**Agronomic amendments drive a diversity of real and apparent priming responses within a grassland soil**

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**Abstract**

Soil carbon (C) sequestration is often viewed as a nature-based solution to help mitigate climate change. Key to realising this potential is a better understanding of which C inputs promote greater long-term C storage. The priming effect (PE) is the change in rates of microbial soil organic matter (SOM) decomposition caused by the addition of organic or mineral amendments to soil. The apparent PE (changes in CO2 from microbial biomass turnover) of substrates is often studied as a confounding factor, however, the real PE (decomposition of native SOM) is rarely measured due to uncertainties in C pool differentiation. Here, we used a 50-day mesocosm study to compare the effect of various common soil amendments (wood biochar and ash, protein, amino acids, glucose, cellulose, cattle farmyard manure (FYM), cattle slurry, inorganic N fertiliser, and different ratios of wheat straw:shoot mixes) on the real and apparent PEs of soil, using 5-year old quasi-stable 14C-labelled SOM and 14C-labelled active microbial biomass, respectively. Our results show that there are often significant differences in the real and apparent PE, for the same amendment, with variance in magnitude and, in some case, direction. We identified few consistent drivers of PE across the two assays, however, there was a negative relationship between the initial C:N ratio of the treatment and PEs, suggesting that while the nutrient stoichiometry of the C amendment is important, the usability and quality of the substrate for the microbial community are key to determining its priming response. Equally, context is important for interpretation, as treatments that elicit positive priming may still be replenishing or increasing soil C stocks.

*Keywords:* priming effect, soil organic matter, carbon turnover, 14C isotope dynamics, soil respiration

**1. Introduction**

Soil represents a major terrestrial carbon (C) store, with topsoil globally containing more than 2300 Pg C (Batjes, 1996; Jobbagy and Kackson, 2000), exceeding the size of the total mass of C contained in vegetation and the atmosphere combined (Paustian et al., 2019). Soil is therefore a key regulator of global climate, and has increasingly been seen as a major asset to help mitigate the effects of climate change through C sequestration (Xu et al., 2020; Bossio et al., 2020). Soil systems can act as a source or sink to atmospheric carbon dioxide (CO2) depending on the balance between above- and below-ground inputs of organic material relative to the rate of decomposition of soil organic matter (SOM; i.e., mineralisation) (Lal, 2004). Most agricultural soils, particularly cropland mineral soils have suffered a reduction in soil C relative to native ecosystems through reduced net primary production and export of harvested biomass, as well as soil erosion, nutrient depletion and intensive soil disturbance (Paustian et al., 2019). Consequently, soil C sequestration, through increasing soil C stocks, is becoming increasingly viewed as a nature-based solution to mitigate climate change. Typically, this is associated with changes in land management such as the addition of organic amendments or nutrients to promote primary productivity and thus C inputs (Mrunalini et al., 2022). Understanding the impact of these inputs on soil functionality, and the net C balance of the soil is therefore vital.

The priming effect (PE) is the change in rates of microbial SOM decomposition caused by the addition of organic or mineral amendments to soil (Kuzyakov et al., 2000). This can result in either positive (mineralisation of C that was already present in the soil that would otherwise have remained as SOM) or negative priming (reduced degradation of SOM), with the net C balance being determined by the magnitude of loss by priming versus the retention of the substrate-C added. Positive priming may take place directly, through co-metabolism of the native SOM with the increased production of extracellular enzymes stimulated by the addition fresh substrate, or indirectly though stimulation of microbial activity though changes in soil nutrient status, aeration, moisture or structure (Kuzyakov et al., 2000). Conversely, negative priming may occur through the diversion of microbial utilisation of SOM to the use of more easily available labile substrates or inhibition of microbial activity due to changes in the soil environment (Kuzyakov et al., 2000). The PE may be further characterised as real (i.e., change in the SOM turnover) or apparent (i.e., change in the C turnover in soil not associated with SOM, through microbial activation and turnover) (Blagodatskaya and Kuzyakov, 2008). Previously, it has been shown that the availability, composition and amount of substrate, as well as the soil microbial community’s biomass and structure, determines the magnitude and type of PE (Blagodatskaya and Kuzyakov, 2008; Liu et al., 2017). The PE is complex, with studies often concluding contradictory findings on the consistency of priming across ecosystems (e.g., consistent; Liu et al. (2020), and inconsistent; Bastida et al. (2019)). It has also been suggested that, due to priming involving a diverse range of soil C substrates, general mechanisms may not exist (Liu et al., 2020). Further, a recent analysis has also suggested that some previous studies may not be valid due to the introduction of experimental artefacts, specifically, the inaccurate measurement of 14C isotopic abundance with some liquid scintillation fluids (Boos et al., 2022, 2023).

Soil nutrient stoichiometry has also been suggested to be a driver of priming of native SOM, particularly the ratio of nutrients in high demand (C and N), and their availability is likely to affect the ability of microorganisms to grow and function (including accessing other nutrients in the SOM) (Brown et al., 2022a; Zhu et al., 2022). The soil microbial community generally breaks down large organic molecules (e.g., cellulose, lignin, proteins) to monomer/oligomer units via extracellular enzymes prior to uptake, a process that can be metabolically costly (Wortel et al., 2018). Addition of more energetically favourable labile C substrates, which are simpler to utilise are likely to be preferentially targeted (Hamer and Marschner, 2005; De Vries et al., 2016), which may induce negative C priming. However, if the soil microbial community, then becomes limited by the availability of N, this may lead to microorganisms accelerating the decomposition of recalcitrant native SOM to acquire N (Chen et al., 2014), potentially leading to positive priming. This negative priming may also be accelerated in the presence of plant roots (Boilard et al., 2019; Murphy et al., 2015). In summary, the diverse range of inputs to soil from the molecular to ecosystem scales can be expected to have substantially differing nutrient contents (e.g., C:N:P ratios), chemical compositions and therefore recalcitrance which may influence the direction and magnitude of the priming response.

The real priming effect of substrates remains a key uncertainty within the soil C cycle, and crucial to understanding the true C storage potential of the soil, particularly, when considering potential greenhouse gas removal or sequestration techniques in an agroecosystem context. However, the comparative impact of diverse substrates on real (decomposition of recalcitrant SOM) and apparent (the increase in microbial C turnover, but not SOM decomposition) priming has been little considered. Here, we use two parallel 14C-labelled assays, to determine the real and apparent PEs of a range of commonly applied organic and inorganic amendments and substrates, at realistic loading rates. The overall aim was to determine the extent of SOC mineralisation priming and reveal possible mechanisms leading to the priming actions as a function of the added substrates.

**2. Material and methods**

*2.1. Experimental setup*

This study consisted of two assays, to examine both the real and apparent PEs of soil. Real PEs (assay 1) of substrates were determined using soil that had been labelled in the field with 14C-labelled glucose five years previously (as previously described in Rousk et al. (2015) and Farrar et al. (2012)), and was therefore considered to contain quasi-stable 14C-labelled SOM. Due to the timescale and likely formation pathways involved in 14C incorporation,it was assumed that the 14C-labelled compounds were distributed primarily in the non-humic fraction of the SOM, with a small amount in the humic fraction. In terms of spatial distribution, it was assumed that the 14C was homogenously distributed throughout the topsoil through the biological perturbation of earthworms (estimated to be ca. 66 kg soil m-2 y-1 (data in supplementary information) and the mass flow of water. The 14C content of the bulk soil (103.0 ± 4.12 Bq g-1 DW; *n* = 8) was quantified using a biological oxidiser (OX400, RJ Harvey Instrument Corp., Hillsdale, NJ), with the 14CO2 evolved collected in Oxosol scintillation fluid (National Diagnostics Ltd, Hessle, UK) and counted using a Wallac 1404 scintillation counter with automated quench correction (PerkinElmer Life Sciences, Boston, MA). To evaluate apparent PEs (assay 2), soil was labelled with a 100 μM solution of glucose to promote microbial metabolism but not growth (Brown et al., 2022b), spiked with 14C-[U]-glucose (Lot 3,632,475; PerkinElmer Inc., Waltham, MA) to give a final activity equivalent to the 14C-labelled SOM in assay 1. The soil was labelled by adding 0.5 ml of the 14C-labelled glucose solution to 5 g of soil 24 h prior to subsequent treatment addition to ensure that the 14C was predominantly in the active microbial pool (Hill et al., 2008). It is acknowledged that the addition of a small amount of 14C-labelled glucose solution may have resulted in its own PE, but as subsequent treatments are measured relative to the control (which also have 14C-labelled glucose solution) this effect was accounted for. In addition, the amount of 14C-glucose added was similar to the intrinsic levels of glucose turnover previously measured in this soil (Boddy et al., 2007). A significant advantage of this study is that labelling soil and microbial biomass carbon (MBC) allowed unlabelled substrates to be used, when in most cases it would be very difficult to produce labelled forms.

*2.2. Soil treatments*

For both real and apparent priming assays, soil was sampled from 0-15 cm depth (Ah horizon) of a Eutric Cambisol with a sandy clay loam texture in a temperate oceanic agricultural grassland at Abergwyngregyn, North Wales (53°14’N, 4°01’W). Soil was passed through a 10 mm sieve (to minimize disruption of soil biological activity; Jones and Willett, 2006), roots removed, and the soil homogenised. Unless otherwise stated, the water content was adjusted to 50% moisture content by volume, with DI H2O. Subsequently, the soil was left for 17 d at 20 °C to allow any early sampling and sieving effects to subside (Kemmitt et al., 2008; Jones et al., 2011).

For each assay, 14 treatments representing commonly used soil amendments were applied (*n* = 4). These were all compared to an unamended control (for the apparent priming assay the unamended control was the 14C-labelled glucose, without additional amendment). Microbial biomass carbon (MBC) was determined on the unlabelled samples using the chloroform fumigation-extraction method of Vance et al. (1987) using the Multi N/C 2100S Analyzer, prior to treatments being applied to determine the amount of C added as a percentage of the MBC (0.465 ± 0.012 mg C g-1; *n* = 8). Treatments were incorporated in 100 g DW equivalent of soil contained within 400 ml airtight plastic vessels.

Soil treatments consisted of four assays; i) wood-derived biochar (10 t ha-1 equivalent; 1.8% (w/w)) and its wood ash equivalent (0.57 t ha-1 equivalent (0.1 % (w/w)) (Reed et al., 2017), ii) complex (Bovine serum albumin (BSA) protein and cellulose) and simple (amino acid mixture to match the amino acid composition of BSA (described in supplementary information), and glucose) C substrates at 1 mg C g-1 soil (equivalent to 218.7% of the microbial biomass C), iii) two organic amendments (cattle FYM and cattle slurry) and one inorganic (ammonium nitrate) fertiliser applied at 120 kg total N ha-1 equivalent (the maximum recommended single application dose in a grassland (AHDB, 2023)), and iv) 5 t ha-1 equivalent of wheat straw:shoot mix at each of the ratios (100:0, 75:25, 50:50, 25:75 0:100). Treatments were designed to reflect realistic agronomic loading rates for the biochar, wood ash, FYM, slurry and inorganic N fertiliser and straw:shoot mixes. All treatments were applied to the surface of the soil and then gently incorporated by hand to avoid disturbance effects; the control samples received the same perturbation. Due to the addition of added water associated with some of the treatments (including the slurry and the amino acid solution), pots with dry treatments were amended with DI H2O, so that each pot equal amounts of water. Full carbon and nitrogen loading rates are contained in Table S1.

*2.3. 14C mineralisation*

After treatment addition, a 1 M NaOH trap (4 ml) was inserted above the soil to trap any 14CO2 produced from the breakdown of either the 14C-labelled SOM (assay 1) or the 14C-labelled microbial biomass (assay 2). The sample containers were then hermetically sealed and incubated at room temperature (20 ± 1 °C) in the dark. The NaOH traps were replaced 14 times at increasing intervals over the 50-day incubation period to ensure that the traps were <25% saturated. The amount of 14C in the NaOH traps was measured by mixing with Optiphase HiSafe 3 liquid scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and placing on a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) with automated quench correction. The overall PE was calculated as the activity of 14CO2 emission from each treatment relative to the control at 50 d (Fig. S1 + S2). Measurements of 14C contained in the microbial biomass were not undertaken as current methods (e.g., chloroform fumigation-extraction) are prone to serious difficulties in interpretation when used in conjunction with isotopes (Glanville et al., 2016). We note that the combination of base molarity and volume, and scintillation cocktail used in this experiment was not shown to be affected by the loss of 14C isotopic abundance described by Boos et al. (2022; 2023).

*2.4. Soil characteristics and analysis*

Soil physicochemical characteristics were measured on both soils from assay 1 and 2 on the final day of the incubation and summarised in Table 1. Briefly, pH and electrical conductivity were determined on 1:5 (w/v) soil-to-DI H2O suspensions using standard electrodes. Gravimetric soil moisture was determined by oven drying (105 °C, > 24 h) and organic matter was quantified by loss-on-ignition in a muffle furnace (450 °C, 16 h) (Ball, 1964). Soil C:N ratio was determined on oven-dried, ground soil using a TruSpec® Analyzer (Leco Corp., St. Joseph, MI, USA). Bioavailable N and P levels in soil were determined using 1:5 (w/v) soil-to-1 M KCl and 1:5 (w/v) soil-to-0.5 M AcOH (acetic acid) extracts, respectively. Soil nitrate (NO3-) and ammonium (NH4+) in the K2SO4 extracts were measured by the colorimetric methods of Miranda et al. (2001) and Mulvaney (1996), respectively. Phosphate (PO4–P) was measured in the AcOH extracts using the colorimetric molybdate blue method of Murphy and Riley (1962). Total dissolved organic carbon (TOC) was determined in the K2SO4 extracts using a Multi N/C 2100S Analyzer (AnalytikJena, Jena, Germany). Phospholipid fatty acid (PLFA) analysis was performed on an unlabelled parallel set of samples for the apparent priming assay, as previously described by Brown et al. (2021). Prior to incubation, the soil used in assay 1 (14C-labelled SOM) was assessed for aggregate fractionation using the method of Gunina et al. (2014). Three aggregate size-classes were isolated; large macroaggregates (>2 mm), small macroaggregates (2-0.25 mm), and microaggregates (<0.25 mm). Briefly, a bulk soil sample (250 g) was air-dried at room temperature and sieved through the 2000 and 250 μm meshes on a shaker for 5 min, at 225 rev min-1. 14C activity of the different soil aggregate sizes was determined using the biological oxidation method described above.

*2.5. Statistical analysis*

All graphical and statistical analysis was performed in R (v 4.2.1; R Core team 2022). Graphical analysis was constructed using the ‘*ggplot2*’ package (Wickham, 2016). All statistics were performed using the ‘*stats*’ package (R Core team 2022). Kruskal-Wallis one-way analysis of variance tests were used to determine the significant differences in PE and soil physical and chemical parameters between treatments. Kendall correlation analysis was used to determine the relationship between soil properties and priming effects. For all analyses the significance threshold was set at *p* ≤ 0.05.

**3. Results**

*3.1. Effect of treatment on PE*

All of the treatments elicited positive apparent priming (i.e., induced microbial C metabolism), of which amino acid, protein, glucose, inorganic N, and wheat straw:shoot, 75:25, 50:50, 25:75, 0:100, mixes addition were significantly (*p* < 0.05) higher than the control. Addition of the amino acid mix induced the most significant positive apparent PE. The only treatment to induce a negative apparent PE was wood ash, however, this was not significantly different from the control (*p* >0.05).

The real PE varied between treatments. Additions of amino acids, glucose, slurry, and all wheat straw/shoot mixes induced positive priming, of which, all but the wheat straw:shoot ratio of 100:0 being significantly higher than the control (*p* < 0.05; Fig. 1B). Treatments that induced a negative real PE (relative to the control) included biochar, wood ash, protein, cellulose, manure and inorganic N fertiliser, of which wood ash, protein, cellulose, manure and inorganic N addition were significantly lower than the control (*p* < 0.05; Fig. 1B). There was also a near linear negative relationship between the real PE and the proportion of wheat straw to shoots, with shoots only, producing on average 4.6 times larger real PE than straw only, and the largest positive PE measured. Wood ash produced the most negative real PE.

*3.2. Soil characteristics*

Differences in soil characteristics were assessed between treatments. There were statistical differences (*p* < 0.001) between all soil treatments in both the apparent and real priming samples, except SOM in the real priming soil samples (Table 1). Soil ammonium was significantly higher in the inorganic N, amino acids and protein treatments than the control samples and soil nitrate, was significantly lower than then control in all the plant ratios, as well as the amino acid, slurry, manure, cellulose and glucose treatments in the real priming soil (*p* < 0.05). In the apparent priming soil, soil ammonium was significantly higher in all treatments except the 100:0 and 25:75 plant ratios, the wood ash and biochar (*p* > 0.05), with the inorganic N, protein and amino acids having the highest concentrations. Soil nitrate was significantly higher than the control in the inorganic N and protein treatments (*p* < 0.05). In the real priming soil available P concentrations were generally lower than the apparent priming soil.Distribution of 14C activity in aggregates of the labelled soil was significantly higher in large macro-aggregates than micro-aggregates (*p* < 0.05).

*3.3. Soil factors affecting C priming*

Kendall correlation analysis was used to assess the relationship between key soil chemical quality in indicators and the priming effect of the different substrates (Table 3). Overall, there were no strong relationships for either the apparent or real PE, and little consistency of relationship across the two assays. The strongest was between the initial C:N ratio of the treatment for both real and apparent PE, implying higher C:N ratios relate to lower PEs.

**4. Discussion**

*4.1. Priming effect by substrate*

*4.1.1 Biochar and wood ash*

Biochar is a recalcitrant, organic C-rich material and its production and use as a soil amendment has been proposed as a nature-based solution to mitigate climate change (Lehmann et al., 2021). Understanding its impact on soil C cycling and the SOC priming is key to widescale deployment. Here, we showed that the effect of biochar addition applied at an equivalent rate of 10 t ha-1 causes a small positive apparent PE, possibly due to the introduction of a small amount of labile C which was subsequently catabolised by the microbial community (Jones et al., 2011). In addition, it caused a negative real PE (Fig. 1A); this is likely due to the surface charges on the char creating a sorption effect, immobilising nutrients, exoenzymes and signalling molecules which play key roles in SOM turnover (Fidel et al., 2018; Foster et al., 2018; Ibrahim et al., 2020). Biochar has previously been shown to have a variable effect on native SOC, with some studies showing a positive PE (Singh et al., 2014; Fang et al., 2019), while others showing a negative PE (Liu et al., 2018; Wang et al., 2020). Due to the low utilisation of C within biochar and the fact that the biochar particles are relatively inhospitable for the soil microbial community, there is, in theory, little biological mineralisation, particularly in the short to medium term (Quilliam et al., 2013). However, application rate, particle size and pyrolysis conditions (lower temperature, reduces stability) are likely to have a significant impact on the availability and stability of the C (Wang et al., 2022).

Wood ash is commonly utilised as a soil improver, providing nutrients (Ca, Mg, P and K as well as trace elements) and increasing pH (Demeyer et al., 2001). Consistent with the results of this study, wood ash has been shown to elicit a negative priming effect (Fig. 1A and B; Reed et al., 2017). Although there is little mechanistic understanding, wood ash only contains negligible amounts of utilizable C so is unlikely to stimulate microbial catabolism (and therefore a PE). Further, it has been posited that that the act of recrystallisation may chemically or physically stabilise (Illikainen et al., 2014) and protect SOM (Reed et al., 2017). Further work should be performed to assess the longevity of this effect, as if prolonged, it may provide a potential, novel C sequestration method.

*4.1.2 Simple and complex molecular substrates*

Glucose, cellulose, amino acids and proteins are all ubiquitous substrates in soil, routinely produced and released through breakdown and metabolism of more complex substrates (Gunina and Kuzyakov, 2015). As such, their availability in most soils is widespread (Brown et al., 2022a). Glucose, being a simple sugar, is a common C substrate and is key in glycolysis, the major energy production pathway in most microorganisms (Sanchez and Demain, 2008). As observed here, it is therefore considered one of the most prevalent molecules to cause a positive PE, due to its labile nature, with addition leading to preferential rapid uptake, and potentially alleviation of microbial dormancy which may also lead to microbial N mining from native SOM (Fig. 1A and B; Gunina et al., 2015; Chen et al., 2019; di Lonardo et al., 2019).

Cellulose is the most abundant organic polymer on earth, and represents a more energetically challenging substrate for microorganisms to utilise in comparison to glucose. Typically, microbial (enzymatic) breakdown of cellulose occurs over weeks to months due to the low concentrations of active cellulases present in soil (Deng and Tabatabai, 1994). Further, the hydrolysis products (cellobiose, glucose) may stimulate further cellulose degradation by fuelling *de novo* synthesis of more cellulases (Chmolowska et al., 2017). In this study, cellulose addition resulted in little change compared to the control regarding microbial utilisation (apparent priming; Fig. 1A). Cellulose has previously been shown to cause a positive real PE (Blagodatskaya et al., 2014), contrary to the results presented in this study (Fig. 1B), which showed that very little cellulose degradation (and associated priming) occurred compared to the control. This may have been due to the microbial community preferentially using more labile substrates compared to the cellulose or due to a lag time that has previously been associated with grassland soils (Chmolowska et al., 2017).

Free amino acids are important nitrogenous substrates in soil and tend to be characterised by a very rapid turnover in soil (half-life ~1.8 h; Jones et al., 2009). This is likely due to their ease of incorporation into common metabolic pathways and their low C:N ratios. In this study, both positive real and apparent PE were observed, however, neither the real or apparent PE was higher than observed for glucose (Fig. 1A and B). Mason-Jones et al. (2018), previously proposed that the N mining hypothesis is not universal as their data suggested that the assumptions of the N mining hypothesis; (i) amino acids should stimulate much less priming than glucose, and (ii) priming should be reduced by higher N availability, whether in organic or mineral form, were violated. While we cannot refute that statement completely, we suggest that the inherent soil and microbial stoichiometry, and microbial community composition may be driving factors of the PE.

Soil proteins are too large to be directly taken up by soil microorganism, and therefore, have been thought to play a lesser role in priming. Due to their size and relative recalcitrance (low utilizablity) they serve as a source of N only when the soil C:N ratio needs redressing (Wild et al., 2019). Our data shows, in contrast to the equivalent free amino acid composition, the real PE of protein was negative (Fig. 1B), suggesting that the recalcitrance of the protein led to little native organic C breakdown. However, our data suggested that protein depolymerisation was beginning to occur (increasing soil NH4+) at day 50 (Table 1), suggesting the full extent of real PE may occur over longer time periods. However, protein addition did stimulate microbial metabolism and associated positive (apparent) priming potentially representing some breakdown to, and utilisation of oligopeptides and amino acids.

*4.1.3. Fertilisers*

Inorganic N addition alone does not stimulate a soil C PE, however, it can be expected to change the soil stoichiometry and thus stimulate microbial biomass growth in the short term, leading to apparent priming (Fig. 1A). Balanced C and N addition has been shown to increase carbohydrates and peptides production and substrate usage (Brown et al., 2022a). However, the increase in available N also alleviates microbial demand for N and therefore reduces microbial N mining from SOM, and thus mineralisation, leading to real negative priming (Hicks et al., 2019).

Kuzyakov and Bol (2006) suggested that livestock slurry is a ‘medium utilisable substrate’, as, while it does not represent a preferential, available substrate, it may be utilised once other preferential substrates are depleted. Livestock excreta are complex substrates containing both labile (e.g., urea) and recalcitrant (e.g., lignocellulosic) factions (Puri et al., 2020; Marsden et al., 2020). Here, a small amount of positive apparent priming occurred (Fig. 1), suggesting, some microbial utilisation of the more labile (likely nitrogenous) compounds. However, co-metabolism of the SOM was low leading to a negligible PE relative to the control. This may have been related to the unbalanced nutrient stoichiometry of the slurry, particularly the N:P ratio, which may be unmatched to biological requirements, which has been shown to affect C partitioning (the balance between growth and respiration) (Brown et al., 2022a; Prado et al., 2022).

FYM is generally considered a more recalcitrant substrate than slurry, due to its higher concentration of lignocellulosic material and C:N ratio (Chadwick et al., 2000). The apparent PE of manure, and turnover through microbial activity was shown to be negligible compared to the control, suggesting it was not a preferential substrate for microbial utilisation (Fig. 1A). While the negative real PE suggested that FYM did not enhance the degradation of native SOM, despite the input of exoenzymes from the FYM itself (Fig. 1B). While little research has focused on the PE of FYM specifically, studies have shown that FYM addition generally leads to higher soil organic C stocks supporting the evidence presented here (Abdalla et al., 2022; Bei et al., 2022; Gross and Glaser, 2021).

*4.1.4. Wheat straw and shoots*

Straw and shoots are substrates with contrasting C:N ratios and nutrient content and represent common inputs to agricultural soils during tillage. Straw, in particular, is often used as a ‘classical’ substrate for soil priming experiments (Chen et al., 2021; Mo et al., 2021; Ye et al., 2015). Here, we showed there was a relationship between the proportion of straw (relatively low utilizablity) to shoots (relatively higher utilizablity), with higher proportions of shoots to straw increasing the real and apparent PE (Fig. 1A and B). Fresh plant biomass input, and its associated input of labile nutrients, has been shown to increase microbial biomass turnover inducing positive apparent priming in the short term (Bernard et al., 2022; Chen et al., 2019). Significant real priming is also likely to have been driven by the fact that many of the extracellular enzymes required to break down the plant matter are also able to mineralise, particularly the labile (particulate) fraction, of SOM (Witzgall et al., 2021). Once the production of these enzymes has been stimulated by the additions of fresh organic matter, their presence continues to mineralise the native SOM.

*4.2. Drivers of the PE*

The results of this study, comparing real and apparent priming effects of a diverse range of common soil amendments as substrates, suggest that no one single soil or substrate quality indicator could adequately explain the observed real or apparent priming effects observed across the substrates. Previously, C:N ratios of substrates have been suggested as a predictor of priming (Qiao et al., 2016; Liu et al., 2020), our results are consistent with this (Table 2). However, the relationship is stronger for the apparent PE than the real PE. We suggest that in addition to the C:N ratio the usability and quality of the substrate (i.e., its immediate accessibility and energy cost and return on investment (Bernard et al., 2022) by the microbial biomass is key to determining its PE. With the more labile material (or utilizable; Kuzyakov and Bol, 2006), there is likely to be less positive priming as microbial communities use this as metabolic fuel rather than native organic matter. However, this will in turn affect the stoichiometry of the soil and microbial biomass, which may lead to priming through continued mineralisation of the native SOM in order to redress the balance. This is difficult to interpret from our data, as it is likely to be more evident as a flux, rather than a snapshot of the soil stoichiometry. Also key to this stoichiometric ratio is the concentration of P, however, in the real priming soils in this study available P levels were low, while there was some addition from P rich substrates (biochar, FYM, slurry and wood ash), there may also be some evidence of P mining from the SOM in treatments with high N loading (inorganic N, and protein) (Meyer et al., 2018).

While priming is generally considered to be a short-term phenomenon, the majority of priming studies including this one, are performed under controlled laboratory conditions and over short time periods and therefore may be only partially representative of real-world conditions. Our data suggests that the real PE of substrates may in fact not be a short-lived phenomenon, in most cases persisting beyond 50 d (Fig. S1 + S2).

Arguably, it is important to contextualise the soil PE with the increase in total soil C and the stability of the initial treatment and the potential for organic matter formation from the mineralisation and metabolic turnover of the treatment, as this will affect the overall stability and permenance of C within the soil system (Raczka et al., 2021). Additionally, we suggest that combining measurements of the PE with functional measurements of microbial activity, for example, untargeted metabolomics, may allow us to elucidate the fundamental biochemical mechanisms of priming and the metabolic products involved (from which stability can be inferred; Kallenbach et al., 2016).

*4.3. Limitations*

The findings of this study must be caveated as SOM is a complex substrate, with different pools possessing varying C residence times. After 5-years, the majority of the 14C-labelled compounds were still assumed to be distributed in less stable and persistent fractions of SOM (Cotrufo et al., 2022; Islam et al., 2022). This was confirmed by the distribution of 14C in soil aggregates (Table 2), with significantly more 14C activity associated with the soil large macro-aggerates, in which C is generally considered to be more susceptible to change than micro-aggregates (Cambardella and Elliot, 1993; Zhou et al., 2020). Thus, the results presented here are representative of priming in relatively young (< 10 years) SOM, but not necessarily of priming of much older SOM (e.g., MAOM). As the specific activity of the MBC was not quantified here, we cannot directly compare the magnitude of real and apparent PE, although this should be the aim of future work.

**5. Conclusions**

To conclude, using a range of common amendments and substrates in soil, we found that the magnitude of effect of real (mineralisation of native SOM) and apparent (microbial C metabolism) priming varied across various common soil treatments. Therefore, care should be taken when interpreting real and apparent priming effects. While there was little evidence of drivers of this effect from our data, a combination of soil and microbial stoichiometric balance, substrate quality and accessibility or ‘microbial energy cost and return on investment’, seem to be broadly driving priming effects. Equally, treatments that illicit priming may still be replenishing or increasing soil C stocks particularly at higher loading rates; however, the extent of priming loss will affect the overall balance of C accumulated from a particular treatment. As such, in future studies more emphasis should be put on the overall stability and permanence of both the C being added, and products formed during microbial metabolism.

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**CRediT author statement**

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**Table and figure captions and labels**

**Fig. 1.** Changes in soil real and apparent priming effects in response to the addition of different C substrates in an agricultural grassland soil (Eutric Cambisol). Panel A – Apparent priming effect, representing changes in microbial C metabolism for a range of common C substrates. The soil microbial biomass was labelled using a 14C-glucose solution 24 h before application of substrates. Panel B – Real priming effect, representing changes in soil organic matter. The soil was 14C-labelled in the field 5 years previously and thus represented quasi-stable 14C-labelled SOM. Priming effects were calculated as the change in 14CO2 production relative to the control soil (no treatment). Letters indicate post hoc significant differences between treatment groups (*p* < 0.05; *n* = 4).

**Table 1.** Characteristics of the soils and treatments used in this study. Values are expressed on a mean dry soil weight basis ± SEM (*n* = 4). Letters denote significant differences between treatments using a Kruskal-Wallis with Dunn post-hoc test and Bonferroni correction (*p* < 0.05). Treatment C:N (*n* = 1).

**Table 2.** 14C activity by aggregate fraction of 14C-labelled in the field 5 years previously (quasi-stable 14C-labelled SOM). Values are expressed on a mean dry soil weight basis ± SEM (*n* = 4). Letters indicate post hoc significant differences between treatment groups (*p* < 0.05).

**Table 3.** Kendall rank correlation of selected soil properties in relation to both the apparent priming and real priming of samples. Numbers represent τ values, NS signifies not significant (i.e., *p* > 0.05).

**Fig. 1.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Apparent priming soil** | | | | | | | | | | | | | | |
|  | **Biochar** | **Wood ash** | **Amino acids** | **Protein** | **Glucose** | **Cellulose** | **FYM** | **Slurry** | **Inorganic N** | **100/0** | **75/25** | **50/50** | **25/75** | **0/100** | **Control** |
| pH | 6.54 ± 0.10a | 4.89 ± 0.06cdefg | 4.26 ± 0.10h | 4.46 ± 0.02gh | 5.04 ± 0.07abcd | 5.05 ± 0.02abcd | 5.18 ± 0.23abcd | 4.99 ± 0.11bcde | 4.50 ± 0.01fgh | 5.23 ± 0.04ab | 5.09 ± 0.05abc | 5.24 ± 0.17abc | 4.90 ± 0.02cdef | 4.83 ± 0.03defgh | 4.83 ± 0.03efgh |
| EC (μS cm−1) | 330 ± 42abcd | 258 ± 8cbe | 467 ± 73ab | 387 ± 8abc | 118 ± 22hi | 86 ± 25i | 284 ± 33bcd | 312 ± 40abcd | 496 ± 24a | 94 ± 4i | 141 ± 9ghi | 165 ± 12fghi | 219 ± 3defg | 254 ± 4def | 189 ± 13efgh |
| SOM (%) | 7.9 ± 0.1abc | 7.5 ± 0.1bc | 8.2 ± 0.3abc | 7.6 ± 0.1abc | 7.4 ± 0.0c | 7.6 ± 0.1bc | 7.8 ± 0.2abc | 7.9 ± 0.2abc | 7.5 ± 0.0c | 8.0 ± 0.3abc | 7.6 ± 0.14abc | 8.2 ± 0.1ab | 8.6 ± 0.4ab | 8.4 ± 0.1a | 7.9 ± 0.2abc |
| Extractable NH4+ (mg N kg−1) | 1.7 ± 0.2fgh | 1.5 ± 0.1gh | 20.4 ± 3.5abc | 20.9 ± 4.1ab | 2.0 ± 0.1efg | 2.3 ± 0.1def | 2.5 ± 0.2de | 2.5 ± 0.2de | 38.9 ± 4.6a | 1.1 ± 0.2h | 2.7 ± 0.3bcde | 3.6 ± 1.3cde | 1.3 ± 0.4gh | 4.5 ± 0.8abcd | 1.3 ± 0.1h |
| Extractable NO3− (mg N kg−1) | 79 ± 2abcd | 78 ± 5abcde | 147 ± 30abc | 169 ± 5ab | 48 ± 9defg | 40 ± 2efg | 63 ± 12cdef | 79 ± 17abcde | 189 ± 3a | 17 ± 2g | 34 ± 5fg | 39 ± 5efg | 56 ± 2defg | 80 ± 4abcde | 77 ± 7bcde |
| Extractable P (mg P kg−1) | 19.3 ± 1.7ab | 4.9 ± 0.2abcd | 28.2 ± 1.6a | 4.4 ± 0.2de | 4.0 ± 0.1de | 3.9 ± 0.1e | 11.0 ± 3.2abc | 7.3 ± 0.7abc | 4.3 ± 0.2de | 4.5 ± 0.1cde | 4.9 ± 0.4bcde | 5.1 ± 0.3abcd | 5.2 ± 0.6abcd | 4.5 ± 0.2cde | 4.6 ± 0.4de |
| C:N ratio | 12 ± 1a | 10 ± 0abc | 10 ± 0bc | 10 ± 0abc | 10 ± 0abc | 10 ± 0bc | 10± 0abc | 11 ± 0abc | 9 ± 0c | 11 ± 0a | 11 ± 0ab | 10 ± 0abc | 10 ± 0abc | 10 ± 0abc | 10 ± 0abc |
| Dissolved organic C (mg C kg−1) | 154 ± 5bcde | 132 ± 10cde | 213 ± 22abcde | 250. ± 30abc | 242 ± 8abcd | 284 ± 26ab | 279 ± 9ab | 265 ± 9abc | 231 ± 12abcd | 295 ± 13a | 310 ± 13a | 295 ± 10a | 167 ± 96abcd | 2 ± 0de | 2 ± 0e |
| Microbial PLFA biomass (μmol PLFA kg−1) | 202 ± 4cdef | 173 ± 2gh | 206 ± 1bcde | 177 ± 1fgh | 211 ± 4abcd | 199 ± 2defg | 204 ± 6cde | 215 ± 5abcd | 173 ± 5h | 218 ± 3abc | 232 ± 3a | 226 ± 7ab | 245 ± 14a | 231 ± 4a | 195 ± 2efgh |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | **Real priming soil** | | | | | | | | | | | | | | |
|  | **Biochar** | **Wood ash** | **Amino acids** | **Protein** | **Glucose** | **Cellulose** | **FYM** | **Slurry** | **Inorganic N** | **100/0** | **75/25** | **50/50** | **25/75** | **0/100** | **Control** |
| pH | 6.37 ± 0.03a | 5.55 ± 0.06cd | 5.69 ± 0.14bc | 4.80 ± 0.02e | 6.00 ± 0.06ab | 5.87 ± 0.06ab | 5.74 ± 0.04abc | 5.70 ± 0.06bc | 5.10 ± 0.20de | 5.70 ± 0.02bc | 5.73 ± 0.02abc | 5.41 ± 0.04cde | 5.39 ± 0.01cde | 5.44 ± 0.13cd | 5.19 ± 0.01de |
| EC (μS cm−1) | 261 ± 7ab | 216 ± 7abc | 144 ± 27cde | 428 ± 17a | 53 ± 4f | 57 ± 4f | 184 ± 6cd | 211 ± 27bc | 433 ± 22a | 72 ± 0ef | 103 ± 2def | 161 ± 18cde | 179 ± 23bcd | 168 ± 15cde | 207 ± 3bc |
| SOM (%) | 7.4 ± 0.2 | 7.1 ± 0.1 | 7.2 ± 0.1 | 7.7 ± 0.2 | 7.5 ± 0.1 | 7.7 ± 0.1 | 8.1 ± 0.6 | 7.5 ± 0.2 | 7.6 ± 0.1 | 7.5 ± 0.2 | 7.6 ± 0.1 | 7.8 ± 0.4 | 7.3 ± 0.2 | 7.9 ± 0.2 | 7.7 ± 0.1 |
| Extractable NH4+ (mg N kg−1) | 0.4 ± 0.1cd | 0.5 ± 0.1cd | 19.3 ± 7.5ab | 4.4 ± 0.8ab | 1.8 ± 0.6abc | 0.3 ± 0.1d | 1.7 ± 0.5abcd | 1.0 ± 0.2abcd | 20.7 ± 6.8a | 0.9 ± 0.22bcd | 1.37 ± 0.4abcd | 1.4 ± 0.5abcd | 0.5 ± 0.4cd | 1.8 ± 1.1abcd | 0.7 ± 0.2cd |
| Extractable NO3− (mg N kg−1) | 49.0 ± 3.4abc | 52.8 ±5.8ab | 14.9 ± 7.3de | 100.1 ± 5.1a | 2.7 ± 0.7ef | 4.1 ± 0.9def | 13.0 ± 2.3bcd | 25.8 ± 9.2bcd | 131.2 ± 12.5a | 1.8 ± 0.5bcd | 4.0 ± 0.8def | 17.7 ± 8.8cd | 24.6 ± 11.6bcd | 12.9 ± 2.9f | 60.5 ± 2.3a |
| Extractable P (mg P kg−1) | 10.1 ± 2.4a | 1.0 ± 0.9abc | 0.0 ± 0.0c | 1.1 ± 0.5abc | 0.0 ± 0.0c | 0.1 ± 0.1c | 8.1 ± 5.4ab | 0.5 ± 0.4abc | 0.4 ± 0.4bc | 0.3 ± 0.3bc | 0.3 ± 0.3bc | 0.0 ± 0.0c | 0.0 ± 0.0c | 0.0 ± 0.0c | 0.0 ± 0.0c |
| C:N ratio | 11 ± 0abc | 10 ± 0abc | 10 ± 0bc | 10 ± 0c | 10 ± 0abc | 10 ± 0abc | 14 ± 4ab | 12 ± 13a | 10 ± 0bc | 12 ± 1a | 11 ± 0ab | 11 ± 1abc | 10 ± 0abc | 10 ± 1abc | 10 ± 0abc |
| Dissolved organic C (mg C kg−1) | 126 ± 9 | 130 ± 6 | 162 ± 19 | 166 ± 8 | 179 ± 19 | 156 ± 4 | 125 ± 4 | 127 ± 9 | 147 ± 3 | 157 ± 9 | 161 ± 10 | 152 ± 11 | 166 ± 23 | 138 ± 12 | 136 ± 4 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | **Treatments** | | | | | | | | | | | | | | |
|  | **Biochar** | **Wood ash** | **Amino acids** | **Protein** | **Glucose** | **Cellulose** | **FYM** | **Slurry** | **Inorganic N** | **100/0** | **75/25** | **50/50** | **25/75** | **0/100** | **Control** |
| Initial C:N ratio | 73 | 90 | 3 | 3 | 0 | 0 | 18 | 17 | 0 | 67 | 26 | 16 | 12 | 10 | N/A |

**Table 1.**

**Table 2.**

|  |  |
| --- | --- |
|  | **Bq g-1** |
| Micro-aggregates (< 0.25 mm) | 72.4 ± 1.8a |
| Small macro-aggregates (2 – 0.25 mm) | 93.0 ± 8.8ab |
| Large macro-aggregates (> 2 mm) | 125.0 ± 14.3b |

|  |  |  |
| --- | --- | --- |
|  | **Apparent priming** | **Real priming** |
| pH | -0.20 | NS |
| EC | 0.23 | -0.33 |
| SOM | NS | NS |
| DOC | NS | 0.17 |
| Soil C:N ratio | -0.24 | NS |
| PLFA microbial biomass | NS | - |
| Initial C:N ratio of treatment | -0.70 | -0.35 |

**Table 3.**