Mixing crop residues induces a synergistic effect on microbial biomass and an additive effect on soil organic matter priming

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Abstract

Applying crop residues is a widely used strategy to increase soil organic matter (SOM) in arable soils because of its recorded effects on increasing microbial biomass and consequently necromass. However, fresh residue inputs could also "prime" the decomposition of native SOM, resulting in accelerated SOM depletion and greenhouse gas (GHG) emission. Increasing the botanical diversity of the crops grown in arable systems has been promoted to increase the delivery of multiple ecological functions, including increasing soil microbial biomass and SOM. Whether mixtures of fresh residues from different crops grown in polyculture contribute to soil carbon (C) pools to a greater extent than would be expected from applying individual residues (i.e., the mixture produces a non-additive synergistic effect) has not been systematically tested and is currently unknown. In this study, we used ^{13}C isotope labelled cover crop residues (i.e., buckwheat, clover, radish, and sunflower) to track the fate of plant residue-derived C and C derived from the priming of SOM in treatments comprising a quaternary mixture of the residues and the average effect of the four individual residues one day after residue incorporation in a laboratory

microcosm experiment. Our results indicate that, despite all treatments receiving the same amount of plant residue-derived C (1 mg C g^{-1} soil), the total microbial biomass in the treatment receiving the residue mixture was significantly greater, by 26% (3.69 µg C g^{-1}), than the average microbial biomass observed in treatments receiving the four individual components of the mixture one day after applying crop residues. The greater microbial biomass C in the quaternary mixture, compared to average of the individual residue treatments, that can be attributed directly to the plant residue applied was also significantly greater, by 132% (3.61 µg C g^{-1}). However, there was no evidence that the mixture resulted in any more priming of native SOM than average priming observed in the individual residue treatments. The soil microbial community structure, assessed using phospholipid fatty acid (PLFA) analysis, was significantly ($P < 0.001$) different in the soil receiving the residue mixture, compared to the average structures of the communities in soil receiving four individual residues. Differences in the biomass of fungi, general bacteria, and Gram-positive bacteria were responsible for the observed synergistic effect of crop residue mixtures on total microbial biomass and residue-derived microbial biomass, especially biomarkers 16:0, 18:2ω6 and 18:3ω3. Our study demonstrates that applying a mixture of crop residues increases soil microbial biomass to a greater extent than would be expected from applying individual residues and that this occurs either due to faster decomposition of the crop residues or greater carbon use efficiency (CUE), rather than priming the decomposition of native SOM. Therefore, growing crop polycultures (e.g., cover crop mixtures) and incorporating mixtures of the resulting crop residues into the soil could be an effective method to increase microbial biomass and ultimately C stocks in arable soils.

Key words: diverse, mixture, synergistic, ^{13}C-PLFA, crop residues

1. Introduction

Soil organic carbon (SOC) plays a critical role in global carbon (C) dynamics in the earth system and is a major property influencing soil functions and health. Applying crop residues to soils is a common strategy used in agroecosystems to enhance SOC stocks (Chapman and Newman, 2010; Chen et al., 2015). When microorganisms decompose plant litter and use the C for metabolism, they catabolise a portion of this C, which is usually released as carbon dioxide (CO_2), and simultaneously assimilate and anabolise a portion of C into their biomass. After their death, microbial necromass returns to soil and contributes to SOC storage (Liang et al., 2020). However, applying fresh crop residues could also stimulate microbial decomposition of native SOM via co-metabolism or mining for nutrients, resulting in a priming effect (Kuzyakov, 2010; Wang et al., 2015). A recent meta-analysis including 2048 individual experimental comparisons from 94 laboratory incubation studies revealed that the addition of exogenous organic C significantly enhanced native SOC decomposition, by 47.5%, in terrestrial ecosystems, with the highest priming effect observed in arable soils (60.9%) (Sun et al., 2019). Therefore, using crop residues to maximize SOC stocks requires a consideration of the impact of amendments on SOC priming.

Previous studies have investigated the C mass balance after the application of plant residues of a single plant species (Rubino et al., 2010; Shahbaz et al., 2018). However, crop residues returned to soils under arable land management practices such as intercropping, rotations, and cover crops include the residues of more than one plant species. Based on studies examining decomposition dynamics in soils receiving mixed species residues, it cannot necessarily be assumed that the application of a crop residue mixture will have the same impact on C dynamics as predicted from observations made on the impact of the individual

residues (Gartner and Cardon, 2004; Porre et al., 2020). If the behaviour or effect of a residue mixture can be predicted from the behaviour of the individual residues, this is classified as an additive effect (Redin et al., 2014). By contrast, a mixture could also deliver an antagonistic non-additive effect (i.e. the mixture's effect is less than the average of individual species) or a synergistic non-additive effect (i.e. the mixture's effect is greater than the average of individual species), which suggests there are interactions, via microbial decomposers, among the constituents of the mixture (Redin et al., 2014).

A majority of the previous studies have explored the effects of mixtures on litter decomposition and associated C cycling and nutrient release (e.g. nitrogen mineralization and immobilization) by focusing on leaf litter decomposition in forest ecosystems (Castro-Díez et al., 2019; Gartner and Cardon, 2004; Mao et al., 2017). Even the most recent meta-analysis on litter mixtures only focused on litter mass loss due to decomposition (Porre et al., 2020). To better understand the mechanisms of plant species diversification on soil C dynamics, we need to fill the knowledge gap regarding mixture effects on the microbial fate of C supplied by individual components of the mixture and the potential for interactions of residue C with older soil organic matter (SOM) via priming effects.

The mechanisms responsible for non-additive effects in residue mixture decomposition dynamics are not fully understood and might depend strongly on the context within which the study was conducted (Porre et al., 2020). To explain non-additive effects, processes relating to nutrient transfer between nutrient-rich (low C:N) and nutrient poor (high C:N) litters, transfer of inhibitory compounds from one species' litter to another, or physical (water retention) effects have been frequently mentioned (Porre et al., 2020). In addition, mixing chemically contrasting crops may provide a greater number of niches for

92 microorganisms to exploit, which allows functionally dissimilar microbial communities to
93 coexist, and thus result in a greater microbial diversity and biomass than might be expected
94 from the average of the individual communities that are supported by monocultures
95 (Chapman and Newman, 2010). Although greater diversity not always linked to greater
96 biomass, a recent meta-analysis found that in temperate regions, where soil is not rich in C,
97 greater microbial diversity is usually associated with greater microbial biomass due to
98 facilitation and niche partitioning by supporting the co-existence of multiple microbial
99 species (Bastida et al., 2021). An increased microbial diversity could increase the probability
100 of including taxa particularly influential in extracellular enzyme production for nutrient
101 mining and SOM degradation through metabolic and co-metabolic processes. Furthermore,
102 an increased microbial biomass could also result in stronger SOM mineralization as a
103 consequence of increased microbial metabolic activity (Bastida et al., 2021). Thus, mixtures
104 of crop residues could enhance the extent to which soil microbial communities mine
105 nutrients for SOM and lead to a greater priming effect. By contrast, some studies showed
106 that mixtures of diverse plant residues have a greater opportunity to provide preferable
107 growth substrates for microbes (e.g., if the average C/N ratio of residues is close to 24),
108 thereby offsetting the extent to which SOM decomposition is primed and decreasing
109 microbial anabolism of primed SOM (Xiao et al., 2015). These two mechanisms (i.e.,
110 increasing or offsetting priming effect) could occur simultaneously and combine to
111 determine the overall magnitude of the priming effect.

112 In this study, we investigated the residues of four functionally dissimilar crops from four
113 different plant families (i.e., buckwheat, clover, radish, and sunflower) which are widely
114 grown in mixtures as cover crops in agricultural systems. We established a microcosm

115 experiment comprising treatments receiving either mixtures or individual (non-mixture) ^{13}C
116 labelled cover crop residues which provided the same amount of residue-derived C (1 mg C
117 g^{-1} soil). Soil phospholipid fatty acid (PLFA) analysis was undertaken one day after
118 incorporating crop residues to quantify the biomass of key soil microbial groups. Gas
119 chromatography-combustion-stable isotope mass spectrometry (GC-C-IRMS) was used to
120 identify the microbial groups that had incorporated residue-derived C and, by mass balance,
121 quantify the amount of primed SOM-derived C which was incorporated into the microbial
122 biomass. The difference between the mixture and the average of four non-mixtures enabled
123 us to determine whether the mixture delivered either a synergistic (mixture > average), an
124 antagonistic (mixture < average), or an additive (mixture = average) effect.

125 We assumed that microbes have no preference for ^{13}C over ^{12}C. Because a mixture of crop
126 residues may increase the niche breadth and provide a more diverse supply of nutrients,
127 thereby creating conditions enhancing the growth and facilitation of multiple co-exist
128 microorganisms. Thus, we hypothesised that the mixture could result in a synergistic effect
129 on total microbial biomass. Compared to individual species, the mixture has higher
130 probability to provide preferable growth substrates which could be more easily assimilated
131 into microbial biomass, and thus we would expect a synergistic effect of the mixture on the
132 microbial biomass derived from plant residues. Given that the cover crop species tested had
133 divergent C:N ratios (ranging between 10 and 32 and spanning the threshold C:N (≈24) for
134 net N mineralization- immobilization (Norton and Schimel, 2011), we hypothesised that
135 adding residues in a mixture (average C/N = 17) would decrease the requirement for
136 microorganisms to prime native SOM to scavenge for N and therefore induce an
137 antagonistic effect on the microbial biomass C derived from primed SOM.

2. Materials and methods

2.1. Soil samples and crop residues

A silty loam Luvisol (World Reference Base classification); pH (H_2O) 6.3, 22.32 g C kg^{-1}, 2.24 g N kg^{-1}, 0.90 mg NH_4^+ -N kg^{-1}, 2.75 mg NO_3^- -N kg^{-1} was collected from an arable field on the University of Reading's research farm at Sonning, Reading, UK (51.481152, -0.902188) in August 2019 after harvesting spring barley (*Hordeum vulgare*). Seven surface soil samples (0-20 cm depth) were randomly sampled and mixed thoroughly to create one homogenous sample, approximately 20 kg in weight.

Four cover crops, buckwheat (*Fagopyrum esculentum*), berseem clover (*Trifolium alexandrinum*), oil radish (*Raphanus raphanistrum*), and sunflower (*Helianthus annuus*), were continuously and uniformly labelled with $^{13}CO_2$ in growth cambers by IsoLife (Wageningen, Netherlands). Buckwheat and clover were harvested 5 weeks after sowing, while radish and sunflower were harvested after 4 weeks. The ^{13}C atom percent of the resulting aboveground biomass was 6.7%, 7.8%, 7.8%, and 8.0% for buckwheat, clover, radish, and sunflower, respectively. Corresponding unlabelled crops were grown under the same conditions in growth chambers by IsoLife (Wageningen, Netherlands), and harvested at the same time. After harvesting, the aboveground residues of both ^{13}C labelled and unlabelled crops were dried at 70 °C and milled to pass through 0.05 mm mesh. The chemical composition of ^{13}C labelled and unlabelled residues is provided in Table S1.

2.2. Experimental design

Soil was sieved to pass a 4 mm mesh and then pre-incubated for 7 days at 26 °C with a soil water content of 60% of the water holding capacity (0.22 g g^{-1}). As indicated in Table 1, the treatments consisted of pure unlabelled residues, labelled non-mixture residues, quaternary

161 mixtures of residues which contained one labelled species and three unlabelled species, and
162 a control without any crop residue additions. For each treatment receiving residues, four
163 replicate microcosms were established by mixing 150 g of fresh soil (equivalent to 122.95 g
164 dry soil) thoroughly with a mass of dry residues to ensure C was added to each microcosm
165 at a rate of 1 mg C g^{-1} soil. In the pure treatments, all the added C was from the same
166 unlabelled residue sample. In the non-mixture treatments, 25% of added C was from the ^{13}C
167 labelled residue and 75% of added C was from the unlabelled residue of the same crop
168 species. In the mixture treatments, 25% of added C was from a ^{13}C labelled crop and 75% of
169 added C comprised unlabelled residues from the other three crop species.

170 For the measurement of soil respiration, a 100 g subsample was transported from each
171 microcosm to a bulk density ring (98 cm^3), stored in a gas-tight plastic jar (365 cm^3), and
172 kept open during incubation. The rest of sample was kept in an opened plastic bag for the
173 measurement of PLFA. Both measurements of PLFA and soil respiration were taken one day
174 after applying cover crop residues.

175 To measure soil respiration, jars were sealed with a Suba-Seal® Septa for 1 h and a 16 ml
176 headspace gas sample was taken from each jar using a syringe and hypodermic needle,
177 transferred into pre- evacuated vials, and analysed with gas chromatography (Agilent 7890B,
178 UK) (Adekanmbi et al., 2020). The universal gas law was used to determine the amount of
179 CO_2 (ng g^{-1} soil h^{-1}) emitted from each jar.

180 **Table 1 Experimental design and the C/N ratio of added crop residues in each treatment**

Treatment	Plant	Abbreviation	Description	Added ^{13}C amount (mg C g^{-1} soil)	C/N ratio
Non-mix	Buckwheat	NB	25% labelled + 75% unlabelled buckwheat	0.0168	10
Non-mix	Clover	NC	25% labelled + 75% unlabelled clover	0.0195	32
Non-mix	Radish	NR	25% labelled + 75% unlabelled radish	0.0195	18
Non-mix	Sunflower	NS	25% labelled + 75% unlabelled sunflower	0.0200	19
Mix	Buckwheat	MB	25% labelled buckwheat, 75% of unlabelled residues (clover, radish, sunflower)	0.0168	17
Mix	Clover	MC	25% labelled clover, 75% unlabelled residues (buckwheat, radish, sunflower)	0.0195	17
Mix	Radish	MR	25% labelled radish, 75% unlabelled residues (buckwheat, clover, sunflower)	0.0195	17
Mix	Sunflower	MS	25% labelled sunflower, 75% unlabelled residues (buckwheat, clover, radish)	0.0200	17
Pure	Buckwheat	PB	%100 unlabelled buckwheat	\	9
Pure	Clover	PC	%100 unlabelled clover	\	30
Pure	Radish	PR	%100 unlabelled radish	\	21
Pure	Sunflower	PS	%100 unlabelled sunflower	\	22
Soil			Soil only without any plant residue addition	\	\

181 *Note: 25% and 75% refers to proportion of total added C. The quantity of C added was the same across all treatments apart from the soil treatment, which*
182 *was 1 mg C g^{-1} soil. The C/N ratio in the mixture treatments were the average of four types of mixture treatments.*

183

2.3. Phospholipid-derived fatty acids (PLFA) extraction

One day after incubation with soil and crop residues, a 10 g aliquot of soil was sampled from each replicate plastic bag and freeze-dried for downstream analysis of PLFA. PLFA was extracted following the method described by Sizmur et al. (2011). Briefly, 4 g of freeze-dried soil was extracted with 7.8 ml of Bligh and Dyer extractant containing chloroform: methanol: citrate buffer (1:2:0.8 v/v/v). The extracted phospholipids were methanolized as fatty-acid methyl esters and dissolved in hexane for analysis by gas chromatography (GC).

2.4. Gas chromatography (GC)

PLFA methyl esters were analysed using an Agilent Technologies 6890N gas chromatography equipped with a Supercowax 10 capillary GC column (60 m × 0.25 mm i.d. × 0.25 µm film thickness) and a Flame Ionisation Detector (FID). Helium was the carrier gas. The temperature programme was 1-minute isothermal at 60 °C, followed by a ramp to 145 °C at 25 °C per minute, followed by an increase to 250 °C at 2.5 °C per minute and then held isothermally at 310 °C for 10 minutes. Data were processed using GC ChemStation (Agilent Technologies). Peaks were identified using a bacterial fatty acid methyl esters (BAME) mix (Sigma Aldrich, UK) and quantified using a 37-component fatty acid methyl esters (FAME) mix (Sigma Aldrich, UK). The biomass of each group of microorganisms was determined using the combined mass of fatty acids to which the group is attributed in Table S2.

2.5. Gas chromatography- combustion-isotope ratio mass spectrometry (GC-C-IRMS)

GC-C-IRMS analysis were performed by injecting a 1 µl sample of fatty-acid methyl esters into an Agilent 7890N GC, upstream of a DELTA V™ Isotope Ratio Mass Spectrometer (electron ionization, 100 eV, 1 mA electron energy, 3 F cup collectors m/z 44, 45 and 46, CuO/Pt Thermofisher GC IsoLink interface maintained at 1000°C). A Nafion membrane was

207 employed to prevent water from reaching the ion source. GC conditions were the same as
208 that described above. Samples were calibrated against reference CO_2 of known isotopic
209 composition, which was introduced directly into the source five times at the beginning and
210 end of every run. Data was processed in the Isodat Gas Isotope Ratio MS Software
211 (ThermoFisher Scientific) to generate $\delta^{13}C$ values representing the ratio of $^{13}C/^{12}C$ in fatty-
212 acid methyl esters, relative to the $^{13}C/^{12}C$ ratio of the international Pee Dee Belemnite (PDB)
213 standard (0.01118).

214 $\delta^{13}C$ values obtained for methylated compounds were corrected for the addition of
215 derivative C using the mass balance equation following equation 1 to 3 (Zhang et al., 2019):

$$\delta^{13}C_{PLFA}(‰) = [(N_{PLFA} + 1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/N_{PLFA} \quad (Eq\ 1)$$

$$R_{sample} = (\delta^{13}C_{PLFA} / 1000 + 1) \times R_{PDB} \quad (Eq\ 2)$$

$$F = R/(R+1) \quad (Eq\ 3)$$

216

217 where $\delta^{13}C_{PLFA}$, $\delta^{13}C_{FAME}$, and $\delta^{13}C_{MeOH}$, were the $\delta^{13}C$ value of PLFA, FAME and methanol
218 ($\delta^{13}C_{MeOH}$ was assumed to equal zero), respectively. N_{PLFA} was the number of C atoms in this
219 fatty acid to which the fatty acid methyl ester corresponds. R_{sample} and R_{PDB} were the ratios
220 of $^{13}C/^{12}C$ in sample and in PDB. F is the fractional isotopic abundance representing the
221 concentration of ^{13}C as a proportion of the total concentration of C in the PLFA.

222 Equation 4 was used to calculate how much ^{13}C (µg ^{13}C g^{-1} soil) was incorporated into PLFA
223 biomass in the non-mixture treatments:

$$13C\ incorporation\ (\mu g\ 13C\ /\ g\ soil)\ in\ non-mixture = F_{non-mixture} \times C_{non-mixture} - F_{pure} \times C_{pure} \quad (Eq\ 4)$$

224 $F_{non\text{-}mixture}$ and F_{pure} were the fractional abundances of ^{13}C in a non-mixture treatment its
225 corresponding pure unlabelled treatment, respectively. $C_{non\text{-}mixture}$ and C_{pure} were the
226 concentration of PLFAs (μg C g^{-1} soil) in the non-mixture and its corresponding pure
227 unlabelled treatment, which were the results of GC-FID analysis. For example, ^{13}C
228 incorporation in the non-mixture of buckwheat (NB) equals to $F_{NB} \times C_{NB} - F_{PB} \times C_{PB}$

229 For each mixture treatment, there was 75% of added C from three different unlabelled
230 residues (25% from each residue). The resulting ^{13}C incorporation (μg ^{13}C g^{-1} soil) of these
231 unlabelled residues was accounted for using equation 5.

$$13C\ incorporation\ (\mu g\ 13C\ /\ g\ soil)\ in\ mix = F_{mix} \times C_{mix} - 0.25 \times (F_{PB} \times C_{PB} + F_{PC} \times C_{PC} + F_{PR} \times C_{PR} + F_{PS} \times C_{PS}) \quad (Eq\ 5)$$

232 where F_{mix} was the fractional abundances of ^{13}C in mixture treatment. F_{PB}, F_{PC}, F_{PR}, F_{PS}, were
233 the fractional abundances of ^{13}C in pure unlabelled buckwheat, clover, radish, and sunflower
234 treatment, respectively. C_{mix}, C_{PB}, C_{PC}, C_{PR}, C_{PS}, were the concentration of PLFAs (μg C g^{-1} soil)
235 in the treatments of mixture, pure buckwheat, pure clover, pure radish, and pure sunflower,
236 which were analysed by GC-FID.

237 The proportion (unitless) of ^{13}C incorporated from total amount of added ^{13}C was calculated
238 using equations 6 and 7:

$$Total\ amount\ of\ added\ 13C\ (\mu g\ 13C\ /\ g\ soil) = Total\ added\ C \times Proportion\ of\ 13C\ labelled\ residue \times 13C\ atom\ percent\ of\ labelled\ residue \quad (Eq\ 6)$$

$$PLFA\ proportion\ of\ added\ 13C = \frac{13C\ incorporation}{Total\ amount\ of\ added\ 13C} \quad (Eq\ 7)$$

where total added C was 1000 µg C g^{-1} soil, the proportion of ^{13}C labelled residue was 0.25. The ^{13}C atom percent was 6.7%, 7.8%, 7.8% and 8.0% for buckwheat, clover, radish, and sunflower residues, respectively. This brings the total amount of added ^{13}C to 16.75, 19.50, 19.50, and 20.00 µg ^{13}C g^{-1} soil, for treatments which received 25% of their added C from ^{13}C labelled buckwheat, radish, clover, and sunflower.

^{13}C is a tracer to track the amount of C derived from crop residues and incorporated into PLFA biomass. Assuming microorganisms have no preference for ^{12}C over ^{13}C, the proportion of PLFA incorporated ^{13}C from added ^{13}C should also be the same to the proportion of PLFA incorporated ^{12}C from total added ^{12}C. Following this logic, in all the non-mixture treatments, the amount of C incorporated into PLFA derived from added crop residue C was calculated using equation 8:

$$PLFA\ derived\ from\ residues\ in\ non-mixture\ (\mu g\ C/g\ soil) = PLFA\ proportion\ of\ added\ 13C * 1000\ \mu g\ C/g\ soil \quad (Eq\ 8)$$

where 1000 µg C g^{-1} soil was the total added amount of C (equal to 1 mg C g^{-1} soil).

In the mixture treatment, the ^{13}C labelled residue contributed 25% of the added C (i.e., 250 µg C g^{-1} soil). The proportion of ^{13}C in each type of mixture (calculated by equation 7) was

multiplied by 250 (μg C g^{-1} soil) to get the amount of PLFA derived from this ^{13}C labelled crop. We then added up the amount of PLFA derived from each type of ^{13}C labelled crop to form the PLFA which was derived from the mixture of crop residues (equation 9).

$$PLFA\ derived\ from\ residues\ in\ mixture\ (\mu g\ C/g\ soil) = (Proportion_{MB} + Proportion_{MC} + Proportion_{MR} + Proportion_{MS}) * 250\ \mu g\ C/g\ soil \quad (Eq\ 9)$$

where $proportion_{MB}$, $proportion_{MC}$, $proportion_{MR}$, and $proportion_{MS}$ represent the proportion (unitless) of ^{13}C incorporated from added ^{13}C in four types of mixture treatments where each type was ^{13}C-labelled by buckwheat, clover, radish, and sunflower, separately.

The PLFA derived from primed SOM was calculated as the difference between the total PLFA (measured using GC-FID), PLFA derived from residues, and PLFA in the control soil without any residue addition using equation 10.

$$PLFA\ derived\ from\ SOM\ priming\ (\mu g\ C/g\ soil) = total\ PLFA - PLFA\ derived\ from\ residues - PLFA\ in\ the\ soil\ only\ treatment \quad (Eq\ 10)$$

where total PLFA and PLFA in the soil only treatment were analysed by GC-FID and converted to μg C g^{-1} soil.

For mixture treatment, because there were only four samples have crop residue-derived PLFA, to calculate SOM-derived PLFA, we created a general mixture of which total PLFA was the average of total PLFA in four different types of mixture in the same replicate as calculated by equation 11.

$$Total\ PLFA\ in\ mixture\ (\mu g\ C/g\ soil) = 0.25 * (total\ PLFA_{MB} + total\ PLFA_{MC} + total\ PLFA_{MR} + total\ PLFA_{MS}) \quad (Eq\ 11)$$

Therefore, there were four replicates for total PLFA, residue derived PLFA, and SOM derived PLFA in the general mixture.

2.6. Data analysis

All the statistics were conducted in R (version 3.5.2) (R Core Team, 2018) except for the analysis of similarity (ANOSIM) which was conducted in Primer 7 (Primer-e, Auckland, New Zealand). We used nested analysis of variance (ANOVA) to compare the effect from the quaternary mixtures with the effect from the average of the four non-mixture treatments on total PLFA biomass, the PLFA biomass incorporated from crop residues, and the PLFA biomass incorporated from primed SOM.

To compare the difference in microbial community structure between the mixture and the average of four individuals, PLFA data measured by GC-FID was "Hellinger" transformed in all treatments. Non-metric multidimensional scaling (NMDS) on Bray-Curtis distance of the transformed data was performed using the "vegan" package in R (Oksanen et al., 2019). Bray-Curtis distance similarity matrices were analysed by a one-way analysis of similarity (ANOSIM) using Primer 7 to test if the differences between the mixture and the average of four non-mixture treatments were significant.

3. Results

3.1 Crop residues increased soil microbial biomass and altered community structure

The incorporation of cover crop residues significantly ($P < 0.001$) shifted the microbial community structure away from the unamended control soil (Figure S1). The incorporation of residues from different crop species lead to significantly ($P < 0.05$) different community structures (Figure S1). The biomass of general bacteria, Gram-positive bacteria, and fungi in the unamended control soil was 1.80, 2.20, and 2.36 μg C g^{-1}, respectively, which was

greater than the biomass of Gram-negative bacteria and protozoa (Table S3). Despite the same rate of C addition applied across all the treatments, total PLFA biomass differed between residue amendment treatments; ranging from 11.66 to 18.93 μg C g^{-1}, which was significantly ($P < 0.05$) greater than that in the control soil (Table S3).

3.2 Mixing crop residues increased total PLFA biomass and altered microbial community structure

Total PLFA biomass was 17.74 and 14.05 μg C g^{-1} for the mixture and the average of four non-mixture treatments, respectively (Figure 1). The soils in the mixture treatments had a significantly ($P < 0.05$) greater total microbial biomass, by 26% (3.69 μg C g^{-1}), compared to the soils in the non-mixture treatments (Figure 1 and Table 2).

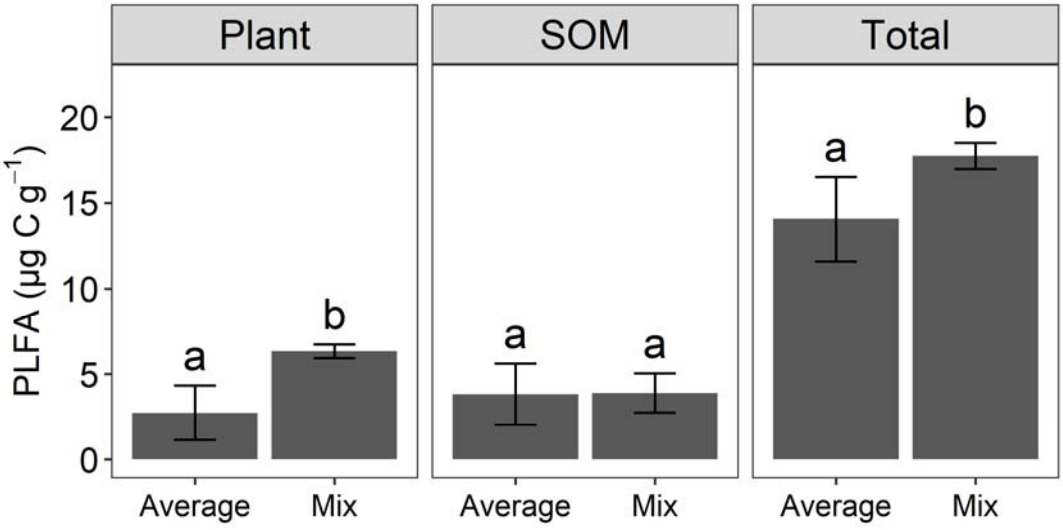


Figure 1 ***Total PLFA biomass (Total), PLFA biomass incorporated from crop residues (Plant), and the PLFA biomass incorporated from soil organic matter (SOM) in the mixture and the average of four non-mixture treatments. Error bars are standard deviations. Different letters within the same panel indicate a significant difference between treatment means at P <0.05.***

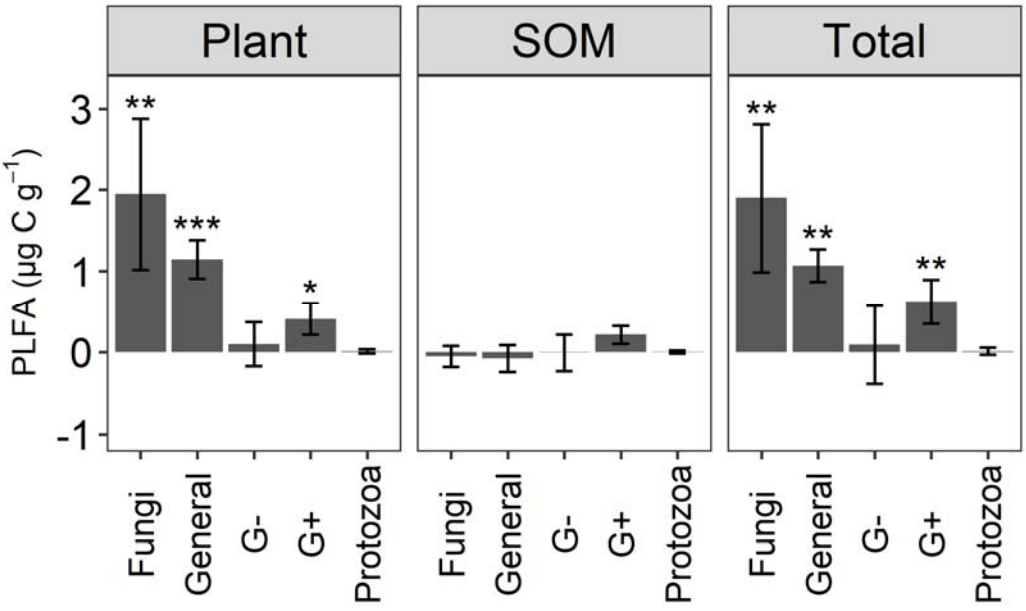
306

307 **Table 2 NEST ANOVA (treatment nested with crop species) to compare the effects of mixture and the species of plant on total PLFA, crop residue-**
308 **derived PLFA, and SOM (soil organic matter)- derived PLFA at different microbial groups. MIX has two levels (mix or non-mix), crop species have five**
309 **levels (radish, clover, buckwheat, sunflower, and mixture). DF is the degree of freedom. Values are F value. *, **, and *** represent significant level at *P***
310 **< 0.05, 0.01, and 0.001. G+ and G- bacteria are Gram-positive and Gram-negative bacteria, respectively.**

Total PLFA									
	DF	Sum of PLFA	General bacteria	G+ bacteria	G- bacteria	Fungi	Protozoa	G+/G-	Fungi/Bacteria
Treatment	1	15.16**	9.16**	12.54**	0.07	9.66**	0.12	0.17	1.80
Treatment:Species	3	5.78**	2.13	9.55***	4.32*	2.69	0.95	3.46*	4.01*
Crop residue- derived PLFA									
Treatment	1	20.62***	16.93***	4.64*	0.19	11.43**	0.22	0.40	0.02
Treatment:Species	3	1.36	0.12	1.34	2.66	1.41	0.85	0.71	0.4
SOM- derived PLFA									
Treatment	1	0.01	0.07	1.33	0.00	0.03	0.01	0.44	0.53
Treatment:Species	3	2.72	2.14	3.97*	3.53*	1.48	0.81	1.08	2.63

311

312 The mixture treatment resulted in a significantly ($P < 0.001$) greater general bacteria, Gram-
313 positive bacteria, and fungi biomass by 31% (1.07 µg C g^{-1}), 18% (0.62 µg C g^{-1}), and 38%
314 (1.90 µg C g^{-1}), respectively, than the average of the non-mixture treatments (Figure 2). In
315 particular, biomarkers i15:0, 16:0, 18:1ω9, 18:2ω6 and 18:3ω3 were significantly ($P < 0.05$)
316 more abundant in the mixture treatment than the average of four non-mixture treatments
317 (Figure 3). The ratios of fungi to bacteria in the mixture and the average of the four non-
318 mixture treatments were 0.64 and 0.57, which was not significantly different (Figure S2).
319 Similarly, there was no significant difference in the ratio of Gram-positive to Gram-negative
320 bacteria between the mixture and the average of four non-mixture treatments (Figure S3).



321

322 ***Figure 2 Differences in PLFA biomass of key microbial groups between the mixture and the***
323 ***average of four non-mixture treatments. Positive value means mixture treatment has greater***
324 ***biomass than the average of non-mixture treatments. The three panels represent the crop derived***
325 ***PLFA biomass (Plant), the primed SOM derived PLFA biomass (SOM), and the total PLFA biomass***
326 ***(Total). Error bars are standard deviations. General, G+, and G- represent general bacteria, Gram-***
327 ***positive and Gram-negative bacteria, respectively. *, **, or *** means significantly different from***
328 ***zero at the level P < 0.05, 0.01 or 0.001.***

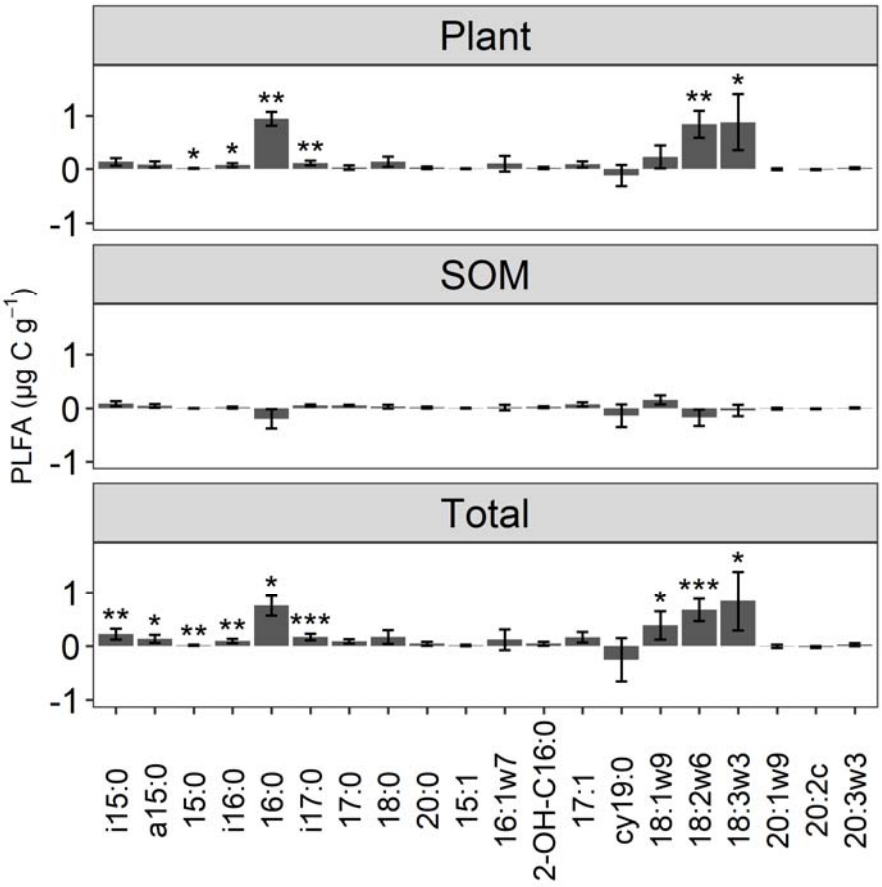


Figure 3 ***Differences in PLFA biomarker biomass between the mixture and the average of four non-mixture treatments. Positive values indicate that mixture has a greater biomass than the average of four non-mixtures. The three panels represent the crop residue derived PLFA biomass (Plant), the primed SOM derived PLFA biomass (SOM), and the total PLFA biomass (Total). Error bars are standard deviations. *, **, and *** indicates significantly different from zero at the level P < 0.05, 0.01, and 0.001, respectively.***

One-way ANOSIM results demonstrated that microbial community structure in the mixture was significantly (P < 0.001, R = 0.326) different from that in the average of four non-mixture (Figure 4).

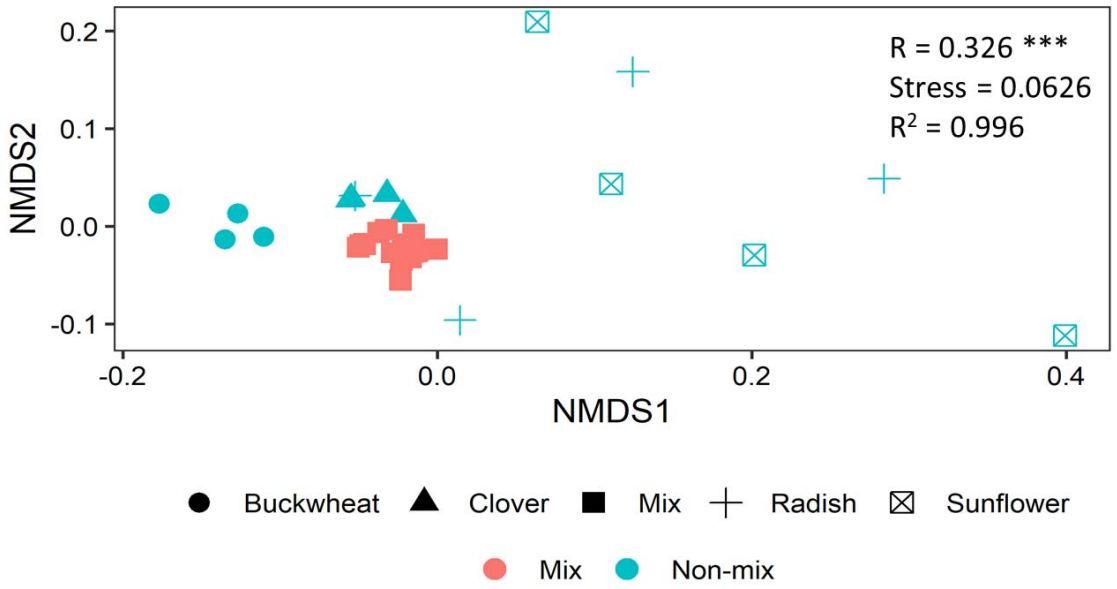


Figure 4 ***Microbial community structure in the mixture and non-mixture treatments. Non-metric multidimensional scaling (NMDS) on the Bray-Curtis distance on the Hellinger transformed PLFA data. Each symbol represents one sample. In the non-mixture treatment, different shapes represent different crop species. R value followed by "***" indicates significant (P < 0.001) difference between the mixture and the average of four non-mixture treatments analysed by one-way ANOSIM.***

3.3 Mixing crop residues increased the PLFA biomass derived from crop residues

Applying ^{13}C labelled cover residues allowed us to distinguish the microbial biomass derived from plant residues from other resources. The results showed that application of crop residues in the mixture and the average of four non-mixture treatments resulted in 6.34 and 2.73 μg C g^{-1} PLFA biomass derived from crop residues, respectively (Figure 1). We observed a significantly ($P < 0.05$) greater crop residue derived PLFA, by 132% (3.61 μg C g^{-1}), in the mixture treatment, compared to the average of four non-mixture treatments (Figure 1).

The mixture exhibited a significantly ($P < 0.01$) greater biomass of general bacteria, Gram-positive bacteria, and fungi derived from crop residues, by 193% (1.15 μg C g^{-1}), 86% (0.41 μg C g^{-1}), and 158% (1.95 μg C g^{-1}), respectively, compared to the average of four non-

358 mixture treatment (Figure 2 and Table 2). The biomass of 16:0, 18:2ω6, and 18:3ω3 in the
359 mixture treatment were significantly ($P < 0.05$) greater than those in the average of non-
360 mixture treatments (Figure 3). Based on the differences in PLFA biomass derived from crop
361 residues, there was no significant differences in either the fungi to bacteria ratio, or the
362 Gram-positive to Gram-negative bacteria ratio, between mixture and the average of four
363 non-mixture treatments (Figure S2 and S3).

364 **3.4 Mixing crop residues did not increase the PLFA biomass derived from primed SOM**

365 The PLFA biomass derived from primed SOM was the difference between total PLFA, PLFA in
366 the control soil, and the PLFA derived from plant residues which was tracked by ^{13}C. The
367 results showed that the PLFA biomass derived from primed SOM was 3.90 and 3.82 μg C g^{-1}
368 for the mixture and the average of four non-mixture treatments, respectively (Figure 1).
369 Therefore, we observed only 2% (0.08 μg C g^{-1}) greater PLFA biomass derived from primed
370 SOM between the mixture and the average of four non-mixture treatments which was not
371 statistically significant (Figure 1 and Table 2). None of the microbial groups or the
372 corresponding PLFA biomarkers exhibited significant differences between the mixture and
373 the average of four non-mixtures in terms of SOM priming (Figure 2 and 3). There were no
374 significant differences in either the fungi to bacteria ratio or the Gram-positive to Gram-
375 negative bacteria of PLFA derived from primed SOM between the mixture and the average
376 of four non-mixture treatments (Figure S2 and S3).

377 **4. Discussion**

378 Following the addition of ^{13}C-labelled residues, we compared the effect of mixing crop
379 residues with the effect of applying the residues of a single crop species, on the soil
380 microbial community composition and attributed the source of the C assimilated by the

381 microbial biomass. Applying crop residues as a mixture resulted in a significantly ($P < 0.05$)
382 greater microbial biomass compared to the average effect of applying each of the residues
383 individually, indicating a synergistic effect of crop residue diversity on soil microbial biomass
384 (Figure 1). This result was consistent with a microcosm experiment which found 38.2%
385 higher soil microbial biomass N in soils receiving a mixture of residues than would be
386 expected from residues of individual species (Mao et al., 2017). The mixture also exhibited
387 greater total microbial biomass than any of the non-mixture treatments (Figure S4),
388 suggesting its synergistic effect is more likely to be caused by facilitation between residues
389 rather than by the disproportionate effect of individual species. The common explanation in
390 the literature for why a mixture induces synergistic effect on decomposition rate is that N is
391 transferred from low C/N residues to high C/N residues to satisfy microbial stochiometric
392 requirement (Gartner and Cardon, 2004; Mao et al., 2017). If the availability of N is the
393 limiting factor in our experiment, we would see the treatment receiving clover residues
394 (which had the highest C/N ratio) exhibiting the smallest microbial biomass. On the contrary,
395 the total microbial biomass in the treatment receiving clover residue (where C/N ratio was
396 32) was significantly ($P < 0.05$) greater than the treatment receiving buckwheat residue
397 (where C/N ratio was 10) (Figure S4), implying that N transfer between high C/N and low
398 C/N residues to satisfy microbial stoichiometric requirement may not be the reason for the
399 observed synergistic effects of the mixture.

400 Although crop residues, such as buckwheat and sunflower in our study, contain high amount
401 of N, they could also contain a considerable amount of plant secondary metabolites (e.g.,
402 tannins and terpenes) that suppress microbial resource assimilation (Gessner et al., 2010) or
403 require specialized enzymes to be degraded (Chomel et al., 2016). When mixing residues,

404 microorganisms could acquire energy via decomposing liable C from other residues to
405 synthesize enzymes to degrade these secondary metabolites, and then anabolise
406 subsequent monomers in their biomass (Wang et al., 2015). Additionally, micronutrients
407 (e.g., Mg and Ca) were identified to play a paramount role in regulating nutrient transfer
408 between residues (García-Palacios et al., 2016). This includes positive effects of Ca on the
409 growth and activity of white rot fungi and significant effects of Mg on invertebrate (e.g.,
410 nematodes) diets which may influence the soil microbial community composition (García-
411 Palacios et al., 2016). Thus, we suggested that the non-additive effect induced by the
412 mixture was not predominantly controlled by residue bulk elemental composition (C/N
413 ratio); instead, it could have been driven by the chemical composition of residues, including
414 plant secondary metabolites and micronutrients.

415 We found that the greater microbial biomass in soils receiving residue mixtures, compared
416 to individual residues, can largely be attributed to C assimilated directly from the plant
417 residues, rather than C obtained by enhanced priming of SOM (Figure 1). Microbial
418 communities preferentially mineralise labile compounds after crop residues are applied to
419 soils to build their biomass rather than decomposing pre-existing SOM (Ball et al., 2014).
420 Significantly greater crop residue-derived C was observed in biomarkers 16:0, 18:2ω6 and
421 18:3ω3 in the soils amended with crop residue mixtures, compared to soils receiving
422 individual residues (Figure 3). This finding is supported by the knowledge that biomarker
423 16:0 represents microbial groups that primarily build their biomass from crop derived C
424 (Wang et al., 2014). Although 16:0 was widely accepted as general bacterial biomarker, it
425 also found in crops (Willers et al., 2015). There is a possibility that this biomarker was
426 present as part of the tissues of our cover crops. Therefore, future studies combining stable

427 isotope probing and DNA fingerprints would overcome this uncertainty associated with the
428 PLFA approach (Blagodatskaya and Kuzyakov, 2013). We found that fungi (e.g., biomarker
429 18:2ω6 and 18:3ω3) were particularly efficient in assimilating a mixture of crop residue-
430 derived C (Figure 3). This could be because fungal hyphae networks allow nutrients to be
431 transported between microsites in the chemically and spatially heterogenous environment
432 resulting from mixed residues (Ball et al., 2014). Furthermore, fungi can produce a wide
433 range of extracellular enzymes that can degrade compounds which are recalcitrant for other
434 microbes (Voriskova and Baldrian, 2013). By contrast, Gram-negative bacteria (e.g., 16:1ω5
435 and 16:1ω7) are good at incorporating SOM derived C into their biomass (Nottingham et al.,
436 2009).

437 Although the application of crop residues did induce microbial assimilation of the native
438 SOM, the magnitude of assimilation was not significantly changed by mixing crop residues
439 (Figure 1 and 2). This observation was contrary to a previous assertion that mixtures of plant
440 residues with a wide spectrum of labile compounds may support a higher microbial biomass
441 and produce more extracellular enzymes, consequently enhancing the potential to prime
442 the decomposition of recalcitrant compounds in SOM (Meier and Bowman, 2008). The lack
443 of a marked mixture-induced priming effect could, however, be because the amount of C
444 added was the same in all treatments. A recent meta-analysis, which analysed studies
445 applying a range of organic C application rates, up to 3 mg C g^{-1}, reported that the
446 magnitude of the priming effect significantly increased with the increasing rate of additions,
447 but was not affected by different residue types (Sun et al., 2019).

448 We made our observations only one day after crop residues were applied to soils because
449 we were interested in identifying the microbial groups which incorporate plant derived C

450 directly into their biomass by anabolism, rather than secondary turnover of C from microbial
451 necromass, which could continue for months or years (Gunina and Kuzyakov, 2015). If
452 incubating longer until the exhaustion of available C, then mixture treatments which have a
453 larger microbial biomass, may facilitate a stronger priming effect because of the increased
454 need for nutrients to maintain microbial survival (Yu et al., 2020).

455 Our study revealed that the incorporation of crop residue mixtures increased the total
456 microbial biomass during the first day after application to a greater extent than would be
457 expected by the addition of the same quantity of C from a single species residue. This
458 additional microbial biomass is mostly derived from the crop residues themselves, rather
459 than primed SOM. It is not clear whether the reason for this greater microbial biomass is
460 due to faster metabolism of the residues, or higher carbon use efficiency (CUE) of the
461 microorganisms responsible. We also found that soil respiration rates one day after applying
462 crop residues were not significantly ($P < 0.05$) different between the mixture and the
463 average of four non-mixtures (Figure S7). If the mechanism of higher CUE is responsible (or
464 even partially responsible), then applying a mixture of crop residues to soils may help to
465 increase SOC stocks in arable soils because more crop residue-derived C enters the microbial
466 biomass and less is respired as CO_2 (Figure S7) and the larger microbial biomass may result
467 in a larger microbial necromass and, ultimately, more SOM. Therefore, land management
468 practices that involve the incorporation of a mixture of crop residues (such as growing cover
469 crop mixtures in polyculture after monoculture crop harvest and before planting the
470 following monoculture crop) may be beneficial for increasing soil C stocks and climate
471 change mitigation.

5. Conclusion

Mixing of crop residues produced a synergistic effect on total soil microbial biomass because fungi, general bacteria, and Gram-positive bacteria were able to incorporate plant-derived C directly into their biomass to a greater extent than when applying individual crop residues. These findings may be due to a better balance of nutrient inputs provided by mixtures, and broader niche for microorganism to colonise. Residue addition primed native SOM, but mixing residues resulted in an additive effect on microbial biomass C resulting from primed SOM. If the greater soil microbial biomass from residue mixtures results in greater microbial necromass and does not result in later priming of native SOM, then applying a mixture of crop residues to soils (e.g., incorporating or growing a cover crop polyculture) could be a sustainable agricultural practice to enhance SOC storage in arable soils.

Acknowledgements

This research was supported by a Biotechnology and Biological Sciences Research Council New Investigator Grant (BB/R006989/1) awarded to Tom Sizmur. The authors wish to thank Paul Brown from Kings Crops, a division of Frontier Agriculture Ltd, for supplying cover crop seed, and the Isolife (Wageningen, Netherland) for growing and labelling the cover crops. We acknowledge the assistance of Andrew Dodson, Anne Dudley, Karen Gutteridge, Fengjuan Xiao, Marta O'Brian, Sean Coole with laboratory analysis, and Dedy Antony, Alex Adetunji Adekanmbi and Chinonso Chukwuma Ogbuagu with soil sampling.

Supplementary information

A Supplementary information document is provided which contains 3 tables and 7 figures

References

494 Adekanmbi, A.A., Shaw, L.J., Sizmur, T., 2020. Effect of Sieving on Ex Situ Soil Respiration of
495 Soils from Three Land Use Types. Journal of Soil Science and Plant Nutrition.
496 doi:10.1007/s42729-020-00177-2

497 Ball, B.A., Carrillo, Y., Molina, M., 2014. The influence of litter composition across the litter-
498 soil interface on mass loss, nitrogen dynamics and the decomposer community. Soil
499 Biology and Biochemistry 69, 71–82. doi:10.1016/j.soilbio.2013.10.048

500 Bastida, F., Eldridge, D.J., García, C., Kenny Png, G., Bardgett, R.D., Delgado-Baquerizo, M.,
501 2021. Soil microbial diversity–biomass relationships are driven by soil carbon content
502 across global biomes. ISME Journal. doi:10.1038/s41396-021-00906-0

503 Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: Critical review of
504 estimation criteria and approaches. Soil Biology and Biochemistry 67, 192–211.
505 doi:10.1016/j.soilbio.2013.08.024

506 Castro-Díez, P., Alonso, Á., Romero-Blanco, A., 2019. Effects of litter mixing on litter
507 decomposition and soil properties along simulated invasion gradients of non-native
508 trees. Plant and Soil 442, 79–96. doi:10.1007/s11104-019-04160-4

509 Chapman, S.K., Newman, G.S., 2010. Biodiversity at the plant-soil interface: Microbial
510 abundance and community structure respond to litter mixing. Oecologia 162, 763–769.
511 doi:10.1007/s00442-009-1498-3

512 Chen, Y., Sun, J., Xie, F., Wang, X., Cheng, G., Lu, X., 2015. Litter chemical structure is more
513 important than species richness in affecting soil carbon and nitrogen dynamics
514 including gas emissions from an alpine soil. Biology and Fertility of Soils 51, 791–800.

515 doi:10.1007/s00374-015-1025-0

516 Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D.,
517 Jackson, B.G., Baldy, V., 2016. Plant secondary metabolites: a key driver of litter
518 decomposition and soil nutrient cycling. Journal of Ecology 104, 1527–1541.
519 doi:10.1111/1365-2745.12644

520 García-Palacios, P., Mckie, B.G., Handa, I.T., Frainer, A., Hättenschwiler, S., 2016. The
521 importance of litter traits and decomposers for litter decomposition: A comparison of
522 aquatic and terrestrial ecosystems within and across biomes. Functional Ecology 30,
523 819–829. doi:10.1111/1365-2435.12589

524 Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos
525 104, 230–246. doi:10.1111/j.0030-1299.2004.12738.x

526 Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H.,
527 Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology & Evolution
528 25, 372–380. doi:10.1016/j.tree.2010.01.010

529 Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: Review of
530 origin, content, composition and fate. Soil Biology and Biochemistry 90, 87–100.
531 doi:10.1016/j.soilbio.2015.07.021

532 Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter.
533 Soil Biology and Biochemistry 42, 1363–1371. doi:10.1016/j.soilbio.2010.04.003

534 Liang, C., Kästner, M., Joergensen, R.G., 2020. Microbial necromass on the rise: The growing
535 focus on its role in soil organic matter development. Soil Biology and Biochemistry 150,

2018–2021. doi:10.1016/j.soilbio.2020.108000

Mao, B., Mao, R., Zeng, D.H., 2017. Species diversity and chemical properties of litter influence non-Additive effects of litter mixtures on soil carbon and nitrogen cycling. PLoS ONE 12, 1–19. doi:10.1371/journal.pone.0180422

Meier, C.L., Bowman, W.D., 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences of the United States of America 105, 19780–19785. doi:10.1073/pnas.0805600105

Norton, J.M., Schimel, J.P., 2011. Nitrogen mineralization-immobilization turnover, in: Huang, P.M. (Ed.), Handbook of Soil Science. CRC Press, pp. 1–51.

Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology 42, 183–190. doi:10.1016/j.apsoil.2009.03.003

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Porre, R.J., van der Werf, W., De Deyn, G.B., Stomph, T.J., Hoffland, E., 2020. Is litter decomposition enhanced in species mixtures? A meta-analysis. Soil Biology and Biochemistry 145, 107791. doi:10.1016/j.soilbio.2020.107791

R Core Team, 2018. R: A Language and Environment for Statistical Computing.

Redin, M., Recous, S., Aita, C., Dietrich, G., Skolaude, A.C., Ludke, W.H., Schmatz, R., Giacomini, S.J., 2014. How the chemical composition and heterogeneity of crop residue

557 mixtures decomposing at the soil surface affects C and N mineralization. Soil Biology
558 and Biochemistry 78, 65–75. doi:10.1016/j.soilbio.2014.07.014

559 Rubino, M., Dungait, J.A.J., Evershed, R.P., Bertolini, T., De Angelis, P., D'Onofrio, A.,
560 Lagomarsino, A., Lubritto, C., Merola, A., Terrasi, F., Cotrufo, M.F., 2010. Carbon input
561 belowground is the major C flux contributing to leaf litter mass loss: Evidences from a
562 13C labelled-leaf litter experiment. Soil Biology and Biochemistry 42, 1009–1016.
563 doi:10.1016/j.soilbio.2010.02.018

564 Shahbaz, M., Kumar, A., Kuzyakov, Y., Börjesson, G., Blagodatskaya, E., 2018. Interactive
565 priming effect of labile carbon and crop residues on SOM depends on residue
566 decomposition stage: Three-source partitioning to evaluate mechanisms. Soil Biology
567 and Biochemistry 126, 179–190. doi:10.1016/j.soilbio.2018.08.023

568 Sizmur, T., Tilston, E.L., Charnock, J., Palumbo-Roe, B., Watts, M.J., Hodson, M.E., 2011.
569 Impacts of epigeic, anecic and endogeic earthworms on metal and metalloid mobility
570 and availability. Journal of Environmental Monitoring 13, 266–273.
571 doi:10.1039/c0em00519c

572 Sun, Z., Liu, S., Zhang, T., Zhao, X., Chen, S., Wang, Q., 2019. Priming of soil organic carbon
573 decomposition induced by exogenous organic carbon input: a meta-analysis. Plant and
574 Soil 443, 463–471. doi:10.1007/s11104-019-04240-5

575 Voriskova, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes
576 rapid successional changes. ISME Journal 7, 477–486. doi:10.1038/ismej.2012.116

577 Wang, H., Boutton, T.W., Xu, W., Hu, G., Jiang, P., Bai, E., 2015. Quality of fresh organic

matter affects priming of soil organic matter and substrate utilization patterns of microbes. Scientific Reports 5, 1–13. doi:10.1038/srep10102

Wang, Q., Wang, S., He, T., Liu, L., Wu, J., 2014. Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils. Soil Biology and Biochemistry 71, 13–20. doi:10.1016/j.soilbio.2014.01.004

Willers, C., Jansen van Rensburg, P.J., Claassens, S., 2015. Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. Journal of Applied Microbiology 119, 1207–1218. doi:10.1111/jam.12902

Xiao, C., Guenet, B., Zhou, Y., Su, J., Janssens, I.A., 2015. Priming of soil organic matter decomposition scales linearly with microbial biomass response to litter input in steppe vegetation. Oikos 124, 649–657. doi:10.1111/oik.01728

Yu, G., Zhao, H., Chen, J., Zhang, T., Cai, Z., Zhou, G., Li, Z., Qiu, Z., Wu, Z., 2020. Soil microbial community dynamics mediate the priming effects caused by in situ decomposition of fresh plant residues. Science of the Total Environment 737, 139708. doi:10.1016/j.scitotenv.2020.139708

Zhang, X., Xin, X., Yang, W., Zhu, A., Ding, S., 2019. Short-term decomposition, turnover and retention of residue-derived carbon are influenced by the fertility level in a sandy loam soil. Geoderma 349, 68–78. doi:10.1016/j.geoderma.2019.04.043