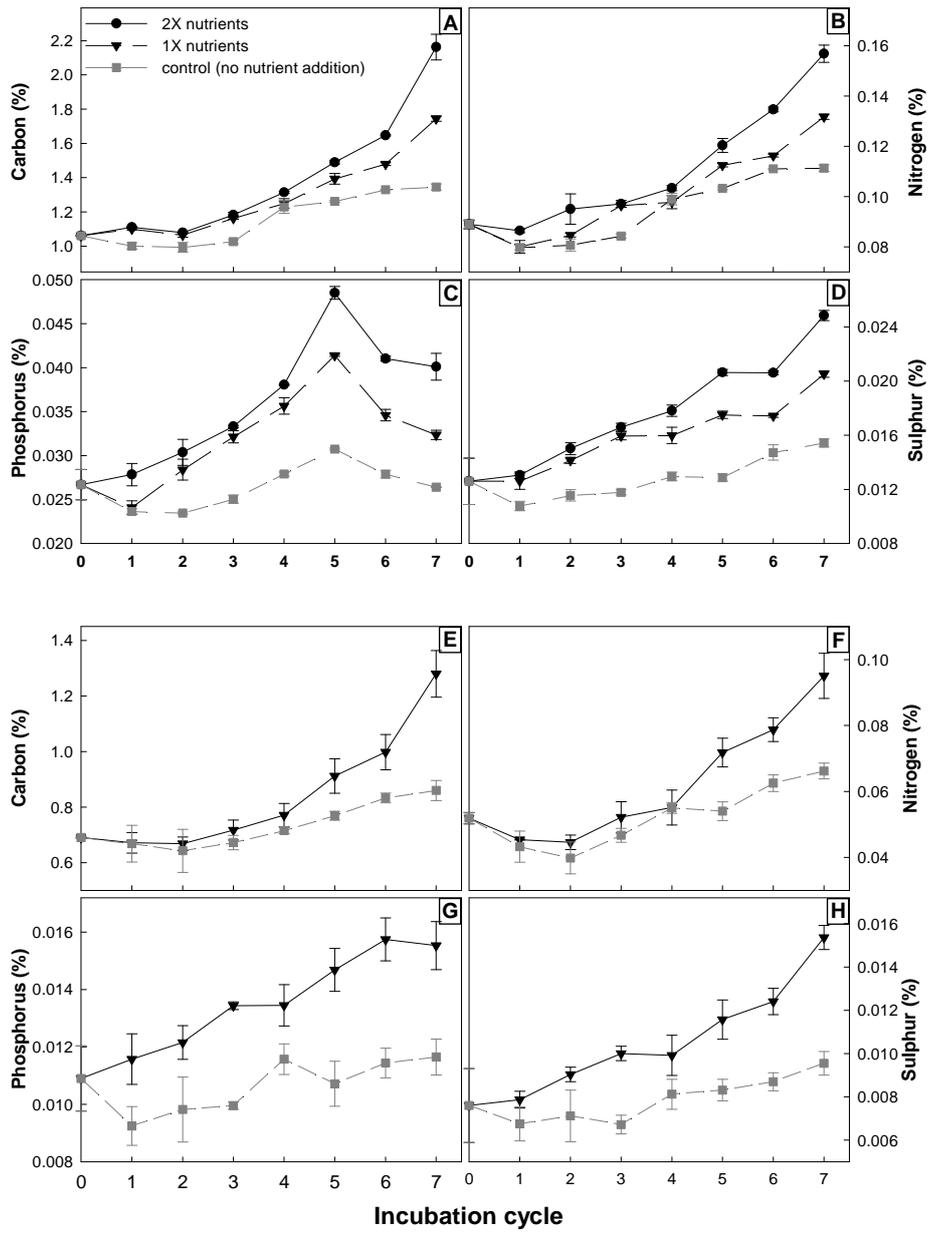


Figure 4

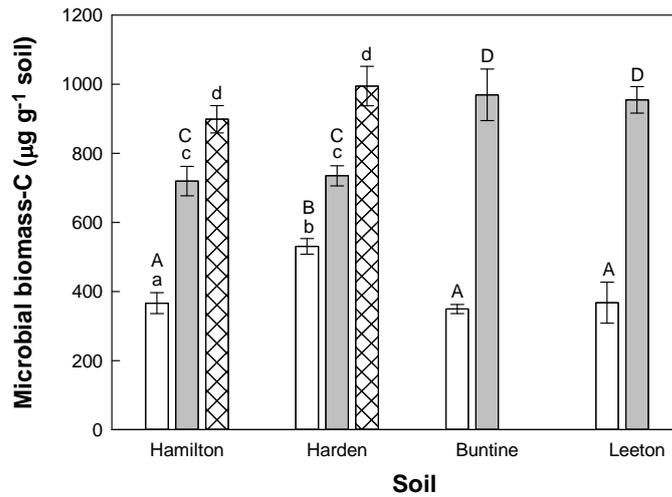
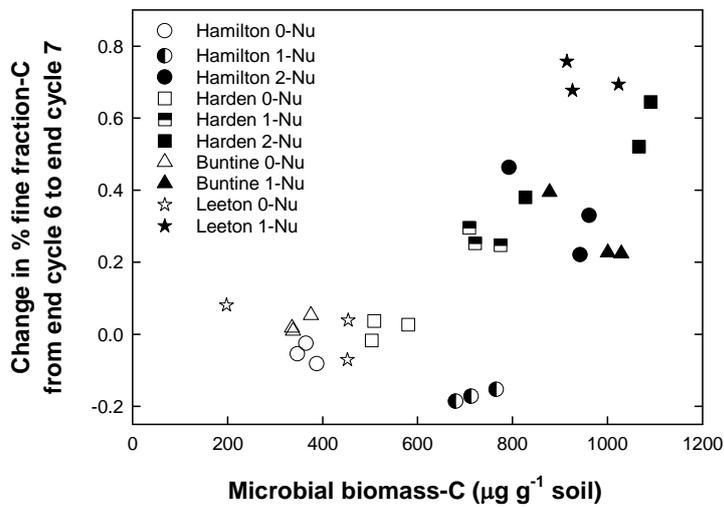


Figure 5.



Tables

Table 1

Geographical position, mean annual rainfall, soil group, selected soil properties (0-15 cm) and initial FF-C (<0.4 mm) -N, -P, -S (mg kg⁻¹ soil) and FF:C:N, C:P, C:S ratios once the CF (>0.4 mm) organic material has been removed.

Site	Geographic Position	Annual Rainfall	Soil Group	Previous land use	% clay	% silt	% sand
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(mm)								
Hamilton, Vic	142.02E	37.65S	686	chromosol	unimproved pasture	25	19	56
Harden, NSW	148.37E	34.56S	610	kandosol	long term cropping	15	10	75
Buntine, W.A.	116.57E	29.99S	357	kandosol	long term cropping	8	3	89
Leeton, NSW	146.41E	34.57S	432	vertisol	long term cropping	60	12	28

Site	C	N	P	S	C:N	C:P	C:S
Hamilton	30790	2503	317	355	12.3	97	87
Harden	10620	890	267	126	11.9	40	84
Buntine	6903	519	109	76	13.3	63	91
Leeton	12860	1217	324	160	10.6	40	80

Table 2

Calculation of the amount of supplementary nutrients (mg) required to humify 30% of the C in 12 g of straw

	C	N	P	S
expected humus C:N:P:S ratios (Himes 1998)	10 000	833	200	143
units N, P, S required per unit humus-C		0.0833	0.02	0.0143
straw (mg) added per kg dry soil	12000			
C, N, P, S concentration of straw (%)	45	0.61	0.0693	0.0629
units N, P, S per unit straw-C		0.0136	0.0015	0.0014
nutrients (mg) in 12000 mg straw	5400	73.2	8.3	7.5
maximum humification (%) expected	30			
C (mg) that will be humified if 30% humified	1620			
total nutrients required (mg) to humify 1620 mg C		134.9	32.4	23.2
supplementary nutrients (mg) required for humification		61.7	24.1	15.6
<i>= (total nutrients required for humification – nutrients in straw)</i>				

Table 3

The FF-N, -P and -S in an unwashed sample can be calculated by assuming the C value in the unwashed sample is the true FF-C value (underlined) and the C:N, C:P and C:S ratios in washed samples are the true FF-C:N, -C:P and C:S ratios (underlined).

Dividing the true FF-C value by the true FF-C:N, -C:P and -C:S ratios enables one to estimate FF-N, -P and -S values for fractionated unwashed samples

C	N	P	S	C:N	C:P	C:S
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measured total-C, -N, -P and -S (and C:N, C:P and C:S ratios) of a fractionated, unwashed sample that received 1x nutrient addition	<u>1.748</u>	0.2427	0.0773	0.0418	7.2	23	42
measured total-C, -N, -P and -S (and C:N, C:P and C:S ratios) of a fractionated, washed sample that received 1x nutrient addition	1.706	0.1288	0.0316	0.0202	<u>13.2</u>	<u>54</u>	<u>84</u>
nutrients lost (%) by washing	2.4	47	59	52			
measured FF-C and calculated FF-N, -P and -S of an unwashed sample that had received 1x nutrient addition	1.748	0.1320	0.0324	0.0207	13.2	54	84

Table 4

Correlation of fine fraction-C with fine fraction-N, -P and -S of four soils receiving three nutrient treatments, (i) control soil, no nutrient addition (ii) 1x nutrient addition and (iii) 2x nutrient addition. Data used for calculation are (i) mean C in unwashed fractionated soils (synonymous with fine fraction-C) and (ii) calculated fine fraction-N, -P and -S values for each soil collected at the end of each of the seven incubation cycles. Cell contents are Pearson correlation coefficient and P value; shaded cells not significant, n = 3.

Nutrient addition	Hamilton			Harden		
	N	P	S	N	P	S
zero (control)	0.782	0.592	0.546	0.994	0.735	0.915
	0.022	0.122	0.162	<0.001	0.038	0.001
1x nutrients	0.965	0.896	0.651	0.985	0.565	0.957
	<0.001	0.003	0.080	<0.001	0.144	<0.001
2x nutrients	0.981	0.932	0.816	0.985	0.668	0.952
	<0.001	<0.001	0.013	<0.001	0.070	<0.001
	Buntine			Leeton		
	N	P	S	N	P	S
zero (control)	0.950	0.764	0.938	0.971	0.876	0.855
	<0.001	0.027	<0.001	<0.001	0.004	0.006
1x nutrients	0.986	0.842	0.966	0.985	0.845	0.942
	<0.001	0.009	<0.001	<0.001	0.008	<0.001