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Soil Research

Supplementary Material

Cover crop residue diversity enhances microbial activity and biomass with additive effects on microbial structure

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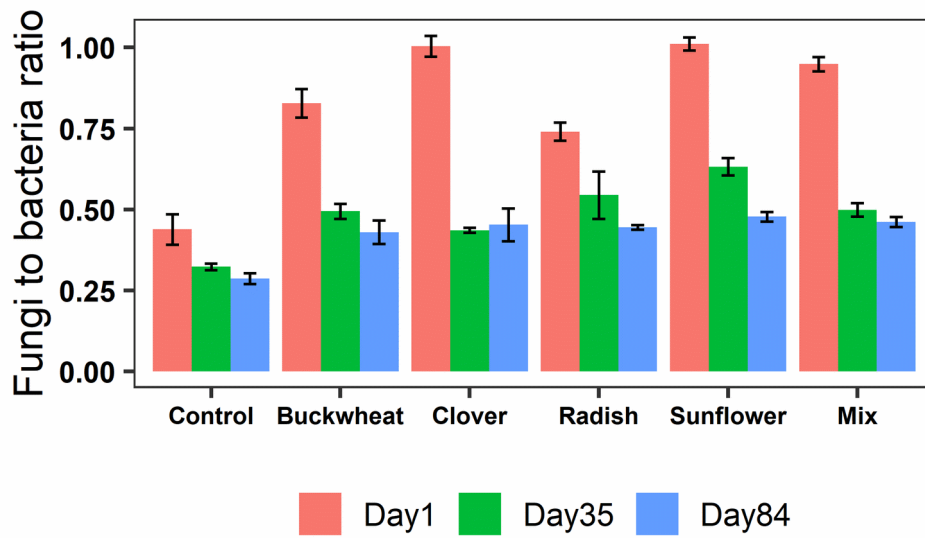


Figure S1 The ratio of fungi to bacteria in all the treatments. Means and standard deviation (error bars).

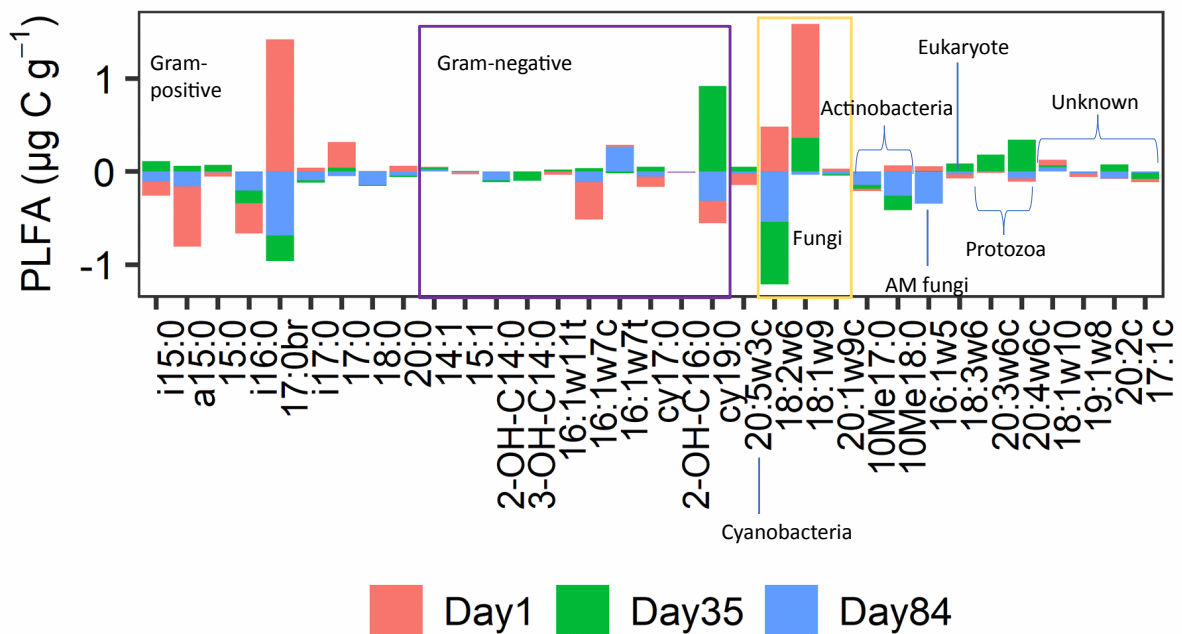


Figure S2 Mass difference of all measured biomarkers between the mixture and the average of the four individuals (buckwheat, clover, radish and sunflower). Positive value means biomarker's biomass is greater in the mixture than the average of the four individuals, and vice versa.

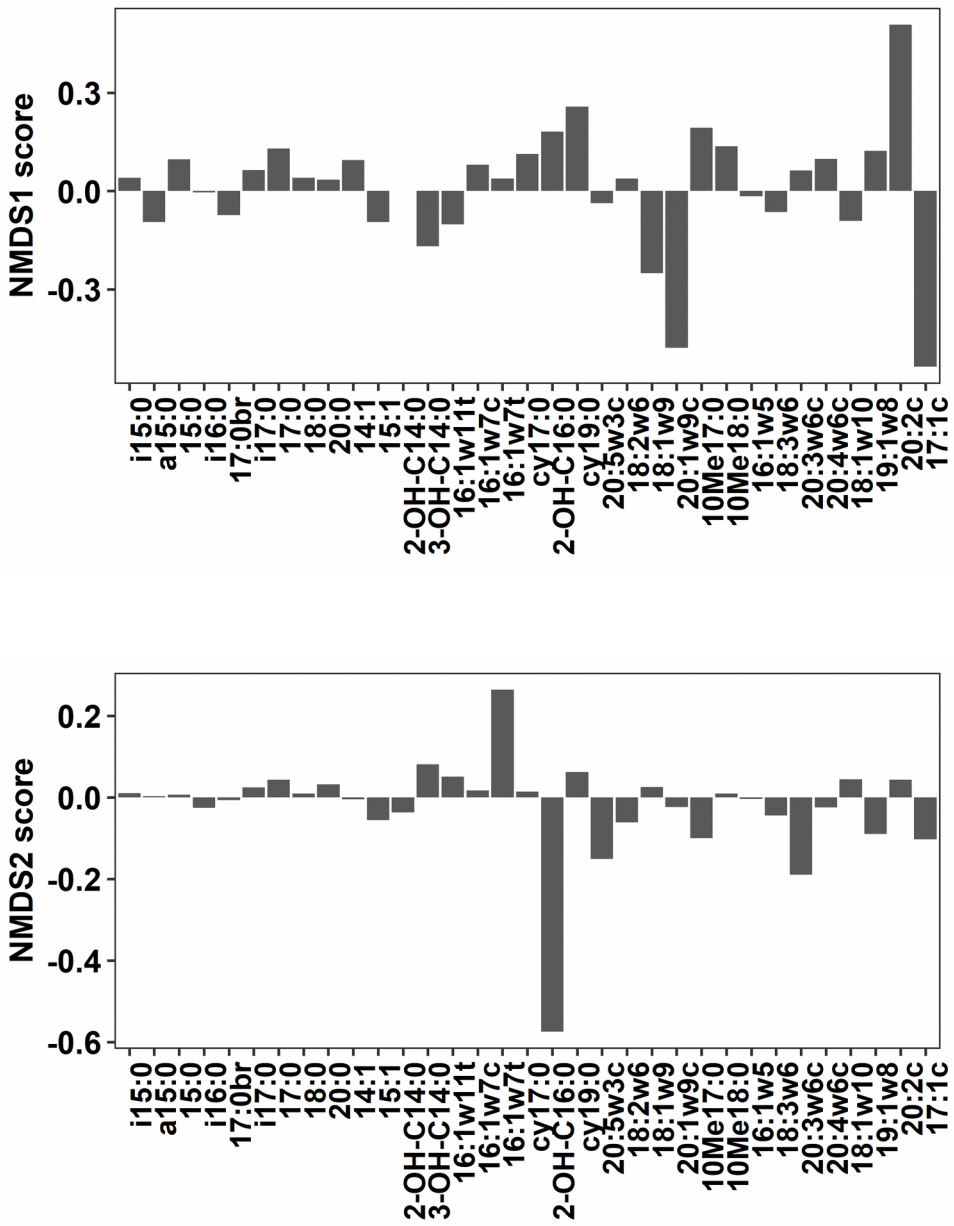


Figure S3 The scores of non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance analysis of Hellinger transformed abundance of phospholipid fatty acid (PLFA) biomarkers.

Table S1 The trace element contents of cover crop residues used in the experiment

Element	Buckwheat	Clover	Radish	Sunflower	Mixture
Al (mg kg ⁻¹)	205	243	381	82.0	229
Cd (mg kg ⁻¹)	0.37	0.08	0.38	0.71	0.09
Co (mg kg ⁻¹)	0.08	0.07	0.37	0.07	0.03
Cr (mg kg ⁻¹)	23.0	21.0	52.0	7.00	25.0
Cu (mg kg ⁻¹)	7.00	10.0	3.00	19.0	9.00
Fe (mg kg ⁻¹)	354	484	816	209	462
Mn (mg kg ⁻¹)	26.0	50.0	38.0	34.0	36.0
Na (mg kg ⁻¹)	91.0	3420	1970	262	1390
Ni (mg kg ⁻¹)	11.0	9.00	21.0	3.00	11.0
Pb (mg kg ⁻¹)	2.06	0.00	2.13	0.71	1.30
Zn (mg kg ⁻¹)	153	47.0	44.0	109	89.0

Table S2 The attribution of PLFA biomarkers to microbial groups

Microbial groups	PLFA biomarkers
Gram-positive bacteria	i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, 17:0br, 18:0, 20:0
Gram-negative bacteria	14:1, 15:1, cy7:0, 16:1 ω11t, 16:1 ω7c, 16:1 ω7t, cy17:0, cy19:0, 2-OH-C14:0, 3-OH-C14:0, 2-OH-C16:0
Fungi	18:2 ω6, 18:1 ω9, 20:1 ω9c
Actinobacteria	10Me17:0, 10Me18:0
Eukaryote	18:3 ω6
AM fungi	16:1 ω5
Protozoa	20:3 ω6c, 20:4 ω6c
Cyanobacteria	20:5 ω3c
Unknown	18:1 ω10, 19:1 ω8, 20:2c, 17:1c

Table S3 Coefficients of linear mixed effects model analysing the fixed effects from different treatments and day as well as their interaction on soil respiration rate ($\mu\text{g C g}^{-1} \text{h}^{-1}$), fungi to bacteria ratio, and the biomass ($\mu\text{g C g}^{-1}$) of total PLFA, fungi, Gram-positive bacteria, Gram-negative bacteria, eukaryote, protozoa, AM fungi, actinobacteria, cyanobacteria, and unknown microbial group. DF means degree of freedom. For all microbial group, the DF are the same as total PLFA. The effects are the effects of different treatments with a comparison to the control which received no cover crop residues. *, **, and *** means significant difference at $P < 0.05$, < 0.01 , and < 0.001 . Abbreviations are F/B ratio (fungi to bacteria ratio), G+ bacteria (Gram-positive bacteria), G- bacteria (Gram-negative bacteria), AMF (arbuscular mycorrhiza fungi).

Fixed term	Respiration		Total PLFA		F/B ratio	Fungi	G+ bacteria	G- bacteria	Eukaryote	Protozoa	AMF	Actinobacteria	Cyanobacteria	Unknown
	Effects	DF	Effects	DF	Effects	Effects	Effects	Effects	Effects	Effects	Effects	Effects	Effects	Effects
Intercept	17.97	426	19.49** *	42	0.42***	4.91*	7.90***	3.97***	0.031	0.138	0.786** *	1.187***	0.015	0.549***
Buckwheat	337.45***	18	45.51** *	18	0.35***	20.47***	20.66** *	1.47	0.236**	0.553*	0.266	0.758	0.196***	0.904***
Clover	340.28***	18	63.77** *	18	0.46***	31.32***	25.77** *	3.2***	0.192**	0.526*	0.346	0.773	0.333***	1.307***
Radish	371.82***	18	53.09** *	18	0.30***	23.46***	25.02** *	3.13***	0.055	0.223	0.032	0.587	0.071*	0.51*
Sunflower	373.48***	18	66.07** *	18	0.54***	34.75***	27.02** *	2.25**	0.135*	0.355	0.11	0.6	0.069*	0.775**
Mix	358.58***	18	59.16** *	18	0.44***	28.83***	25.3***	2.3**	0.159*	0.592*	0.289	0.705	0.096**	0.882***
Day	0.38	426	-0.03	42	-0.002	-0.02	0	0	0	0	-0.007**	-0.003	0	-0.003
Buckwheat*Day	-2.34***	426	-0.33***	42	-0.003*	-0.19**	-0.17***	0.02	0	0	0.009*	0.006	-0.001*	0
Clover*Day	-2.36***	426	-0.54***	42	-0.004**	-0.34***	-0.22***	0.01	-0.001	0.001	0.004	0.016	-0.003***	-0.007
Radish*Day	-2.09***	426	-0.49***	42	-0.002	-0.24***	-0.26***	-0.01	0	0.003	0.002	0.009	0	0
Sunflower*Day	-1.94***	426	-0.61***	42	-0.004**	-0.37***	-0.26***	0.01	-0.002	-0.001	-0.001	0.015	0	-0.005
Mix*Day	-1.77***	426	-0.55***	42	-0.004**	-0.31***	-0.25***	0.01	-0.001	-0.001	-0.001	0.006	0	-0.004

Table S4 Multiple pairwise comparison of the effect between different plant residues on respiration rate ($\mu\text{g C g}^{-1} \text{h}^{-1}$), fungi to bacteria ratio, and the biomass ($\mu\text{g C g}^{-1}$) of total PLFA, fungi, Gram-positive bacteria, Gram-negative bacteria, eukaryote, protozoa, AM fungi, actinobacteria, cyanobacteria, and unknown microbial group. *, **, and *** means significant difference at $P < 0.05$, < 0.01 , and < 0.001 . Abbreviations are F/B ratio (fungi to bacteria ratio), G+ bacteria (Gram-positive bacteria), G- bacteria (Gram-negative bacteria), AMF (arbuscular mycorrhiza fungi).

Comparison	Respiration	Total PLFA	F/B ratio	Fungi	G+ bacteria	G- bacteria	Eukaryote	Protozoa	AMF	Actinobacteria	Cyanobacteria	Unknown
Mix/ Buckwheat	36.93	4.831	0.052	3.650	1.305	0.600	-0.100	0.026	-0.379	-0.046	-0.049	-0.176
Mix/ Clover	34.62	-5.082	0.006	-	-1.658	-0.767	-0.033	0.011	-0.281	-0.480	-0.119	-0.284
Mix/ Radish	-4.32	3.539	0.060	1.470	0.426	-0.065	0.083	0.234	0.120	0.016	0.016	0.229
Mix/ Sunflower	-10.09	-4.589	-0.071	-	-1.452	0.005	0.073	0.260	0.153	-0.233	0.025	0.168
Buckwheat/ Clover	-2.31	-9.913	-0.047	-	-2.963	-1.367	0.068	-0.014	0.099	-0.435	-0.070	-0.107
Buckwheat/ Radish	-41.25	-1.292	0.007	5.120	-0.879	-0.665	0.183**	0.208	0.499*	0.062	0.065	0.405
Buckwheat/ Sunflower	-47.03*	-9.419	-0.123	1.170	-2.757	-0.595	0.173**	0.234	0.532*	-0.188	0.074**	0.344
Clover/ Radish	-38.94	8.621	0.054	7.240	2.084	0.701	0.115	0.223	0.401	0.496	0.135***	0.512*
Clover/ Sunflower	-44.71*	0.494	-0.076	-	0.206	0.772	0.106	0.248	0.434*	0.247	0.143***	0.451*
Radish/ Sunflower	-5.78	-8.127	-0.130	2.110	-1.878	0.070	-0.010	0.026	0.033	-0.249	0.009	-0.061
				6.070								

Table S5 Coefficients of linear mixed effects model analysing the fixed effects from different treatments and day as well as their interaction on relative abundance (proportion) of fungi, Gram-positive bacteria, Gram-negative bacteria, eukaryote, protozoa, AM fungi, actinobacteria, cyanobacteria, and unknown microbial group. For all microbial group, the DF (degree of freedom) are the same. The effects are the effects of different treatments with a comparison to the control which received no cover crop residues. *, **, and *** means significant difference at $P < 0.05$, < 0.01 , and < 0.001 .

Fixed term	D F	Fungi	Gram-positive bacteria	Gram-negative bacteria	Eukaryot e	Protozo a	AM fungi	Actinobacteri a	Cyanobacteri a	Unknow n
Intercept	42	0.255***	0.404***	0.204***	0.001	0.006	0.041***	0.061***	0.001	0.027***
Buckwheat	18	0.141***	0.037***	-0.126***	0.003	0.005	0.026***	-0.032***	0.003***	-0.005
Clover	18	0.168***	0.004	-0.113***	0.001	0.002	0.026***	-0.036***	0.003***	-0.004
Radish	18	0.138***	0.050***	-0.110***	0.000	-0.001	0.029***	-0.035***	0.001	-0.013**
Sunflower	18	0.206***	0.005	-0.131***	0.000	-0.001	0.029***	-0.038***	0.000	-0.012**
Mix	18	0.170***	0.019*	-0.125***	0.001	0.005	0.026***	-0.036***	0.001	-0.009*
Day	42	-0.001	0.001***	0.000*	0.000	0.000	0.000***	0.000	0.000	0.000*
Buckwheat*Day	42	-0.001*	-0.001***	0.001***	0.000	0.000	0.001***	0.000**	0.000	0.000***
Clover*Day	42	-0.002**	-0.001***	0.001***	0.000	0.000	0.000***	0.001***	0.000	0.000*
Radish*Day	42	-0.001	-0.001***	0.001***	0.000	0.000	0.000***	0.001***	0.000*	0.000***
Sunflower*Day	42	-0.002**	-0.001***	0.001***	0.000	0.000	0.000**	0.001***	0.000	0.000*
Mix*Day	42	-0.001*	-0.001***	0.001***	0.000	0.000	0.000**	0.001***	0.000	0.000**

Table S6 Coefficients of linear mixed effects model analysing the fixed effects from mixture plant residues and day as well as their interaction on soil respiration rate ($\mu\text{g C g}^{-1} \text{h}^{-1}$), fungi to bacteria ratio, and the biomass ($\mu\text{g C g}^{-1}$) of total PLFA, fungi, Gram-positive bacteria, Gram-negative bacteria, eukaryote, protozoa, AM fungi, actinobacteria, cyanobacteria, and unknown microbial group. DF means degree of freedom. The effects are the effects of the quaternary mixture of cover crop residues with a comparison to the average of the four individuals. The respiration was compared at two time scales: over 84 days or from day 30 to 84 days. *, **, *** indicates significant at $P < 0.05$, 0.01 , and 0.001 .

Indicators	Intercept		Mix		Day		Mix*Day	
	Effects	DF	Effects	DF	Effects	DF	Effects	DF
Respiration (84 days)	373.35***	358	2.87	18	-1.80***	358	0.42	358
Respiration (30 to 84 days)	305.18***	138	57.61*	18	-0.78***	138	-0.45	138
Total PLFA	76.60***	38	2.05	18	-0.53***	38	-0.06	38
Fungi to bacteria ratio	0.83***	38	0.03	18	-0.005***	38	-0.0004	38
Fungi	32.41***	38	1.33	18	-0.30***	38	-0.03	38
Gram-positive bacteria	32.52***	38	0.69	18	-0.23***	38	-0.03	38
Gram-negative bacteria	6.49***	38	-0.21	18	0.004	38	0.003	38
Eukaryote	0.19***	38	0.004	18	-0.0008	38	0.00003	38
Protozoa	0.55***	38	0.18	18	0.0006	38	-0.001	38
AM fungi	0.97***	38	0.10	18	-0.003*	38	-0.005	38
Actinobacteria	1.87***	38	0.025	18	0.008*	38	-0.005	38
Cyanobacteria	0.18***	38	-0.07	18	-0.001**	38	0.0009	38
Unknown	1.42***	38	0.008	18	-0.006***	38	-0.0006	38

Table S7 Pearson correlation of respiration and PLFA biomass in different group in 3 time points (day 1, 35, and 84). G+ and G- represent Gram-positive and Gram-negative bacteria, respectively. Sum represents total PLFA biomass. FB Ratio is fungi to bacteria ratio. *, **, and *** represent significant correlation at $P < 0.05$, 0.01 , and 0.001 .

	CO ₂	Actinobacteria	Fungi	G+	G-	Cyanobacteria	AM fungi	Unknown	Eukaryote	Protozoa	Sum	FB Ratio
CO ₂		0.06	0.85***	0.89***	0.35**	0.51***	0.27*	0.62***	0.33**	0.18	0.89***	0.84***
Actinobacteria	0.06		-0.14	0.06	0.71***	0.29*	0.44***	0.32**	0.18	0.44***	0.05	-0.20
Fungi	0.85***	-0.14		0.96***	0.13	0.46***	0.27*	0.62***	0.31**	0.06	0.97***	0.97***
G+	0.89***	0.06	0.96***		0.33**	0.51***	0.43***	0.72***	0.37**	0.17	0.99***	0.89***
G-	0.35**	0.71***	0.13	0.33**		0.45***	0.44***	0.55***	0.38***	0.57***	0.33**	0.00
Cyanobacteria	0.51***	0.29*	0.46***	0.51***	0.45***		0.50***	0.69***	0.60***	0.50***	0.54***	0.43***
AM fungi	0.27*	0.44***	0.27*	0.43***	0.44***	0.50***		0.70***	0.56***	0.46***	0.42***	0.19
Unknown	0.62***	0.32**	0.62***	0.72***	0.55***	0.69***	0.70***		0.59***	0.43***	0.73***	0.51***
Eukaryote	0.33**	0.18	0.31**	0.37**	0.38***	0.60***	0.56***	0.59***		0.83***	0.40***	0.26*
Protozoa	0.18	0.44***	0.06	0.17	0.57***	0.50***	0.46***	0.43***	0.83***		0.20	0.00
Sum	0.89***	0.05	0.97***	0.99***	0.33**	0.54***	0.42***	0.73***	0.40***	0.20		0.91***
FB Ratio	0.84***	-0.20	0.97***	0.89***	0.00	0.43***	0.19	0.51***	0.26*	0.00	0.91***	