AMINO ACID N IN HOLOCENE PALEOSOLS AS IDENTIFIED BY IR SPECTROSCOPY AND AMPEROMETRIC TECHNIQUES

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INTRODUCTION

Organic matter in paleosols is investigated to infer the mechanisms by which carbon can be preserved over millennial time scales. However, there has been limited research on the nature of nitrogen associated with the stabilized organic matter in paleosols. The most abundant forms of organic N in soils are the amino acids (AA); they are typically bound in soils and may occur as peptides or part of polymeric structures, such as peptidoglycan or glycoproteins. Calderoni and Schnitzer (1984) have reported the abundance of amino acids (AA) in humic materials of Italian paleosols based on their charge properties. Amino acids can also be grouped based on the polarity of the R group attached to their α-carbon atoms. The alkyl group (R) in AA can act as tracer for the assimilation of C that is associated with N.

This study attempts to utilize the characteristics of the R groups attached to AA to track and understand the preservation of N in paleosols. We coupled the pulse amperometric technique with infrared (IR) signatures in identifying how N and consequently C are preserved over millennial scales.

OBJECTIVES

- Determine the AA content and distribution based on R groups with age or burial of paleosols
- Investigate if IR signals coupled with amino acid identification can provide a better means of understanding the preservation of N as well as C

MATERIALS AND METHODS

I. Study Sites

- Claussen site (along Mill Creek near Lawrence, KS; 740800E, 4326200N)
- Farwell site (along the South Fork of the Big Nemah River in southeast NE; two sub-sites, 64 and 65)

II. Sample Test Materials

- Unfractionated soils
- Clay fractions separated by repeated suspension and exhaustive density separations and then freeze-dried

III. Chemical and Physical Analyses

- Particle size (Gee and Bauder, 1986)
- Total CN analysis via combustion (Vario Micro Cube, Elementar, UK)
- TOC of freeze dried soils following a 24-h 0.5 *M* HCl treatment and 3x water rinses

IV. HF Treatment of Whole Soils

- Soils pre-treated with 0.1 *M* HCl followed by 4x treatment of 5 *M* HF to dissolve inorganics (Fang et al. 2010) and final water rinses before freeze-dried

V. Infrared Analysis

- 2 w/w% in IR-grade KBr
- Collected 200 scans from 4000-600 cm^{-1} via DRIFT using a Nicolet Magna IR spectrometer equipped with a cooled MCT detector
- Reported scans were background corrected and smoothed

VI. Amino Acid Analysis (Martens and Loeffelmann, 2003)

- 250 mg soil (<0.1 mm)/200 mg freeze-dried clay with 2 mL 4 *M* methanesulfonic acid (containing 0.2% tryptamine); soil-acid mixture autoclaved @ 121°C for 16 h (Olk et al. 2008)
- Digest was diluted and then centrifuged before pH adjusted to 4.0-6.0; brought to final 10-mL volume
- Ten-fold diluted with water, and filtered through 0.2 µm nylon filter
- Diluted filtrate analyzed by anion chromatography-pulsed amperometry
- AA grouped based on attached R groups as follows:
  - Charged R -Arg, Lys, Glu, Asp, His
  - Uncharged R - Gly, Thr, Ser, Cys, Tyr
  - Apolar R - Ala, Val, Pro, Leu, Iso, Met, Phe

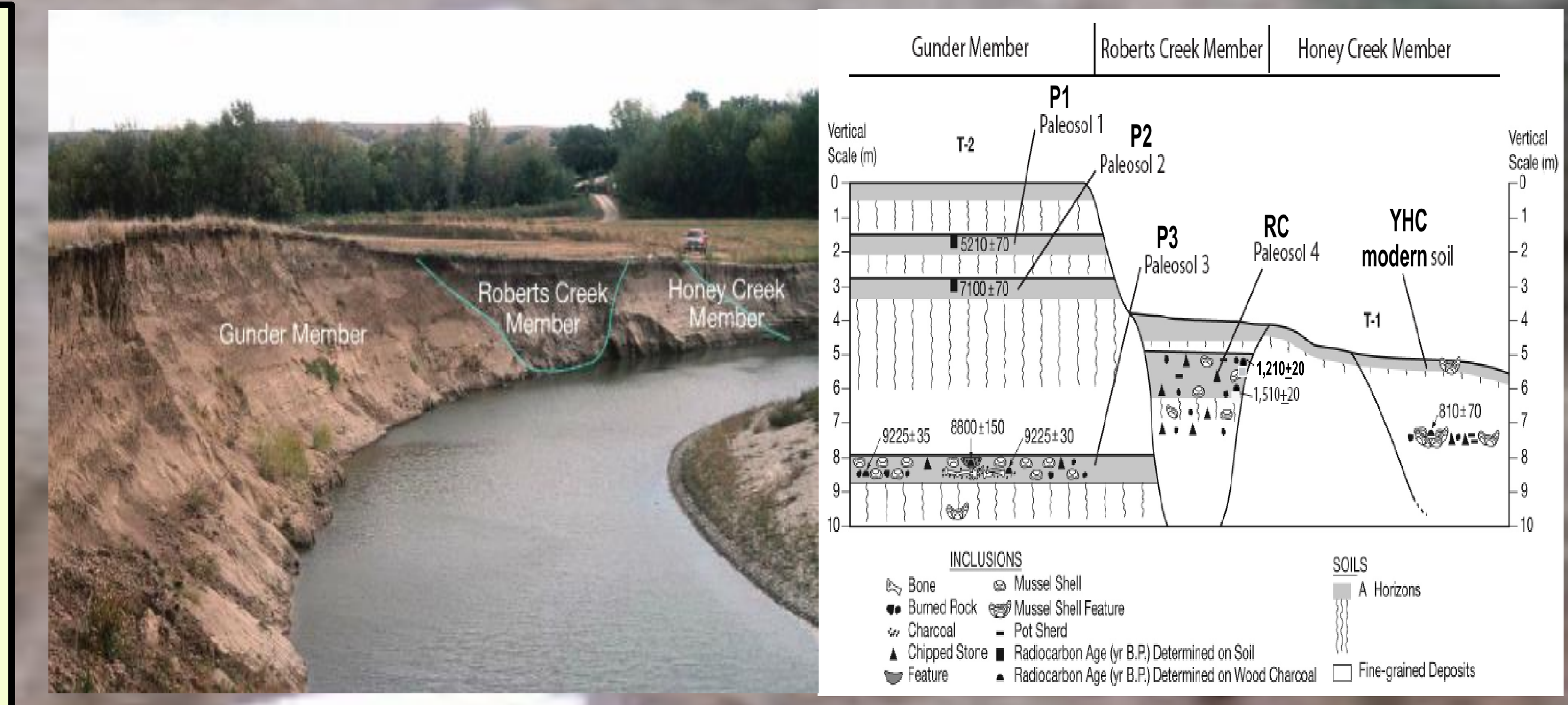

Figure 1. The cutbank at the Claussen site with its cross-sectional view shown on the right, revealing the soil stratigraphy and ages. (from Mandel et. al., 2006)

Table 1. Select chemical and physical characteristics of whole soil samples at the Claussen and Farwell sites.

Site	Depth	TC	TOC§	TN	pH†	Sand	Silt	Clay
	cm	---------- g kg^{-1} ----------				---------- g kg^{-1} ----------		
CLAUSSEN								
Honey Creek (YHC)	0-15	30	14	1.2	7.5	86	531	383
Honey Creek (YHC)	25-40	25	10	1.0	7.6	91	604	305
Robert Creek (RC)	82-107	13	10	0.8	7.7	22	575	404
Robert Creek (RC)	117-132	12	12	1.0	7.5	11	604	385
Gunder Paleosol 1	104-119	5	5	0.5	6.4	3	651	346
Gunder Paleosol 1	129-144	5	5	0.6	6.5	3	623	375
Gunder Paleosol 2	262-277	8	4	0.4	7.6	18	572	410
Gunder Paleosol 2	287-302	10	3	0.4	7.7	13	562	424
Gunder Paleosol 3	525-530	7	4	0.6	7.6	13	682	305
Gunder Paleosol 3	550-565	6	5	0.6	7.6	6	671	323
Gunder Paleosol 3	575-590	10	4	0.5	7.5	30	550	419
FARWELL								
65	0-20	24	21	1.5	7.7	64	680	256
65	20-50	17	15	1.3	6.5	45	625	330
65	90-135	7	6	0.8	6.1	13	621	366
65	135-155	16	13	1.0	6.3	6	644	350
65	155-175	8	7	0.7	6.4	3	621	375
65	175-202	9	8	0.6	6.4	17	644	386
65	202-217	5	5	0.5	6.6	68	606	326
64	0-20	11	9	1.1	6.9	21	544	435
64	20-41	7	6	0.7	7.3	16	555	429
64	109-134	6	5	0.8	8.1	21	627	352
64	134-156	5	4	0.7	7.8	31	638	331

n/a not available
§ HCl-treated samples
† 1:1 soil-water

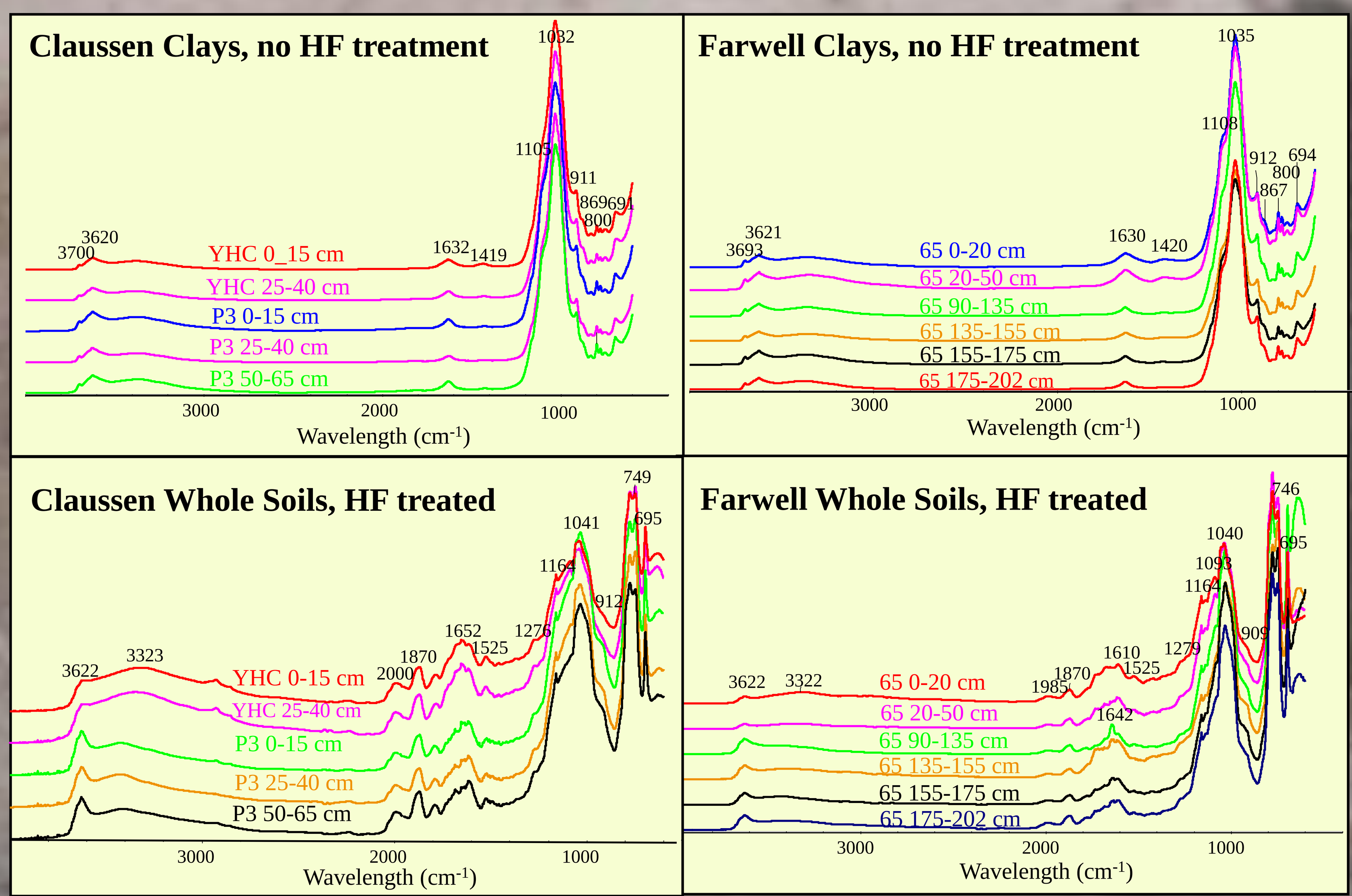

Figure 2. DRIFT scans of clay fractions and HF-treated whole soil samples of modern and buried soils from Claussen and Farwell sites.

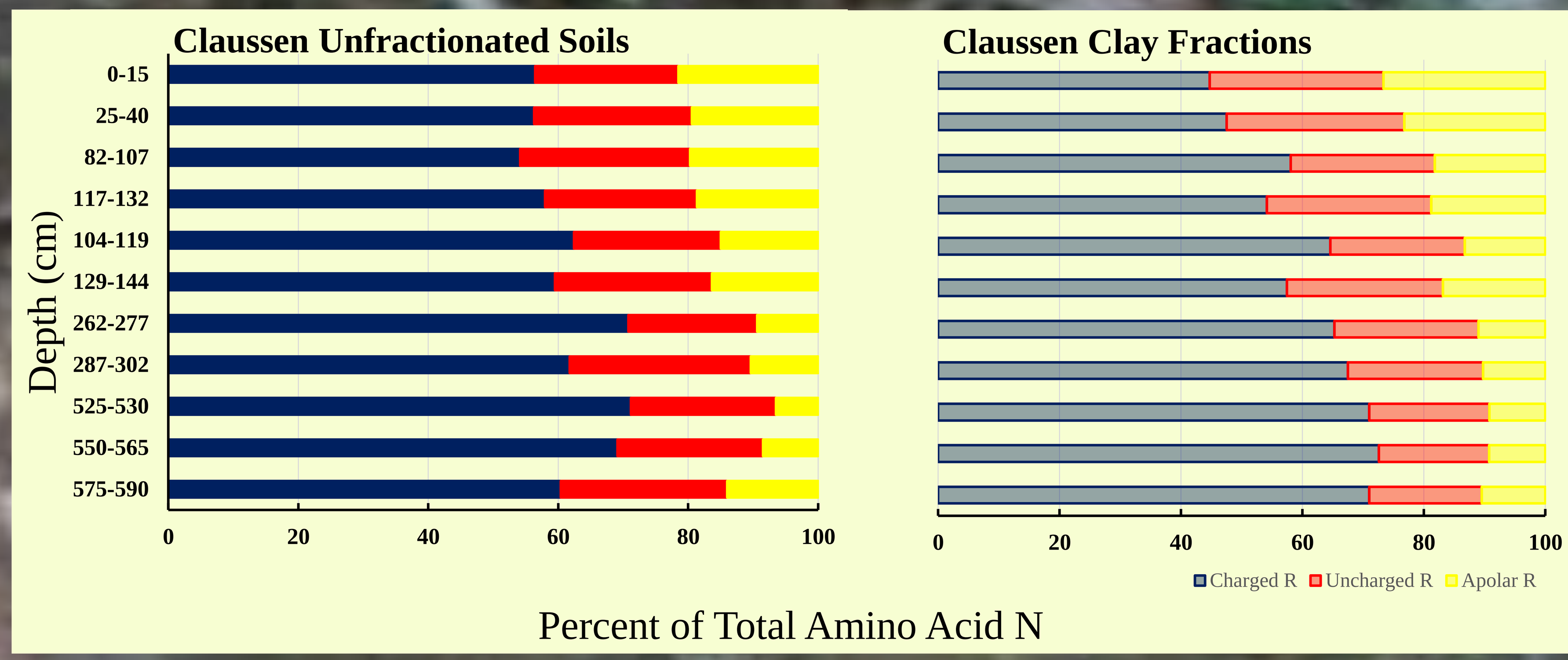

Figure 3. Amino acid distribution based on the attached R groups of Claussen unfractionated soils and their clay fractions.

Table 2. Amino acid distribution based on the attached R groups in Farwell whole soils and clay fractions.

Site	Depth	Total N	Amino Acid-N: Charged R¶	Amino Acid-N: Uncharged R#	Amino Acid-N: Apolar R§	Total Amino Acid N	AA as fraction of total N
	cm	---------- mg kg^{-1} ----------					%
WHOLE SOIL SAMPLES							
65	0-20	1918	296 (51)	145 (25)	137 (24)	578	30
65	20-50	1339	188 (55)	84 (25)	69 (20)	341	26
65	90-135	600	55 (58)	28 (29)	12 (13)	95	16
65	135-155	1104	84 (61)	32 (23)	22 (16)	138	12
65	155-175	674	83 (71)	23 (19)	11 (9)	116	20
65	175-202	739	61 (64)	24 (24)	12 (12)	96	13
64	0-20	1064	125 (54)	57 (25)	48 (21)	230	22
64	20-41	727	89 (55)	42 (26)	31 (19)	163	22
64	109-134	610	66 (68)	21 (22)	10 (10)	96	19
64	134-156	532	55 (72)	15 (20)	6 (8)	76	14
CLAY FRACTIONS							
65	0-20	3475	391 (60)	153 (23)	106 (16)	650	19
65	20-50	3154	316 (64)	111 (22)	70 (14)	497	16
65	90-135	1094	133 (69)	40 (20)	21 (11)	193	18
65	135-155	1498	178 (70)	49 (19)	27 (11)	254	17
65	155-175	1370	159 (72)	40 (18)	23 (10)	222	16
65	175-202	1415	146 (70)	40 (19)	22 (11)	207	15
64	0-20	1716	164 (63)	57 (22)	37 (14)	258	15
64	20-41	1090	138 (62)	50 (23)	33 (15)	221	20
64	109-134	1556	141 (78)	28 (15)	11 (6)	180	12
64	134-156	1349	123 (80)	21 (14)	9 (6)	153	11

Values in parentheses represent the % of total AA.
¶ Arg, Lys, Glu, Asp, His
# Gly, Thr, Ser, Cys, Tyr
§ Ala, Val, Pro, Leu, Iso, Met, Phe

SUMMARY AND CONCLUSION

- Clays at both sites revealed IR signatures at 1630 cm^{-1}, indicative of C=O bonds from carboxylic acid anions (Ellerbrock and Gerke, 2004; Celi et al., 1998) that may be associated with amides. The HF-concentrated whole soils demonstrated IR signals in about the same region of 1600-1650 cm^{-1}. In addition, the 1600 cm^{-1} signal obtained for HF-treated whole soils can be linked to the C=C that are conjugated with the C=O.
- In both Claussen and Farwell sites, AA concentrations decrease with as the period of burial increases. At Farwell, AA ranges from 13-30% of total N in whole soils while it is 11-19% of the total N in the clay fractions. Similarly, the AA in the Claussen whole soils ranged from 10-40% of the total N and was 14-19% of the total N in clays (data not shown).
- In both sites, the percentage of the total AA consisting of apolar R groups was higher in modern than buried soils. Conversely, the percentage of the total AA with charged R groups was higher in the buried than the modern soils.
- The qualitative analysis of AA has the potential in elucidating the historical sequence of the preservation of N as well as C in these Holocene paleosols.

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