DR. LOIC NAZARIES (Orcid ID : 0000-0001-7006-4964)

DR. BHUPINDER PAL SINGH (Orcid ID : 0000-0003-1952-9070)

Article type : Primary Research Articles

Title: Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils

Running head: Microbial mechanisms of SOC priming by wheat residue

Yunying Fang [a], Loïc Nazaries [b], Brajesh K. Singh [b,c], Bhupinder Pal Singh [a,]*

[a] NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568, Australia

[b] Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia

[c] Global Centre for Land-Based Innovation, Western Sydney University, Penrith, NSW 2751, Australia.

*Corresponding author

E-mail: bp.singh@dpi.nsw.gov.au

Tel: +61246406406; Fax: +61246406300;

Key words: ^{13}C isotope, extracellular enzyme activity, enzymatic stoichiometry, copiotrophs, oligotrophs, gene abundance

Paper type: Primary Research

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/gcb.14154

Abstract

Agronomic practices such as crop residue return and additional nutrient supply are recommended to increase soil organic carbon (SOC) in arable farmlands. However, changes in the priming effect (PE) on native SOC mineralization in response to integrated inputs of residue and nutrients are not fully known. This knowledge gap along with a lack of understanding of microbial mechanisms hinders the ability to constrain models and reduce the uncertainty to predict carbon (C) sequestration potential. Using a ^{13}C-labelled wheat residue, this 126-day incubation study examined the dominant microbial mechanisms that underpin the PE response to inputs of wheat residue and nutrients (nitrogen, phosphorus and sulfur) in two contrasting soils. The residue input caused positive PE through "co-metabolism", supported by increased microbial biomass, C and nitrogen (N) extracellular enzyme activities (EEAs), and gene abundance of certain microbial taxa (Eubacteria, β-Proteobacteria, Acidobacteria, and Fungi). The residue input could have induced nutrient limitation, causing an increase in the PE *via* "microbial nutrient mining" of native soil organic matter, as suggested by the low C-to-nutrient stoichiometry of EEAs. At the high residue, exogenous nutrient supply (*cf.* no-nutrient) initially decreased positive PE by alleviating nutrient mining, which was supported by the low gene abundance of Eubacteria and Fungi. However, after an initial decrease in PE at the high residue with nutrients, the PE increased to the same magnitude as without nutrients over time. This suggests the dominance of "microbial stoichiometry decomposition", supported by higher microbial biomass and EEAs, while Eubacteria and Fungi increased over time, at the high residue with nutrients *cf*. no-nutrient in both soils. Our study provides novel evidence that different microbial mechanisms operate simultaneously depending on organic C and nutrient availability in a residue-amended soil. Our results have consequences for SOC modelling and integrated nutrient management employed to increase SOC in arable farmlands.

Introduction

Soil organic matter (SOM) is a key component of ecosystem functionality, and crucial for enhancing soil sustainability and productivity in cropping systems (Pankhurst *et al.*, 2002; Reeves, 1997). Also, SOM represents the largest carbon (C) stock in global terrestrial ecosystems holding approximately 1,550 Gt C (up to 1 m depth) (Lal, 2008). Globally, the return of crop straw residues in soils has been recommended in field practices with the aim to improve soil organic C (SOC) stocks, soil structure, and plant available nutrients (Conteh *et al.*, 1998; Han *et al.*, 2016; Kumar & Goh, 2000; Thompson, 1992). The input of crop residue along with major nutrients [such as nitrogen (N), phosphorus (P) and/or sulfur (S)] in croplands was reported to either increase or decrease SOC stocks (Han *et al.*, 2016; Khan *et al.*, 2007; Russell *et al.*, 2005). Further, small changes in SOC stocks in agroecosystems could result in significant impacts on atmospheric C concentration at a global scale (Stockmann *et al.*, 2013). One mechanism attributed to the change in SOC stocks is that crop residue input in soil affects the magnitude of microbial mineralization of native SOC, an effect known as "priming effect" (PE), *i.e.* an increase or decrease in SOC mineralization, relative to no residue input, termed as positive or negative PE, respectively (Fontaine *et al.*, 2003; Kuzyakov *et al.*, 2000). However, despite the intensive interest in the priming of SOC mineralization, and the importance of microorganisms to soil C cycling (Blagodatskaya & Kuzyakov, 2008; Carney *et al.*, 2007; Rousk *et al.*, 2015), there is still a lack of understanding of the interactive impact of residue and nutrient inputs on the explicit link between PE and changes in soil microbial activity or community composition (Chen *et al.*, 2014; Kirkby *et al.*, 2014).

Accelerated microbial activity with the input of labile organic matter (LOM), such as glucose and crop residues, may enhance SOC mineralization as a result of "co-metabolism" and higher enzyme production (Blagodatskaya & Kuzyakov, 2008). The PE could be positively, if not linearly, related to the amount of LOM added to soil (Liu *et al.*, 2017; Paterson & Sim, 2013). There could also be changes in the direction and magnitude of the PE on SOC mineralization by the input of residue-

derived LOM over time, *e.g.* switching from a negative to a positive PE rate on native SOC, and increased (Wang *et al.*, 2015) or decreased magnitude of positive PE rate (Nottingham *et al.*, 2012). Changes in the magnitude (and direction) of PE were suggested to be due to variations in microbial activity and community as a response to the altered amount of crop residue input in agroecosystems (Blagodatskaya & Kuzyakov, 2008). Further, soil nutrition was reported to have variable impacts on PE induced by crop residue: (i) nutrient inputs (such as N and/or P) had a minimal effect on the magnitude of positive PE induced by maize residue over a short-term period (< 1-month) (Chen *et al.*, 2014), or (ii) urea-N input decreased the magnitude of positive PE caused by maize residue, and the PE then turned to negative after a 2-month period (Qiu *et al.*, 2016). Identifying the direction and magnitude of PE in response to different levels organic C and mineral nutrients in soils are critical for improving management practices to minimize SOC loss and thus to enhance SOC storage (Han *et al.*, 2016), with potential to offset greenhouse gas emissions (Smith *et al.*, 2008).

Furthermore, although there have been a number of studies on the dynamics of PE, the microbial mechanisms underlying PE in response to varied input levels of crop residues and mineral nutrients are still unclear. Two competing microbial theories have been proposed to explain PE caused by the combined inputs of mineral nutrients and LOM: "microbial nutrient mining" and "microbial stoichiometry decomposition". In the case of the former, the nutrient demand for supporting microbial growth in a C-rich and nutrient-poor system leads to an enhanced SOC mineralization (positive PE), called "nutrient mining" (Chen *et al.*, 2014; Fontaine *et al.*, 2011; Hart *et al.*, 1986). That is, when there is not sufficient exogenous nutrient supply but an increase in labile C input, microorganisms would invest in extracellular enzyme nutrient acquisition activities, targeting nutrient (*i.e.* N)-rich SOM. This would then enhance the decomposition of native SOM in response to higher nutrient requirements of microorganisms, for example, following the input of labile residues with a high C-to-nutrient ratio (*i.e.* microorganisms would 'mine' native SOM to obtain nutrients). In other words, the nutrient mining is likely to decrease C-to-nutrient stoichiometry of enzyme activities (Waring *et al.*, 2014). Differing from the "microbial nutrient mining" theory, the "microbial

stoichiometry decomposition" theory suggests that native SOM decomposition can be enhanced when nutrient limitation is alleviated. For example, the combined supply of N, P and/or S (in sufficient quantities) along with C-rich residues may stimulate the growth and activity of contrasting microbial communities (Gulis & Suberkropp, 2003), with changes in microbial C:N:P:S stoichiometry during decomposition (Heuck *et al.*, 2015). In turn, this would have the potential to enhance mineralization of native SOC and residue-C (Hessen *et al.*, 2004; Schneider *et al.*, 2012), while expediting the fate of organic C in stable pools of SOM with a constant C-to-nutrient ratio (Kirkby *et al.*, 2013).

When LOM resources are added and become abundant in soil, there could be an activated growth of both r-strategists (mainly copiotrophs) and K-strategists (such as oligotrophs) from a dormant state (Hu *et al.*, 1999). In the nutrient-poor environment, oligotrophs may facilitate the use of nutrient-rich native SOM, leading to "microbial nutrient mining" (Fierer *et al.*, 2007; Kaiser *et al.*, 2014). Alternatively, "microbial stoichiometry decomposition" assumes that microbial stoichiometry is a driving force, meaning that high nutrient availability may accelerate mineralization of both LOM and native SOM due to an increased growth of all microbial communities, including β-Proteobacteria and oligotrophs (such as Acidobacteria and Fungi), thus contributing to a longer phase of PE (Blagodatskaya & Kuzyakov, 2008; Chen *et al.*, 2014; Fierer *et al.*, 2007; Fontaine *et al.*, 2011; Ramirez *et al.*, 2012). Therefore, positive PE could be dominated by "microbial nutrient mining" of SOM under nutrient limitation, but under sufficient availabe nutrients, either through exogenous input or released *via* mineralization and mobilization from the soil reserves (Kaiser *et al.*, 2014; Sarker *et al.*, 2018), "microbial stoichiometry decomposition" of SOM can become a dominant mechansim over time. Nevertheless, these contrasting microbial theories of PE are not fully validated yet with regards to the linkage between PE and microbial community growth strategies, extracellular enzyme activities (EEAs) under integrated nutrient management in contrasting soils (Blagodatskaya & Kuzyakov, 2008; Chen *et al.*, 2014; Schimel & Weintraub, 2003). Addressing these knowledge gaps relating to microbial mechanisms for the PE is critical to improve prediction of SOC

models and to identify appropriate stubble and nutrient management practices, which can increase SOC in arable farmlands.

The objective of this study was to identify the influence of the concurrent input of crop residue and nutrients at different levels on the PE, and how shifts in soil microbial activity and abundance might affect the PE. A 126-day laboratory-based incubation experiment was conducted using two soils, a Luvisol and a Vertisol, under optimal temperature and moisture conditions. Each of these two soils were collected separately from two long-term management field sites and supplied with wheat stem residue and nutrients (N, P and S) at two input levels (low or high). Three hypotheses were proposed:

(i) Crop residue input is the dominant factor of PE, and the magnitude of PE increases with the increased level of residue input across the two soil types;

(ii) "Microbial nutrient mining" would dominate under nutrient limitation (no nutrient input) to enhance positive PE during the early incubation stage; and

(iii) "Microbial stoichiometry decomposition" would become a dominant mechanism to enhance positive PE with time, particularly under sufficient concurrent inputs of residue and nutrients.

Materials and Methods

Soil collection and processing

Soils were collected at 0−10 cm depth from two long-term trials in Australia: (i) a mixed farming systems trial at Condobolin, New South Wales (33°05'19''S 147°08'58'' E); and (ii) a tillage-stubble-N fertilization trial at Hermitage, Queensland (28°12'S, 152°06'E). The detailed crop rotation and tillage management information is provided in Supporting Information (SI). The soil at the Condobolin site is classified as a Luvisol and the soil at the Hermitage site is classified as a Vertisol as per the World Reference Base (FAO, 2006). Air-dried soils were passed through a 12-mm sieve, followed by a 6.5 mm sieve, by gently breaking the cores along planes of weakness by hand, thus to preserve soil

aggregation. Any recognizable gravels and debris (≥ 2 mm) were carefully removed. Basic soil properties are shown in Table 1.

Soil pH was determined in 1:5 soil:water ratio using a pre-calibrated pH electrode. Soil texture (sand/silt/clay content) was determined using a modified version of the standard hydrometer method with removal of carbonates or organic matter (Gee *et al.*, 1986). The soils were fine-ground to < 53 μm and analyzed for C, N and $\delta^{13}C$ at the University of California's Stable Isotope Facility using an Elementar Vario EL Cube or Micro Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The standard deviations of laboratory analyses were 0.02‰ for $\delta^{13}C$.

Wheat residue and nutrient input

The ^{13}C-enriched wheat stem (*Triticum aestivum* L.) residue was collected from a $^{13}CO_2$ pulse-labelling (99.0 atom% ^{13}C) experiment at Condobolin in Australia, as described in Fang *et al.* (2016). The $^{13}CO_2$ pulse-labelling was conducted during crop flowering and then the aboveground plants were harvested after 50 days (*i.e.* from flowering to grain maturity) and dried at 60°C. Within this time period after labelling, the $\delta^{13}C$ signatures among different aboveground parts reached an equilibrium phase, with stems being highly ^{13}C-enriched, relative to leaves (Fang *et al.*, 2016). Thus, leaves and reproductive parts were removed to minimize non-uniformity of the $\delta^{13}C$ in the selected stem residue for this experiment. The stems were cut into pieces and then ground with a mill (Retsch Ultra Centrifugal Mill ZM 200) to < 2 mm size prior to mixing with soil. A representative portion of the stem residue was finely ground (< 125 μm) using a MM400 Mixer Mill grinder (Retsch GmbH, Haan, Germany), re-dried at 60°C and analyzed for chemical properties (Table 1).

To assess the level of non-uniformity of $\delta^{13}C$ in the stems, a hot water extraction method was used to isolate relatively labile metabolic organic components (extraction at 60°C for 30 min; labile organic C; LOC) and residual structural organic components (recalcitrant organic C; ROC) (Haddix *et al.*, 2016). The extract was filtered through a 20 μm nylon mesh filter and freeze-dried. The remaining residues were rinsed with Reverse Osmosis (RO) water and oven dried at 60°C. The weight proportion of LOC and ROC were 20.07 ± 0.86% and 79.93 ± 0.86%, respectively. Representative subsamples of the ground stems, the LOC, or ROC (< 125 μm) were analyzed for C and $\delta^{13}C$ at the University of California's Stable Isotope Facility. In the stems, total phosphorus (P) was determined by the molybdenum-blue method on a SEAL Discrete Autoanalyser after Kjeldahl digestion, and total nitrogen (N) and sulfur (S) were analyzed by Elementar Vario EL cube CHNS analyzer.

The nutrients (N, P and S) were added along with the C-rich wheat residue at different C-to-nutrient stoichiometric ratios, with a potential to enhance conversion of the residue-C into relatively stable SOC pools (Kirkby *et al.*, 2013). The low or high nutrient input levels of N, P and S (Table 2) were based on the assumption of an additional 10 or 30% conversion of the wheat residue to a stabilized form of SOM with the commonly assumed C: N: P: S stoichiometry of 1: 0.0833: 0.02: 0.0143 (Kirkby *et al.*, 2013; Lal, 2014). Nitrogen, P and S were added from NH_4NO_3, KH_2PO_4 and $(NH_4)_2SO_4$, respectively. The nutrient solution was adjusted to pH 7 using a 10 M NaOH solution. In this study, the inherent availability of nutrients in the residue and soils was disregarded.

Incubation experiment

The soils from the field replicated (n = 4) plots of each site were separately incubated at 22 ± 0.5°C for a total of 126 days after the varied input levels of the residue and nutrients (Table 2). Before the start of the incubation, the wheat residues were gently and uniformly mixed at two levels, *i.e.* 6.7 g

and 20.0 g kg^{-1} (dry soil weight) in each soil, corresponding to 4 and 12 t ha^{-1} to a depth of 5 cm (bulk density of 1.2 t m^{-3}). There were a total of seven treatments for each soil, that is, low and high input levels of residues with and without two levels of nutrients, plus a control without nutrient and residue inputs (Table 2). The RO water without any external nutrient inputs and/or nutrient solution was added to the soil or soil-residue mixtures to obtain a 60% of maximum water holding capacity (WHC). A CO_2 trap (30 mL of 2 or 3 M NaOH in a 70-mL jar) and a jar of RO water (20 mL water in a 40-mL jar) were placed in each incubation bucket to absorb the CO_2 produced and maintain a constant humidity, respectively. The RO water was periodically added to the incubated soils to maintain soil moisture at 60% of WHC. To account for the headspace CO_2, triplicate blank buckets containing only CO_2 trap and RO water were also incubated. The CO_2 traps were changed at days 3, 10, 30, 63, 93 and 126 for total CO_2-C and $\delta^{13}C$ analyses. Soil sub-samples were also taken at days 10, 30 and 126 for microbial biomass C (MBC), enzyme activity and gene abundance analyses.

Total carbon, native SOC mineralization and priming effect

The total mineralized CO_2-C from various treatments was measured by an acid–alkali titration method. The $\delta^{13}C$ analysis of CO_2 trapped in NaOH was performed *via* a Cavity Ring-down Spectrometry using a PICARRO G2131-*i* Analyzer (California, USA). Further details of the procedure for total CO_2-C and $\delta^{13}C$ analysis are reported in the SI and Fang *et al.* (2016).

There was a 3–12% difference between the $\delta^{13}C$ of LOC (433.29 ± 1.25‰) and ROC (510.07 ± 4.55‰) components in the wheat residue, relative to the bulk residue $\delta^{13}C$ (494.04 ± 2.06‰), likely due to the non-uniform ^{13}C labelling of various organic compounds in the residues. This ^{13}C non-uniformity of the labelled stems was similar to the commonly used C_3-plant materials at natural abundance for C-source partitioning, where $\delta^{13}C$ of various organic compounds could differ, *e.g. by* –2 to 4‰, equivalent to 8–16% variations, relative to bulk residue $\delta^{13}C$ (e.g. –25‰) (Fernandez *et al.*, 2003;

Glaser, 2005). Admittedly, the use of initial bulk residues $\delta^{13}C$, as an end member in the two-pool isotope-mixing model, would cause some bias in C-source partitioning due to the isotopic C variations and fractionation (Fernandez *et al.*, 2003; Schweizer *et al.*, 1999). Hence, in the current study, to minimize the bias in C-source partitioning, we used the moving $\delta^{13}C$ of the stem residue (analyzed during incubation in the sand) (Table S1) and soil respired CO_2 from the control treatment over time (Fig. S1), as end members in the two-pool C isotope mixing model (Singh *et al.*, 2015). Further, the alkali trapping of CO_2 released from various organic compounds between the two sampling times, which increased during incubation, would further minimize the effect of $\delta^{13}C$ variations across the compounds on the partitioning of residue–soil C sources.

The two-pool C isotope mixing model used in the current study is:

$$f_S = \frac{{\delta_T}^{13}C_t - {\delta_R}^{13}C_t}{{\delta_S}^{13}C_t - {\delta_R}^{13}C_t} \quad (1)$$

where, f_S is the fraction of CO_2-C evolved from soil in the presence of residues. The ${\delta_T}^{13}C_t$ and ${\delta_S}^{13}C_t$ are the $\delta^{13}C$ value of the total CO_2-C evolved from the residue incorporated and control (soil only) treatments over time, respectively (Fig. S1). The ${\delta_R}^{13}C_t$ was obtained by incubating wheat stem residue (< 2 mm) in a mesh bag in the sand, along with microbial inoculum from the soils (presented in the SI) and nutrient input *via* a Hoagland solution (Sigma-H2395, Sigma-Aldrich), and $\delta^{13}C$ in the residue was analyzed at days 3, 10, 30, 63, 93 and 126. To assess the uncertainty in the C source partitioning, first order Taylor series approximations of the variance of f_S was also calculated using partial derivatives (Phillips & Gregg, 2001); see SI.

The priming effect (PE) of native SOC induced by crop residue input was calculated as:

$$PE = C_{R,SOC} - C_{C,SOC} \quad (2)$$

where, $C_{R,SOC}$ is the amount (mg C kg^{-1} soil) of CO_2–C evolved from soil in the presence of residues (with or without nutrients) and $C_{C,SOC}$ is the amount (mg C kg^{-1} soil) of CO_2–C evolved from the control soil (no residue and nutrients). The primed native SOC mineralization was normalized to per gram of initial native SOC.

To explore the uncertainty that may have occurred while quantifying priming of SOC because of using different residue-$\delta^{13}C$ end members ($\delta_R{}^{13}C$) for C-source partitioning, we did a sensitivity analysis by considering three additional alternative scenarios. That is, we considered two scenarios of extreme positive priming caused by the (a) LOC pool ($\delta^{13}C$: 433.29‰) and (b) ROC pool ($\delta^{13}C$: 510.07‰), and (c) the third scenario was the use of initial bulk residue ($\delta^{13}C$: 494.04‰) throughout. Details of the sensitivity analysis were described in the SI. In particular, the use of initial bulk-residue-$\delta^{13}C$ as an end member is a common approach in C-source partitioning by the two-pool model, for example, the use of natural C_3 and C_4 C-derived sources (Chen *et al.*, 2014).

Residue-C mineralization was calculated by subtracting soil-derived C mineralization from the total C mineralization. The amount of primed SOC per unit of residue-C mineralized over time (126 days) was calculated as a function of nutrient and residue input levels for each soil.

Microbial biomass carbon (MBC)

During the 126-day incubation period, the soil samples were analyzed for MBC on day 10, 30 and 126 by a fumigation-extraction method (Vance *et al.*, 1987; Wu *et al.*, 1990) as per the procedure described in detail in the SI. A conversion factor of 0.45 was applied to determine microbial biomass C (Wu *et al.*, 1990).

Soil microbial community abundance analysis (quantitative PCR)

Soil (~ 0.50 g for Luvisol; ~ 0.25 g for Vertisol) from each sample was used for DNA extraction by using a soil DNA isolation kit (PowerSoil, Mobio). DNA concentrations were measured with a spectrophotometer (Nano-Drop® ND-2000c, NanoDrop Technologies). All samples were then standardized to the same concentration of ~ 10 ng μL^{-1}. A modified method from Fierer *et al.* (2005) was applied to simultaneously quantify the microbial abundance of four major taxa (Eubacteria, Acidobacteria, β-Proteobacteria and Fungi). Optimization procedures were performed in order to simultaneously amplify the four target genes under the same conditions. For the reaction mix, the following final concentrations were used in a 10-µL reaction: 0.7 µM of each primer, 1X of LightCycler® 480 SYBR Green I Master (Roche Applied Science) and 0.8 ng μL^{-1} of soil DNA template. The cycling conditions were like this: pre-incubation at 95°C for 5 min; amplification through 40 cycles of 95°C for 15 sec, 57°C for 30 sec and 72°C for 30 sec; melting curve generation at 95°C for 5 sec and 65°C for 1 min; and cooling period at 40°C for 10 sec. Reactions were prepared under sterile conditions using a QIAgility® automated PCR set-up robotic workstation (Qiagen, software version 4.55.1) in a 384-well plate (FrameStar® 4titude) and run on a LightCycler® 480 Multiwell Plate 384 system (Roche Applied Science). Calibration curves were prepared in triplicate for each of the four target taxon genes from a stock solution generated by cloning PCR fragments obtained from soil samples and insertion into a pUC19 plasmid (TOPO cloning kit, Invitrogen). The R^2 and PCR efficiency

values of the linear regression for each calibration curve were, respectively, the following: Eubacteria (0.96 and 115%), Acidobacteria (0.98 and 83%), β-Proteobacteria (0.98 and 97%) and Fungi (0.93 and 93%). No-template controls (NTCs) were also run in triplicates while duplicates were used for samples. Melt peaks were checked for non-specific amplification. The (average) C_q values for each target taxon gene (and their corresponding NTC) were, respectively, as follows: Eubacteria (12 and 14), Acidobacteria (15 and 34), β-Proteobacteria (19 and 34) and Fungi (23 and 39) (Table S2). The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (Bustin *et al.*, 2009; 2013) were respected for quality and reporting purposes.

Soil extracellular enzyme activities (EEA)

Eight enzymes (see Table S3), including α-Glucosidase (AG), β-Glucosidase (BG), Cellubiosidase (CB), Xylosidase (XYL), Phosphatase (PHOS), Sulfatase (SUL), Glucosaminidase (NAG) and Aminopeptidase (LAP), were simultaneously detected using a 96-well plate assay following a modified method from Bell *et al.* (2013). In brief, soil slurries were prepared by mixing ~1.0 g of air-dried soil with 30 mL of 50 mM sodium acetate buffer (pH=6.0 – chosen in order to accommodate the pH of the two soil types investigated) and shaken for 30 minutes at 200 rpm. The assay was started by adding 800 µL of slurry to 200 µL of 200 µM of substrate (labelled with the Methylumbelliferyl (MUB) or Methylcoumarin (MUC) fluorophore – see Table S3; all reagents from Sigma) in a 2-mL deep 96-well plate. The sample plates were incubated in the dark at 25°C for three hours. In parallel, a 4-Methylumbelliferone (MUB) standard plate and a 7-amido-4-Methylcoumarin (MUC) standard plate were prepared for each soil type, each sampling time (10, 30 and 126 days) and each residue input level. Serial dilutions of a 1 mM stock standard solution were performed to prepare seven different concentrations. For each MUB and MUC standard plate, four soil samples were randomly chosen from each soil type, each sampling time and each residue input level, and used as experimental replicates for the calibration curves. The standard plates were prepared in a similar fashion to the

sample plates, that is, by adding 200 µL of each standard dilution to 800 µL of soil slurry and incubating them alongside the sample plates. After the 3-hour incubation period, the fluorescence was measured (excitation wavelength = 345 nm; emission wavelength = 450 nm), using a different gain for the MUB and MUC plates. A total of 12 calibration curves were produced to cover the range of soil conditions. Their R^2 value was > 0.98; the intercept and slope values were used to convert the fluorescence unit into µmol activity g^{-1} soil h^{-1}.

Statistical analysis

For primed SOC mineralization rate, and ratio of primed SOC-to-residue-C mineralized, a linear mixed model was fitted with fixed effects of soil, residue input level, nutrient input level, and their interaction, and random effects of replicate and replicate by time. The ASReml statistical package (Butler *et al.*, 2009) was used within the R statistical software environment (R Team, 2014). Correlation between successive time points for the same plot was accounted for by either including random plot effects (viz. equal correlation between time points) or assuming auto-regressive order 1 (AR1) correlated errors over time, whichever provided the lowest Akaike information criterion (AIC) value. For the gene abundance, enzymatic activity and stoichiometry data, the significance of the treatment effects was also estimated by linear mixed models, following the procedure described above. Data were log-transformed to achieve normality where needed. In order to better understand the relationship between PE (the response variable) and the soil biotic properties (taxon gene abundance) at the different soil sampling time points, linear regressions using sampling points as a grouping factor, were conducted. The significance of the regressions was tested following 999 random permutations. Residual maximum likelihood (REML), regression and correlation analyses were run under GenStat® 17th edition (VSNI, UK).

Results

Total carbon and residue carbon mineralization

Total C mineralization rates were initially high across residue and nutrient treatments (4.5–290 mg CO_2-C kg^{-1} soil d^{-1}) and then decreased in an exponential manner (2.3–25 mg CO_2-C kg^{-1} soil d^{-1}) over time, in particular within the first two months of incubation (Fig. S2). Total C mineralization rates were significantly ($P < 0.05$) influenced by the main effects of soil type, residue and nutrients input levels, time, and the interactive effects of these four factors (Table S4). Cumulative total C mineralized across the treatments is shown in Fig. S3.

Residue-C mineralization rates (mg CO_2-C g^{-1} residue-C d^{-1}) were significantly affected by the main factors of soil, nutrient, time and the interaction of residue and nutrients ($P < 0.05$) (Fig. S4 and Table S4). On day 126, the cumulative residue-C mineralized ranged from 387 to 549 mg CO_2-C g^{-1} residue-C (Fig. S5), equivalent to 38.7–54.9% of residue-C mineralized across all the treatments. Nutrients input significantly increased residue-C mineralization at the high residue input level (20.0 g kg^{-1} soil), relative to no-nutrient in both soils, but not at the low residue input (Fig. S5). Moreover, at high residue input, increasing nutrients input levels (from Lnu to Hnu) caused an increase in the magnitude of residue-C mineralization throughout in the Vertisol (Fig. S5d), but not in the Luvisol (Fig. S5b).

Native soil organic carbon mineralization

Native SOC mineralization rates (mg CO_2-C g^{-1} SOC d^{-1}) were significantly ($P < 0.001$) influenced by the main factors of residue input levels and time (Fig. S6 and Table S4). There was a significant interaction ($P < 0.05$) of residue, nutrient and time on the native SOC mineralization rates. For example, nutrient input caused lower SOC mineralization rates on days 10 and 30 in the high residue input treatment in both soils (Fig. S6 c, d). The cumulative native SOC mineralized over the 126-day

incubation period was similar in the Luvisol (16−46 mg CO_2-C g^{-1} SOC) and Vertisol (16−33 mg CO_2-C g^{-1} SOC) across the residue and nutrient treatments (Fig. S7 and Table S4, P = 0.07). The cumulative SOC mineralized over the 126-day incubation period followed the same trend across two soils: Re0 (15.3−16.3 mg CO_2-C g^{-1} SOC) < Re6.7 (21−33 mg CO_2-C g^{-1} SOC) < Re20 (30−46 mg CO_2-C g^{-1} SOC) (Fig. S7 and Table S4, P < 0.001).

Priming of native soil organic C mineralization

The input of wheat residue with or without nutrients caused positive priming of SOC mineralization over the 126-day period (Fig. 1). The positive PE rate was the highest initially and then gradually decreased over time but generally remained positive until the end of experiment (except the occurrence of negative rates on days 3 and 10). That is, the PE rates were the highest on day 3 (1.5–2.8 mg g^{-1} SOC d^{-1}) and then decreased to –0.03–0.27 mg g^{-1} SOC d^{-1} after 30 days of incubation (Fig. 1a, b, c, d). The main factors of soil, residue, nutrient and time significantly (P < 0.05) influenced the PE response to the residue treatments (Table S4). In addition, a significant interaction of residue, nutrient and time (P < 0.05) occurred, *i.e.* nutrient input only decreased positive PE at the high residue input during the first 30 days (Fig. 1). The positive PE was consistently higher at the 20.0 g kg^{-1} soil than the 6.7 g kg^{-1} soil residue inputs across the soil types and nutrient treatments.

Over time, the high residue input caused a 1.6–3.1 times increase (*cf.* low residue input) in cumulative positive PE (P = 0.001) across the corresponding nutrient treatments in each soil (Fig. 1e, f). During the early stage of the incubation (*i.e.* 10–60 days), residue without nutrient input caused higher cumulative positive PE at the high residue input, compared to the nutrient treatments, in both soils (Fig. 1e and f). The effect of nutrients on the positive PE peaked on day 30, and was 30–40% (in the Luvisol) and 36–50% (in the Vertisol) lower in the residue with nutrient than without nutrient input treatments. However, the difference in positive PE between no-nutrient and nutrient inputs decreased with time (Fig. 1e, f).

The sensitivity analysis showed that there were variations in the amount of positive PE, resulting from the use of residue-^{13}C from different pools (*e.g.* LOC, ROC, and initial or moving bulk residue-^{13}C) as an end member in the C-source partitioning over the 126-day incubation period. Yet, the pattern of SOC priming was the same across the treatments from the different scenarios (*i.e.* using ROC, and initial or moving bulk-residue δ^{13}C). Moreover, using the moving residue-δ^{13}C values as an end member was the most robust approach to enhance the accuracy of the C-source partitioning because all the residue-C components, which had different ^{13}C values, would be decomposing and contributing to C mineralization over the incubation period (Fig. S8 and SI).

Ratio of primed SOC-to-residue-C mineralized

The ratio of primed SOC-to-residue-C mineralization was 0.1–0.2 on day 3, which decreased to the lowest level on day 30 (or day 10 in the Vertisol with low residue input), followed by an increasing trend during the remaining incubation period (Fig. 2). The ratio of primed SOC-to-residue-C mineralization was significantly ($P < 0.05$) affected by the residue and nutrient input levels, time, and their interaction (Table S4). In the high residue input treatment, this ratio was higher in the no-nutrient treatment on days 10 and 30, relative to the nutrient treatments, and this trend was reversed over time in both soils (*i.e.* 0nu < Lnu < Hnu) (Fig. 2c, d). However, in the low residue input treatment, there was no difference in the ratio of primed SOC-to-residue-C mineralization in different nutrient input treatments (Fig. 2a, b).

Microbial biomass C

Microbial biomass C was significantly higher in the Vertisol than Luvisol, and decreased over time across all treatments (Fig. 3). Microbial biomass C increased significantly ($P < 0.001$) with the residue input in both soils (Table S4). More specifically, the MBC increased with increasing input levels of the

residue in both soils on day 10, whereas the difference in MBC between the two residues input levels diminished with time (Fig. 3). Over time, both nutrient input levels generally increased MBC at the high residue in both soils, but not at the low residue input.

Taxon gene abundance in relation to PE

The input of residue at both low and high levels generally increased ($P < 0.001$) Eubacteria, β-Proteobacteria, Acidobacteria and Fungi, particularly on day 10 (Figs. 4, S9–S12 and Table S5). At the low residue, nutrient inputs usually had no impact on gene abundance of Eubacteria, β-Proteobacteria, Acidobacteria, except Fungi, which increased with nutrients input on days 10 (in both soils) and 126 (in the Vertisol). At the high residue, nutrient inputs decreased gene abundance of Eubacteria (in both soils) and Fungi (in the Luvisol) on days 10; whereas over time, gene abundance of Eubacteria and Fungi in the nutrient treatments increased to the same level as in the no-nutrient treatments. Further, gene abundance of β-Proteobacteria and Acidobacteria showed a general increase trend over time at the high residue with nutrient inputs.

Linear regression between cumulative PE and taxon gene abundances was performed for each soil at each sampling time (Table 3). The cumulative PE was statistically and positively correlated to the gene abundance of Eubacteria and Fungi. In both soils, the PE was generally positively correlated to Acidobacteria and β-Proteobacteria, except on day 10 when PE was negatively correlated to β-Proteobacteria. The gene abundance of certain microbial taxa (Eubacteria, β-Proteobacteria, Acidobacteria, and Fungi) are shown in Figs. S9–S12.

Extracellular enzyme activities

The residue input at both low and high levels increased ($P < 0.001$) BG, CB, XYL, LAP and NAG activities on 10, 30 and 126 days (Figs. 5 and S14–S18). At the low residue, increasing nutrients input levels (from 0nu to Hnu) generally had no significant impact on C, N, P and S EEAs over time. Whereas at the high residue, increasing nutrient input levels (from 0nu to Hnu) significantly increased BG, CB, XYL, LAP and NAG, particularly on days 10 across both soils (Figs. 5 and S14–S18).

The ratios of C-to-N [(AG + BG + CB + XYL):(NAG + LAP)] and C-to-P [(AG + BG + CB + XYL):PHOS] were the highest in the control soil treatments, and they generally decreased with the increasing amounts of residue input (Fig. 6).

Discussion

In the current study, a model conceptualizing the PE over a 4-month period (equating a crop growing season) was built from the perspective of residue and nutrient inputs, changes in microbial activity and abundance, and linking the microbial theories of "co-metabolism", "microbial nutrient mining" and "microbial stoichiometry decomposition" relating to the PE (Fig. 7). We provide novel evidence that the dominant mechanisms varied across the treatments and over time, likely due to the availability of labile C and nutrients in the soils incorporated with the crop residue, which explains the dynamics of PE and its correlation with key microbial variables (*e.g.* microbial biomass, EEAs, C-to-nutrient stoichiometry of EEAs, and copiotroph/oligotroph growth).

Microbial mechanisms of SOC priming in response to crop residue input

The results showed that the crop residue input was one of the major drivers of PE over time in both soils (Chen *et al.*, 2014; Guenet *et al.*, 2010; Pascault *et al.*, 2013; Su *et al.*, 2015), where exogenous LOM inputs could accelerate SOC mineralization *via* positive PE mechanisms (Zhang *et al.*, 2013). The increased positive PE caused by the residue inputs was in the range reported in other studies by various LOM inputs (Chen *et al.*, 2014; Dimassi *et al.*, 2014). Furthermore, the positive PE was almost three times higher in the presence of a relatively high *versus* low residue input level, which is supported by previous reports about a positive relationship between the magnitude of positive PE and LOM input level (Liu *et al.*, 2017; Paterson & Sim, 2013).

The observed positive PE following the input of crop residue could be a result of microbial "co-metabolism" (Fig. 7) (Blagodatskaya & Kuzyakov, 2008; Nottingham *et al.*, 2009). That is, decomposers degrade SOM compounds by using labile C substrate (*e.g.* crop residue) as a source of energy (Fontaine *et al.*, 2003). The theory of "co-metabolism" is supported by the increased MBC, C and N EEAs, and gene abundance of Eubacteria, copiotrophs (β-Proteobacteria) and oligotrophs (Acidobacteria and Fungi) (Figs. 3–5), following the crop residue input, which agreed with previous findings (Fierer *et al.*, 2007; Hu *et al.*, 1999; Naether *et al.*, 2012; Trivedi *et al.*, 2013). The increase in the β-Proteobacteria gene abundance under both residue inputs at the early stages indicated that the copiotrophs were stimulated soon after the input of relatively labile residue-C to induce the positive PE (Bastian *et al.*, 2009; Hu *et al.*, 1999). That is, the copiotrophs produce extracellular enzymes (EEs) to decompose added LOM (Cayuela *et al.*, 2009; Chen *et al.*, 2014) and those EEs probably have the same efficiency in degrading easily available native SOM (but do not use recalcitrant SOM pools). Further, the positive correlation between the PE and the gene abundance of Acidobacteria and Fungi in each soil over time indicated that the oligotrophs would have contributed to degrading recalcitrant C in the SOM (Bell *et al.*, 2003; Hu *et al.*, 1999; Pascault *et al.*, 2013).

Moreover, the decrease of C-to-nutrient stoichiometry of EEAs with residue inputs indicated an increase in nutrient acquisition (N and P) relative to the C acquisition (Fig. 6), which suggested nutrient limitation and the "microbial nutrient mining" of SOM (Allison & Vitousek, 2005; Waring *et al.*, 2014).

Microbial mechanisms of SOC priming in response to the interactive effect of crop residue and nutrient inputs

Nutrient supplementation decreased the magnitude of the positive PE stimulated by the high residue input but not by the low residue input, thus indicating an interactive effect of nutrient and residue input on the PE. At the low residue input level (6.7 g kg^{-1} soil), no impact of nutrient inputs on the magnitude of PE was found in this study. Similarly, Chen *et al.* (2014) reported that maize straw input in soil, with or without nutrients, had the same magnitude of positive PE after a 9-day incubation period. The low residue-C input with extra nutrients generally neither affected the MBC, C, N or P EEAs, enzymatic stoichiometry nor the abundance of Eubacteria, β-Proteobacteria, Acidobacteria and Fungi over time. The results of limited nutrient effects on the microbial properties at the low residue input may imply that microbial growth was constrained by C availability (rather than nutrient availability) (Heuck *et al.*, 2015).

At the high residue along with nutrient inputs, a 30–50% decrease in the positive PE was observed over a 30-day period (Fig. 1), which suggested the alleviation of "microbial nutrient mining" of native SOM (Fig. 7b). This finding is in agreement with previous studies that reported a 37–41% lowering of positive PE by the combined input of cellulose-C and nutrients (such as N and/or P) compared to the input of cellulose-C alone (Dimassi *et al.*, 2014; Fontaine *et al.*, 2011). Here, the lower gene abundance of Eubacteria (in both soils) and Fungi (in the Luvisol) along with the concurrent input of high residue and nutrients (relative to no-nutrient) on day 10 supported our hypothesis of lessened nutrient mining of native SOM (Fontaine *et al.*, 2011). Further, the low ratio of primed SOC-to-

residue-C mineralized in the high residue with nutrients (*cf.* no-nutrients) treatments suggested that microorganisms mineralized less native SOM for nutrients when decomposing the same amount of residue-C at the start of incubation. However, the dominance of "microbial nutrient mining" mechanism relating to the PE in the high residue-amended (no-nutrient) soils seems to have decreased over time. This may be related to an increase in nutrient availability *via* microbial mineralization and chemical mobilization of nutrients from the soil reserves, while the C resource becoming limiting for microbial growth over time.

In our study, the results showed that the exogenous nutrient supply (*cf.* no-nutrient) in the high residue-amended soils increased MBC and enzyme activity (mainly BG, CB, XYL, LAP and NAG) (Fig. 3). However, the nutrient supply (*cf.* no-nutrient) did not increase the PE (and rather decreased the initial positive PE; Fig. 1), possibly because the increased microbial activities mainly increased the mineralization of labile C components in the wheat residue during the early periods (Fig. S5) (Carreiro *et al.*, 2000; Henriksen & Breland, 1999; Keeler *et al.*, 2009; Knorr *et al.*, 2005). Over a longer period (~4 months), although there was a decrease of positive PE rate (Fig.1), the nutrient inputs in the high residue-amended soils (compared with no-nutrient) continued to have higher MBC and C and N EEAs until the end of incubation. Therefore, in the high residue with nutrient (*cf.* no-nutrient) the labile C depleted faster and the gene abundance of all microbial communities was similar throughout, suggesting that the microbes could have adapted to the nutrient availability and therefore enhanced the utilization of native recalcitrant SOC over time (Brooks *et al.*, 2011; Creamer *et al.*, 2015). Moreover, the microbial C and nutrient demands (*i.e.* enzymatic stoichiometry of C-to-N and C-to-P ratios) remained at the same level, while there was an increasing trend of Eubacterial and Fungal abundance over time, thus further supporting the increased utilization of recalcitrant SOC by the microbes. Hence, the positive PE in the high residue-amended soils with nutrient inputs increased to the same magnitude as without nutrients, which may be due to the increased "microbial stoichiometry decomposition" of SOM for C. The higher primed SOC-to-residue-C

mineralized ratio after day 60 to four months in the nutrient (*cf.* no-nutrient) treatments further supported this hypothesis.

Priming of SOC mineralization in response to soil properties

In addition to the exogenous nutrient inputs, the dynamics of PE could have also been affected by the release of residue- and SOM-bound N, P and/or S, *via* microbial mineralization (biotic process), and/or mobilization of mineral-bound nutrient reserves *via* chemical desorption and dissolution reactions (abiotic process) (Bhupinderpal-Singh *et al.*, 2006; Guppy *et al.*, 2005; Manzoni *et al.*, 2010; Sarker *et al.*, 2018). Alternatively, bioavailability of mineral-protected SOM could have increased *via* reductive dissolution of short-range order minerals induced by the release of low-molecular-weight organic acids during residue decomposition (Keiluweit *et al.*, 2015; Kumari *et al.*, 2008). These abiotic processes in the residue-amended soils may have decreased "microbial nutrient mining" and further supported "microbial stoichiometry decomposition" over time. Further, although the microbial mechanisms are the same in the Luvisol and Vertisol, the positive PE was higher in the Luvisol than Vertisol, which is possibly related to differences in clay content and mineralogy. The high, smectite-rich, clay content (63%) in the Vertisol with high surface area could increase organo-mineral associations, thereby limiting physical–chemical accessibility of SOC and nutrients to soil microorganisms and their enzymes (Dungait *et al.*, 2012; Sollins *et al.*, 1996; Torn *et al.*, 1997; von Lützow *et al.*, 2006).

In summary, on the basis of PE responses and microbial mechanisms, the results showed that the return of fresh residue-C provided energy to stimulate microbial growth, C and N extracellular enzyme activities, and microbial gene abundance, which led to an immediate enhancement of the ability of microorganisms to co-metabolize SOM. Further, the dynamics of PE over a longer period, which are usually unpredictable when complex C substrates are added to the soil, could be predicted

in the current study according to the most important drivers, such as residue and nutrient inputs, soil type, and incubation time. The study also showed that increased input of nutrients at certain balanced ratios, aiming to enhance C storage in SOM pools, decreased the magnitude of positive PE at the high residue treatments (but not at the low residue input), while influencing the dynamics of microbial activities and gene abundance (Figs. 4 and 5). Further, the initial suppression of the positive PE by the increasing input level of nutrients under the high residue treatments was alleviated over time. These results provide evidence that both microbial mechanisms can influence the PE but at different times depending on the availability of labile C and nutrients. The "microbial nutrient mining" seems to explain the positive PE response when the native available nutrients in the soils could not satisfy the growth of microorganisms at the high residue input level. However, "microbial stoichiometry decomposition" can predominate over time, particularly in the nutrient-rich soil, and possibly further supported by mobilization of plant available nutrients from the soil reserves (Sarker *et al.*, 2018), where increased microbial biomass would expedite utilization of native SOM for C. Moreover, the level of priming intensity was lower in the Vertisol than Luvisol, thus highlighting the importance of certain soil properties such as clay content and mineralogy in determining the intensity of PE.

In conclusion, this study provides new knowledge to enhance the understanding of underlying microbial mechanisms of priming of SOC mineralization in response to the supply of crop residue and balanced nutrients in soils with different properties (*e.g.* clay conditions) and particularly over a crop growing season. These findings should be explicitly considered to improve process-based models to better predict SOC dynamics and their responses to integrated nutrient management with implications for SOC sequestration in global agroecosystems.

Acknowledgments

This research was supported by Grains Research and Development Corporation in Australia (project number: DAN00169). We are thankful to Meragal Henaka Arachchi's excellent contributions to the laboratory work. Damian Collins' assistance with the statistical analysis is appreciated. BKS laboratory is supported by Australian Research Council (DP170104634). We also extend our gratitude to Marcus Klein for his help with qPCR.

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Table 1 Basic properties of the soils and wheat stem residue.

	Luvisol	Vertisol	Wheat residue
Coordinates	33°05′19′′S 147°08′58′′ E	28°12′S, 152°06′E	–
$pH_{1:5\ water}$	5.8±0.2	7.3±0.1	–
Sand (%)	61.7±2.0	15.2±1.2	–
Silt (%)	11.4±2.3	22.2±0.8	–
Clay (%)	26.9±0.8	62.6±0.3	–
Organic C (%)	1.19±0.08	2.06±0.04	43.52±0.47
^{13}C (‰)	–24.71±0.10	–19.20±0.23	494.04±2.06
TN (%)	0.09±0.01	0.14±0.01	0.40±0.01
TP (%)	0.041±0.005	0.105±0.003	0.19±0.01
TS (%)	0.012±0.001	0.013±0.003	0.024±0.002
C:N	13.1±0.5	14.9±0.7	110±2
C:P	29.0±0.2	19.6±0.1	230±3
C:S	105.8±11.4	162.1±14.7	1839±72
Clay minerals	Mi–Kaol–Sm***, Qtz**, Hem**, Goe**, Ant*	Sm***, Kaol**	–

Mi = mica; Kaol = kaolinite; Sm = smectite; Qtz = quartz; Hem = hematite; Goe = goethite; Ant = anatase. The asterisks ***, **, * represent 60% over, 5−20% and less than 5%, respectively. The number after "±" is the standard error of the mean (n = 3). "–" means it was not applicable or measured.

Table 2 Description of the residue and nutrient (N, P and S) input treatments.

No	Treatments abbreviation	Wheat residue (g kg^{-1} soil)	N (mg kg^{-1} soil)	P (mg kg^{-1} soil)	S (mg kg^{-1} soil)	Expected residue-C stabilized[1] (%)
1	Re0_0nu	0	0	0	0	–
2	Re6.7_0nu	6.7	0	0	0	–
3	Re6.7_Lnu	6.7	25	6	4.3	10
4	Re6.7_Hnu	6.7	75	18	13	30
5	Re20_0nu	20	0	0	0	–
6	Re20_Lnu	20	75	18	13	10
7	Re20_Hnu	20	225	54	39	30

[1]The C, N, P and S nutrients input levels were based on the expectation of residue-C stabilized at 10% or 30% rates and the corresponding C:N:P:S ratio of stabilized SOM at 1: 0.0833: 0.02: 0.0143.

Table 3 Relationship (linear regression) between PE and taxon gene abundance in the Luvisol and Vertisol. The bold numbers represent statistically significant correlations ($P < 0.05$). Abbreviations: s.e.m. = standard error of the mean, V = variable.

Variable (V)	Incubation time (day)	Slope	s.e.m.	Accumulated analysis of variance		R^2_{adj}
Luvisol						
Eubacteria	10	0.065	0.167	Global	**0.001**	0.371
	30	0.503	0.124	+Time	**0.001**	
	126	0.336	0.149	+Time*V	**0.001**	
Acidobacteria	10	-0.021	0.086	Global	**0.001**	0.258
	30	0.189	0.106	+Time	**0.001**	
	126	0.149	0.066	+Time*V	0.052	
β-Proteobacteria	10	-0.336	0.059	Global	**0.001**	0.604
	30	0.429	0.079	+Time	**0.001**	
	126	0.172	0.050	+Time*V	**0.001**	
Fungi	10	0.049	0.101	Global	**0.001**	0.412
	30	0.328	0.076	+Time	**0.001**	
	126	0.262	0.087	+Time*V	**0.001**	
Vertisol						
Eubacteria	10	0.365	0.126	Global	**0.001**	0.290
	30	0.326	0.148	+Time	**0.001**	
	126	0.157	0.194	+Time*V	**0.003**	
Acidobacteria	10	0.141	0.157	Global	**0.001**	0.281
	30	0.205	0.095	+Time	**0.001**	
	126	0.311	0.114	+Time*V	**0.005**	
β-Proteobacteria	10	-0.384	0.146	Global	**0.001**	0.433
	30	0.512	0.141	+Time	**0.001**	
	126	0.581	0.157	+Time*V	**0.001**	
Fungi	10	0.287	0.093	Global	**0.001**	0.356
	30	0.259	0.093	+Time	**0.001**	
	126	0.274	0.128	+Time*V	**0.001**	

Figure Captions

Fig. 1 Rate (mg CO_2-C g^{-1} SOC d^{-1}) and cumulative (mg CO_2-C g^{-1} SOC) priming of native soil organic carbon (SOC) mineralization by the interaction of wheat residue and nutrients in the Luvisol (a, b and c) and Vertisol (d, e and f) over the 126-day incubation period. Error bars represent ± standard errors of the mean (n = 4). Abbreviations: Re6.7 = residue input at 6.7 g kg^{-1} soil; Re20 = residue input at 20.0 g kg^{-1} soil; 0nu = no-nutrient input; Lnu = low nutrient input; Hnu = high nutrient input. Black bars show least significant differences (at 5% level) across soil, residue and nutrient input levels at different time points.

Fig. 2 Ratio of primed soil organic carbon (SOC) to residue carbon (C) mineralized by the interaction of wheat residue and nutrients in the Luvisol (a and b) and Vertisol (c and d) over the 126-day incubation period. Error bars represent ± standard errors of the mean (n = 4). Abbreviations: Re6.7 = residue input at 6.7 g kg^{-1} soil; Re20 = residue input at 20.0 g kg^{-1} soil; 0nu = no-nutrient input; Lnu = low nutrient input; Hnu = high nutrient input. Black bars show least significant differences (at 5% level) across soil, residue and nutrient input levels at different time points.

Fig. 3 Microbial biomass C (mg kg^{-1} soil) after 10, 30 and 126 days of the incubation period in the Luvisol (a) and Vertisol (b) with or without the input of wheat residue and nutrients. Error bars represent ± standard errors of the mean (n = 4). Significant differences between treatments at each time in each soil are marked by lowercase letters. Abbreviations: Re0 = no-residue input; Re6.7 = residue input at 6.7 g kg^{-1} soil; Re20 = residue input at 20.0 g kg^{-1} soil; 0nu = no-nutrient input; Lnu = low nutrient input; Hnu = high nutrient input.

Fig. 4 Radar graphs illustrating taxa gene abundance (gene copy g^{-1} soil) across selected treatments (no and high residue) in the Luvisol (a, c) and Vertisol (b, d) after 10 and 126 days of the incubation period. Details of original data and statistics of gene abundance of tested taxa are provided in Figs. S9–S12 and Table S5. Abbreviations: Re0 = no residue input; Re20 = residue input at 20.0 g kg^{-1} soil; 0nu = no-nutrient input; Lnu = low nutrient input; Hnu = high nutrient input. The asterisk (*) shows that the gene abundance were significantly increased with the residue input.

Fig. 5 Radar graphs illustrating soil extracellular enzyme activities (nmol g^{-1} soil h^{-1}) across selected treatments (no and high residue) in the Luvisol (a, c) and Vertisol (b, d) after 10 and 126 days of the incubation period. Details of original data and statistics of extracellular enzyme activities are provided in Figs. S13–S20 and Table S5. Abbreviations: Re0 = no residue input; Re20 = residue input at 20.0 g kg^{-1} soil; 0nu = no-nutrient input; Lnu = low nutrient input; Hnu = high nutrient input; AG = α-Glucosidase; BG = β-Glucosidase; CB = Cellubiosidase, XYL = Xylosidase, PHOS = Phosphatase; SUL = Sulfatase; NAG = Glucosaminidase; LAP = L-Leucine aminopeptidase. The asterisk (*) shows that the enzyme activities were significantly increased with residue input.

Fig. 6 Organic nitrogen (N) acquisition activity and organic phosphorus (P) acquisition activity in relation to organic carbon (C) acquisition with or without the input of wheat residue and nutrients in the Luvisol (a and c) and Vertisol (b and d) after 10, 30 and 126 days. Organic N acquisition was measured by the activities of Glucosaminidase (NAG) and L-Leucine aminopeptidase (LAP). Organic C acquisition was measured by the activities of α-Glucosidase (AG), β-Glucosidase (BG), Cellubiosidase (CB) and Xylosidase (XYL). Organic P acquisition was measured by the activity of Phosphatase (PHOS). Significant differences between treatments at each time in each soil are marked by lowercase letters.

Fig. 7 A conceptual model showing the dynamics of the priming effect (PE), influenced by the interactive effects of wheat residue and nutrients [nitrogen (N); phosphorus (P); sulfur (S)] at different inputs (low or high), as well as the underlying biotic mechanisms in relation to microbial community growth and extracellular enzyme activities (EEAs). In addition to biotic mineralization, nutrient availability in soil may also be supported by an abiotic mechanism (*e.g.* chemical mobilization of nutrients from the soil reserves). (a) Low residue input level (6.7 g kg^{-1} soil); and (b) High residue input level (20.0 g kg^{-1} soil). Solid black lines show the fluxes induced by native soil. Solid blue lines show the fluxes enhanced by the presence of residue. The solid and broken red line represented that nutrients had an increase and no effect on the fluxes, respectively. The dark green lines indicated the change of gene abundance over time with residue input. The input of residue decreased enzymatic C-to-nutrient stoichiometry. The details of individual EEAs and gene abundance are provided in Figs. S9–S20.