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

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) ± 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.

<table>
<thead>
<tr>
<th>Date</th>
<th>WC (g g⁻¹)</th>
<th>pH(H₂O)</th>
<th>WEOC (g C kg⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td>O-layer</td>
<td>S-layer</td>
<td>O-layer</td>
</tr>
<tr>
<td>NF</td>
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</tr>
<tr>
<td>Jun</td>
<td>1.75 ± 0.37a</td>
<td>0.58 ± 0.04</td>
<td>4.50 ± 0.20ab</td>
</tr>
<tr>
<td>Aug</td>
<td>2.01 ± 0.19ab</td>
<td>0.54 ± 0.06</td>
<td>4.64 ± 0.12a</td>
</tr>
<tr>
<td>Nov</td>
<td>1.38 ± 0.19a</td>
<td>0.55 ± 0.04</td>
<td>4.29 ± 0.11ab</td>
</tr>
<tr>
<td>Dec</td>
<td>1.30 ± 0.18a</td>
<td>0.52 ± 0.04</td>
<td>4.28 ± 0.08b</td>
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<td>Feb</td>
<td>2.77 ± 0.21b</td>
<td>0.58 ± 0.05</td>
<td>4.38 ± 0.12ab</td>
</tr>
<tr>
<td>Apr</td>
<td>2.76 ± 0.27b</td>
<td>0.69 ± 0.05</td>
<td>4.27 ± 0.17b</td>
</tr>
<tr>
<td>Annual</td>
<td>1.99 ± 0.14</td>
<td>0.57 ± 0.02</td>
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1-way ANOVA Season

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<td>Site</td>
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<td>Site x Season</td>
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<tr>
<td>N.S.</td>
<td>***</td>
<td>N.S.</td>
<td>N.S.</td>
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<tr>
<td>***</td>
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<td>*</td>
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NFS-RFS 2-way ANOVA

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<td>N.S.</td>
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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).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC2 Scores*</th>
<th>Soil/mono</th>
<th>Cy/pre</th>
<th>G+/G-</th>
<th>F/B</th>
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<td>O layer</td>
<td>Soil layer</td>
<td>O layer</td>
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<tr>
<td>phenomenon</td>
<td></td>
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</tr>
<tr>
<td>Temperature (7d)</td>
<td>0.92</td>
<td>0.73</td>
<td>0.92</td>
<td>0.86</td>
<td>0.92</td>
</tr>
<tr>
<td>(14d)</td>
<td>0.90</td>
<td>0.76</td>
<td>0.90</td>
<td>0.88</td>
<td>0.90</td>
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<tr>
<td>(21d)</td>
<td>0.93</td>
<td>0.74</td>
<td>0.92</td>
<td>0.87</td>
<td>0.93</td>
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<tr>
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<td>0.73</td>
<td>0.93</td>
<td>0.86</td>
<td>0.94</td>
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<td>0.73</td>
<td>0.73</td>
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<td>0.97</td>
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<td>0.97</td>
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<td>WEOC/WEOG</td>
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<td>Microbial biomarker</td>
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</tr>
<tr>
<td>indices</td>
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</tr>
<tr>
<td>PC2 Scores*</td>
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<td>0.99</td>
<td>0.99</td>
<td>0.97</td>
</tr>
<tr>
<td>Soil/mono</td>
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<td>0.99</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cy/pre</td>
<td>0.97</td>
<td>0.95</td>
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<td>G+/G-</td>
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<td>0.89</td>
<td>0.95</td>
<td>0.92</td>
<td>0.95</td>
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<tr>
<td>F/B</td>
<td>-0.76</td>
<td>-0.95</td>
<td>-0.80</td>
<td>-0.94</td>
<td>-0.84</td>
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</table>

*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 (NH$_4^+$-N + NO$_3^-$-N).

<table>
<thead>
<tr>
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<th>S-layer</th>
</tr>
</thead>
<tbody>
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<td>NF</td>
<td>RF</td>
</tr>
<tr>
<td></td>
<td>VIP</td>
<td>SC</td>
</tr>
<tr>
<td>Meteorological phenomenon</td>
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<td></td>
</tr>
<tr>
<td>Temperature (28d)</td>
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<td>0.110</td>
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<td>Precipitation (28d)</td>
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<td></td>
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<tr>
<td>Sunshine (28d)</td>
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<td></td>
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<tr>
<td>Soil environments</td>
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</tr>
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<td>pH(H$_2$O)</td>
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<td>Fungal-PLFA</td>
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<td>Bacterial-PLFA</td>
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<td>Microbial biomarker indices</td>
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<td>G+/G-</td>
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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

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<td>S-layer</td>
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<td>RF</td>
<td>VIP</td>
<td>SC</td>
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<td>Sunshine (28d)</td>
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<td>Soil environments</td>
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<td>0.134</td>
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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

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<th>Variables</th>
<th>Gross NH$_4^+$-N immobilization potential</th>
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<td>VIP SC</td>
<td>VIP SC</td>
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<td>1.07 -0.139</td>
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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

<table>
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<tr>
<th>Variables</th>
<th>Net (NH$_4^+$-N+NO$_3^-$-N) transformation potential</th>
<th>O-layer</th>
<th>RF</th>
<th>S-layer</th>
<th>RF</th>
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<td>SC</td>
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<td>1.15 0.129</td>
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<td>1.24 -0.113</td>
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</table>

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 ± SE.
Figure S2. Seasonal changes of NH$_4^+$-N, NO$_3^-$-N, water-extractable organic nitrogen (WEON), and inorganic N / WEON in both layers at both sites. Data are presented as mean (n = 5) ± 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) ± 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) ± 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 NH$_4^+$-N production potential: MBN (a, d), gross NH$_4^+$-N immobilization potential: MBN (b, e), and net (NH$_4^+$-N + NO$_3^-$-N) transformation potential: MBN (c, f) at each depth and site among all seasons. Data are presented as mean (n = 5) ± SE.