frontiers

*Supplementary Material for*

# Precipitation-derived effects on the characteristics of proteinaceous organic matter across the continental United States

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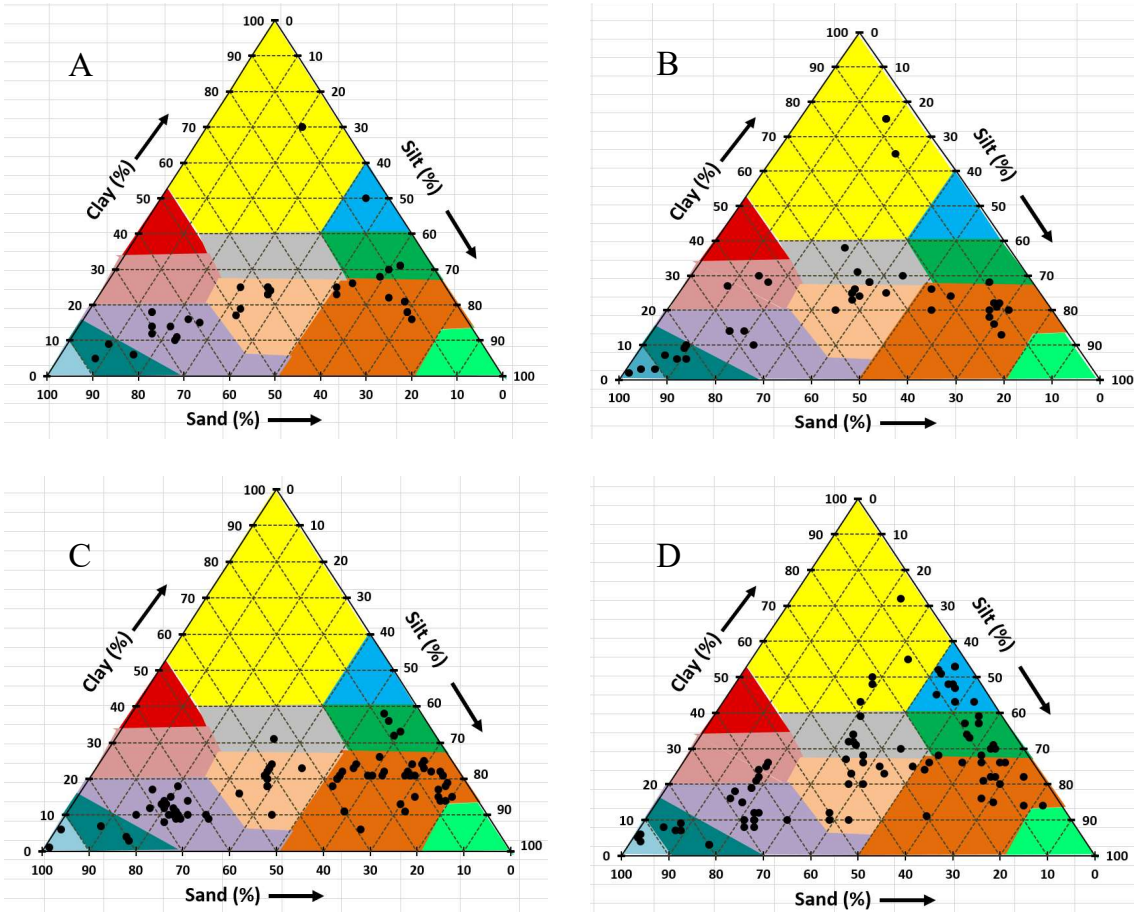


**Figure S1 Soil texture of the investigated subset of USGS samples.**

(A) A-horizon, North-South transect, (B) C-horizon, North-South transect, (C) A-horizon, East-West transect, (D) C-horizon, East-West transect. The soil texture data was collected from The Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx).

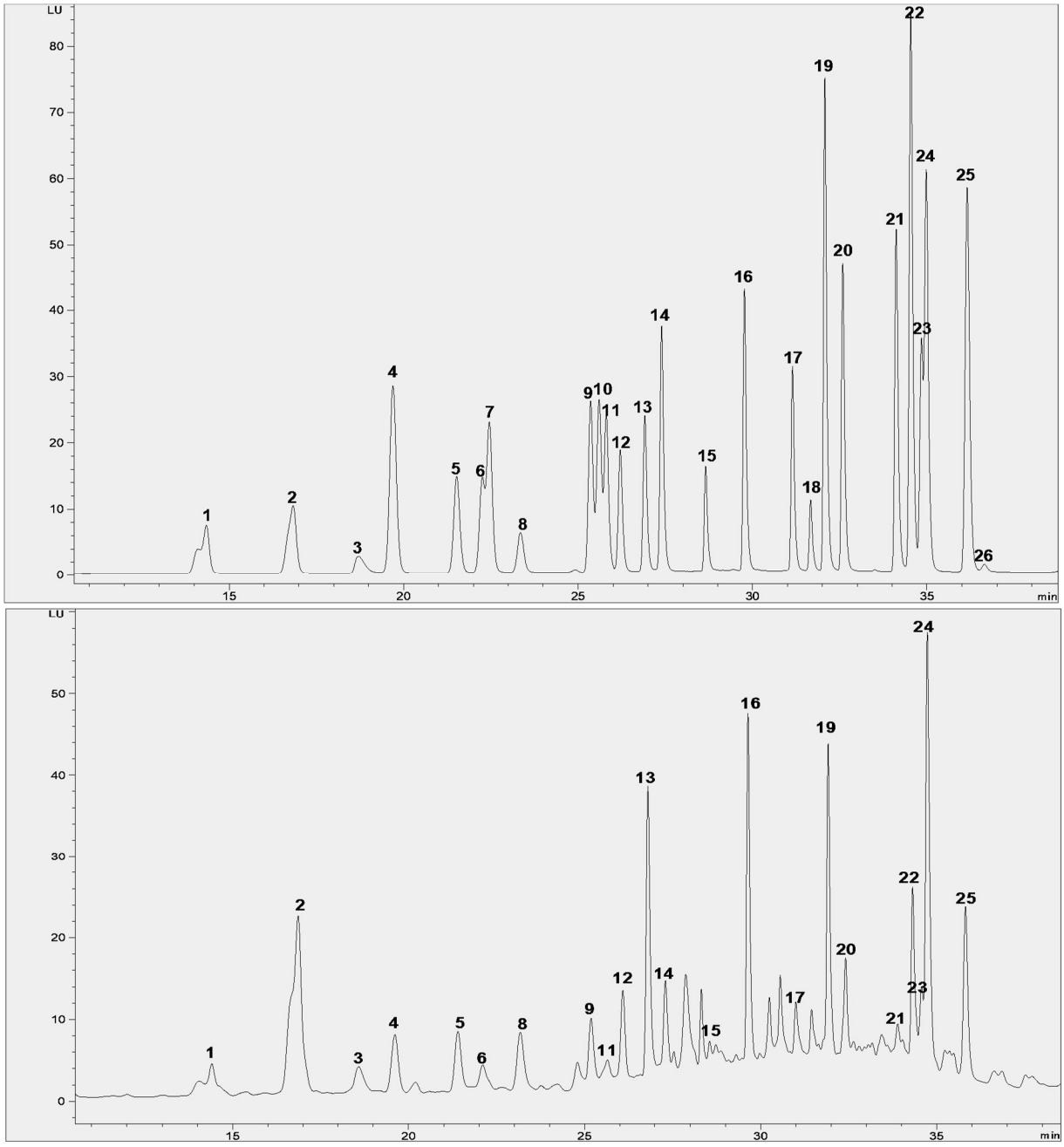


**Figure S2. Chromatograms of derivatized amino acids.**

(above) 10µM standard; (below) an A-horizon soil sample from a grassland site in Minnesota. 1=Asp; 2=Glu; 3= 6-aminoquinoline; 4=Ser+Asn; 5=Gly; 6=Gln; 7=His; 8=$NH_4^+$; 9=Arg; 10=Tau; 11=Cit; 12=Thr; 13=Ala; 14=GABA; 15=Pro; 16=AABA (internal standard); 17=Tyr; 18=Cys; 19=Val; 20=Met; 21=Orn; 22=Ile; 23=Lys; 24=Leu; 25=Phe; 26=Trp.

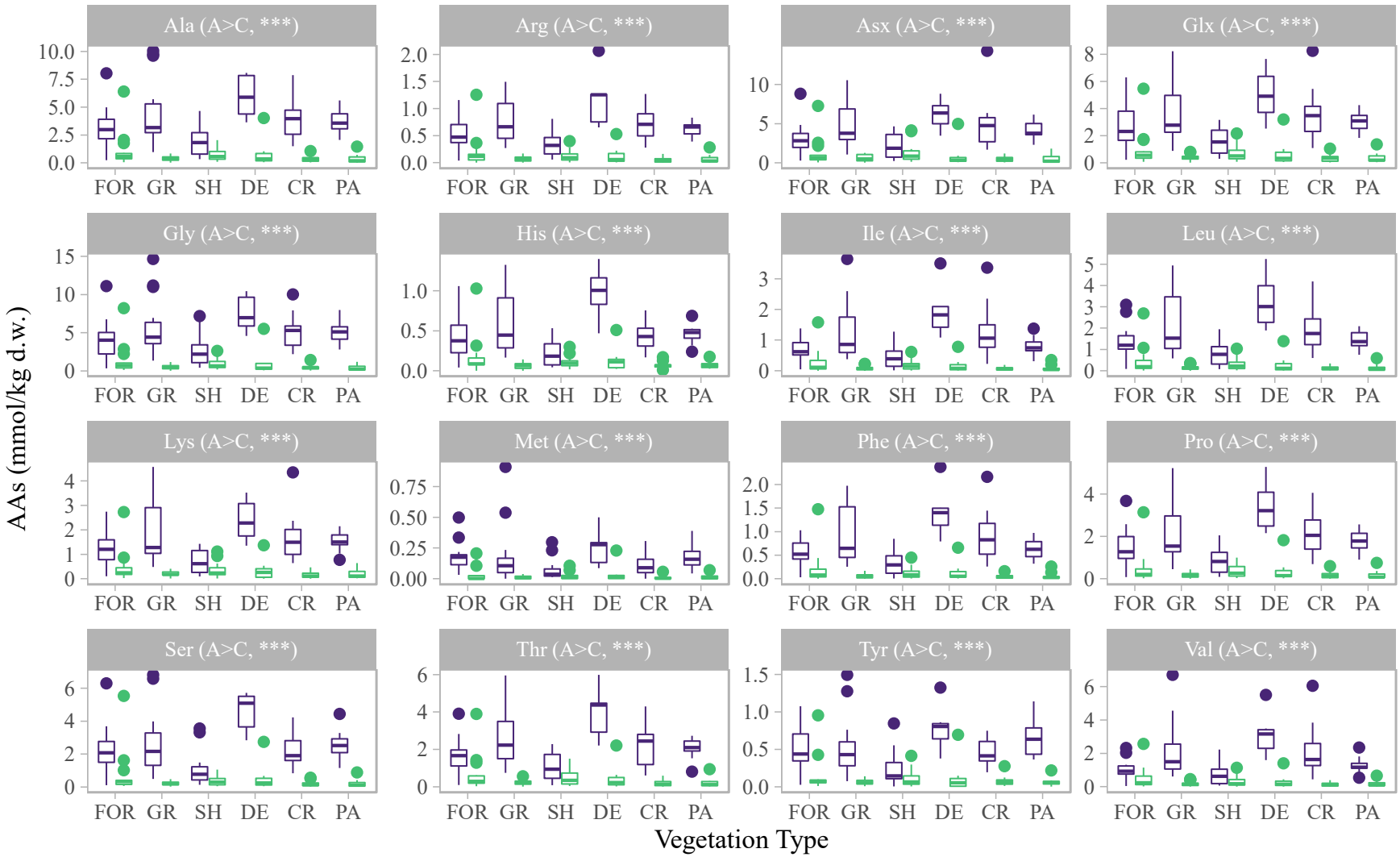


**Figure S3 Comparison of the molar ratios of HAAs in soils of different vegetation (VET).**

Purple and green box/points represent soil in A-horizon and C-horizon, respectively. Significant differences between horizons are depicted for each AA.

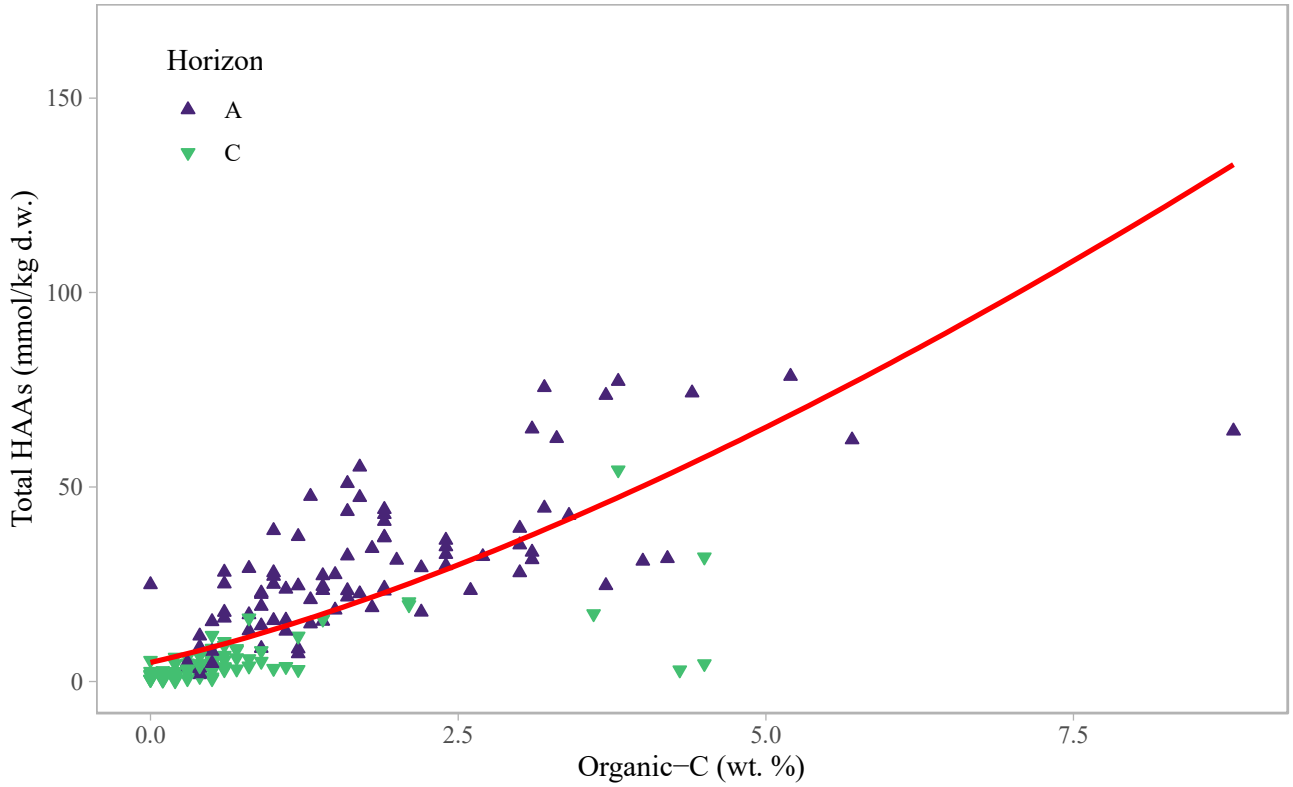


**Figure S4 Relationship between total hydrolyzable amino acids and soil organic carbon.**

Purple and green points represent soil in A-horizon and C-horizon, respectively. The red line depicts the regression of the data.

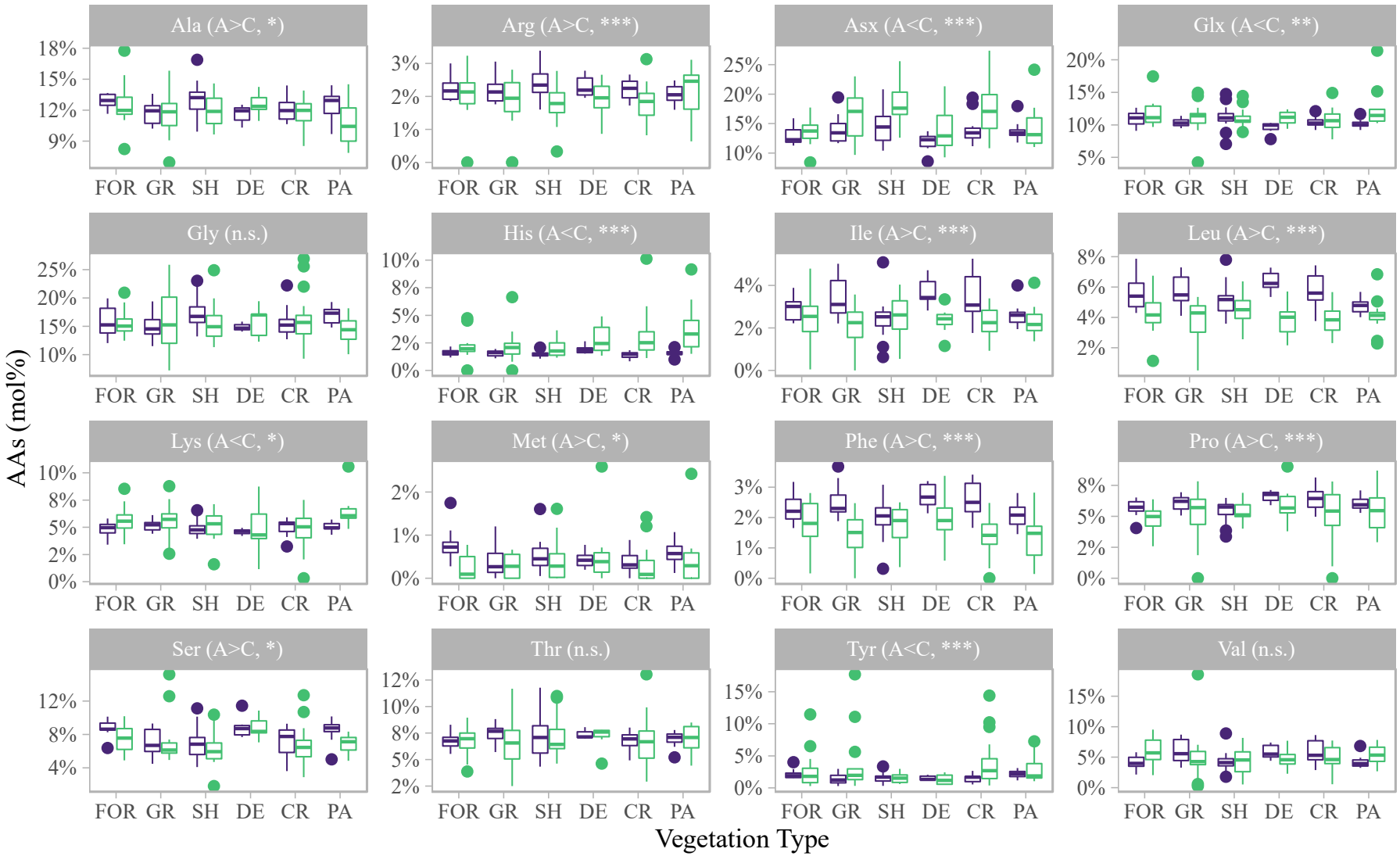


**Figure S5 Comparison of the relative molar ratios of HAAs in soils of different plant types.**

Purple and green box/points represent soil in A-horizon and C-horizon, respectively.

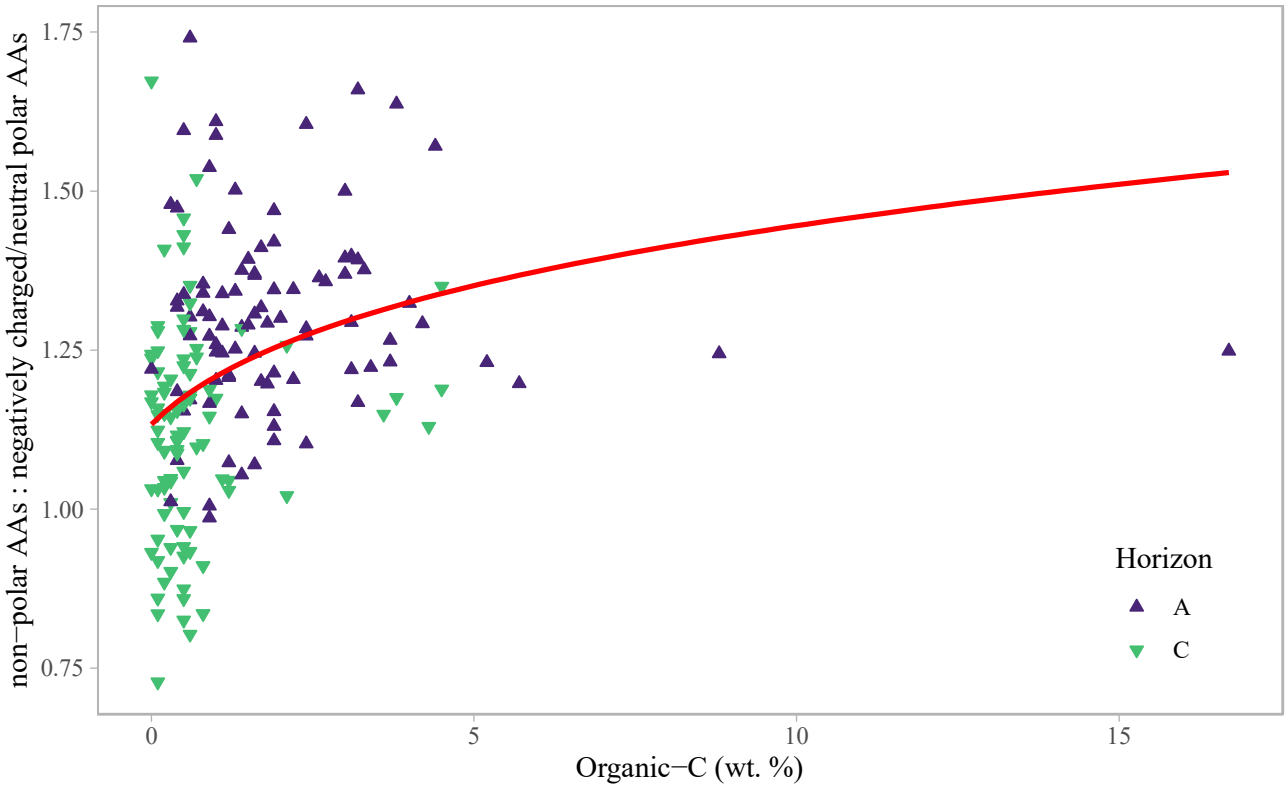


**Figure S6 Relationship between non-polar: polar AA and soil organic carbon.**

Purple and green points represent soil in A-horizon and C-horizon, respectively. The red line depicts the regression of the data.

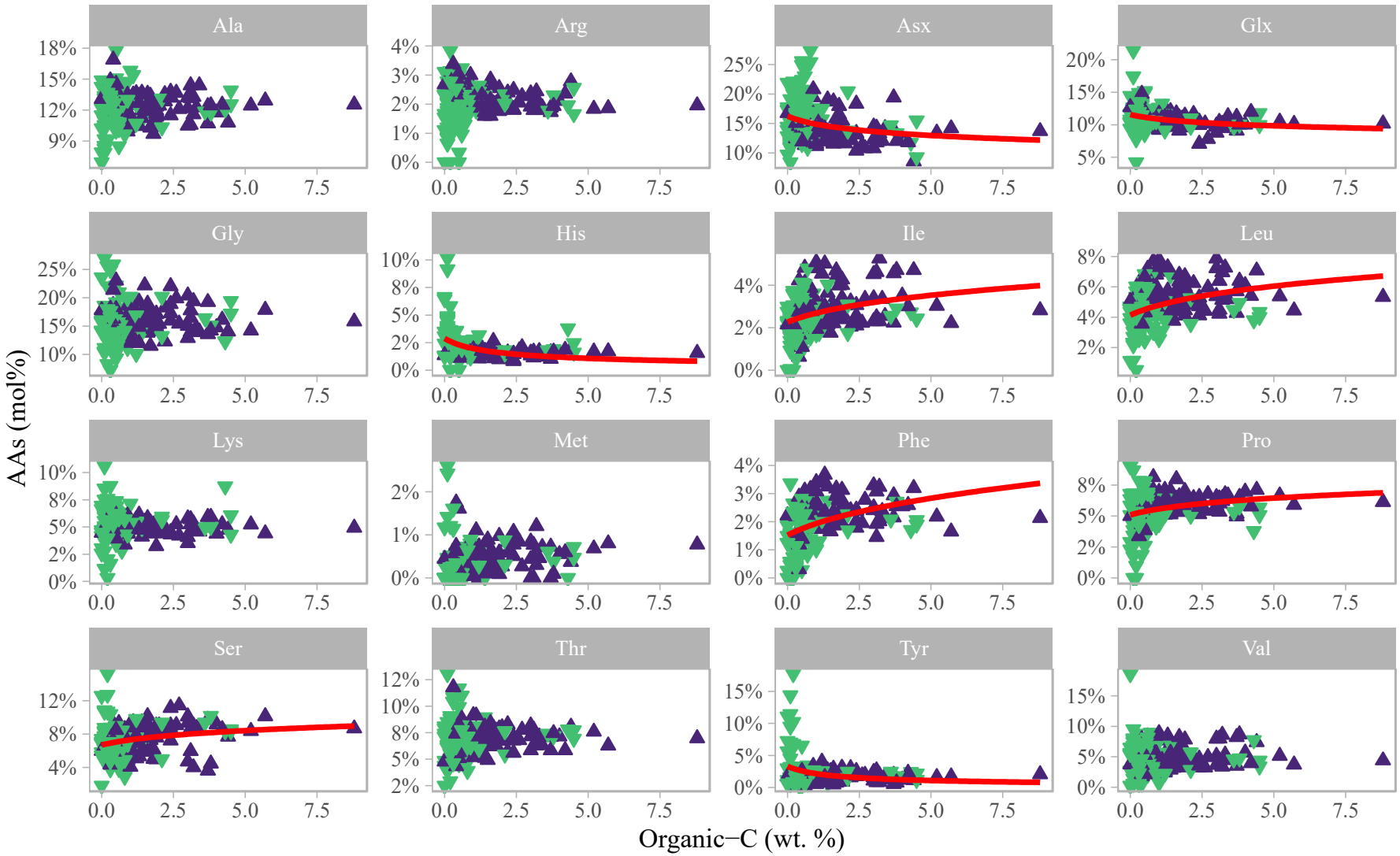


**Figure S7 Relationship between relative molar ratios of HAAs and soil organic carbon.**

Purple and green points represent soil in A-horizon and C-horizon, respectively. Red lines indicate significant regression.

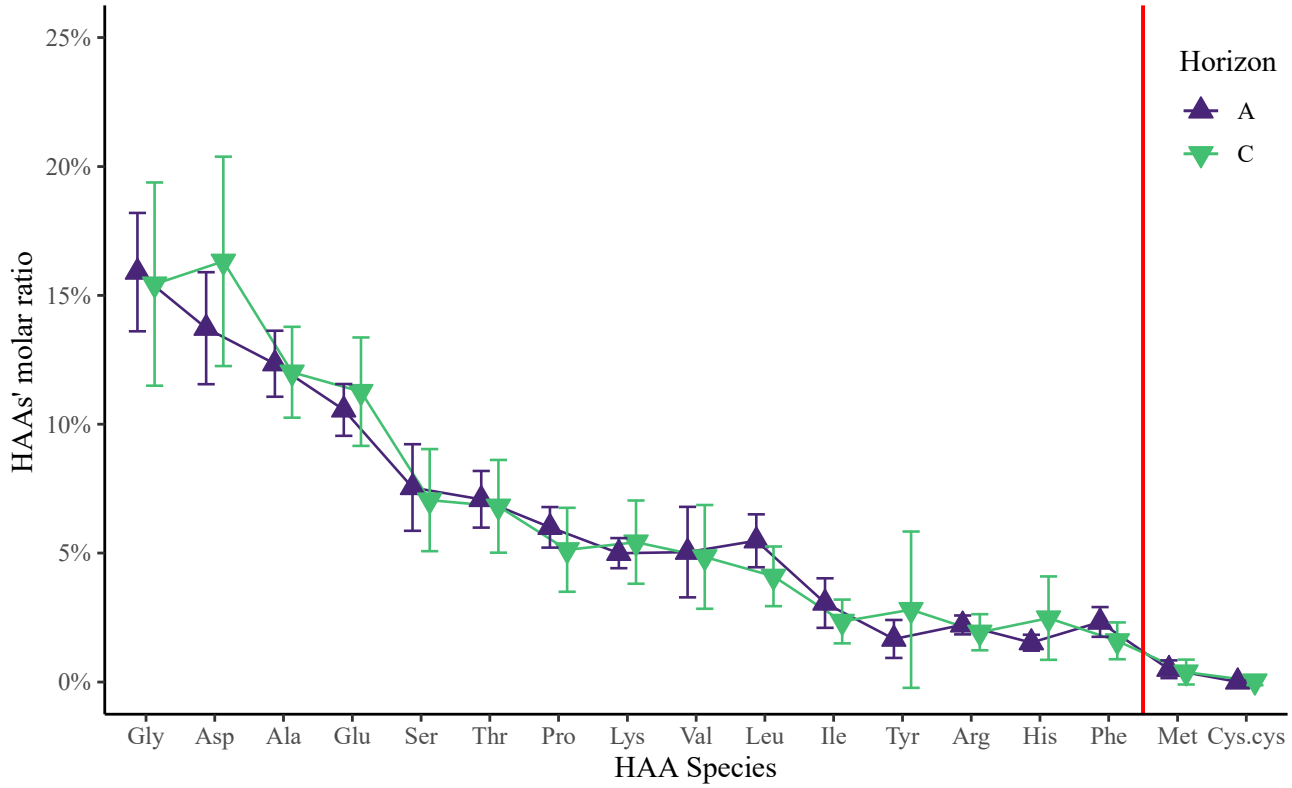


**Figure S8 Mean molar ratio of different HAAs.**

Red line indicated top 15 most abundant amino acids.

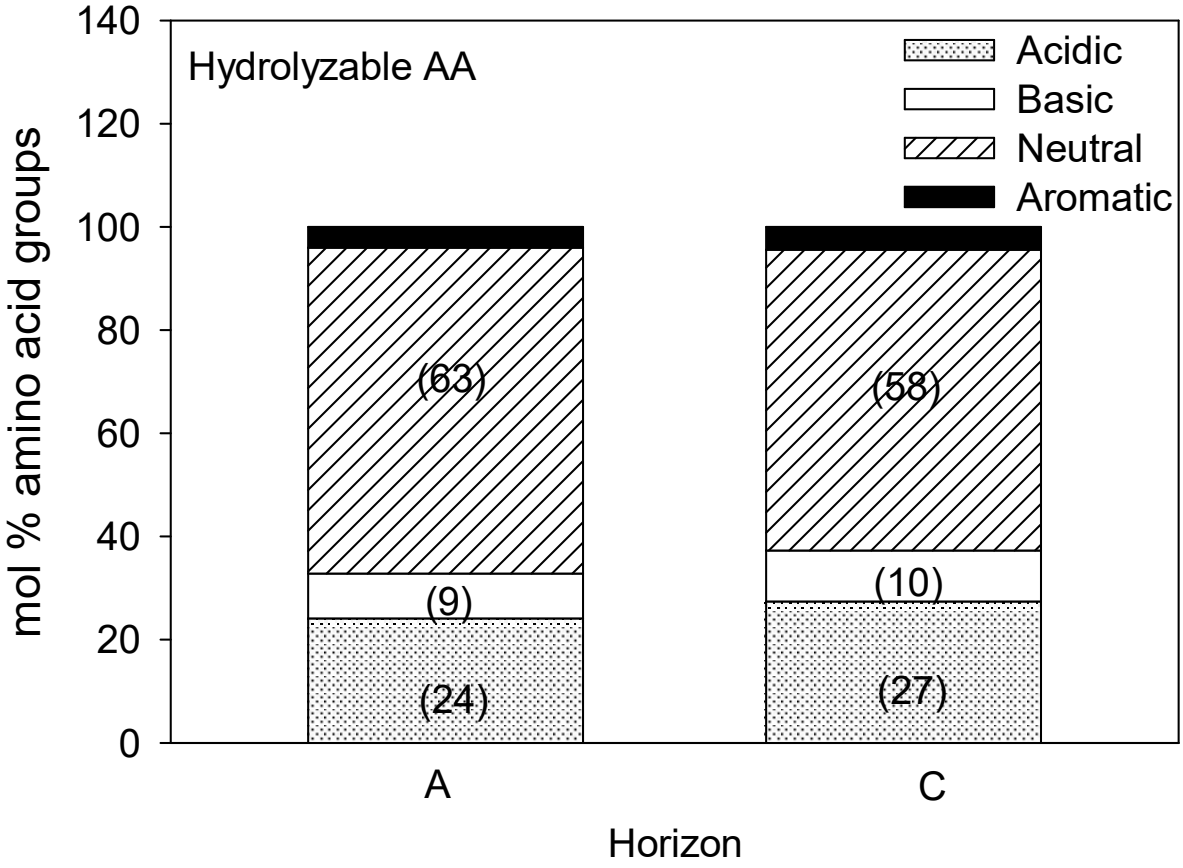


**Figure S9 Mean molar ratio of HAAs in different groups.**

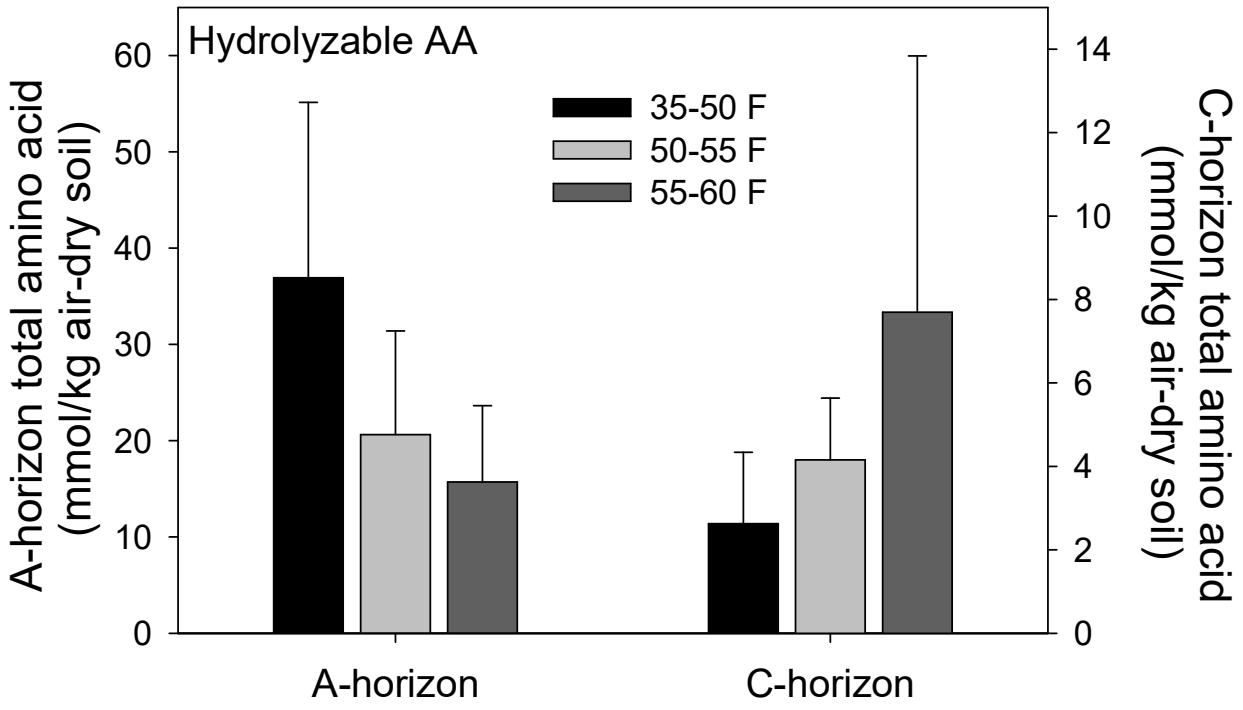


**Figure S10 Changes in soil hydrolysable amino acids show different trends in the A relative to the C horizon along the North-South (MAT) gradient of continental US (Fig. 1).**

There were no significant differences due to MAT, however the opposite trends with horizon are notable. Soils in colder environments may have less movement of carbon via roots and translocation to the C horizons than soils in warmer climates. The greater AA in the soils of the cooler environment follow expectations based on organic matter content.

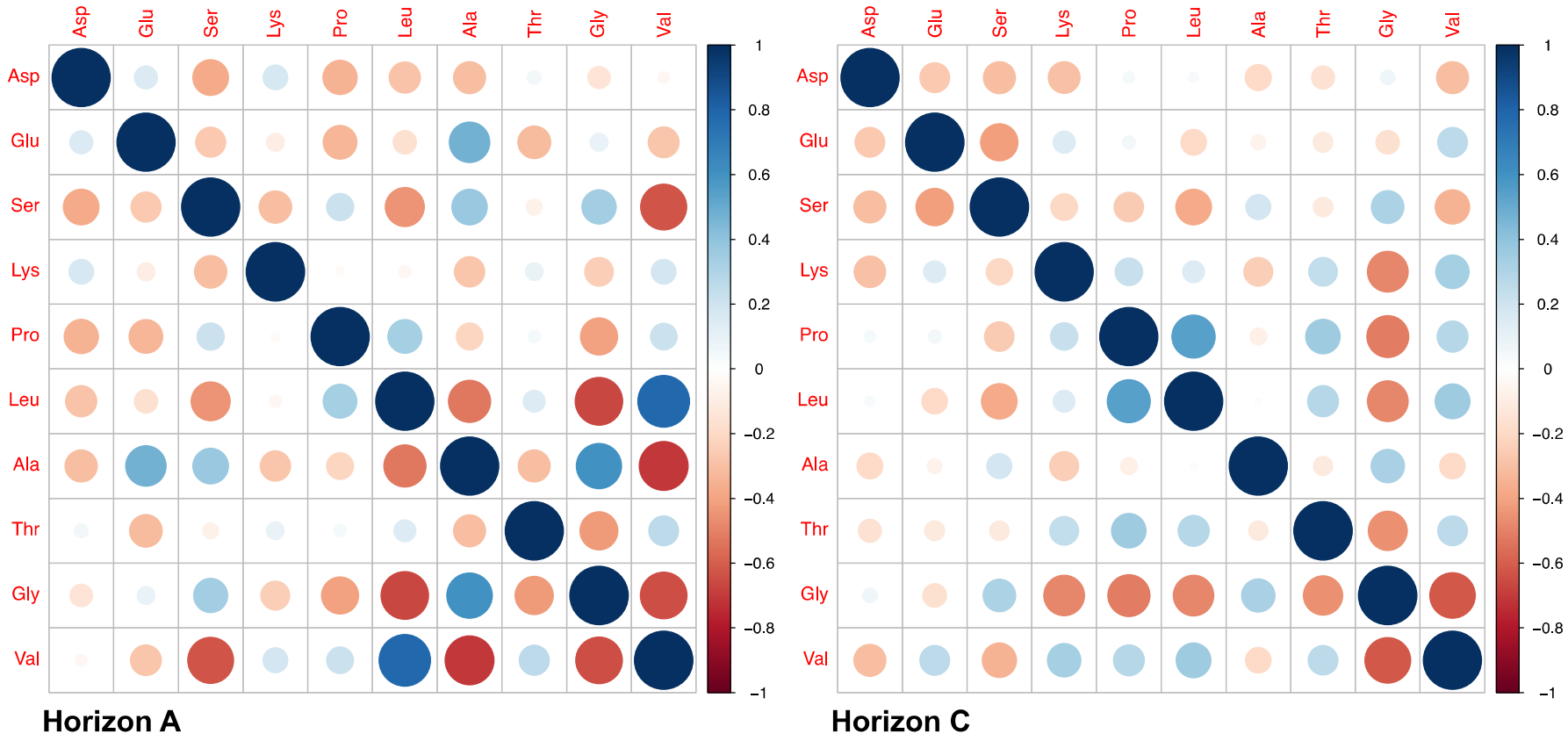


**Figure S11 Correlation matrix between molar ratios of top ten richest HAAs.**

The color and size of the circle represent the magnitude of the correlation coefficients

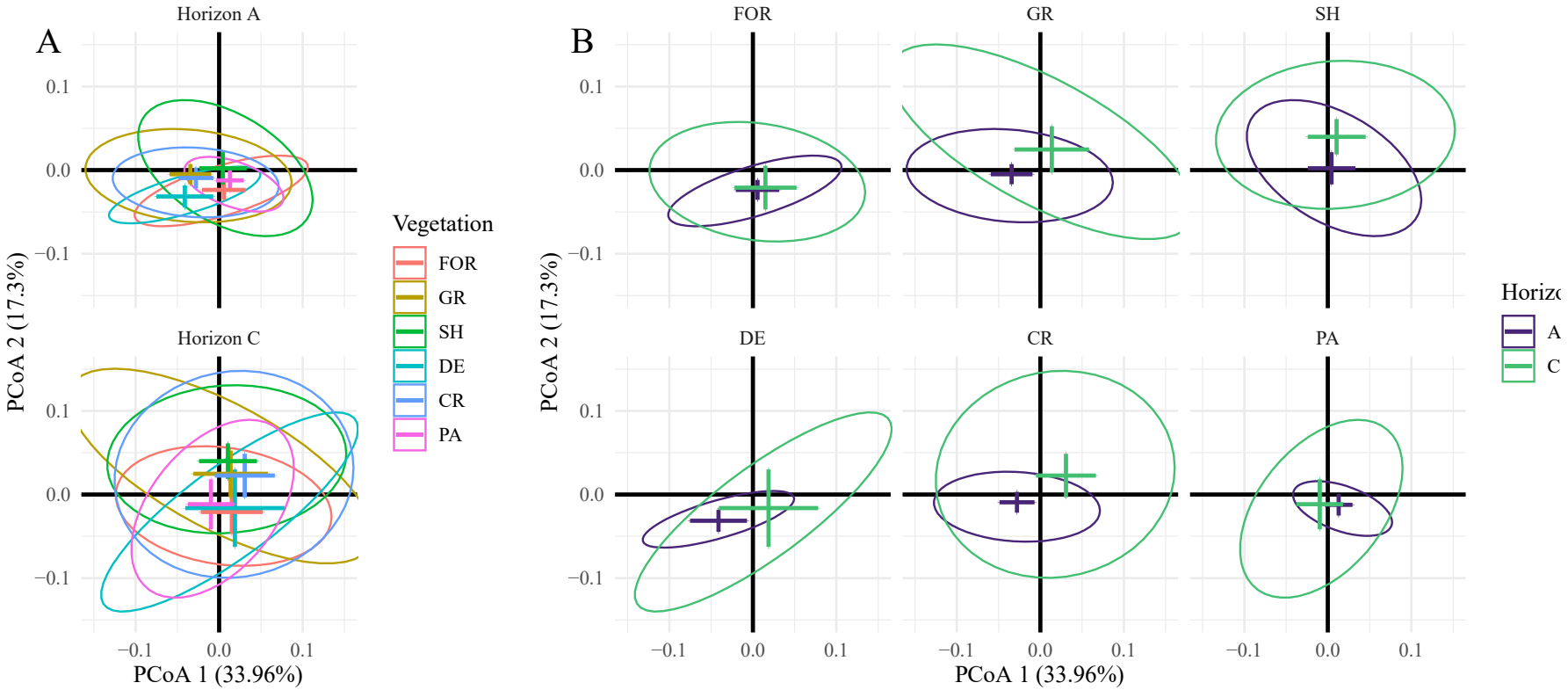


**Figure S12 PCoA-based sorting on the molar ratio of HAAs.**

(A) Samples from different soil layers were exhibited separately. Colors represent different vegetation types. Samples from fallow or low intensity residential are not shown here due to the small sample size. (B) Samples from different vegetation types were exhibited separately. Colors represent different soil layers. FOR: evergreen or mixed forest, GR: grassland/herbaeous, SH: shrubland, DE:decidous forest, CR: small grains or crop, PA: pasture/hay. Samples from fallow or low intensity residential are not shown here due to the small sample size.

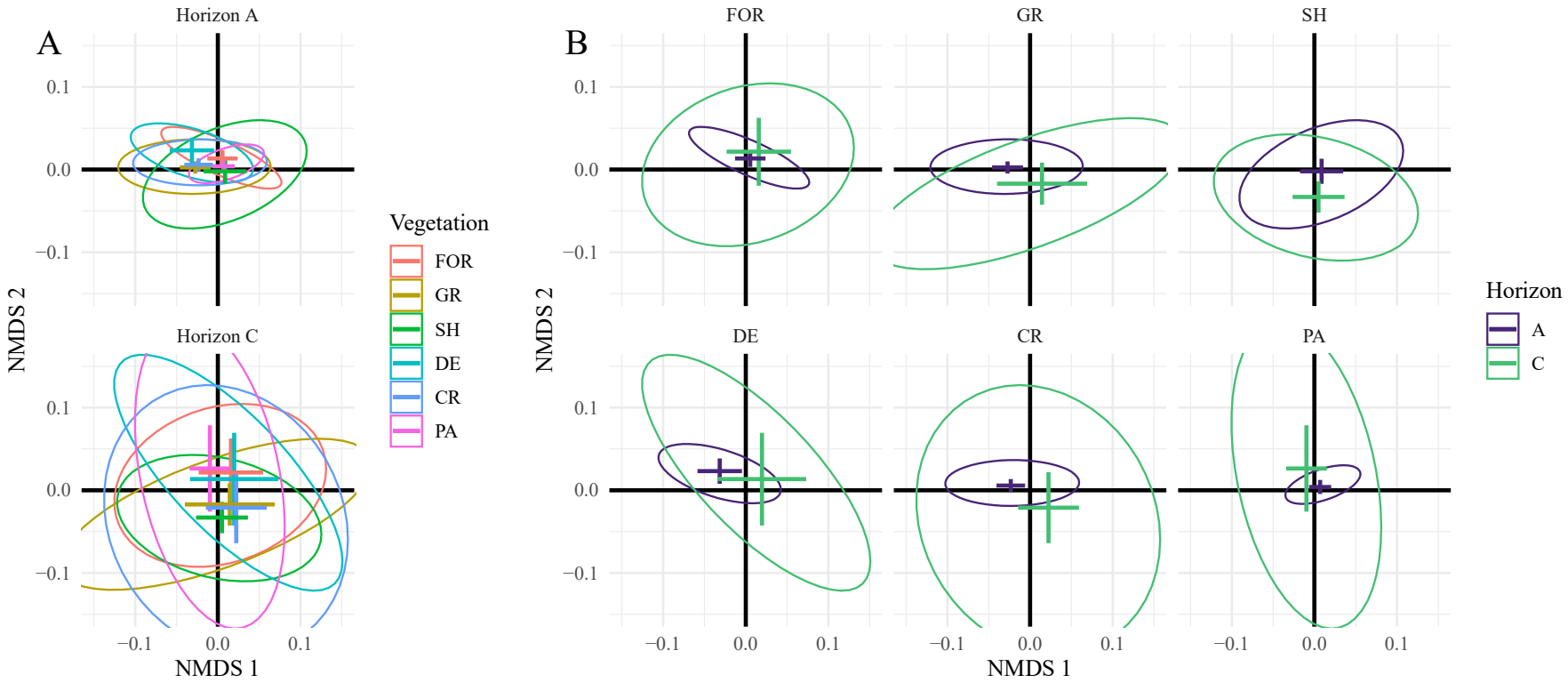


**Figure S13 NMDS-based sorting on the molar ratio of HAAs.**

(A) Samples from different soil layers were exhibited separately. Colors represent different vegetation types. Samples from fallow or low intensity residential are not shown here due to the small sample size. (B) Samples from different vegetation types were exhibited separately. Colors represent different soil layers. FOR: evergreen or mixed forest, GR: grassland/herbaeous, SH: shrubland, DE:decidous forest, CR: small grains or crop, PA: pasture/hay. Samples from fallow or low intensity residential are not shown here due to the small sample size.

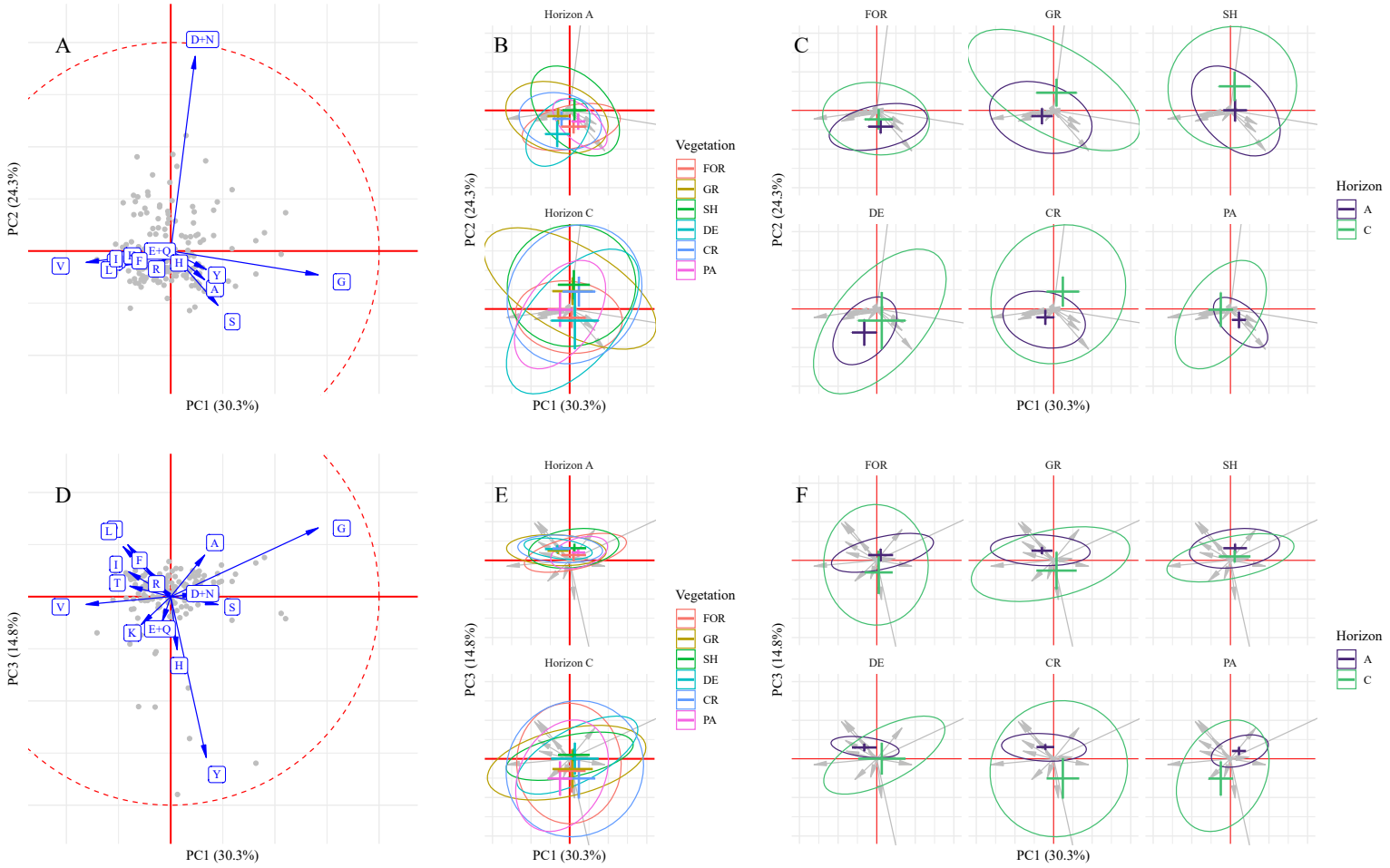


**Figure S14 PCA-based sorting on the molar ratio of HAAs.**

(A,D) PCA loadings of PC1 *v.s.* PC2 and PC1 *v.s.* PC3. (B,E) Samples from different soil layers were exhibited separately. Colors represent different vegetation types. Samples from fallow or low intensity residential are not shown here due to the small sample size. (C,D) Samples from different vegetation types were exhibited separately. Colors represent different soil layers. FOR: evergreen or mixed forest, GR: grassland/herbaeous, SH: shrubland, DE:decidous forest, CR: small grains or crop, PA: pasture/hay. Samples from fallow or low intensity residential are not shown here due to the small sample size.

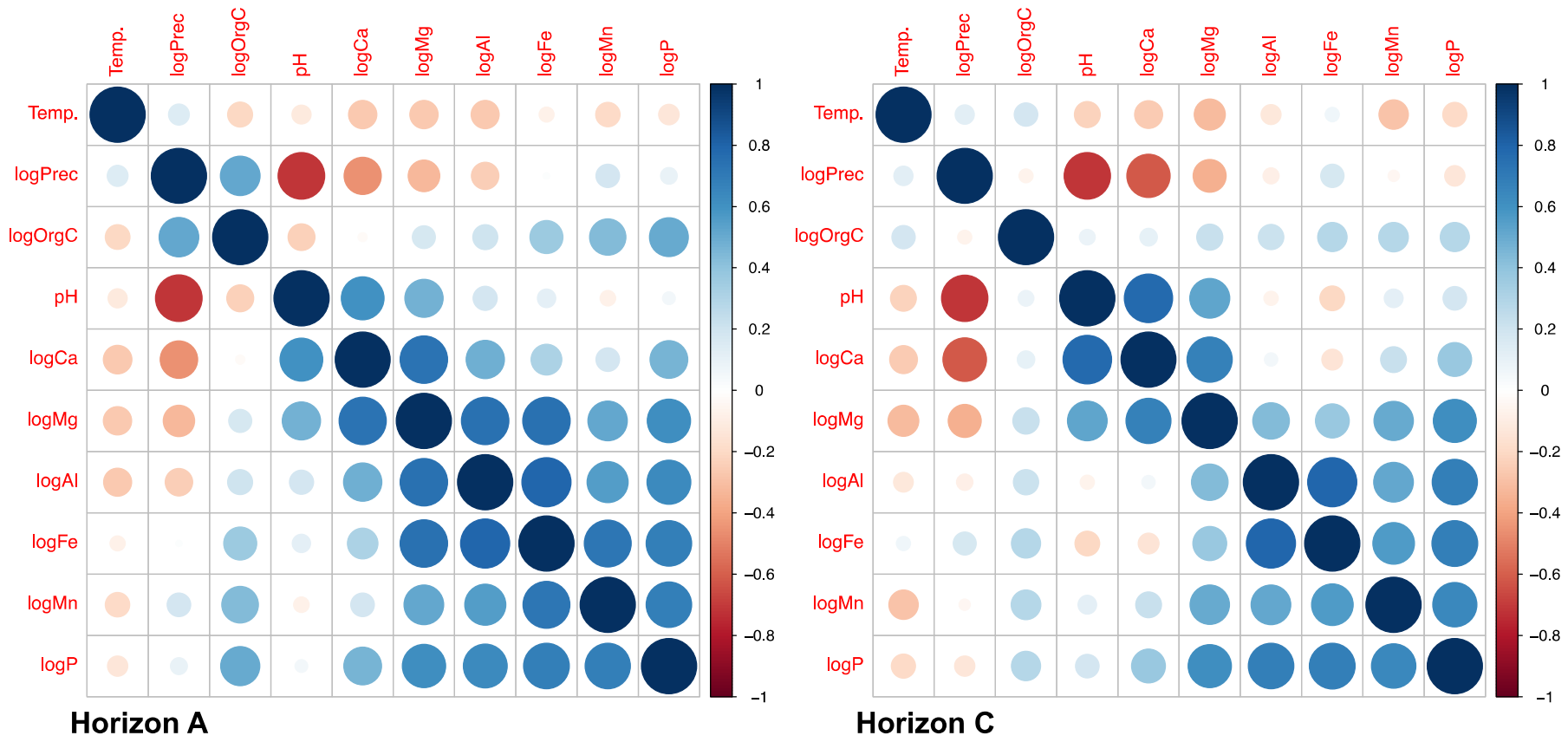


**Figure S15 Correlation matrix between environmental factors.**

The color and size of the circle represent the magnitude of the correlation coefficients.

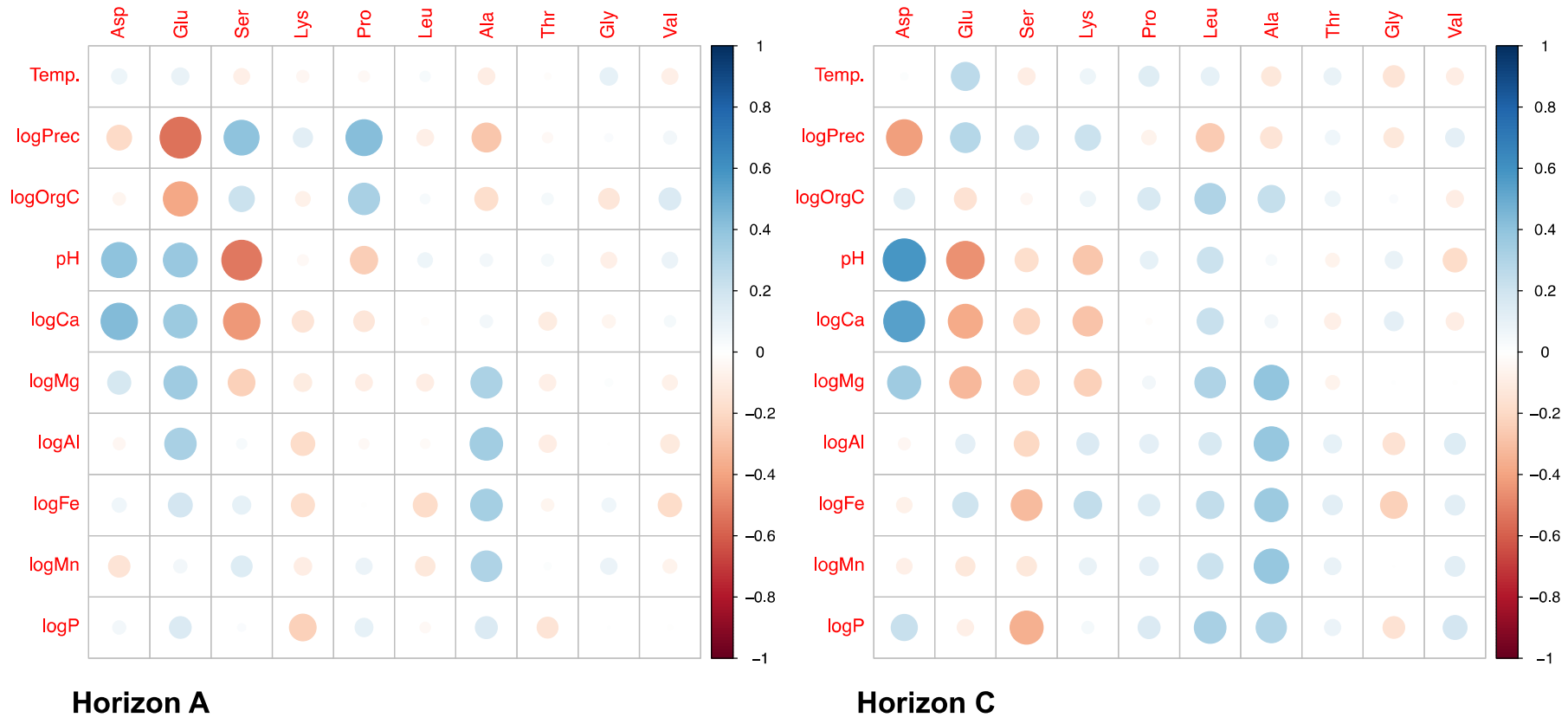


**Figure S16 Correlation matrix between the HAAs' molar ratios and environmental factors.**

The color and size of the circle represent the magnitude of the correlation coefficients

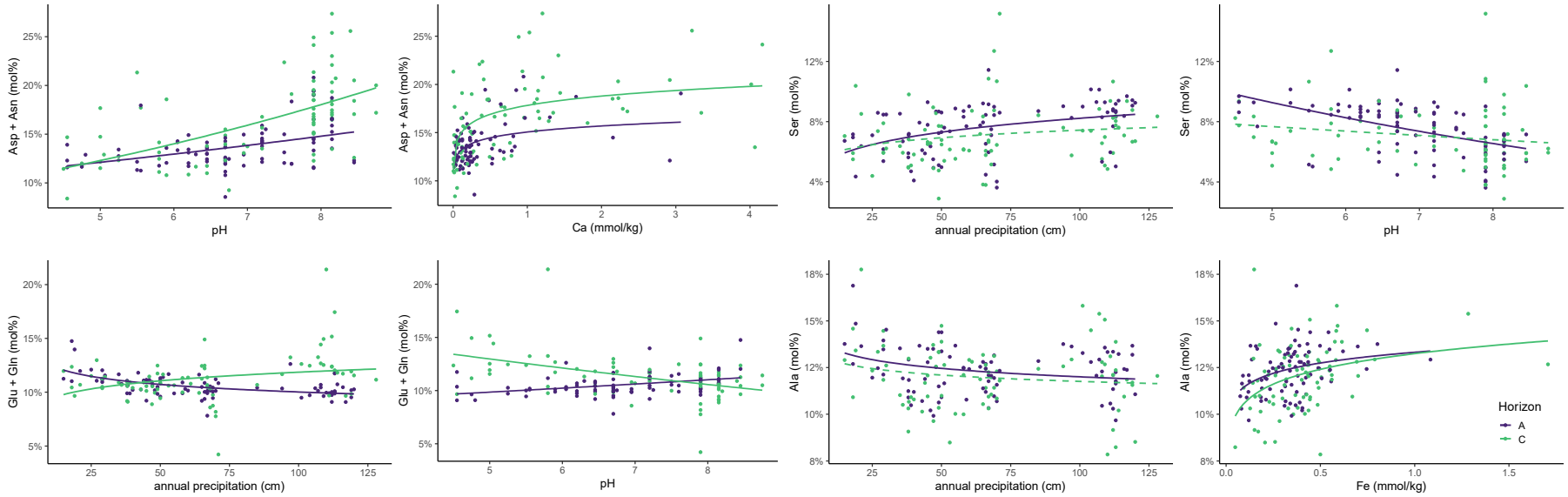


**Figure S17 Correlation between the molar ratios of some representative HAAs (Asx%, Glx%, Ser%, and Ala%) with environmental factors in A- (n = 84) and C- (n = 81) horizon .**

Solid and dashed lines indicate significant and insignificant relationships, respectively.

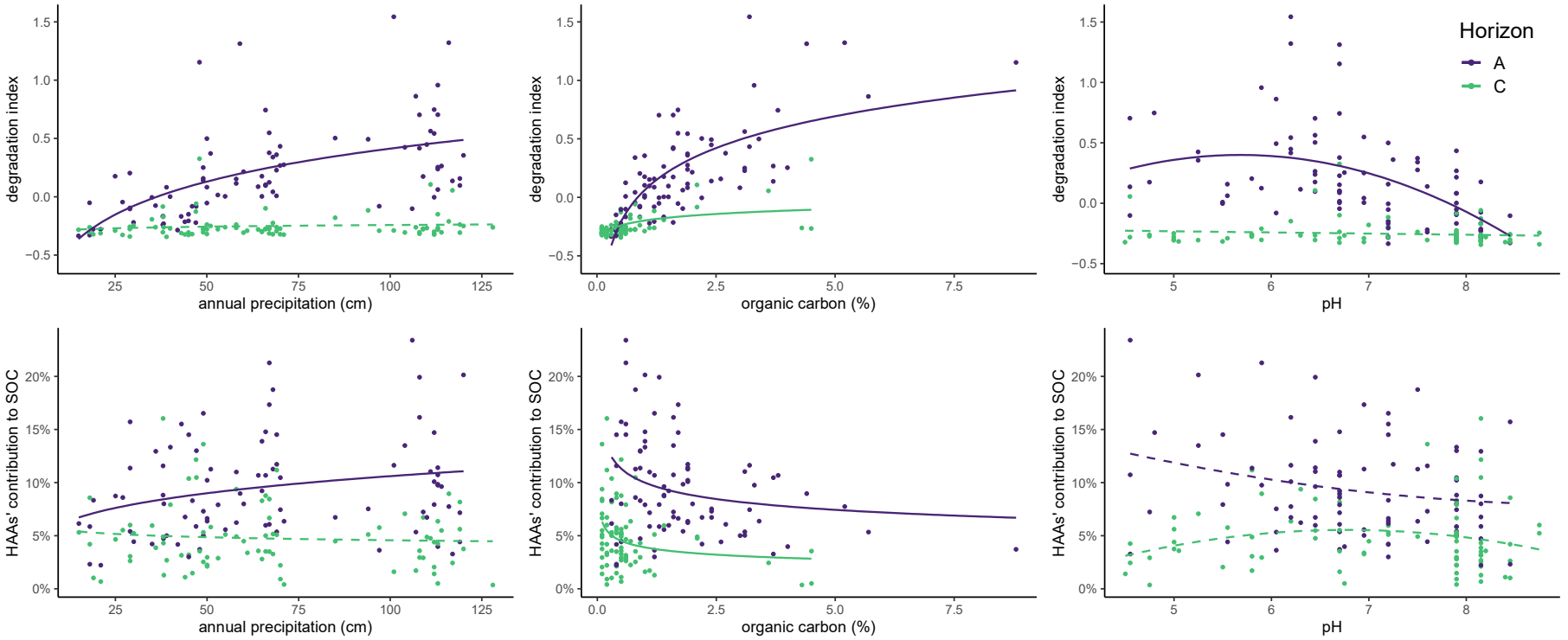


**Figure S18 Correlation of the HAAs' characteristics in carbon cycle (degradation index and contribution to organic carbon pool) with environmental factors (annual precipitation, organic carbon, and pH) in A- (n = 84) and C- (n = 81) horizon.**

Solid and dashed lines indicate significant and insignificant relationships, respectively.

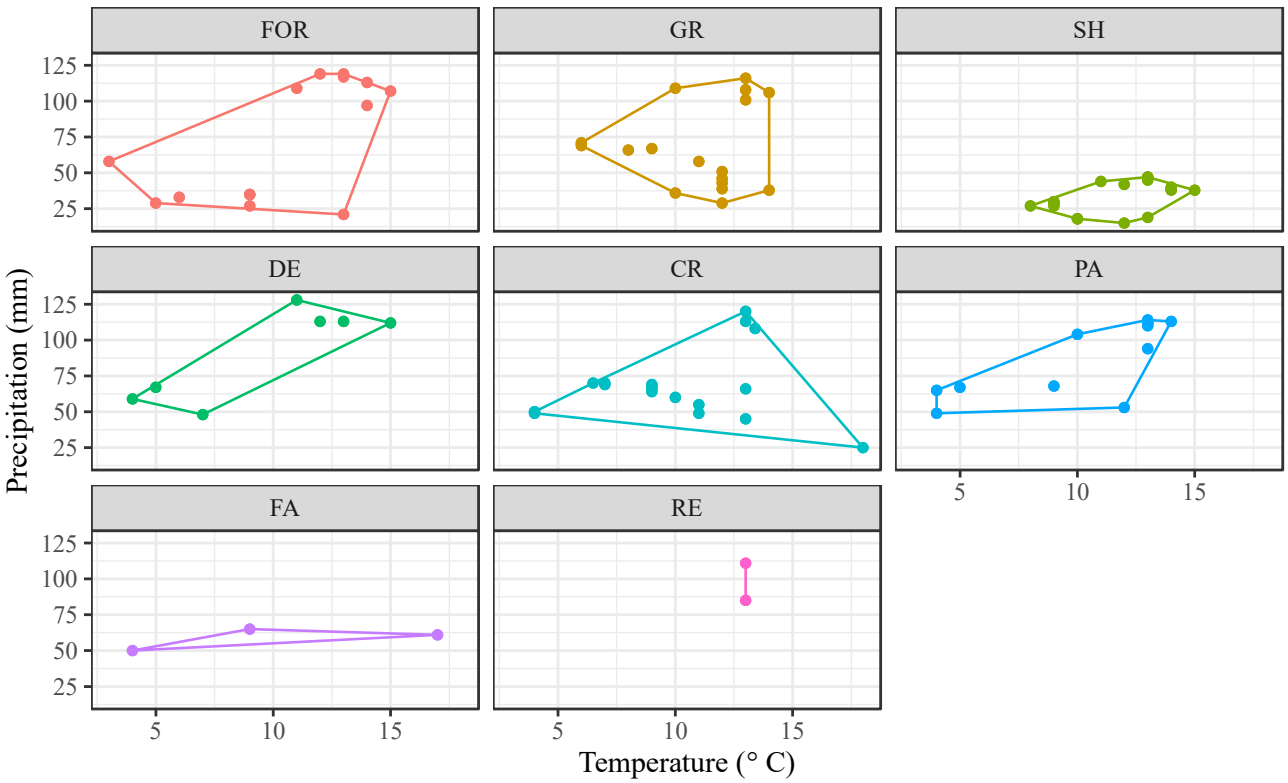


**Figure S19 Distribution of annual mean temperature and precipitation for each VET.**

**Table S1 ANOVA results on 7 HAAs exhibiting strong correlation with the PCoA axes.**

| HAAs | term | df | *F* | *p* |
|---|---|---|---|---|
| Asx | Horizon | 1 | 32.9 | <0.001 |
| | Vegetation | 5 | 6.0 | <0.001 |
| | Horizon:Vegetation | 5 | 1.3 | 0.260 |
| | Residuals | 160 | | |
| Gly | Horizon | 1 | 1.1 | 0.305 |
| | Vegetation | 5 | 0.5 | 0.812 |
| | Horizon:Vegetation | 5 | 1.6 | 0.154 |
| | Residuals | 160 | | |
| Ile | Horizon | 1 | 26.6 | <0.001 |
| | Vegetation | 5 | 1.3 | 0.269 |
| | Horizon:Vegetation | 5 | 3.4 | 0.006 |
| | Residuals | 160 | | |
| Leu | Horizon | 1 | 69.9 | <0.001 |
| | Vegetation | 5 | 0.7 | 0.597 |
| | Horizon:Vegetation | 5 | 2.7 | 0.023 |
| | Residuals | 160 | | |
| Phe | Horizon | 1 | 59.1 | <0.001 |
| | Vegetation | 5 | 1.7 | 0.139 |
| | Horizon:Vegetation | 5 | 3.0 | 0.014 |
| | Residuals | 160 | | |
| Pro | Horizon | 1 | 15.1 | <0.001 |
| | Vegetation | 5 | 1.3 | 0.248 |
| | Horizon:Vegetation | 5 | 1.1 | 0.374 |
| | Residuals | 160 | | |
| Val | Horizon | 1 | 0.0 | 0.840 |
| | Vegetation | 5 | 1.2 | 0.289 |
| | Horizon:Vegetation | 5 | 2.2 | 0.053 |
| | Residuals | 160 | | |

**Table S2 PERMANOVA results on VET and environmental factors influencing HAAs .**

| Horizon | term | df | $r^2$ | *F* | *p* |
|---|---|---|---|---|---|
| A | Vegetation | 5 | 0.181 | 3.6 | <0.001 |
| | Precipitation | 1 | 0.025 | 2.5 | 0.057 |
| | Fe | 1 | 0.026 | 2.6 | 0.054 |
| | Ca | 1 | 0.042 | 4.2 | 0.007 |
| | Al | 1 | 0.018 | 1.8 | 0.132 |
| | Residual | 70 | 0.708 | | |
| C | Vegetation | 5 | 0.095 | 1.7 | 0.021 |
| | pH | 1 | 0.059 | 5.1 | <0.001 |
| | Fe | 1 | 0.057 | 5.0 | 0.001 |
| | Mn | 1 | 0.018 | 1.5 | 0.168 |
| | Residual | 67 | 0.771 | | |