

Seasonal effects on microbial community structure and nitrogen dynamics in temperate forest soil

[Supplementary tables and figures]

Table S1. WC, pH, and WEOC in the O-layer and S-layer on each sampling date at both sites. Values represent the mean (each date n = 5, annual n = 30) $\pm$ SE. Letters indicate significant differences on a sampling date (Tukey's HSD test, $p < 0.05$). Asterisks indicate a significant effect of season in one-way ANOVA and of site, season, and their interaction in two-way ANOVA (** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). N.S., not significant.

Date	WC (g g ⁻¹)		pH(H ₂ O)		WEOC (g C kg ⁻¹)	
	O-layer	S-layer	O-layer	S-layer	O-layer	S-layer
NF						
Jun	1.75 $\pm$ 0.37a	0.58 $\pm$ 0.04	4.50 $\pm$ 0.20ab	4.22 $\pm$ 0.08a	1.17 $\pm$ 0.15ab	0.37 $\pm$ 0.03a
Aug	2.01 $\pm$ 0.19ab	0.54 $\pm$ 0.06	4.64 $\pm$ 0.12a	4.32 $\pm$ 0.12ab	1.07 $\pm$ 0.11abc	0.29 $\pm$ 0.04ab
Nov	1.38 $\pm$ 0.19a	0.55 $\pm$ 0.04	4.29 $\pm$ 0.11ab	4.19 $\pm$ 0.04a	0.87 $\pm$ 0.14bc	0.27 $\pm$ 0.03ab
Dec	1.30 $\pm$ 0.18a	0.52 $\pm$ 0.04	4.28 $\pm$ 0.08b	4.49 $\pm$ 0.04b	0.73 $\pm$ 0.10c	0.25 $\pm$ 0.03b
Feb	2.77 $\pm$ 0.21b	0.58 $\pm$ 0.05	4.38 $\pm$ 0.12ab	4.51 $\pm$ 0.10b	1.50 $\pm$ 0.09a	0.31 $\pm$ 0.04ab
Apr	2.76 $\pm$ 0.27b	0.69 $\pm$ 0.05	4.27 $\pm$ 0.17b	4.21 $\pm$ 0.10a	1.10 $\pm$ 0.13abc	0.29 $\pm$ 0.01ab
Annual	1.99 $\pm$ 0.14	0.57 $\pm$ 0.02	4.39 $\pm$ 0.06	4.32 $\pm$ 0.04	1.07 $\pm$ 0.06	0.30 $\pm$ 0.01
1-way ANOVA	***	N.S.	*	**	***	*
Season						
RF						
Jul	1.04 $\pm$ 0.09a	0.27 $\pm$ 0.07	3.93 $\pm$ 0.24ab	4.43 $\pm$ 0.15	1.45 $\pm$ 0.10	0.13 $\pm$ 0.02
Aug	1.35 $\pm$ 0.10a	0.24 $\pm$ 0.02	4.29 $\pm$ 0.25ab	4.33 $\pm$ 0.10	1.04 $\pm$ 0.11	0.14 $\pm$ 0.02
Nov	1.28 $\pm$ 0.17a	0.20 $\pm$ 0.01	3.79 $\pm$ 0.19a	4.32 $\pm$ 0.09	1.46 $\pm$ 0.32	0.12 $\pm$ 0.01
Dec	1.45 $\pm$ 0.21ab	0.20 $\pm$ 0.01	4.23 $\pm$ 0.32ab	4.36 $\pm$ 0.09	1.24 $\pm$ 0.17	0.13 $\pm$ 0.01
Feb	2.01 $\pm$ 0.11bc	0.29 $\pm$ 0.02	4.12 $\pm$ 0.24ab	4.32 $\pm$ 0.15	1.56 $\pm$ 0.08	0.17 $\pm$ 0.01
Apr	2.15 $\pm$ 0.14c	0.28 $\pm$ 0.03	4.48 $\pm$ 0.31b	4.48 $\pm$ 0.13	1.26 $\pm$ 0.11	0.14 $\pm$ 0.02
Annual	1.55 $\pm$ 0.09	0.25 $\pm$ 0.01	4.14 $\pm$ 0.11	4.37 $\pm$ 0.05	1.33 $\pm$ 0.07	0.14 $\pm$ 0.01
1-way ANOVA	***	N.S.	*	N.S.	N.S.	N.S.
Season						
(NF+RF)						
2-way ANOVA						
Site	N.S.	***	N.S.	N.S.	*	***
Season	***	*	**	N.S.	**	*
Site x Season	*	N.S.	**	*	N.S.	N.S.

Table S2. Pearson correlation coefficients between microbial community structure (MCS) and other variables among all seasons (n = 6). Bold letters indicate significant correlations (p < 0.05).

Variable	PC2 Scores*		Sat/mono		Cy/pre		G+/G-		F/B	
	O layer	Soil layer	O layer	Soil layer	O layer	Soil layer	O layer	Soil layer	O layer	Soil layer
NF										
Meteorological phenomenon										
Temperature (7d)	0.92	0.73	0.92	0.86	0.92	0.77	0.82	0.78	-0.58	-0.24
(14d)	0.90	0.76	0.90	0.88	0.90	0.75	0.83	0.78	-0.59	-0.28
(21d)	0.93	0.74	0.92	0.87	0.93	0.77	0.87	0.75	-0.61	-0.26
(28d)	0.94	0.73	0.93	0.86	0.94	0.80	0.90	0.72	-0.63	-0.25
Precipitation (7d)	0.72	0.67	0.73	0.73	0.64	0.68	0.47	0.20	0.14	0.37
(14d)	0.66	0.64	0.65	0.69	0.57	0.64	0.43	0.15	0.16	0.35
(21d)	0.86	0.80	0.86	0.87	0.80	0.86	0.60	0.40	-0.05	0.22
(28d)	0.83	0.72	0.82	0.80	0.77	0.81	0.65	0.31	-0.08	0.23
Sunshine (7d)	-0.46	-0.29	-0.50	-0.40	-0.39	-0.17	-0.30	-0.13	-0.15	-0.33
(14d)	0.38	0.21	0.38	0.29	0.47	0.27	0.52	0.68	-0.81	-0.62
(21d)	0.03	-0.05	0.02	0.00	0.11	-0.18	0.30	0.51	-0.72	-0.72
(28d)	0.04	0.25	0.03	0.21	0.11	-0.06	0.28	0.72	-0.75	-0.93
Soil environments										
WC	-0.24	-0.05	-0.16	-0.13	-0.34	-0.57	-0.55	0.01	0.73	-0.17
pH(H ₂ O)	0.87	-0.13	0.91	-0.24	0.84	-0.01	0.53	-0.39	-0.19	0.49
Available substrate quality										
WEOC/WEON	-0.68	-0.54	-0.59	-0.66	-0.73	-0.84	-0.91	-0.62	0.71	0.18
Microbial biomarker indices										
PC2 Scores*	—	—	0.99	0.97	0.99	0.79	0.86	0.82	-0.43	-0.26
Sat/mono	0.99	0.97	—	—	0.98	0.88	0.81	0.80	-0.37	-0.19
Cy/pre	0.99	0.79	0.98	0.88	—	—	0.89	0.57	-0.52	0.02
G+/G-	0.86	0.82	0.81	0.80	0.89	0.57	—	—	-0.75	-0.66
F/B	-0.43	-0.26	-0.37	-0.19	-0.52	0.02	-0.75	-0.66	—	—
RF										
Meteorological phenomenon										
Temperature (7d)	0.98	0.59	0.97	0.59	0.95	0.65	0.98	0.68	-0.79	-0.36
(14d)	0.98	0.58	0.98	0.59	0.93	0.67	0.97	0.70	-0.77	-0.33
(21d)	0.99	0.60	0.98	0.60	0.94	0.68	0.98	0.69	-0.76	-0.35
(28d)	0.99	0.62	0.99	0.62	0.94	0.72	0.98	0.69	-0.75	-0.38
Precipitation (7d)	0.61	0.34	0.67	0.36	0.42	0.49	0.56	0.59	-0.41	-0.23
(14d)	0.50	0.30	0.58	0.32	0.29	0.47	0.44	0.54	-0.30	-0.22
(21d)	0.60	0.43	0.66	0.43	0.41	0.54	0.53	0.58	-0.38	-0.36
(28d)	0.56	0.35	0.62	0.35	0.35	0.49	0.50	0.49	-0.26	-0.27
Sunshine (7d)	-0.01	0.53	0.01	0.53	0.24	0.39	-0.07	0.31	-0.42	-0.57
(14d)	0.67	0.40	0.57	0.36	0.79	0.32	0.72	0.21	-0.50	-0.22
(21d)	0.29	-0.15	0.20	-0.15	0.40	-0.10	0.40	-0.20	-0.08	0.37
(28d)	0.14	-0.11	0.15	-0.07	0.16	0.10	0.17	-0.05	0.04	0.34
Soil environments										
WC	-0.72	-0.26	-0.69	-0.20	-0.83	-0.25	-0.69	0.12	0.62	0.29
pH(H ₂ O)	-0.29	-0.09	-0.16	-0.01	-0.43	0.10	-0.37	0.18	0.10	0.23
Available substrate quality										
WEOC/WEON	-0.28	-0.20	-0.22	-0.17	-0.36	-0.17	-0.34	0.05	-0.04	0.04
Microbial biomarker indices										
PC2 Scores*	—	—	0.99	0.99	0.97	0.95	0.99	0.89	-0.76	-0.95
Sat/mono	0.99	0.99	—	—	0.95	0.97	0.95	0.92	-0.80	-0.94
Cy/pre	0.97	0.95	0.95	0.97	—	—	0.95	0.92	-0.84	-0.84
G+/G-	0.99	0.89	0.95	0.92	0.95	0.92	—	—	-0.68	-0.80
F/B	-0.76	-0.95	-0.80	-0.94	-0.84	-0.84	-0.68	-0.80	—	—

*Figure S4

Table S3. Variable importance of projection (VIP) and standardized coefficients of explanatory variables from the PLS regression models for seasonal changes in inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$).

Variables	Inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$)							
	O-layer				S-layer			
	NF		RF		NF		RF	
	VIP	SC	VIP	SC	VIP	SC	VIP	SC
Meteorological phenomenon								
Temperature (28d)	1.20	0.110			1.03	0.096	1.13	0.103
Precipitation (28d)							1.49	0.135
Sunshine (28d)								
Soil environments								
WC			1.75	−0.186	1.30	−0.121	1.20	−0.109
pH(H_2O)	1.48	0.136	1.69	−0.180			1.37	−0.124
Available substrate								
WEON	1.50	0.137	1.25	0.133	1.36	0.127		
WEOC/WEON			1.94	−0.206	1.73	−0.161		
Microbial biomass (MB)								
MB-N	1.54	0.141						
MB-C/N			1.14	0.121	1.28	−0.119		
Fungal-PLFA							1.25	−0.113
Bacterial-PLFA								
Microbial biomarker indices								
PC2 Scores*	1.31	0.119					1.10	0.099
Sat/mono	1.42	0.129						
Cy/pre	1.24	0.114			1.25	0.117	1.06	0.096
G+/G-					1.13	0.105		
F/B							1.06	−0.095

VIP: Variable importance of projection. Only VIPs greater than 1.00 are shown (see Materials and Methods). SC:

Standardized coefficient. *Scores are shown in Figure S4 as a proxy for seasonal changes in PLFA.

Table S4. Variable importance of projection (VIP) and standardized coefficients of explanatory variables from the PLS regression models for seasonal changes in gross NH_4^+ -N production potential

Variables	Gross NH_4^+ -N production potential							
	O-layer				S-layer			
	NF		RF		NF		RF	
	VIP	SC	VIP	SC	VIP	SC	VIP	SC
Meteorological phenomenon								
Temperature (28d)	1.22	0.102	1.47	0.137			1.37	0.178
Precipitation (28d)			1.11	0.104	1.11	-0.152		
Sunshine (28d)								
Soil environments								
WC								
pH(H_2O)	1.38	0.116						
Available substrate								
WEON	1.29	0.108					1.67	0.216
WEOC/WEON							1.96	-0.253
Microbial biomass (MB)								
MB-N	1.39	0.117			1.95	0.267		
MB-C/N								
Fungal-PLFA			1.21	-0.113	1.95	0.267		
Bacterial-PLFA					1.24	0.169	1.29	0.167
Microbial biomarker indices								
PC2 Scores*	1.44	0.121	1.44	0.134	1.38	-0.189	1.02	0.132
Sat/mono	1.52	0.128	1.49	0.139				
Cy/pre	1.41	0.118	1.33	0.123				
G+/G-			1.37	0.127				
F/B			1.36	-0.127				

VIP: Variable importance of projection. Only VIPs greater than 1.00 are shown (see Materials and Methods). SC:

Standardized coefficient. *Scores are shown in Figure S4 as a proxy for seasonal changes in PLFA.

Table S5. Variable importance of projection (VIP) and standardized coefficients of explanatory variables from the PLS regression models for seasonal changes in gross NH_4^+ -N immobilization potential

Variables	Gross NH_4^+ -N immobilization potential							
	O-layer				S-layer			
	NF		RF		NF		RF	
	VIP	SC	VIP	SC	VIP	SC	VIP	SC
Meteorological phenomenon								
Temperature (28d)								
Precipitation (28d)	1.26	0.128						
Sunshine (28d)			1.79	-0.233				
Soil environments								
WC	1.61	0.165			2.11	0.234		
pH(H_2O)								
Available substrate								
WEON	1.62	0.165					1.57	0.186
WEOC/WEON			2.09	0.272	1.61	0.179		
Microbial biomass (MB)								
MB-N	1.34	0.137	1.43	0.186			1.11	0.132
MB-C/N					1.98	0.219		
Fungal-PLFA			1.28	-0.167				
Bacterial-PLFA							1.89	0.225
Microbial biomarker indices								
PC2 Scores*							1.11	0.132
Sat/mono	1.05	0.107					1.14	0.135
Cy/pre					1.41	-0.157		
G+/G-							1.22	0.145
F/B			1.07	-0.139				

VIP: Variable importance of projection. Only VIPs greater than 1.00 are shown (see Materials and Methods). SC:

Standardized coefficient. *Scores are shown in Figure S4 as a proxy for seasonal changes in PLFA.

Table S6. Variable importance of projection (VIP) and standardized coefficients of explanatory variables from the PLS regression models for seasonal changes in net N transformation potential

Variables	Net (NH ₄ ⁺ -N+NO ₃ ⁻ -N) transformation potential							
	O-layer				S-layer			
	NF		RF		NF		RF	
	VIP	SC	VIP	SC	VIP	SC	VIP	SC
Meteorological phenomenon								
Temperature (28d)	1.36	0.123	1.22	0.115			1.18	0.133
Precipitation (28d)	1.23	0.111						
Sunshine (28d)					1.33	0.121		
Soil environments								
WC			1.34	-0.127			1.29	0.145
pH(H ₂ O)	1.40	0.127	1.24	-0.118				
Available substrate								
WEON					1.63	0.148		
WEOC/WEON					1.02	-0.093		
Microbial biomass (MB)								
MB-N							1.87	0.210
MB-C/N							1.23	-0.138
Fungal-PLFA								
Bacterial-PLFA					1.30	0.118	1.55	0.174
Microbial biomarker indices								
PC2 Scores*	1.45	0.132	1.30	0.123	1.00	0.091		
Sat/mono	1.48	0.134	1.18	0.112				
Cy/pre	1.42	0.129	1.50	0.142				
G+/G-	1.11	0.101	1.36	0.129	1.59	0.144	1.15	0.129
F/B			1.18	-0.111	1.24	-0.113		

VIP: Variable importance of projection. Only VIPs greater than 1.00 are shown (see Materials and Methods). SC:

Standardized coefficient. *Scores are shown in Figure S4 as a proxy for seasonal changes in PLFA.

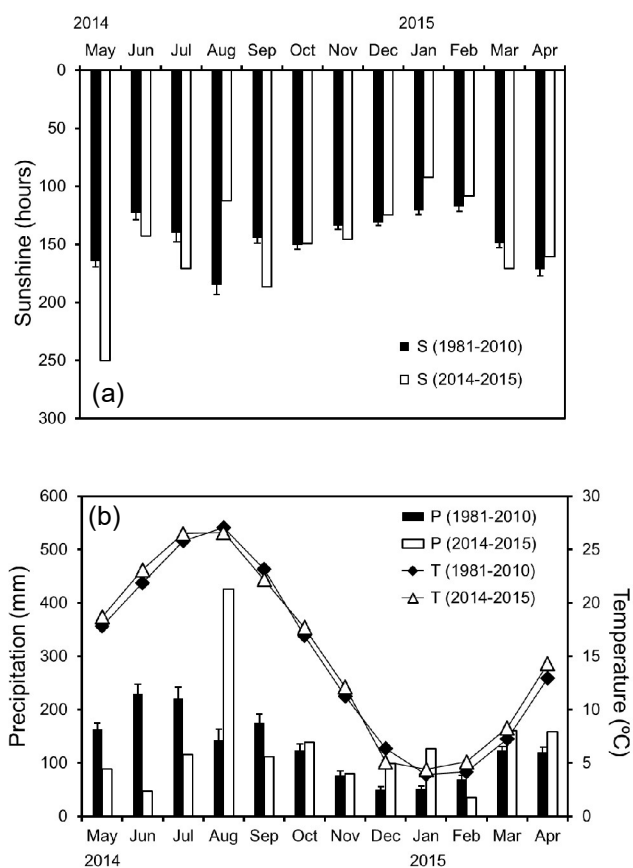


Figure S1. Seasonal dynamics of sunshine (S) (a), precipitation (P), and temperature (T) (b) at Otsu observation station of the Japan Meteorological Agency. Data were obtained from the Japan Meteorological Agency (2015) for the study period (2014-2015) and a long-term average (1981-2010). Long-term average data are presented as mean $\pm$ SE.

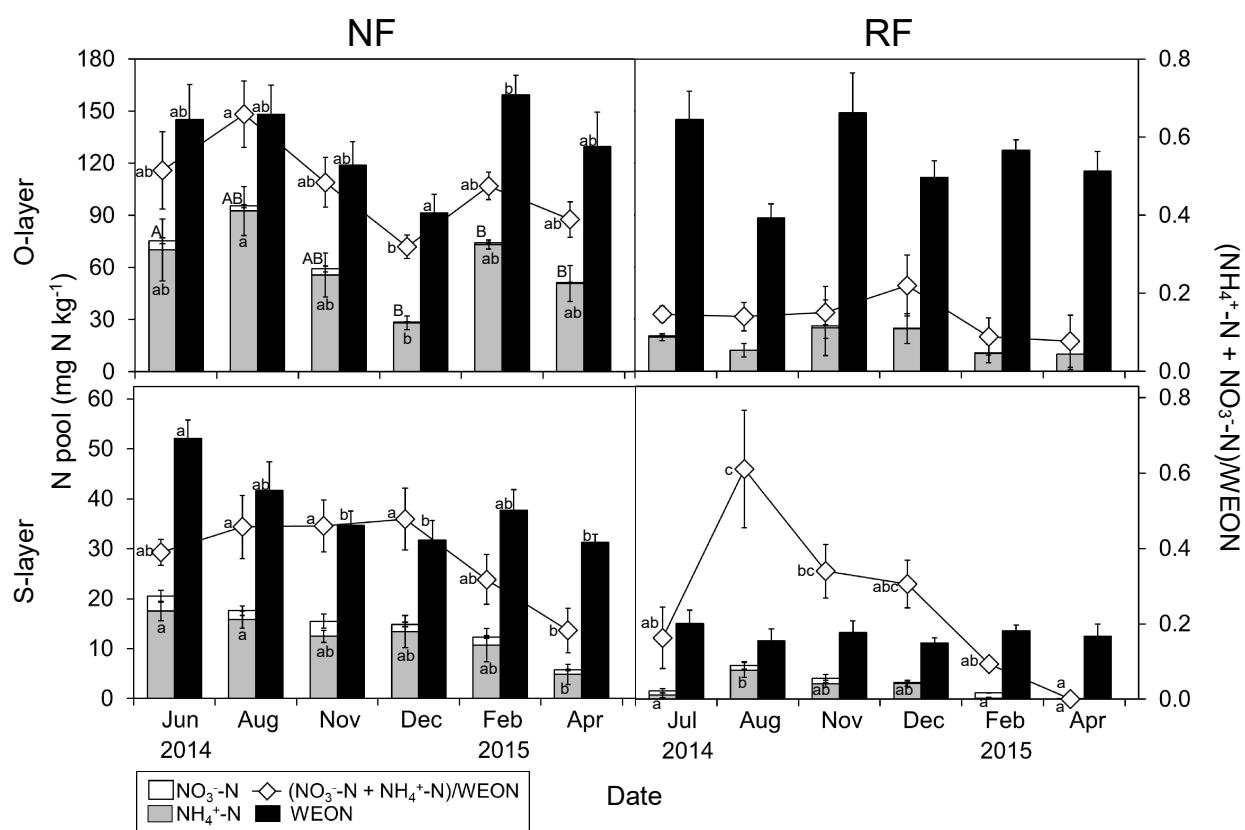


Figure S2. Seasonal changes of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, water-extractable organic nitrogen (WEON), and inorganic N / WEON in both layers at both sites. Data are presented as mean ($n = 5$) $\pm$ SE. Letters indicate significant differences on a sampling date (Tukey's HSD test, $p < 0.05$).

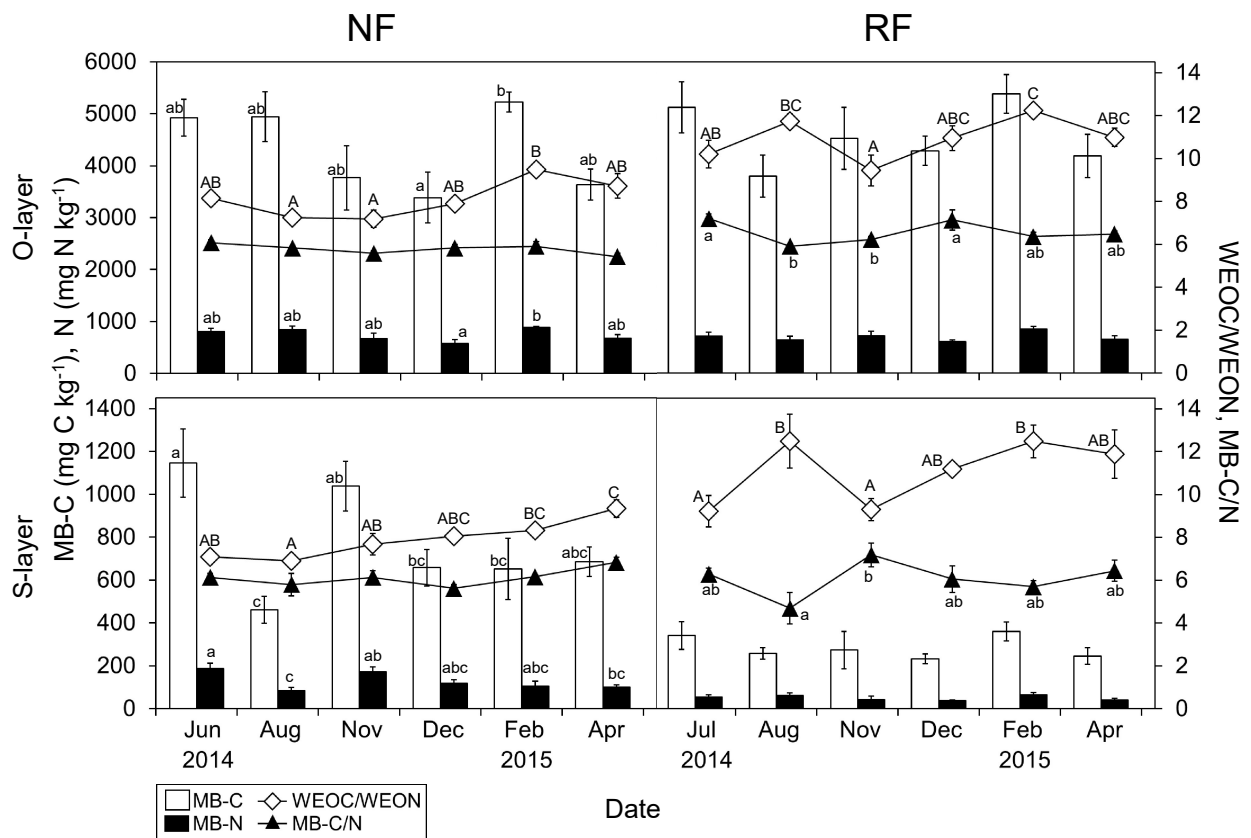


Figure S3. Seasonal changes of microbial biomass (MB)-C, -N, MB-C/N, and WEOC/WEON in both layers at both sites. Data are presented as mean ($n = 5$) $\pm$ SE. Letters indicate significant differences on a sampling date (Tukey's HSD test, $p < 0.05$).

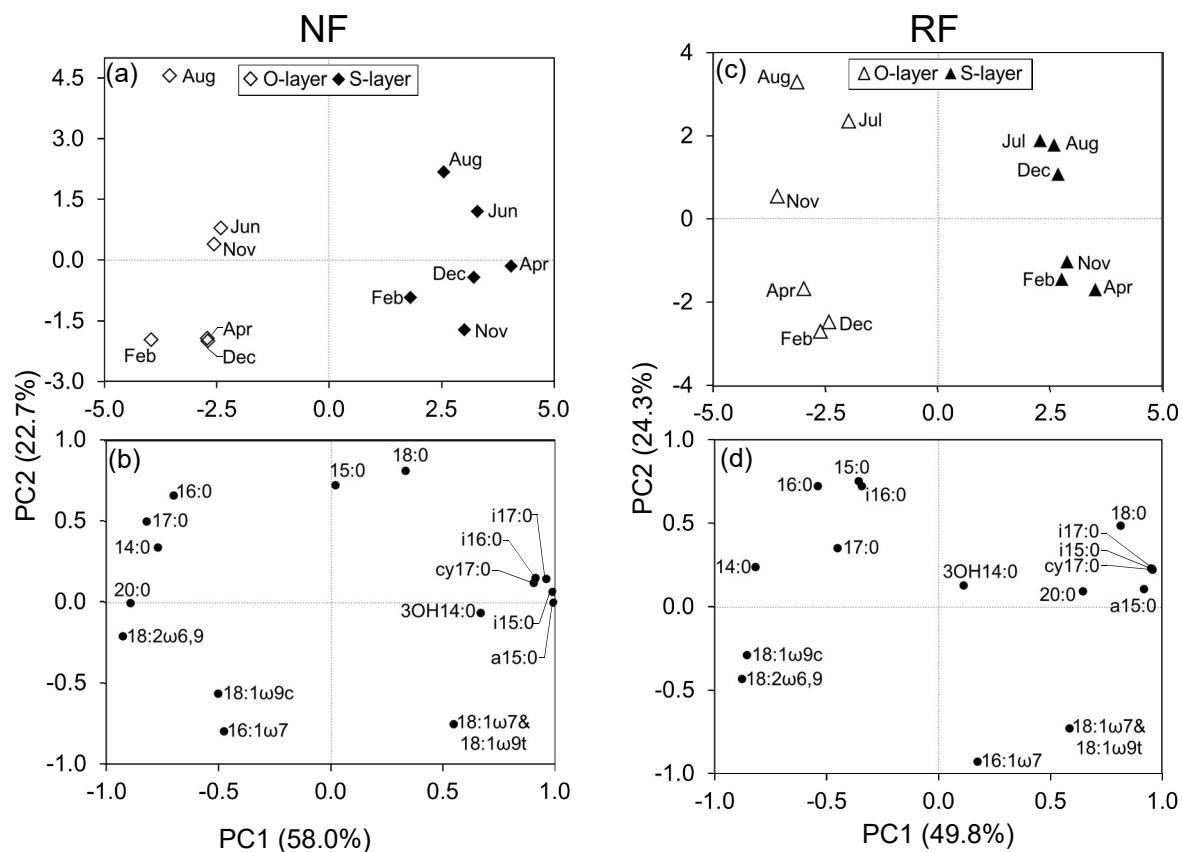


Figure S4. Principal component analysis (PCA) of the phospholipid fatty acid (PLFA) data for both sites among all seasons ($n = 6$), including data for both the O- and S-layers (a, c) and loading scores for individual PLFAs (b, d).

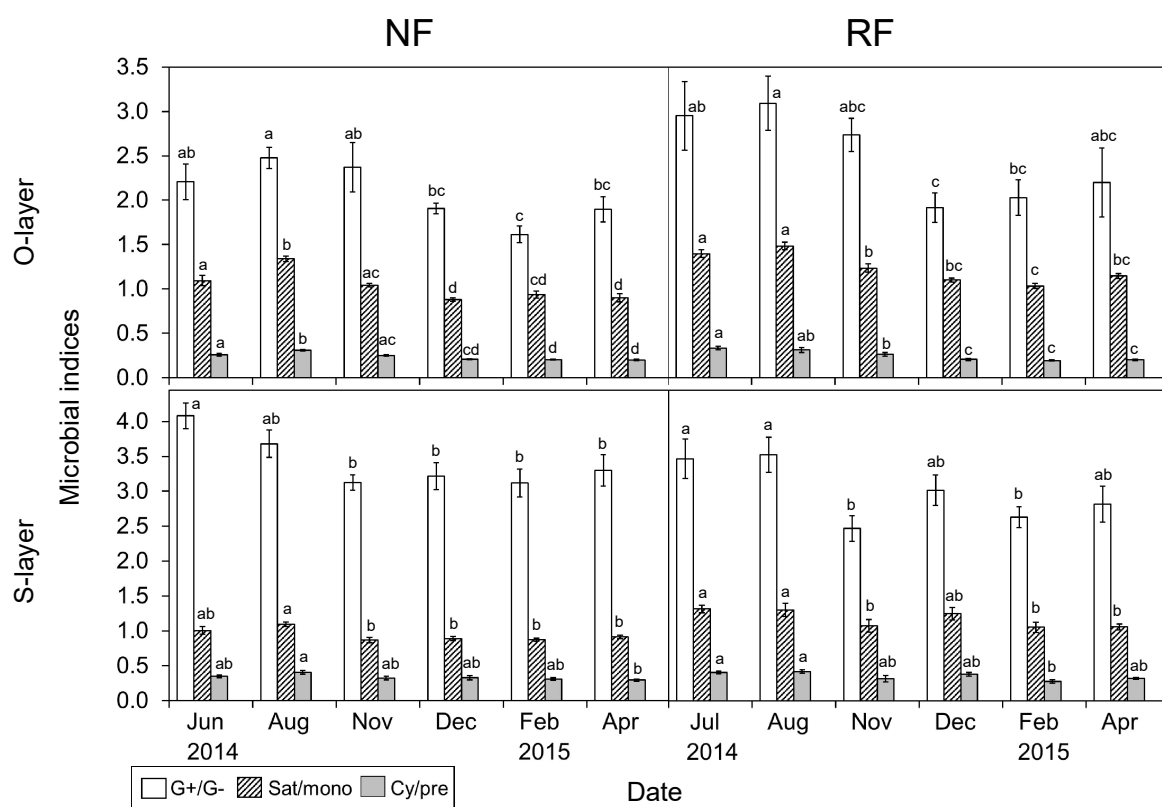


Figure S5. Seasonal changes in microbial indices determined from PLFA data for both sites and both layers. Data are presented as mean ($n = 5$) $\pm$ SE. Letters indicate significant differences on a sampling date (Tukey's HSD test, $p < 0.05$).

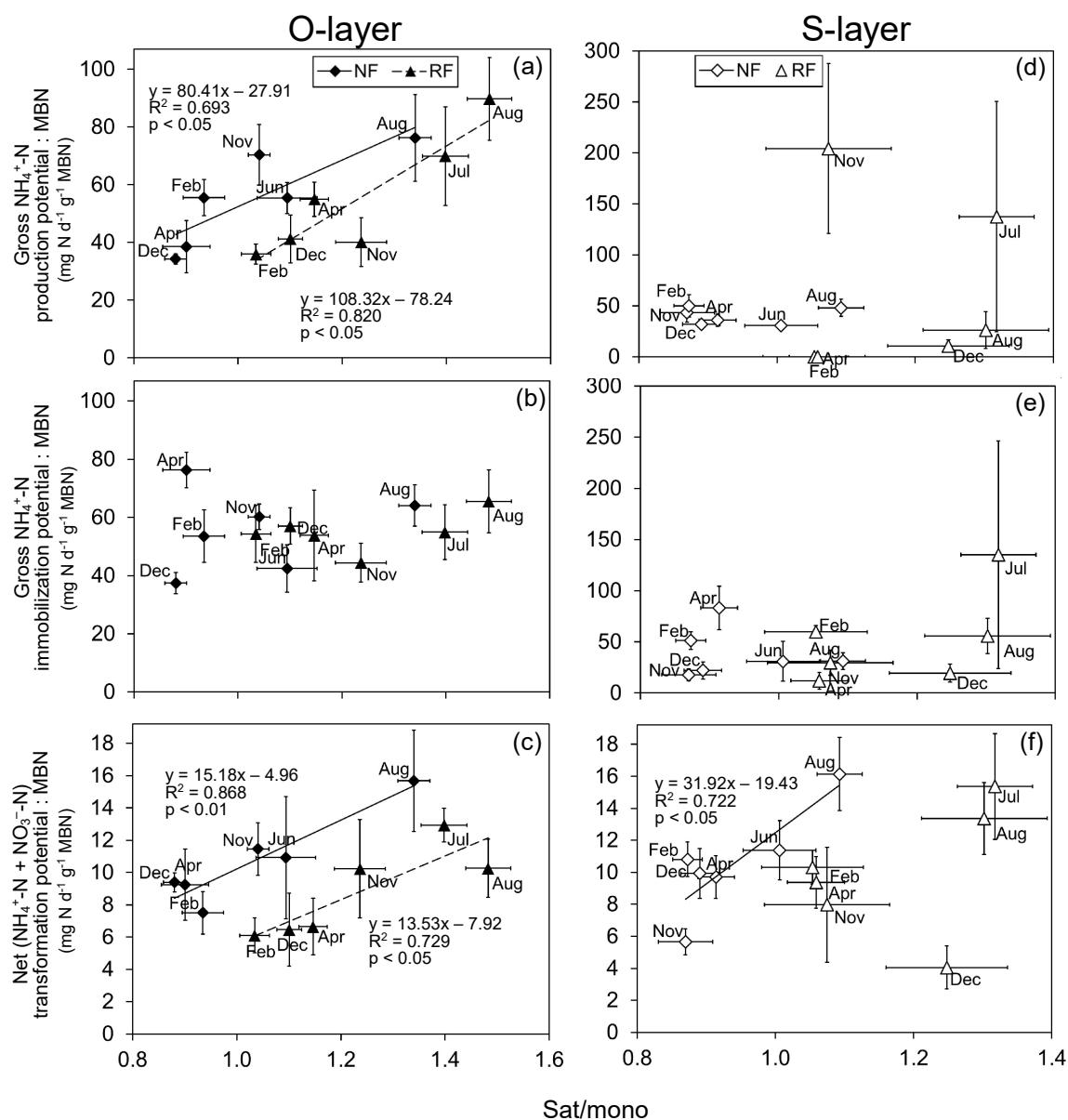


Figure S6. Relationship between the microbial index (Sat/mono) and specific N transformation potentials; gross $\text{NH}_4^+\text{-N}$ production potential: MBN (a, d), gross $\text{NH}_4^+\text{-N}$ immobilization potential: MBN (b, e), and net ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) transformation potential: MBN (c, f) at each depth and site among all seasons. Data are presented as mean ($n = 5$) $\pm$ SE.