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Plant – Microbial and mineral contributions to amino acid and protein organic matter accumulation during 4000 years of pedogenesis

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ABSTRACT

The dynamics and persistence of proteinaceous compounds during pedogenesis are major mechanisms of soil formation and determinants of organic matter (OM) turnover. We investigated the accumulation patterns of proteinogenic amino acids associated with minerals dominated by permanently negative charges (primary silica minerals) and related these to vegetative and belowground microbial succession during soil ecosystem development. Positively-charged amino acids (arginine, lysine, histidine), extracted from whole soil pool using 6 M HCl, showed clear patterns of accumulation, increasing ~65% during 4010 years of development, while negatively charged amino acids (glutamic acid, aspartic acid) decreased ~13%. In the mineral associated sub-pool, positively charged amino acids were approximately ~431% more enriched, while negatively charged amino acids were ~38% depleted as compared to the whole soil pool. The multivariate ordination of soil bacterial community structure based on a 16s ribosomal RNA gene analysis and that of the aboveground plant community structure predicted 71% ($p < 0.0001$) and 66% ($p < 0.0001$) of the amino acid dynamics, respectively, during soil ecosystem development. Ala-rich Actinobacteria abundance declined with the year of development, concomitant with the decrease of Ala content in soil ($r^2 = 0.82$, $p = 0.0019$). His-rich Acidobacteria and His in soil both increased with the year of development ($r^2 = 0.92$, $p = 0.0022$). In support of the main hypothesis, the relative distribution of proteinogenic amino acids changed during pedogenesis with evidence indicating that biological communities and minerals play roles as source and sink of OM in soil, respectively.

1. Introduction

Amino acids (AA), peptides, and proteins are the major form of nitrogen (N) in soil organisms and plants; for example, they comprise approximately 50% and 30% of the cellular weight of bacteria and fungi, respectively (Christias et al., 1975; Neidhardt et al., 1990). These proteinaceous compounds also compose a large fraction of soil organic matter (SOM; ~30%) (Knicker, 2011; Rillig et al., 2007) and are a dominant form of total N (70–90%) in soil (Giagnoni et al., 2010; Knicker and Hatcher, 1997; Miltner et al., 2009; Nannipieri and Eldor, 2009; Schulten and Schnitzer, 1997). The compositions of biotic communities and their proteins, thus, are important determinants of SOM turnover and global biogeochemical cycles of carbon (C) and N.

Cycling of proteinaceous compounds in soil will determine the relative distribution of bioavailable and long-term stabilized pools of N. The breakdown of soil peptides and proteins to amino acids is a primary rate limiting step for N mineralization (Jones and Kielland, 2002). Amino acids, peptides, and proteins are thus an important determinant of available N for plants and pool sizes of SOM. Soil peptides/proteins, through turnover by microorganisms (Hobara et al., 2014) and mineral associations (Mikutta et al., 2006; Peng et al., 2015), also contribute to SOM formation and preservation.

The role and stabilization of proteinaceous compounds have been described in models such as the molecular aggregates model (Wershaw, 1986), onion layering model (Sollins et al., 2006), and encapsulation model (Knicker and Hatcher, 1997). Amphiphilic and

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amphoteric functional groups of proteinaceous compounds are predicted to interact with SOM and minerals so as to be less mobile and more protected from disassociation and decomposition. There remains, however, lack of field-based evidence supportive of protein accrual associated with soil minerals.

The emerging evidence of preferential accumulation and long residence time of proteinaceous compounds in soil (Cotrufo et al., 2013; Schmidt et al., 2011) is counter to the traditional view that peptide bonds are highly labile and readily broken down through heterotrophic-enzymatic activity (Alexander, 1981; Huguet et al., 2008; Kokinos et al., 1998; Schnitzer, 1985; Sollins et al., 1996; Zonneveld et al., 2010) and formerly thought to be an uncommon component of stable SOM. Carbon models have traditionally been dominated by the concept of intrinsic molecular resistance as one of the major controllers of soil C turnover and storage. Accordingly, the molecular structure and lability of organic material has long been thought to determine long-term decomposition rates. However, recent observations have shown that molecular structure is only part of the story. Protein and sugar compounds are more susceptible to chemical attack and biologically labile than aromatic ring structures, but their mean residence times in soil rather tend to be longer (Schmidt et al., 2011). This shift in thinking of the residence time of proteinaceous molecules represents an important change for understanding global biogeochemical C and N cycling.

The distribution of proteinaceous compounds were investigated in a Hawaiian rainforest chronosequence soil (~4.1milion years of development), showing the important role of noncrystalline or short-range ordered minerals in the retention of negatively charged amino acids (aspartic- and glutamic-acid) (Mikutta et al., 2010). The research revealed, furthermore, that at least a portion of mineral associated OM is microbial-derived (Dümig et al., 2012; Mikutta et al., 2010). These results further highlight the connection between mineralogy and some of the molecular details of OM accumulation over tens of thousands of years and more (Torn et al., 1997). It is expected that different dynamics would occur in soils associated with different parent materials over hundreds of years of pedogenesis, which could provide more information relevant to time scales related to anthropogenic induced global change.

In this study, we investigated the variation in the distribution of proteinaceous compounds over hundreds and thousands of years of pedogenesis by analyzing soil amino acids — the structural unit of peptides/proteins — along an eolian sand dune chronosequence adjacent to northern Lake Michigan. We hypothesized that patterns of proteinogenic amino acids in soil will change with pedogenesis; and the changes would be associated with both biotic and abiotic shifts across the chronosequence. The primary objective of this study was to determine pedogensis-related amino acid change in the whole soil pool and the mineral-associated sub-pool. To relate with biotic and abiotic factors, we investigated amino acid change with successional shifts of vegetative and microbial communities (Williams et al., 2013) associated with pedogenesis.

2. Materials and methods

2.1. Site descriptions and sampling

Soil chronosequences are a key tool for studying chemical, biological, and physical changes that occur in soil ecosystems as a consequence of pedogenesis. The study site consisted of a series of beach-dune ridges bordering Lake Michigan (N 45.72729, W84.94076), which was located in the Wilderness State Park. The chronosequence of eolian sediments were derived from intermittent deposition of Lake Michigan over the past ~5000 years. The site was located at the interface of a temperate and boreal climate region. Temperature and precipitation averaged 6.28 °C and 77.2 cm per year, respectively, between 1951 and 1980 at Mackinaw City, 15 km to the east.

The dune ridges had parent material originating from glacial deposits and Paleozoic bedrock underlying the lake basin. The parent material was thought to be similar across the dune sequence. Fine sands deposited on the lake shore are dominated by quartz and contain other minerals in minor quantities (Lichter, 1995). The youngest soils (<100y) were mapped as dunes which then developed into Deer Park sands (soil series) and described taxonomically as mixed, frigid, Spodic Udipsamments. The oldest soils (>1475y) tended to be mapped to the Roscommon series, and were mixed, frigid, Mollic Psammaquents.

The change in aboveground vegetative community structure was greater during early compared to later ecosystem development (Williams et al., 2013). Dune-building grass species were replaced by evergreen shrubs, and these were then replaced by mixed pine forests. This shift in early-to later-succession plant species occurred at 450y when the early-succession species began to disappear and the mixed pine forest began to develop. The compositions of the plant community at 105-155y were completely different from those at 210y, which were again taxonomically different from those at >450y of ecosystem development. Once the forest matured, the plant species composition stabilized and there was no major change in the plant community structure during later ecosystem development.

Belowground bacterial communities changed during early ecosystem development (<450y) but changed little during later ecosystem development (>450y) (Williams et al., 2013). The chronosequence gradients showed a number of changes in phylum composition but were generally dominated by the abundance and dynamics of Acidobacteria, Actinobacteria, and Alphaproteobacteria, comprising 71% of all the sampled sequences. Between early (<450y) and later (>450y) ecosystem development, Acidobacteria increased approximately 6-fold from ~4% to ~30%. Actinobacterial abundance declined, in contrast, from ~60% to ~35% during this same period.

Five replicates of top soil samples were collected from the incipient A-E horizon (0–15 cm, 5-cm dia.) in nine dunes of age 105, 155, 210, 450, 845, 1475, 2385, 3210, and 4010y, using the same method in previous published literature (Williams et al., 2013). Each replicate was separated by 10-m intervals across transects along each dune's crest. The soil samples were stored in sterile Whirlpak bags and frozen immediately in coolers with dry ice. Five replicates of sand samples were also collected along the beach to simulate source materials that formed the eolian deposits of the dunes. Samples were collected in August 2008. The vegetation and soil properties were characterized (Lichter, 2000; Williams et al., 2013).

2.2. Whole soil hydrolysable amino acid analysis

The hydrolysable amino acids in the whole soil were acid digested, purified, and then analyzed by high performance liquid chromatography (HPLC) after derivatization. Two to 5 g (dry weight basis) of moist soil was hydrolyzed in 10 ml of 6 M HCl with an internal standard (L-norvaline) at 110 °C for 24 h (Amelung and Zhang, 2001). After hydrolysis, the soil hydrolysates were centrifuged at 10,000*g* for 10 min. An aliquot of the supernatant (400 μl) was diluted in 55 ml ultra-pure water and cleaned on a preconditioned Dowex 50W X8 resin (hydrogen form, 50–100 mesh; Alfa Aesar, Cat# B22109) (Küry and Keller, 1991; Norman and BOAS, 1953). The interfering metals were removed by rinsing with 0.1 M oxalic acid (pH 1.6–1.7). Amino acids retained on the resin were eluted with 30 ml of 3 M NH_4OH, filtered through a 0.22 μm polyvinylidene fluoride (PVDF; Thermo Scientific™ Target2™

Syringe Filters, Cat# 03377155) membrane syringe filter, vacuum-dried, reconstituted in 10 μl 0.05 M HCl, and finally derivatized using the AccQ Fluor™ reagent kit (Fluorescent 6-Aminoquinoly-*N*-Hydroxysuccinimidyl Carbamate derivatizing reagent; Waters Co. Cat# WAT052880) following the standard protocol from Bosch et al. (2006) and Hou et al. (2009). Chromatographic separation on the HPLC 1260 Infinity system (Agilent Technologies, USA) was carried out on a reversed phase column (Waters X-Terra MS C18, 3.5 μm, 2.1 × 150 mm). The mobile phase consisted of A: an aqueous solution containing 140 mM of sodium acetate, 17 mM of triethylamine (TEA; Fisher Chemical, Cat# O4884100), and 0.1% (g/L, w/v) disodium dihydrogen ethylenediaminetetraacetate dihydrate (EDTA-2Na·2H_2O; Sigma, CAS# 6381-92-6), pH 5.05, adjusted with phosphoric acid solution, and B: Acetonitrile (ACN; HPLC grade, Fisher Chemical, Cat# A998-1): ultrapure water (60:40, v/v). The gradient conditions were 0–17 min 100 - 93% A, 17–21 min 93 - 90% A, 21–30 min 90 - 70% A, 30–35 min 70% A, 35–36 min 70 - 0% A, and then hold for 4 min before restoring to the initial composition at 40.5 min, with the final composition kept for 9 min. The column was thermostated at 50 °C and operated at a flow rate of 0.35 ml/min. The sample injection volume was 5 μL. The analytes detection was carried out using a fluorescence detector (λ_{ex} = 250 nm and λ_{em} = 395 nm) (Bosch et al., 2006; Hou et al., 2009). Hydrolysable amino acids in the samples were qualified and quantified by comparison with amino acid standard solutions. Each amino acid standard solution contained 20 amino acids including alanine (Ala), arginine (Arg), aspartic acid (Asp), asparagine (Asn), cystine (Cys–Cys; more stable form in oxidative condition than monomer cysteine), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), tryptophane (Trp), and valine (Val). During acid hydrolysis, Trp was destructed, Asn and Gln were transformed to Asp and Glu, repectively. The combined peak of Asp and Asn was denoted as Asx (Asp+Asn) and the combined peak of Gln and Glu was denoted as Glx (Gln+Glu). A total of 17 amino acid peaks were quantified for hydrolysable proteinogenic amino acids.

2.3. Soil mineral associated amino acid analysis

The soil mineral associated organic matter fraction (heavy fraction) was isolated by the density fractionation method (Kaiser and Guggenberger, 2007), followed by amino acid analysis in this heavy fraction. Air-dried soils (2.5 g) were fractionated using sodium metatungstate (SMT, $H_2Na_6O_{40}W_{12}$) solutions with a density of 2.4 g/cm^3. The mixture was vigorously agitated on a shaker until the soil was completely dispersed. After dispersion, the sample was centrifuged and the floating particulate matter (light fraction) was carefully separated from the heavy fraction. The heavy fraction was thoroughly cleaned with distilled water and completely dried at 60 °C in an oven overnight. The dried heavy fraction was weighed and hydrolyzed using the same whole soil hydrolysable amino acid analysis, as described above. The amino acid released from the SMT-isolated heavy fraction is interpreted as the mineral associated AA.

2.4. N (1s) K-edge near edge X-ray adsorption fine structure (NEXAFS) analysis

The purpose of using N (1s) K-edge NEXAFS analysis is to describe N functional groups of organic matter on the mineral associated OM fraction. The N (1s) K-edge NEXAFS spectrum for the SMT-isolated mineral associated fraction of each soil sample was collected at room temperature under high vacuum (10^{-8}–10^{-9} Torr) on the soft X-ray beamline U4B at the National Synchrotron Light Sources, Brookhaven National Laboratory, Upton, NY, USA. Approximately a 1 mm layer of the SMT-isolated mineral portion of a soil sample was evenly spread on nitrogen-free adhesive carbon tape mounted on a sample holder. The conductive carbon tape was used to eliminate x-ray interference derived from the substrate. And then the sample holder was loaded into the vacuum spectrum collection chamber. The total electron yield was measured within the photon energy scan range of 390–440 eV. The L_{II} edge of Sc (403.9 eV) was used for energy calibration. The Igor Pro data processing software (Version 5.05A, WaveMetrics, Inc., Lake Oswego, OR) was used for N (1s) K-edge NEXAFS spectra averaging, averaged spectrum background subtraction and normalization. The processed N (1s) K-edge NEXAFS spectrum for each sample was then fitted, using the Solver function of Microsoft EXCEL®, with five Gaussians curves corresponding to N functional groups: Pyridine/Aromatic-N (400.2 eV), Pyridone/Aromatic-N (401.2 eV), Amide/Peptide-N (402.2 eV), Nitro-aromatic-N (405.8 eV), and Mineral fixed-NH_4^+ (407.0 eV) (Lehmann et al., 2009). The abundance of Amide/Peptide-N relative to the total N was calculated based on the curve fitting result.

2.5. Statistical analysis

For the multivariate comparison, molecular species of amino acid concentration were transformed using general relativization to remove the potentially strong influence of absolute abundance on a ordination. Multi-Response Permutation Procedures (MRPP) and Nonmetric Multidimensional Scaling (NMS) ordination were performed using the PC-ORD software version 6.0 (MjM Software, Gleneden Beach, OR, USA) to compare the effect of soil age on the relative abundance of 17 proteinogenic amino acids in whole soil and mineral associated OM hydrolysates. The cutoff of statistical significance for MRPP was $p = 0.01$. Univariate comparisons were conducted by using One-way Analysis of Variance (ANOVA) and Student's t-test on the absolute abundance of amino acids, using SAS JMP pro11 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). The cutoff of statistical significance for ANOVA was $p = 0.05$. SigmaPlot version 11.0 (Systat Software, San José, CA, USA) was used to make graphs.

3. Results

3.1. Abundance of amino acids

The total amino acid content (198 mg/kg-soil) of the whole OM pool in the beach sand without vegetation was significantly lower than those (avg. 923 ± 56 mg/kg-soil) from the dunes with vegetation (Fig. 1a). However, the total amino acid content (102 mg/kg-soil) of the mineral associated fraction in the beach sand was similar to those (avg. 109 ± 7 mg/kg-soil) from the chronosequence soils. It was notable that the beach sand featured a significantly greater percentage of the mineral associated amino acid (54%) compared to the chronosequence soils (avg. 13 ± 1%; Fig. 1b).

Organic C and total N increased and peaked at 450 y; thereafter, both declined (Fig. 1c). The amino acid abundance in the whole soil pool increased during the early years of soil ecosystem development (Fig. 1a). The average amino acid content in the whole soil pool was 623 mg/kg-soil at 105y. Coinciding with vegetative colonization, the values peaked at 1325 mg/kg-soil between 450 and 845y; thereafter, amino acid amounts declined, averaging 749 ± 49 mg/kg-soil. Although the change of the amino acid content was dynamic in the whole soil pool, that in the mineral associated sub-pool was relatively consistent across the chronosequence. Overall, the results indicated a dynamic whole

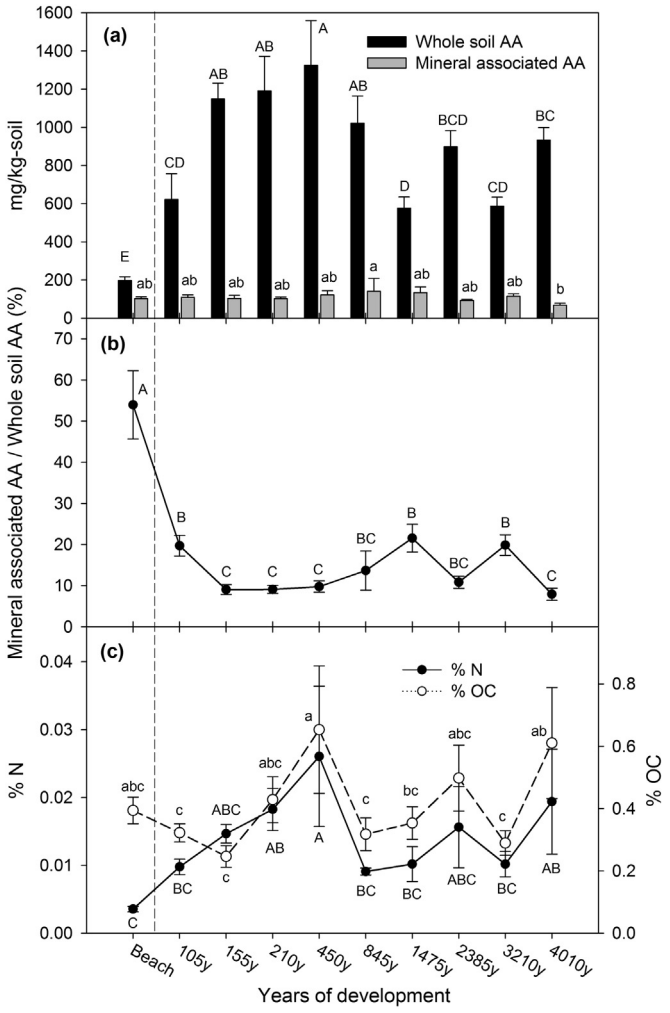

Fig. 1. Sum of 17 proteinogenic amino acids in the whole soil pool (whole soil AA) and mineral associated sub-pool (mineral associated AA) in mg/kg-soil **(a)**, the percentage of the mineral associated amino acid content over amino acid content of whole soil **(b),** the percentage (w/w) of nitrogen (N) and organic carbon (OC) to whole soil **(c)** with the age of sites across the Lake Michigan chronosequence. "Beach" represents the parent material of sand dunes without the influence of vegetation. Letters denote significant differences and the amino acid contents of two pools were separately tested by Student's t ($P < 0.05$) between the years of development: for **(a)**, upper case = whole soil AA, lower case = mineral associated AA and for **(c)**, upper case = %N and lower case = %OC. Error bars represent standard error (n = 5).

soil amino acid pool size compared to a relatively stable mineral associated sub-pool size during ecosystem development.

3.2. Peptide-N in mineral associated fraction

Proteins and peptides were a dominant organic N form on the surface of minerals increasing from 35% at 105y to 68% at 4010y (Fig. 2). The majority of the amino acids that we determined in the mineral associated OM hydrolysates were, thus, expected to be in the form of polymers such as peptides and proteins. Overall, the contribution of polymers linked by peptide bonds to the mineral associated amino acid pool increased with pedogenesis. Polymers, rather than monomers of proteinaceous compounds, became a relatively more abundant component of SOM that interacted with minerals as the chronosequence developed over time.

3.3. Relative distribution of amino acids

Clear patterns of change in the relative distribution of amino

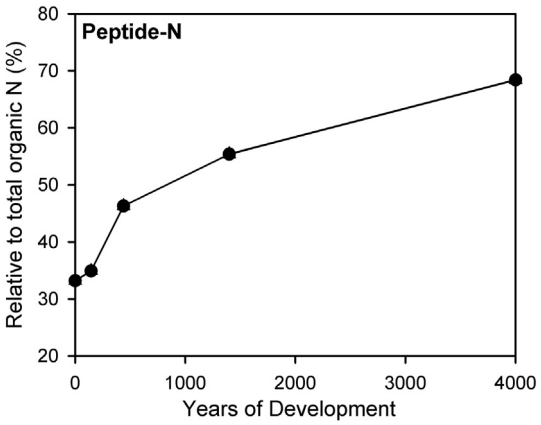

Fig. 2. Abundance of peptide-N relative to total N associated with the mineral portion of the Lake Michigan chronosequence soils at various ecosystem development stages (n = 1). The relative abundance of amide/peptide-N was obtained using the synchrotron based N (1s) K-edge Near Edge X-ray Absorption Fine Structure (NEXAFS) spectroscopy. Beach sand is shown as "0" year.

acids with ecosystem development were shown in both the whole soil pool and mineral associated sub-pool ($p < 0.0001$ from MRPP for both, Fig. 3a,b). For the whole soil pool, two shifts of relative distribution of amino acids were apparent: (1) from 105y to 450y, shown as the solid arrow of "early development" in Fig. 3a and (2) from 450y to 4010y, shown as the dash arrow of "late development". Positively charged amino acids (His, Arg, Lys) and Pro were positively- and Gly, Ala, and Asx were negatively-correlated with age during early ecosystem development. Ser was positively- and Glu was negatively correlated with age during later ecosystem development. For the mineral associated fraction, the shift in relative distribution of amino acids was strongly associated with axis1, shown as a solid arrow in Fig. 3b. Amino acid distributions at the pedogenically younger sites grouped to the right and gradually changed to the left along with the axis1 in Fig. 3b. Those at 4010y were relatively more distinct from the rest of the chronosequence soils where Cys was positively correlated with 4010y and negatively correlated with axis1. The relative abundances of Gly, Ala, Asx, Leu, and Ile were positively correlated with axis1. The relative distribution of amino acid in the whole OM in the beach sand was different from those in the dunes with vegetation (Fig. 3a and Table S3; $p = 0.005$ or less from pairwise MRPP). Despite the distinct amino acid profiles of the beach sand in the whole OM pool, the relative distribution of the mineral associated amino acid in the beach sand was similar to those in younger dunes with vegetation (Fig. 3a and Table S4; $p = 0.027$ or higher from pairwise MRPP). In summary, there were significant shifts of amino acid distributions in both the whole soil pool and the mineral associated sub-pool during ecosystem development.

3.4. Comparison between whole soil pool and mineral associated sub-pool

The overall relative distribution of amino acids differed between the whole soil pool and the mineral associated sub-pool (Fig. 4 and Fig. S1c), although the dominant amino acids were Gly, Ala, Asx, Glx, Ser, and Pro in both pools (Table S6 and Fig. S1a,b). The physicochemical properties of amino acids that were relatively depleted in the mineral associated sub-pool than in the whole soil pool were characterized as three types: (1) amino acids with a carboxyl functional group thus contributing to negative charges on the structure (termed negatively charged amino acids in this paper, including Asp and Glu), (2) those with the side chain of an aliphatic

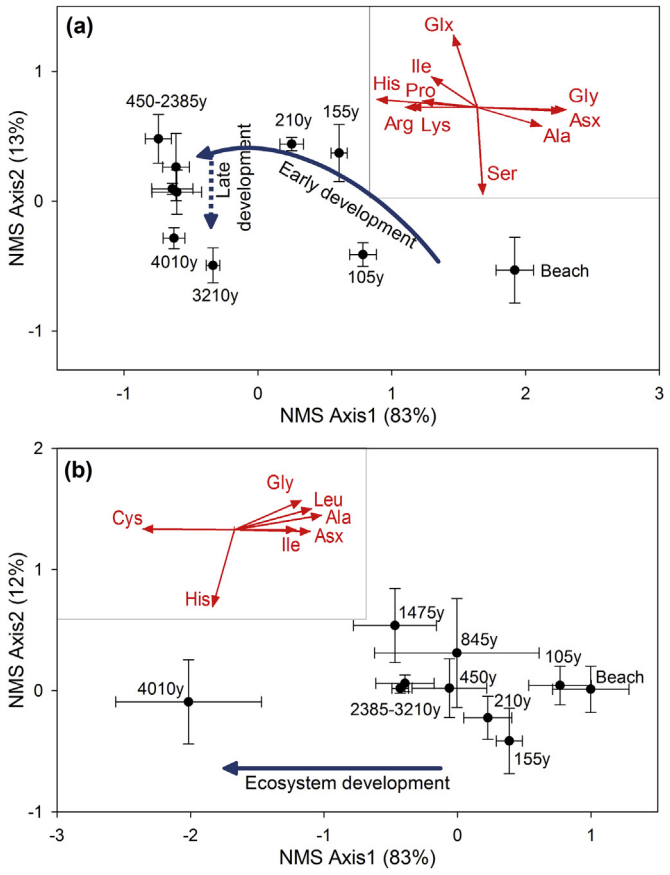

Fig. 3. Relationship between the distribution of 17 proteinogenic amino acids and soil ecosystem development plotted by nonmetric multidimensional scaling (NMS) ordination in the whole soil **(a)**; and in the mineral associated fraction **(b)** in the Lake Michigan sand dune chronosequence. Freshly deposited "beach" sand was also sampled to assess the amino acid distribution of parent material expected to be similar to the source material that formed the eolian deposits of the dune soils. Error bars in **(a)** and **(b)** represent standard error (n = 5). The final stresses are 12 for **(a)** and 10 for **(b)**. Percentages on each axis in each plot denote the amount of variability associated with each axis. Vectors in the small boxes show the direction and strength of the relationship between individual amino acids and ordination scores with the cutoff of $r^2 = 0.5$ for **(a)** and **(b)**. The Pearson and Kendall correlations of the vectors are provided in the supplementary information (Tables S1 and 2).

group (Val, Leu, and Ile), and (3) Thr, which has a hydroxyl functional group. There are three types of amino acid group enriched in the mineral associated sub-pool compared to those from the whole soil pool: (1) amino acids with the side chain of an amino functional group thus contributing to the positive charges associated with the molecular structure (termed positively charged amino acids in this paper, including Arg, His, and Lys), (2) those with a sulfur functional group (Cys and Met), and (3) Tyr, which has both aromatic and hydroxyl functional groups.

The relative abundances of positively charged amino acids were enriched in the mineral associated fraction compared to those in the whole soil pool; for example, His was enriched ~431% in the mineral associated fraction (Fig. 5a). On the other hand, the proportion of the negatively charged amino acids was depleted in the mineral associated fraction compared to that in the whole soil pool; for example, Asp was ~38% less in the mineral associated fraction than the whole soil pool (Fig. 5a). In comparison between the beach sand with the 4010y soil, the mean relative abundance of the positively charged amino acid group increased ~65%, while that of the negatively charged amino acid group decreased 13% (Fig. 5b).

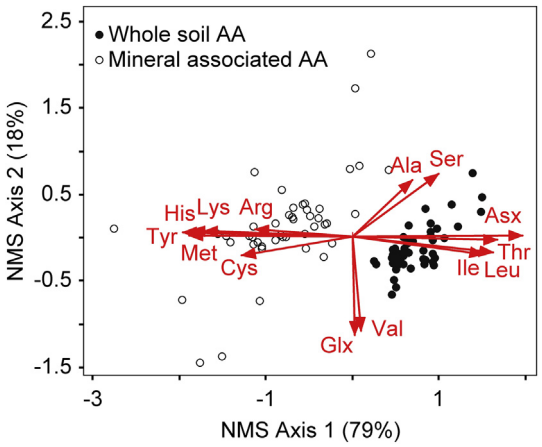

Fig. 4. Differences in 17 proteinogenic amino acid distributions between whole soil and mineral associated fraction in the Lake Michigan sand dune chronosequence, plotted by nonmetric multidimensional scaling (NMS) ordination. The final stress is 8. Percentages on each axis in each plot denote the amount of variability associated with each axis. Vectors show the direction and strength of the relationship between individual amino acids and ordination scores with the cutoff of $r^2 = 0.3$. The Pearson and Kendall correlations of the vectors are provided in the supplementary information (Table S5).

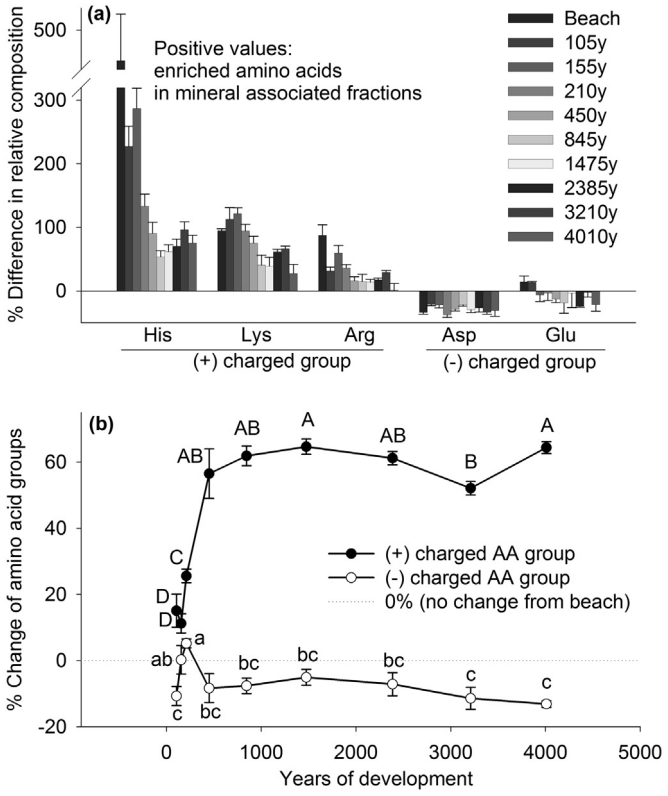

Fig. 5. Percentage of difference in relative abundance of charged amino acids between mineral associated sub-pool and whole soil pool **(a)**; and the percentage change of charged amino acid groups **(b)** during soil development across the Lake Michigan sand dune chronosequence. For **(a)**, the calculation was % Difference = ((mol% of mineral associated AA)-(mol% of whole soil AA))/((mol% of whole soil AA)) × 100%. For **(b)**, the initial abundance of amino acids (Y_0) is at the beach sand and the relative abundance (Y_i) at each year ($_i$). Percentage change is $Y = (Y_i-Y_0)/Y_0$ *100%. In **(b)**, letters denote significant difference and the amino acid contents of two pools were separately tested by Student's t ($P < 0.05$) between the years of development: upper case = whole soil AA, lower case = mineral associated AA. Error bars represent standard error (n = 5).

3.5. Relationship between amino acid dynamics and biotic and abiotic changes during pedogenesis

The variables of biotic and abiotic factors, previously published by Williams at el. (2013), were used as covariates to test the relationship between amino acid dynamics and biotic and abiotic changes. The change of amino acid distribution in the whole soil pool was highly correlated with both aboveground plant ($r^2 = 0.66$, $p < 0.0001$) and belowground bacterial community change ($r^2 = 0.71$, $p < 0.0001$) during ecosystem development (Fig. 6 and Fig. S2). Dune-building grass species were replaced by evergreen shrubs between 155y and 210y, and these were then replaced by mixed pine forests at around 450y. Once the forest matured, the plant species composition was stabilized and there was no major change in the plant community structure during later ecosystem development. Before and after the aboveground establishment of a conifer forest at around 450y, the belowground microbial community also showed a shift in composition. The amino acid distribution as well as the plant and bacterial community compositions rapidly changed from 105y to 450y, but varied less for the next ~3000 years.

Along with the change of biotic communities, the abiotic factors such as pH, cation content, and organic matter content changed (Williams et al., 2013) The change in the distribution of whole soil amino acids was correlated with pH ($r^2 = 0.80$), magnesium (Mg; $r^2 = 0.77$), calcium (Ca; $r^2 = 0.70$), and potassium (K; $r^2 = 0.61$) content during pedogenesis (Table S7). Soil Ca, Mg, and K levels decreased in a log-linear pattern and were concurrent with declining pH (7.6–3.5) as soils aged from younger to older across the chronosequence. Overall, there was a strong association between amino acid and soil chemical changes during pedogenesis.

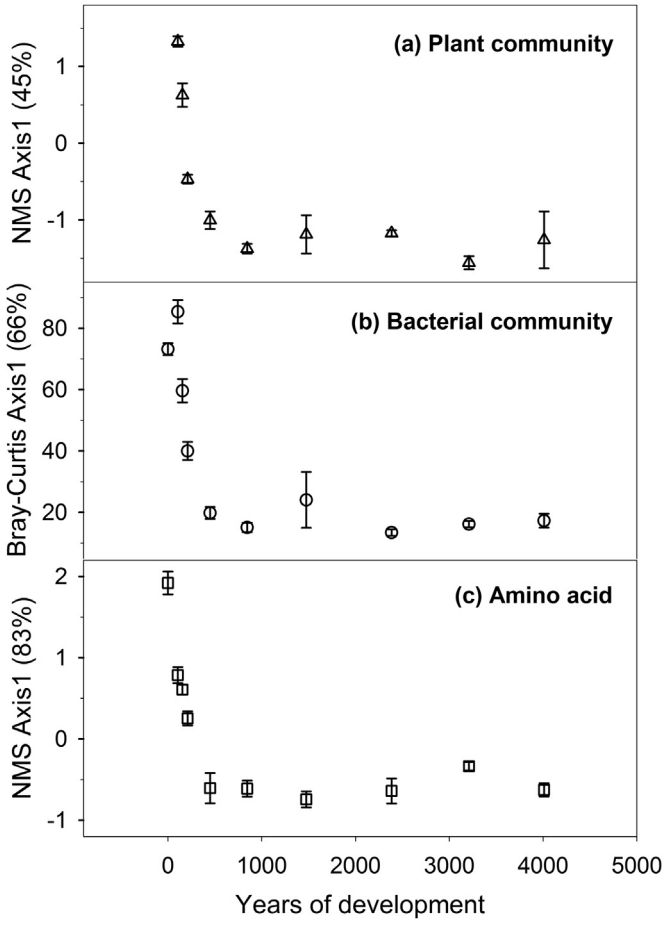

Fig. 6. The relationship between year of development and Axis1 from NMS ordination of plant community **(a)**; from Bray-Curtis ordination of bacterial community **(b)**; and NMS ordination of the relative distribution of 17 proteinogenic amino acids from the whole soil pool **(c)** in the Lake Michigan sand dune chronosequence. **(a)** and **(b)** were reconstructed based on Williams et al., 2013. Error bars represent standard error ($n = 5$). The regression model graphs are provided in the supplementary information (Fig. S2).

4. Discussion

Proteinogenic amino acids from whole soil and mineral-associated OM pools were used as indicators of formation and change of SOM across a 105y to 4010y dune-soil chronosequence. Distinctive shifts in these soil amino acids across the pedogenic gradient (Figs. 3 and 6) support the hypothesis that soil minerals and plant-microbial communities each contribute to SOM accrual. The types of amino acids found to change during soil ecosystem development, furthermore, support the individual roles that organisms provide as organic matter sources, and that mineral binding plays as a sink. Overall, the close relationship in the dynamics of microbial and plant communities and the process of pedogenesis, especially during early ecosystem development, suggests a tight linkage between these factors in formation and accrual of SOM.

4.1. Biological contribution to the amino acid distribution in soil

Soil organisms and plants contribute to SOM formation through their biomass, and physiological- and metabolic-products (Cotrufo et al., 2013). It was expected that the amino acid composition of SOM would resemble its biological sources. At a broad level, amino acid distributions of biological sources resembled those in soil pools, which is in agreement with Amelung et al. (2006) and Friedel and Scheller (2002). For example, Gly, Ala, Asx, Glx, Ser, Val, and Leu accounted for 70% of the total amino acids in eukarya and bacteria (Chen et al., 2013), as well as in the whole soil OM hydrolysates (Table S6). The relative abundance of these common amino acids from soil organisms and plant debris suggests that they are a major source of proteinaceous compounds during SOM accrual.

Although the amino acids of the whole soil hydrolysates share overall common dominant amino acids with their biological sources, the relative abundance of some amino acids varied from biological origins. Two of the smallest amino acids, Gly and Ala, for example, were about 81% and 29% greater in SOM, respectively, than the theoretical average protein of living organisms (Table S6). This might be because these two are the most thermostable amino acids, having been shown to persist for millions of years (Wang et al., 2012). Another reason there is a specific contribution of these amino acids to SOM could be due to the abundance of the peptide interlayer bridges of peptidoglycan. S-layer proteins of *Aeromonas hydrophila* (AN: L37348), for example, are 18% Ala (Thomas and Trust, 1995). Muropeptides from bacteria and cell wall glycoproteins of fungi are key communication pathways (Boudreau et al., 2012), and so the importance of these extracellular cell wall attached peptides and proteins may help to explain their disproportionate contribution to SOM. Therefore, the soil amino acid distribution may be affected by overall turnover and production derived from amino acid metabolism as well as by their structural stability in soil.

4.2. Amino acid shifts associated with microbial and vegetative community changes

Based on a previous study on 16s ribosomal RNA gene-based

bacterial community structure (Williams et al., 2013), shifts in the amino acid profile of the whole soil hydrolysates showed relationships with the proteinogenic amino acid composition of bacterial groups during pedogenesis. For example, the relative abundance of the most dominant phylum Actinobacteria decreased dramatically from 60% to ~35% during early ecosystem development, which coincided with the decline in Ala and Gly during this stage of pedogenesis (relationship between Ala and Actinobacteria: $r^2 = 0.82$, $p = 0.0019$ and between Gly and Actinobacteria: $r^2 = 0.41$, $p = 0.1670$; Table S9). Actinobacterial genomes contain high guanine-cytosine GC and thus code for proteins rich in Ala and Gly (Chen et al., 2013). This would help explain high abundances of Ala and Gly early and declining levels by mid and later periods of pedogenesis, concomitant with the decrease in GC-rich bacterial groups (Fig. S1a). High GC content is also associated with genomes coding scarce Lys and Phe (Chen et al., 2013), and is in agreement with initially low but increasing levels of Phe and Lys in the whole soil hydrolysates during pedogenesis (Fig. S1a). The chemical composition of microbial communities may thus play a role in determining the chemistry of SOM.

Acidobacteria were the second largest bacterial phylum described by a relatively abundant His coding genome (Chen et al., 2013). This again shows agreement with the 120% increase in His when Acidobacteria became more dominant at the intermediate and later stage of soil development ($r^2 = 0.94$, $p = 0.0022$, Table S9). Similarly, the increases of His in the water soluble free (monomer) amino acid pool along a young (105y to 450y) boreal alluvial forest successional sequence (Werdin-Pfisterer et al., 2009) are supportive of our findings. The 120% increase of this amino acid could be related to shifts in the relative biomass of living Acidobacterial groups as well as the turnover of these organisms over the relatively long term periods of soil development. In addition, His is also found in greater amounts in eukaryotic organisms, such as plants and fungi, than in prokaryotes. The dynamics in plant and fungal communities, therefore, also may reflect the dynamics of His during pedogenesis. Overall, the results indicate that biological organisms can have strong and taxa-specific influences on the occurrence and accrual of SOM (Miltner et al., 2009; Schmidt et al., 2015).

Changes in vegetation can affect amino acid pools. Conifers dominated at later ecosystem developmental stages (>450y) and tend to contribute less to soil amino acid compared to deciduous plants (Hobara et al., 2014). This might explain the decline in the abundance of whole soil amino acids coinciding with maturation of conifer forest. Specifically, Glx and Asx might be indicative of the influence of plant debris inputs to SOM. Glx and Asx are common constituents of plant xylem and phloem (Kielland, 1994) and storage amino acids in plant tissues (Nordin and Näsholm, 1997). The decrease in the proportion of Glx and Asx with soil ecosystem development (Fig. S2a) might be the result of selective uptake (Vinolas et al., 2001) due to their roles as precursors in the biosynthesis of many other amino acids (Roberts et al., 2009) and mineralization of these amino acids during decomposition (Vinolas et al., 2001). Gly and Ala also decreased during soil ecosystem development. Plants and mycorrhizal fungi have been shown to preferentially uptake low molecular weight amino acids (Geisseler et al., 2009; Kielland, 1994), which might result in a decrease in the relative proportions of these amino acids in soil during 4010 years of pedogenesis (Fig. S2a). However, the biochemical basis for differences in plant uptake rates among amino acids is unclear. Changes in the dominant vegetation, nevertheless, appear to be linked to chemical changes in SOM during pedogenesis.

4.3. Mineral associated amino acids

The proportion of mineral associated amino acids relative to the whole soil pool was relatively constant, averaging 13% (Fig. 1). This is in agreement with observations of other studies which have shown relatively lower adsorption of SOM on mineral surfaces in sandy soils compared to finer textured soils (Keil and Mayer, 2013; Mikutta et al., 2007) and little change of mineral associated SOM because of saturation of binding sites (Castellano et al., 2015). The rate of weathering of tectosilicates (e.g. quartz and feldspar), which are the most stable structure of primary silicate minerals, tends to be slow (McBride, 1994). Relatively slow mineralogical alteration during pedogenesis might explain the steady pool size of mineral associated AA.

Mineral specific mechanisms such as electrostatic forces between functional groups of amino acids and mineral surfaces can explain stabilizing patterns of proteinaceous compounds in soils. In soils mainly composed of a permanent negative surface charge, generated from mineral isomorphic substitution, the selective association of positively charged amino acids to mineral surface, and thus their limited release to a soil solution likely results in preferential accumulation of these amino acids (Jones and Hodge, 1999; Vieublé Gonod et al., 2006; Vinolas et al., 2001). On the other hand, the decline of negatively charged amino acids is likely because they are weakly adsorbed and thus readily leachable from soil systems (McBride, 1994). The connection between the enrichment on the mineral associations of positively changed amino acids and their preferential accumulation across the chronosequence are consistent with mineral binding as a driver of the types of amino acids that accrue in SOM during pedogenesis.

In contrast to primary silica minerals, poorly crystalline and metal-hydroxide minerals provide positive exchange sites for accrual of negative charged amino acids in soil (Mikutta et al., 2010; Strahm and Harrison, 2008). These results, though showing enrichment of amino acids with different charges, are mechanistically consistent with the results reported herein, and as expected from electrostatic interactions in soil. The two observations tell us that the distribution of amino acids varies depending on mineral composition of parent material and resulting from pedogenesis.

During soil development, the pH of the soil dropped from 7.6 at 105y to 3.5 at 4010y, which coincided with weathering and loss of total soil Ca and Mg from the ecosystem (Williams et al., 2013), while Na remained constant (Lichter, 1998). The dissolution of minerals and leaching of cations may affect the adsorption strength of positively charged amino acids. Divalent cations such as Ca and Mg cations tend to adsorb to cation exchange sites more strongly than monovalent cations such as Na and K (McBride, 1994). Leaching of Ca and Mg, in contrast, may create an opportunity for their replacement by positively charged amino acids on mineral exchange sites. In addition, multivalent cations such as Ca and Mg are responsible for creating the multivalent cation bridging complex between negatively charged mineral surfaces and organic anions (McBride, 1994). The removal of Ca and Mg during the weathering process may result in the disrupting and weakening of the bridging complex and thus affect the chemistry of OM associated with the minerals.

5. Conclusion

The molecular mechanisms contributing to residence times of SOM are fundamental to pedogenesis and soil ecosystem development. There were distinctive shifts in soil amino acids across the pedogenic gradient, supporting the hypotheses that microbial and plant communities and mineral surfaces each contribute to SOM accrual. Biological organisms were shown to have a strong and specific association, related to their genome-encoded chemistry, on the occurrence and accrual of SOM. The patterns of amino acid change also support the concept that mineral binding plays an

important role in determining the types of amino acids and the abundances of protein that accrue in SOM during pedogenesis. Overall, a tight linkage between sink and source factors suggest that there are significant non-random mechanisms that contribute to the formation and accrual of SOM. These results provide a valid alternative model of SOM formation and accrual that can develop beside current source based mechanisms (e.g. structurally complex phenylpropanoid structure of plant lignin) and sink based mechanisms that limit decomposability (e.g. aromatic groups) in support of a conceptual model of OM turnover and persistence in soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.05.011.

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