Supplementary Materials

Table S1 Carbon metabolism activity of rhizosphere soil microbial communities in alfalfa of different ages

Carbon source [a]	*AWCD*			*P*
	L5 [b]	L7	L9	
Carboxylic acids				
D-galacturonic acid	2.27 (0.04)	2.19 (0.07)	2.26 (0.14)	0.806^{ns}
Itaconic acid	1.63 (0.18)	1.28 (0.39)	0.92 (0.09)	0.043*
γ-hydroxy butyric acid	1.01 (0.31)	0.32 (0.14)	1.85 (0.07)	0.005**
D-galactonic acid γ-lactone	0.99 (0.31)	0.96 (0.07)	2.25 (0.15)	0.006**
D-glucosaminic acid	0.96 (0.11)	1.61 (0.07)	0.87 (0.04)	0.001**
4-hydroxy benzoic acid	0.89 (0.05)	0.95 (0.28)	1.29 (0.10)	0.049*
D-malic acid	0.74 (0.11)	1.47 (0.43)	1.58 (0.14)	0.050*
α-keto butyric acid	0.03 (0.03)	0.22 (0.21)	0.34 (0.09)	0.032*
2-hydroxy benzoic acid	0.00 (0.00)	0.00 (0.00)	0.38 (0.02)	0.000***
Carbohydrates				
D-mannitol	2.07 (0.50)	2.18 (0.17)	2.67 (0.06)	0.388^{ns}
N-acetyl-D-glucosamine	1.81 (0.49)	1.37 (0.10)	2.67 (0.08)	0.049*
D-cellobiose	1.63 (0.63)	1.52 (0.12)	2.63 (0.12)	0.047*
β-methyl-D glucoside	1.41 (0.37)	1.32 (0.32)	1.80 (0.15)	0.514^{ns}
α-D-lactose	1.20 (0.25)	2.26 (0.32)	1.77 (0.07)	0.050*
i-erythritol	0.72 (0.04)	0.85 (0.10)	0.48 (0.03)	0.015*
D-xylose	0.70 (0.10)	0.01 (0.01)	2.58 (0.04)	0.000***
Amino acids				
L-serine	2.17 (0.06)	2.18 (0.03)	2.21 (0.08)	0.606^{ns}
L-asparagine	2.10 (0.22)	2.12 (0.10)	2.39 (0.08)	0.347^{ns}
glycyl-L-glutamic acid	1.53 (0.17)	1.38 (0.15)	1.33 (0.05)	0.571^{ns}
L-arginine	1.43 (0.06)	2.10 (0.26)	1.60 (0.14)	0.039*
L-threonine	0.54 (0.02)	0.33 (0.09)	0.63 (0.10)	0.030*
L-phenylalanine	0.29 (0.19)	0.72 (0.20)	1.05 (0.01)	0.039*
Polymers				
Tween 40	1.07 (0.17)	1.74 (0.16)	1.95 (0.15)	0.018*
Tween 80	0.79 (0.18)	1.06 (0.11)	2.32 (0.23)	0.002**
α-cyclodextrin	0.50 (0.31)	0.73 (0.34)	1.29 (0.19)	0.046*
Glycogen	0.19 (0.17)	0.37 (0.27)	1.43 (0.30)	0.027*
Miscellaneous				
Glucose-1-phosphate	1.31 (0.25)	1.14 (0.64)	1.20 (0.06)	0.952^{ns}
Pyruvic acid methyl ester	1.37 (0.22)	1.20 (0.33)	1.55 (0.22)	0.671^{ns}
D, L-α-glycerol phosphate	0.18 (0.10)	0.44 (0.14)	0.25 (0.01)	0.248^{ns}
Amides				
Putrescine	1.14 (0.29)	1.50 (0.60)	1.47 (0.05)	0.545^{ns}
Phenylethylamine	0.01 (0.01)	0.38 (0.38)	0.85 (0.08)	0.042*

Data are expressed as mean (SE). One-way ANOVA with * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; ns = not significant.
[a] The substrates in the Biolog EcoPlates are categorized according to their chemical structure and functional groups.
[b] L5, L7, L9 refer to 5-, 7-, and 9-year-old alfalfa, respectively.

Table S2 Shannon-Weaver diversity index (H) and richness index (S) of carbon metabolism of rhizosphere soil microbial communities in alfalfa of different ages

Incubation time (h)	Shannon-Weaver diversity index (H)				Richness index (S)			
	L5	L7	L9	*P*	L5	L7	L9	*P*
12	1.61(0.81)	2.14(0.35)	2.48(0.06)	ns	0.00(0.00)	0.00(0.00)	0.00(0.00)	–
24	2.36(0.39)	2.45(0.15)	2.73(0.13)	ns	0.33(0.33)	0.00(0.00)	2.67(1.45)	ns
48	2.69(0.06)	2.63(0.03)	2.96(0.04)	0.005	14.33(0.88)	10.33(0.67)	13.33(6.89)	ns
72	2.90(0.08)	2.80(0.03)	3.10(0.02)	0.016	20.00(1.53)	15.67(0.88)	26.33(1.45)	0.004
96	3.04(0.06)	3.04(0.05)	3.21(0.01)	0.045	24.00(1.53)	23.33(0.33)	29.00(0.58)	0.011
120	3.11(0.06)	3.11(0.03)	3.28(0.01)	0.042	25.00(1.53)	25.00(0.58)	29.67(0.33)	0.021
144	3.16(0.05)	3.15(0.02)	3.31(0.01)	0.023	26.33(1.20)	25.33(0.67)	31.00(0.00)	0.005
168	3.16(0.03)	3.19(0.01)	3.30(0.01)	0.012	26.33(1.20)	26.67(0.67)	30.67(0.33)	0.017
192	3.18(0.02)	3.22(0.01)	3.34(0.01)	0.001	26.33(1.20)	26.67(0.67)	31.00(0.00)	0.010
216	3.20(0.01)	3.24(0.01)	3.33(0.01)	0.001	27.00(0.00)	27.00(0.58)	30.67(0.33)	0.001
240	3.22(0.01)	3.25(0.01)	3.35(0.00)	0.000	27.33(0.33)	28.00(1.00)	31.00(0.00)	0.011

Data are expressed as mean (SE). ns = not significant. "–" indicates the P value could not be calculated as all treatments had zero values.

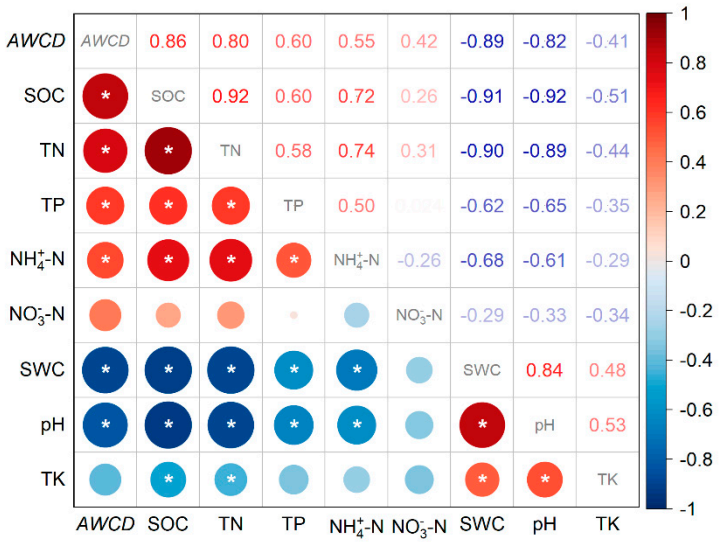

Figure S1 Heat map showing the Pearson correlation between metabolic activity of the alfalfa rhizosphere soil microbial community and soil physicochemical factors. The color intensity represents the strength of correlation, with red indicating positive

correlations and blue indicating negative correlations. Significant correlations are marked with asterisks (* $P < 0.05$).

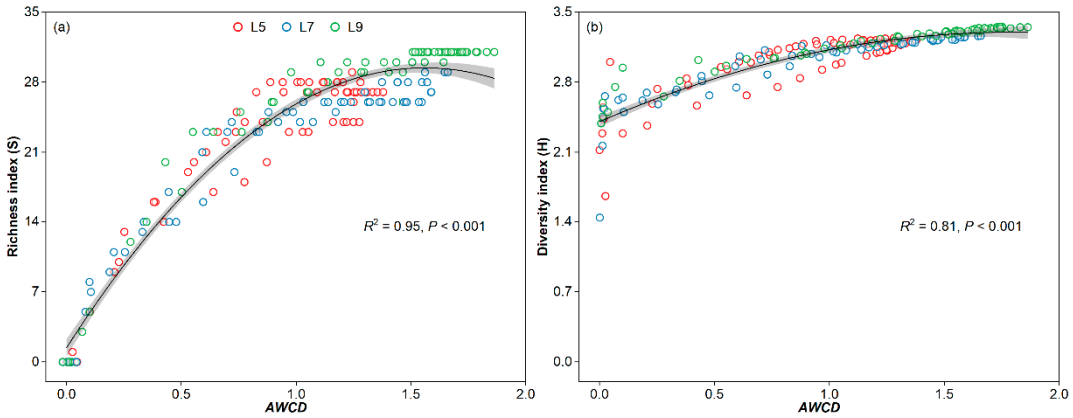

Figure S2 Relationships between the richness index (S), the Shannon-Weaver diversity index (H) of soil microbial communities in the rhizosphere of alfalfa at different age classes, and Average Well Color Development (AWCD). Panel (a) shows the relationship between richness index (S) and AWCD, where S increases rapidly with AWCD initially and then gradually plateaus ($R^2 = 0.95$, $P < 0.001$); Panel (b) shows the relationship between diversity index (H) and AWCD, which exhibits a positive correlation ($R^2 = 0.81$, $P < 0.001$). Data points include samples from 5-year (L5), 7-year (L7), and 9-year (L9) alfalfa rhizosphere soil. AWCD represents the overall carbon utilization capacity of the microbial community.

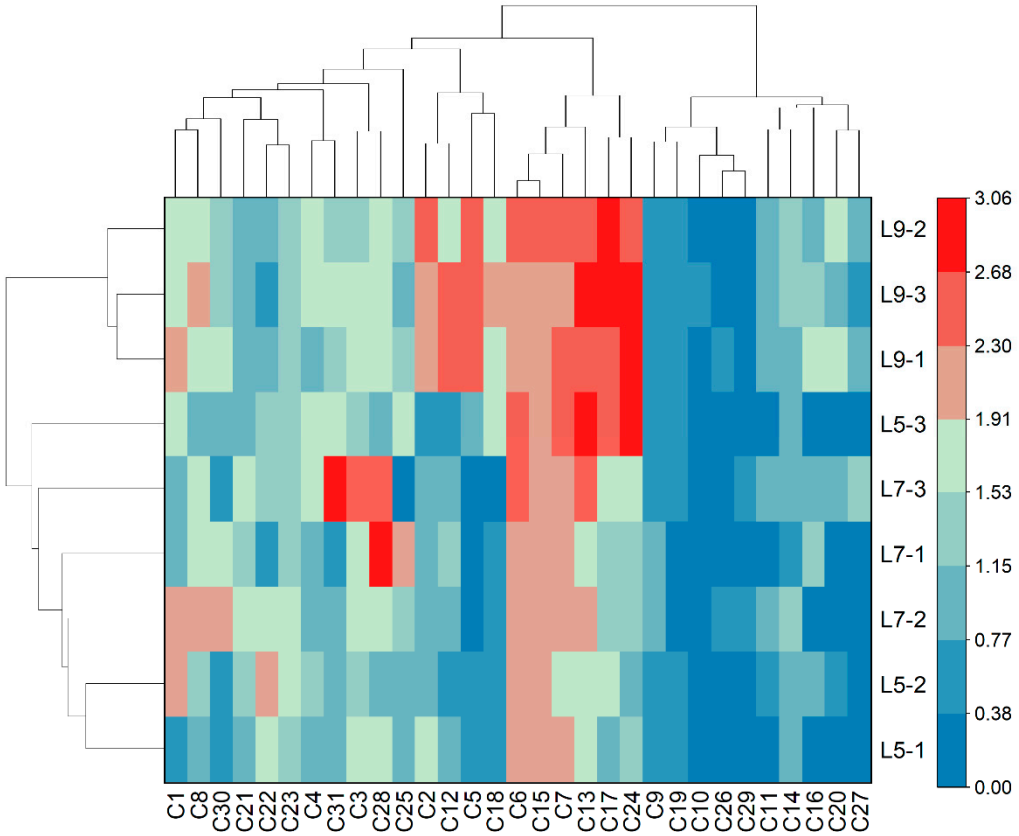

Figure S3 Heat map of carbon utilization clustering in rhizosphere soil microbial communities of alfalfa of different age classes. The carbon sources (C1-C31) are as follows: C1, β-methyl-D-glucoside; C2, D-galactonic acid γ-lactone; C3, L-arginine; C4, pyruvic acid methyl ester; C5, D-xylose; C6, D-galacturonic acid; C7, L-asparagine; C8, Tween 40; C9, i-erythritol; C10, 2-hydroxybenzoic acid; C11, L-phenylalanine; C12, Tween 80; C13, D-mannitol; C14, 4-hydroxybenzoic acid; C15, L-serine; C16, α-cyclodextrin; C17, N-acetyl-D-glucosamine; C18, γ-hydroxybutyric acid; C19, L-threonine; C20, glycyl-L-glutamic acid; C21, D-glucosaminic acid; C22, itaconic acid; C23, glycogen; C24, D-cellobiose; C25, glucose-1-phosphate; C26, α-ketobutyric acid; C27, phenylethylamine; C28, α-D-lactose; C29, D,L-α-glycerol phosphate; C30, D-

malic acid; C31, putrescine. Color intensity indicates the relative utilization level of each carbon source, with red representing higher utilization and blue representing lower utilization.